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Induction of extracellular glycosidases in filamentous fungi and their potential use in chemotaxonomy

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Data on the occurrence and inducibility of extracellular β-*N*-acetylhexosaminidase, α-galactosidase, α- and β-mannosidase and α-L-fucosidase, including inductors, are given for selected *Aspergillus, Penicillium* and *Fusarium* strains. These data represent additional information on the strains in the Culture Collection of Fungi, Department of Botany, Charles University, Prague, and in the Culture Collection of the Institute of Microbiology, Prague, Czech Republic, thus extending their usability in biochemistry and biotechnology. With respect to these biochemical data a taxonomic evaluation of the examined strains is presented. Several strains were re-identified after biochemical and morphological comparisons with the type strains. The strains of *A. niveus* CCF 544, *A. terreus* CCF 76, CCF 869, and CCIM USA were re-identified as *A. flavipes,* the strain *A. oryzae CCF 1301* as *A. wentii.*

Key words: glycosidases, induction, *Aspergillus, Penicillium, Fusarium*

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Pro vybrané kmeny rodů *Aspergillus, Penicillium a Fusarium* jsou uvedeny údaje o výskytu a indukovatelnosti β-*N*-acetylhexosaminidasy, α-galaktosidasy, α- a β-manosidasy a α-L-fukosidasy. Tyto údaje představují další informaci o kmenech ve Sbírce kultur hub při katedře botaniky Přírodovědecké fakulty Karlovy university a ve sbírce mikroorganismů Mikrobiologického ústavu AV ČR, což zvyšuje jejich využitelnost v biochemii a biotechnologii. S ohledem na získaná biochemická data bylo provedeno taxonomické vyhodnocení zkoumaných kmenů. Po biochemickém a morfologickém porovnání s typovými kulturami byly některé zkoumané kmeny přeurčeny. Kmeny *A. niveus* CCF 544, *A. terreus* CCF 76, CCF 869 a CCIM USA byly přeurčeny jako *A. flavipes,* kmen *A. oryzae* CCF 1301 byl přeurčen na *A. wentii.*

INTRODUCTION

Glycosidases from fungi have proved to be very useful in the preparation of many glycosidic structures, such as β-galactosides, β-glucosides, β-mannosides, β-*N*-acetylglucosaminides and β-*N*-acetylgalactosaminides, by transglycosylation or a reversed glycosylation (Křen et al. 1994, Taubken et al. 1993, Crout et al. 1992). Despite of the copious methods developed for the chemical synthesis

of glycosides, the use of enzymes as catalysts is an attractive alternative, since the sugar coupling steps can be performed with high stereoselectivity and certain regioselectivity (Křen and Thiem 1997). There is a continuous effort to identify and to characterise novel glycosidases applicable in biochemistry (Koga et al. 1991, Holazo et al. 1992, Yamamoto et al. 1986a).

Extensive screening to obtain the desired glycosidases from selected fungal strains originating mostly from the Culture Collection of Fungi, Department of Botany, Charles University, Prague was started. Our attention was concentrated mostly on the fungal genera already known for their glycosidase production. Induction by specific inductors (oligosaccharides, glycomimetics) enabled us to obtain the desired glycosidases in sufficient quality and quantity (Huňková et al. 1996a,b, Huňková et al. 1997).

In addition, biochemical characteristics, i.e. production of particular glycosidases, and their inducibility can reveal new chemotaxonomic features for better characterisation and eventual taxonomic re-identification of the strains. So far, profiles of secondary metabolites and isoenzymes were used as an aid in the identification of many fungal genera, e.g. *Penicillium* (Cruickshank and Pitt 1987, Frisvad and Filtenborg 1989, Paterson et al. 1989), *Aspergillus* (Zohri and Ismail 1994, Bridge and Hawksworth 1984), *Fusarium* (Wasfy et al. 1987), *Phoma* (Monte et al. 1991), *Monascus* (Bridge and Hawksworth 1985), and *Beauveria* (Mugnai et al. 1989). Glycosidase activities were also used in some of these studies but only in combination with other features (Bridge and Hawksworth 1984, Bridge and Hawksworth 1985, Bridge et al. 1989). Besides their production, enzyme inductibility and catabolic repression can provide an additional set of data reflecting regulatory systems and physiological typology of the strains studied. Biochemical and physiological differences can, therefore, help to identify species which can be hardly distinguished morphologically. Thus, we have also focused on strains requiring further taxonomic characterization. For comparison we examined in parallel strains derived from the type specimen (ex-type cultures).

