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SHORT COMMUNICATION

Evaluation of Osmotolerant Potential of *Halomonas sulfidaeris* MV-19 Isolated from a Mud Volcano

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Abstract

Salinity is one of the major challenges for cultivation of crops in a sustainable way because it severely affects plant growth and yield. Keeping this challenge in view, in the current study, a salt-tolerant *Halomonas* MV-19 was isolated from an extreme niche of mud volcano of Andaman Nicobar Island, India and identified on the basis of standard morphological, biochemical, and physiological tests and identified as *Halomonas sulfidaeris* strain MV-19 by 16S rRNA gene sequencing. The bacterium can grow on nutrient agar and nutrient broth supplemented with 3.5 M ($\geq$20%) sodium chloride (NaCl). Sugar utilization assay revealed that *H. sulfidaeris* MV-19 utilizes only three sugars (dextrose, fructose, and mannose) from among twenty four tested sugars. The best growth of *H. sulfidaeris* MV-19 was observed in nutrient broth supplemented with 8% NaCl. When the broth was supplemented with dextrose, fructose, and mannose, the *H. sulfidaeris* MV-19 grew maximally in nutrient broth supplemented with 8% NaCl and 5% fructose. This strain produced exopolysaccharides (EPS) in nutrient broth supplemented with 8% NaCl and sugars (dextrose, fructose, and mannose). The EPS production was increased by 350% (three and half time) after addition of 5% fructose in nutrient broth compare with the EPS production in nutrient broth without supplemented with sugars. *H. sulfidaeris* MV-19 strain can produce EPS, which can help aggregate soil particle and reduced osmotic potential in soil, thus, be useful in alleviation of salinity stress in different crops cultivated in saline soils. The findings of the current investigation are expected to contribute towards effective abiotic stress management.

Salinity is one of the main abiotic constraints that reduces growth and yield of different crops [1]. Globally, salinity severely limits the crop productivity in 20% of the arid and semiarid areas which account for 7% of the area on earth [2, 3]. Salinity determines the water potential of each rhizospheric soil. The availability of water, nutrients, and oxygen to plants and microbes is regulated by water potential of soil [4, 5]. The physico-chemical properties of soil can change due to vigorous rhizospheric communications between plant–microbe, soil–plant, microbe–soil, and water–soil [6]. Moreover, different types of polysaccharides secreted by microbes in soil create macro-aggregates (> 250 µm) and micro-aggregates (< 250 µm) and stabilize the physico–chemical properties of soil [7]. The plant roots and fungal hyphae fill the pores of macro-aggregates and micro-aggregates leading to more stabilization of rhizospheric soil [8]. The water availability and structure of rhizospheric soilis indirectly influenced by consumption and secretion of polysaccharides and proteins by microbes [9]. Bacteria are able to produce exopolysaccharides (EPS) which can aggregate around the bacterial cells and protect them in water stress conditions by enhancing the water retention capacity to survive under stressed conditions in saline soil [10]. While EPS secreted by bacteria in rhizospheric soil can be utilized by different microbes and regulate the organic carbon availability under low availability of carbon source [11]. The EPS helps bacteria to colonize on root surface of plants. The EPS binds irreversibly and form a material of fibrillary network which can permanently connect the bacteria to root surface [12]. The role of EPS produced by *Azospirillum*i n aggregation of soil and enhancing its colonization capacity of soil was studied by Pereg et al. [13]. The study showed that *Azospirillum brasilense* Sp245 secreted different polysaccharides, lipids, proteins, and lipopolysaccharides, which

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capsulate the bacteria. The secreted material consisted of a high molecular weight molecules of lipopolysaccharides-protein (LP) complex and polysaccharides-lipid (PL) complex in soil which protect the bacteria as well as plant in stress conditions viz low availability of water and nutrients. Interestingly, decapsulated cells of *A. brasilense* Sp245 survived under abiotic stress condition when LP and PL complexes were used as coating material for decapsulated cells [14]. The concentration and composition of EPS secreted by bacteria change in rhizospheric soil. The plants showed more resistance to water and salt stress when seeds were inoculated with EPS-secreting bacteria before planting in soil [15]. The clay particles of soil adsorb EPS secreted from bacteria and form aggregates of protective capsules around rhizospheric soil particles through different mechanisms like formation of cation bridges, anion adsorption, hydrogen bonding, and Van der Waals forces [11, 13]. It has been reported that wheat plants grew better as compared to control in salt stress condition when plantlets of wheat were inoculated with *Paenibacillus polymyxa* that produces EPS [16]. *Pantoea alhagi* NX-11 has been reported to alleviate the effect of salinity on plants grown in salt amended soil [17]. Hence, for mitigation of salt stress in rhizospheric soil, the EPS-secreting bacteria can be used as bioinoculant to colonize the plant roots and increasing their population to produce more EPS in soil.

