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Production of Dried *Lactobacillus plantarum* HL-15 Culture for Inhibition Growth of Mycotoxin Producing Fungi

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Abstract: *Lactobacillus plantarum* HL-15 is able to inhibit the growth of mycotoxin producing fungi. To support the application of that culture, the research aimed to produce dried *L. plantarum* HL-15 and observe its stability during storage was conducted. Production of dried culture was started by fermentation of *L. plantarum* HL-15 then centrifuged to get the pellet. The pellet was mixed with filler (rice flour or tapioca) with a ratio 1:1 (pellet:filler (10%), v/v) then dried. The temperature of the inlet and outlet of the spray dryer used were 105 °C and 65 °C, respectively. Dried culture was packaged in aluminum foil and sealed then stored at 4 °C. Results showed that viable cells of dried inoculum with rice flour filler was 11.99 ± 0.01 log CFU/g and dried inoculum with tapioca filler was 11.90 ± 0.04 log CFU/g. Spray dried *L. Plantarum* HL-15 was proved being able to inhibit the growth of *Aspergillus niger*. During six months storage, there is a decrease in the viability of spray dried *L. plantarum* HL-15 either with rice flour or tapioca filler. During four months of storage, there was a decrease in the activity of inhibition of spray dried culture of *L. plantarum* HL-15 both with fillers of rice flour and tapioca to the growth of *A. niger*. *L. plantarum* HL-15 spray dried cultures could be stored at 4 °C for four months for tapioca and five months for rice flour as filler. *L. plantarum* HL-15 spray dried cultures could inhibit the growth of *A. niger* so it could be used as a culture for inhibiting the growth of mycotoxin producing fungi in food.

Key words: Spray dried culture, *Lactobacillus plantarum* HL-15 culture, mycotoxin, antifungal in food.

1. Introduction

Mold in agricultural crop is undesired because it defects the quality of food product and potentially produces mycotoxin, for example in chocolate product from cocoa bean [1]. Fungiare found in dried cocoa beans namely *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., *Rhizopus* sp., *Mucor* sp. and *Verticillium* sp. [2]. The existence of a family *Aspergillus* (*A. glaucus*, *A. niger*, *A. flavus* and *A. tamarii*), *Penicillium* and *Mucor* could potentially lead to mycotoxins in cocoa beans and hydrolyze fats into short-chain fatty acids [3, 4]. Another negative impact of the presence of mold in cocoa beans is the emergence of off-flavor due to the activity of lipolysis enzymes [5]. *Aspergillus* has the potential to produce Okratoksin-A (OTA), one of mycotoxins with carcinogenic and immunotoxic effects. Due to health effects, the EU has set a maximum OTA limit on some food which will affect international trade in some food commodities [6].

Control of fungal growth with biological methods can be used as a step to prevent contamination of mycotoxins in the production of food chains. The lactic acid bacteria present in cocoa fermentation have potential inhibitory properties against mycotoxin-producing fungi [7]. *Leuconostoc paramesenteroides* BK 15 and BK 24 and *Pediococcus* spp. BK 14 is able to inhibit the growth of *A. flavus*, *A. niger*, *A. ochraceus* and *Penicillium* spp. [8].

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Lactobacillus fermentum F1L-6an which is able to inhibit the growth of *A. niger*, *A. fumigates* and *Mucor* spp. [9]. *L .plantarum* B4496, *L. brevis* 207 and *L. sanfranciscensis* BB12 showed interesting *in vitro* broad antifungal activities towards the three ochratoxin-producing fungi (*A. carbonarius*, *A. niger* and *A. ochraceus*) [10]. *L. mesenteroides* (L16B, B28C), *L. plantarum* (B40B, B88C), *L. fermentum* (B88C, B40B), *L. paramesenteroides* L16A and *L. casei* (L16A, B28C) produce compounds that are antifungal toward *A. flavus*, *A. ochraceus* and *Penicillium* spp. [11].

