# Literature Study of Lactic Acid Bacteria in Bangka Belitung: Sources, Methodology, Utilization, And Identification

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### Abstract

Bangka Belitung has various types of fermented foods, in the production of which lactic acid bacteria are involved. Lactic acid bacteria plays important role in enhancing the nutritional value of food ingredients, extending their shelf life, producing distinct aromas, and preventing contamination from pathogenic bacteria. Additionally, lactic acid bacteria can also act as probiotics for application in livestock or fisheries. This article examined several studies on lactic acid bacteria that were linked to the biological resources of Bangka Belitung. The aspects studied included the sources of lactic acid bacterial isolates, research methods or approaches employed, utilization, and identification of lactic acid bacteria. Most of the research related to lactic acid bacteria has been conducted on fermented foods, primarily 'rusip' which is a fermented food product made from shrimp or fish. This review expected to provide an overview of the trends and research interest in lactic acid bacteria conducted in Bangka Belitung, while also mapping out aspects that need further exploration in subsequent research

Keywords: Bangka Belitung; Lactic Acid Bacteria; Fermentation; Lactobacillus; Rusip



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### INTRODUCTION

Lactic Acid Bacteria (LAB) are a microbial natural resource that still needs to be explored. Ecologically, this group of bacteria is highly diverse, and the species within it can dominate various foods, drinks, and others (Yuni, 2013). Lactic Acid Bacteria (LAB) are a group of bacteria that produce lactic acid as the main product during fermentation (Masood et al., 2011). LAB are bacteria that produce lactic acid as the

primary product during the fermentation process. The main role of lactic acid bacteria in fermentation is to produce acid in the fermented food.

Fermentation is a chemical change process of organic compounds involving microorganisms under anaerobic conditions, with organic compounds such as lactic acid, alcohol, and other organic acids as the final products. One example of a fermented product from Bangka Belitung is rusip. Rusip is a traditional food product from the Bangka Belitung region made from preserved small marine fish, particularly anchovies, which are fermented with the addition of salt and palm sugar and fermented by lactic acid bacteria (Putri et al., 2014). Other products include shrimp paste (local caleed as Terasi) and Bekasam.

Shrimp paste (terasi) is a processed product made from marine ingredients, usually in a block (solid) form, with shrimp (rebon) or anchovies as the main raw material, which are then processed through fermentation to produce a characteristic aroma (Karim et al., 2014). Meanwhile, bekasam is a fermented product made from local fish, processed with the addition of rice and salt, which gives it a sour taste (Lestari et al., 2018). Bekasam can be made from various types of freshwater fish, such as catfish or snakehead fish. In addition to fish, it can also be made from mushrooms and goat skin (kikil). Lactic acid bacteria have antibacterial properties. As the fermentation process occurs, lactic acid bacteria produce components such as lactic acid, hydrogen peroxide, and bacteriocins, which is why fermented products like bekasam have a sour taste (Piard & Desmazeaud, 1991).

Probiotics are microorganisms that live in the digestive system and offer health benefits to humans and animals (Widayaningsih, 2011). The function of lactic acid bacteria as probiotics is to stimulate immune function, control pathogenic infections, replace antibiotics, and maintain the digestive system by balancing microorganisms in the digestive tract. LAB also produce antimicrobial compounds, such as bacteriocins, which can inhibit spoilage microbes and pathogenic microbes, thus preserving food (Mastuti, 2022). In addition to lactic acid bacteria, other probiotic bacteria include species of *Bacillus, Staphylococcus, Enterococcus, Brevibacterium,* and *Propionibacterium.* Some molds or yeasts, such as *Saccharomyces, Penicillium, Aspergillus,* and *Rhizopus,* are also considered probiotics.

Lactic acid bacteria have general characteristics such as Gram-positive, negative reaction to catalase tests, and do not form spores. Based on the fermentation products, lactic acid bacteria can be classified into two groups: homofermentative bacteria, which produce lactic acid as the main product from sugar fermentation, and heterofermentative bacteria, which produce lactic acid and other fermentation products like ethanol and carbon dioxide (Finanda et al., 2021). One example of a lactic acid-producing bacterium is Lactobacillus, a probiotic bacterium used in food fermentation and preservation (Aini et al., 2021). A literature review study on the sources of lactic acid bacteria from Bangka Belitung has not yet been conducted. Therefore, the purpose of this literature review was to analyze the sources of isolates, methods, applications, and identities of lactic acid bacteria obtained in research.

