



**BIOPROSPECTING OF HALOPHILIC BACTERIA FROM THE COASTAL AREA
OF MARAKANAM SALTPAN**

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ABSTRACT

The saltpan is the resource of cultivating halophilic microbes for their produce diverse metabolites. This study was characterized a total of sixteen halophilic bacteria from Marakanam Saltpan, Tamilnadu, India. Among this isolate only Six isolates having bioprospection such as the production of hydrolytic enzymes, pigments, antibiofilm, secondary metabolites. These are identified by molecular networking sequences showed the *Bacillus aquqmaris* and *Bacillus velezensis*. Secondary metabolites extracted from hexane solvent showed high antimicrobial activity against clinical pathogens. In GC-MS, FT-IR analysis was carried out for the checking of chemical compounds, Besides, all the isolates having great potential sources of biotechnological applications.

Keywords: Saltpan, Hydrolytic Enzymes, Secondary Metabolites, Bio prospectors

1. INTRODUCTION

The hypersaline environment Halophilic microbes with having the represents a source for the isolation of bioprospecting products. Halophiles are

mainly isolated from solar salterns, lakes, sea and ponds. The 3-15% of NaCl containing growth media used for cultivating the moderate Halophilic bacteria [1]. The Protective mechanism of halophiles is very important because it is based on the principle of osmotic pressure. The compatible solutes present in the cells raised by the aggregation of halophiles to counter the osmotic effect. This halophilic microbe that accumulate will create certain enzymes that generate K^+ ions in turn. These ions increase the inner salinity and the outer osmotic pressure accordingly [2]. Traditionally, salterns are used for only salt production for food stuff but halophiles are excellent sources of enzymes production, its useful enzymes are pursuit in industrial applications. *Halococcus* species are produce the extracellular enzymes isolated from the Goa salt pan in India. *Barrientosiimonas* species moderate halophilic bacteria produce protease enzymes with great biotechnological potential [3]. The halophilic bacteria are the ability to produce important enzymes such as an Amylase, Lipase, Protease, Gelatinase and Lipase. Natural salterns are the best source of these enzymes comparative than the artificial salterns [4].

The carotenoid pigment is potential to antioxidant and antibacterial agents, its produced by *Halorubrum* sp to grow at

saline environments. Flavonoid contents found in the halophilic pigments due to good antioxidant scavenging activity. Colours are playing at the important role of major industries like food colouring agents, textile industries and pharmaceutical purpose but halophilic bacteria also producing bright colours pigments, especially *Bacillus* producing pigments have good Potential antioxidant activity [5]. HPLC techniques are used to check the stability of various pigments. *Halorubrum*, *Haloarcula*, *Haloferax*, *Natrinema*, *Halogeometricum*, *Haloterrigena* and *Halopiger* have produced carotenoids pigments, this natural pigment was eco-friendly and potential for biomedical concerns [6]. Bioactive compounds area great novelty source of impact on public concerns, discovered from solar salt pans utilized for anticancer, antimicrobial, antimalarial, antibiofilm and antibiotic production applications. Antibiotic-resistance is the burden of our society in recent years. Natural resources are the best platform to search for novelty compounds [7].

In this research, the genus of *Bacillus* isolated from Marakanam saltern environments are characterized Gram-positive, Rod-shaped bacterium growth with the supplement of NaCl concentration purified simple plate techniques [8]. The

culturable bacteria are screened to the production of enzymes, pigments and bioactive compounds, there is the ability to produce secondary metabolites indicated by good diffusion, antagonistic activity and MIC against the clinical pathogens. Molecular networking was identified by the Genomic DNA kit [9].

The databases of a gene bank to using search for similarity sequencing of clusters and deposited on NCBI Genebank [10]. The full goal of the research work to identify the bioprospecting producers from halophilic bacteria in salterns.

