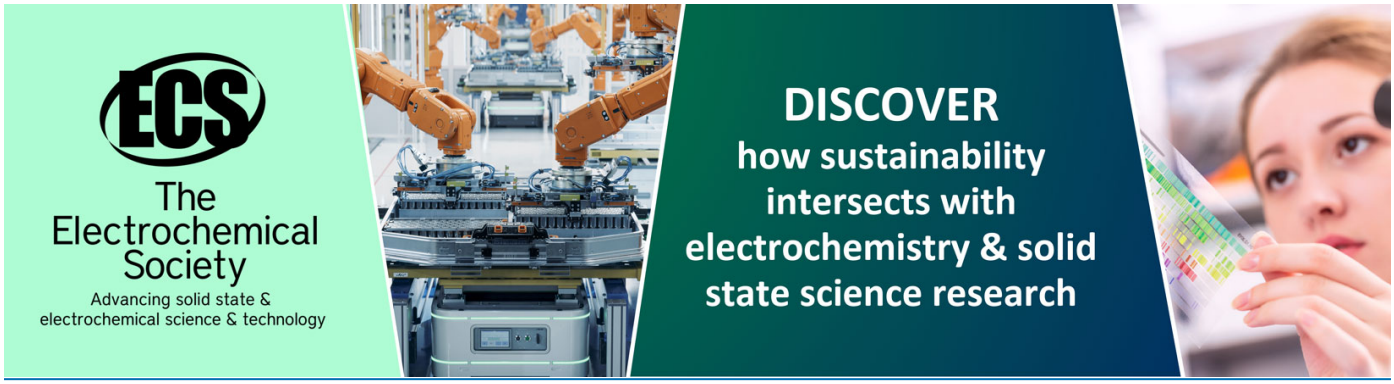
Antifungal activities of lactic acid bacteria and yeast isolated from various types of Tempe



Antifungal activities of lactic acid bacteria and yeast isolated from various types of Tempe

Ema Damayanti[1*], Nur Shabrina[2], Faisal Azis Prihantoro[2] and Maya Shovitri[2]

[1]Research Unit for Natural Product Technology, Indonesian Institute of Sciences (BPTBA-LIPI)
Jl. Jogja Wonosari KM 31.5, Gunungkidul, D.I.Yogyakarta, 55861, Indonesia
[2]Department of Biology, Faculty of Mathematic and Natural Science, Institute Teknologi Sepuluh Nopember, Surabaya, Indonesia

*Corresponding author: ema.damayanti@lipi.go.id

Abstract. Mycotoxin-producing fungi are harmful contaminants in food and feed products. Lactic acid bacteria (LAB) and yeast groups are known to produce antifungals. Indonesia is known as a Tempe-producing country, an important functional fermented food. Soybean Tempe has been commonly known and well-studied, while other types of Tempe such as Tempe Gembus, Tempe Koro, Tempe Lamtoro and Oncom have not been well studied. This study aimed to determine LAB and yeast population in various types of Tempe and to select antifungal activity against mycotoxin-producing fungi. Research methods included total aflatoxin analysis in Tempe products, enumeration and isolation of LAB and yeast, and antifungal assay. The results showed that the total aflatoxins in all Tempe products were 1.99 - 3.84 ppb and it was qualified as food for consumption. The total LABs were 7.16 - 8.25 $\log_{10}$ cfu/g while the total yeast was 4.48 - 7.38 $\log_{10}$ cfu/gram. The highest antifungal activities in mycotoxin-producing fungi such as *Aspergillus parasiticus* and *Penicillium citrinum*, shown by LAB G1 isolates which were identified as *Lactobacillus* sp. and yeast G6K1 and G6K2 which were identified as *Saccharomyces* spp. All the selected isolates were isolated from Tempe Gembus. The clear zone diameter of antifungals was 15-20 mm and 3.5 mm respectively for LAB G1 and G6K1-G6K2. These selected LAB and yeast had the potential to be used as natural bio-preservatives in functional food products to prevent the growth of mycotoxin-producing fungi.

