

Indigenous Lactic Acid Bacteria of *Chao*: A Fermented Fish Product from Pangkep

Nurul Hidayah*, Masyitha Wahid, Musrifah Tahar, Baso Manguntungi, and Mulyati Mulyati

Department of Biology Education, Universitas Sulawesi Barat, Jalan Prof. Dr. Baharuddin Lopa, S.H, Talumung, Kabupaten Majene, Sulawesi Barat 91412, Indonesia

*Corresponding author: nurulhidayah@unsulbar.ac.id

Abstract

Chao is a traditional fermented fish product from Pangkajene and Kepulauan Regency, South Sulawesi, Indonesia. The fermentation process involves lactic acid bacteria (LAB) which produce lactic acid, which lowers the pH of the fish and inhibits the growth of spoilage and pathogenic bacteria. LAB plays an important role in food fermentation by producing various metabolites that contribute to the sensory characteristics of the product. Studies related to the potential of LAB from *chao* are still very limited. This study aimed to isolate LAB from *chao* and characterize it based on the morphology, Gram staining, and catalase test. Isolation of LAB from *chao* were used MRSA + 0.5% CaCO₃. LAB was selected based on a clear zone formed around the bacterial colony. Selected isolates were characterized using colony and cell morphology, Gram staining, and catalase test. The results showed that 16 LAB isolates were originated from *chao* with similar colony morphology, but different colour. There were 3 cocci, 11 rods, and 2 were coccobacillus isolates. All isolates were Gram positive and catalase negative which is the characteristic of LAB.

Keywords: lactic acid bacteria, isolation, fermented fish, *chao*, characterization

Introduction

Chao is a traditional fermented fish product from Pangkajene and Kepulauan Regency, South Sulawesi. It is prepared by soaking raw ingredients such as anchovies (*Stolephorus* sp.) rice, and salt and then fermenting naturally in a controlled condition (Syahriati *et al.*, 2022). The fermentation process involves the activity of lactic acid bacteria (LAB) that produce lactic acid, which lowers the pH of the fish and inhibits the growth of spoilage and pathogenic bacteria (Matti *et al.*, 2019; Song *et al.*, 2021).

LAB are a group of Gram-positive, non-sporulating, and acid-tolerant bacteria that play a crucial role in food fermentation, particularly in dairy and meat products. LAB can produce various metabolites, including organic acids, bacteriocins, and enzymes, which contribute to the flavour, texture, and safety of fermented foods (Abid, *et al.*, 2018; Afriani *et al.*, 2018; Hidayah *et al.*, 2021).

The use of LAB as probiotics has gained popularity due to their ability to confer health benefits to the host. LAB can enhance the immune system, prevent or alleviate gastrointestinal disorders, and lower the risk of certain chronic diseases. Some LAB strains

have also been shown to possess antimicrobial properties that can inhibit the growth of foodborne pathogens and spoilage microorganisms (Abid *et al.*, 2018; Manguntungi *et al.*, 2020b).

The isolation and molecular identification of LAB from *chao* can provide insights into the microbial diversity and dynamics of the fermentation process and determine the potential of the LAB strains as probiotics and food preservatives (Handayani *et al.*, 2018; Matti *et al.*, 2019; Hidayah *et al.*, 2021; Bindu *et al.*, 2021). The isolation and molecular identification of LAB strains will help to understand the fermentation process and ensure the safety and quality of the product. Moreover, the screening of LAB strains for desirable properties will contribute to the selection of potential strains for future applications in the food industry, such as starter cultures or probiotics.

Materials and Methods

Sample Collection. *Chao* samples were obtained from traditional markets in Pangkep Regency. Samples brought in a cooler box to

the Microbiology Laboratory of the Universitas Sulawesi Barat for further investigation.

Isolation of Lactic Acid Bacteria. A total of 1 g of *chao* sample was put into serial dilutions of 9 ml of sterile aquadest then inoculated on Man Rogosa Sharpe Agar media and incubated at 37 °C for 24 hours. Isolates were selected then were grown on MRSA + 0.5% CaCO₃ media by spotting on the surface of the media and incubated at 37 °C for 24 - 48 hours. LAB isolates were selected based on the presence of a clear zone around the bacterial colony.

Characterization of Lactic Acid Bacteria. Characterization of LAB was carried out by observing morphology colony, morphology cells, Gram staining, and catalase test.

Results

Isolation of Lactic Acid Bacteria

The isolation of bacteria from *chao* resulted 109 isolates which were estimated to be LAB. Next, the isolates were selected based on the clear zone formed on MRSA + 0.5% CaCO₃ media and 38 isolates of lactic acid bacteria were obtained (Figure 1).