Fungal food damage caused by fungi is a major concern for the food industry. The use of lactic acid bacteria to reduce fungal growth is a promising solution. Lactic acid bacteria produce antifungal compounds in the form of organic acids (lactic acid, acetic acid and propionate acid), cyclic peptides and acetic acid which can interfere with the growth of pathogenic microorganisms [12, 13]. Inhibition of organic acids on microorganisms is associated with a decrease in pH value. The undissociated acid molecule can diffuse into the cell. The resulting organic acid may inhibit the germination of conidia in the fungus [14]. *L. plantarum* produces a metabolite with a low molecular mass that can inhibit the *P. avenaceum* [15] fungus. *L. reuteri* produces a secondary metabolite called Reuterin which can inhibit fungi. Reuterin is a highly reactive group of aldehydes that can react with the thiol and protein groups causing oxidative stress in the cells so that cells will be inhibited [13].

Liquid starter culture usually has limited shelf life and bulky. Cultures can be preserved in frozen and dry form in order to extend its shelf life. Frozen culture has long shelf-life. However, lactic acid bacteria could lose their viability and ability to produce acid during freezing [16]. In addition, frozen culture need sub-zero temperature for transportation and storage. Compared to liquid and frozen cultures, freeze dried culture has benefits due to the light and compact forms, but it is quite expensive.

Dry culture is a good long-term preservation form as a stock of cultures [17]. There are several drying methods developed such as spray drying, oven drying, drum drying, vacuum drying, fluidized bed drying and water drying. Of the many methods of drying, spray drying began to be widely developed as a technique of preservation of starter culture because it has high speed and continuous production so it is very useful for drying starter culture in large quantities [18]. Removing of water using spray-drying could reduce cost up to six times per kilogram of water compared to the one using freeze-drying [19].

Spray drying method has been widely used for drying of lactic acid bacteria. In the drying of the probiotic strain of *L. acidophilus* using a spray dryer, there is a decrease of approximately one log cycle depending on the temperature of the outlet or filler used [20]. *Lactobacillus* spp. culture which is isolated from kefir could be dried using spray drying method [21]. *L. plantarum* Dad 13, *Streptococcus thermophilus* FNCC 040 and *L. bulgaricus* FNCC 041 could be dried using spray drying method and applied to the production of yogurt [22].

Filler needs to be added to the production of dry cultures. The purpose of adding filler is to support target microbial growth and maintain the desired microbial population over a period of time [23]. Filler must be able to act as a protector for lactic acid bacteria from the effect of heat during drying, safe to consume and cheap price so that final product price becomes economical [24]. Filler for the production of dried cultures can be gelatin, gum Arab, alginate and starch [25]. Starch is a raw material filler that is cheap, available abundant, renewable, biodegradable and nontoxic [26]. Rice flour and tapioca are potentially used as filler because the price is economical and the availability is abundant. Application of rice flour and tapioca on the production of dried culture is also quite a lot done and give good results [27, 28].

L. plantarum HL-15 isolated from fermented cocoa bean in Yogyakarta, Indonesia has the ability to

inhibit the growth of *A. niger* YAC-9 fungi producing mycotoxin [29]. Therefore, to extend the shelf life of the culture of *L. plantarum* HL-15, the research aimed to produce dried *L. plantarum* HL-15 and observe its stability during storage was conducted. The effect of the addition of rice flour and tapioca filler to the viability of *L. plantarum* HL-15 culture after drying process using spray and oven dryer, and the effect of storage on viability, water content and antifungal activity of *L. plantarum* HL-15 dried culture will be studied.

2. Materials and Methods

2.1 Preparation of L. plantarum HL-15 Culture

Culture used for dry culture production in this research was *L. plantarum* HL-15 isolated from fermented cocoa bean in Yogyakarta, Indonesia [29]. Strain was inoculated in MRS Broth (Oxoid) and incubated at 37 °C for 24 h. The cultures were stored in cold room prior to use. Culture used for antifungal assay was *A. niger* YAC-9 as a fungi producing mycotoxin.

