Antagonistic Activity and Surface Decontaminant Potential of Lactic Acid Bacteria from Fermented *Oreochromis niloticus*

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Abstract

Lactic acid bacteria (LAB) have been extensively explored as potential biopreservants. They could produce substances with antimicrobial properties such as bacteriocins and organic acids which can also be the cause of antagonistic activity shown by LAB. Thus, the objective of this study is to assess the antagonistic activity of LAB isolated from fermented *Oreochromis niloticus* against foodborne pathogens and to determine the potential of LAB as a surface decontaminant of raw chicken breast and Tilapia fish fillet. The antagonistic activity of LAB has been shown to affect *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*. When LAB was introduced to the mixed cultures of *E. coli, S. typhimurium*, and *S. aureus*, the growth of those pathogens drastically reduced and this has proven that LAB grows stronger and more stable while eliminating the pathogens. LAB and their cell-free supernatant (CFS) were also introduced into the raw chicken breast and fresh Tilapia fish fillet, where *E. coli* growth was recorded. Both cell cultures and CFS of LAB showed inhibition of *E. coli* on chicken breast and Tilapia fish fillet in the range of 0.16 to 1.28 $\log_{10}$ reduction and 0.12 to 1.12 $\log_{10}$ reduction, respectively. In conclusion, the results above suggest that LAB isolated from fermented *O. niloticus* has the potential to be a surface decontaminant. Additionally, both LAB and their CFS can also be used as biopreservative for both chicken breast and fish fillet due to a very good antagonistic activity shown by the LAB toward the foodborne pathogens.

Keywords: Lactic acid bacteria, antagonistic activity, surface decontaminant, *Oreochromis niloticus*, fermented fish

Introduction

Poultry and seafood are major sources of protein consumed by Malaysian society. Poultry animals contain microorganisms on the skin, feathers, and in their digestive tract, because live animals are processed into meat for consumption, so, carcass contamination during slaughter procedures cannot be completely avoided (Mcleod et al., 2018). Metabolic accumulation by extracellular products or actions can cause bacteria to reproduce in food and causes deterioration such as discoloration, texture change, and formation of off-flavors, off-odors, and slime (Holck et al., 2014). Poultry is vulnerable to contamination by bacteria such as *Salmonella* and *Campylobacter* during processing. Microorganisms are usually distributed among carcasses during de-feathering, evisceration, and chilling. Other than that, contaminated hands and gloves and other tools used by processing plant workers also contribute to the transmission of *Salmonella* (Marriot et al., 2018). Many epidemiological investigations and case-control studies have provided evidence that handling contaminated raw poultry products increases risk factors for infection and illness (Chen et al., 2011; Carrasco et al., 2012; Middleton et al., 2014).

Fresh fish is extremely perishable and the fish industry has made identifying technology to extend shelf-life a priority. Fish spoilage can be defined as a series of biochemical changes, mainly due to microbial growth leading to undesirable sensory changes (Pedrós-Garrido et al., 2018). Microorganisms present in fish may occur naturally, or may be introduced during processing; through cross-contamination, poor handling, and hygiene practice (Møretrø et al., 2016). Seafood is an excellent substrate for microbial growth making it very vulnerable to contamination. Seafood is also an excellent source of proteins and amino acids, vitamin B, and some minerals making it a good source of bacterial nutrition. Other than that, the growth and contamination of spoilage microorganisms and microbes can occur due to improper storage methods (Marriot et al., 2018). In recent years, the application of processing technologies and interventions has been implemented to reduce the incidence of foodborne diseases (Salleh et al., 2017).

Malaysia has produced numerous fermented foods such as fermented durian (Salleh et al., 2021), fermented fruit juice (Lani et al., 2021), fermented dadih (Lani et al., 2021), and many more. Among the fermented food produced, fermented fish is one of the most famous types of fermented goods in Malaysia. Malaysian fermented fish, also known as Pekasam are usually made from freshwater fish with ground roasted uncooked rice (Ezzat et al., 2015). Pekasam is widely consumed in the Peninsular Malaysia because of its special taste. The salting process of fish is one of the oldest methods of fish preservation and it is widely used throughout the world to this day. Traditional fermented fish products are salt fermented products (Kristín, 2010). Salt preserves fresh fish by lowering the rate of humidity to the level at which bacteria and enzyme activity are inhibited. Not only that, but chloride ions are also toxic to some microorganisms (Mustafa, 2019), and LAB were successfully isolated from fermented fish (Zakaria et al., 2018; Ismail et al., 2021). LAB are considered to be a major group of probiotic bacteria and it is believed that these major group of bacteria can assist in keeping the balance between harmful and beneficial bacteria in the stomach consequently maintaining the digestive system (Hugo et al., 2006). Moreover, LAB can also prevent foods from spoilage and pathogenic microorganisms due to the production of lactic acid and acetic acids, hydrogen peroxide, diacetyl, fatty acids, phenyl lactic acid, and bacteriocins (Syed Yaacob et al., 2020; Salleh et al., 2021).

