PLANT SCIENCE TODAY
Vol 12(sp3): 01–08
https://doi.org/10.14719/pst.8712

eISSN 2348-1900

RESEARCH ARTICLE

From microbes to mighty crops: Enhancing soil health and drought tolerance with protein-based EPS biostimulant

K Kiruba, K Kumutha*, N Niranjani & P Vijaya Priya

Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai 625 104, Tamil Nadu, India

*Correspondence email - kkumutha@tnau.ac.in

Received: 06 April 2025; Accepted: 04 September 2025; Available online: Version 1.0: 17 December 2025

Cite this article: Kiruba K, Kumutha K, Niranjani N, Vijaya PP. From microbes to mighty crops: Enhancing soil health and drought tolerance with protein -based EPS biostimulant. Plant Science Today. 2025;12(sp3):01–08. https://doi.org/10.14719/pst.8712

Abstract

The objective of this study is to create a biostimulant formulation that will improve soil health and drought tolerance by utilizing plant-derived protein hydrolysates (PHs) and bacterial exopolysaccharides (EPS). EPS were selected for their potential to enhance soil structure and water retention, while protein hydrolysates were included for their plant growth-promoting properties. This research focuses on EPS extraction from microbial sources and protein hydrolysate preparation, culminating in a combined biostimulant product tested for agricultural efficacy. *Bacillus altitudinis* A1 produced the highest EPS of 70.0 g L^{-1} after 14 days of incubation in Sabouraud Agar Base (SAB) medium among the four cultures evaluated. Since soymeal was identified earlier as having higher protein content, extraction was conducted and the yield was determined to be 43.8 % crude protein, which resulted in 8.6 mg g^{-1} total amino acids upon hydrolysis with papain enzyme. Soil incubation studies conducted with two types of soils viz. sandy loam and clay soils, demonstrated the potential of the EPS based biostimulant in improving physical properties such as pore space, bulk density and water holding capacity. Further this study investigated the effect of the biostimulant on seedling vigor of green gram (*Vigna radiata*) under varying osmotic stress levels ranging from -3 bar to -6 bar induced by polyethylene glycol (PEG). Based on the results, EPS biostimulant performed best in terms of the seedlings' growth and vigor, indicating that it should be used in water-limiting settings to lessen the stress on plant performance. An increase in early vigour of the plants will be helpful for the plants to perform better in the field. The GC-MS analysis of extracted bacterial EPS showed the presence of different types of decanoic acids, indicating potential for biocontrol. The FTIR (Fourier Transform Infrared Spectroscopy) spectrum of EPS revealed the presence of C-H, C=O and O-H stretches. This study provides valuable insights into the potential of EPS-based biostimulants as a sustainable solution to enhance drought resilience in crops, with future research needed to explore long-term effects and to optimize for large-scale applications.

Keywords: bacterial EPS; biostimulant; FTIR; GC-MS; protein hydrolysate; soil properties; vigour index

Introduction

Drought disrupts crop yields, degrades soil quality and exacerbates food insecurity. With the global population projected to exceed 9.7 billion by 2050, sustainable solutions are essential to ensure food security (1). Drought negatively affects soil salinity and organic matter, thereby reducing agricultural productivity. Effective strategies are required to mitigate these effects while improving crop yields. Drought, a major climate hazard, further impacts soil quality, increases salinity and reduces organic matter, thereby exacerbating food insecurity (2). In light of these challenges, there is an urgent need for sustainable agricultural practices that can mitigate drought stress while improving crop yields.

Microbial exopolysaccharides (EPS) are high-molecular-weight polymers that offer promising solutions by enhancing soil structure, nutrient retention and water availability. EPS are produced by bacteria, fungi and other microorganisms, which improve soil aggregation, contribute significantly to soil structure, nutrient retention, water availability, facilitate root growth and support plants in coping with abiotic stresses such as salinity and drought (3, 4). Acting as natural adhesives, EPS bind soil particles into aggregates, enhancing stability and creating pore spaces for root growth and water infiltration (5). The hygroscopic properties of EPS enable microorganisms and plants to withstand drought by maintaining moisture in the rhizosphere.

Protein hydrolysates are gaining attention as a crucial component in biostimulant formulations due to their multifaceted benefits for plant growth and resilience. Derived through enzymatic or chemical hydrolysis of proteins, these hydrolysates are rich in bioavailable peptides and amino acids. They improve nitrogen assimilation in plants, which in turn enhances photosynthetic activity and overall metabolic processes (6). Additionally, they play a role in mitigating oxidative stress in plants by promoting the accumulation of antioxidants and compatible solutes, under abiotic stress conditions such as drought and salinity. Studies have shown that amino acids derived from protein hydrolysates can directly influence plant root architecture, leading to enhanced water and nutrient uptake (7). Furthermore, the integration of protein hydrolysates into biostimulants has been linked to improved seed germination, crop establishment and resistance to environmental stresses (8).

This study focuses on leveraging the synergistic potential of EPS and protein hydrolysates to develop a robust biostimulant capable of enhancing soil health and crop productivity in drought-prone environments to supports sustainable agriculture.

Materials and Methods

Selection of EPS producing bacterial cultures

Based on our previous research, EPS-producing bacterial cultures isolated from drought-affected soils across Tamil Nadu, India, were obtained. These cultures include *Bacillus altitudinis* A1, *Bacillus* sp. *(firmicutes) 183*, *Pseudomonas azotoformans* BJ5 III and *Pseudomonas* sp. AP III. Among these, *B. altitudinis* A1 exhibited the highest EPS production and contained high organic carbon and the presence of the dextransucrase (*dex*) gene led to its selection for the present study (Table 1).

