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Isolation and Identification of Marine Bacteria from Vaitarna Estuary, Palghar (Maharashtra) India

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ABSTRACT

Microbes account for all known life forms for nearly 50 to 90% of Earth's history. Life itself began in the ocean. Among the microbes bacteria are most vital link directly and indirectly with human life. In view of these total three sites along Vaitarna estuary namely Dativare Coast, Vaitarna Railway Bridge and Pargaon Bridge were chosen. From these area total 15 bacterial isolates were collected by standard isolation procedure and then the characterized with respect to cultural and morphological, biochemical aspects and identification by 16S rRNA sequence has been recorded.

Key words: *Marine bacteria, Halophiles, Estuary, Identification, 16S rRNA sequence.*

Introduction

The microorganisms in the coastal waters include bacteria, fungi, algae, protozoa, rotifers, crustacean, worms, bacteriophages and insect larvae (Omiema and Ideriah, 2012). Microorganisms distributed in the marine and brackish environments play an important role in the decomposition of organic matter and mineralization in the system (Seshadri and lgnacimuthu, 2002). Studies indicate that sediment bacteria are more capable of degrading organic matter as compared to their counterparts in the water column (Sinkko *et al*., 2013). Estuary is one of the most productive ecosystems, at the same time one among the least explored ecosystems on earth, which has immense potential as a source of potent microorganisms that produce valuable compounds particularly, enzymes. Bacteria are recognized as important agents in biogeochemical processes in all aquatic ecosystems. It is well known that heterotrophic prokaryotes play relevant roles in the structure and dynamics of trophic web networks and in the re-mineralization of organic matter (Azam and Long, 2001). Bacteria are known to produce many economically important bioactive substances and secondary metabolites like antibiotics.

Among the microbes marine bacteria are known to produce bioactive substances may be to protect themselves from predators, marine bacteria have adapted to extreme physical or chemical parameters like pressure, temperature and salinity. Research into natural products from the marine environment, including microorganisms, has rapidly increased over the past two decades. Despite the enormous difficulty in isolating and harvesting marine bacteria, microbial metabolites are increasingly attractive to science because of their broad ranging pharmacological activities (Azamjon *et al*., 2011). Our aim to study the estuarine water bacterial identification and characterization for future drug development.

Materials and Methods

Identification of halo-tolerant bacteria from water sample collected from Vaitarna estuary.

Collection of water sample

To study bacterial diversity water samples from three different sites of Vaitarna estuary namely Dativare Coast, Vaitarna Railway Bridge and Pargaon Bridge were collected. The sampling depth was about 2 to 3 meters from water surface. The samples were collected in sterilize glass bottles and brought to the laboratory.

Isolation of Bacteria

The samples were labeled according to their collection site. Each sample collected was subjected to 10 fold dilution with sterilize physical saline. 0.1 ml of each dilution for each site was placed on Zobell's Marine Agar plate (Himedia, 2216). Plates were incubated at room temperature for 24 to 72 hours. Isolated colonies were observed for their morphological and cultural characters. Colonies were numbered as Isolate No.1 to Isolate No.15

Colonial characterization of isolated colonies

Isolated colonies were analyzed for the colonial characters. Following colony characters were considered as: 1) Size 2) Shape 3) Colour 4) Surface 5) Elevation 6) Margin 7) Opacity

Consistency and Motility

Morphological characterization of isolated colonies

The morphology of bacterial isolates i.e. the shape of the bacterial cell (cocci, bacilli, vibrio etc) and their arrangement was studied by Gram staining method.

Biochemical tests for bacterial identification

Bacterial species differ in their capacity to attack different carbohydrates, proteins and fats. Most of the biochemical tests are based on -

a) Presence of specific enzymes in bacterial cultures such as coagulase, oxidase, urease, gealtinase, lecithinase, catalase and others.
b) Production of metabolic end products of some compounds like sugar present in the culture media which are the outcome of enzymatic action of bacteria.

The staining is followed by use of various biochemical reagents and test to get closer to the identification of bacteria.

