

THE IDENTIFICATION AND DISTRIBUTION OF NAKED AMOEBAE IN THE WATER AND SEDIMENTS OF SETIU WETLANDS, TERENGGANU

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Abstract : This study was carried out to obtain preliminary data on the amoeba species present in brackish water and sediments at Setiu Wetlands, Terengganu. Water and sediment samples from seven sampling locations in Setiu Wetlands were taken for isolation and identification of the amoebae. The water samples were collected between 5 to 10 cm below the water surface level in sterile 500 mL polyethelene bottles and were filtered through a membrane filtration unit to trap the amoebae. Sediment samples were taken using a shovel grab and were vortex-mixed in Page's Amoeba Solution (PAS) to separate the amoebae from the sediments. Estimation on their abundance in the sediments at each location was done following Singh's method with modifications. The texture of the sediments was also analyzed and correlated with amoeba abundance. The results of this study indicated that at least four species of amoebae were isolated and identified in water at Setiu Wetlands. They were *Acanthamoeba polyphaga*, *Acanthamoeba* sp., *Vahlkamfia* sp. and one unidentified species designated as amoeba A. In sediments of Setiu Wetlands, at least seven species of amoebae were present. They were *Acanthamoeba polyphaga*, *Acanthamoeba* sp., *Vahlkamfia* sp., *Mayorella* sp., Vannella-like amoeba, amoeba A and amoeba B. The amoeba species that were labeled as Amoeba A and B could not be assigned to any genera of amoebae since their morphological characteristics did not fit the standard taxonomic keys for free-living naked amoebae. The most common amoeba found both in water and sediments in Setiu Wetlands belongs to the genus of *Acanthamoeba*. The amoeba distribution and abundance in relation to the sediment texture and water quality are discussed.

KEYWORDS: amoeba species, brackish water, sediments, Setiu Wetlands

Introduction

Setiu Wetlands is a unique habitat in Malaysia that consists of nine interconnected ecosystems such as sea, beach, mudflat, lagoon, estuary, river, islands, coastal forest and mangrove forest (Nakisah and Fauziah, 2003). The lagoon of Setiu Wetlands is a major site for mariculture activities involving brackish water cage culture, pond culture, pen culture and oyster farming. On-going aquaculture activities in that area will eventually give an impact to the water quality in Setiu Wetlands. Free-living, naked amoebae are ubiquitous in nature and they are important bacterial consumers in any ecosystem. Some of them, however can become facultative pathogens to human and animals (Page, 1988). Not many study was conducted worldwide on the occurrence of these amoebae in brackish water or sediments especially in the wetlands. Therefore, in this preliminary study, water and sediment samples from various locations in Setiu Wetlands were taken for the isolation and identification of their amoebae. The presence and distribution of free-living amoebae in water and sediments in Setiu Wetlands will provide us information on the amoeba diversity leading us to understand their importance in the food web of this ecosystem.

Materials and Methods

Sample collection and isolation of amoebae

Sediment samples were taken from seven different locations (designated as C1 to C7, coordinates were determined using GPS) with three replicates, in Setiu Wetlands (see Figure 1) using a shovel grab (GAB 13). Water sample collection was done between 5 to 10 cm below the water surface level using sterile 500mL polyethylene bottles.

For amoeba isolation, water samples were filtered through a membrane filtration unit to trap the amoebae. For sediment samples, method of Singh (1945) with modification was followed. Briefly, each of sediment sample (10 g in weight) was flooded with 50 mL of Page's Amoeba Saline (PAS). The samples were shaken using a vortex mixer, for two minutes to separate the amoebae from the sediment. Only the supernatant part was examined for the presence of amoebae. Later, the membranes containing amoebae (from water samples) and 1 mL of the supernatant of the sediment samples were layered onto non-nutrient agar added with heat-killed *E. coli* and incubated at 30°C. The agar plates were observed daily for the presence of amoebae. Each type of amoebae observed on the agar was carefully transferred onto new agar plates. Sub-passaging of the amoebae was carried out several times until a single species was obtained before species identification was done following Page's key (1983, 1988).

