International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10No. 1s, 2024
https://theaspd.com/index.php

Isolation, Structural Characterization and Antidiabetic Evaluation of Bioactive Exopolysaccharide from *Brevundimonas diminuta* DMW11

Edward C[1], Sangeetha M[2]
[1]Research Scholar), Reg no : 20122102101010 - PG - Research Department of Microbiology, Kamaraj College (Autonomous).Thoothukdi, Tamilnadu, India. Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627012.
email – edwardbenaiah@gmail.com
[2]PG – Research Department of Microbiology, Kamaraj College (Autonomous). Thoothukdi, Tamilnadu, India.Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627012.
email - sangeetha.m@kamarajcollege.ac.in

Abstract
Marine microbes are considered to be a major source of bioactive substances with substantial medicinal promise. In this investigation, samples of seawater from the coastal region of Thoothukudi, South India, were used to extract marine bacteria that produce bioactive exopolysaccharides (EPS). Based on apparent, biochemical, and molecular characterization using 16S rRNA gene sequencing, Brevundimonas diminuta DMW11 was discovered to be a powerful EPS producer among the isolates. FTIR spectroscopy was used to further characterize the EPS after it had been isolated and purified using the cold acetone precipitation procedure. FTIR examination confirmed the polysaccharide beginning of the EPS by revealing the presence of typical polysaccharide functional groups such hydroxyl, carboxyl, aliphatic, and glycosidic linkages. The purified EPS's antidiabetic potential was assessed at amounts between 200 and 1000 μg/mL using α-amylase and α-glucosidase inhibitory tests in vitro.. The potential significance of the EPS in regulating postprandial hyperglycemia is suggested by the reported enzyme inhibitory activity. Overall, the results show that EPS generated by Brevundimonas diminuta DMW11 has encouraging antidiabetic qualities and could be utilized as a natural biomolecule in pharmaceutical and biomedical applications.
Key words: *Exopolysaccharide (EPS); Brevundimonas diminuta DMW11; FTIR analysis; α-amylase inhibition; α-glucosidase inhibition.*

INTRODUCTION

Marine microorganisms have the capacity to produce novel chemical molecules using a variety of biological activity because of their distinct living conditions. Among these, the exopolysaccharides that marine microorganisms make are essential to their survival in these harsh conditions. Because of their structural complexity, biodegradability, biological activity, and biocompatibility, exopolysaccharides from marine microbes—particularly those from extremophiles—have garnered increasing interest to present. An growing number of novel exopolysaccharides are being discovered and studied due to advancements in culture and separation techniques.(Mingxing Qi et al., 2020). Exopolysaccharides are released by some microbes to protect themselves against unfavorable conditions. When cultivated under certain culture conditions, the majority of bacterial species produce slimy substances outside their cell walls; these compounds are frequently polysaccharides with high molecular weight. (Sutherland, 2001a; 2001b). These materials could be capsular polysaccharides (Sutherland, 2001a) or EPSs, which are totally separated from the cell (Sutherland, 2001b). *Xanthomonas campestris, Sphingomonas paucimobilis, Acetobacter xylinum,* and *Rhizobium sp*. are the sources of the commercialized EPSs xanthan, gellan, cellulose, and succinoglycan. Several marine bacteria, such as *Bacillus*, *Rhodococcus*, *Alteromonas*, Enterobacter, *Halomonas*, and *Klebsiella*, are also known as EPS producers (Nicolaus et al., 2000; Maugeri et al., 2002). Marine EPS offers a wide range of biotechnological applications, such as thickening agents, texturizers and stabilizers for the food industry, flocculating agents for wastewater treatment, and anti-aging compounds for the cosmetics sector (Al-Nahas et al., 2011). Microbial marine exopolysaccharides have a complex and diverse structure (Wang et al., 2020). According to Besednova et al. (2018) and Hassan & Ibrahim (2017), they have a wide range of biological actions, including antioxidant, antifreeze, anti-inflammator, anticancer, antibacterial, increase of immunological activity, and decrease of blood pressure and cholesterol. increased blood sugar levels due to insulin resistance, inadequate insulin secretion, or

problems with glucose metabolism are the hallmarks of diabetes mellitus, a multifactorial metabolic disease. Decreased physical activity, greater urbanization, excessive calorie intake, and genetic vulnerability have all contributed to the disease's emergence as a significant global health concern (Cho et al., 2021). According to Forbes and Cooper (2013), chronic hyperglycemia is known to cause oxidative stress and inflammatory responses, which can lead to major microvascular and macrovascular issues such as neuropathy, retinopathy, diabetic nephropathy, cardiovascular illnesses, and delayed tissue repair. Long-term blood glucose rise can also change how proteins, fats, and carbohydrates are metabolized, which can impact the regular physiological processes of several organs (American Diabetes Association, 2023). Inhibition of the enzymes that hydrolyze carbohydrates, such as Alpha-amylase and Alpha-glucosidase, has become a significant therapeutic technique for managing postprandial. Acarbose, a commercially available inhibitor, is frequently used to slow the absorption of glucose; however, its usage is restricted due to gastrointestinal side effects, such as flatulence, diarrhea, and discomfort in the abdomen (Kim et al., 2005). Finding natural and microbially generated bioactive molecules with safer medicinal potential is therefore of greater interest. Among them, microbial exopolysaccharides have drawn a lot of interest because of their various pharmacological actions, such as immunomodulatory, antidiabetic, antioxidant, and antibacterial qualities (Nwodo et al., 2012). Because of their distinct structural variety and biological usefulness resulting from adaption to harsh marine settings, marine bacterial exopolysaccharides in particular are thought to be interesting candidates for biomedical applications (Poli et al., 2010). By postponing the breakdown of carbohydrates and the absorption of glucose, Marine bacteria-derived EPS has shown encouraging inhibitory effect against enzymes that metabolize carbohydrates and could aid in blood glucose regulation. (Patel et al., 2012). Nevertheless there are yet few research examining the antidiabetic potential of EPS produced from *Brevundimonas diminuta*. Therefore, the isolation, structural characterization, and antidiabetic assessment of the bioactive exopolysaccharide generated by *Brevundimonas diminuta* DMW11 are the main objectives of this investigation. The findings of this investigation may aid in the creation of new microbial polysaccharide-based treatments to be treated of diabetes and other biological uses.