Bacterial Identification by 16S rRNA Sequence

In the present study the Bacterial genomic DNA isolation, PCR and sequence analysis was done with the help of Gene Ombio technologies private limited at Pune, Maharashtra in India.

DNA sequencing

Using the gene specific sequencing primers and ABI BigDye® Terminator v3.1 Cycle sequencing reaction kit (Applied Biosystems, USA), the purified PCR amplicon was sequenced.

Instrument: 3130 Genetic Analyser (Applied Biosystems)

Software used for Analysis: Sequencing Analysis.5.2

The 16S rRNA sequences were compared and aligned with sequences deposited in the NCBI GenBank database using BLAST (Altschul *et al.*, 1990)

Results

In the present study about 15 bacterial isolates were obtained. These isolates were selected on the basis of their colonial characters and ability to produce different pigments. These isolates were observed for colony and morphological character Out of these 15 isolates some isolates were observed with pigment producing ability. These isolates were observed to produce yellow, orange pigment. Some isolates were observed to be cream in colour. These isolates also observed for other colony characters such as shape, surface, size, elevation, margin, opacity, motility and consistency (Fig.3.19). Morphological characters were determined by using Gram's staining method. About 11 isolates were observed to be Gram positive and 4 isolates were gram negative. (Fig.3.19). About 10 bacterial isolates were rod shaped, 3 were cocci and 2 were cocco- bacilli (Fig.3.19). Isolates obtained from Vaitarna estuary

Table 1. Universal Primers used in PCR

Sr. No	Name	Seq (5′ - 3′)	Bases	Amplicon size	Reference
1	27 F	AGAGTTTGATCMTGGCTCAG	20 Bases	1450 bp	Applied & Environmental Microbiology, Apr.2008. pp2461-2470
2	1492 R	TACCTTGTTACGACTT	16 Bases		

were assessed for enzyme producing ability. Out of 15 isolates analyzed for enzyme producing ability, following observations were made

14 isolates were capable of producing catalase enzyme, 10 isolates shown positive methyl red test, 4 isolates were showing positive Voges–Proskauer test. About 4 isolates had produced amylase and were positive for amylase production. 7 isolates of 15 shown ability to produce gelatinase, 11 oxidase producers were observed, 8 isolates shown positive test for casienase production. In this study 10 isolates from Vaitarna estuary were observed with ability to ferment Glucose as they shown positive acid–gas production test. Not a single isolate had shown positive indole ring test. Isolates from Vaitarna estuary were tested for their ability to split urea in the medium due to production of urease enzyme, it was observed that 5 isolates were found to be urease producers (Fig.3.20).

Along with the observation of Colony characters, morphological characters, 16s rRNA sequencing were carried out for identification of isolates obtained from Vaitarna Estuary. FASTA format sequences were obtained for 15 isolates. These sequences were analysed with Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology information (NCBI) database to obtain following results.

Table 2. Isolates Nos. and Identified bacteria

Sr. No.	Isolate No.	Identified bacteria as per BLAST of NCBI
1	Isolate No.1	*Bacillus pumilus*
2	Isolate No.2	*Corynebacterium equi*
3	Isolate No.3	*Kokuria flava*
4	Isolate No.4	*Bacillus safensis*
5	Isolate No.5	*Bacillus flexus*
6	Isolate No.6	*Staphylococcus arlettae*
7	Isolate No.7	*Bacillus safensis*
8	Isolate No.8	*Alcaligenes faecalis*
9	Isolate No.9	*Advenella mimigardefordensis*
10	Isolate No.10	*Bacillus safensis*
11	Isolate No.11	*Bacillus flexus*
12	Isolate No.12	*Paracoccus halodenitrificans*
13	Isolate No.13	*Lysinibacillus fusiformis*
14	Isolate No.14	*Halomonas elongate*
15	Isolate No.15	*Halomonas aquamarina*

Conserved Region Gene Sequencing -16s For Bacterial (Halotolerant) Identification