The EPS secretion by bacteria plays a key role in conferring salt tolerance towards higher concentration of sodium chloride. Therefore, the current study is based on (i) EPS producing salt-tolerant *Halomonas sulfidaeris* strain MV-19 which was isolated from extreme niche of mud volcano soils of Car-Nicobar, Island, India, and (ii) the biochemical and molecular approaches were used to characterize the bacterial strain MV-19.

Materials and Methods

Isolation of Bacteria from Mud Volcano Soil Sample

The soil samples of extreme niches were collected at 0–20 cm depth from Mud volcano location (12.18°N, 92.80°E with altitude 9 m) of Car-Nicobar island of Andaman, India. The Physico-chemical analysis of soil of mud volcano was done at ICAR-Institute of Soybean Research, Indore, India. The soil had the following properties: EC-4.9 dS/M, pH 8.5, N-34 ppm, P-6 ppm, K-552 ppm, Zn-5 ppm, Fe-18 ppm, Mn-17 ppm, Cu-6 ppm, OC-0.8%]. For the isolation of halotolerant bacteria, serial dilution of 10 gm of mud volcano soil was done in normal saline solution (NSS) and 100 µl of each dilution was plated on plates containing nutrient agar (g L^{-1}: Peptone 5.0; HM Peptone 1.5; Yeast extract 1.5; Sodium chloride 5.0; Agar 20.0; pH 7.4 ± 0.2) supplemented with 1 M (5.8%), 2 M (11.6%) and 3 M (17.4%) sodium chloride [18]. Simultaneously, these serially diluted samples were also spread on autoclaved Zobell Marine (ZB) agar (g L^{-1}: Peptone 5.0; Yeast extract 1.0; Ferric citrate 0.10; Sodium chloride 19.45; Magnesium chloride 8.8; Sodium sulphate 3.24; Calcium chloride 1.8; Potassium chloride 0.55; Sodium bicarbonate 0.160; Potassium bromide 0.08; Strontium chloride 0.034; Boric acid 0.022; Sodium silicate 0.004; Ammonium nitrate 0.0016; Disodium phosphate 0.008; Sodium fluorate 0.0024; Agar 20.0, Final pH 7.6 ± 0.2) separately followed by incubation at 28 ± 2 °C for 24–72 h for growth of bacteria. After incubation, few colonies appeared on ZB agar and nutrient agar supplemented with 3 M sodium chloride. The colonies were selected and grown on ZB agar plate supplemented with 1, 2, and 3 M sodium chloride and re-streaked for growth on respective nutrient agar plates. The bacterial isolates were preserved in 16% glycerol stock at − 80 °C.

Selection of Osmotolerant Bacterial Isolates

Bacterial isolates were screened for salt tolerance activity to check the growth on nutrient agar plate amended with different concentrations of NaCl. Briefly, all bacterial isolates were grown separately in nutrient broth (g L^{-1}: Peptone 5.0; HM Peptone 1.5; Yeast extract 1.5; Sodium chloride 5.0; pH 7.4 ± 0.2), and each isolate was spot inoculated on Nutrient agar (NA) plate supplemented with different concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M) of NaCl. The growth was observed after every 24 h for 3–5 days. The bacterial isolate grown on NA plate supplemented with 3 M NaCl was selected as best halotolerant isolate and used for subsequent investigation.

Morphological, Biochemical, Molecular, and Functional Characterization

Colony morphology of isolate MV-19 was investigated using stereomicroscope (Olympus SZX10). The isolate was subjected to Gram staining, and the cell shape was observed using compound microscope. The isolate MV-19 was cultivated on NA agar supplemented with 1, 2, and 3 M sodium chloride and ZB agar and incubated at 28 ± 2 °C to study colony morphology.

Cell morphology, motility, and Gram's reaction of the isolate were determined by using standard methods [19]. Isolate MV-19 was analysed for its biochemical properties as per standard microbiological methods [20]. DNA extraction, amplification of 16S rRNA gene was done using method of Henry et al. [21] and the PCR product was sent to Eurofins, Kochi, India for sequencing. The online programme EZ-taxon biocloud was used to find out its exact taxonomic position. The processed nucleotide sequence data with its identity were deposited in the NCBI Gen-Bank sequence database. Phylogenetic and molecular evolutionary analyses of the 16S rRNA gene sequences were done using software MEGA6 and aligned using CLUSTAL-W [22]. Finally, the *H. sulfidaeris* MV-19 (NAIMCC-B-2129) was deposited in National Agriculturally Important Microbial Culture Collection (NAIMCC; WDCM No 1060; https://gcm.wdcm.org/cc?wdcmnumber=1060), ICAR-NBAIM, Mau, Uttar Pradesh, India.