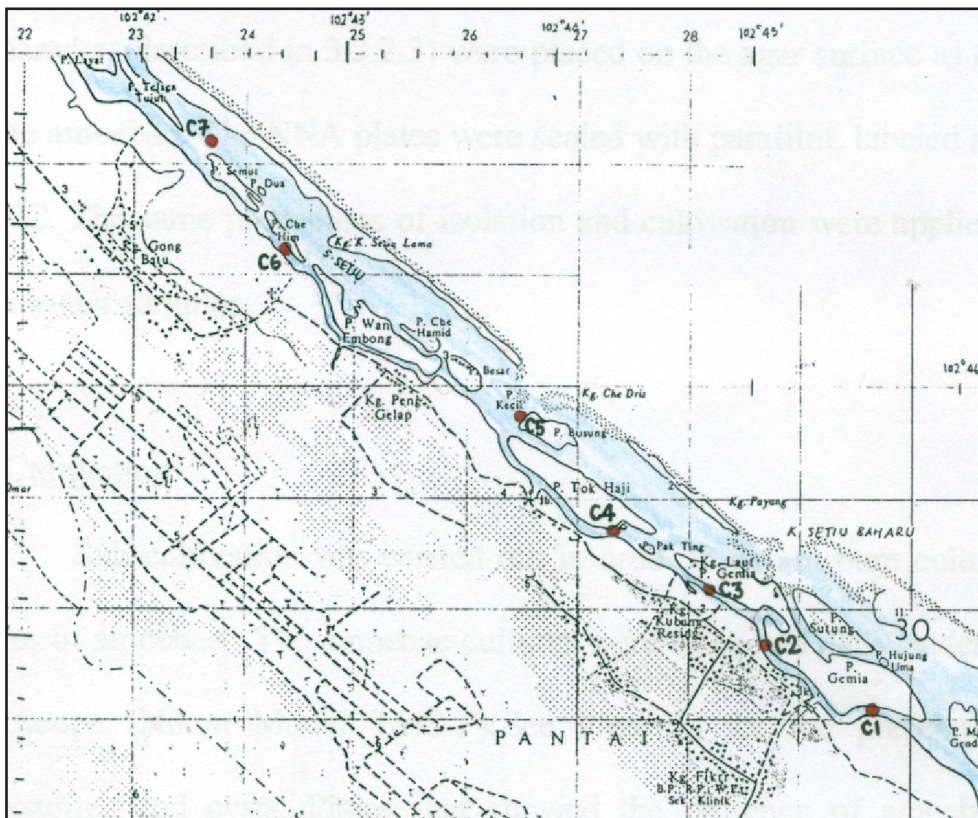


Figure 1. Location of sampling sites (C1 to C7) in Setiu Wetlands, Terengganu. Location C7 is near to the lagoon

Enumeration of amoebae

The number of amoebae present in the sediment was estimated following Singh's Method with modifications (Singh, 1945). Twenty four plates containing non-nutrient agar with heat-killed *E.coli* were used for enumeration of amoebae in sediments taken at Setiu Wetlands. The plates containing amoebae from the sediments were observed daily until day 15 before the presence or absence of amoebae was determined and the number of amoebae per gram of sediment was estimated.

Measurement of physico-chemical parameters of water

The Physico-chemical parameters of water (pH, Dissolve Oxygen (DO), temperature and salinity) were measured in-situ using Multi Probe Systems. For Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Ammonical Nitrogen (AN), their measurement was done in the laboratory following standard protocols.

Analysis of sediment texture

Texture of sediments was analysed following standard protocols described by Pasternack, (2002).

Results and discussion

Four species of free-living amoebae were isolated and identified from water samples of Setiu Wetlands and seven amoeba species were identified from the sediments (Table 1). All amoeba species were identified following descriptions of Page (1983, 1988) but those species that were labeled as amoeba A and amoeba B in this study were the amoebae whose characteristics did not fit the criteria for any genus suggested by Page. One amoeba species was labeled as *Vannella*-like amoeba due to dissimilarity in morphology of this amoeba with the genus *Vannella*. Some free-living amoebae tend to have different morphology as they respond to the water salinity resulting in difficulty to recognize them (Smirnov, 2007). Among the amoeba species found in this study, the genus *Acanthamoeba* was the most frequently encountered in all sampling locations indicating its wide occurrence in Setiu Wetlands.

