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Optimization and characterization of exopolysaccharide produced by *Weissella confusa* isolated from idli batter and study of its application in plant growth promotion

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Abstract: Exopolysaccharides (EPS) are extracellular biopolymers produced by bacteria, particularly under stress conditions. Lactic acid bacteria (LAB) produce high molecular weight, viscous polysaccharides during fermentation, which has wide range of applications in health, food, and environmental sectors. In the present study, *Weissella confusa* isolated from traditional idli batter was identified as the most potent EPS producer, among the 20 isolates obtained from five fermented samples. Optimum production of EPS was obtained in MRS medium (pH 6), containing 7% sucrose, peptone, and beef extract, on incubation at room temperature and static conditions for 24h. Yeast extract also supported significant EPS production. The agricultural potential of the EPS was evaluated through seed coating and EPS-supplemented irrigation experiments using wheat (*Triticum aestivum*) and moong (*Vigna radiata*). EPS treatment significantly enhanced seed germination, plant height, root length, and stress tolerance compared to untreated controls. Additionally, EPS producing LAB improved soil health by increasing microbial activity, water retention, and soil aggregation. These findings demonstrate that EPS produced by *W. confusa* has strong potential as a sustainable, eco-friendly biofertilizer for improving soil quality and crop productivity, particularly in drought-prone regions.

IndexTerms **– biofertilizer, exopolysaccharides, lactic acid bacteria, optimization, sustainable, *Weissella confusa***

I. INTRODUCTION

Agriculture forms the backbone of many developing economies. Presently, the growing global population has placed tremendous pressure on agricultural practices to ensure food security (Pawlak and Kołodziejczak, 2020). In order to meet these increasing agricultural demands, modern farming systems rely heavily on chemical fertilizers to enhance crop productivity (Bisht and Chauhan, 2020). While these fertilizers provide essential nutrients, their excessive and prolonged application has resulted in serious environmental and ecological consequences, including soil degradation, depletion of organic matter, and disruption of beneficial soil microbial communities (Howe et al., 2024). Altogether, these factors have significantly reduced soil fertility. Moreover, fertilizer runoff into water bodies causes eutrophication by supporting the growth of harmful algal blooms. As a result, the aquatic ecosystems are severely compromised (Lan et al., 2024). Chemical fertilizers are also a major source of nitrous oxide emissions, which are potent greenhouse gases, contributing to climate change (Park et al., 2012). These challenges highlight the urgent need for sustainable and eco-friendly alternatives to conventional chemical fertilizers.

Exopolysaccharides (EPS) synthesized by microorganisms show promising potential for sustainable agriculture and environmental management practices (Nguyen et al., 2024). Despite their prominent role in improving soil structure by enhancing water retention and promoting soil aggregation, they remain underexplored for their potential as biofertilizers (Xie et al., 2026; Mouro et al., 2024). The biological role of EPS in protecting cells against desiccation, osmotic stress, extreme pH, and toxic compounds, by facilitating biofilm formation, quorum sensing, and microbial interactions, leads to reduced nutrient leaching and fosters a balanced soil microbiome (Singh and Shivashankar, 2026). They also form protective biofilms around plant roots, facilitating nutrient uptake and improving tolerance to abiotic stresses such as drought and salinity. Thus, they contribute to soil stability, microbial diversity, and plant nutrient uptake, which altogether promote plant growth (Bhagat et al., 2021; Nguyen et al., 2024). In addition to EPS, LAB itself contributes to plant growth by improving nutrient availability, soil structure, root elongation, and stress tolerance, often through the production of plant growth-promoting compounds such as indole-3-acetic acid and organic acids (Jaffer et al., 2023). Thus, microbial EPS represent an eco-friendly and sustainable alternative to chemical fertilizers for plant growth promotion (Lamont et al., 2017).

Several microorganisms are known to produce EPS with diverse functional properties. For instance, *Xanthomonas campestris* produces xanthan gum, widely used as a thickening and stabilizing agent in food and pharmaceutical industries (Oliveira et al., 2025). *Pseudomonas putida* synthesizes EPS, which characteristically binds to heavy metals such as lead and copper, and is thus helpful in bioremediation (Balíková et al., 2022). *Azotobacter vinelandii* contributes to soil fertility through EPS-mediated soil aggregation and nitrogen fixation (Wang et al., 2024). Cyanobacteria such as *Synechocystis* and *Spirulina* produce EPS that enhance soil water retention, biofilm formation, and overall soil health (Gonçalves, 2021). However, compared to these microbial strains, the use of LAB and its by-products, secondary metabolites and structural components including EPS are generally favored in food and pharmaceutical industries because of their Generally Recognized As Safe (GRAS) status granted by the Food and Drug Administration (FDA), and absence of pathogenicity concerns (Hernández-Figueroa et al., 2025; Prete et al., 2021). EPS derived from LAB extensively find application in food texture enhancement, drug delivery systems, and gut health promotion due

to their desirable properties such as water retention, gelling, emulsification and biocompatibility (Juraskova et al., 2022). Many LAB EPS also exhibit prebiotic properties, and thus support beneficial gut microbiota, enhance immunity and contribute to gastrointestinal health (Lee et al., 2022).

