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Optimization of Riau Local Lactic Acid Bacteria Concentration and Evaluation of Cell and Supernatant Antifungal Activity Against *Aspergillus parasiticus*

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Abstract

Lactic acid bacteria (LAB) are capable of producing bioactive metabolites that can inhibit the growth of molds, including *Aspergillus parasiticus*. However, the potential of local LAB strains from Riau as biocontrol agents has not been extensively explored. This study aimed to optimize the concentration of LAB isolated from Riau to obtain the best antifungal activity, evaluate the effectiveness of their supernatants against the growth of *A. parasiticus*, and assess their potential application in food commodities.The antifungal activity of cell suspensions was tested using the well-diffusion method with varying concentrations (OD). Cell-free supernatants were analyzed through dilution assays to determine the percentage of fungal inhibition. Application testing was conducted on corn kernels as a biocontrol model. The results showed that increasing LAB cell density correlated with a larger inhibition zone against the mold. The two best isolates, S28 and AERH7, at OD 0.8 and 48 hours of incubation, produced inhibition zones of 18.67 mm and 17.58 mm, respectively. The supernatants of both isolates also demonstrated strong activity, with inhibition percentages of 72.17% and 64.39%, confirming the role of LAB metabolites in antifungal activity. Application on corn kernels further showed a visible reduction in mold growth compared to the control.This study concludes that LAB strains from Riau possess significant potential as natural biocontrol agents against *A. parasiticus*. These findings provide practical benefits for developing microbial-based biopreservation strategies to enhance food safety and quality.

Keywords: Aspergillus inhibitors; Lactic acid bacteria; Antifungal metabolites; Bacterial supernatant; Food biopreservation.

INTRODUCTION

Food safety is a fundamental aspect of public health protection, particularly because food materials are highly susceptible to contamination during production, storage, and distribution. One of the most serious threats is contamination by toxigenic fungi. Molds from the genus *Aspergillus*, especially *Aspergillus parasiticus*, are frequently found in food products stored under unhygienic conditions and are capable of producing aflatoxins—hazardous secondary metabolites that are carcinogenic, hepatotoxic, and mutagenic. Studies over the last five years have indicated that *A. parasiticus* remains a major source of aflatoxin contamination in peanuts, maize, and various tropical commodities, particularly in hot and humid regions (Pickova, et al., 2021).

Efforts to control pathogenic fungi have long relied on synthetic chemical compounds such as copper sulfate ($CuSO_4$) (Lamichhane et al, 2018). Although effective in inhibiting fungal growth by disrupting metabolic pathways and damaging cell membranes, its use poses toxicity risks to humans. Exposure to $CuSO_4$ has been reported to cause poisoning, digestive disorders, and liver damage (Hajimohammadi et al., 2022). Moreover, recent studies have shown that $CuSO_4$ residues may accumulate in food and the environment, posing long-term health risks (Xu et al., 2022). These concerns highlight the need for safer and more environmentally friendly fungal control strategies.

One increasingly promising approach is the use of non-pathogenic microorganisms as natural biocontrol agents. Lactic acid bacteria (LAB) are highly potential candidates because, aside from their role in fermentation, they produce various bioactive metabolites such as lactic acid, acetic acid, hydrogen peroxide, diacetyl, CO_2, and bacteriocins. These compounds can lower environmental pH and induce fungal cell lysis. Recent studies also demonstrate that LAB produce specific antifungal compounds such as phenyllactic acid and cyclic peptides, which further enhance their antifungal activity (Fugaban et al., 2023). As probiotics, LAB are also considered safe for biopreservation applications compared with chemical preservatives (Shah, et al, 2024).

Previous research consistently reports the ability of LAB to inhibit toxigenic fungi. Local research centers have shown that LAB isolated from silage and livestock gastrointestinal tracts can inhibit *A. flavus* and *A. parasiticus*, producing inhibition zones of approximately 6–9 mm (Damayanti, 2019). Natasia (2020) reported inhibition diameters of up to 16.97 mm against *A. flavus*, while Nasution, et al (2025) demonstrated that LAB inhibited *A. parasiticus* with a zone of 13.5 mm. Additionally, recent studies confirm that LAB species such as *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, and *Pediococcus pentosaceus* exhibit strong antifungal activity through mechanisms of acidification, biosurfactant production, and mycotoxin degradation (Saifur et al., 2025; Pribadhi et al, 2025;Muhialdin et al., 2020).

