ISSN 2808-3229
Journal of Biodiversity and Biotechnology. 5(1), 15–22,
2025 URL: https://jurnal.uns.ac.id/jbb/article/view/109898
doi: http://dx.doi.org/10.20961/jbb.v5i1.109898

Isolation of Lactic Acid Bacteria from Soil and Its Antifungal Activity Against *Aspergillus flavus*

Athiyyah Jihan Tifani[1*], Tjahjadi Purwoko[1], Ari Susilowati[1]

[1]Universitas Sebelas Maret

*Corresponding Author: athiyyahjihant@student.uns.ac.id

Abstract
Food stocks, such as grains, fruits, and vegetables, are vulnerable to spoilage by *Aspergillus flavus*, which is typically controlled using chemical preservatives. Concerns about the health impact of these chemicals highlight the need for safer alternatives, such as biopreservatives. Lactic acid bacteria (LAB) are Generally Recognized as Safe (GRAS) microorganisms and have potential applications as biopreservative agents, as they produce various metabolites with antifungal activity. LAB can be found in nutrient-rich environments, including rhizosphere soil and poultry house soil, where nutrient residues support growth. Research on LAB isolated from soil and their potential application as biopreservatives remains limited; therefore, this study aimed to isolate LAB from soil and evaluate their antifungal activity, with a focus on possible applications as biopreservatives. The LAB isolates from soil were characterized and screened for antifungal activity using the dual culture method. The LAB isolates inhibited A. *flavus* growth, with the most significant inhibition observed with LAB isolate 1, isolated from poultry house soil (17.65%). However, statistical analysis revealed that the inhibition of fungal growth by LAB isolates was not significantly different ($p > 0.05$). Although the result was not statistically significant, LAB-treated fungal growth tended to be smaller than the control, suggesting potential inhibition and aligning with the qualitative observations of inhibited conidia development. This showed potential application of LAB isolates as a biopreservative agent.

Keywords: antifungal, *Aspergillus flavus*, biopreservative, food stock, lactic acid bacteria

Cite this as: Tifani, A.J., Purwoko, T., Susilowati, A. (2025). Isolation of Lactic Acid Bacteria from Soil and Its Antifungal Activity Against *Aspergillus flavus*. Journal of Biodiversity and Biotechnology. 5(1), 15–22. doi: http://dx.doi.org/10.20961/jbb.v5i1.109898

Introduction

Food stocks, including grains, fruits, and vegetables, are frequently compromised by fungal pathogen contamination. Fungal spoilage reduces both the quantity and quality of food stocks, affecting attributes including visual appearance, odor, texture, and taste (1)(2). Pathogenic fungi commonly associated with food contamination are often from the genus *Aspergillus*, particularly *Aspergillus flavus*. During early growth, A. *flavus* displays white mycelium that becomes covered with yellowish-green conidia as incubation progresses (3). After conidiation, A. *flavus* produced mycotoxins that can cause disease in animals and humans (4)(5). These mycotoxins include several types, with aflatoxins B1, B2, G1, and G2 among the most toxic (6). Aflatoxin production begins during the transition from the primary growth phase, marked by active mycelial development, to the differentiation phase, when conidial formation and sporulation occur (7)(8).

Chemical preservatives are commonly used to prevent food spoilage caused by fungal contamination, such as sorbic acid, sulfur dioxide, propionic acid, and benzoate. However, chemical preservatives may pose health risks and can elicit adverse consumer reactions (9)(10). As a result, microorganism-based biopreservatives are being developed as alternatives to food preservation methods. Microorganisms can be applied as biopreservatives either directly, using microbial cells, or indirectly, through their primary and secondary metabolites delivered via spraying, edible coatings, or edible films (11)(2)(12).

LAB is classified as Generally Recognized as Safe (GRAS), thus suitable for food applications, and has demonstrated

antifungal activity against A. flavus, as well as being widely investigated as a biopreservative agent (13)(14). The antifungal activity of LAB is primarily driven by the production of organic acids and secondary metabolites, along with competition for space and nutrients (15)(11)(16). Organic acids are primarily responsible for antifungal effects by disrupting fungal metabolism and suppressing mycelial growth (17). LAB also inhibits gene expression involved in aflatoxin synthesis, adsorbs aflatoxins through their cell wall, and interferes with fungal sporulation (18)(19).

