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MORPHOLOGICAL DOMINANCE OF ISOLATES AT TWO SITES WITH LESS HUMANOID INTERFERENCE IN MAHARASHTRA, INDIA

Priyanka Sawant, Sagarkumar Joshi, Jignasha Thumar*

Department of Microbiology and Biotechnology, Gujarat University, Ahmedabad, Gujarat, India
Email: sawant.priyanka333@gmail.com

ABSTRACT

Products of biological origin are highly demanded in the industrial sector and because of the substantial financial gains made in the enzyme industry; more and more of these businesses are springing up. This study presents the results of processing samples collected in areas with less human interference, such as Jhow Island and the Borivali Monari Creeks. The samples were collected under the supervision of a forest officer after receiving permission from the Mangrove Cell in Maharashtra. Isolate morphology was investigated in this study. Forty-two isolates from the Borivali site shared the following characteristics: circular shape, entire margins, convex, small size colonies, smooth textures, cream pigments, opaqueness, gram positivity, and a major organism group belonging to Coccus. Twenty isolates were collected from Jhow Island, and their predominant features were as follows: circular shape, entire margins, flat elevation, punctiform colonies, smooth textures, tan pigments, opaqueness, Gram positivity, and the organism's major group belonging to Coccus. Protease, amylase, and cellulase were screened for first because of their vital role in industry. Protease producers were chosen for further testing, and using the inverted pyramid technique, the highest protease-producing isolate, Bor S17B13, was chosen for enzymatic activity. 16S rRNA sequencing was used to determine the identity of isolate Bor S17B13, and a phylogenetic tree was constructed to show that Bor S17B13 is a member of the Priestia aryabhattai strain. The gene sequence for Priestia megaterium strain B21 can be found in the National Center for Biotechnology Information database under the accession number OM743775. Protease enzymes can be used for anything from bio-industry to environmental cleanup (bio-remediation). New possibilities for scaling up enzyme production will become available as more research is done.

Key words: Marine, Mangroves, Protease, Halophile, Extremophile

INTRODUCTION

Extremophile microorganisms that have adaptation to a diverse range of conditions in the natural world are subjected to debates in scientific community. Scientists are interested in the bioactive constituents produced by organisms that have adapted to survive under extreme conditions for probable utility in the fields of biofuels, many areas in medicine and agriculture(Amoozegar et al., 2003), (J. Thumar et al., 2010).

Halotolerant organisms are those that can endure high salt concentrations while also surviving in low to zero salt concentrations and producing metabolites (J. T. Thumar & Singh, 2007), (Vijay et al., 2018)(Oliveira de Veras et al., 2018). Both sea sands and green algae have yielded a wide range of halophilic microorganisms. In this way, there are numerous bacteria that are either mildly halophilic or exceptionally halotolerant in the ocean. The predominant varieties of colonies that evolved on agar plates were allotted by numbering taxonomy to the belongs to the genus Salinivibrio , Pseudomonas spp., Alcaligenes group, Acinetobacter, and Flavobacterium in a study of Spanish intermediate salt concentration (between 15% and 30% salts) ponds . They flourished in a salty environment of 10% but were discovered at salt levels of up to 25%. Below 15% salt, Salinivibrio species predominated, but beyond 15% salt, bacteria belonging to the Pseudomonas, Alcaligenes and Alteromonas groups were overwhelmingly prevalent. Below 30% salt, Gram-positive cocci were predominantly observed, whereas Flavobacterium and Acinetobacter were uniformly distributed in lower numbers (Arahal et al., 2002). These organisms are divided into three groups based on the salt concentration required for optimal growth: extreme halophiles, moderate halophiles, and slight halophiles. Various sulfur-oxidizing, sulfate-reducing, homo-acetogenic, methanogenic, hetero-trophic bacteria and archaea, including aerobic representatives of Archaea associated with the genera Halo-bacterium, Natrono-bacterium, Haloferax, and Haloarcula, as well as several species belonging to the Bacteria and Eukarya, are found in the oxygen - deficient areas in the sediment below.

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At concentrations of salt more than 5.1 M NaCl, halophilic archaea take over brine pools and virtually all other forms of life die out (DasSarma & DasSarma, 2012).