2. MATERIALS AND METHODS

2.1. Study Area / Sampling site

Samples were collected from the coastal area of Marakanamsaltpan is located in the Vilupuram District in Tamilnadu, India (12°13'02"N;79°58'12"E). Salt production throughout the summer seasons and end at the monsoon. A sampling of soil and sediments was collected at Marakanam saltpan in February 2020 at ten different location distance between 50-100 meters at the site. Temperature ranges from 30-37°C, Salinity from 0.85% to 0.95%, pH above 7.4 analysed by using a Refractometer. Samples were collected in a sterile Zip-Lock covers and transferred to the laboratory for Halophilic bacteria isolation

with suitable condition with appropriate transportation [10].

2.2. Isolation of cultivatable bacteria

Sediment/soil samples were serially diluted upto the 10^{-7} . Serial dilution technique and Spread Plate Technique was used for the isolation of the Halophilic bacteria, inoculating each dilution in Halophilic agar (Hi media) plates containing 50% of seawater then incubated at 37°C for 24 to 72 hours, to avoid fungal contamination using 50µg/ml Cycloheximide. After the growth of microorganisms, the pure cultures were obtained by sub-culturing at fresh Halophilic agar tubes containing 15% glycerol stock solution and lyophilized at Freezing condition [11].

2.3. Morphology and biochemical properties

The cultivatable Halophiles confirmations was performed and identified according to the Bergey's Manual of Systematic Bacteriology. [12]

2.4. Screening of various enzymes

2.4.1. Amylase

Amylase activity was done by the method described by Drissi Kaitouni *et al.*, (2020) on Starch agar medium. A Plates were incubated at 37°C for 2 days and the activity was revealed using Iodine solution. A positive result indicated by a clear zone

around the bacterial colonies on the medium [4].

2.4.2. Lipase

Lipase activity of all the isolated strains was determined by Nutrient agar medium containing 1% Tributyrin and incubated at 37°C for 2 days. After incubation showed clear zones indicates a lipase production [13].

2.4.3. Protease

Screening of protease activity by using Skim Milk agar and inoculated all strains incubated at 37°C for 2 days. After the incubation period, the clear zone indicates casein hydrolysis as confirmation of protease activity [14].

2.4.4. Cellulase

All the isolated strains inoculated in Nutrient agar containing Carboxymethyl-cellulose and incubated at 37°C for 4-7 days. After incubation, the CMC plates flooded with 0.1% Congo red reagent. The clear zones around the colonies reveals the activity of cellulase [15].

2.5. Pigment extraction

The pure cultures of all pigment-producing halophilic bacteria were grown in halophilic broth. All the pure cultures were inoculated separately and incubated at 37±2°C in an Orbital shaker under 120-150rpm. After three days of incubation, the culture was centrifuged at 10,000 rpm for 10 minutes, the supernatant was discarded.

The pigments were extracted using acetone (intracellular) and ethyl acetate (extracellular). The extracted pigments were subjected to dry under vacuum oven at 50°C for overnight [11].

2.6. Antimicrobial screening

2.6.1. Assay of Antagonistic activity

Pathogens are *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus cereus*, *Streptococcus pyogenes*, *Escherichia coli* were obtained from Rajah Muthiah Medical College and Hospital Chidambaram, Annamalai University. These above-mentioned cultures procured with a proper culture collection procedure. This assay performed by using Halophilic agar. After sterilization, the bacteria were streaked at the right angle of a plate and inoculated pathogens at the same plate. Allow incubating at 37°C for 48 hours. After that checking antagonistic activity by measuring the inhibition area around the isolated bacterial colony [1].

2.6.2. Extraction of bioactive compounds

The potential culture supernatant was extracted with Ethyl acetate, Hexane, Acetone, Chloroform (1:1) by using separating funnel. The organic fraction of metabolites dried under the rotary evaporator. The obtained extracts were used for testing all biological activities and the uninoculated medium used as a control [16].