Given the desirable functional properties associated with EPS-producing LAB strains and their established safety profile, their potential application in sustainable agriculture remains insufficiently explored. Therefore, the present study was undertaken to isolate EPS producing LAB from fermented food samples, optimize physiochemical conditions for maximizing EPS yield, characterize the produced EPS, and evaluate its application in plant growth promotion.

II. RESEARCH METHODOLOGY

2.1 Chemicals and reagents

Sugars, carbohydrates, stains, and agar were procured from Loba Chemicals. Salts, nutrient supplements, detergents, indicators, and reagents were obtained from HiMedia Laboratories Pvt. Ltd. or S.D. Fine-Chem Ltd. All chemicals were of analytical grade. Standard glassware and laboratory consumables were used throughout the experiments.

2.2. Preparation of samples for screening of Lactic acid bacteria

The following samples were processed for screening of LAB.

1. Cheese: Goat milk was curdled using lemon juice. The curdled mixture was strained through a muslin cloth to separate the whey. The solid portion was collected and allowed to ferment in a dry place for two weeks before use.

2. Paneer: It was prepared by curdling cow milk with lemon juice. The resulting curd was strained using a muslin cloth to remove the whey. The solid portion was then refrigerated for several hours before use.

3. Sauerkraut: Fresh cabbage was finely chopped and mixed with 3% brine, with additional table salt sprinkled over the pieces. The mixture was transferred into a sterile glass container, weighted to submerge the cabbage, and tightly sealed. The container was incubated at 30°C until fermentation was complete.

4. Fermented Beetroot Juice: Fresh beetroot was chopped and ground into a fine mixture using a grinder. The juice was extracted by filtering the ground beetroot through a muslin cloth to separate the pulp. The extracted juice was stored in a sterile glass container with a tightly sealed lid and fermented at 30°C in a clean, dry place for 2–3 weeks before use.

5. Idli Batter: Rice and urad dal (black gram) were soaked separately in water for 6–8 h. After soaking, both ingredients were ground into fine pastes and mixed in a 3:1 ratio. The resulting batter was allowed to ferment at room temperature (25–30°C) for 12–16 h.

2.3 Screening of lactic acid bacteria

The prepared samples were diluted using the 10-fold serial dilution method. Isolation was done on Tomato Juice Agar (TJA) plates using the spread plate technique. The plates were incubated in a candle-lit jar at 30°C for 24 h until small colonies with a yellow halo were observed surrounding them. The colonies were then streaked onto sterile De Man, Rogosa, and Sharpe (MRS) agar slants and stored at 4°C until further analysis.

2.4 Characterization of lactic acid bacteria

The LAB strains were characterized based on standard tests described in Bergey's Manual of Determinative Biology (Holt et al., 1994).

2.4.1 Catalase test

Selected LAB cultures (showing yellow colonies on TJA) were tested for catalase activity. A smear of the culture was prepared on a clean, grease-free slide, and a drop of hydrogen peroxide (H_2O_2) was added. Immediate effervescence indicated a positive catalase reaction, whereas the absence of bubbles confirmed a negative result.

2.4.2 Morphology

The isolated LAB cultures were characterized based on colony morphology (including mucoidal nature and string formation) and Gram stain characteristics.

2.4.3 Capsule staining

Capsule staining was performed using the Congo red–Maneval method. A drop of 1% Congo red was placed on a clean slide and mixed with a loopful of LAB culture from the MRS slant to form a smear. While the centre remained wet, the edges were allowed to air-dry. Maneval's stain was then applied to flood the smear. The slide was air-dried and examined under an oil immersion lens at 100× magnification.

2.4.4 Quantification of EPS produced by LAB

EPS production was quantified using a standardized precipitation method. A 0.5% (v/v) formalin solution was added to 20 mL of inoculated MRS broth, followed by refrigeration for 1 h. The broth was centrifuged at 5000 rpm for 30 mins to separate the pellet of bacterial cells. The supernatant containing EPS was collected and stored at 4°C for 2–3 h. EPS was precipitated by adding chilled absolute ethanol to the supernatant in a 3:1 ratio. The precipitate was collected by centrifugation at 3000 rpm for 10 mins,

and the pellet was transferred to a pre-weighed aluminum boat. The EPS was dried in a hot air oven at 80°C for 20–30 min to remove residual moisture and ethanol. The dried EPS was weighed, and the total yield was calculated using Equation 1 (Adesulu-Dahunsi et al., 2018).

