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Antibacterial activity of *Pediococcus acidilacticii* against *Listeria monocytogenes* in Fresh Tilapia (*Oreochromis niloticus*)

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A B S T R A C T

Lactic acid bacteria (LAB) can be used as antibacterial in food, one of them is P. acidilacticii. The purpose of this study was to determine the ability of P. acidilacticii to inhibit the growth of L. monocytogenes in fresh tilapia stored for 4 days at 10oC and to measure the quality of tilapia with the addition of P. acidilacticii. The experimental design used was Randomized Complete Design (RAL) factorial with 2 levels of treatment and 4 days storage. Test parameters were enumeration L. monocytogenes, enumeration P. acidilacticii, organoleptic, and Total Volatile Base Nitrogen (TVBN). The parametric data obtained were tested using ANOVA (P <5%) with advanced test of Honestly Significant Difference (HSD) while nonparametric data was analyzed using Kruskal Wallis (p<5%). The inhibitory zone test was performed as pre-research and resulted in the best amount of P. acidilacticii to be used in the main research of 106 CFU/ml and L. monocytogenes 108 CFU/ml produced a 4 mm inhibition zone. The amount of P. acidilacticii of 3.3 x 105 CFU/g reduced as much as 1.4 x 105 CFU/g L. monocytogenes on 2nd day and P. acicilacticii by 4 x 105 CFU/g was able to reduce L. monocytogenes by 7.8 x 104 CFU/g on 4th day. The results of organoleptic test showed that tilapia with P. acidilacticii addition were received by panelist until 4th day, while the control tilapia fish (without P. acidilacticii) was not received by panelist on the 4th day as well as TVBN levels. The studies revealed that certain fresh fish, biocontrol methods may provide a further obstacle that Listeriae must overcome.

Keywords: antibacterial, *L. monocytogenes*, *O. niloticus*, *P. acidilacticii*, tilapia

1. Introduction

Tilapia (*Oreochromis niloticus*), is a popular farmed fish species that grows quickly, requires simple cultivation methods, and can adapt to a variety of ecological zones. The fish species is also the pride of freshwater aquaculture, generating income, enhancing welfare, considerably minimizing stunting, and delivering animal protein to all societal levels (Taukhid et al, 2021). As a source of protein, tilapia is important in human diets (Boyd et al., 2022). Although this fish is exceedingly perishable, it has a great nutritional value. The presence of critical nutrients, the lack of competing microorganisms, and the ideal water activity and pH make tilapia fish and products an ideal habitat for the growth of a variety of harmful and spoilage microorganisms (Galvez et al., 2008).

One of the most significant pathogenic microbes is *Listeria monocytogenes*, which is the causative agent of listeriosis, an illness primarily affecting the elderly, young children, pregnant women, and people with weakened immune systems (Baka et al., 2014). Numerous nations have implemented a policy of zero tolerance, meaning that ready to eat foods adulterated with *L. monocytogenes* at detectable levels are thought to be tampered with. Pregnant women, newborns, and individuals with compromised immune systems are the primary populations affected by the disease. It is also thought that all raw food including fishery commodities and ingredients may contain the bacterium (Indrotristanto et al., 2022; Martin et al., 2022). It is challenging to control *L. monocytogenes* through food preservation techniques because of its ability to grow in harsh processing or storage conditions, such as low water activity, high salt concentrations, pH ranges of 4.1–9.6, and refrigeration temperatures. Additionally, considering this microorganism can grow at low temperatures (2–4°C), the presence of L. monocytogenes in fish and fish products is particularly concerning (Chen et al., 2019).