2.6.3. Well diffusion method

The selected positive antagonistic bacterial culture is inoculated into the halophilic broth culture and centrifuged at 5000 rpm for 10 min. After centrifugation, supernatant collected through a 0.22 µm membrane filter. Pathogenic microbes were inoculated into Muller Hinton Agar plates and wait for solidification, three wells (6mm) in diameter by using well borer. Adding the supernatant on each well without overflow and incubated at 37°C for 24 hrs and the inhibition zone was measured. The sterile medium without inoculation of culture was served as a negative control [17]. Positive isolates having inhibitory activity were identified using 16S rRNA gene sequencing.

2.6.4. Disc Diffusion

The Different solvents using crude extracts are check to the secondary screening by disc diffusion method. Pathogens are inoculated into the nutrient broth and incubated at 37°C for 24 h. The diameter of zone of inhibition determined and compared to standard techniques. The extracts were tested against pathogens, Using chloramphenicol as a positive control and DMSO as a negative control [17].

2.6.5. MIC Determination

The Minimum Inhibitory Concentration value of potential halophilic

strain against clinical pathogens by used dilution method [18].

2.7. Antioxidant assay

The antioxidant assay was performed according to the method of Sahli *et al.*, 2020. All the isolate was inoculated in nutrient media and incubated on a shaker at RT for 24 h. The bacterial cell mass was separated by centrifugation and cell-free supernatants were lyophilised and stored at 4°C. 100 µg of these lyophilised crude extracts was reconstituted in sterile distilled water, filtered through Millipore filter and used for screening the production of antioxidants using DPPH radical scavenging assay. Two ml of extract was mixed with 2ml of freshly prepared DPPH solution (0.03 mM prepared in methanol) and incubated at room temperature for 30 minutes in dark. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive standard. The percent radical scavenging activity (RSA) was calculated as $RSA (\%) = (A_0 - A_1/A_0) \times 100$ Where A0 is the absorbance of the control and A1 is the absorbance of the test sample [5, 6].

2.8. Assessment of anti-biofilm activity.

The potential strains were added to the wells containing the media and bacterial cells. Five different concentration (25, 50, 75,100,125µg/mL) were tested. Biofilms of selected pathogens were poured into the

Polyvinyl tubes. Cell suspensions of overnight cultures incubated at the 37°C for 48 hours and adjusted to OD 595nm and this suspension (500µL) was dispensed into the Eppendorf tubes. After that, the biofilm was analysed by Crystal violet assay. A 200µL of 0.41% (wt/vol) crystal violet in 12% ethanol was added to polyvinyl tubes containing Biofilm, along well with DMSO (100µL) was used as a negative control and incubated at room temperature. After incubation, the bacterial culture was removed and the tubes were gently washed twice with 200µL of phosphate-buffered saline (PBS) to remove unattached cells. The tubes were washed several times with PBS to remove excess stain. The tubes were left to dry for 10 min at room temperature. Next Quantitative assessment of biofilm formation was obtained by (570nm) using the spectrophotometer [19].

2.9. Molecular Networking of bacterial isolates

The genomic modelling of strain MSPB2& MSPB5 was generated at the DNA Sequencing following the instructions of the CAGL protocol. The 16S ribosomal RNA (rRNA) gene was amplified by polymerase chain reaction (PCR) using archaeal-specific primers: 21F (5'TTCCGGTTGATCC TGCCGGA-3') and 1492R (5'-GGTTACCTTGTTACG ACTT-3'). The *Bacillus aquamaris* & *Bacillus*

velezensis draft Genome was deposited in the NCBI genebank and simultaneously made available to ENA in Europe and the DNA Data Bank of Japan under accession number MT211546 and MT211539. The sequence obtained from the sample was aligned and edited using MEGA software version 9. Phylogenetic tree constructed with the aligned sequences using NCBI-BLAST. The sequence obtained from the sample shows a 99% similarity with *Bacillus aquamaris* and *Bacillus velezensis* [6].

2.10. Gas Chromatography-Mass Spectrometry

GC-MS of the Hexane extracts were recorded using Model Perkin Elmer Clarus 600. An Elite-5 MS silica capillary column (30.0 m × 0.25 mm ID, 250 µm df) was used. The oven temperature was programmed at 60°C for 2 min and then increased to 300°C for 6 min. Helium was used as the carrier gas and injector temperature was set at 250°C. The injection size and flow rates were 1.0 µL and 1.0 mL/min, respectively. The acquired spectra were analysed about the NIST database [20].