LAB were commonly found in nutrient-rich environments such as the animal gastrointestinal tract, fermented food, meat, milk, fruit, and vegetables (14)(20). Still, they can also be found in soil that provides sufficient nutrients for growth (21). Soil contains high microbial diversity, including fungi and bacteria that are naturally capable of competing with fungal phytopathogens like A. *flavus* (22)(23). Research on the antifungal activity of LAB, including studies against A. *flavus*, has been widely conducted; however, research specifically focusing on soil-isolated LAB and its potential as biopreservative agents is currently limited. According to Chen et al. (21), certain soil types, such as the rhizosphere of fruit plants and farm soil, contain abundant nutrients that support the growth of LAB. We hypothesize that LAB isolated from soil can inhibit the growth of A. *flavus*. Therefore, this research focuses on LAB from poultry house soil, the banana plant rhizosphere, and the chilli plant rhizosphere. This study aims to evaluate the antifungal activity of LAB isolated from soil against A. *flavus*, providing primary information for their potential application as a biopreservative agent.

Material and Methods

The study was conducted in the Biology Laboratory at the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, from July 2022 to March 2023. The equipment used included glassware such as micropipettes, Drygalski flasks, Erlenmeyer flasks, test tubes, and petri dishes. Additional equipment comprised a biology safety cabinet, an autoclave for sterilization and disposal, a vortex mixer, an incubator, an inoculation needle, and a ruler.

The materials used in this study included soil samples collected from the poultry house, the rhizosphere of the banana plant, and the rhizosphere of the chili plant. Chemicals were NaCl (Merck), crystal violet and safranin (Merck), Lugol's solution (Merck), $CaCO_3$ (Merck), and H_2O_2 (Merck). Microbial growth media were MRSA (Merck), MRSB (Fluka), and PDA (Himedia). Fungus A. *flavus* (FNCC 6109) was obtained from the Food and Nutrition Culture Collection, Universitas Gadjah Mada, Yogyakarta, Indonesia.

The methodology for this study involved several sequential steps. LAB were first isolated on MRS agar containing 0.3% $CaCO_3$, which functioned as a selective medium. Plates were incubated at 37°C for 48 hours under anaerobic conditions (21)(24). LAB isolates were characterized according to morphological and biochemical properties (25)(26). The isolates were then evaluated for antifungal activity against A. *flavus* using the dual culture method as described by Sharma et al. (27) and Anith et al. (28). A 7 mm diameter disc from a seven-day-old A. *flavus* colony was placed at the center of PDA. Each LAB isolate was streaked on both sides of the fungal disc at a distance of 3 cm (Figure 1). Colony diameters of A. *flavus* were measured on days 3, 5, and 7. The control plate was covered with the fungus A. flavus growing on PDA. The experiment was performed with two replicates.

The inhibitory effect of LAB isolates on A. *flavus* was determined as growth inhibition using the following formula:

$$\% \text{ Growth Inhibition} = [(A-B)/A] \times 100$$

In this formula, A (the mean of D1 and D2) denotes the diameter of the fungal colony in control plates, and B (the mean of D1 and D2) indicates the diameter in test plates. Colony diameter was measured from two separate sides to ensure accurate assessment of fungal growth (29). Data were analyzed using a repeated-measures general linear model at a 5% significance level with IBM SPSS Statistics 26.