Table 1. Exopolysaccharides production by the selected bacterial cultures

S. no.	Bacterial cultures	Quantity of EPS produced ($g L^{-1}$) (wet weight)
1	*Bacillus altitudinis* A1	70 ± 0.21^{a}
2	*Bacillus* sp.(*firmicutes)* 183	64 ± 0.19^{b}
3	*Pseudomonas azotoformans* BJ5 III	57 ± 0.17^{c}
4	*Pseudomonas* sp. AP III	55 ± 1.01^{c}

Extraction of EPS using solvent extraction method

The selected bacterial cultures were grown (14 days) in basal salt broth and the EPS were extracted from the cultures by centrifugation at 10000 rpm for 20 min. The supernatant was collected and thrice the volume of cold acetone was added. In addition, the solution had been refrigerated overnight for precipitation. Following the incubation period, the contents were centrifuged at 6000 rpm for 10 min. The supernatant solution was discarded and the precipitated EPS was collected and the wet weight was taken (9).

Gas Chromatography-mass spectrometry (GC-MS) analysis of crude basal EPS

The EPS from *B. altitudinis* A1 was hydrolyzed into monomeric units and converted into their alditol forms. A crude sample was mixed with 1.25 mL of 72 % sulphuric acid and incubated at 30 °C for 60 min. Following this, the mixture was diluted with 13.5 mL of distilled water and heated in a boiling water bath for 4 hr. After cooling, 3.1 mL of 32 % NaOH (w/v) was added. At the end of the hydrolysis, 0.2 mL of the sample was taken and 2 mL of 2 % sodium borohydride in dimethyl sulfoxide was added. The mixture was shaken at 40 °C for 90 min, after which 0.2 mL of glacial acetic acid was added to neutralize any excess sodium borohydride.

Once cooled, 4 mL of acetic anhydride and 0.4 mL of 1-methylimidazole were introduced. The mixture was incubated at room temperature for 10 min and then 20 mL of distilled water was added to decompose any remaining acetic anhydride. After cooling, 8 mL of dichloromethane was added and solution was shaken vigorously for the extraction of total alditol acetates. The upper layer was discarded and the lower phase was washed thrice with 20 mL of distilled water. The dichloromethane was then evaporated under vacuum at 40 °C and the remaining alditol acetate residues were dissolved in 1 mL of dichloromethane and stored at -20 °C. This was used for GC-MS analysis (10).

Fourier transform infrared spectroscopy (FTIR) analysis of the bacterial EPS

FTIR spectra for EPS of *B. altitudinus* A1 was acquired using a JASCO FTIR – 4600 type A spectrometer (JASCO, Japan), equipped with an attenuated total reflectance (ATR) sensor. The spectral data, covering the range of 400 to 4000 cm^{-1} were collected by averaging with a scanning speed of 2 mm sec^{-1} and a resolution of 4 cm^{-1}. The pellet of EPS was characterized for their molecular structure using FTIR spectroscopy (IRPrestige-21, Shimadzu). The EPS was mixed with potassium bromide (KBr) and examined the structure of EPS using FTIR. Then, the spectra were recorded using IR software.

Selection of plant-based protein sources for biostimulant preparation

To identify cost-effective nitrogen-rich plant sources for deriving proteins, six or five plant-based agricultural byproducts namely, sesame seed cake, soyameal, groundnut seed cake, cotton seed cake and neem seed cake were analyzed for protein content and suitability for extraction (data not shown). Previous research indicated that soyameal exhibited the highest crude protein content among the tested sources. Consequently, commercially available soya meal was selected as the primary protein source for further extraction and application in EPS biostimulant development.

Extraction of protein from soya meal

Crude protein from soya meal was isolated using alkaline extraction and isoelectric precipitation method (11).To prepare the protein isolate, soya meal was ground into fine flour after cleaning and mixed with water at 1:10 ratio (w/v). The mixture was homogenized using a magnetic stirrer and the pH of the suspension was adjusted to 10 with 1 N NaOH and stirred again for 60 min at room temperature. After stirring, the alkaline suspension was allowed to settle at 4 °C for 4 hr and then centrifuged at 5200 rpm for 30 min. The resulting supernatant contains the protein solution was collected and the pH was adjusted to 4.5 using 50 % citric acid to promote isoelectric precipitation of the proteins. The precipitated proteins were allowed to settle for 30 min, after which the supernatant was removed. The protein pellets were collected and subjected to a second centrifugation at 5200 rpm for 30 min. After this, the pellets were neutralized to pH 7.0 and then stored at 4 °C.

Enzymatic hydrolysis of soya protein

The following steps were performed to hydrolyse the protein samples. Initially, 100 g of the protein extracted by isoelectric precipitation was mixed with equal volume of distilled water and heated for 2 min using a hotplate stirrer. Subsequently, 5 % papain enzyme was added to the mixture and the pH was adjusted to 7.0. The sample was then hydrolyzed in a water bath at 55 °C for 6 hr and inactivated by heating at 90 °C for 20 min, followed by centrifuged at -4 °C at 5000 rpm. The supernatant was collected and stored in the refrigerator. The supernatant served as hydrolysate for further analysis.

Development of EPS biostimulant for crop boosting and soil conditioning

An EPS-based biostimulant was formulated by combining the specific components. EPS extracted from the bacterial culture *B. altitudinis* A1 was mixed with protein hydrolysate in a specific proportion along with sterilized glycerol. The resulting mixture was used as biostimulant for evaluation. The ratio of the components in biostimulants are; EPS-25.0 g; protein hydrolysate 100 mL; glycerol 4.0 mL.

Influence of the EPS biostimulant on seeding vigour of green gram under drought stress

The seedling vigour test was performed using the roll towel method, with polyethylene glycol (PEG 6000) solution at varying osmotic pressure (-0.3 bar, -0.5 bar and -0.6 bar) to simulate drought stress. Green gram seeds were surface sterilized with 0.05 % sodium hypochlorite and 70 % ethanol. The surface sterilized seeds were treated with the EPS biostimulant (2.5 % concentration) for 30 min, while control seeds were soaked in water. Treated and untreated seeds were placed on germination towels, which were subsequently incubated in PEG 6000 solutions at -0.3, -0.5 and -0.6 bar concentrations and incubated near the light source.

