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**A GENOMIC AND TRANSCRIPTOMIC APPROACH
TO CHARACTERIZE A NOVEL BIOCONTROL BACTERIUM
*LYSOBACTER CAPSICI AZ78***

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ABSTRACT - English

In the European Union (EU) the authorization of new active substances based on microbial agents is strictly regulated by several Regulations of the European Commission. A large amount of information is needed to achieve the authorization as: a) phylogenetic characterization, b) virulence traits; c) efficacy; d) mechanism of action; e) secondary metabolites production; f) toxic effects on the environment, human and animal. In the last years, there is an increasing interest in biopesticides based on microbial strains, including the bacterial genus *Lysobacter*, because of its biocontrol features. Specifically, *L. capsici* AZ78 is can effectively control phytopathogenic oomycetes, such as *Plasmopara viticola* e *Phytophthora infestans*. Although, some information about the biocontrol activity of the *Lysobacter* genus is available, the data that can be used to complete the registration dossier of *L. capsici* AZ78 are few. Therefore, the aim of the study was to characterize *L. capsici* AZ78. The genome of *L. capsici* AZ78 was sequenced and compared with two close-related pathogenic bacteria to confirm the lack of virulence factors to plant and human. The genome of *L. capsici* AZ78 includes a broad range of specific genes encoding lytic enzyme and *in vitro* trials confirmed the lytic activity on different substrates. The genome annotation showed the presence of genes involved in the biosynthesis of antibiotics and the antimicrobial activity of *L. capsici* AZ78 was tested *in vitro* against bacteria, oomycetes and fungi. The genome mining allowed the identification of genes encoding Type 4 Pilus (T4P), an interestingly motility mechanism that is involved in the host colonization, such as fungal hyphae. Moreover, the identification of a compound that positively modulates the expression of T4P genes and increases the bacterial efficacy against *P. viticola*, could be an important information for the final formulation of the bacterium. Genes involved in the resistance to environmental stressors and in the environmental fitness of *L. capsici* AZ78 under field conditions, were identified. Finally, the analysis of the *L. capsici* AZ78 transcriptome provided a complete overview of the mechanism of action displayed by the bacterium in the interaction with *P. infestans*. In conclusion the scientific literature produced during this work includes information that can speed up the preparation of the registration dossier of *L. capsici* AZ78.

ABSTRACT - Italian

Il processo di registrazione di nuovi principi attivi a base microbiologica è strettamente normato da Regolamenti dall'Unione Europea. Per ottenere l'autorizzazione all'uso come prodotto fitosanitario, nel dossier di registrazione è necessario fornire numerose informazioni relative al microrganismo: *a)* caratterizzazione filogenetica; *b)* caratteri di patogenicità; *c)* efficacia; *d)* meccanismo d'azione; *e)* produzione di metaboliti secondari; *f)* effetti tossici sull'ambiente e sugli organismi viventi. Negli ultimi anni c'è un crescente interesse nei confronti di prodotti fitosanitari di natura microbiologica, tra cui il genere batterico *Lysobacter* per le sue peculiari caratteristiche di biocontrollo. *Lysobacter capsici* AZ78 può controllare efficacemente importanti oomiceti fitopatogeni, come *Plasmopara viticola* e *Phytophthora infestans*. Sebbene siano disponibili informazioni generali sull'attività di biocontrollo del genere *Lysobacter*, poche sono le informazioni specifiche che possono essere utilizzate per la stesura del dossier di registrazione di *L. capsici* AZ78. Lo scopo di questo lavoro è quindi quello di ottenere una profonda caratterizzazione di *L. capsici* AZ78. Il genoma di *L. capsici* AZ78 è stato quindi sequenziato e successivamente analizzato comparandolo con i genomi di due specie patogene al fine di verificare l'assenza di fattori di virulenza verso l'uomo e le piante. Il genoma di *L. capsici* AZ78 include diversi geni specifici codificanti enzimi litici, la cui azione è stata evidenziata mediante prove *in vitro* su diversi substrati. Inoltre, l'annotazione di geni codificanti antibiotici è stata successivamente confermata mediante prove di antagonismo *in vitro* verso batteri, funghi e oomiceti. L'analisi del genoma ha consentito l'identificazione di geni coinvolti nella biogenesi di Pili di Tipo 4, un interessante meccanismo di motilità, utile per l'eventuale colonizzazione del ospite, come il micelio fungino. L'identificazione di un composto che influenza positivamente la produzione di pili e allo stesso tempo aumenta l'efficacia del batterio nel controllo di *P. viticola* è un dato da considerare per la futura formulazione del batterio. Il genoma è inoltre munito di geni relativi alla resistenza agli stress ambientali che possono influire sulla *fitness* del batterio in campo. Infine, l'analisi del trascrittoma di *L. capsici* AZ78 ha consentito l'identificazione di tutti i meccanismi d'azione messi in atto dal batterio nei confronti di *P. infestans*, fornendo un quadro chiaro dell'attività di biocontrollo di *L. capsici* AZ78. I risultati ottenuti durante questo lavoro di tesi contengono informazioni utili da inserire nel dossier per la registrazione di *L. capsici* AZ78, e velocizzarne il processo di registrazione.

CHAPTER 1

Introduction

1.1 Registration of biological control agents as plant protection products

In the European Union (EU) the authorization for the use as pesticide of a new microbial biocontrol agent is strictly regulated by the legislation. A large amount of information related to the specific biological traits of the microorganism of interest is requested for the authorization. This information is commonly included in the registration dossier in a specific section: the biological assessment dossier. The molecular characterization of the strain and the development of molecular markers to follow its fate in the environment or in food are key points to be addressed. The identification of the specific mechanism of action involved in the biocontrol activity and the production of secondary metabolites are other important aspects that shall be clarified. In addition, the efficacy against target organisms must be assessed and unacceptable secondary effects on human and animal health and the environmental impact must be excluded. In this context, the scientific literature can be used in the biological assessment dossier.

The current EU Regulations approved and standardized the use of scientific literature in the registration process. For example, the Regulations (EU) n° 283 and 284 of 2013 reported that information from peer-reviewed published scientific literature can be used in the risk assessment of plant protection products. An indication on the possible reference to peer-reviewed open literature was previously provided in Regulation (EC) 1107/2009, which reported that if the data coming from the peer-reviewed literature are considered relevant and reliable, the individual journal article, published within the last 10 years before the date of dossier submission, can be included in dossier. Scientific papers on the microorganism, microorganism's strains, metabolites, including information on side effects on health, fate in the environment and non-target species, that should be considered relevant may be used in the registration process. Indeed, the Regulation (EU) 284/2013 reported that *the most important and informative information -in literature- is obtained by the characterization and identification of the microorganism of interest. Such information "--" form the basis for an assessment of human health and environmental effects.*

The growing interest on biological control agent was triggered by the Directive 2009/128/CE that established a framework for Community action to achieve a more sustainable use of pesticides. Particularly, the Directive states that sustainable biological, physical and other non-chemical methods must be preferred to chemical

methods if they provide adequate pest control. The Regulation (EC) 889/2008 lists the alternatives to the synthetic chemical products that are allowed in organic production (pheromones, mineral salts, plant extracts and microorganisms).

For the approval of the use of a new microorganism strain as active substance, the Regulation (EC) 1107/2009 states that the substance (the microorganism itself and relevant related metabolites) shall meet specific requirements about toxicological, ecotoxicological and environmental effects. For instance, any effect on honeybee colony survival or development should be assessed and these data must be provided. Moreover, the term 'low-risk active substance' can be used if the active substance complies with all the approval criteria and lack of specific features (e.g. carcinogenic, toxic, persistent in the environment, etc.). Subsequently, the Regulation (EU) 546/2011 reports the complete list of criteria for the evaluation and authorization of a new plant protection product containing microorganisms. For instance, the microorganism identity shall be clearly established (discrimination between closely related strains) and the strain must be deposited in a certified culture collection. Furthermore, the microorganism's resistance to the main drugs used in human and veterinary medicine and the possibility of gene transfer that can mediate resistance to antimicrobial agents must be assessed. The mode of action of the microorganism must be characterized and the possible metabolites/toxins must be identified. Information on the mode of action is in general a valuable tool also to identify potential risks. Scientific literature can be an important source for the necessary data. For instance, the published literature on the maximum temperature of growth of the biocontrol microorganism can be used to exclude pathogenicity to human and animals, when it is below 36°C. The scientific literature can also provide information on the production of secondary metabolites, including their presence or absence in the formulated product and the environmental conditions of application. On the other hand, in the scientific information on specific metabolite toxicity profile, breakdown products or reaction products, and relative metabolite amount produced or not when the product is applied to the crop is not always available. In relation to the evaluation of risk arising from residues (on food and feed), the experimental data on levels of exposure to the microbial residues can be waived when the microorganism or its metabolites are not reported to be hazardous to humans may in the scientific literature. In conclusion, the scientific literature can play a significant to build the registration dossier for the use of a microorganism as plant protection product.

1.2 *Lysobacter* genus as a reservoir of novel candidates for plant protection products

The *Xanthomonadaceae* family (Saddler and Bradbury, 2005) belonging to *Gammaproteobacteria*, includes phylogenetically related Gram-negative bacteria having diverse origin and functions, as the genera *Xylella*, *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, and *Lysobacter* (Hayward et al., 2010). The species belonging to the genera *Xylella* and *Xanthomonas* (Van Sluys et al., 2002; Monteiro-Vitorello et al., 2005) include phytopathogenic or plant-associated bacteria (Hayward, 1993; Rudolph, 1993; Vauterin, 1996). The *Xanthomonas* genus includes many phytopathogenic species such as *X. axonopodis*, *X. campestris*, *X. oryzae* and *X. vesicatoria*, causal agents of severe diseases on economically important crops (e.g. bean, cabbage, citrus, hemp, pepper, rice, sugarcane, cotton or tomato) (Gabriel et al., 1989, Qian et al., 2005; Ezuka and Kaku, 2000; Denancé et al., 2016). The genus *Stenotrophomonas* has received particular attention due to the pathogenic activity of *Stenotrophomonas maltophilia* to humans (Denton and Kerr, 1998; Looney et al., 2009; Brooke, 2012). However, *S. maltophilia* also includes bacterial strains that have biocontrol activity against phytopathogenic microorganisms (Dunne et al., 1997; Kobayashi et al., 2002; Elhalag et al., 2016).

The establishment of the *Lysobacter* genus was proposed by Christensen and Cook (1978). Members of the genus *Lysobacter* are aerobic, non-fruiting, rod-shaped, gliding bacteria with high G + C content (61.7-70.1 %; Hayward et al., 2010), the bacterial colonies appeared mucoid or smooth, yellow-brownish in colour (Christensen and Cook, 1978). The predominant cellular fatty acids in the cells of the genus *Lysobacter* species are C_{16:0}, C_{11:0} iso, C_{11:0} iso 3-OH, C_{15:0} iso, C_{16:0} iso, C_{17:0} iso, and iso C_{17:1}ω9c, although their relative amounts differed between the recognized *Lysobacter* species (Bae et al., 2005, Romanenko et al., 2008). The following polar lipids are present in the genus *Lysobacter*: diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-N-methylethanolamine (Park et al., 2008).

Before the establishment of the *Lysobacter* genus, *Lysobacter* species were grouped into *Myxobacteriales*, and several *Lysobacter* strains were wrongly assigned to the *Stenotrophomonas* and *Xanthomonas* genera (Kobayashi and Yuen, 2007; Nakayama et al., 1999). Initially, the genus includes four species: *L. antibioticus*, *L. brunescens*, *L. enzymogenes* and *L. gummosus*. Currently, 37 species are included in the genus

Lysobacter (<http://www.bacterio.net>), such as the recently described *L. agri* (Singh et al., 2015), *L. hankyongensis*, *L. sediminicola* (Siddiqi and Im, 2016), *L. mobilis* (Yang et al., 2015) and *L. terrae* (Ngo et al., 2015). *Lysobacter* species occur in a wide range of environments (Yassin et al., 2007; Romanenko et al., 2008; Hayward et al., 2010), but they are frequently isolated from soil (*L. thermophilus*, Wei et al., 2012; *L. arseniciresistens*, Luo et al., 2012; *L. ximonensis*, Wang et al., 2009) or are associated to plant-rhizosphere (Park et al., 2008; Aslam et al., 2009; Zhou et al., 2014). Indeed, studies on *Rhizoctonia*-suppressive soils reported the association of soil suppressiveness with the presence of *Lysobacter* members, mainly belonging to *L. gummosus* and *L. antibioticus* species (Postma et al., 2008).

Members of *Lysobacter* genus are efficient antagonists and have been used as biological control agents of plant diseases (Kobayashi and Yuen, 2007; Hayward et al., 2010). In particular, some *Lysobacter* members, for example *L. enzymogenes* strains 3.1T8, C3 and OH11, have antifungal and antimicrobial properties, mainly linked to the production of extracellular lytic enzymes and antibiotics (Yuen et al., 2006; Sullivan et al., 2003; Folman et al., 2003; Palumbo et al., 2005; Qian et al., 2009). Biological control features were reported also for other *Lysobacter* species, such as *L. antibioticus* (Ji et al., 2008; Ko et al., 2009), *L. capsici* (Park et al., 2008; Puopolo et al., 2014a) and *Lysobacter* sp. SB-K88 (Islam et al., 2005).

Studies on biocontrol activity carried out on *Lysobacter* members were mainly focused on the suppression of soil-borne diseases caused by *Phytophthora* spp., *Pythium* spp., *Fusarium* spp. and *Rhizoctonia solani* (**Table 1**) and root-knot nematodes (Chen et al. 2006; Nour et al., 2003). A considerable number of works focused on *L. enzymogenes* C3 and its lytic potential against *Magnaporthe poae* (Kobayashi and Yuen, 2005), *P. ultimum* (Palumbo et al., 2005) and *P. capsici* (Kim et al., 2008). Some studies report the application of *Lysobacter* members in leaf pathosystems, such as *L. capsici* AZ78 and *L. enzymogenes* C3 for the biocontrol of grapevine downy mildew and fusarium head blight, respectively (Puopolo et al., 2014a,b; Yuen et al. 2003). The *L. antibioticus* is another relevant species in term of biocontrol activity and different strains, HS124, MAD009 and YFY 02, were studied against *Plasmodiophora*, *Phytophthora* and *Pythium* (Rondon et al., 1999; Ko et al. 2009; Zhou et al., 2014). Noteworthy, only one *in vivo* study was carried out to assess the antibacterial activity of *L. antibioticus* 13-1 on *X. oryzae* pv. *oryzae* (Ji et al. 2008).

Table 1. Reports of biological control of plant disease by *Lysobacter* species

Bacterial strain	Plant	Disease	Pathogen	Reference
<i>Lysobacter</i> sp. SB-K88	<i>Beta vulgaris</i> L.	Damping-off of sugar beet	<i>Pythium</i> sp. <i>Aphanomyces cochlioides</i>	Nakayama et al. 1999; Islam et al. 2005
<i>L. antibioticus</i> 13-1	<i>Oryza sativa</i> L.	Bacterial leaf blight	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Ji et al. 2008
<i>L. antibioticus</i> HS124	<i>Capsicum annuum</i> L.	Phytophthora blight	<i>Phytophthora capsici</i>	Ko et al. 2009
<i>L. antibioticus</i> MAD009	<i>Solanum lycopersicum</i> L.	Pythium damping-off Fusarium wilt	<i>Pythium torulosum</i> <i>Fusarium oxysporum</i>	Rondon et al. 1999
<i>L. antibioticus</i> YFY 02 and HY	<i>Brassica campestris</i> L.	Club root of crucifers	<i>Plasmodiophora brassicae</i>	Zhou et al. 2014
<i>L. capsici</i> AZ78	<i>Vitis vinifera</i> L.	Grapevine downy mildew	<i>Plasmopara viticola</i>	Puopolo et al. 2014 a, b
	<i>Solanum lycopersicum</i> L.	Tomato late blight	<i>Phytophthora infestans</i>	
<i>L. capsici</i> PG4	<i>Solanum lycopersicum</i> L.	Tomato crown and root rot	<i>Fusarium oxysporum</i>	Puopolo et al. 2010
<i>L. capsici</i> YS1215	<i>Solanum lycopersicum</i> L.	Plant root-knot	<i>Meloidogyne incognita</i>	Lee et al. 2014 a,b;
<i>L. enzymogenes</i> 3.1T8	<i>Cucumis sativa</i> L.	Damping-off of cucumber	<i>Pythium aphanidermatum</i>	Folman et al. 2003; Postma et al. 2009
<i>L. enzymogenes</i> C3	<i>Festuca arundinacea</i> Schreb	Leaf spot on tall fescue	<i>Bipolaris sorokiniana</i>	Giesler and Yuen 1998; Zhang and Yuen 2000; Li et al., 2008
		Tall fescue brown patch	<i>Rhizoctonia solani</i>	Zhang et al. 2001; Palumbo et al. 2005; Kobayashi et al. 2005
	<i>Poa pratensis</i> L.	Summer patch disease	<i>Magnaporthe poae</i>	Kobayashi and Yuen 2007
	<i>Triticum aestivum</i> L.	Fusarium head blight	<i>Fusarium graminearum</i>	Yuen et al. 2003; Jochum et al. 2006;
	<i>Beta vulgaris</i> L.	Pythium damping-off	<i>Pythium ultimum</i> var. <i>ultimum</i>	Palumbo et al. 2005
	<i>Phaseolus vulgaris</i> L.	Bean rust	<i>Uromyces appendiculatus</i>	Yuen and Zhang 2001
	<i>Capsicum annuum</i> L.	Phytophthora blight	<i>Phytophthora capsici</i>	Kim et al., 2008

1.3 Bioactive natural compounds produced by *Lysobacter* members

In the recent years, the production, by the *Lysobacter* members, of a large number of lytic enzymes and secondary metabolites with antibiotic activity was reported (Folman et al., 2003; Xie et al., 2012), making this bacterial genus an interesting source for the discovery of new compounds, suitable both for plant protection products and pharmaceutical uses (e.g. lysoamidase complex; Stepnaya et al., 2004).

1.3.1 Compounds protective against sunlight

The compounds of the *Lysobacter* genus responsible for the yellow-brownish appearance of the bacterial colonies serve as cell protectant against UV-light and they were identified in *L. enzymogenes* OH11 (Wang et al., 2013). The main *Lysobacter* pigment is a xanthomonadin-like aryl polyene metabolite (**Fig. 1**). These are distinctive pigments produced by species belonging to the *Xanthomonas* genus, which are also used as chemotaxonomic markers to distinguish *Xanthomonas* species based on the different patterns of bromination and methylation on the aryl polyene skeleton (Starr et al., 1977).

The biosynthetic gene cluster involved in the pigment production of *L. enzymogenes* OH11 is composed of 17 putative open reading frames (ORF) and includes also transport-related genes (Wang et al., 2013). The main biosynthetic complex is the Type II polyketide synthase (PKS) that is an unusual pathway for polyene compounds biosynthesis (Wang et al., 2013). The polyene-type polyketides are usually synthesized by the modular type I Polyketide Synthase (PKS) (Staunton and Weissman, 2001), such as the PKSs involved in the production of antifungal compounds, i.e. amphotericin B (*Streptomyces noursei*; Brautaset et al., 2000), olygomycin (*S. avermitilis*; Ikeda et al., 2003) and cephabacin (*L. lactamgenus*; Lee et al., 2008).

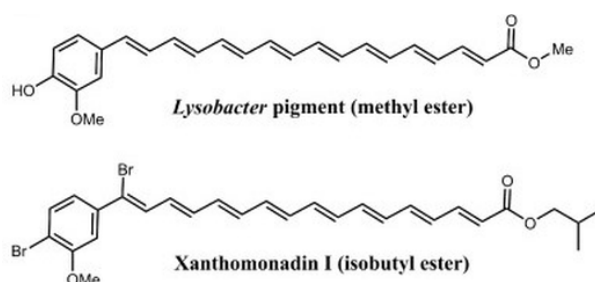


Figure 1: The proposed chemical structure of the yellow *Lysobacter*'s pigment. (yellow pigment of *L. enzymogenes* OH11) and the xanthomonadin I from *Xanthomonas campestris* pv. *campestris*. (Modified from Wang et al., 2013).

Similarly to *Xanthomonas*, *Lysobacter* spp. are ubiquitous environmental bacteria, for instance the *L. enzymogenes* OH11 was isolated from pepper rhizosphere (Qian et al., 2009), while the strain C3 was recovered from the surface of grass leaves (Giesler et al., 1998). When *Lysobacter* cells are colonizing leaves, they are exposed to photo-oxidative stress and therefore the biosynthesis of photoprotective compounds is crucial to allow the survival of the bacterial cells in the phyllosphere (Poplawsky et al., 2000). Indeed, the pigment deficient mutants generated by Wang et al. (2013) were significantly more sensitive to UV and visible light radiations, as well as H₂O₂ treatment.

1.3.2 Organic acids

Lactic acid was identified in the bacterial culture filtrate of *L. capsici* YS1215 by Lee et al. (2014a). This organic acid has an ovicidal activity against root-knot nematodes, such as *Meloidogyne incognita*, which can cause severe damages to several crops. The study reports a significant inhibition effect on nematode egg hatching due to the increasing concentrations of bacterial culture filtrate applied. However, the nematicidal activity of this bacterial strain could be due not only to organic acids, but also to other active compounds (Lee et al., 2014a,b).

1.3.3 Lytic enzymes

The name of the *Lysobacter* genus is inspired by its lytic effects on a broad range of (micro)organisms, such as algae, bacteria, fungi, nematodes and oomycetes (Christensen and Cook, 1978). The *Lysobacter*'s lytic activity has been correlated with the production of a wide-spectrum of extracellular enzymes, which are active in the degradation of cellulose, chitin, glucans, lipids and proteins (**Table 2**). Despite these traits are commonly well-studied, few information is available on the specific hydrolytic enzymes that the *Lysobacter* members produce and the role played by each enzyme class in the biocontrol activity of the *Lysobacter* species (Palumbo et al., 2003).

Glucanases

Several studies demonstrated the inhibitory effects of exogenous bacterial β -1,3-glucanases against fungi or oomycetes; indeed glucans are essential structural cell wall components of both the microorganisms (Bartnicki-Garcia, 1968; Mérida et al., 2013).

The strains *L. enzymogenes* C3 (Palumbo et al., 2005) and N4-7 (Palumbo et al., 2003) produced three extracellular β -1,3-glucanases encoded by *gluA*, *gluB* and *gluC* genes. The creation of knockout mutants allowed defining that the total β -1,3-glucanase activity of *L. enzymogenes* C3 cells depended only on the three identified genes (Palumbo et al., 2005) (**Fig. 2**). The purification and the subsequent characterization of the three enzymes revealed that the hydrolysing activity is optimal at pH 4.5-5.0 (Palumbo et al., 2003). These optimal pH values are consistent with the acid fungal hyphae environment, which is the possible site of action of biocontrol *Lysobacter* strains (Griffen, 1994). In support of the hypothesis of a direct role of glucanases on the biological activity of *L. enzymogenes* C3, the antagonism activity of the knockout mutant for *gluABC* was negatively affected in the biocontrol assays against *Pythium* damping-off of sugar beet and *Bipolaris* leaf spot of tall fescue (Palumbo et al., 2005).

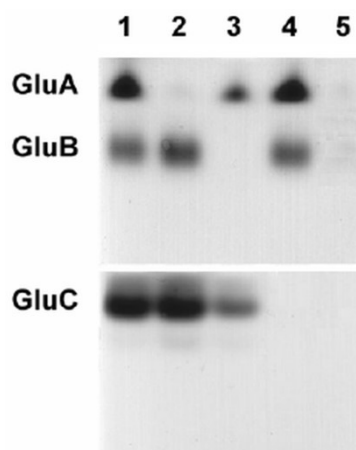


Figure 2: β -1,3-glucanases activity from culture filtrates of *Lysobacter enzymogenes* wild-type and knockout mutant strains. 1, wild-type strain C3; 2, *gluA* mutant; 3, *gluB* mutant; 4, *gluC* mutant; 5, *gluABC* mutant. (Modified from Palumbo *et al.*, 2005).

In addition, Ogura et al. (2006) purified from *Lysobacter* sp. IB-9374 a cellulase enzyme (β -1,4-glucanase) that also display chitinases activity (Cel8A). The Cel8A showed from 41.5 to 46.2% homology at the amino acid level with the conserved sequence of GFH-8 (chitinase-glucanase) of *Bacillus* sp. and *Peanibacillus fukuinensis*. Specifically, Cel8A showed the maximum activity in the hydrolysis of carboxymethylcellulose (CMC) compared to other chitosan compounds.

Chitinases

The role of chitinase production in the biocontrol mechanism of *Lysobacter* members was firstly investigated by Zhang and Yeun (2000). These authors proved that the chitinolytic activity is one of the mechanisms involved in the biological control of *B. sorokiniana* by *S. maltophilia* strain C3 (later named *L. enzymogenes* C3), both *in vivo* and *in vitro* experiments. The chitinolytic fractions of *L. enzymogenes* C3 caused

conidial deformation and abnormal germ tube formation in fungi (Zhang et al., 2001), and two specific proteins of 32 and 48 kDa were identified. Zhu et al. (2008) isolated a chitinase gene (*chiA*) from *L. enzymogenes* OH11, which was cloned and expressed in *Escherichia coli*. The *chiA* homologues were identified in the genome of *L. antibioticus* strain ATCC 29479 and 76, *L. gummosus* 3.2.11 (de Bruijn et al., 2015). Besides the biocontrol of phytopathogenic fungi, the activity of a secreted chitinase from *L. capsici* YS1215 was evaluated against nematodes. Results showed that the purified enzyme degraded the chitin layer of the eggshells and affected the second-stage juveniles in *M. incognita* (Lee et al., 2014b).

Proteases

Different studies focused on the broad range proteolytic activity of the genus *Lysobacter*. The first research focused on α -lytic proteases (extracellular serine protease) purified from the culture filtrate of *L. enzymogenes* 495 in 1965 by Whitaker, followed by the investigation of the mechanism and action of an inhibitory complex of the enzyme (Bone et al., 1987). Vasilyeva et al. (2008) isolated and characterized the α -lytic protease L5 from *Lysobacter* sp. XL1. The enzyme L5 is released from the bacterial cell through the formation of outer membrane vesicles filled with the proteases. Besides, another secreted lytic endopeptidase (L1) was previously identified and, conversely to L5, is directly secreted into the medium (Lapteva, 2012). The gene cluster involved in the biosynthesis of these enzymes was indicated as *alpAB*, moreover the analysis of the relative expression level of both the genes revealed that the production of L1 was much higher than L5 in *Lysobacter* sp. XL1 cells (Lapteva, 2012). The biosynthetic gene *alpA* was also found in *L. antibioticus* ATCC29479, 76, *L. gummosus* 3.2.11 and *L. enzymogenes* C3 (de Bruijn et al., 2015). Furthermore, the bacteriolytic activity of α -lytic proteases was demonstrated against several soil bacteria, such as *Erwinia carotovora* (Gökçen et al., 2014, Vasilyeva et al., 2014).

Achromobacter lyticus together with *L. enzymogenes* produced lysine-specific proteases (A-LEP), such as Lys-C, useful in biotechnology to determine the protein primary structures (Masaki et al., 1978; Jekel et al., 1983). Moreover, *Lysobacter* sp. IB-9374 produces a lysyl endopeptidase (LepA) identical to A-LEP. Interestingly, the amount of enzyme secreted into the medium was 6-12 fold higher than the one produced by *L. enzymogenes* ATCC 29487 (Chohnan et al., 2002). A second lysyl endopeptidase (LepB) and the correlated biosynthetic gene *lepB* was found upstream of *lepA* (Chohnan

et al., 2004). Moreover, *Lysobacter* sp. IB-9374 produces β -lytic protease (metalloendopeptidases) initially discovered in the culture medium of *L. enzymogenes* 495 (Whitaker, 1965) and active against Gram-positive bacteria, such as *B. subtilis* and *Staphylococcus aureus* (Ahmed et al., 2003).

Lysobacter capsici YS1215 produces gelatinolytic protein, probably belonging to the group of metallopeptidases, which displayed a significant role as a nematicidal compound Lee et al. (2013a). The purified enzyme is active against the second-stage juveniles of *M. incognita*. Another metallopeptidase with keratinolytic activity was isolated and characterized from *Lysobacter* NCIMB 9497. This was the first detailed report of the keratinolytic activity of bacterial metalloprotease. The novel reaction mechanism has a great biotechnological potential in the leather industry, and in the processing of poultry waste (Allpress et al., 2002).

The *L. gummosus* DSMZ 6980 genome includes different genes encoding five α -lytic proteases, one β -lytic protease and three lysis endopeptidases. The effective role of these enzymes in the degradation of bacterial cell wall and bacterial biofilm could be a starting point for the development of new products useful in the sanitization of industrial and medical devices from pathogenic bacteria, such as *S. aureus* and *S. epidermidis* (Gökçen et al., 2014).

Lipases

The ability to degrade polysorbate-type nonionic surfactants, such as Tween 20 or Tween 80, is usually tested to characterize phenotypically the new *Lysobacter* sp. nov. (Smibert and Krieg, 1994): for instance *L. terrae* THG-A13^T, *L. niabensis* KACC 11587^T and *L. oryzae* KACC 14553^T were reported to hydrolyze both the detergent compounds, instead of *L. yangpyeongensis* KACC 11407^T and *L. enzymogenes* KACC 10127^T that are able to degrade only Tween 20 (composition: lauric acid $\geq 40\%$) or Tween 80 (composition: oleic acid $\geq 58.0\%$), respectively (Ngo et al., 2015).

Moreover, the lipolytic activity is a specific feature that differs between strains belonging to the same *Lysobacter* species, as in the case of *L. enzymogenes*. Indeed, the strain 3.1T8 cannot degrade Tween 80 (Folman et al., 2003), while the strain C3 shows hydrolysing activity (Kobayashi et al., 2005). Von Tigerstrom and Stelmaschuk (1989) identified two major extracellular esterases produced by *L. enzymogenes* ATCC 2987, one released in the culture's broth and the other was associated to the cell wall.

However, both the lipolytic enzymes, were not further investigated due to the issues occurred during the purification steps.

Table 2. List of bioactive compounds produced by *Lysobacter* members

Bioactive compound	Biosynthetic pathway	Species	Activity	Reference
xanthomonadin	<i>PKS</i>	<i>L. enzymogenes</i> OH11	UV-light Visible light H ₂ O ₂	Wang et al., 2013
Lactic acid		<i>L. capsici</i> YS1215	<i>Meloidogyne incognita</i>	Lee et al., 2014b
β-1,3-glucanase	<i>gluABC</i>	<i>L. enzymogenes</i> C3	<i>Pythium ultimum</i> <i>Bipolaris sorokiniana</i>	Palumbo et al., 2005
		<i>L. enzymogenes</i> N4-7		Palumbo et al., 2003
β-1,4-glucanase chitinase	<i>cel8A</i> <i>chiA</i>	<i>Lysobacter</i> sp. IB-9374 <i>L. enzymogenes</i> C3 <i>L. enzymogenes</i> OH11	<i>Bipolaris sorokiniana</i>	Ogura et al., 2006 Zhang and Yeun, 2000 Zhang et al., 2001 Zhu et al., 2008
		<i>L. capsici</i> YS1215	<i>Meloidogyne incognita</i>	Lee et al., 2014a
α-lytic proteases L1 and L5	<i>alpAB</i>	<i>Lysobacter</i> sp. XL1	<i>Soil bacteria</i>	Lapteva, 2012 Vasilyeva et al., 2008 Vasilyeva et al., 2014
lysine-specific protease	<i>lepA</i> <i>lepB</i>	<i>Lysobacter</i> sp. IB-9374 <i>L. enzymogenes</i> ATCC 29487		Chohnan et al., 2002 Chohnan et al., 2004
β-lytic protease		<i>Lysobacter</i> sp. IB-9374 <i>L. capsici</i> YS1215 <i>Lysobacter</i> NCIMB 9497	<i>Bacillus subtilis</i> <i>Staphylococcus aureus</i> <i>Meloidogyne incognita</i>	Ahmed et al., 2003 Lee et al., 2013b Allpress et al., 2002
esterase		<i>L. enzymogenes</i> ATCC 2987		Von Tigerstrom and Stelmaschuk, 1989

The lysoamidase complex

The lysoamidase is a drug isolated from the culture liquid of *Lysobacter* sp. XL (Stepnaya et al., 1993). Specifically, lysoamidase is a complex of exopolysaccharide and lytic enzymes, which includes an extremely active metalloprotease and two enzymes having proteinase and bacteriolytic activity (endopeptidases L1 and L5; muramidase, and *N*-acetylmuramoyl-l-alanine amidase) (Lapteva et al., 2012). Due to the characteristic of the lytic complex, this drug was mainly used to control Gram-positive external infection. The antibacterial activity of lysoamidase did not boost the impairment of patient immune status, rather showed an immunostimulating effect on human. Moreover, the efficacy against a broad range of microorganisms, such as Gram-negative bacteria, yeast cells and fungi, was reported (Ryazanova et al., 2005).

1.3.4 Antibiotics

The *Lysobacter* genus is a source of bioactive small secondary metabolites active as antibiotics. This characteristic can be suitable for the research of new bioactive compounds in human medicine. The genomes of *Lysobacter* spp. contain a pool of genes encoding non-ribosomal peptide synthetases (NRPSs) and PKS that are mainly involved in the *Lysobacter* antibiotic production and the biocontrol activity against fungi and bacteria (**Table 3**) (Xie et al., 2012).

Cyclodepsipeptides

The lysobactin (katanosin B) is a macrocyclic depsipeptide compound isolated from *Lysobacter* sp. ATCC 53042 (Tymiak et al., 1898). Lysobactin has strong antibacterial activity against many Gram-positive and Gram-negative bacteria. In particular, the compound is highly effective against human infection caused by Gram-positive bacteria, such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). For instance, the effective dose of lysobactin was two to fifty fold lower than the vancomycin one (Maki et al., 2001). The lysobactin mode of action is based on the inhibition of the bacterial cell wall biosynthesis by impairing the peptidoglycan and lipid intermediate formation (Maki et al., 2001). The gene cluster responsible for the antibiotic biosynthesis in *Lysobacter* sp. ATCC 53042 (**Fig. 3**) is composed by two genes encoding NRPSs (LybA and LybB), one ABC-transporter permease (ORF79) putatively involved in the secretion of lysobactin and three proteins conferring resistance to antibiotics (ORF78, ORF80, and ORF82) (Hou et al. 2011). The lysobactin gene cluster was also identified in *L. gummosus* 3.2.11 (de Bruijn et al., 2015). The features of lysobactin make it a promising new compound especially in the view of creating analogs with improved pharmacological properties (Hou et al., 2011).

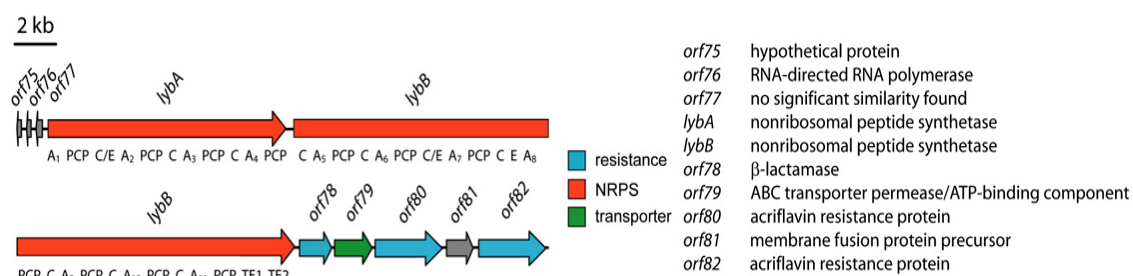


Figure 3: Lysobactin biosynthetic gene cluster of *Lysobacter* sp. ATCC 53042. The putative functions of the proteins encoded within the region are based on BLAST analysis and are reported in the figure on the right. (Modified from Hou *et al.*, 2011).

Cyclic lipodepsipeptides

Cyclic lipodepsipeptides differ from cyclodepsipeptides because of the presence of a β -hydroxyl fatty acid chain. Strains belonging to *Lysobacter* genus produce two families of cyclic lipodepsipeptides: tripropeptins and WAP-8294A family (Xie et al., 2012). The tripropeptin C was isolated from *Lysobacter* sp. BMK333-48F3 by Hashizume et al. (2001) and the putative mechanism of action involves the impairment of the lipid cycle of cell wall biosynthesis (Hashizume et al., 2011). The WAP-8294A was initially isolated from *L. staphylocidin* in Japan in 1997 (Blackledge, 2011). The genes involved in the biosynthesis of WAP-8294A were identified and characterized in the genome of *L. enzymogenes* OH11; the gene cluster containing two NRPSs (*WAPSI* and *WAPS2*) was found to be the genetic core of the antibiotic production. Both the cyclic lipodepsipeptides described, have a strong efficacy against MRSA and VRE (Hashizume et al., 2011; Zhang et al., 2011).

Cephem-type β -lactams

The cephem-type β -lactam antibiotics encompassed the cephabacins, which was firstly isolated from *L. lactamgenus* YK90 (Harada et al., 1984). The biosynthetic pathways of cephem-antibiotics were initially studied in actinomycetes and fungi (Brakhage et al., 2009). These antibiotics are composed of a cephem nucleus, an acetate residue and an oligopeptide side chain. The particular chemical structure makes the cephabacins resistant to the hydrolysis of various β -lactamases. In *L. lactamgenus* (IFO-14288), the genes involved in the biosynthesis of cephabacins are *pcbC* and *pcbAB* encoding a key enzyme in penicillin/cephalosporin production and an NRPS, respectively (Sohn et al., 2001). Additionally ORFs located upstream *pcbAB*-cluster were found to encode a hybrid PKS-NRPS (*cpbI*) and an NRPS (*cpbK*), responsible for the oligopeptide chain incorporation in the cephabacin biosynthesis (Lee et al., 2008; Demirev et al., 2006).

Cyclic tetramate macrolactams

Dihydromaltophilin was isolated from *Streptomyces* and *Lysobacter* strains. This antibiotic has a strong inhibitory activity against a broad range of fungi and oomycetes. When complexed with macrocyclic lactams, the dihydromaltophilin produced by *Lysobacter* genus are named Heat Stable Antifungal Factor (HSAF) (Yu et al., 2007). The HSAF activity was initially studied against *Aspergillus nidulans* (Li et al., 2006). These compounds interfere in the biosynthesis of sphingolipids by blocking a

filamentous fungi's specific enzyme (ceramide synthase), the final effect is the accumulation of intermediates and the subsequent stop of elongation of the hyphal tips. Moreover, the HSAF purified from *L. enzymogenes* C3 can inhibit the germination of *B. sorokiniana* conidia. In experiments on plants, the mutant strains for PKS-NRPS domains had a reduced efficacy to control disease incidence and severity of *Bipolaris* leaf spot (Li et al., 2008). Thus, gene locus including PKS-NRPS is responsible for the HSAF production in both in *L. enzymogenes* C3 and *L. enzymogenes* OH11. The presence of the dihydromaltophilin gene cluster was reported also in *L. gummosus* 3.2.11 (de Bruijn et al., 2015). Another group of antibiotics included in cyclic tetramate macrolactame family is xanthobaccin. Specifically, in *Lysobacter* (previously *Stenotrophomonas*) sp. strain SB-K88 were identified three xanthobaccins A, B, and C involved in the suppression of damping-off disease of sugar beet (Nakayama et al., 1999). These antibiotics showed growth inhibitory activity on *Aphanomyces cochlioides*, *P. ultimum*, *R. solani* causal agents of damping-off and other seven plant pathogens (*P. viciae*) *in vitro* experiments. Interestingly, xanthobaccin A was directly recovered from sugar beet rhizosphere of bacterized seeds, and the application of purified molecule suppressed damping-off disease in *Pythium* spp. infected soil. Islam et al. (2005) focused on the lytic effect of xanthobaccin A on *Peronosporomycetes* zoospores.

Other bioactive compounds isolated from Lysobacter members

In addition to the four previously reported classes, other natural products synthesized by members of the *Lysobacter* genus were reported. The production of phenazine N-oxide myxin was initially reported by Peterson et al. (1966), long before the establishment of the *Lysobacter* genus (Christensen and Cook, 1978). Subsequently, other studies showed that *L. antibioticus* UASM 3C can produce 1-hydroxy-6-methoxyphenazine 5,10-dioxide (myxin), which has antioomycete activity (Cook et al., 1971; Christensen and Cook, 1978). Putative phenazine biosynthetic genes were predicted for *L. antibioticus* strain ATCC 29479 and 76 (de Bruijn et al., 2015). The myxin chelated with copper ion is a commercial antibiotic product (Unitrop®) used in animal-skin infection treatment. The inhibition of the DNA synthesis is the possible mechanism of action involved, however the biosynthetic pathway of myxin was not fully investigated so far (Xie et al., 2012).

The production of 4-hydroxyphenylacetic acid in *L. antibioticus* HS124 is related to antioomycete and antifungal activity against *P. capsici* (Ko et al., 2009), while the antifungal compound identified in *L. gummosus* AB161361 is 2,4-diacetylphloroglucinol (Brucker et al., 2008). Furthermore, the metabolite lactivicin was isolated in *L. albus* YK-422 (Xie et al., 2012). The lactivicin is mainly active against Gram-positive bacteria; the mode of action is similar to the one of the β -lactams, but the antibiotic biosynthesis has not been described yet.

Lysobacter capsici AZ78, is involved in the biosynthesis of a proline derivatives of 2,5-diketopiperazine family [cyclo(L-Pro-L-Tyr)] and it is probably encoded by a genomic region which includes a PKS-NRPS. Specifically, the compound displayed toxic activity against sporangia of phytopathogenic oomycetes, such as *P. infestans* and *P. viticola*. Moreover, the application of purified antibiotic on tomato leaves prevented the occurrence of late blight lesions (Puopolo et al., 2014b).

Table 3. List of antibiotic compounds produced by *Lysobacter* members

Antibiotic	Biosynthetic pathway	Species	Activity	Reference
lysobactin (katanosin B)	NRPS (<i>lybA</i> , <i>lybB</i>)	<i>Lysobacter</i> sp. ATCC 53042	Antibacterial	Hou et al., 2011 Maki et al., 2001 Tymiak et al., 1898
tripropeptin C		<i>Lysobacter</i> sp. BMK333-48F3	Antibacterial	Hashizume et al., 2001 Hashizume et al., 2011
WAP-8294A	NRPS (<i>WAPS1</i> , <i>WAPS2</i>)	<i>L. staphylocidin</i> <i>L. enzymogenes</i> OH11	Antibacterial	Blackledge, 2011 Zhang et al., 2011
cephabacins	PKS-NRPS (<i>pcbC</i> , <i>pcbAB</i> , <i>cpbI</i> , <i>cpbK</i>)	<i>L. lactamgenus</i> strain YK90 and IFO-14288	Antifungal Antioomycete	Demirev et al., 2006 Harada et al., 1984 Lee et al., 2008 Sohn et al., 2001
dihydromaltophilin (HSAF)	PKS-NRPS	<i>L. enzymogenes</i> C3 <i>L. enzymogenes</i> OH11	Antifungal Antioomycete	Li et al., 2006 Li et al., 2008
xanthobaccins A, B, and C		<i>Lysobacter</i> sp. strain SB-K88	Antifungal Antioomycete	Islam et al., 2005 Nakayama et al., 1999
1-hydroxy-6-methoxyphenazine 5,10-dioxide (myxin)		<i>L. antibioticus</i> UASM 3C		Christensen and Cook, 1978 Cook et al., 1971;
4-hydroxy phenylacetic acid		<i>L. antibioticus</i> HS124	Antioomycete	Ko et al., 2009
2,4-diacetyl phloroglucinol		<i>L. gummosus</i> AB161361	Antifungal	Brucker et al., 2008
lactivicin		<i>L. albus</i> YK-422	Antibacterial	Stawikowska et al., 2009
2,5-diketo piperazine[cyclo(L-Pro-L-Tyr)]	PKS-NRPS	<i>L. capsici</i> AZ78	Antioomycete	Puopolo et al., 2014b

1.4 The regulation of biocontrol activity in *Lysobacter* genus

To date, the background of information about the regulatory mechanisms of *Lysobacter* biocontrol activity was mainly focused on the role of the *clp* gene. Kobayashi et al. (2005) characterized for the first time a *clp* homologue in *L. enzymogenes* C3. The *clp* gene belongs to the global regulator gene family Crp and is responsible for the biosynthesis of Clp a cyclic adenosine monophosphate (cAMP)-like protein. The Crp-regulator role in the pathogenic mechanisms of phytopathogenic bacteria (e.g. *X. campestris* pv *campestris* and *E. chrysanthemi*) has been extensively studied (Reverchon et al., 1997; Chin et al., 2010). In *Pseudomonas aeruginosa*, a member of the Crp family, the *yfr* gene, is responsible for several virulence traits, such as twitching motility, quorum sensing and toxin production (Beatson et al., 2002). The *clp* has a role in the antimicrobial activity and lytic enzyme production (chitinases, β -1,3-glucanases, proteases) (Kobayashi et al. (2005). In fact, the *L. enzymogenes* C3 *clp*-mutant showed reduction in fungal antagonism against *B. sorokiniana* and *P. ultimum*, in addition to changes in pigmentation and extracellular polysaccharide production. Interestingly, the *clp*-mutant conserved the same leaf-colonization rate of the wild-type strain, confirming a direct role of *clp* only in the biocontrol activity. In addition, the chitinase production of *Lysobacter* spp. depends on Clp by the regulation of *chiA* transcription through the direct binding of Clp to the promoter gene region (Xu et al., 2016a).

The influence of *clp* on the antibiotic biosynthesis and surface motility in *Lysobacter* members was recently discovered (Wang et al., 2014). Specifically, the biosynthesis of HSAF and WAP-8294A2 is controlled by the expression of *clp* in *L. enzymogenes* OH11 and a downstream component of the Clp signalling pathways, named Lat, is probably involved as a regulator in the HSAF production. In fact, Lat belongs a family of N-acetyltransferase, named GNAT, which has a significant role in gene regulation (Neuwald and Landsman, 1997). The role of a GNAT-acetyltransferase in the biocontrol activity of *Lysobacter* genus was previously hypostatized by Kobayashi et al. (2005). Besides the antibiotic production, *clp* has a role on the bacterial motility and specifically on the production of type 4 pilus (T4P) (Wang et al., 2014). In fact, the mutation of *clp* affected the expression of *pilA* encoding the major subunit of T4P, which are involved in colonization and infection of fungal hyphae (Patel et al., 2011), and impaired the surface motility of *L. enzymogenes* OH11.

The *pilG* has a role as a regulator of T4P biogenesis as demonstrated by the study on *pilG*-deletion mutants (Zhou et al., 2015). The study demonstrated that the production of HSAF was significantly enhanced by the inactivation of *pilG*, suggesting a regulatory role of PilG proteins into the antibiotics biosynthesis.

The diffusible signal factor (DSF), is an upstream regulator of Clp transcription into cell-to-cell signalling and is responsible for the production of different enzymes in *X. campestris* pv. *campestris* (He et al., 2007). A functional DSF system was identified in *L. enzymogenes* OH11 (Qian et al., 2013), however the *Lysobacter* chitinase production mediated by Clp is independent of DSF signal, in contrast with what was previously demonstrated for *X. campestris* (Xu et al., 2016a). The DSF genes are usually located in a conserved *rpf* (regulation of pathogenicity factor) cluster as reported for *X. campestris* (He et al., 2006). *Lysobacter enzymogenes* OH11 has an *rpf*/DSF signalling system, which is involved in the HSAF biosynthesis modulation (Qian et al., 2013). RpfC/RpfG two-component system is responsible for DSF sensing and transduction. It specifically controls DSF3 (13/methyltetradecanoic acid) production which together with Clp is involved in HASF production (Wang et al., 2014; Han et al., 2015). The study of Xu et al. (2016b) confirmed that the Clp regulatory pathways for the biosynthesis of proteases, chitinases and twitching motility (mediated by T4P) are DSF-independent (**Fig. 4**). In conclusion, the DSF signalling and Clp are commonly present in the genomes of *Lysobacter* species and are associated with the capacity to produce a broad range of lytic/enzyme and antibiotics (de Bruijn et al., 2015).

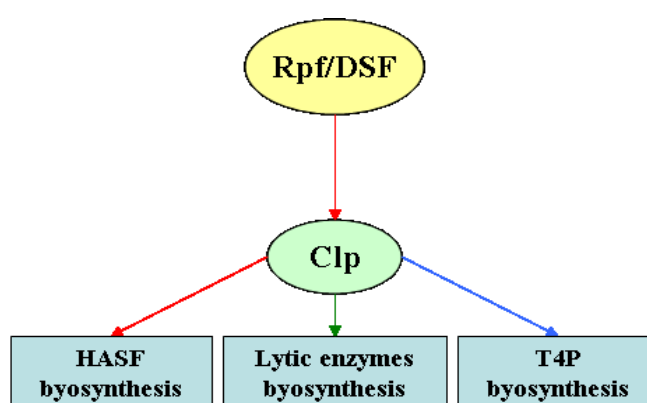


Figure 4: The role of the global regulator Clp in *Lysobacter* genus. In the cell-to-cell signalling system the Clp is a downstream regulator of Rpf/DSF signalling in the biosynthetic pathways of HASF antibiotics. Moreover, Clp regulates the biosynthesis of lytic enzymes, such as chitinases and proteases, and type 4 pilus (T4P) in a DSF-independent manner. (Modified from Xu et al., 2016b).

1.5 The *Lysobacter capsici* species

The species *L. capsici* was recently established by Park et al. (2008). The bacterial strains *L. capsici* YC5194^T (type strain, also known as =KCTC 22007^T =DSM 19286^T) was isolated from the pepper rhizosphere (*Capsicum annuum* L.) and the high 16S rRNA gene sequence similarity (>97%) together with physiological and chemical characteristics (e.g. polar lipids, major lipids, antifungal activity) confirm the affiliation to the genus *Lysobacter* (Fig. 5). The characterisation of *L. capsici* species at the chemical level highlights the peculiarities of the new species, such as the α -galactosidase activity, the major fatty acid ratio and the detection of specific fatty acids (C_{9:0} and C_{16:1}ω11c). The *L. capsici* YC5194^T showed biocontrol activity a broad range of (micro)organism, such as fungi (*Botrytis cinerea*, *Botryosphaeria dothidea*, *Colletotrichum gloeosporioides*, *Colletotrichum coccodes*, *Colletotrichum orbiculare*, *F. oxysporum*, *R. solani*, *Sclerotinia sclerotiorum* and *A. fumigatus*), yeasts (*Candida albicans* and *Cryptococcus neoformans*) and a Gram-positive bacterium (*B. subtilis*). Moreover, *L. capsici* YC5194^T displays antagonistic activity against the oomycete *Pythium ultimum* (Park et al., 2008).

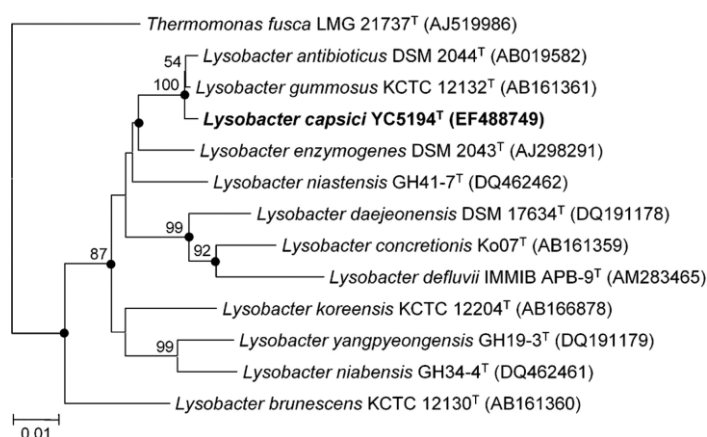


Figure 5: Phylogenetic tree of strain YC5194^T showing the relationships with other related species. The tree was constructed from the comparative analysis of 16S rRNA gene sequences. Bar, 1 substitution per 100 nucleotide positions (Park et al., 2008).

The potential use of strain belonging to the *Lysobacter* genus has stimulate the research and the identification of other *L. capsici* strains. Postma et al. (2010) isolated, from *Rhizoctonia* suppressive-soils, four bacterial strains belonging to the *L. capsici* species, named 1.33, 6.2.3, 10.4.5 and 55. These *L. capsici* strains exhibited a strong inhibition of *R. solani* AG2 growth (Postma et al., 2010) and the presence of a combined population of closely related *Lysobacter* (*L. antibioticus*, *L. capsici* and *L. gummosus*)

species could be involved in soil suppressiveness (Postma et al., 2008). The bacterization of tomato seeds with *L. capsici* PG4, a bacterial strain isolated from tobacco rhizosphere (*Nicotiana tabacum* cv. Burley), reduced the incidence of tomato crown and root rot diseases caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Puopolo et al., 2010). The *L. capsici* strain X2-3 was isolated from the wheat rhizosphere and showed antimicrobial activities against a broad range of plant pathogenic fungi and oomycete *in vitro* (e.g. *B. sorokiniana*, *P. myriotylum*, *Verticillium dahliae*, *Valsa mali* and *P. parasitica* var. *nicotianae*) (Yi et al., 2015). The antioomycete activity is the main biological control trait of *L. capsici* AZ78, that can effectively control *P. viticola* and *P. infestans*, two important phytopathogens of grapevine, tomato and potato (Puopolo et al., 2014a,b). The remarkable trait of *L. capsici* AZ78 is the compatibility with copper-based fungicides related to the genomic mediated resistance of this bacterial strain to heavy metals (Puopolo et al., 2014a; Puopolo et al., 2016). Next to the antimicrobial activity against phytopathogenic fungi and bacteria, the *Lysobacter* members show nematicidal activity (Chen et al., 2006; Lee et al., 2013a), such as the *L. capsici* strain YS1215 (Lee et al., 2013b). Indeed, *L. capsici* YS1215 is able to control soil-borne pathogens (*P. capsici*, *R. solani* and *F. oxysporum*) as well as root-knot nematodes (*Meloidogyne* spp.) by the secretion of lytic enzymes and lactic acid (Lee et al., 2013; Lee et al., 2014a,b).