Lactic acid bacteria (LAB) and microbiology can be used as alternatives to suppressing *L. monocytogenes*. Lactic acid bacteria, which can be utilized to inhibit pathogenic bacteria like *L. monocytogenes*, have been the subject of numerous investigations. Previous study stated that *Pediococcus acidilactici* isolated from silage, producing bacteriosin strains with high specificity againts Listeria (Fugaban et al., 2022). *P. acidilacticii* is one of the lactic acid bacteria that has antimicrobial properties (Moslem et al., 2023). In addition, Papagianni and Anastasiadou (2009), states that *P. acidilacticii* grows at 41°C, and is able to grow at temperatures from 10°C to 45°C. Another approach involves adding commercially accessible, industrially or laboratory-produced pediocin directly into food products (Espitia et al., 2016). The purpose of this study was to determine the ability of *P. acidilacticii* to inhibit the growth of *L. monocytogenes* in fresh tilapia (*O. niloticus*) stored for 4 days.

2. Material and Methods

Material

The sample used in this study is fresh tilapia (*O. niloticus*) obtained from the Rejomulyo Semarang Market. *P. acidilacticii* F-11 and *Listeria monocytogenes* FNCC-ATCC 7644 isolates, as well as selective media of Listeria agar base (Oxoid) obtained from the Inter-University Centre (PAU) of the University of Gajah Mada Yogyakarta. The equipment used in this study is laminar water flow (Thermo Scientific), incubator (Labnet 311DS), glass measurement (Pyrex) and other equipment used for detailed analysis purposes. Methods

1. Prior Works

The bacterial isolates *L. monocytogenes* and *P. acidilacticii* were enriched by growing 1 ose in nutrient broth medium and then incubated for 48 hours at 37 °C. The inhibition zone test was conducted using a paper disc method to determine the antibacterial activity possessed by *P. acidilacticii* to inhibit the growth of L. monocytogenes. Target bacteria as much as 100µL inserted into sterile petri dish, then added nutrient media for as much as 12–15 ml and moved to form number 8 so that bacteria flatten. After blocking, *P. acidilacticii* bacteria in disc paper were placed on agar medium and incubated at 37 °C for 48 hours.

2. Main Study

The implementation of this research started with fresh tilapia (*O. niloticus*) being washed out. Some tilapia were immersed in 1.5 L of aquadest solution plus 1% isolate *L. monocytogenes* for 10 minutes as a control. Other samples were immersed in 1.5 L of aquadest solution, then added with 1% isolate of *P. acidilacticii* as a Lactic Acid Bacteria (LAB) and 1% isolate of *L. monocytogenes* for 10 min. Samples were stored in a refrigerator at a temperature of 10 ℃ for 4 days, with observation every 48 hours. Tests carried out include counting the number of *L. monocytogenes*, counting the amount of *P. acidilacticii*, organoleptic, and Total Volatile Base Nitrogen (TVBN). The implementation procedure refers to Mulkyte et al. (2017), which state that the inoculated sample with *L. monocytogenes* is immersed in 3 L of aquadest solution already added with 2% BAL, and as a control, the sample is only inoculated with L. monocytogenes.

3. The Enumeration of *L. monocytogenes* (Bacteriological Analitycal Manual, 2022)

Enumeration of *L. monocytogenes* can be done using TPC (total plate count). A total of 25 g of sample was dissolved in 225 mL of BLEB (Buffered Listeria Enrichment Broth) and then diluted to 10 dilutions. Samples that have become solution are taken in 1 mL, then streaked into Listeria Agar Base media shaped for the plate.

4. The Enumeration of *P. acidilacticii* (National Standardization Bureau, 2015)

The protocol for *P. acidilacticii* was referred to in Indonesian National Standardization No. 2332.3:2015. The diluted sample was 1 ml, then poured into a petri dish and added 12–15 ml of medium (MRS agar). The cup is moved slowly to form the number 8. After the stub, the cup is incubated in the upside position.

5. Total Volatile Base Nitrogen Level (TVBN) (National Standardization Bureau, 2009).

The protocol for TVBN measurements was referred to in Indonesian National Standard No. 2354.8:2009. The sample of 10 g was blended and then added to 90 mL of 6% perchloric acid. The samples are homogenized and then filtered. The obtained filtrate is fed into a distillation tube and coupled with a phenolphthalein and silicon anti-foaming indicator of 2-3 drops each. The sample is added to 10 mL of 20% NaOH, and the color turns pink. A total of 100 mL of 3% H_3BO_3 was included in Erlenmeyer, and Tashiro added 3 drops, and the color changed to purple. The sample is distilled until the volume reaches 200 mL and the solution is green. The sample was titrated with 0.02 N HCl to a purple-red color.