Despite the extensive reports on LAB antifungal activity, several research gaps remain. Most studies assess inhibition activity without determining the optimal concentration or cell density, even though defining the effective concentration is essential for biocontrol application. Furthermore, the potential of LAB derived from traditional fermented foods of Riau has not been thoroughly investigated, despite the fact that local microbes often possess unique physiological characteristics. Previous studies have also focused predominantly on cell suspensions, while the antifungal activity of cell-free supernatants containing antimicrobial metabolites has not been comprehensively explored. Recent literature highlights significant differences between antifungal mechanisms derived from whole cells and those from supernatants (Mani_Lopez et al., 2022; Rohman et al., 2025).

Addressing these research gaps, the present study aims to optimize the concentration of LAB strains isolated from Riau to obtain the highest antifungal activity against *A. parasiticus* using the well-diffusion method; to evaluate the antifungal effectiveness of cell-free supernatants through dilution assays; and to determine the potential application of both forms as natural biocontrol agents in food systems. The novelty of this research lies in the exploration of LAB from Riau traditional fermented foods, which remain understudied; the optimization of effective LAB concentration, which has not been performed in previous studies; and the combined evaluation of both cell and supernatant activity, including their application in a food model. This study is expected to provide valuable scientific contributions to the development of safe, effective, and locally based biopreservation strategies.

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MATERIALS AND METHODS

Materials

Petri dishes, inoculating loop, spirit lamp, incubator (Memmert®), autoclave (Gea®), oven (Memmert®), cork borer, spatula, stirring rod, analytical balance (Shimadzu®), micropipettes (Nesco®), microtips, caliper, Erlenmeyer flasks (Iwaki Pyrex®), test tubes (Iwaki®), beakers (Iwaki Pyrex®), Eppendorf tubes, vortex mixer (Asone®), centrifuge, biosafety cabinet (Esco®), UV-Vis spectrophotometer (Shimadzu®), hot plate, and magnetic stirrer. Local Riau isolates of lactic acid bacteria (LAB) with isolate codes S28, AERH7, TKHT2, and TKHT3; *Aspergillus parasiticus* (IPBCC 08 0611); fresh corn kernels; deMann Rogosa Sharpe Agar (MRSA); deMann Rogosa Sharpe Broth (MRSB); Potato Dextrose Agar (PDA); Potato Dextrose Broth (PDB); H_2SO_4 98%; $BaCl_2$; sodium hypochlorite 12%; distilled water; physiological NaCl; 70% ethanol; spiritus; plastic wrap; aluminum foil; cotton plugs; gauze; corn silk; 0.45 μm syringe filters; and label paper.

Methods

Samples

The samples used in this study were local Riau LAB isolates (S28, AERH7, TKHT2, and TKHT3) obtained from traditional Riau fermented foods, and *Aspergillus parasiticus* obtained from the Bogor Agricultural Institute Culture Collection (IPBCC).

Sterilization of Instruments and Media

All instruments were washed and dried. Glassware with an opening was covered with cotton wrapped in gauze and aluminum foil, then wrapped tightly with parchment paper. The glassware was sterilized in an oven at 160 °C for 2 hours. Spatulas, stirring rods, tweezers, and inoculating loops were sterilized by flaming over a spirit lamp for 20 seconds. Media, microtips, and Eppendorf tubes were sterilized using an autoclave at 121 °C for 15 minutes. All antifungal assays were performed aseptically inside a biosafety cabinet that had been disinfected with 70% ethanol [8].

Preparation of Culture Media

MRSA (34.1 g/500 mL), MRSB (26.1 g/500 mL), PDA (3.9 g/100 mL), and PDB (2.65 g/100 mL) were each prepared by dissolving the respective media in distilled water, heating while stirring until homogeneous, and covering the flasks appropriately. All media were sterilized using an autoclave at 121 °C for 15 minutes. After cooling, MRSA and PDA were poured into Petri dishes to solidify, with PDA supplemented with chloramphenicol (0.05 g/L). MRSB and PDB were distributed into sterile test tubes for subsequent use.

Subculture of Lactic Acid Bacteria (LAB)

LAB isolates were subcultured anaerobically in 10 mL MRSB by adding 5–6 loopfuls of cells from pure stocks using a sterile inoculating loop. Cultures were incubated at 37 °C for 24 hours (Pritom et al, 2022).

Subculture of *Aspergillus parasiticus*
The fungus was subcultured by streaking 3–4 loopfuls onto PDA in Petri dishes and test tubes using a sterile loop, then incubated at room temperature for 7 days.

Preparation of LAB Suspension
Subcultured LAB were homogenized using a vortex mixer. The turbidity of each LAB suspension was measured using a UV-Vis spectrophotometer at 625 nm to obtain LAB concentrations with OD 0.2, 0.4, 0.6, and 0.8.

