ISOLASI BAKTERI ASAM LAKTAT DARI TEMPOYAK DAN AKTIVITAS ANTIMIKROBA TERHADAP *Escherichia coli*

(Isolation Of Lactic Acid Bacteria From Tempoyak And Its Antimicrobial Activity On The *Escherichia coli*)

Eliya Mursyida*, Fifi Candita, Muhammad Faisal, Deinike Wanita Marwan

Program Studi Pendidikan Dokter, Universitas Abdurrab

Keywords:
Diarrhea, Escherichia coli, Lactic Acid Bacteria (LAB), Probiotics, Tempoyak

ABSTRACT

Diarrhea is a potential extraordinary event that can cause death. The most common microbes that cause diarrhea in developing countries are Rotavirus and *Escherichia coli*. One of the treatment of diarrhea is by giving antibiotics. However, the use of antibiotics is known to disrupt the balance of normal gastrointestinal flora, thereby changing the composition of the microbiota. Probiotics can be given to balance the normal flora during or after treatment with antibiotics. Lactic acid bacteria (LAB) is one of the probiotics that can inhibit the growth of pathogenic microbes and is commonly found in fermented foods. This study aimed to isolate LAB from tempoyak made from kampar durian, Riau Province, Indonesia and test its antimicrobial activity against Escherichia coli. The study was initiated by isolating LAB from tempoyak using the multilevel dilution method, followed by characterizing LAB by colony and cell morphology, and testing its antimicrobial activity against *E. coli* using the agar well diffusion method. The antimicrobial activity test results were analyzed using one-way ANOVA and Bonferroni follow-up test. The results of LAB characterization showed that four LAB isolates had different colony morphology, including spherical Gram-positive bacteria and catalase-negative bacteria. The results of the antimicrobial activity test showed that LAB1 isolates had the highest ability to inhibit Escherichia coli with an average inhibition zone diameter of 15.21 mm and the lowest inhibition zone was found in LAB 4 isolates, which was 10.80 mm. The results of the one way ANOVA test showed a significant difference between the four LAB isolates in inhibiting *E. coli* with P value <0.05. In the Bonferroni test, there were significant differences between LAB 1 and LAB 4 isolates. It can be concluded that LAB isolated from tempoyak has the potential to be a source of probiotics.

Kata Kunci:
Diare, *Escherichia coli*, Bakteri Asam Laktat (BAL), Probiotik, Tempoyak.

ABSTRAK

Diare merupakan penyakit potensial Kejadian Luar Biasa yang dapat menyebabkan kematian. Mikroba penyebab diare yang paling umum di negara berkembang yaitu Rotavirus dan *E. coli*. Salah satu pengobatan diare adalah dengan pemberian antibiotik. Namun, penggunaan antibiotik diketahui dapat mengganggu keseimbangan flora normal gastrointestinal sehingga mengubah komposisi mikrobiota. Probiotik dapat diberikan untuk keseimbangan flora normal selama atau setelah pengobatan dengan antibiotik. Bakteri asam laktat (BAL) termasuk salah satu probiotik yang dapat menghambat pertumbuhan mikroba patogen dan banyak ditemukan pada makanan fermentasi. Penelitian ini bertujuan untuk mengisolasi BAL dari tempoyak yang dibuat dari durian kampar, Provinsi Riau, Indonesia dan uji aktivitas antimikroba terhadap *E. coli*. Penelitian diawali dengan mengisolasi BAL dari tempoyak dengan metode pengenceran bertingkat, dilanjutkan karakterisasi BAL secara morfologi koloni dan sel, dan uji aktivitas antimikroba terhadap *Escherichia coli* dengan metode difusi sumur agar. Hasil uji aktivitas antimikroba dianalisis menggunakan one-way ANOVA dan uji lanjut Bonferroni. Hasil karakterisasi BAL menunjukkan empat isolat BAL memiliki perbedaan secara morfologi koloni, termasuk bakteri Gram positif bentuk bulat dan katalase negatif. Hasil uji aktivitas antimikroba didapatkan bahwa isolat BAL1 memiliki kemampuan tertinggi dalam menghambat *E. coli* dengan diameter zona hambat rata-rata yaitu 15.21 mm dan zona hambat terendah terdapat pada isolat BAL 4 yaitu 10.80 mm. Hasil uji one way ANOVA menunjukkan adanya perbedaan yang signifikan antara keempat isolat BAL dalam menghambat *Escherichia coli* dengan P *value* <0.05. Pada uji Bonferroni diperoleh perbedaan yang bermakna antara isolat BAL 1 dengan BAL 4. Dapat disimpulkan bahwa BAL yang diisolasi dari tempoyak berpotensi menjadi sumber probiotik.