6. Sensory Test of Fresh Tilapia (National Standardization Bureau, 2013).

Sensory test was performed with 25 samples, 1 sample per panelist. Parameter tested by panelists by grouping texture, smelling the smell, and seeing the color of the fish provided. Furthermore, the sample is assessed by the panelists on the score sheet. The data obtained will be analyzed descriptively.

3. Results and Discussions

Enrichment of Isolate and Inhibition Zone Test Pediococcus acidilacticii to Listeria monocytogenes

The results of the *P. acidilacticii* inhibitory zone test against *L. monocytogenes* are presented in Table 1.

Table 1 – Inhibitory zone of *P. acidilacticii* against *L. monocytogenes*

***P. acidilacticii* (*CFU/mL*)**	**Inhibition Zone of *P. acidilacticii* against *L. monocytogenes* in different dilution (mm)**		
	10^8 (*CFU/ml*)	**10^7 (*CFU/ml*)**	**10^6 (*CFU/ml*)**

10^8	-	2	4
10^7	-	1.5	1.7
10^6	-	1	0.5

The density of *P. acidilacticii* used was 106 CFU/mL and L. monocytogenes 108 CFU/ml and it is showed the widest inhibition zone when compared to other inhibition zones. Based on these results, it can be said that the amount of *P. acidilacticii* used as an antibacterial is inversely proportional in suppressing to the presence of L. monocytogenes. Recently, Lee et al. (2022), stated that the adherence and invasion of intestinal epithelial cells by *L. monocytogenes* was not inhibited by *P. acidilacticii* in a dose-dependent manner. In many previous studies, it is found that *P. acidilacticii* has the potential to become a bioprotective organism against Listeria (Abbasiliasi et al., 2017). Although Listeriae can grow under vacuum conditions, at low temperatures and BAL it is able to reduce the amount of Listeria. This LAB may also be added as bioprotective with an anti-listerial compound in the fermentation product.

Antibacterial Activity of P. acidilacticii against the Growth of L. monocytogenes on Tilapia Fish Storage for 4 Days with 10°C Temperature

P. acidilacticii enumeration in the fresh tilapia (*O. niloticus*) stored for 4 days at 10°C was presented in **Figure 1**.

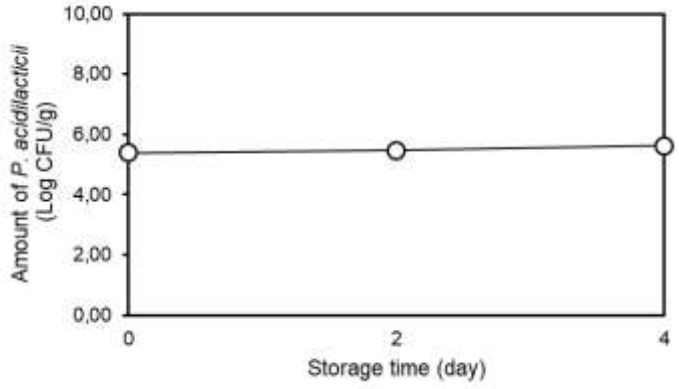

Fig. 1. Enumeration of *P. acidilacticii* enumeration in the fresh tilapia (*O. niloticus*) stored for 4 days at 10°C

