

## Studies On Species Of Lactic Acid Bacteria Isolate Sr 12 From Bali Cattle Gastric With Conventional Method And Api Kit 50ch

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**Abstract.** Lactic acid bacteria (LAB) are Gram positive bacteria that characterized by non-motil, non-spore, negative catalase, coccus or bacillus cell shape, and live in microaerophilic condition with CO<sub>2</sub> aeration 5-7%. The purpose of study was to identify LAB isolate SR 12 isolated from gastrointestinal tract of Bali cattle that previously known have potency as a source of probiotic. The study initiated by cultivation of isolate on de Man, Rogosa, Sharpe (MRS) medium, confirmation of isolate with Gram staining and catalase test as a main procedure for Gram positive identification. The next identification in order to know the species of isolate was conducted both conventional test and kit API 50CH test. The result of study showed the LAB SR12 isolate as a genus of *Pediococcus* or *Enterococcus* according to its growth in temperature 10°C, pH9,6 and it can't grow in halophilic condition. On the other hands, the use of API kit 50CH test showed the LAB SR12 was detected as *Pediococcus pentosaceus*.

**Keyword:** Bali cattle, Identification, Lactic Acid Bacteria, SR 12.

### I. INTRODUCTION

Food processing through fermentation has been done by humans since prehistoric times. Pure culture of Lactic Acid Bacteria was found by J. Lister in 1873 and use in cheese making as fermenter bacteria in 1890. Lactic Acid Bacteria (LAB) is a bacterium that can fermentate carbohydrate to lactic acid. Lactic Acid Bacteria can be divided into two groups, such as homofermentative LAB produce 95% lactic acid as a result of its fermentation and heterofermentative LAB produce not only lactic acid but ethanol and carbon dioxide as byproduct[1]. It is known that cows gastrointestinal track are the habitat of several types of lactic acid bacteria which have good potential as probiotics[2-

5]. Based on the results of previous research conducted by Suardana *et al* in 2012 (unpublished data), it was found that from some strains of rumen origin of the Bali cattle, especially SR 12 is known to have the potential to be developed as a source of probiotics. This is based on the ability of isolate SR 12 to inhibit pathogenic bacteria with large inhibitory zones.

### II. RESEARCH METHOD

#### *Cultivation of LAB SR 12 isolate*

The cultivation of BAL isolate SR 12 was done by isolate reculture that characterized by turbidity on MRS broth media.

### Gram Staining

Gram stain on SR 12 isolate was done by coloring preparations that have been fixed with crystal violet 2%, lugol solution, acetone alcohol, and safranin. The staining results can be seen under a microscope with 1000x magnification.

### Catalase Test

The catalase test was conducted to determine the activity of catalase enzyme. Positive results are characterized by bubbles.

### Conventional Test

#### Growth Test on 10°C Temperature

A 10°C temperature test is conducted to determine the ability of bacteria to grow in low temperatures. Isolates that grow at this temperature are classified as *Pediococcus*, *Lactobacillus*, *Enterococcus* or *Tetragonococcus*. On the contrary, isolate that do not grow at this temperature are classified as *Streptococcus*.

#### Growth Test on NaCl 15%

A 15% NaCl test is conducted to determine the ability of bacteria to grow in hypersaline conditions. Isolates that grow at this condition are classified as *Tetragonococcus*. On the contrary, isolate

that do not grow at this condition are classified as *Pediococcus*.

#### Growth Test on pH 9,6

A pH 9.6 test was conducted to determine the ability of bacteria to grow in alkaline conditions. Isolates that grow at this pH are classified as *Enterococcus*. On the contrary, isolate that do not grow at this pH are classified as *Lactococcus*.

#### API Kit 50CH Test

The API test kit aims to identify species of lactic acid bacteria by utilizing the ability of LAB to ferment carbohydrates. The test was carried out with the Analytical Profiler Index kit.

## III. RESULT AND ANALYSIS

### Confirmation of Isolate BAL SR 12

#### Gram Staining

Gram staining results (Figure 1) show that LAB isolates SR 12 are Gram positive bacteria, and bacterial cells are coccus shaped. Based on its form, lactic acid bacteria SR 12 isolates belong to the genus *Lactococcus*, *Aerococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc* [6].



Figure 1. The results of Gram stain LAB isolate SR 12. The results of Gram staining bacteria showed that bacteria were Gram positive bacteria with a coccus shape

### Catalase Test

The principle of the catalase test is to detect active enzymes in bacteria that are useful in breaking down hydrogen

peroxide (H<sub>2</sub>O<sub>2</sub>) into 2 molecules of water (H<sub>2</sub>O) and 1 molecule of oxygen (O<sub>2</sub>). Catalase test results on LAB SR 12 isolates are presented in Figure 2.