MATERIALS AND METHOD

Collection of Seawater Sample

The containers were made of sterile polypropylene to gather seawater samples from the coastal region of Thoothukudi, South India. The samples were obtained and transported aseptically to the laboratory for additional microbiological investigation after being promptly stored within an ice box. The indigenous population of microbes can be preserved and contamination can be reduced with proper cold-chain maintenance throughout transportation (Kumar et al., 2021).

Isolation of Marine Bacteria

After carefully mixing the seawater sample, Aseptically, 1 mL was added to 9 mL of sterile physiological saline (0.85% NaCl) to create a 10^{-1} dilution. Subsequently, sterile serial dilutions were made up to 10^{-2}. To isolate culturable marine bacteria from environmental samples, serial dilution procedures are frequently used (Subramanian et al., 2020).

Plating and Incubation

Using a sterile L-shaped spreader, an aliquot (0.1 mL) from the proper dilutions was equally distributed onto Zobell Marine Agar (ZMA) plates. For 48–72 hours, the inoculation plates were incubated at 28 ± 2 °C in an inverted position. Due to its balanced nutritional composition and salt concentration, which are ideal for the expansion of marine microorganisms, Zobell Marine Agar was chosen (Saranraj & Stella, 2021).

Selection and Purification of Isolates

After being incubated, colonies that were morphologically unique in terms of size, shape, pigmentation, margin, elevation, and texture were chosen. Pure cultures were obtained by repeatedly streaking individual colonies on new marine agar plates. For additional research, pure isolates were kept on marine agar slants at 4 °C (Aneja, 2020).

Screening for Exopolysaccharide Production

Purified isolates were inoculated into EPS production medium containing (g/L) glucose 20, yeast extract 1.0, NH_4NO_3 0.8, K_2HPO_4 0.6, KH_2PO_4 0.05, $MnSO_4 \cdot 4H_2O$ 0.1, and $CaCO_3$ 0.1 in order to screen for bacteria that produce exopolysaccharide (EPS). The inoculation cultures were shaken and incubated for 48–72 hours at 30 °C. Potential EPS producers were identified in isolates with slimy or mucoid colony shape (Raveendran et al., 2021).

Morphological and Biochemical Characterization

Initially bacterial
isolates were first characterized using colony morphology, which includes form, color, elevation, opacity, and surface appearance. Common biochemical assays, such as catalase, oxidase, citrate consumption, motility, and carbohydrate fermentation assays, were performed to determine the isolates' physiological and metabolic characteristics in compliance with accepted microbiological practices (Cheesbrough, 2019).

Molecular Identification of *Brevundimonas diminuta DMW11*

The potent EPS-producing bacterial isolate was identified using 16S rRNA gene sequencing. As directed by the manufacturer, genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit. The universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) were used to amplify the 16S rRNA gene. A 5-minute initial denaturation at 95 °C was 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 55 °C for 45 seconds, extension at 72 °C for 90 seconds, and a final extension at 72 °C for 10 minutes followed. Agarose gel electrophoresis analysis and sequencing were performed on the amplified product. The acquired sequences were assessed using the BLASTn tool in the National Center for Biotechnology Information database in order to identify similarities. To verify taxonomic status, phylogenetic analysis was carried out using the neighbor-joining approach in MEGA X program (Tamura et al., 2021).

Isolation and Purification of Exopolysaccharide

A modified cold acetone precipitation procedure was used to isolate EPS. To eliminate bacterial cells, the bacterial culture broth was centrifuged at 10,000 rpm for 10 minutes at 4 °C. In order to precipitate EPS, the clear supernatant was mixed with two volumes of cold acetone and incubated at 4 °C for the entire night. The precipitated EPS was recovered by centrifugation at 12,000 rpm for 15 minutes, after which it was dried for further characterization studies (Singha, 2020).

FTIR Analysis of EPS

Fourier Transform Infrared Spectroscopy (FTIR) was used to investigate the pure EPS from DMW11 in order to identify the primary functional groups. Potassium bromide (KBr) and dried EPS powder were properly combined before being compacted into pellets. An FTIR spectrophotometer with 64 scans and a resolution of 4 cm^{-1} was used to record FTIR spectra in the wavelength range of 4000–400 cm^{-1}. The acquired spectra were examined for distinctive absorption peaks that matched the functional units of polysaccharides (Zhao et al., 2022).