As seen in the **Fig. 1**, LAB of *P. acidilacticii* increased from day 0 to day 4. Increment of *P. acidilacticii* may resulted reduction of growth of *L. monocytogenes* (**Fig. 2)**. The primary target of the bacteriocins produced by the LAB is most likely the cytoplasmic membrane, as the bacteriocin initiates reactions that alter the membrane permeability and thus interfere with membrane transport, resulting in impaired energy production and biosynthesis of proteins or nucleic acids (Mokoena, 2017). On the 2nd day, the amount of LAB was 3.3 x 10^5 CFU/g and was able to reduce the number of *L. monocytogenes* 1.4 x 10^5 CFU/g. While on 4th day, the amount of *P. acidilacticii* was 4 x 10^5 CFU/g and was able to reduce the amount of *L. monocytogenes* by 7.8 x 10^4 CFU/g. The amount of *L. monocytogenes* in the control sample increased as much as 1.5 x 10^5 CFU/g on the 2nd day and as much as 1.8 x 10^5 CFU/g on the 4th day. According to Li et al. (2018), pediocin from the bacteriocin group included in bacteriocin that has antilisterial properties. This bacteriocin inhibited Gram positive bacteria and pathogenic bacteria. Pediocin produced by Pediococcus will be used as food additives such as nisin.

The addition of *P. acidilacticii* was considered capable of inhibiting the growth of *L. monocytogenes* because it was able to suppress and reduce the amount of *L. monocytogenes* in tilapia stored at 10°C. The results obtained in accordance with the research undertaken by Nietolozano et al. (2010), that low temperature storage and addition of bacteriocin and bacteriocin-producing strains are the most appropriate method for inhibiting *L. monocytogenes* bacteria. Pediocin PA-1 produced from *P. acidilacticii* can inhibit the growth of *L. monocytogenes* in fermented sausages. The amount of *P. acidilacticii* was also observed in the sample given the addition of *P. acidilacticii.*

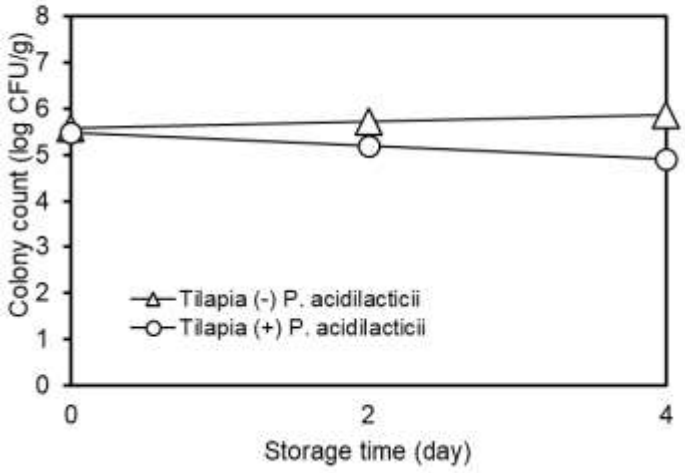

Fig. 2. Enumeration of *L. monocytogenes* in fresh tilapia stored for 4 days at 10℃

The result of enumeration *P. acidilacticii* presented in **Fig.2** is an evident that *P. acidilacticii* pediocins can be applied into fresh tilapia (*O. niloticus*). Pediocins are sensitive to most protease enzymes such as papain, pepsin, and trypsin; however, they keep their antimicrobial activity during heat treatment, at low temperatures even at −80°C, and after treatment with lipase, lysozyme, phospholipase C, DNase, or RNase (Khorsidian et al., 2021). This is in accordance with the research of Yuliana et al. (2015), which states that at the time of immersion process using lactic acid bacteria, active lactic acid bacteria isolate biochemical processes that produce antimicrobial compounds such as lactic acid, bacteriocin and hydrogen peroxide. These antimicrobial compounds cause death in target bacteria.

Total Volatile Base Nitrogen (TVBN) of Fresh Tilapia stored for 4 days at 10℃

TVBN value on tilapia *O. niloticus* fish stored for 4 days at 10℃ temperatures presented in Figure 3.