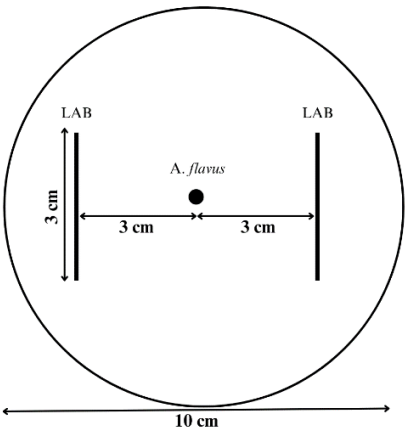

Figure 1. Dual Culture Assay Method

Results and Discussion

Five LAB isolates were obtained from soil samples collected in a poultry house, the rhizosphere of a banana plant, and the rhizosphere of a chilli plant. Table 1 summarizes the morphological traits of the colonies and cells of these LAB isolates, while Table 2 details their biochemical characteristics. The observed morphological data align with the characteristics described by Dwidjoseputro (30) and Sari et al. (31). Specifically, Table 1 indicates that the LAB isolates in this study share similarities with those reported by Tolieng et al. (32) and Husain et al. (33), who characterized LAB colonies as circular and ranging in color from white to cream. The cells of the LAB isolates were identified as cocci and bacilli, and were classified as gram-positive bacteria.

Table 1. Morphological Characteristics of LAB Isolated from Soil

Isolates	Source	Colony				Cell	
		Color	Shape	Margin	Elevation	Shape	Gram Staining
1	A	Translucent	Circular	Irregular	Raised	Rod	Positive
2	A	Milky white	Circular	Entire	Raised	Coccus	Positive
3	B	Milky white	Circular	Undulate	Convex	Coccus	Positive
4	C	Milky white	Circular	Undulate	Raised	Coccus	Positive
5	C	Creamy white	Circular	Irregular	Raised	Rod	Positive

Note: A= poultry house soil; B= rhizosphere of banana plant; C= rhizosphere of chilli plant

Table 2 showed that isolates 1, 2, 3, and 4 were homofermentative bacteria, confirmed by the absence of gas formation. In contrast, isolate 5 was classified as a heterofermentative bacterium based on gas production. Homofermentative bacteria yield two molecules of lactic acid through the EMP (Embden-Meyerhof-Parnas) pathway; meanwhile, heterofermentative bacteria yield one molecule of lactic acid and one molecule of ethanol or acetic acid, and one molecule of CO_2 through the pentose phosphate pathway (34)(35). The catalase test was performed to determine the bacteria's ability to produce catalase. Generally, catalase is produced by aerobic bacteria, whereas LAB are classified as facultative anaerobes. As shown in Table 2, five isolates yielded negative catalase test results, indicated by the absence of bubble formation after treatment with 3% hydrogen peroxide (H2O2). This result was consistent with the findings of Husain et al. (33) and Ekundayo (36). The Triple Sugar Iron Agar (TSIA) test was performed to determine the ability of the bacteria to ferment glucose, sucrose, and lactose, as indicated by a color change of the medium from reddish-orange to yellow in the slant and butt of the reaction tube as a result of the decrease in medium pH to an acidic condition (37). Table 2 showed that all isolates fermented three different types of sugar. Our results were consistent with previous research (38). Morphological and biochemical characteristics provide a fast and affordable preliminary approach for identification before proceeding to molecular analysis (40)(41). Our results showed that five isolates were gram-positive, catalase-negative, facultative anaerobes, and had coccoid and bacillary shapes. Isolate 1 and isolate 5 were included *in the Lactobacillaceae family,* characterized by their bacilli-shaped cells and arranged in chains, gram-positive bacteria, catalase-negative, and facultative anaerobes. Isolate 2, isolate 3, and isolate 4 were included *in the Streptococcaceae family,* characterized by their cocci-shaped cells, gram-positive, catalase-negative, and facultative anaerobes.

Table 2. Biochemical Characteristics of LAB Isolated from Soil

Isolates	Fermentation Test		Catalase Test	TSIA Test
	Gas	Result		(*slant*/*butt*)
1	Negative	Homofermentative	Negative	yellow/yellow
2	Negative	Homofermentative	Negative	yellow/yellow
3	Negative	Homofermentative	Negative	yellow/yellow
4	Negative	Homofermentative	Negative	yellow/yellow
5	Positive	Heterofermentative	Negative	yellow/yellow

Antifungal activity of LAB isolates against A. *flavus* was shown as the percentage of growth inhibition, which was measured based on the diameter of the fungal colony (Table 3). Table 3 showed that the diameter growth of A. *flavus* on the side without LAB (D1) was larger than the diameter growth of A. *flavus* on the LAB-confronted side (D2) at days 3, 5, and 7. This is due to the inhibition of the LAB isolates against A. *flavus* growth. Meanwhile, control did not show differences between D1 and D2 because no LAB affected A. *flavus* growth. These results are further presented in Figure 2. Based on Figure 2, the average diameter growth of fungi treated with LAB isolates tended to be smaller than the control over the observation period (days 3, 5, and 7), indicating a potential inhibition of the tested LAB isolates against A. *flavus*.

