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Short Communication

The Numerical Phenetic of Taxonomy Vibrio in Shellfish (*Meretrix meretrix*) at Edu-Tourism Mangrove Cengkrong Beach Trenggalek

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Abstract

Meretrix meretrix is one type of shellfish that is potential, and economic value, which high animal protein content as well as seafood that is widely consumed in Indonesia. This study aims to determine the diversity and similarity of *Vibrio* in *M. meretrix* at eduwisata mangrove Cengkrong Beach, Trenggalek. Isolation and identification of *Vibrio* was conducted by using the media TCBSA (Thiosulfate Citrate Bile Salt Sucrose Agar) streat plate through morphological observation, physiological and biochemical tests based Bergey's Manual of Systematic Bacteriology conducted in the laboratory of Microbiology Faculty of Biology, Universitas Gajah Mada. Data analysis was performed using the MVSP (Multivariate Statistical Package) 3.1 in the form of a dendrogram showing the similarity relationship *Vibrio* bacteria. The results of the identification of *Vibrio* bacteria in *M. Meretrix* are *V. herveyi, V. parahemolyticus, V. nereis and V. proteolyticus. V. herveyi* has similarities with *V. parahemolyticus* 66% and 62% with *V. proteolyticus* while with *V. nereis* bacterium 54%.

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1. Introduction

Edu-Tourism Mangrove Cengkrong Beach is one of the tourist attractions in Trenggalek with a diversity of mangrove vegetation that is quite high and has a marine fauna in the form of shells (*Meretrix meretrix*). These mussels are consumed by many people around and sold throughout Indonesia. In research (Arnanda *et al*., 2005), stated that the protein content in shells ranged from 6.97-11.92%. Therefore, shells are a fairly high source of animal protein.

Along with changes in lifestyle and eating patterns, the consumption needs of the community for shells increased. An increase follows the increase in the consumption needs of the Indonesian people in the production value of shells. *Meretrix* spp. Production in Indonesia reported an increase of an average of 22.10% during 2001-2011 (KKP, 2012).

Meretrix meretrix is a member of the Bivalvia class by eating filter feeders (Nybakken, 1992). How to eat that is a filter feeder can cause the absorption of various kinds of dangerous heavy metals and some pathogenic bacteria into the body.

Pathogenic bacteria are bacteria that have the genetic ability to cause disease, produce metabolic material, or cause tissue changes that endanger the host. Pathogenic microbes that cause disease have specific characteristics that saprophytic microbes do not have (Suharni *et al*., 2008). Pathogenic bacteria play a role in marine biota, especially in shellfish that can cause disease for humans, one of which is the *Vibrio* bacteria.

Vibrio is an aquatic bacteria that can be found in rivers, ponds, and seas that are pathogenic opportunistic (Widowati, 2008). Member of *Vibrio* bacteria as a cause of disease for humans, among them, *V. cholerae* and *V. parahaemolyticus* (Mailoa and Setha, 2011). *V.cholerae* and *V. parahaemolyticus*, respectively cause cholera and gastroenteritis diseases. Patients with severe cholera can experience diarrhea 20-30 times a day and similar with18 liters of fluid loss. Therefore, information about the presence and diversity of *Vibrio* bacteria in *M. meretrix* is very necessary to be done through a phenetic numerical taxonomic analysis approach.

The purpose of this study was to determine the diversity and the similarity of the intraspecies of *Vibrio* spp. at *M. meretrix* in the Eduwisata Mangrove Cengkrong Beach, Trenggalek.

2. Materials and Methods

2.1 Isolation and screening

The tools used include micropipettes, LAF, autoclaves, incubators, digital scales, vortices, shakers, mortars, and various glassware such as: test tubes, Petri dishes, inoculating loop test tube, racks Erlenmeyer, and drigalski. Materials include M. meretrix, aquadest, NA, NB, TCBS, MIO, oxidase paper.

A sampling of *M. meretrix* was done by using a purposive sampling method or random sampling with various sizes, large or small. A total of 25 grams of *M. meretrix* sample that had been pulverized was then homogenized with 225 ml of peptone water (APW) alkaline media and incubated for 6-8 hours. The homogenized samples were then isolated on the selective media Thiosulfate Citrate Bile Salt Sucrose Agar (TCBSA) by streak plate method then incubated for 24-48 hours at 30^0C. Colonies of bacteria that grow on selective media are thought to be Vibrio spp (Onarheim *et al*., 1994).