The experiment consisted of eight treatments. Treatment T_1 included the biostimulant alone. Treatment T_2 consisted of the biostimulant combined with PEG adjusted to -0.3 bar, while T_3 included the biostimulant with PEG at –0.5 bar and T_4 consisted of the biostimulant with PEG at -0.6 bar. Treatment T_5 contained no biostimulant. Treatment T_6 included no biostimulant but was supplemented with PEG at -0.3 bar, whereas T_7 included no biostimulant with PEG at -0.5 bar and T_8 consisted of no biostimulant with PEG at -0.6 bar.

On day 7, germination percentage, root length, shoot length and plant height were measured. Seedling vigor was calculated using the formula:

$$\text{Vigor index} = \text{Total plant height} \times \text{Germination percentage}$$

Influence of EPS based biostimulant on physical properties of soil

A soil incubation study was conducted with and without the addition of EPS biostimulant and incubated for 15 days. Two different soil samples such as sandy loam and clay were used for the treatment. The soil samples were inoculated with the prepared biostimulant to achieve the soil moisture content of 40 %. Each treatment was replicated five times and a CRD was followed. The different soil parameters such as bulk density, pore space and water holding capacity were measured after incubation using the formula:

$$\text{Bulk density} = (b - a) / v$$

$$\text{Water holding capacity (\%)} = \frac{(c-a)-(b-a)}{(b-a)} \times 100$$

$$\text{Porosity} = \frac{(d-e)}{v} \times 100$$

Where,

a - weight of the keen box with filter paper; b- weight of the keen box with filter paper + soils treated with EPS cultures; c- weight of the keen box with wet saturated soil; d- weight of the keen box with wet residual soil, after removing the wet expanded soil; e- weight of the box with residual soil after drying the soil at 105 °C; v - The box's internal volume.

Results and Discussion

Extraction of EPS from identified cultures

The selected four bacterial cultures were inoculated in SAB broth and cultivated for fourteen days to evaluate EPS production. EPS was subsequently extracted and quantified. Among the four cultures, *B. altitudinis* A1 produced the highest EPS yield (70 ± 0.2 g L^{-1}) followed by *Bacillus* sp. (Firmicutes) (64 ± 0.1 g L^{-1}). The EPS production by *Pseudomonas azotoformans* BJ5 III (57 ± 0.2 g L^{-1}) and *Pseudomonas* sp. AP III (55 ± 0.2 g L^{-1}) was comparable but lower than that of the *Bacillus* strains. Out of all 99 isolates obtained from different natural ecosystems, maximum EPS (44.6 ± 0.63 mg/50 mL) was produced by *Azotobacter chroococcum* at 24 hr, corresponding to the sub-stationary growth phase ($7 \times 10^8 \pm 0.29$ CFU/mL). While screening the cultures for maximum production of EPS, it was estimated that EPS yield of 3 mg mL^{-1} was produced by the culture *B. velezensis* KY471306 and depicted in Table 1 and Fig. 1 (12).

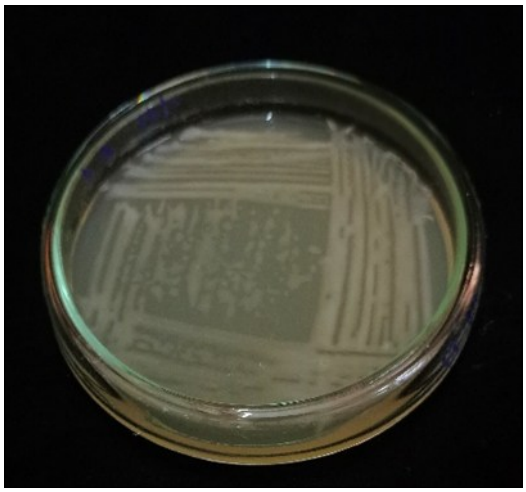
GC-MS analysis of crude basal EPS

The GC-MS analysis of the bacterial EPS revealed the presence of a variety of bioactive compounds, each contributing to potential functional roles in microbial and plant systems. By comparing the mass spectra of the constituents with the NIST library, the EPS constituents were characterized and identified as octadecenoic acid and 9,12-octadecadienoic acid with significant areas of 39.096 % and 11.09 %, respectively, indicating their abundance. Both 9,12-octadecadienoic acid methyl ester and 6-octadecenoic acid methyl ester are known components in microbial EPS that play roles in plant defense and stress responses, acting as elicitors of signalling pathways like jasmonic acid and ethylene. These compounds are well-known for their antioxidant, anti-inflammatory and antimicrobial activities, underscoring their potential applications in plant defense and health. Additionally, compounds like 13-Hexyloxacyclotridec-10-en-2-one and 6-octadecenoic acid were detected, which are known for antimicrobial properties and roles in biotic stress responses. The presence of 10-undecenoic acid, methyl ester, associated with seed germination and stress tolerance highlights the potential for agricultural applications.

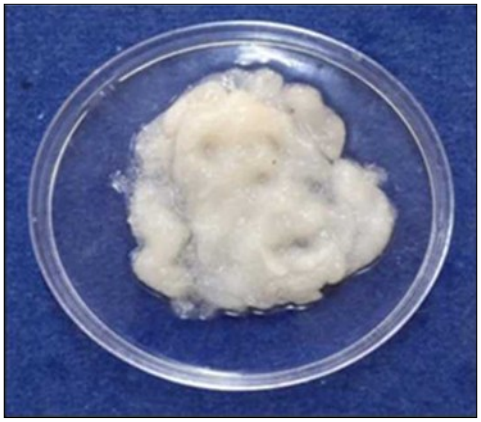
These results are in corroboration with the findings of the GC -MS analysis of bacterial secondary metabolites, which revealed nine compounds with different retention times and peak areas, namely: 2,6,10-trimethyl-12-oxatricyclo[7.3.1.0(1,6)]tridec-2-ene; nicotinic acid; 1,6-dihydro-4-hydroxy-6-oxo-2-propyl ethyl ester; benzoic acid; 2-amino-6-chloro-methyl ester; heptadecanoic acid, 9-methyl-, methyl ester; 2-(2,6,6-trimethylcyclohex-1-enyl)cyclopropane carboxylic acid, methyl ester; 9-octadecenoic acid (E)-; and pentadecanoic acid, 13-methyl-, methyl ester. The presence of this wide variety of compounds in bacterial EPS supports their relevance for plant growth, stress mitigation and plant defense (Table 2; Fig. 2).

