

Comprehensive evaluation of the multifaceted bioactivities of exopolysaccharides derived from marine bacteria *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3

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Received 07 October 2025; revised 02 March 2026

The increasing demand for biodegradable, sustainable, and multifunctional biopolymers has intensified research on microbial exopolysaccharides (EPSs), particularly from extremophilic microorganisms. However, the bioactive potential of EPSs derived from halophilic bacteria remains underexplored. Therefore, the present study aimed to investigate. Exopolysaccharides (EPSs) are high molecular weight biopolymers composed predominantly of sugar monomers, exhibiting diverse chemical structures and properties. The structural diversity of EPSs from halophilic bacteria underpins their utility in environmental remediation and cosmetic formulations, owing to their physicochemical stability and functional properties. In a comprehensive study, two bacterial isolates, *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 were examined for their capacity to synthesize EPSs with bioactive potential. The extraction and purification of EPSs from these isolates revealed significant functional activities. The EPS derived from both *Halomonas* sp. DU-15-9-1 (52.45%) and *S. roseus* S-5-9-3 (55.45%) demonstrated an emulsifying activity with coconut oil and groundnut oil, respectively, suggesting their potential as an emulsifying agent. Additionally, the antioxidant assays exhibited 52.68% and 64.82% radical scavenging activity, suggesting their usage as natural antioxidants. While the flocculating activity, a noteworthy feature for particulate aggregation in environmental processes, was 93% and 92%, respectively. The IC₅₀ values against HeLa cells of 104.10 ± 9.26 µg/mL and 96.05 ± 5.54 µg/mL and against MCF-7 breast cancer cells 31.09 ± 0.63 µg/mL and 24.19 ± 0.22 µg/mL EPSs, respectively, demonstrate their cytotoxic effect and potential use as an anticancer agent. Thus, the multifaceted applications of microbial EPSs will certainly pave the way for futuristic research and biotechnological applications in the areas of food, cosmetics, environmental management, and pharmaceuticals.

Keywords: Anticancer, Antioxidants, Emulsification activity, Flocculation, Hydrocarbons

Exopolysaccharides (EPS) are complex polymers that are produced by different microorganisms. Their unique chemical and physical properties have drawn significant attention, making them potential biopolymers for a broad spectrum of industrial and biomedical uses¹. Moreover, their functional properties such as emulsification, viscosity, stabilization, gelling, and foaming capacity EPS are extremely versatile². The structural diversity of EPS is an important characteristic that allows for customized applications, rendering to particular requirements. Fermentation methods and genetic modification can be used to synthesize or optimize these microbial polymers, which are made up of various monosaccharide units and molecular topologies³. This flexibility shows EPS can

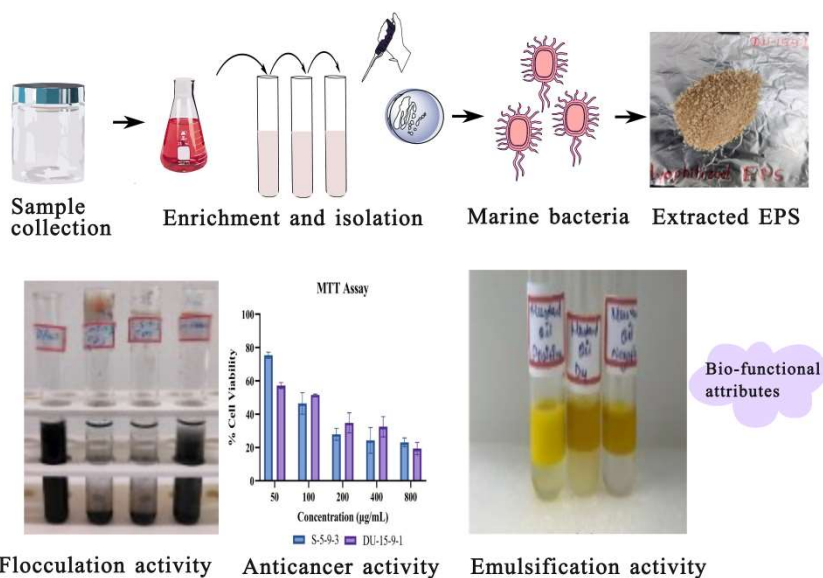
be used for targeted roles across several industries. In the pharmaceutical field, they are used in controlled drug delivery systems due to their capacity to encapsulate and safeguard bioactive compounds⁴. Furthermore, in cosmetics, EPS play a key role in stabilizing and enhancing the performance of emulsions and gels found in skincare products⁵. As research progresses, we can expect the range of EPS applications to expand even further, paving the way for sustainable and innovative solutions across both scientific and industrial domains.