2.2 Spray Drying of L. plantarum HL-15 Culture

Solution of 10% rice flour or tapioca was added to *L. plantarum* HL-15 pellet and stirred vigorously. Starter culture and filler (rice flour or tapioca) solution ratio was 1:1 (v/v). Culture was spray dried in a laboratory plant spray dryer (model SD-05). The inlet and outlet temperatures were 105 °C and 65 °C, respectively. Spray-dried powder of *L. plantarum* HL-15 was collected and vacuum-packed in polyethylene bags, and stored at 4 °C. Viable cells of *L. plantarum* HL-15 was enumerated before and after spray drying, and during storage of the spray-dried cultures at 4 °C. Water content and antifungal activity of spray dried *L. plantarum* HL-15 during storage at 4 °C was analyzed every month periodically.

2.3 Enumeration of Spray Dried L. plantarum HL-15 Culture

Enumeration of *L. plantarum* HL-15 was carried out using dilution and plating method. MRS agar was used to count the viable cells of *L. plantarum* HL-15. Plates were incubated at 37 °C for 48 h. Results of plate counting were expressed as colony forming units (CFU) per milliliter suspension or per gram solid.

2.4 Determination of the Water Content of Spray Dried L. plantarum HL-15 Culture

Water content of spray dried of *L. plantarum* HL-15 culture was determined by thermogravimetry [30].

2.5 Antifungal Assay of Spray Dried L. plantarum HL-15 Culture

The mycotoxin-producing fungus used in this study was *A. niger* YAC-9. Assay for antifungal activity of *L. plantarum* HL-15 was carried out using overlay method [31] with modification. *L. plantarum* HL-15 inoculated in MRS agar media, then overlayed with PDA media containing *A. niger* YAC-9, then incubated at 27 °C for 3 d. Antifungal activity was determined by calculating the fungal biomass through the width of the fungi diameter. The smallest fungi biomass shows the largest antifungal activity of *L. plantarum* HL-15.

3. Results and Discussion

3.1 The Viability of L. plantarum HL-15 during Biomass Production, before and after Spray Drying

The production of dried culture *L. plantarum* HL-15 begins with biomass production. Biomass production is done by growing *L. plantarum* HL-15 culture on MRS media. The viability of *L. plantarum* HL-15 during biomass production, before and after spray drying can be seen in Table 1. The initial cell count of *L. plantarum* HL-15 for biomass production was

Table 1 The viability of *Lactobacillus plantarum* HL-15 during biomass production, before and after spray drying

Filler	Initial cell number (log CFU/mL)	Cell number after biomass production (log CFU/4,000 mL)	Cell number before spray drying (log CFU/300 mL)	Cell number after spray drying (log CFU/g)
Rice flour	10.89 ± 0.13	14.49 ± 0.13	13.37 ± 0.13	11.99 ± 0.01
Tapioca	10.99 ± 0.03	14.59 ± 0.03	13.47 ± 0.03	11.90 ± 0.04

Values are the mean of three determinations.

10.89 log CFU/mL and 10.99 log CFU/mL. After harvesting the cell increased by about 3.6 log CFU/4,000 mL to 14.49 log CFU/4,000 mL and 3.6 log CFU/4,000 mL to 14.59 log CFU/4,000 mL (Table 1). The increase in cell number is high because the media used is MRS that contains complete nutrition for the growth of lactic acid bacteria. Another study reported that the production of *L. plantarum* cells during biomass production only increased cell approximately 1 log cycle because the medium used was coconut water. MRS has more nutrients than coconut water [32].

Table 1 shows that there is a decrease of cell number after drying with spray dryer either on rice flour or tapioca filler with decreasing value of 1.38 log and 1.57 log. The decrease in viability after drying is caused by the high temperatures used during the spray drying process. The optimum temperature of lactic acid bacteria especially *L. plantarum* is 37 °C [33], therefore high temperature spray drying conditions can decrease cell resistance. In thermal drying processes such as spray drying, lactic acid bacteria cultures are atomized through hot air flow resulting in cell inactivation due to high temperature exposure, as well as dehydration inactivation [17]. Bacterial cells comprise 70%-95% of water that contributed to maintaining the stability of proteins, DNA and lipids. The drying process will evaporate water therefore the cell becomes unstable.