1. Introduction

Mold is one of the main biological agents causing damage on food and feed products because it produces mycotoxins. Mycotoxins are produced by *Aspergillus*, *Fusarium*, and *Penicillium* spp. Mold is a natural contaminant that is generally present in grain-based foods (cereals) [1]. *Aspergillus* and *Penicillium* are two types of mold that dominant produce mycotoxins such as aflatoxin, ochratoxin, and patulin [2]. Aflatoxin is one of the important mycotoxins and it receives a lot of attention because it can cause health problems in humans and animals. In human and animal populations, aflatoxin is associated with both toxicity and carcinogenicity [2]. Health problems caused by the accumulation of aflatoxin in the body include carcinogens, mutagens, teratogens, immunosuppressants [3].

Lactic acid bacteria and yeasts are known to be potential natural agents that can inhibit the growth of mycotoxin-producing molds. Lactic acid bacteria especially *Lactobacillus* strains have the ability to

inhibit fungal growth and aflatoxin production [4]. Yeast is also reported to have potential antimicrobial activity against molds in food spoilage [5]. Some yeast species are also known to have enzymes that can degrade mycotoxins [1]. LAB and yeast are two groups of bacteria that are widely used in food processing because of their ability to improve food quality through metabolic processes such as fermentation.

Indonesia is a tropical country that has a large variety of traditional foods. Bean and legume-based foods are usually made by fermentation techniques using mold. Various types of *Rhizopus* mold are used in the fermentation process of soybean to make Tempe. Tempe that is commonly known is soybean (*Glicin max* L.) Tempe. The traditional process of making Tempe is by either steaming or boiling soybeans, dehulling the soybeans, mixing them with rice flour and fermenting them semi-aerobically using yeast. The most often used Tempe yeasts are *R. oligosporus* and *R. oryzae*, fermented for 1-2 days at room temperature. In addition to soybean Tempe, another type of fermented legume-based food commonly found in Java regions is Tempe Lamtoro (Klandingan or Manding) from *Leucaena leucocephala* bean. Tempe Koro made from tropical sword beans with white seeds (*Canavalia gladiata*) and jack beans with red seeds (*Canavalia ensiformis*) [6]. Tempe Gembus and also Oncom produced from by-products include peanut (*Arachis hypogaea*) press cake, solid waste from soybean curd production, solid waste from tapioca production (*onggok*) and dried coconut press cake [6,7] and fermented by *Neurospora sitophila* [8].

Lactic acid (LAB) bacteria and yeasts in Tempe have long been reported to be part of Tempe microbiota [9-11]. The types of yeast, *Candida* sp. and *Trichosporon* sp. isolated from Tempe are known to be able to inhibit the production of alfatoxin by *Aspergillus flavus* [12]. The information about LAB and yeast associated with various types of Tempe, apart from soybean Tempe, has not been widely reported. This study aimed to isolate lactic acid bacteria and yeast associated with various types of Tempe and to select LAB and yeast isolates which have antifungal activities against mycotoxin-producing molds.

2. Material and Method

2.1. Lactic acid bacteria and yeast enumeration

Samples of Tempe Koro, Tempe Lamtoro and Tempe Gembus were obtained from individual producers in Gading village, Gunungkidul, Yogyakarta, Indonesia, while Oncom sample was obtained from traditional markets in Bantul, Yogyakarta. Figure 1 shows the tempe sample used in this study. Five g of Tempe sample were mashed and dissolved in 45 ml of sterile NaCl 0.86% and then homogenized. Serial dilution was carried out up to 10^6. The total LAB and total yeast were enumerated by plating out 100 µL of sample using spread plate technique on agar media from three series of last dilution. The enumeration of LAB used de Man, Rogosa and Sharpe Agar (MRSA) + CaCO3 0.2%, incubated at 37 °C for 48 hours. The enumeration of yeast used Chloramphenicol Yeast Glucose Agar (CYGA), incubated at 28 °C for 48 hours, aerobics. The total colonies were counted using a colony counter.