Osmotolerance Assay

The strain *H. sulfidaeris* MV-19 was tested for its sensitivity/tolerance to salt agar plate dilution method. The nutrient agar plate was amended with increasing concentration of sodium chloride (0–4.0 M at 0.5 M increasing interval)). While, in ZB broth, the salt present in broth was assumed as 1 × (Sodium chloride 19.45; Magnesium chloride 8.8; Sodium sulphate 3.24; Calcium chloride 1.8; Potassium chloride 0.55; Sodium bicarbonate 0.160; Potassium bromide 0.08; Strontium chloride 0.034; Boric acid 0.022; Sodium silicate 0.004; Ammonium nitrate 0.0016; Disodium phosphate 0.008; Sodium fluorate 0.0024) and 2 ×, 3 ×, and 4 × increasing concentrations of salts were manually prepared. Subsequently, 10 µl of 10^8 cells mL^{-1} of *H. sulfidaeris* MV-19 was spot inoculated on plates. Broth was incubated in shaking incubator at 28 ± 2 °C with shaking at 120 rpm, and plates were also incubated 28 ± 2 °C in BOD incubator. The highest concentration of sodium chloride and combination of different salts which supported the growth of *H. sulfidaeris* MV-19 was referred to as maximum tolerance level (MTL). The experiment was repeated thrice.

Sugar Utilization Assay

A 5 ml autoclaved phenol red broth base (g L^{-1}: Protease peptone 10.0 g; HM peptone B # 1.0 g; NaCl 5.0 g; Phenol red 0.018) supplemented with 1 M sodium chloride supplemented with 23 different discs of carbohydrate (Hi-media) having concentration of 25 mg $disc^{-1}$, namely, dextrose, sucrose, lactose, fructose, raffinose, arabinose, cellobiose, sorbitol, galactose, trehalose, xylose, mannose, melibiose, inulin, rhammanose, mannitol, maltose, salicin, adinitol, dulcitol, and inositol was inoculated with freshly grown broth culture of *H. sulfidaeris* MV-19 (10^8 cfu mL^{-1}) and incubated at 28 ± 2 °C for 24–48 h [23]. Change of colour from red to yellow for production of acid and gas by Durham tubes was observed.

Amino Acid Utilization Assay

For amino acid utilization assay, Moeller decarboxylase broth base (g L^{-1}: Protease peptone 5.0 g; HM peptone B # 5.0 g; Dextrose 0.5 g; Bromocresol purple 0.010; Cresol red 0.005; pyridoxal 0.005) supplemented with 1 M sodium chloride was used as growth medium for *Halomonas sulfidaeris* MV-19. Further, six different discs of amino acids (Hi-media) having concentration of 25 mg/disc, namely lysine, ornithine, citruline, proline, serine, and histidine were separately added in each vial of 5 ml autoclaved Moeller decarboxylase broth base supplemented with 1 M sodium chloride and inoculated with freshly grown broth culture of *Halomonas sulfidaeris* MV-19 (10^8 cfu ml^{-1}). Incubation was done for 24–48 h at 28 ± 2 °C, and the change in colour was observed.

Plant Growth Promoting (PGP) Activity Assay and Pathogenicity Test

The *H. sulfidaeris* MV-19 was screened for different PGP traits including phosphate solubilization, ACC deaminase activity, IAA biosynthesis, siderophore production, cyanide synthesis, exopolysaccharides secretion, and antifungal activity by methods described by Ahmad et al. [24, 25]. To check the pathogenicity of *H. sulfidaeris* MV-19, the β-haemolysis test and DNAse activity were performed as described by Blanco-Vargas et al. [26]. These tests were required for selection of non-pathogenic bacteria to establish the beneficial nature of microbes for farmers.

Extraction, Purification, and Estimation of Exopolysaccharide Production Under Salt Stress

For estimation of EPS production under salt stress, 100 µl of 24-h-old culture of *H. sulfidaeris* was inoculated (0.1% v/v) into 250 ml Erlenmeyer flasks containing 100 ml of Nutrient broth (NB) supplemented with 1.5 M NaCl and 5 g L^{-1} dextrose, fructose, or mannose was separately added and the flasks were incubated at 28 ± 2 °C for 7 days in rotatory shaker with 125 rpm agitation. The seven-day-old grown cultures were centrifuged at 7000 rpm for 20 min, and pellet was washed twice with 0.85% KCl for extraction of EPS from all treated bacterial cultures. The presence of DNA was assayed by DPA reagent to check the extraction of intracellular polysaccharides [27]. The proteins were checked and estimated by Folin's reagent in supernatant [28]. For extraction of EPS from cell-free supernatant, chilled ethanol (Merck) was added in 1:3 ratio. The supernatant–ethanol mixture was shaken and incubated for 24 h at 4 °C. After overnight incubation, 0.45 µm nitrocellulose membrane was used for filtration of supernatant which was dialysed against double distilled water at 4 °C. The dialysate was centrifuged for 25 min at 20,000×*g* to remove insoluble material if any. This procedure was repeated three times, and the precipitated form of purified EPS was extracted. For additional purification of EPS, the method described by Bales et al. [29] was followed. In this method, chilled Trichloroacetic acid

(TCA) was mixed with EPS in 20% (v/w) for precipitation of nucleic acids and proteins. After centrifugation of solution mixture at 15,000 rpm for 1 h, 95% ethanol was added in supernatant and kept at − 20 °C for 24 h to remove fatty acids after precipitation. The purified EPS was kept at 60 °C for determination of yield of crude EPS [30]. The extracted EPS was dried at 60 °C for 24 h. Carbohydrate content in EPS was assayed and estimated by Dubois method [31].