### METHOD

The method used in this article was a literature study (library research) through a systematic literature review, which is a method of collecting data by understanding and studying theories from various literatures related to the research (Adlini et al., 2022). This research is based on a qualitative approach, where the author examines and reviews the sources of data without conducting analysis and hypotheses. The data collection technique is a way to search for and find information and facts based on credible sources. The data collection technique for this journal review is library research. Library research is a method of collecting data without conducting direct field research (Prastowo, 2011), by gathering and analyzing data from journals that are relevant to the topic discussed. Data collection for this article review was carried out by gathering data from various research journals online through websites such as SINTA, Science Direct, Google Scholar, PubMed, and ResearchGate, with search keywords including Lactic Acid Bacteria, Isolation of Lactic Acid Bacteria from Bangka Belitung, Identification of LAB from Bangka Belitung, and etc. The journals related to this theme were used as references for writing this review. From this search, 19 journals were selected as reference data for the article review.

#### **RESULT AND DISCUSSION**

The literature search found several studies on lactic acid bacteria that are related to the biological resources from the Bangka Belitung Province. Some of the studies that serve as sources for this review regarding lactic acid bacteria in Bangka Belitung are presented in Table 1.

### LAB Isolate Source

Several research results showed that the findings interpreted in Table 1 indicate that the sources of lactic acid bacteria isolates in Bangka Belitung are obtained from various sources, including anchovy rusip, aruk rice, chicken intestines from Merawang, red snapper fish intestines, *Chana* sp. fish intestines, catfish intestines (kelik/lembat), cow feces, and shrimp rusip. According to the literature review on lactic acid bacteria, the most commonly used source of lactic acid bacteria isolates comes from anchovy rusip. According to Yuliana (2007), rusip is a traditional fermented product from Bangka Belitung. The main ingredient of rusip is anchovy or bilis (*Stolephorus* sp.) or other small fish, which are given 25 % salt and about 10 % palm sugar, then fermented anaerobically for about two weeks. The fish preservation process through fermentation involves enzymatic, chemical, and microbial processes during fermentation, which ultimately determines the microbiological and chemical characteristics of the fermented fish. From the research above, several bacteria involved in the fermentation process of rusip are lactic acid bacteria, with different genera found in each study.

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Table 1. Research related to lactic acid bact	eria in Bangka Belitung
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Reference	Isolate Source	Method	Potetial/aplication	Identification	Journal index
Kurniawan et al.,(2015)	Waste	Experimental Method	Liquid organic fertilizer EM4	Lactobacillus sp. Saccharomyces sp. Rhodopseudomonas sp. Actinomycetes	SINTA
Kusmarwati et al., (2014)	Commercial "Rusip" in Bangka and Kalimantan	Methods: serial dilution and pour plate	Bacteriocin producer	Pediococcus pentosaceus	SINTA
Kusmarwati et al., (2011)	Rusip ( <i>Stolepherus</i> sp.)	The method used was bacterial culture in the preparation of anchovy rusip	Fermentation starter	Pediococcus acidilactici	SINTA
Ahsaniyah et al., (2023)	"Ayam Merawang" intestine	Isolation using serial dilution on Media MRSA + CaCO3 1% with scatter method.	Potential probiotics	Lactobacillus	SINTA
Putri et al. (2014)	Rusip ( <i>Stolepherus</i> sp.)	Pour plate method	Food presevation	Streptococcus Pediococcus Leuconostoc Lactobacillus	-
Fauziyah et al. (2023)	"Beras Aruk"	Isolation and identification through macroscopic, microscopic, biochemical, and molecular tests based on 16S rRNA	Has the potential as a probiotic bacterium and, if further developed, as a starter to improve	Lactobacilus plantarum	SINTA