Dry weight of EPS=B-A … Eq. 1

Where A is the weight of the aluminum boat and B is the weight of EPS in the aluminum boat

2.4.5 Molisch Test for EPS Detection

To detect the presence of carbohydrate in EPS, 1 mL of extracted EPS solution was mixed with 1 mL of saturated α-naphthol in ethanol. Carefully, 1 mL of concentrated H_2SO_4 was added along the sides of the tube wall. The formation of a purple or violet ring at the interface indicated a positive test.

2.5 Identification of potential EPS producing LAB

Potential EPS producing LAB isolate was identified based on morphological characteristics and 16S rRNA gene sequencing, which was done at HiMedia Laboratories Pvt. Ltd.

2.6 Optimization of EPS production

To improve EPS production by the potential isolate, the standard MRS medium was systematically optimized by modifying carbon and nitrogen sources, media composition and growth conditions. A 24 h old culture was used to prepare a suspension adjusted to an optical density (OD_{540}) of 0.5, and 1 mL was inoculated into 20 mL of modified MRS medium. It was incubated up to 120h under various experimental conditions described below. After incubation, EPS was extracted using the ethanol precipitation method and quantified to evaluate the effects of each modification.

2.6.1 Optimization of carbon and nitrogen sources

Carbon sources were optimized by testing the effect of different monosaccharides (glucose, fructose, galactose, xylose, arabinose, ribose, mannose), disaccharides (lactose, sucrose, maltose) and polysaccharides (starch). Initially, 2% sugars were tested and the concentration of the best carbon source was further optimized (2-10%) in MRS broth. Nitrogen sources tested in this study included both organic (yeast extract, beef extract, peptone, tryptone, meat extract, casein hydrolysate, urea) and inorganic (ammonium chloride, ammonium citrate, ammonium sulfate) sources.

2.6.2 Optimization of MRS media composition

The optimization of MRS media composition was done by studying the effect of the deletion of media components. For the analysis, 2 setups were designed to study the effect of individual MRS components on EPS production. In setup 1, the original MRS medium containing ammonium citrate, peptone, beef extract, and yeast extract was modified by systematically removing one nutrient source in separate tubes, and the effect of each deletion was evaluated. In setup 2, two nitrogen sources were simultaneously removed in different combinations to further evaluate their combined effect on EPS synthesis. Two control tubes were also set up with 2% glucose or 2% sucrose containing the original nutrient composition of MRS. In addition, the MRS medium was modified by altering the concentrations of the optimal components, while keeping other components (and their concentrations) constant to assess their effect on EPS production.

2.6.3 Optimization of physicochemical and growth conditions

The physicochemical and growth conditions optimized in this study included pH (4–7), incubation temperature (room temperature, 37°C, 45°C), aeration (shaking vs. static; aerobic vs. microaerophilic), inoculum size (0.5–2 mL) and incubation time (24–120 h).

2.7 Purification of EPS

EPS were purified from the culture supernatant using a combination of chemical precipitation and dialysis (Sorensen et al., 2022). First, 0.5 mL of formaldehyde was added to 20 mL of culture to fix the bacterial cells, followed by centrifugation at 5,000 rpm for 30 mins to separate cells from the supernatant. Proteins were removed by adding trichloroacetic acid (TCA) to a final concentration of 12% (w/v) and centrifuging at 12,000 rpm for 20 mins. EPS was precipitated from the clarified supernatant using chilled absolute ethanol and collected by centrifugation at 5,000 rpm for 20 mins. The collected EPS pellet was dissolved in deionized water and dialyzed at 4°C for 3–4 days using an EDTA-activated dialysis membrane to remove low-molecular-weight impurities. The dialyzed EPS solution was recovered, reprecipitated with chilled ethanol, and the final purified EPS was obtained by freeze-drying.

2.8 Determination of Total Carbohydrate Content of EPS

The total carbohydrate content of the purified EPS was determined using the Phenol-Sulfuric Acid (PSA) and anthrone methods.

