Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 10 (8): 652-657, 2010
ISSN 1812-5654

Bacterial Communities in Kuantan Estuary of Pahang Malaysia

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Abstract: Physicochemical parameters influence the vertical spatial distribution of microbial communities in any water bodies. Based on this perspective, a study on physicochemical parameters and bacterial community was carried out from five stations on January-June 2009 at the Kuantan estuary of Pahang, Malaysia. The temperature was ranged from 25.01-27.48°C, salinity fluctuation observed 0.03-25.84 ppt, Dissolved Oxygen (DO) 6.10 to 10.73 mg L^{-1}, specific conductivity ranged from 0.10 to 42.43 mS cm^{-1}, Total Dissolved Solids (TDS) ranged from 0.05 to 26.36 g L^{-1}) and pH varied from 5.69 to 8.11 and chlorophyll a ranged 0.01 to 1.14 μg L^{-1}. The nitrite concentration was higher at St. 5 (0.19 mg L^{-1}) followed by St. 4 (0.16 mg L^{-1}) and it was lowest at St. 1 (0.13 mg L^{-1}). Similarly, high phosphorus content (0.17 mg L^{-1}) was found at St. 4 followed by St. 5 (0.16 mg L^{-1}) while, it was lowest at St. 1 (0.08 mg L^{-1}). Out of 19 isolated bacteria most dominant bacteria were *Citrobacter freundii* followed by *Leuconostoc* sp. and *Staphylococcus xylosus*. High bacterial colony (cfu mL^{-1}) was observed at St. 4 (570 cfu mL^{-1}) in water column followed by St. 5 (490 cfu mL^{-1}). In contrary, it was lowest at St. 2 (213 cfu mL^{-1}). Meanwhile, the highest bacterial colony in sediment was observed at St. 4 (390 cfu mL^{-1}) followed by St. 5 (333 cfu mL^{-1}). It was lowest observed at St. 2 (167 cfu mL^{-1}). Nevertheless, a continuous monitoring of water quality is needed in this estuary especially at St. 4 and 5 which could be alarming in the long run due to deposition of nutrients from the outlets of fishing villages and industry sources.

Key words: Bacterial community, physicochemical parameters, kuantan estuary, primary productivity, nutrients

INTRODUCTION

Microorganisms constitute a huge and almost unexplained reservoir of resources likely to provide innovative applications useful to man and thriving in almost every habitat (Jain *et al*., 2005). Bacterial diversity constitutes the most extraordinary reservoir of life in the biosphere that people have only just begun to explore and understand. The study of bacterial diversity is important to solve new and emerging disease problems. Though, the negative effects of bacteria such as disease are well known, their often subtle functions explain why their biodiversity positively affects humans. Distribution of bacteria depends on changes in water temperature, salinity and other physicochemical parameters (Alavandi, 1990). Bacteria not only maintain the pristine nature of the environment, but also serve as biological mediators through their involvement in the biogeochemical processes. Bacterial populations have different metabolic and enzymatic properties and the composition and dynamics of bacterial populations, therefore, affect the overall cycling of organic matter (Martinez *et al*., 1996; Garrity, 2001).

Bacteria typically represent more than 90% of microorganisms in non-extreme aquatic habitats. In the 1980s, it was believed that bacterial species inhabiting brackish water systems do not differ taxonomically from bacteria inhabiting the surrounding terrestrial environments (Rheinheimer, 1980). Only gram positive bacteria (i.e., *Actinobacteria* and *Firmicutes*) were considered to be absent from the water column (pelagic zone) of water habitats (Hahn, 2006). These assumptions were based on cultivation experiments that resulted, on the one hand, in the isolation of the same opportunistic taxa (e.g., *Pseudomonas* sp.) from soil and brackish water samples and on the other hand, in the isolation of only a few gram positive bacteria (*Actinobacteria* and *Firmicutes*) from brackish water sites (Hahn, 2006).