Polysaccharides have various industrial applications, including food, paper, biotechnology, textiles, cosmetics, and pharmaceuticals. This is due to their unique properties, like mechanical strength, adhesion, emulsification properties, rheological characteristics, metal-complexing capabilities, and their role as hydrocolloids^{1,6}. Moreover, polysaccharides are

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Graphical abstract

biodegradable, non-toxic, and can be broken down through enzymatic or chemical processes to produce biofuels and a range of simple molecules that can be used to produce industrial polymers and fine chemicals. The food (emulsifier, stabilizer, viscosifier, and moisture retention), cosmetic (anti-aging activity and reduction of allergic reaction), pharmaceutical (blood flow improvement and drug delivery system), and textile (better water holding capacity and flame retardancy) industries have all made extensive use of EPS, which can form thick pseudoplastic liquids. Along with the technological benefits, some EPS improve human health through a variety of processes, including immune response modulation, blood cholesterol reduction, heavy metal detoxification, and the provision of a fermentable substrate for intestinal bacteria^{6,7}.

Materials and Methods

Sample collection and isolation of Bacteria

Water samples and soil samples were collected from the Diu and Somnath coast, Gujarat, India. Water samples were collected in sterile glass bottles. The samples were transferred to the laboratory within 6 h and stored at 4°C until further analysis. Inoculation of 5% (v/v) marine water sample was proceeded into flask containing Zobell Marine Broth (ZMB) medium supplemented with 5–25% (w/v) NaCl. The flasks after inoculation were incubated on a rotatory shaker at 180 rpm for 72 h at 37°C. The enriched culture was consecutively diluted to prepare aliquots ranging from 10⁻¹ to 10⁻⁵ using distilled water

as diluent, and 100 µL from each aliquot was spread on ZMB agar plates, which were supplemented with 5–25% (w/v) NaCl. After incubation at 37°C for 24 h, morphologically diverse colonies were selected and sub-cultured to acquire a pure culture of isolates.

Characterization of the organisms

Morphological and Biochemical Characterization

The colony characteristics of the isolated bacteria were evaluated after their pure cultures were streaked on the ZMB agar plate under the corresponding enrichment conditions of pH 9 and NaCl (5–25%, w/v). In the activated culture with nutrients at the appropriate enrichment conditions, the Gram reaction, cell morphology, and cell arrangement were observed. The isolates were studied for their biochemical and metabolic activities. The biochemical tests included the production of catalase, oxidase, H₂S, ammonia, indole, hydrolysis of urea, reduction of nitrate and litmus; fermentation of the sugars such as sucrose, fructose, glucose, maltose, lactose, and xylose. All the biochemical media and their test reagents were prepared as mentioned by Prabhakar and coworkers⁸. Due to the halophilic nature of the organisms, all the biochemical media were supplemented with 5% (w/v) NaCl. The individual isolate was inoculated into the respective biochemical medium and incubated at 37°C for 24 h, and the results were later observed.

Molecular identification using 16S rDNA

Based on the results, DU-15-9-1 and S-5-9-3 were selected for further studies. Molecular identification through 16S rDNA sequence analysis reveals the

species identification of the isolates. Two isolates, DU-15-9-1 and S-5-9-3 was sequenced at the National Center for Biotechnology Information (NCBI), Gene Bank Database using the Basic Local Alignment Search Tool for Nucleotide BLASTn tool (<http://www.ncbi.nlm.nih.gov/blast>). DU-15-9-1 and S-5-9-3 sequences are submitted to NCBI GenBank, & Phylogenetic tree was constructed by the Neighbor-Joining Method using MEGA 11 Software¹⁷.

Emulsification activity

The emulsification activity of EPS was assessed using various oils, including castor oil, groundnut oil, cottonseed oil, mustard oil, coconut oil, sunflower oil, and paraffin oil, as well as hydrocarbons such as benzene, hexane, toluene, and xylene. In each test, 2 mL of oil or hydrocarbon was mixed with 2 mL of EPS in a test tube. A positive control consisted of 2 mL of 1% SDS and 2 mL of oil or hydrocarbon, while a negative control involved adding 2 mL of oil or hydrocarbon to 2 mL of distilled water. The tubes were vigorously vortexed for 2 min and then incubated for 24 h. Following incubation, the height of the emulsion layer was measured to calculate the emulsifying activity⁹.

$$\text{Emulsification activity} = \frac{\text{height of emulsion layer after 24 h}}{\text{total height}} \times 100$$

Flocculation Activity

The flocculating activity of exopolysaccharides (EPS) was evaluated as described by Yadav and coworkers¹⁰. The flocculating activity was conducted in a test tube containing a mixture of 10 mL of 0.7% activated carbon and 200 μL 1% CaCl_2 . In addition to this, 500 μL of 0.02% EPS solution was then added to the tube and shaken well for mixing. The resulting suspension was observed for flocculation of activated carbon for 1 h. The experiment was carried out at room temperature. Gum Arabic and distilled water were used as positive and negative controls, respectively, in a similar experiment. After setting down, the absorbance of the supernatant was measured at 550 nm (A_{550}) using a UV-Visible spectrophotometer. Flocculation activity (%) was calculated using the following equation^{11,12}. In the equation, ABS and ABS_0 are the optical densities of the sample and the negative control, respectively.

$$\text{Flocculation activity} = \frac{\text{ABS}_0 - \text{ABS}}{\text{ABS}_0} \times 100$$

Scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Scavenging of DPPH radicals by the EPS was measured according to the previously described method¹³. In brief, 2 mL of EPS solution (1-6 mg/mL) was added to 1.0 mL DPPH solution (0.004 % w/v) and incubated in the dark for 20 min. Radical scavenging was measured by the decrease in absorbance at 517 nm.