Table 3. Colony Diameters of A. *flavus*

Treatment	Day 3		Day 5		Day 7	
	D1 (mm)	D2 (mm)	D1 (mm)	D2 (mm)	D1 (mm)	D2 (mm)
Control	35.50	35.50	52.00	52.00	67.00	69.00
1	34.50	35.00	50.50	49.00	59.00	53.00
2	37.50	34.00	52.00	50.50	67.00	58.50
3	34.50	31.00	53.00	46.00	69.00	52.50
4	35.50	34.00	52.50	50.00	64.00	60.50
5	33.00	32.00	53.00	48.00	68.00	60.50

Note: D1 (Fungal diameter on the side without LAB confrontation); D2 (Fungal diameter on the LAB-confronted side).

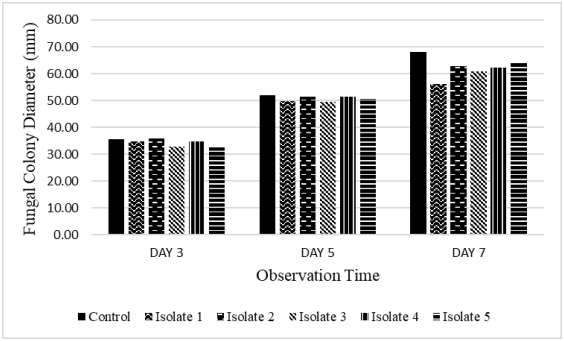

Figure 2. Effect of LAB Isolates on A. *flavus* Average Colony Diameter

The percentage of growth inhibition is shown in Table 4 and Figure. 3. The most significant growth inhibition on day 7 incubation was shown by isolate 1 (17.65%), and the smallest one was shown by isolate 5 (5.51%). The antifungal activity of LAB is associated with the production of primary and secondary metabolites. The different levels of growth inhibition observed across the study period are likely due to variations in the active metabolites produced by each LAB isolate, which depend on culture conditions such as pH, temperature, and carbon availability in the medium (42). Figure 3 showed that on day 7 of incubation, variations in the growth inhibition of LAB against A. flavus were observed, with relatively minor differences across isolate treatments, except for isolate 1, which exhibited a clearly distinct difference compared to the other isolates.

Table 4. Growth Inhibition of LAB toward A. *flavus*

Isolates	Day 3	Day 5	Day 7
	Growth Inhibition (%)	Growth Inhibition (%)	Growth Inhibition (%)
1	2.11	4.33	17.65
2	nd*	1.44	7.72
3	7.75	4.81	10.66
4	2.11	1.44	8.46
5	8.45	2.88	5.51

Note: *nd (not detected) growth inhibition ≈ 0%

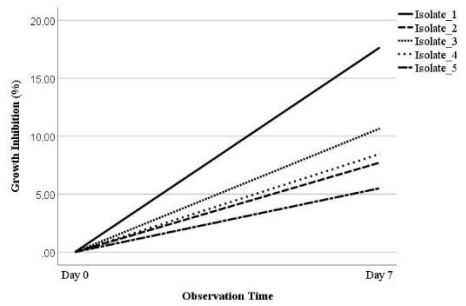

Figure 3. Growth Inhibition of LAB against A. *flavus* on Day 7

Figure 4 showed the qualitative antifungal activity of LAB isolates against A. *flavus* growth on day 7 of incubation. As shown in the Figure. 4, the diameter growth of all A. *flavus* treated with LAB isolates was slightly different compared to the diameter growth of the control. Previous studies have reported that viable LAB cells can inhibit the growth of A. flavus by up to 51.67% (13), although inhibiting A. flavus growth proved to be challenging (43). The LAB's ability to inhibit fungal growth may be due to its antimicrobial compounds (44).