Fasta Format Sequence

Isolate No.1 - *Bacillus pumilus*

TACAGACCAGAGAGTCGCCTTCGCCACTG GT
GTTCCTCCACATCTCTACGCATTTCACCG CTA
CACGTGGAATTCCACTCTCCTCTTCTG CAC
TCA AGTTTCCCAGTTTCCAATGACCC TCCCC
GGTTGAGCCGGGGGCTTTCACATCAGACTTA
AGAAACCGCCTGCGAGCCCTTTACGCCCAA
TAATTCCGGACAACGCTTGCCACCTACGTA
TTACCGCGGCTGCTGGCACGTAGTTAG CCG
TGGCTTTCTGGTTAGGTACCGTCAAGGT GCGA
GCAGTTACTCTCGCACTTGTT CTTCC CTAA CA
ACAGAGCTTTACGATCCGAAAACCTTCAT
CACTCACGCGGCGTTGCTCCGTCAGACTTT
CGTCCATTGCGGAAGATTCCCTACTGCTGCC
TCCCGTAGGAGTCT GGG CCG TGTC TCA GTC
CCAGTGTGGCCGATCACCCTCTCAGGTCGG
CTACGCATCGTCGCCTTGGTGAGCCATTA
CCCCACCAACTAGCTAATGCGCCG CGGGTCC
ATCTGTAAGTGACAGCCGAAACCGTCTT
TCATCCTTGAACCATGCGGTTCA

Isolate No.2 - *Corynebacterium equi*

TCGCCACCGGTGTTCCTCCTGATATCTGCG
CATTTCACCGCTACACCAGGAATTCCAGTCT
CCCCTGCAGTACTCAAGTCTGCCCGTATCG
CCCGCAAGCTTGGGGTTGAGCCCCAAGT TTTC
ACGGACGACGCGACAAACCGCCTACGAGC
TCTTTACGCCCAGTAATTCCGGACAACGCT
CGCACCCTACGTATTACCGCGGCTGCTGGC
ACGTAGTTGGCCGGTGCTTCTTCTGCAGG
TACCGTCACTCTCGCTTCGTCCCTGCTGAAA
GAGGTTTACAACCCGAAGGCCGTCATCCCTC
ACGCGGCGTCGCTGCATCAGGCTTTCGCCCA
TTGTGCAATATTCCCCACTGCTGCCTCCCGT
AGGAGTCTGGGCCGTGTCTCAGTCCCAGTG
TGGCCGGTCGCCCTCTCAGGCCGGCTACCC
GTCGTCGCCTTGGTAGGCCATTACCCCACC
AACAAGCTGATAGGCCGCGGGCCCATCCT
GCACCAGTAAACCTTTCCAACCCCCGCCAT
GCGACAGGAGCTCATATCCGGTATTAGAC
CCAGTTTCCCAGGCTTATCCCAGAGTGCAG
GGCAGATCACCCACGTGTTACTCACCCGT
TCGCCACTCGTGTACCCCCGAA

Isolate No.3 - *Kokuria flava*

GGGTAGCCGGCCTGAGAGGGTGACCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCA
CAATGGGCGCAAGCCTGATGCAGCGA CG CC
GCGTGAGGGATGACGGCCTTCGGGTTGTAA

ACCTCTTTCAGCAGGGAAGAAGCCACAA
GTGACGGTACCTGCAGAAGAAGCGCCGGC
TAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGGCGCAAGCGTTGTCCGGAATTATT
GGGCGTAAAGAGCTCGTAGGCGGTTTGTC
GCGTCTGCTG TGAAAGCCCG GGGCTCAAC
CCCGGGTCTGCAGTGGGTACGGGCAGACT
AGAGTGCAGTAGGGGAGACTGGAATTCCT
GGTGTAGCGGTGAAATGCGCAGATATCAG
GAG GAA CAC CGATGGC GAAGGCAGGTCTC
TGGGCTGTTACTGACGCTGAGGAGC GAAAG
CATGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCATGCCGTAAACGTTGGGCAC
TAGGTGTGGGGGACATTCCACGTTCTCCGC
GCCGTAGCTAACGCATTAAGTGCCCCGC

