IDENTIFICATION OF LACTIC ACID BACTERIA ISOLATED FROM MILKFISH INTESTINE (*Chanos chanos*)

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ABSTRACT

The intestinal microflora of milkfish (*Chanos canos*) from Pohuwato district was studied to isolate and identify lacted acid bacteria. The lactic acid bacteria were isolated used selective media MRS agar for lactic acid bacteria. The determination original the genus was performed according to their morfological and biochemical characteristics by the procedures described in the Bergey' manual. From the tested samples ten lacted acid bacterial cultures were isolated. After origional characterization, seven of them were determined as representatives of lactic acid cocci as refferred to genus *Leoconostoc* and others were representatives of lactic acid rood reffered to genus *Lactobacillus.*

Keywords : Lactic Acid Bacteria, Milkfish, Intestine

1. Introduction

The milkfish that breed in brackish waters have slim bodied characteristics, fin forked , fleshy scales like glass and white. In addition it has a uniqueness , that his mouth is toothless and food plants seabed. In addition, intestinal length milkfish 9 times body length (Murtidjo, 2002). In the long intestine that there are many different types of bacteria including lactic acid bacteria (LAB), which helps the process of digestion of food. In addition to these functions BAL antagonistic against pathogenic bacteria. Lactic acid bacteria can be isolated and tested antagonistic activity against pathogenic bacteria and developed as a new atibotik .

Lactic acid bacteria (LAB) are known microorganisms that have probiotic properties. They can produce inhibitory compound such as lactic acid, hydrogen peroxide, diacetyl, acetal dehyde and bacteriocin. These compounds are able to inhibit the growth of harmful microorganisms (Ringo and Gatesoupe, 1998; Gatesoupe, 1999). Lactic acid bacteria are widely distributed in the nature. In this group are included representatives of the genus Lactobacillus, Lactococcus, Pediococcus and Leuconostoc. According to many reports lactic acid bacteria are normal flora in gastrointestinal (GI) tract of healthy animals like mammals and aquaculture animals (Nikoslulainen et al, 2001) with no harmful effects (Ringo et al, 2005).

The present experiment aimed to isolation and identify lactic acid bacteria as new antibiotic from intestine of milkfish (*Chanos chanos*).

2. Material and Methods

2.1 Material

This research used intestine milkfish from Pohuwato District. The medium used for the first phase include fever deMan Rogosa Sharp (MRS) Agar, Nutrient Agar (NA), physiological saline, distilled water, plastic, cotton, paper labels, Nutrient Broth (NB), solution of crystal violet, safranin, lugol, IMViC media, 95 percent alcohol, spritus, glucose and lactose.

2.2 Methodology

The determination of genus lactic acid bacteria was performed according to their morphologycal, cultural, physiological and biochemical characteristics by the procedures described in Bergey's Manual.

2.2.1 Collection the Samples

A total of 35 milkfish were collected from three diffrent farms from Pohuwato District. The surface of fish bodies were disinfected by alcohol (70%), dissected under anticeptic condition; intestines taken out and washed three times with normal saline (NaCl) 0.85%). The intestines were then homogenized in 10 mL on sterile solution (NaCl 0.85%) with a mechanic homogenizer.

2.2.2 Purification and isolation of Lactic Acid Bacterial Culture

The measurement has been done according to method illustrated by (Cappuccino and Sherman (2005).

One bacterial colony was taken with the characteristics that are most dominant in the scratches of the media that has been inoculated with used a loopful sterile , inoculated again on MRSA medium aseptically by means of scraping, petri dish placed upside down and labeled statements, for ± 24 hours incubated at a temperature of 30 - 35ºC. If after colony purification has not obtained a homogeneous (same) be returned to the purification steps for the same .

2.2.3 Phyisiology and Morphology of Bacteria Test

The measurement has been done according to method illustrated by (Cappuccino and Sherman (2005)

The purpose of physiological and morphology test was to determine the morphological characteristics of bacteria and forms of bacteria through the Gram staining and microscopy observations. Object glass that have been prepared cleaned with 70 % alcohol and labeled,, one drop of distilled water was dripped in to the surface of glass objects, isolates taken with a sterile needle ose, mix distilled water and pillowcase evenly on the glass surface of the object, fixation by passing the above preparations fire (approximately 15 cm) several times until it dry. Crystal violet solution dripped on preparations until evenly , and let stand for one minute . Preparations were washed with running water, lugol iodine solution was dripped on the preparations thoroughly, and let stand for one minute, preparations were then washed with water and dried -aired . Alcohol- acetone solution was dripped on preparations until evenly and let stand for thirty seconds, preparations were then washed with water and dried -aired, safranin solution dropped into the preparations until evenly and let stand for two minutes. Preparations were then washed with water and dried -aired, preparations were subjected to microscopy. Gram-negative bacteria are marked with red color while the bacteria Gram- positive marked with purple color , shape round the Coccus bacteria and Basil the stem .

