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"Innovation on Marine and Fisheries Product Processing and Biotechnology Towards the Asean Economic Community in 2015"

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FOREWORD

It is with deep satisfaction that we present the Proceeding of the International Seminar on Marine and Fisheries Product Processing and Biotechnology 2014 held on 26 September 2014 at the Pullman Hotel, Jakarta, Indonesia. The seminar was organized by Research and Development Center for Marine and Fisheries Product Processing and Biotechnology.

The Seminar entitled "Innovation on Marine and Fisheries Product Processing and Biotechnology Towards the Asean Economic Community in 2015" aims to communicate the state of the art of marine and fisheries product processing and biotechnology innovation and to support industrialization based on blue economy concept. In addition, it also aims to strengthen partnerships with research and development institutions, universities, industries, government and other stakeholders in achieving the national goals for marine and fisheries.

The Seminar was attended by more than 150 participants from 6 different countries and 51 papers have been presented. In addition to the contributed papers, three invited keynote presentations were given by Prof. Dr. Jose Alberto Ramirez de Leon of the Universidad Autonoma de Tamaulipas Mexico, who spoke about production of microbial transglutaminase enzyme from agroindustrial by-products and its use in the production of restructured meat and fish products, by Prof. Martin Cole of the CSIRO Division of Animal, Food and Health Science Australia, who spoke about issues in food safety and by Mr. Ansen Ward of the Food and Agriculture Organization who spoke about global trends and issues related to products and food processing.

These Proceedings will furnish the scientists with an excellent reference book. This will also be an impetus to stimulate further study and research in all these areas.

We thank all authors and participants for their contributions.

Editor

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PROCEEDING OF INTERNATIONAL SEMINAR ON MARINE AND FISHERIES PRODUCT PROCESSING AND BIOTECHNOLOGY 2014

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

Rieny Sulistijowati S*)

ABSTRACT

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

Keywords : Lactic acid bacteria, milkfish, intestine

INTRODUCTION

The milkfish that breed in brackish waters has slim bodied characteristics, fin forked, fleshy scales like glass and white. It has a uniqueness, that his mouth is toothless and seaweed base food consumtion. In addition, the intestinal length of the milkfish 9 times longer than the body length (Murtidjo, 2002). In the long intestine, there are many different types of bacteria including lactic acid bacteria (LAB), which helps the food digestion process. LAB also function as antagonistic bacteria against pathogenic bacteria. Lactic acid bacteria can be isolated and tested its antagonistic activity against pathogenic bacteria and can be developed as a new atibiotic .

Lactic acid bacteria (LAB) are known as 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 & Gatesoupe, 1998; Gatesoupe, 1999). Lactic acid bacteria are widely distributed in the nature. Representatives of the genus Lactobacillus, Lactococcus, Pediococcus and Leuconostoc are belongs this group. 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 isolate and to identify lactic acid bacteria from intestine of milkfish (*Chanos chanos*).

MATERIAL AND METHODS

Material

This research used milk fish intestine from Pohuwato District. The materials used for the first phase were 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.

Methods

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

Samples Collections

A total of 35 milkfish were collected from three diffrent farms from Pohuwato District. The surface of fish bodies were disinfected by alcohol (70%), the fish were under antiseptic condition; the fish's intestines were

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taken out and washed three times with normal saline (NaCl) 0.85%). The intestines were then homogenized in 10 mL of sterile solution (NaCl 0.85%) with a mechanic homogenizer.

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 with must dominant characteristics amongs bacterial colonies on the media was aseptically inoculated on MRSA medium and incubated for ± 24 hours at temperature of 30 - 35°C. The colony purification steps were repeated until pure isolates (homogeneous isolate) were obtained.

Phyisiology and Morphology test of Bacteria

The measurement has been done according to the method illustrated by Cappuccino and Sherman (2005). The purpose of physiological and morphological test was to determine the morphological characteristics of bacteria and to determine the forms of bacteria through Gram staining and microscopy observations. Glass object was cleaned with 70 % alcohol and labeled, one drop of distilled water was dripped into the surface of glass objects, the isolates was then taken with a sterile ose needle, mixed with distilled water and spreaded evenly on the glass surface of the object and fixated by passing the glass above the fire (approximately 15 cm) several times until it dry. Crystal violet solution was evenly dripped on the microscopic slide, and let it stand for one minute. The microscopic slides were washed with running water, and lugol iodine solution was dripped on the slide thoroughly, and let it stand for one minute and the microscopic slides with water and air dried. Next, alcohol- acetone solution was dripped evening on this microscopic slide until evenly and let it stand for thirty seconds, the slides were then washed with water and air dried, safranin solution was then evenly dropped into the slides and let it stand for two minutes. The microscopic slides were then washed with water and air dried. Finally the microscopic slides preparations were subjected to microscop for examination Gram-negative bacteria are marked with red color while the Gram- positive bacteria marked with purple color.

Biochemia test (Catalase, glucose and lactose test)

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

Microscopic slide was cleaned with 70% alcohol, one use of pure bacterial isolate was placed into the slide and then etched with 3% H_2O_2 solution. After that any changes was observed. If any bubble was observed indicating bacteria containing catalase enzyme (positive), but if no bubbles were do served indicating no catalase enzyme produced by the bacteria (negative).

Hydrogen peroxide (H_2O_2 3 %) reagent was used to inactivated the enzyme in the cells. Catalase is an enzyme used by microorganism to decompose hydrogen peroxide into H_2O and O_2.

Sugar test aimed to determined 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 and some bacteria will produce gas that can be observed with sugars medium. Confectionery medium is a liquid medium. Pure bacterial isolates was taken with sterile ose then inoculated on glucose and lactose liquid medium, and incubated for 24 hours at 30-35°C . After 24 hours incubation color change of the medium was observed. If the medium color changed into yellow indicating positive carbohidrates fermenting bacteria, if there was no color change of the medium indicating negative. When the glucose medium changed to yellow, it means that the bacteria able to produce acid from glucose fermentation. If the glucose medium formed bubbles in Durham tubes, that were placed upside down in the medium tube. Indicating that the bacteria was produce fermentation gas.

RESULT AND DISCUSSION

Ten lacted acid bacterial cultures were isolated. After characterization of the cell morphologycal, seven isolates (RS1,RS2,RS3,RS4,RS5,RS8 and RS10) are classified as cocci and three isolates (RS6,RS7 and RS9) are rod. Gram staining of all isolates shows that all isolates are Gram positive bacteria. The color of all culture were yellow white and the size changed from 1 mm to 5 mm, all isolates were nonspore forming. Biochemistry test of all isolates shawed non catalase production. Glucose fermentation test shawed that 9 isolates were positive except RS 8. Lactose fermentation test of all isolates were negative, and the isolates

can growth at 30-35°C. The morphological, cultural and physiological characteristics data of the isolates was showed in Table 1.

After characterization, based on Bergey's manual, seven of the isolates (RS1,RS2,RS3,RS4,RS5,RS8 and RS10) were determined as representatives of lactic acid cocci 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. Morphological, 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°C	+	+	+	+	+	+	+	+	+	+

Table 2. Characteristics of Acid Lactic Bacteria Isolate to Genera

Characteristics	ISOLATE LAB	
	RS1, RS2, RS3, RS4, RS5, RS8, RS10	RS6, RS7, RS9
Cell morphology	Cocci	Rod
Gram Staining	+	+
Gas production	+/-	+
Catalase	-	-
Fermentation tipe	Hetero/Homo	Hetero
GENERA	*Leoconostoc*	*Lactobacillus*

CONCLUSIONS

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

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