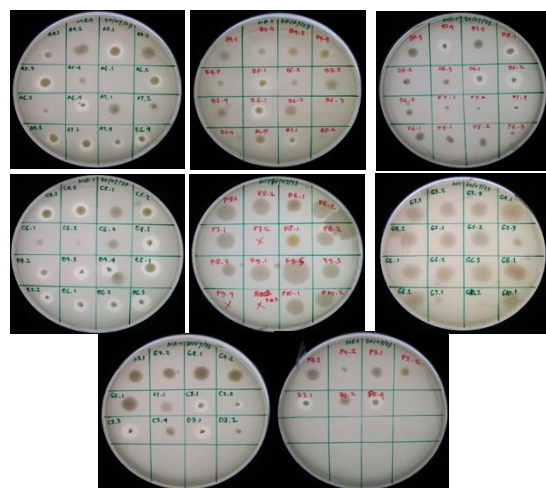


Figure 1. Lactic acid bacteria formed a clear zone around the colony

Characterization of Lactic Acid Bacteria

These bacteria were then selected by catalase test and Gram staining, isolates with catalase negative and Gram positive characteristics were selected and 16 isolates were obtained. Colony morphological characteristics were also observed and it was

found that 4 isolates were milky white, 5 were yellowywhite, 5 were dark white, 1 yellow, and 1 shine clear white with the same form, edges, and elevation, namely circular, entire, and convex, respectively. Observation of cell morphology showed that 3 isolates were cocci, 11 were rods, and 2 were coccobacillus (Table 2).

Table 1. Characteristics of lactic acid bacteria isolated from *chao*

No	I	CM					CS	GR	CTL
		C	F	M	E				
1	A4.1	MW	Ci	En	Co	Cc	+	-	
2	A4.3	YW	Ci	En	Co	Rd	+	-	
3	A5.1	DW	Ci	En	Co	Rd	+	-	
4	A5.3	DW	Ci	En	Co	Rd	+	-	
5	A7.3	YW	Ci	En	Co	Rd	+	-	
6	B4.3	YW	Ci	En	Co	Rd	+	-	
7	B5.1	Y	Ci	En	Co	Rd	+	-	
8	B7.4	YW	Ci	En	Co	Cb	+	-	
9	C3.1	SCW	Ci	En	Co	Rd	+	-	
10	C4.1	DW	Ci	En	Co	Rd	+	-	
11	C4.2	MW	Ci	En	Co	Rd	+	-	
12	C5.2	DW	Ci	En	Co	Cb	+	-	
13	D3.1	YW	Ci	En	Co	Rd	+	-	
14	E4.1	DW	Ci	En	Co	Cc	+	-	
15	E5.1	MW	Ci	En	Co	Cc	+	-	
16	E6.3	MW	Ci	En	Co	Rd	+	-	

Note: I: isolate, CM: colony morphology, CS: cell shape, GR: gram, CTL: catalase, C: colour, F: form, M: margin, E: elevation, MW: milky white, YW: yellowywhite, DW: dark white, Y: yellow, SCW: shine clear white, Ci: circular, En: entire, Co: convex, Cc: cocci, Rd: rod, Cb: coccobacillus

Discussion

In this study, selection of lactic acid bacteria was based on their growth on MRSA + 0.5% CaCO₃ medium and resulted 38 isolates. The recommended medium for growing LAB is MRSA medium. Meanwhile, the addition of CaCO₃ aims to select LAB that will grow in the medium and after incubation for 1 x 24 hours a transparent zone will be visible around the bacterial colony. LAB produces organic acids such as lactic acid and acetic acid as the main products. Lactic acid produced by LAB reacts with CaCO₃ (Calcium carbonate) which is insoluble in the media to produce soluble calcium lactate, thus forming a clear zone around the growing bacterial colony (Sandi *et al.*, 2018; Putri *et al.*, 2020).

Based on Gram staining and catalase test, showed that 16 isolates were Gram positive and catalase negative (Table 1). In general, lactic acid bacteria are known as bacteria that produce lactic acid as the final product of

carbohydrate fermentation. Other characteristics of LAB are Gram positive bacteria, catalase negative, usually non motil, do not sporulate, and do not produce gas from glucose (Karyawati *et al.*, 2018; Nofiani *et al.*, 2022).

All the colony bacteria exhibited the same shape, margin, and height, as demonstrated in Table 1, but they vary in colour. LAB colonies commonly appear circular in shape, white to yellowish in colour, with an entire edge, convex elevation, and a smooth surface (Siregar *et al.*, 2014; Manzoor *et al.*, 2016; Rahayu & Qurbaniah, 2019; Nasution *et al.*, 2020; Nasri *et al.*, 2021).

The cell shape characteristic of LAB found in this study were cocci, coccobacillus, and rods (Table 1). *Lactobacillus plantarum*, *Streptococcus thermophilus*, and *Pediococcus pentosaceus* dominate LAB identified in Indonesian fermented foods. Meanwhile, the majority of the *Streptococcus*, *Enterococcus*, and *Leuconostoc* species have been detected mostly from salty fermented fish and shrimp (Rahayu, 2003).

Matti *et al.* (2019) and Nurhikmayani *et al.* (2019) also reported that LAB strains were discovered from *chao* produced in Pangkep Regency. Many fermented fish products, such as bekasam (Desniar *et al.*, 2013), plaa-som (Kopermsub & Yunchalard, 2018), som-fak (Piayura *et al.*, 2017) have been shown to contain high levels of LAB in their microbial communities. LAB plays an essential role in fish fermentation and is responsible for the distinct features of fermented fish (Ngasotter *et al.*, 2020).

In conclusion, our investigation found that 16 LAB isolates were originated from *chao* with similar colony morphology, but different colour. There were 3 cocci, 11 rods, and 2 were coccobacillus isolates. All isolates were Gram positive and catalase negative which is the characteristic of LAB.

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