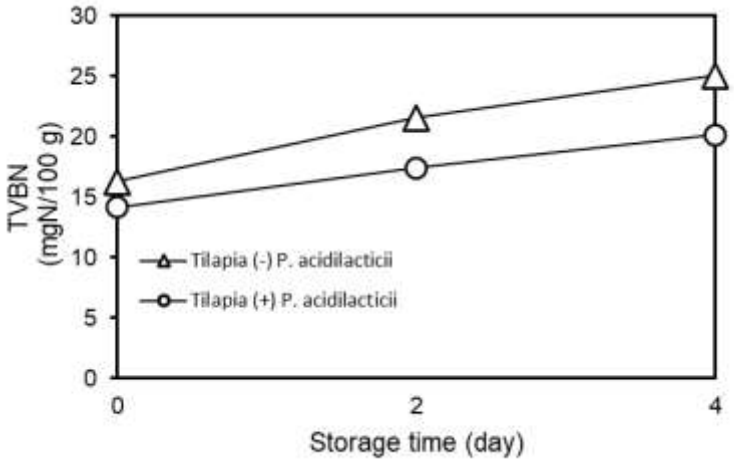

Fig. 3. Enumeration of *L. monocytogenes* in fresh tilapia stored for 4 days at 10℃.

Data is the result of an average of 3 repeatation

Fig. 3 shows, on day 0 the control tilapia has TVBN value of 16.257 mgN / 100 g and tilapia which was given the addition of *P. acidilacticii* of 14.142 mgN / 100 g, the value indicates that the sample tilapia fish in fresh condition. There was a rise in the value of TVBN during the storage process. Tilapia control on day 2 has a value of 21.578 mgN / 100 g and 25.053 mgN / 100 g on 4th day. While the tilapia with *P. acidilacticii* had a lower value than the control tilapia fish of 17.403 mgN / 100 g on day 2 and 20.126 mgN / 100 g on the 4th day. The findings show that the TVBN index rises as storage duration increases, indicating a consistent decline in the quality of the fish meat throughout the studies. Susanto et al. (2011), mackerel treated with natural ingredients has lower values than controls, natural-treated mackerel has a different rate of change in TVBN values during storage but has the same improvement pattern. To date, TVBN index used as one of prediction tools using gas sensor array system to determine a freshness index of Tilapia stored into chilling room (Sun and Zhang, 2022).

Sensory Acceptance of Tilapia (O. niloticus) Stored for 4 Days with 10°C Temperature

The organoleptic of tilapia (*O. niloticus*) stored at 10oC for 4 days are presented in **Table 2**.

Table 2 – Sensory score of fresh tilapias added with *P. acidilacticii*

Storage (*day*)	**Fresh tilapias**	
	(-) *P. acidilacticii*	**(+) *P. acidilacticii***
0	8.90^{f}	8.75^{e}
2	7.42^{c}	8.46^{d}
4	5.42^{a}	7.13^{b}

Values are reported as the mean of four replicate groups and error bars indicate standard deviation. Different lowercase letters on the bar charts indicate statistically significant differences at each sample were assessed by the Tukey at $p < 0.05$

The acceptance score based on sensory test presented in Table 2, which all treatments on fresh tilapia (with and without *P. acidilacticii*) have a value above 7, therefore feasible accepted by the panelist. Assessment on the 2nd day showed that tilapia was still accepted by the panelists on the control and tilapia with *P. acidilacticii*. The sensory assessment on the 4th day showed that the control tilapia was not accepted by the panelists while the tilapia with P. acidilacticii had a value above 7 so it was still received by the panelists. According to the National Standardization Bureau (2013), the requirements for quality and safety of fresh fish sensory are at least 7 (score 1-9).

4. Conclusion

P. acidilacticii were able to inhibit the growth of *L. monocytogenes* on tilapia (*O. niloticus*) stored in low temperature and the combination between *P. acidilacticii* isolate at low temperatures were able to maintain the fish freshness through microbiology aspect (TPC, LAB), chemically aspect (*Total Volatile Base Nitrogen*) and physically aspect (sensory). The studies revealed that certain fresh fish, biocontrol methods may provide a further obstacle that Listeriae must overcome.

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