*Corresponding Author : eliya_mursyida@univrab.ac.id

INTRODUCTION

Diarrhea is a communicable disease and become a threat to society due to throw fearful number of mortalities in children under five years old. This disease has been nominated as the second rank deadly threat. Globally, there were 1.7 billion cases of diarrhea disease that kills approximately 525.000 toddlers annually (World Health Organization, 2017). In Indonesia diarrhea is the third rank of deadly disease, after the tuberculosis and pneumonia. In 2017, 179.764 people of Riau province has suffered by diarrhea. Diarrhea is an endemic to Indonesia and also a potentially fatal outbreak. In addition, 2017 was the worst period since the diarrhea outbreak was sadly happening in mostly province of Indonesia, 21 times of diarrhea outbreak into 12 provinces and 17 districts/cities. Polewali Mandar, Pohuwato, central Lampung and Merauke districts were suffered due to twice diarrhea outbreaks which thrown 1.725 cases and deaths of 34 people (KemenKes, 2018).

The clinical symptoms of diarrhea have been investigated, an increasing stool volume leading to high frequency of bowel movements. Poor sanitation causes emerging of pathogenic such as bacteria, viruses, and parasites, then contaminate food or drink, or from person to person (McPhee and Hammer, 2014). Rotavirus and *E. coli* were reported as the main pathogens of diarrhea outbreak in the developing countries, basically having poor sanitation (World Health Organization, 2017). *E. coli* is found as a healthy indigenous microflora in the human colon, unfortunately, it occasionally emerges as the primary infection of diarrhea. *E. coli* has a short rod-shaped and belongs to Gram negative bacteria, in size 0.4-0.7x1.4µm, mostly motile and some strains have capsules. *E. coli* can produce enterotoxin namely LT toxin (thermolabile) and ST toxin (thermostable). LT toxin stimulates the enzyme adenyl cyclase (ADCY) contained in the epithelial cells of the fine intestinal mucosa. The enzymes ADCY activation can overflow the permeability of intestinal epithelium cells, then leading to the accumulation of fluid in the intestines recognized as diarrhea (Syahrurachman, 2019).

One treatment of diarrhea is by administering antibiotics. Approximately 15-30% of patients suffer recurrence of symptoms after discontinuing of antibiotics (Anadón *et al.*, 2016). However, inappropriate administration of antibiotics induces bacterial resistance. According to the statistical report by Kemenkes (2018), on the results of monitoring the coverage and the quality of diarrhea treatment in 2009 reported that the percentage of antibiotics using without indications in the diarrhea sufferers ranged between 45.6-100%. Aceh,

Lampung and Papua are the provinces with the highest percentage of 100% and the provinces with the lowest percentage (45.6%) namely West Sumatera. Treatment with antibiotics can interfere with the balance of healthy gastrointestinal flora due to the alteration of the composition of microbiota. Probiotics can nourish and recover the intestinal microecology by administering concordantly or after treatment with antibiotics to reduce the duration of clinical symptoms and severity of diarrhea.

Probiotics are an active microbe (yeast and LAB which benefits to the human colon when consumed adequately (Anadón *et al.*, 2016). LAB is a group of Gram-positive bacteria in the form of coccus or bacil, not spores, an aerobic facultative often found in the fermented foods. Bacteria that include LAB are Lactobacillus, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. LAB produce lactic acid by fermenting carbohydrates. Moreover, LAB also produces other compounds such as acetic acid, hydrogen peroxide and bacteriocin that can inhibit enteropathogenic microbes and also *E. coli* invasion through the intestines (Quinto *et al.*, 2014).