Reference	Isolate Source	Method	Method Potetial/aplication		Journal index
			the quality of aruk rice		
Adibrata et al., (2023)	Slender walking fish ( <i>Clarias nieuhofii</i> )	The Total Plate Count (TPC) method is used to count the number of microbes. The isolation method used is the spread plate method, or spreading method using a spreader rod	Probiotics	Lactobacillus Bifidobacterium	SINTA
Lingga et al, (2022)	Barramundi intestine	Isolation and selection of LAB by culturing using the pour plate method	Probiotics	Lactobacillus sp.	SINTA
Harmiatun et al., (2022)	Pepetek fish ( <i>Leiognathus equulus</i> )	Fermentation of preservation through preparation stages and treatment application stages, followed by the descriptive statistical approach method and the hedonic method.	Rusip Pepetek	Lactobacillus bulgaricus.	SINTA
Bayu et al., (2023)	<i>Channa</i> sp. intestine	Sample preparation, isolation of LAB, characterization and purification of bacterial isolates, biochemical tests, temperature resistance tests, antimicrobial tests,	Antimicrobial metabolite and probiotics	Lactobacillus	SINTA

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Reference	Isolate Source	Method	Potetial/aplication	Identification	Journal index
		hemolysis tests, lactic acid production tests, and cholesterol-lowering activity tests			
Indriati et al., (2014)	Rusip	The agar well diffusion method is used to detect the antibacterial spectrum of the crude bacteriocin from L. lactis ssp. lactis CN1.10a	Bacteriocin producer	Lactococcus lactis ssp. lactis CN1.10a	SINTA
Kurniawan & Syila, (2021)	Anchovy fish ( <i>Stolephorus</i> sp.)	Experimental method	Fermentation of anchovy fish ( <i>Stolephorus</i> Sp.)	-	SINTA
Batubara et al., (2019)	Anchovy fish (Stolephorus commersoni)	The addition of a probiotic LAB starter combination to the chemical and microbiological changes in rusip	Fermentation of anchovy fish	Lactobacillus plantarum SK(5) Lactobacillus plantarum NS(5)	SINTA
Lingga et al., (2022)	Cow Feces	Methods: serial dilution and spread plate.	Probiotics	Lactobacillus	-
Kusumarwati et al., (2013)	Rusip	Isolation dan Screening	Bacteriocin producer	Lactococcus lactis subsp lactis	SINTA
Purwadi et al., (2019)	Shrimp Rusip	Isolation dan Screening	Protease producer	Bacillus cereus	-

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Reference	ReferenceIsolate SourceMethod		Isolate Source Method H		Potetial/aplication	Identification	Journal index	
Helmi et al., (2022)	Shrimp paste	Metagenomic	Terasi fermentation	Tetragenococcus, Aloicoccus, Alkalibacillus, Atopostipes, Alkalibacterium	SCOPUS			
Lestari et al., (2018)	Shrimp Rusip	The method used is sample collection and laboratory testing/analysis, followed by chemical quality tests including pH value, salt content, protein content, and peptide content.	Fermentation starter	Lactobacillus Streptococus Leuconostoc	SINTA			
Lingga et al, (2023)	Slender walking fish ( <i>Clarias nieuhofii</i> ) intestine	Isolation and selection of lactic acid bacteria from the intestines of catfish ( <i>Clarias</i> <i>nieuhofii</i> ), morphological characterization, purification and Gram staining, biochemical property tests, lactic acid production, temperature and acid resistance tests, antimicrobial tests, hemolysis tests, DNA genome isolation of a bacterial isolate, and 16S rRNA gene identification of the bacterial isolate	Probiotics potential	Lactobacillus fermentum, Limosilactobacillus fermentum, Lactobacillus vaginalis, Levilactobacillus brevis, Bacillus cereus, Streptomyces avermitilis, Streptomyces yokosukanensis	SCOPUS			

### METHOD

There were various methods used in research from several data sources related to lactic acid bacteria, including the isolation of lactic acid bacteria using dilution methods cultured on various media, including MRSA media, TGE Broth, VRBA, experimental methods with preservation fermentation, spread plate methods, pour plate, total plate count, starter culture addition methods, sampling methods, chemical testing methods, agar well diffusion methods, and screening. Among the various methods used, the most frequently used method for isolating LAB was the dilution isolation method cultured on agar media. Bacterial isolation is a process of taking bacteria from a medium or its original environment and then growing them in an artificial medium to obtain a pure culture.

Several methods for obtaining pure cultures from a mixed culture exist. Two of the most commonly used are the spread plate and pour plate methods. In the spread plate method, microorganisms are grown on agar media by pouring the bacterial culture stock or spreading it over the solidified agar medium (Arini, 2016). The pour plate method is a technique for growing microorganisms in agar media by mixing liquid media with bacterial culture stock, allowing the cells to spread evenly and settle well on the agar surface or inside the agar (Damayanti et al., 2020). This method requires dilution before being cultured on agar medium in Petri dishes. It is based on the dilution principle, aiming to obtain individual species, assuming that each colony can be separated from a single type of cell that can be observed (Afrianto, 2004). In the study by Helmi et al., (2022), a metagenomic analysis approach was conducted to understand the dynamics of microbial populations in shrimp paste, including lactic acid bacteria.