2.2 Characterization and identification

Characterization and identification of *Vibrio* bacteria were analyzed by looking at morphological and biochemical properties based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) and Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

2.3 Data Analysis

Vibrio bacterial isolates in *M. meretrix* were analyzed for phenetics numerically. Positive and negative phenetic characters are coded respectively as + and -. This phenetic numerical analysis was carried out by using the MVSP 3.1 Program, therefore the dendrogram construction is obtained (Kovach, 2007). Generating similarity between strains was estimated by Simple Matching Coefficient (SSM) and Jaccard's Coefficient (Sj) (Sokal and Meichener, 1958), and cluster building was carried out using Unweighted Pair-Group Method of Association (UPGMA) (Sneath and Sokal, 1973). Analysis of cophenetic correlations was then calculated to determine the level of dendrogram reception statistically (Sneath and Johnson, 1972).

3. Result and Discussion

3.1 Isolation and screening of Vibrio bacteria

Isolation of *Vibrio* bacteria from *M. meretrix* located in Edu-Tourism Mangrove Cengkrong Beach, Trenggalek Regency, there were 48 isolates, which were isolated on TCBSA media. In making this TCBSA media, it does not go through a sterilization process on the autoclave because, in TCBSA media, there is content or composition of thiosulphate which serves to inhibit other bacteria that grow on TCBSA media, After all, TCBSA media are included as selective media to grow *Vibrio* bacteria. Colonies of bacteria that grew on TCBSA colored media; green, transparent green, and yellow (Figure 1). The difference in the color of the bacterial colony shows the difference in the ability of sucrose fermentation contained in TCBSA media. Colonies with a green appearance indicate the inability to ferment sucrose, conversely colonies with a yellow appearance indicate the ability to ferment sucrose (Mailoa and Setha, 2011).

The results of isolation and screening of *Vibrio* bacteria from *M. meretrix* contained five strains. These isolates show the diversity of colony shape, elevation edges, color, and cell shape. Analysis of cell Morphology characters found that all strains were gram-negative and stem-shaped, and part of the stem was bent (Table 2). In accordance with research (Ihsan and Retnaningrum, 2017) states that the bacteria *Vibrio* sp. has various forms of colony morphology such as colony colors (yellow, orange, green, and bluish-green), circular colony forms, the edge of the entire colony and various colony elevations (low convex, convex and effuse).

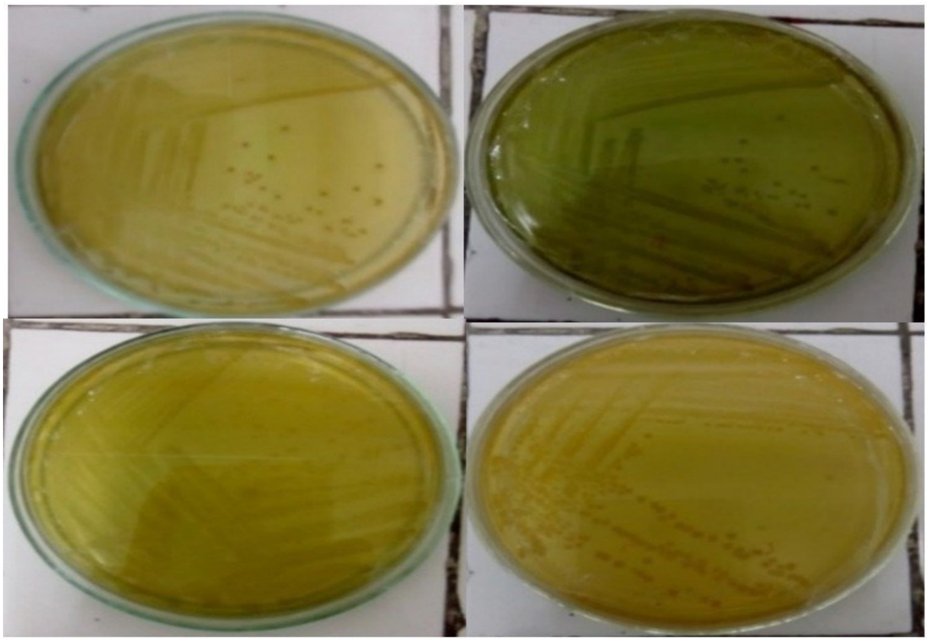

Figure 1. Bacterial colony color

Table 1. Isolation *Vibrio.*

No	Characterization	Isolate				
		BI 11	BI 35	BI 17	BI 53	BI 26
1	Morphology colony					
	Circular	+	+	+	+	+
	Effuse	–	–	–	–	–
	Low convex	+	+	–	–	+
	Convex	–	–	+	+	–
	Entire	+	+	+	+	+
	Yellow	+	–	–	+	+
	Orange	–	–	–	–	–
	Green	–	+	–	–	–
	Transparent green	–	–	+	–	–
2	Morphology cell					
	Gram staining	–	–	–	–	–
	Straight stem cell	–	–	+	+	+
	Crooked stem cell	+	+	–	–	–