2.8.1 Phenol-Sulfuric Acid method

A 1% EPS solution was prepared and diluted to different concentrations (1:10, 1:50, 1:100, and 1:200). Standard assay tubes containing 20-100 µg/mL glucose were also prepared using 100µg/mL glucose as a stock solution. To each of these test tubes, 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4 was added. Tubes were incubated in a water bath at 25°C for 15 mins. Ice-cold conditions were maintained while adding H_2SO_4 to prevent caramelization. Absorbance was measured

spectrophotometrically, and the unknown concentration in the sample was calculated using a standard graph of Absorbance v/s glucose concentration.

2.8.2 Anthrone Method

EPS solutions and standard glucose assay tubes were prepared as described previously. The anthrone assay was performed using freshly prepared anthrone reagent (0.2 g anthrone per 100 mL concentrated H_2SO_4). The contents of the tubes were mixed with 4 mL of anthrone reagent and incubated in a hot water bath at 100°C for 10 mins. Ice-cold conditions were maintained while adding the reagent to prevent sugar degradation. The resulting color intensity was measured spectrophotometrically at 620nm, and the concentration of EPS in the samples was determined from a standard graph of Absorbance v/s glucose concentration.

2.9 Ninhydrin test for amino acid detection

The presence of amino acids in purified EPS was tested using a 0.2% ninhydrin solution prepared in acetone. A 0.1% glycine solution served as the standard. For the assay, 1 mL of purified EPS (0.1%) was mixed with 2–5 drops of ninhydrin solution and placed in a boiling water bath for 5 mins. The development of a pink, purple, or violet-blue color indicated the presence of free amino acids.

2.10 Biuret Test for Peptide/Protein Detection

Peptides or proteins in EPS were detected using the Biuret test. The reagents included 1% (w/v) copper sulfate solution and 40% (w/v) NaOH solution. Bovine serum albumin (0.1%) was used as the standard. To 1 mL of EPS solution (0.1%), 0.5 mL of NaOH was added and mixed, followed by the addition of 2–5 drops of copper sulfate solution. Formation of a pink or violet color indicated the presence of peptides or proteins in the EPS.

2.11 Component Analysis of Exopolysaccharide

2.11.1 Hydrolysis

Purified EPS (1% solution) was hydrolyzed by adding an equal volume of 8 M HCl and incubating in a boiling water bath for 1 h. The hydrolyzed mixture was neutralized to pH 7 using NaOH before further analysis.

2.11.2 Thin-Layer Chromatography (TLC)

Silica gel TLC plates were activated at 110°C for 30 mins to remove any moisture before use. Hydrolyzed EPS (2–3 drops) and 1% solutions of standard sugars (mannose, glucose, maltose, fructose, galactose, xylose, arabinose) were applied to the plates. A solvent system consisting of Ethyl acetate:Isopropanol:H_2O:Pyridine (26:14:7:2, v/v/v/v) was prepared and allowed to saturate in the TLC chamber for 3 h. The spotted TLC plates were carefully placed in the chamber, ensuring proper contact with the solvent, and the chromatographic separation was allowed to proceed until the solvent front reached the desired height. Once the solvent front reached the desired height, the TLC plates were removed from the chamber, air-dried, and sprayed uniformly with the developing reagent (Diphenylamine:Aniline:Acetone:Ortho-phosphoric acid in a 2:2:100:10, g/v/v/v ratio) to visualize the separated compounds. The chromatograms were then heated at 85°C for 10 mins for the development of colored spots. The EPS components were identified by comparing the Rf values with those of the standard sugars (Ziadi et al., 2018).

2.12 Applications of EPS

2.12.1 Water retention capacity of EPS

Two clean, dry petri plates were labeled as Plate 1 (Control) and Plate 2 (EPS-treated). The initial weight of each empty plate was recorded as W_1 (Plate 1) and W_2 (Plate 2). Equal amounts of cocopeat were added to each plate, and the weight of each plate with cocopeat was measured and recorded. In plate 1 (Control), 20 mL of RO water was added, ensuring even distribution and the weight was noted. In plate 2 (EPS-treated), 20 mL of 1% EPS solution was added evenly and the weight was recorded. Both plates were placed in direct sunlight for 6 days to observe water retention differences. After 6 days, the plates were weighed again to assess moisture loss and the effect of EPS on water retention.

2.12.2 Effect of EPS on microbial load

From the above experimental setup, 1 g of cocopeat was collected from each plate before and after incubation to assess microbial growth differences between the control and EPS treated samples.

2.12.3 Seed germination and plant growth promotion

Wheat (*Triticum aestivum*) and moong (*Vigna radiata*) seeds were soaked overnight. These plants were selected for the study since they represent monocotyledons and dicotyledons, respectively. A portion of seeds was coated with 1% EPS solution, air-dried and used as test samples, while uncoated seeds were used as controls. Seeds were sown in cocopeat-filled cups (150 g per cup) with small drainage holes. Four treatment groups were established, including (i) EPS-coated + RO water, (ii) EPS-coated + EPS solution, (iii) Non-coated + RO water (control), and (iv) Non-coated + EPS solution. Each cup was watered daily with 10 mL of the respective solution, and germination, shoot/root development, and plant growth were monitored daily.