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Although, efforts have been made to reveal the microbial ecosystems in brackish water environment on the basis of traditional cultivation methods, it is now widely recognized that only 0.001 to 15% of the total cell counts in environmental samples can be cultured. However, cultivation-based study remains important, since the ecological role of prokaryotes in natural environments can be estimated only when they are successfully cultivated and characterized. Bacteria are important components of coastal microbial communities which plays an important role in nutrient and carbon cycling trophic dynamics of aquatic food webs and in the remineralization of dissolved and particulate organic matter (Pierce and Turner, 1992; Chau, 2006). The abundance, biomass and composition of coastal microbial communities should be much more variable than those of open sea communities, due to the greater variability in factors influencing their growth characteristic of coastal water. Environmental parameters do not only influence the size and composition of microbial communities, but also the activity and viability of these bacteria (Nielsen and Kiorboe, 1991).

The study of physicochemical parameters is also important as these factors determine the distribution and composition of bacterial community (Hahn, 2006), temporarily and spatially within habitat (Lindstrom, 2001; Dominik and Hofle, 2002; Yannarell and Triplett, 2004) as well as between habitats (Yannarell and Triplett, 2004; Lindstrom 2001; Wu and Hahn, 2006). Hence, it is very important to study the vertical and spatial distribution of the microbes and try to determine the range of physico-chemical parameters in order to understand the dynamic of microbial communities.

Kuantan estuary is the outskirts of Pahang River that is important for aquaculture and feeding area for brackish water fish and shrimps. With the mangrove area surrounding part of its terrestrial area, the ecosystem is considered to be diverse in nature. Thus, this study endeavor the information on bacteria vertical and spatial distribution for future study; including bacteria that can degrade hydrocarbons, pathogenic microbes for molluscs and aquaculture potential fishes. Fisherman along the Kuantan estuarine areas occasionally suffered gastro intestinal illness due the consumption of fisheries resources. Besides, it is evident that fishes cultured in cages were contaminated with numbers of bacterial diseases. It is hypothesized that Kuantan estuary might have significant varieties of pathogenic bacteria which could be detrimental for aquaculture and as well human utilization of existing sea foods.Furthermore, knowledge of the physico-chemical, biological and chemical processes in coastal waters are essential in order to understand the mechanisms involved in the distribution of pollutants from their points of entry in the open sea.Till date no profound study has been conducted on water quality and microbial diversity in this astounding eutrophic estuary. In these contexts, the study was aimed to determine the spatio-temporal distribution of bacterial communities pertinent to existing physicochemical parameters along the Kuantan estuary.

MATERIALS AND METHODS

Study area: Kuantan estuary is located within the capital state of Pahang, Malaysia. Kuantan estuary was chosen as the sampling site because it is believed to have a diverse species of bacterial communities, as it consists of brackish water ecosystem that is surrounded by mangrove swamps. Owing to the rapidly increasing importance of the sustainable management of brackish water resources, detailed knowledge on the diversity, specific functions and ecology of microorganisms inhabiting brackish water ecosystems is urgently needed.

Location of sampling sites: Five different stations of Kuantan estuary were chosen which represented the Kuantan estuary (Fig. 1). The distance between each station was 500 m. Those stations were selected based on different locations and ecosystem. Station 1 is the area that near to the open sea. Station 2 near the fishing village occupied with fishing activity. Station 3 near the mangrove swamp. Station 4 is the most disturbed area because all the pollutants from non-point sources enters into this area. Station 5 is the last station, which can be considered as undisturbed area and it is near the fresh water zone.

Physicochemical parameters: Field observation on physico-chemical parameters (temperature, pH, salinity, conductivity, TDS, dissolved oxygen and chlorophyll a) of Kuantan estuary was recorded by using Hydrolab DataSonde 5 and Hydrolab DataSonde 4 from 5 different stations. The analysis procedures for total phosphorus nitrate and nitrite was adopted from methods as described (Parsons *et al*., 1992). Water samples were collected at different depths by using Van Dorn water sampler. Both water and sediment samples were kept in the Falcon Tubes prior to laboratory analysis.