Tempoyak is traditionally made by fermenting durian by adding salt and kept in a sealed container for 2-7 days. According to Aisyah *et al*., (2014) findings, isolation of LAB originated of the tempoyak has the characteristic Gram-positive, bacil or coccus shaped, non-motile, positive carbohydrate fermentation test (+), and negative reaction of catalase enzyme (-). In the form of coccus, it was identified to be the genus *Streptococcus*, *Pediococcus*, and *Leuconostoc*, while the bacil-shaped bacteria were declared as a derivative of the genus *Lactobacillus*. Thus, this study aims to isolate and characterize LAB from tempoyak and determine its antimicrobial activity to *E. coli* growth.

MATERIAL AND METHOD

Materials

The materials used in the study were tempoyak with a salt concentration of 2% (w/w) from the weight of durian from Kampar district. The pathogenic bacteria used in this study was *E. coli* obtained from the Laboratory of Microbiology and Parasitology, Abdurrab University. The medium used for LAB growth was deMann Rogosa Sharpe Agar (MRSA) (Oxoid, UK).

Fermented durian preparation (Nizori *et al*., 2017)

Durian was separated from the seeds and weighed 100 g, then added salt with a concentration of 2% of the weight of durian meat. Mixture of durian meat and salt were stirred evenly and stored in a glass jar for 7 days. After 7 days of the fermentation

process, tempoyak is ready to be used for the LAB isolation process.

Lactic acid bacteria isolation *(*Aisyah *et al*., 2014)

Isolation of LAB from tempoyak was carried out on the 7th day of fermentation by taking 1 g of tempoyak and mixed with 9 mL of 0.9% NaCl solution (Merck, Germany), then homogenized. The dilution is carried out up to 10^{-6}. The results of the 10^{-4} to 10^{-6} dilutions were each taken as much as 1 mL using a micropipette and planted on MRSA medium using the pour plate method in duplicate, then incubated using an incubator (Memmert IN55, Germany) at 37^{o}C for 48 hours. After incubation time, the growing colonies were counted using a colony counter (570 Suntex) and qualified about 30-300 colonies with CFU/g units. Furthermore, LAB isolates were purified to obtain a single isolate.

Lactic acid bacteria characterization (Nizori *et al*., 2017)

LAB characterization was carried out based on the morphological characteristics of the colonies and cells, as well as the catalase test. The morphology of bacterial colonies was carried out on nutrient agar (NA) medium (Oxoid, UK) with the streak quadrant method, then incubated at 37^{o}C for 48 hours. Colony morphology observed included shape, size, elevation, margin, and color (pigmentation). Bacterial cell morphology was observed using the Gram stain method to determine the shape and type of cells under a microscope (Olympus CX21, Japan), and followed by catalase test.

Antimicrobial suspension preparation (modification from Antari *et al*. 2020)

LAB isolates were inoculated into 9 mL of deMann Rogosa Sharpe Broth (MRSB) medium (Oxoid, UK), then incubated at 37^{o}C for 24 hours using an incubator. After incubation time, 1 mL of LAB isolate suspension was centrifuged (PLC 03 Gemmy, Taiwan) at 6000 rpm for 1 hour to separate the bacterial cells (pellet) from the filtrate (supernatant). Furthermore, the supernatant was used to test the antimicrobial activity.

Antimicrobial activity of Lactic Acid Bacteria (modification from Antari *et al*. 2020)

The antimicrobial activity of LAB against *Escherichia coli* was tested by the well diffusion method. *E. coli* which has met the McFarland 0.5% standard was spread on mueller hinton agar (MHA) medium (Oxoid, UK). Next, a hole with a diameter of 6 mm was made using a cork borrer. Supernatant from each LAB (LAB1, LAB2, LAB3 and LAB4), 20 L of positive and negative control was taken and poured into wells, then

incubated at 37°C for 24 hours. After incubation time, the inhibition zone formed around the well was measured using a caliper (Tricle, China) and calculated using the formula for the diameter of the inhibition zone minus the diameter of the well.