III. RESULTS AND DISCUSSION

3.1 Screening and isolation of potential LAB strains

A total of 20 isolates were obtained on TJA plates (Fig. 1) from five samples analyzed in the present study. Among these, nine isolates were catalase-negative, indicative of LAB strains, of which only five isolates exhibited distinctly mucoid and slimy colony morphology, suggestive of extracellular EPS production. These isolates were represented as E1- E5. Quantitative

estimation of EPS production by these isolates using the ethanol precipitation method revealed the highest EPS yield in E1 (30 g/L), followed by E5 (25 g/L), E3 (20 g/L), E2 (15 g/L), and E4 (14 g/L).

EPS production by LAB isolates shows considerable variations under unoptimized conditions. The yield of EPS typically ranges between 0.1 and 10 g/L in the published literature. For instance, some LAB isolates obtained from plant-based fermented foods produced 10–400 mg/L EPS (Angelov et al., 2023) under un-optimized condition. *Lacticaseibacillus rhamnosus* RW-9595 M and *L. kefiranofaciens* WT-2B have been reported to yield up to 2.5–5 g/L EPS (Prete et al., 2021). Similarly, the EPS yield of *Leuconostoc mesenteroides* SJC113 was 7.4 ± 0.9 g/L in unoptimized MRS media (Jurášková et al., 2024). Compared to these strains, isolates E1-E5 show significantly high yield under unoptimized conditions, indicating their potential application in various industrial fields.

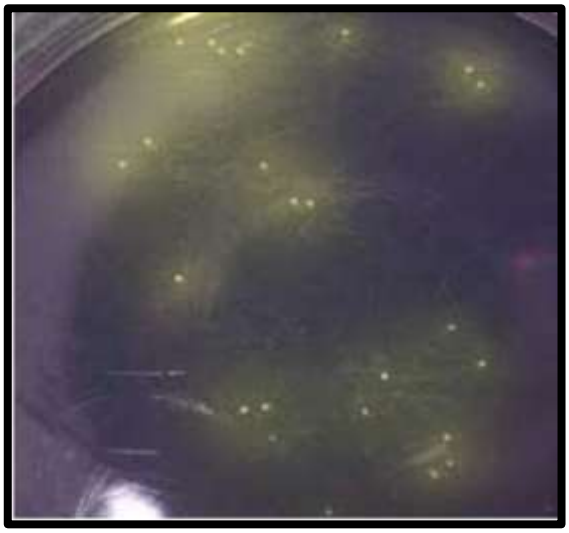

Fig. 1: Colonies of LAB showing a yellow halo on Tomato Juice Agar plates

3.2 Characterization and identification of potential EPS producers

The E1 isolate showed a very pronounced sticky, string-like consistency on contact with the inoculating loop, which is a characteristic phenotypic indicator of EPS production (Fig. 2a). The formation of a purple ring at the interface of the reaction mixture in Molisch test indicated the presence of carbohydrate moieties, which is consistent with typical polysaccharide structures of microbial EPS (Fig. 2d). Capsule staining further confirmed the presence of a well-defined capsule surrounding the cells of E1 (Fig. 2b). Gram staining revealed the isolate to be Gram-positive coccobacilli (Fig. 2c). No cell-bound EPS was detected in the cell pellet of E1, suggesting that EPS production by this isolate is predominantly extracellular.

The phenotypic and biochemical tests confirmed the E1 isolate to belong to the Weissella genus. Species identification, done based on 16S rRNA gene sequencing and BLAST analysis (Fig. 3), identified the isolate as *Weissella confusa* (NCBI accession no. AB494723.1). Phylogenetic analysis (Fig. 4) further confirmed the identification by showing a close evolutionary relationship with other known *W. confusa* strains. The short branch lengths between the isolate and other *W. confusa* strains suggested minimal genetic divergence and a recent common ancestor. In contrast, species such as *Weissella cibaria* and *Weissella viridescens* appeared more distantly related.

W. confusa is a hetero-fermentative LAB commonly found in a wide range of natural and fermented foods. It has been frequently isolated from cereals and legumes-based fermented foods, dairy products, vegetables, sourdough, and traditional fermented batters (Kavitake et al., 2020). Its widespread occurrence in spontaneous fermentations is attributed to its ability to efficiently utilize diverse sugars and to produce non-toxic metabolites (Liu et al., 2025). This strain is also well known for its ability to synthesize structurally diverse polysaccharides that contribute to texture, viscosity, and stability in fermented foods (Du et al., 2025; Lahmar et al., 2024).