The water physico-chemical parameters measured at each sampling location are presented in Table 2. In general, the values of water parameters measured at all sampling locations are quite uniform except for BOD, TSS and salinity. Since free-living amoebae isolated in the present study were from brackish water, their presence therefore are very much affected by water salinity. In the present study, *A. polyphaga*, was found in water at most sampling locations indicating that this amoeba has the ability to tolerate a wide range of BOD, TSS and salinity. *Acanthamoeba* sp on the other hand, was encountered at locations 1, 3 and 6. Although BOD, TSS and salinity values varied at these locations to indicate its wide tolerance as *A. polyphaga*, other factors (biological or physical factors) might influence its occurrence in this study. *Vahlkampfia* sp was present at locations 1 to 5 and was absent at locations 6 and 7. Amoeba A was encountered only at locations 3 and 4. At these locations, the values of TSS are high indicating that this amoeba can tolerate high TSS in water. Some of the amoeba species isolated in this study are also common found in soils such as *A. polyphaga*, *Vahlkampfia* and *Acanthamoeba* sp (Page, 1988). Their occurrence in water in Setiu Wetlands to suggest further their significance in our environment. In this preliminary study, the

relationship between different amoeba species and their distribution with water quality was not clearly shown. Further study should be conducted to obtain more information on amoeba distribution and water quality.

Table 1. Distribution of amoeba species in water and sediments at various locations in Setiu Wetlands Terengganu.

Sampling Locations/Locations	Species of amoebae in water	Species of amoebae in sediments
C1 005°38'30N, 102°45'27E	<i>A. polyphaga</i> <i>Acanthamoeba</i> sp. <i>Vahlkampfia</i> sp.	<i>Acanthamoeba</i> sp., <i>A. polyphaga</i> , Amoeba B
C2 005°38'53N, 102°45'09E	<i>A. polyphaga</i> <i>Vahlkampfia</i>	<i>A. polyphaga</i> , Amoeba A
C3 005°39'10N, 102°44'46E	<i>Acanthamoeba</i> sp. <i>Vahlkampfia</i> sp. Amoeba A	<i>Acanthamoeba</i> sp., <i>A. polyphaga</i> , <i>Mayorella</i> sp., Amoeba B
C4 005°39'21N, 102°44'17E	<i>A. polyphaga</i> <i>Vahlkampfia</i> sp. Amoeba A	<i>Acanthamoeba</i> sp., <i>Mayorella</i> sp., <i>Vahlkampfia</i> sp.
C5 005°40'10N, 102°43'49E	<i>A. polyphaga</i> <i>Acanthamoeba</i> sp. <i>Vahlkampfia</i> sp.	<i>Vahlkampfia</i> sp. Amoeba A, <i>Vannella-like amoeba</i>
C6 005°40'55N, 102°42'41E	<i>A. polyphaga</i>	<i>Vannella-like amoeba</i>
C7 005°41'16N, 102°42'26E	<i>A. polyphaga</i>	<i>Vannella-like amoeba</i>

Table 2. Water physico-chemical parameters at various sampling locations

Sampling Locations	BOD (mg/L)	AN (ppm)	TSS (mg/L)	DO (mg/L)	pH	Salinity (ppt)	T°C
C1	1.50	0.27	32	5.42	7.41	24.60	30.00
C2	0.97	0.24	50	5.67	7.52	28.50	30.41
C3	1.37	0.27	67	5.50	7.53	30.01	30.57
C4	1.27	0.28	81	6.04	7.65	32.10	30.75
C5	1.08	0.20	48	5.95	7.67	32.08	30.63
C6	1.20	0.23	58	5.78	7.64	31.81	31.09
C7	1.90	0.29	56	5.37	7.58	30.73	31.00

Results for sediment texture analyses and estimation of the number of amoebae in sediments at seven locations at Setiu Wetlands are shown in Table 3. The highest number of amoebae was found in sampling location 7 whereas the number of amoebae was relatively low at locations 2, 3, 5 and 6. Locations 1 and 4 had the second and third highest amoeba count, respectively. Sediment texture did not seem to contribute significantly to the abundance and types of amoebae at each sampling location. The direct enumeration on number of amoebae in water was not done due to no proper protocol available. Indirect enrichment cultivation method gives underestimation of the true count (Rogerson and Gwaltney, 2000).