Bacterial isolation from water and sediment samples: Water samples were collected by using Van Dorn water sampler and sediment sample by using an Ekman dredge bottom sampler. Water samples were collected at each site at different depths (surface, middle and bottom). All

Fig. 1: Kuantan Estuary and its designated sampling areas (Adapted from: Google-Map data © 2009 and Map IT, Europa)

samples were kept in Falcon Tubes and ice chest was used to kept the samples for transportation to laboratory. Serial dilution was prepared by pipetted 10 mL pure sample of water into centrifuge tube and from pure sample, 1 mL was pipetted into another centrifuge tube where, 9 mL sterile distilled water was added. The samples were prepared until dilution 10^{-3}. Then, 100 µL of each samples were pipetted with different serial dilution according to designated stations onto Tryptic Soy Agar (TSA) media plate and spreaded using hockey stick. After 24 h of incubation at 37°C, the plates were observed under coulter counter and the colony forming unit (cfu) was recorded.

Meanwhile, 10 g of each sediment was weighted and 20 mL sterile distilled water was added into centrifuge tube and vortexed until homogenous. Then, the samples were incubated 30 min at 37°C and 200 rpm in orbital shake. Samples were centrifuged at 5000 rpm for 20 min. The supernatant was poured into new centrifuge tube and only 10 mL supernatant was taken for serial dilution. Serial dilution was prepared by pipetted 10 mL pure sample into centrifuge tube and from pure sample; 1 mL was pipetted into another centrifuge tube where, 9 mL sterile distilled water was added. The samples were prepared until dilution 10^{-3}. One hundred microliter of each samples were pipetted with different serial dilution according to designated stations onto TSA media plate and spreaded using hockey stick. After 24 h of incubation at 37°C the (cfu) was recorded.

After direct counting, the morphology of colonies was observed and colonies with different morphology were isolated from TSA media using sterile tooth pick and patched on new TSA agar plates. The particular plates were incubated at 37°C. After 24 h incubation, the individual colony that showed growth on all plates was picked and streaked on fresh TSA plates and incubated for 37°C. Each of these colonies was subcultured again on fresh TSA plates to obtain pure culture. The cultures were maintained on TSA plates. For longer storage, the cultures were preserved in glycerol stock at -80°C. Morphology of each single colony was observed for classification by observing at different criteria such as, color, optical density, shape, edge, elevation and also texture by using naked eyes and also using dissecting microscope. All morphological characteristics of bacteria samples were recorded. Gram staining test was performed to differentiate between two major taxonomic groups, the Gram positive and Gram negative bacteria. Identification of isolates was done using API 20E, API STAPH and API 20 STREP. All the results obtained from the API 20E, API STAPH and API 20 STREP tests were analysed by using respective Stand Alone Version 1.1 Software (Biomeriux, France).

RESULTS AND DISCUSSION

The values in the Table 1 show the physicochemical parameters condition for different stations (ST1, ST2, ST3,

