The Characteristics and Potential of Lactic Acid Bacteria as Probiotics in Silage Made from *Hymenachne acutigluma* and *Neptunia oleracea lour*

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Abstract. This study aims to determine the characteristics of lactic acid bacteria (LAB) obtained from the silage made from *Hymenachne acutigluma* and *Neptunia oleracea Lour* to be used as probiotics. This study used a completely randomized design (CRD), which consists of 3 treatments and 5 replications. The treatment consisted of P1 (silage made from *Hymenachne acutigluma*), P2 (silage made from 50% of *Hymenachne acutigluma* and 50% of *Neptunia oleracea lour*), and P3 (silage made from *Neptunia oleracea lour*). The variables measured were LAB characteristics including shape, gram staining, catalase, endospore, motility, growth at different temperatures, TSIA, MR, VP, and identification using API Kit. The data were analyzed descriptively. The results showed that there were 9 selected isolates. All isolates are rod-shaped, gram-positive, catalase, endospore, and negative motility, growth at 15 °C negative and at 45 °C positive. TSIA slant and butt test show yellow and negative colors on gas and H_2S production, while MR-VP is red and yellow. The conclusions of this study were all isolates belonging to the *Lactobacillus plantarum* strain with a similarity level of 87.3-99.9 %.

1 Introduction

The soil's pH of swamps is generally less than 5.9 [1] and water pH of swamps below 4 [2]. The potential for swamp forage in South Sumatra is large enough to be used as animal feed. This is because the area of swamp in South Sumatra is about 9,159,200 ha, with coverage of forest and peatland of 1,055,447 ha. The types of swamp forage that have potential as animal feed include *Hymenachne acutigluma* and *Neptunia oleracea Lour*. The way to utilize swamp forages is by making silage. Silage is freshly preserved forage into a place called silo and fermented under anaerobic conditions by the activity of lactic acid bacteria. Carbohydrate content in forage is needed to produce silage with good quality because carbohydrate is used as a substrate source for bacterial growth. Silase has a secondary metabolite product, one of which is lactic acid bacteria (LAB). Lactic acid bacteria is a group of gram-positive bacteria that have the ability to convert carbohydrates into lactic acid and can produce antimicrobial components such as bacteriocin[1].

Therefore, this study aims to determine the characteristics of lactic acid bacteria obtained from the silage made from *Hymenachne acutigluma* and *Neptunia oleracea Lour.*

2 Materials and Methods

2.1 Source of Lactic Acid Bacteria (LAB)

The swamp forages, *Hymenachne acutigluma* and *Neptunia oleracea lour,* were cut with the size 2-5 cm and stored for 24 h for the process of withering. It was then weighed as many as 500 g for each treatment and mixed with molasses, which had been dissolved in water as many as 3% by weight of forage, then stirred until blended. After that, each treatment forage was put into a plastic bag of 3 layers and then compacted to airtight. It was then tied tightly and stored for 21 days in a dry place and not exposed to direct sunlight. After 21 days, the silage was opened and taken as a sample to be tested in the laboratory.

2.2 Isolation and Identification of LAB

Isolation of LAB was done gradually to get various types of bacteria. Method of isolation of bacteria based on Hayakawa method (1992). Bacterial isolation was carried out by suspending 10 g of swamp forages silage into 90 ml of 0.85% NaCl solution (dilution 10^{-1}). It was then made a series dilution to 10^{-6} into a physiological saline solution. Three series of the last dilution was plated as 1 ml into sterile petri dish then added 15-20 ml MRSA media to see the growth of lactic acid bacteria. Subsequently, it was carried out horizontally and after freezing it was incubated at 37 °C for 48-72 hours. The colonies were observed with flat and colored appearance. Colonies of different colors and sizes were traced back to the same medium as the quadrant scratches. Incubation was done under the same conditions as before. The scratching process continues until a uniform colony was obtained. Pure colonies were characterized by cell morphology, physiology and biochemistry based on standard taxonomic descriptions. Identification of lactic acid bacteria isolate was done using API Kit 50 CHL (Biomerieux). Bacterial cultures used were 48-hour-old cultures in petri dishes containing MRSA.