Damage to the cellular structure during cell drying can be prevented by the addition of a protectant or filler that will replace the water-binding side of the cell [34]. Dry culture of *L. plantarum* HL-15 with rice flour filler has higher viability than tapioca, since it has higher protein and sugar content. According to the United States Department of Agriculture, rice protein flour content is 5.95%, while tapioca is 0%, and sugar content of rice flour is 0.12%, while tapioca is 0%.

3.2 The Viability and Water Content of Spray Dried L. plantarum HL-15 during Storage at 4 °C

One of the benefits of the process of drying bacterial culture is that it can produce stable products that can be stored at low cost [20]. To maintain high viability, it is important to keep the dried culture with the appropriate packaging materials and storage conditions in order to protect from exposure to oxygen, moisture and light [22]. Storage temperature is also important parameter that could affect dry culture resistance. The viability of bacteria will decrease with increasing storage temperatures [34]. Store the dry culture at 4 °C may inhibit lipid oxidation and cell damage therefore cell viability can be maintained [24]. Water content is also an important parameter in maintaining cell stability [35]. The viability and water content of spray dried *L. plantarum* HL-15 during storage at 4 °C were shown in Table 2.

During six months storage, there is a decrease in the viability of spray dried *L. plantarum* HL-15 either with rice flour or tapioca filler (Table 2). Decrease in viability of spray dried *L. plantarum* HL-15 may be due to cell damage [36]. In this research, packing dried culture does not use vacuum sealer so it is possible that oxygen causes oxidation during storage process. Similar studies of dry culture storage have also been performed. During storage at 4 °C for 12 months, the reduced viability of dry culture *L. plantarum* TISTR 2075 with spray drying was 0.53-0.95 log with fillers of trehalose, protein concentrate, acacia gum and fiber sol [34].

Table 2 The viability and water content of spray dried *L. plantarum* HL-15 during storage at 4 °C.

Storage time (month)	Viability (CFU/g)		Water content (%)	
	Spray-dried culture using rice flour filler	Spray-dried culture using tapioca filler	Spray-dried culture using rice flour filler	Spray-dried culture using tapioca filler
0	11.99 ± 0.01	11.90 ± 0.04	9.08 ± 0.05	10.11 ± 0.08
1	11.91 ± 0.04	11.81 ± 0.03	11.29 ± 0.20	12.60 ± 0.48
2	11.86 ± 0.02	11.78 ± 0.08	11.35 ± 0.08	12.53 ± 0.11
3	11.31 ± 0.07	11.06 ± 0.07	11.40 ± 0.14	12.52 ± 0.12
4	8.79 ± 0.14	8.43 ± 0.17	11.67 ± 0.13	12.61 ± 0.07
5	7.90 ± 0.05	6.55 ± 0.04	11.93 ± 0.09	12.78 ± 0.05
6	7.90 ± 0.00	6.24 ± 0.06	12.15 ± 0.05	13.05 ± 0.08

Values are the mean of three determinations.

Table 3 The antifungal activity of spray dried *L. plantarum* HL-15 against *Aspergillus niger* YAC-9 during storage at 4 °C.

Storage time (month)	Diameter of *A. niger* YAC-9 (cm)					
	Spray-dried culture using filler rice flour			Spray-dried culture using filler tapioca		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0	0.47 ± 0.04	1.24 ± 0.24	2.03 ± 0.47	0.53 ± 0.06	2.04 ± 0.31	2.75 ± 0.59
1	0.41 ± 0.05	0.88 ± 0.04	1.76 ± 0.26	0.37 ± 0.03	1.11 ± 0.1	2.38 ± 0.10
2	0.50 ± 0.03	1.12 ± 0.07	2.12 ± 0.39	0.37 ± 0.04	0.92 ± 0.25	2.70 ± 0.62
3	0.48 ± 0.05	0.94 ± 0.05	1.66 ± 0.28	0.46 ± 0.02	1.01 ± 0.10	1.92 ± 0.20
4	0.63 ± 0.12	1.17 ± 0.26	1.66 ± 0.48	0.73 ± 0.12	1.49 ± 0.45	2.01 ± 0.25

Diameter of control (*A. niger* YAC-9); Day 1 (1.21 ± 0.26), Day 2 (2.63 ± 0.32), Day 3 (3.66 ± 0.31).
Values are the mean of three determinations.

