



RESEARCH ARTICLE

A STUDY ON THE ROLE OF MICROBIAL DIVERSITY AND THEIR INTERFERENCES WITH THE ECOSYSTEM OF MANAKUDY ESTUARY

¹*Ajithamol, A., ²Michael Babu, M., ²Saraswathy, S. and ¹Venkadesh, B.

¹Muslim Arts and Science College, Thiruithancode, S. India

²Centre for Marine Science and Technology, M.S. University, S. India

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ABSTRACT

Water samples were collected from the six stations of Manakudy estuary. During the present investigation of bacterial species presented in the water samples of estuary were *Zobellia russellii*, *Pseudomonas tolaasii*, *Staphylococcus sp.*, *Helicobacter sp.*, *Escherichia sp.*, *Paracoccus sp.*, *Proteus sp.*, *Maraxella sp.*, *Halmonas sp.*, *Aquasalimonas sp.*, *Neisseria sp.* and *Marinobacter sp.* The dominant bacterial strain *Zobellia russellii* were finally identified by 16S rRNA sequence analysis. The objective of the study of microbial diversity in the estuarine environment is the vital beginning to estimate the major compartment of biodiversity and effective causes of human interferences.

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INTRODUCTION

Microorganisms can be defined as life forms that cannot be seen with the unaided eye. They are widely distributed in the aquatic environment. The broad definition encompasses an extensive and diverse assemblage of organisms, which exhibit widely different morphological, ecological, physiological characteristics. The microbial diversity encompasses vast number of species and the number of species of these groups known to science (Groombridge, 1992). In estuarine areas, tides are natural phenomena that cause changes in the densities of bacterial populations and heterotrophic activities (Cunha et al., 2001). The distribution of microbial activities in estuarine systems is clearly complex and variable. The distributions of microbial activities and the major factors involved in controlling these distributions in estuaries. In water depth, the proportion of organic matter deposited in the sediments which is degraded by microbial processes appears to be similar in fresh and salt waters (Wollast and Billen, 1981). Studies on bacterial ecology in Indian waters, as elsewhere, have in the past been carried out with the culturable fractions of microorganisms. Although these viable fractions from only a small fraction of total microorganisms, they can still be used

for a comparison of data representing different times of the year or different areas (Van Es and Meyer-Reil, 1982). Estuaries are heterotrophic systems, with overall respiration, mainly bacterial, exceeding primary production (Heip et al., 1995).

MATERIALS AND METHODS

The surface water samples were collected from the Manakudy estuary once in the first week of month for one year extending from May 2012- April 2013.

Collection of water sample

Water sample were collected separately in the sterile bottles and brought to the laboratory for microbiological analysis

Total heterotrophic bacterial count

1ml of water sample was mixed with 9ml distilled water and serially diluted. 1gm of soil sample was dissolved in 99ml distilled water and serially diluted using distilled water. 0.1ml of sample was taken from each dilution and poured into the surface of nutrient agar and spread uniformly by using sterilized 'L' rod. After inoculation the plates were incubated in an inverted position at 37 °C for 24hrs. For each dilution three

*Corresponding author: Ajithamol, A.

Muslim Arts and Science College, Thiruithancode, S. India.

replicates were maintained. After incubation, petriplates with 30-300 colonies were selected for the total viable counts. The bacterial population was expressed as number of Colony Forming Units (CFU) per ml of water.

Isolation and identification of heterotrophic bacterial flora of water

Morphologically dissimilar, well –isolated colonies were randomly selected and streaked on the nutrient agar slants. It was done after observing the morphology and pigmentation of the colony. The slant cultures were then stored at 4°C refrigerator.

Gene sequencing of heterotrophic bacteria

The bacterial strain *Zobellia russellii* were finally identified by 16S rRNA sequence analysis. All the PCR reaction was carried out by Genei Bio tech Ltd (Bangalore).