Observations of the biochemical properties of the five strains showed facultative anaerobes, motile, able to reduce nitrates/nitrites, produce enzymes oxidase and catalase, able to grow in medium containing methyl red. The five strains also showed an inability to produce indole, H_2S, and Voges-Proskauer. The biochemical properties of the five strains, on the contrary, showed diversity in growth ability at various temperatures and different NaCl levels, as well as the ability to ferment several types of carbohydrate sources (Table 2). Similarities and differences in the phenotypic characteristics of these bacterial isolates are due to the static and dynamic nature of the phenotypic characters. The dynamic nature of the phenotypic characters is due to changes in the environment and microbial growth conditions (Ochman, 2005).

Table 2. Characterization and identification of *Vibrio* bacteria.

No	**Characterization**	**Isolate**				
		BI 11	**BI 35**	**BI 17**	**BI 53**	**BI 26**
1	Carbohidrate fermentation					
	D-Glucose	+	–	–	+	–
	Arabinose	–	–	–	–	–
	Galactose	–	+	+	–	+
	Lactose	+	+	+	+	–
	Maltose	–	–	–	–	+
	Glucose	+	+	+	+	+
	Mannitol	+	–	+	+	–
	Raffinose	–	–	–	–	–
	Sucrose	+	–	–	–	–
	Xilosa	–	–	–	–	+
2	Growth on temperature					
	Temperature 30^0C	+	+	+	–	–
	Temperature 35^0C	+	+	–	–	–
	Temperature 40^0C	–	+	–	–	–
3	Growth in NaCl					
	NaCl 0%	–	–	–	–	–
	NaCl 0.1%	–	–	–	–	–
	NaCl 0.2%	–	+	+	–	–
	NaCl 0.3%	–	+	+	–	–
	NaCl 0.4%	–	+	+	–	–
	NaCl 0.5%	+	+	+	–	+
	NaCl 1.0%	+	+	+	+	+
	NaCl 2.0%	+	+	+	+	+
	NaCl 3.5%	+	+	+	+	+
	NaCl 6.0%	+	+	+	+	+
	NaCl 8.0%	+	+	+	+	+
	NaCl 10.0%	+	–	–	+	+
	NaCl 12.0%	–	–	–	–	–
4	Anaerobic Facultative	+	+	+	+	+
5	Motility	+	+	+	+	+
6	Reduction nitrate/nitrite	+	+	+	+	+
7	Oxidase	+	+	+	+	+
8	Indole formation	–	–	–	–	–
9	Voges–Proskauer	–	–	–	–	–
10	Methyl red	+	+	+	+	+
11	Catalase	+	+	+	+	+
12	H_2S	–	–	–	–	+
Identification		*V. hervey*	*V. parahemoliticus*	*V. proteoliticus*	*V. nereis*	*Vibrio* sp.