Chemical additives are commonly used as preservatives in food products to inhibit lipid peroxidation and microbial growth as well as to prolong their shelf life (Fernandez-Lopez et al., 2005). However, consumers are increasingly concerned about health-related issues associated with the use of these chemical additives driving the food industry to find natural alternatives that exhibit strong antimicrobial and/or antioxidant properties (Salleh et al., 2021). Biopreservation is defined as the use of antagonistic microorganisms or their metabolic products to inhibit or destroy unwanted microorganisms in food (Jeevaratnam et al., 2005; Lani et al., 2019). Biopreservatives are an alternative that can be used for fresh product preservation. LAB and their metabolites are potentially used as biopreservatives. The antagonistic capabilities include adhesion to the intestine, reduction of pathogenic bacterial adhesion to the intestine, aggregation and co-aggregation as well as production of antimicrobial substances such as bacteriocins (Khan & Kang, 2016; Russo et al., 2017). LAB can antagonize pathogens by competing for nutrients and by secretion of substances with antimicrobial activities including organic acids, peroxides, and antimicrobial polypeptides termed bacteriocins (Gálvez et al., 2007; Cizeikiene et al., 2013; Syed Yaacob et al., 2020). Infectious diseases caused by resistant enteric bacteria including *Klebsiella, Escherichia coli, Pseudomonas, Salmonella, Shigella, Proteus, Vibrio cholerae,* and *Staphylococcus aureus* are responsible for health problems (Kolling et al., 2012).

Several researchers reported the application of LAB on food products such as those used in pork carcasses (Pipek et al., 2006) and raw shrimp (Lani et al., 2019). Despite the promising antibacterial properties, to the best of our knowledge, so far limited studies are elucidating the antagonistic effects of lactic acid bacteria against raw food commodities. LAB have very high potential as a natural decontaminant and bioprotective agent. Moreover, the productions of lactic and acetic acids, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes are important in enhancing shelf life, microbial safety, and improving texture (Blagojev et al., 2012). This study aimed to assess the activity of a LAB antagonist which has been isolated from fermented *Oreochromis niloticus* against foodborne pathogens and to determine the LAB potential as a surface decontaminant of raw chicken breast and Tilapia fish fillet.

Material and Methods

Fermented *Oreochromis niloticus* Preparation

The fermented *O. niloticus* was prepared following the method from Noor 'Izzati (2013). The Tilapia weight of between 300-350 g was obtained alive from the local fresh market and placed directly in iced water to

Table 1. The ingredients to prepare the fermented Tilapia fish

Ingredients	Amount of ingredients (% of fish weight)	Weight (g)
Fish	100	~300-350
Salt	20	65
Crushed roasted rice	20	65
Asam gelugur (*Garcinia atroviridis*)	5	16.25
Brown sugar	5	16.25
Spices:		
Black pepper	9	29.25
Chilli	6	19.50
Turmeric	9	29.25

weaken it. The fermented Tilapia was prepared following the procedures carried out by the fermented fish industry as stated in Awang (2001) but the amount of salt used was reduced to 20%. Fresh spices such as black pepper, chili, and turmeric were also added to the fish. These spices were purchased from a local Hock Kee Seng market in Gong Badak, Kuala Terengganu, Malaysia (5.3814° N, 103.0811° E). The fermented fish was prepared according to the ingredients listed in Table 1.

Fermented Tilapia with 0% spices was used as a control. The spices had undergone UV treatment under a laminar airflow cabinet (ERLA-CFM SERIES, VFM-4, Malaysia) for 30 minutes before being mixed with the fish to reduce microbial contamination. After that, the fermented Tilapia was placed in a sterile-labeled plastic container (previously sterile with hot water) and fermented at ambient temperature (25-28 °C) for 15 days. During the fermentation process, the sample was withdrawn (300-350 g) from an individual plastic container, then homogenized and placed individually inside a sterile container with Lid 1000 mL (115 mm x 165 mm x 71 mm) on a predetermined day from day 1 until day 15. The samples of fermented Tilapia were placed into a sterile container and transported immediately to the laboratory for microbiological analysis.

Isolation of Lactic Acid Bacteria (LAB) from Fermented Tilapia

For isolation purposes, 10 grams of the sample was homogenized in 90 mL of sterile normal saline (0.85% NaCl) solution. The homogenates were aseptically prepared using a stomacher (BagMixer 400, Interscience, Singapore) at high speed for 3 minutes. Then, 1 mL of sample from the stomacher bag was added to 10 mL of de Mann Rogosa Sharpe (MRS) broth (110661, Merck Millipore, Germany) and the sample solution was incubated at 30 °C for 48 h in anaerobic conditions. LAB was confirmed based on their microscopic and biochemical characterizations by performing the Gram stain and catalase test (Zakaria et al., 2018). The stock cultures of LAB were maintained in MRS broth or nutrient broth supplemented with 15% glycerol and stored at -80 °C (Liasi et al., 2009).