Brine Shrimp Lethality Assay (BSLA)

Brine shrimp lethality assay was accomplished for EPS¹⁴. Approximately 5 mg of *Artemia salina* eggs, also known as brine shrimp eggs, were kept in a petri dish with artificial saline water for a duration of 24 h to hatch. After 24 h, 10 nauplii and 200 μL of artificial saline water were added to each well and treated with varying concentrations of the EPS (20-600 $\mu\text{g}/\text{mL}$). Artificial saline water was added to each well to make up the final volume of 2000 μL . The nauplii were then incubated for 24 h at 37°C. The percentage mortality of each well was calculated after 24 h and compared with the test control, potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$). The LC_{50} was determined using simple linear regression on the concentration versus percent mortality curve.

In vitro cytotoxicity assay using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

The *in vitro* anticancer activity of EPS extracted from *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 were determined using MTT assay on Human epithelia cervical carcinoma cell line (HeLa) and Michigan Cancer Foundation-7 (MCF-7) breast cancer cell lines. HeLa and MCF-7 cells were seeded in a 96-well plate (10^4 cells/well in 200 μL of media) and allowed to adhere for 24 h. HeLa cells were exposed to different concentrations of EPS (50, 100, 200, 400, 800 $\mu\text{g}/\text{mL}$). Simultaneously, MCF-7 cells were exposed to varying concentrations of EPS (12.5, 25, 50, 100, 200, 400, 800 $\mu\text{g}/\text{mL}$). After 24 h and 48 h treatment with test compounds, the culture medium was aspirated, followed by PBS wash. Subsequently, 50 μL of MTT reagent (5 mg/ mL in PBS) was added to each well and incubated for 4 h at 37°C in a humidified CO_2 (5%) incubator under dark conditions to facilitate formazan crystal formation. Further, MTT was removed, followed by adding 100 μL Dimethyl Sulfoxide (DMSO) to solubilize the formazan crystals^{15,16}. The plate was then incubated for an additional 30 min, and the OD was measured at 570 nm using the Epoch Microplate Spectro-photometer (Epoch BioTek Instruments, Winooski, Vermont, United States).

$$\text{Percentage cell viability} = \frac{\text{Absorbance of treated group}}{\text{Absorbance of untreated group}} \times 100$$

Results and Discussion

Sample Collection and Isolation of Bacteria

The bacteria were isolated from the seawater samples collected from the Somnath (pH 8.5) (20°53'16.9"N 70°24'5.0" E), and Diu region (pH 8.0) (20°42'36"N 70°58'48" E). The temperature was 33 °C at the time of sample collection. The salinity and pH of the samples varied from 2.5- 4% and 8.0-8.9, respectively. Numerous studies show that the type of source and its micro and macro components have a significant impact on the growth of microorganisms. In a similar vein, the occurrence of specific microorganisms and their biosynthesis of primary and secondary metabolites are similarly caused by the nutrients present in the corresponding environment¹⁷.

Characterization of the organisms

Morphological and Biochemical Characterization

The isolates were primarily screened based on the colony appearance on the ZMB agar plates. The isolates varied in their cell morphology, cell arrangement, Gram reaction, spore formation ability, and motility. The isolate DU-15-9-1 cellular morphology, *i.e.*, Gram-negative short rods and colony morphology is round with entire margin, moist consistency, mucoid texture, semi-translucent, and creamy appearance with string formation in the KOH test, while the cellular morphology of the isolate S-5-9-3 *i.e.*, Gram-negative thick rods and colony morphology is round with entire margin, mucoid texture, opaque, and orange pigmented. Further characterization was carried out by studying the biochemical and metabolic profiling of both isolates. The isolate DU-15-9-1 shows the positive result for citrate utilization, methyl red, catalase, oxidase, and gelatin liquefaction test, while all the other biochemical tests showed negative results. Furthermore, the isolate was able to utilize 7 sugars out of 21, such as Raffinose, Salicin, Fructose, Galactose, Rhamnose, Dextrose, and Sucrose, while negative test for other sugars (Table 1). The isolate S-5-9-3 shows the positive result for citrate utilization, indole, catalase, oxidase, and the oxidase fermentative test, while all the other biochemical tests showed negative results. Furthermore, the isolate was able to utilize 11 sugars out of 21, such as Dulcitol, Raffinose, Salicin, Fructose, Galactose, Rhamnose,