Fourier transform infrared spectroscopy (FTIR) analysis of the bacterial EPS

FTIR spectroscopy of the EPS produced by *B. altitudinis* A1 revealed distinct functional groups characteristic of microbial polysaccharides and bioactive biomolecules. A broad and strong absorption peak at 3432.67 cm^{-1} corresponded to the O-H stretching vibrations of hydroxyl groups, typically present in polysaccharides and other hydrophilic compounds. This peak is indicative of the hydrophilic nature of exopolysaccharides, which reflects their high water-retention capacity and the presence of high molecular weight sugars in EPS (14). A prominent C–H stretching peak at 2926.45 cm^{-1} suggested the presence of aliphatic hydrocarbon chains. Additionally, a distinct absorption band at 1641.13 cm^{-1} was attributed to C=O stretching vibrations, indicating the presence of carboxylic acid groups in the EPS matrix.



Bacillus altitudinis A1 culture in SAB medium.



EPS extracted from *Bacillus altitudinis* A1 culture.

Fig. 1. *Bacillus altitudinis* A1 culture and EPS extracted from the culture.

Table 2. GC-MS analysis of crude basal EPS from *Bacillus altitudinis* A1

S. no.	Name of the compound	Retention time	Area	Area (%)	Height	Height (%)	Uses
1.	Octadecenoic acid	39.096	3024897	39.88	871452	41.86	Self-defence in plants
2.	13-hexyloxacyclotridec–10–en-2-one	33.886	829299	10.93	245747	11.80	Used in plants as an alternative to agrochemicals
3.	9,12–octadecadienoic acid	35.886	841448	11.09	76840	3.69	Antioxidant, anti-inflammatory and antimicrobial activity
4.	6-octadecenoic acid	34.745	450989	5.95	116688	5.60	A precursor to jasmonic acid, which helps plants respond to biotic stresses
5.	10-Undecenoic acid, methyl ester	16.188	164697	2.17	37542	1.80	Root elongation and increases root biomass, improve seed germination rates and accelerate seedling emergence, helps plants cope with water stress by regulating stomatal closure and improving water use efficiency, increases plant resistance to high temperatures, potential as a biopesticide, influences plant hormone signalling pathways, particularly those related to jasmonic acid and ethylene
6.	Heptadecanoic acid,16-methyl	35.372	110231	1.45	45624	2.19	Antioxidant
7.	11,14-eicosadienoic acid	36.831	115356	1.52	30280	1.45	Antioxidant activity
8.	Ricinoleic acid	40.139	133203	1.76	40152	1.93	Control pests, provide essential nutrients to plants
9.	1- hexadecene	27.029	105360	1.39	43194	2.07	antioxidant properties

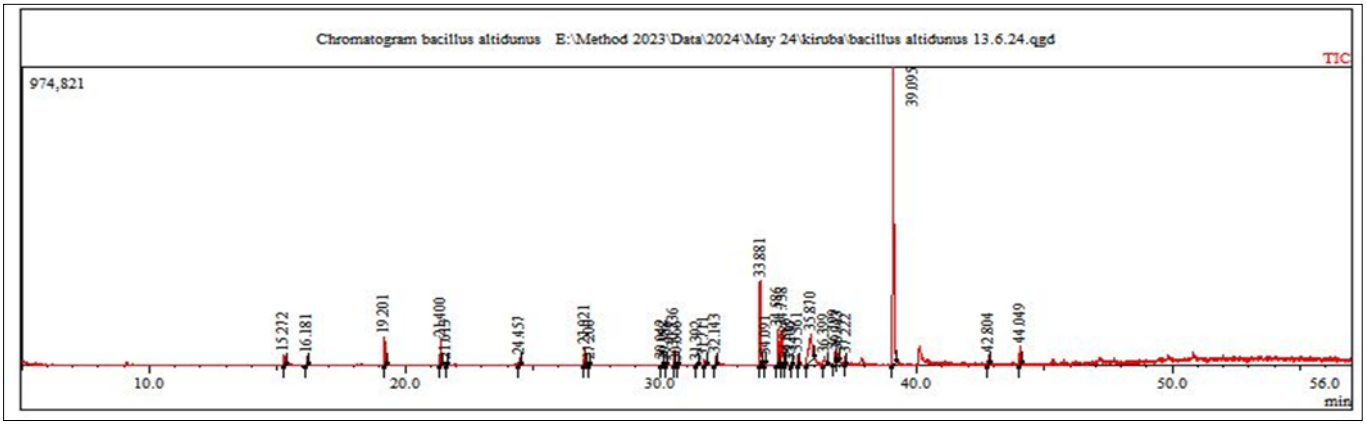


Fig. 2. Chromatogram of GC-MS analysis of crude basal EPS of *Bacillus altitudinis* A1.

These functional groups also play a role in enhancing the affinity for oppositely charged molecules such as heavy metals (15). The FTIR spectrum also showed a peak at 1072.23 cm^{-1}, which is characteristic of 1α-glucosidic linkages, a defining feature of microbial extracellular polysaccharides (Table 3; Fig. 3). This reflects the presence of sugar monomers such as glucose, mannose and galactose, commonly found in bacterial EPS. Notably, no absorption band corresponding to C-I stretching vibrations, typically associated with halogenated compounds, was observed. This suggests the absence of halogenated biomolecules in the EPS under the tested conditions. These findings are in agreement with earlier reports on the FT-IR profiles of EPS from *Bacillus* and *Stenotrophomonas* species, which demonstrated the presence of amide, hydroxyl and carboxyl groups in their respective EPS structures (16).