2.3 Experimental Design and Data Analysis

The treatment consisted of P1 (silage made from *Hymenachne acutigluma*), P2 (silage made from 50% of *Hymenachne acutigluma* and 50% of *Neptunia oleracea lour*), and P3 (silage made from *Neptunia oleracea lour)*. The variables measured were LAB characteristics including shape, gram staining, catalase, endospore, motility, growth at different temperatures, TSIA, MR, VP, and identification using API Kit. The data were analyzed descriptively. Data in analysis a sort of descriptive

3 Results and Discussions

The results showed that on the swamp forage silage, there were 9 selected isolates showing clear zone on 1% CaCO3 + MRSA medium. According to[2], lactic acid bacteria having the nature of its ability to give zone clear in medium skim that.The establishment of the zone clear shows that the bacteria is produce a metabolite secondary (lactic acid) excess so excess lactic acid be made clear zone around the bacteria colonies[3]. Next[4] reported that the ability of forming clear zone vary depending on bacteria type , bakteriosin

concentration and of the womb nutrients in media .The medium through which recommended to grow lactic acid bacteria is medium MRSA.While the addition of 1 percent CaCO3 aimed at to select lactic acid bacteria which grows on medium and when incubation period is complete 1 x 24 hours will look clear zone around bacteria colonies that grows . It is influenced during incubation period is complete the growth of bacteria lactic acid produces lactic acid that react with CaCO3 that is not soluble in in the medium so as to form calcium lactate that is soluble , with shows that there has been clear regions or zones around bacteria colonies growing[5]

Furthermore, the results showed that all isolates had gram-positive characters with purple and rod-shaped cells, while catalase, endospore, and motility were negative.[6] reported the characteristics of lactic acid bacteria is of a gram positive and the form of of his cell of which the stem. According[7], lactic acid bacteria have characteristics such as gram positive, non spore, catalase negative, and non motile. Next [8], lactic acid bacteria does not form spores

The result of experiment to the sample isolates silage forage swamp it negative on all conditions of temperature 150C subjects are characterized by not existence of white colonies in a media MRS broth and showing positive results on all conditions of temperature 45^0C subjects are characterized by existence of white colonies in a media MRS broth .According to[9] characteristic of fenotif lactic acid bacteria growing optimal at a temperature of 45°C but was not growing optimal at a temperature of 15oC .This is in accordance with statement of the court was [10] stated that one of a breed of lactic acid bacteria can grow with the less the temperature of 37°C . This indicates that kind of isolates is both in mesofilik .Because according to *Bergey's Manual of Systematic Bacteriology*, the characteristics of lactic acid bacteria mesofilik is ca not grow in 15°C temperature , but can grow on 45°C.

Isolates E, H and I with TSIA media were still red in the slant. While the isolates A, B, C, D, F and G changed in color to yellow. None of the isolates produce H_2S, which is indicated by the absence of black deposits on the base of the media. All isolates showed positive results on MR tests marked by the formation of red color on the media after added methyl red as indicator solution. Furthermore, the negative results are shown by all isolates with the VP test.

Table 1. Identification of isolates on swamp forage silage

isolate code	shape	color	catalase	motility	endospore	growth on temperature		TSIA test				MR	VP
						15	45	Slant	butt	gas	H_2S		
A	stem	+	-	-	-	+	-	yellow	yellow	-	-	red	yellow
B	stem	+	-	-	-	+	-	Yellow	yellow	-	-	red	yellow
C	stem	+	-	-	-	+	-	Yellow	yellow	-	-	red	yellow
D	stem	+	-	-	-	+	-	Yellow	yellow	-	-	red	yellow
E	stem	+	-	-	-	+	-	Red	yellow	-	-	red	yellow
F	stem	+	-	-	-	+	-	Yellow	yellow	-	-	red	yellow
G	stem	+	-	-	-	+	-	Yellow	yellow	-	-	red	yellow

H	stem	+	-	-	-	+	-	Red	yellow	-	-	red	yellow
I	stem	+	-	-	-	+	-	Red	yellow	-	-	red	yellow

Description: Isolates A, B, C, D are isolates from silage of *Hymenachne acutigluma*. Isolates E, F, G are isolates from combination of 50% of *Hymenachne acutigluma* and 50% of *Neptunia oleracea lour*. Isolate H, I are isolates from silage of *Neptunia oleracea lour*. Slant: slope on media, Butt: upright, MR: methyl red, VP: voges proskeur, (-): negative, (+): positive

Moreover, the results of identification using the API CH 50 device and continued with the analysis showed that all isolates belonged to the *Lactobacillus* group, similar to *Lactobacillus plantarum*, with a good category indication of 87.3-99.9%. The results of this study are supported by previous research that *Lactobacillus plantarum* is commonly found in king grass[11] .

4 Conclusions

The conclusions of this study were all isolates incorporated into the *Lactobacillus plantarum* strain with a similarity level of 87.3-99.9%.

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