In vitro Antidiabetic Assay of EPS from *Brevundimonas diminuta* DMW11

α-Amylase Inhibitory Assay

A slightly modified in vitro enzyme inhibition technique was used to investigate the α-amylase inhibitory activity of isolated EPS derived from *Brevundimonas diminuta* DMW11. Distilled water was used to prepare various EPS concentrations (200, 400, 600, 800, and 1000 µg/mL). In short, 500 µL of EPS solution and 500 µL of α-amylase enzyme solution made in phosphate buffer (0.02 M, pH 6.9, containing 0.006 M NaCl) were mixed together, and the mixture was incubated at 37 °C for ten minutes. Following the addition of 500 µL of a 1% solution, the reaction mixture was incubated at 37 °C for 15 minutes soluble starch solution was added. It was boiled in a boiling water bath for five minutes after DNS reagent was added to halt the process. Absorbance was measured using a UV-visible spectrophotometer at 540 nm after cooling. The normal reference medication was acarbose. By comparing the absorbance of treated and untreated samples, the inhibitory potential of DMW11-EPS was ascertained (Zhang et al., 2022).

α-Glucosidase Inhibitory Assay

A modified in vitro experiment was used to measure DMW11-EPS's α-glucosidase inhibitory activity. Phosphate buffer (0.1 M, pH 6.8) was employed to produce various amounts of EPS (200–1000 μg/mL). 50 μL of EPS solution and α-glucosidase enzyme solution were combined within the assay mixture, which was subsequently pre-incubated for ten minutes at 37 °C. Following the addition of substrate solution, p-nitrophenyl-α-D-glucopyranoside (pNPG) the mixture was incubated for 20 minutes at 37 °C. After stopping the reaction with sodium carbonate solution, absorbance at 405 nm was measured using a spectrophotometer. The positive control standard was acarbose. The decrease in enzyme activity relative to the control was employed to evaluate the inhibitory activity of EPS (Liu et al., 2022).

Standard Acarbose Assay

The standard antidiabetic medication for the α-amylase and α-glucosidase inhibitory experiments was acarbose. To generate concentrations ranging from 200 to 1000 μg/mL, a stock solution of acarbose (1 mg/mL) was made in distilled water and serially diluted. In all enzyme inhibition tests, the produced acarbose solutions were subjected to the identical experimental conditions as the EPS samples. For α-amylase inhibition, absorbance measurements were taken at 540 nm, and for α-glucosidase inhibition, at 405 nm. The antidiabetic potential of DMW11-EPS was assessed by evaluating its inhibitory action with the conventional acarbose activity (Wang et al., 2022). The potential applications of marine microbe products in the culinary, pharmaceutical, and cosmetic industries define them. They can produce a range of specific active chemicals, such as exopolysaccharides, in harsh conditions of oligotrophic conditions, high pressure, and high salt, and low temperature. (L´opez-Ortega et al., 2021). Additionally, exopolysaccharides—a major active component of marine microorganisms resources have become a new field of strong research focus (Banerjee et al., 2021).

RESULTS AND DISCUSSION

Collection of Water Samples and Isolation of Marine Bacteria

Several microbial communities were discovered in seawater samples taken from the Thoothukudi Coastal Region's coastal waters, demonstrating the marine habitat's biological richness. Numerous bacterial colonies with a range of morphological traits were seen after incubation on Zobell Marine Agar (ZMA) following serial dilution and inoculation. The seawater samples' total number of live bacteria found dense microbial abundance, which is consistent with earlier research that bacterial populations in marine ecosystems range from 10^{0} to 10^{1} CFU/mL, depending on environmental factors and nutrient availability (Kumar et al., 2021). The growth of marine-adapted and halotolerant microorganisms was promoted by the selected composition of ZMA, especially its balanced salt concentration and nutritional profile (Li et al., 2020). Studies on marine bacterial diversity from coastal habitats, where nutrient-rich seawater enabled widespread microbial colonization, have reported similar findings (Wang et al., 2022).

Morphological Diversity and Purification of Isolates

The existence of varied marine bacterial communities was indicated by the isolated colonies' significant morphological variety in terms of colony size, hue, elevation, opacity, texture, and margin features. To further assess the synthesis of exopolysaccharides (EPS), distinct colonies were chosen and purified. Previous studies demonstrating varied bacterial populations in marine environments are supported by the observed phenotypic variety among marine isolates (Chen et al., 2021). Axenic cultures were produced by repeated streak plate purification, guaranteeing the separation of genetically stable bacterial strains for further biochemical and molecular analysis. For dependable and repeatable experimental results, standard microbiological purification techniques are still crucial (Aneja, 2019).

Screening for Exopolysaccharide (EPS) Production

On marine agar plates, a number of pure isolates showed notable mucoid and viscous colony shape, indicating their possible capacity to produce extracellular polysaccharides. Due to the buildup of polysaccharide matrices surrounding the cells, mucoidal appearance is widely recognized as a significant phenotypic signal of EPS secretion in marine bacteria (Patel et al., 2020). By promoting biofilm formation, osmotic balance, and cellular protection, extracellular polysaccharide synthesis is essential for microbial adaptation to saline and harsh marine environments (Suresh et al., 2021). Previous study showing that

carbohydrate-rich substrates promote polysaccharide biosynthesis in marine bacterial species is supported by the increased EPS production shown in glucose-supplemented media (Zhao et al., 2022).