Isolate No.4 - ***Bacillus safensis***

CAGTTACAGACCAGAGAGTCGCCTTCGCCA
CTGGTGTTCCTCCACATCTCTACGCATTTC
ACCGCTACACGTGGAATTCCACTCTCCTCTT
CTGCACTCAAGTTTCCCAGTTTCCAATGACCC
TCCCCGGTTGAGCCG GGGGCTTTCACATCA
GACTTAAGAAACCGCCTGCGAGCCCTTTAC
GCCCAATAATTCCGGACAACGCTTGCCAC
CTACGTATTACCGCGGCTGCTGGCACGTA
GTTAGCCGTGGCTTTCTGGTTAGGTACCG
TCAAGGTGCGAGCAGTTACTCTCGCACTT
GTTCTTCCCTAACAACAGAGCTTTACGATCCG
AAAACCTTCATCACTCACGCGGCGTTGCTCC
GTCAGACTTTCGTCCATTGCGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGG
GCCGTGTCTCAGTCCCAGTGTGGCCGAT
CACCCTCTCAGGTCGGCTACGCATCGTCGC
CTTGGTGAGCCATTACCCCACCAACTAGCTA
ATGCGCCGCGGGTCCATCTGTAAGTGA CAG
CCGAAACCGTCTTTCATCCTTGAACCATGCGG
TTCAAGGAACTATCCGGTATTAGCTCCG
GTTTCCCGGAGTTATCCCAGTCTTACAGGC
AGGTTACCCACGTGTTACTCACCCGTCCGC
CGCTAACATCCGGGAGCAAGCTCCCTTCT
GTC CGC TCGACT TGC ATGTATTA

Isolate No.5 - ***Bacillus flexus***

GTCGCCTTCGCCACTGGTGTTCCTCCACAT
CTCTACGCATTTCACCGCTACACGTGGAA
TTCCACTCTCCTCTTCTGCACTCAAGTTT CC
CAGTTTCCAATGACCCTCCCCGGTTGAG
CCGGGGGCTTTCACATCAGACTTAAGAAA
CCGCCTGCGAGCCCTTTACGCCCAATAA
TTCCGGACAACGCTTGCCACCTACGTA
TTACCGCGGCTGCTGGCACGTAGTTAGCC
GTGGCTTTCTGGTTAGGTACCGTCAAGG TG
CGAGCAGTTACTCTCGCACTTGTTCTTCCC
TAACAACAGAGCTTTACGATCCGAAAACC
TTCATCACTCACGCGGCGTTGCTCCGTCA
GACTTTCGTCCATTGCGGAAGATTCCCTA
CTGCTGCCTCCC GTAG GAGTC TG GGCC GT
GTCTCAGTCCCAGTGTGGCCGATCACCCT
CTCAGGTCGGCTACGCATCGTCGCCTTGGT
GAGCCATTACCCCACCAACTAGCTAATG
CGCCGCGGGTCCATCTGT

Isolate No. 6 –

TTACAGACCAGAAAGTCGCCTTCGCCACTGG
TGTTCCTCCATATCTCTGCGCATTTCACCGCT
ACACATGGAATTCCACTTTCCTCTTCTGCAC
TCAAGTCTCCCAGTTTCCAATGACCCTCCA
CGGTTGAGCCGTGGGCTTTCACATCAGACTTA
AGAAACCGCCTACGCGCGCTTTACGCCCA
ATAATTCCGGATAACGCTTGCCACCTACGTA
TTACCGCGGCTGCTGGCACGTAGTTAGCCG
TGGCTTTCTGATTAGGTACCGTCAAGACGTG
CACAGTTACTTACACGTTTGTTCTT CCC TAAT
AACAGAGTTTTACGAGCCGAAACCCTT CATC
ACTCACGCGGCGTTGCTCCGTCAGGCTTTCG
CCCATTGCGGAAGATTCCCTACTGCTGCCTC
CCGTAGGAGTCTGGACCGTGTCTCAGTTC
CAGTGTGGCCGATCACCCTCTCAGGTCGG
CTACGTATCGTTGCCTTGGTAAGCCATTA CC
TTACCAACTAGCTAATACGGCGCGGGTCCA
TCTATAAGTGATAGCAAAACCATCTTTCACT
TTAGAACCATGCGGTTCTAAATGTTATCCGG
CATTAGCCCCGGTTTCCCGGAGTTATTCC
AGTCTTATAGGTAGGTTACCCACGTGTTAC
TCA CCCGTCCGCCGCTAACGTCAAAGGAG
CAAGCTCCTTATCTG TTC GCT CGAC TTGC
ATGTATTAGGCACGCCGCCAGCGTTCAT
CCTGAGC