Data Analysis

The experiments were repeated thrice with each treatment having three replications. The comparison of difference among treatments means was performed by high-range statistical domain (HSD) using Tukey test at 5% probability level.

Results

Morphological and Molecular Characterization of *H. sulfidaeris* MV-19

A total of twenty one bacterial cultures were isolated NA supplemented with 1 M sodium chloride. Out of these cultures, only one bacterial strain was able to grow on nutrient agar containing 3 M sodium chloride, and this bacterial culture was selected for further study as an osmotolerant bacterial strain. To validate the osmotolerant capacity of this isolate, 1 ×, 2 ×, and 3 × concentrations of ZB broth were used to check its growth. This isolate survived and grew in 3X ZB broth at 28 ± 2 °C after 3 days of incubation period. Furthermore, the isolate was found to survive in nutrient agar as well as in nutrient broth supplemented with 3.5 M sodium chloride.

The colony morphology of the osmotolerant MV-19 bacterial strain was creamy white, smooth, and circular with an entire margin on NA plate supplemented with 1 M sodium chloride. The strain was Gram negative and rod shaped. It was positive for nitrate reduction and catalase, and negative for citrate utilization, methyl red, indole production, and oxidase and negative for starch, gelatin, cellulose, and chitin hydrolysis (Supplementary Table 1). The 16S rRNA gene analysis by using EZ-Taxon (https://www.ezbiocloud.net/taxonomy) of strain MV-19 showed 99.6 similarity to *H. sulfidaeris*, and hence, it was identified as *H. sulfidaeris* strain MV-19. The 16S rRNA gene sequence was submitted to NCBI (accession No. MW282893). The phylogenetic analysis also suggested that this strain was closely related to *H. sulfidaeris*$^{(T)}$ BAA-803 (Fig. 1) deposited in National Agriculturally Important Microorganisms Culture Collection (NAIMCC), Mau, India for its long-term preservation with accession no. NAIMCC-B-2129.

Salt Tolerance Assay of Osmotolerant *H. sulfidaeris* MV-19

Out of 21 different carbohydrates tested, C-source utilization assay showed that *H. sulfidaeris* MV-19 was able to utilize dextrose, fructose, and mannose. However, amino acid utilizing test of this bacterial strain showed that it utilized only proline and serine, out of six tested amino acids (Supplementary Table 2).

The growth rate of *H. sulfidaeris* MV-19 was standardized in NB by using various concentration of sodium chloride and different sugars (dextrose, fructose, and mannose). Among different carbohydrates added in nutrient broth, the best growth of *H. sulfidaeris* MV-19 was in mannose containing nutrient broth (Fig. 2). However, nutrient broth containing 1.5 M sodium chloride was found to be best for the growth of *H. sulfidaeris* MV-19 when only sodium chloride is added to nutrient broth without supplementing it with any carbohydrate. The lag phase of *H. sufaedris* MV-19 was 72 h when it was grown in NB supplemented with 3 M NaCl and mannose, while the log phase of this stain was recorded after 96 h when it was grown in nutrient broth supplemented with 3 M sodium chloride and dextrose (Fig. 2).

Plant Growth Promoting Trait Activity of *H. sulfidaeris* MV-19

The evaluation of the MV-19 strain for plant growth-promoting activity showed that it was shown positive activity for ACC deaminase enzyme, synthesized low amount of IAA and produced very significant amount of EPS secretion while the *H. sulfidaeris* MV-19 strain shows negative for Zn and P solubilization, siderophore production, and cyanide production (Supplementary Table 3).

Exopolysaccharide Assay of *H. sulfidaeris* MV-19 Under Salt Stress

The optimum growth of *H. sulfidaeris* MV-19 was observed in nutrient broth containing 1.5 M sodium chloride and by adding dextrose, fructose, and mannose. In general, the EPS production by *H. sulfidaeris* MV-19 was increased by adding dextrose, fructose, and mannose in NB containing 1.5 M NaCl as compared to without addition of any sugar (Fig. 3). Among the three sugars, fructose was best utilized by *H. sulfidaeris* MV-19 and had optimum growth in NB at different concentrations of NaCl as compared to dextrose and mannose (Fig. 4). *H. sulfidaeris* MV-19 produced 32.5 mg/ml EPS in NB containing 1.5 M sodium chloride