Antagonistic Activity of Isolated LAB

LAB antagonistic activity was performed against selected foodborne pathogens to understand cell activity and availability of LAB to thrive in a competitive environment. Therefore, both LAB and pathogens were grown in a liquid medium; MRS broth (Catalog no. 1106610500, Merck, Germany) and nutrient broth (Catalog no. 1054430500, Merck, Germany) for 24 h. The cell-free supernatant (CFS) was obtained from the MRS broth for 24 h as well and centrifuged at 8,000 x g for 10 minutes and washed twice with Phosphate Buffer Saline. The CFS was the remaining solution after centrifugation and washed of the pellet.

Ten mililiters of grown LAB and cell-free supernatant (CFS) were transferred into a new sterile flask followed by the pathogens of the same quantity. The cell LAB and cell-free supernatant were fixed at 0.5 McFarland standard and confirmed by plate count method at 1 x 10^8 CFU/mL. The flask was slightly swirled to make sure the bacteria were competing for nutrients. The flask was labeled as mix 1 representing isolate 12 against pathogens of interest. The mixed flasks were incubated at 37 °C together with a monoculture of LAB isolates. The colony-forming units of both bacteria were recovered at 0-hour, 6-hour, 12 h, and 24 h of the incubation period. One mililiters of mixed culture bacteria was transferred into a 9 mL saline solution to perform a series of ten-fold dilutions. 0.1 mL of higher dilution was plated onto both MRS agar and Eosin Methylene Blue (EMB) agar for *E. coli*, Baird-Parker Agar (BPA) for *S. aureus*, and Xylose Lysine Deoxycholate (XLD) medium for *S. Typhimurium*. The media were incubated at 37 °C for 24 h and the colony-forming units were recorded (Hütt et al., 2006).

Artificial Contamination of Chicken Breast and Tilapia Fillet with *E. coli* ATCC11775

The fish were purchased from a supermarket and immediately taken to Food Microbiology Laboratory. The preparation of fish was done by removing the head, gut, and fillet. The filleted fish were then rinsed with 1 L of sterile distilled water and left for approximately

five minutes to dry at room temperature. The fish fillet was placed on a tray covered with aluminum foil which has been sprayed with 95% alcohol. Four fish fillets for each treatment were prepared to determine bacterial counts at 0, 1, 2, and 3 h. The preparation of a bacterial suspension of *E. coli* ATCC11775 was initiated with 50 mL of 24 h of freshly grown *E. coli* ATCC11775 in a nutrient broth that has been centrifuged for 15 minutes at 8,000 rpm at 4°C. The supernatant was removed and 50mL of 0.1% buffered peptone water (BPW) (CM0509B, Oxoid, UK) was placed in the tube, vortex to mix, and centrifuged to clean the pallet. The bacterial suspension was adjusted to 0.5 McFarland standards and used as final inoculums for artificial contamination. The 50mL of bacterial suspension was placed into a sterile spray bottle. The suspension was sprayed onto the surface of 8 samples of prepared fish fillets on the trays. The trays were covered with aluminum foil to prevent air contamination. The samples were then left for 3 h in contact with bacterial cells. The same methodology was used for chicken breast samples.

Preparation of LAB Suspension and Cell-Free Supernatant of LAB

Firstly, 50 mL of 24 h freshly grown and labeled Isolate 12, Isolate 14 and Isolate 28, these three LAB isolates were cultured in MRS broth and centrifuged at 8,000 rpm and 4°C for 15 minutes. The supernatant was poured into another 50 mL sterile tube and set aside to allow enough supernatant for experiments. The experiments were done in triplicate. Then, 50 mL of 0.1% buffered peptone water (BPW) was placed in the tube with the cell pellet, vortex to mix, and centrifuged to purify the pallet. The supernatant was then discarded and 50 mL of 0.1% BPW was added and mixed with the pallet. The suspensions were adjusted to McFarland standard no. 2 (6.0×10^8 CFU/mL). Meanwhile, the previously released CFS was filtered with a 0.45 µm microfilter and used as a decontaminant.

Application of LAB Suspension and CFS of LAB onto Contaminated Chicken Breast and Tilapia Fillet

After 3 h of the artificial contamination, the LAB suspension was applied to the fish fillet by spraying method. The 25 mL of LAB suspension was inserted into a sterile spray bottle and sprayed evenly on 4 fish fillets on the trays. These steps were repeated for another treatment of LAB and CFS of LAB. A set of 4 fish fillets was left as control (without LAB application). The *E. coli* counts were determined by microbiological analysis. The same step is also used for the chicken breast.