Although the *in vitro* trials demonstrated the positive effect of *L. capsici* in the control of phytopathogens, few information is available on the specific mechanism of action of the strains belonging to that species. Recently the genomes of two *L. capsici*, strain X2-3 and 55, have been sequenced to provide new insights into the antimicrobial activity of these strains (Yi et al., 2015; de Bruijn et al., 2015). The information originating from the draft genome of *L. capsici* X2-3 highlights the presence of genes involved in the biosynthesis of antibiotics and lytic enzymes (Yi et al., 2015). Moreover, different biocontrol-related genes are present in the genome of *L. capsici* 55: *i*) *chiA* homolog responsible for the biosynthesis of chitinases; *ii*) gene cluster *alpAB* involved in the production of lytic endopeptidases L1 and L5; *iii*) biosynthetic gene clusters encoding PKS-NRPS responsible for the production of WAP-8294A and HSAF antibiotics (de Bruijn et al., 2015). Despite all the information collected with the studies discussed above, the role of each mechanism of action, such as the production of lytic enzyme and antibiotic, in the biocontrol activity of *L. capsici* is not yet investigated.

1.6 Aim of the study

Lysobacter capsici AZ78 is a good biocontrol agent and a promising strain for the development of a new microbiological-based fungicide. In fact, *L. capsici* AZ78 can control of *P. viticola* and can be applied in combination with low doses of copper-based fungicides commonly used in vineyard (Puopolo et al., 2014a). Moreover, *L. capsici* AZ78 is active against *P. infestans*, both *in planta* and *in vitro* conditions (Puopolo et al., 2014 a,b). However, the persistence of *L. capsici* AZ78 in vineyard, is mainly correlated to the bacterial capacity to overcome the unfavourable temperature (Segarra et al., 2015, 2016). The designing of an efficient formulation for *L. capsici* AZ78 improved its persistence on the grapevine leaves more than 10 times compared to the unformulated cells. The environmental fate of *L. capsici* AZ78 can be evaluated thanks to a strain-specific primer in quantitative PCR analysis (Segarra et al., 2015). To date only few information is available about the possible mechanisms of action displayed by *L. capsici* AZ78, such as the production of cyclo(L-Pro-L-Tyr) and its effect on the viability of oomycete sporangia (Puopolo et al., 2014 b).

Although a lot of information on the *Lysobacter* genus is already available, most of the specific descriptions needed for the registration processes of *L. capsici* AZ78 are still missing. For example, a key requirement for the registration of a microorganism as active substance is the ‘low risk’, such as the non-human pathogenicity and the absence of adverse effects to non-target organisms. Thus, the bacterial strain shall be well identified and characterized. The mechanism of action against phytopathogens and production of secondary metabolites shall be clarified.

The aim of this study was to better characterize *L. capsici* AZ78, in order to support the registration of this strain as a new active substance and to achieve a more robust knowledge about the physiology of this bacterial species.

To obtain a complete overview of the biocontrol potential of *L. capsici* AZ78, the bacterium genome was sequenced and compared with two closely related pathogenic bacteria to verify the absence of possible pathogenic traits. Moreover, the biocontrol features of *L. capsici* AZ78 were investigated by the genome mining and supported by *in vitro* trials. This approach was also applied to identify the genomic basis of the bacterial resistance to environmental stressors. A particular behaviour of *L. capsici* AZ78 related to the production of T4P and a subsequent positive effect on biocontrol activity, was investigated via molecular, *in vitro* and *in vivo* approaches. Finally, the

transcriptional profiling of *L. capsici* AZ78 interacting with *P. infestans* was applied in order to investigate the mechanism of action displayed by the bacterium in a possible pathosystem.

CHAPTER 2

The *Lysobacter capsici* AZ78 genome has a gene pool enabling it to interact successfully with phytopathogenic microorganisms and environmental factors

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The *Lysobacter capsici* AZ78 Genome Has a Gene Pool Enabling it to Interact Successfully with Phytopathogenic Microorganisms and Environmental Factors

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Lysobacter capsici AZ78 has considerable potential for biocontrol of phytopathogenic microorganisms. However, lack of information about genetic cues regarding its biological characteristics may slow down its exploitation as a biofungicide. In order to obtain a comprehensive overview of genetic features, the *L. capsici* AZ78 genome was sequenced, annotated and compared with the phylogenetically related pathogens *Stenotrophomonas malthophilia* K729a and *Xanthomonas campestris* pv. *campestris* ATCC 33913. Whole genome comparison, supported by functional analysis, indicated that *L. capsici* AZ78 has a larger number of genes responsible for interaction with phytopathogens and environmental stress than *S. malthophilia* K729a and *X. c.* pv. *campestris* ATCC 33913. Genes involved in the production of antibiotics, lytic enzymes and siderophores were specific for *L. capsici* AZ78, as well as genes involved in resistance to antibiotics, environmental stressors, fungicides and heavy metals. The *L. capsici* AZ78 genome did not encompass genes involved in infection of humans and plants included in the *S. malthophilia* K729a and *X. c.* pv. *campestris* ATCC 33913 genomes, respectively. The *L. capsici* AZ78 genome provides a genetic framework for detailed analysis of other *L. capsici* members and the development of novel biofungicides based on this bacterial strain.

Keywords: *Lysobacter*, biological control, lytic enzymes, siderophores, environmental stress

INTRODUCTION

Genome sequencing represents an excellent tool for biological characterization of bacterial species; especially in the case of species that have been largely underexplored. In the family Xanthomonadaceae (Saddler and Bradbury, 2005), attention has been paid particularly to members that are pathogenic to humans (*Stenotrophomonas*) and plants (*Xanthomonas* and *Xylella*; Simpson et al., 2000; da Silva et al., 2002; Crossman et al., 2008). In contrast, other bacterial genera have been underexplored, as in the case of the genus *Lysobacter*, which was established in 1978 (Christensen and Cook, 1978). Since most *Lysobacter* spp. were wrongly classified as *Myxobacteriales* and

Cytophagales, and several *Lysobacter* strains were wrongly assigned to *Stenotrophomonas* and *Xanthomonas* spp. (Christensen and Cook, 1978; Giesler and Yuen, 1998; Sakka et al., 1998; Nakayama et al., 1999), the importance of this genus was underestimated for a long time. The increasing number of 16S rDNA gene sequences available in public databases and the polyphasic approach to the identification of bacterial strains has led to an increase in the identification of new *Lysobacter* species. So far the genus has expanded to include 37 species (Singh et al., 2015) from the initial four: *L. antibioticus*, *L. brunescens*, *Lysobacter enzymogenes*, and *L. gummosus* (Christensen and Cook, 1978).

Some bacterial strains of the *Lysobacter* species act as biological control agents (BCAs) of plant diseases (Kobayashi and Yuen, 2007; Hayward et al., 2010). To date, most of the BCAs characterized have belonged to *L. enzymogenes* (Folman et al., 2003; Sullivan et al., 2003; Qian et al., 2009). Antagonistic mechanisms have received most attention in recent years, with the production of antibiotics and lytic enzymes by *L. enzymogenes* 3.1T8, C3, and OH11 and the related regulatory mechanisms being studied in some of these bacterial strains (Folman et al., 2004; Kobayashi et al., 2005; Palumbo et al., 2005; Yu et al., 2007; Zhang et al., 2011; Qian et al., 2012, 2013). Similarly to *L. enzymogenes*, *L. capsici* strains possess characteristics exploitable for the control of phytopathogenic microorganisms (Park et al., 2008). For example, the type strain *L. capsici* YC5194 produces secondary metabolites that inhibit the growth of phytopathogenic fungi (Park et al., 2008) and the *L. capsici* strain PG4 controls tomato foot and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Puopolo et al., 2010). Some *L. capsici* strains have been isolated from soils suppressive to *Rhizoctonia solani* and have been shown to be involved in the control of other phytopathogenic fungi (Postma et al., 2010). Other *L. capsici* strains can control nematodes, as in the case of *L. capsici* YS1275, used against *Meloidogyne incognita* (Lee et al., 2014), or oomycetes, as in the case of *L. capsici* AZ78 (*Lc* AZ78) used to control *Phytophthora* (*P.*) *infestans* and *Plasmopara* (*Pl.*) *viticola* (Puopolo et al., 2014a,b), indicating their high potential as broad spectrum BCAs. *Lc* AZ78's resistance to copper is an additional positive feature for a BCA, because it can be integrated within plant protection strategies including the use of copper fungicides (Puopolo and Pertot, 2014).

In comparison to *L. enzymogenes*, much less is known about the biological features of *L. capsici* (Puopolo et al., 2015). As understanding the biological characteristics of a microorganism is crucial for its development as a biopesticide, we sequenced the *Lc* AZ78 genome using PacBio technology and carried out functional experiments to assess the biological properties predicted by the genome analysis. To obtain a comprehensive overview of the genetic cues of *L. capsici*-specific biological characteristics, we compared the *Lc* AZ78 genome with the genome of two phylogenetically similar bacteria (Kobayashi and Yuen, 2007; Hayward et al., 2010): the opportunistic human pathogen *S. malthophilia* K729a (*Sm* K729a) and the phytopathogen *X. campestris* pv. *campestris* ATCC 33913 (*Xcc* ATCC 33913).

MATERIALS AND METHODS

Microorganisms

L. capsici AZ78 was stored at length in glycerol 40% at -80°C and routinely grown on Luria-Bertani Agar at 27°C (LBA, Sigma-Aldrich, USA). In all the experiments *Lc* AZ78 cell suspensions were prepared by flooding LBA dishes with 5 ml of sterile saline solution (0.85% NaCl) after 72 h growth at 27°C . *L. capsici* AZ78 cells were then scraped from the medium surface using sterile spatulas and collected in sterile 15 ml tubes. The resulting *Lc* AZ78 cell suspensions were centrifuged (11,200 g, 5 min) and the pelleted cells were suspended in sterile distilled water to a final absorbance of 0.1 at 600 nm, corresponding to 1×10^8 Colony Forming Units (CFU)/ml. *L. capsici* AZ78 was used at this concentration in all experiments, except when otherwise indicated.

The phytopathogenic bacteria and fungi used in this work (Table S1) were grown respectively on Nutrient Agar (NA, Oxoid, United Kingdom) at 28°C and Potato Dextrose Agar (PDA, Oxoid) at 25°C . Bacterial strains were stored at length in glycerol 40% at -80°C , while fungal strains were stored on PDA slants at room temperature. *P. infestans* isolate was maintained on Pea Agar Medium (PAM, 12.5% frozen peas and 1.2% agar in distilled water) at 17°C and stored at length in glycerol 20% at -80°C .

DNA Extraction, Genome Sequencing, and Assembly

L. capsici AZ78 genomic DNA was extracted with a PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Invitrogen, USA) according to the manufacturer's instructions. Once extracted, DNA integrity and the absence of RNA contamination was checked on a 1% agarose gel. Subsequently, the whole genomic DNA of *Lc* AZ78 was sequenced using PacBio technology at Baseclear B.V. (Leiden, Netherlands). A 10-kb PacBio single-molecule real-time (SMRT) cell was employed (Chin et al., 2013). The generated subreads were *de novo* assembled using the RS hierarchical genome assembly process (HGAP) protocol version 3.0, as available in SMRT Portal v2.0 (<https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP-in-SMRT-Analysis>). The SMRT Portal was configured and used with a public machine image that Pacific Biosciences maintains and upgrades on Amazon Cloud (<https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/%22Installing%22-SMRT-Portal-the-easy-way---Launching-A-SMRT-Portal-AMI>).

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JAJA00000000. The version described in this paper is version JAJA02000000.

Genome Annotation and Comparative Analysis

The genome of *Lc* AZ78 was annotated with the online platform Rapid Annotation using Subsystem Technology (RAST) version 2.0 (Aziz et al., 2008). For genome comparison, the genomes of *Sm* K729a (AM743169) and *Xcc* ATCC 33913 (AE008922) were submitted to the same online platform to eliminate bias deriving

from the different annotation systems employed. Genetic content comparison between genomes was conducted at nucleotide and amino acid level using BLASTN and BLASTP, respectively. Both minimum length > 70 and identity \geq 70% at amino acid level were used as threshold parameters.

Proteolytic Activity in the Interaction between *Lysobacter Capsici* AZ78 and *Phytophthora infestans*

Plugs (5 mm) were cut from the edge of 7-day-old *P. infestans* colonies and transferred onto cellophane film overlying PAM dishes and incubated at 20°C for 7 days. After the incubation period, *P. infestans* macrocolonies originating from the plugs were transferred into 2 ml sterile tubes containing 500 μ l of Phosphate Buffer Solution (PBS, 0.8% NaCl; 0.02% KCl; 0.145% NaH₂PO₄; 0.025% KH₂PO₄). Subsequently, the tubes were inoculated with 50 μ l of a *Lc* AZ78 cell suspension (1 \times 10⁹ CFU/ml). Tubes containing PBS, PBS with *Lc* AZ78 and PBS with *P. infestans* were used as controls.

All the tubes were incubated at 25°C for 48 h and were processed at 6, 24, and 48 h to determine proteolytic activity. Briefly, tubes were centrifuged (16,100 g, 5 min) and 225 μ l of supernatants were mixed with 150 μ l of 1% Casein stock solution (50 mM Tris-HCL, pH 8.8) in new sterile 2 ml tubes. Subsequently, the tubes were incubated at 37°C for 1 h and undigested substrates were precipitated by adding 375 μ l of 5% Trichloroacetic acid. The tubes were then centrifuged at 16,100 g for 3 min. The resulting supernatants were transferred into new sterile 2 ml tubes containing 400 μ l of 1 M NaOH, and absorbance at 405 nm ($A_{OD405\text{ nm}}$) was assessed using a spectrophotometer. At each time point, three 2 ml tubes (replicates) for each treatment were used, and the experiment was repeated.

Production of Lytic Enzymes and Siderophores

L. capsici AZ78 was evaluated in terms of its ability to degrade cellulose, chitin, laminarin and proteins using classic methods (Cowan, 1974; Sambrook and Russell, 2001). The occurrence of a clear halo surrounding *Lc* AZ78 colonies was checked after 48 h incubation at 27°C.

To determine siderophore production, LBA dishes were overlaid with CAS agar medium (Schwyn and Neilands, 1987). The final medium looked dark blue. Five microliter of *Lc* AZ78 cell suspension were spot inoculated onto these dishes. Siderophore production associated with the change in the color of CAS agar medium (Schwyn and Neilands, 1987) was assessed after 72 h incubation at 27°C.

In vitro Antifungal and Antibacterial Activity

The antifungal activity of *Lc* AZ78 against 23 phytopathogenic fungi (Table S1) was evaluated by using the classic dual-culture method. Briefly, 50 μ l of *Lc* AZ78 cell suspension were spotted on two opposite edges of a PDA plate. After 24 h incubation at 27°C, plugs of mycelium (5 mm) were cut from the edge of young fungal colonies grown on PDA and placed at the center

of the plates containing the *Lc* AZ78 macrocolonies. PDA plates seeded only with mycelium plugs were used as controls. After 4 days incubation at 25°C, inhibition of mycelial growth was evaluated by scoring the diameters of fungal colonies. Each test was performed in triplicate and the experiment was repeated.

In vitro antibacterial activity of *Lc* AZ78 against eight phytopathogenic bacteria (Table S1) was evaluated. NA dishes were spot inoculated with 50 μ l of *Lc* AZ78 cell suspension and incubated for 72 h at 27°C. *L. capsici* AZ78 cells were then killed by exposure to chloroform vapor for 60 min. The plates were subsequently aerated under the laminar flow for 60 min. Dishes were overlaid with 8 ml of 0.4% agar PBS, mixed with 2 ml of a suspension containing 1 \times 10⁸ CFU/ml of the test bacterial strains. NA dishes not seeded with *Lc* AZ78 and NA dishes overlaid with 0.4% agar PBS only were used as controls. Each test was performed in triplicate and the experiment was repeated. The diameters of inhibition haloes were scored after 48 h incubation at 28°C.

Determination of *Lysobacter capsici* AZ78 Resistance to Cobalt and Zinc

To determine the ability of *Lc* AZ78 to resist heavy metals, *Lc* AZ78 cells were grown on LBA amended with CoCl₂ and ZnSO₄ (Sigma-Aldrich). Briefly, filter-sterilized CoCl₂ and ZnSO₄ solutions were added to LBA to obtain the final concentrations of 0.5 and 1 mM, respectively. Subsequently, 100 μ l of a serial dilution, from 10⁻¹ to 10⁻⁷, of *Lc* AZ78 cell suspension were spread onto LBA and LBA amended with CoCl₂ and ZnSO₄ using sterile spatulas. *L. capsici* AZ78 CFU were counted after 4 days of incubation at 27°C. Three replicates (Petri dishes) of each combination (dilution and heavy metal concentration) were prepared and the experiments were repeated. *Bacillus amyloliquefaciens* FZB42 was used as control. The number of *Lc* AZ78 and *B. amyloliquefaciens* FZB42 CFU were log₁₀ transformed before statistical analysis.

Resistance of *Lysobacter capsici* AZ78 to Chemical Fungicides and Insecticides

Thirty-two plant protection products commonly applied for the chemical control of grapevine plant diseases were used in this experiment (Table S2). Each plant protection product was dissolved in distilled water, filter-sterilized and added to LBA to achieve the maximum concentration commonly applied in the field (Table S2). A volume of 100 μ l of *Lc* AZ78 cell suspension (1 \times 10³ CFU/ml) was spread onto LBA and LBA amended with plant protection products with sterile spatulas. *L. capsici* AZ78 CFUs were counted after 72 h of incubation at 27°C. Viability reduction was calculated according to this formula:

$$\text{CFU grown on LBA} - \text{CFU grown on LBA amended with the plant protection product} / \text{CFU grown on LBA}$$

Three Petri dishes (replicates) were used for each plant protection product, and the experiments were repeated.

Sensitivity of *Lysobacter capsici* AZ78 to Antibiotics

The standardized disc susceptibility testing method (Andrews et al., 2011) was used to determine the sensitivity of

Lc AZ78 to ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline, tobramycin, trimethoprim, and vancomycin. Briefly, Mueller Hinton Agar (MHA, Sigma-Aldrich) was poured into sterile Petri dishes to reach a depth of 4 mm (Atlas, 2004). A volume of 100 μ l of *Lc* AZ78 cell suspension (2×10^8 CFU/ml) was spread over the entire surface of MHA dishes using sterile spatulas. Subsequently, three discs of the same antibiotic (Oxoid) were placed on each inoculated Petri dish. After 48 h incubation at 27°C, the diameters of the inhibition zones were measured with a caliper. Three Petri dishes (replicates) were used for each antibiotic and the experiments were repeated. Resistance or sensitivity to antibiotics was assigned on the basis of the inhibition zone diameters (Andrews et al., 2011; Anonymous, 2011).

Statistical Analysis

The data obtained in the experiments aimed at assessing (i) proteolytic activity; (ii) antifungal and antibacterial activity; (iii) resistance to cobalt and zinc, were subjected to two-way ANOVA. The data on experimental repetitions were pooled when no significant differences were found according to the *F*-test ($\alpha > 0.05$). In the case of proteolytic activity, antibacterial activity, and resistance to cobalt and zinc, the data were subsequently analyzed using one-way ANOVA, and mean comparisons were performed

with Tukey's test ($\alpha = 0.05$). Student's *T*-test ($\alpha = 0.05$) was used as a *post hoc* test for mean comparison in the case of antifungal activity. All these statistical tests were carried out using Statistica 7.0 (StatSoft, USA).

RESULTS

Genome Assembly and Sequence Comparison

The PacBio SMRT cell yielded output data with average genome coverage of $\sim 44\times$ to generate a *de novo* assembly of the complete genome sequence of *Lc* AZ78. The PacBio RSII sequencing system generated 298,751,307 base pairs (bp) through 67,899 reads (N50 read length 6848 and mean read length 4399). The genome of *Lc* AZ78 (Figure 1) consists of 6,272,844 bp assembled into three contigs, and the G+C content is 66.4% (Table 1), similarly to the *L. capsici* type strain (65.4%; Park et al., 2008). The *Lc* AZ78 genome contains 5292 predicted coding sequences and 93 predicted non-coding RNAs, including one transfer-messenger RNA (tmRNA), seven rRNAs, and 85 tRNAs (Table 1).

The *Lc* AZ78 genome was compared with the genomes of the opportunistic human pathogen *Sm* K729a and the phytopathogen *Xcc* ATCC 33913 (da Silva et al., 2002; Crossman et al., 2008). Genome characteristics such as chromosome size, G+C content and the number of predicted coding sequences

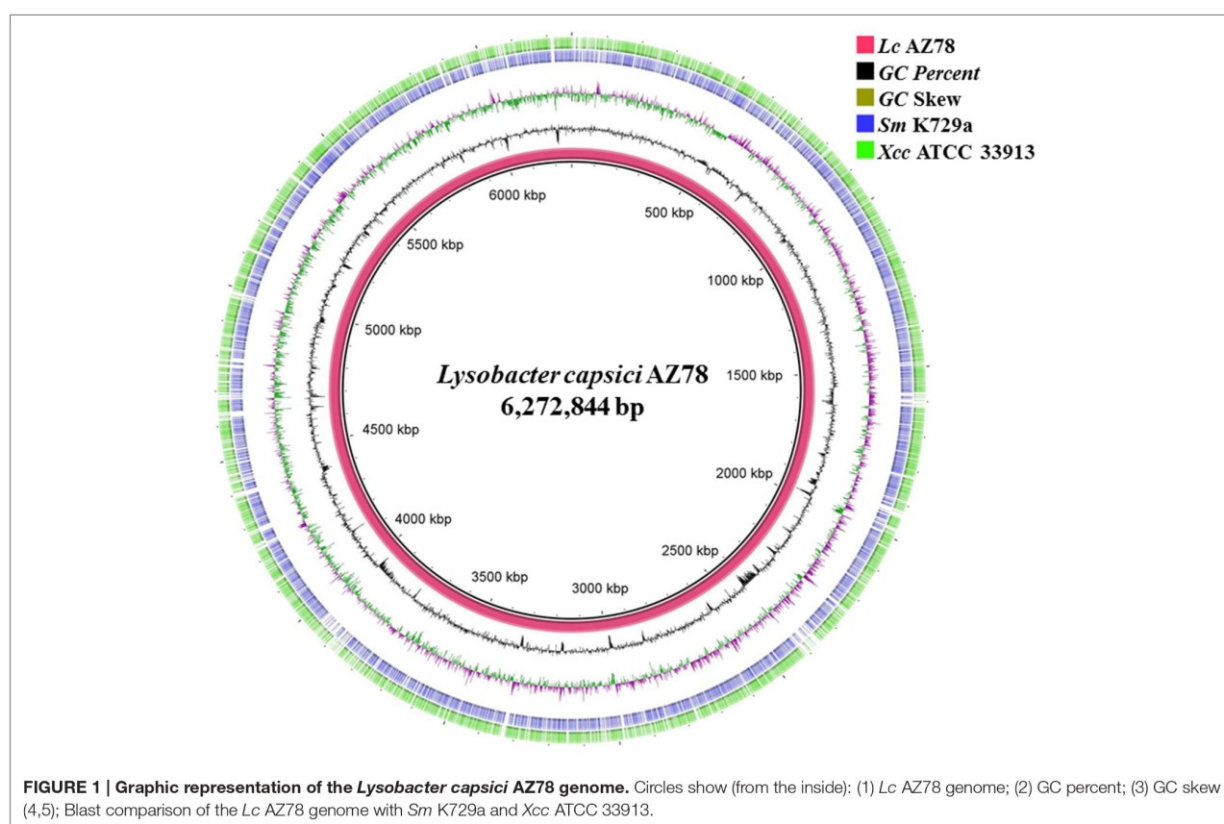
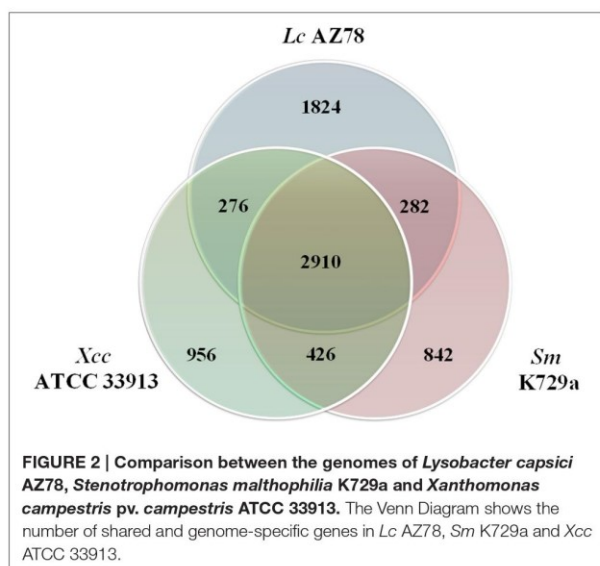


TABLE 1 | Summary of the genomic characteristics of *Lysobacter capsici* AZ78, *Stenotrophomonas malthophilia* K729a and *Xanthomonas campestris* pv. *campestris* ATCC 33913.

	<i>Lysobacter capsici</i> AZ78	<i>Stenotrophomonas malthophilia</i> K729a	<i>Xanthomonas campestris</i> pv. <i>campestris</i> ATCC 33913
Contigs	3	1	1
Number of bases (bp)	6,272,844	4,851,126	5,076,187
G+C content (%)	66.43	66.70	65.00
Number of predicted coding sequences	5292	4386	4182
Coding percentage	82.8	88.8	84.34
rRNA	7	4	2
tRNA	85	74	53



differed in the three bacterial strains (Table 1). They share a core genome of 2910 orthologs, mostly involved in primary metabolism (Figure 2). Genes that are associated with the capacity of *Sm* K729a to infect humans, such as the *smlt0598*, *smlt3048*, *smlt4452*, and *wbpv* genes (Crossman et al., 2008), are absent in the genome of *Lc* AZ78 and *Xcc* ATCC 33913. Similarly, the *avrBs1*, *avrBs1.1*, *avrBs2*, *avrXccA1*, *avrXccA2*, *avrXccB* and *avrXccC* genes involved in the pathogenic interaction (da Silva et al., 2002; Wang et al., 2007) are specific for the *Xcc* ATCC 33913. The *Lc* AZ78 genome contains several unique genes involved in interaction processes with other microorganisms and environmental factors (Table 2).

Interaction with Microorganisms: Production of Lytic Enzymes

The presence in *Lc* AZ78 genome of 79 genes encoding proteolytic enzymes represents a substantial difference with *Sm* K729a and *Xcc* ATCC 33913 genomes. A region of 40,829

bp in length is specific to the *Lc* AZ78 genome and is missing from the genome of the other two bacterial species (Proteolytic Region, Table 2). Although this region contains five genes (AZ78_4489, 4492, 4498, 4503, and 4505) encoding extracellular proteases homologous to an extracellular protease present in the genome of *Sm* K729a (*expR*, Smlt0861; Table 2) and *Xcc* ATCC 33913 (XCC0851), it has other *Lc* AZ78-specific genes. In particular, four genes (AZ78_4508, 4509, 4511, and 4512) encoding extracellular zinc proteases (EC 3.4.24.26), and two genes (AZ78_4514 and 4516) encoding bacterial leucyl aminopeptidase (EC 3.4.11.10) have no orthologs in the *Sm* K729a and *Xcc* ATCC 33913 genomes. Another *Lc* AZ78-specific region (Metalloendopeptidase Region, Table 2) contains three *Lc* AZ78-specific genes (AZ78_269, 271 and 272) encoding metalloendopeptidases that do not share homology with any gene of *Sm* K729a and *Xcc* ATCC 33913. However, peptidase genes that have already been characterized in other *Lysobacter* strains were found in *Lc* AZ78, such as three genes encoding endopeptidases and a peptidyl Asp-metalloendopeptidase homologous to the *lepA* (AB045676) and *lepB* (AB094439) genes of *Lysobacter* sp. IB-9374, respectively (Chohnan et al., 2002, 2004).

Other genes located in these two regions share homology with protease genes (KF738078, KF738082, KF738069, and KF738070) of *L. gummosus* UASM 402 (Gökçen et al., 2014). The high number of proteases found in *Lc* AZ78 genome, led to investigate whether proteolytic activity could be involved in the interaction between *Lc* AZ78 and the phytopathogen *P. infestans*. A significant increase in proteolytic activity occurred when the two microorganisms were co-cultured at 25°C for 24 h (Figure 3).

Other lytic enzymes of *Lc* AZ78 may be involved in the degradation of other components of *P. infestans* cell wall such as cellulose that was degraded *in vitro* by *Lc* AZ78 (Figure 4A). Cellulose degradation is related to the presence in *Lc* AZ78 genome of the AZ78_3681 gene encoding a cellulase belonging to glycosyl hydrolase family 5 that has no homology with cellulases included *Sm* K729a and *Xcc* ATCC 33913 genomes (Table 2). However, cellulase from *Lc* AZ78 shows homology at amino acid level with the Cel5G, a putative cellulase found in the genome of *Cellvibrio japonicus* Ueda107 (CP000934; DeBoy et al., 2008).

L. capsici AZ78 was able to degrade laminarin *in vitro* (Figure 4B) and this lytic activity was confirmed by the presence in its genome of three genes encoding two enzymes (GluA, GluC) belonging to glycosyl hydrolase family 16, and one enzyme (GluB) belonging to glycosyl hydrolase family 64. Specifically, three genes (AZ78_3675, 4722, 1531) are orthologs of *gluA*, *gluB* and *gluC* previously characterized in *L. enzymogenes* C3 (AY667477; AY667478; AY667479) and N4-7 (AY157838; AY157839; AY157840; Palumbo et al., 2003, 2005). The *Sm* K729a genome lacks genes encoding endo β -1,3 glucanases, while *Xcc* ATCC 33913 has a gene (XCC1188) encoding an endo β -1,3 glucanase homologous to *gluA* of *Lc* AZ78 (Table 2). Unlike *L. enzymogenes* C3, N4-7 and *Xcc* ATCC 33913, *Lc* AZ78 has a second gene (AZ78_406) encoding an endo β -1,3 glucanase homologous to GluA, a gene (AZ78_4157) encoding an enzyme belonging to glycosyl hydrolase family 16 showing considerable similarity with the KF738079 gene identified in *L. gummosus*

TABLE 2 | List of genes specific to *Lysobacter capsici* AZ78 or shared with *Stenotrophomonas malthophilia* K279a and *Xanthomonas campestris* pv. *campestris* ATCC 33913.

	Shared features		Specific features			
	Name	Biological activity	Name	Biological activity		
Interaction with microorganisms	<i>expR</i> (AZ78_4489, 4492, 4498, 4503, 4505)	Production of extracellular protease	Proteolytic Region (AZ78_4508, 4509, 4511, 4512)	Production of zinc extracellular proteases		
	<i>gluA</i> (AZ78_3675)	Degradation of glucans	Proteolytic Region (AZ78_4514, 4516)	Production of bacterial leucyl aminopeptidase		
	<i>chiA</i> (AZ78_1828)	Degradation of chitin	Metalloendopeptidase Region (AZ78_269, 271, 272)	Production of metalloendopeptidases		
	<i>feoABC</i> (AZ78_5035-5037)	Uptake of ferrous iron		<i>cel5G</i> (AZ78_3681)	Degradation of cellulose	
				<i>gluB</i> (AZ78_4722); <i>gluC</i> (AZ78_1531); KF738079 (AZ78_4157); <i>cel8A</i> (AZ78_4006); <i>celA₁</i> (AZ78_4352)	Degradation of glucans	
				<i>chiB</i> (AZ78_54)	Degradation of chitin	
				NPR-PKS (AZ78_1098)	Production of antifungal compounds	
				<i>lanL</i> (AZ78_848)	Production of lantibiotics	
				<i>csbC-entEBF-viuB-entA</i> (AZ78_407-412)	Synthesis of catechol siderophores	
				<hr/>		
Interaction with environment				<i>pigABCDEFG</i> (AZ78_3467-3472)	Biosynthesis of xanthomonadin	Catalase/Peroxidase (AZ78_681, 1116, 1469)
	<i>copA, copB</i> (AZ78_402-403)	Resistance to copper ions	<i>cphA-cphB</i> (AZ78_4099-4100)	Cyanophycin metabolism		
	<i>czcCBA</i> (AZ78_3809-3811)	Resistance to heavy metals	Copper efflux region (AZ78_560-562)	Resistance to copper ions		
	Resistance-Nodulation-Division (AZ78_451, 452, 826)	Resistance to toxic compounds	SMR protein (AZ78_906, 1192, 2446)	Resistance to toxic compounds		
	ABC transporters (AZ78_929, 967, 3014)	Resistance to β -lactams	MFS protein (AZ78_266, 1103, 3068, 3698, 3949, 4767)	Resistance to toxic compounds		
	<i>blaL2</i> (AZ78_3946)	Resistance to β -lactams		Kanamycin nucleotidyltransferase (AZ78_3393)	Resistance to aminoglycosides	
				B-lactamases (AZ78_238, 2665, 3448, 3627, 4028)	Resistance to β -lactams	

UASM 402 (Gökçen et al., 2014), and another gene (AZ78_4006; **Table 2**) which is homologous to *cel8A* of *Lysobacter* sp. IB-9374 (AB244037) encoding an enzyme belonging to glycosyl hydrolase family 8 with β -1,4 glucanase and chitosanase activity (Ogura et al., 2006). *L. capsici* AZ78 also has a gene (AZ78_4352) that encodes a β -1,4 endoglucanase belonging to glycosyl hydrolase family 6, which has never been characterized to date in *Lysobacter* members. This is missing in the genome of *Sm* K729a and *Xcc* ATCC 33913 (**Table 2**), although it is homologous to *celA₁* (Z12157) described for *Streptomyces halstedii* JM8 (Fernández-Abalos et al., 1992).

As regards phytopathogenic fungi, *Lc* AZ78 degraded chitin *in vitro* (**Figure 4C**), and this activity is related to the presence in its genome of the AZ78_1828 gene encoding a chitinase A present in the *Sm* K729a genome (Smlt0682, **Table 2**) and not in the *Xcc* ATCC 33913 genome. Unlike *Sm* K729a, the *Lc* AZ78 genome contains another gene (AZ78_3859) encoding a chitinase A that shares high similarity with the *chiA* gene (AB014770) described in

Xanthomonas sp. AK (Sakka et al., 1998). *L. capsici* AZ78 genome also has a gene (AZ78_54) encoding a chitinase B (*chiB*, **Table 2**) sharing homology with *chiB* genes identified in the two strains *Burkholderia gladioli* CHB101 (AB038998) and BSR3 (CP002600; Shimosaka et al., 2001; Seo et al., 2011).

Interaction with Microorganisms: Production of Antibiotics

L. capsici AZ78 released secondary metabolites with antifungal activity *in vitro* and reduced the *in vitro* mycelial growth of 22 phytopathogenic fungi, with the sole exception of *Pyrenochaeta (Py.) lycopersici* (**Table 3**). *L. capsici* AZ78 genome was mined for genes potentially involved in the production of antibiotics and a genomic region of 9489 bp missing in *Sm* K729a and *Xcc* ATCC 33913 was identified. Specifically, the AZ78_1098 gene (**Table 2**) has homology with a gene encoding a hybrid polyketide synthase and a non-ribosomal peptide synthetase (NPR-PKS) involved in

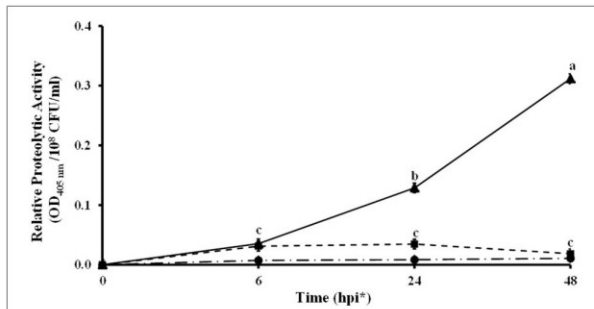


FIGURE 3 | Proteolytic activity in the *in vitro* interaction between *Lysobacter capsici* AZ78 and *Phytophthora infestans*. Proteolytic activity was monitored 6, 24, and 48 h after the following treatments: Lc AZ78 (●), *P. infestans* (■) and Lc AZ78 + *P. infestans* (▲). Mean and standard error values for six replicates (2 ml-tubes) pooled from two experiments are reported for each condition. Different letters indicate significant differences according to Tukey's test ($\alpha = 0.05$).

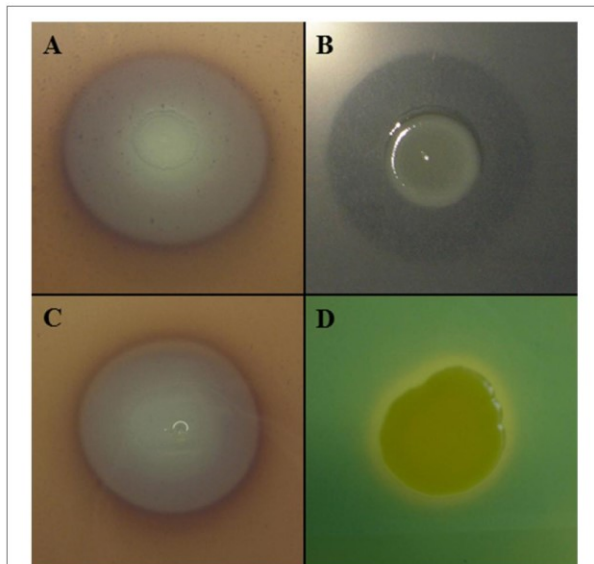


FIGURE 4 | Characterization of *Lysobacter capsici* AZ78. Lc AZ78 produces (A) cellulases; (B) β -glucanases; (C) chitinases, and (D) siderophores.

the biosynthesis of Heat Stable Antifungal Factor (HSAF) in *L. enzymogenes* C3 (EF028635; Yu et al., 2007).

In our antibacterial activity tests, Lc AZ78 released compounds that are toxic to the Gram-positive phytopathogenic bacteria *Clavibacter michiganensis* subsp. *michiganensis* LMG 7333, *C. michiganensis* subsp. *sepedonicus* LMG 2889, *Rhodococcus fascians* LMG 3605 and *Streptomyces turgidiscabies* DSM 41997, while no toxic activity was shown against the tested Gram-negative phytopathogenic bacteria (Table 4). *L. capsici* AZ78 genome hosts genes involved in the biosynthesis of ribosomally encoded antibacterial peptides named lantibiotics

TABLE 3 | Antifungal activity of *Lysobacter capsici* AZ78.

Phytopathogenic fungus	Untreated	Lc AZ78
<i>Alternaria alternata</i>	4.50 ± 0.05	1.50 ± 0.16*
<i>Ascochyta rabiei</i>	6.10 ± 0.12	1.36 ± 0.06*
<i>Aspergillus flavus</i>	2.32 ± 0.07	0.65 ± 0.03*
<i>Aspergillus niger</i>	2.50 ± 0.02	0.58 ± 0.02*
<i>Aspergillus ochraceus</i>	2.68 ± 0.09	0.48 ± 0.04*
<i>Botrytis cinerea</i>	7.45 ± 0.10	2.54 ± 0.12*
<i>Colletotrichum gloeosporioides</i>	7.86 ± 0.21	1.84 ± 0.14*
<i>Fusarium acuminatum</i>	7.92 ± 0.12	3.72 ± 0.16*
<i>Fusarium avenaceum</i>	3.56 ± 0.13	1.77 ± 0.24*
<i>Fusarium oxysporum</i> f. sp. <i>asparagi</i>	7.56 ± 0.34	4.34 ± 0.18*
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	7.34 ± 0.25	3.10 ± 0.15*
<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	7.12 ± 0.21	3.08 ± 0.04*
<i>Fusarium sambucinum</i>	7.11 ± 0.12	2.24 ± 0.08*
<i>Fusarium semitectum</i>	7.83 ± 0.08	4.42 ± 0.12*
<i>Fusarium solani</i>	7.95 ± 0.09	2.66 ± 0.11*
<i>Penicillium</i> sp.	3.52 ± 0.20	1.76 ± 0.06*
<i>Phoma tracheiphila</i>	3.92 ± 0.21	2.23 ± 0.06*
<i>Pyrenochaeta lycopersici</i>	7.84 ± 0.08	7.73 ± 0.13*
<i>Rhizoctonia solani</i>	7.89 ± 0.11	1.73 ± 0.16*
<i>Sclerotinia maior</i>	7.86 ± 0.14	1.84 ± 0.08*
<i>Sclerotinia minor</i>	7.92 ± 0.15	1.86 ± 0.20*
<i>Sclerotinia sclerotiorum</i>	7.94 ± 0.13	1.46 ± 0.27*
<i>Thielaviopsis basicola</i>	7.89 ± 0.17	3.50 ± 0.16*

The antifungal activity of Lc AZ78 was assessed through dual-culture assay. Mean and standard errors values of mycelial growth diameters (cm) with six replicates (Petri dishes) pooled from two experiments are reported for each fungus. Values followed by asterisks differ significantly according to Student's t-test ($\alpha = 0.05$).

TABLE 4 | Antibacterial activity of *Lysobacter capsici* AZ78.

Phytopathogenic bacterium	Halo of inhibition zone (mm) ^a
<i>Agrobacterium tumefaciens</i>	0 ± 0
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	0 ± 0
<i>Ralstonia solanacearum</i>	0 ± 0
<i>Xanthomonas campestris</i> pv. <i>campestris</i> DSM 3586	0 ± 0
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> LMG 3690	12.44 ± 0.55 ^a
<i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i> LMG 3690	12.77 ± 0.16 ^a
<i>Rhodococcus fascians</i> LMG 3605	10.38 ± 1.06 ^a
<i>Streptomyces turgidiscabies</i> DSM 41838	11.05 ± 0.65 ^a

^aThe production of secondary metabolites with antibacterial activity by Lc AZ78 was assessed against Gram-negative and Gram-positive phytopathogenic bacteria. Antibacterial activity is expressed as the mean value of the halo inhibition zone ± standard errors. Six replicates (Petri dishes) pooled from two experiments are reported for each bacterium. No significant differences were present in inhibition zones according to Tukey's test ($\alpha = 0.05$).

(Chatterjee et al., 2005), and these genes are missing in the genomes of Sm K729a and Xcc ATCC 33913 (Table 2). *L. capsici* AZ78 contains a gene (AZ78_848) homologous to the *venL* gene (WP_015031826) identified in *Streptomyces venezuelae* ATCC 10712 (Goto et al., 2010). As for *venL* in *S. venezuelae* ATCC 10712, the AZ78_848 gene is followed by two genes (AZ78_847

and AZ78_846) encoding an ATP-binding protein and an efflux transporter, indicating synteny of this region in the two bacterial species.

Interaction with Microorganisms: Production of Siderophores

L. capsici AZ78 produced siderophores on CAS agar plates (Figure 4D) and has genes involved in uptake and transport of iron ions that are homologous to genes present in the genome of *Sm* K729a and *Xcc* ATCC 33913. For example, the *Lc* AZ78 genome includes the *feoABC* operon (AZ78_5035-5037, Table 2) involved in the uptake of ferrous iron, which shares high homology with the *feoABC* operon present in *Sm* K729a (Smlt2211-2213), *Xcc* ATCC 33913 (XCC1834-1836). *L. capsici* AZ78 contains an additional gene cluster (AZ78_407-AZ78_412, Table 2) whose genes show homology with the *entAFBE-csbC* (CP001157) genes responsible for the production of catechol siderophores in *Azotobacter vinelandii* strains DJ and ATCC 12837 (Setubal et al., 2009; Yoneyama et al., 2011). The corresponding region in *Lc* AZ78 contains one gene (AZ78_411) encoding a protein similar to ViuB (CP001235), involved in the utilization of exogenous ferric vibriobactin complex in *Vibrio cholerae* 0395 (Butterton and Calderwood, 1994).

Interaction with the Environment: Tolerance to Environmental Stressors

The genetic information needed for resistance to environmental stressors is shared by the *Lc* AZ78, *Sm* K729a and *Xcc* ATCC 33913 genomes. For instance, *Lc* AZ78 genome has genes involved in the biosynthesis of xanthomonadin (AZ78_3467-3472, Table 2), a pigment responsible for protection against UV light irradiation in *Xanthomonas* spp. (Rajagopal et al., 1997). Moreover, *Lc* AZ78 includes several genes related to reactive oxygen species (ROS) resistance and shared with *Sm* K729a and *Xcc* ATCC 33913 and genes encoding a cytochrome c551 peroxidase (EC 1.11.1.5) (AZ78_681), a catalase (EC 1.11.1.6)/Peroxidase (EC 1.11.1.7) (AZ78_1116) and a superoxide dismutase (EC 1.15.1.1) (AZ78_1469) with no orthologs in the *Sm* K729a and *Xcc* ATCC 33913 genomes (Table 2).

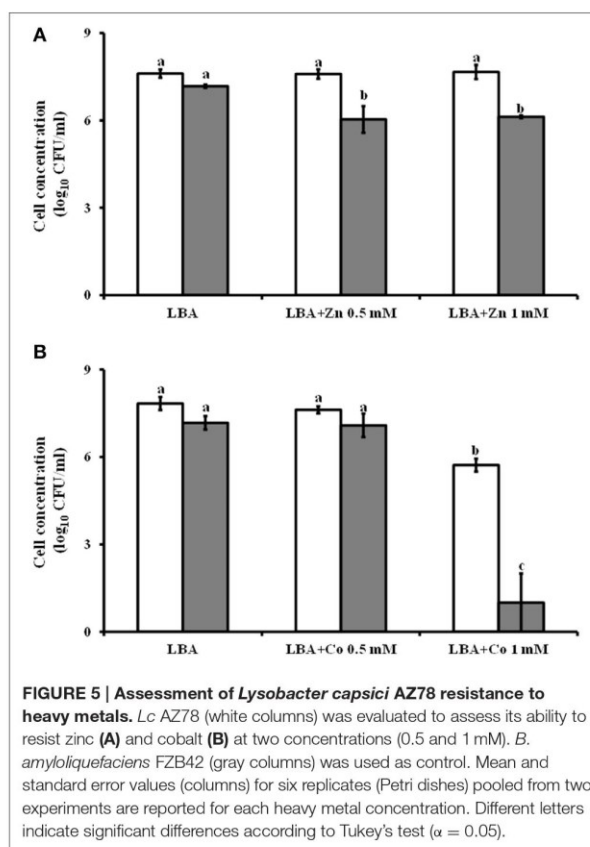
Another specificity of *Lc* AZ78 genome is represented by a two-gene cluster (AZ78_4099-4100) responsible for the biosynthesis and degradation of cyanophycin with no orthologs in *Sm* K729a and *Xcc* ATCC 33913 (Table 2). The synthesis and degradation of cyanophycin are catalyzed respectively by cyanophycin synthetase and cyanophycinase encoded by the two-gene cluster *cphA-cphB* (Li et al., 2001; Krehenbrink et al., 2002). *L. capsici* AZ78 genome hosts a cyanophycinase (AZ78_4099), followed immediately by a cyanophycin synthetase (AZ78_4100).

Interaction with the Environment: Tolerance to Heavy Metals

The *Lc* AZ78, *Sm* K729a, and *Xcc* ATCC 33913 genomes have the *copA* and *copB* genes, encoding a multicopper oxidase and the copper resistance protein B respectively (AZ78_402-403, Table 2). Unlike the other two Xantomonadaceae members, *Lc*

AZ78 has a genomic region encoding proteins involved in the efflux of copper ions (Table 2). This region contains two Cu²⁺ exporting ATPases (EC 3.6.3.4; AZ78_560-561), homologous with CP002600 of *B. gladioli* BSR3 (Seo et al., 2011). The product of the gene AZ78_562, located downstream of the AZ78_560-561 genes, belongs to the MerR superfamily of transcriptional activators (Hobman and Brown, 1997) and is homologous to a Cu(I)-responsive transcriptional activator (*cueR*) of the copper efflux system in γ -proteobacteria (Stoyanov et al., 2001).

The *Lc* AZ78 genome has a 4396 bp region containing three genes (AZ78_3809-3811) that are homologous with the *czcCBA* operon involved in resistance to cadmium, cobalt and zinc (Nies, 2003). This operon (Table 2) is also present in the genomes of *Sm* K729a (Smlt2456-2458) and *Xcc* ATCC 33913 (XCC4036-4038). The *Lc* AZ78 genome also hosts a gene (AZ78_3808) encoding an ortholog of the CzcD protein involved in the expression regulation of *czcCBA* in *Ralstonia* sp. CH34 (Anton et al., 1999). The presence of these genes was associated with the ability of *Lc* AZ78 to grow on LBA amended with different concentrations of cobalt and zinc (Figure 5). In this *in vitro* experiments, *B. amyloliquefaciens* FZB42 was used as control since its genome is missing of *czcCBA* operon (CP000560; Chen et al., 2007). The viability of *Lc* AZ78 was not negatively affected by ZnSO₄ at the concentrations tested while *B. amyloliquefaciens* FZB42 viability



was reduced of an order of magnitude at both the concentrations tested (Figure 5A). CoCl₂ resulted more toxic against *Lc* AZ78 cells and a decrease of two orders of magnitude was registered on LBA amended with 1 mM CoCl₂ (Figure 5B). However, a more drastic reduction in *B. amyloliquefaciens* FZB42 was observed at the same conditions (Figure 5B).

Interaction with the Environment: Tolerance to Fungicides and Antibiotics

L. capsici AZ78 was resistant *in vitro* to several fungicides and insecticides commonly applied in viticulture (Figure 6). This resistance may rely on the presence of efflux systems such as ABC transporters, Resistance-Nodulation-Division (RND), Small Multidrug Resistance protein (SMR), and proteins belonging to the Major Facilitator Super-family (MFS) and the Multidrug Toxic compound Extrusion family (MTE; Poole, 2001), which *Lc* AZ78 shares with *Sm* K729a and *Xcc* ATCC 33913 (Table 2). Moreover, there are some *Lc* AZ78-specific genes related to fungicide and antibiotic resistance, such as three genes (AZ78_906, 1192, and 2446) encoding putative SMR proteins and six genes (AZ78_266, 1103, 3068, 3688, 3949, and 4767) encoding putative MFS proteins (Table 2).

Since all these efflux systems can also contribute to resistance to antibiotics, we evaluated the sensitivity of *Lc* AZ78 to several antibiotics *in vitro* (Table 5). *L. capsici* AZ78

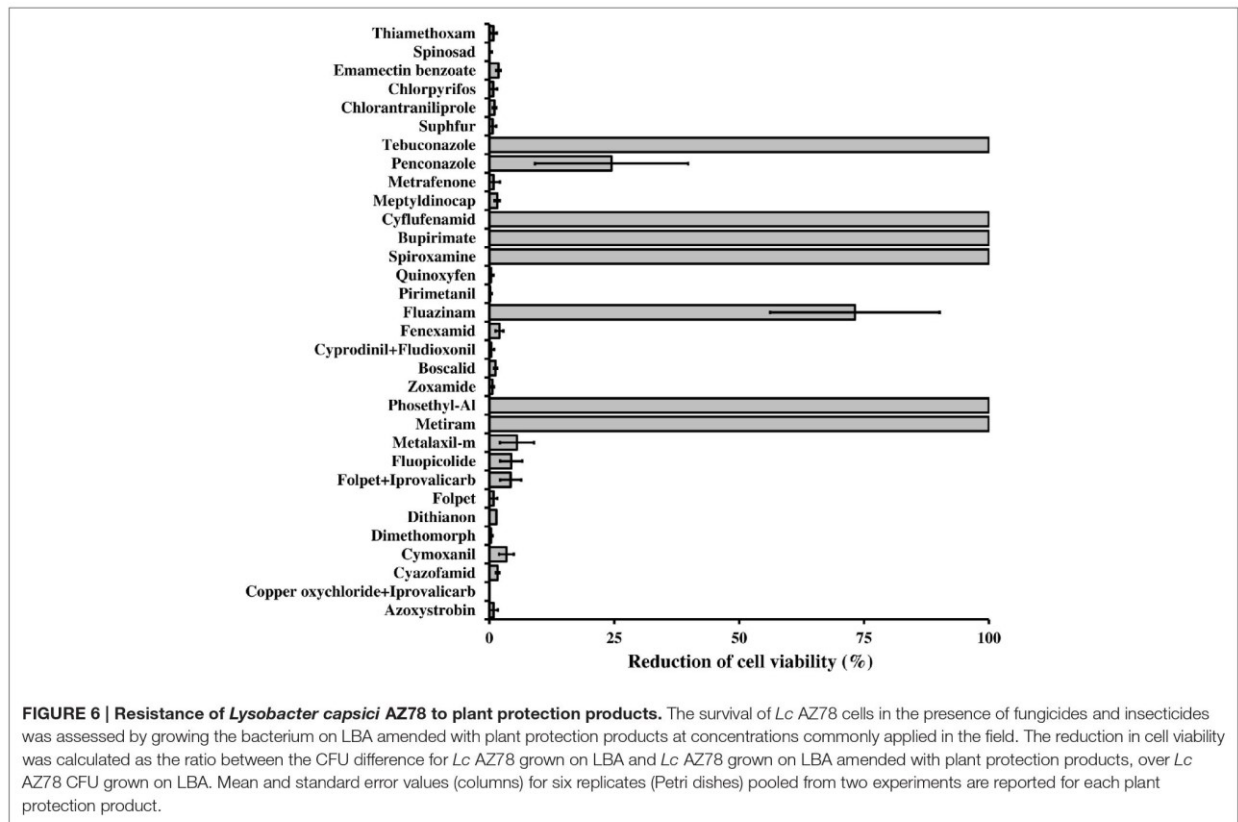
is sensitive to chloramphenicol, erythromycin, gentamicin, tetracycline, trimethoprim and vancomycin whereas it is resistant to ampicillin, kanamycin, streptomycin, and tobramycin. The

TABLE 5 | Assessment of *Lysobacter capsici* AZ78 resistance to antibiotics.

Antibiotic	Disc content (µg)	Diameter of inhibition zone (mm)	Zone diameter breakpoint ^a (mm)
Ampicillin	10	0.00 ± 0.00	<14
Chloramphenicol	30	52.10 ± 1.01	<19
Erythromycin	30	45.83 ± 0.81	<17
Gentamicin	30	23.33 ± 0.21	<17
Kanamycin	30	0.00 ± 0.00	<12
Streptomycin	25	0.00 ± 0.00	<12
Tetracycline	30	43.90 ± 0.55	<17
Tobramycin	10	0.00 ± 0.00	<16
Trimethoprim	5	23.23 ± 0.67	<13
Vancomycin	30	23.70 ± 0.21	<13

Lc AZ78 was evaluated for its resistance to different antibiotics. The results are the mean diameters ± standard errors of pooled data for a repeated experiment with three replicates of each antibiotic for each experiment. The diameters of inhibition zone were determined to assign resistance or sensitivity to each antibiotic.