MATERIAL AND METHODS

Sites of Sampling

Jhow Island and Borivali monari creek in Maharashtra, both known for their abundance of mangrove trees, were chosen as sampling sites. These both sites comes under conservative area and human interference is restricted here. Samples were collected from these sites by taking prior permission from Mangrove Cell Maharashtra and sample were collected under guidance of forest officer.

Figure 1. Jhow Island, Maharastra (19.5005° N, 72.8176° E)

Figure 2. Jhow Island site and sampling under guidance of authorities

Borivali monari creek:

Figure 3. Borivali monari creek, Maharastra (19.2179° N, 72.8087° E)(image adapted from google maps)

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Figure 4. Sample collection from Borivali site under guidance of forest officer

Samples were collected from Jhow island and Borivali coastline monari creek as show in figure 1 and 3 respectively. These Island was rich in mangrove diversity and it falls into low humanoid area or we can say human interference was very less so this site was selected special permission is to be taken to enter this reserved area from government of Maharashtra.
For serving the purpose of obtaining more species of bacteria of our interest, samples were collected from this mangrove rich and reserved area.

Sample Collection and Sample Type

The samples were collected in sterile plastic containers; the pH and temperature of all samples were measured manually at time of sampling. Records of physical character of soil-samples were also collected.

Figure 5. Sample collected from both the sites

Sample type - As shown in the figure 5. samples collected were mangrove Root, Soil, Sea Water.
Root and soil samples collected from land and water sample was collected via boat and latitude and longitude for all samples were recorded. All samples were stored at 4˚C ±2 further processes.

Sample Processed by Enrichment technique
Sample were processed by taking 1gm soil samples and inoculating them in enrichment media, and similar process was done for water sample and root sample which were taken 1ml and 1gm respectively. Samples were inoculated in flask containing 50ml broth, which were kept at 100RPM shaking condition. Enrichment media (Complete Medium Broth{CMB}) includes glucose, 10g; peptone, 5g; yeast extract, 5g; K_2HPO_4, 5g and NaCl, 100g and 200g as per the experiment. The pH of the medium was adjusted by adding separately autoclaved Na_2CO_3 (20%, w/v).
First, samples from Jhow Island were processed. All processes samples were inoculated in two flasks. One flask contains 10% NaCl, the other 20% NaCl along with rest of the media from CMB, for each sample processed. Jhow samples contain pH 9 and Borivali samples contain pH 7 in broth. To get more number of isolate and wider the spectra for potential organisms pH 7 was used for second site of sampling.

Isolation and Cultivation
After inoculation, flasks were incubated at 37˚C ±2 for 48 h. After growth appeared, one loop–full of culture was transferred to Complete Medium Plate (CMP). CMP contain glucose, 10g; peptone, 5g; yeast extract, 5g; K_2HPO_4, 5g; Agar-agar 30g and NaCl, 100g and 200g as per the experiment. Plates were incubated for 3days at 37˚C ±2. Different types of colonies were observed on single plates so further transferred of selected colonies was done to get isolates.

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Preservation and Maintenance
The pure cultures were preserved on CMP and stored at 4˚C. Isolates obtained were regularly sub-cultured.

Characterization of Isolates
Isolates were further characterized based on gram staining and colony morphology. The colony characters were studied on Complete Medium Plate, pH of medium was pH 9 for Jhow Island isolates and pH 7 for Borivali isolates. All the isolates obtained from 10% NaCl broth were streaked on Complete Medium Plate containing 10% NaCl concentration and same processes was followed for 20% NaCl concentration broth isolates at 37°C±2 temperature.

Identification of Organisms
Isolates were observed under light microscope, then screen for protease, amylase , cellulose enzymes, from these potential isolate Bor S17B13 was identified by molecular identification (Sawant & Thumar, 2022), ("Microb. Enzym. Biotechnol.," 1990), (Kasana et al., 2008).

Bacterial selection inverted pyramid approach
30 samples were processed for getting Isolates, these isolates were further narrow down based on fast growing isolates and on the basis of industrially important enzyme production. Moreover isolates were selected based on wider zone of hydrolysis for protease, amylase and cellulase enzymes as primary screening. Demand for Protease enzyme is increasing day by day in Industries and in mol-biology research, so best from them was selected for secondary screening of protease enzyme.