Determination of *E. coli* ATCC11775 In Applied Samples

E. coli counts were determined at each LAB contact time of 0, 1, 2, and 3 h with contaminated samples to observe the effect of LAB. The surface of the fish fillet was swabbed with a wet sterile cotton swab. The cotton swab was then dipped into 1 mL of 0.1% buffered peptone water and the suspension was considered as 10^0. Six sets often-fold serial dilutions from 10^{-1} to 10^{-6} were done by transferring 1 mL of 10^0 into 9 mL of 0.1% buffered peptone water to prepare the 10^{-1} dilution series and continued till 10^{-6}. Then, 1 mL of dilution 10^{-4}, $10^{-5,}$ and 10^{-6} onto a 3M Petrifilm™ *E. coli*/Coliform count plate and incubated at 37 °C for 24 h.

Statistical Analysis

The data were expressed as mean ± standard error. Results were analyzed by multiple comparisons and two-way analysis of variance (ANOVA) using Tukey's test, Graph Pad Prism where $p<0.05$ is considered a statistically significant difference.

Results and Discussion

Overall, about 37 isolates were obtain over the 15 days of fermentation where only 7 isolates, namely, Isolates 1, 2, 4, 10, 12, 14, and 28 were used for further analyses. These isolates were chosen due to their highest activities observed after several analyses. The colonies count Log_{10} CFU/g of *E. coli* ATCC11775 in monoculture and mixed culture with LAB isolates for 4 h of incubation period was shown in Figure 1.

Figure 1 shows a large decrease in *E. coli* after 4 h of the incubation period for all mixed cultures. At 0 h of the incubation period, the growth of *E. coli* was the highest and unaffected by LAB isolates. However, the growth of *E. coli* in all mixed cultures began to decline after 2 h of the incubation period. Mix 1 (plus isolate 1), mix 10 (plus isolate 10), mix 12 (plus isolate 12), mix 14 (plus isolate 14) and mix 28 (plus isolate 28) shows a total inhibition after 4 h. After 4 h of the incubation period, all mixed cultures showed no signs of *E. coli* growth. Conversely, the growth of monoculture *E. coli* remained stable during the incubation period.

Furthermore, Figure 2 shows the growth of LAB isolates in mixed culture with *E. coli* ATCC11775 for 24 h of the incubation period. The growth of LAB isolates in mixed cultures remained unchanged and

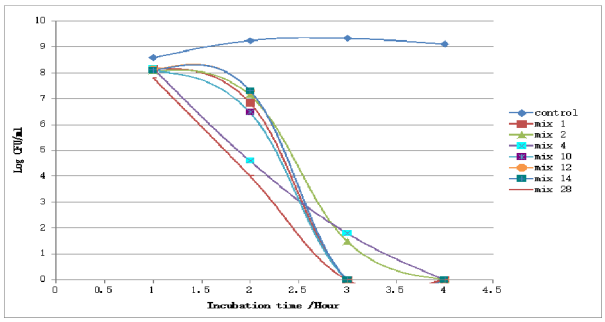

Figure 1. The growth of *E. coli* ATCC11775 against incubation time in monoculture and mixed culture with LAB isolates for 4 h. The control represents the growth of monoculture *E. coli* without LAB. (Note: Mix 1 represents *E. coli* + Isolate 1; Mix 2, *E. coli* + Isolate 2; Mix 4, *E. coli* + Isolate 4; Mix 10, *E. coli* + Isolate 10; Mix 12, *E. coli* + Isolate 12; Mix 14, *E. coli* + Isolate 14; Mix 28, *E. coli* + Isolate 28)

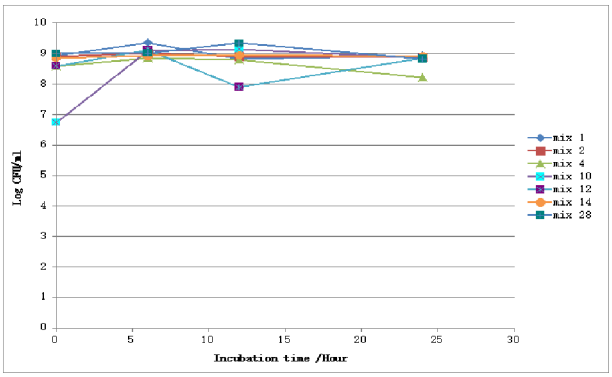

Figure 2. The growth of LAB isolates against incubation time in mixed culture with *E. coli* ATCC11775 for 24 h. The control represents the growth of monoculture *E. coli* without LAB. (Note: Mix 1 represents *E. coli* + Isolate 1; Mix 2, *E. coli* + Isolate 2; Mix 4, *E. coli* + Isolate 4; Mix 10, *E. coli* + Isolate 10; Mix 12, *E. coli* + Isolate 12; Mix 14, *E. coli* + Isolate 14; Mix 28, *E. coli* + Isolate 28)