2.2.4 Biochemia test (Catalase, glucose and lactose Test)

The measurement has been done according to method illustrated by (Cappuccino and Sherman (2005)

Using reagent hydrogen peroxide (H_2O_2 3 %), hydrogen peroxide is toxic to cells because to inaktif enzymes in the cell . Catalase an enzyme that is used microorganisms to decompose hydrogen peroxide into H_2O and O_2 . The way it works is provided a glass object has been cleaned with 70 % alcohol and then take a pure bacterial isolates ose using sterilized needles and placed into the object glass and etched with a solution of 3 % H_2O_2 , observed changes, if there are the bubble is positive bacteria containing the enzyme catalase, but if there are no bubbles then say negative bacteri , does not produce enzyme catalase.

Sugar test aimed to determiner the bacteria 's ability to degrade the sugar and produce organic acids derived from each types of sugar . The process of fermentation of sugars large

amounts of acid (acid) and somebacteria will produce gas that can be observed denagn Durham tubes were placed on media sugars . Media confectionery is a liquid medium (liquid)red . The way it works is pure bacterial isolates taken with ose sterile then inoculated on each medium is glucose and lactose in a manner dipped loopful half . Once completed then stored in an incubator for 24 hours . After 24 hours, observe the color change , if bacteria on each of these media color changes to yellow the bacteria are positive and if no change is said to be negative. This test is performed for identifying bacteria capable of fermenting carbohydrates . In confectionery test only the color changes in glucose medium color changed to yellow , meaning these bacteria to form acid from glucose fermentation . On glucose medium also formed bubbles in Durham tubes were placed upside down in the tube media , meaning fermented gaseous .

3. Result and Discussion

From the tested samples ten lacted acid bacterial cultures were isolated. After origional characterization, cell morphological seven isolates (RS1,RS2,RS3,RS4,RS5,RS8 and RS10) are cocci dan three isolates (RS6,RS7 and RS9) are rod. Gram staining all isolates are positive. The color all culture yellow white and size 1 mm to 5 mm, nonspore forming all isolates. Biochemia test all isolates non catalase production. Glucose fermentation nine isolates positive without RS 8. Lactose fermentation all isolates negative, and can growth on 30-35^{o}C. The data morphological, cultural and physiological characteristics of isolates shown in Table 1.

After origional characterization, based on Bergey's manual seven of them (RS1,RS2,RS3,RS4,RS5,RS8 and RS10) were determined as representatives of lactic acid cocci as refferred to genus *Leoconostoc* and others (RS6, RS7 and RS9) were representatives of lactic acid rood reffered to genus *Lactobacillus.* The Characteristics Acid Lactic Bacteria Isolate to Genera shown in Table 2.

Table 1. Morphologycal, cultural and physiological characteristics of isolates

Characteristic	ISOLATE									
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RS9	RS10
Cell morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Rod	Rod	Cocci	Rod	Cocci
Gram stain reaction	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+
Spores formation	-	-	-	-	-	-	-	-	-	-
Colony morphology	Yellow-white, 1 mm,slightly	Yellow-white, 4 mm	Yellow-white, 1mm,snowflake	Yellow-white, 5 mm	Yellow - white, 1 mm	Yellow-white, 5 mm	Yellow-white, 5 mm	Yellow-white, 5 mm	Yellow-white, 5 mm	Yellow-white, 3 mm
Catalase activity	-	-	-	-	-	-	-	-	-	-
Glucose fermentation	+	+	+	+	+	+	+	-	+	+
Lactose fermentation	-	-	-	-	-	-	-	-	-	-
Growth at temperature 30;35^{o}C	+	+	+	+	+	+	+	+	+	+

Table 2. Characteristics Acid Lactic Bacteria Isolate to Genera

Characteristics	ISOLATE LAB	
	RS 1, RS 2, RS 3, RS 4, RS 5, RS 8, RS 10	RS 6, RS 7, RS 9
Cell morphology	Cocci	Rod
Gram Staining	+	+
Gas production	+/-	+
Catalase	-	-
Fermentation tipe	Hetero/Homo	Hetero
GENERA	*Leoconostoc*	*Lactobacillus*

4. Conclusions

The present study conclused that genus *Leoconostoc* and genus *Lactobacillus* was normal in microflora acid lactic bacteria in intestine milkfish. *Leoconosctoc* as dominant genera.

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