Preparation of *Aspergillus parasiticus* Suspension
The fungus was streaked (3–4 strokes) into a test tube containing physiological saline, homogenized using a vortex mixer, and its turbidity measured at 625 nm to obtain an OD of 0.1.

Inoculation of Fungal Culture
A total of 100 µL *A. parasiticus* suspension was pipetted into a Petri dish, followed by the addition of 25 mL MRSA, gently swirled, and allowed to solidify.

Optimization of Antifungal Activity of LAB Against *Aspergillus parasiticus*
Antifungal activity was evaluated using the well diffusion method. Six wells (5 mm diameter) were made in MRSA plates containing *A. parasiticus* OD 0.1, with three replications. LAB suspensions at OD 0.2, 0.4, 0.6, and 0.8 (40 µL each) were added into separate wells. The negative control was 40 µL MRSB, and the positive control was ketoconazole. Plates were incubated at 37 °C. Clear zone diameters around the wells were measured using a caliper at 24, 48, and 72 hours.

Antifungal Activity of LAB Supernatant Using UV-Vis Spectrophotometry

- **Preparation of LAB Supernatant**

The LAB strain with the highest antifungal activity was inoculated into MRSB and incubated at 37 °C for 48 hours. Cultures were centrifuged at 10,000 rpm for 10 minutes to obtain cell-free supernatant (CFS), which was filtered through a 0.45 µm syringe filter and heated at 100 °C for 15 minutes to inactivate cells.

- **Preparation of Fungal Suspension**

Fungal cultures were streaked into PDB, homogenized, and turbidity was adjusted to OD 0.1 at 625 nm.

- **Antifungal Assay of Cell-Free Supernatant (CFS)**

CFS was mixed with *A. parasiticus* suspension (OD 0.1) at a 1:1 ratio, vortexed, and the 0-hour OD value was recorded. The mixture was incubated at 37 °C for 72 hours, and OD was measured again at 625 nm. The same procedure was performed for the control (Muhialdin et al., 2020).
The inhibition percentage was calculated using the formula:
$\%\ \text{Inhibition}=[(Ak^{72}-Ak^{0})-(As^{72}-As^{0})/Ak^{72}-Ak^{0}]\times 100\%$
Description:
Ak = absorbance of control
As = absorbance of sample

Biocontrol of *Aspergillus parasiticus* on Fresh Corn Kernels
Corn was obtained from local farmers, manually shelled, and sterilized with 2% sodium hypochlorite for 5 minutes, then rinsed with sterile distilled water. Corn kernels were placed in four sterile Petri dishes, each containing 20 kernels (≈10 g).

- Petri dish 1: each kernel was treated with 50 µL *A. parasiticus* suspension + 50 µL LAB supernatant (most effective).
- Petri dish 2: each kernel was treated with 50 µL LAB suspension (OD 0.8) + 50 µL *A. parasiticus*.
- Petri dish 3: each kernel was treated with 100 µL fungal suspension (positive control).
- Petri dish 4: each kernel was treated with 100 µL MRSB (negative control).
- Petri dish 5: untreated kernels (comparison).

All dishes were incubated at 30 °C. Texture and color of kernels were observed organoleptically on day 0, day 5, and day 7 (Muhialdin et al., 2020).

RESULTS AND DISCUSSION

Result

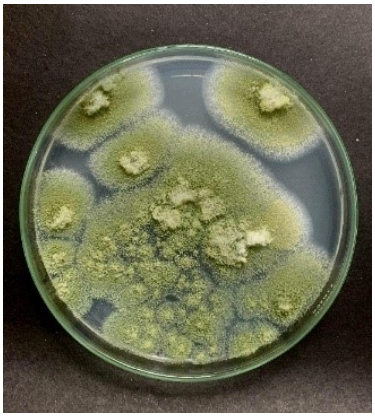

Figure 1. Results of *Aspergillus parasiticus* Rejuvenation Over 7 Days

Tabel 1. OD and Average Inhibition Zone Diameter (mm) of LAB Isolates at 24, 48, and 72 Hours.