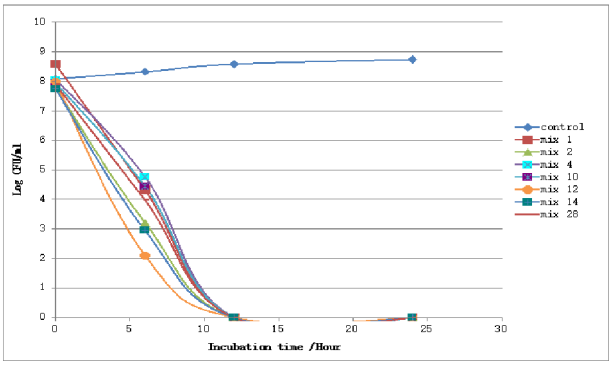

Figure 3. The growth of Salmonella Typhimurium ATCC14128 against incubation time in monoculture and mixed culture with LAB isolates for 24 h. The control represents the growth of monoculture S. Typhimurium ATCC14128 without LAB. (Note: Mix 1 represents S. Typhimurium + Isolate 1; Mix 2, *S. Typhimurium* + Isolate 2; Mix 4, S. Typhimurium + Isolate 4; Mix 10, S. Typhimurium + Isolate 10; Mix 12, S. Typhimurium + Isolate 12; Mix 14, S. Typhimurium + Isolate 14; Mix 28, S. Typhimurium + Isolate 28)

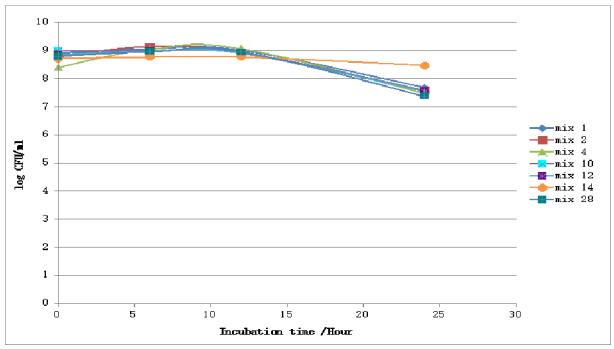

Figure 4. The growth of LAB isolates against incubation time in mixed culture with S. Typhimurium ATCC14128 for 24 h. (Note: Mix 1 represents S. Typhimurium + Isolate 1; Mix 2, S. Typhimurium + Isolate 2; Mix 4, S. Typhimurium + Isolate 4; Mix 10, S. Typhimurium + Isolate 10; Mix 12, S. Typhimurium + Isolate 12; Mix 14, S. Typhimurium + Isolate 14; Mix 28, S. Typhimurium + Isolate 28)

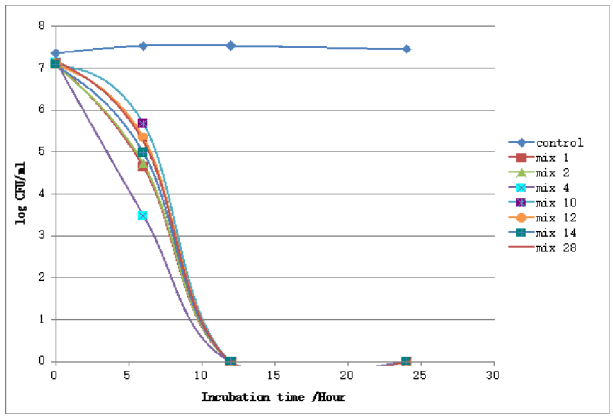

Figure 5. The growth of *Staphylococcus aureus* ATTC25923 against incubation time in monoculture and mixed culture with LAB isolates for 24 h. The control represents the growth of monoculture *S. aureus* ATTC25923 without LAB. (Note: Mix 1 represents *S. aureus* + Isolate 1; Mix 2, *S. aureus* + Isolate 2; Mix 4, *S. aureus* + Isolate 4; Mix 10, *S. aureus* + Isolate 10; Mix 12, *S. aureus* + Isolate 12; Mix 14, *S. aureus* + Isolate 14; Mix 28, *S. aureus* + Isolate 28)