Table 3. FTIR wave number of EPS extracted from *Bacillus altitudinis* A1

Functional group	Wave number (cm^{-1})
	***Bacillus altitudinis* A1**
O-H stretch	3432.67
C-H stretch	2926.45
C=O stretch	1641.13
Amide I	-
Amide II	-
C-H stretch	-
1α-glucosidic linkage	1072.23
P-O-C stretch	-
C-I stretch	-

Extraction and estimation of protein from soya meal and hydrolysate preparation

Total crude protein content of soya meal was estimated to be 43.75 %. All the extracted plant proteins were subjected to hydrolysis using 5 % papain enzyme. Degree of hydrolysis recorded was 95.0 %, which showed that most of the proteins are degraded into amino acids and short chain peptides. Hydrolysis using papain at the concentration of 5 % at 55 °C for 6 hr yielded the soluble proteins content of 1.2 % and a total amino acid content of 8.6 mg/g with 95 % degree of hydrolysis. The results are in accordance with the findings, papain enzymes for the hydrolysis of protein and reported that the hydrolysis process with 3 % papain for three hours produced the highest soluble protein concentration of 6.56 ± 0.815 % (17).

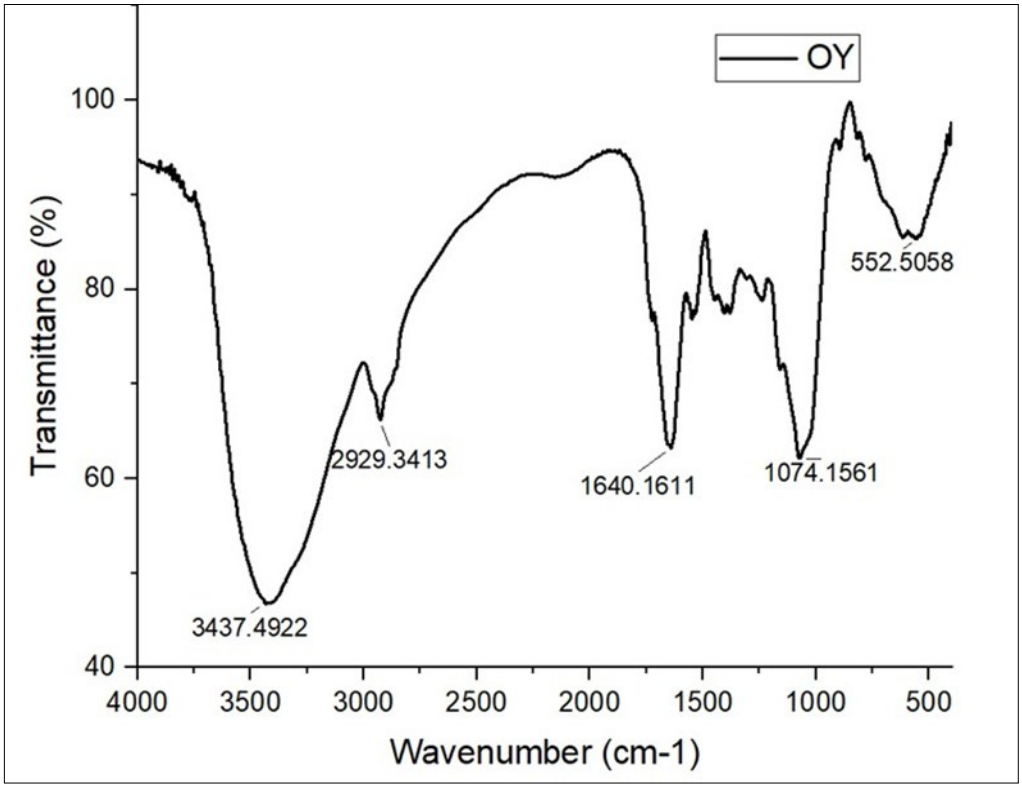


Fig. 3. Representation of absorption peaks in FTIR spectra in EPS from *Bacillus altitudinis* A1.

Preparation and evaluation of EPS based biostimulant in green gram under PEG induced stress condition

The protein hydrolysate contains amino acids and peptides and the extracted EPS was used in proportion to prepare the biostimulant. Biostimulant was used to treat the seeds and the response was studied in green gram under PEG induced stress.

The application of EPS-based biostimulants significantly enhanced seedling vigor in green gram under drought stress, as evidenced by various growth parameters. *In vitro* tests demonstrated that biostimulant-treated seedlings exhibited a higher vigour index, with the highest value recorded was 4763 for the biostimulant alone, followed by 4550 for the biostimulant combined with PEG at -3 bar and 2988 and 1343 for the treatments with PEG at -5 bar and -6 bar osmotic pressure respectively. The untreated seedlings and those with only PEG treatment showed much lower vigour indices, emphasizing the positive impact of biostimulant application, especially under stress conditions.

The biostimulant-treated seedlings also exhibited a higher germination percentage of 91.6 %, compared to 79 % in untreated seedlings exposed to various osmotic stress levels. Biostimulant application enhanced the rate of germination and recorded 87.5 % at -3 bar, 83.0 % at -5 bar and 79 % at -6 bar osmotic pressure, significantly higher than the untreated plants. These results indicated that the biostimulant application enhanced the germination, growth of green gram and doubled the vigour of seedlings under different osmotic potential, showcasing its potential to withstand the moisture stress.

The results are line with earlier studies with the corn and tomato treated with vegetable derived protein hydrolysate, significantly increased the rate of coleoptile elongation compared to untreated control and also didn't show any significant differences among the different concentrations used as well as the auxin treatment in corn. The shoot and root growth were significantly influenced by the protein hydrolysate treatment in tomato from 21-35 % over untreated control. The same bio-stimulant has also significantly influenced the shoot length in gibberellin deficient pea plants (18). Chicken feather meal and fish meal hydrolysates have also promoted the root as well as shoot growth of corn at early stages of growth and also positively influenced the germination and growth of sugarcane setts demonstrated the hormonal action of the protein hydrolysate (19). Similarly, the positive influence of EPS producing bacterial strains on growth of plants under stress has been reported earlier.

