29

by Identificat Identificat

Submission date: 05-Nov-2022 12:59PM (UTC+0800)
Submission ID: 1945148717
File name: 29._EJABF_Volume_25_Issue_5.doc (151.5K)
Word count: 2324
Character count: 12970

Identification of Bacterial Symbionts from the Marine Sponge *Aaptos suberitoides* (Demospongiae: Suberitidae)

ARTICLE INFO

Article History:
Received: June 18, 2021
Accepted: Aug. 17, 2021
Online: Oct. 25, 2021

Keywords:
Sponges,
Symbiotic,
Bacteria,
Coastal water,
Diversity

ABSTRACT

The sponges are primitive multicellular animals (metazoans) that belong to the phylum of Porifera. They are symbiotic with bacteria, archaea, microalgae and fungi, and the common symbionts organisms. Symbionts bacteria are obtained vertically through gametes and horizontal through the filter feeder process. This study aimed to isolate and the species of symbionts bacteria in *Aaptos suberitoides* sponge from Pasir Putih Beach waters Situbondo, East Java. Six symbionts bacterial were isolated from the sponge *Aaptos suberitoides*; namely, *Corynebacterium hofmanni*, *Vibrio damsela*, *Oligella urethralis*, *Bacillus coagulan* and *Bordetella parapertusis*. Biochemical tests confirmed that the symbiotic bacteria can perform several processes: nitrification process, citrate, urease, Voges Proskauer, decarboxylase lysine, and decarboxylase ornithine and hydrolysis aesculin. In conclusion, excluding *Bacillus coagulant* bacteria, symbionts bacterial were proved mostly pathogenic to humans and fish.

INTRODUCTION

Sponges are filter feeders known to be symbiotic with bacteria, archaea, microalgae and fungi. These microorganisms can contribute significantly to spongy metabolism (**Taylor *et al*., 2007**). Bacteria found to be symbiotic with sponges *Aaptos* sp. dominated by bacteria of the class Actinobacteria, Flavobacterium, Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria (**Chasanah *et al*., 2013**). Sponges can produce chemical compounds as a form of self-defence response to predators and competitors (**Taylor *et al*., 2007**). The resulting chemical compounds can induce

symbiotic microorganisms to produce specific secondary metabolites (**Nofiani *et al.*, 2009**). The sponge *Aaptos suberitoides* produces aaptamine and demethylaaptamine compounds with cytotoxic activity (**Hanif *et al.* 2019**). Bacteria *Rhodobacteraceae bacterium* (**Murniasih *et al.* 2014**), *Halomonas aquamarina, Alphaproteobacterium* and *Pseudoalteromonas luteviolaceae* symbiotic with sponges og genus *Aaptos* sp. are known to produce antibacterial compounds (**Radjasa *et al.*, 2007**). Identification of symbiotic bacterial species with sponges is essential for assessing the composition and function of the microbial community (**Martin, 2002**).

This study aimed to identify bacteria species that are symbiotic with marine sponges. The symbiotic bacteria are expected to be developed and become bioremediation candidates in cultivation activities such as shrimp pond processing and waste fish processing industry, which can decrease the water quality around the activity area.

MATERIALS AND METHODS

Samples Collection

Samples were taken from Pasir Putih beach water Situbondo at a depth of 12-14 meters. After the sponge was taken and cut, the samples were put in a cool box and seawater with fixed aeration. The samples were then taken to the laboratory to be identified and analyzed (**Abubakar *et al.*, 2011**). Sponge identification refers to the world sponge database accessed through online database (www.marinespecies.org/porifera) and based on sponge morphology (**Subagio & Aunurohim, 2013**).

Isolation Bacterial Symbiont Sponge

The sterilisation of the sponges surface was done with ethanol (70%) and rinsed thrice with sterile seawater. The rinsed sponges were taken as much as one gram, smoothed using a mortar, and five (5) mL of sterile seawater was added on each sampled gram. The homogeneous solution was diluted by adding one (1) mL of the solution into nine (9) mL of sterile seawater, then the outcome was diluted to 10^{-3} of the initial concentration (**Rua et al., 2014**). The sample planting was done by surface/spread plate method [11] on Tryptic Soy Agar Sea Water (TSA-SW) media, and the TSA medium diluted with sterile seawater (**Whitman *et al.*, 2012**). The cultured sample was an outcome of 10^{-1} to 10^{-3} dilution and was incubated at 30°C for one to three days (**Cicirelli, 2007**). The bacterial colonies were collected using a sterile ose. They were scraped on TSA-SW mediumand incubated for 48 hours at 30°C (**Setyati & Subagiyo, 2012**)until a single colonywas obtained.