Data analysis (Safitri, 2018)

The results of the antimicrobial activity test were analyzed using SPSS version 20 software. One-Way ANOVA test was used to determine the differences in each treatment group and continued with the Bonferroni Post-hoc test. The data is presented in the form of tables and pictures.

RESULT AND DISCUSSION

Lactic acid bacteria isolation

The results showed that the number of bacterial colonies that met the requirements were 30-300 colonies with a total of $1.46x10^8$ CFU/g. LAB isolates grown on MRSA medium, four LAB isolates showed differences in colony morphology such as color, edge, and elevation (Table 1). Another study also found that LAB isolated from tempoyak Padang Pariaman showed a colony count of $16x10^8$ CFU/g (Juliyarsi *et al.*, 2018).

Characterizing selected isolates

Four isolates were observed as Gram-positive with coccus-shaped (Figure 1). Previous study of Aisyah *et al*., 2014) reported that LAB is a Gram-positive bacteria with a rod or round shape, where the rod shape is expected to be the genus *Lactobacillus*, while the round shape is suspected to be the genus *Streptococcus*, *Pediococcus*, and *Leuconostoc*. In addition, Rahayu and Qurbaniah (2019) also found that LAB isolated from tempoyak was observed as coccus Gram-positive bacteria. Another study, Juliyarsi *et al.*, (2018) found that LAB isolated from tempoyak Padang Pariaman showed Gram-positive bacteria with a rod shape. This is because Gram-positive bacteria are able to maintain the purple color of crystal violet despite being given an alcohol solution (Nurhidayati *et al*., 2015).

Subsequently, we catalase test on the LAB isolates. The four LAB isolates negatively showed of catalase enzyme. Briefly, the LAB isolates have not possessed the catalase enzyme to break down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). This finding is in accordance with the report

of Rizqiati *et al.*, (2015), Mardalena (2016), and Juliyarsi *et al.*, (2018), they found that lactic acid bacteria (LAB) isolates showed negatively in the catalase test. Rahayu and Qurbaniah (2019) also found that LAB isolated from tempoyak exhibited negative catalase characteristics, did not have endospores and was not motile.

Table 1. Characterization of the four lactic acid bacteria (LAB) isolates

Isolate	Morphological characteristics			
	Color	Shape	Edge	Elevation
LAB 1	Cream	Circular	Entire	Flat
LAB 2	White	Circular	Undulate	Flat
LAB 3	White	Circular	Entire	Flat
LAB 4	White	Circular	Entire	Raised

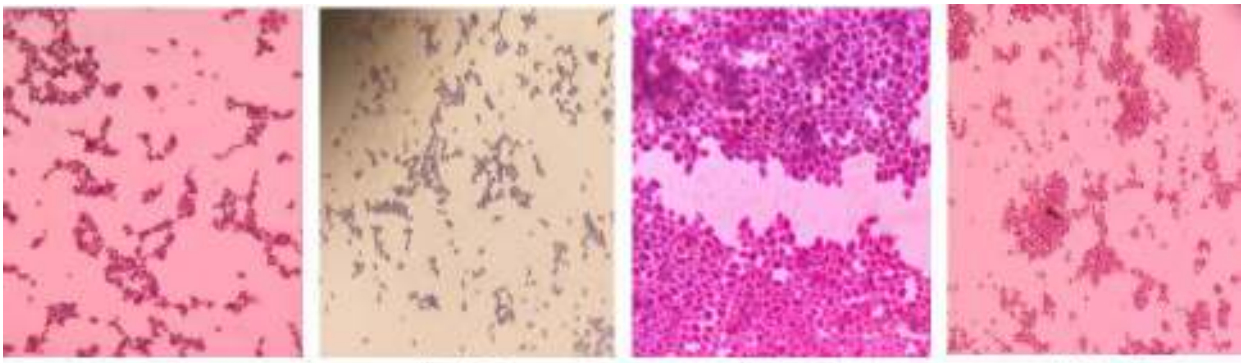

Figure 1. Gram Staining of Lactic Acid Bacteria (LAB)