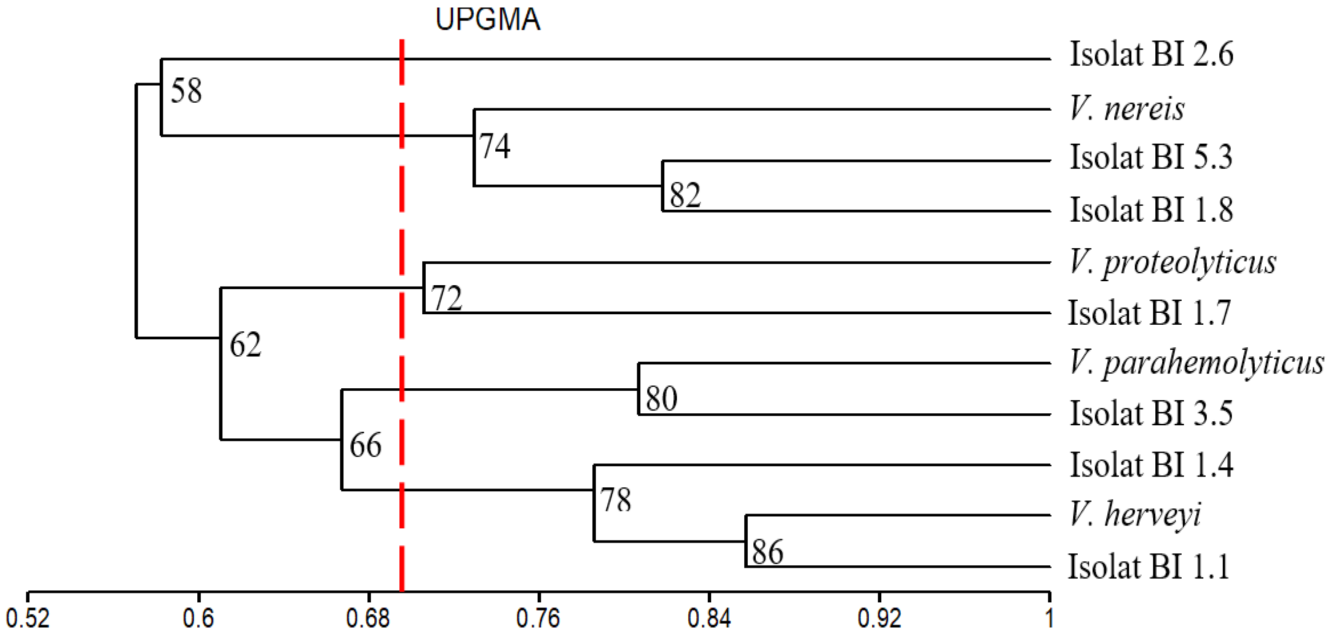

Figure 2. Dendogram Sj

3.2 Biochemical characterization of Vibrio bacteria

The five *Vibrio* bacterial strains were characterized and identified based on Bergey's Manual of Systematic Bacteriology, there were four strains of bacteria identified as *V. harveyi, V. parahemolyticus V. proteolitycus* and *V. nereis* and one bacterial strain could not be identified at the species level, possibly strains the new species.

3.3 Relationship similarity of Vibrio bacteria

The results of the characterization and identification of the five bacteria were analyzed using the PFE and MVSP program resulting in two dendrograms; Simple Matching Coefficient (Ssm) and Jaccard's Coefficient (Sj) to determine the similarity and taxonomic status of the identified bacteria and analysis of cophenetic correlations.

3.4 Dendrogram construction

The dendrogram is a phylogeny tree that describes a category hierarchy based on the degree of similarity of several characteristics in taxonomy (Dransfield *et al*., 2005). The results of bacterial characterization were entered into the NxT table for analysis with the PFE program. The results of the characterization data were clustering by using the MVSP program. Based on the analysis of Sj, similarity produced five species at a similarity level of 70%.

According to the taxo-species concept, all isolates and comparative strains are said to be one species with another strain if it has a similarity index of ≥ 70% (Priest and Goodfellow, 2000). In accordance with the opinion (Sneath *et al*., 1986) which states that a strain can be grouped in the same species if it has a similarity in phenetic properties of ≥ 70%. In (Figure 2), the results of dendrogram analysis illustrate the similarity between bacterial species. *V. harveyi* bacteria have similarities with *V. parahemolyticus* 66%, and 62% with *V. proteolyticus* bacteria, and 54% with *V. nereis* bacteria.

3.5 Analysis of cophenetic correlations

Analysis of cophenetic correlations produces sorted and unsorted similarity matrices in Ssm and Sj, from the similarity matrix will produce cophenetic correlation values or r values with values of 0.786 on Ssm and 0.834 on SJ, respectively. The acceptable cophenetic correlation index is ≥ 0.7 or ≥ 70%. The lower the value of the cophenetic correlation index produced, the dendrogram formed will not represent the similarity matrix on which the construction is based. The results of the second dendrogram cophenetic correlation analysis show that similarity formed after the application of the UPGMA algorithm can still be accepted statistically, considering that the resulting r value is greater than 0.7.

4. Conclusion

There are four Vibrio bacteria in *M. Meretrix*, namely; bacteria *V. herveyi, V. proteolitycus V. nereis* and *V. parahemolyticus* and one unidentified bacterium *Vibrio* sp. *V. herveyi* has similarities with *V. parahemolyticus* 66% and 62% *V. proteolyticus* while *V. nereis* bacterium 54%.

Authors' Contributions

All authors discussed the results and contributed to from the start to the final manuscript; BI: responsible for the overall data collection and writing the initial until the final draft of this paper, ER: besides being involved in a few field works, is also in the discussion of this paper.

Conflict of Interest

The authors declare that they have no competing interests.

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This research was conducted independently.

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