2.2. Aflatoxin analysis

Supernatant samples (500 µL) were collected by extraction of Tempe using methanol (Merck, Germany) and analyzed by Elisa Reader (Multiscan, Thermo Scientific). The measurement of aflatoxin content was performed by ELISA method using AgraQuant Total Aflatoxin Assay procedure [13].

2.3. Lactic acid bacteria and yeast isolation

LAB and yeast colonies that grew as a single colony were isolated and refined on the same medium. LAB isolate was characterized through cell morphology observation including catalase test, motility test and Gram staining. Lactic acid bacteria have rod or cocci cell, negative catalase, non-motile and Gram positive. Yeast cells are usually larger than bacteria, vary in size with a width ranging from 1 to 5 µm and length ranging from 5 to 30 µm or larger. Yeast are usually egg-shaped, but some are elongated or ball-shaped and are positive catalase. Pure isolate was collected and stored in 20% glycerol at -20 ° C.

2.4. Antifungal assay

Antifungal assay was carried out on *Aspergillus parasiticus* FNCC 6033 and *Penicillium citrinum* FNCC 6111 obtained from the Food and Nutrition Culture Collection (FNCC), Gadjah Mada University Yogyakarta, Indonesia. *A. parasiticus* and *P. citrinum* were grown on Malt Extract Agar (MEA; Oxoid) medium at 30 °C for 5-7 days. The spores were then harvested from the plate by dripping 0.05% of Tween 80 solution on the surface of the mold spores and homogenized using sterile triangular stems. Dissolved spores were taken aseptically using a micropipette. The concentration of the mold spores was then calculated using a Haemocytometer (Marienfeld, Germany) and adjusted to 10^7/mL of the total spores [14]. LAB and yeast isolates were selected for qualitative antifungal assay using the overlay method [15]. LAB isolates were grown in MRSA medium using a streak plate method, incubated for 48 hours at 37 °C, while yeasts were grown on CYGA medium with a streak plate method and incubated for 48 hours at 30 °C. After growing, soft MEA (15%) contained mold spores was added to the plate, then incubated aerobically at 30 °C for 2-3 days. Antifungal activity was detected from the clear zone that appeared around the LAB or yeast colony. LAB and yeast isolates showing antifungal were selected for quantitative antifungal assay using the disc diffusion method. Secondary metabolites were obtained by separating the supernatant and biomass from the fermentation product in MRSB for LAB and YGB for yeast. A total of 50 µL supernatant containing secondary metabolites were dripped in a sterile disc paper and then placed on the surface of the MEA media containing mold spores. The inhibition zone diameter was measured after incubation at 30 ºC for 3 days.

3. Result and Discussion

Various types of Tempe samples used in this study and mold and yeast population found on CYEA media are shown in Figure 1. Various types of mold and yeast grew on the Tempe samples. There were differences in morphology, especially mold in various Tempe. Molds as a primary inoculum used to produce Tempe vary in types, depending on the raw materials. Tempe Gembus is Tempe derived from tofu pulp (soybeans) fermented with mold *Rhizopus* sp. [16], while Tempe Lamtoro (or referred to as Tempe Manding by the Javanese people) is made from lamtoro (*Leucaena leucocephala*) [6]. Tempe Koro is Tempe derived from velvet beans (*Mucuna pruriens* L) fermented with mold *Rhizopus* sp., while Oncom is a fermented food derived from groundnut or sometimes a mixture of groundnut cake and tofu waste, fermented by *Neurospora sitophila* [17]. The main genus for tempe production is *R. microsporus*, with varieties *microsporus*, *oligosporus*, *rhizopodiformis* and *chinensis*. In traditional process, cottonwood leaves (*Hibicus tiliaceus*) are used as a carrier for Tempe mould starter locally known as *usar* in Indonesia [18].