Table 1 — Biochemical test and sugar fermentation test of isolates

Biochemical tests	DU-15-9-1	S-5-9-3
Citrate utilization test	+	+
Indole production	-	+
Urea hydrolysis test	-	-
Methyl red test	+	-
Voges-Proskauer test	-	-
Deamination Test	-	-
Hydrogen sulfide production	-	-
Ammonia Production	-	-
Nitrate production	-	-
Starch Hydrolysis	-	-
Casein Hydrolysis	-	-
Catalase	+	+
Oxidase	+	+
Oxidative Fermentative Test	-	+
Lipid Hydrolysis	-	-
Gelatin Hydrolysis	+	-
Triple sugar iron agar test	Butt: Alkaline Slant: Acidic	Butt: Alkaline Slant: Acidic
Sugar fermentation test	DU-15-9-1	S-5-9-3
Dulcitol	-	+
Raffinose	+	+
Salicin	+	+
Fructose	+	+
Galactose	+	+
Rhamnose	+	+
Dextrose	+	+
Lactose	-	+
Mannose	-	-
Trehalose	-	-
Mannitol	-	+
Melibiose	-	-
Inulin	-	-
Xylose	-	-
Inositol	-	-
Maltose	-	-
Cellobiose	-	-
Adonitol	-	-
Arabinose	-	+
Sucrose	+	+
Sorbitol	-	-

Dextrose, Lactose, Mannitol, Arabinose, and Sucrose, while negative test for other sugars (Table 1).

Molecular identification using 16S rDNA

The isolates DU-15-9-1 and S-5-9-3 were identified based on the 16S rRNA gene sequence. The query sequence and the database's sequence match, according to the sequencing analysis report. By comparing the rRNA sequence with reference strains, NCBI BLAST further analyzes the consensus sequence to find the closest homologs. The

phylogenetic tree of the isolate was constructed using the Neighbor-Joining Method by MEGA 11 software (Fig. 1a & b). The 16S rRNA sequence of *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 are submitted to NCBI GenBank with Accession numbers OP077301 and ON261556, respectively.

Emulsification activity

Emulsification activity (% EA) of the EPS produced was checked by using coconut oil, castor oil, cottonseed oil, groundnut oil, mustard oil, paraffin oil, and sunflower oil. Emulsification activity of EPS with different hydrocarbons was also studied. After 24h incubation period, the height of the emulsion layer was measured. EPS produced from *Halomonas*

sp. DU-15-9-1 showed the highest 52.45% emulsification activity with coconut oil (Table 2), while EPS produced from *Salinicoccus roseus* S-5-9-3 showed 55.45% emulsification activity with groundnut oil (Table 2). Results are summarized and illustrated in (Table 2 and Fig. 2a & b).

Emulsification activity of both the isolates *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 were studied using different hydrocarbons such as Xylene, Toluene, Benzene, and Hexane. *Halomonas* sp. DU-15-9-1 showed the highest 45.17 % emulsification activity with hexane (Table 3 and Fig. 3a), while EPS produced from *Salinicoccus roseus* S-5-9-3 showed 41.17% emulsification activity

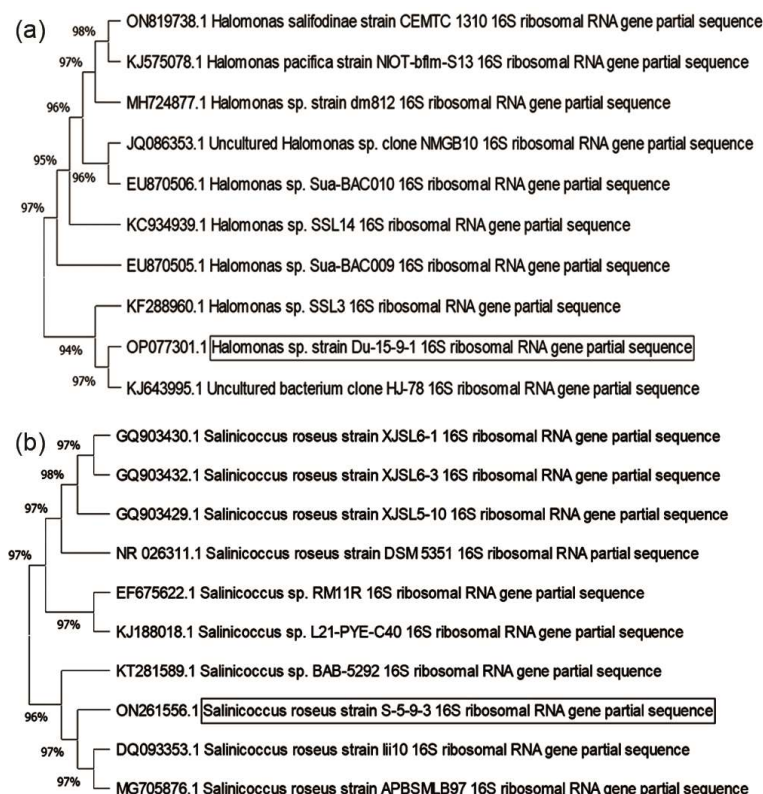


Fig. 1 — Phylogenetic tree of (a) *Halomonas* sp. DU-15-9-1; and (b) *Salinicoccus roseus* S-5-9-3