Lactic Acid Bacteria Isolate	OD	Average Inhibition Zone Diameter (mm)		
		24 h	48 h	72 h
S28	0,2	14,44 ± 0,51	12,33 ± 0,29	11,83 ± 0,29
	0,4	14,52 ± 1,05	14,08 ± 0,52	12,22 ± 0,19
	0,6	17,13 ± 0,53	17,50 ± 0,87	13,43 ± 0,13
	0,8	18,55 ± 0,08	18,67 ± 0,58	15,25 ± 0,65
	control (+)	28,00 ± 0,87	28,33 ± 2,25	30,75 ± 0,67
	control (-)	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00
AERH7	0,2	14,14 ± 0,12	10,91 ± 0,80	12,15 ± 0,60
	0,4	14,33 ± 0,19	13,95 ± 0,66	12,54 ± 0, 07
	0,6	16,26 ± 0,23	15,92 ± 0,30	13,18 ± 0, 08
	0,8	18,11 ± 0,34	17,58 ± 0,14	14,53 ± 0,46
	control (+)	27,08 ± 0,56	28,61 ± 0,35	32,04 ± 0,07
	control (-)	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00
TKHT2	0,2	8,20 ± 0,17	7,42 ± 0,14	7,97 ± 0,05
	0,4	8,54 ± 0,36	8,27 ± 0,82	8,17 ± 0,17
	0,6	10,17 ± 0,07	10,21 ± 0,26	10,15 ± 0,06
	0,8	11,27 ± 0,25	10,65 ± 0,47	10,79 ± 0,62
	control (+)	28,00 ± 0,87	30,16 ± 0,14	31,83 ± 0,29
	control (-)	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00
TKHT3	0,2	9,15 ± 0,13	8,17 ± 0,29	7,49 ± 0,02
	0,4	11,24 ± 0,30	10,20 ± 0,18	7,54 ± 0,36
	0,6	11,80 ± 0,61	10,93 ± 0,69	8,34 ± 0,14
	0,8	12,07 ± 0,95	11,66 ± 0,73	8,76 ± 0,25
	control (+)	27,17 ± 0,29	31,49± 1,29	31,67 ± 1,44
	control (-)	5,00 ± 0,00	8,17 ± 0,29	5,00 ± 0,00

Description:
Positive Control (+): 1% Ketoconazole positive control; Negative Control (−): MRSB media negative control (5.00 mm well diameter); Category: Strong (≥20 mm), Moderate (15–19 mm), Weak (≤14 mm) (CLSI, 2020)

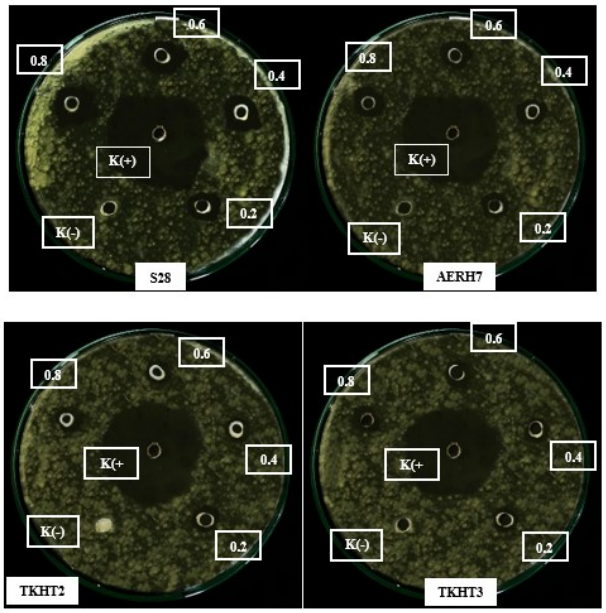

Figure 2. Inhibition Zone Diameter of Lactic Acid Bacteria Isolates Against *Aspergillus parasiticus* at 37 °C for 24 Hours.

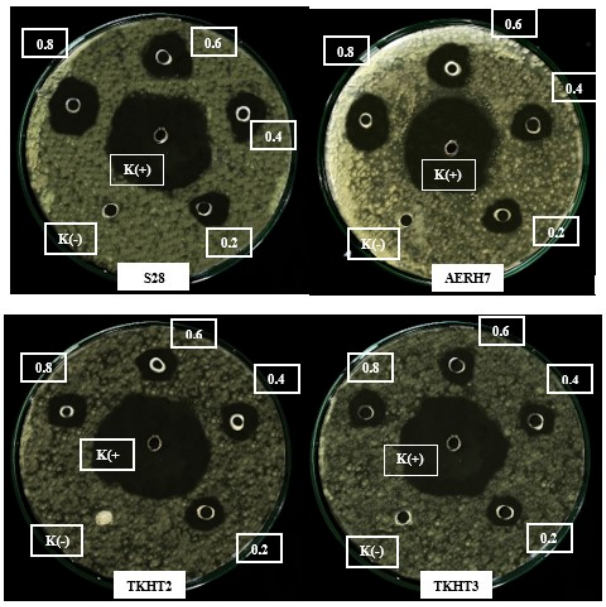

Figure 3. Inhibition Zone Diameter of Lactic Acid Bacteria Isolates Against *Aspergillus parasiticus* at 37 °C for 48 Hours