Isolate No. 7 - ***Bacillus safensis***

AGAGTCGCCTTCGCCACTGGTGTTCCTCCA
CATCTCTACGCATTTCACCGCTACACGTGG
AATTCCACTCTCCTCTTCTGCACTCAAGTTT
CCCAGTTTCCAATGACCCTCCCCGGTTGA
GCCGGGGGCTTTCACATCAGACTTAAGA
AACCGCCTGCGAGCCCTTTACGCCCAATA
ATTCCGGACAACGCTTGCCACCTACGTATT
ACCGCGGCTGCTGGCACGTAGTTAGCCGT
GGCTTTCTGGTTAGGTACCGTCAAGGTGC
GAGCAGTTACTCTCGCACTTGTTCTTCCC
TAACAACAGAGCTTTACGATCCGAAAACCT
TCATCACTCACGCGGCGTTGCTCCGTCAG

ACTTTCGTCCATTGCGGAAGATTCCCTACT
GCTGCCTCCCGTAGGAGTCTGGGCCGT
GTCTCAGTCCCAGTGTGGCCGATCACCCTC
TCAGGTCGGCTACGCATCGTCGCCTTGGTG
AGCCATTACCCCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGACAGCCGA
AACCGTCTTTCATCCTTGAACCATGCGGTTC
AAGGAACTATCCGGTATTAGCTCCGGTTT
CCCGGAGTTATCCCAGTCTTACA

Isolate No. 8 - ***Alcaligenes faecalis***

TATTATCCCAGGGGGCTGCCTTCGCCATCGG
TATTCCTCCACATCTCTACGCATTTCACTGC
TACACGTGGAATTCTACCCCCCTCTGACATA
CTCTAGCTAGGTAGTTAAAAATGCAGTTCCA
AGGTTGAGCCCTGGGATTTCACATCTTTCTT
TCCTAACCGCCTGCGTACCCTTTACGCCCAG
TAATTCCGATTAACGCTTGCACCCTACGTATT
ACCGCGGCTGCTGGCACGTAGTTAGCCGGTG
CTTATTCTACAGGTACCGTCATCTACAGA
AGTTATTAGCTCCTGTCATTTCTTCCCTGTCA
AAAGTGCTTTACAACCCGAAGGCCTTCATCA
CACACGCGGGATGGCTGGATCAGGGTTTCC
CCCATTGTCCAAAATTCCCCACTGCTGCCTCC
CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAG
TGTGGCTGGTCGTCCTCTCAAACCAGCTAC
GGATCGTCGCCTTGGTAGGCCTTTACCC
CACCAACTAGCTAATCCGATATCGGCCGCTC
CAATAGTGAGAGGTCTTGCGATCCCCCCCT
TTCCCCCGTAGGGCGTATGCGGTATTAGCC
ACTCTTTC