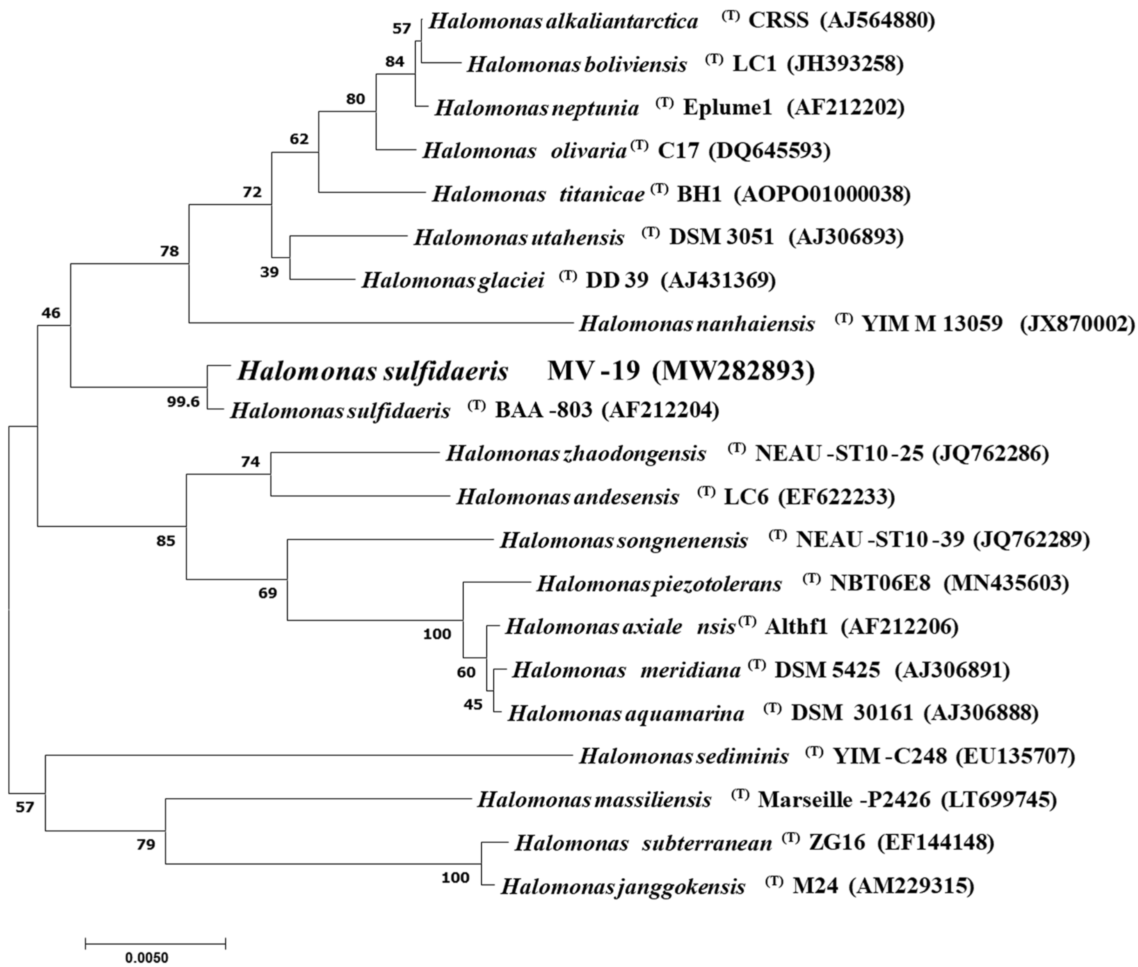

Fig. 1 Phylogenetic relation of 16SrRNA gene sequences of *H. sulfidaeris* MV-19 strain with different members of *Halomonas* genus used by neighbour-joining method

and supplemented with 5 g ml^{-1} fructose after 7 days of incubation at 28 ± 2 °C (see Fig. 5).

Discussion

In our study, *H. sulfidaeris* MV-19 exhibited extreme tolerance towards higher concentration of sodium chloride. Its osmotolerance capacity ranged from 0.5 M to 3.5 M. In bacteria, the osmotic tolerance against sodium chloride is a very complex physiological and biochemical process. Additionally, the osmotolerance capacity involves different physiological and biochemical mechanisms which are regulated both genetically and phenotypically [32, 33]. The main physiological mechanism is to synthesize different osmotolerant molecules including ecotine and exopolysaccharides [34, 35]. While the ecotine is one of the essential biochemical molecules synthesized by osmotolerant bacteria to maintain the equilibrium between osomotic pressure on the outside and inside in cytosol environment of bacterial cell [36, 37].

Some physiological changes in halotolerant bacteria temporarily affect its osmotolerance potential, while the EPS secretion and ecotine synthesis in bacteria play a key role in permanent osmotolerant activity of bacteria for inside and outside environment of bacterial cell, respectively. Other genetic modifications also affect osmotolerant activity of *Halomonas* [38]. Therefore, the study was focussed on EPS secretion by *H. sulfidaeris* MV-19 in saline environment to overcome the stress created in saline soils.