Table 1: Trends of physicochemical parameters at Kauntan estuary

Stations	Temperature (°C)	Conductivity (mS cm^{-1})	TDS (g L^{-1})	Salinity (ppt)	DO (mg L^{-1})	pH	Chl a (μg L^{-1})
St. 1 (S)	27.48±0.02	8.14±0.02	6.64±0.02	6.63±0.01	10.73±0.03	7.45±0.01	0.02±0.01
St. 1 (M)	27.32±0.05	42.43±0.01	26.36±0.01	25.84±0.01	10.30±0.02	7.95±0.02	0.01±0.01
St. 1 (B)	27.05±0.03	42.43±0.02	26.31±0.01	25.80±0.02	10.25±0.01	8.11±0.01	0.01±0.01
St. 2 (S)	27.38±0.01	0.60±0.02	0.37±0.02	0.28±0.02	10.49±0.01	7.04±0.01	0.07±0.02
St. 2 (M)	27.14±0.02	27.45±0.01	17.47±0.03	16.74±0.02	9.90±0.02	7.68±0.01	1.03±0.01
St. 2 (B)	26.49±0.04	41.23±0.01	25.61±0.02	25.01±0.03	9.73±0.02	8.11±0.02	0.91±0.04
St. 3 (S)	27.14±0.03	0.40±0.01	0.25±0.01	0.16±0.05	10.00±0.02	6.84±0.01	0.05±0.01
St. 3 (M)	26.64±0.03	24.45±0.02	15.12±0.01	13.89±0.04	9.44±0.01	7.20±0.02	0.11±0.02
St. 3 (B)	26.14±0.05	37.79±0.01	24.21±0.02	23.80±0.04	9.35±0.02	7.93±0.01	0.09±0.02
St. 4 (S)	27.07±0.05	0.32±0.04	0.21±0.02	0.17±0.05	8.19±0.02	6.48±0.01	1.04±0.01
St. 4 (M)	25.67±0.04	3.93±0.01	2.40±0.03	1.98±0.04	6.86±0.02	6.31±0.00	1.14±0.05
St. 4 (B)	25.96±0.03	22.05±0.02	14.31±0.03	13.50±0.02	6.10±0.01	6.68±0.01	0.92±0.01
St. 5 (S)	25.80±0.02	0.15±0.00	0.05±0.01	0.03±0.01	7.87±0.00	6.53±0.01	0.10±0.01
St. 5 (M)	25.35±0.02	0.13±0.01	0.05±0.01	0.03±0.01	7.14±0.02	5.82±0.023	1.04±0.01
St. 5 (B)	25.01±0.04	0.10±0.02	0.05±0.01	0.03±0.01	6.95±0.01	5.69±0.03	1.04±0.01

ST4 and ST5) for January-June 2009. Salinity showed the highest values (25.84 ppt) at the middle part of St. 1 and the lowest values (0.03 ppt) were observed at St. 5 from surface to bottom. Total dissolved solids showed the highest values 26.36 g L^{-1} at middle of St. 1, while middle of the ST 5 showed the lowest values 0.05 g L^{-1}. There could be many factors that affected TDS values such as geology and soil in the watershed and siltation from mangrove sources. Besides, the rocks of the nearby mountains and soil may release ions when water flows over them especially during rainy seasons. The wastewater effluent might enter from outlets of fishing village and Jetty areas (used by fishing boats) which contains both suspended and dissolved solids that mixed into water bodies of Kuantan estuary. Apart from this decaying plants from mangroves and dissolved organic particles might contribute to increase the higher TDS concentration. Conductivity was 42.43 mS cm^{-1} was the highest at the middle and bottom part of St. 1 and it was lowest (0.10 mS cm^{-1}) at St. 5. Temperature varied from 25.01 to 27.48°C at St. 5 (bottom) and St. 1 (surface), respectively. The ST5 bottom area showed the lowest temperature because area nearer to the mouth river where the depth is around 20 m. Earlier study (Bartram and Ballance, 1996) has supported this statement, in which the differences in temperature between 0 to 2°C indicate that the water body maintains the temperature fluctuations. So, that the amount of total dissolved solids in those stations were higher compared to St. 5 due to the overloading of nutrients and urban and rural runoff.

Mean values of pH of all stations ranged from 5.69 to 8.11. The pH value lower at St. 5 may be due to the water comes from fresh water and run off from the bank of the river soil. Dissolved oxygen showed the highest value (10.73 mg L^{-1}) at the surface of St. 1 while, it was lowest (6.10 mg L^{-1}) at the bottom of St. 4. Actually, the concentration of dissolved oxygen in water is affected by many factors and not only ambient temperature, but also atmospheric pressure, ion activity, the volume and velocity of water flowing in the water body and the amount of organisms using oxygen for respiration of decomposers. As such St. 4 was the most polluted areas among the sampling stations. Probably high amount of microbial communities at the bottom level were utilized DO at that station compared to other stations. Furthermore, many chemical and biological reactions in ground water and surface water depend directly or indirectly on the amount of available oxygen. Dissolved oxygen is necessary in aquatic systems for the survival and growth of many aquatic organisms and important parameter of the water bodies. The results showed that there were no significant variations on physico–chemical parameters of water among the five different stations of Kuantan estuary. It indicates that this estuary is well mixed.