Manakudy estuary were *Zobellia russellii*, *Pseudomonas tolaasii*, *Staphylococcus* sp., *Helicobacter* sp., *Escherichia* sp., *Paracoccus* sp., *Proteus* sp., *Maraxella* sp., *Halmonas* sp., *Aquaslimonas* sp., *Neisseria* sp. and *Marinobacter* sp. In water samples of different stations bacterial population based on numerical counts showed wide fluctuations in their distribution.

Seasonal distribution of total bacterial counts in water sample

Seasonal variation of bacterial counts in estuarine water samples were low at station 1 (9.95×10^5 CFU/ml) of non-monsoon season and high in northeast monsoon season of station 5 (20.35×10^5 CFU/ml) (Table 1). In the present study showed the maximum bacterial counts during the monsoon season and minimum bacterial counts during non-monsoon of the study period. It is similar to that of the seasonal variation of higher counts occurs in wet season than dry in Imo river, Nigeria (Ogbonna, 2010). Environmental stress brought by the contamination for the reduction in microbial population and

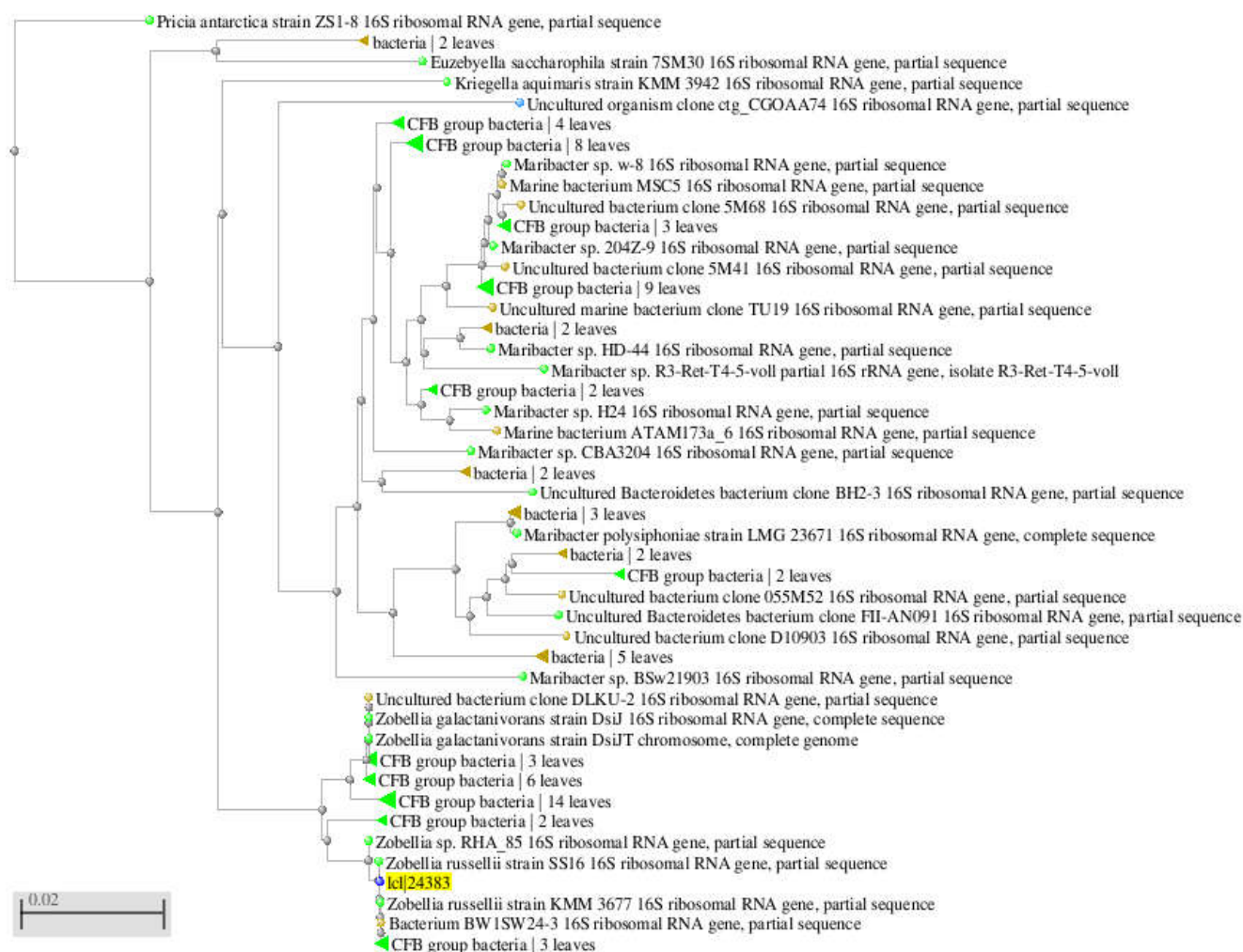


Figure: Dendrogram of the sequenced data and retrieved sequence data *Zobellia russellii*

RESULTS AND DISCUSSION

During the present investigation of bacterial genera encountered in the water samples of the six stations of

diversity. The total heterotrophic bacteria of the estuary system revealed high counts in the monsoon season than non-monsoon season, this may be due to the more favourable physico chemical conditions such as DO and the nutrients nitrate, phosphate, silicate.

Table 1. Seasonal variation on total heterotrophic bacterial count (CFU×10⁵) in the water sample during the study period May 2012 - April 2013

	Southwest monsoon	Northeast monsoon	Non-monsoon
Station 1	12.20±0.26	17.30±0.58	9.95±0.43
Station 2	13.65±0.51	13.10±0.78	12.70±0.40
Station 3	13.58±0.66	12.03±0.37	13.60±0.12
Station 4	16.93±0.41	11.48±0.66	11.38±0.32
Station 5	14.25±0.21	20.35±0.26	11.45±0.30
Station 6	15.98±0.19	13.38±0.14	10.33±0.16