Some publication about indigenous fermented foods of Southeast Asia showed several processes to make various types of Tempe that exist in Indonesia, especially in Java regions. Tempe lamtoro is made by boiling the seeds with ash for about 2 hours. The cooked seeds are then dried overnight followed by dehulling process. The seeds are then soaked overnight, washed and soaked again until foam appears on the surface and boiled again for around 2.5 hours. After boiling, the seeds are dried, cooled and inoculated with mold wrapped in leaves and incubated for 36 - 48 hours [6].

Tempo Koro is also made in a similar way. *C. gladiata* beans are boiled in water containing wood ash. This method functions to absorb the odor and the bitter taste of the beans and prevent them from sticking back after dehulling. After boiling for about 1 hour, beans are dried and dehulled. The hull is soaked for 3 days by replacing the water daily to remove any poisonous content. The beans are then washed and cooked for about 30 minutes until the beans are soft. After cooking, the beans are dried again, ground and cut into small pieces and then inoculated with mold and wrapped in banana leaves. The fermentation process takes around 48 hours. Tempe Gembus is made from tofu waste. Waste from the process of making tofu is not only used for animal feed but also still consumed by humans. Tofu pulp is steamed for 1 hour, dried, cooled and inoculated with *R. oligosphorus* or mixed molds, wrapped in plastic or banana leaf and incubated at room temperature for around 24 hours. Tempe gembus is widely produced in both Central Java and Yogyakarta [6].

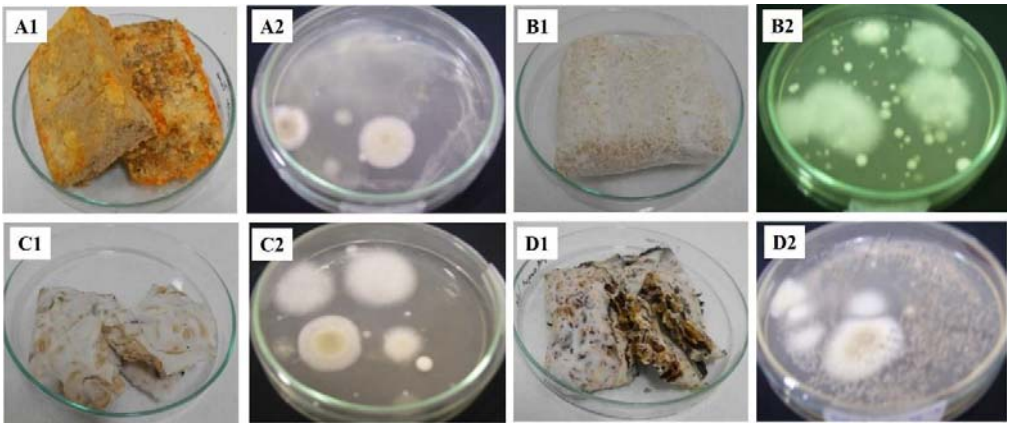


Figure 1. Oncom (A), Tempe Gembus (B), Tempe Koro (C), and Tempe Lamtoro (D). Number 1 shows the image of Tempe sample and number 2 shows various types of mold and yeast that grew on agar medium.

Oncom is a traditional food from West Java which is made from food waste such as peanut cake, and a mixture of tofu waste and sometimes added with tapioca waste (*onggok*) and dried coconut pulp. There are two types of oncom, namely black oncom which uses *R. oligosporus* and red oncom that uses *Neurospora intermedia* var. *oncomensis*. Oncom is made by soaking groundnut cake for 3-4 hours and soaking *onggok* for 1 hour, then mixing the two and boiling them for 1 hour, after which they are made into a thin layer. After cooling down, the mixture is inoculated with oncom yeast and placed in a bamboo container with a banana leaf. Incubation is carried out at room temperature 25-30 °C for 36-48 hours [7].