Molecular Identification of selected Isolates
DNA Extraction then DNA QC was done , followed by PCR amplification with 16s Primer Sample loading on Agarose-Gel Sequencing using ABI-3130xl platform then data was Analysed and Phylogenetic-tree was prepared and isolate was identified. Here genomic-DNA was isolated from the Bor S17B13. Then approximate ~1.5 k base pair, 16s-rDNA fragment amplified by using High-fidelity PCR-polymerase. PCR product obtained was sequenced Bi-directionally. Sequence obtained was analyzed for identification of isolate and its relative closeness with other Bacteria.

The sample was taken out, put in a mortar, and homogenised with 1 ml of DNA extraction buffer. The tubes were filled with an phenol: chloroform: isoamyl alcohol (25:24:1), and the homogenate mixture was then transferred to 2 ml microcentrifuge tube. A fresh tube was used to extract the uppermost aqueous phase, and an equivalent volume of chloroform and isoamly was added. The tubes were thoroughly mixed by gently shaking them before the centrifuge, which was run for 15 minutes at 14,000 rpm at room temperature. The topmost aqueous layer that was left after centrifuging was incubated at room temperature for 10 minutes at 14,000 alcohol (24:1), was added and mixed. By slowly adding volume of 3 M Sodium Acetate of pH 7.0 to the solution, the DNA was precipitated out of it and transferred to a new tube. The tubes were centrifuged at 4°C for 15 min with 0.7 volume of isopropanol after 15 minutes of incubation at room-temperature. The DNA in form of pellet was washed three times in 70% ethanol, followed by a quick rinse in 100% ethanol at 14,000 rpm. After that 5 µl of DNAse free RNAse A, was add to remove RNA and then DNA was dissolved for further process in TE(Tris-Cl 10 mM pH 8.0, EDTA 1 mM) for drying .
PCR condition- 25 cycling

RESULTS AND DISCUSSION

Soil characteristics
Soil sample were collected and their texture was noted as shown in table No. 1 below

Table No. 1. – Describes soil texture of sample collected

Sample ID	Soil Texture	Colour/shade	Sample ID	Soil Texture	Colour/shade
Jho_S1	Moist	Muddy brown	**Bor_S5**	Moist	Muddy brown
JHO_S2	Moist	Black to grey	**Bor_S6**	Moist	Muddy brown
Jho_S3	Semi-dry	Muddy brown	**Bor_S7**	Semi-dry	Muddy brown
Jho_S4	Semi-dry	Muddy brown	**Bor_S8**	Moist	Reddish-black
Jho_S5	Moist	Black to grey	**Bor_S9**	Semi-dry	Muddy brown
Jho_S6	Moist	Muddy brown	**Bor_S10**	Moist	Muddy brown
Jho_S7	Moist	Muddy brown	**Bor_S11**	Moist	Reddish-black
Jho_S8	Moist	Muddy brown	**Bor_S12**	Moist	Muddy brown

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Jho_S9	Dry	Grey	**Bor_S13**	Moist	Muddy brown
Jho_S10	Dry	Grey	**Bor_S14**	Moist	Reddish-black
Bor_S1	Moist	Muddy brown	**Bor_S15**	Moist	Reddish-red
Bor_S2	Dry	Yellowish	**Bor_S16**	Moist	Reddish-black
Bor_S3	Moist	Muddy brown	**Bor_S17**	Moist	Reddish-black
Bor_S4	Moist	Brown -black	**Bor_S18**	Moist	Muddy brown

In table No. 1. - sample ID represent site and 'S' indicate soil samples, above show table gives a brief about texture of soil and colouration of soil sample from where samples were collected.
Most soil texture from Jhow Island have moist texture and very few have dry and semi-dry texture and shade were majorly muddy brown colour.
For Borivali samples major texture style was moist and few were having semi-dry texture. While shade of sample have two major style one muddy brown and other rusti reddish –black.