Water content of spray dried *L. plantarum* HL-15 either with rice flour or tapioca filler increased during six months storage (Table 2). Changes in water content can be caused by cytoplasmic cell changes, changes in tension in the cell wall and changes in environmental osmolality around it [37]. The stability of cells during storage can be improved by maintaining water content, low use of outlet temperatures and storage at refrigeration temperatures [35]. Differences of filler used cause differences in water content of dried culture [20]. Water content of spray dried *L. plantarum* HL-15 with tapioca filler was higher than with rice flour filler. This result is also similar to previous research that water content of dried culture with tapioca filler is 13.71% and rice flour is 11.38%. Water content is related to the characteristics of starch granules [37]. The oval tapioca shaped oval with the cut end and has a larger size than the rice flour granules so that the water binding on the tapioca is larger therefore the water difficult to evaporate [27].

3.3 The Antifungal Activity of Spray Dried L. plantarum HL-15 during Storage at 4 °C

The smallest diameter of *A. niger* YAC-9 fungi shows the highest antifungal activity of spray dried *L plantarum* HL-15, which means it is able to inhibit fungal growth. Spray dried *L. plantarum* HL-15 with rice flour or tapioca filler has the ability to inhibit the growth of *A. niger* YAC-9 fungi (Table 3). This is indicated by the control (*A. niger* YAC-9) fungi having the largest diameter compared to the diameter of *A. niger* YAC-9 which treated by spray dried *L. plantarum* HL-15.

The results of this study are supported by some previous researches. *L. plantarum* 21B as a sourdough starter can inhibit the growth of *A. niger* FTDC3227 mushrooms for more than 7 d of bread storage [38]. With dual plate agar methods, *L. brevis* KR cultures can inhibit the growth and sporogenesis of *A. niger* [39]. The mixture of *L. pentosus* G004 with *L. fermentum* Te007 may inhibit the growth of *A. niger*

