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Bioprospecting for moderately halophilic eubacteria for potential biotechnological applications from Sambhar Lake, Rajasthan, India

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Abstract: Sambhar Lake an athalassohaline habitat located at 27°58′N 75°55′E, Rajasthan, India is a major source of salt production in the country. From surface lake water, soil, and shore sediments, fifty-nine moderate halophiles were isolated which were subsequently grouped according to shape, colony characteristics, and staining into twenty-two isolates. Fourier transform infrared spectroscopy profiling identified these isolates as eubacterial with characteristic C=O stretching of ester functional groups. Observations further indicated similarity within some *Halomonas* isolates indicating potential phylogenetic lineages. The FASTA sequences obtained after sequencing with universal bacterial primers were processed for phylogenetic analysis. Predominantly Gram-positive genera like *Alkalibacillus*, *Amphibacillus*, *Marinococcus*, *Piscibacillus*, *Planococcus*, *Salinicoccus*, *Staphylococcus* and *Virgibacillus* with only two Gram-negative strains of *Halomonas* were identified. The genus *Amphibacillus* was recognized for the first time in the study of Sambhar Lake. Despite being moderately halophilic, several isolates exhibited high salt tolerance with growth in 25% salt. All isolates were mesophilic with growth observed between 18-42°C which matches the temperature profile of the region. Analysis of hydrolytic potential identified eighteen isolates as protease producers, thirteen as lipase producers, and ten as cellulase-producing strains. Further evaluation showed the dominance of C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C18:1 FAMEs, among which presence of C16:0 and C18:1 fatty acid indicated probable antimicrobial potentials of these strains.

Keywords: moderate halophiles; eubacteria; diversity; phylogenetic analysis; salt range; enzymes

1. Introduction

Increasing demand for novel products is focused on the unexplored microbial diversity present ubiquitously. In this search, habitats yet unexplored or relatively unexplored are garnering a lot of attention with respect to the microbes they may host, and the range of biotechnological benefits harnessed which include enzymes, pigments, solutes, and fatty acids [1]. Additional ecological niches that have not yet been explored should be investigated to meet the demands of the market. The underexplored, hypersaline environments are now recognized as areas harboring novel organisms with unique biotechnological potential [2]. Sambhar Lake, situated at 27°58′N 75°55′E, stands as the largest inland halo-alkaline lake in India, boasting salinity levels ranging from 5 to 35% and consistently alkaline pH values between 7.15 and 9. Sambhar Lake contributes significantly to India's overall salt production, meeting a substantial portion of the domestic demand. The salt industry around Sambhar Lake not only serves as an economic lifeline for the local communities but also contributes to the nation's self-sufficiency in this essential mineral [3].

Moderately halophilic bacteria are one of the most important bacterial groups adapted to hypersaline environments that grow optimally in media containing between 5% and 25% salt. Because of the wide range of salinity in this grow, they are widely distributed in different saline habitats such as hypersaline lakes, desert and saline soils, saltern ponds, salt mines, salted foods, and others [4]. The culturable bacterial diversity analysis is mostly dependent on biochemical, morphological, and phylogenetic tests. Considerably less focus is given to using FTIR for categorization and grouping prior to 16s rRNA studies Moderately halophilic bacteria have the capacity to

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produce a variety of biologically active molecules, including antibiotics, enzymes, pigments, fatty acids and more [5]. These compounds have many applications in biotechnological fields [6-9]. Halophilic enzymes possess distinct attributes that provide stability and solubility in environments with high salt concentrations. There has been a significant increase in interest in a wide range of extracellular enzymes and other substances produced by halophilic microorganisms, including fatty acids, proteases, lipases, amylases, and cellulases [10]. This interest stems from their potential applications in industries and biotechnological processes. These halophilic enzymes exhibit resistance to high salt concentration, as well as the ability to function effectively throughout a wide pH range and survive extreme temperatures [11]. Enzymes derived from halophiles have distinct characteristics compared to regular enzymes. Consequently, they provide significant uses in areas of environmental bioremediation, the food industry, and waste-water treatment [12, 13]. In the current study, the culturable diversity of Sambhar Lake was targeted to identify new strains of moderately halophilic prokaryotic diversity. The work aims towards generating a potential microbial library for further explorations of biotechnological relevance.