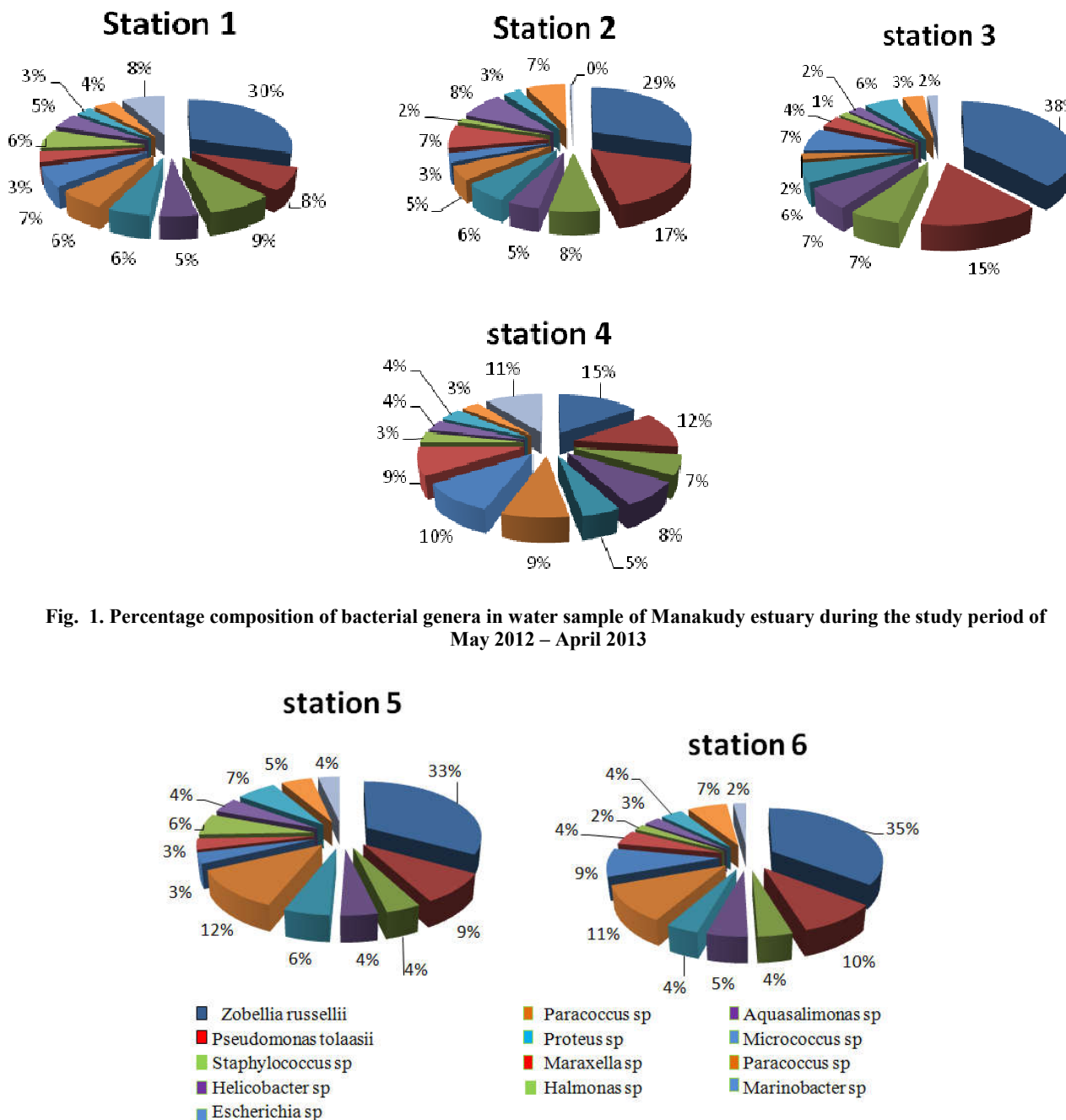


Fig. 1. Percentage composition of bacterial genera in water sample of Manakudy estuary during the study period of May 2012 – April 2013

Kuch (1974) worked out the distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour.

Percentage occurrence of microflora in the water sample

The annual percentage occurrence of microflora in the water sample of the estuarine study stations were presented in the

(Fig. 1). Among the bacterial genera in the water sample, the dominated species is *Zobellia russellii* (35.45%) in station 6 throughout the study period. Higher number of pathogenic bacteria was identified in the sampling areas. Bacteriological examination of water is therefore a powerful and foremost tool in order to foreclose the presence of microorganisms that might

constitute a health hazard (Bonde, 1977). Wastewaters may become seriously dangerous leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystems (Beg et al., 2003). The man made pollution are transmission of diseases, eutrophication of natural water bodies, accumulation of toxic or recalcitrant chemicals and destabilization of ecological balance and negative effects on human health (Amisu et al., 2003). The potable water is one that does not contain chemical substances or microorganisms in amounts that could cause hazard to health (Alonge, 1991). Also improper management practices have largely being responsible for the gross pollution of the aquatic environment with concomitant increase in water borne diseases especially typhoid, diarrhoea and dysentery (Osibanjo and Adie, 2007). In the present study of the water and soil samples are observed with bacterial species like *Proteus species* can cause health problems while the *Staphylococcus spp*s can cause skin infections and respiratory related problems. In Manakudy estuary, *Escherichia* were noted in sampling stations areas of human activities disposal of wastages. *Escherichia* is a common bacterium that is found in water that has been contaminated with fecal matter.

Gene Sequencing of Heterotrophic Bacteria

The 16s ribosomal RNA gene was sequenced for selected strains about 1370bp and submitted in the National Center for Biotechnology Information (NCBI) in the following Accession Number KT992778.

Conclusion

In Manakudy estuary, the dominated species of *Zobellia russellii* were found at station 6 of the water sample. They were observed in the areas of human interferences, which will affect the health of the people in the surrounding area. So preventive measures should be taken to avoid contaminations in the Manakudy estuary.

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