### Application

Based on the review of several previous studies, it can be seen that the applications and potential uses of lactic acid bacteria were diverse. Lactic acid bacteria (LAB) are a group of microorganisms that produce lactic acid as the main product from sugar fermentation. Below are some applications and potentials of utilizing lactic acid bacteria based on previous research reviews conducted in Bangka Belitung, including organic liquid fertilizer EM4, bacteriocin and protease enzyme producers, rusip fish fermentation starters, and probiotics.

The microorganisms contained in the specifications of the EM4 organic liquid fertilizer product include lactic acid bacteria (*Lactobacillus* sp.), fermentation fungi (*Saccharomyces* sp.), photosynthetic bacteria (*Rhodopseudomonas* sp.), and Actinomycetes bacteria. The EM4 product is a mixture of selected microbes that work synergistically in the decomposition process. According to the research by Wididana (1994); Hastuti (2012); Yuniati et al., (2025) the use of selected EM4 microbes can accelerate the decomposition of organic material from 3 months to 7-14 days.

Based on several applications and potential uses of lactic acid bacteria, the next is as bacteriocin producers. Bacteriocin is defined as an antimicrobial compound synthesized by various bacterial species, including lactic acid bacteria (LAB), in ribosomes. Bacteriocin is a protein compound showing antibacterial activity and is effective against sensitive bacteria. This protein is often active against other species that are closely related to the producing microorganism (Klaenhammer, 1988). Several LAB produce bacteriocins with broad inhibition spectra. Some bacteriocins also have potential as food preservatives. The use of bacteriocin in the food industry can help reduce the use of chemical preservatives or intensive heat treatments. Bacteriocins derived from lactic acid bacteria are considered very interesting because they are safe and non-toxic food additives and can prevent food spoilage caused by Gram-positive pathogenic bacteria (Hatta et al., 2010). Based on the review of previous research, it was found that fermented products associated with lactic acid bacteria can produce bacteriocins.

Several bacteriocins from fermented products include bacteriocins isolated and explored from lactic acid bacteria originating from rusip in Bangka and Kalimantan (Kusmarwati et al., 2014). The bacteriocin produced by *Lactococcus lactis* ssp. lactis CN1.10a from rusip (Indriati et al., 2014) and "Production and Characterization of Bacteriocin Produced By Lactic Acid Bacteria Isolated From Rusip (Kusumarwati et al., 2013). From rusip samples originating from Bangka and Kalimantan, 12 LAB isolates were obtained. From these 12 LAB isolates, one isolate (RK4) from Kalimantan was found to be capable of producing bacteriocin. The LAB isolate that was successfully identified as a bacteriocin producer was Pediococcus pentosaceus.

Bacteriocin produced by *Pediococcus pentosaceus* can be utilized as a food preservative because bacteriocin only inhibits Gram-positive microbes, while Gram-negative bacteria are not inhibited by bacteriocin. *Pediococcus pentosaceus* can inhibit several foodborne pathogens such as Listeria monocytogenes, which can cause Listeriosis (Kusmarwati et al., 2014). Furthermore, Indriati et al., (2014) mentioned that certain bacteria capable of producing lactic acid, such as *Lactococcus lactis* ssp., have the potential to produce bacteriocin. This bacterium can produce bacteriocin. According to Indriati et al., (2014) the characteristics of this bacteriocin are sensitive to proteolytic enzymes such as proteinase K and papain but not to RNAse. Bacteriocin has a broad inhibition activity against both Gram-positive and Gram-negative bacteria such as *Escherichia coli, Listeria monocytogenes*, and *Lactobacillus plantarum*. Lactococcus lactis ssp. can produce bacteriocin on CMC medium with lactose and sucrose as carbohydrate sources, optimally produced at 35 °C and pH 5.

Several other applications and potentials of lactic acid bacteria include their use as a fermentation starter for rusip fish. The addition of Pediococcus acidilactici in the study by Kusmarwati et al., (2011) as a starter culture improved the quality of rusip, providing the best sensory value, especially in taste and texture, during salting with a fermentation time of 9 days. The rusip produced had lower total LAB coliform content than rusip without the starter.