Isolate No. 9 - ***Advenella mimigardefordensis***

GGTGTTCCTCCACATATCTACGCATTTCACTGC
TACACGTGGAATTCCACCCCCCTCTGACAT
ACTCTAGTTCGGTAGTTAAAAATGAAG
TTCCAAGGTTGAGCCCTGGGATTTCACAT
CTTACTTTC CAAACCGCCTGCGCACGCTTT
ACGCCCAGTAATTCCGATTAACGCTTGCA
CCCT AC GTATT ACCGC GGCTGCTG GCACG
TAGTTAGCCGGTGCTTATTCTTCAGGTAC
CGTCATCATTTCCGGGTATTATCCGAAACC
TTTTCTTCCCTGACAAAAGTGCTTTACAAC C
CGAAGGCCTTCATCGCACACGCGGGA
TGGCTGGATCAGGGTTTCCCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAG
GAGTCTGGGCCGTGTCTCAGTCCCAGTG TG
GCTGG TCGTCCTC TCAAACCA GCTACG GA
TCGTCGCCTTGGTAGGCCTTTACCCCACC
AACTAGCTAATCCGATATCGGCCGCTCCA
ATAGTGAGAGGTCCTAA

Isolate No. 10 - *Bacillus safensis*

CATTTCACCGCTACACGTGGAATTCCACTCT C
CTCTTCTGCACTCAAGTTTCCCAGTTTCCA
ATGACCCTCCCCGGTTGAGCCGGGGGCT
TTCACATCAGACTTAAGAAACCGCCTGC
GAGCCCTTTACGCCCAATAATTCCGGACAA
CGCTTGCCACCTACGTATTACCGCGGCTG
CTGGCACGTAGTTAGCCGTGGCTTTCTGGTTA
GGTACCGTCAAGGTGCGAGCAGTTACTCT
CGCACTTGTTCTTCCCTAACAACAGAGCTT
TACGATCCGAAAACCTTCATCACTCACGCG
GCGTTGCTCCGTCAGACTTTCGTCCATTGCG
GAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTCTGGGCCGTGTCTCAGTCCCAGTGTGG
CCGATCACCCTCTCAGGTCGGCTACGCATC
GTCGCCTTGGTGAGCCATTACCCCACCAAC
TAGCTAATGCGCCGCGGGTCCATCTGTAA
GTGACAGCCGAAACCGTCTTTCATCCTTG
AACCATGCGGTTCAAGGAACTATCCGGTATT
AGCTCC GGTTTCCC

Isolate No. 11 - ***Bacillus flexus***

CCTCAGCGTCAGTTACAGACCAAAAAGCCGC
CTTCGCCACTGGTGTTCCTCCACATCTCTAC
GCATTTCACCGCTACACGTGGAATTCCGCTT
TTCTCTTCTGCACTCAAGTTCCCCAGTTTCCA
ATGACCCTCCACGGTTGAGCCGTGGGCTTTC
ACATCAGACTTAAGAAACCGCCTGCGCG
CGCTTTACGCCCAATAATTCCGGATAACGCT
TGCCACCTACGTATTACCGCGGCTGCTGGC
ACGTAGTTAGCCGTGGCTTTCTGGTTAGGTA
CCGTCAAGGTACAAGCAGTTACTCTTGTAC
TTGTTCTTCCCTAACAACAGAGTTTTACGACC
CGAAAGCCTTCATCACTCACGCGGCGTTGC
TCCG TCAGACTTTCGT CCATTGCGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGG
CCGTGTCTCAGTCCCAGTGTGGCCGATCACCC
TCTCAGGTCGGCTATGCATCGTTGCCTTGGT
GAGCCGTTACCTCACCAACTAGCTAATGCA
CCGCGGGCCCATCTGTAAGTGATAGCCGAAA
CCATCTTTCAATTTTCTCTTATGCAAGAGAA A
ATGTTATCCGGTATTAGCTCCGGTTTCCCG
GAGTTATCCCAGTCTTACAGGCAGGTTGCCC
ACGTGTTACTCACCCGTCCGCCGCTAACGTC
ATAGAAGCAAGCTTCTAATCAGTTCGCTCGA
CTTGCATGTATTAGGCACGCCG CCA G CGTTC
ATCCTGAGCCAG