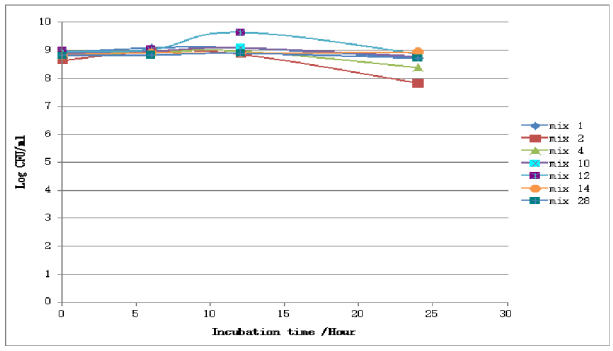

Figure 6. The growth of LAB isolates against incubation time in mixed culture with *S. aureus* ATTC25923 for 24 h. (Note: Mix 1 represents *S. aureus* + Isolate 1; Mix 2, *S. aureus* + Isolate 2; Mix 4, *S. aureus* + Isolate 4; Mix 10, *S. aureus* + Isolate 10; Mix 12, *S. aureus* + Isolate 12; Mix 14, *S. aureus* + Isolate 14; Mix 28, *S. aureus* + Isolate 28)

stable from 0 h to 24 h of the incubation period the graph exhibits the range of log 8 to log 9 for all mixed cultures except for mix 10 (i.e. log 6.8 CFU/mL) having the lowest concentration of bacteria at 0 h of the incubation period.

The $\log_{10}$ CFU/mL of Salmonella Typhimurium ATCC14128 for 24 h of the incubation period is shown in Figure 3. Monoculture of S. Typhimurium shows a constant growth from 0 h to 24 h. However, after 12 h of the incubation period, mixed cultures of S. Typhimurium were eliminated.

Figure 4 indicates the colonies count $1\log_{10}$ CFU/g of LAB isolates in mixed cultures with S. Typhimurium ATCC14128 for 24 h of the incubation period. The graph shows a stable and unaffected growth of LAB isolates in the mixed cultures with S. Typhimurium. In terms of LAB-isolated growth, no effect is shown after being incubated with S. Typhimurium for 24 h.

The following Figure 5 shows the growth of *Staphylococcus aureus* ATTC25923 in monoculture and mixed cultures with LAB isolates. Stable growth of monoculture of *S. aureus* was observed for 24 h incubation period. On the other hand, the growth of *S. aureus* in mixed cultures began to decline after 6 h of the incubation period. After 12 h of incubation, *S. aureus* was eliminated. At 24 h of the incubation period, *S. aureus* was not detected in all mixed cultures. The LAB isolates appear to dominate the culture and deplete rapidly growing *S. aureus* thus stopping their reproduction process.

Figure 6 describes the growth of LAB isolates in mixed cultures with *S. aureus* ATTC25923 for 24 h of the incubation period. The stable growth of isolated LAB in all mixed cultures for 24 h can be observed. The LAB isolated remains unaffected with the *S. aureus* in mixed culture. The stable growth of LAB isolated eliminated *S. aureus* during 12 h of the incubation period. LAB isolates can multiply and produce metabolites that dominate the environment during stable growth.

Lactic acid bacteria (LAB) not only provide flavors, odors, textures, and nutritional changes and effects in fermented foods but also display an antagonistic effect against pathogenic bacteria. Fermented Tilapia can produce LAB. Moreover, Mohammad et al. (2017) also found that LAB such as *Pediococcus* and *Lactobacillus* spp. have been isolated in traditional fermented yogurt. Other than that, it has been shown that LAB inhibits the *in vitro* growth of many pathogenic bacteria and has been used in both humans and animals to treat gastrointestinal disorders (Fernandez et al., 2003). LAB are successfully formed from fermented products due to the abundance of important nutrient sources contained such as carbohydrate and minerals from cereals and dairy which have been used as fermentation ingredients. The low pH of fermented food was due to the production of different acid that also induces LAB growth (Iñiguez-Palomares et al., 2007). In fermented foods processing, LAB display various antimicrobial activities, including bacteriocin (De Vuyst & Leroy, 2007; Mduduzi et al., 2016) and hydrogen peroxide (Syed Yaacob et al., 2020)

The reduction of *E. coli*, S. Typhimurium, and *S. aureus* can be seen after the co-incubation with the LAB isolates. Meanwhile, LAB colony counts remained stable and was unaffected by those foodborne pathogens. There have been several studies showing that the LAB tested exhibited antagonistic activity against the indicator microorganisms (Sahraoui, et al., 2015; Da Costa et al., 2018). The ability of LAB to suppress foodborne bacteria has been proven in many studies. According to Forestier et al. (2001) and Marianelli et al. (2010), *L. casei* subsp. *rhamnosus* strains and probiotic bacteria (*L. rhamnosus* and *L. reuteri*) have appropriate antimicrobial activity against human pathogenic bacteria (*E. coli, Enterobacter cloacae, Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Clostridium difficile*). According to the result obtained by Alireza et al. (2018), the antimicrobial activity of some *L. plantarum* and *L. fermentum* isolates were highly resistant to the indicator bacteria tested. Similar results were also reported by Anas et al. (2008), who found that the supernatant of *L. plantarum, and L. paracasei* subsp. *paracasei* or *L. rhamnosus* was able to inhibit *S. aureus* growth and the supernatants of *L. plantarum* were more effective in reducing pathogenic populations.