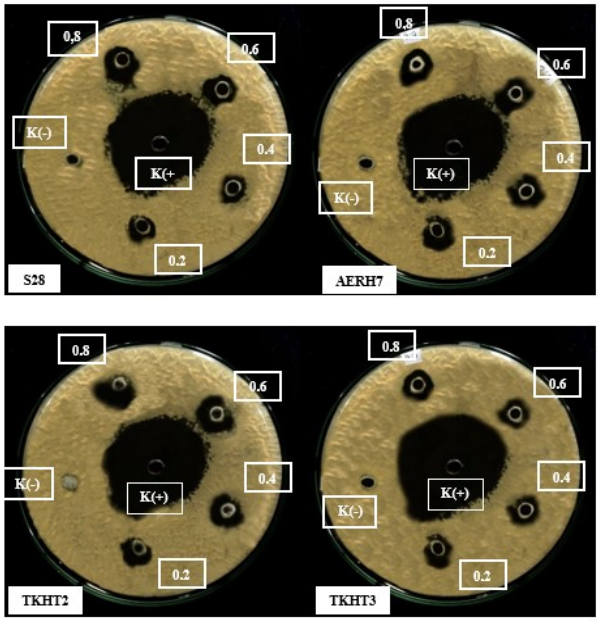

Figure 4. Inhibition Zone Diameter of Lactic Acid Bacteria Isolates Against *Aspergillus parasiticus* at 37 °C for 72 Hours.

Table 2. Tukey Test Results for Isolates S28, AERH7, TKHT2, and TKHT3 at OD 0.8 After 48 Hours.

Tukey HSD[a]

Optical Density	N	Subset for alpha = 0.05	
		1	2
TKHT2	3	10.6533	
TKHT3	3	11.6600	
AERH7	3		17.5800
S28	3		18.6667
Sig.		.166	.128

Table 3. Tukey HSD Test Results for Isolate S28 at 48-Hour Incubation.

Optical Density	N	Subset for alpha = 0.05			
		1	2	3	4
K-	3	5.0000			
0,2	3		12.3333		
0,4	3		14.0800		
0,6	3			17.5000	
0,8	3			18.6667	
K+	3				28.3333
Sig.		1.000	.370	.743	1.000

Table 4. Tukey HSD Test Results for Isolate AERH 7 at 48-Hour Incubation.

Inhibitory Diameter.

Tukey HSD[a]

Optical Density	N	Subset for alpha = 0.05				
		1	2	3	4	5
K-	3	5.0000				
0,2	3		10.9133			
0,4	3			13.9467		
0,6	3				15.9233	
0,8	3					17.5800
K+	3					
Sig.		1.000	1.000	1.000	1.000	1.000

Table 5. Results of Cell-Free Supernatant Assay of Lactic Acid Bacteria Isolates S28 and AERH7 at 37 °C.

Treatment	Absorbance				Mean ± SD		% Inhibition
	0 h		72 h				
	I	II	I	II	0 Jam	72 Jam	
S28	0,155	0,157	0,268	0,272	0,156 ± 0,001	0,270 ± 0,003	72,19
AERH7	0,131	0,135	0,279	0,281	0,133 ± 0,003	0,280 ± 0,001	64,39
Control (+)	0,167	0,172	0,577	0,583	0,170 ± 0,004	0,580 ± 0,004	-

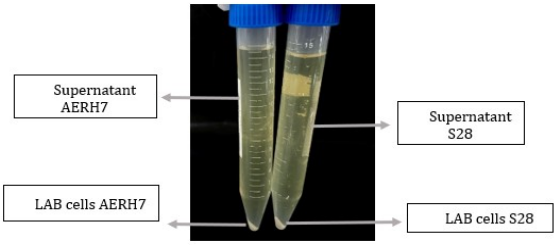

Figure 5. Cell-Free Supernatant of Lactic Acid Bacteria After Centrifugation.

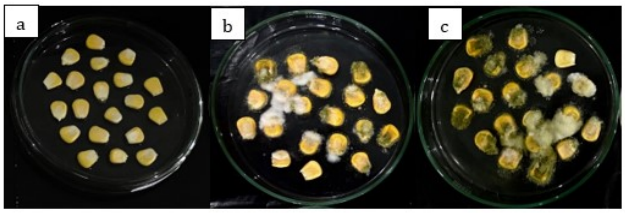

Figure 6. Biocontrol assay results on maize kernels treated with 50 µL of S28 cell suspension + 50 µL of *Aspergillus parasiticus* spore suspension, incubated at 30 °C. a. Day 0, b. Day 5, c. Day 7.