The nutrient value of various types of Tempe is shown in Table 1. Tempe Koro has the highest carbohydrate content and Oncom has the highest protein content. The aflatoxin content of all Tempe is less than 5 ppb. The Indonesian food and drug authority (BPOM RI) set a maximum limit of total aflatoxin in food products at 35 ppb [19]. Meanwhile the US FDA and EU's public health division limit the mycotoxin levels in food and animal feed at 20 ppb (US) and 4-15 ppb (EU) [20]. Based on these regulations, the total levels of aflatoxin in the four types of Tempe are within the safe limits for consumption. The low aflatoxin content in all the types of Tempe is possible due to the presence of lactic acid bacteria as shown in Figure 2. A study by Ghanbari shows that the inhibitory effect of LAB against *A. parasiticus* growth occurs in a population of 2 x 10^3 cfu/ mL of *L. plantarum* and *L. delbrueckii* subsp. Aflatoxin production is significantly inhibited, seen from the fact that the expression of the aflR gene greatly decreases after fungi is exposed to LAB [21]. Therefore, LAB acts as an antifungal against *A. parasiticus* and is effective in reducing aflatoxin production.

Table 1. Nutrition and aflatoxin content on various types of Tempe.

Parameter	Oncom	Tempe Gembus	Tempe Koro	Tempe Lamtoro
Carbohydrate (%)*	4	12	23	21
Crude protein (%)*	20 – 30	3,4	10	1
Fat (%)*	-	0.2	1.3	0.5
Crude fiber (%)*	2	3.9	-	-
Total aflatoxin (ppb)**	3.84 ± 1.00	2.15 ± 0.27	1.99 ±1.16	3.27 ± 1.62

*source: [22]
**source: this study

The number of LAB and yeast colonies from the samples of Tempe Gembus, Tempe Lamtoro, Tempe Koro and Oncom is shown in Figure 2. The highest number of LAB was obtained from Oncom with 8.25 $\log_{10}$ cfu/g while the lowest one was in Tempe Gembus (7.16 $\log_{10}$ cfu/g sample). The highest number

of yeast was found in Tempe Gembus (7.38 $\log_{10}$ cfu/g) while the lowest number of yeast was found in Oncom i.e. 4.48 $\log_{10}$ cfu/g sample. The variation in the number of LAB and yeast populations in various types of Tempe is due to substrate, mold types and processes in each type of Tempe. Another study mentioned that LAB and yeast are part of a common microbial community during the production of soybean Tempe and remain in fresh Tempe products. Different Tempe production methods affect the presence of LAB and yeast. The highest population of LAB and yeast was found in fresh soybean Tempe, which was between 6.54 - 9.70 $\log_{10}$ cfu/g. Filotype identification found during the production of Tempe, there are several species and LAB strains that belong to the genus *Aerococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Weissella* [9]. Another study stated that a high number of LAB at the initial step of Tempe processing is correlated with low pH at the initial step and this may also contribute to inhibiting the growth of *Enterobacteriaceae* and bacterial spores. The presence of yeasts and LAB up to 7 - 8 $\log_{10}$ cfu/g during fermentation indicates that those microorganisms contribute to fermentation process of Tempe [10]. In other studies, the LABs identified in the process of Tempe fermentation are the heterofermentative *Lactobacilli* group and *Streptococcus* non enterococci. *Lactobacillus* is the dominant LAB found in each stage of Tempe fermentation using *Rhizopus* mold [23].

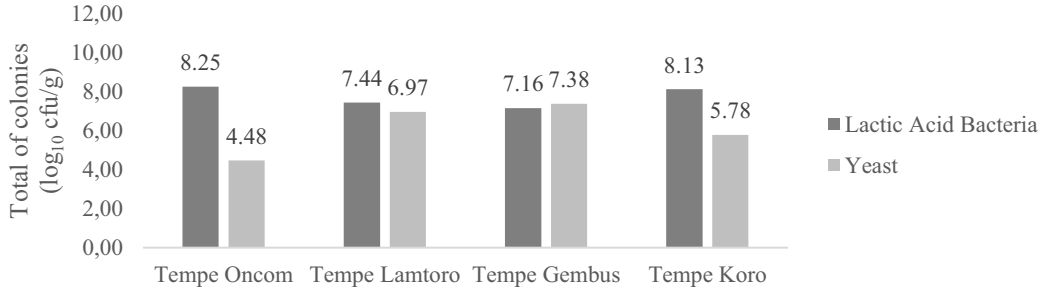


Figure 2. The number of lactic acid bacteria and yeast colonies in various types of Tempe samples.