Here, we summarize the data on the occurrence of the most important extracellular glycosidases and their inducibility. These results represent further biochemical information on the strains in the Collections and they can also serve as the basis for their chemotaxonomy.

Some glycosidases from our screening were already successfully employed for glycoside synthesis (Weignerová et al. 1996, Rajnochová et al. 1997, Huňková et al. 1997, Křen et al. 1998, Weignerová et al. 1998).

Material and methods

Strains and cultivation conditions

The strains used originated from the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, from the Culture Collection of the Institute of Microbiology (CCIM), Prague, Czech Republic, from the American Type Culture Collection (ATCC), Rockville, Maryland, U. S. A., and from the International Mycological Institute (IMI), Egham, U. K.

Before the glycosidase assay, the cultures were maintained on the slants [g/l]: agar-agar, 20; bacto-peptone, 5; malt extract, 35. Conical flasks (500 ml) with 100 ml of medium were inoculated with a suspension of spores in 0.1% Tween 80 solution. The flasks were cultivated on a rotary shaker at 28 C. Media used: Sabouraud's medium [g/l]: mycological peptone, 10; glucose, 40; pH 5.6. Casamino-acid medium [g/l]: yeast extract, 0.5; mycological peptone, 5; KH_2PO_4, 3; $NH_4H_2PO_4$, 5; casein hydrolysate, 7.5; pH 6.0. After sterilization each flask was supplemented with 0.5 ml of 10 % $MgSO_4.7H_2O$. The inductor supplemented medium (production medium) was the same as the casamino-acid medium, the casamino-acids were replaced by respective amounts of inductor. Concentrations of inductors are given in the respective cases – see Tab. 1–3.

For morphological examination of the *Penicillia* and *Aspergilli* three agar media were used: Czapek yeast-extract agar (CYA) and malt-extract agar (MEA) according to Pitt (1980), and wort-beer agar. *Fusarium* strains were cultivated on potato-glucose agar and on synthetic nutrient agar according to Nirenberg (1976).

Enzyme activity assay

Glycosidases were assayed using corresponding *p*-nitrophenyl glycosides as substrates according to Mega et al. (1970). Proteins were determined according to Bradford (1976) using the kit from Bio-Rad (München, Germany) with bovine serum albumin as a standard.

Morphological examination

The identity of the *Penicillia* and *Aspergilli* strains was checked according to Raper and Thom (1949), Raper and Fennell (1965), Pitt (1980), Klich and Pitt (1988), Tzean et al. (1990), and Samson and Pitt (1990). *Fusarium* strains were identified according to Burgess et al. (1988).

Preparation of crude chitin hydrolysate

Crude chitooligomers were prepared by the acid hydrolysis of chitine according to Rupley (1964).

Preparation of α-mannooligomers

Mannooligomers were prepared by the condensation of mannose catalysed by α-mannosidase from *Canavalia ensiformis* (jack bean) under conditions as

published (Ajisaka et al. 1995) and separated by gel filtration on BioGel P2 (BioRad, U. S. A.).

RESULTS AND DISCUSSION

Constitutive production of glycosidases

Many fungal strains have low basal levels of glycosidases. For their purification and characterisation constitutively produced glycosidases were used, e.g. *β-N*-acetylhexosaminidase from *Trichoderma harzianum* (Koga et al. 1991) and *Penicillium oxalicum* (Yamamoto et al. 1985), α-galactosidase from *Trichoderma reesei* (Savel'ev et al. 1996) and *Mortierella vinacea* (Shibuya et al. 1997), α-galactosidase and *β-N*-acetylglucosaminidase from *Aspergillus niger* (Bahl and Agraval 1969) . *A. niger* is frequently used as a source microorganism for purification and characterisation of numerous glycosidases or for enzymatic syntheses (e.g., Jones and Kosman 1980, Bahl 1970, Ajisaka and Skirakabe 1992, Itoh and Kamiyama 1995).

