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## Isolation of Marine Bacteria along Vasai Coast (M.S.) India

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Marine bacteria,  
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### A B S T R A C T

Microbes account for all known life forms for nearly 50 to 90% of Earth's history-Life itself began in the ocean. Marine microbes play significant role in the Earth system. Among the microbes bacteria are most vital link directly and indirectly with human life. In view of these total three sites along Vasai coast which is a part of Arabian Sea chosen viz.kalamb, Rangaon and Bhuigaon sea sites were selected. From these area total 227 bacterial isolated were collected by standard isolation procedure and then the characterization with respect to cultural and morphological characters were recorded. Among these the ratio of Gram positive and Gram negative were found to be varied as the dominance of Gram negative was comparatively more in all the three sites. Among the pigmented colonies yellow, red, pink, brown, white, cream etc. are preserved. The pigmentation and incubation temperature has correlation as pigmentation was observed at incubation temperature 25<sup>0</sup>-28<sup>0</sup> C.

## Introduction

The Earth's surface consists of 70% water, which is inhabited by 80% of all the life forms and consequently aquatic life has a greater diversity than their terrestrial counterparts. Marine environment exploration for research is still in early phase, many mysteries associated with aquatic flora and fauna have yet to be discovered. Hence it is very much an important area of research to many investigators to explore rich biodiversity. Despite being comprised of a

diverse ecosystem, the marine habitat is rich with nutrients and metabolites and the challenge for the investigators is to access and culture most of the bacteria in laboratory conditions to study their metabolic activities.

Heterotrophic bacteria play a significant role in the biogeochemical cycle of carbon and other materials such as nitrogen and sulphur in the ocean because of their high

abundance and ubiquity. (Copley, 2002, Karl, 2002). Most of the marine environment contains only few substances which can be used for metabolism and growth. In contrast the natural surfaces tend to collect and concentrate nutrient by charge-charge or hydrophobic interactions (Beveridge *et al.*, 1997). It suggests that the colonization on substratum may be biotic or abiotic has interrelation for growth of bacteria and metabolism.

The main problem in performing investigation into autecology and physiological properties of marine bacteria are currently the isolation and cultivation of dominant marine bacterial species. By knowing cultivability of such bacteria would not give all types of bacteria in sea but large population of bacteria which cannot be culture in artificial growth medium as they are non culturable. Pigmented bacteria occur frequently in aquatic samples. However, these colourful organisms often arouse only transient interest.

The aim of this study is to isolate dominant marine bacteria by culture method from the Vasai coast. Vasai Coast which lies between latitude 19.315°N longitudes 72.875°E, forms the northern boundary of Salsette Island, and empties west into the Arabian Sea.

## **Material and Methods**

Materials used for the isolation of marine bacteria are as follows, Zobells marine agar and broth, Modified nutrient agar

## **Methods**

**Sampling sites:** Sea water samples were collected from three different pollution free

site of Vasai coast zone namely Kalamb, Rangaon and Bhuigaon area. The sampling dept was 10 m. The samples were collected in sterile glass bottles and brought to laboratory in the ice box and processed within one hour of collection.

**Serial dilution method:** The three samples were labeled according to their location and from each 100 ul was introduced into 900 ul of saline and serially diluted. Each of the dilution was used to isolate the bacteria by using Zobells marine broth and Modified nutrient broth procured from Hi-Media ltd, Mumbai

**Isolation of bacteria:** The broth enriched samples then using spread plate method isolated on Zobells marine agar media and incubated for 24 and 48 hrs at 28 degree Celcius in bacteriological incubator.

**Characterization of isolates:** The well isolated colonies of marine bacteria were studied for their colony characterization including the pigmentation produce by them.

**Sub culturing and Preservation:** Studied isolates were restreaked on the Marine agar plates and incubated for 24 hours and after 24 hours the colonies were checked for purity by Grams staining and streaked on slope of marine agar which was over layered by sterile paraffin for preservation and kept at 4 degree Celsius for further use.

## **Result and Discussion**

In view of isolation and study of characteristics of marine bacteria along Vasai coast three stations were selected namely Kalamb, Rangaon and Bhuigaon. Samples collected were brought to laboratory and the serial dilutions were prepared and enriched using Zobell's

marine broth procured from Hi-Media, Mumbai. Each dilution of different sites were plated for growth of marine bacteria and after 24 hours of incubation the colonies were observed, Each station gave different count viz. Kalamb sample gave 72 isolates, Rangaon-57 isolates and Bhuigaon gave 64 isolates, from soil sediment 20 and from marine animals associated 14 respectively (table no.1).

In all 125 isolates were collected from these three stations. Each of the isolates was studied for routine colony characteristics like size, colour, elevation, consistency etc. and Grams nature. Among all from Kalamb out of 72 total 39 were found to be Grams negative, 36 from Bhuigaon and 32 isolates from Rangaon were found to be Gram negative, this dominant Gram negative marine bacteria presence is supported by the study of Soliev *et.al*, 2011, which is because of the ability to survive and grow in the water environment with low nutrient, high salinity and high pressure. Therefore we can say that on the basis of habitat they can be divided as psychrophiles (Living at low pressure), halophiles (Living at high salinity) and barophile (living under high pressure). Although these characteristics highlight the differences between marine and terrestrial microorganism, it remains difficult to separate bacterial genera on the basis of habitat due to the ubiquitous presence of similar species in both the environment.