2.11. FT-IR

The infrared (IR) spectrum of the purified crude extract was determined by EXI- Spectrum One Model. The spectrum was obtained using potassium bromide

(KBr) pellet techniques in the range of 4000 to 400 cm^{-1} at a resolution of 1.0 cm^{-1} . Potassium bromide (AR grade) was dried under vacuum at 100°C and 100 mg of KBr pellet was used. The spectrum was plotted between intensity and wave number. The FT-IR spectra were analysed for the presence of various functional groups [21].

3. RESULTS

3.1. Isolation and characterization of isolates

In this study, Marakanam Saltpan area was investigated to characterize and isolate of cultivatable bacteria. Six strains of cultivatable halophilic bacteria were isolated. These isolates are namely MSPB1 to MSPB6 (**Figure 1**). Out of all two isolates only carried out morphological and biochemical characterization shown in (**Table 1**). The isolated halophilic bacteria are grown at 30°C, temperature, 7.5 pH and 1.5-30% salinity.

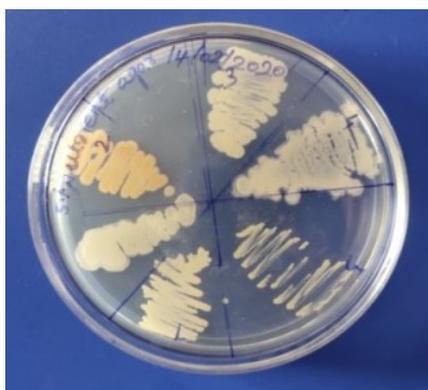


Figure 1: Pure cultures of Halophilic bacteria

3.2 Physiological, Biochemical characterization

All the isolates are carried out morphological and biochemical

characterization. Halophilic agar supplemented with 1-25% (w/v) NaCl and incubated at 24h. Morphology of the isolates was observed by compound microscope. Gram-staining was performed as described previously by the Bergey's manual. Cells were gram-positive, rod-shaped, smooth, and opaque colonies are established the *Bacillus* sp. A summary of the physiological and biochemical features of two isolates such as colony morphology, pigment, cell morphology, motility, has been shown in **Table 1**.

3.3. Screening Hydrolytic Enzymes

The selected six strains were screened for enzyme production. In this study, three isolates MSPB1, MSPB2 & MSPB6 halophilic bacteria showed the clear zone to produce the Amylase, Cellulase, Protease and Gelatinase enzymes. Another three strains did not show zone (**Table 2**).

3.4. Extraction of Pigments

The pigment-producing halophilic bacterial cells were extracted by centrifugation. The Pale yellow, Dark Brown, Light Pink and Dark yellow coloured pigments produced from the Four isolates (**Figure 1**).

3.5. Antimicrobial activity

3.5.1. Antagonistic activity

Out of the six tested strains, only two strains showed high potential activity.

The inhibition zone was observed after the incubation period. The isolate MSPB2 and MSPB5 showing high zone of inhibition in diameter against bacterial pathogens was tabulated in **Table 4** and **Figure 2**.

3.5.2. Screening of the Bioactive compounds

The Secondary metabolite from the pure culture of the isolate MSPB2 & MSPB5 was extracted. The secondary metabolite was extracted from an equal volume of Chloroform and Hexane as a solvent.

3.5.3. Antibacterial activity of crude

In secondary screening, the extracellular metabolites produced by MSPB2 and MSPB5 was screened for the antibacterial activity by agar Well Diffusion method. The metabolites from MSPB2 showed maximum zone of inhibition against all the pathogens viz., *S.Pyogenes*, *E.coli*, *B.cereus*, *S.aureus*, *P.vulgaris* (**Figure 4**).