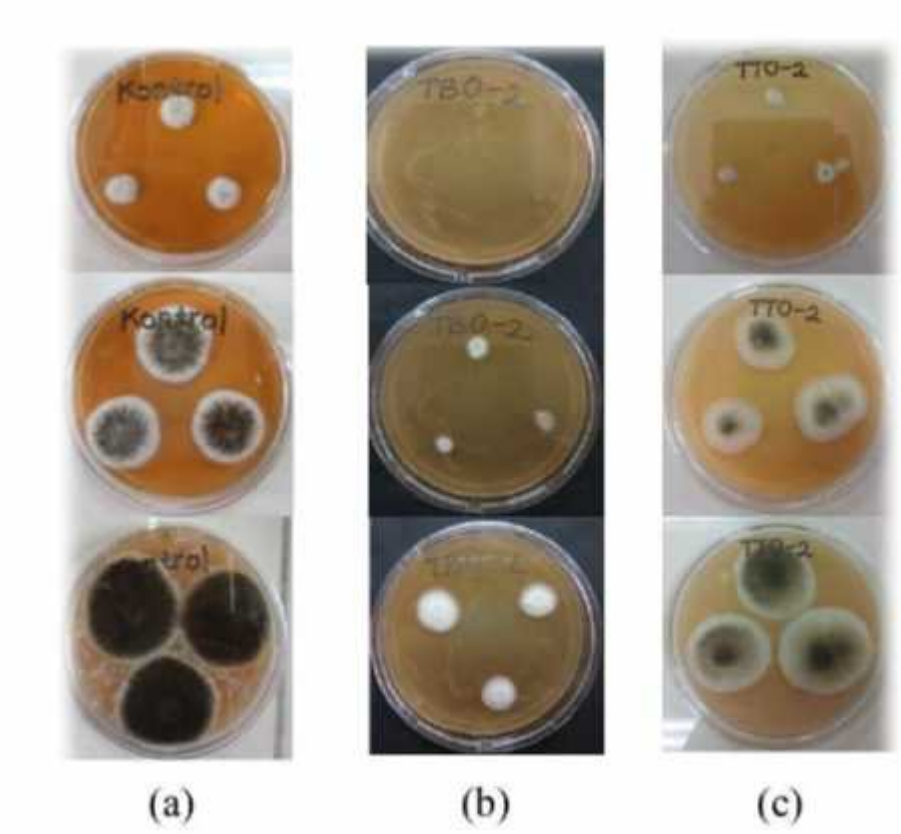

Fig. 1 Growth of *Aspergillus niger* YAC-9 control (a), growth of *A. niger* YAC-9 treated with spray dried *Lactobacillus plantarum* HL-15 using rice flour filler (b) and growth of *A. niger* YAC-9 treated with spray dried *L. plantarum* HL-15 using tapioca filler (c).

and *A. oryzae* [14]. The growth of *A. niger* YAC-9 treated with spray dried *L. plantarum* using rice flour filler is smaller than the growth of *A. niger* YAC-9 treated with spray dried *L. plantarum* using tapioca filler (Fig. 1). This is because the viability of rice flour is higher than tapioca so it has higher growth inhibition toward *A. niger*.

4. Conclusions

L. plantarum HL-15 can be produced as spray dried cultures using rice flour and tapioca filler with viability 6.57×10^{12} CFU and 1.62×10^{12} CFU, respectively. Viability loss of spray dried *L. plantarum* HL-15 culture using rice flour as filler is lower than dried culture using tapioca as filler. *L. plantarum* HL-15 spray dried cultures could be stored at 4 °C for four months for tapioca and five months for rice flour as filler. *L. plantarum* HL-15 spray dried cultures could inhibit the growth of *A. niger* so it could be used as a culture for inhibiting the growth of mycotoxin producing fungi in food.

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References

[1] Ho, V. T. T., Zhao, J., Srzednicki, and Fleet, G. 2013. "The Functional Role of Microorganisms in Cocoa Bean Fermentation." In *Proceedings of the International Symposium on Agri-Foods for Health and Wealth*, 83-91.

[2] Asrul. 2009. "Mycotoxic Fungal Populations and Afflation Content in some Sample of Cocoa Beans (*Theobroma cacao* L.) from Central Sulawesi." *Agroland* 16: 258-67. (in Indonesia)

[3] Christensen, C. M. 1987. "Field and Storage Fungi." In *Food and Beverage Mycology*, 2nd edition, Beuchat, L. R. (ed.), New York: AVI Book, Van Nostrand Reinhold.

[4] Harrison, T. 2000. "The Importance of Quality from International Confectionery Industry View Point." Present at the ICCO International Seminar on Quality and Financial Aspect of the Cocoa Campaign, Abidjan.

[5] Lund, B. M. 2000. *The Microbiological Safety and Quality of Food.* Volume II. Maryland: Aspen Publisher Inc.

[6] Nugroho, A. D., Setyabudi, F. M. C. S., Salleh, B., and Rahayu, E. S. 2013. "Ochratoxigenic Black *Aspergilli* Isolated from Dried Agricultural Products in Yogyakarta, Indonesia." *J. Food Sci. and Eng.* 3: 472-80.

[7] Sardjono, S. 2003. "Occurrence and Detoxification of Mycotoxins in Food." *Agritech* 23 (2): 97-102.

[8] Suwarsono and Susijahadi. 2013. "Exploration of Antifungal Agents from Lactic Acid Bacteria in Cocoa Fermentation and Their Potential to Improve the Quality of Cocoa for Export." Competitive Research Report for Universities in 2007-2008, University Jember. Accessed November 12, 2018. http://repository.unej.ac.id/handle/123456789/3056.

[9] Fitriana, N. I. 2012. *Isolation and Identification of Indigenous Lactic Acid Bacteria with Antifungal Potential from Cacao Fermentation at PTPN XII Kebun Banjarsari, Jember.* Technical Report.

