

## Lactic Acid Bacteria Isolated From *Cangkuak* Riau: Characterization of Antibacterial Activity

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**Abstract:** This paper reports on the *Cangkuak* from the fermentation of meat and bones using bamboo shoots, added rice and salt stored in a container (anaerobic conditions). The *cangkuak* was obtained from the Riau (Indonesia). The *cangkuak* may contain lactic acid bacteria (LAB), which are essential in the fermentation process. Most LAB is gram-positive bacteria with some health benefits, such as antibacterial. This study investigated the isolate and characterized LAB from *cangkuak* by an antibacterial activity test. The bacteria used *Bacillus Cereus*, *S. Aureus*, *E. Colli*, and *Salmonella sp.* Bacterial isolates were grown on MRS agar media, to which 0,5% CaCO<sub>3</sub> was added and then purified using the streak method. The results obtained were pure isolates. The results of the *cangkuak* isolation showed 8 LAB isolates. The eight isolates were characterized by standard Bergey's manual of systemic bacteriology (genus *Lactobacillus sp.*). Furthermore, an antibacterial test revealed differences in ineffectiveness against the inhibition zone. Cangkuak (CK) 3.1 isolate was most effective in inhibiting bacterial growth. The size of the inhibition zone for *Aureus*, *E. Colli*, and *Salmonella sp.* were 10.82 mm, 12.65 mm, and 12.29 mm, respectively. This result could indicate the presence of LAB isolates from fermented foods that have the potential to be antibacterial.

**Keywords:** Antibacterial Cangkuak; Fermentation; LAB; Riau.

### Introduction

The Kuantan Singingi Regency lies in Riau Province, which is known for its fermented foods. Fermentation takes place naturally, without the use of a microbial starter. *Cangkuak* is one of the fermented foods found in the Kuantan Singingi Regency. *Cangkuak* is a traditional meal cooked from cow or buffalo flesh and bones, bamboo shoots, rice, and slat, and then preserved anaerobically in a container. *Cangkuak* can be stored for over a month without deterioration [1]. Fermented foods have been demonstrated to provide many health benefits, including lowering the risk of certain diseases [2]. Irritable diseases, tumors, bowel disorders, and even colon cancer are examples of such conditions [3].

Lactic acid (LAB) is a gram-positive bacteria that does not make spores is catalase-negative, and can create lactic acid as a by-product of the fermentation process. In food fermentation, LAB plays a significant role in producing antimicrobial compounds. LAB can create antimicrobial compounds such as organic acids, bacteriocin, and hydrogen peroxide, all of which have the potential to be used as probiotics [4]. LAB possesses antibacterial properties because of the metabolites it creates. LAB metabolites can impair the permeability of cell membranes and the cell walls of microbes, inhibiting microbial cell development [5].

Several researchers have identified LAB as an antibacterial generated by local Indonesian microbes. Samual reported the existence of LAB as an antibacterial agent for Romain lettuce fermentation [6]. *Lactobacillus curvatus* and *Enterococcus Faecium* have also recovered from *bekasam* [7] and LAB from *lemea* [8]. However, no research on the *cangkuak* microorganisms has been published. Therefore, this study aimed to isolate and characterize lactic acid bacteria from *Cangkuak*, Riau, by testing their antibacterial activity.

### Research Methods

#### Material and Methods

The *cangkuak* used in this research came from the Kuantan Singingi Regency in Riau. *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella sp.*, and *E. Colli* were used as indicator bacteria in the antibacterial test.

#### Isolation Procedure

According to the study, the LAB isolation process was carried out [9]. *Cangkuak* as much as 5 grams was carried out in graded dilutions from 10<sup>-1</sup> to 10<sup>-5</sup>. Then, the last three dilutions were inoculated onto MRS Agar media using the Duplo technique and incubated at 37°C for 24-48 hours. Bacterial colonies grew on the medium, inoculated

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MRS Agar + CaCO<sub>3</sub> 0.5%, then incubated at 37°C for 24-48 hours.

**Antibacterial Activity Test**

Antibacterial activity test of LAB against indicator bacteria using the suitable method [9]. The indicator bacteria used were *E. Colli*, *Salmonella sp*, *Bacillus cereus*, and *S. Aureus*. BAL was grown in MRS broth and incubated for 24 hours at 37°C. LAB was 24 hours old and was centrifuged (3000 rpm for 30 minutes) to obtain supernatant. The indicator bacteria grown on NB media for 24 hours were poured together with NA media, and then a well was made with a diameter of 7 mm. Drop 50 microliter of each supernatant into the wells. Then, incubate for 24 hours at 37°C.