3.5.4. Determination of MIC Values

The Chloroform and Hexane crude extracts of the MSPB2 & MSPB8 isolate were analysed for the MIC by broth micro dilution method and resulted tabulated in **Table 5 & 6**. The Hexane extract showed microbes in the Phylogenetic tree. It is an important tool for identifying bacterial species for further research investigation. In

the lowest MIC ranging from 0.165-0.33 mg/ml. From the results, the Hexane extract of MSPB2 isolate was chosen for further characterization.

3.6. Antioxidant assay

To evaluate the antioxidant activity of extracted Bioactive compounds of the isolates *Bacillus aquamaris* sp MSPB2 and MSPB5 using DPPH assay. These molecules act as a potential antioxidant activity at a scavenging capacity. Comparative studies used as an ascorbic acid showing low scavenging activity than the bioactive compounds from *Bacillus aquamaris* sp. (**Table 7**).

3.7. Result of Anti-Biofilm activity

The comparisons of the crude extract are penetrated through biofilm was tested and the result of penetration of crude extract was lower than without adding crude extract. The zones of two comparisons significantly reduced through the biofilms of isolates in compared with the respective control assemblies (**Figure 7, Table 8 & 9**).

3.8. Phylogenetic tree analysis

The cultivatable bacteria genome sequencing (16S rRNA) from PCR amplification used to identify similarities this technique, gene sequencing revealed the presence of *Bacillus* groups. MSPB2 and MSPB5 isolates were associated with

the members of the diverse *Bacillus* spectrum. Isolate MSB2 *Bacillus velezensis* had a 99% MSB8 *Bacillus aquamaris* had a 100% Similarity to NCBI database for comparison with other sequences.

3.9. GC-Ms analysis of MSPB2

GC-Ms analysis of crude extracts from chloroform and hexane detected for the presence of chemical compounds as shown in **Table 10**.

Table 1: Physiological and Biochemical features of halophilic Bacteria

S. No.	Test	Results	
		MSPB2	MSPB5
1.	Gram Staining	+ve	+ve
2.	Morphology	Rod	Rod
3.	Motility	+	+
4.	Colony colour	Pale Yellow	Creamy White
5.	NaCl Concentration	1-20%	1-15%
6.	pH	9	7.5
7.	Temperature	37°C	37°C
8.	Indole	+	-
9.	Methyl Red	+	+
10.	Voges Proskauer	-	-

Table 2: Qualitative Enzyme Screening

S. No.	Enzymes	MSB1	MSB2	MSB6
1	Amylase	+	-	-
2	Cellulase	+	+	-
3	Protease	+	+	+
4	Lipase	+	-	-

+ Presence of zone of hydrolysis, - Absence

Table 3: Characteristics of Pigments

S. No.	Isolates	Colour	Morphology	Colonies
1	MSPB1	Dark Brown	+ve Rod	Irregular, Smooth
2	MSPB3	Yellow	+ve Rod	Circular, shiny
3	MSPB4	Creamy Pink	-ve Rod	Small, Smooth
4	MSPB6	Orange	+ve Cocci	Circular