Table 3. Sediment Texture Analyses and number of amoebae present at various sampling locations

Sampling Location	Sediment type	%	Type	Number of amoebae (x 10 ³ /g)
C1	Sand Silt clay	8.59 12.35 79.06	Clay	2.45
C2	Sand Silt clay	79.18 5.95 14.87	Sandy loam	2.77
C3	Sand Silt clay	87.16 2.88 9.96	Sand	1.8
C4	Sand Silt clay	10.00 79.30 10.70	Silt loam	8.57
C5	Sand Silt clay	98.46 1.22 0.32	Sand	0.56
C6	Sand Silt clay	9.42 13.40 77.18	Clay	0.82
C7	Sand Silt clay	0.54 7.91 91.55	Clay	92.0

The amoebae identified from the sediments of Setiu Wetlands in this study were *A. polyphaga*, *Acanthamoeba* sp., *Mayorella* sp., *Vannella*-like amoeba and two unidentified amoeba species that were labeled as amoeba A (similar amoeba that was encountered in water in the present study) and amoeba B. The genus *Acanthamoeba* was isolated from most sediment samples (in Locations 1, 2, 3 and 4) indicating it is a dominant amoeba species in Setiu Wetlands. *Mayorella* sp was observed both in sampling locations 3 and 4, and this amoeba is known as euryhaline species that can tolerate salt water (Page, 1983) so its presence in this area especially in sediments is expected. Its presence in the water at this sampling location, however was not detected and this was probably due to its number is too small in the water. The amoebae that were isolated from the sediment were

cultivated and fed with *E. coli* before their identification was done. Through this technique, some of the amoeba species disappeared and some of them reappeared as noted by Smirnov (2007) in his study using brackish-water sediment.

Amoeba A was encountered in sediment in locations 2 and 5, suggesting that this amoeba species prefers sediments with sandy loam and sand texture but detailed analyses of its ecological preference should be conducted. Amoeba B was found in Locations 1 and 3, without showing any preference or association with the sediment texture. The amoeba was present either in sediments with clay or sand texture to suggest that its presence in the sediments were influenced by other factors, not by sediment texture alone.

Vannella-like amoeba was observed in Locations 5, 6 and 7 but their number was relatively high in Location 7. This location is near to the brackish water cage cultures activities that supply nutrients and perhaps provide favorable environment for the amoeba to grow abundantly at that area.

Higher abundance of amoebae has been associated with fine sediment with greater porosity, organic carbon content and bacterial abundance, whereas lower densities of amoebae have been associated with higher concentration of Acid Volatile Sulphides (AVS) (Burkholder *et al.*, 1998). From this study, the abundance of amoebae at each sampling locations may be associated with the fine structure of the sediment but this relationship is less obvious for most locations. Other relevant factors such as bacterial presence and nutrients should also be investigated.

Free-living amoebae are the controller of bacteria population in soil, so the abundance of *E. coli* or other bacteria affect the abundance of amoebae. In Location 7 where amoebae was most abundant (9.2×10^4 amoebae /g sediment), the clay texture of the sediment perhaps is one of the contributing factors since small particles will provide protection to the amoebae and also clay contains more nutrient compared with silt and sand. Location 7 is also near to cage culture activities at Gong Batu, therefore this area maybe rich in bacteria that serve as food for the amoebae. The abundance of amoebae in sand was lower than in clay and silt loam (Pasternack, 2002). Factors such as nutrient availability and the presence of other microorganisms perhaps contribute to the presence of amoebae at each sampling location.

Conclusion

Four species of free-living amoebae were isolated from brackish water taken at various locations in Setiu Wetlands, Terengganu. They were *A. polyphaga*, *Acanthamoeba* sp., *Vahlkampfia* sp. and unidentified amoeba, amoeba A. Seven species of free-living amoebae were isolated and identified in sediments of Setiu Wetlands, Terengganu. They are *Acanthamoeba polyphaga*, *Acanthamoeba* sp., *Mayorella* sp., *Vannella*-like amoeba, amoeba A and amoeba B. The number of amoeba species present at each sampling location varied, and their estimated number was observed high in sampling location with clay texture and which is close to the food sources (such as mariculture activities).

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