**Identification and Determinasi Lactic Acid Bacteria**

The isolates that were most effective in producing antibacterial activity were then subjected to biochemical tests to determine the physiologic properties of LAB. The test results were included in the observation list and adjusted according to *Bergey's Manual of Systemic Bacteriology*. The book contains all the known properties of bacteria.

**Results and Discussion**

**Isolation of LAB From Cangkuak**

LAB was isolated by planting cultures from a dilution of 10<sup>-5</sup> on MRS Agar media to obtain single colonies. A single colony that has been received and then grown back on MRS Agar added 0.5% CaCO<sub>3</sub> to see how lactic acid produced showed their inhibitory zone around the colony. The research results to get 17.8 × 10<sup>6</sup> colonies with eight LAB isolates are based on a clear zone. A catalase test and gram stain were performed to ensure that eight isolates were LAB. Based on the precise zone analysis, the eight isolates, the catalase test, and gram staining were gram-positive isolates. It can be seen in table 1.

**Table 1.** Data on the LAB from isolates *cangkuak*

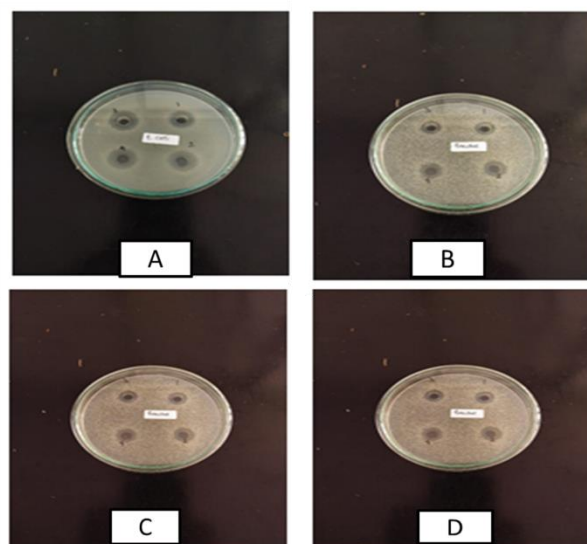
No	Isolate name	Clear zone	Catalase test	Gram stain tets
1	CK.1	Positive	Negative	Positive
2	CK.2	Positive	Negative	Positive
3	CK.3	Positive	negative	Positive
4	CK.4	Positive	negative	Positive
5	CK.5	Positive	negative	Positive
6	CK.6	Positive	negative	Positive
7	CK.7	Positive	negative	Positive
8	CK.8	Positive	negative	Positive

The presence of LAB isolates in *cangkuak* fermentation was due to the bamboo shoots used to make it. It is supported by Bahera and Balaji [10], which state that bamboo shoots are fermented food products that are a source of LAB. Additionally, Das et al. [11], Chen. et al. [12] 2022). Lactic acid bacteria play a significant role in the fermentation of bamboo. LAB found that can be seen from

the formation of clear zones, catalase-negative and gram-positive. According to Mokoena *et al.*[13], LAB is included in bacteria that are gram-positive and catalase-negative. LABs are rod- or cocci-shaped Gram-positive, catalase-negative microorganisms that do not produce spores [14].

**Antibacterial Activity Test**

Eight LAB isolates obtained through selection by Gram stain test and catalase test were then cultured for 24 hours for further antibacterial activity testing. Antibacterial tests were carried out to see whether the eight isolates could inhibit gram-negative bacteria (*E.Coli*, *Salmonella sp*) and gram-positive bacteria (*Bacillus cereus*, *S. Aureus*). Fig. 1 shows the antibacterial activity inhibition zones, which appear as transparent areas.



**Figure 1.** Antibacterial activity test against test bacteria (A: *S.Aereus*; B: *E.Coli*; C: *Salmonella sp*; D: *Bacillus cereus*).

The results of the antibacterial activity test showed that the apparent zone diameter differed for each isolate. The largest clear zone was found in isolate CK.1, which could inhibit pathogenic bacteria with the highest area of inhibition zone, namely *E.Coli* 12.65 mm and *Salmonella sp* 12.29 mm, which could inhibit gram-positive bacteria *S.Aureus* 10.82 mm. The inhibition zone research produced in this study was higher than the research reported by Sari and Suryanto [15], which stated that the inhibition zone of LAB isolates against test bacteria was *S. Aereus* 18.1, *E.Coli* 9.6. and *Salmonella sp* 7.5 mm. Meanwhile, isolates CK.3 and CK.5 showed no inhibition zones around the wells for pathogenic test bacteria. The ability of the isolate to inhibit the test bacteria can be seen in Table 2.