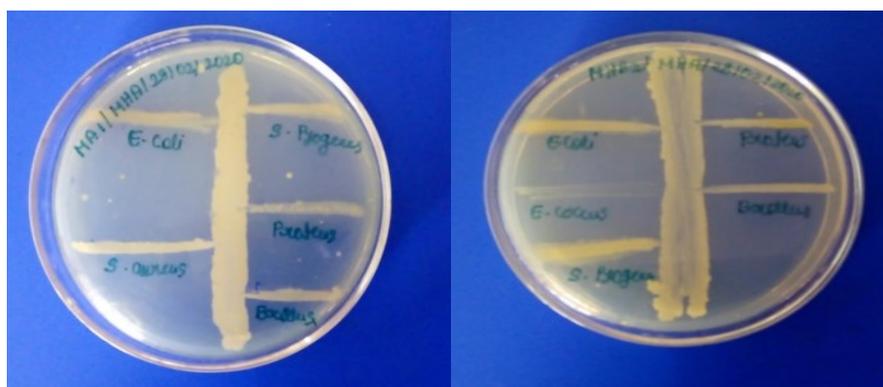


Figure 2: Assay of antagonistic activity

Table 4: Antagonistic activity against bacterial pathogens

S. No.	Isolates	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Proteus vulgaris</i>	<i>Escherichia coli</i>	<i>Bacillus cereus</i>
1.	MSPB1	+	+	-	-	-
2.	MSPB2	++	++	++	++	+
3.	MSPB3	-	+	+	+	+
4.	MSPB4	+	+	-	-	+
5.	MSPB5	++	+	+	++	++
6.	MSPB6	++	-	-	-	+