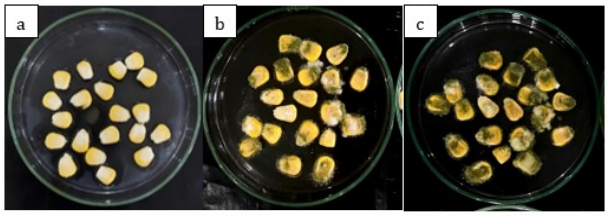

Figure 7. Biocontrol assay results on maize kernels treated with 50 µL of AERH7 cell suspension + 50 µL of *Aspergillus parasiticus* spore suspension, incubated at 30 °C. a. Day 0, b. Day 5, c. Day 7.

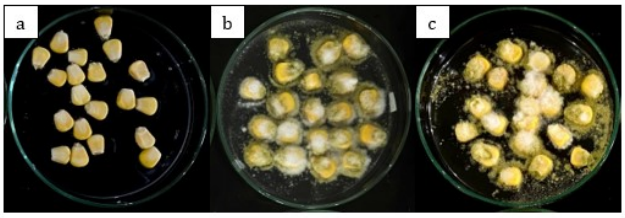

Figure 8. Biocontrol assay results on maize kernels treated with 50 µL of S28 supernatant + 50 µL of *Aspergillus parasiticus* spore suspension, incubated at 30 °C. a. Day 0, b. Day 5, c. Day 7.

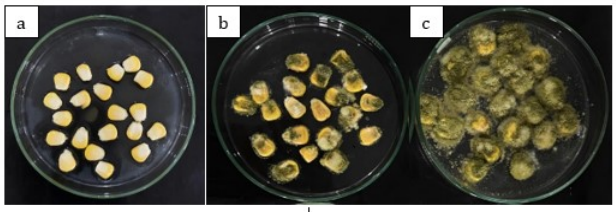

Figure 9. Biocontrol assay results on maize kernels treated with 50 µL of AERH7 supernatant + 50 µL of *Aspergillus parasiticus* spore suspension, incubated at 30 °C. a. Day 0, b. Day 5, c. Day 7.

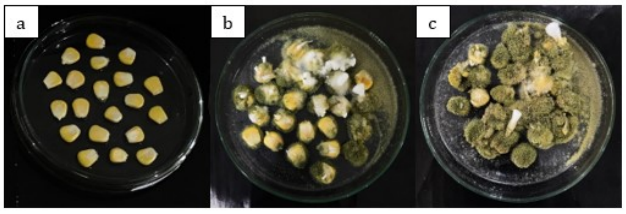

Figure 10. Biocontrol assay results on maize kernels treated with 100 µL of *Aspergillus parasiticus* spore suspension, incubated at 30 °C as the positive control. a. Day 0, b. Day 5, c. Day 7.

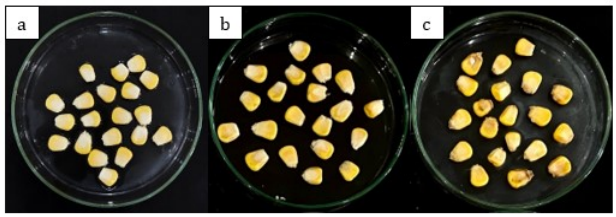

Figure 11. Biocontrol assay results on maize kernels treated with 100 µL of MRSB medium, incubated at 30 °C as the negative control. a. Day 0, b. Day 5, c. Day 7.

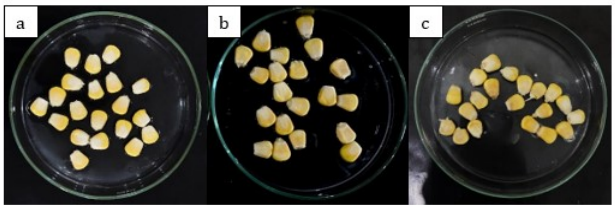

Figure 12. Biocontrol assay results on untreated maize kernels incubated at 30 °C as a comparison. a. Day 0, b. Day 5, c. Day 7.