EPS are natural polysaccharides from microorganisms play crucial roles in protecting plants under water stress by forming hydrophilic biofilms on root surfaces, reducing water loss (20). Additionally, EPS-based biostimulants aid in water retention, nutrient supply and plant-microbe interactions, thereby promoting plant growth under adverse conditions (21). The combined use of bacterial EPS producer with protein hydrolysates in a formulation further enhances seedling growth by improving water and nutrient-use efficiency, boosting tolerance to abiotic stresses and improving crop yield and quality (Table 4; Fig. 4) (22, 23).

Incubation study for evaluating EPS based biostimulant on soil physical properties

The soil incubation study involving the EPS-based biostimulant revealed its significant impact on physical properties. Analysis of the physical properties of the soils, including porosity, bulk density and water holding capacity, demonstrated the beneficial effects of EPS. After 10 days of incubation, the application of the EPS biostimulant resulted increased pore space with sandy loam soil showing a significant improvement of 41.94 %, reaching 87.66 % pore space, while the control sandy loam soil registered only 45.72 %. In clay soil, although the increase in pore space was minimal, it still demonstrated some enhancement due to the biostimulant. The bulk density of the soil was reduced as a result of EPS application, with sandy loam soil showing a reduction from 1.48 g/cc to 1.24 g/cc, reflecting a 16.2 % decrease. Similarly, in clay soil, the bulk density was reduced from 1.37 g/cc to 1.30 g/cc, indicating a 5.1 % decrease. Moreover, the water holding capacity of both soils was increased by 18 % with the application of the biostimulant (Table 5).

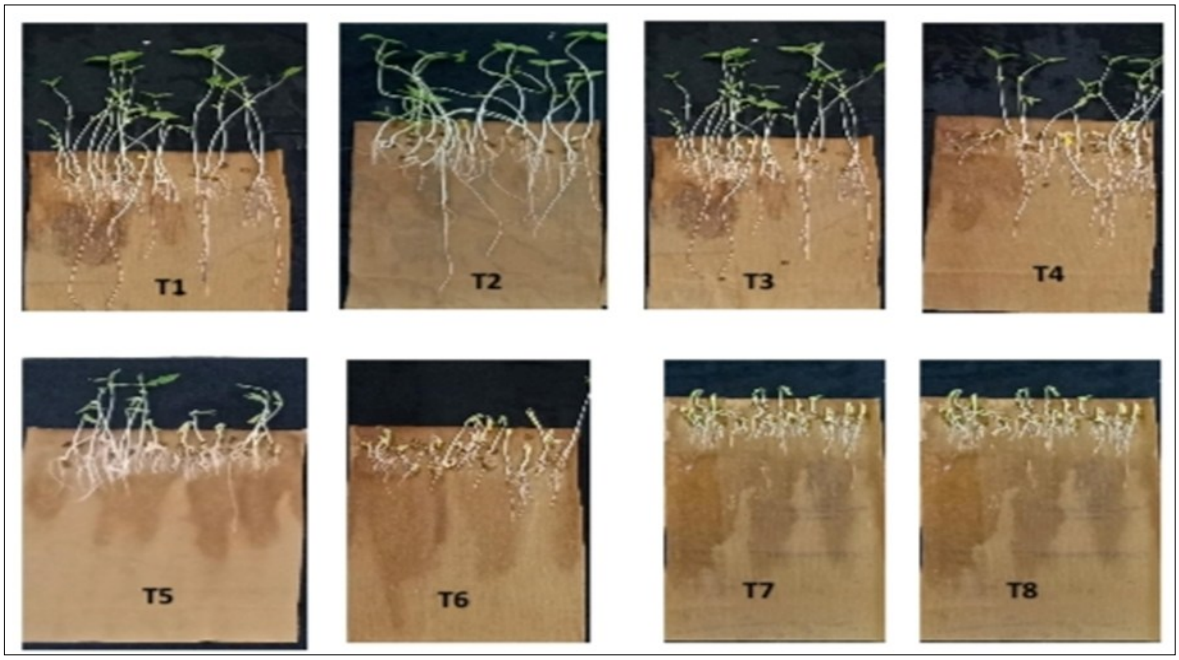


Fig. 4. Effect of EPS based biostimulant on seedling vigour of green gram.

T1-biostimulant+, T2-biostimulant+ (+) PEG – (0.3 bar), T3-biostimulant+ (+) PEG– (0.5 bar), T4-biostimulant+ (+) PEG- (0.6 bar), T5-biostimulant -, T6-biostimulant (+) PEG -(0.3bar), T7-biostimulant (+) PEG-(0.5bar), T8-biostimulant (+) PEG-(0.6 bar).

Table 4. Influence of EPS based biostimulant on the physical properties of different type of soil