The wider the clear zone around the well, the higher the bacterial activity produced. The clear zone around the well indicates that the isolate has antibacterial compounds such as bacteriocin. *Bacteriocin* is a protein in bacterial cells. In line with Mobolaji and Wuraola's [16] opinion, LAB can produce various compounds that can inhibit the growth of pathogenic bacteria, such as lactic acid, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), diacetyl, and bacteriocin. Inhibition of the development of pathogenic bacteria can be done by lowering the pH produced by the

secretion of organic compounds, which can be seen in Table 2. Organic acid molecules can enter through the cell membrane of pathogenic bacteria, thereby changing the permeability of the cell membrane and causing instability of proteins in cells and the material transport system in

bacteria. The pathogen becomes disturbed [17]. In vitro, bacteriocins can eliminate or suppress pathogenic bacteria, including multidrug-resistant bacteria (MDR pathogens) [18,19, 20, 21].

**Table 2.** Data on antibacterial activity test

Isolate code	pH Medium	A clear zone of inhibition (mm)			
		<i>Bacillus cereus</i>	<i>S. Aureus</i>	<i>E.Coli</i>	<i>Salmonella sp</i>
CK3.1	3.38	-	10.82	12.65	12.29
CK3.2	2.70	-	10.76	-	10
CK3.3	2.27	-	8.6	-	-
CK3.4	3.02	-	7.52	9.38	-
CK3.5	3.42	9.05	-	-	-
CK3.6	3.91	-	-	-	6
CK3.7	3.05	-	9.4	8.20	-
CK3.8	2.07	7.9	9.1	-	7.45

Antimicrobial tests on gram-positive bacteria: Each isolate had different results, with eight capable of inhibiting gram-positive bacteria (*S. aureus* isolates, six isolates). In comparison, for gram-negative bacteria, there were only three isolates. The most comprehensive clear zone in the gram-positive bacteria test was found in isolate CK.1, which could inhibit bacteria *S. aureus* by 10.82 mm. In this study, the inhibition of gram-positive bacteria was more effective than that of gram-negative bacteria. This is due to the different cell structures between gram-positive and gram-negative bacteria. According to Breijyeh et al. [22], gram-negative bacteria have a higher peptidoglycan content than gram-positive bacteria, causing secondary metabolites found in the LAB to be challenging to penetrate gram-negative. Furthermore, the same results were conveyed by Rahayu et al. [23], who stated that there was an inhibition zone produced in gram-positive bacteria that was larger than the inhibition zone, which is gram-negative.

**Characterization LAB**

The observation results from the identification that has been carried out are then matched with *Bergey's manual of systemic bacteriology*, which is used as a guide for determining bacteria. The observation results of the CK1 isolate above are based on the characteristics of the genus *Lactobacillus sp*, a group of rod-shaped and gram-positive bacteria. The genus *Lactobacillus sp* has morphological characteristics of milky white or cream color colonies, large round or round colonies, gram-positive rods, and measuring 0.5-1.2 x 0.5-1.5 m [24]. The bacteria *Lactobacillus spp.* are gram-positive, do not form spores or rods, and do not react negatively to the catalase test [25]. The same was stated by Ramadhanti et al. [26] and Maslami et al. [27]. *Lactobacillus* bacteria are rod-shaped, homofermentative, gram-positive, and catalase-negative.

**Table 3.** The results of the identification of the LAB characteristics

Test	CK.1
Gram	+
Anaerobic	-

Gas	-
H <sub>2</sub> S	-
Catalase	-
Motility	-
Laktosa	-
Glukosa	-
Mannitol	-
Aeaculin	-
Melezitose	-
Melibrose	-
Raffinose	-
Sorbitol	-
Trehalase	-
Indol	-
TSIA	R/R
VP	-
OF	-
Nitrate	-
Strain	<i>Lactobacillus sp</i>

Description: R= Read, Y= Yellow

**Conclusion**

Eight LAB were found by isolates *cangkuk* from Kuantan Singingi Regency, Riau Province. The eight LAB isolates can produce antibacterial compounds that can inhibit the growth of pathogenic bacteria. Of the eight LAB isolates produced, only CK.1 isolate was most effective in inhibiting pathogenic bacteria. CK.1 is the LAB of the genus *Lactobacillus sp*.

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