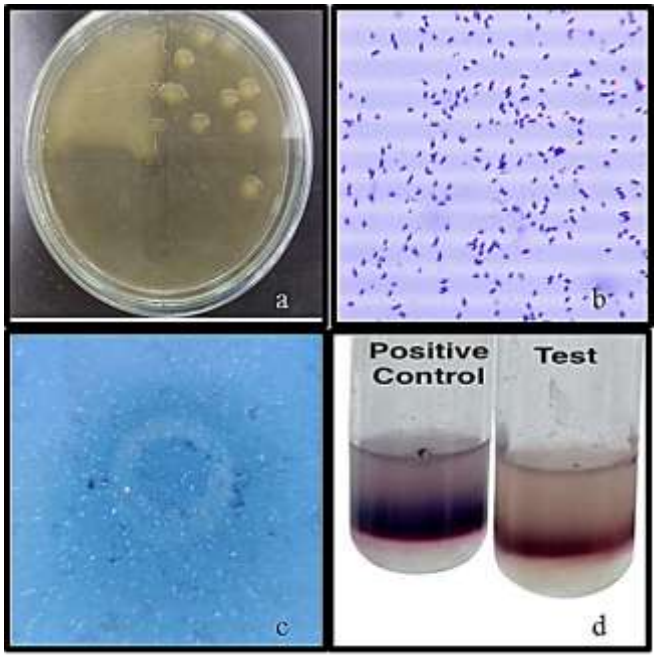

Fig. 2: Characteristics of isolate E1

The figure shows (a) mucoid colonies on MRS plates, (b) capsule staining, (c) Gram staining, and (d) Molisch test