2. Experimental

2.1 Sample collection

The sampling site was Sambhar Lake, which has an average summer temperature of 40–45°C and a winter temperature of 6–8 °C in Rajasthan, India (26° 57′ 59.99″ N, 74° 35′ 40″ E). Samples were taken from various locations of Sambhar Lake in March 2020. These included the main lake, the lake's eastern division, which serves as a reservoir for brine, salterns, and tiny brine pools. Mostly samples taken were water samples from main lake, brine pools, and salterns (Figure 1).

2.2 Isolation and purification

Samples obtained were plated out directly on petri plates containing PPYG medium agar plates [14], Luria Bertani (LB) Medium agar plates [15], and Halophilic Medium (HM) agar plates [16] with pH maintained between 7.2–7.5 and supplemented with 5% (w/v) NaCl. 2% (w/v) agar was used as solidifying agent and incubated for 5 days. The colonies were purified and maintained in the medium of isolation. The glycerol stock of bacterial cultures was kept at −20 °C.

2.3 Morphological characterization

Cell morphology was examined using light microscopy on liquid culture in the exponential growth phase. Gram staining was performed on air-dried slides, which were fixed with a loopful of culture and desalted in 2% (w/v) acetic acid for 5 minutes. Subsequently, the slides were dried before staining, involving the creation of a thin smear from the bacterial colony [17]. The stained slides were then dried and scrutinized under oil immersion at a magnification of 100x.

2.4 Biochemical and Physiological characterization

2.4.1 Metabolic activity tests

According to Cappuccino and Sherman, 10th ed, the halophilic isolates were screened for many metabolic profiling activities through biochemical tests (citrate utilization, indole, MR-VP, gelatine, oxidase, catalase and urease test) [18].

2.4.2 Effect of physiological parameters on the growth of isolates

To detect the salt tolerance range of the isolates, different sets of LB broth (pH 7.2-7.5) were prepared and supplemented with variable salt concentrations to achieve 1%, 2.5%, 5%, 20%, 25%, and 30% (w/v) salt levels. One set with no salt was used as a control. For each isolate, inoculation was carried out using 5µl of freshly growing culture in a set of culture tubes with a salt range from 0-30% (w/v) and inoculated at 37 °C with sampling carried out on 5th day for estimating growth at 620nm [19].

Each isolate was tested for growth under different temperatures. Multiple sets of LB broth supplemented with 5% (w/v) salt and pH 7.2-7.5 were prepared. For every isolate 7 culture tubes containing medium were used. After inoculating each tube with 5µl fresh culture, for every isolate, incubation was carried out at different temperatures (12°C, 18°C, 25°C, 30°C, 37°C, 42°C and 50°C). Samples

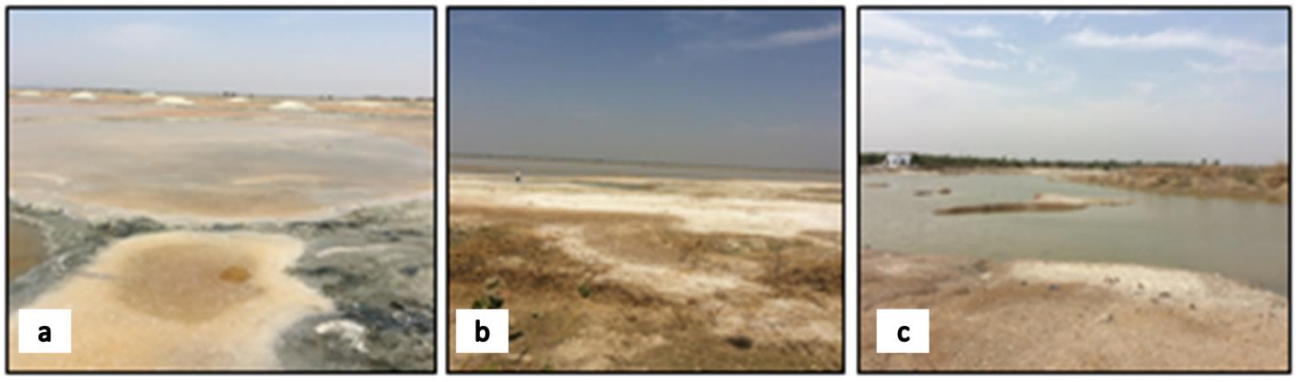

Figure 1. Various sampling sites in the Sambhar Lake, (a) Saltpan; (b) Soil from Lake Shore and (c) Reservoir water.

were withdrawn on 5th day of incubation and growth was turbidometrically estimated at 620 nm [20].