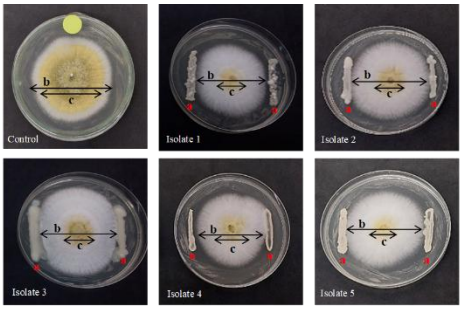

Figure 4. Dual Culture Assay of LAB against A. *flavus* on Day 7

Note: (a) LAB isolates; (b) fungal mycelial area; (c) fungal conidial area

Statistical analysis revealed that the growth of all A. flavus treated with LAB isolates was not significantly different ($p > 0.05$). However, LAB-treated fungal growth tended to be smaller than that of the control, as shown in Figure 2. In addition, the qualitative observations show that the conidia area of all A. *flavus* treated with LAB isolates was small compared to the conidia area of the control (Figure 4). That showed LAB isolates inhibited A. *flavus* growth, especially conidial growth. This might have been caused by lactic acids and bacteriocins produced by LAB isolates, which affected A. *flavus* conidiation. Zhang et al. (19) reported that the antifungal effect of LAB could be demonstrated by disruption of fungal sporulation, as indicated by morphological changes in the mycelia and by shrinkage or deformation of the conidia. Additionally, previous studies have shown that fungal conidial development is inhibited by the LAB metabolite phenyllactic acid (45). In addition, LAB isolates and A. *flavus* competed for nutrients and space. Furthermore, LAB isolates could influence fungal physiological activity, including the reduction of mycotoxin production by inhibiting the expression of aflatoxin biosynthetic regulatory genes in A. *flavus*. Although A. *flavus* produced aflatoxin, LAB isolates could adsorb it through their cell walls (18). Other organic acids, such as acetic acid and propionic acid, as well as reutericyclin and peptides, could improve acidity, creating unfavorable conditions for fungal growth. An acidic environment can impair cell membrane function, thereby reducing fungal viability (46)(47)(48). Therefore, the inhibition of conidia formation might reduce aflatoxin production (7)(8).

This study acknowledges several limitations. The number of samples and replicates was relatively small, as the study was designed as an initial screening. These limitations may restrict the generalization of the findings. Despite this, the present results provide preliminary information on the antifungal potential of the LAB isolates, notably their ability to inhibit fungal conidia, and highlight the need for further studies with larger sample sizes and more comprehensive experimental approaches.

Conclusion

Despite LAB isolates affecting the mycelial growth of A. flavus by 17.65%, statistical analysis did not show a difference in the mycelial growth of all A. *flavus* treated with LAB isolates. Nevertheless, LAB isolates affected conidial growth of A. *flavus*, as evidenced by a small yellowish-green area on conidia. This indicated that LAB isolates were potentially suitable for biopreservation applications. However, further research will be necessary to explore this potential application, particularly for food biopreservation purposes.

Conflict of Interest

All authors declare no conflicts of interest.

References

[1] Agnis FR, Wantini S. Gambaran Jamur *Aspergillus flavus* Pada Bumbu Pecel Instan Dalam Kemasan Tanpa Merek yang Dijual di Pasar Gedong Tataan Kabupaten Pesawaran. Jurnal Analis Kesehatan. 2017;4(2):456-460.

