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Bacterial composition in the gastrointestinal tract of *Uca* spp crabs fed on *Avicennia marina* leaf litter

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Abstract.*Uca* spp, found in mangrove areas, has a role in sediment reworking by making a burrow for air circulation. The nutrient content remains stable and sediment fertility for vegetation growth is maintained. *Uca* spp has the feeding behavior of eating the residue of plants, separating the detritus and microbes and removing the residue of the plant. The presence of bacteria in the mangrove ecosystem has an important place in the decomposition of the mangrove leaf litter into organic material, used as a source of nutrients for mangrove forest organisms. This study aims to determine the composition of bacteria in the digestive tract of*Uca* spp., after the consumption of*A. marina*leaf litter.The results of the bacterial identification shows that the bacterial composition contained in *Uca spp's*digestive tract is *Acinetobacter* sp. and *Actinobacillus* sp, which are bacteria contained in the leaf litter of*A. marina*.

1. Introduction

The fiddler crab habitat is closely related to the mangroves, but it is not entirely dependent on mangroves for shelter or food. Decaying mangrove leaves can enrich the muddy substrates that provide the main food for crabs [1]. Most species of fiddler crabs ingest benthic microalgae and bacteria [2]. As a feeder on detritus, fiddler crabs eat the microheterotrophs (bacteria and protozoa) and meiofauna (nematodes) present in the mud or on the surface of sand particles [1]. Crabs handle a large amount of detritus in the mangrove environment, but the oxidation of organic carbon is mediated by anaerobic microbial processes [3].

The source of organic carbon in the mangrove environment is due to the accumulation of litter that falls on the surface of the sediment and creates an anoxic sediment layer. The activity of digging fiddler crabs causes the translocation of oxygen to the anoxic layer and increases aerobic respiration, iron reduction and nitrification [4]. Transportation and sediment mixing occurs during burrow maintenance and feeding activities change the dynamics of the sediment and the availability of food sources for microbial, faunal and mangrove communities and adjacent ecosystems [5]. Excavation activities by fiddler crabs can improve drainage and substrate oxidation, increase the rate of debris and plant decay and promote the growth of micro-organisms [6].

The mangrove litter causes the mangrove ecosystem to have a very high level of productivity. The mangrove litter consisting of leaves, twigs, flowers, fruits and other biomass falls into the category of nutrient sources for aquatic biota and this determines the productivity of marine fisheries [7]. Leaf litter has fallen and begun the decomposition process is one of the factors of fertility in the mangrove ecosystem. The rate of the decomposition process contributes to the amount of organic matter, which in turn contributes to the growth and development of vegetation, fish, shrimp, crabs and other

microorganisms in the mangrove forests [8]. The presence of bacteria in the mangrove ecosystem has a very important place in decomposing the litter of mangrove leaves into organic material that is then used as a source of nutrients for mangrove forest organisms[9].

Microbial detritus complexes are the most prevalent in the *Uca* spp species diet. The detritus complex is widely capable of providing suitable sources of energy for detritivores [10]. This laboratory study aims to determine the bacterial composition contained in the digestive tract of *Uca spp* crab associated with *Uca* spp crabs being a detritus feeder.

2. Materials And methods

2.1. Materials

The materials used in this study were the digestive tract *Uca* spp. females before and after being fed *A. marina* litter, living substrate *Uca* spp., substrate water, *A. marina* leaf litter, aquadest and a physiological solution (NaCl 0.85%). The materials used for bacterial identification were Gram A (gentian violet), Gram B dye (iodine Lugol), Gram C dye (acetone alcohol), D gram dye (safranin), Tryptone Soya Agar (TSA) media, Triple Sugar media Ion Agar (TSIA), Lysine Iron Agar (LIA) medium, citrates medium, urea medium, gelatin nutrient medium, methyl red reagent, naphtol, KOH 3%, KOH 40%, emersion oil, H_2O_2 3%, distilled water, paper oxidase, liquid paraffin, Kovac reagents and oxidative fermentative media.

2.2. Methods

The samples were taken randomly from the digestive tract of the *Uca spp* females in the study tank. Sampling was done before they were fed *A. marina* and after 30 days of being fed on *A. marina*. This was done by taking part of the digestive tract of *Uca* spp., the leaf litter of *A. marina*, substrates where they lived and the substrate water.