Discussion

This study utilized four lactic acid bacteria (LAB) isolates native to Riau, namely S28 derived from fermented *cangkuak semaung* (Lovenia, 2023), AERH7 from *nira enau* (toddy palm sap), and TKHT2 and TKHT3 isolated from *tapai ketan hijau* (Nasution et al., 2025). These four isolates were selected as potential anti-mold agents due to their morphological and physiological characteristics, indicating their ability to produce antifungal metabolites such as organic acids, hydrogen peroxide (H_2O_2), phenyllactic acid (PLA), and peptide compounds. In the context of biocontrol studies targeting aflatoxin-producing molds, the use of local LAB isolates is highly important, not only in terms of biological effectiveness but also as a strategy to develop local biological resources into sustainable food-safety solutions. Local isolates often have better adaptability to the environmental conditions of local food ecosystems, making their potential as biopreservative agents stronger compared to commercial LAB strains cultivated under standardized laboratory conditions (Giwa et al., 2025).

The primary objective of this study was to optimize the anti-mold activity of the four LAB isolates against *Aspergillus parasiticus*, one of the major producers of aflatoxins B1, B2, G1, and G2, which are well-known for their carcinogenicity, hepatotoxicity, and immunosuppressive effects in humans and animals. Controlling *A. parasiticus* is therefore crucial, as aflatoxin contamination represents one of the most serious global food safety issues, particularly in cereal commodities such as maize, which is a staple food and animal feed in many tropical countries. The use of biocontrol agents such as LAB is increasingly studied because they are safer than synthetic fungicides and align with the global trend toward “clean-label” foods (Liu et al., 2018).

Optimization was performed by varying LAB cell concentrations using optical density (OD 0.2–0.8). OD measurements were conducted using a UV–Vis spectrophotometer at a wavelength of 625 nm, based on the Lambert–Beer law which states that absorbance is proportional to the concentration of particles in a solution, in this case LAB cell density. The use of OD to standardize cell concentration is widely applied and recommended in modern antimicrobial studies because it provides a strong correlation between viable cell numbers and active metabolite production (Matsue et al., 2019). Thus, OD-based optimization allowed assessment

of the relationship between LAB population density and the resulting anti-mold activity.

Once concentration standards were established, anti-mold activity was assessed using the well diffusion assay. This method was chosen because it distinguishes inhibitory effects produced by live cells from those produced by metabolites in the cell-free supernatant (CFS). In this assay, LAB suspension or supernatant is dispensed into wells made on agar media previously inoculated with *A. parasiticus*, followed by observation of inhibition zone formation around the wells. These zones represent the effectiveness of antifungal activity of metabolites diffusing into the media. Numerous previous studies have shown that this method is highly effective for evaluating the antifungal potential of LAB, particularly in examining the roles of metabolites that actively suppress spore and hyphal growth (Trinh et al., 2025).

In this study, the diffusion assay was conducted to compare two LAB components simultaneously: (1) live cells that perform competition and produce metabolites *in situ*, and (2) cell-free supernatant (CFS) containing fermentation-derived metabolites. CFS typically contains various antifungal compounds, including lactic acid, acetic acid, phenylacetic acids, bacteriocins, volatile compounds, and H_2O_2. This "dual approach" is crucial because LAB may inhibit fungi through direct interaction or through metabolite contribution alone. By separating these components, the study could clearly identify the dominant inhibitory mechanism.

All assays were performed in triplicate and compared against positive and negative controls. In this study, the positive control used Ketomed® shampoo containing 2% ketoconazole, a synthetic fungicide proven effective against dermatophytes and food-spoilage molds. Ketoconazole was selected because it is stable, exhibits strong antifungal activity, and provides a clear benchmark standard. Meanwhile, the negative control (K−) received no LAB or antifungal treatment, thus representing maximal fungal growth. The function of these controls is essential: the positive control indicates the maximum achievable inhibitory effect, whereas the negative control indicates baseline growth without treatment. Therefore, the inhibition zones produced by each LAB isolate could be proportionally evaluated.

The *A. parasiticus* culture used in the study was rejuvenated on Potato Dextrose Agar (PDA) before use. Its typical morphology is presented in Figure 1, showing colonies with yellowish-green to dark green pigmentation, filamentous surfaces, and dense sporulation. These characteristics confirm that the culture was in an active growth phase, where aflatoxin production is typically high. Ensuring the quality of fungal cultures strengthens the reliability of anti-mold assays.

Results of anti-mold activity are presented in Table 1 and Figures 2–4, showing a consistent pattern in which increasing LAB OD correlates positively with inhibition zone diameter. At low OD (0.2), inhibition zones were modest but still detectable. At OD 0.4 and 0.6, inhibition increased significantly, reaching the highest values at OD 0.8.

Isolates S28 and AERH7 demonstrated the strongest activity, producing inhibition zones approaching 18 mm, while TKHT2 and TKHT3 generated only 10–12 mm under the same conditions. These results indicate substantial variability in anti-mold abilities among LAB isolates, likely driven by differences in their capacity to produce bioactive metabolites.