The results of antifungal assay of LAB and yeast isolates obtained in this study are shown in Table 2. Lactic acid bacteria (LAB) isolates that showed qualitative and quantitative antifungal activity included O2 and O5 isolates from Oncom, KL2 and KL5 from Tempe Lamtoro and G1 from Tempe Gembus. The highest antifungal activity was produced by LAB G1, namely 15-20 mm. Yeast isolates showed higher antifungal activity. Antifungal activity produced by LAB in this study was relatively lower than the antifungal activity produced by other *Lactobacillus* sp. isolated from Kunu against *A. flavus* which was 5-20 mm [24]. Another study also revealed that *Lactobacillus* from some Nigerian fermented food resulted in 6 – 18 mm antifungal activity against *P. citrinum* [25]. In fact, this study showed that there were 8 yeast isolates which showed antifungal activity both in qualitative and quantitative method. Yeast G6K1 and G6K2 isolates from Tempe Gembus showed the highest antifungal activity (3.5 mm). The clear zone of LAB and yeast antifungals on overlay method is shown in Figure 3 (A1 and A2). Antifungals from lactic acid bacteria appear to be higher than those from yeast isolates.

Morphological analysis of LAB G1 isolates showed negative catalase non-motile gram-positive rod. Based on these characteristics, LAB G1 is the genus of *Lactobacillus* as in Figure 3 (B2). LAB is mostly used in the making of various fermented foods, where they contribute to improving their shelf life, organoleptic properties, and nutritional value. One of the most commonly used LABs in fermented foods is *Lactobacillus* [5]. LAB is widely used as a bio-preservative in food because it is known to produce bacteriocin compounds and antifungal compounds [26].

Table 2. Antifungal activities of lactic acid bacteria and yeast isolated from various types of Tempe.

Lactic acid bacteria	Clear zone*		Average of inhibition zone (mm)**		Yeast	Clear zone*		Average of inhibition zone (mm)**	
	Ap	Pc	Ap	Pc		Ap	Pc	Ap	Pc
O2	+	-	2	0	O5K1	-	-		
O4	-	-	0	0	G5K1	+	+	3	4
O5	+	-	10	0	G5K2	+	-	3	0
O6	-	-	0	0	G6K1	+	+	3.5	3.5
KL1	-	-	0	0	G6K2	+	+	3.5	3.5
KL2	-	+	0	10	G6K3	-	+	0	4
KL3	-	-	0	0	G6K4	+	-	0.3	0
KL4	-	-	0	0	KO5K1	-	-	0	0
KL5	++	-	15	0	KO5K2	-	-	0	0
KL6	-	-	0	0	KL15K1	+	+	0	4
G1	++	++	15	20	KL15K2	-	-	0	0
G3	-	-	0	0	KL15K3	+	-	2.5	0
G5	-	-	0	0	KL15K4	+	-	2.5	0
KO2	-	-	0	0	KL15K5	-	-	0	0
KO3	-	-	0	0					
KO4	-	-	0	0					
KO5	-	-	0	0					

* overlay method (qualitative)
** diffusion method (quantitative)
Ap = *Aspergillus parasiticus* FNCC 6033; Pc = *Penicilium citrinum* FNCC 6111
O = Oncom; G = tempe gembus; Ko = tempe koro; K; = tempe klandingan
–: 0 cm; +: 0,1-1 cm; ++: 1,1-2 cm; +++: 2,1-3 cm; dan ++++: ≥ 3,1 cm.