We have found low constitutive production of extracellular glycosidases in many fungal strains, e.g. *β-N*-acetylhexosaminidase in *A. oryzae* strains 0.1–1.3 U/mg prot. and in *A. terreus* strains 0.5–2.2 U/mg prot. (Huňková et al. 1996a), in *P. vinaceum* CCF 2384 2.2 U/mg prot., and in *P. oxalicum* strains 0.22–6.3 U/mg prot.; α-galactosidase in *A. terreus* strains 0.25–7.0 U/mg prot.; α-fucosidase in *Fusarium oxysporum* strains 0.25–0.44 U/mg prot. and in *A. flavipes* IMI 171885 0.10–0.15 U/mg protein. Low constitutive production of α-mannosidase was found in many strains and slight constitutive production of β-mannosidase was observed in several strains (see Table 1, 2). Such activities are, however, rather low for practical use. Higher constitutive production of *β-N*-acetylhexosaminidase was found in *P. chrysogenum* CCF 1269 (9.2 U/mg prot.). High constitutive production of *β-N*-acetylhexosaminidase (11.0–24.6 U/mg prot.) and α-galactosidase (7.3–32.9 U/mg prot.), and slight production (under 0.5 U/mg prot.) of α- and β-mannosidase and α-L-fucosidase was found in culture filtrates of *A. niger* CCIM K1 and CCIM K2, *A. awamori* CCF 763 and *A. phoenicis* CCF 61. However, contamination of the main activity by other glycosidases complicates their purification and use in bioorganic chemistry.

Induction and inductors

Some glycosidases in fungi are known to be inducible, e.g. α-galactosidase in *Penicillium ochrochloron* was induced by galactomannan from guar *(Cyamopsis tetragonobola)* gum (Dey et al. 1993), α-fucosidase in *Fusarium oxysporum* was substantially induced by L-fucose and slightly by D-arabinose (Yamamato et al. 1986b), *β-N*-acetylhexosaminidase in *Aspergillus oryzae* was strongly induced

Table 1 Glycosidases in *Aspergillus*

STRAIN (original name)	β-HexNAc		α-Gal	α-Man	β-Man	α-Fuc
	β-GlcNAc	β-GalNAc/ β-GlcNAc rate				
A. oryzae CCF 147	I+++ cho	0.26	I++ raf	I mo, MeMan C ca	0	0
A. oryzae CCF 172	I+++ cho	0.34	I+ raf	I mo	ND	ND
A. oryzae CCF 1063	I+++ cho	0.27	ND	ND	ND	ND
A. oryzae CCF 1065	I+++ cho	0.29	ND	ND	ND	ND
A. oryzae CCF 1066	I+++ cho I++ GlcNAc I+ chi I GlcN	0.39	I++ raf	I MeMan, mo C ca	0	0
A. oryzae CCIM NZS	I+++ cho	ND	ND	ND	ND	ND
A. oryzae CCIM NZZ	I+++ cho	ND	ND	ND	ND	ND
*A. oryzae CCF 1301**	C Glc	ND	0	0	0	ND
A. oryzae CCF 1602	I+ cho	0.26	I++ raf	I MeMan C ca	0	0
A. oryzae T IMI 16266ii	I++ cho	0.43	I raf	I MeMan C ca	0	0
A. flavus CCF 146	I cho	0.23	I++ raf	C ca, GlcN	0	0
A. flavus CCF 642	C ca, GlcN NI cho	0.54	I+++ raf	C ca	0	0
A. flavus CCF 814	I+ cho	0.60	I++ raf	I MeMan C ca	0	0
A. flavus CCF 1129	I++ cho I GlcN	0.66	I++ raf I GlcN	C ca	0	ND
A. flavus CCF 1624	C ca, GlcN NI cho	ND	I+ raf	C ca	0	0
A. flavus T IMI 124930	I++ cho I GlcN	0.57	I+++ raf I+ GlcN	C ca	0	0
A. parasiticus CCF 141	I cho	0.25	I+ raf I dGlc	C ca	0	0
A. parasiticus CCF 1298	I+++ cho I GlcN	0.59	I+++ raf I GlcN	I MeMan, mo	0	ND
A. parasiticus T IMI 15957lx	I+ cho	0.32	I++ raf I+ dGlc, GlcN	I MeMan, mo	0	0
A. sojae T IMI 191300	I cho	0.51	I++++ raf I dGlc, GlcN	C Glc, ca	0	0
A. flavofurcatis CCF 107	I+ cho	0.22	I+ raf	C ca	C- ca	0