The negative control (K−) exhibited the smallest inhibition zone, approximately 5 mm, reflecting maximum fungal growth without intervention. Conversely, the positive control containing ketoconazole produced the largest inhibition zones (28–32 mm), serving as the standard for optimal antifungal activity. These extreme reference values allow more objective evaluation of LAB effectiveness.

Further analysis using the Tukey HSD test is presented in Table 2. The test revealed that at OD 0.8 with 48-hour incubation, isolates S28 and AERH7 belonged to the highest-activity subset (18.67 and 17.58 mm, respectively). These two isolates were not significantly different from each other but were statistically superior to TKHT2 and TKHT3, which formed separate subsets with lower inhibition zones (10.65–11.66 mm). This confirms that anti-mold activity is highly isolate-specific and that S28 and AERH7 are the best candidates for biocontrol development. Findings are consistent with Nasution et al. (2025), who reported that effective local LAB isolates typically produce inhibition zones of 15–20 mm.

Results in Table 3 and Table 4 show that across all OD intervals, anti-mold activity increased linearly with LAB concentration. OD 0.2–0.4 formed the first subset (12.33–14.08 mm), while OD 0.6–0.8 formed the second subset with higher inhibition zones (17.50–18.67 mm). This demonstrates a positive and significant relationship between LAB cell density and antifungal activity, consistent with studies showing that production of bioactive metabolites increases directly with cell population (Shehata et al., 2019).

The cell-free supernatant (CFS) assay was performed on the two best isolates, S28 and AERH7, using supernatants obtained from OD 0.8 cultures incubated for 48 hours, considered optimal for metabolite production. At this stage, the assay employed the turbidity-based dilution method (OD turbidity assay) to evaluate the ability of CFS to suppress *A. parasiticus*, quantified by measuring decreases in optical density of fungal suspensions. The results in Table 5 and Figure 5 show that CFS from S28 produced an inhibition percentage of 72.19%, while AERH7 produced 64.39%.

These findings indicate that LAB metabolites contribute significantly to inhibiting *A. parasiticus*. Previous studies reported that LAB metabolites such as PLA, lactic acid, bacteriocins, and volatile compounds

play crucial roles in disrupting fungal cell membranes, interfering with carbohydrate metabolism, and inhibiting spore formation (Rohman et al., 2025; Giwa et al., 2025; Muhialdin et al., 2020).

Biocontrol application tests were performed on fresh maize kernels to simulate real food-storage conditions. Results in Figures 6–12 show that live LAB cells more effectively inhibited mold growth compared to CFS. This occurs because live cells not only produce metabolites continuously but also compete directly with fungi for nutrients and ecological niches.

This is consistent with findings by Mani Lopez et al. (2022), which state that live cultures are more stable and effective in complex food environments compared to CFS alone. LAB are also capable of forming protective biofilms and creating acidic environments unfavorable for fungal growth (Muhialdin et al., 2020).

Overall, this study demonstrates that Riau-native LAB isolates—particularly S28 and AERH7—exhibit strong potential as biocontrol agents against *Aspergillus parasiticus* and represent a promising strategy to reduce aflatoxin contamination risks in maize commodities. The combined use of OD optimization, separation of cell- and metabolite-based activity, and direct biocontrol tests on food materials illustrates high implementation potential for modern biopreservation. Moreover, the use of local microbial isolates supports conservation of regional microbial biodiversity and the development of safe, effective, and sustainable domestically sourced biopreservatives.

CONCLUSIONS

The results of this study demonstrate that lactic acid bacteria (LAB) isolates originating from local Riau sources possess strong potential as antifungal agents against *Aspergillus parasiticus*. Concentration optimization showed that increasing LAB cell density correlated with greater inhibition effectiveness, with antifungal activity consistently rising across OD 0.2–0.8 and reaching its highest effectiveness at OD 0.8 after 48 hours of incubation. Isolates S28 and AERH7 were identified as the most active strains, both in cell-based assays and cell-free supernatant (CFS) tests. The supernatants obtained after 48 hours of incubation at OD 0.8 exhibited strong inhibitory activity, with inhibition rates of 72.19% for S28 and 64.39% for AERH7, confirming the crucial role of LAB-derived bioactive metabolites in antifungal activity. In addition, both live LAB cells and their supernatants effectively reduced fungal colonization on maize kernels in situ, as indicated by visibly lower mold growth compared with the controls. These findings support the application of local LAB isolates as natural biocontrol agents with promising potential for use in food systems.

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