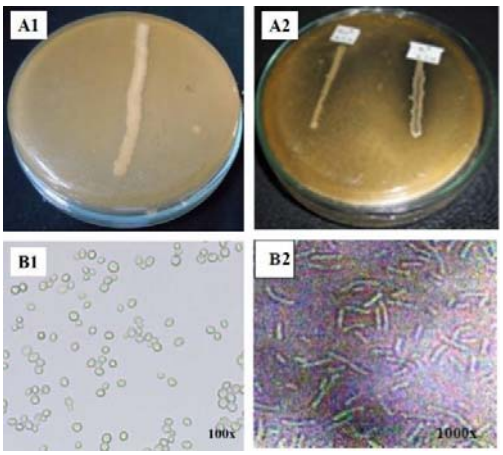


Figure 3. Clear zone on agar media indicated antifungal activities of yeast (A1) and lactic acid bacteria (A2). Morphological microscopic of yeast (B1) and lactic acid bacteria (B2).

Yeast G6K1 has characteristics categorized as the genus *Saccharomyces* as shown in Figure 3 (B1) which has budding and egg-shape and unicellular cell. *Saccharomyces* is known as a probiotic because it is non-toxic and able to survive in food products in the long-term condition [27]. Antagonistic ability

of yeast has been attributed mainly to competition for nutrients, pH changes in the medium as a result of growth cells and organic acid production, tolerance to high concentrations of ethanol and the secretion and release of antimicrobial compounds, such as killer toxins or "mycocins" [28].

Thus, the presence of lactic acid bacteria and yeast in various types of Tempe products has a role as an inhibitor of the growth of mycotoxin-producing fungi. This is indicated by the low content of aflatoxin in Tempe products such as Tempo Koro, Tempe Lamtoro, Tempe Gembus or Oncom (Table 1). In addition, other studies mentioned that the total aflatoxin content in whole and fresh groundnuts was 42.3 ppb while those could reach 108 ppb in contaminated groundnuts [29,30]. After fermentation process to produce Oncom, the total aflatoxin decreased to 3.8 ppb as shown in this study. In addition to being able to inhibit the growth of aflatoxin-producing fungi, LAB and yeast are also known to have aflatoxin binding. Previous studies showed that *L. plantarum* G7 and *S. cerevisiae* B18 have aflatoxin binding ability [31,32].

4. Conclusion

The total aflatoxins in all the Tempe products were 1.99 - 3.84 ppb, thus qualified as food for consumption. The total LAB in Tempe product was 7.16 - 8.25 $\log_{10}$ cfu/g while the total yeast was 4.48 - 7.38 $\log_{10}$ cfu/gram. *Lactobacillus* sp. G1 and *Saccharomyces* spp. G6K1 and G6K2 which were isolated from Tempe Gembus showed the highest antifungal activities against mycotoxin-producing fungi *Aspergillus parasiticus* and *Penicillium citrinum*. These selected LAB and yeast were potential to be used as natural bio-preservatives in functional food products to prevent the growth of mycotoxin-producing fungi.

Acknowledgments

This research was funded by DIPA BPTBA LIPI 2014. Authors thank Sri Endartini and Madina Nurohmah for sample preparation, and Lusty Istiqomah for technical assistance in aflatoxin analysis.