Morphological and Biochemical Identification

On marine agar, the isolate *Brevundimonas diminuta* DMW11 displayed distinctive colony morphology, such as round colonies with smooth borders, convex elevation, and a clearly mucoidal texture. These physical characteristics suggest potential extracellular polymeric substance release and are frequently linked to marine EPS-producing bacteria (Ramesh et al., 2020). The isolate's aerobic metabolic nature was confirmed by biochemical characterisation, which showed that it was oxidase and catalase positive. In keeping with the metabolic versatility commonly seen in marine heterotrophic bacteria, the organism also showed the capacity to use and ferment specific carbohydrates (Singh and Gupta, 2021). Such physiological variability may have a major impact on EPS biosynthesis efficiency as well as survival in changing marine environments.

Fig 1 : Morphological and Biochemical identification

Morphological features		Cultural features	
Size	Small (1–2 mm)	Gram's staining	Gram- positive
Shape	Circular	Shape	Cocci (tetrads/clusters)
Elevation	Convex	Capsule staining	– (non-capsulated)

Texture	Smooth, butyrous	Spore staining	– (non-sporing)
Colour	Yellow to creamy	Motility	Non-motile
Margin	Entire (smooth)	Flagella	Absent

Table 1 : Observation of morphological and biochemical characteristics of *Bravidomonas* sp. DMW11

Biochemical test	
Indole	–
Methyl Red (MR)	–
Voges–Proskauer (VP)	–
Citrate Utilization	–
Catalase	+
Oxidase	+
Urease	+

Molecular identification of *Brevundimonas diminuta* strain DMW11

The 16S rDNA sequence (~1400 bp) of the EPS-producing strain (DMW11) was successfully amplified and sequenced; BLASTn analysis verified the isolate's taxonomic affiliation, confirming the highest sequence similarity (≥99%) with members of the genus *Brevundimonas*; The sequence was sent to the NCBI GenBank database with the accession number (OQ772248.1). Phylogenetic analysis using the neighbor-joining method revealed that strain DMW11 was closely related to other *Brevundimonas species*, forming a separate clade with strong bootstrap support that demonstrated its evolutionary kinship within the genus. Molecular identification verified the isolates' morphological and biochemical characteristics. *Brevundimonas species* have been extensively documented from marine and aquatic environments and are renowned for their metabolic diversity, which includes the formation of exopolysaccharides with potential biotechnological uses (Ryan and Pembroke, 2018). Strong sequence similarity confirms the genus *Brevundimonas,* suggesting that it may be involved in the production of marine EPS and associated bioactivities.

CGCGGCAGCTACACATGCAGTCGAACGGACCCTTCGGGGTTAGTGGCGGACGGGTGAG
TAACACGTGGGAACGTGCCTTTAGGTTCGGAATAGCTCCTGGAAACGGGTGGTAATGCC
GAATGTGCCCTTCGGGGGAAAGATTTATCGCCTTTAGAGCGGCCCGCGTCTGATTAGCT
AGTTGGTGAGGTAACGGCTTCAACCAAGCGACGATTCAGTAGCTTGTCTGGAGATGACC
AGTCACACTGGGACTGAGACACGTCCAGACTCTACGGGAGGCAGCAGTGGGGGAATCT
GCGCAATGGGCGAAAGCCTGACGCAGCCATGCCGCGTGAATGATGAAGGTCTTAGGATT
GTAAAATTCTTCACCGGGGACGATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAACT
TCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCG
TAAAGGGCGCGTAGGCGGATCGTTAAGTCAGAGGTGAAATCCCAGGGCTCAACCCTGG
AACTGCCTTTGATACTGGCGATCTTGAGTATGAGAGAGGTATGTGGAACTCCGAGTGTA
GAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACATACTGGCTCA
TTACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
CACGCCGTAAACGATGATTGCTAGTTGTCGGGCTGCATGCAGTTCGGTGACGCAGCTAA

CGCATTAAGCAATCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTT
ACCACCTTTTGACATGCCTGGACCGCCACGGAGACGTGGCTTTCCCTTCGGGGACTAGG
ACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTCGCCATTAGTTGCCATCATTTAGTTGGGAACTCTAATGGGACTG
CCGGTGCTAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACAGGG
TGGGCTACACACGTGCTACAATGGCAACTACAGAGGGTTAATCCTTAAAAGTTGTCTCAG
TTCGGATTGTCCTCTGCAACTCGAGGGCATGAAGTTGGAATCGCTAGTAATCGCGGATC
AGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG
AGTTGGTTCTACCCGAAGGCGGTGCGCTAACCAGCAATGGAGGCAGCCGACCACGCGT
ACAG