Sample collected and Sample type

Soil and root sample were collected from mangrove plants like, Avicennia marina, Bruguiera cylindrical, Salvadora persica, Sonneratia alba etc. Sample were stored in 4˚C temperature till they were processed. Sample were collected and 30 sample were processed from which 8 samples were from Jhow Island and 22 samples from Borivali. Each sample inoculated flask contain NaCl concentration of 10% and 20%(2 flask for each sample). Jhow Island samples were screened with 9 pH while Borivali samples were screened with 7 pH growth medium. After 48hr serial dilution was carried out and culture was spread on complete medium plate.
Sample type: Random sampling was done for both the sites.
Sample nomenclature was 'Jho'- indicates site of sampling while 'S' indicate soil sample, 'W' indicates water sample and 'R' indicates root sample.
Sample processed from Jhow Island were- S1, S3, S5, S6, S8, S9, S10, W16.
Sample processed from Borivali monari creek- S1, S2, S4, S5, S7, S8, S9, S10, S12, S13, S14, S16, S17, R1, R3, R8, R14, W1, W2, W3, W4, W8.

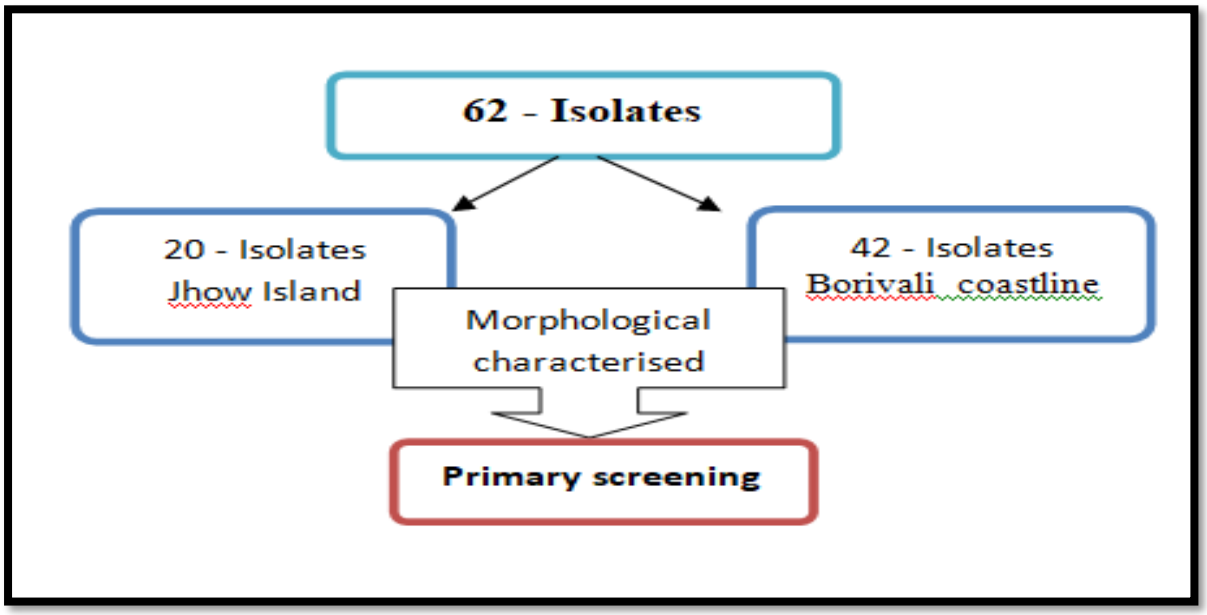
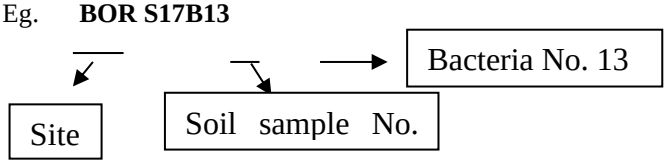


Figure 6. Isolation was carried out from mixed culture obtained after enrichment method.
As shown in figure 6. after processing 30 samples, 62 Isolated colony was obtained from which 20 isolates were from Jhow site and 42 Isolates were from Borivali site. Their morphology characteristics were studied and all of them where screened for Industrial important enzymes like Protease, Amylase, Cellulase
Nomenclature of samples: Eg. **BOR S17B13**