^aCut-off diameters to define resistance to the tested antibiotic (Andrews et al., 2011; Anonymous, 2011).



resistance to kanamycin and streptomycin may depend on inactivation mediated by aminoglycoside phosphotransferase or adenyltransferase enzymes (Shaw et al., 1993). The *Lc* AZ78 genome contains a gene (AZ78_3072) encoding putative streptomycin 3'-kinase (EC 2.7.1.87) that does not show homology with *aph(3'')* and *aph(3')-IIc* involved in streptomycin and kanamycin resistance in *Sm* K729a (Okazaki and Avison, 2007; Crossman et al., 2008). Moreover, the *Lc* AZ78 genome includes another gene (AZ78_3393) encoding a putative kanamycin nucleotidyltransferase (Table 2). Other putative antibiotic resistance genes in *Lc* AZ78 genome are responsible for resistance to β -lactams such as ampicillin: 12 genes showed homology with the β -lactamases of *Sm* K729a and *Xcc* ATCC 33913 and five β -lactamases genes (AZ78_238, 2665, 3488, 3627, and 4028) were unique to *Lc* AZ78 (Table 2).

DISCUSSION

Development of new biofungicides based on BCAs are becoming an important task to reduce the use of chemical fungicides for the control of phytopathogenic microorganisms. Recently, *Lc* AZ78 was shown to effectively control two important phytopathogenic oomycetes and a first formulation prototype was designed for its application in vineyards for the control of *Pl. viticola* (Puopolo et al., 2014a,b; Segarra et al., 2015a,b). However, information regarding the biological characteristics of *Lc* AZ78 are needed for the registration process and to foster the development of this bacterial strain as the main ingredient of novel biofungicides. Thus, the genome of *Lc* AZ78 was sequenced by PacBio sequencing system. This technology was chosen because the advantage of longer reads during assembly ensures a higher quality final result (Roberts et al., 2013). The drawback of higher error rate for PacBio sequences can be overcome with a higher genome coverage, a choice that already proved to be the best strategy nowadays for bacterial sequencing projects (Booher et al., 2015; Cameron et al., 2015; Lee et al., 2015). Accordingly, PacBio RSII sequencing system resulted more effective than the Illumina GAIIx system previously used for obtaining a first draft genome of *Lc* AZ78 (Puopolo et al., 2014c) and number of contigs was reduced from 142 achieved with Illumina to three achieved with PacBio in this work.

Since comparison of the genomes highlighted important similarities and differences between microorganisms (Studholme et al., 2009; Straub et al., 2013), the *Lc* AZ78 genome was compared with the genomes of the opportunistic human pathogen *Sm* K729a and the phytopathogen *Xcc* ATCC 33913 originally sequenced using Sanger sequencing technology (da Silva et al., 2002; Crossman et al., 2008). This technology is no longer used for whole genome assembly projects, however the quality of Sanger sequencing technology is comparable to the quality achieved with PacBio technology (Eid et al., 2009). The comparative analysis of the three genomes revealed high diversity between the three bacterial strains and showed that *Lc* AZ78 does not have the genetic basis for pathogenic interaction with humans and plants. Particularly, the comparison highlights the lack in *Lc* AZ78 genome of genes responsible for the instauration of human diseases present in *Sm* K729a genome. Similarly, the lack of these genes represents one of the factors

differentiating the opportunistic human pathogen *Sm* K729a from the closely related beneficial bacterium *Stenotrophomonas rhizophila* DSM14405^T (Alavi et al., 2014). This comparative analysis also allowed to identify several *Lc* AZ78-specific genes that are related to bacterial responses to other microorganisms and environmental factors.

A vast number of *Lc* AZ78-specific genes are involved in competition with other microorganisms highlighting the biocontrol properties of *Lc* AZ78 associated with the release of extracellular enzymes that can lyse the cell wall of both fungi and oomycetes. Members of the genus *Lysobacter* are known to produce a plethora of extracellular enzymes with lytic activity capable of degrading the cell wall components of several phytopathogenic microorganisms (Kobayashi and Yuen, 2007; Hayward et al., 2010). Accordingly, the *Lc* AZ78 genome includes a repertoire of genes encoding lytic enzymes capable of degrading cellulose, chitin, laminarin and proteins *in vitro*.

One of the main differences between *Lc* AZ78, *Sm* K729a and *Xcc* ATCC 33913 genomes, relies on the presence of a large number of genes encoding extracellular proteases in the *Lc* AZ78 genome that may display specific proteolytic activities. Particularly, *Lc* AZ78 genome has four gene sharing homology with proteases characterized in *L. gummosus* UASM 402, which are involved in the digestion of biofilm produced by *Staphylococcus epidermidis* (Gökçen et al., 2014). The presence of these genes in the genome of *Lc* AZ78 highlights the importance of members of the *L. capsici* species as potential new sources of enzymes exploitable for the control of important human pathogens through degradation of their extracellular matrix. Proteolytic activity of *Lc* AZ78 increased by *in vitro* incubation with *P. infestans*, and this may represent a key process in *Lc* AZ78's biocontrol activity as shown for *L. enzymogenes* 3.1T8 (Folman et al., 2003, 2004). Furthermore, *Lc* AZ78 degraded *in vitro* β -glucans (laminarin) and cellulose other components of the oomycete cell wall. The degradation of these polymers is associated with the presence in the *Lc* AZ78 genome of genes encoding cellulases and a vast array of β -glucanases that clearly indicates that phytopathogenic oomycetes represent an optimal target of this BCA.

The *Lc* AZ78 genome also includes unique genes encoding chitinases that are commonly involved in the control of phytopathogenic fungi and they play a significant role in the biocontrol activity of *L. enzymogenes* C3 against *Bipolaris sorokiniana* (Zhang and Yuen, 2000). The *Lc* AZ78 genome has a gene encoding for a ChiA enzyme already characterized in the *L. enzymogenes* C3 (AY667480), N4-7 (AY667481) and OH11 (DQ888611) strains (Zhang et al., 2001; Sullivan et al., 2003; Qian et al., 2009). Moreover, the analysis of genes encoding chitinases in *Lc* AZ78 genome revealed the presence of a second gene with a high level of identity with the *chiA* gene previously characterized in *Xanthomonas* sp. AK (Sakka et al., 1998). This homology, and the 16S rDNA analysis carried out by Folman et al. (2003), represents strong evidence for misidentification in the case of *Xanthomonas* sp. AK, which in our opinion should be considered a *Lysobacter* sp. Analysis of the *Lc* AZ78 genome highlighted the presence of a gene encoding a chitinase B, a novelty for the genus *Lysobacter* and Xanthomonadaceae. Indeed, enzymes such as ChiB belong to glycosyl hydrolase family 19, which includes

chitinases mostly identified in actinomycetes (Watanabe et al., 1999). Within Proteobacteria, *chiB* genes were only identified in the two strains *B. gladioli* CHB101 and BSR3 (Shimosaka et al., 2001; Seo et al., 2011). The presence of both ChiA and ChiB chitinases in the *Lc* AZ78 genome supports the potential of this bacterial strain to attack and degrade the cell wall of phytopathogenic fungi.

The genomic and functional information provided in this work demonstrates that *Lc* AZ78 has unique genes responsible for the synthesis of macrocyclic lactams toxic against phytopathogenic microorganisms. *L. enzymogenes* C3 produces HSAF a compound consisting of dihydromaltophilin and related macrocyclic lactams, toxic for several phytopathogenic fungi and oomycetes (Yu et al., 2007; Li et al., 2008). *Lysobacter* sp. SB-K88 synthesizes the macrocyclic lactams Xanthobaccin A, B and C, which are highly active *in vitro* against *Aphanomyces cochlioides* and *Pythium ultimum* (Nakayama et al., 1999). The *Lc* AZ78 genome includes regions involved in the production of macrocyclic lactams and the *in vitro* experiments let to hypothesize that the putative toxic compounds released by *Lc* AZ78 have probable similarities with xanthobaccins. Indeed, Nakayama et al. (1999) reported that *Py. lycopersici* was not sensitive to xanthobaccins produced *in vitro* by *Lysobacter* sp. SB SB-K88. Similarly, this phytopathogenic fungus was not sensitive to the toxic compounds released *in vitro* by *Lc* AZ78 in our experiments. Future work will be aimed at determining the chemical structure of the secondary metabolites with antifungal activity produced by *Lc* AZ78 to study the involvement of this class of antibiotics in the biological control of phytopathogenic fungi and oomycetes.

L. capsici AZ78 also released secondary metabolites toxic to four phytopathogenic Gram-positive bacteria and the presence of genes involved in their biosynthesis differed in *Lc* AZ78, *Sm* K729a and *Xcc* ATCC 33913 strains. Particularly, *Lc* AZ78 genome contains genes involved in the production of lantibiotics, compounds toxic to Gram-positive bacteria (Chatterjee et al., 2005) that are missing in the genome of the other two bacterial strains. Little is known about lantibiotic production in *Lysobacter* members, whereas it was reported that *L. enzymogenes* OH11 produces the cyclic lipodepsipeptide WAP-8294A2 active against the human pathogenic Gram-positive bacterium *Staphylococcus aureus* (Zhang et al., 2011). Therefore, we cannot rule out the possibility that the antibacterial activity of *Lc* AZ78 could be associated with the production of other toxic secondary metabolites, and chemical analysis is needed to further investigate this topic.

The comparison of the three genomes highlighted genetic informations regarding the ability of *Lc* AZ78 to scavenge ferrous ions from the environment through the production of siderophores (Neilands, 1995; Chu et al., 2010). Although the production of these secondary metabolites is known to be important for human pathogenic, plant pathogenic and plant beneficial bacteria (Hamdan et al., 1991; Pandey and Sonti, 2010; Skaar, 2010), few information are available about siderophore production in *Lysobacter* spp. Differently from *Sm* K729a and *Xcc* ATCC 33913, the genome of *Lc* AZ78 is provided with the *entAFBE-csbC* operon responsible for the production of catechol

siderophores indicating that *Lc* AZ78 may also compete with other microorganisms for iron ions in the environment.

Our results also highlight key genes involved in the resistance of *Lc* AZ78 to UV-light irradiation and starvation. Previously, we have shown that *Lc* AZ78 resisted to starvation stress for 15 days and can be stored at 4°C in distilled water for a year (Puopolo et al., 2014a; Segarra et al., 2015a). This ability may be associated with the presence of the *cphA-cphB* operon responsible for the production and degradation of cyanophycin, that is missing in the genome of *Sm* K729a and *Xcc* ATCC 33913. This compound is a branched non-ribosomally synthesized polypeptide that accumulates in cyanobacteria and proteobacteria (Allen et al., 1980; Krehenbrink et al., 2002) and acts as a temporary nitrogen and carbon reserve (Li et al., 2001; Krehenbrink et al., 2002). Therefore, *L. capsici* members could have the genetic basis to promptly adapt to environments lacking in nutrients. *L. capsici* AZ78 is able to resist UV-light irradiation (Puopolo et al., 2014a) and this ability may be associated with the presence of genes involved in the biosynthesis of xanthomonadin, in agreement with the production of a xanthomonadin-like aryl polyene group reported for *L. enzymogenes* OH11 (Wang et al., 2013). *L. capsici* AZ78 has a pronounced resistance to copper ions, which renders this BCA a prime candidate for combination with copper-based fungicides for more efficient control of *Pl. viticola* on grapevine plants (Puopolo and Pertot, 2014). Resistance to copper frequently arises in phytopathogenic xanthomonads (Stall et al., 1986; Behlau et al., 2011), and resistant *Stenotrophomonas* strains have been isolated from copper-polluted soils (Altimira et al., 2012). Some of the genes involved in the resistance to copper are shared among the *Lc* AZ78, *Sm* K729a and *Xcc* ATCC 33913 genomes, whereas genes encoding copper exporting ATPases are specific for *Lc* AZ78 genomes. Based on these differences, *L. capsici* members seem to have the genome makeup necessary to guarantee better survival in an environment with greater concentrations of copper. *L. capsici* AZ78 is also resistant to other heavy metals such as cobalt and zinc and this phenotype is associated with the presence of the *czcCBA* operon and several genes involved in the efflux of cadmium, cobalt and zinc. Most of these genes encode RND proteins known as key multidrug efflux transporters for resistance to antibiotics, dyes, fungicides and solvents in Gram-negative (Kumar and Schweizer, 2005; Bazzini et al., 2011; Yamaguchi et al., 2015). Accordingly, the presence of a high number of RND proteins and other efflux systems (SMR, MFS, and MTE) is also associated with the *Lc* AZ78 resistance to fungicides and insecticides *in vitro*. Regarding resistance to antibiotics, *Lc* AZ78 was sensitive to chloramphenicol and erythromycin although several genes involved in antibiotic resistance are shared with *Sm* K729a. Indeed, this opportunistic human pathogen is resistant to these antibiotics, making it very risky for treating infections in immunocompromised patients (Crossman et al., 2008; Ryan et al., 2009). Thus, it is conceivable that *Sm* K729a genome has genes involved in chloramphenicol and erythromycin resistance with no orthologs in *Lc* AZ78 genome.

In conclusion, the sequence and annotation of the *Lc* AZ78 genome provide a genetic framework for detailed analysis of potential biocontrol mechanisms against phytopathogens. In

particular, the comparison of *Lc* AZ78, *Sm* K729a, and *Xcc* ATCC 33913 genomes allows to state that *Lc* AZ78 is missing of the genetic information needed to establish pathogenic interaction with humans and plants, an aspect that is crucial for the registration of new BCAs. This comparative approach highlights the genetic basis determining the *Lc* AZ78 aptitude to compete with phytopathogenic microorganisms through the release of (i) extracellular lytic enzymes; (ii) secondary metabolites with antibacterial and antifungal activity; and (iii) catechol siderophores. Furthermore, the *Lc* AZ78 genome contains a vast number of genes involved in resistance to environmental stress, antibiotics, heavy metals and plant protection products. Analysis of the *Lc* AZ78 genome will help to provide more accurate characterization of bacterial strains belonging to the *Lysobacter* genus and lead to important advances in the further development of *Lc* AZ78 as an active ingredient in new biofungicides.

AUTHOR CONTRIBUTIONS

GP conceived the work, designed the experiments, carried out annotation of the genome and the experiments, analyzed the

data, and wrote and edited the manuscript. ST carried out annotation of the genome and the experiments, analyzed the data, and wrote and edited the manuscript. PS, MM, and KE assembled the genome, wrote and edited the manuscript. MP and IP contributed to the conception of the work, designed the experiments and edited the manuscript. All the authors have read the manuscript and agree with its content.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmicb.2016.00096>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table ST1: Bacterial and fungal strains used to assess the production of antibacterial and antifungal compounds by *Lysobacter capsici* AZ78.

Phylum	Strain
Bacteria	<i>Agrobacterium tumefaciens</i>
	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>
	<i>Ralstonia solanacearum</i>
	<i>Xanthomonas campestris</i> pv. <i>campestris</i> DSM 3586
	<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> LMG 3690
	<i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i> LMG 3690
	<i>Rhodococcus fascians</i> LMG 3605
	<i>Streptomyces turgidiscabies</i> DSM 41838
Fungi	<i>Alternaria alternata</i>
	<i>Ascochyta rabiei</i>
	<i>Aspergillus flavus</i>
	<i>Aspergillus niger</i>
	<i>Aspergillus ochraceus</i>
	<i>Botrytis cinerea</i>
	<i>Colletotrichum gloeosporioides</i>
	<i>Fusarium acuminatum</i>
	<i>Fusarium avenaceum</i>
	<i>Fusarium oxysporum</i> f. sp. <i>asparagi</i>
	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
	<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>
	<i>Fusarium sambucinum</i>
	<i>Fusarium semitectum</i>
	<i>Fusarium solani</i>
	<i>Penicillium</i> sp.
	<i>Phoma tracheiphila</i>
	<i>Pyrenochaeta lycopersici</i>
<i>Rhizoctonia solani</i>	
<i>Sclerotinia maior</i>	
<i>Sclerotinia minor</i>	
<i>Sclerotinia sclerotiorum</i>	
<i>Thielaviopsis basicola</i>	

Supplementary Table ST2: Fungicides and insecticides used to determine the resistance of *Lysobacter capsici* AZ78 to plant protection products.

Active Ingredient (AI)	Commercial Product	Target Grapevine Disease	Dose (AI ml or g /hl)
Azoxystrobin	Amistar		23.2 ml/hl
Copper oxychloride +	Melody-		124.6 + 14.7 g/hl
Cyazofamid	Mildicut		10.4 ml/hl
Cymoxanil	Cymbal 20		22.8 g/hl
Dimetomorph	Quantum		25.3 g/hl
Dithianon	Delan 70 WG		84.0 g/hl
Folpet	Solofol	Downy mildew	121.2 g/hl
Folpet + Iprovalicarb	Melody Care		93.75 + 16.5 g/hl
Fluopicolide	Profiler		11.0 g/hl
Metalaxil-m	Ridomil Gold SI		9.8 g/hl
Metiram	Polyram DF		142.4 g/hl
Phosethy-AI	Prodeo 80 WG		200 g/hl
Zoxamide	Zoxium 240 SC		16.35 ml/hl
Boscalid	Cantus		60.6 g/hl
Cyprodinil + Fludioxonil	Switch		30.0 + 20.4 g/hl
Fenexamid	Teldor	Grey mould	75.5 g/hl
Fluazinam	Ohayo		59.3 ml/hl
Pyrimethanil	Scala		74.8 ml/hl
Bupimirate	Nimrod 250 EW		35.7 ml/hl
Cyflufenamid	Cidely		2.6 ml/hl
Meptyldinocap	Karantane 3D		21.4 ml/hl
Metrafenone	Vivando		10.6 ml/hl
Penconazole	Support 10 EC	Powdery mildew	5.1 ml/hl
Quinoxifen	Arius		6.8 ml/hl
Spiroxamine	Batam		20.2 ml/hl
Tebuconazole	Fezan		9.87 ml/hl
Sulphur	Thiamon 80 Plus		565.6 g/hl
Chlorantraniliprole	Coragen		3.3 ml/hl
Chlorpyrifos	Dursban		53.0 g/hl
Emamectin benzoate	Affirm	European grapevine moth	1.4 g/hl
Spinosad	Laser		8.4 ml/hl
Thiamethoxam	Actara 25 WG		5.1 g/hl

CHAPTER 3

**Pea broth enhances the biocontrol efficacy
of *Lysobacter capsici* AZ78 by triggering
cell motility associated with biogenesis
of Type IV Pilus**

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Pea Broth Enhances the Biocontrol Efficacy of *Lysobacter capsici* AZ78 by Triggering Cell Motility Associated with Biogenesis of Type IV Pilus

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Bacterial cells can display different types of motility, due to the presence of external appendages such as flagella and type IV pili. To date, little information on the mechanisms involved in the motility of the *Lysobacter* species has been available. Recently, *L. capsici* AZ78, a biocontrol agent of phytopathogenic oomycetes, showed the ability to move on jellified pea broth. Pea broth medium improved also the biocontrol activity of *L. capsici* AZ78 against *Plasmopara viticola* under greenhouse conditions. Noteworthy, the quantity of pea residues remaining on grapevine leaves fostered cell motility in *L. capsici* AZ78. Based on these results, this unusual motility related to the composition of the growth medium was investigated in bacterial strains belonging to several *Lysobacter* species. The six *L. capsici* strains tested developed dendrite-like colonies when grown on jellified pea broth, while the development of dendrite-like colonies was not recorded in the media commonly used in motility assays. To determine the presence of genes responsible for biogenesis of the flagellum and type IV pili, the genome of *L. capsici* AZ78 was mined. Genes encoding structural components and regulatory factors of type IV pili were upregulated in *L. capsici* AZ78 cells grown on the above-mentioned medium, as compared with the other tested media. These results provide new insight into the motility mechanism of *L. capsici* members and the role of type IV pili and pea compounds on the epiphytic fitness and biocontrol features of *L. capsici* AZ78.

Keywords: *Lysobacter*, plant components, type IV pilus, flagellum, biological control

INTRODUCTION

Motility is a key trait that allows bacteria to reach nutrients, colonize natural niches and display pathogenic and antagonistic aptitudes (Dörr et al., 1998; Harshey, 2003). Henrichsen (1972) first classified bacterial motility into six distinct types, namely darting, gliding, spreading, swarming, swimming, and twitching. Whereas darting and spreading indicated different forms of passive dispersal of bacterial cells, the other motility types indicated active propagation of bacterial cells on inert surfaces (Henrichsen, 1972). Swimming motility indicates bacterial cell dispersal that

is dependent on the presence of rotating flagella and allows bacteria to move in environments characterized by a high water content (Harshey, 2003; Kearns, 2010). Swarming motility is a dispersal mechanism for bacterial cells on semi-solid surfaces; it is made possible by functional rotating flagella, and in some cases by secreted amphiphilic compounds (surfactants) that reduce the tension between bacterial cells and inert surfaces (Kearns, 2010). Surfactants and rhamnolipids are examples of surfactants involved in the swarming motility of strains belonging to *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively (Caiazza et al., 2005; Julkowska et al., 2005). Additional external cell appendages can also be involved in swarming motility, such as type IV pili (T4P). Köhler et al. (2000) proved that T4P play a key role in the swarming motility of *P. aeruginosa* PAO1, suggesting that bacterial swarming motility is not exclusively flagella-dependent. Furthermore, the involvement of T4P in swarming motility was also confirmed in the phytopathogenic bacterial strain *P. syringae* pv. *tabaci* 6605 (Taguchi and Ichinose, 2011). However, the extension and retraction of T4P are considered key processes, generally associated with the twitching motility observed in several bacterial species, such as *P. aeruginosa*, *Ralstonia solanacearum*, and *Vibrio cholerae* (Mattick, 2002). Henriksen (1972) also introduced another type of motility, called gliding, which does not need the presence of flagella and T4P, and originates from movement along the long cell axis characterizing bacterial strains belonging to the *Cytophaga* and *Flavobacterium* genera (McBride, 2001; Kearns, 2010). However, the involvement of T4P has been demonstrated in so-called 'social gliding' in some bacterial species, as in the case of *Mixococcus xanthus* (Wu et al., 1997, 1998; McBride, 2001). Movement on surfaces through gliding motility was also attributed to bacteria belonging to the genus *Lysobacter*, and most of the bacterial species in this genus lack flagella (Christensen and Cook, 1978; Hayward et al., 2010). However, the presence of a single polar flagellum was reported for some recently proposed new species, such as *L. spongicola*, *L. arseniciresistens*, and *L. mobilis* (Romanenko et al., 2008; Luo et al., 2012; Yang et al., 2015).

Sequencing and annotation of the genomes of *Lysobacter* strains shed light on the presence of the genes involved in the biogenesis of flagella and T4P in this bacterial genus (de Bruijn et al., 2015; Kwak et al., 2015; Liu et al., 2015). The presence of genes related to the flagellar machinery was reported in the genome of strains belonging to *L. arseniciresistens*, *L. capsici*, *L. enzymogenes*, and *L. gummosus* (Wang et al., 2014; de Bruijn et al., 2015; Liu et al., 2015). However, the biosynthesis pathway of the flagellum was not functional in some strains, such as *L. capsici* 55, *L. enzymogenes* C3, and *L. gummosus* 3.2.11 (de Bruijn et al., 2015). Moreover, the genomes of *L. antibioticus* ATCC 29479, *L. antibioticus* 76, *L. capsici* 55, *L. dokdonensis* DS-58^T, *L. enzymogenes* C3, *L. enzymogenes* OH11, and *L. gummosus* 3.2.11 encompass several genes involved in T4P biogenesis (Patel et al., 2011; Wang et al., 2014; de Bruijn et al., 2015; Liu et al., 2015). Despite all this structural and functional information, characterisation of the mechanisms involved in the motility of *Lysobacter* cells is still very poor.

Recently, Zhou et al. (2015) observed that *L. enzymogenes* C3 cells located at the edge of the colonies were able to move,

possibly using some sort of twitching motility. Some *L. capsici* and *L. enzymogenes* strains were reported to be able to disperse on agar surface after 12 days of incubation, and this movement was associated with gliding motility (Gómez Expósito et al., 2015). Similarly, we observed that dispersal of *L. capsici* AZ78, a biocontrol agent of *Phytophthora infestans* and *Plasmopara viticola* (Puopolo et al., 2014a,b), occurred when the bacterium was grown on a medium containing pea broth used in a dual-culture assay with *P. infestans* (Supplementary Figure S1).

Based on this observation, trials were carried out under greenhouse conditions to assess the contribution of the motility due to the pea broth in the biocontrol activity of *L. capsici* AZ78 against *P. viticola*. Subsequently, we investigated the ability of several strains belonging to various *Lysobacter* species to move on inert surfaces through swimming, swarming and twitching motility and we observed specific medium-dependent motility in several *L. capsici* strains. The availability of the *L. capsici* AZ78 genome (Puopolo et al., 2016) made it possible to identify genes encoding proteins involved in the flagellum and T4P biogenesis. Furthermore, relative gene expression analysis revealed that *L. capsici* AZ78 motility on pea broth jellified medium is associated with the upregulation of genes related to T4P machinery.

MATERIALS AND METHODS

Bacterial Strains and Growth Media

The bacterial strains used in this study (Table 1) were stored in 40% glycerol at -80°C and routinely grown on Luria Bertani Agar [LBA; LB broth (Sigma Chemical-St. Louis, MO, USA), 1.6% (w/v) Agar Technical No.3 (Agar, Oxoid-Columbia, MD, USA)] at 27°C . Bacterial cultures originating from 72 h incubation at 27°C were used in all the experiments, unless otherwise indicated.

Agar (Oxoid) was added at different concentrations in all the growth media used. Swimming Agar [SWM; 1.0% Tryptone (Oxoid), 0.5% NaCl (Sigma-Aldrich), 0.3% (w/v) Agar, pH 7.00] and Swarming Agar [SWR, 0.8% Nutrient broth (NB) No.2 (Fluka analytical), 0.5% D-(+)-Glucose (Sigma-Aldrich), 0.5% (w/v) Agar] were used for swimming and swarming motility assays, respectively, (Rashid and Komberg, 2000; Déziel et al., 2001). LB amended with 0.5% (LBA 0.5) and 1% Agar (w/v) (LBA 1) were used for the swarming and twitching motility assays, respectively, (Rashid and Komberg, 2000; Dunger et al., 2014). Pea broth (PB; 12.5% frozen peas in distilled water) amended with 0.3% [Pea Agar Medium (PAM) 0.3]; 0.5% (PAM 0.5) and 1% Agar (w/v) (PAM 1) were used for the swimming, swarming and twitching motility assays, respectively.

Analysis of Pea Broth Effects on the *In vivo* Activity of *Lysobacter capsici* AZ78

The PB effect on the efficacy of *L. capsici* AZ78 against *P. viticola* under controlled greenhouse conditions was tested according to Puopolo et al. (2014b). Two-year-old *Vitis vinifera* cv. Pinot Noir grapevine plants, grafted onto Kober 5BB, were treated with distilled water (H_2O), PB, PB augmented with *L. capsici*

AZ78 (1×10^8 cells/ml) or *L. capsici* AZ78 (1×10^8 cells/ml). Each treatment (40 ml/plant) was applied on adaxial and abaxial leaf surfaces 24 h before inoculation with *P. viticola* (2.5×10^5 sporangia/ml). Both treatments and inoculum were sprayed with a hand sprayer. Inoculated plants were incubated overnight at $25 \pm 1^\circ\text{C}$ and 80–99% Relative Humidity (RH) in the dark, and then maintained at $25 \pm 1^\circ\text{C}$ and 60–80% RH with a 16/8-h day/night light regime. Seven days after inoculation, plants were incubated overnight in the dark at $25 \pm 1^\circ\text{C}$ and 80–99% RH to induce *P. viticola* sporulation. Disease severity (percentage of abaxial leaf area covered with sporulating lesions) and disease incidence (percentage of leaves with visible sporulation) were evaluated visually according to the standard guidelines of the European and Mediterranean Plant Protection Organization (EPPO, 2001). A randomized complete block design (six plants per treatment) was used. The presence of *L. capsici* AZ78 cells on grapevine leaves was assessed 24 h after the treatment with the dilution plating method (Puopolo et al., 2014b).

Motility Assays on Inert Surfaces

The motility of the *Lysobacter* strains (Table 1) was evaluated on different media. The experiments to assess swimming and swarming motility were carried out according to Köhler et al. (2000), with modifications. Briefly, 18 ml of each medium (SWM, PAM 0.3, SWR, LBA 0.5, and PAM 0.5) were poured into Petri dishes (90 mm) and dried for 1 h on the bench at room temperature and 20 min under laminar flow. Once dry, the dishes were inoculated with each bacterial strain with a toothpick and maintained at 27°C for 20 h.

The twitching motility assay was carried out according to Dunger et al. (2014), with modifications. Briefly, the bacterial strains were inoculated with a toothpick at the bottom of the dishes containing LBA 1 and PAM 1. After 7 days of incubation at 27°C , the agar was removed and the zone of twitching motility was dyed with 0.1% (w/v) crystal violet (CV) at room temperature

for 15 min. *Bacillus amyloliquefaciens* S499 (Pertot et al., 2013) and *Pseudomonas chlororaphis* M71 (Puopolo et al., 2011) were used as a control in all the assays. For each motility assay, three Petri dishes of each medium were used for each bacterial strain.

To evaluate whether PB may foster *L. capsici* AZ78 motility *in planta*, autoclaved PB (200 ml) cooled down to room temperature was applied with a hand sprayer to the leaves of three grapevine plants (replicates) and left to dry for 1 h under greenhouse conditions. Grapevine plants treated with 200 ml of distilled water (H_2O) were used as a control. Subsequently, ten leaves from each grapevine plant were collected and placed in sterile plastic boxes containing 100 ml of double distilled water and washed for 1 h with orbital shaking (100 rpm) at room temperature. The resulting leaf-washing suspensions were filtered with sterile cheesecloth and collected in sterile bottles. Agar was added to each leaf-washing solution to reach a final concentration of 0.5% (w/v) and subsequently autoclaved. The resulting media were poured into Petri dishes (18 ml each) and dried as described previously. *L. capsici* AZ78 was inoculated with a toothpick and colony areas were measured after 20 h of incubation at 27°C as described above. Three Petri dishes were used for each replicate.

For each assay, Petri dishes were visualized with a Bio-Rad Geldoc system and images were digitally captured using Bio-Rad Quantity One software. The motility of bacterial strains on inert surfaces was subsequently quantified by scoring the colony areas (mm^2) using Fiji software (ImageJ 1.49; Dunger et al., 2014).

Genome Mining

The sequenced genome of *L. capsici* AZ78 (version JAJA00000000.2; Puopolo et al., 2016) was mined to identify putative genes involved in bacterial motility mechanisms using nucleotide and protein sequence comparison. To identify putative *L. capsici* AZ78 genes responsible for flagellum and T4P, annotated products of genes encoding regulatory and structural

TABLE 1 | Bacterial strains.

Species	Strain	Origin	Reference
<i>Lysobacter antibioticus</i>	DSM 2044 ^T	Soil	DSMZ
<i>Lysobacter arseniciresistens</i>	DSM 2723 ^T	Soil	DSMZ
<i>Lysobacter brunescens</i>	DSM 6979 ^T	Lake water	DSMZ
<i>Lysobacter capsici</i>	DSM 19286 ^T	Pepper rhizosphere	DSMZ
<i>Lysobacter capsici</i>	DSM 23109 ^T	Clay soil (grass crop)	DSMZ
<i>Lysobacter capsici</i>	AZ78	Tobacco rhizosphere	Puopolo et al., 2014b
<i>Lysobacter capsici</i>	M143	Tomato rhizosphere	Puopolo et al., 2014b
<i>Lysobacter capsici</i>	55	Clay soil (cauliflower crop)	Postma et al., 2010
<i>Lysobacter capsici</i>	6.2.3	Clay soil (grass crop)	Postma et al., 2010
<i>Lysobacter daejeonensis</i>	DSM 17634 ^T	Greenhouse soil	DSMZ
<i>Lysobacter enzymogenes</i>	DSM 2043 ^T	Soil	DSMZ
<i>Lysobacter gummosus</i>	DSM 6980 ^T	Soil	DSMZ
<i>Lysobacter spongiicola</i>	DSM 21744 ^T	Deep-sea sponge	DSMZ
<i>Bacillus amyloliquefaciens</i>	S499	Soil	Pertot et al., 2013
<i>Pseudomonas chlororaphis</i>	M71	Tomato rhizosphere	Puopolo et al., 2011

^T Type strain; DSMZ, Leibniz Institute DSMZ-German Collection of Microorganism and Cell Cultures.

components of flagella and T4P of *L. capsici* 55 (CP011130), *P. aeruginosa* PAO1 (AE004091), *Stenotrophomonas maltophilia* (*Sm*) K279a (AM743169), and *Xanthomonas campestris* (*Xc*) pv. *campestris* ATCC 33913 (AE008922; Stover et al., 2000; da Silva et al., 2002; Crossman et al., 2008; de Bruijn et al., 2015) were aligned against the *L. capsici* AZ78 genome, using the RAST server (Aziz et al., 2008), and a cut-off of $1e-10$ at amino acid level was applied. The putative *L. capsici* AZ78 genes identified were then analyzed with BLATP (Johnson et al., 2008), and length >70 and identity $\geq 50\%$ at amino acid level were used as thresholds.

Primers specific (Supplementary Table S1) for flagellum and T4P biogenesis in *L. capsici* AZ78 were designed using Primer3 software¹ (Untergasser et al., 2012) and their specificity was assessed through PCR and Sanger sequencing before gene expression analysis using quantitative real-time polymerase chain reaction (qRT-PCR).

Assessment of *Lysobacter capsici* AZ78 Cell Growth in Different Media

The influence of the medium on the cell growth of *L. capsici* AZ78 was assessed in LB, PB, and SWR broth [NB; 0.5% D-(+)-glucose (Sigma–Aldrich) (w/v)]. Cell growth was measured as optical density at 600 nm (OD_{600}) using a Synergy 2 agitated multiwell plate reader (Biotek, Winooski, VT, USA) according to Segarra et al. (2015). The OD_{600} was monitored each hour for 36 h at 27°C. The initial bacterial cell concentration was 1×10^7 cells/ml (Puopolo et al., 2014b). Non-inoculated media were used as a control. Seven technical replicates were carried out.

Gene Expression Analysis

Lysobacter capsici AZ78 was inoculated on LBA 0.5, PAM 0.5, and SWR with a toothpick and afterward incubated at 27°C for 20 h. Then plugs (5-mm diameter) were collected from the bacterial macrocolony, immediately frozen in liquid nitrogen and ground to a fine powder. Three Petri dishes for each medium were inoculated (replicates). Each replicate was composed of three plugs from *L. capsici* AZ78 macrocolonies originating from the same dish.

Total RNA was extracted with Tri Reagent (Sigma–Aldrich, St. Louis, MO, USA), according to the manufacturer's instructions, with slight modifications. The final pellet was re-suspended in 50 μ l of RNase-free water. Total RNA was subsequently purified using the RNeasy Mini Kit (Qiagen Sciences, Valencia, CA, USA) and DNase treatment was performed with the RNase-Free DNase set (Qiagen). RNA integrity and concentration were assessed using electrophoresis in agarose gel and a Qubit 3.0 Fluorometer (Invitrogen), respectively. First-strand cDNA was synthesized from 100 ng of purified RNA with the SuperScript III Reverse Transcriptase RT kit (Invitrogen), according to the manufacturer's instructions. All qRT-PCR reactions were carried out with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and specific primers (Supplementary Table S1) using LightCycler 480 software (Roche Diagnostics, Mannheim,

Germany). The qRT-PCR reactions consisted of 50 amplification cycles (95°C for 15 s and 60°C for 45 s) and melting curve analysis. Cycle threshold (C_t) values were extracted with LightCycler 480 SV1.5.0 software (Roche Diagnostics) using the second derivative calculation and reaction efficiency was calculated with LinRegPCR 11.1 software (Ruijter et al., 2009). Relative expression levels were determined with the $2^{-\Delta\Delta C_t}$ method (Pfaffl, 2001) based on three replicates per sample using *L. capsici* AZ78 growth on LBA 0.5 as the calibrator. The housekeeping gene *recA* (AZ78_1089) was used as the constitutive gene for normalization, because its expression was not significantly affected by growth media and conditions (Takle et al., 2007; Florindo et al., 2012). qRT-PCR reactions were carried out for the two independent experiments.

Influence of Pea Concentrations on the Motility of *Lysobacter capsici* AZ78

The influence of nutrient concentration on *L. capsici* AZ78 motility was evaluated using PAM with 0.5% Agar (w/v) and frozen peas at four concentrations: 1.5, 3.0, 6.0, and 12.5% (w/v). Swarming motility experiments were carried out as described above. Three Petri dishes were used as replicates.

Transmission Electron Microscopy

Drops (50 μ l) containing a suspension of *L. capsici* AZ78 cells (1×10^8 cells/ml), following 20 h incubation at 27°C on PAM 0.5 and LBA 0.5, were adsorbed to transmission electron microscopy (TEM) carbon-formvar coated nickel grids for 10 min, at room temperature. The bacterial cells were then stained in 3% (w/v) uranyl-acetate for 8 min and rinsed three times (10 s each) in sterile distilled water. The grids were examined under a Philips CM 10 TEM (Eindhoven, The Netherlands) operating at 80 kV (Cowles and Gitai, 2010).

Statistical Analysis

Swarming, swimming and twitching assays and the experiments regarding the influence of pea concentration on the *L. capsici* AZ78 motility were carried out three times while the remaining experiments were carried out twice. All statistical tests were carried out using Statistica 9.0 (StatSoft, USA). For each assay the data obtained from the repeated experiments were subjected to two-way analysis of variance (ANOVA) and data were pooled when no significant differences were found, according to the *F*-test ($\alpha > 0.05$). Data on disease incidence and severity were log transformed, while the fold change values of gene expression analysis were transformed using the equation $y = \log_{10}(1+x)$ (Casagrande et al., 2011). The data for swarming, qRT-PCR, disease severity, disease incidence and cell density were analyzed using one-way ANOVA, after validation of normal distribution (*K-S* test, $\alpha > 0.05$) and variance homogeneity of the data (Levene's test, $\alpha > 0.05$), Tukey's test ($\alpha = 0.05$) was applied to detect significant differences. Student's *t*-test ($\alpha = 0.05$) was applied in pairwise comparison of the colony areas reached in the swimming, twitching, and *in planta* motility assays.

¹<http://bioinfo.ut.ee>

RESULTS

Pea Broth Enhances the Plant Protection Efficacy of *Lysobacter capsici* AZ78

Based on the occurrence of motility observed when *L. capsici* AZ78 was grown on jellified PB (Supplementary Figure S1), greenhouse trials were set up to assess the impact of PB on the efficacy of *L. capsici* AZ78 in controlling *P. viticola*. Although an effect of the experiment was present (F -test, $P = 0.004$), the application of *L. capsici* AZ78 alone and *L. capsici* AZ78 with PB significantly reduced disease severity and incidence, as compared with H₂O and PB-treated plants (Table 2). In particular, disease severity and incidence were significantly lower in plants treated with *L. capsici* AZ78 and PB than with *L. capsici* AZ78 alone. As expected, no *L. capsici* AZ78 cells were isolated from leaves of H₂O and PB-treated plants. *L. capsici* AZ78 cells recovered from leaves treated with *L. capsici* AZ78 alone were lower than those recovered from leaves treated with *L. capsici* AZ78 and PB (Table 2).

The Motility of *Lysobacter capsici* Depends on Medium Composition

Bacillus amyloliquefaciens S499 and *P. chlororaphis* M71, used as controls, were able to move on all the media employed in the motility assays (Supplementary Table S2). No significant differences were found between the swimming motility on SWM and PAM 0.3 in type strains of *L. antibioticus*, *L. arseniciresistens*, *L. brunescens*, *L. daejeonensis*, *L. enzymogenes*, *L. gummosus*, and *L. spongiicola* (Figure 1A). The colony area of *L. capsici* strains grown on PAM 0.3 [ranging from 125.8 ± 22.0 mm² of *L. capsici* M143 (mean \pm SE) to 684 ± 99.7 mm² of *L. capsici* AZ78], was significantly larger than the area measured on SWM [ranging from 12.5 ± 0.8 of *L. capsici* M143 to 52.2 ± 3.7 mm² of *L. capsici* AZ78; Figures 1A,D,E]. However, the colony morphology of *L. capsici* strains grown on PAM 0.3 did not show the typical swimming morphology registered in the case of *B. amyloliquefaciens* S499 and *P. chlororaphis*

M71 (Supplementary Figure S2). In particular, the colony morphology of all *L. capsici* strains on PAM 0.3 was characterized by the presence of multiple dendrites originating from the inoculation spot and elongating toward the edge of the Petri dish (Figure 1E).

Lysobacter antibioticus, *L. arseniciresistens*, *L. brunescens*, *L. daejeonensis*, *L. enzymogenes*, *L. gummosus*, and *L. spongiicola* type strains did not swarm on LBA 0.5, PAM 0.5, and SWR. They developed a circular macrocolony and the growth area was limited to the inoculation spot on the three media (Figures 1B,F,G). All the *L. capsici* strains showed swarming motility and spread on PAM 0.5, forming dendrites as in the swimming test (Figure 1G). Moreover, significant differences between the areas observed on PAM 0.5 and the two other media were observed (Figure 1B). For example, *L. capsici* AZ78 grown on PAM 0.5 had a colony area of 399.1 ± 45.7 mm², significantly larger than the area covered on LBA 0.5 (45.0 ± 8.7 mm²) and SWR (77.5 ± 13.5 mm²). Interestingly, a ring halo caused by the production of biosurfactant compounds was observed in the case of *L. capsici* strains grown on PAM after 20 h of incubation at 27°C (Figure 1H).

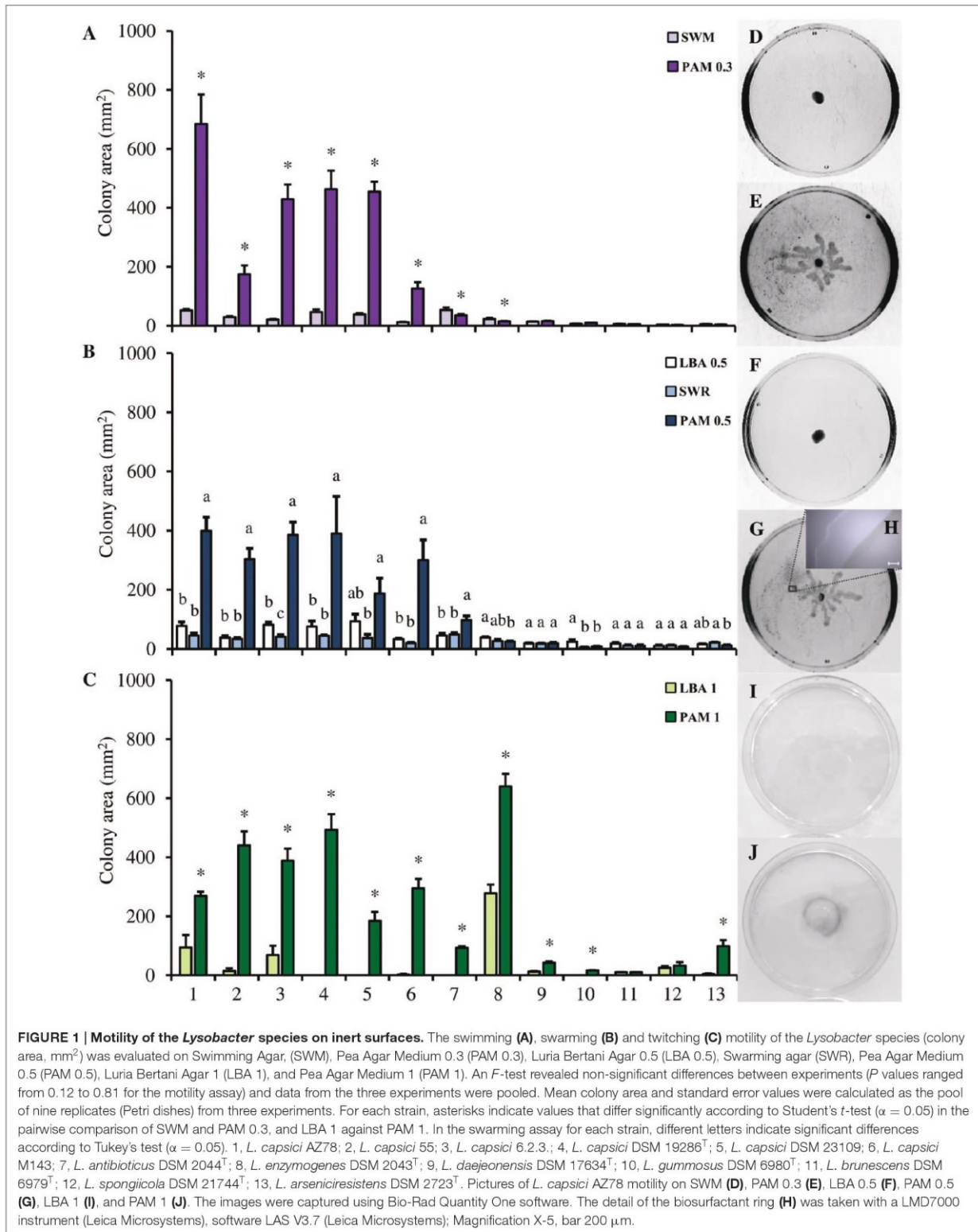
When LBA 1 was used, *L. antibioticus*, *L. arseniciresistens*, *L. brunescens*, *L. capsici*, *L. daejeonensis*, *L. gummosus*, and *L. spongiicola* strains did not produce a visible twitching zone. *L. enzymogenes* DSM 2043^T was the only strain that developed a visible twitching zone, with an area of 277.4 ± 29.2 mm² (Figures 1C,I,J). However, *L. antibioticus*, *L. arseniciresistens*, *L. daejeonensis*, *L. enzymogenes*, *L. gummosus*, and all the *L. capsici* strains developed a visible twitching zone on the plastic surface when PAM 1 was used. On this medium, the largest areas were developed by *L. enzymogenes* DSM 2043^T (639.6 ± 42.7 mm²) and the *L. capsici* strains (ranging from 184.0 ± 30.9 mm² of *L. capsici* DSM 23109 to 9388.1 ± 41.1 mm² of *L. capsici* 6.2.3) (Figure 1J).

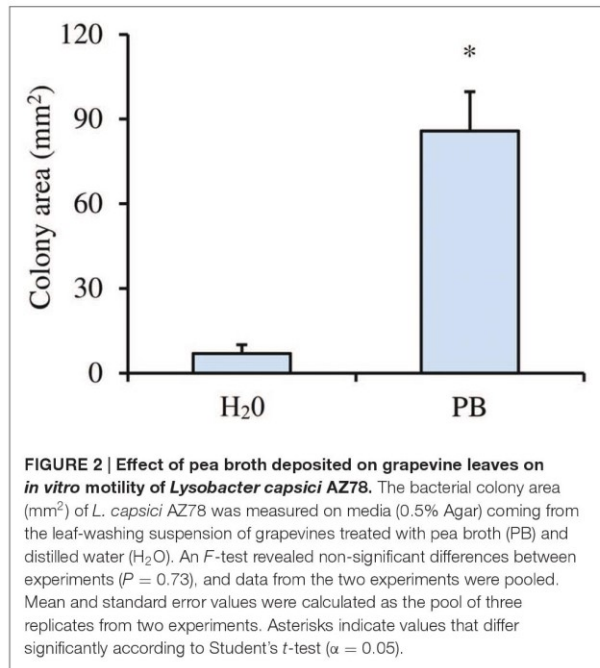
Moreover, leaf-washing suspensions deriving from plants treated with PB allowed significantly greater motility of *L. capsici* AZ78 cells, as compared with those deriving from plants treated with H₂O (Figure 2).

TABLE 2 | Effect of pea broth on the plant protection efficacy of *Lysobacter capsici* AZ78.

Experiment 1	Disease Severity (%)	Disease Incidence (%)	Cell Density (log ₁₀ CFU g ⁻¹ of leaf)
H ₂ O	24.7 \pm 2.3 ^a	100 \pm 0 ^a	0 \pm 0 ^c
PB	21.7 \pm 4.2 ^a	100 \pm 0 ^a	0 \pm 0 ^c
PB + <i>L. capsici</i> AZ78	3.4 \pm 0.8 ^c	47.9 \pm 6.2 ^c	5.5 \pm 0.3 ^a
<i>L. capsici</i> AZ78	7.9 \pm 1.4 ^b	87.0 \pm 1.4 ^b	4.2 \pm 0.1 ^b
Experiment 2	Disease Severity (%)	Disease Incidence (%)	Cell Density (log ₁₀ CFU g ⁻¹ of leaf)
H ₂ O	29.3 \pm 5.0 ^a	100 \pm 0 ^a	0 \pm 0 ^c
PB	30.2 \pm 4.4 ^a	100 \pm 0 ^a	0 \pm 0 ^c
PB + <i>L. capsici</i> AZ78	0.8 \pm 0.2 ^c	10.7 \pm 2.7 ^c	6.2 \pm 0.1 ^a
<i>L. capsici</i> AZ78	2.5 \pm 0.3 ^b	43.1 \pm 5.2 ^b	5.3 \pm 0.1 ^b

The treatments applied to grapevine plants were: distilled water (H₂O), pea broth (PB), *L. capsici* AZ78 (1×10^8 cells/ml) and the combination of pea broth and *L. capsici* AZ78 (1×10^8 cells/ml). Disease severity (% of abaxial leaf area covered with sporulating lesions) and disease incidence (% of leaves with visible sporulation) were evaluated seven days after *P. viticola* inoculation. The density of *L. capsici* AZ78 cells residing on grapevine leaves was evaluated using a dilution plating method. Two separate trials (Experiment 1 and Experiment 2; [F -test; $P = 0.004$]) were carried out and six plants (replicates) were used for each treatment. Mean \pm standard error values are reported in the table. Different letters indicate significant differences according to Tukey's test ($\alpha = 0.05$).





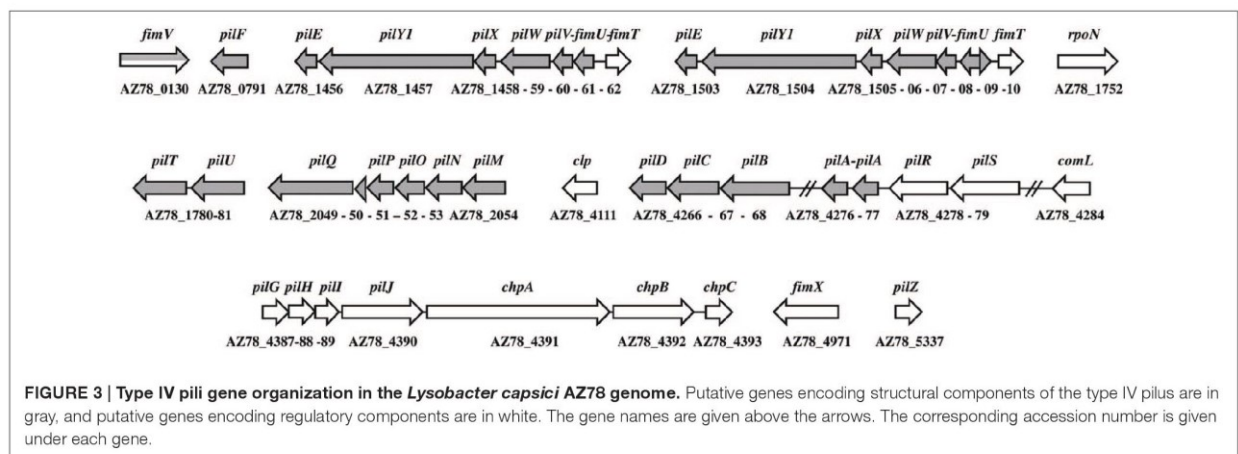
The Genome of *Lysobacter capsici* Az78 Includes Genes Responsible for Flagellum and Type IV Pilus Biogenesis

The flagellar gene organization of *L. capsici* AZ78 matched that of *L. capsici* 55, *L. enzymogenes* C3, *L. enzymogenes* OH11, *L. gummosus* 3.2.11, and the oceanic γ -Proteobacteria *Haella chejuensis* KCTC 2396^T (CP000155; Jeong et al., 2005; Wang et al., 2014; de Bruijn et al., 2015). In particular, 21 putative genes encoding components of flagellar apparatus are present in the *L. capsici* AZ78 genome (Supplementary Table S3). These genes are highly conserved in *L. capsici* 55 (de Bruijn et al., 2015) and members of the two phylogenetically related flagellated

species *Xc* and *Sm*. For instance, the amino acid sequence of the basal body components *flgI* and *flgH* (AZ78_1627, 1628) are 100 and 99% identical with the flagellar P-ring protein and L-ring protein of *L. capsici* 55 (ALN84757, ALN84756), respectively. The same amino acid sequences are 75 and 71% identical with the basal body proteins of *Sm* (KOO80985, KOO78078). The putative *flgE* (AZ78_1616) shared 99 and 60% of identity at amino acid level with the flagellar hook protein of *L. capsici* 55 (ALN84768) and *Sm* (KOO78088), respectively. Through BLASTP analysis, a gene (AZ78_1611) including two sigma-70 conserved regions was identified in the *L. capsici* AZ78 genome. This transcriptional factor was 99 and 39% identical with the RNA polymerase sigma-70 factor of *L. capsici* 55 (ALN84773) and *L. enzymogenes* C3 (ALN59990), respectively. No ortholog of *fliC* was found in the genome of *L. capsici* AZ78.

Genome mining allowed identification of 43 genes related to T4P biogenesis, and they were organized into six main gene clusters (Supplementary Table S4), as reported for other *Lysobacter* strains (de Bruijn et al., 2015). The putative T4P genes of *L. capsici* AZ78 had high sequence identity with the orthologs found in members of the *Xc* and *Sm* species. The genome of *L. capsici* AZ78 had two genes encoding type IV major pilin PilA (AZ78_4276, 4277), and their amino acid sequence identity ranged from 42 to 56% with the *Xc* ortholog (KOB01592) and 31 to 73% with the protein products of *xac3240*–*3241* of *X. axonopodis* pv. *citri* 306 (AE008923). Moreover, the PilA proteins (AZ78_4276, 4277) shared 59 and 68% amino acid identity with the pilin ortholog of *L. capsici* 55 (ALN87506). Interestingly, the *L. capsici* AZ78 genome included a third putative pilin gene (AZ78_3612) that was 39% identical with XAC3805 of *X. axonopodis* pv. *citri* 306 at amino acid level, and it was located far from the *pilRS* (5,121,276–5,124,387 bp) and *pilABCD* (5,105,118–5,120,729 bp) regions.