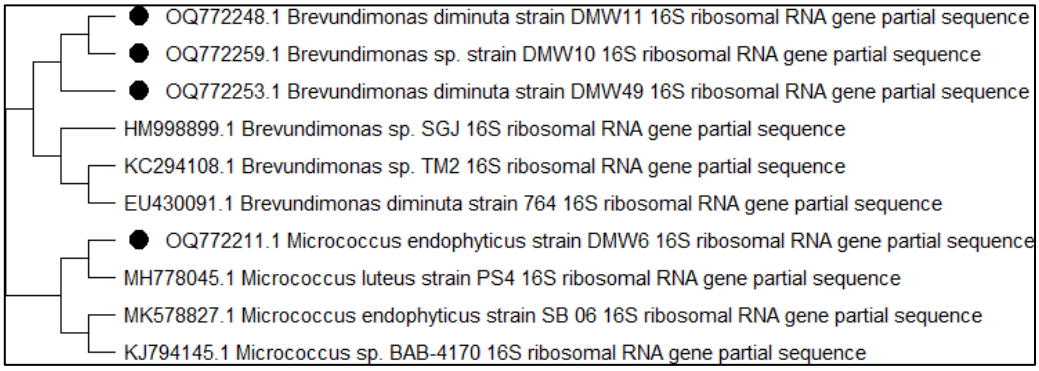


Fig 2 : Phylogenetic tree

Fourier Transform Infrared (FTIR) Spectral Analysis of EPS

Utilizing Fourier Transform Infrared (FTIR) spectroscopy, the functional groups in the isolated exopolysaccharide (EPS) generated by *Brevundimonas diminuta* was studied. The isolated biomolecule's polysaccharide origin was confirmed by the FTIR spectrum, which displayed multiple distinctive absorption peaks. Microbial EPS with hydroxyl, carboxyl, and glycosidic groups have been shown to have comparable FTIR spectra (Wang et al., 2021). The presence of hydroxyl (–OH) groups, which are characteristic of carbohydrate structures and hydrogen-bonded polysaccharides, was revealed by a wide absorption band around 3312 cm^{-1}. Liu et al. (2020) reported similar hydroxyl stretching vibrations in microbial EPS. Aliphatic components inside the polysaccharide backbone were indicated by the peak near 2944 cm^{-1}, which matched the C–H stretching vibrations of methyl and methylene groups (Zhao et al., 2022). The existence of uronic acids or protein-associated polysaccharides was suggested by an absorption peak at 1641 cm^{-1} that was ascribed to C=O stretching or amide I vibrations. Singh et al. (2021) observed similar findings with marine bacterial EPS. C–H bending and carboxylate group vibrations were reflected by peaks about 1455 cm^{-1} and 1395 cm^{-1}, respectively, suggesting acidic polysaccharide residues that can support biological activity (Chen et al., 2021). The EPS's polysaccharide structure was confirmed by strong absorption bands between 1147 cm^{-1} and 957 cm^{-1}, which correlated to C–O–C and glycosidic linkage vibrations. Kumar et al. (2022) previously observed similar glycosidic bond-associated peaks in bacterial exopolysaccharides. Kumar et al. (2022) previously identified similar glycosidic bond-associated peaks in bacterial exopolysaccharides. Overall, the FTIR analysis supported the possible biological and physicochemical features of the EPS isolated from *Brevundimonas diminuta* by confirming the presence of main polysaccharide-associated functional groups together with minor proteinaceous and acidic components (Roca et al., 2019).