From the results obtained from this study, the selected LAB isolates became stronger and more stable while eliminating the selected pathogens. All pathogens showed reduction pattern when when co-incubated with LAB isolates. These results prove that LAB isolated from fermented *O. niloticus* can potentially be a surface decontaminant. The potential of LAB isolates as surface decontaminant agents in the form of cell culture as well as cell-free supernatant (CFS) are shown in Table 2 and Table 3, respectively. From the observations, the cell culture of LAB shows a higher reduction of the bacteria colonies compared to the CFS of LAB. For Isolate 12, the highest $\log_{10}$ reduction can be observed at 0 h and slowly decreases after 2 h of contact time (Table 2). However, during the 2 to 3 h of contact time, the drastic decrease from 0.45 CFU/mL to 0.03 CFU/mL of *E. coli* can also be seen. As for Isolates 14, an increase in $\log_{10}$ reduction can be observed with the increase of contact time from 0 to 3 h from no reduction to 1.28 CFU/mL. Log_{10} reduction of *E. coli* after the application of Isolates 28 was

Table 2. The mean number of *E. coli* colonies after the artificial contamination of cell culture and cell-free supernatant (CFS) of LAB isolates onto the chicken breast for 3 h of contact time

LAB isolates	Log_{10} reduction of *E. coli* (CFU/mL)			
	0 h	1 h	2 h	3 h
Cell culture				
12	0.53^{a*}	0.49^{b*}	0.45^{c*}	0.93^{d*}
14	0.21^{a*}	0.54^{a*}	0.58^{b*}	1.28^{c*}
28	0.16^{a*}	0.62^{b*}	0.79^{c}	0.15^{d*}
Cell-free supernatant (CFS)				
12	0.59^{e*}	0.63^{f*}	0.81^{g*}	0.65^{h*}
14	0.58^{d*}	0.20^{e*}	0.40^{f*}	0.59^{g*}
28	0.74^{e*}	0.31^{f*}	0.79^{g}	0.007^{h*}

Note: Different letters after the value indicate statistically significant between LAB isolates while the asterisk (*) indicates statistically significant between the cell culture and CFS within similar contact time.

Table 3. The mean number of *E. coli* colonies after the artificial contamination of cell culture and cell-free supernatant (CFS) of LAB isolates onto the Tilapia fillet for 3 h of contact time.

LAB isolates	Log_{10} reduction of *E. coli* (CFU/mL)			
	0 h	1 h	2 h	3 h
Cell culture				
12	0.65^{a*}	0.58^{b*}	0.48^{c*}	0.73^{d*}
14	0.03^{a*}	0.20^{b*}	0.21^{b*}	0.52^{c*}
28	0.12^{a}	0.66^{b*}	0.34^{c*}	1.12^{d*}
Cell-free supernatant (CFS)				
12	0.23^{e*}	0.60^{e*}	0.94^{f*}	0.91^{f*}
14	0.60^{d*}	0.16^{e*}	0.41^{f*}	0.65^{g*}
28	0.46^{e*}	0.16^{f*}	0.53^{g*}	0.43^{h*}

Note: Different letters after the value indicate statistically significant between LAB isolates while the asterisk (*) indicates statistically significant between the cell culture and CFS within similar contact time.

increased from 0.16 CFU/mL at 0 h to 0.79 CFU/mL at 2 h but declined dramatically to 0.15 CFU at 3 h of contact time.

The log_{10} reduction in *E. coli* CFU/mL after the application of CFS of LAB onto the surfaces of artificially contaminated chicken breast at 0, 1, 2, and 3 h of contact time was shown in Table 2. The increasing log_{10} reduction in *E. coli* can be observed from 0.60 CFU/mL at 0 h to 0.81 CFU/mL at 3 h of contact time. However, the reduction of *E. coli* after the application of CFS Isolates 12 decreased at the end of the contact time. For Isolates 14, the log_{10} reduction of *E. coli* is 0.58 CFU/mL at 0 h but slowly decreases to 0.20 CFU/mL at 1 h of contact, but the log_{10} reduction of *E. coli* slowly increases from 2 h up to 3 h of contact time. The fluctuation trend can be observed on the application of Isolates 28 and the highest log_{10} reduction of *E. coli* can be observed at 2 h of contact time.