Table 1 — Continued

STRAIN (original name)	β-HexNAc		α-Gal	α-Man	β-Man	α-Fuc
	β-GlcNAc	β-GalNAc/ β-GlcNAc rate				
A. flavofurcatis T IMI 124938	I+ cho I dGlc, GlcN	0.35	I++ raf I+ GlcN I dGlc	C ca	0	0
A. tamarii CCF 1665	I+ cho I dGlc, GlcN	0.30	I+++ raf	I MeMan C ca	0	0
A. tamarii CCF 2492	I++ cho	ND	I+ raf	ND	ND	ND
A. terreus CCF 55	I+ cho	0.50	I++ raf I++ mel I Gal	ND	C- Glc	ND
A. terreus CCF 57	NI cho	1.00	I raf	ND	ND	ND
A. terreus CCF 58	I cho	ND	I++ mel I++ raf	ND	ND	ND
A. terreus ATCC 20542	ND	ND	I+ raf, mel I Gal	ND	ND	ND
A. terreus T IMI 17294	I cho	0.46	I+ raf I dGlc, GlcN	0	0	0
A. terreus CCF 2539	I+ cho	1.01	I+ raf, mel I+ Gal	ND	C- Glc	ND
*A. terreus CCF 76**	I++++ cho	0.54	C ca NI raf, dGlc	ND	C- ca	ND
*A. terreus CCF 869**	I++++ cho I+++ GlcNAc	0.56	C ca NI raf, dGlc	ND	C- ca	ND
A. terreus CCIM LM	I cho	ND	I dGlc NI raf	ND	ND	ND
*A. terreus CCIM USA**	I++ cho	0.68	I dGlc NI raf	ND	ND	ND
A. flavipes CCF 1895	I++ cho NI dGlc, GlcN	0.70	I dGlc NI raf	I MeMan C ca	0	0
A. flavipes CCF 2026	I+++ cho NI dGlc, GlcN	1.00	I++ dGlc NI raf	0	0	0
A. flavipes T IMI 171885	I+++ cho NI dGlc, GlcN	0.90	NI raf, dGlc (C- Glc)	C ca	C Glc	C Glc
A. niveus CCF 544	I++++ cho	0.75	I dGlc NI raf	I MeMan C ca	0	0
A. niveus T IMI 171878	I cho C+ Glc	0.15	NI raf, dGlc C Glc	C- ca	C- Glc	0
A. niger CCIM K1	C++ Glc	0.40	C++ ca	C- ca	C ca	ND

Table 1 — Continued

STRAIN (original name)	β-HexNAc		α-Gal	α-Man	β-Man	α-Fuc
	β-GlcNAc	β-GalNAc/ β-GlcNAc rate				
A. niger CCIM K2	C++ Glc	0.42	C++ ca	C ca	C ca	ND
A. awamori CCF 763	C++ ca	ND	C+++ ca	C- ca	ND	ND
A. phoenicis CCF 61	C++ ca	0.49	C++ ca	C Glc	C ca, GlcN	C- Glc
A. versicolor CCF 2491	I++ cho I+ GlcN	0.09	I raf, GlcN	0	0	0

Abbreviations:

T	type strain
*	re-identified strain, see chapter Taxonomic evaluation
ND	not determined
	Constitutive production (basal levels are not given in case of unquestionable induction)
C-	specific activity below 0.02 U/mg prot.
C	spec. act. 0.02–2 U/mg prot.
C+	spec. act. 2–10 U/mg prot.
C++	spec. act 10–20 U/mg prot.
C+++	spec. act. more than 20 U/mg prot.
	Inductive production (compared with basal level)
I	induction less than 5×
I+	5–10×
I++	10–20×
I+++	20× — 50×
I++++	more than 50×
NI	no induction
β-HexNAc	β-*N*-acetylhexosaminidase
β-GlcNAc	β-*N*-acetylglucosaminidase
β-GalNAc	β-*N*-acetylgalactosaminidase
α-Gal	α-galactosidase
α-Ma	α-mannosidase
β-Man	β-mannosidase
α-Fuc	α-L-fucosidase
	Inductors and substrates used (concentrations)
Ara	D-arabinose (0.1 %)
Gal	galactose (1 %)
Glc	glucose (4 %; Sabouraud medium)
GlcNAc	*N*-acetylglucosamine (0.5 %)
dGlc	6-deoxyglucose (0.1 %)
GlcN	glucosamine (0.1 %)
Fuc	L-fucose (0.1 %)
MeMan	α-methylmanoside (0.1 %)
cho	chitooligomers (0.2 %; crude chitin hydrolysate)
chi	chitosan (MW 70000, 0.25 %)
raf	raffinose (0.2 %; raffinose pentahydrate)
mel	melibiose (0.2 %; melibiose monohydrate)
mo	α-mannooligomers (0.1%; crude α-mannooligomers)
ca	casamino acids (0.75 %; casein hydrolysate)

Table 2 Glycosidases in *Penicillium* (For legend and abbreviations see Table 1)