Table 2 — Emulsification activity of EPS with different oils

Oils	Emulsification activity (%)			
	Positive Control	Negative control	<i>Halomonas</i> sp. DU-15-9-1	<i>Salinicoccus roseus</i> S-5-9-3
Coconut oil	58.33	36.36	52.45	34.61
Castor oil	33.33	29.16	36.36	29.16
Cottonseed oil	40.74	0	38.46	29.16
Groundnut oil	53.84	0	30.43	55.45
Mustard oil	52.00	0	34.78	34.78
Paraffin oil	54.16	0	34.78	30.76
Sunflower oil	58.33	0	36.00	37.56

Table 3 — Emulsification activity of EPS with different hydrocarbons

Hydrocarbons	Emulsification activity (%)			
	Positive control	Negative control	<i>Halomonas</i> sp. DU-15-9-1	<i>Salinicoccus roseus</i> S-5-9-3
Benzene	52.17	0	38.88	41.17
Hexane	42.10	0	45.17	18.75
Toluene	59.25	0	27.27	36.36
Xylene	53.84	0	12.50	11.53



Fig. 2 — Emulsification activity of (a) *Halomonas* sp. DU-15-9-1; and (b) *Salinicoccus roseus* S-5-9-3 isolates by using different oils: A=Mustard oil, B=Cottonseed oil, C=Groundnut oil, D=Sunflower oil, E=Paraffin oil, F=Coconut oil, G=Castor oil.

with benzene (Table 3 and Fig. 3b). Currently, many research works are carried out on EPS to exploit these salient features for application in the food, pharma, and bioremediation sectors. The emulsification activity of some EPS, such as those produced by *Halomonas* sp. DU-15-9-1 was similar to the earlier reported strains of the same genus. In therapeutics, emulsifiers are widely used to increase the dispersion and wetting properties used for topical applications, and oil-in-water emulsions are used in drug delivery systems for lipophilic bioactive compounds¹⁶.

Emulsifiers are also used in the food industry to modify the texture of preparations containing immiscible compounds and in the bioremediation of oil spills¹⁸.

Flocculation Activity

The flocculation activity of EPS produced by *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 were determined by reacting the EPS with activated carbon and CaCl₂. The results of flocculating activity of the EPS after 5, 10, 15, 30, 45, and 60 min are illustrated in (Fig. 4). Literature

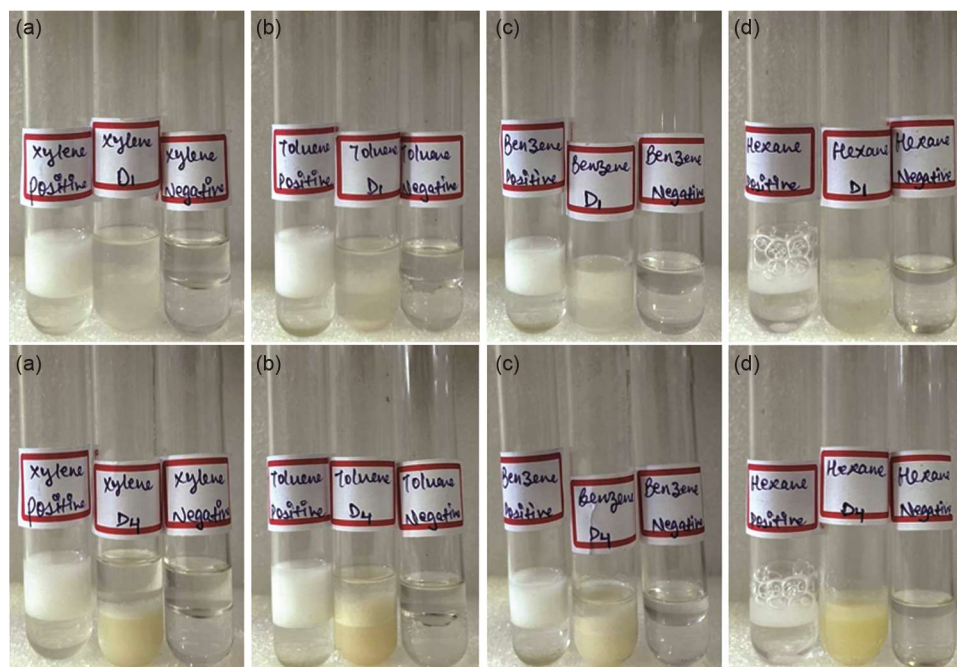


Fig. 3 — Emulsification activity of (a) *Halomonas* sp. DU-15-9-1; and (b) *Salinicoccus roseus* S-5-9-3 isolates by using different hydrocarbons A=Xylene, B=Toluene, C=Benzene, D=Hexane

studies showed that the highest flocculating activity was by *Bacillus cereus* with 93%, followed by *Bacillus pumilus* with 92%. *Nitratireductor aquimarinus* showed 89% of flocculating activity, and *Pseudoalteromonas* sp. showed 86% of flocculating activity. *Bacillus subtilis* recorded 79% of flocculating activity, while *Halomonas venusta* showed the lowest record, 59% of flocculating activity¹⁹. The flocculating activity of the EPS produced by *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 have been studied. In the present study, EPS from *Halomonas* sp. DU-15-9-1 showed the highest flocculation activity with 93%, followed by *Salinicoccus roseus* S-5-9-3 with 92% flocculation activity. The water and Gum Arabic were used as controls. The produced EPS showed flocculating activity after 30 min, whereas Gum Arabic exhibited flocculation activity after 45 min.