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Morphological characteristics

Table No. 2. Morphological diversity of borivali monari creek isolates

SR. NO	SAMPLE NAME	SHAPE	MARGINE	ELEVATION	SIZE	TEXTURE	PIGMENT	OPACITY	TYPE	GRAM REACTION
1	Bor R1B1	circular	entire	convex	small	smooth	cream	opaque	Short rod	Gram -ve
2	Bor R1B2	circular	entire	convex	small	smooth	cream	trasparent	Cocci	Gram -ve
3	Bor S1B3	circular	entire	convex	small	smooth	cream	opaque	Bacilli	Gram -ve
4	Bor S1B4	circular	entire	flat	punctiform	smooth	cream	opaque	Cocci	Gram +ve
5	Bor S2B5	circular	entire	flat	punctiform	rough	tan	translucent	Short rod	Gram -ve
6	Bor S8B6	circular	entire	convex	small	smooth	cream	opaque	Short rod	Gram -ve
7	Bor S9B7	circular	entire	convex	small	smooth	cream	opaque	Bacilli	Gram +ve
8	Bor R3B8	circular	entire	convex	punctiform	smooth	white	trasparent	Bacilli	Gram -ve
9	Bor S7B9	circular	entire	convex	small	smooth	cream	opaque	Bacilli	Gram -ve
10	Bor S9B10	circular	entire	convex	small	smooth	cream	opaque	Short rod	Gram -ve
11	Bor S16B11	circular	entire	convex	punctiform	smooth	tan	opaque	Cocci	Gram -ve
12	Bor S16B12	circular	entire	flat	small	smooth	cream	opaque	Bacilli	Gram +ve
13	Bor S17B13	circular	entire	convex	moderate	Mucoid	tan/Peach	opaque	Bacilli	Gram +ve
14	Bor S1B14	irregular	filamentous	raised	moderate	smooth	tan	opaque	Bacilli	Gram +ve
15	Bor R3B15	circular	entire	convex	small	smooth	white	opaque	Bacilli	Gram +ve
16	Bor R8B16	circular	entire	convex	small	smooth	Cream	translucent	Cocci	Gram -ve
17	Bor R14B17	irregular	undulate	raised	moderate	smooth	tan	opaque	Bacilli	Gram +ve
18	Bor W3B18	circular	entire	convex	small	smooth	yellow	opaque	Cocci	Gram +ve
19	Bor W3B19	irregular	undulate	flat	small	rough	cream	opaque	Bacilli	Gram +ve
20	Bor S14B20	circular	entire	convex	small	smooth	cream	opaque	Cocci	Gram -ve
21	Bor S13B21	circular	entire	convex	small	smooth	cream	opaque	Short rod	Gram -ve
22	Bor W4B22	circular	entire	convex	moderate	smooth	cream	opaque	Short rod	Gram +ve
23	Bor S12B23	circular	entire	flat	small	rough	tan	translucent	Short rod	Gram -ve

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24	Bor S13B24	circular	entire	convex	small	smooth	cream	opaque	Cocci	Gram +ve
25	Bor S12B25	circular	entire	convex	small	smooth	cream	trasparent	Cocci	Gram +ve
26	Bor S17B26	circular	entire	convex	small	smooth	cream	trasparent	Cocci	Gram +ve
27	Bor S12B27	circular	entire	convex	small	smooth	cream	opaque	Short rod	Gram +ve
28	Bor W4B28	circular	entire	convex	small	smooth	cream	opaque	Short rod	Gram -ve
29	Bor W2B29	circular	entire	convex	small	smooth	cream	opaque	Cocci	Gram +ve
30	Bor S10B30	circular	entire	flat	punctiform	rough	tan	translucent	Short rod	Gram -ve
31	Bor S10B31	circular	entire	convex	small	smooth	white	opaque	Cocci	Gram +ve
32	Bor W1B32	circular	entire	convex	small	rough	cream	trasparent	Short rod	Gram +ve
33	Bor S8B33	circular	entire	convex	punctiform	smooth	tan	opaque	Cocci	Gram -ve
34	Bor S5B34	circular	entire	convex	punctiform	smooth	cream	opaque	Cocci	Gram -ve
35	Bor S4B35	circular	entire	convex	punctiform	smooth	cream	trasparent	Cocci	Gram +ve
36	Bor S5B36	circular	entire	flat	punctiform	rough	tan	opaque	Bacilli	Gram +ve
37	Bor W8B37	circular	entire	convex	small	smooth	white	opaque	Cocci	Gram +ve
38	Bor R14B38	circular	entire	flat	punctiform	rough	tan	translucent	Cocci	Gram -ve
39	Bor R14B39	circular	entire	flat	punctiform	rough	tan	translucent	Short rod	Gram -ve
40	Bor S15B40	circular	entire	flat	punctiform	rough	tan	translucent	Bacilli	Gram +ve
41	Bor S18B41	circular	entire	flat	punctiform	rough	tan	translucent	Cocci	Gram +ve
42	Bor S18B42	circular	entire	flat	punctiform	rough	tan	translucent	Bacilli	Gram +ve