-: No inhibition, +: moderate inhibition, ++: high inhibition

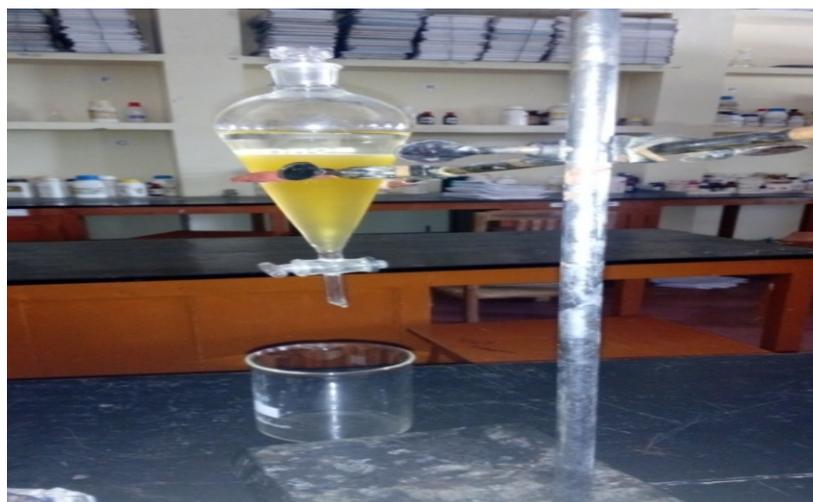


Figure 3: Bioactive compounds separation

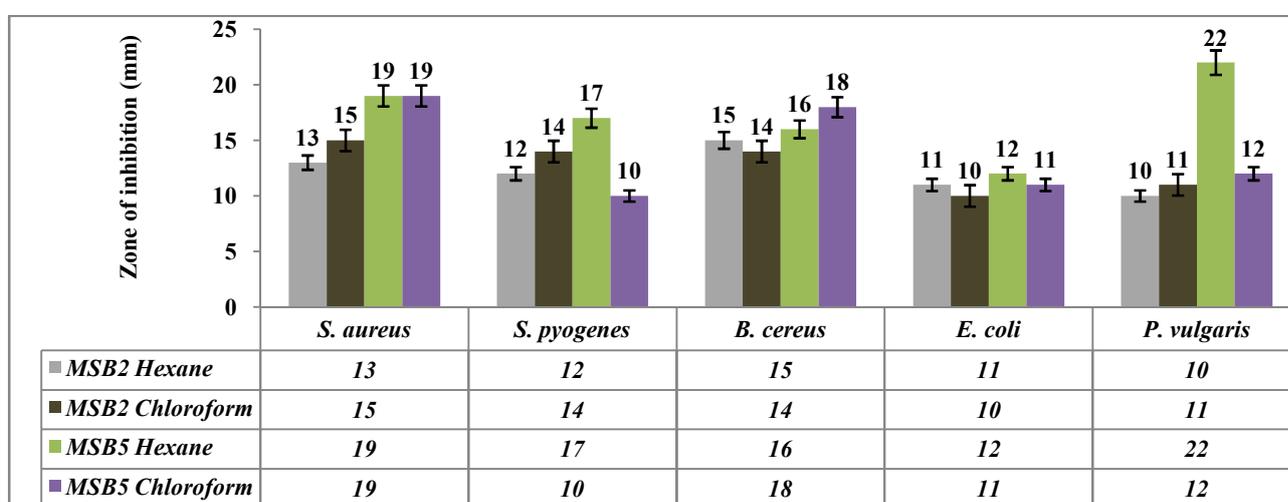


Figure 4: Antibacterial activity of cell-free- supernatant

Table 5: MIC value of crude extracts of *Bacillus aquamaris* sp. MSPB2

Extract	Concentration of Crude extracts (mg/ml)				
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Proteus vulgaris</i>	<i>Escherichia coli</i>	<i>Streptococcus Pyogenes</i>
Chloroform	0.93	1.87	-	1.80	-
Hexane	0.33	0.36	0.90	0.165	1.90

Table 6: MIC value of crude extracts of *Bacillus velenzsis* sp MSPB5

Extract	Concentration of Crude extracts (mg/ml)				
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Proteus vulgaris</i>	<i>Escherichia coli</i>	<i>Streptococcus pyogenes</i>
Chloroform	0.36	0.21	1.90	-	-
Hexane	0.20	1.99	0.90	0.165	1.98

Table.7: Antioxidant activities

S. No	Isolates	% Antioxidant Activity
1	MSPB1	32.01
2	MSPB2	50.49
3	MSPB3	39.84
4	MSPB4	32.61
5	MSPB5	49.57
6	MSPB6	40.10

Table 8: Quantification of biofilm by CV assay of Chloroform Extract

S. No	Pathogens	(OD 570 nm)			
		MSPB2		MSPB5	
		Control	Test	Control	Test
1	<i>Staphylococcus aureus</i>	0.237	0.109	0.305	0.240
2	<i>Streptococcus pyogenes</i>	0.138	0.053	0.241	0.102
3	<i>Bacillus cereus</i>	0.184	0.087	0.150	0.70
4	<i>Escherichia coli</i>	0.147	0.049	0.256	0.100
5	<i>Proteus vulgaris</i>	0.243	0.082	0.130	0.50

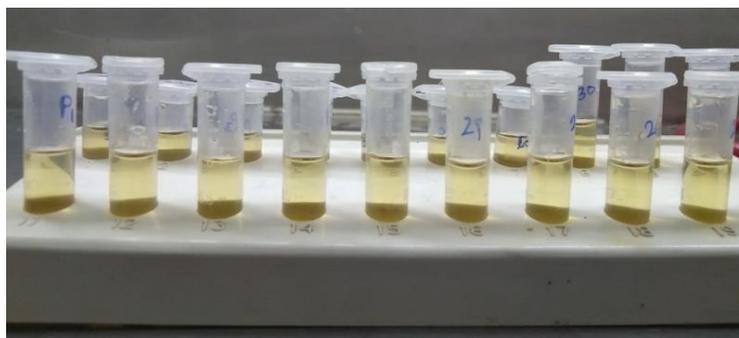


Figure 7: Biofilm activity Zone of Inhibition (mm)

Table 9: Biofilm activity Zone of Inhibition (mm)

S. No	Pathogens	Biofilm activity zone of Inhibition (mm)	
		MSPB2	MSPB5
1	<i>Staphylococcus aureus</i>	>16	15
2	<i>Streptococcus pyogenes</i>	>20	<21
3	<i>Bacillus cereus</i>	<24	19
4	<i>Escherichiacoli</i>	19	21
5	<i>Proteus vulgaris</i>	21	17