The minor pilin operon (*fimUpilVWXY1E*) was repeated in the genome of *L. capsici* AZ78 (1,769,261–1,776,246 and 1,814,313–1,821,233 bp; **Figure 3**), similarly to *L. capsici* 55, *L. enzymogenes* C3, and *L. gummosus* 3.2.11 (de Bruijn et al., 2015). The genes were highly conserved at amino acid level through *Lysobacter* members with an identity value ranging



from 59 to 100% (Supplementary Table S4). The *L. capsici* AZ78 pilus-specific chemotaxis system (Pil-Chp) was composed of seven *pilGHIJchpABC* genes (5,263,810–5,277,356 bp) and was missing a *pilK* ortholog. Interestingly, the putative assembly protein PilG (AZ78_4387) shared 86% identity with the *P. aeruginosa* ortholog (KSF29090) and more than 90% with the corresponding orthologs in *L. enzymogenes* C3 (ALN59304; Zhou et al., 2015), *Xc* (KOB02299) and *Sm* (KIP84116). The alignment of the subcomplex operon *pilMNOP* (2,509,989–2,513,122 bp) and *pilQ* (AZ78_2049) revealed that these genes were highly conserved through the *Lysobacter* members (from 83 to 100% amino acid identity) and the genes shared from 61 to 82% of amino acid sequence identity with *Xc* and *Sm*, respectively.

The T4P transcriptional factor RpoN (\mathcal{E}^{54}) (AZ78_1752) was 67 and 65% identical to the ortholog protein of *Xc* (CDN18987) and *Sm* (KOQ68432), respectively. The amino acid identity of this transcriptional factor ranged from 88 to 100% through the *Lysobacter* species (e.g., *Lysobacter antibioticus* ATCC 29479, ALN61594; *L. capsici* 55, ALN84614). Moreover, the two-component system PilR–PilS, expressed by the gene operon AZ78_4278–4279, showed high amino acid identity (from 58 to 73%) with the PilR–PilS proteins of *Xc* (NP_638443–NP_638442) and *Sm* (WP_049405671–WP_017356171). The *pilR–pilS* operon was highly conserved in *L. capsici* AZ78 and *L. capsici* 55 (ALN87505, ALN87504), indeed the protein sequences were 100 and 99% identical, respectively. The derived amino acid sequence of the global regulator *clp* (AZ78_4111) was 84 and 45% identical to the ortholog Clp of *Xc* (NP_635866) and Vfr of *P. aeruginosa* PAO1 (NP_249343), respectively, (Beatson et al., 2002; He et al., 2007; Burrows, 2012). The Clp amino acid sequence was conserved in *Lysobacter* members with an identity value of 99% (e.g., *L. capsici* 55, ALN87652; *L. enzymogenes* C3, AAP83141).

Upregulation of Genes Involved in Type IV Pilus Biogenesis is Associated with *Lysobacter* Az78 Medium-Dependent Motility

Lysobacter capsici AZ78 grew in a similar way in LB, PB and SWR broth (Supplementary Figure S3). The bacterial strain entered the logarithmic phase after 6 h in all the media. However, the cell mass produced in PB was lower than LB and SWR (Supplementary Figure S3).

Six and 14 genes, respectively, responsible for flagellum and T4P biogenesis in *L. capsici* AZ78 were selected for gene expression analysis using qRT-PCR (Supplementary Table S1). The relative expression levels of the structural and regulatory components of flagellum and T4P were calculated for *L. capsici* AZ78 cells grown on LBA 0.5, PAM 0.5, and SWR. qRT-PCR analysis revealed the absence of transcriptional regulation of the structural flagellar genes *flgI* (AZ78_1627), *flhB* (AZ78_1633), *fliH* (AZ78_1621), and *fliR* (AZ78_1634) in the three media tested (Figure 4A). The expression of putative *flgE* (AZ78_1616) was down regulated (0.5-fold) when *L. capsici* AZ78 was grown on both PAM 0.5 and SWR, compared with LBA 0.5 (Figure 4A). The putative RNA-polymerase \mathcal{E}^{70}

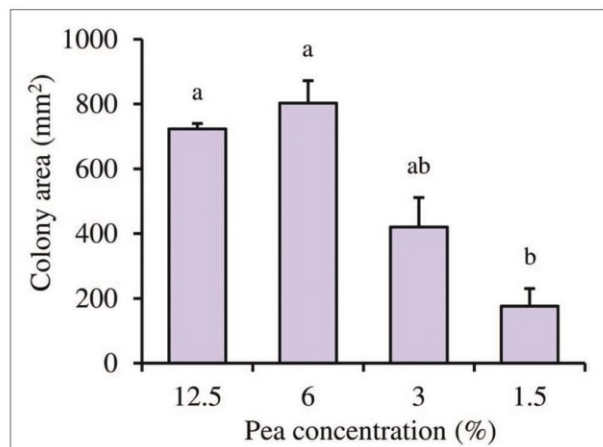
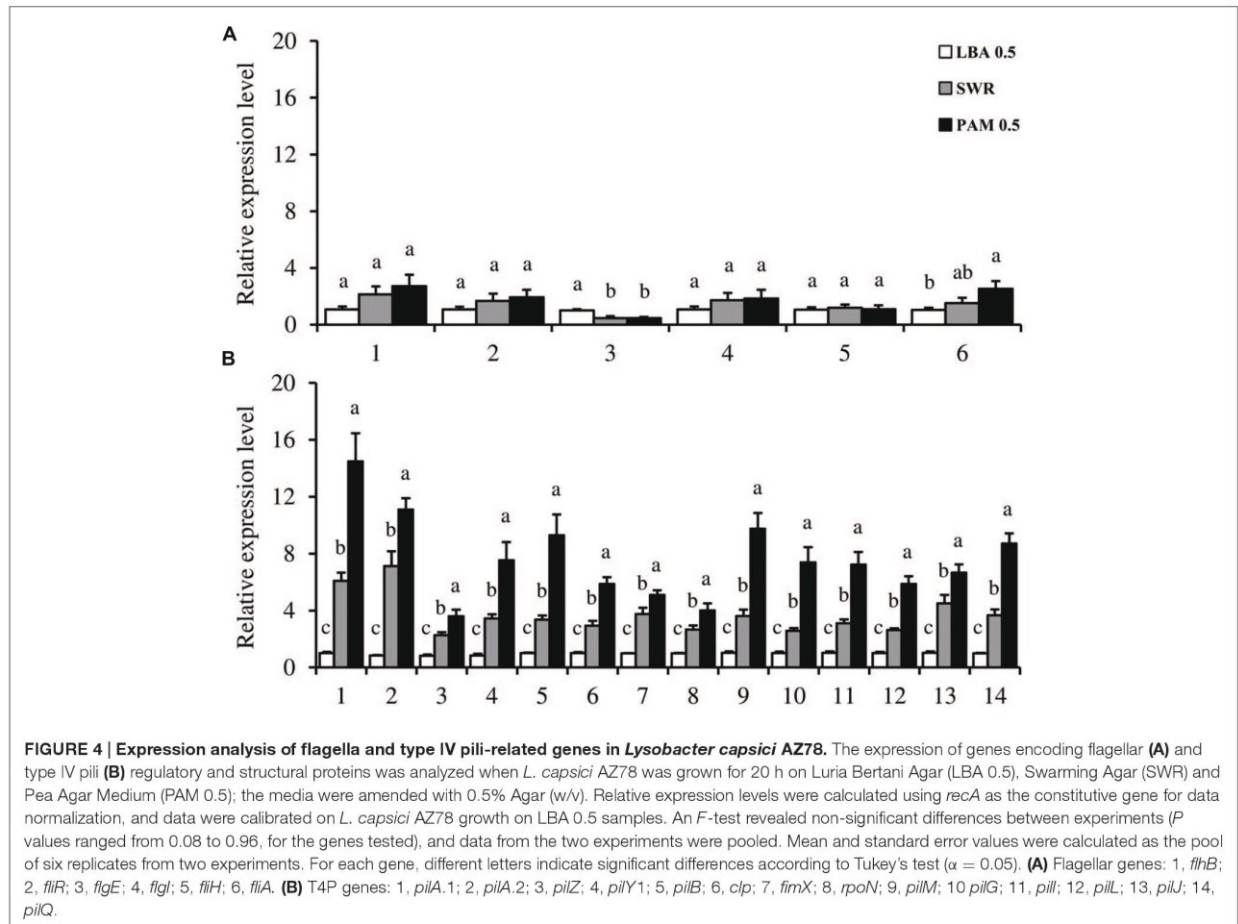


FIGURE 5 | Influence of pea concentrations on *Lysobacter capsici* AZ78 motility. The motility of *Lysobacter capsici* AZ78 was monitored on Pea Agar Medium (0.5% Agar) containing 12.5, 6.0, 3.0, and 1.5% frozen peas. An *F*-test revealed non-significant differences between experiments ($P = 0.84$), and data from the three experiments were pooled. Mean colony area (mm²) and standard error values were calculated as the pool of nine replicates (Petri dishes) from three experiments. Different letters indicate significant differences according to Tukey's test ($\alpha = 0.05$).

regulatory factor (*fliA*, AZ78_1611) was induced more than twofold on PAM 0.5 compared with LBA 0.5, but the gene expression level on PAM 0.5 was comparable to that on SWR (Figure 4A).

Gene expression analysis revealed significant upregulation of all the T4P biogenesis genes when *L. capsici* AZ78 was grown on PAM 0.5, compared with LBA 0.5 and SWR (Figure 4B). The gene expression levels of the four apparatus sub-complexes were induced by growth on PAM 0.5 medium as compared with SWR and LBA 0.5. In particular, the putative *pilQ* (AZ78_2049) and *pilM* (AZ78_2054) genes were upregulated on PAM 0.5 (more than ninefold) and on SWR (more than fourfold), compared with LBA 0.5. The expression level of the putative *pilB* (AZ78_4268) on PAM 0.5 was nine and threefold higher than on LBA 0.5 and SWR, respectively. The putative *pilY1* (AZ78_1457) was upregulated on PAM 0.5 (ninefold) and SWR (fourfold), compared with LBA 0.5. The major pilin genes *pilA* (AZ78_4276, 4277) were induced more than 13-fold when *L. capsici* AZ78 was grown on PAM 0.5, as compared to LBA 0.5 (Figure 4B). Moreover, the expression levels of *pilA* genes on PAM 0.5 were twice as high as those on SWR (sevenfold). As regards the regulatory system, the putative *pilJ* (AZ78_4390), encoding the single methyl-accepting chemotaxis protein (MCP) of the Pil-Chp system, was positively regulated when the bacterium was grown on PAM 0.5 (6.5-fold) and SWR (1.5-fold), as compared with LBA 0.5. Moreover, the other components of the Pil-Chp system tested [*pilG* (AZ78_4387), *pilI* (AZ78_4389), and *chpA* (AZ78_4391)] were upregulated on PAM 0.5 (five to sevenfold) and SWR (two to threefold), as compared with LBA 0.5. The gene encoding the regulatory factor RpoN (\mathcal{E}^{54}) (AZ78_1752) was induced (fourfold) on PAM 0.5, as compared to LBA 0.5.



In addition, growth on PAM 0.5 increased the *clp* (AZ78_4111) expression level sixfold as compared with LBA 0.5.

Based on the qRT-PCR analysis, the possible implication of the pea concentration on *L. capsici* AZ78 motility was assessed. *L. capsici* AZ78 bacterial motility was negatively influenced by low nutrient availability. Indeed, a reduction in the *L. capsici* AZ78 colony area was registered in the assays, with a significant difference between the highest (12.5%) and lowest (1.5%) pea concentration (from $723.4 \pm 16.4 \text{ mm}^2$ to $175.7 \pm 54.9 \text{ mm}^2$; Figure 5).

Finally, TEM analysis confirmed the presence of surface appendages on *L. capsici* AZ78 cells. The negative staining of *L. capsici* AZ78 cells revealed pilus-like structures localized at the poles of bacterial cells grown on PAM 0.5 (Figures 6A,C). In contrast, these structures were not present when *L. capsici* AZ78 was grown on LBA 0.5 (Figures 6B,D).

DISCUSSION

The efficacy of bacterial biocontrol agents is strictly associated with their ability to actively colonize the same ecological niches

occupied by phytopathogenic microorganisms (Weller, 1988; Compant et al., 2005). To reach these niches, bacteria move in the environment thanks to external appendages, such as flagella and T4P (Mattick, 2002; Harshey, 2003; Kearns, 2010). Thus, determination of the mechanisms involved in the motility of bacterial biocontrol agents and understanding the factors that affect these mechanisms should be taken in consideration in the development of novel biopesticides. Unfortunately, little is known about the motility of *Lysobacter* members, a bacterial genus of increasing relevance in the development of biocontrol agents for important plant pathogens (Ji et al., 2008; Postma et al., 2010; Puopolo et al., 2014a,b). Recently, low control of *Rhizoctonia solani* *in vivo* by *Lysobacter* strains was associated with the poor ability of these strains to actively colonize the rhizosphere of different plants (Gómez Expósito et al., 2015). Understanding the factors and mechanisms involved in *Lysobacter* spp. motility is therefore crucial in order to improve their biocontrol efficacy.

Recently, we observed the dispersal of *L. capsici* AZ78 cells when grown on a medium containing PB used in tests aimed at evaluating *in vitro* inhibition of *P. infestans* (Supplementary Figure S1; Puopolo et al., 2014a). Based on these observations,

we carried out greenhouse trials to discover whether PB could enhance the efficacy of *L. capsici* AZ78 against *P. viticola* by fostering cell movement on grapevine leaves. Interestingly, co-application of PB increased the quantity of *L. capsici* AZ78 cells residing on grapevine leaves and its efficacy against the phytopathogen significantly more than the bacterial strain applied alone. We carried out independent greenhouse trials to assess whether PB applied to grapevine leaves may lead to *L. capsici* AZ78 cell movement *in planta*. The results of the greenhouse experiments clearly showed that the quantity of PB remaining on grapevine leaves was sufficient to allow *L. capsici* AZ78 motility on agar surfaces.

Since these results could depend on the production of external appendages involved in the attachment of bacterial cells to the leaf and hyphae of phytopathogenic fungi and oomycetes (Van Doorn et al., 1994; Ojanen-Reuhs et al., 1997; Islam et al., 2005; Patel et al., 2011), we carried out further *in vitro* motility trials to better characterize the cell dispersal observed in *L. capsici* AZ78. We included other bacterial strains belonging to various *Lysobacter* species to have a more comprehensive analysis of cell motility in the *Lysobacter* genus.

The swimming and swarming tests carried out on SWM and SWR media confirmed that the type strains of *Lysobacter* spp. tested were unable to move on agar surfaces. Inability to move on these media containing 0.3 (SWM) and 0.5% (SWR) of agar was also observed in the case of type strains of the *L. arseniciresistens* and *L. spongiicola* species, although they have a single polar flagellum (Romanenko et al., 2008; Luo et al., 2012). The inability to move can be explained by the tendency of these bacterial

strains to easily lose the flagella (Romanenko et al., 2008; Luo et al., 2012).

Inability to move on agar surfaces was also confirmed when most of the *Lysobacter* type strains were grown on PAM 0.3 and 0.5. In contrast, all the *L. capsici* strains moved on these two media, giving rise to macrocolonies characterized by the production of dendrites, which allowed rapid colonization of the medium surface after 20 h of incubation. Although the formation of dendrites is a typical trait of swarming motility in other bacterial species belonging to other genera (Harshey, 1994), to the best of our knowledge, this is the first report regarding dendrite formation in members of the *L. capsici* species.

In addition, we observed the formation of a biosurfactant ring surrounding the macrocolony in all *L. capsici* strains grown on PAM 0.5. Interestingly, the biosurfactant ring surrounding the macrocolony is another typical trait of swarming motility characterizing other bacterial strains belonging to other genera, as in the case of *B. subtilis* 3610 (Julkowska et al., 2004). Biosurfactants are involved in the swarming motility of many bacteria, such as *P. aeruginosa* (Köhler et al., 2000; Déziel et al., 2003), *B. subtilis* (Kearns and Losick, 2003) and *Serratia liquefaciens* (Lindum et al., 1998), where they help to overcome surface tension for efficient bacterial surface colonization (Matsuyama and Nakagawa, 1996; Harshey, 2003; Daniels et al., 2004). In contrast with other bacterial species, little attention has been paid to biosurfactant production in *Lysobacter* members. The only evidence of biosurfactant production was provided by Folman et al. (2004), who proved surfactant production in *L. enzymogenes* 3.1T8 cells grown

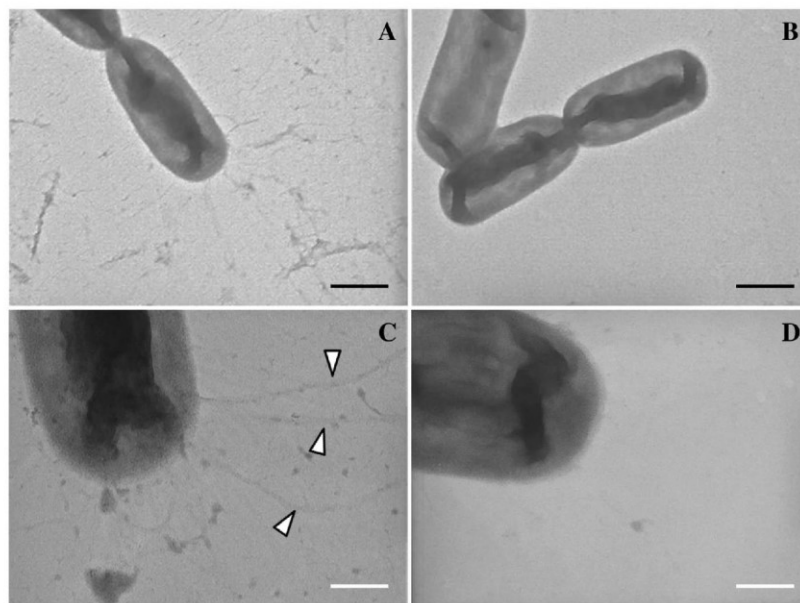


FIGURE 6 | Visualization of type IV pili of *Lysobacter capsici* AZ78 using Transmission Electron Microscopy (TEM). Pilus-like structures (arrows) emerge at the pole of *L. capsici* AZ78 cells grown on Pea Agar Medium amended with 0.5% Agar (w/v; **A,C**) and these structures are absent in cells grown on Luria Bertani Agar amended with 0.5% Agar (w/v; **B,D**). **A** and **B**, magnification X-19000, black bar 500 nm. **(C,D)**, magnification X-64000, white bar 150 nm.

in 1/2 and 1/10 Tryptone Soy Broth. However, biosurfactant compounds have not yet been identified and characterized in any *Lysobacter* member, and their characterisation merits additional studies.

Conventionally, swarming motility is described as flagella-related motility that allows the rapid colonization of semisolid surfaces (Harshey, 1994; Daniels et al., 2004). However, Köhler et al. (2000) and Overhage et al. (2007) reported the involvement of T4P in swarming motility of *P. aeruginosa* PAO1. A *fliC* mutant of *P. aeruginosa* PAO1 (PT690), unable to synthesize any flagellum, showed the ability to propagate on a semisolid medium while maintaining swarming motility (Köhler et al., 2000). T4P is also involved in twitching motility (Mattick, 2002) and this motility was recently reported in *L. enzymogenes* C3 and OH11 (Zhou et al., 2015). Interestingly, we observed that *L. enzymogenes* DSM 2043^T was moving through twitching on LBA 1, and a further increase in the colony area was recorded when this type strain was grown on PAM 1. These data thus confirm the twitching motility of members of the *L. enzymogenes* species and also show that PAM has some effect on the twitching motility of the *L. enzymogenes* type strain. Most of the other tested *Lysobacter* type strains did not move through twitching on either LBA 1 or PAM 1, with the sole exception of *L. capsici* strains. Indeed, a colony growth zone was observed on the bottom of plastic dishes when *L. capsici* strains were inoculated on PAM 1. Overall, the results of swimming, swarming and twitching assays indicated that *L. capsici* strains move on inert surfaces and this motility depended on the composition of the medium. This medium-dependent behavior was already observed in medium-dependent biofilm production by *L. capsici* AZ78 (Puopolo et al., 2014b).

Since the observed medium-dependent motility of *L. capsici* strains could be associated with the presence of both flagella and T4P, we mined the *L. capsici* AZ78 genome for genes involved in the production of these bacterial external appendages. We also assessed the involvement of these genes in the *L. capsici* AZ78 medium-dependent motility using qRT-PCR, since attempts to generate knock-out mutants have not been successful to date in the case of *L. capsici* strains (de Bruijn et al., 2015). The flagellar regulon in *L. capsici* AZ78 encompasses genes sharing a high amino acid sequence identity with flagellar proteins of *L. capsici* 55 (de Bruijn et al., 2015) and the related *Xc* and *Sm* species (Hayward et al., 2010). However, the *L. capsici* AZ78 genome lacked genes involved in regulation of the flagellar assembly pathway, such as the master operon *flhDC* (Liu and Matsumura, 1994), the anti-sigma factor *flgM* (Ohnishi et al., 1992) and a gene encoding the flagellin protein *FliC*. The lack of a functional regulon responsible for flagellum biogenesis was recently reported in other strains belonging to the *L. capsici*, *L. enzymogenes*, and *L. gummosus* species (de Bruijn et al., 2015). In spite of lack of functionality of the flagellum regulon, the *L. capsici* AZ78 genome has the protein export system of flagellar proteins (e.g., *flhA*, *flhB*, *fliH*, *fliI*, *fliP*, *fliQ*, and *fliR*). The presence of this export system, associated with the low expression level of putative *flhB*, *fliH*, *fliR*, and a non-functional flagellar biosynthesis pathway, led to the hypothesis that the flagellar system in *L. capsici* AZ78 evolved in a new function connected to

protein export (Toft and Fares, 2008). This point may be involved in other aspects of their lifestyle, such as the establishment of pathogenic interaction with the microbial host (de Bruijn et al., 2015).

The T4P biogenesis system of *L. capsici* AZ78 is highly conserved in members of *Xc*, *Sm*, and other *Lysobacter* species. *L. capsici* AZ78 encompasses two *pilA* genes that were next to the putative *pilR-pilS* two-component system and followed by putative *pilB*, *pilC*, and *pilD* in a conserved gene cluster. The presence of two *pilA* genes has already been reported in *X. axonopodis* pv. *citri* strain 306 (*xac3240* and *xac3241*; da Silva et al., 2002; Dunger et al., 2014). Moreover, another gene (AZ78_3612) encoding a pilin-like protein was identified in *L. capsici* AZ78, but it is probably not a *bona fide* T4P pilin, as previously reported for *X. axonopodis* pv. *citri* strain 306 (XAC3805) (Dunger et al., 2014).

In *P. aeruginosa* strains, the regulation of *pilA* transcription is controlled by RpoN (ϵ⁵⁴) and the two-component system PilR–PilS (Ishimoto and Lory, 1989, 1992; Burrows, 2012). Interestingly, the growth of *L. capsici* AZ78 on PAM 0.5 was associated with the induction of both *rpoN* and *pilA* genes, as compared with LBA 0.5 and SWR 0.5, indicating a direct effect of the medium composition on T4P biogenesis. The movement of *L. capsici* AZ78 on PAM 0.5 was also associated with upregulation of a transcription factor (*clp*) involved in flagellar and T4P biogenesis of *Xc* and *L. enzymogenes*, respectively, (Lee et al., 2003; He et al., 2007; Wang et al., 2014).

Since the chemosensory system is responsible for regulating pilus assembly and retraction (Burrows, 2012; Leighton et al., 2015), the expression of genes belonging to the chemosensory Pil–Chp system (*pilI*, *pilG*, *pilJ*, and *chpA*) was assessed in *L. capsici* AZ78. The expression of *pilG* was upregulated on PAM 0.5, compared with the other two media tested. PilG is a CheY-like regulator that influences T4P biogenesis in *P. aeruginosa* (Fulcher et al., 2010) and *L. enzymogenes* C3 (Zhou et al., 2015). Similarly, the *pilJ* gene was induced in *L. capsici* AZ78 grown on PAM 0.5 and this gene encodes a MCP protein responsible for sensing environmental stimuli and inducing pilus extension (DeLange et al., 2007; Burrows, 2012). The flagellar chemotaxis-system plays an important role in swarming motility in response to environmental signals (Harshey, 2003; Daniels et al., 2004). For instance, swarming in *P. aeruginosa* PAO1 is induced by specific amino acids (glutamate and aspartate) and carbon sources (glucose and glycerol) (Köhler et al., 2000). Likewise, T4P-dependent “social gliding” is controlled by the chemotaxis-like system in *M. xanthus* and *Synechocystis* spp. (Shi and Zusman, 1995; Bhaya et al., 2001). Based on the expression profiles of *L. capsici* AZ78 genes involved in the chemotaxis-system, it is conceivable that peas release some compounds that are perceived by *L. capsici* strains, and then the expression of genes involved in T4P biogenesis favoring cell motility is triggered. This is also supported by the fact that the quantity of peas used in PAM affects *L. capsici* AZ78 motility and stimulates dendrite production. Similarly, *B. amyloliquefaciens* S499 can sense components of the plant cell wall (xylan and arabinogalactan) and this perception stimulates the production of the cyclic lipopeptide surfactin

involved in biofilm formation and swarming motility (Debois et al., 2015).

Finally, the high expression level of genes responsible for the structure and regulation of T4P genes was associated with the presence of external appendages in *L. capsici* AZ78 grown on PAM 0.5 and visualized through TEM. In agreement with the gene expression levels, these structures were not visible on LBA 0.5-grown *L. capsici* AZ78 cells. The presence of external appendages (polar brush-like fimbriae) has already been reported in another biocontrol *Lysobacter* strain (*Lysobacter* sp. SB-K88) when grown on the roots of sugar beet seedlings (Islam et al., 2005), supporting the theory that the medium-dependent motility of *L. capsici* strains possibly relies on T4P.

CONCLUSION

This work is a first step in deciphering motility mechanisms in *L. capsici* AZ78. The medium-dependent motility observed is associated with the release of a surfactant and with upregulation of the genes responsible for T4P biogenesis. This type of movement seems to be on the narrow borderline separating the swarming motility observed in *P. aeruginosa* (Köhler et al., 2000) and the “social gliding” of *M. xanthus* (Wu et al., 1997). From a practical point of view, the application of PB on grapevine plants increased efficacy against downy mildew, which is associated with an increase in leaf colonization by *L. capsici* AZ78. These results demonstrate that nutritional components could be used to improve the poor plant colonization observed in some *Lysobacter* strains (Gómez Expósito et al., 2015), and future studies aiming at identifying PAM piliation factors to be included in the formulation of *L. capsici* AZ78 may help to improve biocontrol efficacy under field conditions.

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AUTHOR CONTRIBUTIONS

ST carried out all the experiments, analyzed the data and wrote and edited the manuscript. GP conceived the work, designed the experiments, carried out the greenhouse experiments, analyzed the data and wrote and edited the manuscript. RM carried out TEM analysis and wrote and edited the manuscript. MP, NL, and IP contributed to the conception of the work, designed the experiments and edited the manuscript. All the authors have read the manuscript and agreed to its content.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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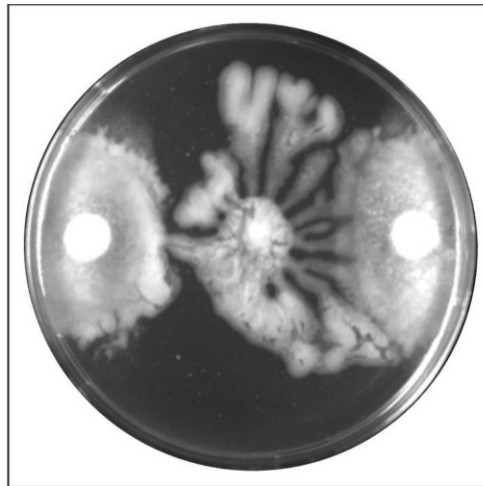


Figure S1: *Lysobacter capsici* AZ78 dendrite-like macrocolony in dual-culture assay with *Phytophthora infestans*. The dual-culture assay of *P. infestans* and *L. capsici* AZ78 (centre) on Pea Agar Medium (1.2% Agar). Images was captured using Bio-Rad Quantity One software.

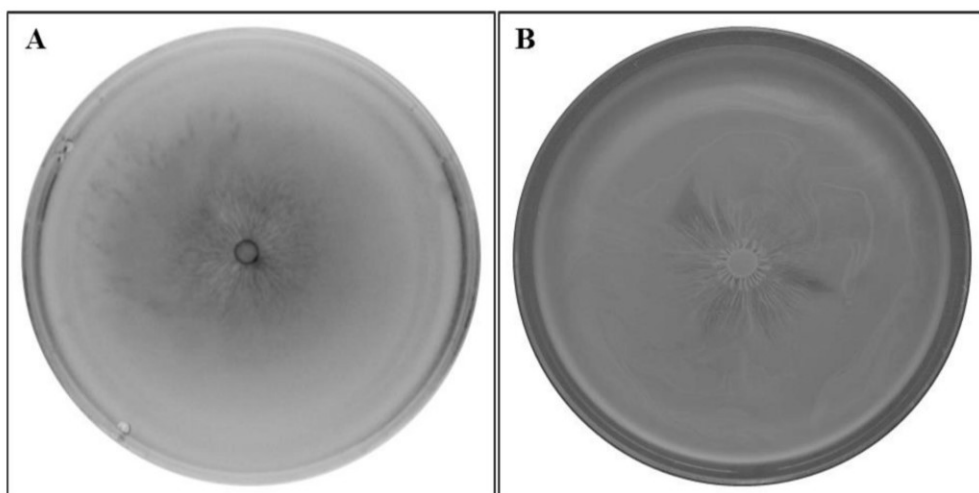


Figure S2: Morphology of *Bacillus amyloliquefaciens* S499 and *Pseudomonas chlororaphis* M71 macrocolonies in swimming motility assays. The swimming motility was evaluated on Swimming Agar (SWM). Images of macrocolonies were taken with Bio-Rad Quantity One software after 20 h incubation at 27°C. **A**, *B. amyloliquefaciens* S499; **B**, *P. chlororaphis* M71.

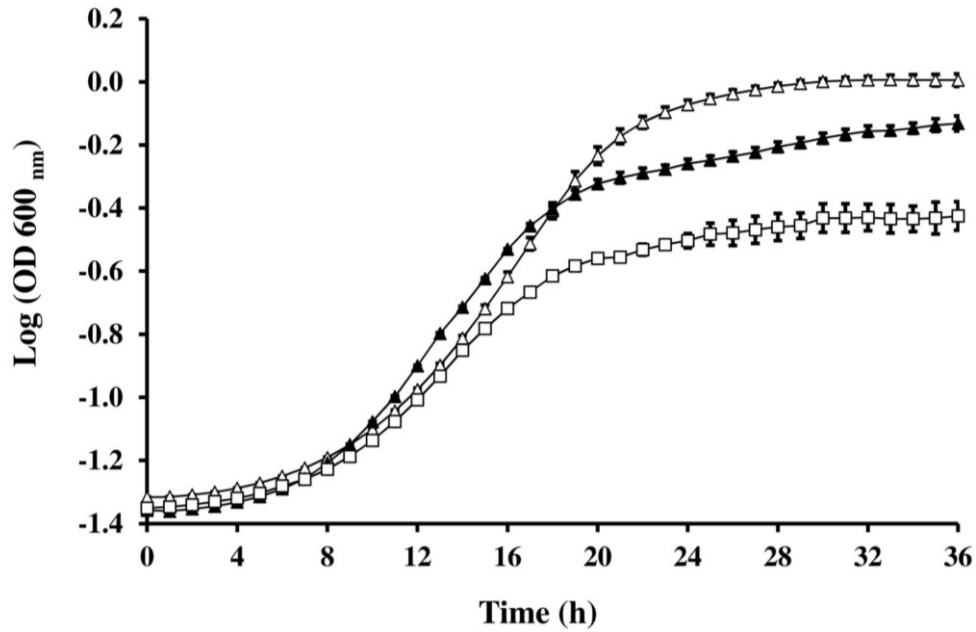


Figure S3: Growth curves of *Lysobacter capsici* AZ78 in different liquid media. Cell growth of *L. capsici* AZ78 was monitored in LB (Δ), PB (□) and SWR (▲) broth. The OD₆₀₀ value was taken each hour for 36 h at 27°C. Not-inoculated media were used as controls. Mean and standard error values, for seven replicates pooled from two experiments, are reported for each time and media.

Table S1. Primers used in quantitative real-time polymerase chain reaction

Gene	Gene ID ¹	Primer Name	Primer Sequence (5'-3')	Amplicon Size (bp)
<i>recA</i>	AZ78_1089	recAF	GAGCCAGATCGACAAGCAAT	159
		recAR	GGACCGTAGATCTCGACCAC	
<i>chpA</i>	AZ78_4391	chpAF	GACCCGTCAGTACGAAAC	147
		chpAR	CAGTTCACCTCGAAGCCTG	
<i>clp</i>	AZ78_4111	clpF	ACTCGGCCTATTCATCGAAA	108
		clpR	GTCAGCAGCAGGTCGTACAG	
<i>rpoN</i>	AZ78_1752	rpoNF	GAATGCCTCAACCTGCAACT	139
		rpoNR	TGTCGAGCTTGAGTTCGTTG	
<i>fliA</i>	AZ78_1611	fliAF	TGGAAGCATCGTCGACTC	182
		fliAR	CTGGATGATCAGCCTCAGC	
<i>flgE</i>	AZ78_1616	flgEF	GTCGGATTACGAGGTGATGG	159
		flgER	TCGATCGAGGTGATCGTGTA	
<i>flgI</i>	AZ78_1627	flgIF	ATCAGTTCGAAGGCGTTACC	151
		flgIR	GTGAAATCCGGCTCGTTTAG	
<i>flhB</i>	AZ78_1633	flhBF	ACCTCGGTCTACGTGTTGCT	169
		flhBR	GCAATTGCTGTTTCTGCTTG	
<i>fliH</i>	AZ78_1621	fliHF	ATAACGCCAACGCACAACCTC	163
		fliHR	CACAAGCGCTTCATCAGTTC	
<i>fliR</i>	AZ78_1633	fliRF	GAACTGAGCATCGTTCCTCGT	200
		fliRR	AGGTCCAGGTTGAAGAACAG	
<i>fimX</i>	AZ78_4971	fimXF	AGTCGCGCGTCTACCACTA	121
		fimXR	ATGTAGCTGCGGTCGATCTT	
<i>pilA</i>	AZ78_4276	pilA1F	CAAGAACATGGAGTGCCTCA	123
		pilA1R	GAGGCGCTGAAGCTATAACC	
	AZ78_4277	pilA2F	CGTACCAAGAACGCTGAGTG	120
		pilA2R	GAAGTGGTAGCCGCTCAGAG	
<i>pilB</i>	AZ78_4268	pilBF	GGTTCACCCACGAGGAGAT	103
		pilBR	ACCTGATAGATGCCGCTACG	
<i>pilG</i>	AZ78_4387	pilGF	ACGATTCCAAGACCATCCGT	129
		pilGR	CGAAAATGATCTGTGGCTGC	
<i>pilI</i>	AZ78_4389	pilIF	ACCTCAAGCAGTTCCTCG	107
		pilIR	CAGTTCGTCGATCAGCAC	
<i>pilJ</i>	AZ78_4390	pilJF	GAAAAGGTGTCGTCGGATC	104
		pilJR	ATCGTGTTCATCGTCTGGGT	
<i>pilM</i>	AZ78_2054	pilMF	CTACGGTCTGAGCTACGAGG	104
		pilMR	GGACCATCGCTTCCTTGAAC	
<i>pilQ</i>	AZ78_2049	pilQF	GAAGCTGGGCAGAACGAAAA	118
		pilQR	GCTCCTTCATCTGGGTCACT	
<i>pilY1</i>	AZ78_1457	pilYF	CAGTACAACAAGGCGATCCA	113
		pilYR	CGCATCTTCTCGGTCTTTTC	
<i>pilZ</i>	AZ78_5337	pilZF	TCGTTGACGATCAAGGACAA	126
		pilZR	GGTCAGCAGCAGGAACACTT	

¹ Gene codes of the *Lysobacter capsici* AZ78 genome (JAJA02000000; Puopolo et al., 2016)

Table S2. Motility on inert surface of *Bacillus amyloliquefaciens* S499 and *Pseudomonas chlororaphis* M71.

Bacterial strain	Motility Assay ¹	Medium	Colony area (mm ²) ²
<i>B. amyloliquefaciens</i> S499	Swimming	SWM	6233.7 ± 64.3
		PAM 0.3	6172.3 ± 74.2
	Swarming	LBA 0.5	279.9 ± 28.9 ^c
		SWR	461.0 ± 51.8 ^b
	Twitching	PAM 0.5	6266.8 ± 54.4 ^a
		LBA 1	218.4 ± 71.5
<i>P. chlororaphis</i> M71	Swimming	PAM 1	519.7 ± 104.6*
		SWM	1230.2 ± 154.8
	Swarming	PAM 0.3	5525.5 ± 427.5*
		LBA 0.5	48857.4 ± 542.5 ^a
	Twitching	SWR	4332.0 ± 378.6 ^a
		PAM 0.5	5543.1 ± 327.2 ^a
Twitching	LBA 1	176.1 ± 15.6	
	PAM 1	361.1 ± 76.9*	

¹The swimming, swarming and twitching motility of *B. amyloliquefaciens* S499 and *P. chlororaphis* M71 was evaluated on Swimming Agar (SWM), Pea Agar Medium 0.3 (PAM 0.3), Luria Bertani Agar 0.5 (LBA 0.5), Swarming agar (SWR), Pea Agar Medium 0.5 (PAM 0.5), Luria Bertani Agar 1 (LBA 1), Pea Agar Medium 1 (PAM 1).

² Mean colony area ± standard error values are calculated as the pool of nine replicates (Petri dishes) from three experiments. Value followed by asterisks indicate values that differ significantly according to Student's T test ($\alpha = 0.05$) in the pairwise comparison of SWM against PAM 0.3, and LBA 1 against PAM 1. Different letters indicate significant differences according to Tukey's test ($\alpha = 0.05$).

Table S3. Gene cluster of flagellum in *Lysobacter capsici* AZ78. Genetic organization of the gene cluster that includes putative genes encoding for the flagellar apparatus and hypothetical protein.

Gene	Function	Gene ID	Identity	E-value	Homologs/Orthologs	Gene ID
-	hypothetical protein	AZ78_1605			No putative conserved domains have been detected	KMU66257
-	hypothetical protein	AZ78_1606			No putative conserved domains have been detected	WP_049443893
-	hypothetical protein	AZ78_1607	86%	0	hypothetical protein STRNTR1_1505 [<i>Stenotrophomonas maltophilia</i>]	WP_049397666
-	putative enzyme; Integration, recombination (Phage or Prophage Related)	AZ78_1608	81%	0	hypothetical protein [<i>Stenotrophomonas maltophilia</i>]	KGM56886
-	putative site specific recombinase	AZ78_1609	87%	0	integrase [<i>Stenotrophomonas maltophilia</i>]	KOO78064
-	Single-stranded DNA-binding protein	AZ78_1610	96%	0	single-stranded DNA-binding protein [<i>Lysobacter arseniciresistens</i> ZS79]	WP_005989636
			86%	0	single-stranded DNA-binding protein [<i>Stenotrophomonas maltophilia</i>]	ALN84773
<i>fliA</i>	RNA polymerase sigma factor for flagellar operon	AZ78_1611	79%	0	MULTISPECIES: single-strand-binding protein [<i>Xanthomonas</i>]	KOO80989
			99%	3E-162	RNA polymerase sigma factor, sigma-70 family protein [<i>Lysobacter capsici</i> 55]	CRD57622
			48%	4E-54	hypothetical protein VO93_05635 [<i>Stenotrophomonas maltophilia</i>]	ALN59990
			40%	3.8E-02	RNA polymerase, sigma 70 (sigma D) factor [<i>Stenotrophomonas maltophilia</i>]	KOB02991
			39%	7E-40	RNA polymerase sigma factor, sigma-70 family [<i>Lysobacter enzymogenes</i> C3]	ALN84772
<i>fliP</i>	Flagellar biosynthesis protein FliP	AZ78_1612	37%	7E-33	RNA polymerase sigma-70 factor [<i>Xanthomonas campestris</i>]	
			99%	6E-164	flagellar biosynthetic protein fliP	

-	hypothetical protein	AZ78_1618	41%	7E-25	[<i>Xanthomonas campestris</i>] Flagellar hook capping protein [<i>Hahella chejuensis</i> KCTC 2396]	ABC30785
-	hypothetical protein	AZ78_1619			No putative conserved domains have been detected	
<i>fliI</i>	Flagellum-specific ATP synthase FliI	AZ78_1620	100%	0	No putative conserved domains have been detected ATPase FliI/YscN family protein [<i>Lysobacter capsici</i> 55]	ALN84764
			67%	0	ATP synthase [<i>Stenotrophomonas maltophilia</i>]	KOO78084
			59%	4E-168	ATP synthase [<i>Xanthomonas campestris</i>]	KOB02983
			55%	3E-152	flagellum-specific ATP synthase [<i>Hahella chejuensis</i> KCTC 2396]	ABC30788
<i>fliH</i>	Flagellar assembly protein FliH	AZ78_1621	97%	1E-148	flagellar assembly FliH family protein [<i>Lysobacter capsici</i> 55]	ALN84763
			36%	2E-15	hypothetical protein VO93_05585 [<i>Stenotrophomonas maltophilia</i>]	KOO78083
			31%	2E-14	hypothetical protein AE921_04185 [<i>Xanthomonas campestris</i>]	KOB02982
-	hypothetical protein	AZ78_1622	31%	4E-07	flagellar assembly protein FliH [<i>Pseudomonas</i> sp. GM48]	WP_007988851
<i>fliF</i>	Flagellar M-ring protein FliF	AZ78_1623	99%	0	No putative conserved domains have been detected flagellar M-ring protein FliF [<i>Lysobacter capsici</i> 55]	ALN84761
			61%	0	hypothetical protein VO93_05575 [<i>Stenotrophomonas maltophilia</i>]	KOO80986
			44%	4E-103	flagellar M-ring protein FliF [<i>Xanthomonas campestris</i>]	KOB03079
<i>fliE</i>	Flagellar hook-basal body complex	AZ78_1624	34%	2E-68	flagellar M-ring protein FliF [<i>Hahella chejuensis</i> KCTC 2396]	ABC30791
			100%	2E-68	flagellar hook-basal body complex protein	ALN84760

protein FlIE	AZ78_1625	50%	3E-25	FlIE [<i>Lysobacter capsici</i> 55]	KOO78081	
				hypothetical protein VO93_05570	WP_016849718	
				[<i>Stenotrophomonas maltophilia</i>]	ABC30792	
<i>flgC</i>	AZ78_1625	100%	2E-86	MULTISPECIES: flagellar hook-basal body protein FlIE [<i>Xanthomonas</i>]	ALN84759	
				flagellar hook-basal body complex protein FlIE [<i>Hahella chejuensis</i> KCTC 2396]	KOO78080	
				Flagellar basal body rod FlIEFG	WP_003488392	
				C-terminal family protein	WP_042727962	
				[<i>Lysobacter capsici</i> 55]	ALN84758	
				hypothetical protein VO93_05565	WP_039814212	
				[<i>Stenotrophomonas maltophilia</i>]	KOO78079	
				MULTISPECIES: hypothetical protein [<i>Xanthomonas</i>]	ABC30794	
				MULTISPECIES: flagellar basal-body rod protein FlgC [<i>Pseudomonas</i>]	ALN84757	
				hypothetical protein LC55x_1476	KOO80985	
<i>flgB</i>	AZ78_1626	98%	8E-78	[<i>Lysobacter capsici</i> 55]	KOB02978	
				MULTISPECIES: hypothetical protein [<i>Xanthomonas</i>]	Q2SEX9	
				hypothetical protein VO93_05560	ALN84756	
				[<i>Stenotrophomonas maltophilia</i>]		
				Flagellar basal body protein		
				[<i>Hahella chejuensis</i> KCTC 2396]		
				flagellar P-ring protein FlgI		
				[<i>Lysobacter capsici</i> 55]		
				flagellar P-ring protein FlgI		
				[<i>Stenotrophomonas maltophilia</i>]		
<i>flgI</i>	AZ78_1627	75%	0	flagellar P-ring protein FlgI		
				[<i>Stenotrophomonas maltophilia</i>]		
				flagellar P-ring protein FlgI		
				[<i>Xanthomonas campestris</i>]		
				Flagellar P-ring protein 1; AltName: Basal body P-ring protein [<i>Hahella chejuensis</i> KCTC 2396]		
				100%	0	
				70%	3E-170	
				55%	8E-125	
				99%	4E-143	
				99%	4E-143	
<i>flgH</i>	AZ78_1628	99%	4E-143	flagellar L-ring protein FlgH		
				flagellar L-ring protein		

<i>flhB</i>	Flagellar biosynthesis protein FlhB	AZ78_1633	99%	0	component FlhA [<i>Hahella chejuensis</i> KCTC 2396]	ALN84751					
					flhB HrpN YscU SpasS family protein [<i>Lysobacter capsici</i> 55]	KRG61253					
					flagellar biosynthesis protein FlhB [<i>Stenotrophomonas maltophilia</i>]	WP_039814221					
					flagellar biosynthesis protein FlhB [<i>Xanthomonas arboricola</i>]	KWU52851					
					flagellar biosynthesis protein [<i>Pseudomonas fluorescens</i>]	ALN84750					
					bacterial export, 1 family protein [<i>Lysobacter capsici</i> 55]	KRG61254					
					hypothetical protein ARC02_03350 [<i>Stenotrophomonas maltophilia</i>]	KOB02972					
					UDP kinase [<i>Xanthomonas campestris</i>]	KWU52850					
					flagellar biosynthesis protein [<i>Pseudomonas fluorescens</i>]	ABC30802					
					Flagellar biosynthesis pathway, component FlhR [<i>Hahella chejuensis</i> KCTC 2396]	ALN84749					
<i>fliQ</i>	Flagellar biosynthesis protein FliQ	AZ78_1635	79%	2E-40	Bacterial export, 3 family protein [<i>Lysobacter capsici</i> 55]	KRG61255					
					flagellar biosynthetic protein FliQ [<i>Stenotrophomonas maltophilia</i>]	KOB03078					
					export protein FliQ [<i>Xanthomonas campestris</i>]	ABC30803					
					flagellar biosynthetic protein FliQ [<i>Hahella chejuensis</i> KCTC 2396]	KIQ61315					
					export protein FliQ [<i>Pseudomonas fluorescens</i>]	ALN84748					
					TPR repeat family protein [<i>Lysobacter capsici</i> 55]	KRG61256					
					hypothetical protein ARC02_03360 [<i>Stenotrophomonas maltophilia</i>]						
					-	TPR domain protein, putative component of TonB system	AZ78_1636	99%	0		
					<i>fliR</i>	Flagellar biosynthesis protein FliR	AZ78_1634	44%	1E-82		
<i>fliB</i>	Flagellar biosynthesis protein FliB	AZ78_1633	66%	5E-150	component FlhA [<i>Hahella chejuensis</i> KCTC 2396]	ALN84751					
					flhB HrpN YscU SpasS family protein [<i>Lysobacter capsici</i> 55]	KRG61253					
					flagellar biosynthesis protein FlhB [<i>Stenotrophomonas maltophilia</i>]	WP_039814221					
					flagellar biosynthesis protein FlhB [<i>Xanthomonas arboricola</i>]	KWU52851					
					flagellar biosynthesis protein [<i>Pseudomonas fluorescens</i>]	ALN84750					
					bacterial export, 1 family protein [<i>Lysobacter capsici</i> 55]	KRG61254					
					hypothetical protein ARC02_03350 [<i>Stenotrophomonas maltophilia</i>]	KOB02972					
					UDP kinase [<i>Xanthomonas campestris</i>]	KWU52850					
					flagellar biosynthesis protein [<i>Pseudomonas fluorescens</i>]	ABC30802					
					Flagellar biosynthesis pathway, component FlhR [<i>Hahella chejuensis</i> KCTC 2396]	ALN84749					

-	hypothetical protein	AZ78_1637	48%	8E-45	hypothetical protein AE923_03890, partial [<i>Xanthomonas campestris</i>] No putative conserved domains have been detected	KOB11384
-	hypothetical protein	AZ78_1638	60%	2E-53	hypothetical protein ARC02_03370 [<i>Stenotrophomonas maltophilia</i>] hypothetical protein AE921_13040 [<i>Xanthomonas campestris</i>]	KRG61258
			45%	6E-30		KOA99158

Table S4. Gene clusters of Type 4 pilus in *Lysobacter capsici* AZ78. Genetic organization of the gene clusters that includes putative genes encoding for T4P and hypothetical protein.