Table No. 3. Morphological diversity of jhow island isolates

SR. NO	SAMPLE NAME	SHAPE	MARGINE	ELEVATION	SIZE	TEXTURE	PIGMENT	OPTICAL	TYPE	GRAM REACTION
1	Jho S1B1	circular	entire	flat	punctiform	smooth	cream	opaque	Cocci	Gram +ve

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2	Jho S1B2	circular	entire	flat	punctiform	smooth	cream	opaque	Short rod	Gram +ve
3	Jho S1B3	circular	entire	flat	punctiform	rough	cream	opaque	Bacilli	Gram +ve
4	Jho S5B4	irregular	filamentous	convex	medium	smooth	tan	trasparent	Cocci	Gram +ve
5	Jho W16B5	circular	entire	flat	punctiform	rough	tan	opaque	Bacilli	Gram +ve
6	Jho S3B6	circular	entire	flat	punctiform	smooth	tan	opaque	Cocci	Gram -ve
7	Jho S3B7	irregular	filamentous	raised	medium	smooth	cream	opaque	Short rod	Gram +ve
8	Jho S5B8	circular	entire	convex	punctiform	smooth	tan	opaque	Bacilli	Gram +ve
9	Jho S5B9	irregular	filamentous	raised	medium	smooth	brown	translucent	Cocci	Gram +ve
10	Jho S6B10	irregular	filamentous	raised	medium	smooth	cream	opaque	Bacilli	Gram +ve
11	Jho S6B11	circular	entire	flat	punctiform	smooth	white	opaque	Short rod	Gram +ve
12	Jho W16B12	circular	entire	flat	punctiform	rough	tan	translucent	Bacilli	Gram -ve
13	Jho S5B13	irregular	filamentous	raised	medium	smooth	dark brown	opaque	Bacilli	Gram +ve
14	Jho S8B14	circular	entire	flat	punctiform	smooth	tan	translucent	Bacilli	Gram +ve
15	Jho S10B15	circular	entire	flat	punctiform	smooth	cream	opaque	Cocci	Gram +ve
16	Jho S8B16	circular	entire	flat	punctiform	rough	tan	translucent	Short rod	Gram +ve
17	Jho S9B17	circular	entire	convex	small	smooth	white	opaque	Cocci	Gram -ve
18	Jho S9B18	circular	entire	flat	punctiform	rough	tan	translucent	Cocci	Gram +ve
19	Jho S10B19	circular	entire	flat	punctiform	rough	tan	translucent	Short rod	Gram -ve
20	Jho S10B20	circular	entire	flat	punctiform	rough	tan	translucent	Cocci	Gram +ve

Primary screening protease enzymes

There were 11 protease producers from Jhow and 13 from the Borival site. Total amylase producers were 17 from Jhow and 20 from Borival site. Total cellulase producers were 9 from Jhow and 12 from Borival site.