Antimicrobial activity

The LAB was hypothesized to possess antimicrobial properties. Antimicrobial activity of LAB on *E. coli* growth was done by obtaining that four LAB isolates inhibit the growth *of E. coli* by forming an inhibition zone around the well (Figure 2). The highest inhibition zone was produced by LAB1 isolates with an average diameter of 16.85 mm which was included into a strong category. In this study, LAB4 isolates showed the lowest antimicrobial activity, with an average diameter of 10.80 mm for

inhibition. However, according to the inhibition zone, the LAB4 is nominated into a medium category (Table 2). Previous research by Juliyarsi *et al.*, (2018) found that LAB isolates isolated from tempoyak Padang Pariaman were able to inhibit the growth of *E. coli* with an inhibition zone diameter of 12.3 mm. Somashekaraiah *et al*. (2019) also found that from 7 selected LAB isolates isolated from neera were able to inhibit *E. coli*. The antimicrobial activity is apparently triggered by secondary metabolites manufactured by the LAB. The LAB was reported able to produce secondary metabolites namely bacteriocins (Mora-Villalobos *et al.*, 2020).

Lactic acid bacteria are identified to have active compounds such as organic acids (lactic and acetic), hydrogen peroxide, and bacteriocins. Interestingly, lactic acid and acetic acid secreted by LAB are importantly considerable as antimicrobials and have a high spectrum of inhibition. Lactic acid is a water-soluble molecule that can permeate into the periplasm of *E. coli* through the protein porin on the outer membrane. In addition, lactic acid can impair the permeability of *E. coli* by disrupting the outer membrane. The outer membrane permeability barrier of *E. coli* is a layer of lipopolysaccharide (LPS) located on the surface of the membrane. As a result of damage to the lipopolysaccharide layer, it will cause a damage to the outer cell membrane, hence other antimicrobial compounds, including hydrogen peroxide and bacteriocin, can enter the cytoplasmic membrane and damage intracellular activity which can eventually lyse *E. coli* cells (Khikmah, 2015).

Table 2. Descriptive zone inhibitory analysis results

Treatment	Mean ± SD (mm)	Min (mm)	Max (mm)
LAB 1	15.21±1.48	13.30	16.85
LAB 2	13.30±1.67	11.00	15.00
LAB 3	13.46±1.93	11.30	15.70
LAB 4	10.80±1.28	9.00	12.00
Control +	27.25±1.92	25.00	29.30
Control -	0±0	0	0

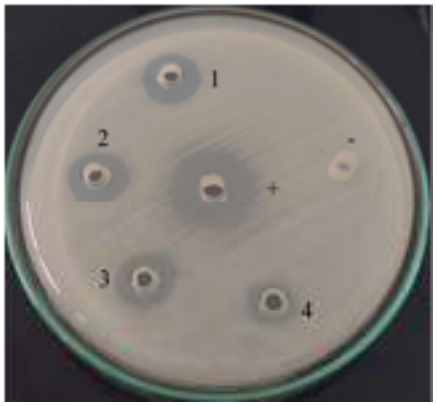

Figure 2. Inhibitory zone of *E. coli* growth induced by LAB of tempoyak. (1). LAB1, (2). LAB2, (3). LAB3, (4). LAB4, (+). Ciprofloxacin, and (-). Aquadest

Hydrogen peroxide is a compound that possess antimicrobial properties. These compounds are capable to oxidize cells and cause damage to protein molecules of the bacterial cell structure, resulting in the cell death. However, this compound is unstable and not heat resistant. The exceeding of heat exposure will be easily decomposed hydrogen peroxide into water and oxygen, then the antimicrobial effect will be degraded. Linked to our result, our LAB from tempoyak is non-producing catalase. Our finding is concordant with Anadón *et al.*, (2016) stated incapability of LAB in generating catalase enzyme hence the hydrogen peroxide produced cannot be degraded and acts as an oxidant by forming free radicals.