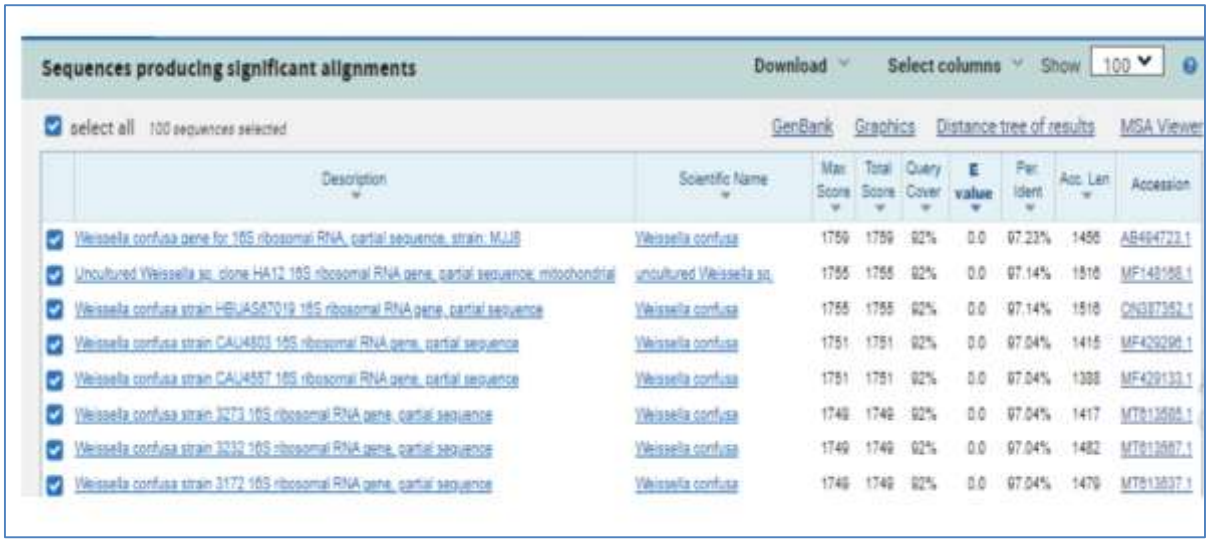

Fig. 3: BLAST analysis of E1 isolate

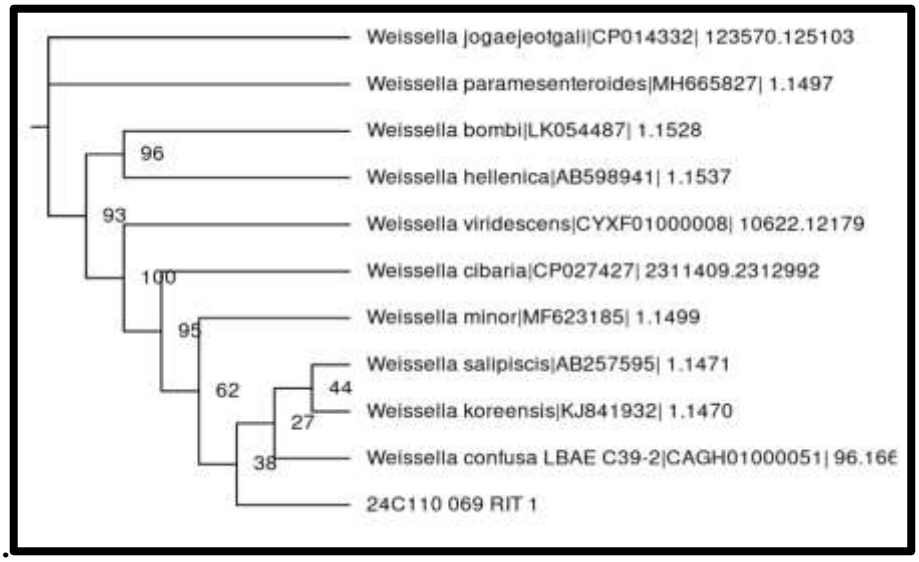

Fig. 4: Phylogenetic tree of isolate E1

3.3 Optimization of EPS production

3.3.1 Optimization of carbon and nitrogen sources

Carbon sources influence both cellular growth and the biosynthesis of secondary metabolites such as EPS (Yilmaz et al., 2012). Similarly, nitrogen sources support microbial growth, enzyme synthesis, and metabolic regulatory pathways (Hernández-Rosas et al., 2021). Hence, selection of appropriate sugars and nitrogen sources plays an important role in optimization studies.

The effect of different carbon and nitrogen sources on EPS production by *W. confusa* is demonstrated in Fig. 5. Among the eleven carbon sources tested, sucrose supported the highest EPS yield (21 g/L). In the absence of sucrose, EPS production was limited to 13 g/L. Additionally, the effect of sucrose on EPS production was observed to be concentration-dependent. A progressive rise in EPS yield was noted up to 7% sucrose concentration, yielding 100 g/L EPS. However, further increase in sucrose concentration resulted in a decline in EPS production. This decline may be attributed to factors such as osmotic stress, substrate inhibition, or repression of EPS biosynthetic enzymes under high sugar conditions (Huang et al., 2024). Among the nitrogen sources, organic compounds significantly enhanced EPS production compared to inorganic sources. Yeast extract supported the highest EPS yield (55 g/L), followed by peptone (31.5 g/L) and beef extract (17.5 g/L). In contrast, inorganic nitrogen sources such as ammonium citrate, ammonium chloride, ammonium sulfate, and urea negatively affected EPS yields. This may be due to the inefficiency of LAB strains to utilize these sources. In contrast, it is possible that inorganic nitrogen sources are rapidly assimilated and may preferentially support primary metabolism and biomass formation rather than secondary metabolite production, thereby limiting EPS synthesis (Biswas and Paul, 2017).

Similar to this study, sucrose has been reported as a preferred carbon source for many EPS producing LAB strains, due to its direct involvement in glucosyltransferase- and fructosyltransferase-mediated polymer synthesis (Sørensen et al., 2022). For instance, sucrose was reported as best carbon source for EPS production by most of the 43 LAB strains isolated from fermented plant based foods. Optimization of EPS production by *Lactiplantibacillus plantarum* ZE2, in the same study, resulted in 211.53 mg/L yield on supplementation of media with sucrose (Angelov et al., 2023). Even among other bacteria, *Bacillus altitudinis* strain EPBAS.1 produced highest EPS yield in presence of sucrose (Jacob et al., 2026). Among nitrogen sources, yeast extracts being rich in amino acids, peptides, vitamins, and nucleotides support microbial growth and secondary metabolite production (Sørensen et al., 2022). This may be the reason for highest EPS yield by *W. confusa* in presence of yeast extracts. Similar observations are reported by other studies. *Lacticaseibacillus rhamnosus* ZFM216 showed maximum EPS yield in basal media with the following composition in g/L; yeast extract (7.5), beef extract (12.5), peptone (10), maltose (21.23), yeast nitrogen base (5.51), K_2HPO_4 (2), anhydrous sodium acetate (5), ammonium citrate (2), $MgSO_4{\cdot}7H_2O$ (0.58), $MnSO_4{\cdot}H_2O$ (0.25), and L–1 Tween 80 (1), which was optimized using the Plackett–Burman design and Box–Behnken design (Chen et al., 2022). In contrast, optimum EPS production by a bacterial consortium of three strains i.e. *Klebsiella pneumonia* strain, *Pseudomonas aeruginosa* strain and *Burkholderia cepacian* showed optimum yield of 17.84 g/L and 21.07 g/L when ammonium sulfate and glucose were employed as nitrogen and carbon supplements, respectively (Jyoti et al., 2024).