Figure 2. Result of catalase test on LAB SR 12 isolates. SR 12 isolates showed negative results.

### Isolate Identification of LAB SR 12

#### Conventional test

Conventional tests are carried out with the aim of detecting BAL through its

physiological characteristics. Conventional test results on LAB SR 12 isolates are presented in table 1.

TABLE 1. CONVENTIONAL TEST RESULT OF LAB ISOLATE SR 12

No	Type of Test	Result
1.	Gram Staining	Positive
2.	Shape	Coccus
3.	Catalase Test	Negative
4.	10°C Temperature Test	Positive
5.	NaCl 15% Test	Negative
6.	pH 9,6 Test	Positive
Conclusion		<i>Pediococcus/Enterococcus</i>

Based on the results of conventional tests, the LAB SR 12 isolates can be identified as the genus *Pediococcus* or *Enterococcus* based on the LAB classification through morphological and physiological characteristics [7].

#### API Kit 50CH Test

The API kit test was carried out to detect LAB isolates SR 12 by utilizing biochemical characteristics of isolate by testing 50 type of sugar. The API 50CH kit test results are presented in Figure 3.



Figure 3. Results of API 50CH kit test on LAB SR 12isolates. (A). Positive results. (B). Negative results. (C). Positive control.

(A). Positive results (microtube no. 11) are marked by changes in color from blue to yellow; (B). Negative results (micotube no. 20) are marked with no discoloration; (C). On microtube no. 25 positive results were marked by reddish-black discoloration, this was due to hydrolysis of esculin ferric citrate to esculitin and glucose. Color changes caused by exculitin. The test results in Figure 3 are briefly as shown in Figure 4.

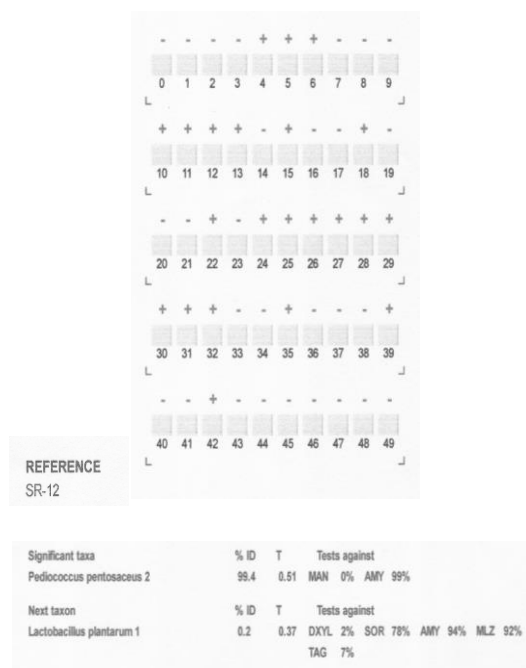


Figure 4. Test results of API Kit 50CH on apiweb

Based on the results of the API 50CH kit test, LAB isolate SR 12 was identified as *Pediococcus pentosaceus* 2. The results of this study were the same as conventional tests. *Pediococcus pentosaceus* bacteria have various benefits, especially as probiotics. Borges *et al* in his study said *Pediococcus pentosaceus* in the form of frozen dry powder has the ability to inhibit pathogenic bacteria such as *Listeria monocytogenes* [8]. These bacteria can also be used as anti-obesity drugs by reducing the Body mass Index (BMI), waist circumference, and fat in the body [9]. *Pediococcus pentosaceus* bacteria is very useful in the process of fermentation of mackerel fish sausages by suppressing the growth of *Enterobacteriaceae* and *Staphylococcus* bacteria [10]. Sadeghi *et al* in his study found that *Pediococcus pentosaceus* has antifungal abilities [11]. *Pediococcus pentosaceus* bacteria also have benefits as a growth promoter in tilapia (*Oreochromis niloticus*) [12]. *Pediococcus pentosaceus* can also increase growth, quality of meat, and reduce the content of ammonia in the digestive tract of wild chickens [13].

#### IV. CONCLUSION

The results showed that LAB SR 12 isolates in conventional tests as *Pediococcus* / *Enterococcus* whereas in the API 50CH test kit showed *Pediococcus pentosaceus* 2.

#### SUGGESTION

Identification of LAB isolates SR 12 by utilizing physiological and biochemical characteristics still has some disadvantages, namely identification is limited to conventional tests and API 50CH kits. Therefore, it is necessary to

carry out advanced conventional tests with more parameters.

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