2.4.3 Scanning Electron Microscopic (SEM) studies

Morphological analysis of the culture isolates was performed according to the method of Castillo et al. [21]. Samples for SEM were prepared as follows16-18hr cultures were taken and centrifuged for 10 min at 8000rpm to form a pellet. The SEM produces a largely magnified image by using electrons instead of light to form an image.

2.4.4 FTIR studies

The functional groups analysis of prokaryotes using Fourier Transform Infrared (FTIR) spectrophotometer. The methodology given by Ghosh et al. [22], was followed for KBr pellet preparation.

2.5 Molecular characterization

2.5.1 Bacterial DNA preparation and 16S rRNA amplification

Overnight grown culture was extracted for genomic DNA isolation using the HiPurA® Genomic DNA Purification Kit with the protocol suggested by HiPurA® HiMedia. Bacterial 16S rRNA genes were amplified using universal bacterial primers, 27F 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R 5′-GGTTACCTTGTTACGACTT-3′ [23].

All PCR products were verified on a 1% (w/v) agarose gels and ran in 1X TBE buffer at 50-100 V for approximately 45-50 min. Gels were stained with ethidium bromide and bands visualized under U.V. light to confirm the amplification of the desired fragment. The amplified products were purified by using a HiPurA® PCR Product Purification Kit with the protocol suggested by HiPurA® HiMedia. The purified PCR products were sent to Eurofins (India) for sequencing and the obtained FASTA sequences were further validated by NCBI BLAST.

2.6 Screening of halophilic isolates for hydrolytic enzyme production

2.6.1 Amylase activity

The isolated halophilic bacterial species were point inoculated onto sterile starch agar medium and incubated at 37°C for 5 days after which iodine solution (0.2 percent iodine, 0.4 percent KI, 100 ml of H2O) was flooded on to the plates. After holding for few minutes, the excess stain was decanted, and the presence of a clear zone was observed [24].

2.6.2 Protease activity

The isolates were point inoculated on sterile skimmed milk agar at 2% (w/v) to investigate protein hydrolysis. After incubation at 37°C for 5 days, the plates were checked for the formation of a halo or clearance [25].

2.6.3 Lipase activity

The isolates were point inoculated on nutritional medium (pH 8.0) containing olive oil (2.0% v/v), tween 80 (1.0% v/v), and rhodamine B (0.001% w/v) and incubated under conditions mentioned above to detect a zone around the colonies indicating lipolytic behavior, the plates were subjected to UV rays [26].

2.6.4 Cellulase activity

Carboxymethyl cellulose agar medium was used for this plate assay. After 5 days on incubation of point inoculated plates at 37°C, 0.1% (w/v) congo red solution was used to saturate the plates [27]. The formation of a clear zone surrounding the culture was a sign of cellulase production.

2.7 Fatty Acid Methyl Esters (FAME) studies

The methodology given by Sasser (2006) was followed for FAME preparation [28]. The evaluation was carried out through GC-MS. The selected cultures were inoculated in respective media and incubated at temperatures ranging from 20°C to 50ºC for a maximum of 48 hours. FAME analysis was carried out as per method described by MIDI (Newark, De, USA) on Agilent 6980N GC system.

3. Results & Discussion

3.1 Isolation and phenotypic characterization of the strains

Using different media with salt concentration of 5% (w/v) resulted in fifty-nine isolates. Based on the colony morphology, staining pattern and cell morphology, these were grouped into twenty-two moderate halophilic isolates. Out of these, 14 were found to be pigmented with orange, yellow and pink/red pigmentation. Refer to the Supplementary Table 1 for the results of the morphological analysis of bacterial halophilic isolates. The colonies were mostly circular with some variations. Gram staining identifies them mostly as gram positive with only strains BVMH012 and BVMH013 being gram negative (Supplementary Figure 1). The cells were mostly cocci with different cell arrangements like single cells, double cells, clusters in rods, or cocci. Using SEM, a detailed examination could be done of the structure of these isolates further highlighting variations in cell size (Supplementary Figure 2). The biochemical characterization of these isolates presents the metabolic activity profiling of these isolates. Predominantly, the isolates exhibit negative results of indole, methyl red, Voges-Proskauer tests and gelatin liquefaction with a greater number of isolates testing positive for citrate, oxidase and catalase tests (Supplementary Table 2).