[2] Salas ML, Mounier J, Valence F, Coton M, Thierry A, Cotton E. Antifungal Microbial Agents for Food Biopreservation. Microorganisms. 2017;5(37):1-35.
[3] Khan R, Mohamad GF, Mahyudin NA, Samsudin NIP. Morphological Characterization and Determination of Aflatoxigenic and Non-aflatoxigenic *Aspergillus flavus* Isolated from Sweet Corn Kernels and Soil in Malaysia. Agriculture. 2020;10(10):450-463.
[4] Jiang Y, Wang J, Yang B, Wang Q, Zhou J, Yu W. Molecular Characterization of Debilitation-Associated Partitivirus Infecting the Pathogenic Fungus *Aspergillus flavus*. Frontiers in Microbiology. 2019;10(626):1-11.
[5] Klich MA. *Aspergillus flavus*: The Major Producer of Aflatoxin. Molecular Plant Pathology. 2007;8(6):713-722.
[6] Tantio LN. A Study on *Aspergillus flavus*: Biochemical Characterization of Aspergillus flavus. Norderstedt: GRIN Verlag; 2011.
[7] Ramadan NA, Al-Ameri HA. Aflatoxins. In: Abdulra'uf LBB, editor. Aflatoxins Occurrence, Detoxification, Determination, and Health Risks. London: IntechOpen; 2022. p. 3-40.
[8] Reverberi M, Beccaccioli M, Zaccaria M. The Role of Aflatoxins in *Aspergillus flavus* Resistance to Stress. In: Razzaghi-Abyaneh and Rai M, editors. The Genus *Aspergillus*-Pathogenicity, Mycotoxin Production and Industrial Applications. London: IntechOpen; 2022. p. 71-82.
[9] Muhialdin BJ, Algboory HL, Kadum H, Mohammed NK, Saari N, Hassan Z, Hussin ASM. Antifungal Activity Determination for the Peptides Generated by *Lactobacillus plantarum* TE10 Against *Aspergillus flavus* in Maize Seeds. Food Control. 2020;109(106898):1-6.
[10] Silva MM, Lidon F. Food Preservatives–An Overview on Applications and Side Effects. Emirates Journal of Food and Agriculture. 2016;28(6):366-373.
[11] Sadiq FA, Yan B, Tian F, Zhao J, Zhang H, Chen W. Lactic Acid Bacteria as Antifungal and Anti-mycotoxigenic Agents: A Comprehensive Review. Comprehensive Reviews in Food Science and Food Safety. 2019;18(5):1403-1436.
[12] Agriopoulou S, Stamatelopoulou E, Sachadyn-Król M, Varzakas T. Lactic Acid Bacteria as Antibacterial Agents to Extend The Shelf Life of Fresh and Minimally Processed Fruits and Vegetables: Quality and Safety Aspects. Microorganisms. 2020; 8(6):952-978.
[13] Taheur FB, Mansour C, Kouidhi B, Chaieb K. Use of Lactic Acid Bacteria for the Inhibition of *Aspergillus flavus* and *Aspergillus carbonarius* Growth and Mycotoxin Production. Toxicon. 2019;166(16):15-23.
[14] Natasia N, Jannah SN, Rukmi MI. Potensi Antifungi Bakteri Asam Laktat dari Saluran Pencernaan Ayam Kampung terhadap Kapang *Aspergillus flavus*. Bioma: Berkala Ilmiah Biologi. 2020;22(1):91-102.
[15] Bintsis T. Lactic Acid Bacteria as Starter Cultures: An Update in Their Metabolism and Genetics. AIMS microbiology. 2018;4(4):665-684.
[16] Olonisakin OO, Jeff-Agboola YA, Ogid CO, Akinele BJ. Isolation of Antifungal Lactic Acid Bacteria (LAB) from "Kunu" against Toxigenic *Aspergillus flavus*. Prev. Nutr. Food Sci. 2017;22(2):138-143.
[17] Guimarães A, Santiago A, Teixeira JA, Venâncio A, Abrunhosa L. Anti-aflatoxigenic Effect of Organic Acids Produced by *Lactobacillus plantarum*. International Journal of Food Microbiology. 2018;264:31-38.
[18] Wang Y, Jiang L, Zhang Y, Ran R, Meng X, Liu S. Research Advances in The Degradation of Aflatoxin by Lactic Acid Bacteria. Journal of Venomous Animal and Toxins Including Tropical Diseases. 2023;29:1-11.
[19] Zhang Y, Li B, Fu M, Wang Z, Chen K, Du M, Zalan Z, Hegyi F, Kan J. Antifungal Mechanisms of Binary Combinations of Volatile Compounds Produced by Lactic Acid Bacteria Strains Against *Aspergillus flavus*. Toxicon. 2024;243:1-12.
[20] Pundir RK, Rana S, Kashyap N, Kaur A. Probiotic Potential of Lactic Acid Bacteria Isolated from Food Samples: An In Vitro Study. Journal of Applied Pharmaceutical Science. 2013;3(3):85-93.
[21] Chen YS, Yanagida F, Shinohara T. Isolation and Identification of Lactic Acid Bacteria from Soil Using an Enrichment Procedure. Letters in Applied Microbiology. 2005;40(3): 195-200.
[22] Devi S, Soni R. Relevance of Microbial Diversity in Implicating Soil Restoration and Health Management. In: Meena R, editor. Soil Health Restoration and Management. Singapore: Springer; 2020.