Isolate No. 12 - ***Paracoccus halodenitrificans***

ATCGAGCCAGTGAGCC GCCTTCG CCACTGGT

GTTCCTCCGAATATCTA CGAATTT CACCTCTA
CACTCGGAATTCCACTCACCTCTCTCGAACTC
CAGACCGATAGTTTCAAAGGCAGTTCCAA
GGTTGAGCCCTGGGATTTCACCTCTGACTTT
CCGGTCCGCCTACGTGCGCTTTACGCCCAGT
AATTCCGAACAACGCTAGCCCCCTCCGTA
TTACCGCGGCTGCTGGCACGGAGTTAGCC
GGGGCTTCTTCTGCTGGTACCGTCATTATC
TTCCCAGCTGAAAGAGCTTTACAACCCTAAG
GCCTTCATCACTCACGCGGCATGGCTAGAT
CA GGGTTGCCCCCATTGTCTAAGATTCCCCA
CTGCTGCCTCCCGTAGGAGTCTGGGCCG
TGTCTCAGTCCCAGTGTGGCTGATCATCCTCT
CAAACCAGCTATGGATCGTCGGCTTGGTAG
GCCATTACCCCACCAACTACCTAATCCAA
CGCGGGCTGATCCTTCTCCGATAAATCTTTC
CCCAA

Isolate No. 13 - *Lysinibacillus fusiformis*
GTGTCAGTTACAGACCAGATAGTCGCCTTCG
CCACTGGTGTTCCTCCAAATCTCTACGCAT
TTCACCGCTACACTTGGAATTCCACTATCCTC
TTCTGCACTCAAGTCTCCCAGTTTCCAAT
GACCCTCCACGGTTGAGCCGTGGGCTTTCA
CATCAGACTTAAGAAACCACCTGCGCGCG
CTTTACGCCCAATAATTCCGGACAACGCTT
GCCACCTACGTATTACCGCGGCTGCTGGCA
CG TAGTTAGCCGTGGCTTTCTAATAAGGTAC
CGTCAAGGTACAGCCAGTTACTACTGTACT
TGTTCTTCCCTTACAACAGAGTTTTACGAA
CCGAAATCCTTCTTCACTCACGCGGCGTTG CT
CCATCAGGCTTTCGCCCATTGTGGAAGA
TTCCCTACTGCTGCCTCCCGTAGGAGTCT
GGGCCGTGTCTCAGTCCCAGTGTGGCCGAT
CACCCTCTCAGGTCGGCTACGCATCGTCGCC
TTGGTGAGCCGTTACCTCACCAACTAGCTAA
TGCGCCGCGGGCCCATCCTATAGCGACAGCC
GAAACCGTCTTTCAATATTTCACCATGAGG
TGAAACAGATTATTCGGTATTAGCCCCGGTT
TCCCGGAGTTATCCCAAACTATAA

Isolate No. 14 - *Halomonas elongate*
TCAGTGTCAGTGTCAGTCCAGAAGGCCGCCT
TCGCCACTGGTATTCCTCCCGATCTCTAC
GCATTTCACCGCTACACCGGGAATTCTACCT
TCCTCTCCTGCACTCTAGCCTGACAGTTCCG
GATGCCGTTCCCAGGTTGAGCCCGGGGCTTT
CACAACCGGCTTATCAAGCCACCTACGCGC
GCTTTACGCCCAGTAATTCCGATTAACGCTT
GCACCCTCCGTATTACCGCGGCTGCTGG
CACGGAGTTAGCCGGTGCTTCTTCTGCGAG
TGATGTCTTTCCTAATGGGTATTAACCACTAG
GCGTTCTTCCTCGCTGAAAGTGCTTTA
CAACCCGAGGGCCTTCTTCACACACGCGGCA
TGGCTGGATCAGGGTTCCCCCCATTGTCC
AATATTCCCCACTGCTG CCTCCCGTAGGAG
TT CGGGCCGTGTCTCAGTCCCGATGTGGCT
GATCATCCTCTCAGACCAGCTACGGATC
GTTGCCTTGGTGAGCCTTTACCTCACCAAC
CAGCTAATCCGACATAGGCTCATCCAATAG
CGGGAGCCAAAGCCCCCTTTCTCCCGTA
GGACGTATGCGGTATTAGCCTGGGTTTCCCC
AGGTTATCCCCCACTACCGGGCAGATTCCT
ATGCATTACTCACCCGTCCGCCGCTCGTC
AGCATCTAGCAAGC TA