3.2 Effect of salt on growth

Results show (Figure 2) that among 22 isolates, only 5 isolates (BVMH002, BVMH005, BVMH021, BVMH023 and BVMH024) could not grow above 15% (w/v) of salt. Conversely, isolates BVMH003, BVMH006, BVMH014, BVMH016, BVMH017, BVMH019 exhibited growth up to 20% salt showing their higher range of adaptability to salt stress. Isolates BVMH004, BVMH007, BVMH008, BVMH009, BVMH010, BVMH011, BVMH012,

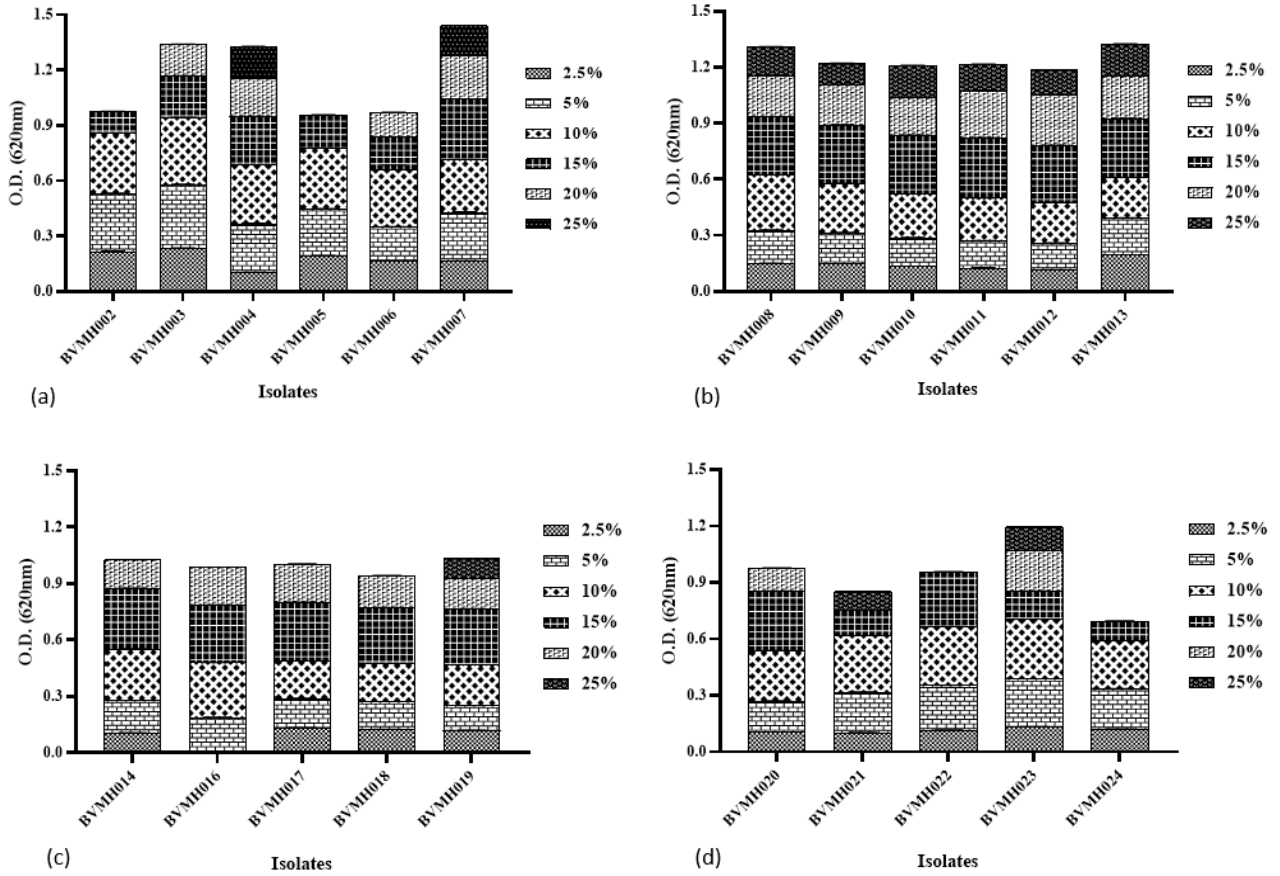

Figure 2. Effect of different salt (NaCl) concentrations on bacterial halophilic isolates. (a). isolate BVMH002 and BVMH005 (2.5 to 15% (w/v)), BVMH003 and BVMH006 (2.5 to 20%), BVMH004 and, BVMH007 (up to 25% NaCl) (b). isolate BVMH008, BVMH009, BVMH010, BVMH011, BVMH012 and BVMH013 (25%) (c). BVMH014, BVMH017 and BVMH018 (2.5 to 20%), BVMH016 (5 to 20%) BVMH019 (25%). (d). isolate BVMH020, BVMH021 (2.5 to 20%), BVMH022 and BVMH024 (2.5 to 15%), BVMH023 (upto 25%).