[10] Ngang, J. J., Yadang, G., Kamdem, S. L. S., Kouebou, C. P., Fanche, S. A. Y., Kougan, D. L. T., Tsoungui, A., and Etoa, F. X. 2015. "Antifungal Properties of Selected Lactic Acid Bacteria and Application in the Biological Control of Ochratoxin A Producing Fungi during Cocoa Fermentation." *J. Biocontrol Sci. and Tech.* 25: 245-59.

[11] Putri, B. C. P. 2015. "Identification of Lactic Acid Bacteria as Anti-Fungi from Fermented Cocoa in Gunung Kidul Yogyakarta." Undergraduate Thesis, University of Jember.

[12] Ross, R. P., Morgan, S., and Hill, C. 2002. "Preservation and Fermentation: Past, Present and Future." *Intl. J. Food Microb.* 79: 3-16.

[13] Crowley, S., Mahony, J., and Sinderen, D. V. 2013. "Current Perspectives on Antifungal Lactic Acid Bacteria as Natural Bio- Preservatives." *Trends in Food and Sci. Tech.* 33: 93-109.

[14] Muhialdin, B. J., Hassan, Z., Sadon, S. K., Zulkifli, N. A., and Azfar, A. A. 2011. "Effect of pH and Heat Treatment on Antifungal Activity of *Lactobacillus fermentum* TE007, *Lactobacillus pentosus* G004 and *Pediococcus pentosace*us TE010." *Innovative Romanian Food Biotechnology* 8: 41-53.

[15] Niku-Paavola, M. L., Laitila, A., Mattila-Sandholm, T., and Haikara, A. 1999. "New Types of Antimicrobial Compounds Produced by *Lactobacillus plantarum*." *J. Appl. Microb.* 86: 29-35.

[16] To, B. C. S., and Etzel, M. R. 1997. "Spray Drying, Freeze Drying, or Freezing of Three Different Lactic Acid Bacteria Species." *J. Food Sci.* 62 (3): 576-85.

[17] Santivarangkna, C., Kulozik, U., and Forest, P. 2008. "Inactivation Mechanism of Lactic Acid Starter Cultures Preserved by Drying Processes." *J. Appl. Microb.* 105: 1-13.

[18] Peighambardoust, S. H., Tafti, A. G., and Hesari, J. 2011. "Application of Spray Drying for Preservation of Lactic Acid Starter Cultures: A Review." *Trends in Food Sci. and Tech.* 22: 215-24.

[19] Desmond, C., Stanton, C., Fitzgerald, G. F., Collins, K., and Ross, R. P. 2001. "Environmental Adaptation of Probiotic *Lactobacilli* towards Improvement of Performance during Spray Drying." *Int. Dairy J.* 12: 183-90.

[20] Riveros, B., Ferrer, J., and Borquez, R. 2009. "Spray Drying of Vaginal Probiotic Strain of *Lactobacillus acidophilus.*" *Drying Technol.* 27: 123-32.

[21] Golowczyc, M. A., Silva, J., Teixeria, P., Antoni, G. L. D., and Abraham, A. G. 2011. "Cellular Injuries of Spray-Dried *Lactobacillus* spp. Isolated from Kefir and Their Impact on Probiotic Properties." *Intl. J. Food Microb.*144: 556-60.

[22] Utami, T., Kasmiati, Harmayani, E., and Rahayu, E. S. 2016. "Survival of *Lactobacillus plantarum* Dad 13 during Spray Drying and Its Application for Yoghurt Fermentation." *Intl. Res. J. Biol. Sci.* 5 (2): 16-22.

[23] Stephens, J. H. G., and Rask, H. M. 2000. "Inoculant Production and Formulation." *Field Crops Res.* 65 (2-3): 249-58.