Gene	Function	Gene ID	Identity	E-value	Homologs/Orthologs	Gene ID
<i>pilE</i>	Type IV pilus biogenesis protein PilE	AZ78_1456	100%	3E-102	prepilin-type N-terminal cleavage/methylation domain protein [<i>Lysobacter capsici</i> 55]	ALN84946
					putative type IV pilin [<i>Lysobacter antibioticus</i> 76]	ALN79896
					type IV pilin [<i>Stenotrophomonas maltophilia</i>]	KRG55821
					Pilus assembly protein PilE [<i>Xanthomonas campestris</i>]	WP_039407505
<i>pilY1</i>	Type IV fimbrial biogenesis protein PilY1	AZ78_1457	96%	0	neisseria PilC beta-propeller domain protein [<i>Lysobacter capsici</i> 55]	ALN84945
					neisseria PilC beta-propeller domain protein [<i>Lysobacter gummosus</i> 3.2.11]	ALN92492
					putative PilY1 protein [<i>Xanthomonas campestris pv. viticola</i>]	CDN19294
					PilY1 protein [<i>Stenotrophomonas maltophilia</i> AU12-09]	EMI50639
<i>pilX</i>	Type IV fimbrial biogenesis protein PilX	AZ78_1458	99%	1E-105	Tfp pilus assembly protein, tip-associated adhesin PilY1 [<i>Pseudomonas aeruginosa</i>]	CRQ55241
					pilX N-terminal family protein [<i>Lysobacter capsici</i> 55]	ALN84944
					pilX N-terminal family protein [<i>Lysobacter antibioticus</i> 76]	ALN79894
					protein PilX [<i>Stenotrophomonas maltophilia</i>]	ALA90019
			43%	3E-23	PilX protein [<i>Xanthomonas campestris pv. campestris</i> str. ATCC 33913]	NP_637842

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E	R	1	<i>piIW</i>	Type IV fimbrial biogenesis protein PiIW	AZ78_1459	42%	2E-18	Tfp pilus assembly protein PiIX [<i>Pseudomonas aeruginosa</i>]	CRQ55258
						98%	0	putative piIW [<i>Lysobacter capsici</i> 55]	ALN84943
						69%	0	putative piIW [<i>Lysobacter gummosus</i> 3.2.11]	ALN92494
						44%	4E-89	pilus assembly protein PiIW [<i>Xanthomonas campestris</i>]	KOA99459
						44%	1E-82	pilus assembly protein PiW [<i>Stenotrophomonas maltophilia</i>]	KRG52456
						43%	1E-81	PiIW-related protein [[<i>Pseudomonas</i>] <i>geniculata</i>]	WP_010486095
						91%	1E-83	type IV pilus modification protein PiIV [<i>Lysobacter capsici</i> 55]	ALN84942
						70%	3E-57	type IV pilus modification protein PiIV [<i>Lysobacter gummosus</i> 3.2.11]	ALN92495
						49%	3E-31	pilus assembly protein PiIV [<i>Stenotrophomonas maltophilia</i>]	WP_049466992
						45%	4E-32	pilus assembly protein PiIV [<i>Xanthomonas campestris</i> pv. <i>campestris</i>]	AKS15873
						44%	5E-26	type IV pilus modification protein PiIV [<i>Pseudomonas aeruginosa</i>]	CRQ55296
						98%	3E-113	type II transport GspH family protein [<i>Lysobacter capsici</i> 55]	ALN84941
						E	R	1	<i>fimU</i>
43%	2E-35	pre-pilin like leader sequence [<i>Stenotrophomonas maltophilia</i>]	W_049466990						
40%	2E-28	putative type IV pilus assembly protein FimT [<i>Stenotrophomonas maltophilia</i>]	CRD47827						
40%	3E-28	type IV pilus assembly protein FimT [<i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> str. GSPB2388]	EKQ62306						

T E R 4	<i>pilO</i>	Type IV pilus biogenesis protein PilO	AZ78_2052	73%	1E-84	fimbrial protein [<i>Stenotrophomonas maltophilia</i>]	WP_046274019			
				72%	5E-84	fimbrial protein [<i>Xanthomonas campestris</i>]	AKC79290			
				95%	9E-161	pilO protein [<i>Lysobacter capsici</i> 55]	ALN84331			
				90%	8E-132	pilO protein [<i>Lysobacter antibioticus</i> ATCC 29479]	ALN65006			
				87%	2E-127	type IV assembly protein [<i>Lysobacter enzymogenes</i> C3]	ALN56292			
				86%	4E-127	pilO protein [<i>Lysobacter gummosus</i> 3.2.11]	ALN89953			
	<i>pilN</i>	Type IV pilus biogenesis protein PilN	AZ78_2053	69%	2E-104	fimbrial protein [<i>Stenotrophomonas maltophilia</i>]	WP_053518455			
				75%	5E-112	Fimbrial assembly membrane protein [<i>Xanthomonas campestris</i> pv. <i>campestris</i>]	CAP50304			
				99%	0	fimbrial assembly family protein [<i>Lysobacter capsici</i> 55]	ALN84330			
				92%	1E-116	fimbrial assembly family protein [<i>Lysobacter antibioticus</i> 76]	ALN82271			
				90%	4E-104	Type IV pilus assembly protein [<i>Lysobacter enzymogenes</i> C3]	ALN56291			
				65%	1E-74	fimbrial protein [<i>Xanthomonas campestris</i> pv. <i>campestris</i>]	AKS19290			
	<i>pilM</i>	Type IV pilus biogenesis protein PilM	AZ78_2054	61%	8E-73	fimbrial protein, partial [<i>Stenotrophomonas maltophilia</i>]	WP_019338751			
100%				0	type IV pilus assembly PilM family protein [<i>Lysobacter capsici</i> 55]	ALN84329				
98%				0	type IV pilus assembly PilM family protein [<i>Lysobacter antibioticus</i> ATCC 29479]	ALN65008				
97%				0	type IV pilus assembly protein [<i>Lysobacter enzymogenes</i> C3]	ALN56290				
82%				0	pilus assembly protein PilM	WP_042614422				

<i>pilA.2</i>	Type IV pilin PilA	AZ78_4277	73%	9E-07	[<i>Xanthomonas axonopodis</i> pv. <i>citri</i> str. 306]	AAM38085
					Fimbrillin	
					[<i>Xanthomonas axonopodis</i> pv. <i>citri</i> str. 306]	ALN87506
					Prepilin-type N-terminal cleavage/methylation domain protein [<i>Lysobacter capsici</i> 55]	KOB01592
					pilin [<i>Xanthomonas campestris</i>] fimbriillin	AAM38084
<i>pilR</i>	Type IV fimbriae expression regulatory protein PilR	AZ78_4278	100%	0	bacterial regulatory, Fis family protein [<i>Lysobacter capsici</i> 55]	ALN87505
					PilR [<i>Xanthomonas campestris</i> pv. <i>campestris</i>]	AAP43028
					two-component system regulatory protein [<i>Xanthomonas campestris</i> pv. <i>campestris</i> str. ATCC 33913]	NP_638443
					two-component system response regulator [<i>Stenotrophomonas maltophilia</i>]	WP_049405671
					his Kinase A domain protein [<i>Lysobacter capsici</i> 55]	ALN87504
<i>pilS</i>	Two-component sensor PilS	AZ78_4279	99%	0	two-component system sensor protein [<i>Xanthomonas campestris</i> pv. <i>campestris</i> str. ATCC 33913]	NP_638442
					PilS [<i>Xanthomonas campestris</i> pv. <i>campestris</i>]	AAP43027
					two-component system sensor histidine kinase [<i>Stenotrophomonas maltophilia</i>]	WP_017356171
					competence protein [<i>Lysobacter capsici</i> 55]	ALN87499
					competence protein	ALN62868
<i>comL</i>	putative component of the lipoprotein assembly complex	AZ78_4284	88%	0		

<i>chpA</i>	Signal transduction histidine kinase CheA (EC 2.7.3.-)	AZ78_4391	67%	0	PilJ/methyl accepting chemotaxis protein [Stenotrophomonas maltophilia K279a] pilus biogenesis protein [Xanthomonas campestris pv. campestris str. ATCC 33913]	NP_638270	
						transcriptional regulator [Lysobacter capsici 55]	ALN87392
						transcriptional regulator [Stenotrophomonas maltophilia]	WP_019185135
						MULTISPECIES: sensor histidine kinase [Xanthomonas]	WP_022558749
						PilL	ACF16411
						[Xanthomonas campestris pv. campestris] chemotaxis protein	WP_043399184
						[Stenotrophomonas maltophilia] chemotaxis protein	WP_039412175
						[Xanthomonas campestris] putative chemotaxis CheW protein	CAQ47084
						[Stenotrophomonas maltophilia K279a] Chemotaxis-related protein	CAP50583
						[Xanthomonas campestris pv. campestris] fimV N-terminal domain	ALN86296
<i>chpB</i>	glutamate methyltransferase	AZ78_4392	53%	2E-39	chemotaxis protein [Stenotrophomonas maltophilia]	WP_043399184	
						chemotaxis protein [Xanthomonas campestris]	WP_039412175
						putative chemotaxis CheW protein [Stenotrophomonas maltophilia K279a]	CAQ47084
						Chemotaxis-related protein [Xanthomonas campestris pv. campestris]	CAP50583
<i>chpC</i>	Chemotaxis signal transduction protein	AZ78_4393	55%	7E-42	putative chemotaxis CheW protein [Stenotrophomonas maltophilia K279a]	CAQ47084	
						Chemotaxis-related protein [Xanthomonas campestris pv. campestris]	CAP50583
<i>fimV</i>	Tfp pilus assembly protein FimV	AZ78_0130	97%	0	fimV N-terminal domain [Lysobacter capsici 55]	ALN86296	
						fimV protein [Lysobacter antibioticus ATCC 29479]	ALN61076
						fimV protein [Stenotrophomonas maltophilia]	WP_019336664
						FimV protein [Xanthomonas campestris pv. raphani 756C]	AEL07747
<i>pilF</i>	Type IV pilus biogenesis protein PilF	AZ78_0791	100%	0	type IV pilus biogenesis/stability protein [Lysobacter capsici 55]	ALN85602	
						fimbrial protein, partial [Xanthomonas]	KFA34954

<i>fimX</i>	two-component system response regulator					str. ATCC 33913] transcriptional regulator [<i>Stenotrophomonas maltophilia</i>] cAMP-regulatory protein [<i>Stenotrophomonas maltophilia</i> R551-3] vfr cAMP-regulatory protein [<i>Pseudomonas aeruginosa</i> PAO1]	WP_017354543 WP_010482649 NP_249343.1	
						diguanylate cyclase domain protein [<i>Lysobacter capsici</i> 55] putative transmembrane protein [<i>Stenotrophomonas maltophilia</i> K279a] Putative sensor protein [<i>Xanthomonas campestris</i> pv. <i>campestris</i>] diguanylate cyclase/phosphodiesterase [<i>Xanthomonas citri</i> subsp. <i>citri</i> A306]	ALN86593 CAQ45526 CAP51240 AJD68992	
						type IV fimbriae assembly protein [<i>Lysobacter capsici</i> 55] pilus assembly protein PilZ [<i>Lysobacter daejeonensis</i> GH1-9] type IV pilus assembly PilZ [<i>Stenotrophomonas maltophilia</i> R551-3] type IV fimbriae assembly protein [<i>Xanthomonas campestris</i> pv. <i>campestris</i> str. ATCC 33913] type IV pilus assembly protein PilZ [<i>Xanthomonas translucens</i>]	ALN86700 KGM55504 KRG40644 NP_636400 WP_003479797	
	<i>pilZ</i>	Type IV pilus biogenesis protein PilZ						

CHAPTER 4

Dual RNA-Seq of the *Lysobacter capsici* AZ78 - *Phytophthora infestans* interaction indicates the implementation of attack strategies by the bacterium and unsuccessful defence responses of the oomycete

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Dual RNA-Seq of the *Lysobacter capsici* AZ78 - *Phytophthora infestans* interaction indicates the implementation of attack strategies by the bacterium and unsuccessful defence responses of the oomycete

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Summary

Microbial communities, in the rhizosphere, are characterized by a network of biological interactions that continuously shape the gene expression patterns of each single microorganism. A dual RNA-Seq approach was applied to obtain a comprehensive picture of the molecular mechanisms activated in the interaction between the biocontrol rhizobacterium *Lysobacter capsici* AZ78 and the soilborne phytopathogenic oomycete *Phytophthora infestans*.

The RNA-Seq transcriptional profile of *L. capsici* AZ78 is characterized by the up-regulation of genes involved in the biogenesis of type 4 pilus and lytic enzymes, involved in the host colonization and the subsequent attack of *P. infestans* cell wall, respectively. The activation of detoxification processes allowed *L. capsici* AZ78 to overcome the defense activity of *P. infestans*. Moreover, genes involved in the antibiotic biosynthesis were up-regulated in *L. capsici* AZ78 causing the activation of programmed cell death via apoptotic process in *P. infestans*. The consequences of the activation of this process resulted in the overall down-regulation of primary metabolic pathways, such as carbohydrate, nucleic acids and protein metabolisms. Overall, the mechanism of action of *L. capsici* AZ78 is related to parasitism and predatory activities and it is so aggressive to cause the death of *P. infestans* cells.

Introduction

Rhizosphere is a nutrient-rich environment for microorganisms due to the release of organic compounds by plant roots (Pieterse et al., 2016). This ecological niche is occupied by large numbers of different microorganisms and it is the place where complex microbial interactions occur between plant roots, microflora and microfauna (Huang et al., 2014; Whipps, 2001). Among these large numbers of microbial interactions, the confrontation between biocontrol bacteria and phytopathogens also occurs and its outcome contributes quite often to the health of the plant root (Haas and Défago, 2005; Raaijmakers et al., 2009). Although microbial interactions have been studied for long time with classical microbiological methods, the next generation sequencing techniques have widely fostered our knowledge on their specific mechanisms (Knief et al., 2014; Massart et al., 2015). However, most of the focus has generally been posed either on the biocontrol agent or the phytopathogen. For example at the early stage of their interaction only the modulation of *Pseudomonas fluorescens* Pf29Arp transcriptome in response to *Gaeumannomyces graminis* var. *tritici* was described (Barret et al., 2009). Similarly, the antagonist activity of *Serratia plymuthica* against *Rhizoctonia solani* and the response of *R. solani* to two *Serratia* species were investigated in two separate studies (Gkarmiri et al., 2015; Neupane et al., 2015). Few studies took in account simultaneously the whole interaction (both the phytopathogen and the biocontrol bacteria). Only the interaction between fungi and bacteria, as in the case of *Aspergillus niger*-*Collimonas fungivorans* or *A. niger*-*Bacillus subtilis* (Mela et al., 2011; Benoit et al., 2015), was taken into consideration so far and no studies addressed the transcriptional changes occurring in the intereaction between a phytopathogenic oomycete and a biocontrol bacterium.

Soilborne oomycetes, such as *Phytophthora infestans* (Pi), are major plant pathogens worldwide (Blackman et al., 2014; Nowicki et al., 2012). Since the sequencing of Pi genome (Haas et al., 2009) attention was mainly given to the determination of Pi pathogenicity factors *in planta* and the possible plant resistance mechanisms (Gao et al., 2013; Zuluaga et al., 2016), whereas the Pi response to attacks of biocontrol microorganisms was scarcely investigated.

On the other hand, the antagonism mechanisms against Pi of several biocontrol bacteria, such as *Bacillus* spp. and *Pseudomonas* spp., was studied, both *in vitro* and *in vivo* conditions (Islam and Hossain, 2013). Members of the genus *Lysobacter* can be

efficient antagonists of phytopathogenic microorganisms such as bacteria, fungi, nematodes and oomycetes (Hayward et al., 2010). For example, *Lysobacter capsici* AZ78 (AZ78), which is a bacterium isolated from the tobacco rhizosphere, can effectively control Pi on tomato plants by releasing cyclo L-Pro L-Tyr in the liquid growth medium (Puopolo et al., 2014a). The antagonistic behavior of *Lysobacter* spp. strains is confirmed by the presence in their genome of a gene pool involved in the release of extracellular lytic enzymes and secondary metabolites with antibacterial and antifungal activity (De Bruijn et al., 2015; Puopolo et al., 2016). Although the biocontrol mechanisms of *Lysobacter* spp. strains were largely investigated (Folman et al., 2004; Kobayashi et al., 2005; Ko et al., 2009), a complete overview of the transcriptional interplay occurring during the *Lysobacter* spp.-phytopathogen interaction has not been described yet. Solely the candidate genes of fungal defense response of *Magnaporthe oryzae* to the biocontrol strain *L. enzymogenes* C3 have been identified (Mathioni et al., 2013), but the transcriptional changes occurring in the biocontrol bacterium are unknown.

Our aim was therefore to understand the simultaneous transcriptome changes of both AZ78 and Pi occurring at two stages of the interaction, specifically after 6 and 24 h. These two time points were chosen based on previous experiments aimed at assessing the proteolytic activity during the interaction between AZ78 and Pi. These results showed a low proteolytic activity in the early stage of interaction (6 h) and an increase was registered after 24 h (Puopolo et al., 2016). Transcriptional profiles were obtained using a dual RNA-Seq approach, which can provide a comprehensive understanding of host-pathogen interactions (Wang et al., 2009; Westermann et al., 2012).

Results

*Dual RNA-Seq analysis of *Lysobacter capsici* AZ78 and *Phytophthora infestans* interaction*

The interaction between AZ78 and Pi was investigated at two time points. The transcriptome of AZ78 (Lc6 and Lc24) and Pi (Pi6 and Pi24) was characterised after 6 and 24 hours of respective interaction (hoi) at 25°C. To assess the changes in the total transcriptome of AZ78 and Pi, the data of these treatments were compared to their respective controls, specifically AZ78 or Pi incubated alone for 6 h at 25°C named Lcc and Pic, respectively.

The total number of raw read pairs (sum of reads of two sequencing technical replicates), obtained in dual RNA-Seq methodology (Fig. S1), ranged from approximately 22 and 38 million (Table S1). The total number of read pairs that mapped uniquely to AZ78 genome or *P. infestans* T30-4 genome ranged from 1,812 to 26,000,000 and from 5,893,253 to 20,000,000, respectively. The Lc6 and Lc24 sample resulted in 290 (5.5% of total genes) and 548 (10.4% of total genes) differentially expressed genes (DEGs) as compared with Lcc using a P-value of ≤ 0.001 and \log_2 Fold Change (\log_2FC) ≥ 2 , respectively (Table 1, Table S2 and Table S3). The proportion of up-regulated genes was 55.5 and 63.7% in Lc6 and Lc24, respectively. The number of DEGs for Pi was 337 (1.9% of total genes) and 1,076 (5.9% of total genes) in Pi6 and Pi24 as compared with Pic ($P \leq 0.001$ and $\log_2FC \geq 2$), respectively (Table 1 and Table S4). Contrarily to Lc6 and Lc24, the 93.2 and 64.1% of Pi DEGs were down-regulated at 6 and 24 hoi, respectively.

Modulation of Lysobacter capsici AZ78 transcriptome during interaction with Phytophthora infestans

The changes in gene expression levels should be mainly ascribed to transcriptional reprogramming, because no significant differences were observed after 6 and 24 hoi between the number of AZ78 cells grown alone in the control (8.4 ± 0.1 and $10.4 \pm 0.4 \log_{10}$ CFU/dish) and in the interaction with Pi (8.6 ± 0.3 and $10.3 \pm 0.1 \log_{10}$ CFU/dish)(Fig. S2).

Only the transcriptomic data related to AZ78 biological responses to Pi after 24 h of interaction are reported here (Lc24), while the AZ78 transcriptome at 6 hoi was analyzed separately due to the low sequencing depth of AZ78 RNA-Seq sequencing (Haas et al., 2012) and presented in the Supplementary information. DEGs for Lc24 were divided in two clusters (up and down-regulated genes) and each gene group was subjected to enrichment analysis in order to retrieve the over-represented Gene Ontology (GO) categories belonging to biological process terms (Fig. 1). The enriched GO data were additionally classified by a manual annotation on the basis of the NCBI gene description and Blast2GO description, which indicated a positive modulation of motility, antagonism activity, bacterial metabolism and transport (Fig. S3A).

Regarding motility, functional annotation of the Lc24 up-regulated genes (Table S5) resulted in the enrichment of the GO functional category pilus assembly (Fig 1A) and two genes, *pilQ* (AZ78_2049) and *pilB* (AZ78_4268), respectively part of the outer

membrane subcomplex and motor subcomplex of Type 4 Pilus (T4P), were up-regulated. Manual annotation identified other thirteen up-regulated genes involved in T4P biogenesis, such as the gene-cluster AZ78_1780-AZ78_1781, encoding PilT-PilU ATPases of T4P motor subcomplex, and *pilMNOP* (AZ78_2054-AZ78_2051), part of the T4P alignment subcomplex. It is worth noting that the motility pathways were already activated in Lc6 with the presence of eight up-regulated T4P genes (Supplementary information).

Regarding antagonism, the manual annotation showed also the up-regulation of the gene AZ78_1098 (Table S6), encoding a non-ribosomal peptide synthase/polyketide synthase (NRPS-PKS) involved in the antagonistic mechanisms. Expression of the genomic region AZ78_1097-AZ78_1109 was up-regulated in its entirety. This region includes AZ78_1098 and genes encoding a putative sterol desaturase (AZ78_1097), phytoene dehydrogenases (AZ78_1099, AZ78_1100 and AZ78_1101), an oxidoreductase YncB (AZ78_1102), and two transporter proteins (AZ78_1093, AZ78_1096). Other genes probably involved in the biosynthesis of NRPS (AZ78_3544) and lantibiotics (AZ78_2411) were up-regulated as well (Table S6). The GO enrichment analysis revealed the over-representation of the GO carbohydrate metabolic process, corresponding to 20 up-regulated genes (Table S5). Thanks to the manual annotation process, eight of these genes (AZ78_0055; AZ78_0407; AZ78_1531; AZ78_1535; AZ78_1827; AZ78_3679; AZ78_3685 and AZ78_3864) were identified as lytic enzymes with chitinolytic and glucanolytic activity. The bacterial antagonism was also associated with the up-regulation of 38 genes in Lc24 (Table S6) recovered by manual annotation and encoding hydrolytic enzymes, such as three chitinases, 11 glucanases and 24 proteases. The over-represented GO category chitin catabolic process encompassed three up-regulated genes AZ78_0055 (*chiB*), AZ78_1827 (*chiA*) and AZ78_3864 (Table S6) involved in the chitin degradation. The hydrolysis of glucans and cellulose by AZ78 was associated with the up-regulation of *gluA* (AZ78_3679), *gluB* (AZ78_4728), *gluC* (AZ78_1535), *celA* (AZ78_4360), *cel8A* (AZ78_4012) and *cel5G* (AZ78_3685). The genes *gluA* (AZ78_3679) and *cel5G* (AZ78_3685) were already up-regulated at 6 hoi (Supplementary information). Two genes (AZ78_1556 and AZ78_1567) related to the production of pectate lyase were also up-regulated (Table S6). Regarding the proteolytic activity, three genes encoding proteases were up-regulated more than six-fold in Lc24 compared to Lcc (AZ78_0270, AZ78_0272, AZ78_2802). Expression of an entire genome region (AZ78_4515-AZ78_4522)

encoding several metallocoarboxypeptidases was up-regulated. Some of these genes were also up-regulated in Lc6 (Supplementary information).

Genes related to carbohydrate metabolic process, galactose metabolic process, D-galactonate catabolic process and glycosaminoglycan catabolic process were up-regulated (Fig. 1A). Up-regulated genes belonging to these GO classes were AZ78_0300 (β -galactosidase), AZ78_0303 (galactonate dehydratase), AZ78_0305 (2-dehydro-3-deoxygalactonokinase), and AZ78_3684 (glucokinase) involved in the catabolism of galactose, and AZ78_0637 (β -hexosaminidase) related to the peptidoglycan recycling. Three genes involved in the peptidoglycan biosynthetic process, as shown by the manual annotation, were up-regulated in Lc24 (AZ78_0477, AZ78_2357, AZ78_2811). Two genes, encompassed by the over-represented GO categories galactose transport and glucose transport, were up-regulated in Lc24 and specifically encoded a N-acetyl glucosamine transporter (AZ78_2826) and a putative mannose transporter (AZ78_3634) involved in the transmembrane transport of these compounds. Transcription of the entire gene cluster AZ78_1558-AZ78_1560, encoding a putative tripartite ATP-independent periplasmic (TRAP)-type C4-dicarboxylate transport system, was up-regulated and identified by manual annotation. Cellular amino acid metabolism was activated, and genes involved in serine biosynthesis (cysteine biosynthetic process and glycine decarboxylation via glycine cleavage system), branched amino acid biosynthesis (leucine biosynthetic process and valine biosynthetic process) and energy metabolism (sulfate assimilation) were up-regulated (Fig. 1A). In addition to the stimulated bacterial metabolism the GO enrichment analysis revealed the up-regulation of 28 genes related to signal transduction (Table S6), 19 of them specifically encoding TonB-dependent receptors.

The GO enrichment analysis showed the activation of the 'response to stress' category, associated with the up-regulation of AZ78_1581, AZ78_1588 and AZ78_1600 encoding proteins of the universal stress family (UspA domain). Five putative genes related to the oxidoreductase activity were also up-regulated, such as AZ78_1116 (catalase) recovered by manual annotation. Expression of genes encoding proteins involved in drug and toxic compound extrusion were also up-regulated, such as two permeases of major facilitator superfamily (MFS; AZ78_3401, AZ78_5171). The up-regulation of genes involved in response to stress and detoxification process was already observed in Lc6 (Supplementary information).

The biofilm formation category was over-represented among the down-regulated Lc24 gene group (Fig. 1B). A gene cluster (AZ78_5013-AZ78_5015) involved in the biosynthesis/transport of poly-beta-1,6 N-acetyl-D-glucosamine was down-regulated. Nine genes involved in flagella biogenesis processes (bacterial-type flagellum-dependent cell motility, bacterial-type flagellum assembly, bacterial-type flagellum organization) were down-regulated (Fig. 1B, Table S6). The manual annotation identified other three down-regulated genes related to the flagellar protein export system in Lc24.

Bacterial pathways linked to DNA replication, DNA repair and double-strand break repair via non homologous end joining were down-regulated in Lc24 (Fig. 1B). For instance, AZ78_0555 (ATP-dependent DNA ligase), AZ78_0699 (DNA polymerase-like protein), AZ78_3913 (Endonuclease V) and AZ78_3942 (ATP-dependent DNA ligase) involved in the DNA repair were down-regulated. AZ78_1090 was down-regulated both in Lc24 and L6 and is involved in negative regulation of transcription (SOS-response repressor and protease LexA) involved in DNA repair and DNA replication processes. AZ78_0566, encoding a Ku domain protein clustered with ATP-dependent DNA ligase (AZ78_0555), was down-regulated and associated to double-strand break repair processes.

The dual RNA-Seq results were validated by the analysis of relative expression level of 11 selected AZ78 genes by real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR; Table S7). A close correlation (Pearson's $r = 0.89$) was observed between \log_2 FC measured by RNA-Seq and qRT-PCR (Fig. 2A). Although Lc6 was sequenced with low sequencing depth, the expression profiles of all the selected genes were confirmed (Table S7).

Transcriptional response of Phytophthora infestans during the interaction with Lysobacter capsici AZ78

DEGs of Pi6 and Pi24 samples were divided into six groups according to gene expression profiles (Table S4). The groups included down-regulated DEGs at 6 h with positive trend at 24 h (G1, 115 genes), down-regulated DEGs at 6 h with negative trend at 24 h (G2, 216 genes), not DEGs at 6 h with negative trend at 24 h (G3, 411 genes), not DEGs at 6 h with positive trend at 24 h (G4, 349 genes), up-regulated DEGs at 6 h with positive trend at 24 h (G5, 37 genes) and up-regulated DEGs at 6 h with negative trend at 24 h (G6, 2 genes). Over-represented GO terms resulting from the GO

enrichment analysis revealed a general down-regulation of the Pi transcriptional response to AZ78 (G1, G2, G3, Fig 3A-C, Table S8) related to transport process, carbohydrate metabolic process, cell adhesion, and DNA metabolism. The manual annotation allowed the further identification of genes involved in stress response, defense and antagonism (Fig. S3B, C).

The enriched GO term related to protein ADP-ribosylation processes (Fig. 3A) characterized G1; specifically three genes (PITG_12939, PITG_21255 and PITG_21256) encoding poly(ADP-ribose) polymerases were strongly down-regulated in Pi6. The transcription of 12 genes, related to transmembrane transport, was down-regulated in Pi with negative trend from 6 hoi to 24 hoi with AZ78 (G2). Other transport-related GO categories were also over-represented (Fig. 3B). For instance, the expression of a two-gene cluster (PITG_07710- PITG_07711), involved in sugar-proton symporter activity inside the proton transport GO category, was down-regulated. The cation transmembrane transport was negatively modulated and four genes encoding calcium-transporting P-type ATPase Superfamily proteins (PITG_02353, PITG_02358, PITG_02359 and PITG_02364) were down-regulated. Twelve down-regulated genes of G2 involved in toxic compound extrusion mechanisms were identified by manual annotation, such as ATP-binding cassette (ABC) superfamily (PITG_13558, PITG_16912, and PITG_22087) and MFS (PITG_00329, PITG_007723 and PITG_13473). In G2 the negative effect on cell adhesion was highlighted (GO term homophilic cell adhesion via plasma membrane adhesion molecules) by the down-regulation of protocadherin-like protein (PITG_09982), and the gene cluster involved in the biosynthesis of immunoglobulin-like plexin transcription factors (IPT, PITG_05441, PITG_05442, and PITG_05443).

The G3 group contained four down-regulated genes (PITG_00571, PITG_12300, PITG_19656, PITG_07024) related to purine nucleotide biosynthetic process of the DNA metabolism and GMP biosynthetic process (Fig. 3C). G3 also had four genes (PITG_00748, PITG_09374, PITG_11796 and PITG_16107) involved in the ribosomal RNA maturation process. Manual annotation identified down-regulation in Pi24 of 15 and 25 genes involved in DNA and RNA metabolism, respectively.

The metabolic processes of Pi were negatively modulated for G3. Eleven genes of the carbohydrate metabolic process, and nine genes involved in both starch and sucrose metabolic processes were down-regulated. The GO enrichment analysis highlighted over-representation of the cell wall organization term by four polygalacturonase genes

(PITG_10255, PITG_19623, PITG_19625 and PITG_19625). Another two down-regulated genes (PITG-01029 and PITG_08914) were identified by manual annotation and they encode a pectinesterase related to cell wall modification and plant infection mechanisms.

The intracellular transport of Pi was negatively affected during the interaction period and genes involved in GO terms of post-Golgi vesicle-mediated transport, Golgi to endosome transport, Golgi to vacuole transport, endosomal vesicle fusion, late endosome to vacuole transport and endocytosis were down-regulated (Fig. 3C).

In response to AZ78, two genes PITG_09169 and PITG_09173 (negative regulation of endopeptidase activity) encoding cystatin-like effector proteins (EPICs), were strongly down-regulated. Heat shock protein (hsp) 90 (PITG_06415) and hsp 70-like (PITG_6415) involved in the response to stress are part of G3, showing a negative expression trend during the interaction period.

The programmed cell death via regulation of apoptotic process, as reported by GO enrichment analysis of G4, was positively regulated in Pi interacting with AZ78 (Fig. 3D). Two up-regulated genes involved in the signal transduction (PITG_00124 and PITG_15917) and a O(6)-methylguanine-induced apoptosis (PITG_0700) were up-regulated in Pi24 and associated to apoptotic processes (Table S9). The GO enrichment analysis of G4 DEGs showed other over-represented categories: phosphate ion transmembrane transport (three genes), sodium-dependent phosphate transport (three genes), system development (two genes) and cellular glucan metabolic process (two genes). Concerning the latter GO term, PITG_10217 and 16965, encoding glucan 1,3-beta-glucosidase related to the hydrolysis of β -D-glucose units from β -glucans, were up-regulated in Pi24.

Manual annotation of G4 showed the up-regulation of genes involved in the oxidative stress response, defense and antagonism activity during the AZ78-Pi interaction. The gene PITG_08902, encoding a transport protein of pleiotropic drug resistance (PDR) family, was positively expressed at 24 hoi (Table S9). Genes encoding extracellular serine proteases (PITG_00392, PITG_00393, PITG_11807), metalloprotease (PITG_11607) and cysteine protease (PITG_22022) were also specifically up-regulated in Pi24. As a response to the biotic and abiotic stress, Pi up-regulated the expression of genes related to the Kazal-like protease inhibitor in G4 (PITG_23012, PITG_22942, PITG_22940) and G5 (PITG_23147; Table S9). During AZ78-Pi interaction, the over-represented category of the aromatic amino acid metabolic process was activated (Fig.

3E) and a maleylacetoacetate isomerase (PITG_05380) was up-regulated in Pi6 and Pi24. The over-represented GO term of protein processing in G6 (Fig. 3F) is possibly not statistically reliable due to the reduced gene number of this cluster.

Manual annotation revealed the up-regulation of three genes in G4 (PITG_04744, PITG_12989 and PITG_15687) and one gene in G5 (PITG_09016) related to the oxidoreductase activity in Pi interacting with AZ78, while the production of a manganese superoxide dismutase was down-regulated in Pi24 (PITG_07328; G3). About the possible activation of Pi virulence mechanism, 14 DEGs encoding proteins belonging to the cytoplasmic effectors RXLR family, were up-regulated during the interaction.

The dual RNA-Seq results were validated by the analysis of relative expression level of nine Pi genes (Table S10). A close (Pearson's $r = 0.95$) was observed between \log_2 FC measured by RNA-Seq and qRT-PCR (Fig. 2B) and expression profiles generated by qRT-PCR and RNA-Seq agreed completely for eight genes and slightly differed for one gene (PITG_07661, MFS) (Table S10) possibly due to differences in method sensitivity and in the discrimination capacity of multigene families (Perazzolli *et. al.*, 2016).

Discussion

In this study, we aimed at understanding the molecular mechanisms underlying the interaction between the biocontrol rhizobacterium *L. capsici* AZ78 and the soilborne phytopathogenic oomycete *P. infestans* by using a dual RNA-Seq approach. Although this technology was already applied to characterise interactions between pathogenic bacteria and mammal cells (Tierney *et al.*, 2012; Westermann *et al.*, 2012), to our knowledge this is the first time that dual RNA-Seq was applied to study the interaction between a beneficial bacterium and a phytopathogenic oomycete.

Based on the transcriptional profilings obtained for AZ78 and Pi allowed we formulated a model (Fig. 4) that summarizes the attack strategies implemented by AZ78 and their outcome on the Pi transcriptome. AZ78 starts the attach and spread over the Pi mycelium with the upregulation of genes involved in the biogenesis of T4P, cell external appendages that are associated to AZ78 cell motility (Tomada *et al.*, 2016). The T4P is required for host colonization and pathogenesis in human and plant pathogenic bacteria (Burrows, 2012; Dunger *et al.*, 2016) and, in *L. enzymogenes* C3, T4P promoted the hyphae colonization and infection of the phytopathogenic fungus

Cryphonectria parasitica (Patel et al., 2011). The up-regulation of T4P genes is also associated to the impairment of poly- β -1,6-*N*-acetyl-D-glucosamine (PNAG), an exopolysaccharide involved in biofilm formation in Gram-negative and Gram-positive bacteria (Vuong et al., 2004; Choi et al., 2009; Little et al., 2015). In addition, a gene encoding β -hexosaminidase, an enzyme involved in bacterium-biofilm degradation through the PNAG depolymerization (Kaplan et al., 2003; Itoh et al., 2005), was up-regulated in AZ78 in the interaction with Pi. Therefore, once entered in contact with Pi mycelium, AZ78 starts to spread over it by using T4P and contemporaneously represses its biofilm formation in order to efficiently colonize the Pi mycelium.

Once the contacts between AZ78 cells and Pi mycelium have been established, the AZ78's genes involved in direct antibiosis are up-regulated. Indeed, RNA-Seq profiling of AZ78 revealed the up-regulation of a pool of genes involved in the antibiotic biosynthesis such as AZ78_1098 sharing homology with the hybrid NRPS-PKS deputed to the synthesis of a polycyclic tetramate macrolactam named heat stable antifungal factor (HSAF) in *L. enzymogenes* C3 (Yu et al. 2007; Puopolo et al., 2016). This up-regulation is also associated to the positive modulation of the metabolic pathways related to the biosynthesis of cysteine and valine which are some of the basic building blocks of many non-ribosomal peptide antibiotics (Hancock and Chapple, 1999). HSAF has recently been found to induce apoptosis by binding the β -tubulin and triggering the over production of ROS in *Candida albicans* SC5314 (Ding et al., 2016). Interestingly, RNA-Seq transcriptional profiling of Pi was characterized by the up-regulation of genes involved in apoptotic activity pattern. Particularly, an up-regulated gene encoding O⁶-methylguanine involved in DNA-damage can activate the apoptotic process (Roos et al., 2006). The up-regulation of two Pi genes encoding a mitogen-activated protein kinase (PITG_15917 and PITG_00124), inside the serine/threonine kinases family, could be associated with the terminal stage of signaling cascade and promotion of apoptosis (Cross et al., 2000). The up-regulation of a receptor-type tyrosine-protein phosphatase (PITG_09671) recently emerged as a drug target site (van Huijsduijnen et al., 2002) and a hypothetical protein encompassing a tyrosine kinase domain (PITG_12002) could act as an initiator factor upstream of ser/thr kinases (Cross et al., 2000). The up-regulation of these genes was also associated to a perturbation of the homeostasis of the Pi cells deriving from the down-regulation of genes respectively involved in proton and potassium transport and in the active export of Ca²⁺ through the plasma membrane (Krebs et al., 2015). The perturbation of intracellular calcium concentration in Pi cells

can cause cytotoxicity and trigger programmed cell death (PCD) via apoptotic process (Orrenius et al., 2003).

The PCD occurring in Pi is also supported by the suppression of the whole oomycete metabolism during the interaction with AZ78. The enrichment analysis indicated the down-regulation of genes encoding poly (ADP-ribose) polymerase (PARP), which plays a key role in DNA repair and genome integrity (Citarelli et al., 2010). During the sporulation process of *A. nidulans* a PARP-like protein was detected in mycelia before the conidial formation, and it was then degraded together with an increased apoptotic activity (Thrane et al., 2004). The breakdown of DNA repair mechanisms and the general down-regulation of DNA and RNA metabolic pathways in Pi let to hypothesize an inactivation of nuclear activity.

Although the apoptotic processes involved in mammalian cells are deeply investigated, little is known about the molecular pathways related to PCD via apoptotic process in oomycetes. Galiana et al. (2005) reported that hypersensitive response of tobacco leaves can induce PCD in *P. parasitica* zoospores by inducing the production of numerous large vacuoles. In contrast to these findings, the PCD mechanism observed in *P. parasitica* zoospores seems not be present in Pi mycelium. In Pi the intracellular transport to the vacuoles was negatively affected as demonstrated by the down-regulation of related genes. Two hypothesis can explain the results: the PCD mechanism is different in the two *Phytophthora* species or the PCD mechanism in zoospores is different compared to PDC mechanism in mycelium. However, at the best of our knowledge, the results reported in our work represent a first step in understanding the molecular pathways deputed to PCD in Pi and, in future, these information will be helpful to select new compounds that can trigger the PCD in this important plant pathogen and used as novel fungicides.

Indeed, the progressive Pi cell death was associated with the impairment of pathogenic mechanisms through the down-regulation of plant cell wall-degrading enzymes, such as polygalacturonase and pectinesterase, well characterized in *P. parasitica* (Blackman et al., 2014). Moreover, the expression of EPICs, which interfere with the cysteine proteases produced by the plant host (Hardham and Cahill, 2010; Tian et al., 2007), was down-regulated. Similarly, *hps90* and *hps70*, two highly conserved molecular chaperone essential for viability in eukaryotes and expressed in Pi during the early stage of potato infection (Picard, 2002; Avrova et al., 2003) were also down-regulated. Conversely, Pi interacting with AZ78 showed the up-regulation of 14 of the 57 DEGs

encoding RXLR effectors that can mediate host targeting, interaction with host protein and virulence mechanisms (Morgan and Kamoun, 2007; Kamoun, 2006).

A consequence of PCD could be represented by the down-regulation of genes encoding protocadherin-like protein (PITG_09982), positioned downstream to PITG_09983 the most notable example of the Pi cadherin family (Lévesque et al., 2010) and three genes encoding cell-surface plexin protein probably involved in signal transduction via semaphorin binding (Ohta et al., 1995; Hutter et al., 2000). The outcome of this down-regulation could be represented by the disaggregation of cell to cell junctions with negative effects on Pi cell-adhesion.

At this stage of interaction, the progressive Pi cell death nullify the possible efforts to counteract the AZ78 attacks as supported by the down-regulation of genes encoding a manganese superoxide dismutase involved in ROS detoxification (Apel and Hirt, 2004) and ABC and MFS transporters (Del Sorbo et al., 2000).

On the other side, AZ78 takes advantages from this condition and strengthens its attack of Pi mycelium by up-regulating 38 DEGs responsible for the biosynthesis and release of enzymes involved in the digestion of 1,3- β - glucans, cellulose and proteins the main components of Pi cell-wall (Bartnicki-Garcia, 1968; Mélida et al., 2013). The products deriving from the degradation of Pi cell wall were converted into energy available for AZ78 cells as supported by the up-regulation of AZ78 genes involved in catabolism of galactose, a monosaccharide sugar associated with oomycete cell-wall (Mélida et al., 2013). Moreover, AZ78 also up-regulated TRAP-type C4-dicarboxylate transport system involved in uptake of carbon energy sources in *P. aeruginosa* PAO1 under low-nutrient condition (Valentini et al., 2011). Furthermore, in response to external stimuli deriving by the presence of Pi, AZ78 activated the signal transduction pathway by up-regulating 19 genes encoding TonB-dependent receptors involved in the uptake of iron-siderophore complexes or vitamins which plays a key role in the microbial competitions (Braun, 1995).

Despite of the progressive cell death, Pi tries to respond to the AZ78 lytic activity. For instance, four genes encoding Kazal-like protease inhibitors involved in the inactivation of potato and tomato proteolytic activity (Tian et al., 2005) were up-regulated together with five genes encoding proteins with extracellular lytic activity (París and Lamattina, 1999). The products of these genes could be involved in the inactivation of AZ78 extracellular lytic enzymes as reported in the case of *R. solani* challenged with *Serratia* spp. (Gkarmiri et al., 2015). To scavenge the free radicals and try to increase the

resistance to oxidative stress caused by AZ78, Pi showed the up-regulation of five genes related to oxidoreductase activity, such as the gene encoding for glutathione-S-transferase protein that showed the same pattern in *R. solani* interacting with *Serratia* spp. (Gkarmiri et al., 2015). Interestingly, the up-regulated PITG_08902 gene encoding a protein of PDR family in Pi could be involved in drug and toxin extrusion and the stress-induced pathways (Connolly et al., 2005). Moreover, a gene encoding maleylacetoacetate isomerase involved in the aromatic amino acid metabolic pathways was up-regulated in Pi during the interaction. Similarly, the maleylacetoacetate isomerase of *Saprolegnia parasitica* (SPRG_01336) was shown to be up-regulated under abiotic stress (Hu et al., 2016). However, little is known about the role in maleylacetoacetate isomerase in the bacteria-oomycete interaction and further studies are needed to further determine its functions.

Similarly, AZ78 also activates defense mechanisms against the Pi response, such as the transcription of UspA family protecting the cell against DNA-damaging (Kvint et al., 2003, Liu et al., 2007) in response to the stress condition mediated by the Pi interaction (oxidative radicals). In the same way, the detoxification of the AZ78 cells from reactive oxygen species (ROS) was mediated by the production of catalase and putative oxidoreductase enzymes UcpA and YncB (Wang et al., 2012; Kim et al., 2010). The down-regulation of *lexA* could be also associated to the defenses of AZ78 cell against toxic compounds released by Pi. Indeed, LexA-depletion mutants of the close related bacterial strain *X. axonopodis* pv. *citri* XW47 were more resistant to mitomycin C and methylmethane sulfonate (Yang et al., 2005). Interestingly, AZ78 *lexA* showed respectively 56 and 77% homology at the amino acid level with LexA1 (XAC1196) and LexA2 (XAC1739) of *X. axonopodis* pv. *citri* XW47. The down-regulation of *lexA* in AZ78 could be also involved in the activation of the SOS system for DNA repair mediated by RecA (Butala et al., 2009). However, the enrichment analysis showed the down-regulation of genes involved in DNA integrity by repair of double-strand breaks (Ramsden and Gellert, 1998). It is tempting to speculate that an increased mutation rates could occur in AZ78 during the interaction with Pi and this behavior could improve AZ78 cell survival under stress conditions through adaptive mutagenesis process (Bjedov et al., 2003; Harris et al., 1997).

In conclusion, the attack strategy implemented by AZ78, in our opinion, could be ascribed to bacterial mycophagy (Levau and Preston, 2008) which would be the first report of such behavior for *Lysobacter* members. Regarding Pi, these results represent a

first step in elucidating how *P. infestans* may respond to the attacks of bacterial biocontrol agents and provided the first evidences of PCD via apoptotic process in *P. infestans*.

Experimental procedures

Strain and growth conditions

Lysobacter capsici AZ78 was stored in 40% glycerol at -80°C and routinely grown on Luria Bertani Agar [LBA, LB Broth (Sigma-Aldrich, St. Louis, MO, USA), 1.6% (w/v) Agar Technical No.3 (Oxoid-Columbia, Md, USA)] at 27°C. *Lysobacter capsici* AZ78 cells originating from 72 h incubation at 27°C were used in all the experiments. *Phytophthora infestans* isolate 11/10, kindly provided by M. Finckh and A. Butz (University of Kassel, Germany), was stored at length in 20% glycerol at -80°C and grown on Pea Agar Medium [PAM, 12.5% frozen peas in distilled water, 1.2% (w/v) Agar] at 20°C for seven days before the experiments.

Bacterium-oomycete interaction

The experimental design to evaluate the AZ78-Pi interaction mechanisms was composed by four treatments: AZ78 and Pi interacting for 6 h and 24 h at 25°C (LcPi6 and LcPi24) and two controls, AZ78 and Pi incubated alone for 6 h at 25°C (Lcc and Pic). The controls collected after 6 h represent the control for all treatments, since no significant changes in the transcriptome of the two microorganisms occurred between 6 h and 24 h in preliminary experiments (data not shown). More specifically Pi plugs (5 mm) were transferred onto cellophane film overlying PAM dishes and grown 96 h at 20°C. AZ78 cell suspensions (1×10^9 cells/ml) were obtained according to Puopolo et al. (2014b). The mycelium of Pi was inoculated with 80 µL of AZ78 cell suspension and incubated at 25°C. Samples consisting of Pi mycelium with AZ78 cells were collected at 6 and 24 h, immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. PAM dishes inoculated with Pi or AZ78 alone and incubated 6 h at 25°C were used as control. For each treatment, three replicates of six dishes each were processed.

Six additional dishes for LcPi and Lcc treatments were used to quantify the growth rate of AZ78. The AZ78 cells were collected by adding 5 ml of sterile saline solution (0.85% NaCl) after 6 and 24 h of incubation at 25°C. The amount of AZ78 cells for

each dish was then quantified by serial dilution plating method on LBA. The Student's T test ($\alpha = 0.05$) was applied to identify significant differences within Lcc and LcPi at the two time points (Statistica 13.1; StatSoft, Tulsa, OK, USA).

RNA extraction

The frozen samples were ground to a fine powder in liquid nitrogen and total RNA was extracted using Tri-Reagent (Sigma-Aldrich) according to manufacturer's instructions. Isolated RNA was purified using the RNeasy Mini Kit (Qiagen Sciences, Valencia, CA, USA) and subjected to DNase treatment with an RNase-Free DNase set (Qiagen) during the RNA purification, following manufacturer's instructions. The RNA integrity and concentration were assessed using a BioAnalyzer RNA 6000 (Agilent Technologies, Santa Clara, CA, USA) and a Qubit 3.0 Fluorometer (Invitrogen, Life Technologies) with Qubit RNA BR assay (Invitrogen, Life Technologies), respectively.

Illumina sequencing and mapping to the reference genomes

Library construction and Illumina Sequencing was carried out at Fasteris (Plan-les-Ouates, Switzerland) following the scheme summarized in Fig. S1. Each sample of total purified RNA was diluted with RNase-free water to a final concentration of 50 ng/ μ L. The rRNA depletion was performed using the Ribo-Zero Gold rRNA Removal Kit (Epidemiology, Illumina, San-Diego, CA, USA). The PolyA depletion step was then applied to the samples derived from treatments LcPi6 and LcPi24. Then, these two treatments were split in Lc6 and Lc24, consisting in total PolyA depleted mRNA respectively originated from LcPi6 and LcPi24, and in Pi6 and Pi24 consisting in total PolyA enriched mRNA respectively originated from LcPi6 and LcPi24. Subsequently, these treatments were sequenced separately. The PolyA depletion treatment was not carried out for the control treatments Lcc and Pic (Fig. S1).

The mRNA-Seq libraries were multiplexed (two libraries per lane) and sequenced with Illumina HiSeq High Output (HO) Version 4 - paired reads, according to the manufacturer's instructions. Complementary DNA (cDNA) libraries were synthesized using TruSeq RNA Sample preparation Kit v2 (Illumina) and the process was carried out according to the Standard protocol mRNA Stranded sample preparation (Illumina). Paired-end reads of 125 nucleotides were obtained using an Illumina HiSeq 2500. Raw sequences were deposited at the Sequence Read Archive of the National Center for

Biotechnology (www.ncbi.nlm.nih.gov/sra) under accession number SRP080721 and BioProject number PRJNA329480.

The Illumina HiSeq data were assessed for quality using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Illumina paired-end (2 x 125bp) reads for each sample were merged to increase the overall read length using the Illumina PEAR (Paired-End reAd mergeR version 0.9.8) software (Zhang et al., 2014). The resulting merged reads of Lcc, Lc6 and Lc24 samples were aligned separately to the AZ78 genome (version JAJA00000000.2 deposited at DDBJ/EMBL/GenBank) (Puopolo et al., 2016) and those of Pic, Pc6 and Pc24 samples were aligned to the reference genome of *P. infestans* T30-4 (http://protists.ensembl.org/Phytophthora_infestans/Info/Index) (Haas et al., 2009) using the Subread aligner with default parameters (Liao et al., 2013). Read counts were extracted from the Subread alignments using the feature Count read summarization program (Liao et al., 2014).

Identification of differentially expressed genes

Changes in gene expression levels were analyzed with the voom method (Law et al., 2014) which estimates the mean-variance relationship of the log counts, generating a precision weight for each observation that is fed into the limma empirical Bayes analysis pipeline (Smyth, 2004). A Volcano Plot generated using the ShinyVolcanoPlot Web App (<http://www.showmeshiny.com/volcano-plot/>) was used to select sets of DEGs for each comparison based on both p-value and expression fold change. A $\log_2FC \geq 2$ and $P\text{-value} \leq 0.001$ were imposed to identify DEGs through pairwise comparisons. One (Lcc vs Lc24) and two (Pic vs Pi6 and Pic vs Pi24) pairwise comparisons were analyzed to identify AZ78 and Pi DEGs, respectively. An additional comparison (Lcc vs Lc6) was analyzed for AZ78, but it was considered unreliable due to the low sequencing depth of AZ78 reads at the early interaction stage LcPi6.

Functional annotation

The protein sequences of the corresponding selected DEGs lists were functionally characterized using Blast2GO program (Conesa et al., 2005; <http://www.blast2go.org>). Default settings were applied and the minimal Blast Expected Value was set to 1×10^{-5} . A Gene Ontology Analysis was performed to identify over-represented GO categories of the biological process terms using the goseq R Bioconductor package (Young et al.,

2010) with the Wallenius approximation followed by Benjamini–Hochberg correction (Bacher and Kendzioriski, 2016). The over-represented GO categories were represented as in the Biological Networks Gene Ontology (BiNGO) tool (Maere et al., 2005) of Cytoscape 3.4.0 software (Shannon et al., 2003). The DEGs were additionally classified by a manual annotation on the basis of the NCBI gene description and Blast2GO description in 21 functional classes (Table S2-S4).

RNA-Seq validation

The RNA-Seq was validated by qRT-PCR. First-strand cDNA synthesis and qRT-PCR reactions were carried out as reported in Tomada et al. (2016). Specific primers for AZ78 and Pi (Table S11) were designed using the Primer3 software (<http://bioinfo.ut.ee>; Untergasser et al., 2012), and their specificity was assessed by PCR and Sanger sequencing before the gene expression analysis. Relative expression levels were determined by the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001) based on three technical replicates per sample using Lcc or Pic as the calibrator. The housekeeping gene *recA* (AZ78_1089, (Tomada et al., 2016) and *actA* (PITG_09284, Avrora et al., 2003) were used as constitutive genes for the normalization of AZ78 and Pi data, respectively. The RNA-Seq validation was done using the same samples that were subjected to the RNA-Seq analysis. Pearson correlation test was applied to measure the linear relationship between the RNA-Seq \log_2FC values and qRT_PCR \log_2FC values of selected genes (Statistica 13.1).

Conflict of interest statement

All authors declare no conflict of interest.

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Table 1. Differentially expressed genes of *Lysobacter capsici* AZ78 and *Phytophthora infestans* after 6 and 24 hours of interaction.

	Blast2GO	Sample			
		Lc6*	Lc24	Pi6	Pi24
Induced	Annotated	96	237	15	193
	Hypothetical	65	112	8	193
		161	349	23	386
Repressed	Annotated	77	123	191	346
	Hypothetical	52	76	123	228
		129	199	314	690
Total	Annotated	173	360	206	655
	Hypothetical	177	188	131	421
		290	548	337	1076

Samples were collected after 6 and 24 h of bacterium-oomycete interaction. The resulting treatments after RNA-Seq analysis were: *L. capsici* AZ78 (AZ78) at 6 h (Lc6) and 24 h (Lc24), *P. infestans* (Pi) at 6 h (Pi6) and 24 h (Pi24). The derived expressed genes lists were subjected to a pairwise comparison with AZ78 control (Lcc) and Pi control (Pic) treatments. A \log_2 -Fold Changes ≥ 2 and P-value ≤ 0.001 were applied to identify the differentially expressed genes per treatment. Annotated genes indicate the number of genes having a putative function after Blast2Go analysis. *Low sequencing depth of AZ78 reads was obtained at the early interaction stage (6 h).

Supplementary Information

Transcriptional response of Lysobacter capsici AZ78 after 6 hours interaction with Phytophthora infestans

The relative expression levels of 11 selected genes of AZ78 assessed by qRT-PCR at 6 hours of interaction (6 hoi) agreed with those assessed by RNA-Seq analysis (Fig. 2A; Table S7) and Lc6 DEGs list provided interesting data regarding the early stage of the bacterial-oomycete interaction. The modulated genes of Lc6 were divided into two groups (up- and down-regulated genes) that were subjected to GO enrichment analysis (Table 1) and manual annotation. Overall, the data analysis revealed the up-regulation of biological pathways related to bacterial motility, antagonism activity and transport. Particularly, Lc6 up-regulated genes resulted in an over-represented GO related to type IV pilus (T4P) biogenesis, including the AZ78_1459 (*PilW*). Moreover, other seven manually annotated genes involved in the T4P motility mechanism were up-regulated in Lc6 (AZ78_1456, AZ78_1457, AZ78_1458, AZ78_1460, AZ78_1461, AZ78_1781 and AZ78_2052), such as the operon *pilVWY1E* (from AZ78_1456 to AZ78_1460).

The over-represented carbohydrate metabolic process encompassed two up-regulated genes related to antagonism and lytic activity of AZ78, such as *gluA*, (AZ78_3679) and *cel5G*, (AZ78_3685) that are involved in the degradation of glucans and cellulose, respectively. Another over-represented GO related to the bacterial lytic activity was proteolysis that encompassed eight up-regulated genes (AZ78_0270, AZ78_0395, AZ78_2137, AZ78_2802, AZ78_4497, AZ78_4519, AZ78_4791, AZ78_4903) classified as serine-endopeptidase and metalloproteases. Moreover, 14 up-regulated genes related to the antagonism activity were identified by manual annotation, such as AZ78_0849 and AZ78_3544 encoding respectively a lanthionine biosynthesis protein LanL and a siderophore biosynthesis non-ribosomal peptide synthetase module involved in the biosynthesis of antibiotics. The biosynthesis of polar amino acids was represented by the up-regulation of AZ78_1207 (asparagine synthetase), AZ78_2151 (threonine dehydratase) AZ78_2811 (glutamine synthetase type I), AZ78_5034 (histidinol-phosphatase) and AZ78_5035 (imidazole glycerol phosphate synthase amidotransferase subunit) that are possibly implicated also in the antibiotic biosynthesis.

Signal transduction processes were activated in Lc6, by the up-regulation of 13 and three genes encoding TonB-dependent receptor proteins and outer membrane receptor

proteins, respectively. TonB-receptor genes were also assigned to the GO term transport, due to the possible double function (receptor/channel) of these membrane proteins. Three TonB-receptor genes (AZ78_1685, AZ78_2691, AZ78_3798) were additionally classified in the siderophore transport category. Transport processes were also related to the up-regulation of genes involved in toxic compound extrusion, such as ABC transporter (AZ78_0717; AZ78_1097, AZ78_2688, AZ78_2689 and AZ78_2690), permease (AZ78_1597) and major facilitator superfamily (AZ78_5171). In Lc6, the response to stress was another over-represented category that encompassed three up-regulated genes (AZ78_1581, AZ78_1588 and AZ78_1600) encoding proteins of universal stress family (UspA domain). The manual annotation allowed the identification of superoxide dismutase gene (AZ78_3722) involved in response to stress as well.

The GO enrichment analysis of the down-regulate Lc6 genes resulted in the over-representation of two terms related to the DNA and transcription (negative regulation of transcription DNA-templated and double-strand break repair via non homologous end joining). The negative regulation of transcription encompassed two down-regulated genes AZ78_1090 (SOS-response repressor LexA) and AZ78_1864 (Flavoprotein WrbA). Regarding the down-regulated genes AZ78_4056, encoding a putative entericidin protein, was highlighted in the over-represented GO term of response to toxic substances.

Table 1. Over-represented gene ontology (GO) categories after enrichment analysis of *Lysobacter capsici* AZ78 differentially expressed genes (DEGs) after 6 hours of interaction with *Phytophthora infestans*. DEGs of *L. capsici* AZ78 after 6 h of interaction (Lc6) with the *P. infestans* were distinguished in up-regulated and down-regulated genes. The total genes in category represent the total number of genes grouped in each specific category in the annotated genome of AZ78.