Isolate No. 15 - *Halomonas aquamarina*
CTCAGTGTCAGTGTCAGTCCAGAAGGCCGCCTT
CGCCACTGGTATTCCTCCCGATCTCTACGC
ATTTCACCGCTACACCGGGAATTCTACCTT
CCTCTCCTGCACTCTAGCTTGACAGTTCCG
GATGCCGTTCCCAGGTTGAGCCCGGGGC
TTTCACAACCGGCTTATCAAGCCACCTAC
GCGCGCTTTACGCCCAGTAATTCCGATTAA
CGCTTGCACCCTCCGTATTACCGCGGCTG
CTGGCACGGAGTTAGCCGGTGCTTCTTCT
GCGAGTGATGTCTTTCCTAATGGGTATTAA
CCACTAGGCGTTCTTCCTCGCTGAAAGT
GCTTTACAACCCGAGGGCCTTCTTCACACA
CGCGGCATGGCTGGATCAGGCTTTCGCCCA
TTGTCCAATATTCCCCACTGCTGCCTCCCG T
AGGAGTTCGGGCCGTGTCTCAGTCCCGA
TGTGGCTGATCATCCTCTCAGACCAGCTAC
GGATCGTCGCCTTGGTGAGCCTTTACCTC
ACCAACTAGCTAATCCGACATAGGCTCAT C
CAATAGCGGGAGCCGGAGCCCCCTTTCT
CCCGTAGGACGTATGCGGTATTAGCCTGG
GTTTCCCCAGGTTATCCCCCACTATCGG
GCAGATTCCTATGCATTACTCACCCGTCCG C
CGCTCGTCAGCGGGTAGCAAGCTACCCC
TGTTACCGCTCGACTTGCATGTGTTA

Discussion

In the present study about 15 isolates were obtained from Vaitarna estuary. About 11 isolates were observed to be Gram positive and 4 isolates were gram negative, about 10 bacterial isolates were rod shaped, 3 were cocci and 2 were cocco- bacilli. Isolates obtained from Vaitarna estuary were assessed for enzyme producing ability. Out of 15 isolates analyzed for enzyme producing ability, following ob-

servations were made 14 isolates were capable of producing Catalase enzyme, 10 isolates shown positive methyl red test, 4 isolates were showing positive Voges–Proskauer test. About 4 isolates had produced amylase and were positive for amylase production. 7 isolates 15 shown ability to produce gelatinase, 11 oxidase producers were observed, 8 isolates shown positive test for casienase production. In this study 10 isolates from Vaitarna estuary were observed with ability to ferment Glucose as they shown positive acid–gas production test. Not a single isolate had shown positive indole ring test. Isolates from Vaitarna estuary were tested for their ability to split urea in the medium due to production of urease enzyme; it was observed that 5 isolates were found to be urease producers. 16s r RNA sequencing was used to identify the bacterial isolates. The identified isolates were *Bacillus pumilus, Corynebacterium equii, Kokuria flava, Bacillus safensis, Staphylococcus arlettae, Bacillus flexus, Alcaligenes faecalis, Advenella mimigardefordensis, Halomonas aquamarina, Paracoccus halodenitrificans, Halomonas elongate, Lysinibacillus fusiformis*. All the isolates identified are pigment producers and halo tolerant, i.e. could thrive with the high salinity. Marine bacteria along Vasai coast were isolated and screened for enzymatic activity by Pimpliskar and Jadhav (2014).

They obtained marine bacteria from 3 different sites and screened on the basis of colony and morphological characters. Bacterial diversity of water and sediment at marine salterns near Bhavnagar, Gujarat (Dave and Desai, 2006). Bacterial diversity of water and sediment in the Changjiang estuary and coastal area of the East China Sea by (Feng *et al*., 2009). They used 16s r RNA method to identify bacterial isolates. The most abundant species obtained were belonging to phylum Proteobacteria then followed by Firmicutes, Bacteroidetes and Actinobacteria.

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