The bacteria was isolated from the digestive tract sample in the TSA medium. Media incubation was conducted at 20-24^{o}C for 24-48 hours [11]. The next growing colony was identified. The identification of the bacteria was performed to determine the bacteria isolated from the digestive tract of *Uca* spp., from within the litter of the *A. marina'* leaves, from the subsector of *Uca* spp., and from the substrate water. This was done through 1) a morphological test of both the colony and cells, 2) Gram staining of the bacterial colony and 3) biochemical tests.

The macroscopic morphological test was done by looking at the shape and color of the colony. The cell morphological tests were performed with the aim of observing the microscopic cell forms. Biochemical tests were performed through several stages: catalase test, oxidase test, Triple Sugar Iron Agar (TSIA) test, oxidative fermentative (O/F) test, motility indole ornithine (MIO) test, Indole test, citrate test, gelatin test, urea, Lysine Iron Agar (LIA) test, Methil Red (MR) test and the Voges-Proskauer (VP) test. Furthermore, to identify the bacterial isolates, the researcher used Cowan and Steel's Manual for the Identification of Medical Bacteria[12].

3. Result

The results of the isolation sample obtained from the gastrointestinal tract of *Uca* spp. before and after being given *A. marina* leaf litter, from the leaf litter of *A. marina*, from the substrates where they lived and the substrate water showed there to be a number of colonies growing on each medium. The growing colony had a convex elevation with flat edges and was colored red and cream. The results showed that most of the bacteria were short and Gram-negative (Table 1). All of the bacteria in the oxidase and catalase test were positive. The catalase test was characterized by the incidence of gas bubbles in the preparation whereas oxidase was indicated by a color change on the oxidase paper. In the motility test, most of the bacteria were not motile. The Indole test results showed that most of the bacteria were not able to produce indole. In the oxidative/fermentative testing, most of the bacteria exhibited fermentative properties in which the bacteria were able to live without free oxygen. The results of the identification of the three dominant bacterial isolate samples in *were* that the samples were fermentative, so it can be deduced that they can live without utilizing free oxygen / anaerobic respiration (Table 1).

All bacteria produce catalase and oxidase enzymes characterized by positive oxidase and catalase test results (Table 1). The results of the oxidative/fermentative test showed positive and positive fermentative oxidation. This means that some bacteria have oxidative properties related to glucose, and that some bacteria have fermentative properties. From the results, the identification found there to be seven isolates that were not motile and two isolates of bacteria that were motile.

Table 1. The results of the bacterial identification test on the sample.

Sample	Cell Morphology	Gram Staining	Catalase	Oxidase	Motile	Indole	O/F
P1	Short rod	-	+	+	-	-	F
P2	Rod	-	+	+	-	-	O/NR
P3	Short rod	-	+	+	-	-	F
K1	Short rod	-	+	+	-	-	F
K2	Short rod	-	+	+	+	-	F
K3	Short rod	-	+	+	-	-	F
Litter	Rod	-	+	+	-	-	NR
Water	Short rod	-	+	+	+	-	F
Substrate	Short rod	-	+	+	-	-	F

Description: P: *Uca* spp. after littering, K: *Uca* spp. before littering of O / F: oxidative/ fermentative, NR: Non- Reagents

Table 2. Types of bacteria in the gastrointestinal tract and place of life of *Uca* spp.

Sample	Type of Bacteria
P1	*Actinobacillus lignieresii*
P2	*Acinetobacter* sp.
P3	*Actinobacillus lignieresii*
K1	*Actinobacillus* sp.
K2	*V. alginolyticus*
K3	*Actinobacillus* sp.
Litter	*Acinetobacter* sp.
Water	*Vibrio* sp.
Substrate	*Actinobacillus* sp.

Description: P1, P2, P3: the digestive tract *Uca* spp. after littering, K1, K2, K3: the digestive tract *Uca* spp. before littering

4. Discussion

A. marina is a type of mangrove that is tolerant to a wide salinity range and is a a vegetation pioneer that determines mangrove quality at an early stage of growth [13]. The results of the bacteria identification fromt he leaf litter samples of *A. marina* found *Acinetobacter* sp. which is a Gram-negative bacterium with a short stem shape that is positive in oxidase, positive in catalase, not motile and fermentative. According to Shovitri et al [14], bacteria identified from the *A. marina* samples on the Wonorejo coast had a more diverse composition that genera *Bacillus*, *Amphibacillus*, *Lampropedia*, *Acinetobacter*, and *Planococcus*. According to Wijiyono [15], the bacterial colonization dominating the decomposition of *A. marina* leaf litter was *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa*. The genus *Acinetobacter* was successfully identified from the microbial flora in *A. marina* mangrove leaves and rotting Rhizophora apiculata, in addition to the genera *Flavobacterium*, *Vibrio*, *Pseudomonas*, and *Corynebacterium*. Mangrove samples were collected from the mangrove forests in Pichavaram located on the southeast coast of India [16].