Treatment	GO category	Term	Over-represented P value	DEGs in category	Total genes in category	
Lc6 up-regulated genes	GO:0007165	signal transduction	4.36E-12	16	72	
	GO:0006810	transport	3.48E-07	16	126	
	GO:0015891	siderophore transport	1.10E-03	3	8	
	GO:0005975	carbohydrate metabolic process	2.15E-03	7	65	
	GO:0006950	response to stress	2.52E-03	3	9	
	GO:0006529	asparagine biosynthetic process	2.79E-02	1	1	
	GO:0006542	glutamine biosynthetic process	2.79E-02	1	1	
	GO:0009399	nitrogen fixation	2.79E-02	1	1	
	GO:0043683	type IV pilus biogenesis	2.79E-02	1	1	
	GO:0006565	L-serine catabolic process	2.79E-02	1	1	
	GO:0000105	histidine biosynthetic process	3.19E-02	2	10	
	GO:0015990	electron transport coupled proton transport	3.68E-12	1	1	
	GO:0006508	proteolysis	4.81E-02	8	143	
	Lc6 down-regulated genes	GO:0045892	negative regulation of transcription, DNA-templated	1.76E-02	2	9
		GO:0006051	N-acetylmannosamine metabolic process	2.35E-02	1	1
GO:0006303		double-strand break repair via non homologous end joining	2.35E-02	1	1	
GO:0061077		chaperone-mediated protein folding	2.35E-02	1	1	
GO:0044238		primary metabolic process	4.64E-02	1	2	
GO:0006559		phenylalanine catabolic process	4.64E-02	1	2	
GO:0009636		response to toxic substance	4.64E-02	1	2	
GO:0034220		ion transmembrane transport	4.64E-02	1	2	

Figure Captions

Figure 1: Over-represented gene ontology (GO) functional categories of *Lysobacter capsici* AZ78. Differentially expressed genes (DEGs) of *L. capsici* AZ78 after 24 h of interaction with *Phytophthora infestans* were divided in two groups (up- and down-regulated genes). GO categories from the biological process were analysed with Bioconductor R package goseq for each group. **A**, up-regulated DEGs; **B**, down-regulated DEGs.

Figure 2: Scatterplot between RNA-Seq and qRT-PCR relative expression levels. Pearson correlation test was applied to \log_2 FC values assessed by RNA-Seq and qRT-PCR of selected genes (Table S11). Graph and correlation value (R) of **A**, *Lysobacter capsici* AZ78 and **B**, *Phytophthora infestans* differentially expressed genes. Different colors in each graph correspond to 6 h (light) and 24 h (dark) of interaction.

Figure 3: Over-represented gene ontology (GO) functional categories of *Phytophthora infestans*. Differentially expressed genes (DEGs) of *P. infestans*, after 6 h and 24 h of interaction with *Lysobacter capsici* AZ78 were listed in six groups (G) and the over-represented GO categories were calculated using the Bioconductor R package goseq. Over-represented enriched GO categories are reported for **A**, G1; **B**, G2; **C**, G3; **D**, G4; **E**, G5, **F**, G6 of DEGs. Gene expression profiles are schematized for **G**, down-regulated DEGs at 6 h with positive trend at 24 h; **H**, down-regulated DEGs at 6 h with negative trend at 24 h; **I**, not DEGs at 6 h with negative trend at 24 h; **J**, not DEGs at 6 h with positive trend at 24 h; **K**, up-regulated DEGs at 6 h with positive trend at 24 h (K); **L**, up-regulated DEGs at 6 h with negative trend at 24 h.

Figure 4: Schematic overview of *Lysobacter capsici* AZ78 and *Phytophthora infestans* interaction. The scheme reported the microorganism interaction as interpreted from the transcriptional profiling of both the partners.

Supplementary Figure Captions

Figure S1: Scheme of the RNA-Seq experiment. Summary of the procedures applied for the dual RNA-Seq experiment: sample collection, total RNA extraction, cDNA library preparation, rRNA depletion, PolyA depletion and sequencing. Samples of *L. capsici* AZ78 (AZ78) and *Phytophthora infestans* (Pi) were collected after 6 h (LcPi6) and 24 h (LcPi24) of interaction at 25 °C. Control treatments of AZ78 (Lcc) and Pi (Pic) were incubated for 6 h at 25 °C. After PolyA depletion, AZ78 samples were indicated as Lc6 and Lc24, corresponding to AZ78 interacting with Pi for 6 h and 24 h, respectively. Samples from Pi were indicated as Pi6 and Pi24 consisting in Pi interacting with AZ78 for 6 h and 24 h, respectively.

Figure S2: Growth rate of *Lysobacter capsici* AZ78 (AZ78). The amount of AZ78 developing cells expressed as log₁₀ CFU/dish was evaluated for AZ78 interacting with *Phytophthora infestans* (dark grey) after 6 and 24 h of interaction (hoi) at 25 °C. AZ78 incubated alone (light grey) at 25°C was used as a control. Mean and standard deviation values are reported for each treatment. No significant differences between AZ78 interacting with *P. infestans* and AZ78 incubated alone were found according to Student's T test ($\alpha \leq 0.05$)

Figure S3: Manual annotation of differentially expressed genes (DEGs). DEGs were classified by a manual annotation on the basis of the NCBI gene description and Blast2GO description in 21 biological process classes (Table S2-S4). The number of DEGs for each functional category (except for the unknown function) is reported for **A**, *Lysobacter capsici* AZ78 genes modulated after 24 h of interaction (Lc24); **B**, *Phytophthora infestans* genes modulated after 6 h of interaction (Pi6); **C**, *P. infestans* genes modulated after 24 h of interaction (Pi24). Green, up-regulated DEGs; Red, down-regulated DEGs. The total number of DEGs is shown per for each biological process class.

List of Supporting Information

Figure S1. Scheme of RNA-Seq experiment.

Figure S2: Growth rate of *Lysobacter capsici* AZ78 (AZ78).

Figure S3. Manual annotation of differentially expressed genes (DEGs).

Table S1. Number of sequenced read pairs and mapping results to the *Lysobacter capsici* AZ78 or *Phytophthora infestans* T30-4 genome.

Table S2. List of differentially expressed genes of *Lysobacter capsici* AZ78 after 6 h of interaction with *Phytophthora infestans*.

Table S3. List of differentially expressed genes of *Lysobacter capsici* AZ78 after 24 h of interaction with *Phytophthora infestans*.

Table S4. List of differentially expressed genes of *Phytophthora infestans* interacting with *Lysobacter capsici* AZ78 for 6 h and 24 h.

Table S5. Over-represented gene ontology (GO) categories after GO enrichment analysis of *Lysobacter capsici* AZ78 differentially expressed genes (DEGs) after 24 hours of interaction with *Phytophthora infestans*.

Table S6. Differentially expressed genes of *Lysobacter capsici* AZ78 related to the antagonism activity against *Phytophthora infestans* after 24 hours interaction.

Table S7. Comparison of RNA-Seq and qRT-PCR results of *Lysobacter capsici* AZ78.

Table S8. Over-represented gene ontology (GO) categories after GO enrichment analysis of *Phytophthora infestans* differentially expressed genes (DEGs) during the interaction with *Lysobacter capsici* AZ78.

Table S9. Relevant differentially expressed genes of *Phytophthora infestans* interacting with *Lysobacter capsici* AZ78.

Table S10. Comparison of RNA-Seq and qRT-PCR results of *Phytophthora infestans*.

Table S11. Primers used in quantitative real-time polymerase chain reaction.

Figure 1. Over-represented gene ontology (GO) functional categories of *Lysobacter capsici* AZ78.

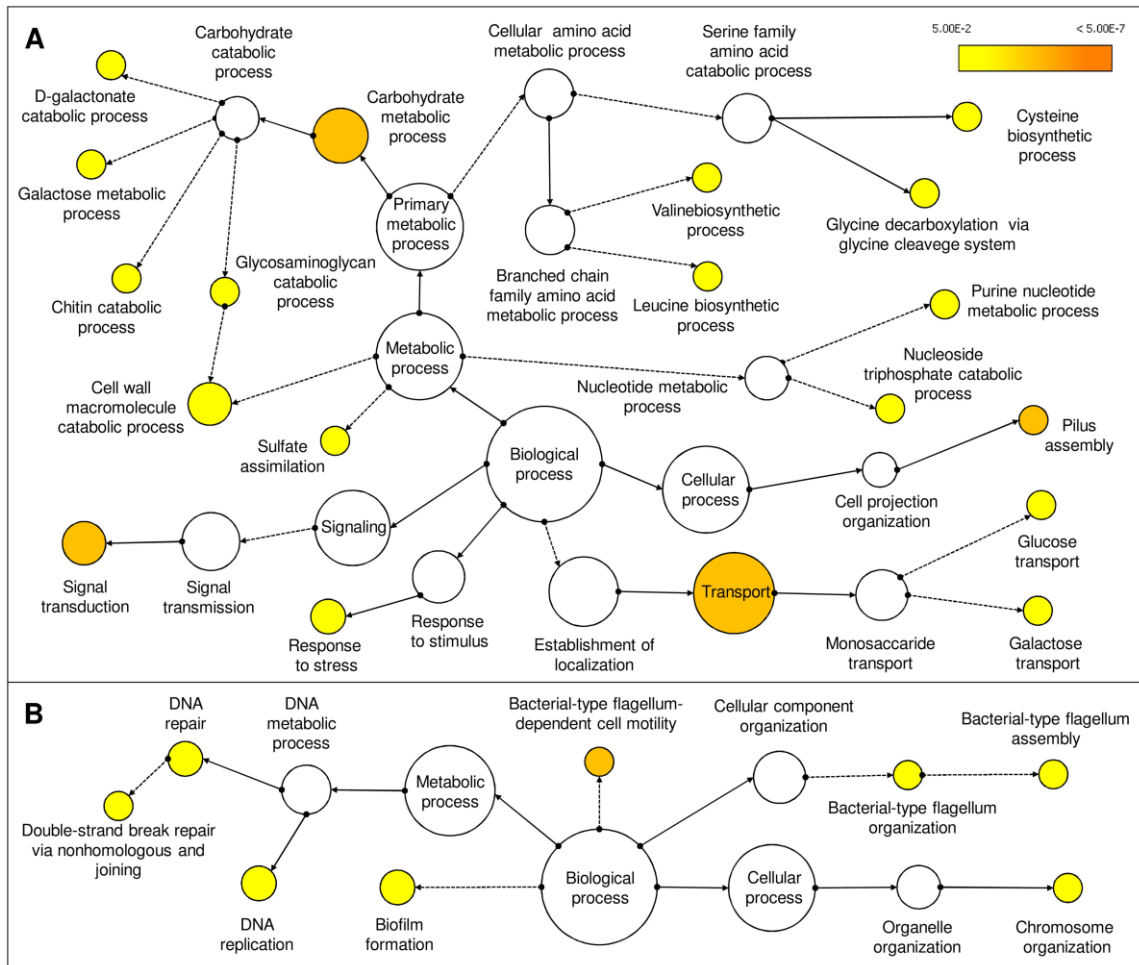


Figure 2. Scatterplot between RNA-Seq and qRT-PCR relative expression levels.

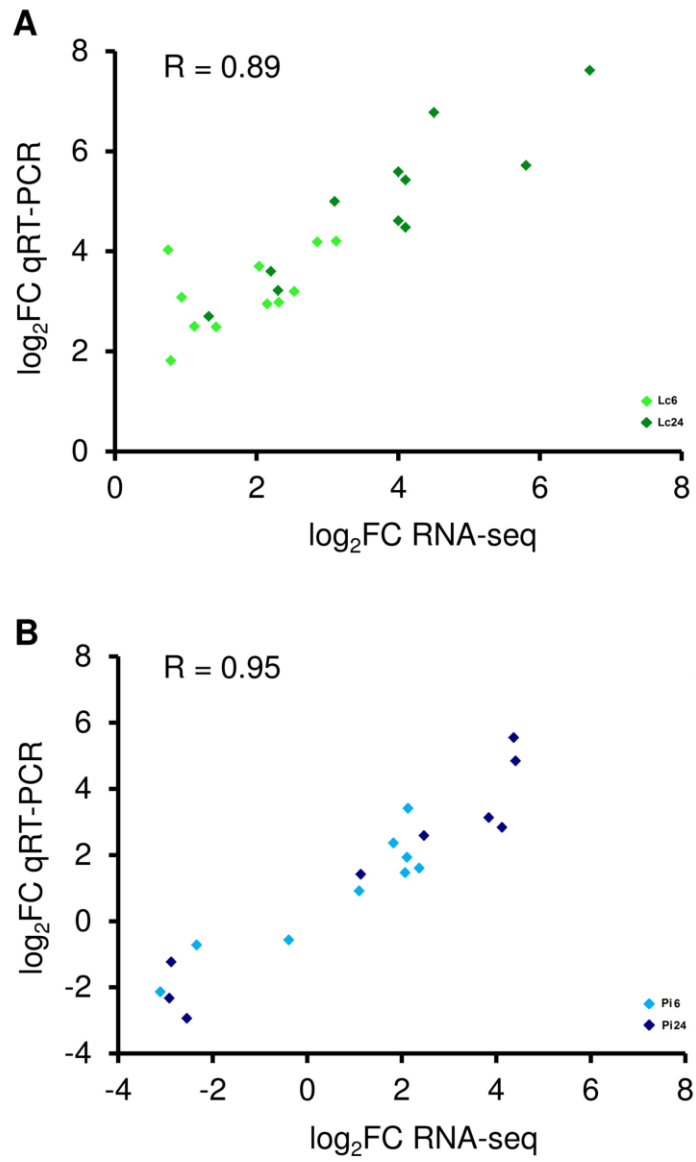


Figure 3. Over-represented gene ontology (GO) functional categories of *Phytophthora infestans*.

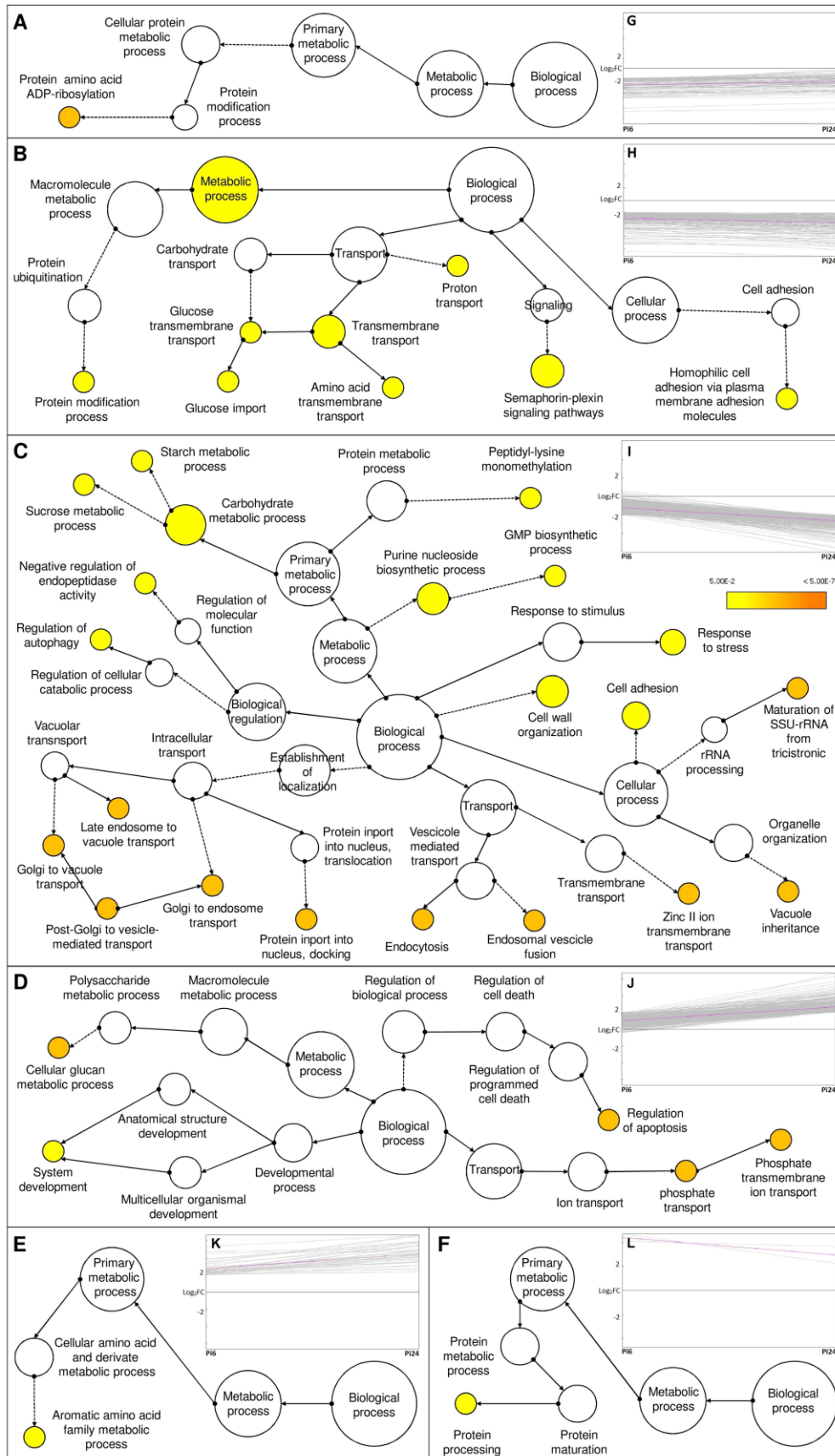


Figure 4. Schematic overview of *Lysobacter capsici* AZ78 and *Phytophthora infestans* interaction.

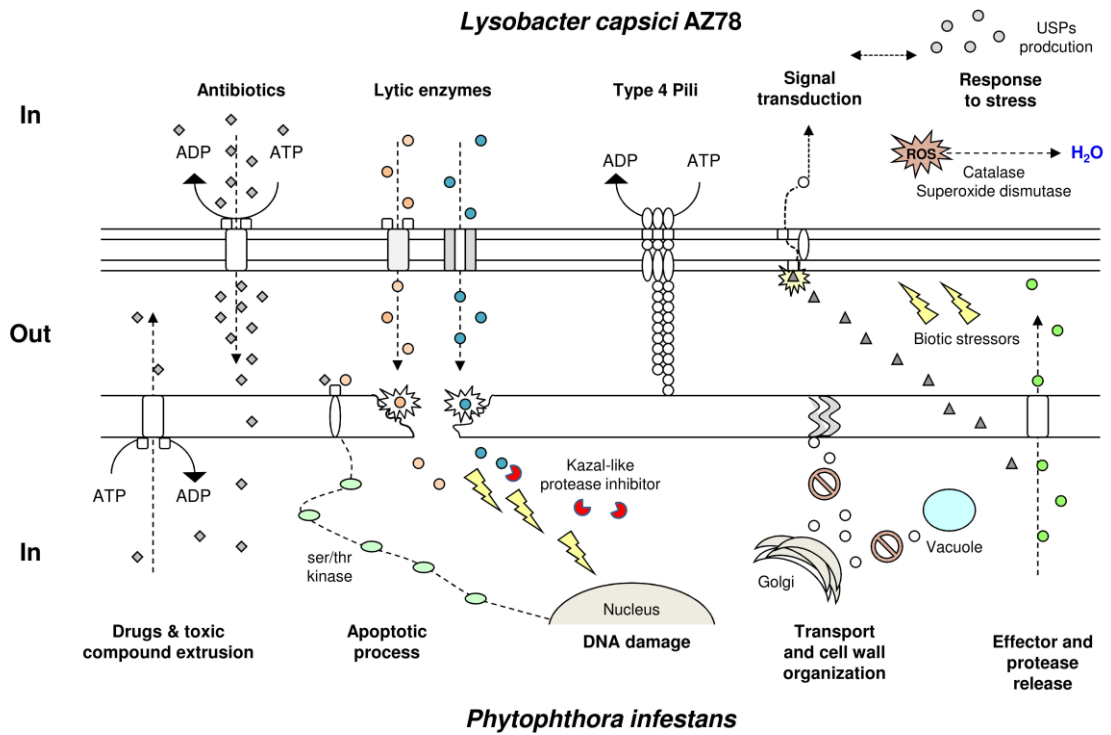


Figure S1. Scheme of RNA-Seq experiment.

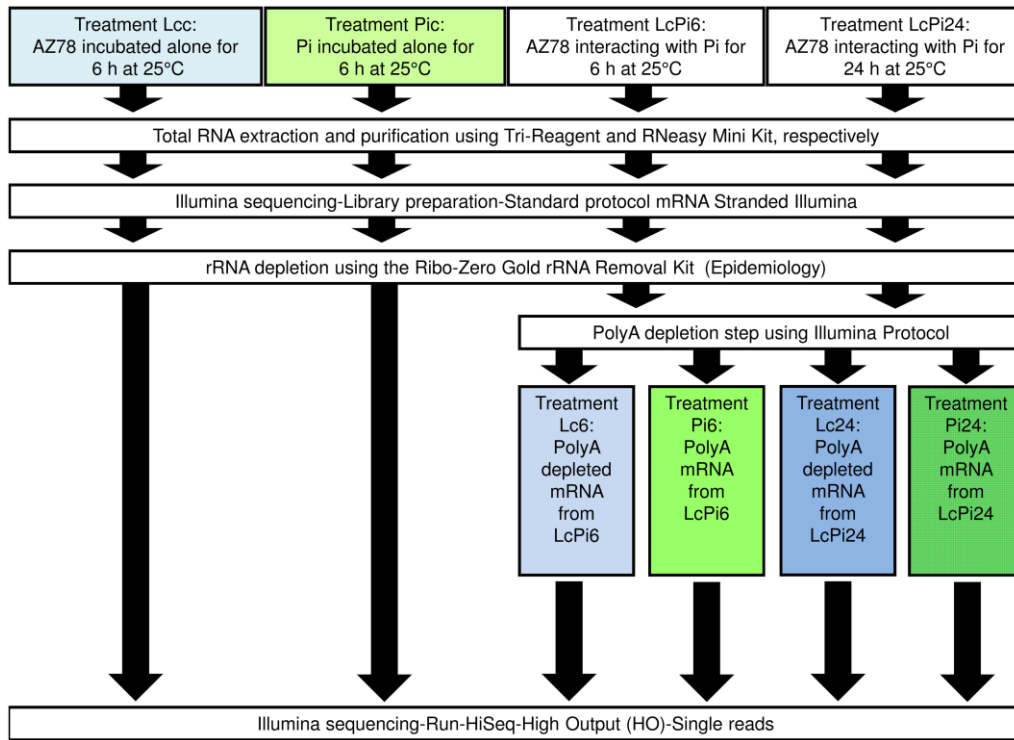


Figure S2. Growth rate of *Lysobacter capsici* AZ78 (AZ78).

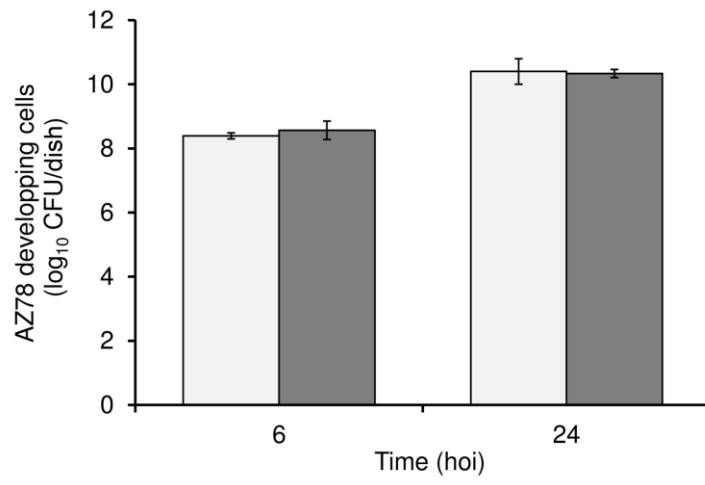


Figure S3. Manual annotation of differentially expressed genes (DEGs).

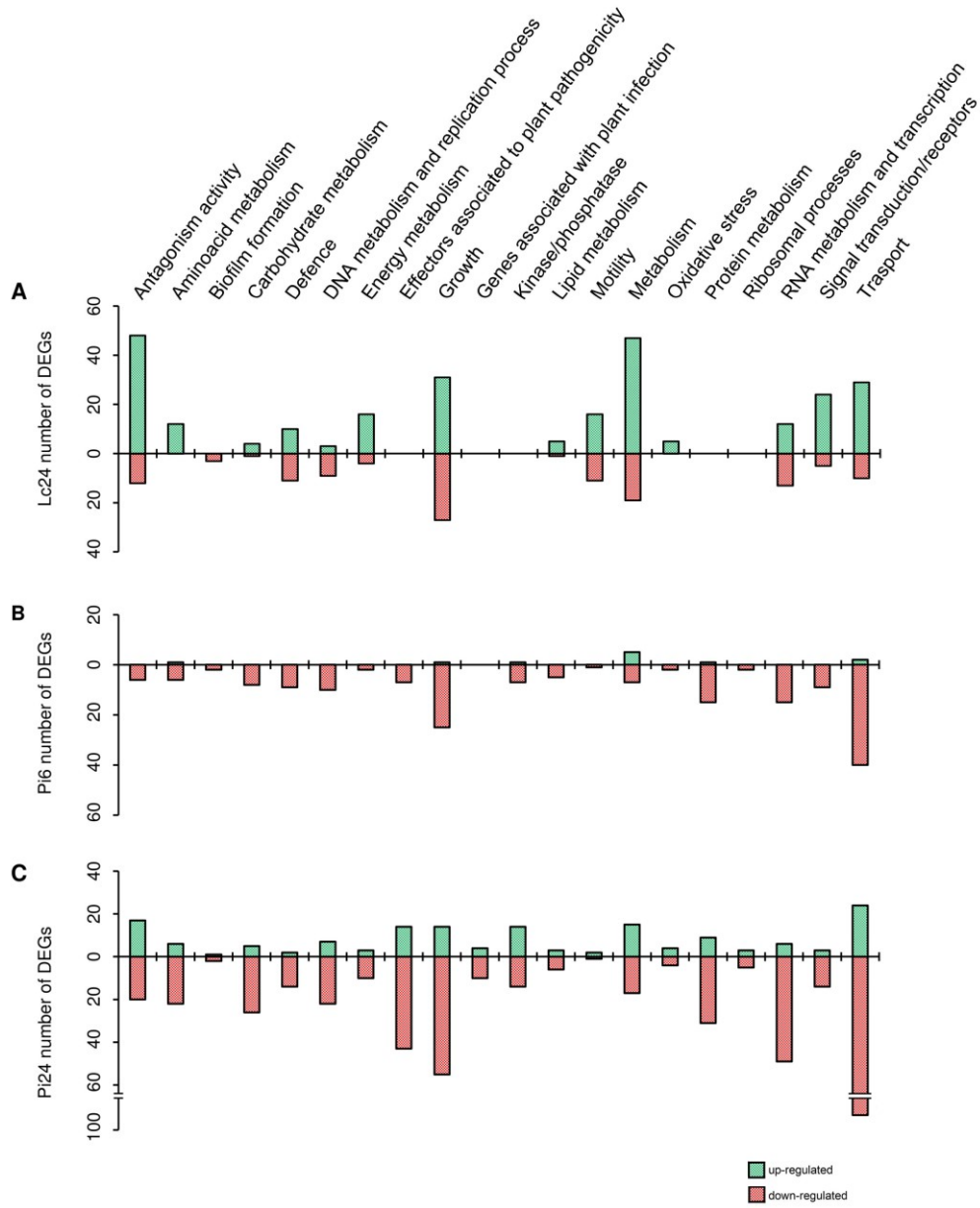


Table S1. Number of sequenced read pairs and mapping results to the *Lysobacter capsici* AZ78 or *Phytophthora infestans* T30-4 genome.

Lane	Sample ¹	Fasteris library tag	Read pairs before adapter removal	Read pairs after adapter removal	Total mapped reads <i>P. infestans</i>	Total mapped reads <i>L. capsici</i> AZ78	Uniquely mapped reads <i>P. infestans</i>	Uniquely mapped reads <i>L. capsici</i> AZ78
A	Pic 1	HVH-1	15068804	12250657	8586051	3659	7415769	2394
B	Pic 1	HVH-1	15002345	12191957	8546351	3420	7371722	2263
A	Pic 2	HVH-2	17933876	13897080	9683592	3158	8535357	2490
B	Pic 2	HVH-2	17900913	13866576	9661118	3024	8511461	2373
A	Pic 3	HVH-3	22053371	17250497	11820342	3564	10214030	2342
B	Pic 3	HVH-3	22007235	17206100	11778866	3541	10172504	2235
A	Lcc 1	HVH-4	16528174	11640448	7248	11389595	2951	9735948
B	Lcc 1	HVH-4	16562122	11662993	6757	11414635	2642	9755198
A	Lcc 2	HVH-5	17215783	12648553	6668	12350608	3014	10464012
B	Lcc 2	HVH-5	17315684	12715861	5750	12415424	2830	10516714
A	Lcc 3	HVH-6	25188738	17017415	19215	16226652	4733	13018656
B	Lcc 3	HVH-6	25211715	17025746	19391	16236472	4175	13027173
A	Pi6 1	HVH-7	14834300	11612589	10509082	2430	9158478	2006
B	Pi6 1	HVH-7	14858333	11622437	10536993	2191	9176120	1812
A	Lc6 1	HVH-8	16831526	13567912	7446882	659690	6516187	486653
B	Lc6 1	HVH-8	16776412	13515828	7413409	659767	6479918	486505
A	Pi6 2	HVH-9	15299139	10899341	9937813	2104	8910214	1755
B	Pi6 2	HVH-9	15322381	10909249	9944902	2070	8904651	1698
A	Lc6 2	HVH-10	16700006	12305664	6613144	654159	5928603	518502
B	Lc6 2	HVH-10	16631059	12251071	6579151	654802	5893253	518794
A	Pi6 3	HVH-11	15689381	11719026	10733258	2637	9798815	2232
B	Pi6 3	HVH-11	15681665	11708005	10713723	2549	9776052	2133
A	Lc6 3	HVH-12	21233821	16093441	7969451	876508	7270533	714032
B	Lc6 3	HVH-12	21180268	16047872	7944623	880087	7243427	716219
A	Pi24 1	HVH-13	16674094	11265921	10127131	39130	9017260	28275
B	Pi24 1	HVH-13	16695943	11262286	10118787	39232	8999322	28405
A	Lc24 1	HVH-14	18924851	13703674	2832692	7030668	2413436	5327811
B	Lc24 1	HVH-14	19030140	13777252	2844542	7085462	2422040	5364933
A	Pi24 2	HVH-15	17980880	12174975	10978851	19625	9657028	12763
B	Pi24 2	HVH-15	17913012	12116869	10937924	18877	9611882	12386
A	Lc24 2	HVH-16	18126777	13135033	3357214	5903207	2894355	4406492
B	Lc24 2	HVH-16	18205302	13179018	3361607	5936917	2896089	4427192
A	Pi24 3	HVH-17	15376607	11025383	9974286	22625	9546655	17163
B	Pi24 3	HVH-17	15333370	10981236	9937477	22683	9498974	17154
A	Lc24 3	HVH-18	25326749	19212386	4227903	10046156	3693230	8257525
B	Lc24 3	HVH-18	25366231	19235173	4222952	10085971	3678980	8281077

¹ Technical replicates (lane A and B) were summed up together before the downstream analysis.

Pic 1, Pic 2, Pic 3: 3 biological replicates with *Phytophthora infestans* alone

Lcc 1, Lcc 2, Lcc 3: biological replicates with *Lysobacter capsici* AZ78 alone

Pi6 1, Pi6 2, Pi6 3, Lc6 1, Lc6 2, Lc6 3: 3 biological replicates of *Phytophthora infestans* and *Lysobacter capsici* AZ78 after 6 h of interaction

Pi24 1, Pi24 2, Pi24 3, Lc24 1, Lc24 2, Lc24 3: 3 biological replicates of *Phytophthora infestans* and *Lysobacter capsici* AZ78 after 24 h of interaction

In Additional Files:

Table S2. List of differentially expressed genes of *Lysobacter capsici* AZ78 after 6 h of interaction with *Phytophthora infestans*.

Table S3. List of differentially expressed genes of *Lysobacter capsici* AZ78 after 24 h of interaction with *Phytophthora infestans*.

Table S4. List of differentially expressed genes of *Phytophthora infestans* interacting with *Lysobacter capsici* AZ78 for 6 h and 24 h.

Table S5. Over-represented gene ontology (GO) categories after enrichment analysis of *Lysobacter capsici* AZ78 differentially expressed genes (DEGs) after 24 hours of interaction with *Phytophthora infestans*. DEGs of *L. capsici* AZ78 after 24 h interaction (Lc24) with the oomycete were distinguished in up-regulated and down-regulated genes. The total genes in category represent the total number of genes grouped in each specific category in the annotated genome of AZ78.

Group	GO category	Term	Over-represented P value	DEGs in category	Total genes in category
Lc24 up-regulated genes	GO:0007165	signal transduction	1.98E-09	28	72
	GO:0005975	carbohydrate metabolic process	3.13E-07	20	65
	GO:0006810	transport	5.91E-07	31	126
	GO:0006032	chitin catabolic process	2.63E-03	3	4
	GO:0034194	D-galactonate catabolic process	4.57E-03	2	2
	GO:0016998	cell wall macromolecule catabolic process	4.92E-03	3	5
	GO:0006012	galactose metabolic process	5.57E-03	4	10
	GO:0019344	cysteine biosynthetic process	7.18E-03	2	2
	GO:0015757	galactose transport	7.67E-03	2	2
	GO:0015758	glucose transport	7.67E-03	2	2
	GO:0006950	response to stress	1.26E-02	3	9
	GO:0000103	sulfate assimilation	1.38E-02	2	2
	GO:0009297	pilus assembly	1.42E-02	2	2
	GO:0009098	leucine biosynthetic process	3.56E-02	4	14
	GO:0009099	valine biosynthetic process	3.56E-02	4	14
	GO:0006027	glycosaminoglycan catabolic process	4.34E-02	2	3
	Lc24 down-regulated genes	GO:0019464	glycine decarboxylation via glycine cleavage system	4.51E-02	1
GO:0006163		purine nucleotide metabolic process	4.88E-02	1	1
GO:0009143		nucleoside triphosphate catabolic process	4.88E-02	1	1
GO:0071973		bacterial-type flagellum-dependent cell motility	3.65E-06	5	8
GO:0042710		biofilm formation	5.01E-04	3	5
GO:0044780		bacterial-type flagellum assembly	1.42E-03	2	2
GO:0006260		DNA replication	1.15E-02	4	24
GO:0044781		bacterial-type flagellum organization	1.32E-02	2	5
GO:0006281		DNA repair	1.47E-02	5	39
GO:0006303		double-strand break repair via non-homologous end joining	3.79E-02	1	1
GO:0051276	chromosome organization	3.79E-02	1	1	

Table S6. Differentially expressed genes of *Lysobacter capsici* AZ78 related to the antagonism activity against *Phytophthora infestans* after 24 hours interaction. The table reported the characteristic DEGs expressed in *L. capsici* AZ78 after 24 h interaction (Lc24) with *P. infestans* and the relative expression levels, expressed as log₂ Fold Changes (log₂FC) for each gene.

Antagonism activity	Gene ID	NCBI gene name	Blast2GO description	Lc24 log ₂ FC
Chitin hydrolysis	AZ78_0055	Secreted chitinase [<i>Lysobacter capsici</i> AZ78]	Chitinase	4.5
	AZ78_1827	Chitinase [<i>Lysobacter capsici</i> AZ78]	Chitinase	4.4
	AZ78_3864	Chitinase [<i>Lysobacter capsici</i> AZ78]	Chitinase	2.7
	AZ78_0407	endo-1,3-beta-glucanase precursor [<i>Lysobacter capsici</i> AZ78]	---NA---	5.5
Glucan & cellulose hydrolysis	AZ78_1531	Endo-1,4-beta-xylanase A precursor [<i>Lysobacter capsici</i> AZ78]	Chitosanase	4.8
	AZ78_1535	putative exported glycosyl hydrolase family 31 protein [<i>Lysobacter capsici</i> AZ78]	Concanavalin A-like lectin glucanases superfamily	2.3
	AZ78_1556	Pectate lyase [<i>Lysobacter capsici</i> AZ78]	Endoglucanase	2.6
	AZ78_1567	Pectate lyase [<i>Lysobacter capsici</i> AZ78]	Beta-1,3-glucanase	3.6
	AZ78_3679	Endo-1,4-beta-xylanase A precursor [<i>Lysobacter capsici</i> AZ78]	Glycosyl hydrolase family 16	5.8
	AZ78_3685	hypothetical protein AZ78_3685 [<i>Lysobacter capsici</i> AZ78]	Glycosyl hydrolase family 5	4.0
	AZ78_4012	Endoglucanase Y [<i>Lysobacter capsici</i> AZ78]	Glycosyl hydrolase family 16	4.1
	AZ78_4325	LamG domain protein jellyroll fold domain protein [<i>Lysobacter capsici</i> AZ78]	Glycosyl hydrolase	5.5
	AZ78_4360	Endoglucanase [<i>Lysobacter capsici</i> AZ78]	Pectate lyase	2.4
	AZ78_4728	putative secreted sugar hydrolase [<i>Lysobacter capsici</i> AZ78]	Pectate lyase	5.1
Protein hydrolysis	AZ78_0051	interphotoreceptor retinoid-binding protein [<i>Lysobacter capsici</i> AZ78]	Peptidase S41 family	2.0
	AZ78_0061	peptidase S8 and S53, subtilisin, kexin, sedolisin [<i>Lysobacter capsici</i> AZ78]	Subtilase family	4.4
	AZ78_0270	Vibriolysin, extracellular zinc protease [<i>Lysobacter capsici</i> AZ78]	Peptidase M4	6.7
	AZ78_0272	Vibriolysin, extracellular zinc protease [<i>Lysobacter capsici</i> AZ78]	Peptidase M4	6.3
	AZ78_0415	Lysyl endopeptidase [<i>Lysobacter capsici</i> AZ78]	---NA---	3.2
	AZ78_0956	Extracellular protease precursor [<i>Lysobacter capsici</i> AZ78]	Protease	3.5
	AZ78_1931	peptidyl-Asp metalloendopeptidase [<i>Lysobacter capsici</i> AZ78]	Peptidyl-Asp metalloendopeptidase	3.2
	AZ78_2137	LasA protease precursor [<i>Lysobacter capsici</i> AZ78]	Beta-lytic metalloendopeptidase	4.5
	AZ78_2802	extracellular protease [<i>Lysobacter capsici</i> AZ78]	Peptidase S8	6.0
	AZ78_3091	putative neutral zinc metalloprotease [<i>Lysobacter capsici</i> AZ78]	Thermolysin metallopeptidase	2.1
	AZ78_3093	putative neutral zinc metalloprotease [<i>Lysobacter capsici</i> AZ78]	Peptidase M4	2.7
	AZ78_3312	putative secreted protein [<i>Lysobacter capsici</i> AZ78]	igA Peptidase M64 family	4.4
	AZ78_3414	Cold-active serine alkaline protease [<i>Lysobacter capsici</i> AZ78]	Trypsin-like peptidase domain	5.3

AZ78_4463	S1D (lysyl endopeptidase) subfamily C-terminal domain protein [<i>Lysobacter capsici</i> AZ78]	Lysyl endopeptidase	2.1
AZ78_4497	extracellular protease [<i>Lysobacter capsici</i> AZ78]	Peptidase S8	2.6
AZ78_4515	peptidyl-Asp metalloendopeptidase [<i>Lysobacter capsici</i> AZ78]	Peptidyl-Asp metalloendopeptidase	2.1
AZ78_4517	Extracellular zinc protease [<i>Lysobacter capsici</i> AZ78]	Leupeptin-inactivating enzyme 1	3.2
AZ78_4519	Extracellular zinc protease [<i>Lysobacter capsici</i> AZ78]	Peptidase M14	4.0
AZ78_4520	Extracellular zinc protease [<i>Lysobacter capsici</i> AZ78]	Peptidase M14	2.5
AZ78_4522	Bacterial leucyl aminopeptidase [<i>Lysobacter capsici</i> AZ78]	Peptidase M20 M25 M40 family	5.0
AZ78_4791	putative neutral zinc metalloprotease [<i>Lysobacter capsici</i> AZ78]	Thermolysin metalloprotease	3.5
AZ78_4903	Streptogrisin-C precursor (Serine protease C) (SGPC) [<i>Lysobacter capsici</i> AZ78]	Alpha-lytic protease	5.3
AZ78_4905	Streptogrisin-C precursor (Serine protease C) (SGPC) [<i>Lysobacter capsici</i> AZ78]	Alpha-lytic protease	2.6
AZ78_4905	Serine protease, subtilase family [<i>Lysobacter capsici</i> AZ78]	Protease domain-containing	3.4
AZ78_1098	hybrid non-ribosomal peptide synthase/polyketide synthase [<i>Lysobacter capsici</i> AZ78]	hybrid non-ribosomal peptide synthase polyketide synthase	3.1
AZ78_1099	Carotenoid cis-trans isomerase [<i>Lysobacter capsici</i> AZ78]	phytoene dehydrogenase	2.8
AZ78_1100	Carotenoid cis-trans isomerase [<i>Lysobacter capsici</i> AZ78]	phytoene dehydrogenase	2.7
AZ78_1101	Carotenoid cis-trans isomerase [<i>Lysobacter capsici</i> AZ78]	phytoene dehydrogenase	2.3
AZ78_2411	Lanthionine biosynthesis protein LanM [<i>Lysobacter capsici</i> AZ78]	lanthionine synthetase	2.1
AZ78_3544	Siderophore biosynthesis non-ribosomal peptide synthetase module [<i>Lysobacter capsici</i> AZ78]	non-ribosomal peptide synthase	4.8
AZ78_5069	3-oxoacyl-[acyl-carrier-protein] synthase, KASIII [<i>Lysobacter capsici</i> AZ78]	3-oxoacyl-ACP synthase	2.5

Table S7. Comparison of RNA-Seq and qRT-PCR results of *Lysobacter capsici* AZ78. The relative expression levels, expressed as log₂ Fold Changes (log₂FC) coming from RNA-Seq and qRT-PCR, are reported for each selected genes of *L. capsici* AZ78 challenged with *Phytophthora infestans* for 6 h (Lc6) and 24 h (Lc24.)

Gene ID	Gene name	RNA-Seq		qRT-PCR	
		Lc6*	Lc24	Lc6*	Lc24
AZ78_0055	Secreted chitinase	0.8	4.5	4.0±0.5	6.8±0.0
AZ78_0270	Vibriolysin, extracellular zinc protease	3.1	6.7	4.2±0.1	7.6±0.1
AZ78_1098	hybrid non-ribosomal peptide synthase/polyketide synthase	2.3	3.1	3.0±0.1	5.0±0.0
AZ78_1116	Catalase	1.1	2.2	2.5±0.1	3.6±0.0
AZ78_1457	Type IV fimbrial biogenesis protein PilY1	2.9	4.1	4.2±0.0	5.4±0.0
AZ78_2049	Type IV pilus biogenesis protein PilQ	1.4	2.3	2.5±0.1	3.2±0.0
AZ78_3679	Endo-1,4-beta-xylanase A precursor	2.1	5.8	2.9±0.3	5.7±0.1
AZ78_3685	hypothetical protein AZ78_3685	2.5	4.0	3.2±0.1	4.6±0.1
AZ78_4012	Endoglucanase Y	0.9	4.1	3.1±0.5	4.5±0.0
AZ78_4111	Cyclic AMP receptor protein, clp	0.8	1.3	1.8±0.1	2.7±0.1
AZ78_4519	Extracellular zinc protease	2.0	4.0	3.7±0.0	5.6±0.0

*Low sequencing depth of AZ78 reads at the early interaction stage (6 h).

Table S8. Over-represented gene ontology (GO) categories after enrichment analysis of *Phytophthora infestans* differentially expressed genes (DEGs) during the interaction with *Lysobacter capsici* AZ78. The DEGs lists of *P. infestans* (Pi), interacting with *L. capsici* AZ78 for 6 and 24 hours, were splitted in six groups: G1, down-regulated DEGs at 6 h with positive trend at 24 h; G2, down-regulated DEGs at 6 h with negative trend at 24 h; G3, not DEGs at 6 h with negative trend at 24 h; G4, not DEGs at 6 h with positive trend at 24 h; G5, up-regulated DEGs at 6 h with positive trend at 24 h; G6, up-regulated DEGs at 6 h with negative trend at 24 h. The total genes in category represent the total number of genes grouped in each specific category in the annotated genome of Pi.

Group	GO category	Term	Over-represented P value	DEGs in category	Total genes in category
G1	GO:0006471	protein ADP-ribosylation	1.87E-05	3	7
	GO:0015992	proton transport	1.01E-04	5	46
	GO:0071526	semaphorin-plexin signaling pathway	4.69E-04	3	4
	GO:0016567	protein ubiquitination	7.88E-04	6	47
G2	GO:0046323	glucose import	1.06E-03	3	20
	GO:1904659	glucose transmembrane transport	1.06E-03	3	20
	GO:0098655	cation transmembrane transport	1.53E-03	4	36
	GO:0055085	transmembrane transport	2.79E-03	12	344
	GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	3.09E-03	4	6
	GO:0003333	amino acid transmembrane transport	3.11E-03	4	60
	GO:0008152	metabolic process	3.11E-03	27	987
	GO:0071577	zinc II ion transmembrane transport	5.32E-05	6	31
	GO:0006164	purine nucleotide biosynthetic process	1.86E-04	3	5
	GO:0000011	vacuole inheritance	5.22E-04	12	150
G3	GO:0034058	endosomal vesicle fusion	5.22E-04	12	150
	GO:0006895	Golgi to endosome transport	5.43E-04	12	151
	GO:0006896	Golgi to vacuole transport	5.82E-04	12	151
	GO:0045324	late endosome to vacuole transport	7.32E-04	12	153
	GO:0018026	peptidyl-lysine monomethylation	8.84E-04	3	8
	GO:0006897	endocytosis	1.06E-03	12	165
	GO:0071555	cell wall organization	1.45E-03	4	24
	GO:0006892	post-Golgi vesicle-mediated transport	1.83E-03	2	3
	GO:0006177	GMP biosynthetic process	1.98E-03	2	3
	GO:0006950	response to stress	2.70E-03	2	11
		maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	3.62E-03	4	20
	GO:0010506	regulation of autophagy	4.64E-03	2	5

	GO:0005975	carbohydrate metabolic process	4.71E-03	11	160
	GO:0005985	sucrose metabolic process	4.72E-03	9	106
	GO:0010951	negative regulation of endopeptidase activity	5.13E-03	2	8
	GO:0005982	starch metabolic process	5.58E-03	9	109
	GO:0000059	protein import into nucleus, docking	8.29E-03	3	8
	GO:0007155	cell adhesion	9.14E-03	3	14
	GO:0035435	phosphate ion transmembrane transport	3.24E-06	3	3
	GO:0044341	sodium-dependent phosphate transport	3.24E-06	3	3
G4	GO:0042981	regulation of apoptotic process	1.59E-03	3	17
	GO:0048731	system development	2.34E-03	2	6
	GO:0006073	cellular glucan metabolic process	6.90E-03	2	9
G5	GO:0009072	aromatic amino acid family metabolic process	5.23E-03	1	2
G6	GO:0016485	protein processing	1.77E-03	1	20

Table S9. Relevant differentially expressed genes of *Phytophthora infestans* interacting with *Lyso bacter capsici* AZ78. The relative expression levels, expressed as log₂ Fold Changes (log₂FC) coming from RNA-Seq are reported for some selected genes of *P. infestans* challenged with *L. capsici* AZ78 for 6 h (Pi6) and 24 h (Pi24).

Gene ID	NCBI gene name	Blast2GO description	Pi6 log ₂ FC	Pi24 log ₂ FC	Group
PITG_03546	translational activator GCN1, putative [<i>Phytophthora infestans</i> T30-4]	translational activator GCN1	-1.3	-2.2	G3
PITG_05367	hsp70-like protein [<i>Phytophthora infestans</i> T30-4]	heat shock 90-2	-2.2	-2.7	G4
PITG_06415	heat shock protein 90 [<i>Phytophthora infestans</i> T30-4]	conserved hypothetical protein	-1.6	-2.1	G3
PITG_07328	manganese superoxide dismutase [<i>Phytophthora infestans</i> T30-4]	---NA---	-0.6	-2.2	G4
PITG_08902	pleiotropic drug resistance protein, putative [<i>Phytophthora infestans</i> T30-4]	pleiotropic drug resistance	1.0	3.0	G4
PITG_09169	protease inhibitor EpiC1 [<i>Phytophthora infestans</i> T30-4]	extracellular cystatin-like protease inhibitor	-1.4	-5.6	G3
PITG_09173	protease inhibitor EpiC2B [<i>Phytophthora infestans</i> T30-4]	extracellular cystatin-like protease inhibitor	-0.4	-2.6	G3
PITG_12989	glutathione S-transferase theta, putative [<i>Phytophthora infestans</i> T30-4]	Glutathione S-transferase T1	0.8	2.1	G4
PITG_22940	Kazal-like protease inhibitor [<i>Phytophthora infestans</i> T30-4]	Kazal-like protease inhibitor	2.0	3.5	G4
PITG_22942	Kazal-like protease inhibitor [<i>Phytophthora infestans</i> T30-4]	Kazal-like protease inhibitor	1.3	3.1	G4
PITG_23012	Kazal-like protease inhibitor [<i>Phytophthora infestans</i> T30-4]	Kazal-like protease inhibitor	1.2	2.7	G4
PITG_23147	Kazal-like protease inhibitor [<i>Phytophthora infestans</i> T30-4]	Kazal-like protease inhibitor	2.1	4.1	G5
PITG_00392	serine protease family S33, putative [<i>Phytophthora infestans</i> T30-4]	serine protease family S33	0.5	2.1	G4
PITG_00393	conserved hypothetical protein [<i>Phytophthora infestans</i> T30-4]	serine protease family S33	1.1	2.8	G4
PITG_11607	metalloprotease family M48X, putative [<i>Phytophthora infestans</i> T30-4]	---NA---	1.2	2.0	G4
PITG_11807	metalloprotease family M48X, putative [<i>Phytophthora infestans</i> T30-4]	serine protease	1.5	2.6	G4
PITG_22022	cysteine protease family C01A, putative [<i>Phytophthora infestans</i> T30-4]	cysteine protease family C01A	1.5	2.9	G4
PITG_00124	ser/thr kinase [<i>Phytophthora infestans</i> T30-4]	---NA---	1.4	2.2	G4
PITG_03205	pro-apoptotic serine protease, putative [<i>Phytophthora</i>]	pro-apoptotic serine protease	-2.6	-1.7	G1

PITG_00700	<i>infestans</i> T30-4] hypothetical protein PITG_00700 [<i>Phytophthora infestans</i> T30-4]	mma111 O(6)-methylguanine-induced apoptosis 2	1.1	2.5	G4
PITG_15917	mitogen-activated protein kinase kinase, putative [<i>Phytophthora infestans</i> T30-4]	serine threonine kinase	1.8	4.4	G4

Table S10. Comparison of RNA-Seq and qRT-PCR results of *Phytophthora infestans*. The relative expression levels, expressed as log₂ Fold Changes (log₂FC) coming from RNA-Seq and qRT-PCR, are reported for each selected genes of *P. infestans* challenged with *Lysobacter capsici* AZ78 for 6 h (Pi6) and 24 h (Pi24).

Gene	Gene name	RNA-Seq Log ₂ FC			qRT-PCR Log ₂ FC		
		Pi6	Pi24	Pi6	Pi24	Pi6	Pi24
PITG_00700	O(6)-methylguanine-induced apoptosis 2	1.1	2.5	0.9±0.2	2.6±0.3		
PITG_04748	mitochondrial-processing peptidase subunit alpha	2.4	1.1	1.6±0.5	1.2±0.1		
PITG_05380	maleylacetoacetate isomerase	2.1	3.8	1.9±0.3	3.1±0.3		
PITG_07661	Major Facilitator Superfamily	-3.1	-2.9	-2.1±0.2	-2.3±0.2		
PITG_07711	Major Facilitator Superfamily	-2.3	-2.9	-0.7±0.1	-1.2±0.1		
PITG_09173	protease inhibitor EpiC2B	-0.4	-2.6	-0.6±0.2	-2.9±0.1		
PITG_15917	mitogen-activated protein kinase kinase, putative	1.8	4.4	2.4±0.4	4.8±0.3		
PITG_20857	secreted RxLR effector peptide protein	2.2	4.4	3.4±0.2	5.5±0.1		
PITG_23147	Kazal-like protease inhibitor	2.1	4.1	1.5±0.0	2.8±0.1		

Table S11. Primers used in quantitative real-time polymerase chain reaction.

Gene ID	Primer Sequence (5'-3')	Amplicon Size (bp)	
AZ78_1089	GAGCCAGATCGACAAGCAAT GGACCGTAGATCTCGACCAC	159	Tomada <i>et al.</i> , 2016
AZ78_0055	GAATTGAACCAGGCCAACTG GCCGAGGCGTAGTTGTAGTT	116	This study
AZ78_0270	ACTACTTCGGCAAGGTCGTG AACGCGTTCTCGTAGTTGCT	112	This study
AZ78_1098	TACAACATTCTGCCGCTGAC GTGTAGGCGAGGTCCTTCAG	122	This study
AZ78_1116	TCAAACACCGAATCGAAGTG CCTTGAGGTTGAGCTGGTTC	91	This study
AZ78_1457	CAGTACAACAAGGCGATCCA CGCATCTTCTCGGTCTTTTC	113	Tomada <i>et al.</i> , 2016
AZ78_2049	GAAGCTGGGCAGAACGAAAA GCTCCTTCATCTGGGTCACT	118	Tomada <i>et al.</i> , 2016
AZ78_3679	CGACTTCTTCTTGCTGCTGA GTAGTCCACGTGCATCTTCG	97	This study
AZ78_3685	CACATCGTCATCATCGAAGG GGTGCCGTTCCAGTATTTGT	114	This study
AZ78_4012	CTCGACAAGCTGTGGACCTA CTTGAGCCAGTTGTTGGACA	105	This study
AZ78_4111	ACTCGGCCTATTCATCGAAA GTCAGCAGCAGGTCGTACAG	108	Tomada <i>et al.</i> , 2016
AZ78_4519	ACGACACGATGTACGGTCTG CGCAGGGTCTTGAGATTGTT	121	This study
PITG_09284	CATCAAGGAGAAGCTGACGTACA GACGACTCGGCGGCAG	69	Anvrora <i>et al.</i> , 2003
PITG_00700	AAAGATCGTGACGGATGGAG CCCTCGACGTTTTCGACTTA	116	This study
PITG_04748	ATGATGCTGCGGTAAGTCGT CAGCTTGTCATCACTGGTC	89	This study
PITG_05380	GTGGGTGACGAAGTGAGCTT CCCACGATACGTGAGATCG	110	This study
PITG_07661	GCGTACGTGGACAGCATCTA TGCCGAACAACTGTAGTGG	124	This study
PITG_07711	AGTGTGGGTGGTTGGTTCAT CACACCCAATGGGAAAATCT	114	This study
PITG_09173	GTCTCGGGCGAGAACTACAA CCACGACTGCGAGTAGATGA	129	This study
PITG_15917	CGCAATTGACGCTGAAGATA GTTTCGAGCCCTGTGACCTT	107	This study
PITG_20857	AAAAGCGGTAAAGCCCTCAT TTCGTCTCTGGGTAGCTGT	80	This study
PITG_23147	TCCAGTGTGTGGATCGAATG GTTTCAGGCCAGCGTTGTT	82	This study

CHAPTER 5

Discussion

The *L. capsici* AZ78 is a promising microbial biocontrol agent that could be a suitable active substance for novel biofungicides, due to its effective control of phytopathogenic oomycetes, such as *P. infestans* and *P. viticola* (Puopolo et al., 2014a,b; Segarra et al., 2015). The scientific interest on the *Lysobacter* genus is increasing as demonstrated by the large amount of published studies on specific traits of its biocontrol activity, such as lytic enzymes or antibiotics production (Hayward et al., 2010; Xie et al., 2012). However, the positive results obtained with *Lysobacter* members in small-scale experiments are not always confirmed under practical conditions. The inconsistent efficacy under field condition is often due to the wrong application of the microorganism, due to the lack of information on the effect of limiting environmental factors (Folman et al., 2004). In addition the scientific data available on the biological and physiological traits of *Lysobacter* members are insufficient to complete the registration dossier for the use as plant protection product of *L. capsici* AZ78. Indeed, the current EU legislation requires an extensive characterization of the bacterial strain. For these two reasons, the thesis focused on the specific characterization of *L. capsici* AZ78.