Bacteriocins are a potent antimicrobial peptide synthesized by the LAB group. The site of action of bacteriocins is the cytoplasmic membrane of pathogenic bacteria and targets the energy of the vesicle membrane to disrupt the proton motive force of the pathogenic bacteria. Bacteriocins are classified into three classes, namely class I bacteriocins, also called lanbiotics. Class I bacteriocins contain the amino acids lanthionine and methyllanthionine and have a molecular weight of less than 5 kDa, for example nisin, lactocin and mersacidin. Class II bacteriocins have molecular weight less than 10 kDa, heat-resistant, cationic, hydrophobic peptides, e.g pediocin, sakicin, leucocin. Class III bacteriocins have a molecular weight more than 30 kDa and are not

heat-resistant, for example, lysostaphin, enterolycin, helveticin (Toomula, 2011; Alvarez-Sieiro *et al*., 2016; Mokoena, 2017).

More insight of bacteriocins, it can be produced by *Lactococcus*, *Lactobacillus*, and *Pediococcus* derived from various foodstuffs, for example *Lactococcus* lactis produces nisin and pediocin is produced by *Pediococcus acidilactic* (Toomula, 2011). Bacteriocins are synthesized following a protein synthesis pattern regulated by plasmid DNA. In general, non-lantibiotic bacteriocins are synthesized via the ribosomal pathway, while the lantibiotic group is synthesized by a ribosome as pre-peptides and then undergoes modification. Bacteriocins are produced in the exponential phase and the optimum is produced in the stationary phase (Angkuna *et al*., 2019). Gaspar *et al*. (2018) found that the production of bacteriocins clearly increased in the exponential phase and was followed by a decrease during the stationary phase.

The results of the One-Way ANOVA test showed that the four LAB isolates showed statistically significant differences in inhibiting the growth of *E. coli* with p value <0.05. The Bonferroni Post hoc test showed that there was a significant difference between LAB1 isolates and LAB4 isolates indicated by p value <0.05.

REFERENCES

Aisyah, A., Kusdiyantini, E. and Suprihadi, A. 2014. Isolasi, Karakterisasi Bakteri Asam Laktat, dan Analisis Proksimat dari Pangan Fermentasi Tempoyak. *Jurnal Biologi*, 3(2):31–39.

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., and Kuipers, O. P. 2016. Bacteriocins of lactic acid bacteria: extending the family. *Appl Microbiol Biotechnol*, 100:2939-2951.

Anadón, A. *et al.* 2016. Probiotics: Safety and Toxicity Considerations. *Nutraceuticals: Efficacy, Safety and Toxicity*, pp. 777–798. doi: 10.1016/B978-0-12-802147-7.00055-3.

Angkuna, S. A., Apridamayanti, P., and Sari, R. 2019. Penentuan Waktu Optimum Produksi Bakteriosin *dari Lactobacillus casei* Terhadap Bakteri Patogen *Staphylococcus aureus* dan *Escherichia coli*. J Mahasiswa Farmasi Fakultas Kedokteran UNTAN 4(1):1-18.

Antari, M. J., Puspawati, N. N., and Wipradynyadewi, P. A. S. 2020. Aktivitas antimikroba bakteri asam laktat dari air susu ibu (ASI) terhadap *Listeria monocytogenes* FNCC 0156. *Jurnal Ilmu Teknologi Pangan*, 9(1): 96-107.

Gaspar, C., Donders, G. G., Palmeira-de-Oliveira, R., Queiroz, J. A., Tomaz, C., Martinez-de-Oliveira, J., and Palmeira-de-Oliveira, A. 2018. Bacteriocin

production of the probiotic *Lactobacillus acidophilus* KS400. *AMB Expr*, 8(153):1-8.