STRAIN (original name)	β-HexNAc		α-Gal	α-Man	β-Man	α-Fuc
	β-GlcNAc	β-GalNAc/ β-GlcNAc rate				
P. spinulosum CCF 2159	I cho	0.92	I raf, dGlc I GlcN	ND	0	0
P. vinaceum CCF 2384	C+ Glc	ND	I+++ raf	I MeMan C ca	C Glc	0
P. daleae CCF 2365	I+ cho I dGlc, GlcN	ND	I+ raf I dGlc, GlcN	0	0	0
P. melinii CCF 2440	I++ cho I GlcN	0.12	I+ raf	C- ca	0	0
P. brasilianum CCF 2155	I cho	0.75	I raf	I MeMan, mo	0	0
P. brasilianum CCF 2171	I++++ cho I++ dGlc	ND	I raf, dGlc	0	0	0
P. ochrochloron CCF 2379	C Glc	ND	I raf, mel	0	ND	ND
P. commune CCF 2962	0	ND	I+ mel	C- ca	ND	ND
P. chrysogenum CCF 1269	C++ Glc	ND	I+++ mel	C ca	ND	ND
P. funiculosum CCF 1994	I++ cho I dGlc, GlcN	1.20	I raf I+ dGlc	I MeMan, mo	0	I dGlc
P. funiculosum CCF 2325	I+ cho I dGlc, GlcN	0.76	I raf I+ dGlc	C- ca	0	I dGlc
P. purpurogenum var. *rubrisclerotium CCF 2984*	I+ cho	1.22	I raf I++ dGlc	0	C- Glc	I dGlc
P. purpurogenum var. *rubrisclerotium CCF 2985*	I cho	1.15	I raf I+ dGlc	I MeMan, GlcN	C- Glc	I dGlc
P. pitii CCF 2277	I cho	0.63	C+ ca	0	0	0
Talaromyces flavus CCF 2324	C Glc, ca	ND	I raf I++ dGlc	0	C Glc	0
P. oxalicum CCF 1659	I cho	2.8	ND	ND	ND	ND
P. oxalicum CCF 1667	I++ cho	2.0	ND	ND	ND	ND
P. oxalicum CCF 1959	I+ cho I dGlc	1.6	ND	I MeMan	ND	ND

Table 2 — Continued

STRAIN (original name)	β-HexNAc		α-Gal	α-Man	β-Man	α-Fuc
	β-GlcNAc	β-GalNAc/ β-GlcNAc rate				
P. oxalicum CCF 2315	I++++ cho	1.4	ND	ND	ND	ND
P. oxalicum CCF 2430	I++++ cho	2.3	ND	ND	ND	ND
P. oxalicum CCF 3009	I+ cho	1.4	ND	ND	ND	ND
*P. asturianum CCF 2062**	I cho	1.5	ND	ND	ND	ND

Table 3 Glycosidases in *Fusarium oxysporum* (for legend and abbreviations see Table 1)

STRAIN (original name)	β-HexNAc		α-GlcNAc	α-Gal	α-Man	β-Man	α-Fuc
	β-GlcNAc	β-GalNAc/ β-GlcNAc rate					
F. oxysporum CCF 377	I cho, Fuc, GlcN	0.18	ND	I+ raf I Fuc I dGlc, GlcN	0	0	I+++ Fuc I Ara
F. oxysporum CCF 483	I Fuc	ND	ND	ND	ND	ND	I++ Fuc
F. oxysporum CCF 906	I Fuc	ND	ND	ND	ND	ND	NI Fuc C ca
F. oxysporum CCF 1389	I Fuc	ND	ND	ND	ND	ND	NI Fuc C ca
F. oxysporum CCF 1414	I Fuc	ND	ND	ND	ND	ND	I++ Fuc

by aminosugar derivatives (Huňková et al. 1996a). For obtaining the glycosidases in higher amount, we tested glycosidase induction susceptibility of all examined strains. Extracellular production of certain glycosidases can be remarkably increased by induction (see Tables 1–3). The data in the tables represent induction in comparison with the basal level (casamino-acids medium without any supplement).

β-N-Acetylhexosaminidase

Overproduction of extracellular β-*N*-acetylhexosaminidase from *A. oryzae* by induction with aminosugar-containing inductors was already demonstrated by us

(Huňková et al. 1996a). Induction of this enzyme in *A. oryzae* strains was triggered by crude chitin hydrolysate (chitooligomers containing 2–10 glycosyl units together with ca 75% NaCl produced by neutralization of HCl used for hydrolysis), by *N*-acetylglucosamine and by chitosan. Because the best results were obtained with the crude chitin hydrolysate, we have chosen this preparation as an inductor for all tested strains. Production of a large series of *β-N*-acetylhexosaminidases can be improved in this way (Tab. 1). Moreover, by induction we obtained *β-N*-acetylhexosaminidases in high specific activity without a significant amount of other glycosidases in some strains, e.g. *A. terreus* CCF 76* and 869*, *A. terreus* CCIM USA*, *A. tamarii* CCF 2492, *P. oxalicum* CCF 1959 and with all strains of *A. oryzae* (except the strain CCF 1301*). Thus, culture filtrate containing *β-N*-acetylhexosaminidase could, after precipitation with for example ammonium sulphate, directly be used for many biochemical procedures (Rajnochová et al. 1997, Weignerová et al. 1996, Křen et al. 1998, Weignerová et al. 1997, Weignerová et al. 1998).