The amount of chlorophyll a in the water column is indicative of the biomass of phytoplankton, which can indicate the nutrient levels in the water column (United States Environmental Protection Agency, 2002). Excessive nutrients and plant growth can in turn decrease DO levels and increase turbidity. The range of chlorophyll a at all stations is between 0.01 to 1.14 μg L^{-1}. It was evident from the result that the amount of chlorophyll a in the surface water of St. 4 is relatively higher compared to other stations. Station 4 is located near the Hospital Tengku Ampuan Afzan (HTAA), where there is a rapid growth of development. Phytoplankton blooms and their obnoxious smell at St. 4 due to the amount of sewage and nutrients discharged both from riverbank restaurants and urban settlement.

Nitrite determination in water samples: The lowest value of nitrite was 0.13 mg L^{-1}. The highest value of nitrite was observed at St. 5 (0.18 mg L^{-1}) due to the influx of fresh rainwater and probably heavy runoff from the upstream mixed with the downstream of Kuantan estuary (Fig. 2).

The highest value of total phosphorus concentration was 0.15 mg L^{-1} at St. 4 (Fig. 3). This was possibly

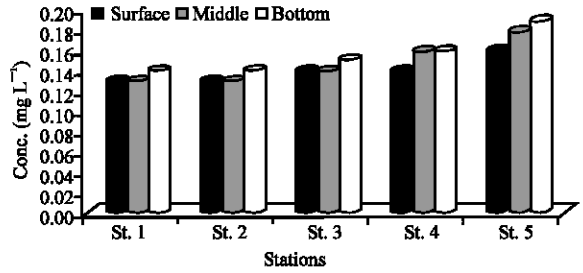

Fig. 2: Nitrite concentration in each station of Kuantan estuary

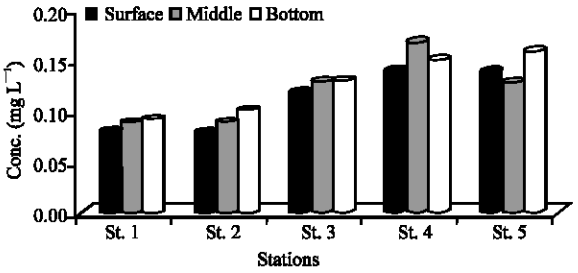

Fig. 3: Total phosphorus in each station of Kuantan estuary

due to the decomposition of macrophyte and phytoplankton releasing phosphorus to the water column. Besides, St. 4 receives high amount of pollutants due to its location which is highly disturbed by human activities. So, high concentration of total phosphorus in surface water of St. 4 might be due to the variety of sources including domestic wastewater, detergents, industrial process wastes and urban runoff. High concentration of phosphorus at St. 4 was due to the geochemical changes that occurred when fresh water enters the estuary by the rainfall. Previous study performed by Smith (1984) have mentioned that geochemical arguments can be made to argue for phosphorus limitation of phytoplankton growth in pristine marine environments.

Bacterial community: The higher number of bacterial colony (cfu mL^{-1}) was observed at St. 4 (~570 cfu mL^{-1} in water coloumn, followed by St. 5 (490 cfu mL^{-1}) and St. 3 (367 cfu mL^{-1}). It was lowest at St. 2 (213 cfu mL^{-1}) (Fig. 4). Interesting fact that sediment sample at St. 4 has shown highest number (390 cfu mL^{-1}) of bacterial colony followed by St. 5 (333 cfu mL^{-1}). The St. 2 showed lowest among the stations. This might be presence of high organic matter which could be the sources of bacteria at St.s 4 and 5. High amount of nutrients phosphorus and nitrites has supported this findings.