Comparatively, the log_{10} reduction of *E. coli* on the surface of artificially contaminated Tilapia fish fillet at 0, 1, 2, and 3 h of contact time is shown in Table 3. The decline of log_{10} reduction of *E. coli* from 0.65 CFU/mL to 0.48 CFU/mL can be observed from 0 h to 2 h. However, the log_{10} reductions for Isolate 12 increased to 0.73 CFU/mL at the end of the contact time. As for Isolates 14, the log10 reduction of *E. coli* increased from no reduction at 0 h to 0.52 CFU/mL after 3 h. The *E. coli* reduction increased to 0.66 CFU/mL (after 1 h) from no reduction using Isolates 28. Then the log_{10} reduction slowly declines to 0.34 CFU/mL in 2 h but drastically increased to 1.12 CFU/mL within 3 h. Application of CFS of Isolates 12 showed an increase in the log_{10} reduction of *E. coli* colonies from 0 to 3 h (Table 3). Fluctuated trends can be seen after the application of CFS of Isolates 14, and the highest log_{10} reduction of *E. coli* can be seen within the 3 h. Finally, for Isolates 28, no reduction at all was observed from 0 h but 0.16 CFU/mL of log_{10} reduction at 1 h of contact time. However, log_{10} reduction of *E. coli* increased to 0.53 CFU/mL after 2 h but declined to 0.43 CFU/mL after 3 h.

Based on the results above, CFS of LAB showed a higher reduction of *E. coli* in chicken breast compared to LAB. The research conducted by Mahmoud et al. (2017) also showed that the CFS of all *Lactobacillus* isolates had remarkably higher antimicrobial activity compared to their microbial suspensions (Forestier et al., 2001; Vaseeharan & Ramasamy, 2003; Marianelli et al., 2010; Mahmoud et al., 2017). However, for Tilapia fillets, the reduction of *E. coli* was slightly higher for LAB compared to CFS of LAB. This is probably due to some of the CFS tested does not have the desired activity to inhibit the tested indicator bacteria; some CFS perform poorly (null or small inhibition halos) and/or only act against one or few of the tested indicator microorganisms as agreed by Arrioja-Breton et al. (2020). The application of pure pediocin also might be the most effective for the Tilapia fillet. The effectiveness of treatment using pediocin has been anticipated by several researchers, who verified its ex situ efficiency by applying it to a variety of meat, against numerous contaminant species and foodborne pathogens (Chen & Hoover, 2003; Santiago-Silva et al., 2009; Espitia et al., 2013; da Silva Sabo et al., 2017).

E. coli reduction after LAB and CFS of LAB application to chicken breast and Tilapia fish fillet was at the peak after 2 h and 3 h of contact time. Different types of LAB show different effects on both chicken

breast and fish fillet. The antagonistic activity that varied between LAB and indicator strains is consistent with previous reports (Sahraoui et al., 2015; Giles-Gómes et al., 2016; Almeida da Costa et al., 2018). Further analyses using 16S rRNA gene sequencing confirmed the 1, 4, 10, 12, 14, and 28 isolates were 97-98 % genetic similarity to *Lactobacillus plantarum*, and isolate 2 was *Lactobacillus pentosus* (data not shown). *L. plantarum* is known as one of the strongest LAB, supported by the previous study by Valerio et al. (2013) and Coman et al. (2014) that also detected the strongest inhibitory effects of *L. plantarum* and *L. paracasei* against *S. enterica* and *L. monocytogenes* compared to other LAB species

LAB and CFS of LAB exhibit excellent antimicrobial effects against the foodborne microorganism due to the LAB secrete compounds including bacteriocin, lactic acid, and hydrogen peroxide exhibiting antibacterial activity against pathogens (Mobin et al., 2018). As reported by Almeida da Costa et al. (2018), the highest organic acid contents detected in CFS of *L. plantarum* 49, *L. paracasei* 108, and *L. plantarum* 201 were related to the strongest antagonistic activity resented by these strains against *L. monocytogenes* and *S. enteritidis* PT4. From the results above, there are a few times that the reduction of *E. coli* may be due to the instability of the bacteriocin molecule when applied to food. The inactivation by proteolytic enzymes, oxidative processes, adsorption of bacteriocin molecules to food components, and low solubility and/or inadequate distribution in the food can be the cause of the instability of the bacteriocin (Gálvez et al., 2007).

Conclusion

The antagonistic activity of lactic acid bacteria (LAB) isolated from fermented *Oreochromis nilolticus* showed an effect against *Escherichia coli*. The mixed culture of *E. coli*, *Salmonella Typhimurium*, and *Staphylococcus aureus* with LAB showed a significant decrease in growth over 24 h period. This finding also suggests that LAB has antimicrobial activity against common foodborne pathogens. Moreover, the cell culture and cell-free supernatant (CFS) of LAB demonstrate the LAB's ability to inhibit the production and growth of *E. coli* on chicken breast and Tilapia fish fillets. CFS of LAB showed higher inhibition of *E. coli* in chicken breast compared to cell culture. However, a reverse behavior was observed when these two forms of LAB were used in the surface decontamination of Tilapia fish fillets. For future study, the characteristics of cell culture and CFS-LAB can be further quantified using more recent techniques such as proteomics and applying this concept to other raw commodities.

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Supplementary Material

Supplementary material is not available for this article.

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