With some strains slight improvement of *β-N*-acetylhexosaminidase activity was also reached using 6-deoxyglucose, glucosamine or L-fucose.

β-N-*acetylhexosaminidase* with high *β*-N-*acetylgalactosaminidase* activity

As *β-N*-acetylhexosaminidase having high *β-N*-acetylgalactosaminidase activity and low *β-N*-acetylglucosaminidase activity was required, we have also determined the rate of these two activities. Most of *β-N*-acetylhexosaminidases from fungal sources have a low *β*-GalNAcase/*β*-GlcNAcase rate, being below 0.50 (Table 1–3). A higher *β*-GalNAcase/*β*-GlcNAcase rate was found only in several strains, e.g. *P. brasilianum* CCF 2155: 0.75, *P. funiculosum* CCF 2325: 0.76, *A. terreus* CCF 57: 1.00, *A. terreus* 2539: 1.01, *A. flavipes* CCF 2026: 1.00, *A. flavipes* IMI 171885: 0.90, *P. spinulosum* CCF 2159: 0.92, *P. funiculosum* CCF 1994: 1.20 and *P. purpurogenun* var. *rubrisclerotium* CCF 2985: 1.15 and CCF 2984: 1.22. Unique *β-N*-acetylhexosaminidase with the *β*-GalNAcase/*β*-GlcNAcase rate of about 1.50 was found only in culture filtrates of some strains of *P. oxalicum* (Huňková et al. 1997). These findings are in agreement with those of Yamamoto et al. (1985) obtained with the strain *P. oxalicum* IFO 5748 not exposed to induction. A high *β*-GalNAcase/*β*-GlcNAcase rate seems to be characteristic of the species *P. oxalicum*. Similar *β-N*-acetylhexosaminidase biochemical pattern was found only with *P. asturianum* CCF 2062[1)] (1.50). This species seems to be very close to *P. oxalicum*.

[1)] Strains with an asterisk (*) were re-identified due to the results obtained – see the part "Taxonomic evaluation".

α-Galactosidase

α-Galactosidase catalyzes hydrolysis of terminal α-galactosidic linkages of glycosides (Savel'ev et al. 1996, Kaneko et al. 1990). Both raffinose and melibiose can serve as inductors of extracellular α-galactosidase, giving similar induction rates (Huňková et al. 1996b). Cheaper raffinose was chosen for most of the experiments. After induction, extracellular enzyme was produced in several strains (e.g. *A. terreus* CCF 55, CCF 58, CCF 2539, ATCC 2052 and *P. commune* CCF 2962) in high specific activity and void of contaminating glycosidases (Huňková et. al 1996b). Thus, this enzyme could be directly precipitated by, e.g., ammonium sulphate and then directly used for further reactions (Weignerová et al. 1996, Weignerová et al. 1997, Weignerová et. al. 1998).

Weak induction of α-galactosidase was achieved using galactose. In some strains, a slight improvement of production was observed after adding of 6-deoxyglucose or glucosamine and in *F. oxysporum* also after adding of L-fucose.

In culture filtrates of the strains *Talaromyces flavus* CCF 2324, *P. funiculosum* CCF 2325, *P. purpurogenum* var. *rubrisclerotium* 2984 and CCF 2985, *A. fumigatus* CCF 1059, *A. flavipes* CCF 1895 and CCF 2026, *A. terreus* CCIM LM and CCIM USA*, and *A. niveus* CCF 544* induction of α-galactosidase was triggered by 6-deoxyglucose whilst raffinose induced slightly or not at all. Induction of α-galactosidase by 6-deoxyglucose in fungi is described here for the first time.