The first part of the study was focused on *L. capsici* AZ78's genome sequencing and mining. The genome of *L. capsici* AZ78 was compared with two phylogenetically close related pathogens, as *S. malthophilia* K729a and *X. campestris* pv. *campestris* ATCC 33913. The results showed the absence in *L. capsici* AZ78 of genetic bases for virulence to animals and plants, such as *smlt4452* encoding a hemolysin protein of *S. malthophilia* K729a or the *avr* genes of *X. campestris* involved in gene-for-gene resistance (Crossman et al., 2008; da Silva et al., 2002). This result is crucial to exclude the pathogenicity of *L. capsici* AZ78, which is an important criterium for the registration of this biocontrol bacterium as plant protection product.

In addition, the genome analysis provided a picture of the potential biocontrol mechanisms displayed by *L. capsici* AZ78 against phytopathogenic bacteria, fungi and oomycete and in particular showed the presence of a pool of genes encoding external appendages, extracellular lytic enzymes and secondary metabolites with antibacterial and antifungal activity.

The bacterial motility mechanisms displayed by *L. capsici* AZ78 were investigated and the complete set of gene encoding type 4 pilus (T4P), mainly involved in bacterial twitching motility (Barrows, 2012), was identified. The RNA-Seq data analysis revealed the up-regulation of T4P genes from the early stage of *L. capsici* AZ78-*P. infestans*

interaction, suggesting the colonization and parasitism of the oomycete mycelium (Patel et al., 2011). Moreover, the expression of T4P genes on a specific growth medium composed with pea broth (PB), suggested the possible combined application of *L. capsici* AZ78 amended with PB to increase the bacterium biocontrol activity. Indeed, the data achieved by *in vivo* trials highlighted the positive effect of PB on *L. capsici* AZ78 efficacy against grapevine downy mildew and leaf colonization. The results coming from the characterization of *L. capsici* AZ78 motility mechanism and related piliation factor (PB) enlighten the important role of nutrient compounds in the formulation of new microbial-based biofungicides, which can improve the efficacy of the microbial active substance related to a better hosts colonization (Tomada et al., 2016). In conclusion, this part of the study provided new insights to implement the applicative use in field of *L. capsici* AZ78.

The genome of *L. capsici* AZ78 let to hypothesize a high proteolytic ability due to the presence of seventy-nine genes encoding proteases, between these genes *Lysobacter*-specific proteolytic regions were found (e.g metalloendopeptidase region AZ78_269-272). Subsequently, the *in vitro* assays confirmed the proteolytic activity of *L. capsici* AZ78. Moreover, an increasing proteolytic activity was observed when *L. capsici* AZ78 was co-cultured with *P. infestans*, suggesting a strong lytic activity mediated by the bacterium during the biocontrol interaction (Puopolo et al., 2016). Indeed, the RNA-Seq analysis allowed identifying up-regulation of 24 genes encoding extracellular proteases during *L. capsici* AZ78-*P. infestans* interaction. The RNA-Seq data confirmed the hypothesis of a *L. capsici* AZ78's biocontrol activity mediated by proteolysis, which emerged as one of the main biocontrol mechanisms carried out by *L. capsici* AZ78. Thus, this bacterial strain could be the new object of study as a source of enzymes suitable for the control of human pathogens through the degradation of microorganism extracellular matrix, as in the case of *S. epidermidis* biofilm digestion by *L. gummosus* DSMZ 6980 (Gökçen et al., 2014). Inside the broad range of lytic enzyme produced by *L. capsici* AZ78 the *in vitro* experiments showed the production of gluconolytic and cellulolytic enzymes. Indeed the bacterial genome encompassed specific genes related to these lytic activities, such as *gluA*, *gluB* and *gluC* previously described in *L. enzymogenes* C3 (Palumbo et al., 2005) or a homologue of *celA* of *Myxococcus xanthus*, species phylogenetically distant from *Xanthomonadaceae* (Puopolo et al., 2016). Moreover, the RNA-Seq data analysis showed the up-regulation of 11 genes related to β -1,3 glucanase and β -1,4 glucanase production during the *L. capsici* AZ78-*P. infestans*

interaction. These results support the hypothesis that the optimal target-site of *L. capsici* AZ78 can be the cell wall of phytopathogenic oomycete, which includes glucan compounds (Mélida et al., 2013). The genome of *L. capsici* AZ78 has genes related to chitinolytic activity, such as *chiB* a novelty for the *Xanthomonadaceae* family (Puopolo et al., 2016). Moreover, the expression of three genes encoding chitinases were observed in *L. capsici* AZ78 interacting with *P. infestans*, although the *Phytophthora* spp. cell wall does not encompass this polymer (Mélida et al., 2013). This observation suggested the activation in *L. capsici* AZ78 of a non-specific mechanism of action during the biocontrol interaction with *P. infestans*. Indeed, the production of chitinases could be a major mechanism of action in the control of phytopathogenic fungi and nematodes (Zhang et al., 2001; Lee et al., 2014b). The lytic activity of *L. capsici* AZ78 was supported by the antimicrobial and antifungal activity related to the production of antibiotics. Specifically, the genome mining allowed to identify a gene AZ78_1098, encoding an NPR-PKS compound, that shared 85% homology with the biosynthetic HASF gene of *L. enzymogenes* C3 (de Bruijn et al., 2015). The RNA-Seq data analysis showed the up-regulation in *L. capsici* AZ78 of a pool of genes that may be involved in the biosynthesis of NRPS and lantibiotics active against fungi, oomycete and bacteria (Puopolo et al., 2016). The data on the production of lytic enzymes and secondary metabolites provided a clear picture of the specific mode of action displayed by *L. capsici* AZ78. Moreover, this information is useful to complete the biological assessment dossier of *L. capsici* AZ78 and is a valuable tool to identify potential risks related to secondary metabolites production. However, further studies will be necessary to deep investigate the spectrum of antibiotics produced by *L. capsici* AZ78 and the related biochemical features (e.g. chemical structure and concentration), which shall be included in the registration dossier.

The environmental fitness of *L. capsici* AZ78 was evaluated. The capacity to compete for iron was investigated by genome mining and confirmed by *in vitro* experiments. Specifically, the biosynthesis of catechol siderophores through the expression of two gene clusters *feoABC* and *entAFBE-csbC*, shared with *Xanthomonadaceae* members and *Azotobacter vinelandii*, respectively (Pandey and Sonti, 2010; Setubal et al., 2009). Moreover, the *L. capsici* AZ78 genome includes a broad range of genes involved in resistance to environmental stress, such as UV light and reactive oxygen species (ROS) by the production of xanthomonadin (AZ78_3467-3472) and ROS detoxification enzymes. The up-regulation of genes encoding catalase and superoxide dismutase

enzymes were observed during the interaction with *P. infestans* in RNA-Seq experiment, suggesting a bacterial defence response to the oxidative rush activated by the oomycete activity. At the same time the transcription, under stress conditions, of genes encoding universal stress proteins that act to protect DNA from damages was observed in *L. capsici* AZ78. These data reinforced the biocontrol potential traits of *L. capsici* AZ78 and its capacity to effectively compete whit phytopathogens.

Moreover, the *L. capsici* AZ78 cell growth was assessed during *in vitro* experiments in the presence of antibiotics, heavy metals and plant protection products. The genomic basis related to tolerance of *L. capsici* AZ78 to these active compounds was found in the presence of genes encoding extrusion protein of ABC transporter family, Major facilitator superfamily, Resistance-Nodulation-Division and Small Multidrug Resistance protein. Some of the gene encoding efflux pump systems were up-regulated in the transcriptome of *L. capsici* AZ78 interacting with *P. infestans*, suggesting the implementation of detoxification mechanism to stem the oomycete response to the bacterial biocontrol activity. Additionally, *L. capsici* AZ78 is resistant to ampicillin, kanamycin, streptomycin and trobamycin due to the presence of genes encoding β -lactamases, streptomycin phosphotransferase/kinase and kanamycin nucleotidyltransferase (Puopolo et al., 2016). The achieved data of *L. capsici* AZ78 resistance to the main drugs used in human and veterinary medicine is an important information, which is mandatory to submit the registration dossier. Moreover, the compatibility of *L. capsici* AZ78 with a broad range of synthetic plant protection products and copper-based fungicides showed the ability of this bacterium to resist in the field-environment and the possibility of a direct application of *L. capsici* AZ78, as an active substance, in integrated pest management strategies. Particularly, the combination of *L. capsici* AZ78 whit copper based fungicides could be an interesting strategy to reduce the doses of this heavy metal in field, as previously explore by Puopolo et al. (2014a).

CHAPTER 6

Conclusions

The current study provides an accurate characterization of *L. capsici* AZ78 genome and its biocontrol activity, producing data useful for the registration process of this bacterial strain as a new active substance. Specifically, the sequencing and comparison of *L. capsici* AZ78 genome with two phylogenetically close pathogenic bacterial species have been proven that *L. capsici* AZ78 genome does not include information needed to establish pathogenic interactions with animals, humans and plants. These data could be included in the registration dossier together with the scheme of the *L. capsici* AZ78 mechanism of action.

A clear picture of the mechanism of action displayed by *L. capsici* AZ78 was obtained thanks to the genome mining and the subsequent deep investigation of *L. capsici* AZ78-*P. infestans* interaction. *Lysobacter capsici* AZ78 cells secreted bioactive compounds such as antibiotics and lytic enzymes, which are responsible for the lysis of fungal hyphae and the releasing of nutrient useful for the bacterial growth. Overall, the strategy implemented by *L. capsici* AZ78 could be ascribed to bacterial mycophagy.

These data could be the starting point for future studies on *L. capsici* AZ78 secondary metabolites, such as the isolation of its bioactive compounds and residues. The isolated compounds could be tested in toxicological tests on mammals, fishes and bees (exposure assessment, sensitisation potential, etc.) or environmental fate studies required during the registration process. Moreover, the pool of *L. capsici* AZ78 bioactive compounds could be a source of active molecules useful in medicine or biotechnology.

This study also provides new insights into the applicative perspectives of *L. capsici* AZ78 for the control of phytopathogenic oomycetes. In particular, the role of the nutrient substance Pea Broth (PB), a possible coformulant, on the biocontrol efficacy of *L. capsici* AZ78 was explored. The data showed that PB could improve the colonization of plant tissues and bacterial environmental fitness, two key aspects of a microorganism-based plant protection product. Moreover, the possibility to combine *L. capsici* AZ78 with commercial plant protection products, such as copper-based fungicides, reinforced the evidences of a successful persistence of the bacterium in the environment and its application in integrated pest management strategies. Thanks to the data produced in this study, it is possible to hypothesize the future development of this bacterial strain as an active substance against phytopathogenic (micro)organisms, such as fungi or nematodes, due to the multiple antagonism mechanisms that can be implemented during the interaction with phytopathogenic microorganisms

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Additional Files

List of differentially expressed genes of *Lyso bacter capsici* AZ78 after 6 h of interaction with *Phytophthora infestans*.

Sequence ID	NCBI Gene description	GenBank accession number	6 h log ₂ FC	6 h P Value	DEG at 6 hpi	Blast2GO Description	Blast2GO GO	Blast2GO Enzyme Codes list	Internal code	Internal code Legend
AZ78_0090	ribosomal subunit interface protein, putative [Lyso bacter capsici AZ78]	KW502546.1	-4.6	0.0008	-	ribosomal subunit interface	P:primary metabolic process	-	R	Unknown
AZ78_0097	L-lysine dehydrogenase [Lyso bacter capsici AZ78]	KW502553.1	-3.0	0.0005	-	L-lysine dehydrogenase	P:carbohydrate metabolic process; F:oxidoreductase activity, acting on the CH-OH group of donors, quinone or similar compound as acceptor; F:quinone binding; P:oxidation-reduction process	-	CM	Antagonism activity
AZ78_0177	hypothetical protein AZ78_0177 [Lyso bacter capsici AZ78]	KW502633.1	-3.0	5.93E-05	-	hypothetical protein	C:integral component of membrane	-	AM	Amino acid metabolism
AZ78_0178	hypothetical protein AZ78_0178 [Lyso bacter capsici AZ78]	KW502634.1	-3.2	0.0004	-	hypothetical protein	C:membrane; C:integral component of membrane	-	-	Biotin formation
AZ78_0183	hypothetical protein AZ78_0183 [Lyso bacter capsici AZ78]	KW502635.1	-2.7	4.47E-05	-	trans-1,6-bisphosphoglycerate [Lyso bacter capsici AZ78] pathway signal sequence domain	F:catalytic activity; P:metabolic process	-	CM	Carbohydrate metabolism
AZ78_0184	hypothetical protein AZ78_0184 [Lyso bacter capsici AZ78]	KW502640.1	-3.1	4.19E-05	-	hypothetical protein AZ78_0184	-	-	-	Defense (detoxification pumps)
AZ78_0186	tonB-dependent receptor [Lyso bacter capsici AZ78]	KW502642.1	2.5	1.68E-05	-	-dependent receptor	P:signal transduction; C:cell outer membrane; F:receptor activity; P:transport	-	S	DNA metabolism and
AZ78_0247	hypothetical protein AZ78_0247 [Lyso bacter capsici AZ78]	KW502703.1	-0.4	0.0001	-	hypothetical protein AZ78_0247	-	-	AG	Effectors associated to plant
AZ78_0270	Vibriovisin, extracellular zinc protease [Lyso bacter capsici AZ78]	KW502725.1	3.1	1.97E-05	-	proteolysis; F:serine-type endopeptidase activity; F:metalloendopeptidase activity	EC:3.4.21; EC:3.4.24	-	AG	EF
AZ78_0315	ABC transporter, permease protein, putative [Lyso bacter capsici AZ78]	KW502769.1	-2.3	1.51E-05	-	ABC transporter permease	C:integral component of membrane	-	G	Growth
AZ78_0359	putative exported protein [Lyso bacter capsici AZ78]	KW502847.1	-2.6	3.05E-06	-	outer membrane protein containing lipid membrane domain	-	-	T	Gene associated with plant
AZ78_0395	Alkaline serine protease [Lyso bacter capsici AZ78]	KW502849.1	2.1	1.20E-05	-	proteolysis; F:serine-type endopeptidase activity	EC:3.4.21	-	AG	Kinase/phosphatase
AZ78_0398	outer membrane protein [Lyso bacter capsici AZ78]	KW502852.1	-2.7	2.73E-06	-	cell envelope biogenesis	C:integral component of membrane; C:cell outer membrane	-	G	Lipid metabolism
AZ78_0401	Nucleoside diphosphate-sugar epimerase [Lyso bacter capsici AZ78]	KW502855.1	-3.1	2.02E-05	-	ATP-dependent DNA ligase clustered with Ku protein, LigD [Lyso bacter capsici AZ78]	F:oxidoreductase activity; C:integral component of membrane; P:oxidation-reduction process	-	AG	Motility
AZ78_0407	endo-1,3-beta-glucanase precursor [Lyso bacter capsici AZ78]	KW502861.1	2.6	9.01E-06	-	1,3-beta-glucanase	P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds	-	AG	Metabolism generic
AZ78_0427	SlyP-like protein [Lyso bacter capsici AZ78]	KW502881.1	2.3	0.000132497	-	taurine catabolism diacylglycerol	F:hydrolase activity; P:oxidation-reduction process	-	AM	Oxidative stress
AZ78_0501	Polysome synthetase I [Lyso bacter capsici AZ78]	KW502951.1	3.2	1.98E-06	-	polysome synthetase I	-	-	AG	Protein synthesis and
AZ78_0506	Cytochrome c ubiquinol oxidase subunit IV [Lyso bacter capsici AZ78]	KW502960.1	2.3	0.000715728	-	cytochrome c ubiquinol oxidase subunit IV	F:cytochrome c ubiquinol oxidase activity; P:oxygen electron transport; C:cytochrome c ubiquinol oxidase complex; P:electron transport coupled proton transport; P:oxidation-reduction process	EC:1.10.3	Met	Ribosomal processes
AZ78_0527	hypothetical protein AZ78_0527 [Lyso bacter capsici AZ78]	KW502981.1	4.4	2.03E-05	-	hypothetical protein	-	-	Met	RNA synthesis and
AZ78_0544	putative signal peptide protein [Lyso bacter capsici AZ78]	KW502986.1	-3.0	6.07E-05	-	hypolase	-	-	AG	Signal transduction and
AZ78_0545	Transcriptional regulator, AraC family [Lyso bacter capsici AZ78]	KW502999.1	-2.2	5.47E-05	-	family transcriptional regulator	P:metabolic process; F:hydrolase activity	-	-	RNA/TR
AZ78_0553	Mg2+ transport ATPase protein C [Lyso bacter capsici AZ78]	KW503007.1	-4.4	1.10E-05	-	transporter	C:integral component of membrane	-	-	-
AZ78_0554	ATP-dependent DNA ligase clustered with Ku protein, LigD [Lyso bacter capsici AZ78]	KW503008.1	-4.6	4.50E-07	-	DNA ligase	P:DNA metabolic process; F:ligase activity	-	-	DNA
AZ78_0555	ATP-dependent DNA ligase clustered with Ku protein, LigD [Lyso bacter capsici AZ78]	KW503009.1	-4.6	3.51E-07	-	DNA ligase	F:ATP binding; P:DNA repair; F:DNA ligase (ATP) activity; P:DNA replication; P:DNA recombination	EC:6.5.1; EC:6.5.1.1	DNA	Carbohydrate metabolism
AZ78_0556	Ku domain protein [Lyso bacter capsici AZ78]	KW503010.1	-4.1	1.32E-07	-	Ku	F:double-strand DNA binding; P:DNA recombination; F:helicase activity; F:double-strand break repair via nonhomologous end joining	EC:3.8.1; EC:3.6.1.15	DNA	-
AZ78_0557	putative signal peptide protein [Lyso bacter capsici AZ78]	KW503011.1	-4.2	6.26E-07	-	low affinity iron permease family	-	-	-	-
AZ78_0558	hypothetical protein AZ78_0558 [Lyso bacter capsici AZ78]	KW503012.1	-4.3	2.36E-06	-	hypothetical protein	-	-	-	-
AZ78_0565	hypothetical protein AZ78_0565 [Lyso bacter capsici AZ78]	KW503039.1	-4.4	2.03E-05	-	hypothetical protein	-	-	-	-
AZ78_0566	Outer membrane lipoprotein carrier protein LoaA [Lyso bacter capsici AZ78]	KW503040.1	-2.4	3.32E-05	-	outer membrane lipoprotein carrier	F:lipoprotein transfer activity; C:periplasmic space; P:lipoprotein transport	-	-	T
AZ78_0635	tonB-dependent receptor [Lyso bacter capsici AZ78]	KW503089.1	2.5	4.16E-05	-	tonB dependent receptor family	P:signal transduction; C:membrane; F:receptor activity; P:transport	-	S	-
AZ78_0636	hypothetical protein AZ78_0636 [Lyso bacter capsici AZ78]	KW503096.1	4.1	0.000818333	-	hypothetical protein AZ78_0636	-	-	-	-
AZ78_0637	beta-hemiamidase [Lyso bacter capsici AZ78]	KW503091.1	-2.7	6.44E-05	-	beta-hemiamidase	-	-	-	-
AZ78_0638	hypothetical protein AZ78_0638 [Lyso bacter capsici AZ78]	KW503092.1	3.3	0.000695344	-	hypothetical protein AZ78_0638	-	-	-	-
AZ78_0639	alpha-1,2-mannosidase [Lyso bacter capsici AZ78]	KW503093.1	3.3	1.42E-05	-	alpha-mannosidase	P:glycolytic metabolic process; P:carbohydrate metabolic process; P:amino sugar metabolic process; F:beta-N-acetylhexosaminidase activity; P:glycosaminoglycan catabolic process	EC:3.2.1.52; EC:3.2.1	CM	-
AZ78_0640	tonB-dependent receptor [Lyso bacter capsici AZ78]	KW503094.1	3.1	2.50E-07	-	hypothetical protein	-	-	-	-
AZ78_0655	hypothetical protein AZ78_0655 [Lyso bacter capsici AZ78]	KW503109.1	3.9	0.00149116	-	hypothetical protein AZ78_0655	-	-	-	-
AZ78_0717	ABC transporter involved in cytochrome c biogenesis, CcmB subunit [Lyso bacter capsici AZ78]	KW503171.1	5.9	0.000494116	-	-NA--	-	-	-	-
AZ78_0728	hypothetical protein AZ78_0728 [Lyso bacter capsici AZ78]	KW503182.1	-3.6	5.74E-06	-	MULTISPECIES: hypothetical protein	F:heme transporter activity; C:membrane; P:cytochrome complex assembly; P:heme transport	-	-	-
AZ78_0729	hypothetical protein AZ78_0729 [Lyso bacter capsici AZ78]	KW503183.1	-3.2	2.01E-05	-	hypothetical protein	-	-	-	-
AZ78_0780	hypothetical protein AZ78_0780 [Lyso bacter capsici AZ78]	KW503234.1	3.4	0.000317068	-	hypothetical protein	-	-	-	-
AZ78_0835	FerriC siderophore transport system, periplasmic binding protein TonB [Lyso bacter capsici AZ78]	KW503289.1	3.9	0.000216706	-	energy transducer	P:transport	-	-	-
AZ78_0849	Lanthionine biosynthesis protein LanL [Lyso bacter capsici AZ78]	KW503303.1	2.0	0.000724335	-	Membrane translocator	F:ATP binding; F:protein kinase activity; P:protein phosphorylation	-	AG	-
AZ78_0903	transglycosylase associated protein [Lyso bacter capsici AZ78]	KW503357.1	-5.2	0.000693941	-	transglycosylase associated protein	F:oxidoreductase activity; C:integral component of membrane	-	AM	Met
AZ78_0904	Pyridoxamine 5-phosphate oxidase [Lyso bacter capsici AZ78]	KW503358.1	-4.3	0.000334414	-	general stress	F:FMN binding; F:oxidoreductase activity; P:oxidation-reduction process	-	OX	-
AZ78_0905	hypothetical protein AZ78_0905 [Lyso bacter capsici AZ78]	KW503359.1	-3.2	9.61E-06	-	hemerythrin	-	-	-	-
AZ78_0951	Two-component hybrid sensor kinase [Lyso bacter capsici AZ78]	KW503375.1	-3.0	0.001788452	-	histidine kinase	F:phosphorylase sensor kinase activity; F:ATP binding; C:protein histidine kinase complex; P:phosphorelay signal transduction system; P:signal transduction by protein phosphorylation; C:intracellular	EC:2.7.3; EC:2.7.13.3	OX	-
AZ78_0954	hypothetical protein AZ78_0954 [Lyso bacter capsici AZ78]	KW503407.1	-3.0	5.64E-05	-	cytochrome C family	P:oxidoreductase activity; C:integral component of membrane	-	G	C:outer membrane
AZ78_0955	hypothetical protein AZ78_0955 [Lyso bacter capsici AZ78]	KW503408.1	-3.5	3.66E-05	-	putative iron-acyl-associated outer membrane lipoprotein precursor	P:oxidoreductase activity; C:integral component of membrane	-	Met	-
AZ78_0963	hypothetical protein AZ78_0963 [Lyso bacter capsici AZ78]	KW504161.1	3.4	0.000958572	-	hypothetical protein AZ78_0963	-	-	-	-
AZ78_0975	hypothetical protein AZ78_0975 [Lyso bacter capsici AZ78]	KW504249.1	4.4	2.03E-05	-	hypothetical protein	-	-	-	-
AZ78_1054	COS response repressor and repressor [Lyso bacter capsici AZ78]	KW504542.1	0.7	0.000391553	-	response repressor	F:DNA binding; P:DNA repair; P:negative regulation of transcription; DNA templated; P:DNA replication; Proteolysis; F:serine-type endopeptidase activity; P:SOS response	EC:3.4.21	RNA/TR	-
AZ78_1095	Arginase [Lyso bacter capsici AZ78]	KW505471.1	2.3	0.000766203	-	arginase	P:arginine catabolic process; F:metabolism	EC:3.5.3.1	AM	-
AZ78_1096	Ferredoxin-NAD(P)+ reductase [Lyso bacter capsici AZ78]	KW505488.1	2.3	0.000773547	-	ferredoxin-NAD(P)+ reductase	F:oxidoreductase activity; F:metal ion binding; P:oxidation-reduction process	-	Met	-
AZ78_1097	de novo desaturase [Lyso bacter capsici AZ78]	KW505489.1	3.6	0.000723381	-	C14-6-oxoacyl-ABC transporter	F:iron ion binding; F:oxidoreductase activity; C:integral component of membrane; P:oxidation-reduction process; P:atty acid biosynthetic process	-	AM	-
AZ78_1142	hypothetical protein AZ78_1142 [Lyso bacter capsici AZ78]	KW505941.1	-2.9	3.50E-05	-	MULTISPECIES: hypothetical protein	-	-	-	-
AZ78_1171	hypothetical protein AZ78_1171 [Lyso bacter capsici AZ78]	KW506263.1	2.9	0.000307061	-	hypothetical protein	-	-	-	-
AZ78_1189	Carboxypeptidase [Lyso bacter capsici AZ78]	KW506911.1	-2.0	0.000429077	-	carboxypeptidase	F:hydrolase activity; P:cell wall macromolecule catabolic process; P:cellin catabolic process	EC:3.2.1.14	AG	-
AZ78_1194	3'-5'-exoribonuclease RNase R [Lyso bacter capsici AZ78]	KW506461.1	-2.1	4.92E-06	-	ribonuclease R	F:exoribonuclease II activity; P:RNA phosphodiester bond hydrolysis, exonucleolytic; F:RNA binding; C:cytoplasm; P:regulation of RNA metabolic process	EC:3.1.1.1; EC:3.1.1.3; EC:3.1.15; EC:3.1.13.1	AG	RNA/TR
AZ78_1197	Metallo-beta-lactamase [Lyso bacter capsici AZ78]	KW506499.1	-2.5	6.40E-05	-	MBL fold metallo-hydrolase	-	-	-	D
AZ78_1200	Asparagine synthetase [Lyso bacter capsici AZ78]	KW506499.1	2.0	5.73E-05	-	asparagine synthetase II	F:asparagine biosynthetic process; P:aspartate metabolic process; F:asparagine synthase (glutamine-hydrolyzing) activity; P:alanine metabolic process	EC:6.3.5.4	AG	-
AZ78_1256	Putative alpha helix protein [Lyso bacter capsici AZ78]	KW507071.1	3.4	0.000613361	-	Y2605_XANCP_ane_Full-UPF007_KCC2605	-	-	-	RNA/TR
AZ78_1277	hypothetical protein AZ78_1277 [Lyso bacter capsici AZ78]	KW503728.1	3.9	0.00149116	-	hypothetical protein AZ78_1277	-	-	-	-
AZ78_1364	Cysteine betaane transporter CysB [Lyso bacter capsici AZ78]	KW503784.1	4.9	0.000376954	-	ABC transporter permease	C:integral component of membrane; P:transport; C:plasma membrane; F:transporter activity	-	-	RNA/TR
AZ78_1365	General secretion pathway protein C [Lyso bacter capsici AZ78]	KW503816.1	-2.5	0.00067026	-	dehydroquinase class II	F:3-dehydroquinate dehydrogenase activity; P:1-phenylalanine biosynthetic process; P:tryptophan biosynthetic process; P:tyrosine biosynthetic process	EC:4.2.1.10	AG	-
AZ78_1366	Metallo-beta-lactamase family protein [Lyso bacter capsici AZ78]	KW503817.1	-2.1	0.000329627	-	general secretion pathway	C:membrane; C:dehydrogenase component of membrane; F:protein transporter activity; P:protein secretion by the type II secretion system; C:type II protein secretion system complex	-	T	-
AZ78_1401	hypothetical protein AZ78_1401 [Lyso bacter capsici AZ78]	KW503852.1	-2.9	0.000333559	-	hypothetical protein	-	-	-	-
AZ78_1422	hypothetical protein AZ78_1422 [Lyso bacter capsici AZ78]	KW503873.1	-3.7	0.001783446	-	hypothetical protein	-	-	-	-
AZ78_1424	hypothetical protein AZ78_1424 [Lyso bacter capsici AZ78]	KW503875.1	-2.2	6.66E-05	-	hypothetical protein	-	-	-	-
AZ78_1432	HNH endonuclease family protein [Lyso bacter capsici AZ78]	KW503883.1	-2.3	3.85E-05	-	HNH endonuclease	F:nucleic acid binding; P:nucleic acid phosphodiester bond hydrolysis; F:endonuclease activity	EC:3.1	DNA	-
AZ78_1456	Type IV pilus biogenesis protein PHE [Lyso bacter capsici AZ78]	KW503907.1	-2.5	0.000303609	-	type IV pilus	C:integral component of membrane	-	M	-
AZ78_1457	Type IV fimbriae biogenesis protein FXY1 [Lyso bacter capsici AZ78]	KW503908.1	-2.9	2.29E-06	-	cellulose beta-propeller domain	-	-	-	M
AZ78_1458	Type IV fimbriae biogenesis protein PIX1 [Lyso bacter capsici AZ78]	KW503909.1	-3.0	3.73E-06	-	pilQ N-terminal family	C:membrane; C:integral component of membrane	-	M	-
AZ78_1459	Type IV fimbriae biogenesis protein PIW [Lyso bacter capsici AZ78]	KW503910.1	-2.5	3.39E-06	-	proton-type N-terminal cleavage methylation domain-containing	-	-	-	M
AZ78_1460	Type IV fimbriae biogenesis protein PIV [Lyso bacter capsici AZ78]	KW503911.1	2.2	1.76E-05	-	type IV pilus biogenesis	C:integral component of membrane	-	M	-
AZ78_1461	Type IV fimbriae biogenesis protein FmT [Lyso bacter capsici AZ78]	KW503912.1	2.4	6.44E-06	-	pre-pilin leader sequence	P:protein transporter activity; P:protein secretion by the type II secretion system; C:type II protein secretion system complex	-	M	-
AZ78_1464	hypothetical protein AZ78_1464 [Lyso bacter capsici AZ78]	KW503915.1	4.4	2.03E-05	-	hypothetical protein	-	-	-	-
AZ78_1533	hypothetical protein AZ78_1533 [Lyso bacter capsici AZ78]	KW503984.1	2.8	1.19E-05	-	hypothetical protein AZ78_1533	-	-	-	-
AZ78_1580	Oxidoreductase [Lyso bacter capsici AZ78]	KW504031.1	2.6	3.01E-06	-	family transcriptional regulator	-	-	-	-
AZ78_1581	hypothetical protein AZ78_1581 [Lyso bacter capsici AZ78]	KW504032.1	4.0	3.72E-07	-	hypothetical protein	-	-	-	-
AZ78_1582	hypothetical protein AZ78_1582 [Lyso bacter capsici AZ78]	KW504033.1	4.6	1.07E-05	-	hypothetical protein	-	-	-	-
AZ78_1583	hypothetical protein AZ78_1583 [Lyso bacter capsici AZ78]	KW504034.1	5.2	0.000290791	-	outer membrane lipoprotein	C:membrane; C:integral component of membrane	-	-	-
AZ78_1584	cytochrome c ubiquinol oxidase subunit II [Lyso bacter capsici AZ78]	KW504035.1	6.1	1.01E-07	-	cytochrome c ubiquinol oxidase subunit II	C:integral component of membrane; P:oxidation-reduction process	-	Met	-
AZ78_1585	Cytochrome c ubiquinol oxidase subunit I [Lyso bacter capsici AZ78]	KW504036.1	5.9	4.68E-08	-	cytochrome c terminal oxidase subunit I	C:integral component of membrane	-	Met	-
AZ78_1586	hypothetical protein AZ78_1586 [Lyso bacter capsici AZ78]	KW504037.1	4.9	1.55E-05	-	hypothetical protein AZ78_1586	-	-	-	-
AZ78_1587	homocitric acid repressor Y precursor [Lyso bacter capsici AZ78]	KW504038.1	4.7	5.78E-08	-	cellulose synthase	-	-	-	-
AZ78_1588	Universal stress protein UspA [Lyso bacter									

AZ78_2933	Phenylalanine-4-hydroxylase [Lysobacter capsici AZ78]	KW505382.1	-2.2	9.04E-05	phenylalanine-4-hydroxylase	P-L-phenylalanine catabolic process; P-L-phenylalanine biosynthetic process; P-tryptophan biosynthetic process; F-phenylalanine 4-monooxygenase activity; F-iron ion binding; P-tyrosine biosynthetic process; P-oxidation-reduction process	EC:1.14.16.1; EC:1.14.16	AM
AZ78_2950	hypothetical protein AZ78_2950 [Lysobacter capsici AZ78]	KW505398.1	4.4	2.03E-05	hypothetical protein AZ78_2950	-	-	-
AZ78_2955	Response regulator [Lysobacter capsici AZ78]	KW505407.1	-2.2	6.84E-05	two-component system response regulator	P-phosphorelay signal transduction system; C:intracellular	-	RNA/TR
AZ78_2960	Sensory box histidine kinase [Lysobacter capsici AZ78]	KW505408.1	-2.7	6.14E-05	PAS domain-containing sensor histidine kinase	F-phosphorelay sensor kinase activity; FATP binding; C:protein histidine kinase complex; P-phosphorelay signal transduction system; P:regulation of transcription, DNA-templated; P:signal transduction by protein phosphorylation; C:intracellular	EC:2.7.3; EC:2.7.13.3	K
AZ78_2961	hypothetical protein AZ78_2961 [Lysobacter capsici AZ78]	KW505409.1	4.4	2.03E-05	hypothetical protein LC25x_0033	-	-	-
AZ78_2962	sulfur deprivation response regulator [Lysobacter capsici AZ78]	KW505410.1	-2.4	0.00029484	transcription factor	P:potassium ion transport; P:zinc ion transport; C:integral component of membrane; F:cation transmembrane transporter activity	-	Met
AZ78_3026	hypothetical protein AZ78_3026 [Lysobacter capsici AZ78]	KW505474.1	-2.5	4.17E-05	hypothetical protein	C:integral component of membrane	-	-
AZ78_3173	Phosphoglucosyltransferase [Lysobacter capsici AZ78]	KW505621.1	-2.8	8.30E-05	phosphoglucosyltransferase	F:magnesium ion binding; P:gluconogenesis; F:phosphoglucosyltransferase activity; P:peptidase-phosphate shunt; P:glycolytic process; P:serotonin biosynthetic process; P:galactose metabolic process; P:sucrose metabolic process	EC:5.4.2.2	Met
AZ78_3181	hypothetical protein AZ78_3181 [Lysobacter capsici AZ78]	KW505628.1	-2.3	0.00069096	hypothetical protein LC25x_5548	-	-	-
AZ78_3213	hypothetical protein AZ78_3213 [Lysobacter capsici AZ78]	KW505661.1	-2.3	0.00052349	transcriptional regulator	F:sequence-specific DNA binding	-	-
AZ78_3290	hypothetical protein AZ78_3290 [Lysobacter capsici AZ78]	KW505738.1	-2.5	0.000470714	antibiotic biosynthesis monooxygenase	P:oxidation-reduction process; F:monooxygenase activity	-	AG
AZ78_3301	Short-chain type dehydrogenase [Lysobacter capsici AZ78]	KW505745.1	3.9	0.000149116	NADH-dependent aldehyde reductase	F:oxidoreductase activity; P:oxidation-reduction process	-	OK
AZ78_3322	hypothetical protein AZ78_3322 [Lysobacter capsici AZ78]	KW505770.1	-3.7	5.78E-06	hypothetical protein	-	-	-
AZ78_3333	putative exported protein [Lysobacter capsici AZ78]	KW505781.1	-2.6	2.85E-05	peptidase inhibitor 178 family	-	-	G
AZ78_3361	hypothetical protein AZ78_3361 [Lysobacter capsici AZ78]	KW505809.1	2.0	4.17E-05	hypothetical protein AZ78_3361	-	-	-
AZ78_3372	hypothetical protein AZ78_3372 [Lysobacter capsici AZ78]	KW505819.1	-2.3	0.000794835	ligand-binding SH3	-	-	-
AZ78_3428	transacylase-associated protein [Lysobacter capsici AZ78]	KW505874.1	-3.3	8.48E-07	transacylase	C:integral component of membrane	-	-
AZ78_3463	hypothetical protein AZ78_3463 [Lysobacter capsici AZ78]	KW505909.1	3.9	0.000216706	hypothetical protein AZ78_3463	-	-	G
AZ78_3511	hypothetical protein AZ78_3511 [Lysobacter capsici AZ78]	KW505957.1	3.9	0.000149116	hypothetical protein LC25x_5039	-	-	-
AZ78_3543	UDP-glucose 6-epimerase [Lysobacter capsici AZ78]	KW505989.1	3.1	0.000198151	steroid 3 beta-glucosyltransferase	F:transferase activity, transferring heptosyl group; P:lipid glycosylation	-	LM
AZ78_3545	ribosome biogenesis non-ribosomal peptide synthetase module [Lysobacter capsici AZ78]	KW505993.1	3.5	0.00025504	non-ribosomal peptide synthetase	F:oxidoreductase activity; P:oxidation-reduction process	-	AG
AZ78_3545	hypothetical protein AZ78_3545 [Lysobacter capsici AZ78]	KW505991.1	4.3	0.000107115	hypothetical protein AZ78_3545	-	-	-
AZ78_3583	N-acetylmannosamine-6-phosphate 2-epimerase [Lysobacter capsici AZ78]	KW506029.1	-2.3	1.08E-05	N-acetylmannosamine-6-phosphate 2-epimerase	P:carbohydrate metabolic process; F:N-acylglucosamine-6-phosphate 2-epimerase activity; P:N-acetylneuraminate catabolic process; P:N-acetylmannosamine metabolic process	EC:5.1.3.9	Met
AZ78_3594	putative hypothetical Oxy11 phosphage protein [Lysobacter capsici AZ78]	KW506037.1	-2.9	0.000113993	hypothetical protein	-	-	-
AZ78_3605	hypothetical protein AZ78_3605 [Lysobacter capsici AZ78]	KW506051.1	-2.1	0.000332247	hypothetical protein	-	-	S
AZ78_3649	Outer membrane receptor protein [Lysobacter capsici AZ78]	KW506095.1	2.3	1.69E-05	-dependent receptor	P:signal transduction; C:membrane; F:receptor activity; P:transport	-	-
AZ78_3671	hypothetical protein AZ78_3671 [Lysobacter capsici AZ78]	KW506134.1	-2.1	2.1E-05	superoxide dismutase	-	-	-
AZ78_3674	hypothetical protein AZ78_3674 [Lysobacter capsici AZ78]	KW506120.1	3.4	0.000365872	hypothetical protein AZ78_3674	-	-	-
AZ78_3679	Enid-1,4-beta-xylosylase A precursor [Lysobacter capsici AZ78]	KW506125.1	2.1	8.47E-05	glycosyl hydrolase family 16	P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; F:carbohydrate binding	-	GM
AZ78_3685	hypothetical protein AZ78_3685 [Lysobacter capsici AZ78]	KW506131.1	-2.5	0.00069288	tryptophan halogenase	P:oxidoreductase activity; P:oxidation-reduction process	-	-
AZ78_3686	Tryptophan halogenase [Lysobacter capsici AZ78]	KW506132.1	2.9	0.000124339	tryptophan halogenase	-	-	AM
AZ78_3687	Pass-1-related protein [Lysobacter capsici AZ78]	KW506133.1	3.0	6.99E-05	cupin	-	-	-
AZ78_3688	Peptide transport system permease protein sacP [Lysobacter capsici AZ78]	KW506134.1	3.2	2.1E-05	peptide	-	-	AG
AZ78_3689	putative b-glucosidase-specific TonB-dependent outer membrane receptor [Lysobacter capsici AZ78]	KW506135.1	4.4	4.55E-06	-dependent receptor	P:signal transduction; C:cell outer membrane; F:receptor activity; P:transport	-	S
AZ78_3722	Manganese superoxide dismutase [Lysobacter capsici AZ78]	KW506168.1	-4.5	8.09E-05	superoxide dismutase	F:metal ion binding; F:removal of superoxide radicals; P:oxidation-reduction process; F:superoxide dismutase activity	EC:1.15.1.1	OK
AZ78_3743	acyltransferase 3 [Lysobacter capsici AZ78]	KW506189.1	2.8	8.35E-05	acyltransferase	F:transferase activity, transferring acyl groups other than amino-acyl groups; P:metabolic process; C:integral component of membrane	-	Met
AZ78_3766	putative sarcosine oxidase alpha subunit, 2Fe-2S domain [Lysobacter capsici AZ78]	KW506212.1	4.4	2.03E-05	(2Fe-2S) binding	-	-	-
AZ78_3789	Outer membrane vitamin B12 receptor BldB [Lysobacter capsici AZ78]	KW506228.1	2.3	0.00059305	iron-dependent siderophore receptor family	-	-	Met
AZ78_3806	hypothetical protein AZ78_3806 [Lysobacter capsici AZ78]	KW506251.1	-2.4	0.000253131	hypothetical protein	-	-	-
AZ78_3809	isoquinoline 1-oxidoreductase alpha subunit [Lysobacter capsici AZ78]	KW506254.1	-2.8	0.000103194	(2Fe-2S) binding	-	-	-
AZ78_3822	Iron-sulfur cluster transport system, periplasmic binding protein TonB [Lysobacter capsici AZ78]	KW506263.1	2.2	0.000797028	C-terminal domain	F:isoquinoline 1-oxidoreductase activity; P:proton electron transport; F:metal ion binding; F:electron carrier activity; P:oxidation-reduction process; F:iron 2 sulfur cluster binding	EC:1.3.99.16	Met
AZ78_3877	isoformyltransferase [Lysobacter capsici AZ78]	KW506322.1	-2.2	0.000792629	cystine hydrolase	P:metabolic process; F:hydrolase activity	-	-
AZ78_3895	tail-specific protease [Lysobacter capsici AZ78]	KW506340.1	-2.2	9.30E-05	peptidase S41	F:protein binding; P:proteolysis; F:serine-type peptidase activity	-	-
AZ78_3914	transport protein [Lysobacter capsici AZ78]	KW506358.1	-2.5	3.32E-05	transport protein	-	-	AG
AZ78_3915	hypothetical protein AZ78_3915 [Lysobacter capsici AZ78]	KW506359.1	-3.0	3.97E-05	hypothetical protein	-	-	-
AZ78_3916	hypothetical protein AZ78_3916 [Lysobacter capsici AZ78]	KW506360.1	-4.0	0.000483807	membrane	C:integral component of membrane	-	G
AZ78_3917	hypothetical protein AZ78_3917 [Lysobacter capsici AZ78]	KW506361.1	-2.7	6.01E-06	hypothetical protein	-	-	-
AZ78_3918	Trypsin-like serine protease [Lysobacter capsici AZ78]	KW506362.1	-2.5	3.85E-06	heat-shock	F:protein binding; P:proteolysis; F:serine-type endopeptidase activity	EC:3.4.21	AG
AZ78_3925	ERK/YbsY/CybYmoG family protein [Lysobacter capsici AZ78]	KW506369.1	-3.3	9.81E-05	heptadecan-binding	F:transferase activity	-	Met
AZ78_3929	Flavinase: azomycin oxidoreductase, methyltransferase, methyltransferase [Lysobacter capsici AZ78]	KW506373.1	-2.1	8.02E-05	iron-dependent oxidoreductase	F:oxidoreductase activity; P:oxidation-reduction process	-	Met
AZ78_3943	mRNA 3'-end processing factor [Lysobacter capsici AZ78]	KW506387.1	-5.0	6.00E-05	DNA ligase-associated DEX1 box helicase	P:metabolic process; F:hydrolase activity	-	RNA/TR
AZ78_3945	hypothetical protein AZ78_3945 [Lysobacter capsici AZ78]	KW506389.1	-5.9	8.55E-05	hypothetical protein	-	-	-
AZ78_3954	hypothetical protein AZ78_3954 [Lysobacter capsici AZ78]	KW506398.1	4.2	0.0007181	hypothetical protein	-	-	-
AZ78_4009	6-phosphogluconate dehydrogenase, decarboxylating [Lysobacter capsici AZ78]	KW506453.1	2.4	6.39E-05	6-phosphogluconate dehydrogenase	F:phosphogluconate dehydrogenase (decarboxylating) activity; P:acetate phosphate shunt; P:oxidation-reduction process; P-D-glucuronate metabolic process	EC:1.1.1.44	Met
AZ78_4023	PQQ-dependent oxidoreductase, gp88 family [Lysobacter capsici AZ78]	KW506467.1	2.1	3.01E-05	glucose dehydrogenase	P:carbohydrate metabolic process; F:oxidoreductase activity, acting on the CH-OH group of donors, quinone or similar compound as acceptor; F:quinone binding; P:oxidation-reduction process	-	Met
AZ78_4028	hypothetical protein AZ78_4028 [Lysobacter capsici AZ78]	KW506473.1	6.1	2.38E-05	hypothetical protein	-	-	-
AZ78_4031	hypothetical protein AZ78_4031 [Lysobacter capsici AZ78]	KW506475.1	-2.7	0.000290647	hypothetical protein	-	-	-
AZ78_4038	Putative ribonucleoprotein related protein TRDVE Domain [Lysobacter capsici AZ78]	KW506482.1	-2.5	0.000418164	RNA-binding	F:RNA binding	-	-
AZ78_4041	Two-component system regulatory protein [Lysobacter capsici AZ78]	KW506490.1	-2.9	1.84E-05	two-component system regulatory protein	F:DNA binding; P-phosphorelay signal transduction system; P:regulation of transcription, DNA-templated; C:intracellular	-	RNA/TR
AZ78_4047	Sensor protein PhoQ [Lysobacter capsici AZ78]	KW506491.1	-2.1	2.58E-05	ATPase	F:phosphorelay sensor kinase activity; F:ATP binding; C:protein histidine kinase complex; P:phosphorelay signal transduction system; C:integral component of membrane; P:signal transduction by protein phosphorylation; C:intracellular; P:peptidyl-histidine phosphorylation	EC:2.7.3; EC:2.7.13.3	S
AZ78_4056	hypothetical protein AZ78_4056 [Lysobacter capsici AZ78]	KW506500.1	-3.4	1.31E-07	entrieldin	C:membrane; P:response to toxic substance	-	-
AZ78_4084	calcium ion antiporter [Lysobacter capsici AZ78]	KW506528.1	3.4	0.000741297	calcium ion antiporter	P:transmembrane transport; C:integral component of membrane	-	T
AZ78_4103	hypothetical protein AZ78_4103 [Lysobacter capsici AZ78]	KW506547.1	2.1	0.000797776	polysaccharide biosynthesis	C:cytoplasm; P:antibiotic biosynthetic process; P:oxidation-reduction process; F:monooxygenase activity	-	AG
AZ78_4165	Membrane lipoprotein lipid attachment site containing protein USSD80 [Lysobacter capsici AZ78]	KW506609.1	-2.3	0.000354402	ABC transporter	P:transport	-	T
AZ78_4169	ABC transporter substrate-binding protein [Lysobacter capsici AZ78]	KW506613.1	-2.6	5.03E-06	polysaccharide permease	-	-	-
AZ78_4244	hypothetical protein AZ78_4244 [Lysobacter capsici AZ78]	KW506688.1	-2.8	0.000233094	polyketide cyclase	-	-	Met
AZ78_4296	hypothetical protein AZ78_4296 [Lysobacter capsici AZ78]	KW506739.1	3.1	2.44E-05	-NA-	-	-	-
AZ78_4316	hypothetical protein AZ78_4316 [Lysobacter capsici AZ78]	KW506758.1	3.4	0.000849676	hypothetical protein AZ78_4316	-	-	-
AZ78_4325	LamG domain protein jlytJ1 fold domain protein [Lysobacter capsici AZ78]	KW506767.1	2.7	2.40E-06	concanavalin A-like lectin glucanases superfamily	-	-	-
AZ78_4328	hypothetical protein AZ78_4328 [Lysobacter capsici AZ78]	KW506768.1	3.5	4.96E-05	MUL1SPC/D63; beta-galactosidase	-	-	-
AZ78_4364	hypothetical protein AZ78_4364 [Lysobacter capsici AZ78]	KW506806.1	-2.3	0.00073398	hypothetical protein AZ78_4364	-	-	GM
AZ78_4373	hypothetical protein AZ78_4373 [Lysobacter capsici AZ78]	KW506815.1	3.3	2.38E-05	hypothetical protein AZ78_4373	-	-	-
AZ78_4453	hypothetical protein AZ78_4453 [Lysobacter capsici AZ78]	KW506893.1	-3.9	0.000216706	peptidase S8	-	-	-
AZ78_4467	extracellular protease [Lysobacter capsici AZ78]	KW506937.1	2.2	9.6E-05	peptidase S8	-	-	-
AZ78_4509	putative oxidoreductase/short-chain dehydrogenase [Lysobacter capsici AZ78]	KW506949.1	-3.3	0.00037905	short-chain dehydrogenase	P:oxidoreductase activity; P:oxidation-reduction process	-	-
AZ78_4519	Extracellular zinc protease [Lysobacter capsici AZ78]	KW506959.1	2.0	0.000171828	peptidase M14	F:zinc ion binding; F:metallocarboxypeptidase activity; P:proteolysis; F:serine-type endopeptidase activity	EC:3.4.21; EC:3.4.17	AG
AZ78_4533	Putative outer membrane protein [Lysobacter capsici AZ78]	KW506973.1	4.2	5.89E-05	outer membrane	-	-	-
AZ78_4534	hypothetical protein AZ78_4534 [Lysobacter capsici AZ78]	KW506974.1	-5.8	0.000479868	hypothetical protein	-	-	-
AZ78_4613	Phosphate-specific outer membrane porin OmpP [Lysobacter capsici AZ78]	KW507023.1	2.9	2.70E-05	porin	-	-	T
AZ78_4621	Suppressor of fused [Lysobacter capsici AZ78]	KW507061.1	-2.5	0.000385996	suppressor of fused family	F:transaminase activity; P:metabolic process; F:transferase activity	-	G
AZ78_4661	Mobile element protein [Lysobacter capsici AZ78]	KW507101.1	2.1	0.000896042	transposase	F:DNA binding; F:transposase activity; P:transposition, DNA-mediated	-	-
AZ78_4682	hypothetical protein AZ78_4682 [Lysobacter capsici AZ78]	KW507132.1	4.4	2.03E-05	hypothetical protein AZ78_4682	-	-	-
AZ78_4695	hypothetical protein AZ78_4695 [Lysobacter capsici AZ78]	KW507135.1	-2.8	0.000759451	aspartyl protease family	-	-	-
AZ78_4723	hypothetical protein AZ78_4723 [Lysobacter capsici AZ78]	KW507162.1	-2.6	0.000184173	hypothetical protein AZ78_4723	-	-	-
AZ78_4728	putative secreted sugar hydrolase [Lysobacter capsici AZ78]	KW507167.1	2.6	1.45E-05	beta-1,3-glucanase	-	-	-
AZ78_4734	hypothetical protein AZ78_4734 [Lysobacter capsici AZ78]	KW507173.1	-2.2	0.000539228	hypothetical protein AZ78_4734	-	-	AG
AZ78_4736	Kynurenine formamidase, bacterial [Lysobacter capsici AZ78]	KW507175.1	2.2	7.86E-05	cyclase	F:arylformamidase activity; P:glyoxylate metabolic process; P:tryptophan catabolic process to kynurenine	EC:3.5.1.9	Met
AZ78_4753	TonB-dependent receptor [Lysobacter capsici AZ78]	KW507192.1	2.6	3.02E-06	-dependent receptor	P:signal transduction; C:membrane; F:receptor activity; P:transport	-	-
AZ78_4756	Spermidine N1-acetyltransferase [Lysobacter capsici AZ78]	KW507195.1	-2.5	4.02E-05	spermidine acetyltransferase	P:acyl-carrier-protein biosynthetic process; F:N-acetyltransferase activity	EC:2.3.1	Met
AZ78_4791	putative neutral zinc metalloprotease [Lysobacter capsici AZ78]	KW507230.1	2.4	6.79E-06	thermolysin metalloprotease, catalytic domain	P:proteolysis; F:metalloendopeptidase activity	EC:3.4.24	AG
AZ78_4792	hypothetical protein AZ78_4792 [Lysobacter capsici AZ78]	KW507231.1	2.5	0.000464572	-NA-	-	-	-
AZ78_4805	hypothetical protein AZ78_4805 [Lysobacter capsici AZ78]	KW507244.1	-2.6	0.000766541	hypothetical protein AZ78_4805	-	-	-
AZ78_4806	Coproporphyrinogen III oxidase [Lysobacter capsici AZ78]	KW507245.1	3.5	5.64E-05	Coproporphyrinogen III oxidase	F:oxygen binding; F:heme binding	-	OK
AZ78_4903	Streptogramin C precursor (Serine protease C) (SDPC) [Lysobacter capsici AZ78]	KW502296.1	2.0	0.000122034	alpha-lytic protease	P:proteolysis; F:serine-type endopeptidase activity; C:extracellular region	EC:3.4.21	AG
AZ78_4925	Glutamate synthase [NADPH] large chain [Lysobacter capsici AZ78]	KW502258.1						

List of differentially expressed genes of *Lysobacter capsici* AZ78 after 24 h of interaction with *Phytophthora infestans*.