The optimal growth of *H. sulfidaeris* MV-19 was checked by growing this strain in NB supplemented with different concentrations of sodium chloride and ZM broth and studied its growth kinetics. The strain grew optimally in NB containing 1.5 M sodium chloride with 5% sucrose. Similarly, in a recent study, *Halomonas campisalis* has been reported to grow at different concentrations of sodium chloride ranging from 0 to 260 gm L^{-1} [39]. The growth kinetics of different osmotolerant bacteria were evaluated for their optimum

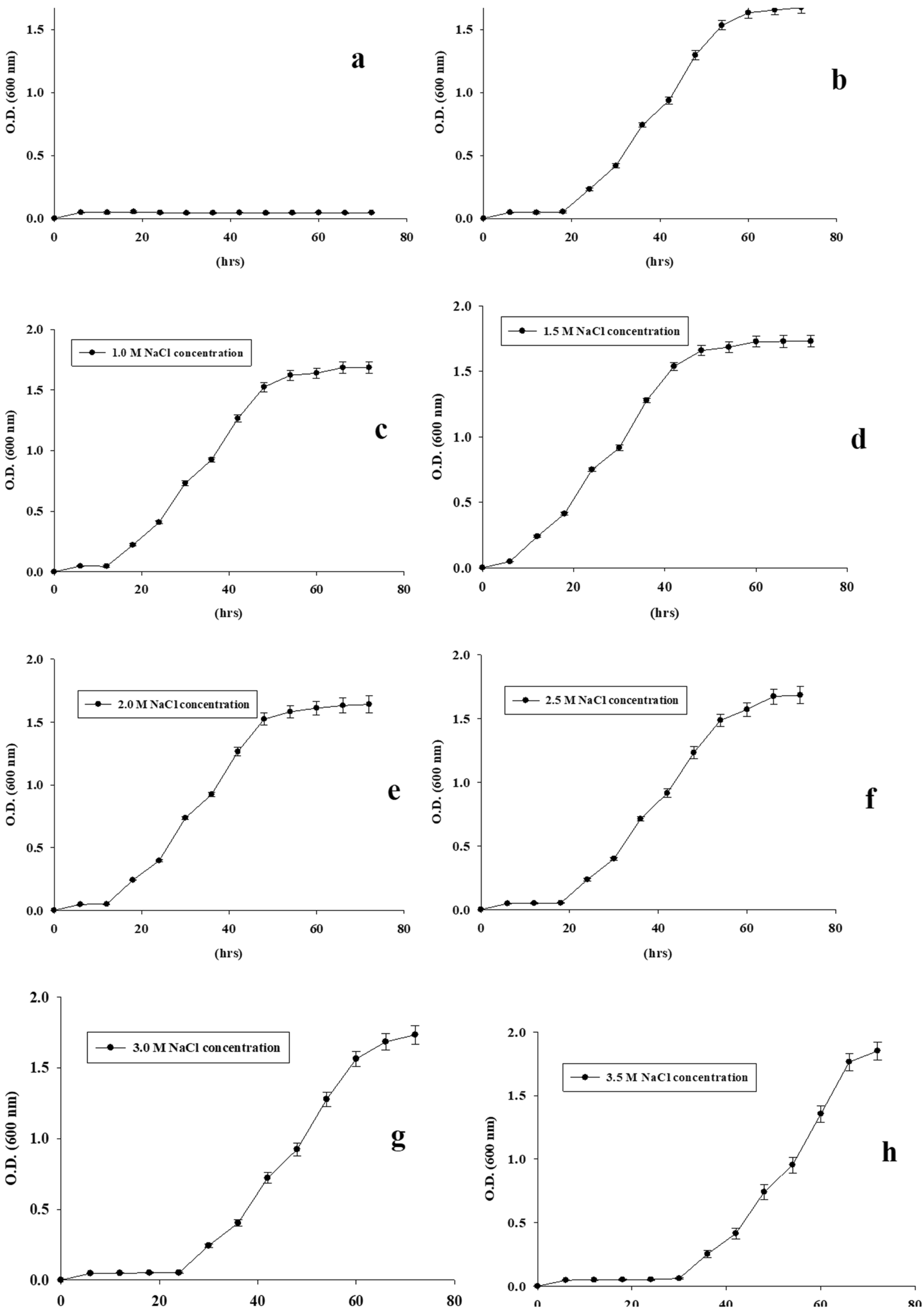

Fig. 2 Growth kinetics of osmotolerant *H. sulfidaeris* MV-19 in ZM Broth with different concentrations **a** I× **b** 2× **c** 3× **d** 4×; OD indicates the mean values ± SD of three replicates