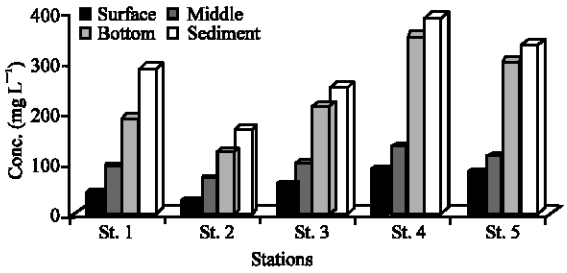

Fig. 4: Spatial and vertical distribution of bacteria at Kuantan estuary

Table 2: Bacterial Community at Kuantan Estuary

Name of bacteria	No. of species
Staphylococcus xylosus, *Aeromonas hydrophila*, *Serratia ficaria*	3
Enterobacter sakazakii, *Citrobacter freundii*, *Streptococcus bovis* II 1, *Enterococcus faecium*	4
Serratia odorifera 1	1
Klebsiella oxytoca, *Escherichia coli* 1, *Serratia liquefaciens*, *Klebsiella terrigena*, *Leuconostoc* sp., *Lactococcus lactis* sp. *lactis*, *Enterococcus avium*, *Staphylococcus simulans*	8
Enterobacter cloacae, *Staphylococcus carnosus*, *Staphylococcus aureus*	8

The most dominant gram negative bacterial species was *Citrobacter freundii*, followed by *Enterobacter sakazakii* and *Klebsiella oxytoca*. *Citrobacter freundii* and *Enterobacter sakazakii* were found in the surface water of St. 2, while *Klebsiella oxytoca* was found on the surface water of the St. 4. However, the gram negative bacteria were found predominantly at St. 4 and 5 followed by St. 2. Station 4 which experiences high human activity and due to the domestic sewage run off the pathogenic bacteria like *Enterobacter cloacae* and *Staphylococcus aureus* present in huge quantity. There were also some beneficial bacteria like *Staphylococcus carnosus* present at St. 5. The source of these bacteria in the Kuantan estuary might be from food industries located near the estuary. The floating ships near the Jetty and restaurants always produced nutrients around the area. As such greenish color of water and obnoxious smell always present at St. 4 (Table 2).

CONCLUSION AND FUTURE DIRECTION

The findings showed that the study area reasonably diverse with bacterial communities. The identified bacteria were *Enterobacter sakazakii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella terrigena*, *Serratia odorifera*, *Serratia ficaria*, *Serratia liquefaciens*, *Aeromonas hydrophila*, *Leuconostoc* sp., *Enterococcus faecium*, *Lactococcus*

lactis sp., *lactis Enterococcus avium*, *Streptococcus bovis*, *Staphylococcus simulans*, *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Staphylococcus aureus*. This study aimed to describe the relationships between physicochemical parameters with the nutrient contents and bacterial communities that flourish around the Kuantan estuary. Hence, this study could serve as foundation for identifying microorganisms for specific use such as hydrocarbon degraders and pharmaceutical purposes. The understanding of the bacterial diversity gives us a basic idea of microbe's potential activity. Eventhough, the data did not show any indication of severe pollution around the study area.But essential steps still need to be taken by using ISO standard water quality guidelines for tropical environments where considerable variation in temperature and salinity can occur at different times of the year. Nevertheless, a long term extensive monitoring program can be conducted which could enable us to know the spatio-temporal patterns of bacterial standing stocks along with the primary productivity and their interactions at Kuantan estuary. In addition, the study need to be focused towards molecular study 16S RNA of the potential microbes at Kuantan estuary in the near future. The sequencing results may perhaps give the probable microbial ecology of fascinating coastal water Pahang, Malaysia.

ACKNOWLEDGMENTS

The authors extended their heartfelt thanks to the Research Management Centre, International Islamic University Malaysia for granting funds to conduct this research. Authors also express their gratitude to technical staffs (Mr. Azfar, Mr. Nahar, Mr. Lazuardi and Azizul), Institute of Oceanography of Maritime Studies, Kulliyyah of Science for their invaluable assistance throughout the sampling period.

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