Table 10: GC-MS analysis of MSPB2

Name	Area %	Height %	A/H	Retention Time
9-Octadecenoic acid (Z)- (CAS) Oleic acid	7.82	8.96	6.48	14.006
3-Cyclohexen-1-ol (CAS) Cyclohexen-4-ol	0.98	0.88	8.32	14.392
9,12-Octadecadienoic acid (Z,Z)- (CAS)Linol	30.07	26.14	8.53	15.878
Hexadecanoic acid (CAS) Palmitic acid	17.88	10.86	12.21	16.090
METHANETRIAMINE,N,N,N',N',N'',N''-HE	1.69	4.30	2.92	16.375
7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl	1.98	4.30	3.42	16.429
DIHYDROTORULOSOL	1.55	3.92	2.93	16.483
1-methyl-2-acetoxycyclohexane	1.14	3.56	2.38	16.517
2-Propenoic acid, 6-methylheptyl ester (CAS)	14.71	8.89	12.28	16.689
6,9,12-Octadecatrienoic acid, methyl ester(CA	1.95	1.95	5.61	16.907
Hexanoic acid, 2-hexenyl ester, (E)- (CAS)tra	1.15	1.00	8.55	17.192
1,4-CYCLOHEXANEDIOL	1.44	1.25	8.55	20.408
Silane, (2-ethoxycyclohexyl)trimethyl- (CAS)	1.07	2.40	3.33	20.617
Octadecanoic acid, 3-hydroxy-, methyl ester	1.36	2.83	3.55	20.650
Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-met	3.99	3.46	8.56	20.729
Methyl-O-(2-methylpropyl) ester of carbamoth	1.45	3.15	3.42	20.883
ACETAMIDE,N-(3-METHYLTRICYCLO[2.	2.39	3.47	5.11	20.976
7-Thiabicyclo[4.1.0]heptane, 1-methyl-(CAS)	3.25	3.60	6.68	21.083
STEARIC ACID	2.55	3.37	5.62	21.125
1-Dodecanol (CAS) n-Dodecanol	1.58	1.10	10.61	22.759

4. DISCUSSION

The saltpan is the highly intensive terms of saline environment and they can harbour truly halophilic bacteria. However, the potential halophilic bacteria present in these environments have several Bioactive compounds from a halophilic bacterium had antimicrobial activity, Antioxidant activity, Antibiofilm and production of Hydrolytic enzymes, Pigments and Bioactive compounds. The isolation of Halophilic bacteria followed by using the protocol reported by Mariana, 2018. In this study, reported on cultivatable bacteria isolate from Saltpan by Common Spread plate technique, and potential for bioprospecting applications was analysed [10].

Halophiles are reported to produce hydrolytic enzymes are Protease, Amylase, Cellulase and Gelatinase were isolated. Krishna reported on the halophilic bacteria is a source of important industrial enzymes isolated from the saline environments, Marakanam saltpan. *Marinobacter* sp. and *Bacillus* produce lipase and Protease enzymes for the high industrial purpose. Amylase, Lipase, Protease also reported from the *Halomonas* sp. [14].

Pigments are the source of antioxidants and antibacterial activity, *Halorubrum* species are highly produced the carotenoid Production and had good

scavenging activity. Sahli *et al*, reported that moderate halophilic Bacteria of pigments are a response to the salt effect and scavenging property [6].

The first lipolytic microbes report on *Halomonas* sp. are producing biosurfactant for an antibiofilm activity [5]. Halophilic bacteria can be developed as an innovative source of secondary metabolites, isolated from saltpan, Bibi revealed the *Halodule universis* from Saudi to screening the antimicrobial activity by the antagonistic activity method. Bioactive compounds are useful for the control of bacterial diseases [22]. The new strategy of mixed halophilic bacteria to develop the compounds. *Bacillus subtilis* showing highest antibacterial activity from solar salterns [16].

In this study, we have used 16s rRNA sequencing for the identification of *Bacillus aquamaris* and *Bacillus vezensills*.

It's revealed the isolation of halophilic *Bacillus* species was successfully produced the hydrolytic enzymes, pigments and Bioactive Compounds. To our knowledge, using molecular network analysis for isolating these Potential Halophilic Bacteria from Marakanam Saltpan, Therefore further studied carried out the antimicrobial

activity followed by Antagonistic Activity, the primary screening extends to Separation of Bioactive Compounds. The antimicrobial activity of the solvent extracts of Hexane against the Clinical pathogens, As the results revealed the *Bacillus aquamaris* and *Bacillus venzensills* are having the antioxidant activity, bioactive Compounds are characterized for GC-MS and FT-IR analysis.

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