Juliyarsi, I. *et al.* 2018. Characterization of lactic acid bacteria and determination of antimicrobial activity in tempoyak from Padang Pariaman district, West Sumatra, Indonesia. *Pakistan Journal of Nutrition*, 17(10):506–511. doi: 10.3923/pjn.2018.506.511.

KemenKes, R. I. 2018. *Data dan Informasi Profil Kesehatan Indonesia Tahun 2017*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Khikmah, N. 2015. Uji Antibakteri Susu Fermentasi Komersial Pada Bakteri Patogen. *Jurnal Penelitian Saintek*, 20(1):45–52. doi: 10.21831/jps.v20i1.5610.

Mardalena. 2016. Fase Petumbuhan Isolasi bakteri Asam Laktat (BAL) Tempoyak Asal Jambi yang Disimpan Pada suhu Kamar. *Jurnal Sain Peternakan Indonesia*, 11(1):58–66. Available at: file:///C:/Users/User/Downloads/fvm939e.pdf.

McPhee, S., and Hammer, G. 2014. *Pathophysiology of Disease - An Introduction to Clinical Medicine*. 7th edn. United States: McGraw-Hill Education.

Mokoena, M. P. 2017. Lactic acid bacteria and their bacteriocins: Classification, biosynthesis and applications against uropathogens: A mini-review', *Molecules*, 22(8). doi: 10.3390/molecules22081255.

Mora-Villalobos, J. A. *et al.* 2020. Multi-Product Lactic Acid Bacteria Fermentations. *Fermentation*, pp. 1–21.

Nizori, A., Prayogi, N. and Mursalin. 2017. Isolasi dan Identifikasi Bakteri Asam Laktat Tempoyak asal Jambi dari Berbagai Kosentrasi Garam. *Prosiding Seminar Nasional FKPT-TPI*, (September), pp. 20–21.

Nurhidayati, S., Faturrahman, F., and Ghazali, M. 2015. Deteksi Bakteri Patogen yang Berasosiasi dengan Kappaphycus alvarezii (Doty) Bergejala Penyakit Ice-ice. *Jurnal Sains Teknologi & Lingkungan*, 1(2):24–30. doi: 10.29303/jstl.v1i2.53.

Quinto, E. J. *et al.* 2014. Probiotic Lactic Acid Bacteria: A Review. *Food and Nutrition Sciences*, 05(18):1765–1775. doi: 10.4236/fns.2014.518190.

Rahayu, H. M., and Qurbaniah, M. (2019) 'Selection of Tempoyak Lactic Acid Bacteria As Candidate Strain for Yoghurt Starter Culture ?', *Biosaintifika*, 11(1):39–46. doi: http://dx.doi.org/10.15294/biosaintifika.v11i1.16769 Program.

Rizqiati, H. *et al.* 2015. Isolation and identification of indigenous lactic acid bacteria from North Sumatra river buffalo milk', *Jurnal Ilmu Ternak dan Veteriner*, 20(2):87–94. doi: 10.14334/jitv.v20i2.1163.

Safitri L. 2018. Uji efektivitas antibiotik ekstrak daun kayu manis (*Cinnamomum burmannii*) terhadao pertumbuhan *Salmonella typhi* secara in vitro. [Skripsi].

Somashekaraiah, R., Shruthi, B., Deepthi, B. V., and Sreenivasa, M. Y. 2019. Probiotic Properties of Lactic Acid Bacteria Isolated From Neera: A Naturally Fermenting Coconut Palm Nectar. Front in Microbiol 10(1382):1-11. https://doi.org/10.3389/fmicb.2019.01382.

Syahrurachman, A. 2019. *Buku Ajar Mikrobiologi Kedokteran*. Jakarta:

Universitas Indonesia.

Toomula, N. 2011. Bacteriocin Producing Probiotic Lactic acid Bacteria', *Journal of Microbial & Biochemical Technology*, 03(05):121–124. doi: 10.4172/1948-5948.1000062.

World Health Organization. 2017. *Diarrhoeal disease*. Available at: http://www.who.int/mediacentre/factsheets/fs330/en.

ISSN-P : 1978-6417; ISSN-E : 2580-5991