[23] Chauhan P, Sharma N, Tapwal A, Kumar A, Verma GS, et al. Soil Microbiome: Diversity, Benefits, and Interactions with Plants. Sustainability. 2023;15(19):14643.
[24] Xu SJ, Kim BS. Biocontrol of Fusarium Crown and Root Rot and Promotion of Growth of Tomato by *Paenibacillus* Strains Isolated from Soil. Mycobiology. 2014; 42(2):158-166.
[25] Fitriyana NI, Suwasono S, Kusnadi J. Isolasi dan Identifikasi Bakteri Asam Laktat Indigenous dari Fermentasi Alami Biji Kakao Sebagai Kandidat Agen Antikapang. AGROINTEK. 2015;9(1):33-41.
[26] Ismail YS, Yulvizar C, Putriani. Isolasi, Karakterisasi dan Uji Aktivitas Antimikroba Bakteri Asam Laktat dari Fermentasi Biji Kakao (*Theobroma cacao* L.). BIOLEUSER. 2017;1(2):45-53.
[27] Sharma A, Kaushik N, Sharma A, Bajaj A, Rasane M, Shouche YS, Marzouk T. Djébali N. Screening of Tomato Seed Bacterial Endophytes for Antifungal Activity Reveals Lipopeptide Producing *Bacillus siamensis* strain NKIT9 As A Potential Bio-control Agent. Frontiers in Microbiology. 2021;12:1228-1245.
[28] Anith KN, Nysanth NS, Natarajan C. Novel and Rapid Agar Plate Methods for In Vitro Assessment of Bacterial Biocontrol Isolates' Antagonism Against Multiple Fungal Phytopathogens. Letters in Applied Microbiology. 2021;73(2):229-236.
[29] Hendricks KE, Christman MC, Roberts PD. Statistical Evaluation of Methods of In-vitro Growth Assessment for *Phyllosticta citricarpa*: Average Colony Diameter vs. Area. PloS one. 2017;12(1):1-7.
[30] Dwidjoseputro D. Dasar-Dasar Mikrobiologi. Jakarta: Djambatan; 1978.
[31] Sari YES, Azizah F, Tumbol MVL, Prayekti E, Nurbidayah N, Wijayanti DR, Rohayati R, Astuti RAW, Sartika F, Situmeang SMF, Inayah M, Rini CS, Juniawan MF, Darmo K, Dewi YR, Sahroni M, Sanatang S, Patricia V, Arimurti ARR, Dewi NPSP. Bakteriologi 1: Teknologi Laboratorium Medis. Cilacap: PT. Media Pustaka Indo; 2025.
[32] Tolieng V, Prasirtsak B, Sitdhipol J, Thongchul N, and Tanasupawat S. Identification and Lactic Acid Production of Bacteria Isolated from Soils and Tree Barks. Malaysian Journal of Microbiology. 2017;13(2):100-108.
[33] Husain A, Hassan Z, Huda-Faujan N, Lani MN. Antifungal Activity of Lactic Acid Bacteria Isolated From Soil Rhizosphere on *Fusarium* Species Infected Chilli Seeds. American Scientific Research Journal for Engineering, Technology, and Sciences. 2017;29(1):182-202.
[34] Raman J, Kim J, Choi KR, Eun H, Yang D, Ko Y, Kim S. Application of Lactic Acid Bacteria (LAB) in Sustainable Agriculture: Advantages and Limitations. International Journal of Molecular Sciences. 2022;23(7784):1-22.
[35] Mora-Villalobos JA, Montero-Zamora J, Barboza N, Rojas-Garbanzo C, Usaga J, Redondo-Solano M, Schroedter L, Olszewska-Widdrat A, López-Gómez J P. Multi-Product Lactic Acid Bacteria Fermentations: A Review. Fermentation. 2020; 6(1):23.
[36] Ekundayo FO. Isolation and Identification of Lactic Acid Bacteria from Rhizosphere Soils of Three Fruit Trees, Fish, and Ogi. International Journal of Current Microbiology and Applied Sciences. 2014;3(3): 991-998.
[37] Hutahaean AJN, Silalahi J, Suryanto D, Satria D. Characterisation of Lactic Acid Bacteria from Dengke Naniura of Common Carp (*Cyprinus carpio*) with α-glucosidase Inhibitory Activity. Open Access Macedonian Journal of Medical Sciences. 2019;7(22):3794.
[38] Finanda A, Mukarlina, dan Rahmawati. Isolasi dan Karakterisasi Genus Bakteri Asam Laktat dari Fermentasi Daging Buah Pisang Kepok (*Musa paradisiaca* L.). PROTOBIONT. 2021;10(2):37-41.
[39] Uvarova YE, Bryanskaya AV, Roznov AS, Shlyakhtun VN, Demidov EA, Starostin KV, Goryachkovskaya TN, Shekhovtsov SV, Slynko NM, Peltek SE. An Integrated Method for Taxonomic Identification of Microorganisms. Vavilovskii Zhurnal Genet Selektsii. 2020;24(4):376-382.
[40] Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST, editors. Bergey's Manual of Determinative Bacteriology. 9th Edition. Baltimore: William and Wilkins; 2000.
[41] De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K, Whitman WB, editors. Bergey's Manual of Systematic Bacteriology Volume 3: Firmicutes. New York: Springer; 2009.