References

[1] Reddy K R N, Salleh B, Saad B, Abbas H K, Abel C A and Shier W T 2010 *Toxin. Rev.* **29**
[2] Bennett J and Klich M 2003 *Clin. Microbiol. Rev.* **16**, 497–516
[3] Topcu A, Bulat T, Wishah R and Boyac I H 2010 *Int. J. Food. Microbiology* **139** 202–5
[4] Gomah N H, Ragab W S and Bullerman L B 2010 *Assiut. J. Agric. Sci.* **40** 27-36
[5] Salas M L, Mounier J, Valence F, Coton M, Thierry A and Coton E 2017 *Microorganism* **5**
[6] Astuti M 2015 Tempe from other Pulses in *Indigenous Fermented Foods of Southeast Asia*, edited by J. D. Owens. (Ner York: CRC press)
[7] Kuswanto K R 2015 Indonesian oncom (fermented food processing by product)" in *Indigenous Fermented Foods of Southeast Asia*, edited by J. D. Owens. (New York: CRC press)
[8] Nout M J R and Aidoo K E 2010 "Asian Fungal Fermented Food Industrial Applications", 2nd Edition *the Mycota X* edited by M. Hofrichter (Springer-Verlag Berlin Heidelberg)
[9] Efriwati, Suwanto A, Rahayu G and Nuraida L 2013. *Hayati J. Biosci.* **20** 57-64
[10] Nurdini A L, Nuraida L, Suwanti A and Suliantari 2005 *Int Food Res. J.* **22** 1668-74
[11] Pangastuti A, Alfisah R K, Istiana N I, Sari S L A, Setyaningsih R, Susilowati A, *et al.* 2019 *Biodiversitas* **20**
[12] Purwijantiningsih E, Hariyadi RD, Nurwitri C C and Istiania 2005 *Biota* **X** 146-53
[13] AgraQuant Total Aflatoxin. https://www.romerlabs.com/shop/inter_en/agraquant-r-total-aflatoxin-elisa-test/
[14] Yang E J and Chang H C 2010 *Int. Food Microbiol.* **139** 56-63

[15] Ström K 2005 "Fungal inhibitory lactic acid bacteria characterization and application of *Lactobacillus plantarum* MiLAB 393", Doctoral thesis, Swedish University of Agricultural Sciences Uppsala
[16] Sulchan M and Rukmi M G I 2007 *Med. J. Indones.* **16** 205
[17] Sastraatmadja D D and Saono S 1984 *Berita Biologi* **2** 9-10
[18] Nout M J R and Kier J L 2005 *J. Appl. Microbiol.* **98** 789–805
[19] BPOM RI (Badan Pengawas Obat dan Makanan RI), Keputusan Nomor HK.00.05.01.4057 Tentang Batas Maksimum Alfatoksin Dalam Produk Pangan 2004
[20] Alshannaq A and Yu J H 2017 *J. Environ. Res. Public*. Health. **14** 632
[21] Ghanbari R, Aghaee E M, Rezaie S, Khaniki G J, Alimohammadi M, Soleimani M *et al.* 2018 *J. Food Saf.* **38** e12413
[22] Owen J D 2015 "Indonesian oncom (fermented food processing by product)" in *Indigenous Fermented Foods of Southeast Asia*, edited by J. D. Owens. (New York: CRC Press)
[23] Pisol B, Nuraida L, Abdullah N, Suliantarari and Khalil K A 2013 *Proceeding 4th International Conference on Biology, Environ. Chem IPCBEE* **58**
[24] Olonisakin O O, Jeff-Agboola Y A, Ogidi O C and Akinyele B J 2017 *Prev. Nutr. Food Sci.* **22** 138-43
[25] Adebayo C O and Aderiye B I 2010 *Res. J. Microbiol.* **5** 1070–80
[26] T Bintsis 2018 *J. Bacteriol. Mycol.* **6** 89–94
[27] Lara-Hidalgo C E, Hernández-Sánchez H, Hernández-Rodríguez C and Dorantes-Álvarez L 2017 *J. Nutr. Metab.* **4** 1045
[28] Muccilli S and Restuccia C 2015 *Microorganisms* **3** 588-611
[29] Rubak Y T and Purawisastra S 2011 *PGM* **34** 21-8
[30] Windyarani A "Populasi *Aspergillus flavus* dan kandungan aflatoksin B1 pada biji kacang tanah mentah dan produk olahannya di Kecamatan Bogor Tengah, Kotamadya Bogor", Undergraduate Thesis, Department of Biology, Faculty of Mathematic and Natural Science, Institute Pertanian Bogor, 2009
[31] Damayanti E, Istiqomah L, Saragih J E, Purwoko T and Sardjono 2017 *IOP Conf. Series: Earth and Environmental Science* **101** 012030
[32] Istiqomah L, Damayanti E, Arisnandhy A, Setyabudi F M C S and Anwar M 2019 *AIP Conference Proceedings* **2099** 020009