Additionally, based on several applications and potentials of lactic acid bacteria, they are used as probiotics. Probiotics are live microorganisms that, when given in adequate amounts, provide health benefits to their host. Bacteria that naturally reside in the bodies of living organisms are called normal flora and are generally considered beneficial bacteria with potential as probiotics. Probiotic bacteria can enhance human health. Probiotic bacteria are able to survive during processing, storage, and within the digestive tract ecosystem, even in extreme environments like saliva, stomach acid, and bile. Furthermore, probiotic bacteria can multiply, are non-toxic, and non-pathogenic (Sunarlim, 2009).

Characteristics of lactic acid bacteria that can be classified as probiotics include being safe for consumption, able to withstand acidic pH conditions, capable of inhibiting pathogenic bacteria (Rahmiati et al., 2020), easy to cultivate, and have high viability under anaerobic conditions (Lestari et al., 2018). Probiotic bacteria found in previous research articles include *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus bifidobacterium*, *Lactobacillus* sp., *E. coli*, *Salmonella typhi*, and *Staphylococcus aureus*. From the list of bacteria obtained, most are considered probiotics with known health benefits. However, it should be noted that *E. coli*, *Salmonella typhi*, and *Staphylococcus aureus* are not probiotics, but rather pathogenic or potentially pathogenic bacteria.

#### Identification

Based on the results of morphological observation of LAB in previous studies, which included color, edge, and shape, along with Gram staining to confirm whether the bacteria were indeed LAB, it can be seen that the identification process in these previous studies was carried out by examining the characteristics of the bacteria that grew. Several isolates used in the research on lactic acid bacteria from Bangka Belitung in this identification were pure cultures. The observation of colony morphology was conducted macroscopically and microscopically. Macroscopically, it involved examining the shape, color, edge, and elevation of the colony. Microscopically, the shape and arrangement of the cells were observed using a microscope. Gram staining was performed on 24-hour-old bacterial cultures in MRSA media.

Based on the review of several previous studies, it was found that research on the isolation and identification of lactic acid bacteria (LAB) from Bangka Belitung showed that various species of LAB had been identified from diverse sources of isolates. The LAB genus that dominated, according to the studies presented, was the genus Lactobacillus, which appeared in almost all research on lactic acid bacteria, such as in fish waste in the study by Kurniawan et al., (2015). It was found to be a potential probiotic bacteria and was discovered in the intestines of chickens in Merawang village (Ahsaniyah et al., 2023). It was also found in Aruk rice and showed potential as a probiotic and starter culture to improve the quality of Aruk rice (Fauziyah et al., 2023).

This shows that the genus Lactobacillus has a wide functional diversity and can be found in various environments. These findings provide evidence that LAB from the genus Lactobacillus have broad potential applications, ranging from waste management to improving food product quality. The species of Lactobacillus found in several studies show that there are various species, including *Lactobacillus* sp., *Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus bifidobacterium, Lactobacillus bulgaricus, Lactobacillus plantarum* SK(5), and *Lactobacillus plantarum* NS(5).

Next, the bacteria that have the potential to produce lactic acid are dominated by the genus Pediococcus. Pediococcus is a genus of bacteria that belongs to the lactic acid bacteria (LAB) group, characterized by being non-motile (not moving) and having a spherical shape. The cells of this bacteria are divided into two fields, forming pairs, tetrads (arranged in fours), or larger spherical cell clumps. The species of Pediococcus found in several studies include *Pediococcus pentosaceus, Pediococcus acidilactici*, and *Pediococcus leuconostoc. Pediococcus pentosaceus* is one of the LAB that is potential in producing lactic acid. In previous studies, isolation of LAB with potential use as probiotics for chickens was conducted (Hamida et al., 2015).

## CONCLUSION

Based on the review conducted on 19 literatures, the average research on lactic acid bacteria in Bangka Belitung used isolates sourced from rusip, which is a traditional fermented product from Bangka Belitung. The dominant method used in the research is the isolation method using MRSA media. The application of LAB is not only as a food fermentation ingredient but can also be applied as probiotics, bacteriocins, and organic fertilizers. The bacteria found were from the genera *Lactobacillus*, *Pediococcus*, *Bacillus*, and *Streptococcus*.

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