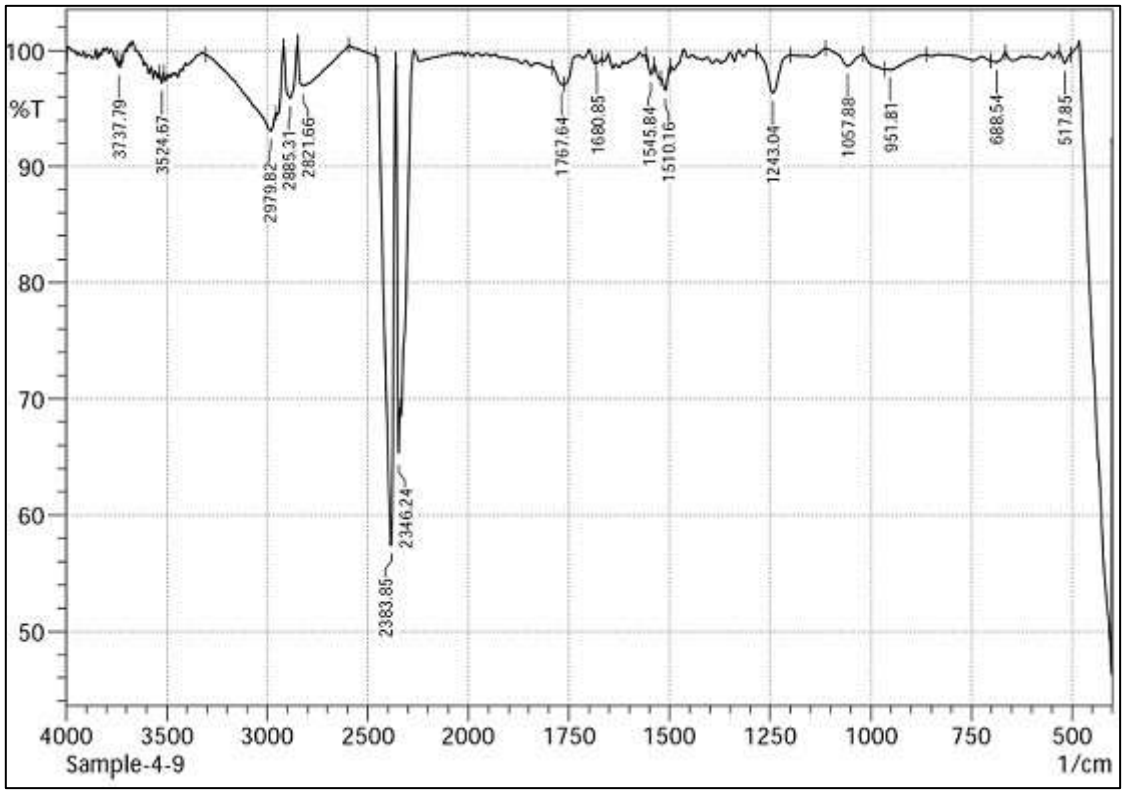


Fig 3 : FTIR Analysis

In vitro Antidiabetic Assay of EPS from *Brevundimonas diminuta* DMW11

α-Amylase Inhibitory Assay

Exopolysaccharide (EPS) isolated from *Brevundimonas diminuta* was tested at concentrations between 200 to 1000 µg/mL for its α-amylase inhibitory activity. The results showed a concentration-dependent increase in enzyme inhibition. The findings showed that enzyme inhibition increased in a concentration-dependent manner. At 200 µg/mL, the EPS displayed an inhibition of 29.55 ± 0.45%, which gradually increased to 43.35 ± 0.39%, 57.03 ± 0.39%, and 66.73 ± 0.40% at 400, 600, and 800 µg/mL, respectively. At 1000 µg/mL, the greatest inhibition of 76.43 ± 0.45% was noted. The potential ability of the EPS to block α-amylase's hydrolysis of starch is indicated by the increase in inhibitory activity with increasing concentration. Postprandial hyperglycemia and glucose release may be lessened by this inhibition. Singh et al. (2021) showed similar concentration-dependent α-amylase inhibitory effects of microbial polysaccharides, suggesting that EPS's hydroxyl and carboxyl functional groups play a major role in enzyme binding activity. According to Wang et al. (2022), marine bacterial EPS shown significant antidiabetic effect by inhibiting the enzymes that hydrolyze carbohydrates. According to our results, the EPS generated by *Brevundimonas diminuta DMW11* may be utilized as a natural medicinal biomolecule in order to treat diabetes and has promising antidiabetic potential.

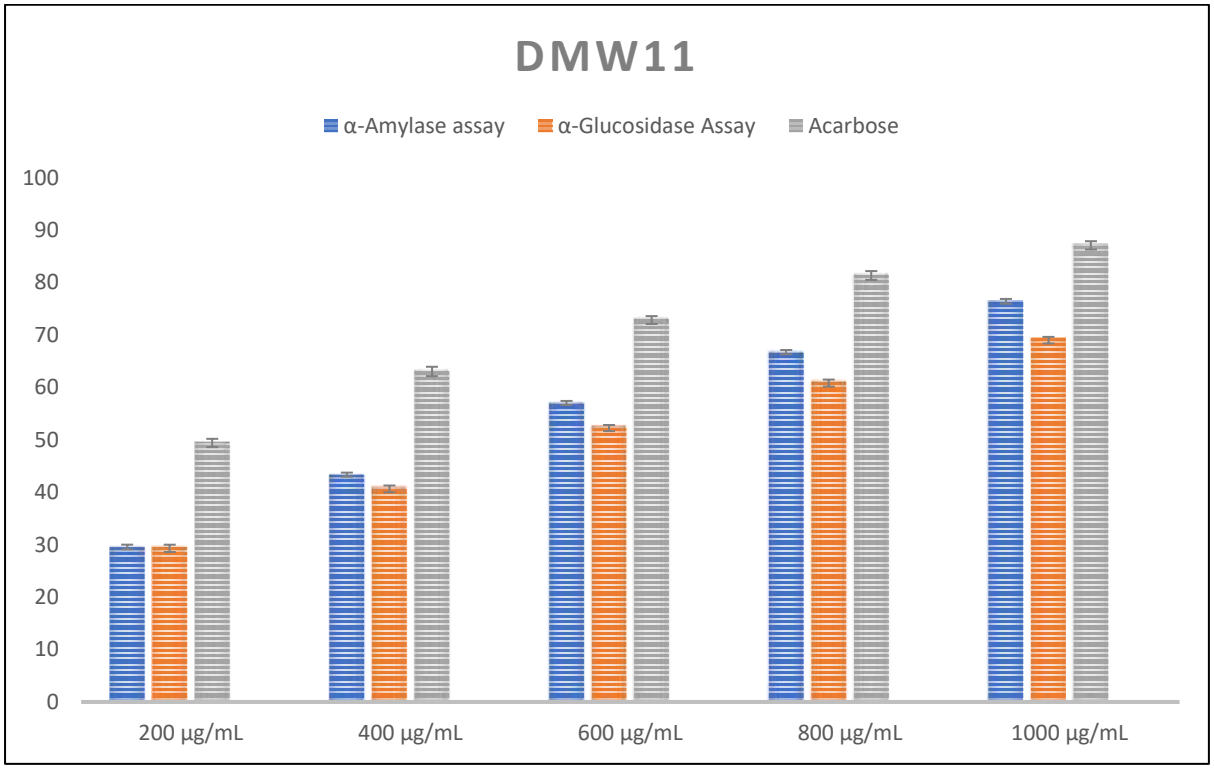
α-Glucosidase Inhibitory Assay

With various concentrations between 200 and 1000 µg/mL, the EPS isolated from *Brevundimonas diminuta* was evaluated for its α-glucosidase inhibitory action. The α-glucosidase enzyme was significantly inhibited by the EPS in a dose-dependent manner. At doses of 400, 600, and 800 µg/mL, the percentage inhibition increased from 29.61 ± 0.38% to 41.00 ± 0.28%, 52.66 ± 0.16%, and 61.16 ± 0.33%, respectively. At 1000 µg/mL, the maximum inhibition of 69.38 ± 0.25% was observed. The findings show that the EPS successfully suppressed α-glucosidase activity, which may have delayed the absorption of glucose and the digestion of carbohydrates. Similar findings were reported by Patel et al. (2020), who showed that the presence of uronic acids and glycosidic residues in marine bacterial polysaccharides resulted in strong α-glucosidase inhibitory characteristics. According to previous studies by Chen et al. (2021), microbial EPS with carboxyl and hydroxyl groups had increased antidiabetic efficacy via methods of enzyme inhibition. The therapeutic potential of the EPS produced by *Brevundimonas diminuta* DMW11

as a natural antidiabetic drug is confirmed by the relatively high inhibitory activity seen at higher doses in the current investigation.