BVMH013, BVMH018, BVMH020 and BVMH022 could even grow at 25% of salt while retaining some growth even at 2.5% salt concentration. In presence of such high concentration though the growth rate was not very high, but it is substantial enough to allow for the organism to survive. A suitable designation for such isolates could be "Facultative moderate halophiles," acknowledging their ability to adapt and flourish across a broad range of salinity conditions. In this study, three groups of moderately halophiles could be identified based on the upper limit of salt concentration affecting growth.

3.3 Effect of temperature on growth

Results show (Figure 3) that all isolated strains exhibit growth within the temperature range of 25-42°C, demonstrating their adaptability to a broad spectrum of temperatures. Notably, a subset of strains, namely BVMH003, BVMH007, BVMH009, BVMH011, BVMH014, BVMH017, BVMH018, BVMH020, BVMH021, and BVMH023, display additional growth capability at the lower temperature of 18°C. The ability of the mentioned strains to grow at both 18°C and higher temperatures indicates their mesophilic nature. No growth shown on 12°C and 50°C indicates that strains can be considered as mild mesophiles.

3.4 FTIR analysis

The infrared analysis of all isolates revealed a consistent and strong bond at 1740-1742 cm^{-1}, indicating the presence of ester bonds. This shared characteristic strongly suggests that the isolates all belong to the eubacterial domain. The specific bond at this wavelength aligns with the C=O stretching of ester functional groups. Therefore, FTIR was employed as an identification tool for domain classification for subsequent phylogenetic analysis (Supplementary Table 3, Supplementary Figures 3 to 24). Additionally, the data shows that BVMH006, BVMH008, BVMH013, BVMH016, BVMH018, BVMH019, BVMH021 and BVMH022 are very closely related due to the similarity in the FTIR profile. These isolates may belong to similar genera.

3.5 Molecular characterization

The twenty-four moderate halophilic isolates were categorized into two phyla and nine genera. Supplementary Table 4 illustrates the evolutionary distance as well as