According to Abdel-El-Haleem [17], *Acinetobacter* bacteria is a Gram-negative bacteria that is negative in oxidase, not motile and aerobic. *Acinetobacter* sp. includes bacteria that have the ability to ferment, resulting in the production of a number of products such as lipase, protease and cyanophycine.

Acinetobacter is one of the bacteria types that can reduce and eliminate various organic and inorganic compounds. According to Shovitri et al [14], *Acinetobacter* is included in Rhizobacteria, which includes bacteria that live on plant roots or rhizosphere. The function of Rhizobacteria is as a growth promoter (biostimulant) by synthesizing and regulating the concentration of growth-regulating substances (phytohormone).

Based on the results of the identification, *Acinetobacter* sp. was also found in the digestive tract of Uca spp. after being given *A. marina* leaf litter. The presence of *Acinetobacter* sp found in both the mangrove leaf litter and *Uca* spp digestive tract proves that the mangrove litter feeds Uca spp. Longonje and Raffaelli [18] proved that mangrove material is a major component of the crab's diet. Crab plays a significant ecological role in the functioning of the mangrove ecosystems by influencing mangrove recruitment and biogeochemical functions.According to Natania [19], violin crabs (*Uca* spp) are one of the crustaceans that have an important role in the mangrove ecosystem as detritus based on the observations conducted on Enggano Island, which is the outermost small island of Bengkulu Province. The mangrove ecosystem can still be classified as natural.

Uca spp's eating habits involve taking leaf litter that has been mixed in mud containing algae, detritus, fungi, and microbes and then putting it into its mouth, allowing the *Acinetobacter* sp. contained in the substrate to enter the digestive tract. According to Kwok and Tang [1], crabs repeatedly erode substrate pieces with minor chelipeds and then put it into its mouth, so then it looks like it is eating mud. Even the maxillipeds of the mouth section function in a complex way, separating edible matter from inorganic particles. Maxilliped crabs have spoon-tipped setae that serve to sort out the sand particles. Inorganic material is released back into the ground in pellet form. At mealtimes, the crabs move slowly forward, leaving the pelleted lines of food behind them, visible from the burrow.

Fiddler crabs are important consumers of the benthic detritus, bacteria, fungi and microalgae present in coastal swamps, mangroves, sand flats and mudflathabitats [20]. This is evidenced by the discovery of *Actinobacillus* sp bacteria in the substrate samples, and in the gastrointestinal tract of *Uca spp* before and after littering. The result of the identification of *Actinobacillus* sp. showed Gram-negative bacteria with a short stem shape, positive in oxidase, positive in catalase, not motile and fermentative.

Bacteria *Vibrio* sp. was found in the water samples and gastrointestinal tract of *Uca spp* before consuming the litter. The characteristics of *Vibrio* sp bacteria are Gram-negative with a short rod shape, positive in oxidase, positive in catalase, motile and fermentative properties. According to Kaysner [21], the genus *Vibrio* is a Gram-negative, asporogenous rod-shape which is straight and motile with a single polar flagellum when grown in a liquid medium.

Vibrio spp. can be found naturally in brackish water and estuarine ecosystems with optimal salinity and temperature conditions [22]. *Vibrio* spp. is widespread in marine and estuarine environments, and some Vibrio species are pathogenically associated with outbreaks of Vibrio infection [23]. The results of the study identified the presence of *V. alginolyticus* in the digestive tract of *Uca spp*. before being given the litter of *A. marina*. The presence of *V. alginolyticus* in the *Uca* spp digestive tract is in accordance with Gomathi [24].

5. Conclusion

The composition of the bacteria in *Uca* spp's digestive tract, which feeds on *A. marina* leaf litter, has been identified as *Acinetobacter* sp. and *Actinobacillus* sp.

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