Thus, the EPS produced by *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 were found to be more efficient in flocculation activity than the conventional Gum Arabic. The EPS produced by *Halomonas* sp. DU-15-9-1 showed 93% of flocculating activity, while the EPS from *Salinicoccus roseus* S-5-9-3 showed 92% of flocculating activity. The EPS produced by the *Salinicoccus roseus* is not reported for flocculation activity. Results showed

strong potential for the use of EPS produced by *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 in industry sectors like wastewater treatment, sludge dewatering, food processing, and bioremediation with high flocculating efficiency, as well as their faster activity when compared to conventional gum Arabic. These marine-derived EPS's capacity to function as effective and natural bio-flocculants offers a sustainable substitute for chemical flocculants, meeting the increasing demand for environmentally friendly industrial processes.

Scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Antioxidant activity reduces the stable DPPH radical (purple) to the nonradical DPPH-H (yellow) form. The ability of the reference antioxidant and the isolated EPS to reduce DPPH was studied. It was found that the EPS was a more potent antioxidant than ascorbic acid. The maximum scavenging activity was revealed with the concentration of 1 mg/mL, 52.68% for EPS produced by *Halomonas* sp. DU-15-9-1 and 64.82% for EPS produced by *Salinicoccus roseus* S-5-9-3 (Fig. 5). The activity was found to increase with an increase in dosage. Even at very low concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/mL), our studies found that EPS's DPPH scavenging activity was greater than that of the reference. Along with EPS, the presence of reducing sugars or

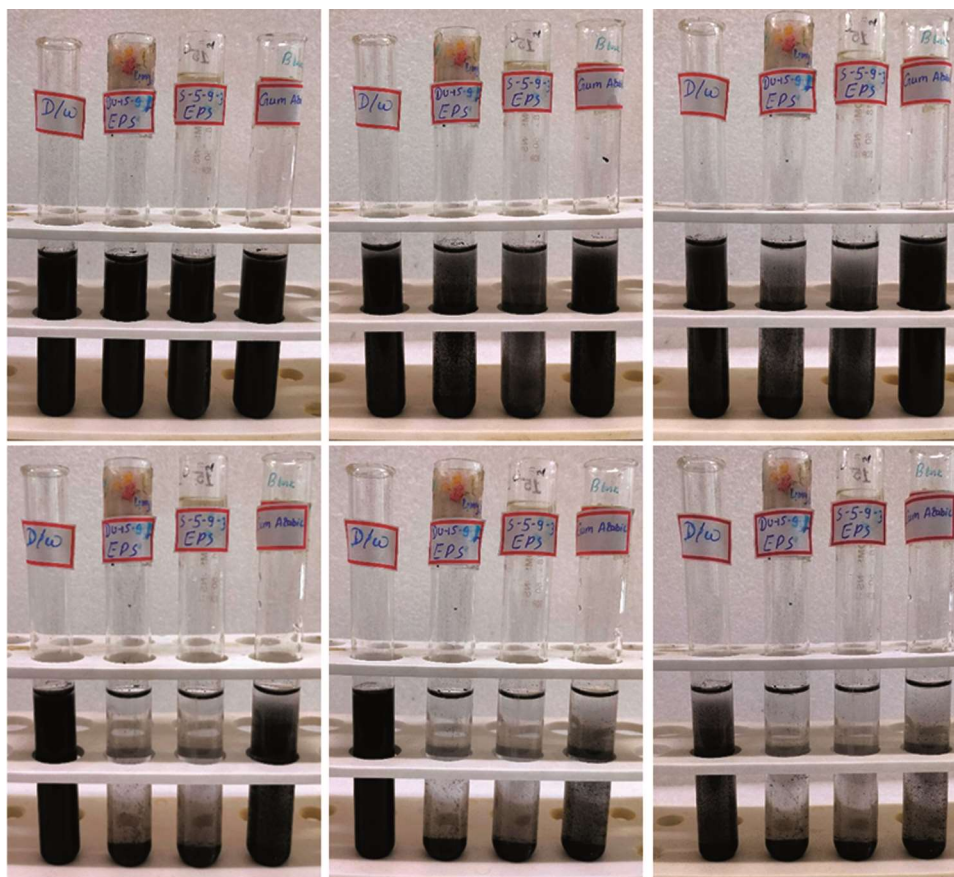


Fig. 4 — Flocculation activity of the EPS after 5, 10, 15-, 30-, 45- and 60-min. Tube 1- Negative control (D/W), Tube 2,3- EPS sample tubes, and Tube 4- Positive control (Gum Arabic). The EPS showed flocculating activity after 15 min

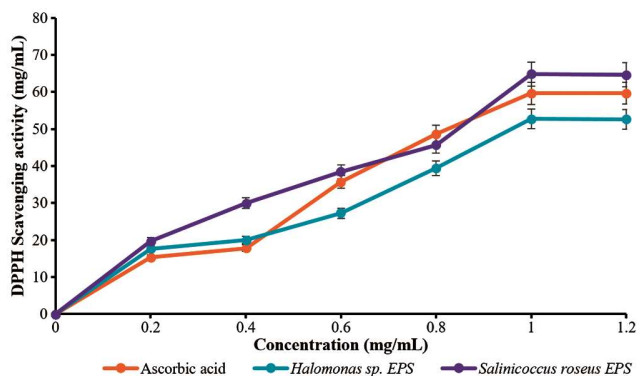


Fig. 5 — DPPH scavenging activity of EPS produced by *Halomonas sp.* DU-15-9-1 and *Salinicoccus roseus* S-5-9-3

monosaccharides, proteins, peptides, amino acids, and other microelements appear to be the cause of the reducing activity²⁰.