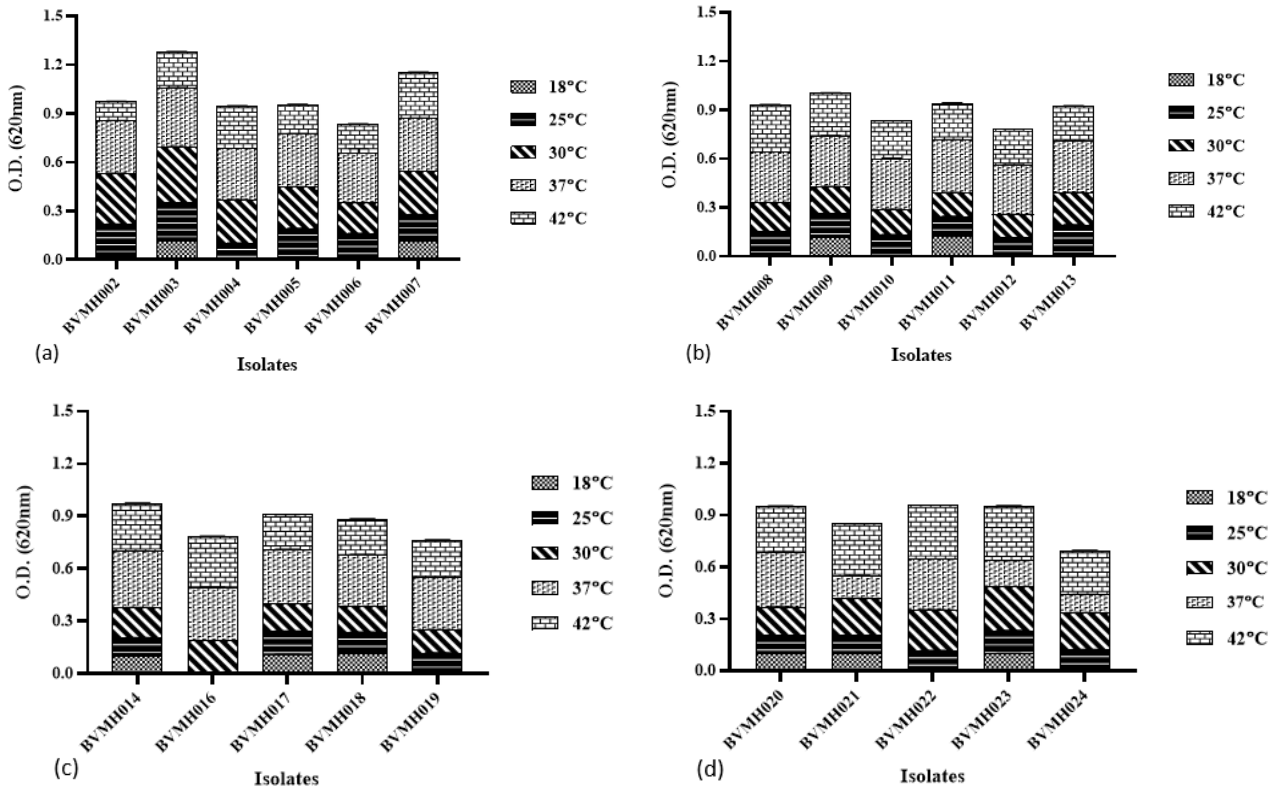

Figure 3. Effect of various temperatures on the growth of obtained bacterial halophilic isolates. (a) isolates BVMH002, BVMH004, BVMH005, BVMH006 (25-42°C), and BVMH003, BVMH007 (18-42°C); (b) isolates BVMH008, BVMH010, BVMH012, BVMH013 (25-42°C) and BVMH009, BVMH011 (18-42°C); (c) isolates BVMH014, BVMH017, BVMH018 (18-42°C), BVMH016 (30-42°C) and BVMH019 (25-42°C); and (d) BVMH020, BVMH021, BVMH023 (18-42°C), and BVMH022, BVMH024 (25-42°C).

phylogenetic relationships of isolates and a sequence comparison using the Basic Local Alignment Search Tool (BLAST) programmed with the GenBank database. Among the isolates BVMH002, BVMH005, BVMH006, BVMH021, and BVMH023, the closest relation was found with *Staphylococcus* sp. Notably, BVMH021 exhibited a sequence similarity of 92.13%, while BVMH023 showed a similarity of 94.45%. This suggests that BVMH021 and BVMH023 can be regarded as belonging to the same genus but likely represent different species within the *Staphylococcus* genus.

The acquired 16S ribosomal RNA sequences in GenBank were assigned the following accession numbers (in brackets): BVMH002 (OR965024), BVMH003 (OR965025), BVMH004 (OR965027), BVMH005 (OR965026), BVMH006 (OR965028), BVMH007 (OR965046), BVMH008 (OR965047), BVMH009 (OR965050), BVMH010 (OR965049), BVMH011 (OR965052), BVMH012 (PP101310), BVMH013 (OR965053), BVMH014 (OR965056), BVMH016 (OR965059), BVMH017 (OR965061), BVMH018 (OR965063), BVMH019 (OR965067), BVMH020 (OR965069), BVMH021 (PP101311), BVMH022 (OR965089), BVMH023 (PP101322), BVMH024 (OR965092) (Supplementary Table 4).

3.6 Screening for isolates for production of extracellular hydrolytic enzymes

Twenty-two halophilic cultures were evaluated to produce amylase, lipase, protease, and cellulase using plate assays with media containing 5% NaCl. Apart from BVMH008 and BVMH022, all the identified halophilic isolates produced at least one extracellular hydrolytic enzyme. Eleven isolates produced the enzyme cellulase, twelve isolates produced lipase, and eighteen isolates produced protease (Supplementary Table 5).

The prevalence of protease production in isolated strains from Sambhar Lake suggests a possible correlation with the presence of amino acid substrates in this saline and alkaline environment. Lipase production strains indicate adaptation to lipid-rich conditions, while cellulase production suggests the availability of cellulose substrates. It is essential to note that this information is not from a detailed enzyme study in the Sambhar Lake, and definitive statements on microbial capabilities and specific substrates require further investigation.

3.7 Fatty Acid Methyl Ester (FAME) analysis

The isolates showed the presence of C16:0 and C18:1 in all the strains and showed significant concentrations within the total lipid composition, particularly in *Planococcus*, *Virgibacillus*, *Salinicoccus*, *Halomonas*, and *Alkalibacillus*

species (Supplementary Table 6). This specific fatty acid stands out due to its higher abundance compared to other fatty acids identified, highlighting its importance in the lipid profiles of these microbial strains.