[24] Erdiandini, I., Sunarti, T. C., and Meryandini, A. 2015. "Selection of Lactic Acid Bacteria and Its Use as a Dry Starter Using Tapioca Acid Matrix." *Biological Journal of Resources* 1 (1): 26-33

[25] Lian, W. C., Hsiao, H. C., and Chou, C. C. 2002. "Survival of Bifidobacteria after Spray-Drying." *Intl. J. Food Microb.* 74: 79-86.

[26] Winarti, C., Sunarti, T. C., Mangunwidjaja, D., and Richana, N. 2013. "Potential and Application of Modified Starch as Active Ingredients of Bioactive Encapsulation Matrix." *Bulletin of Agricultural Postharvest Technology* 9 (2): 83-94.

[27] Arisanti, D. 2012. "Viability of Lactic Acid Bacteria during the Preparation and Storage of Mocaf Yeast and Its Application to the Fermentation of Fresh Cassava." Undergraduate thesis, Gadjah Mada University, Yogyakarta.

[28] Nurfiana, D. A. 2016. "Development of *Lactobacillus plantarum* HL-15 Dry Inoculum with Oven Drying

Method for Cocoa Beans Fermentation." Undergraduate thesis, Ganja Made University, Yogyakarta.

[29] Marwati, T., Khulna, R. N. B., Deafer, T. F., and Rahway, E. S. 2017. "Inhibition Growth of Mycotoxin Producing Fungi by Lactic Acid Bacteria Isolated from Fermented Cocoa Bean (*Theobroma cacao* L.) in Indonesia." In *Proceeding of the 15th ASEAN Conference on Food Science and Technology, November 14-17, 2017, Ho Chi Minh City, Vietnam,* 295-302.

[30] Association of Official Analytical Chemists (AOAC). 2005. *Official Method of Analysis of AOAC International.* 18th ed. Gathersburg, MD, USA Official Methods.

[31] Hassan, Y. I., and Bullerman, L. B. 2008. "Antifungal Activity of *Lactobacillus paracasei* ssp. *tolerans* Isolated from a Sourdough Bread Culture." *Intl. J. Food Microb.* 121: 112-5.

[32] Harmayani, E., Ngadirah, Rahayu, E. S., and Utami, T. 2001. "The Resistance and Viability of Probiotics from Lactic Acid Bacteria during the Process of Making Dry Culture Using the Freeze and Spray Drying Method." *Journal of Technology and Food Industry* 7 (2): 126-32. (in Indonesian)

[33] Matejčeková, Z., Liptáková, D., Spodniaková, S., and Valík, Ľ. 2016. "Characterization of the Growth of *Lactobacillus plantarum* in Milk in Dependence on Temperature." *Acta Chimica Slovaca* 9 (2): 104-8.

[34] Lapsiri, W., Bhandari, B., and Wanchaitanawong, P. 2012. "Viability of *Lactobacillus plantarum* TISTR 2075 in Different Protectants during Spray Drying and Storage." *Drying Tech.* 30: 1407-12.

[35] Fu, W. Y., and Etzel, M. R. 1995. "Spray Drying of *Lactococcus lactis* ssp. *lactis* C2 and Cellular Injury." *J. Food Sci.* 60 (1): 195-200.

[36] Selmer-Olsen, E., Sorhaug, T., Birkeland, S. E., and Pehrson, R. 1999. "Survival of *Lactobacillus helveticus* Entrapped in Ca-Alginate in Relation to Water Content, Storage and Rehydration." *J. Ind. Microbe. and Biotech.* 23: 79-85.

[37] Imanningsih, N. 2012. "Gelatinization Profile of Some Flour for Estimating Cooking Properties." *Food Nutrition Research* 35 (1): 13-22. (in Indonesian)

[38] Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., and Gobbetti, M. 2000. "Purification and Characterization of Novel Antifungal Compounds from the Sourdough *Lactobacillus plantrum* Strain 21B." *Appl. and Environ. Microb.* 66: 4084-90.

[39] Tropcheva, R., Nikolova, D., Evstatieva, Y., and Danova, S. 2014. "Antifungal Activity and Identification of *Lactobacilli*, Isolated from Traditional Dairy Product 'Katak'." *Anaerobe* 28: 78-84.