Standard Acarbose Assay

The α-amylase inhibitory assay was employed to assess the antidiabetic activity of the EPS with the conventional medication Acarbose. At optimum concentrations of 200, 400, 600, 800, and 1000 µg/mL, acarbose showed significant concentration-dependent inhibition, with inhibition percentages of 49.60 ± 0.58%, 63.15 ± 0.78%, 73.11 ± 0.49%, 81.51 ± 0.68%, and 87.30 ± 0.58%, respectively. The EPS produced by *Brevundimonas diminuta* DMW11 had significant enzyme inhibitory action at higher concentrations, although it showed relatively less inhibition when compared to the usual medication. The well-known α-amylase and α-glucosidase inhibitor acarbose is frequently used to regulate postprandial blood glucose levels. Kumar et al. (2022) showed similar inhibitory patterns for acarbose and discussed its efficacy as a standard reference molecule in antidiabetic investigations. Kumar et al. (2022) showed similar inhibitory patterns for acarbose and discussed its efficacy as a standard reference molecule in antidiabetic investigations. The natural origin and bioactive polysaccharide composition of the EPS could provide further therapeutic advantages. with possibly fewer adverse effects, despite the fact that acarbose's inhibitory effectiveness was greater.. For the formation of novel antidiabetic formulations, the EPS derived from *Brevundimonas diminuta DMW11* may therefore be regarded as a promising natural substitute.



CONCLUSION

Overall, the findings of this research indicate that EPS obtained from *Brevundimonas diminuta* DMW11 has promising antidiabetic qualities and could be a viable natural substitute for the creation of new therapeutic agents for the treatment of diabetes. The study also emphasizes the significance of marine microorganisms as important sources of bioactive substances with potential uses in medicine. To better understand the molecular structure and bioactive components of the EPS, future research should concentrate on detailed structural clarification utilizing contemporary analytical techniques such as Nuclear Magnetic Resonance (NMR), Gas Chromatography–Mass Spectrometry (GC–MS), High Performance Liquid Chromatography (HPLC), and Scanning Electron Microscopy (SEM). Furthermore, to confirm the EPS's therapeutic efficacy, bioavailability, toxicity, and safety profile, in vivo antidiabetic investigations employing experimental animal models are required. Further understanding of its pharmacological potential would come from examining the molecular processes that underlie insulin

signaling pathways, antioxidant action, and glucose management. Additionally, the EPS may be investigated for multifunctional biomedical drug delivery methods, for example, wound healing, antibacterial, anti-inflammatory, and antioxidant. The present investigation provides new avenues for the use of marine bacterial exopolysaccharides as ecologically conscious and sustainable biomolecules in the biomedical, pharmacological, and nutraceutical fields.

REFERENCES

1. American Diabetes Association. (2023). Classification and diagnosis of diabetes: Standards of care in diabetes—2023. Diabetes Care, 46(Suppl. 1), S19–S40. https://doi.org/10.2337/dc23-S002
2. Aneja, K. R. (2020). Experiments in microbiology, plant pathology and biotechnology (5th ed.). New Age International Publishers.
3. Banerjee, S., Das, D., & Dey, P. (2021). Marine microbial exopolysaccharides: Biosynthesis, characterization and biomedical applications. International Journal of Biological Macromolecules, 183, 1637–1650.
4. Besednova, N. N., Zaporozhets, T. S., Kuznetsova, T. A., Makarenkova, I. D., Fedyanina, L. N., Kryzhanovsky, S. P., & Zvyagintseva, T. N. (2018). Metabolites of seaweeds as potential agents for the prevention and therapy of influenza infection. Marine Drugs, 16(3), 55–72.
5. Cheesbrough, M. (2019). District laboratory practice in tropical countries (Part 2, 3rd ed.). Cambridge University Press.
6. Chen, X., Zhao, R., Liu, Y., & Wang, J. (2021). Structural characterization and biological activities of microbial exopolysaccharides: A review. International Journal of Biological Macromolecules, 189, 111–123.
7. Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., Fernandes, J. D. R., Ohlrogge, A. W., & Malanda, B. (2021). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2021 and projections for 2045. Diabetes Research and Clinical Practice, 183, 109119. https://doi.org/10.1016/j.diabres.2021.109119
8. Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. Physiological Reviews, 93(1), 137–188. https://doi.org/10.1152/physrev.00045.2011
9. Hassan, S. W., & Ibrahim, A. M. (2017). Biological activities of microbial exopolysaccharides: A review. Journal of Applied Pharmaceutical Science, 7(6), 221–228.
10.Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y., & Rhee, H. I. (2005). Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. Nutrition, 21(6), 756–761. https://doi.org/10.1016/j.nut.2004.10.014
11.Kumar, A., Singh, S., & Sharma, P. (2021). Isolation and characterization of marine bacteria from coastal environments. Journal of Marine Biology Research, 15(2), 101–110.
12.Kumar, V., Patel, R., & Singh, P. (2022). Structural characterization and biological activities of bacterial exopolysaccharides. International Journal of Biological Macromolecules, 194, 289–301.
13.Kumar, C. G., Joo, H. S., Choi, J. W., Koo, Y. M., & Chang, C. S. (2007). Purification and characterization of an exopolysaccharide from haloalkalophilic Bacillus sp. I-450. Enzyme and Microbial Technology, 40(4), 902–907.
14.Li, W., Zhang, Y., & Chen, H. (2020). Marine bacterial diversity and cultivation using marine agar media. Marine Biotechnology, 22(4), 455–466.
15.Liu, J., Wang, Y., Zhao, L., & Chen, H. (2020). FTIR characterization of bacterial exopolysaccharides from marine microorganisms. Carbohydrate Polymers, 229, 115481.
16.Liu, X., Zhang, J., Wang, Q., & Li, Y. (2022). Evaluation of α-glucosidase inhibitory activity of microbial polysaccharides. International Journal of Biological Macromolecules, 209, 1165–1174.
17.López-Ortega, M. J., Delattre, C., Pierre, G., & Michaud, P. (2021). Marine exopolysaccharides: A sustainable source of bioactive molecules. Marine Drugs, 19(12), 664.
https://doi.org/10.3390/md19120664
18.Maugeri, T. L., Gugliandolo, C., Caccamo, D., & Stackebrandt, E. (2002). A polyextremophilic halophilic bacterium producing exopolysaccharides. Systematic and Applied Microbiology, 25(3), 404–411.
19.Nicolaus, B., Kambourova, M., & Oner, E. T. (2000). Exopolysaccharides from extremophilic microorganisms: Production and applications. Environmental Technology, 21(11), 1111–1124.