The isolates are also studied for their visual pigmentation characteristics, as bacterial light absorption is largely ignored for a long time as a result of the general presumption that marine heterotrophic bacteria do not contain pigments with significant absorption in the visible spectra. Therefore in present study different pigments were observed among isolate irrespective of the site of collection viz. yellow, red, blue, brown, cream, white (Table no.2).

Similar level studies were done by Hailian du *et.al*. (2006) from china to study the pigmentation and Pane *et.al*. (1996). In the present study the distribution of pigmented bacteria is represented in Graph.2 from that we can relate our studies with the role of pigments in different potential as literature gives mention of red pigment shows the presence of active compound like prodigiosin and its derivatives, yellow pigment with phenazine and its derivatives these are few to name bioactive natural compounds from marine bacteria.

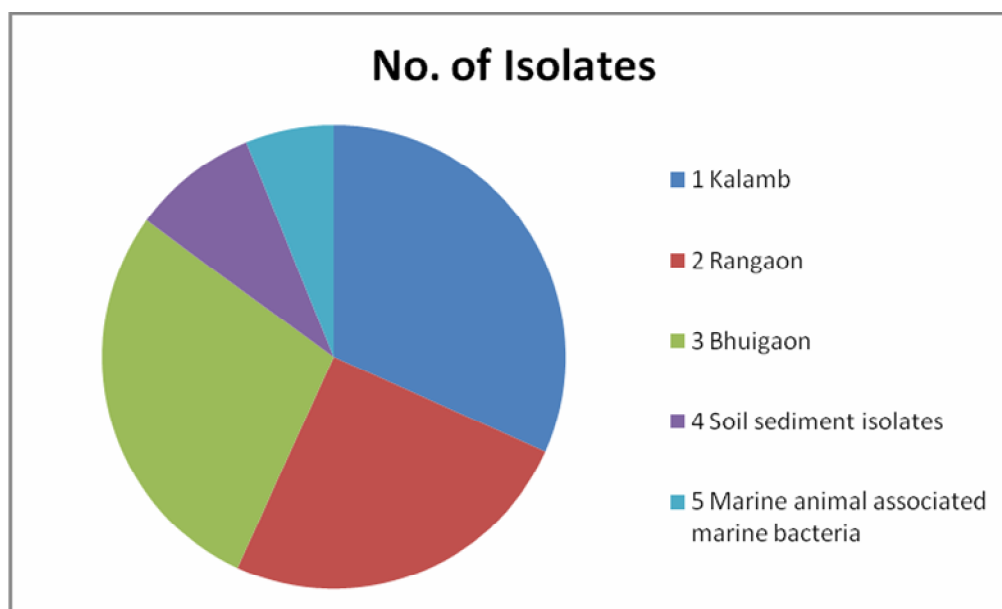
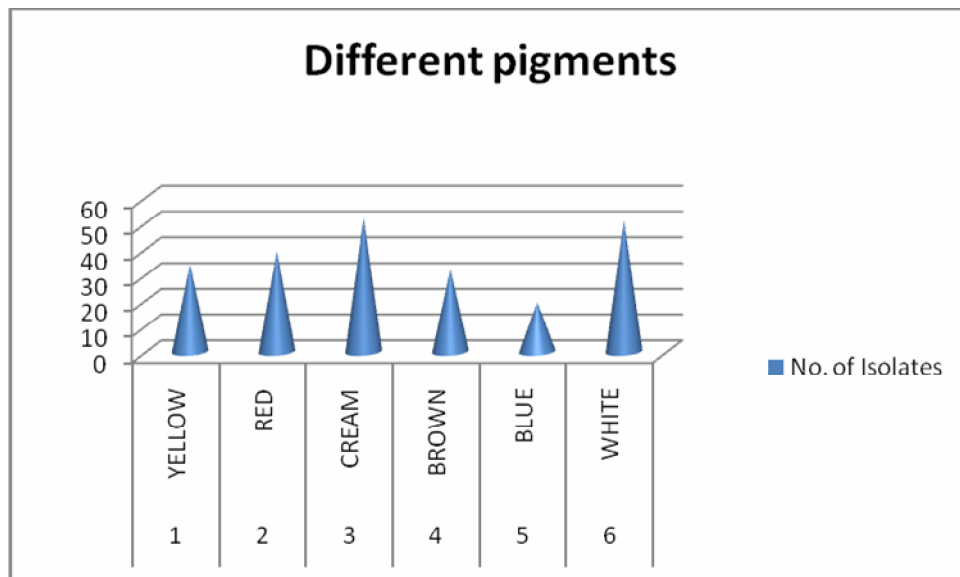
In conclusion, our initial work of isolation on the abundance distribution of marine bacteria along Vasai coast, gives us an idea about diversity among the bacteria with reference to their nature, growth characteristics and pigmentation patterns gives a baseline work for studying different metabolic activities of isolates from marine environment.

**Table.1** Distribution of Isolates according to station of collection

Sr.No	Site of collection	No. of Isolates
1	Kalamb	72
2	Rangaon	57
3	Bhuigaon	64
4	Soil sediment isolates	20
5	Marine animal associated marine bacteria	14

**Table no.2** Pigments and no. of bacterial isolates

Sr.No	Pigments	No. of Isolates
1	YELLOW	23
2	RED	19
3	CREAM	24
4	BROWN	16
5	BLUE	09
6	WHITE	34



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