Bacterial selection inverted pyramid approach

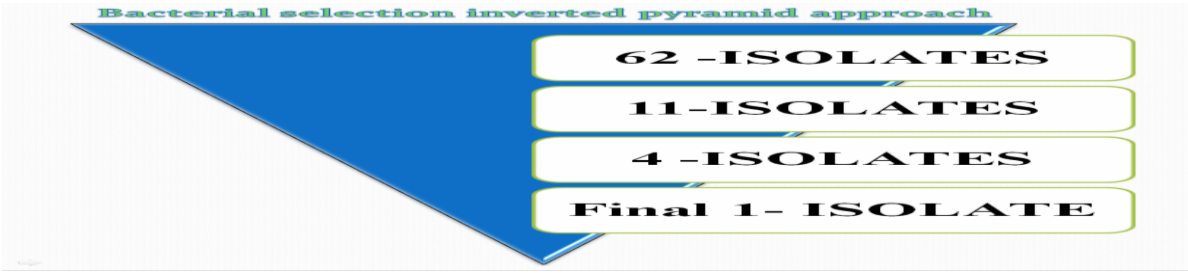

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Figure 7: Selection funnel

Total of 62 Isolates obtained from the samples processed, from them based on enzymatic screening of isolates they were screen down to 11 isolates. From them 4 isolates were selected based fast growing, from them best one protease producer was selected (Bor S17B13). This isolate showed peach colour when grown in higher salt concentration then its usual tan coloration. This isolate also produce biofilm. This isolate was further carried for optimization of media and identification of organism.

Molecular Identification of selected Isolate

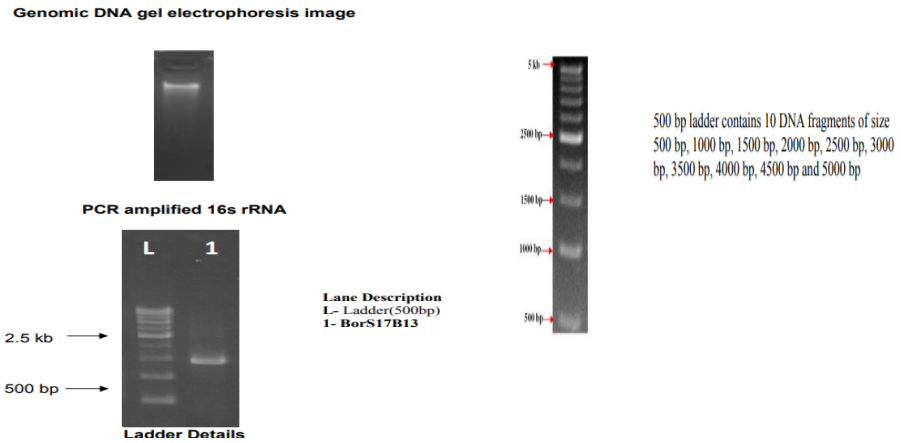

Figure 8. Molecular Identification

Primer Details - The PCR product size ~1.5 kb No. Oligo Name Sequence (5`à 3`) Tm (°C) GC- Content 1 16s Forward GGATGAGCCCGCGGCCTA 57 72.22% 2 16s Reverse CGGTGTGTACAAGGCCCGG 58 68.42% Aligned Sequence Data of Sample – BorS17B13 (1362bp)

- BorS17B13

GGCCCTTCCTATAGACGTTAGCGCGGCGGGCGCTAACCGGGGAACTGCTGAAATGGGTAATTCGG
GAACCGAGTATACCGGTAGGTCTCTCCTTCTGGGAGATGATTGAAAGTGGTTCGGCTATCACTTAC
AGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC
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CTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAAAGCGGAATTCCA
CGTGTAGCGGTGAAATGCGTAAAGATGTGGAGGAGAACACCAGTGGCGAAGGCGGCTTTTTGGTC
TGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAG
CACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAC
AACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCAT
TTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATC
ATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACC
GCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATG
AAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACAC
ACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCCCGTGGCAGTCAACT

Above mention is the sequence of Bor S17B13 isolate. This isolate also contain plasmid