Treatment	Treatment details	Root length (cm)	Shoot length (cm)	Plant height (cm)	Germination percentage (%)	Vigour index
T1	Biostimulant^{+}	29.0 ± 0.205^{a}	23.0 ± 0.18^{a}	52.0 ± 0.13^{a}	91.6± 0.37^{a}	4763.2 ±0.23^{a}
T2	Biostimulant^{+} (+) PEG- (0.3bar)	25.0 ± 0.197^{b}	27.0 ± 0.19^{b}	52.0 ± 0.13^{a}	87.5 ± 1.30^{b}	4550.0 ± 0.26^{b}
T3	Biostimulant^{+} (+) PEG- (0.5 bar)	19.5 ± 0.196^{c}	16.5 ± 0.09^{c}	36.0 ± 0.08^{b}	83.0 ± 1.10^{b}	2988.0 ± 0.19^{c}
T4	Biostimulant^{+} (+) PEG - (0.6 bar)	10.0 ± 0.189^{d}	7.0 ± 0.13^{d}	17.0 ± 0.90^{d}	79.0 ± 0.10^{c}	1343.0 ± 0.11^{cd}
T5	Biostimulant^{-}	20.0 ± 0.191^{c}	18.5 ± 0.11^{c}	38.5 ± 0.18^{c}	79.0 ± 1.24^{c}	3041.5 ±0.17^{c}
T6	Biostimulant^{-} (+) PEG - (0.3 bar)	14.0 ± 0.098^{d}	17 ± 0.90^{c}	31.0 ± 0.18^{c}	70.8 ± 1.66^{d}	2194.8 ±0.18^{c}
T7	Biostimulant^{-} (+) PEG- (0.5 bar)	5.5 ± 0.016^{e}	9 ± 0.11^{d}	14.5 ± 0.09^{d}	37.5 ± 0.11^{e}	543.7 ±0.10^{d}
T8	Biostimulant^{-} (+) PEG-(0.6 bar)	4.5 ± 0.069^{e}	3 ± 0.04^{e}	7.5 ± 0.13^{c}	20.8 ± 0.11^{f}	156.0 ± 0.11^{e}

These results are in line with previous studies, which demonstrated that the EPS treatment significantly enhanced soil physical properties, including improved porosity and reduced bulk density, contributing to better water retention and soil aggregation (24). The improved porosity and lower bulk density in EPS-treated soils, enhanced their ability to hold more water (25). Improving soil aggregation enhances water permeability and retention, which is crucial for plant survival during drought (26, 27). Collectively, these results emphasize the role of EPS-based biostimulants in improving soil structure, enhancing water retention and fostering healthier, more productive soils.

Conclusion

This study demonstrated the potential for exploiting EPS produced by bacterial strains as a promising source of biostimulant. Application of protein-based EPS biostimulant result in a significant enhancement in seedling vigor, particularly under drought stress conditions, as evidenced by improved germination rates, higher vigour indices and increased drought tolerance in green gram seedlings. The EPS-based biostimulant also improved the physical properties such as pore space and water holding capacity of the soils evaluated. The positive impact on water retention and enhanced plant growth under osmotic stress showcases its potential as an effective tool for improving plant resilience to abiotic stresses.

The amino acid content of protein hydrolysate serves as nitrogen sources for the plant to improve the growth of the seedlings at early stages of crop growth. Further, the amino acids may also contribute to enhanced plant performance under stress condition. In order to improve plant growth, yield and soil health-particularly in drought-prone areas-EPS mixed with protein hydrolysates will be the biostimulant of the future. It will also be an efficient part of sustainable agricultural systems. Particularly in areas that are prone to drought, this formulation in conjunction with NPK inoculants offers a sustainable solution to agricultural problems. This formulation, when combined with NPK inoculants, represents a sustainable approach to agricultural challenges, especially in drought-prone regions.

Acknowledgements

The authors acknowledge the support of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu for organizing the technical writing workshops to facilitate the students for writing this review article.

Authors' contributions

KK[1] developed the overall framework of this review article, providing the core structure that guided its organization and flow. KK[2] contributed to idea conceptualization, manuscript correction and editing, ensuring coherence and clarity throughout the document. NN and PVP reviewed the manuscript and supported the refinement process by assisting with editing and offering critical feedback to strengthen the final version. All authors read and approved the final manuscript [KK[1] stands for K Kiruba and KK[2] for K Kumutha].

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

Ethical issues: None

References

1. Capps R, Castaneda RM, Chaudry A, Santos R. Paying the price: the impact of immigration raids on America's children. Washington (DC): National Council of La Raza. 2007.
2. Ahmed T, Noman M, Manzoor N, Shahid M, Abdullah M, Ali L, et al. Nanoparticle-based amelioration of drought stress and cadmium toxicity in rice via triggering the stress-responsive genetic mechanisms and nutrient acquisition. Ecotoxicol Environ Saf. 2021;209:111829. https://doi.org/10.1016/j.ecoenv.2020.111829
3. Bibi A, Xiong Y, Rajoka MS, Mehwish HM, Radicetti E, Umair M, et al. Recent advances in the production of exopolysaccharide (EPS) from *Lactobacillus* spp. and its application in the food industry: a review. Sustainability. 2021;13(22):12429. https://doi.org/10.3390/su132212429
4. Paul S, Parvez SS, Goswami A, Banik A. Exopolysaccharides from

Table 5. Influence of EPS based biostimulant on the physical properties of different type of soils

Treatments	Treatment details	Pore space %	Bulk density (g/cc)	% decrease of bulk density over control	Water holding capacity (%)
T1	Biostimulant^{-} + S1	45.72 ± 1.13^{c}	1.73 ± 0.01^{a}	-	24.88 ± 0.30^{a}
T2	Biostimulant^{+} + S1	87.66 ± 1.53^{a}	1.45 ± 0.04^{b}	16.5	33.22 ± 0.28^{b}
T3	Biostimulant^{-} + S2	71.54 ± 0.37^{b}	1.61 ± 0.10^{b}	-	23.68 ± 0.87^{a}
T4	Biostimulant^{+} +S2	76.12 ± 1.35^{b}	1.52 ± 0.05^{b}	5.1	48.11 ± 0.31^{b}

S1 - sandy loam soil; S2 - clay soil.

agriculturally important microorganisms: conferring soil nutrient status and plant health. Int J Biol Macromol. 2024;262:129954. https://doi.org/10.1016/j.ijbiomac.2024.129954