α-Mannosidase

Although the occurrence of α-mannosidase in different natural sources was described (Dey and Del Campillo 1984), no effective inductor for this enzyme was found yet. In several strains a slight improvement of extracellular α-mannosidase was observed in the growth phase of cultivation when using α-methylmannoside or α-mannooligomers, but in the lytic phase constitutive production of this enzyme predominated (*A. oryzae* CCF 1066, CCF 147, CCF 172 and IMI 16266ii, *A. flavus* CCF 814, *A. tamarii* CCF 1665, *A. flavipes* CCF 1895, *A. niveus* CCF 544*, *P. vinaceum* CCF 2384 – Table 1, 2). Slight induction both in the growth and lytic phases of cultivation was observed only in the strains *A. parasiticus* CCF 1298 and IMI 15957ix, *P. funiculosum* CCF 1994 and *P. purpurogenum* var. *rubrisclerotium* CCF 2985.

In some strains improvement of α-galactosidase and α-mannosidase was achieved using glucosamine. In our opinion, this improvement cannot be explained in terms of induction, similarly as the improvement of β-*N*-acetylhexosaminidase.

β-Mannosidase

No effective inductor for β-mannosidase was found so far. Brown copra meal (residual cake of coconut oil containing about 50% β-mannan) was used as a substrate in the screening test where *A. niger, A. awamori, A. sojae, P. wort-*

manni and *Emericella nidulans* were found to produce a detectable level of β-mannosidase (Holazo et al. 1992). For purification and characterization studies, the enzyme from *A. niger* produced constitutively was used (Monttreuil 1975, Elbein et al. 1977). In our study we observed slight constitutive production of extracellular β-mannosidase in several strains (*A. flavipes* IMI 171885, *A. niger* CCIM K1 and K2, *A. phoenicis* CCF 61, *P. vinaceum* CCF 2384, *P. purpurogenum* var. *rubrisclerotium* CCF 2984 and 2985 and *T. flavus* CCF 2324).

α-L-Fucosidase

Production of extracellular α-L-fucosidase was slightly induced by 6-deoxyglucose in *P. funiculosum* CCF 1994 and CCF 2325 and *P. purpurogenum* var. *rubrisclerotium* CCF 2984 and CCF 2985. Effect of 6-deoxyglucose as inductor for α-L-fucosidase was observed here for the first time.

On the contrary, in *F. oxysporum* extracellular α-L-fucosidase was not induced by 6-deoxyglucose but its production was improved by L-fucose addition in some strains. The best results were reached with *F. oxysporum* CCF 377, good induction was observed in *F. oxysporum* CCF 483 and CCF 1414. No induction was observed in the strains CCF 906 and CCF 1389. Our results obtained with *F. oxysporum* CCF 377 are consistent with the results of Yamamoto et al. (1986b) obtained with *F. oxysporum,* strain S252. Just as these authors we also found that the amount of induced α-L-fucosidase was increased proportionally with the concentration of L-fucose added (0.1–0.5%) and that the enzyme is slightly induced by D-arabinose.

Taxonomic evaluation

The data on the occurrence and inducibility of selected glycosidases represent additional information on the strains in the Collections. These data will appear in the addendum to the CCF Catalogue of filamentous fungi (Kubátová and Huňková 1998).

From the results given in the Tables 1–3 partial taxonomic conclusions can be deduced in such where more representatives of a particular species were examined, i.e. for *A. oryzae, A. flavus, A. terreus, P. oxalicum* and *F. oxysporum.*

The species *A. flavus, A. parasiticus, A. oryzae* and *A. tamarii* belong to economically important fungi, two first species producing aflatoxins, the other two species being used in food industry. All these species are included in the section *Flavi* of the genus *Aspergillus* and their morphological characters are very similar (Klich and Pitt 1988). The identification of these species based only on morphological features is difficult and quite often incorrect. Therefore, any aid in clarifying their taxonomy and their identification is very useful. Among our strains of *A. flavus, A. parasiticus* and *A. oryzae* large morphological variability was observed, especially in shape and surface texture of the conidias and in colony

habits. Some variability was found in the production of glycosidases as well. Contrary to *A. flavus,* the induction of *β-N*-acetylhexosaminidase in *A. oryzae* was higher than the induction of α-galactosidase, only the strain *A. oryzae* CCF 1602 differed by a higher induction of α-galactosidase. The morphological features of this strain were similar to the strains designated here as *A. tamarii.* Nevertheless, its correct placement in the species *A. oryzae* was confirmed by Dr. Z. Lawrence (IMI, Egham, U. K.). In *A. flavus* strains we found constitutive production of α-mannosidase (except the strain CCF 814) whilst in *A. oryzae* slight induction was observed in the growth phase of cultivation and constitutive procuction prevailed in the lytic phase. However, the fact that no effective inductor exists for α-mannosidase together with the fact that *A. flavus* CCF 814 exhibited typical morphological features makes definite conclusions impossible. In *A. oryzae* a slight induction of α-mannosidase in the growth phase was observed, in *A. flavus* (except the strain CCF 814) constitutive production of this enzyme was found and in *A. parasiticus* CCF 1298 and IMI 15957ix induction of α-mannosidase was observed both in the growth and lytic phase. Current results suggest that clarification of this situation requires detailed comparative study. Anyhow, one of the *A. oryzae* strains (CCF 1301) exhibited strong differences in the production of glycosidases manifested in the absence of induction of *β-N*-acetylhexosaminidase and α-galactosidase and in the absence of constitutive α-mannosidase production. After detailed morphological examination this strain was re-identified as *A. wentii.*