Phylogenetic tree

Sample: Bor S17B13

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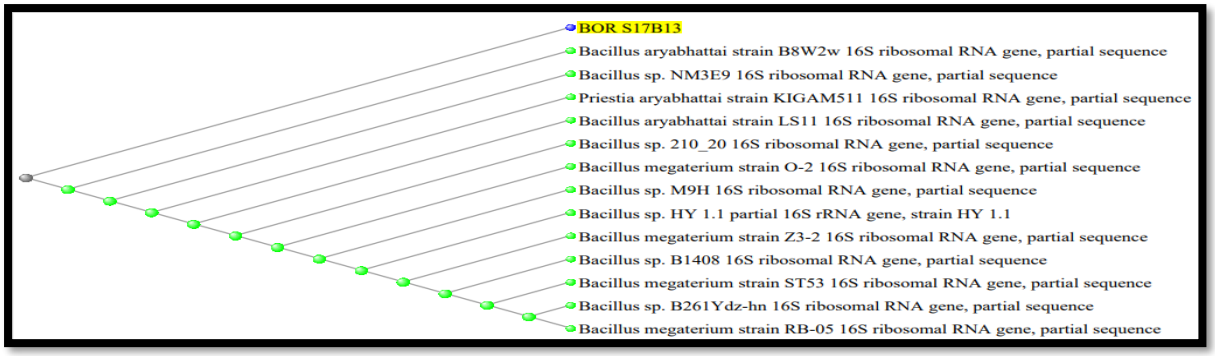

Figure 9. Phylogenetic tree of Bor S17B13

These indicate the phylogenetic tree of isolate Bor S17B13. The closest homologue was found with Priestia aryabhattai strain-based 16s ribosomal RNA gene sequencing. The above-mentioned strain, whose scientific name is Priestia aryabhattai strain. Bor S17B13, has characteristics such as strain mension. The peach colour and other colony morphology characters are of Indian origin and are found in western India, where the sample was collected. The data above was presented with the help of the BLAST-NCBI tool, and the method employed was neighbor joining.

NCBI submission

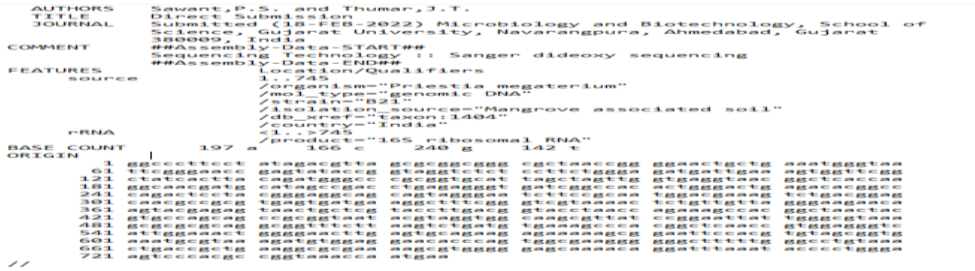

Figure 10. NCBI Sequence submission ID.

The above mention sequence was submitted to NCBI and Gene accession No. SUB11100631BorS17B13 OM743775, Priestia megaterium strain B21.

CONCLUSION

The present study displays the samples processed from areas with less human interference, including Jhow Island and Borivali Monari Creeks and Forest. Here, the morphological characteristics of isolates were studied. The following characteristics were dominant in 42 isolates from the Borivali site: circular shape, entire margins, convex, small size colonies, smooth textures, cream pigments, opaqueness, gram positive, and a major organism group belonging to Cocci. On Jhow Island, from 20 isolates, the dominant characteristics were: circular shape, entire margins, flat elevation, punctiform colonies, smooth textures, tan pigments, opaqueness, Gram positivity, and the organism major group belonging to Cocci. Primary screening was done for industrially important enzymes like protease, amylase, and cellulase. For secondary screening, protease producers were selected, and by applying the inverted pyramid method, isolate Bor S17B13, which was the maximum protease producer, was selected for enzyme kinetics. Isolate Bor S17B13 was identified by 16S rRNA sequencing, and a phylogenetic tree was prepared, which shows that the isolate belongs to the Priestia aryabhattai strain. The sequence was submitted to NCBI gene accession no. OM743775, Priestia megaterium strain B21. Protease enzymes have a wide range of applications, ranging from Bio-industry to Bioremediation. Further studies will open up new horizons for up scaling enzyme production.

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