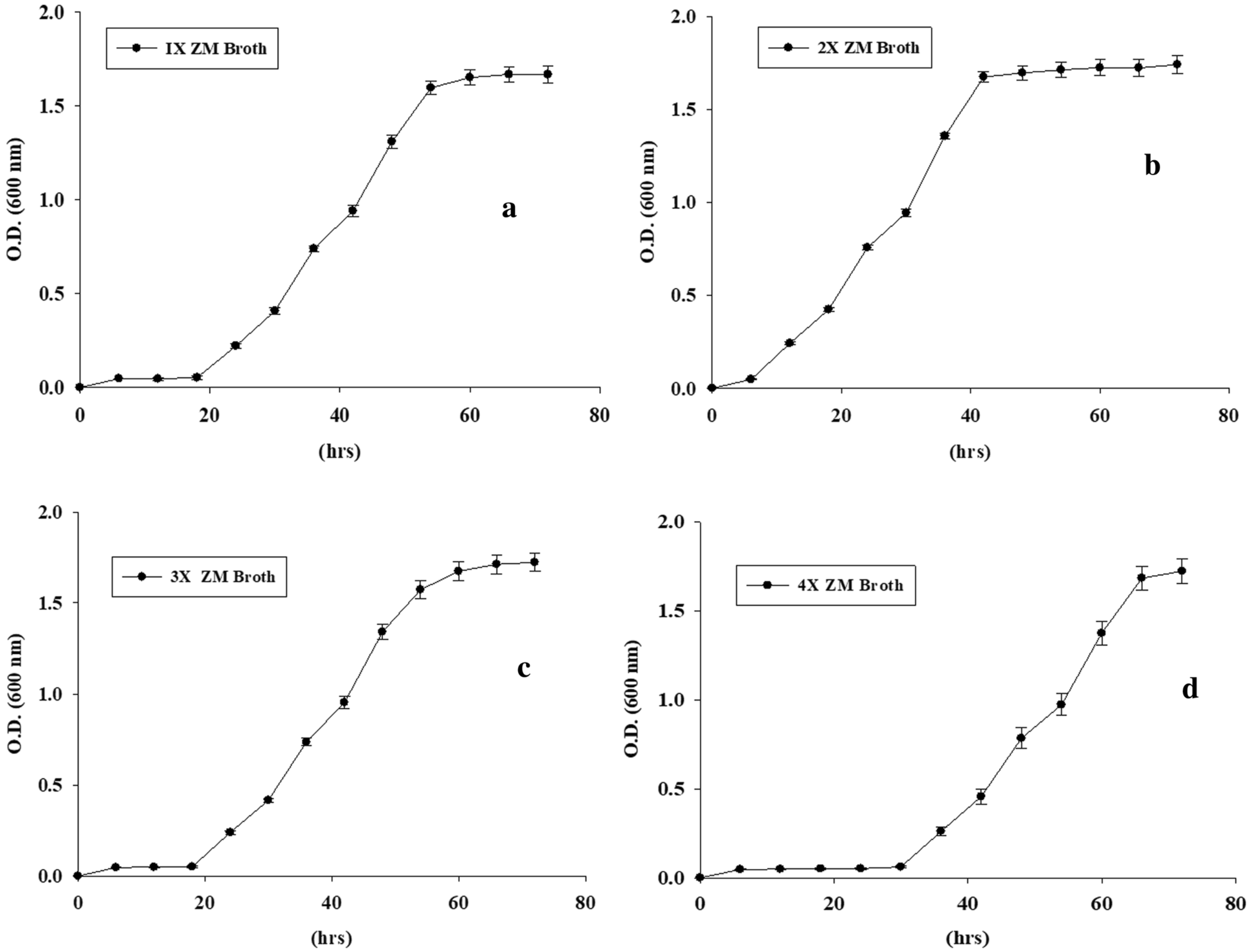

Fig. 3 Effect of fructose, dextrose, and mannose on efficacy of EPS secretion by *H. sulfidaeris* MV-19 in NB at different concentrations of Sodium chloride; Bars indicate the mean values ± SD of three replicates followed by above different letters are significantly different in each set of incubation periods at 0.05 ≤ *P* according to Tukey test

growth conditions by growing them in different concentrations of sodium chloride various researchers [31, 40]. In addition, the EPS secretion by osmotolerant bacteria is a key feature. Moreover, the sugars are essentially required for the bacterial EPS biosynthesis where the sugars are first converted to nucleoside diphosphate sugar [41]. Interestingly, our results showed that EPS secretion was mainly at exponential growth phase of H. *sulfidaeris* MV-19 after utilization of sugars during its growth. The growth *H. sulfidaeris* MV-19 was influenced by utilization of different sugars like mannose, sucrose, and dextrose. The sugar composition of EPS depends as much on the carbon source [42, 43] as on kinetic and physical chemical parameters [44, 45], the influence of growth conditions on the carbohydrate composition of the polymer was studied.

The EPS was released in stationary phase of *H. sulfidaeris* MV-19, while the EPS adhered on bacterial surface in exponential phase during growth curve. These findings are in consonance with the results obtained for different halotolerant bacteria such as *Halomonas maura* [46], *H. Ventosae* and *H. eurihalina* [38], *H. anticariensis* [45], and *Alteromona shispanica* [47]. The highest quantity of EPS was obtained after 120 h, while after stationary phase, it declined due to degradation of EPS by different hydrolytic enzymes [48]. This phenomenon has been reported in some lactic acid bacteria which produced EPS during stationary phase [49]. The EPS synthesis increased during exponential growth phase and declined during stationary phase of *H. sulfidaeris* MV-19. In our study, the optimum EPS was released after 120 h incubation period at 28 ± 2 °C when *H. sulfidaeris* MV-19 was grown in NB containing 1.5 M sodium chloride and 5% sucrose was also added. Similarly, *Halomonas almeriensis* also synthesized EPS during stationary phase [50]. The utilization of different C sources can influence the synthesis of EPS and can change the chemical composition and amount of EPS by the bacterial cell