For the cultures of *A. terreus* (compared with the type strain IMI 17394) high induction of α-galactosidase by raffinose or melibiose was typical. In the studied strains of *A. flavipes* (including the type strain IMI 171885) slight constitutive production of α-galactosidase was observed, raffinose and melibiose being non-effective. Inducibility of *β-N*-acetylhexosaminidase in *A. flavipes* was very high but low or absent in *A. terreus.* In spite of the overall similarity of *A. terreus* and *A. flavipes*, these species have several distinct morphological features. *A. terreus* has remarkably shorter stipes, its phialides are more closely arranged and its colonies are growing faster. With respect to the differences in biochemical evaluations and distinct morphological features the strains *A. terreus* CCF 76, CCF 869 and CCIM USA were re-identified as to be *A. flavipes.*

Strain *A. niveus* CCF 544 was found to exhibit other biochemical characteristics than the type strain *A. niveus* IMI 171878 (see Table 1). Morphological examination revealed close resemblance of the strain CCF 544 to *A. flavipes* and the strain was therefore transferred to this species.

Black aspergilli, e.g. *A. niger, A. awamori* and *A. phoenicis,* are closely related to the section *Nigri* of the genus *Aspergillus.* Due to their morphological similarity, *A. awamori* and *A. phoenicis* were considered varieties of *A. niger* (Al-Musallam 1980, Klich and Pitt 1988, Kozakiewicz 1989). However, recent

molecular studies treated *A. awamori* and *A. phoenicis* as synonyms (Pařenicová et al 1997, Varga et al. 1997). Pařenicová et al. (1997) divided the studied strains of *A. awamori* and *A. phoenicis* into three groups: *A. niger, A. tubingensis* and *A. foetidus* varieties. Our results dealing with the glycosidase activity of four strains (constitutive production of all examined glycosidases) confirmed their close relationships. Nevertheless, correct placement of the four strains used in our study can be carried out only after a more detailed study involving comparative strains and molecular methods.

The slight induction of α-L-fucosidase by 6-deoxyglucose in *P. purpurogenum* var. *rubrisclerotium* CCF 2984 and CCF 2985 as well as in *P. funiculosum* CCF 1994 and CCF 2325 together with other biochemical similarities correspond with the fact that *P. purpurogenum* var. *rubrisclerotium* is a species close to *P. funiculosum*. Both species belong to the series *Miniolutea* of the subgenus *Biverticillium*.

P. oxalicum strains represent a group which could be biochemically characterized by induction of unique β-*N*-acetylhexosaminidase with a very high β-*N*-galactosaminidase/β-*N*-glucosaminidase rate. *P. asturianum* CCF 2062, which possesses the same β-*N*-acetylhexosaminidase, has been considered as synonym of *P. oxalicum* since 1990 when Frisvad and Filtenborg (1990) and Frisvad et. al. (1990) made a revision of *Penicillium* subgenus *Furcatum* based on secondary metabolites and conventional characters. Our results fully corroborate their conclusions.

F. oxysporum strains were preferentially examined for the production of α-L-fucosidase and β-*N*-acetylhexosaminidase, and no other glycosidases were studied yet. For chemotaxonomic evaluation it would be necessary to examine all glycosidases, not only in *F. oxysporum* but also in closely related species. *F. oxysporum* is related to *F. solani*. Therefore, besides a morphological study a test of ammonium salts agar with sorbitol according to Brayford and Bridge (1989) was carryed out. All *F. oxysporum* strains showed a red-vinaceous pigment on the colony reverse, one strain (CCF 1389) produced this pigment after a prolonged period of time (two weeks). Although the test confirmed the correct identification, the strains CCF 906 and CCF 1389 still differ from other strains by an absence of α-L-fucosidase induction.

Differences in the production and inducibility of glycosidases certainly cannot serve as a the only criterion for taxonomic determination of strains. Nevertheless, striking biochemical differences among strains of the same species drew our attention to a more detailed morphological examination of disputable strains and led us to eventual re-identification of some of them.

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