Bacterial Isolate Identification

Identification of spongy symbiont bacteria includes morphological and biochemical tests. Morphological tests include colony shape, colony colour, colony edge, elevation or

colony surface, colony and Gram staining structures. Biochemical tests included catalase, oxidase, O/F, TSIA, nitrate reduction, gelatin, MR-VP, phenylalanine deaminase, urease, citrate utilisation, glucose, lactose, sucrose, maltose, mannitol, dulcitol, inositol, sorbitol, arabinose, raffinose and xylose, lysine, malonate, MIO and aesculine. The results of further identification tests were compared to Cowan and Steel's Manual for the Identification of Medical Bacteria (**Cowan and Steel, 1965**) and Bergey's Manual of Determinative Bacteriology (**Whitman *et al*., 2012**), which was used for the analysis and determination species from isolates of symbiotic bacteria.

RESULTS

Isolation of Bacterial Symbiont

Bacterial isolation from the *Aaptos suberitoides* spongy tissue was grown on Tryptic Soy Agar-Sea Water (TSA-SW) medium, selected by colour, size and colony shape. Based on the colony morphology, though seven (7) bacterial isolates were planted on TSA-SW media, only six (6) isolates were grown (Table 1). The six bacterial isolates were purified with the same medium for the identification process.

Identification of Bacterial Symbiont

The six isolate symbiont bacteria were then conventionally identified, including morphological and biochemical tests (Table 2). The identification results of each isolate of the symbiotic bacteria were compared with identification manuals, Cowan and Steel's Manual for the Identification of Medical Bacteria (**Cowan & Steel, 1965**) and Bergey's Manual of Determinative Bacteriology (**Whitman *et al*., 2012**) to determine the species of bacteria.

Table 1. Colony morphology of bacterial symbionts on *Aaptos suberitoides*

No.	isolate code	color	elevate	Edge	form	size (mm)	isolate growth (+/-)
1.	SC1	white	Convex	Flat	rounded	1.5	+
2.	SC2	cream	Convex	Flat	rounded	1.0	-
3.	SC3	cream	Convex	Flat	amorf	6.0	+
4.	SC4	cream	Convex	Flat	amorf	2.0	+
5.	SC5	white	Convex	Flat	rounded	1.0	+
6.	SC6	white	Convex	Flat	rounded	2.0	+
7.	SC7	cream	Convex	Flat	rounded	1.0	+

DISCUSSION

Bacteria that are found and symbiotic with sponges have different characteristics. The *Corynebacterium hofmanni* bacterium has another name; *Corynebacterium pseudodiphtheriticum* (**Cowan & Steel, 1965; Whitman *et al*., 2012**). *Corynebacterium hofmanni* bacteria are normal bacteria in the human nasopharynx (**Whitehouse *et al*., 2018**). The bacteria of this genus are found to live in seawater (**Mudryk, 1998**), and one of the species of this genus; *Corynebacterium maris*,is found in *Fungia granulosa* coral

mucus (**Ben-Dov *et al.*, 2009**). *Corynebacterium hofmanni* bacteria rarely cause infections, but they can cause endocarditis, urinary tract infection (UTIs) and may infect skin lesions (**Whitehouse *et al.*, 2018**).

Table 2. Morphological and biochemical tests of symbiotic sponge bacteria

Parameter	Isolate					
	SC1	SC3	SC4	SC5	SC6	SC7
Gram Characteristic						
Bacterial form	coccus *^	coccus *^	coccus *^	coccus *^	coccus *^	coccus *^
Colour/Gram	+*^	-*^	-*^	-*^	+*^	-*^
Biochemistry Test						
TSI Agar	K/K	A/A	A/A	K/K	A/A	K/K
Gas	-	-	-	-	-	-
H_2S	-	-^	-^	-^	-	-
Catalase	+*	+*	+*	+*^	+*^	+*
Oxidase	-*	+*^	+*^	4*^	-	-*^
O/F	NR*	F*	F	NR*	NR	NR*
Nitrate Reduction	+*^	-*^	-*^	-*^	+*d	W*^
Gelatin	-*	-^	-^	-*^	-^d	-*
Motility	-*	+*^	+*^	-*^	+*^	-*^
Indole	-	-*^	-*^	-^	-*	-
Simmons Citrate	+	+^	+^	-.*d	-d	-*^
Malonate	-	-^	-^	-	-	-
Christine's Urease	+*^	-*^	-*^	-*^	+*^	-*^
Methyl Red (MR)	-^	-	-	-	-	-
4ges Proskauer (VP)	-*	+*^	+*^	-	-dd	-
Lysine Decarboxylase	-	-*^	-*^	+	-^	+^
Ornithine Decarboxylase	+	-	-*^	+	+^	+
Phenylalanine Deaminase	-	-^	-^	-	-	-
Aesculin Hydrolysis	-*^	+^	+^	-	-^d	-
9**rbohydrate Fermentation**						
Glucose	-*	+*^	+*^	-*	+*^	-*
Lactose	-*	-^	-^	-	+^d	-
Sucrose	-*	+*^	+*^	-	+^d	-
Maltose	-*	+^	+^	-*	+^	-*
Mannitol	-*	+^	+^	-	-^	-
Dulcitol	-	-^	-^	-	-^	-
Inositol	-	-^	-^	-	-	-
Sorbitol	-	-^	-^	-	-^d	-
Arabinose	-	-*^	-*^	-	-^d	-
Raffinose	-	-^	-^	-	-*+^d	-
Xylose	-	-*^	-*^	-	-*^	-
Genus	*Corynebacterium*	*Vibrio*	*Vibrio*	*Oligella*	*Bacillus*	*Bordetella*
Species	*C. hofmannii*	*V.damsela*	*V.damsela*	*O.uretralis*	*B. coagulans*	*B. Parapertussis*