High salt concentration, alkalinity, and low oxygen stress combine to limit biodiversity in hypersaline environment [29] but reports of microbial colonisation of several hypersaline environments [30-32] have been reported. To isolate moderate halophiles from water, soil and shore sediments of Sambhar Lake in India, twenty-two eubacterial halophiles belonging to nine different genera were isolated with more strains from *Staphylococcus* and *Salinicoccus*. In different isolation studies carried out previously with the Sambhar Lake samples, many different eubacterial and archaeal taxa have been recorded. Some studies have focussed on highlighting gamma proteobacterial diversity [33] while others have focused on isolation of some lesser reported taxa like *Nesterenkonia* sp. [34]. In a detailed study reported by *Kajale* et al. (2020), significant diversity belonging to Sphingobacteriales, proteobacteria (dominated by gamma and alpha proteobacteria) were noted [35]. They also reported members of *Piscibacillus* and *Virgibacillus* among other eubacterial and archaeal members. In the current study, the genera *Amphibacillus* have been identified in the Sambhar Lake ecosystem which has not been previously reported.

Certain isolates are robust rods, while others have distinctive bacillus or coccoid *morphologies*. *Planococcus* sp., *Salinicoccus* sp., *Marinococcus* sp., and *Staphylococcus* sp. dominate the microbial ecosystem of the Sambhar Lake with their lesser-known counterparts being *Virgibacillus*, *Piscibacillus*, *Halomonas*, *Alkalibacillus*, and *Amphibacillus* sp. In this work, fourteen of the isolated strains exhibited pigmentation with orange, yellow and pink/red pigmentation. Carotenoids are the major pigments synthesised by halophiles. β-carotene, lycopene, phytoene, haloxanthin, and bacterioruberin (which includes monoanhydrobacteriorubeerin, bisanhydrobacteriorubeerin, and epoxymonoanhydrobacterioruberin) are the main carotenoid compounds synthesised by halophilic archaea and bacteria [36, 37]. Numerous carotenoids, such as phytoene, lycopene, and haloxanthin, are produced by halophiles. Nonetheless, the primary focus of researchers is to investigate the potential uses of pigments called β-carotene, bacteriorhodopsin, and bacterioruberin in many domains [38, 39].

These eubacterial isolates were tested for their capacity to grow at varied NaCl and temperature tolerance levels to determine their biotechnological interest. The halotolerance and temperature tolerance test revealed that most of the isolates were mesophilic, moderate halophiles as they showed growth between 25 to 42°C and in 2.5% to 15% NaCl respectively. Isolate BVMH004, BVMH007, BVMH008, BVMH009, BVMH010, BVMH011, BVMH012, BVMH013, BVMH018, BVMH020 and BVMH022 showed extreme resistance to high salt concentration which is very relevant for study of the nature of compatible solutes produced by osmoregulatory mechanism in halophilic bacteria enabling survival in extreme environments [40-42]. When it comes to temperature tolerance, strain BVMH004 which was identified as a strain of *Virgibacillus kimchii* reached growth at 42°C but previous study showed that the *V. kimchii* could grow at 50°C [43] indicating that this species has a previously reported thermophilic biovar. An important reason for these variations can be the heterogenous distribution of the microbial diversity within the Sambhar Lake and its adjacent land. Due to fluctuations in salt levels, there might be generation of gradients which might generate variabilities in stress pattern resulting in variable strains being formed. Additionally, the microbial community in the Sambhar Lake and the associated salterns are probably amenable to change over the period due to evaporation weather patterns and other environmental fluxes leading to diversification.

To distinguish the isolates, several biochemical assays were carried out. Microorganisms have potential in biotechnology due to a few of their biological characteristics. Interpreting these data indicates that most of the isolates showed positive catalase, oxidase, and citrate activity, showing they could synthesise catalase enzymes, use citrate as a carbon source, and go through oxidative processes. On the other hand, indole, MR-VP, gelatine liquification, and urease activities were predominantly absent. The positive MR test for BVMH008 and BVMH022 suggests their ability to produce mixed acids, while the positive VP test for BVMH021 implies the absence of acetoin production in this isolate. To explain, the fact that these isolates use citrate as a carbon source suggests that they can integrate citrate into the metabolic pathways involved in growth and energy production [44]. Like this, the existence of cytochrome c oxidase in microorganisms is indicated by the measurement of catalase activity in isolates, which shows that they can survive and adapt to oxygen-rich environments [45]. During aerobic respiration, cytochrome c oxidase is an essential enzyme in the electron transport chain [46]. The distinctive traits and metabolic capabilities of the isolated strains can be understood through these biochemical characteristics.

