Isolation, Characterisation and Identification of Sea Urchin-Associated *Bacillus* in Mentigi Beach, West Lombok

Novi Febrianti[A], Bambang Fajar Suryadi[B]
[A)]Biology Education Department, Faculty of Teacher Training and Education,
Ahmad Dahlan University, Yogyakarta
[B)]Biology Department, Faculty of Mathematics and Science, Mataram University, Mataram

The genus *Bacillus* is gram-negative bacterium that can be found in the variety of habitat and can be isolated from many sources. This genus have many potential, mainly as a new source of antibiotic substance. The objectives of the study are to isolate, characterize and identify sea urchin-associated *Bacillus* in Mentigi Beach, West Lombok. Sea urchin-associated *Bacillus* were isolated from the external and internal part of the body of sea urchin that had been heated at 80^{o} C. This research was able to obtain four *Baclllus* isolates, namely 1A, 2J, and 3L. On the basis of phenotypic characterizationed by using profile matching method isolates 1A was assigned to *Bacillus spahericus*, isolate 2J to *Bacillus carotarum*, and isolate *3L* to *Bacillus cereus.*

Key words: Sea urchin, *Bacillus*, Mentigi beach

INTRODUCTION

The genus *Bacillus* is gram-negative bacterium that widely distributed in the nature. This group can be found in the variety of habitat and can be isolated from many sources. This genus have many potential, mainly as a new source of antibiotic substance.

Bacillus species produce many kinds of antibiotics which share a full-range of antimicrobial activity such as bacitracin, pumulin and gramicidin (Todar, 2009). *Bacillus* species polypeptide antibiotics which constitute the *Bacillus* bacteria have been gaining importance as a result of studies.The *Bacillus* species that produce antibiotics are *B. subtilis, B.polymyxa, B. brevis, B. licheniformis, B. circulas,* and *B. cereus*. The *bacillus* species have a wide range of antimicrobial activities since they are used as anti-fungal agents, anti-viral agents, anti-ameobocytic agents, and anti-mycoplasma agents (Yilmaz *et al*., 2006).

Marine microbial live in a biologically competitive environment with unique condition of pH, temperature, oxygen, light, nutrient and salinity. There in no wonder that marine microbial metaboliets exhibit special biological activities compared with terrestrial bacteria (Zhang *et al.*, 2005). Researched by Kanagasabhapathy *et al.* (2005) found *Bacillus* that live on the surface of marine invertebrates produce chemicals that are having potential antibacterial and antifouling activities. In this study we isolate sea urchin-associated *Bacillus*, characterize and identify them. This *Bacillus* isolates will be used in our next research to study their antibiotic potential.

MATERIALS AND METHODS

Sea Urchin Collection

Sea urchin samples were collected from depth of 2-5 metre along the coastal waters of Mentigi beach, West Lombok . The collected specimens were washed with sterile water to remove adhering debris and associated biota. Then the sea urchin samples were dissected after cut their prickles. Intestinum and prickles of the sea urchin were crushed into smaller pieces, after that 5 ml of NaCl was added to it.

Isolation of *Bacillus*

The extracts were heated in water bath at 80^0C for 30 minutes to induce endospore forming. Serial dilution ($10^{-1} – 10^{-7}$) was done using sterile physiologic water. 100 µL of the bacterial suspensions (10^{-3}, 10^{-5}, 10^{-7}) were poured on Nutrien Agar (NA) medium plates. Plates were incubated at 30^0C for 24-48 hours.

Characterisation and Identication of *Bacillus*

The isolated bacteria were characterized according to Gram characteristics, cell and spore morphology, and motility. In addition, the following characterized tests were carried out: urea hydrolis, catalase, H_2S, sugar fermentation, citrate, oxidase,Voges-Proskauer, mehyl red, and indol tests.

Identification by using *Profile matching* method based on *Bargey's Manual of Determinative Bacteriology* (Holt et al., 1984) and Pibwin Ver. 1.9.2 Build 29 software with *Bacillus* matrix (downloaded from University of Sothampton, UK website).

RESULT AND DISCUSSION

This research was found three species of sea urchin from Mentigi beach, West Lombok, namely: *Echinotrix calamaris*, *Echinometra mathei*, and *Diadema setosum*. These species is the general type of sea urchin and abundance in this beach.

From external and internal parts of three sea urchin specien that was collected, we successfully isolated three species of *Bacillus.* These three isolates grew slowly (3-4 x 24 hrs) at 25-30^0C incubation temperature but showed faster growth rate at 33-37^0C (only 1-2x 24 hrs)

The unique characteristic of that three isolates that they are can not growth in NA medium supplemented by sea water. It just resistence to maximal 10% NaCl and grew very well in standard NA media (without supplemented by sea water). This case maybe because the isolated bacteria live in the tissue of sea urchin (not in sediment) that have rather low salinity.

The characteristics of colony and cell morphological is showed in Table 1 and Figure 1. All isolates have circle form colony and entire edge but they have vary elevation and colour.