Remark: (+) = Positive; (-) = Negative; A = Acid; K = Alkaline; NR = Non Reaction; F = Fermentative; W = weak;
d = 16-84 % strain positive

The bacterium *Vibrio damsela* or now known as *Photobacterium damselae* subsp. *damselae* (**Rivas *et al.*, 2011**) can be found in some damselfish sea fish, yellowtail, seabream, and brown shark. Another report explained that these bacteria are also common in lemon sharks, dolphins, turtles, octopus, uninfected fish and wounds in

humans. These bacteria can cause ulcers in *Chromis punctipinnis* fish and cause infection in human wounds (**Brenner *et al*., 2005**). Bacteria *Photobacterium damselae* has been isolated from the marine sponges, family Geodiidae and Halichondriidae (**Sfanos *et al*., 2005**). Other photobacterium bacterial species known to be symbiotic with marine sponges are known as *Photobacterium rosenbergii* (**Thompson *et al*., 2005**), *Photobacterium jeanii* (**Chimetto *et al*., 2010**) and *Photobacterium phosphoreum* (**Sfanos *et al*., 2005**). In addition, *Vibrio* sp. bacteria are present in the water of white shrimp (*Litopenaeus vannamei*). If the abundance of bacteria *Vibrio* sp. in white shrimp exceed the minimum threshold of bacteria in the waters of 10^4 CFU/ml, it becomes susceptible to the attack of Vibriosis (**Kharisma & Manan, 2012**).

Oligella uretralis, formerly known as *Moraxella urethralis* (**Brenner *et al*., 2005**), is a bacterium that is generally susceptible to most antibiotics. It was observed that this bacterium is susceptible to penicillin (**Welch *et al*., 1983**). *Oligella urethralis* is widespread (**Rossau *et al*., 1987**) but it is primarily isolated from the human urogenital tract and has been reported to cause urosepsis (**Pugliese *et al*., 1993**). Some *Oligella urethralis* are also isolated from ear, blood and leg wounds (**Rossau *et al*., 1987; Brenner *et al*., 2005**) . These bacteria have also been isolated from rabbit conjunctiva (**Marini et al., 1996**). This genus, *Oligella ureolytica,* has been isolated from Gokceada-Turkey island in the coastal and offshore areas (**Türetken & Altuğ, 2016**).

The *Bacillus coagulant* has been found in corneal infections, bacteremia and cow abortions. These bacteria are also found in compost, milk, paper, cardboard and silage (**De Vos & Garrity, 2009**). They are also found in gemstones (**Khan *et al*., 2001**). In addition, they were isolated from Gokceada-Turkey Island in the coastal and offshore areas (**Türetken & Altuğ, 2016**). *Bacillus coagulan* bacteria have an essential role in food spoilage as a producer of commercially valuable products such as lactic acid, thermostable enzymes, and antimicrobial peptides coagulin, and are used as probiotics (**De Vos & Garrity, 2009**). This bacterium was known to play a role in degrading chitin (**Clements *et al*., 2002**). Several Bacillus species have been isolated from marine sponges; namely, *Bacillus benzoevorans*, *Bacillus cereus*, *Bacillus firmus*, *Bacillus gibsonii*, *Bacillus methanolicus*, *Bacillus niacini*, *Bacillus pumilus*, *Bacillus* sp., *Bacillus anthracis* and *Bacillus vietnamensis* (**Sfanos *et al*., 2005**). Furthermore, *Bordetella parapertusis* bacteria have been found in the human respiratory tract (**Wolfe *et al*., 2005**) and sheep (**Martin, 1996**). One species of this genus *Bordetella petrii* isolated from the sea sponges of the family Coelosphaeridae and Pseudoceratinidae (**Sfanos *et al*., 2005**). *Bordetella parapertusis* bacteria can cause mild pertussis in humans and cause chronic progressive pneumonia in sheep (**Brenner *et al*., 2005**).