Accordingly, the study demonstrated that antioxidants' capacity to donate hydrogen is responsible for their capacity to scavenge DPPH²¹. In this study, EPS obtained from *Halomonas sp.* DU-15-

9-1 and *Salinicoccus roseus* S-5-9-3 show significant antioxidant activity, offering promising opportunities across multiple sectors such as cosmetic and personal care products. Antioxidant activity of EPS can serve as natural health-promoting ingredients and clean-label alternative to synthetic additives in products like dairy, sauces, and baked goods as well as a natural health-promoting ingredient.

Brine Shrimp Lethality Assay (BSLA)

The brine shrimp test showed that the shrimp survival increased at decreasing doses of EPS solution. This suggests that EPS produced by the isolate *Halomonas sp.* DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 are not toxic. Assay was performed by using 20-600 µg/mL EPS concentration. Up to 200 µg/mL, there was no mortality observed.

A popular and affordable bioassay for determining the overall toxicity of natural extracts and compounds is BSLA. *Artemia salina* (brine shrimp) nauplii mortality is tracked over 24 h at various concentrations, and the results are frequently reported

as LC_{50} (lethal concentration to kill 50%). Up to 200 $\mu\text{g/mL}$, no mortality was observed in the assay, which tested EPS concentrations ranging from 20 to 600 $\mu\text{g/mL}$ (Fig. 6). As the EPS dose dropped, the survival rate increased, indicating a dose-dependent but generally non-toxic action at those concentrations. Up to 200 $\mu\text{g/mL}$, EPS from *Salinicoccus roseus* S-5-9-3 and *Halomonas* sp. DU-15-9-1 exhibits no discernible toxicity in BSLA. The effect of EPS from the microalgae *Porphyridium cruentum* (also known as *P. purpureum*) on shrimps *Litopenaeus vannamei* was examined to find out the way this immunostimulant affected the shrimps' non-specific immune response and whether it could be utilized as a protective agent against *Vibrio* infection. The outcome demonstrates that EPS is not harmful, as evidenced by the mortality data and normal embryonic development²².

In vitro cytotoxicity assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Anticancer effects of the produced EPS were investigated on HeLa and MCF-7 cell lines. The EPS concentration was used at different concentrations (50–800 $\mu\text{g/mL}$) for HeLa cell lines (Fig. 7a) and (12.5–800 $\mu\text{g/mL}$) concentration in MCF-7 cell lines (Fig. 7b). IC_{50} ($\mu\text{g/mL}$) was calculated. In the present study, two bacterial EPS showed significant cytotoxic potential against HeLa cell lines and MCF-7 cell lines. EPS produced by *Salinicoccus roseus* S-5-9-3 shows IC_{50} ($\mu\text{g/mL}$) 96.05 ± 5.54 , while EPS produced by *Halomonas* sp. DU-15-9-1 showing IC_{50} ($\mu\text{g/mL}$) 104.10 ± 9.26 against HeLa cell lines. EPS produced by *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 with the IC_{50} ($\mu\text{g/mL}$) 31.09 ± 0.63 and 24.19 ± 0.22 , respectively, against MCF-7 cell lines. According to these results, exopolysaccharides (EPS) obtained from *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 show significant anticancer potential, especially against MCF-7 breast cancer cell lines. Their potential as attractive candidates for the development of natural, EPS-based anticancer agents are highlighted by the reduced IC_{50} values obtained against MCF-7 cells. To confirm and build on these findings, more mechanistic research and *in vivo* experiments are necessary. The EPS extracts have a much lower IC_{50} value (~ 24 – 31 $\mu\text{g/mL}$) for MCF-7 cells (breast cancer) than for HeLa cells (cervical cancer), which need much higher concentrations (~ 96 – 104 $\mu\text{g/mL}$) to inhibit growth. Studies revealed that *Porphyridium*

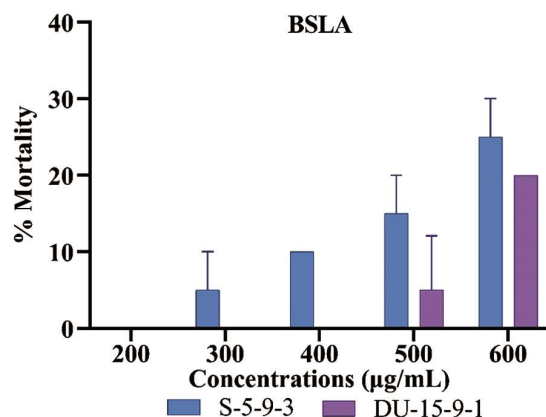


Fig. 6 — Mortality of brine shrimp analyzed against different concentrations of EPS via Brine shrimp lethality assay