5. Alami Y, Champolivier L, Merrien A, Heulin T. The role of *Rhizobium* sp., a rhizobacterium that produces exopolysaccharide, in the aggregation of the rhizospheric soil of sunflower: Effects on plant growth and resistance to hydric constraints. Oleagineux Corps Gras Lipides. 2000;6:524–8.
6. Colla G, Nardi S, Cardarelli M, Ertani A, Lucini L, Canaguier R, et al. Protein hydrolysates as biostimulants in horticulture. Sci Hortic. 2015;196:28-38. https://doi.org/10.1016/j.scienta.2015.08.037
7. Bhat ZF, Kumar S, Bhat HF. Bioactive peptides of animal origin: A review. J Food Sci Technol. 2015;52(9):5377-92. https://doi.org/10.1007/s13197-015-1731-5
8. Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, et al. Humic and fulvic acids as biostimulants in horticulture. Sci Hortic. 2015;196:15-27. https://doi.org/10.1016/j.scienta.2015.09.013
9. Gupta A, Thakur IS. Optimization of wastewater contaminant removal along with EPS production by thermotolerant *Bacillus* sp. ISTVK1 isolated from heat-shocked sewage sludge. Bioresour Technol. 2016;213:21-30. https://doi.org/10.1016/j.biortech.2016.02.040
10. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci Technol. 2015;52 (2):1212-7. https://doi.org/10.1007/s13197-013-1105-9
11. Sivasankari R, Hemalatha G, Amutha S, Murugan M, Vanniarajan C, Uma Maheswari T. Extraction of protein concentrates from faba beans. Int J Chem Stud. 2019;7:3430-4.
12. Moghannem SA, Farag MM, Shehab AM, Azab MS. Exopolysaccharide production from *Bacillus velezensis* KY471306 using statistical experimental design. Braz J Microbiol. 2018;49 (3):452-62. https://doi.org/10.1016/j.bjm.2017.05.012
13. Anantha PS, Deventhiran M, Saravanan P, Anand D, Rajarajan S. Comparative GC-MS analysis of bacterial secondary metabolites of *Pseudomonas* species. Pharma Innov J. 2016;5(4B):84.
14. Negi R, Kaur T, Devi R, Kour D, Yadav AN. Nitrogen-fixing endophytic and mineral-solubilizing rhizobacteria as multifunctional microbial consortium for growth promotion of wheat and *Aegilops kotschyi*. Heliyon. 2022;8(12):e12345. https://doi.org/10.1016/j.heliyon.2022.e12579
15. Shultana R, Kee Zuan AT, Yusop MR, Saud HM. Characterization of salt-tolerant plant growth-promoting rhizobacteria and their effect on saline-affected rice. PLoS One. 2020;15(9):e0238537. https://doi.org/10.1371/journal.pone.0238537
16. Verhoef R, Schols HA, Blanco A, Siika-aho M, Rättö M, Buchert J, et al. Sugar composition and FT-IR analysis of exopolysaccharides from microbial isolates of paper mill slime deposits. Biotechnol Bioeng. 2005;91(1):91-105. https://doi.org/10.1002/bit.20494
17. Agustin V, Husni A, Putra MM. Antioxidant activity of protein hydrolysate from snakehead fish (*Channa striata*) viscera obtained by enzymatic process. IOP Conf Ser Earth Environ Sci. 2021;919:012046. https://doi.org/10.1088/1755-1315/919/1/012046
18. Colla G, Rouphael Y, Canaguier R, Svecova E, Cardarelli M. Biostimulant action of a plant-derived protein hydrolysate obtained through enzymatic hydrolysis. Front Plant Sci. 2014;5:448. https://doi.org/10.3389/fpls.2014.00448
19. Fitriyah F, Kresnawaty I, Santoso D. Protein hydrolysates enhance germination and early growth of maize and sugarcane. IOP Conf Ser Earth Environ Sci. 2022;974:012066. https://doi.org/10.1088/1755-1315/974/1/012066
20. Kaushal M. Portraying rhizobacterial mechanisms in drought tolerance: A way forward toward sustainable agriculture. In: Singh AK, Kumar A, Singh PK, editors. PGPR Amelioration in Sustainable Agriculture. Cambridge: Woodhead Publishing. 2019. p.195-216. https://doi.org/10.1016/B978-0-12-815879-1.00010-0
21. Sandhya VZ, ZA SK, Grover M, Reddy G, Venkateswarlu BS. Alleviation of drought stress in sunflower seedlings by exopolysaccharide-producing *Pseudomonas putida* GAP-P45. Biol Fertil Soils. 2009;46 (1):17-26. https://doi.org/10.1007/s00374-009-0401-z
22. Colla G, Hoagland L, Ruzzi M, Cardarelli M, Bonini P, Canaguier R, et al. Biostimulant action of protein hydrolysates: effects on plant physiology and microbiome. Front Plant Sci. 2017;8:2202. https://doi.org/10.3389/fpls.2017.02202
23. Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, et al. Humic and fulvic acids as biostimulants in horticulture. Sci Hortic. 2015;196:15-27. https://doi.org/10.1016/j.scienta.2015.09.013
24. Deng J, Orner EP, Chau JF, Anderson EM, Kadilak AL, Rubinstein RL, et al. Synergistic effects of soil microstructure and bacterial EPS on drying rate in emulated soil micromodels. Soil Biol Biochem. 2015;83:116-24. https://doi.org/10.1016/j.soilbio.2014.12.006
25. Obia A, Mulder J, Martinsen V, Cornelissen G, Borresen T. *In situ* effects of biochar on aggregation, water retention and porosity in tropical soils. Soil Tillage Res. 2016;155:35-44. https://doi.org/10.1016/j.still.2015.08.002
26. Naseem H, Ahsan M, Shahid MA, Khan N. Exopolysaccharide-producing rhizobacteria and their role in plant growth and drought tolerance. J Basic Microbiol. 2018;58(12):1009-22. https://doi.org/10.1002/jobm.201800309
27. Masondo NA, Kulkarni MG, Finnie JF, Van Staden J. Influence of biostimulant seed-priming on *Ceratotheca triloba* germination and seedling growth under stress conditions. Ecotoxicol Environ Saf. 2018;147:43-8. https://doi.org/10.1016/j.ecoenv.2017.08.017

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.