Table 1. Colony and cell morphology of bacterial isolates

Characters	**Isolate**		
	1A	2J	3L
A. Colony morphology			
Form	Circle	Circle	Circle
Edge	Entire	Entire	Entire
Elevation	Flat	Convex	Convex
Colour	White	Yellow	Yellow

B. Cell morphology			
Form	Rod	Rod	Rod
Arrangement	Chain	Single	Single
Gram stain	Positive	Positive	Positive
Spore form	Oval	Oval	Oval

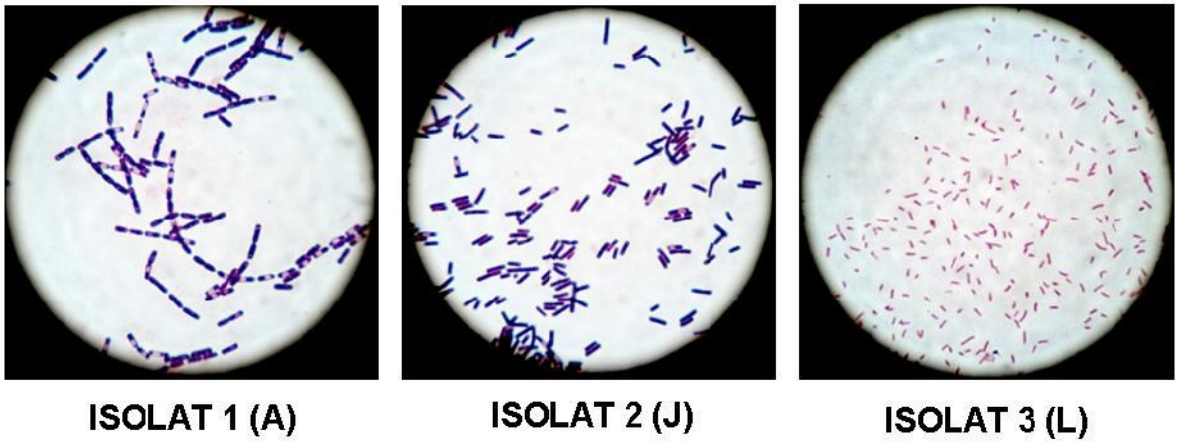

Figure 2. Gram stain of bacterial isolates (1000x)

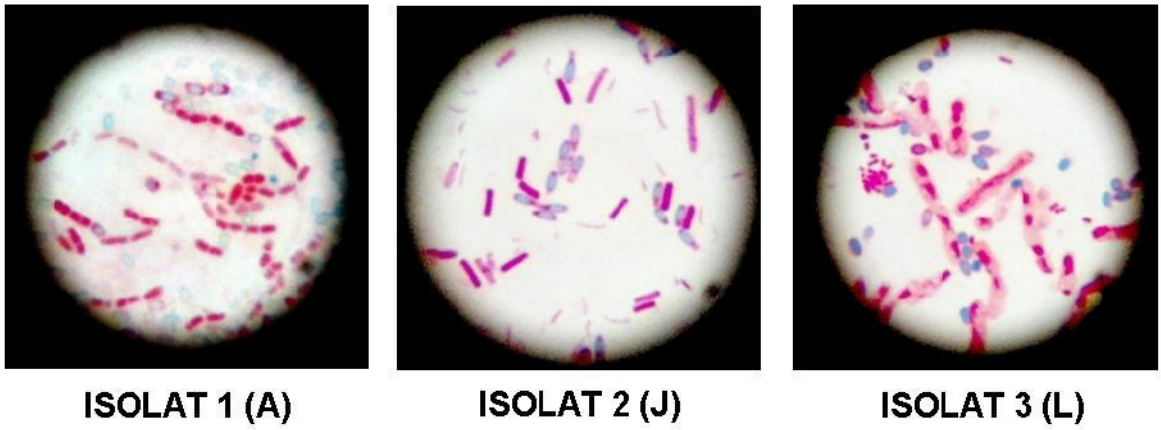

Figure 3. Endospore stain of bacterial isolates (1000x)

We can see from the table and figures that all isolates are the gram positive bacteria, rod form and have oval endospore.These characteristics make sure that all bacterial isolates are member of *Bacillus* genus.

Biochemistry characteristics of bacterila isolates are showed in Table 2.

Table 2. Biochemistry characteristics of bacterial isolates

Characteristics	**Isolate**		
	1A	**2J**	**3L**
Carbohydrate fermentation: – Glucose	+	+	+
- Maltose	+	+	+
- Manitol	+	+	+
- Sucrose	-	-	-
Motility	+	+	+
Catalase	+	+	+

Simmons Citrate	-	-	-
Urease	+	+	+
TSI	+	+	+
Indol	+	+	+
Methyl Red	-	-	+
Voges Proskauer	-	+	+
Oxidase	+	+	-
Cloramphenicol	+	+	+
Nalidixic Acid	+	+	+
Polymyxin	+	+	+
Growth in NaCl 10%	+	+	+
Growth in anaerobic condition	-	+	-

Identification by using *Profile matching* method based on *Bargey's Manual of Determinative Bacteriology* (Holt et al., 1984) and Pibwin Ver. 1.9.2 Build 29 software with *Bacillus* matrix (downloaded from University of Sothampton, UK website) identify that isolates 1A was assigned to *Bacillus spahericus*, isolate 2J to *Bacillus carotarum*, and isolate *3L* to *Bacillus cereus.*

Bacillus sphaericus is member of *Bacillus* genus that produce toxin for mosquito larvae (larvasidae). This species mainly found in soil and freshwater sediment (Anonim, 2009). *B. carotarum* found in the soil that was used as ornament in Spanyol (Heyrman *et al.*, 2005). *Bacillus cereus* produce exotoxin that gave occasion to intoxication (Todar, 2009)

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