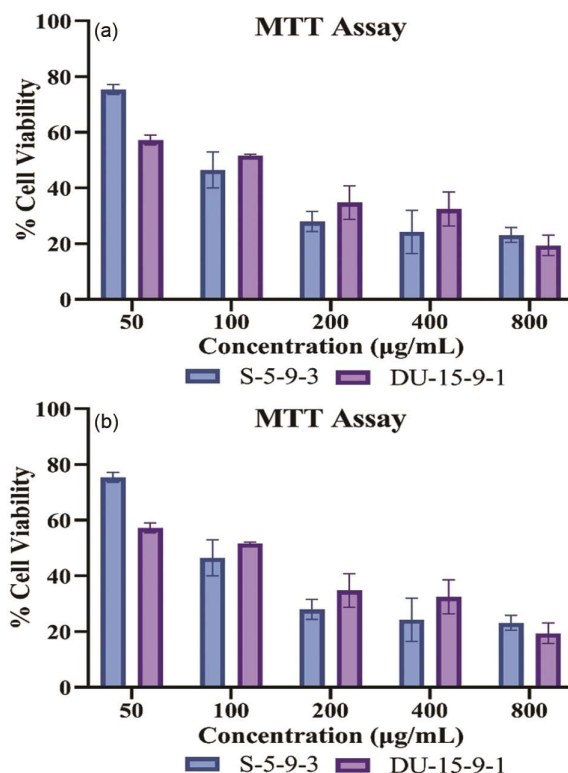


Fig. 7 — Anticancer activity of EPS against the (a) HeLa cell line; and (b) MCF-7 cell line

aeruginosa EPS showed specific anticancer activity: It had no discernible effect on HeLa or normal BJ fibroblast cells, but it significantly lowered MCF-7 cell viability with IC_{50} values of 1139 ± 74 $\mu\text{g/mL}$ (48 h) and 807 ± 76 $\mu\text{g/mL}$ (72 h). The EPS from S-5-9-3 is marginally more effective than that from DU-15-9-1 within each cell line. This demonstrates a selective anticancer effect that may be more therapeutically relevant for breast cancer (MCF-7)

than cervical cancer (HeLa). Due to their potent inhibitory potential against MCF-7 cells, EPS (particularly from S-5-9-3) are appealing candidates for additional research in breast cancer therapies^{23,24}.

Conclusion

The studies showed that EPS from *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3, exhibit good water holding capacity, flocculating activity, significant DPPH-scavenging activity, and anticancer activity. Such EPS have demonstrated promise as natural, clean-label antioxidant ingredients with versatile applications in food, cosmetics, and personal care industries. A comparative evaluation of both isolates demonstrates that the exopolysaccharides (EPS) produced by *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3 exhibit promising multifunctional properties with slight variations in bioactivity. While both isolates showed comparable emulsifying potential, *S. roseus* S-5-9-3 (55.45%) exhibited marginally higher emulsification activity than *Halomonas* sp. DU-15-9-1 (52.45%), indicating a relatively stronger surface-active capacity. Similarly, the antioxidant activity of EPS from *S. roseus* S-5-9-3 (64.82%) was notably higher than that of *Halomonas* sp. DU-15-9-1 (52.68%), suggesting superior free radical scavenging potential. In contrast, both isolates demonstrated almost equivalent and remarkably high flocculating activity, with *Halomonas* sp. DU-15-9-1 showing 93% and *S. roseus* S-5-9-3 showing 92%, indicating strong and comparable efficiency in particulate aggregation processes. Overall, although both isolates exhibit significant biotechnological potential, *S. roseus* S-5-9-3 appears to possess relatively enhanced emulsifying and antioxidant properties, whereas both strains perform similarly in terms of flocculating efficiency. These findings highlight their suitability for diverse industrial and environmental applications. The results of this study indicate that EPSs from *Halomonas* sp. DU-15-9-1 and *Salinicoccus roseus* S-5-9-3, particularly the former, are promising candidates for additional research in breast cancer therapies due to their selective anticancer effects. Their potential to develop into natural, EPS-based anticancer agents is indicated by their lower IC₅₀ values against MCF-7 cells.

Acknowledgement

Authors DMB (201901380189) and NYP (202001380174) acknowledge the SHODH (Scheme of Developing High Quality Research) fellowship,

Education Department, Government of Gujarat, India. DMB also acknowledges CSIR-HRDG, Direct SRF Award No. 112-2158-501/2k23/1 at Gujarat University. VHR generously acknowledges the funding provided by the UGC-Basic Science Research (BSR) Start-up Research Grant No. F.30-52/2020. All authors acknowledge the infrastructural support and facilities provided by the DST-FST-sponsored Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad.

Conflict of interest

All authors declare no conflict of interest.

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