[42] Ouattara HG, Yao W, Ouattara H, Germain K, Sebastien N. Fungal Inhibition by Lactic Acid Bacteria (LAB) is modulated in Vitro by Cocoa Fermentation-related Conditions: Towards a Biocontrol of Fungi in Processing Cocoa. Emirates Journal of Food and Agriculture. 2023;35(9):853-861.

[43] Abbaszadeh S, Tavakoli R, Sharifzadeh A, Shokri H. Lactic Acid Bacteria as Functional Probiotic Isolates for Inhibiting the Growth of *Aspergillus flavus*, A. *parasiticus*, A. *niger* and *Penicillium chrysogenum*. Journal de Mycologie Médicale. 2015;25:263-267.

[44] Zhao S, Hao X, Yang F, Wang Y, Fan X, Wang Y. Antifungal Act of *Lactobacillus plantarum* ZZUA493 and Its Application to Extend The Shelf Life of Chinese Steamed Buns. Foods. 2022;11(2):195.

[45] Svanström Å, Boveri S, Boström E, Melin P. The Lactic Acid Bacteria Metabolite Phenyllactic Acid Inhibits Both Radial Growth and Sporulation of Filamentous Fungi. BMC Research Notes. 2013;6(1):464.

[46] Ashwini M, Kamble MV, Babar AR, Jahagirdar S, Jagdeesh KS, Mulla JA. Evaluation of Antifungal Activity of Lactic Acid Bacteria Against *Aspergillus flavus*. Journal of Pure and Applied Microbiology. 2015;9(4):1-9.

[47] Damayanti E, Suryani AE, Sofyan A, Karimy MF, Julendra H. Seleksi Bakteri Asam Laktat dengan Aktivitas Anti Jamur yang diIsolasi dari Silase dan Saluran Cerna Ternak. AGRITECH. 2015;35(2): 164-169.

[48] Siedler S, Balti R, Neves AR. 2019. Bioprotective Mechanisms of Lactic Acid Bacteria Against Fungal Spoilage of Food. Biotechnology. 2019;56:138-146.