The acquired bacterial symbionts can perform several processes in the body of the sponge. Collectively the microbes in the sponge can perform many processes, such as photosynthesis, methane oxidation, nitrification, nitrogen fixation, sulfate reduction, and dehalogenation (**Taylor *et al*., 2007**). Bacterial symbionts are also involved in various

processes of sponge metabolism, such as vitamin production, nutrient transport, utilisation, redox sensing and response (**Thomas *et al*., 2010**).

The data obtained showed that the mechanisms of these bacteria in the sponges body are vertical. Vertical transmission is transmitted through sponge gametes through oocytes or sponge larvae **(Aknin *et al*., 2010)**. The vertical transmission process of the sponge begins with the inclusion of the symbiotic bacteria present in mesohyl into the oocyte by phagocytosis. During the process of the division of the embryo, bacteria exist between blastomeres or are found in vacuoles. In the blastula, all bacteria are removed in the blastocoele **(Ereskovsky *et al*., 2005)**. The presence of bacterial cells in the central cavity of the embryo (blastocele) is consistent with the description of bacteria present in the larval cavity **(Sharp *et al*., 2007)**.

Based on the sponge properties of the filter feeder, there is a possibility of horizontal transmission. Horizontal transmission is the bacterial selective absorption process of bacterial diversity in the surrounding water column that passes through the sponge during the filter feeder process **(Taylor *et al*., 2007)**. Known bacteria found in coastal waters of Pasir Putih Situbondo are *Pseudomonas cepacia, Bacilus subtilis* and *Vibrio alginolyticus* **(Andriyono *et al*., 2015)**. *Pseudomonas cepacia* or *Burkholderia cepacia* was found in *Aplysina fulva* marine sponges in coastal waters of Brazil **(Hardoim *et al*., 2009)**. *Bacilus subtilis* bacteria have also been isolated from marine sponges *Haliclona simulans* **(Phelan *et al*., 2013)** and *Fasciospongia cavernosa* **(Pandey *et al*., 2014)**. The *Vibrio algilinolitycus* is known as symbiotic bacteria with a sea sponge *Algelas* sp. and *Spongia* sp. **(Hassanzadeh *et al*., 2014)**. However, the current study did not find the three species of bacteria on the body of the *Aaptos suberitoides* sponge as a symbiont. This condition is possible due to the selection process in the body of the sponge during the filter feeder process. Sponges have an effective defence system against microbes and parasites involving bacterial engulfment into specialised cells, beside the fact that sponges also use signal pathways that actively kill bacteria transduction. The archaeocyte cell in the sponge body can be thought of as the macrophage of the sponge, where it is used for self-defense against foreign microorganisms **(Müller & Müller, 2003)**.

CONCLUSION

This study has obtained isolates that are symbiotic with *Aaptos suberitoides*. Symbiotic bacteria with *Aaptos suberitoides* spongewere isolated and identified as *Corynebacterium hofmanni, Vibrio damsela, Oligella urethralis, Bacillus coagulant* and *Bordetella parapertusis*. The bacteria can not be used as bioremediation agents because most bacteria are pathogenic to humans and fish, except *Bacillus coagulant*. However, further research is required to determine the effect of bacteria as a probiotic agent candidate on bioremediation.

ORIGINALITY REPORT

8%	6%	7%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Yamada, S., E. Baba, and A. Arakawa. "Proliferation of Pasteurella pneumotropica at oestrus in the vagina of rats", Laboratory Animals, 1983. Publication	2%
2	www.ncbi.nlm.nih.gov Internet Source	2%
3	onlinelibrary.wiley.com Internet Source	1%
4	Noel R. Krieg, Wolfgang Ludwig, Jean Euzéby, William B. Whitman. "Chapter 3 Phylum XIV. Bacteroidetes phyl. nov.", Springer Science and Business Media LLC, 2010 Publication	1%
5	lib.unnes.ac.id Internet Source	1%
6	Ibrahim Musa Moi, Noordiyanah Nadhirah Roslan, Adam Thean Chor Leow, Mohd Shukuri Mohamad Ali et al. "The biology and	1%

the importance of Photobacterium species", Applied Microbiology and Biotechnology, 2017
Publication

7	scholar.unair.ac.id Internet Source	1 %
8	files.eric.ed.gov Internet Source	<1 %
9	Natalie I. Novikova, Elena A. Pavlova, Elena V. Limeshchenko. "Phage sensitivity and host range of Rhizobium strains isolated from root nodules of temperate legumes", Plant and Soil, 1993 Publication	<1 %
10	Zena Werb, Patrice Tremble, Zena Werb, Caroline H. Damsky, Patrice Tremble, Caroline H. Damsky. "Regulation of extracellular matrix degradation by cell—extracellular matrix interactions", Cell Differentiation and Development, 1990 Publication	<1 %

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		