Sequence ID	NCBI Gene description	GeneBank accession number	24 h logFC	24 h P.Value	DEG at 24 hpi	Blast2GO Description	Blast2GO GO	Blast2GO Enzyme Codes list	Internal code	
AZ78_0032	lipase family protein [Lysobacter capsici AZ78]	KWS02488	2.0	0.000218164	-	lipase	Lipid metabolic process			
AZ78_0051	interphotoreceptor retinoid binding protein [Lysobacter capsici AZ78]	KWS02507	2.0	2.87E-05	-	peptidase S41 family	Proteolysis; F:serine-type peptidase activity			
AZ78_0055	ketonolase [Lysobacter capsici AZ78]	KWS02511	4.5	6.57E-07	-	chitinase	Chitin catabolic process	EC:3.2.1.4	AG	
AZ78_0061	peptidase S8 and S53, subtilisin, kefir, sedoimin [Lysobacter capsici AZ78]	KWS02517	4.0	1.20E-07	-	subtilase family	Proteolysis; F:serine-type endopeptidase activity	EC:3.4.21	AG	
AZ78_0062	Para-aminobenzoate synthase, amidotransferase component [Lysobacter capsici AZ78]	KWS02518	2.8	0.00083398	-	glutamine amidotransferase class I family	Glutamine metabolic process; F:hydrolase activity			
AZ78_0063	Microcystin dependent protein [Lysobacter capsici AZ78]	KWS02520	2.4	1.97E-06	-	DNA dependent				
AZ78_0084	Microcystin dependent protein [Lysobacter capsici AZ78]	KWS02540	2.8	7.50E-07	-	phage tail				
AZ78_0085	Microcystin dependent protein [Lysobacter capsici AZ78]	KWS02541	2.7	1.18E-06	-	microcystin-dependent				
AZ78_0088	Nucleoside binding outer membrane protein [Lysobacter capsici AZ78]	KWS02545	3.2	0.000700705	-	Nucleoside-binding outer membrane				
AZ78_0100	Multimodular transpeptidase-transglycosylase [Lysobacter capsici AZ78]	KWS02556	-2.5	3.19E-05	-	penicillin-binding 1C	Cell wall metabolism; Penicillin-binding; F:peptidoglycan biosynthetic process; C:peptidoglycan-based cell wall; F:penicillin binding; F:peptidoglycan glycosyltransferase activity	EC:2.4.1.129	AG	
AZ78_0108	hypothetical protein AZ78_0108 [Lysobacter capsici AZ78]	KWS02564	-3.2	0.00040926	-	roadblock LC7 domain				
AZ78_0177	hypothetical protein AZ78_0177 [Lysobacter capsici AZ78]	KWS02617	0.4	3.44E-06	-	hypothetical protein			RNA:TR	
AZ78_0178	hypothetical protein AZ78_0178 [Lysobacter capsici AZ78]	KWS02634	-0.1	1.08E-06	-	hypothetical protein				
AZ78_0182	Transcriptional regulator [Lysobacter capsici AZ78]	KWS02628	2.9	1.92E-06	-	transcriptional regulator	F:DNA binding; C:transcription factor complex; F:transcription factor activity, sequence-specific DNA binding; F:regulation of transcription, DNA-templated; P:regulation of transcription, DNA-templated			
AZ78_0183	hypothetical protein AZ78_0183 [Lysobacter capsici AZ78]	KWS02638	5.9	2.93E-07	-	tail (protein translocation) pathway signal sequence domain			RNA:TR	
AZ78_0184	hypothetical protein AZ78_0184 [Lysobacter capsici AZ78]	KWS02640	2.9	7.30E-07	-	hypothetical protein AZ78_0184				
AZ78_0186	TorB-dependent receptor [Lysobacter capsici AZ78]	KWS02643	2.8	6.80E-06	-	-dependent receptor				
AZ78_0233	putative trehalase permease, MFS family, FucP subfamily [Lysobacter capsici AZ78]	KWS02689	3.8	1.71E-07	-	MFS transporter	Paramembrane transport; C:integral component of membrane			
AZ78_0234	Trehalase-regulated TorB-dependent outer membrane receptor [Lysobacter capsici AZ78]	KWS02690	4.4	3.83E-08	-	-dependent receptor				
AZ78_0235	Substrate phosphotransferase [Lysobacter capsici AZ78]	KWS02691	4.0	2.36E-08	-	beta-phosphoglucomutase	Phosphatase activity; F:beta-phosphoglucomutase activity; F:carbohydrate binding; P:arabinose metabolic process; F:hydrolase activity	EC:5.4.2.6	E	
AZ78_0247	hypothetical protein AZ78_0247 [Lysobacter capsici AZ78]	KWS02703	-2.7	1.90E-05	-	hypothetical protein AZ78_0247				
AZ78_0270	Vibriofurin, extracellular zinc protease [Lysobacter capsici AZ78]	KWS02725	6.2	3.44E-07	-	peptidase M4	Proteolysis; F:serine-type endopeptidase activity; F:metalloendopeptidase activity	EC:3.4.21; EC:3.4.24	AG	
AZ78_0271	hypothetical protein AZ78_0271 [Lysobacter capsici AZ78]	KWS02726	6.0	1.39E-07	-	beta-galactosidase				
AZ78_0272	Vibriofurin, extracellular zinc protease [Lysobacter capsici AZ78]	KWS02727	6.3	1.94E-09	-	peptidase M4	Proteolysis; F:serine-type endopeptidase activity; F:metalloendopeptidase activity	EC:3.4.21; EC:3.4.24	AG	
AZ78_0299	N-acetylglucosamine-regulated TorB-dependent outer membrane receptor [Lysobacter capsici AZ78]	KWS02753	5.1	5.62E-08	-	-dependent receptor				
AZ78_0300	hypothetical protein AZ78_0300 [Lysobacter capsici AZ78]	KWS02754	5.3	7.49E-09	-	beta-galactosidase	Proteolysis; F:serine-type endopeptidase activity; F:metalloendopeptidase activity	EC:3.2.1.23; EC:3.2.1	S	
AZ78_0301	Oxidoreductase ucpA [Lysobacter capsici AZ78]	KWS02755	4.1	4.87E-08	-	short-chain dehydrogenase	F:oxidoreductase activity; P:oxidation-reduction process		OX	
AZ78_0302	2-dehydro-3-deoxyphosphogluconate aldolase [Lysobacter capsici AZ78]	KWS02756	4.2	2.24E-05	-	2-dehydro-3-deoxyphosphogluconate aldolase	F:metabolic process; F:ase activity		Met	
AZ78_0303	Galactonate dehydratase [Lysobacter capsici AZ78]	KWS02757	4.1	5.48E-08	-	galactonate dehydratase	F:magnesium ion binding; P:cellular amino acid catabolic process; F:galactonate dehydratase activity; P:D-galactonate catabolic process; F:galactate metabolic process	EC:4.2.1.6	Met	
AZ78_0304	L-arabinofuranose [Lysobacter capsici AZ78]	KWS02758	4.3	1.94E-07	-	regucalcin				
AZ78_0305	2-dehydro-3-deoxygluconate aldolase [Lysobacter capsici AZ78]	KWS02759	4.0	1.18E-07	-	MFS transporter	P:D-galactonate catabolic process; F:2-dehydro-3-deoxygluconate activity; F:galactate metabolic process; F:carbohydrate phosphorylation	EC:2.7.1.58	Met	
AZ78_0306	putative sodium-dependent galactose transporter [Lysobacter capsici AZ78]	KWS02760	4.1	7.58E-07	-	sodium transporter	Paramembrane transport; C:integral component of membrane; C:plasma membrane; F:transporter activity		T	
AZ78_0307	hypothetical protein AZ78_0307 [Lysobacter capsici AZ78]	KWS02761	2.9	5.06E-07	-	hypothetical protein AZ78_0307				
AZ78_0308	lipase epimerase family protein [Lysobacter capsici AZ78]	KWS02762	2.9	1.33E-08	-	lipase				
AZ78_0315	ABC transporter, permease protein, putative [Lysobacter capsici AZ78]	KWS02769	2.0	8.67E-06	-	ABC transporter permease	C:integral component of membrane		T	
AZ78_0334	Transcriptional regulator, Anst family [Lysobacter capsici AZ78]	KWS02788	-2.2	0.00035166	-	family transcriptional regulator	C:integral component of membrane; F:DNA binding; F:transcription factor activity, sequence-specific DNA binding; F:regulation of transcription, DNA-templated; F:methyltransferase activity; F:regulation of transcription, DNA-templated; P:methylation		RNA:TR	
AZ78_0346	hypothetical protein AZ78_0346 [Lysobacter capsici AZ78]	KWS02820	-2.2	0.00049286	-	hypothetical protein				
AZ78_0389	hypothetical protein AZ78_0389 [Lysobacter capsici AZ78]	KWS02843	4.2	2.63E-06	-	16S rRNA methyltransferase				
AZ78_0394	hypothetical protein AZ78_0394 [Lysobacter capsici AZ78]	KWS02844	4.2	2.63E-06	-	16S rRNA methyltransferase				
AZ78_0391	Oxycine oxidase THO [Lysobacter capsici AZ78]	KWS02845	2.4	1.62E-06	-	FAD-dependent oxidoreductase				
AZ78_0398	Outer membrane protein [Lysobacter capsici AZ78]	KWS02852	-2.6	4.37E-05	-	cell envelope biogenesis				
AZ78_0399	Nucleoside binding capillary synthesis regulator RucH [Lysobacter capsici AZ78]	KWS02853	-2.5	0.000202471	-	family transcriptional regulator			RNA:TR	
AZ78_0400	sensor histidine kinase-response regulator GacS [Lysobacter capsici AZ78]	KWS02854	-2.5	4.10E-06	-	---NA---			EC:2.7.3; EC:2.7.13.3	S
AZ78_0401	nonreducing diphosphate sugar epimerase [Lysobacter capsici AZ78]	KWS02855	-2.7	1.00E-05	-	---NA---			RNA:TR	
AZ78_0407	endo-1,3-beta-glucanase precursor [Lysobacter capsici AZ78]	KWS02861	5.5	7.25E-08	-	---NA---			AG	
AZ78_0415	Leyt endopeptidase [Lysobacter capsici AZ78]	KWS02869	3.2	1.86E-05	-	---NA---				
AZ78_0427	SlyP-like protein [Lysobacter capsici AZ78]	KWS02881	2.5	0.00119447	-	---NA---				
AZ78_0428	Lipid A export ATP-binding/permease protein MABA [Lysobacter capsici AZ78]	KWS02882	2.6	3.57E-05	-	---NA---				
AZ78_0452	putative Cu/Zn/Cd efflux system membrane fusion protein [Lysobacter capsici AZ78]	KWS02906	2.7	8.34E-05	-	---NA---				
AZ78_0454	Opa-like protein [Lysobacter capsici AZ78]	KWS02908	2.8	6.70E-07	-	---NA---			Met	
AZ78_0455	hypothetical protein AZ78_0455 [Lysobacter capsici AZ78]	KWS02909	2.7	2.71E-08	-	---NA---				
AZ78_0456	hypothetical protein AZ78_0456 [Lysobacter capsici AZ78]	KWS02910	3.0	1.32E-05	-	---NA---				
AZ78_0457	Glutaryl endopeptidase precursor, MAGE [Lysobacter capsici AZ78]	KWS02911	3.1	2.20E-05	-	---NA---				
AZ78_0477	UDP-N-acetylglucosamine 2-epimerase [Lysobacter capsici AZ78]	KWS02931	-2.7	2.87E-05	-	---NA---				
AZ78_0478	UDP-glucose dehydrogenase [Lysobacter capsici AZ78]	KWS02932	-2.2	1.55E-06	-	---NA---				
AZ78_0480	Chromophenol acetyltransferase [Lysobacter capsici AZ78]	KWS02934	-2.2	4.76E-05	-	---NA---				
AZ78_0482	hypothetical protein AZ78_0482 [Lysobacter capsici AZ78]	KWS02936	-2.1	2.28E-05	-	glycosyl transferases group 1 family				
AZ78_0489	benzoate transporter protein [Lysobacter capsici AZ78]	KWS02943	2.2	0.0001337	-	membrane				
AZ78_0544	putative signal peptide protein [Lysobacter capsici AZ78]	KWS02998	2.5	3.64E-05	-	hydroxylase	Benzoate transporter activity; C:integral component of membrane; P:benzoate transport			
AZ78_0551	Ornithinyl inducible protein Y precursor [Lysobacter capsici AZ78]	KWS03005	-5.8	0.000230728	-	BCN domain-containing	Metabolic process; F:hydrolase activity		D	
AZ78_0552	Transcriptional regulator [Lysobacter capsici AZ78]	KWS03006	-6.3	0.00049190	-	plasmid stabilization			RNA:TR	
AZ78_0553	Mg2p1 transporter ATPase protein C [Lysobacter capsici AZ78]	KWS03007	-4.3	1.53E-06	-	transporter				
AZ78_0554	ATP-dependent DNA ligase clustered with Ku protein, LigD [Lysobacter capsici AZ78]	KWS03008	-3.6	2.98E-06	-	DNA ligase	DNA metabolic process; F:ligase activity		DNA	
AZ78_0556	ATP-dependent DNA ligase clustered with Ku protein, LigD [Lysobacter capsici AZ78]	KWS03009	-3.1	1.43E-07	-	DNA ligase	F:ATP binding; F:DNA repair; F:DNA replication; F:DNA recombination	EC:6.6.1.1; EC:6.6.1.11	DNA	
AZ78_0558	Ku domain protein [Lysobacter capsici AZ78]	KWS03010	-3.4	2.87E-05	-	Ku	F:double-strand DNA binding; F:DNA recombination; F:helicase activity; P:double-strand break repair via nonhomologous end joining	EC:3.6.1.1; EC:3.6.1.15	G	
AZ78_0557	protein of unknown function DUF1452 [Lysobacter capsici AZ78]	KWS03011	-4.1	0.000132687	-	low affinity ion permease family	Paramembrane transport; C:integral component of membrane		T	
AZ78_0559	hypothetical protein AZ78_0559 [Lysobacter capsici AZ78]	KWS03012	-3.2	1.80E-05	-	---NA---				
AZ78_0573	Putrescine transport system permease protein PutJ [Lysobacter capsici AZ78]	KWS03027	2.0	7.03E-05	-	permease	Arginine metabolic process; P:arginine transport; P:ornithine metabolic process; C:integral component of membrane; F:ornithine carbamoyltransferase activity; P:transport; C:plasma membrane; C:ornithine carbamoyltransferase complex	EC:2.1.3.3	T	
AZ78_0592	Beta-ketoadipate enol-lactonase [Lysobacter capsici AZ78]	KWS03046	-2.1	0.00092296	-	3-oxoadipate enol-lactonase	F:3-oxoadipate enol-lactonase activity; F:benzoate metabolic process; P:beta-ketoadipate pathway	EC:3.1.1.24	Met	
AZ78_0595	Outer membrane lipoprotein carrier protein Lck [Lysobacter capsici AZ78]	KWS03048	2.5	1.11E-05	-	lipoprotein carrier	F:lipoprotein transporter activity; C:periplasmic space; P:lipoprotein transport	EC:3.1.1.24	Met	
AZ78_0606	hypothetical protein AZ78_0606 [Lysobacter capsici AZ78]	KWS03060	-3.6	2.10E-05	-	hypothetical protein				
AZ78_0607	hypothetical protein AZ78_0607 [Lysobacter capsici AZ78]	KWS03061	-3.4	5.35E-07	-	hypothetical protein				
AZ78_0608	hypothetical protein AZ78_0608 [Lysobacter capsici AZ78]	KWS03062	3.8	9.49E-08	-	hypothetical protein AZ78_0608				
AZ78_0634	Beta-mannosidase [Lysobacter capsici AZ78]	KWS03088	5.1	3.34E-07	-	beta-mannosidase	Carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds		Met	
AZ78_0635	TorB-dependent receptor [Lysobacter capsici AZ78]	KWS03089	5.7	1.65E-07	-	torB-dependent receptor family			S	
AZ78_0636	hypothetical protein AZ78_0636 [Lysobacter capsici AZ78]	KWS03090	5.6	8.69E-08	-	hypothetical protein			Met	
AZ78_0637	Beta-hexosaminidase [Lysobacter capsici AZ78]	KWS03091	5.3	4.05E-07	-	Beta-hexosaminidase	Polysaccharide metabolic process; P:carbohydrate metabolic process; P:amino sugar metabolic process; F:beta-N-acetylhexosaminidase activity; P:glycosaminoglycan catabolic process	EC:3.2.1.52; EC:3.2.1	G	
AZ78_0638	hypothetical protein AZ78_0638 [Lysobacter capsici AZ78]	KWS03092	6.2	2.05E-08	-	hypothetical protein AZ78_0638				
AZ78_0639	Alpha-1,2-mannosidase [Lysobacter capsici AZ78]	KWS03093	6.1	1.91E-07	-	alpha-mannosidase	Carbohydrate metabolic process		Met	
AZ78_0640	TorB-dependent receptor [Lysobacter capsici AZ78]	KWS03094	6.1	3.64E-09	-	membrane			S	
AZ78_0680	Plastin-TorB dependent hemime receptor [Lysobacter capsici AZ78]	KWS03136	2.2	3.05E-05	-	---NA---				
AZ78_0699	DNA polymerase-like protein [Lysobacter capsici AZ78]	KWS03137	-2.0	0.00030112	-	DNA repair nucleotidyltransferase			DNA	
AZ78_0727	hypothetical protein AZ78_0727 [Lysobacter capsici AZ78]	KWS03181	-2.8	0.000923074	-	membrane			G	
AZ78_0728	hypothetical protein AZ78_0728 [Lysobacter capsici AZ78]	KWS03182	-3.2	2.64E-06	-	MAL TOPEICES; hypothetical protein				
AZ78_0729	hypothetical protein AZ78_0729 [Lysobacter capsici AZ78]	KWS03183	-2.7	7.29E-06	-	hypothetical protein				
AZ78_0730	SAM-dependent methyltransferase [Lysobacter capsici AZ78]	KWS03184	-3.0	0.00114845	-	methyltransferase type 12	Phosphorylation; F:kinase activity		DNA	
AZ78_0731	hypothetical protein AZ78_0731 [Lysobacter capsici AZ78]	KWS03185	-3.4	4.00E-05	-	hypothetical protein				
AZ78_0732	hypothetical protein AZ78_0732 [Lysobacter capsici AZ78]	KWS03234	-2.5	0.00031147	-	hypothetical protein				
AZ78_0903	transglycosylase associated protein [Lysobacter capsici AZ78]	KWS03257	4.4	0.000291477	-	transglycosylase				
AZ78_0904	Pyridoxamine 5-phosphate oxidase [Lysobacter capsici AZ78]	KWS03258	-3.6	0.00025266	-	general stress			Met	
AZ78_0905	hypothetical protein AZ78_0905 [Lysobacter capsici AZ78]	KWS03259	-2.8	0.000597811	-	hemerythrin				
AZ78_0909	hypothetical protein AZ78_0909 [Lysobacter capsici AZ78]	KWS03263	2.5	6.72E-05	-	hypothetical protein				
AZ78_0921	Two-component hybrid sensor and regulator [Lysobacter capsici AZ78]	KWS03275	-2.8	2.99E-05	-	histidine kinase				
AZ78_0954	hypothetical protein AZ78_0954 [Lysobacter capsici AZ78]	KWS03407	-2.1	4.88E-05	-	cytochrome C family				
AZ78_0955	lipopolysaccharide-associated outer membrane lipoprotein [Lysobacter capsici AZ78]	KWS03408	2.5	1.20E-05	-	periplasmic carrier			G	
AZ78_0956	Extracellular protease precursor [Lysobacter capsici AZ78]	KWS03409	3.5	1.25E-06	-	protease	Proteolysis; F:metalloendopeptidase activity	EC:3.4.24	AG	
AZ78_0983	DNA-binding response regulator, OrpH family [Lysobacter capsici AZ78]	KWS03436	2.0							

Accession	Gene Name	Score	Value	Category	GO Term	GO ID
AZ78_1634	Flagellar biosynthesis protein FIR [Lyso bacter capsici AZ78]	KWS04085	-3.0	2.23E-05	UDP kinase	
AZ78_1652	Ribulose 5-phosphate 4-epimerase [Lyso bacter capsici AZ78]	KWS04103	-2.3	2.96E-05	class II aldolase	
AZ78_1653	Ribulose 5-phosphate 4-epimerase [Lyso bacter capsici AZ78]	KWS04104	-4.0	6.43E-05	aldolase	
AZ78_1666	hypothetical protein AZ78_1666 [Lyso bacter capsici AZ78]	KWS04117	-2.8	0.00084579	hypothetical protein	
AZ78_1667	hypothetical protein AZ78_1667 [Lyso bacter capsici AZ78]	KWS04118	-2.1	0.00102099	hypothetical protein	
AZ78_1668	hypothetical protein AZ78_1668 [Lyso bacter capsici AZ78]	KWS04119	-0.7	0.00051485	leucine	
AZ78_1670	integral membrane protein [Lyso bacter capsici AZ78]	KWS04121	3.4	4.97E-07	eamA-like transporter family	
AZ78_1671	Outer membrane receptor protein [Lyso bacter capsici AZ78]	KWS04122	4.7	4.94E-07	T	
AZ78_1672	hypothetical protein AZ78_1672 [Lyso bacter capsici AZ78]	KWS04123	4.5	2.04E-07	Regulator hook-length control	
AZ78_1673	hypothetical protein AZ78_1673 [Lyso bacter capsici AZ78]	KWS04124	2.3	3.41E-06	Carbohydrate catabolic process; Phytolase activity; hydrolyzing O-glycosyl compounds; F-carbohydrate binding	
AZ78_1677	Protein containing SET domain [Lyso bacter capsici AZ78]	KWS04128	2.5	2.01E-05	SET domain	
AZ78_1685	Ferrioxamine iron receptor [Lyso bacter capsici AZ78]	KWS04136	3.3	1.06E-05	Ferrioxamine iron receptor	
AZ78_1706	Plastine membrane protein [Lyso bacter capsici AZ78]	KWS04151	0.1	0.00064697	transglycosylase associated family	
AZ78_1707	Sulfate adenylyltransferase subunit 1 [Lyso bacter capsici AZ78]	KWS04158	3.5	2.53E-07	bifunctional sulfate adenylyltransferase subunit 1 adenylylsulfate kinase	
AZ78_1708	Sulfate adenylyltransferase subunit 2 [Lyso bacter capsici AZ78]	KWS04159	2.8	3.31E-05	sulfate adenylyltransferase, small subunit	
AZ78_1710	Sulfite reductase [NADPH] hydrogenase alpha component [Lyso bacter capsici AZ78]	KWS04160	0.5	0.00059649	Sulfite reductase [NADPH] beta-, alpha-component	
AZ78_1710	Sulfite reductase [NADPH] hemoprotein beta-component [Lyso bacter capsici AZ78]	KWS04161	3.6	1.28E-07	sulfite reductase subunit beta	
AZ78_1711	Phosphoenolpyruvate reductase [Lyso bacter capsici AZ78]	KWS04162	3.9	3.63E-07	phosphoenolpyruvate reductase	
AZ78_1715	hypothetical protein AZ78_1715 [Lyso bacter capsici AZ78]	KWS04166	2.2	0.00094762	hypothetical protein AZ78_1715	
AZ78_1737	Plastine nucleotide pyrophosphatase [Lyso bacter capsici AZ78]	KWS04189	3.5	4.31E-07	nucleotide pyrophosphatase	
AZ78_1748	3-deoxy-D-manno-oxulosone-8-phosphate phosphatase [Lyso bacter capsici AZ78]	KWS04199	-2.2	1.28E-05	Fremetal ion binding; F-3-deoxy-manno-oxulosone-8-phosphate activity; P-3-oxopropylacetate biosynthetic process; P-dephosphorylation	
AZ78_1768	Twelching motility protein PIT [Lyso bacter capsici AZ78]	KWS04221	2.4	7.97E-06	FATP binding; P-transport	
AZ78_1781	Twelching motility protein PIT [Lyso bacter capsici AZ78]	KWS04232	2.8	0.00201711	type IV pil twelching motility	
AZ78_1781	Lipase precursor [Lyso bacter capsici AZ78]	KWS04238	2.5	4.12E-05	lipase	
AZ78_1826	hypothetical protein AZ78_1826 [Lyso bacter capsici AZ78]	KWS04277	4.4	1.38E-05	hypothetical protein AZ78_1826	
AZ78_1827	Chitinase [Lyso bacter capsici AZ78]	KWS04278	4.4	2.00E-08	chitinase	
AZ78_1829	reported protein, contained [Lyso bacter capsici AZ78]	KWS04295	3.0	0.00203461	asmA family	
AZ78_1847	TouB-dependent receptor [Lyso bacter capsici AZ78]	KWS04298	3.8	4.94E-05	asmA family	
AZ78_1849	TouB-dependent receptor [Lyso bacter capsici AZ78]	KWS04300	4.4	2.29E-08	asmA family	
AZ78_1861	Major facilitase family transporter [Lyso bacter capsici AZ78]	KWS04312	0.8	0.00031263	MFS transporter	
AZ78_1880	Glycine cleavage system H protein [Lyso bacter capsici AZ78]	KWS04331	2.2	4.12E-06	glycine cleavage system H membrane	
AZ78_1897	putative transmembrane protein [Lyso bacter capsici AZ78]	KWS04348	-2.3	3.03E-05	membrane	
AZ78_1914	Plastine hemoprotein [Lyso bacter capsici AZ78]	KWS04349	-0.6	0.00031497	transferrinase activity; transferring acyl groups; P-metabolic process	
AZ78_1931	peptidyl-Asp metalloendopeptidase [Lyso bacter capsici AZ78]	KWS04382	3.2	1.30E-06	peptidyl-Asp metalloendopeptidase	
AZ78_1942	hypothetical protein AZ78_1942 [Lyso bacter capsici AZ78]	KWS04390	2.5	0.00029026	hypothetical protein AZ78_1942	
AZ78_1955	Plastine chaperone [Lyso bacter capsici AZ78]	KWS04407	2.4	1.71E-05	Plastine chaperone	
AZ78_1956	hypothetical protein AZ78_1956 [Lyso bacter capsici AZ78]	KWS04407	2.4	6.28E-06	serine threonine phosphatase	
AZ78_1957	hypothetical protein AZ78_1957 [Lyso bacter capsici AZ78]	KWS04408	2.4	3.08E-05	von Willebrand factor type A	
AZ78_1960	hypothetical protein AZ78_1960 [Lyso bacter capsici AZ78]	KWS04411	-2.6	0.00263263	hypothetical protein	
AZ78_1970	hypothetical protein AZ78_1970 [Lyso bacter capsici AZ78]	KWS04421	3.6	2.66E-07	hypothetical protein	
AZ78_1988	hypothetical protein AZ78_1988 [Lyso bacter capsici AZ78]	KWS04429	-2.1	0.00070017	hypothetical protein AZ78_1988	
AZ78_1997	hypothetical protein AZ78_1997 [Lyso bacter capsici AZ78]	KWS04448	3.5	7.93E-06	hypothetical protein	
AZ78_1998	hypothetical protein AZ78_1998 [Lyso bacter capsici AZ78]	KWS04449	3.7	1.89E-06	hypothetical protein AZ78_1998	
AZ78_2011	hypothetical protein AZ78_2011 [Lyso bacter capsici AZ78]	KWS04462	4.8	3.39E-06	hypothetical protein AZ78_2011	
AZ78_2012	hypothetical protein AZ78_2012 [Lyso bacter capsici AZ78]	KWS04463	2.5	0.00132885	hypothetical protein AZ78_2012	
AZ78_2013	5-methyltetrahydropyridopyridylamine-homocysteine methyltransferase [Lyso bacter capsici AZ78]	KWS04464	3.3	3.42E-06	5-methyltetrahydropyridopyridylamine-homocysteine S-methyltransferase activity; P-methylation	
AZ78_2014	hypothetical protein AZ78_2014 [Lyso bacter capsici AZ78]	KWS04466	2.1	0.00025841	hypothetical membrane	
AZ78_2049	Type IV plus biogenesis protein P10 [Lyso bacter capsici AZ78]	KWS04500	2.3	2.43E-05	infralipid	
AZ78_2051	Type IV plus biogenesis protein P10 [Lyso bacter capsici AZ78]	KWS04502	2.2	0.0017162	infralipid	
AZ78_2051	Type IV plus biogenesis protein P10 [Lyso bacter capsici AZ78]	KWS04503	2.7	0.0024581	infralipid	
AZ78_2053	Type IV plus biogenesis protein P10 [Lyso bacter capsici AZ78]	KWS04504	2.9	0.00038345	infralipid	
AZ78_2054	Type IV plus biogenesis protein P10 [Lyso bacter capsici AZ78]	KWS04505	2.8	0.00091124	infralipid assembly	
AZ78_2080	Nucleoside 5-phosphatase RldB (rHPATP, dTP, XTP-specific) [Lyso bacter capsici AZ78]	KWS04530	3.3	0.00023265	non-canonical purine NTP pyrophosphatase	
AZ78_2108	Acetylornithine decarboxylase/Succinyl-diaminopimelate decarboxylase [Lyso bacter capsici AZ78]	KWS04558	2.1	8.57E-06	peptidase M20	
AZ78_2108	Pyrophosphate energized system [Lyso bacter capsici AZ78]	KWS04571	2.7	1.86E-06	Ferrous diphosphate activity; Pyridoxate phosphorylation; P-transport; P-transmembrane transport; C-integral component of membrane; F-hydrogen-translocating pyrophosphatase activity	
AZ78_2127	Steryl desaturase [Lyso bacter capsici AZ78]	KWS04577	4.3	4.18E-05	fatty acid hydrolyase	
AZ78_2136	hypothetical protein AZ78_2136 [Lyso bacter capsici AZ78]	KWS04596	2.3	6.93E-06	hypothetical protein AZ78_2136	
AZ78_2137	LiAsi proteinase [Lyso bacter capsici AZ78]	KWS04597	2.4	2.46E-07	proteolysis; F-metalloendopeptidase activity	
AZ78_2143	Threonine synthase [Lyso bacter capsici AZ78]	KWS04593	2.2	1.68E-05	threonine synthase	
AZ78_2148	Acetylacetyl synthase large subunit [Lyso bacter capsici AZ78]	KWS04596	2.9	0.00049495	acetylacetyl synthase	
AZ78_2148	Acetylacetyl synthase small subunit [Lyso bacter capsici AZ78]	KWS04597	3.6	4.95E-06	acetylacetyl synthase	
AZ78_2150	Branched-chain amino acid aminotransferase [Lyso bacter capsici AZ78]	KWS04600	4.4	2.84E-07	branched-chain amino acid aminotransferase	
AZ78_2150	Threonine dehydratase [Lyso bacter capsici AZ78]	KWS04601	3.5	1.03E-05	threonine dehydratase	
AZ78_2152	2-isopropylmalate synthase [Lyso bacter capsici AZ78]	KWS04602	3.5	4.23E-05	2-isopropylmalate synthase	
AZ78_2153	3-isopropylmalate dehydratase large subunit [Lyso bacter capsici AZ78]	KWS04603	3.3	0.00049412	isopropylmalate isomerase	
AZ78_2153	3-isopropylmalate dehydratase large subunit [Lyso bacter capsici AZ78]	KWS04607	2.4	0.29E-05	isopropylmalate isomerase	
AZ78_2202	Biotin carboxyl carrier protein of acetyl-CoA carboxylase [Lyso bacter capsici AZ78]	KWS04652	2.7	1.73E-05	acyl-CoA carboxylase biotin carboxyl carrier subunit	
AZ78_2203	Plastine four helix bundle protein [Lyso bacter capsici AZ78]	KWS04653	2.4	1.40E-05	four helix bundle	
AZ78_2207	Sialylase hydrolase [Lyso bacter capsici AZ78]	KWS04677	-0.8	0.00031733	polysialidase	
AZ78_2228	hypothetical protein AZ78_2228 [Lyso bacter capsici AZ78]	KWS04678	-3.6	4.06E-05	hypothetical protein AZ78_2228	
AZ78_2229	Membrane protein involved in cation uptake [Lyso bacter capsici AZ78]	KWS04679	-4.0	3.07E-05	surface layer	
AZ78_2251	hypothetical protein AZ78_2251 [Lyso bacter capsici AZ78]	KWS04701	-1.7	1.85E-06	membrane	
AZ78_2282	hypothetical protein AZ78_2282 [Lyso bacter capsici AZ78]	KWS04712	-3.5	0.00140311	uncharacterised	
AZ78_2294	putative nucleoside-diphosphate-sugar epimerase [Lyso bacter capsici AZ78]	KWS04718	-1.5	0.00157251	family transcriptional regulator	
AZ78_2274	hypothetical protein AZ78_2274 [Lyso bacter capsici AZ78]	KWS04724	-0.5	0.00181443	hypothetical protein	
AZ78_2276	ABC transporter ATP-binding protein [Lyso bacter capsici AZ78]	KWS04728	-3.0	4.93E-05	ABC transporter ATP-binding	
AZ78_2277	ABC transporter permease [Lyso bacter capsici AZ78]	KWS04729	3.1	5.31E-07	ABC transporter permease	
AZ78_2278	protein of unknown function DUF885 [Lyso bacter capsici AZ78]	KWS04728	3.8	6.66E-07	protein of unknown function DUF885	
AZ78_2281	3-oxoacyl-carrier protein reductase [Lyso bacter capsici AZ78]	KWS04731	-2.7	9.99E-06	oxido-reductase	
AZ78_2282	intra-biotin N-acylglucosaminidase [Lyso bacter capsici AZ78]	KWS04735	2.8	7.66E-05	beta-lytic metalloendopeptidase	
AZ78_2297	hypothetical protein AZ78_2297 [Lyso bacter capsici AZ78]	KWS04747	-2.0	0.000102961	outer membrane beta-barrel domain	
AZ78_2302	Outer membrane protein W precursor [Lyso bacter capsici AZ78]	KWS04752	-5.9	8.80E-07	membrane	
AZ78_2303	putative hydrolase [Lyso bacter capsici AZ78]	KWS04756	-2.3	1.03E-05	membrane	
AZ78_2305	hypothetical protein AZ78_2305 [Lyso bacter capsici AZ78]	KWS04765	-2.5	2.86E-05	membrane	
AZ78_2312	hypothetical protein AZ78_2312 [Lyso bacter capsici AZ78]	KWS04762	-2.1	0.00026226	hypothetical protein AZ78_2312	
AZ78_2331	Phosphoenolpyruvate synthase [Lyso bacter capsici AZ78]	KWS04781	3.3	1.49E-06	phosphoenolpyruvate synthase	
AZ78_2336	stomatin-prohibitin-family membrane protease subunit [Lyso bacter capsici AZ78]	KWS04786	-4.5	2.86E-07	stomatin-prohibitin-family membrane protease subunit	
AZ78_2338	hypothetical protein AZ78_2338 [Lyso bacter capsici AZ78]	KWS04789	3.0	0.00013774	hypothetical protein	
AZ78_2337	Sialidase [Lyso bacter capsici AZ78]	KWS04807	4.5	6.65E-07	N-acetylneuraminyl-L-alanine amidase A domain	
AZ78_2358	condensation domain protein [Lyso bacter capsici AZ78]	KWS04808	4.1	6.99E-08	condensation domain	
AZ78_2359	cTDP:thymosin transferase RibF [Lyso bacter capsici AZ78]	KWS04809	3.4	1.35E-07	glycyl-tRNA synthetase family metalloendopeptidase	
AZ78_2363	Membrane protein [Lyso bacter capsici AZ78]	KWS04813	-2.3	8.97E-06	membrane	
AZ78_2395	Cyclic beta-1,2-glucan synthase [Lyso bacter capsici AZ78]	KWS04845	4.3	3.92E-07	cyclic beta-1,2-glucan synthetase	
AZ78_2398	secreted and surface protein [Lyso bacter capsici AZ78]	KWS04846	2.6	2.05E-07	secreted and surface protein	
AZ78_2401	putative transcriptional regulator for fatty acid degradation FadL, TerF family [Lyso bacter capsici AZ78]	KWS04851	2.7	1.32E-06	RNA polymerase regulator	
AZ78_2402	Sigma factor, ECF subfamily [Lyso bacter capsici AZ78]	KWS04852	2.5	2.25E-06	RNA polymerase subunit sigma-24	
AZ78_2403	transcriptional regulator, caduA [Lyso bacter capsici AZ78]	KWS04853	4.0	4.94E-06	transcriptional regulator	
AZ78_2409	hypothetical protein AZ78_2409 [Lyso bacter capsici AZ78]	KWS04859	3.6	6.91E-06	hypothetical protein AZ78_2409	
AZ78_2410	hypothetical protein AZ78_2410 [Lyso bacter capsici AZ78]	KWS04860	2.1	0.00020208	hypothetical protein	
AZ78_2411	Lanthionine biosynthesis protein LanM [Lyso bacter capsici AZ78]	KWS04861	2.1	6.65E-06	lanthionine synthase	
AZ78_2418	Transcriptional regulator, AraC family [Lyso bacter capsici AZ78]	KWS04868	2.7	1.46E-05	family transcriptional regulator	
AZ78_2419	Nodulation protein N [Lyso bacter capsici AZ78]	KWS04869	4.0	7.95E-08	lectin	
AZ78_2434	xylosyltransferase [Lyso bacter capsici AZ78]	KWS04884	3.9	5.95E-08	regulator	
AZ78_2503	FmrR, Negative transcriptional regulator of formaldehyde detoxification operon [Lyso bacter capsici AZ78]	KWS04953	-2.6	7.70E-05	regulator	
AZ78_2518	hypothetical protein AZ78_2518 [Lyso bacter capsici AZ78]	KWS04968	2.2	0.00080443	hypothetical protein AZ78_2518	
AZ78_2519	Outer membrane receptor protein [Lyso bacter capsici AZ78]	KWS04969	2.7	0.00274785	hypothetical protein	
AZ78_2520	TouB-dependent receptor [Lyso bacter capsici AZ78]	KWS04980	3.2	1.47E-06	hypothetical protein	
AZ78_2521	TouB-dependent receptor [Lyso bacter capsici AZ78]	KWS04981	3.1	3.51E-06	hypothetical protein	
AZ78_2541	TouB-dependent receptor [Lyso bacter capsici AZ78]	KWS04991	5.0	4.41E-07	hypothetical protein	
AZ78_2550	hypothetical protein AZ78_2550 [Lyso bacter capsici AZ78]	KWS05009	2.7	1.45E-05	hypothetical protein	
AZ78_2610	Phosphomannomutase [Lyso bacter capsici AZ78]	KWS05050	3.9	6.13E-05	phosphomannomutase	
AZ78_2616	hypothetical protein AZ78_2616 [Lyso bacter capsici AZ78]	KWS05055	3.7	9.04E-06	hypothetical protein	
AZ78_2623	Plastine membrane protein VmH [Lyso bacter capsici AZ78]	KWS05072	3.3	6.47E-06	membrane	
AZ78_2626	hypothetical protein AZ78_2626 [Lyso bacter capsici AZ78]	KWS05075	-2.2	6.75E-05	membrane histogenetic activity family	
AZ78_2627	Magnesium chelatase family protein [Lyso bacter capsici AZ78]	KWS05076	-2.9	0.000150109	ATP-dependent protease	
AZ78_2657	plastine membrane protein [Lyso bacter capsici AZ78]	KWS05106	4.8	8.79E-06	membrane	
AZ78_2682	MFS superfamily export protein YoeL [Lyso bacter capsici AZ78]	KWS05131	3.1	1.98E-05	multidrug resistance	
AZ78_2683	hypothetical protein AZ78_2683 [Lyso bacter capsici AZ78]	KWS05132	4.3	1.69E-08	hypothetical protein	
AZ78_2684	hypothetical protein AZ78_2684 [Lyso bacter capsici AZ78]	KWS05133	4.5	8.15E-09	carboxyl-terminus ligase	
AZ78_2685	Cysteine synthase [Lyso bacter capsici AZ78]	KWS05134	4.2	5.10E-08	cysteine synthase	
AZ78_2686	Threonine kinase in B12 biosynthesis [Lyso bacter capsici AZ78]	KWS05135	4.1	1.11E-07	kinase	
AZ78_2687	hypothetical protein AZ78_2687 [Lyso bacter capsici AZ78]	KWS05136	3.7	8.15E-07	hypothetical protein	
AZ78_2688	hypothetical protein AZ78_2688 [Lyso bacter capsici AZ78]	KWS05137	3.9	8.28E-08	ABC-2 transporter family	
AZ78_2689	hypothetical protein AZ78_2689 [Lyso bacter capsici AZ78]	KWS05138	3.8	2.31E-08	ABC-2 transporter family	
AZ78_2690	ABC transporter ATP-binding protein [Lyso bacter capsici AZ78]	KWS05139	3.8	4.74E-07	ABC transporter ATP-binding	
AZ78_2691	Outer membrane vitamin B12 receptor BtuB [Lyso bacter capsici AZ78]	KWS05140	3.5	1.55E-07	torB-dependent siderophore receptor family	
AZ78_2693	hypothetical protein AZ78_2693 [Lyso bacter capsici AZ78]	KWS05142	-0.3	0.00010083	pyruvate hydrolyase	
AZ78_2694	hypothetical protein AZ78_2694 [Lyso bacter capsici AZ78]	KWS05143	-3.8	6.87E-06	Au1 family	
AZ78_2721	hypothetical protein AZ78_2721 [Lyso bacter capsici AZ78]	KWS05170	-2.4	8.87E-05	RMS Repeat family	
AZ78_2740	Asn protein [Lyso bacter capsici AZ78]	KWS05189	-2.3	3.92E-05	membrane	
AZ78_2743	alkaline phosphatase precursor (APASE) [Lyso bacter capsici AZ78]	KWS05192	3.7	5.52E-07	alkaline phosphatase	
AZ78_2745	FKBP-type peptidyl-prolyl cis-trans isomerase [Lyso bacter capsici AZ78]	KWS05194	-2.9	7.17E-07	FKBP-type peptidyl-prolyl cis-trans isomerase family	
AZ78_2746	hypothetical protein AZ78_2746 [Lyso bacter capsici AZ78]	KWS05196	-2.7	8.29E-06	substrate ABC transporter permease	
AZ78_2753	ABC-type anion transport system, duplicated permease component [Lyso bacter capsici AZ78]	KWS05202	-2.7	6.04E-05	nitrate ABC transporter ATP-binding	
AZ78_2754	ABC-type nitrate/sulfate/bicarbonate transport system, ATPase component [Lyso bacter capsici AZ78]	KWS05203	-2.5	0.00027581	nitrate ABC transporter ATP-binding	
AZ78_2780	Outer membrane receptor protein [Lyso bacter capsici AZ78]	KWS05204	-2.4	0.00214985	hypothetical protein	
AZ78_2790	Outer membrane receptor protein [Lyso bacter capsici AZ78]	KWS05209	2.1	6.87E-06	phosphotransferase activity; P-methylation	
AZ78_2795	Cytochrome oxidase biogenesis protein CcoI/Sox-C/ProC, putative copper metallochaperone [Lyso bacter capsici AZ7					

List of differentially expressed genes of *Phytophthora infestans* interacting with *Lysobacter capsici* AZ78 for 6 h and 24 h.

Contig	Sequence ID	NCBI Gene description	GenBank accession number	6 h logFC	6 h P Value	24 h logFC	24 h P Value	BLAST Description	BLAST E-Value	Internal index	Class name	Acronym
G3	PT1_0017	calyculin A phosphatase	EF17478	-0.8	2.6E-03	-	-	Calyculin A phosphatase	1.1E-05	-	Phosphatase	Ph
G3	PT1_0021	Mutator/Organotin-binding domain	EF17479	-0.51	6.13E-03	-	-	Mutator/Organotin-binding domain	1.1E-05	-	Mutator	M
G1	PT1_0026	conserved hypothetical protein	EF17513	-1.20	2.16E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0027	conserved hypothetical protein	EF17520	-1.20	6.16E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0030	conserved hypothetical protein	EF17531	-1.20	2.16E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0034	phospholipase C, putative	EF17550	-1.21	6.65E-04	-	-	phospholipase C, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0034	earth lipoase	EF17565	-1.40	3.66E-06	-	-	earth lipoase	1.1E-05	-	Phosphatase	Ph
G1	PT1_0035	conserved hypothetical protein	EF17566	-1.20	2.16E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0035	protein kinase	EF17567	-0.83	4.93E-04	-	-	protein kinase	1.1E-05	-	Phosphatase	Ph
G4	PT1_0037	hyphal protein PTO_0037	EF17561	0.95	3.66E-04	-	-	hyphal protein PTO_0037	1.1E-05	-	Phosphatase	Ph
G4	PT1_0037	DEAD/DEAF like RNA helicase, putative	EF17562	-0.83	3.20E-03	-	-	DEAD/DEAF like RNA helicase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0035	conserved hypothetical protein	EF17565	1.26	7.02E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0035	conserved hypothetical protein	EF17566	1.14	6.37E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0028	conserved hypothetical protein	EF17573	-1.20	6.16E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0028	conserved hypothetical protein	EF17573	-1.20	6.16E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0039	Major Facilitator Superfamily (MFS)	EF17576	0.96	2.49E-01	-	-	Major Facilitator Superfamily (MFS)	1.1E-05	-	Phosphatase	Ph
G2	PT1_0039	conserved hypothetical protein	EF17576	0.96	2.49E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0039	serine protease family S33, putative	EF17589	0.54	1.58E-01	-	-	serine protease family S33, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0039	conserved hypothetical protein	EF17589	1.11	7.97E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0040	lysin	EF17587	-1.01	8.41E-05	-	-	lysin	1.1E-05	-	Phosphatase	Ph
G3	PT1_0040	conserved hypothetical protein	EF17587	-1.01	8.41E-05	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0044	RNA (cytosine 5'-methyltransferase NSUN2, putative)	EF17581	-1.10	3.42E-04	-	-	RNA (cytosine 5'-methyltransferase NSUN2, putative)	1.1E-05	-	Phosphatase	Ph
G3	PT1_0044	lysine-specific aminopeptidase	EF17588	-1.04	1.60E-05	-	-	lysine-specific aminopeptidase	1.1E-05	-	Phosphatase	Ph
G4	PT1_0044	histone lysine N-methyltransferase, putative	EF17587	-0.76	1.90E-04	-	-	histone lysine N-methyltransferase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0044	conserved hypothetical protein	EF17587	-0.76	1.90E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0052	protein kinase, putative	EF17590	1.47	2.07E-04	-	-	protein kinase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0052	conserved hypothetical protein	EF17590	1.47	2.07E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0054	formaldehyde lyase	EF17596	-1.06	1.38E-04	-	-	formaldehyde lyase	1.1E-05	-	Phosphatase	Ph
G3	PT1_0054	conserved hypothetical protein	EF17596	-1.06	1.38E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0057	bifunctional purine biosynthesis protein PURH, putative	EF17572	-1.00	3.45E-03	-	-	bifunctional purine biosynthesis protein PURH, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0057	conserved hypothetical protein	EF17572	-1.00	3.45E-03	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0068	conserved hypothetical protein	EF17580	-3.33	3.21E-05	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0068	conserved hypothetical protein	EF17580	-3.33	3.21E-05	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0042	conserved hypothetical protein	EF17583	-3.59	1.87E-03	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0042	12-oxophorbate oxidase, putative	EF17583	-3.59	1.87E-03	-	-	12-oxophorbate oxidase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0070	hyphal protein PTO_0070	EF17588	1.10	1.83E-03	-	-	hyphal protein PTO_0070	1.1E-05	-	Phosphatase	Ph
G4	PT1_0070	12-oxophorbate oxidase, putative	EF17588	1.10	1.83E-03	-	-	12-oxophorbate oxidase, putative	1.1E-05	-	Phosphatase	Ph
G2	PT1_0072	acetyl-coenzyme A synthetase	EF17588	-1.87	7.75E-09	-	-	acetyl-coenzyme A synthetase	1.1E-05	-	Phosphatase	Ph
G2	PT1_0072	acetyl-coenzyme A synthetase	EF17588	-1.87	7.75E-09	-	-	acetyl-coenzyme A synthetase	1.1E-05	-	Phosphatase	Ph
G1	PT1_0074	conserved hypothetical protein	EF17583	-1.59	1.38E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0074	conserved hypothetical protein	EF17583	-1.59	1.38E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0078	conserved hypothetical protein	EF17573	4.09	3.21E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0078	conserved hypothetical protein	EF17573	4.09	3.21E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0082	phosphatidylinositol kinase (PK-3)	EF17584	-1.78	1.27E-07	-	-	phosphatidylinositol kinase (PK-3)	1.1E-05	-	Phosphatase	Ph
G3	PT1_0082	conserved hypothetical protein	EF17584	-1.78	1.27E-07	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G6	PT1_0084	conserved hypothetical protein	EF17583	2.42	1.60E-08	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G6	PT1_0084	conserved hypothetical protein	EF17583	2.42	1.60E-08	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0089	hyphal protein PTO_0089	EF17584	0.79	2.59E-02	-	-	hyphal protein PTO_0089	1.1E-05	-	Phosphatase	Ph
G3	PT1_0089	conserved hypothetical protein	EF17584	0.79	2.59E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0086	conserved complex subunit CD, putative	EF17584	1.41	7.24E-08	-	-	conserved complex subunit CD, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0086	conserved complex subunit CD, putative	EF17584	1.41	7.24E-08	-	-	conserved complex subunit CD, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0099	dyein heavy chain	EF17582	-1.53	3.07E-06	-	-	dyein heavy chain	1.1E-05	-	Phosphatase	Ph
G3	PT1_0099	conserved hypothetical protein	EF17582	-1.53	3.07E-06	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0109	peckiosinase, putative	EF17588	-1.11	2.76E-04	-	-	peckiosinase, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0109	conserved hypothetical protein	EF17588	-1.11	2.76E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0102	5-methyltetrahydropteroylglutamate-homocysteine methyltransferase	EF17587	-1.43	1.51E-04	-	-	5-methyltetrahydropteroylglutamate-homocysteine methyltransferase	1.1E-05	-	Phosphatase	Ph
G4	PT1_0107	conserved hypothetical protein	EF17580	-2.49	2.00E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0107	conserved hypothetical protein	EF17580	-2.49	2.00E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0107	lysine-specific histone demethylase, putative	EF17580	-2.49	2.00E-01	-	-	lysine-specific histone demethylase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0107	conserved hypothetical protein	EF17580	-2.49	2.00E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0119	Foliate-Biotin Transporter (FBT) Family	EF17589	1.00	3.81E-02	-	-	Foliate-Biotin Transporter (FBT) Family	1.1E-05	-	Phosphatase	Ph
G1	PT1_0121	conserved hypothetical protein	EF17589	1.00	3.81E-02	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0148	conserved hypothetical protein	EF17580	1.59	1.22E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0148	glycylglycyl hydrolase, mitochondrial precursor	EF17580	1.59	1.22E-01	-	-	glycylglycyl hydrolase, mitochondrial precursor	1.1E-05	-	Phosphatase	Ph
G4	PT1_0148	ATP-binding cassette (ABC) superfamily	EF17580	1.59	1.22E-01	-	-	ATP-binding cassette (ABC) superfamily	1.1E-05	-	Phosphatase	Ph
G2	PT1_0124	hydroxy acid lyase (esterase) superfamily	EF17583	2.55	6.74E-06	-	-	hydroxy acid lyase (esterase) superfamily	1.1E-05	-	Phosphatase	Ph
G2	PT1_0124	lysine, putative	EF17583	2.55	6.74E-06	-	-	lysine, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0136	conserved hypothetical protein	EF17583	2.69	2.65E-06	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0136	conserved hypothetical protein	EF17583	2.69	2.65E-06	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0134	hyphal protein PTO_0134	EF17587	0.43	2.44E-01	-	-	hyphal protein PTO_0134	1.1E-05	-	Phosphatase	Ph
G4	PT1_0134	conserved hypothetical protein	EF17587	0.43	2.44E-01	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0132	conserved hypothetical protein	EF17587	1.46	3.22E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0132	conserved hypothetical protein	EF17587	1.46	3.22E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0137	conserved hypothetical protein	EF17583	-1.73	8.85E-09	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0137	conserved hypothetical protein	EF17583	-1.73	8.85E-09	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0136	glycosyl hydrolase, putative	EF17583	-2.02	4.27E-05	-	-	glycosyl hydrolase, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0136	glycosyl hydrolase, putative	EF17583	-2.02	4.27E-05	-	-	glycosyl hydrolase, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0138	beta-glucosidase, putative	EF17583	-1.70	2.11E-01	-	-	beta-glucosidase, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0138	beta-glucosidase, putative	EF17583	-1.70	2.11E-01	-	-	beta-glucosidase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0145	HCT1 E3 ubiquitin ligase, putative	EF17584	-2.41	8.41E-08	-	-	HCT1 E3 ubiquitin ligase, putative	1.1E-05	-	Phosphatase	Ph
G4	PT1_0145	conserved hypothetical protein	EF17584	-2.41	8.41E-08	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0140	conserved hypothetical protein	EF17584	-2.11	3.88E-08	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G1	PT1_0140	conserved hypothetical protein	EF17584	-2.11	3.88E-08	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0143	conserved hypothetical protein	EF17587	-2.89	5.93E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G2	PT1_0143	conserved hypothetical protein	EF17587	-2.89	5.93E-04	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G3	PT1_0152	Cu(I)-associated NEDD8 dissociated protein, putative	EF17584	-1.61	2.78E-06	-	-	Cu(I)-associated NEDD8 dissociated protein, putative	1.1E-05	-	Phosphatase	Ph
G3	PT1_0152	conserved hypothetical protein	EF17584	-1.61	2.78E-06	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	Ph
G4	PT1_0152	Zinc (Zn2+-ion F42) Peptidase (ZPF) Family	EF17580	-1.20	8.78E-05	-	-	Zinc (Zn2+-ion F42) Peptidase (ZPF) Family	1.1E-05	-	Phosphatase	Ph
G4	PT1_0152	conserved hypothetical protein	EF17580	-1.20	8.78E-05	-	-	conserved hypothetical protein	1.1E-05	-	Phosphatase	