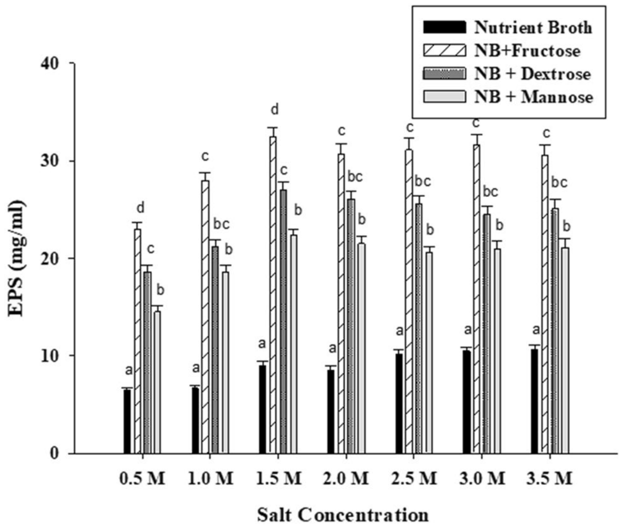

Fig. 4 Growth kinetics of osmotolerant *H. sulfidaeris* MV-19 in nutrient broth amended with different concentrations of Sodium chloride **a** 0 M **b** 0.5 M **c** 1.0 M **d** 1.5 M **e** 2.0 M **f** 2.5 M g 3.0 M h 3.5 M NaCl, OD indicate the mean values ± SD of three replicates

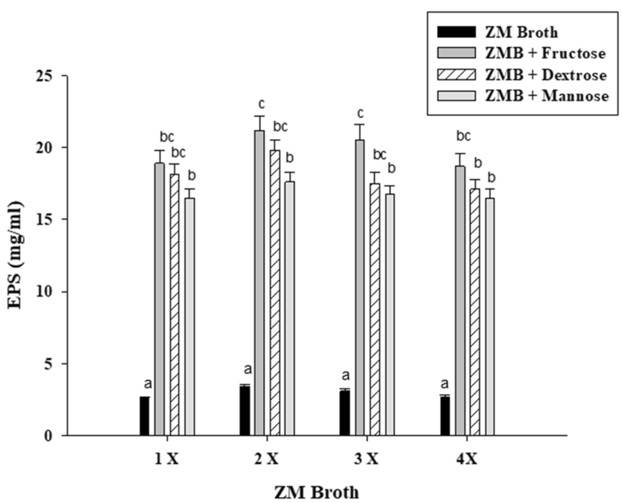

Fig. 5 Effect of fructose, dextrose, and mannose on efficacy of EPS secretion by *Halomonas sulfidaeris* MV-19 in at different concentrations of ZM Broth (I×, 2×, 3×, 4×); Bars indicate the mean values ± SD of three replicates followed by above different letters are significantly different in each set of incubation periods at $0.05 \leq P$ according to Tukey test

[51]. However, it may also depend on the metabolic pathway operating in different bacteria for EPS synthesis [52]. The *H. sulfidaeris* MV-19 grown in NB amended with 1.5 M NaCl and 0.5% fructose as carbon source produced maximum amount of EPS. In a similar study, the osmotolerant bacteria *Saccharophagus degradans* produced maximum amount of EPS (1.5 mg ml^{-1}) when it was grown in mineral medium amended with galactose [53]. Additionally, the osmotolerance ability of plants refers to their capability to regulate the uptake of ions and differentiate between ions of essential elements and non-essential elements [54]. The bacterial EPS bind positively charged ions including Na^+, thereby, limiting the uptake of Na^+ in plants and maintain the osmotic balance Na^+/K^+ ratio in plants [7].

Conclusion

In present study, *H. sulfidaeris* MV-19 was isolated from a mud volcano, which is an extreme niche for isolation of osmotolerant bacteria. The isolate showed maximum tolerance level (MTL) towards salt stress and could grow in NB, NA amended with 3.5 M sodium chloride. It could also grow in 4X ZM broth and ZM agar. The *H. sulfidaeris* MV-19 secreted high amount of EPS to enhance its osmotolerance activity. This strain also has multiple PGP activity including synthesis of ACC deaminase enzyme and IAA. This isolate has the potential to be used for mitigation of salinity which adversely affects physico–chemical properties and microbial diversity of rhizospheric and non-rhizospheric soils and also negatively impacts plant growth, and yield.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-023-03202-6.

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Author Contributions EA contributed to collect the data and performed the experiments. SKS performs sampling of the materials from mud volcano. PKS, AK and NM helped in the writing and checking the Manuscript. PKS and UBS, HVS analysed the data.

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Declarations

Conflict of interest The author and co-authors declare that the current investigation involved no conflict of interest.

Research Involving Human and Animal Participants This research does not involve Human and Animal Participants.

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