Phylogenetic analysis showed that twenty-two moderate halophilic isolates were categorized into two phyla and nine genera and Firmicutes was the most abundant phylum across all the samples while only two strains belonged to Proteobacteria (Supplementary Figure 25). Although many isolates had similarities greater than 95%, it is proposed that these may be considered as different strains of the same species. Among the isolates BVMH002, BVMH005, BVMH006, BVMH021, and BVMH023, the closest relation was found with *Staphylococcus* sp. Notably, BVMH021 exhibited a sequence similarity of 92.13%, while BVMH023 showed a similarity of 94.45%. This

suggests that BVMH021 and BVMH023 can be regarded as belonging to the same genus but likely represent different species within the *Staphylococcus* genus. *Staphylococcus* genus showed lowest G+C content (50-52%).

Furthermore, salt-tolerant enzymes synthesized by these strains have been offered industrial processes that take place in high salinity environments. In this study, *Planococcus* sp., *Staphylococcus* sp., *Virgibacillus* sp., *Salinicoccus* sp., *Piscibacillus* sp., *Halomonas* sp., and *Marinococcus* sp., all showed protease activity in minimal medium supplemented with 2% skimmed milk agar. Staphylococcus sp., *Salinicoccus* sp., *Piscibacillus* sp., *Halomonas* sp., *Alkalibacillus* sp., *Marinococcus* sp., and *Amphibacillus* sp., exhibited lipolytic behavior, which is of relevance in the food, paper, detergent industries, and, most notably in biotransformation as biocatalysts [47-50]. *Virgibacillus* sp., *Salinicoccus* sp., *Piscibacillus* sp., Halomonas sp., *Alkalibacillus* sp., and *Amphibacillus* sp., produced cellulase enzyme, which is a globally acclaimed industrial enzyme, is capable of hydrolysing glycosidic bonds of cellulose and cellulose derivatives into soluble oligosaccharides and glucose [51, 52] without producing hazardous byproducts [53, 54]. Microbes that undergo hydrolytic activity can catalyse the cycling of organic matter in their environment and extract energy and nutrients from complex polymers. Since halophilic proteins are acidic and hydrophilic, soluble in water, and fold reversibly, halophilic bacteria can generate a broad spectrum of bioactive chemicals with a variety of applications in various industries [19]. The encouraging outcomes could be applied to the development of industrially useful biotechnologically significant enzymes [55]. The search for novel and innovative sources of these enzymes is necessary due to their versatile applications. In this context, marine organisms are promising candidates for many industrial processes, because of their ability to thrive in nutrient-limited and hostile environment [56, 57].

In response to salt stress, bacteria alter the content of fatty acids in membranes and cause lipid build-up [58]. Certain species create polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (EPA), during under salt stress [59]. This study used GCMS to determine the fatty acid profile of twelve isolates from the Sambhar Lake. *Salinicoccus* sp. produced many of the fatty acids among these strains. long chain fatty acids (C16:0-C18:1) which can be of use in healthcare industries as they have antimicrobial properties [60, 61]. These microbial strains might be able to produce antimicrobial compounds, which could be useful for a variety of uses, such as food preservation and medications, according to previous studies [62]. *Arthrospira platensis* has also been discovered to contain bioactive fatty acids, such as linolenic acid, oleic acid, and palmitic acid [63]. *Clostridium perfringens*, *E. coli*, and *Staphylococcus pyogenes* were all inhibited by fatty acids, including oleic, palmitic, stearic, myristic, linoleic, and linolenic acids [64]. It is necessary to conduct additional research to clarify the processes underlying their antibacterial action and investigate their possible uses in biotechnology and medicine.

4. Conclusion

The study concludes that understanding the bioactivity of these organisms can be facilitated by considering the bioprospecting of moderate halophilic bacteria that were isolated from Sambhar Lake. This research showed that the Sambhar Lake ecosystem is potential hotspot for a variety of halophilic bacteria with variable physiological characters. Future bioprospecting investigations may find these isolates to be a viable option due to their potential bioactivity, which includes fatty acid synthesis and enzyme hydrolysis.

Declarations

Author Contribution: The complete methodology and experimental design were conceptualised and approved by KD and experimentally performed by SS. While AG has read, edit, improved, and approved the final manuscript.

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