20.Nwodo, U. U., Green, E., & Okoh, A. I. (2012). Bacterial exopolysaccharides: Functionality and prospects. International Journal of Molecular Sciences, 13(11), 14002–14015. https://doi.org/10.3390/ijms131114002
21.Patel, S., Majumder, A., & Goyal, A. (2012). Potentials of exopolysaccharides from lactic acid bacteria. Indian Journal of Microbiology, 52(1), 3–12.
22.Poli, A., Anzelmo, G., & Nicolaus, B. (2010). Bacterial exopolysaccharides from extreme marine habitats. Marine Drugs, 8(6), 1779–1802. https://doi.org/10.3390/md8061779
23.Ramesh, R., Kumar, P., & Rajasekaran, R. (2020). Characterization of marine exopolysaccharide-producing bacteria from coastal ecosystems. Journal of Applied Microbiology, 128(5), 1455–1468.
24.Raveendran, S., Parameswaran, B., & Pandey, A. (2021). Production and applications of microbial exopolysaccharides. Bioresource Technology Reports, 15, 100764.
25.Roca, C., Alves, V. D., Freitas, F., & Reis, M. A. M. (2019). Exopolysaccharides enriched in rare sugars: Bacterial sources, production and applications. Frontiers in Microbiology, 10, 1953.
26.Ryan, M. P., & Pembroke, J. T. (2018). Brevundimonas spp.: Emerging global opportunistic pathogens. Virulence, 9(1), 480–493.
27.Saranraj, P., & Stella, D. (2021). Marine microbiology: Isolation and characterization techniques. Journal of Marine Science Research, 8(2), 45–56.
28.Singha, T. K. (2020). Microbial exopolysaccharides: Biosynthesis, purification and applications. International Journal of Biological Macromolecules, 150, 1097–1110.
29.Singh, R., & Gupta, N. (2021). Physiological and biochemical diversity of marine bacteria. Journal of Applied Biology & Biotechnology, 9(4), 55–63.
30.Subramanian, G., Kumar, S., & Ravi, V. (2020). Isolation and cultivation of marine bacteria from seawater samples. Journal of Environmental Microbiology, 22(1), 35–44.
31.Suresh, P., Kannan, R., & Balakrishnan, S. (2021). Role of exopolysaccharides in marine bacterial adaptation and biofilm formation. Marine Biotechnology, 23(5), 789–801.
32.Sutherland, I. W. (2001a). Biofilm exopolysaccharides: A strong and sticky framework. Microbiology, 147(1), 3–9.
33.Sutherland, I. W. (2001b). The biofilm matrix – An immobilized but dynamic microbial environment. Trends in Microbiology, 9(5), 222–227.
34.Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120
35.Wang, J., Zhao, X., Tian, Z., & Yang, Y. (2020). Marine microbial exopolysaccharides: Sources, structures and bioactivities. Marine Drugs, 18(6), 321.
36.Wang, L., Zhang, H., & Li, X. (2021). Structural characterization of bacterial exopolysaccharides by FTIR and spectroscopic techniques. Carbohydrate Polymers, 251, 117090.
37.Wang, Y., Liu, J., Chen, H., & Zhang, Q. (2022). Antidiabetic activity of marine bacterial exopolysaccharides through carbohydrate-hydrolyzing enzyme inhibition. International Journal of Biological Macromolecules, 209, 1880–1890.
38.Zhang, X., Liu, H., Wang, Q., & Chen, Y. (2022). Evaluation of α-amylase inhibitory activity of natural polysaccharides. Food Chemistry, 367, 130742.
39.Zhao, C., Wu, S., Wang, Q., & Li, Y. (2022). Characterization and biological activities of marine bacterial exopolysaccharides. International Journal of Biological Macromolecules, 203, 552–563.