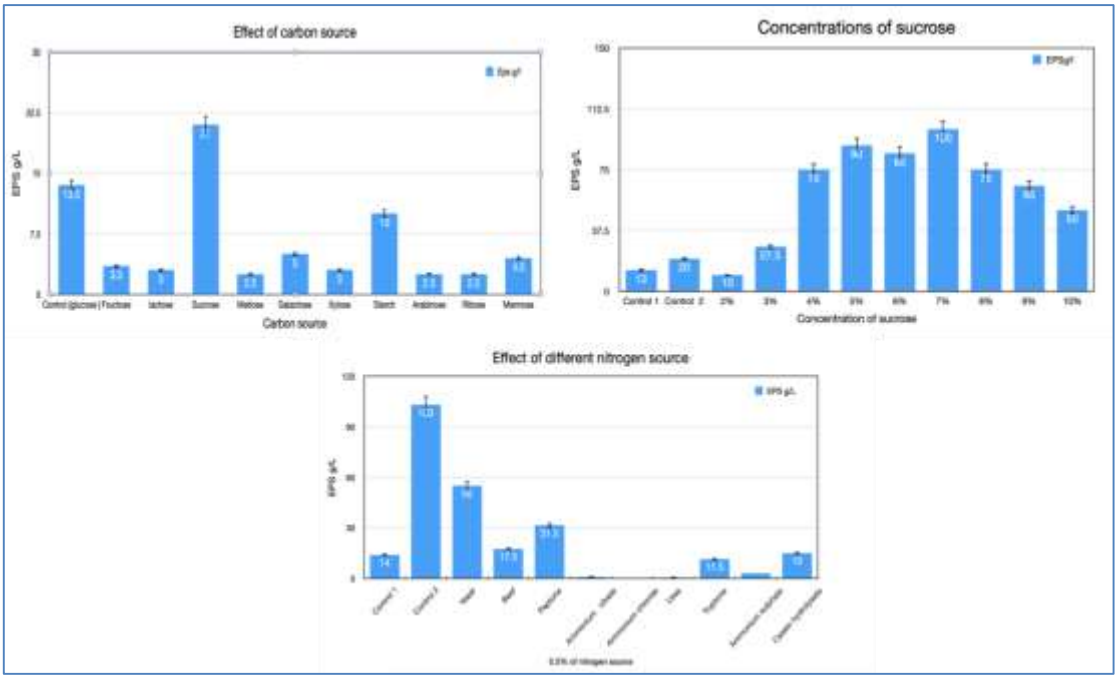

Fig. 5: Optimization of carbon and nitrogen sources for EPS production by *Weissella confusa*

3.3.2 Optimization of MRS media composition

The effect of different combinations of MRS media composition on EPS production by *W. confusa* is demonstrated in Table 1. Among the tested combinations in experimental setup 1, Tube 4 containing all MRS components except ammonium citrate yielded the highest EPS concentration (110 g/L). In experimental setup 2, Tube 2 (beef extract + peptone) produced the highest EPS yield (115 g/L), followed by tubes containing yeast extract with one other nitrogen source (80 - 100 g/L). This observation was rather interesting, given that yeast extract supported optimum EPS production when used in the absence of other nutrient sources. It suggests that beef extract and peptone, when used together, likely exert a synergistic effect in supporting EPS

production. Additionally, MRS supplemented with 2% sucrose (control 2) showed a markedly high yield of EPS (~105 g/L). Altogether, the observations of set- up 1 as well as set-up 2 (Table 1) indicated that ammonium citrate contributed minimally to EPS biosynthesis, and confirmed the importance of sucrose supplementation. Similar findings have been reported for other LAB strains, where inorganic nitrogen sources were effective in supporting EPS synthesis compared to complex organic nitrogen sources (Wang and Bi, 2008; Zajsek et al., 2013). Lastly, studying the effect of varying concentrations of peptone and beef extract indicated that adding 1g/L of each of these components is optimum for EPS production. Overall, the findings indicated that along with the type of nutrient, their concentration is equally important in optimization studies. Lower nitrogen concentrations resulted in reduced EPS production, likely due to metabolic limitations affecting enzyme synthesis and polysaccharide synthesis (Guerin et al., 2020).

Table 1: Optimization of MRS composition for EPS production by *Weissella confusa*

Tube no.	Tube composition	EPS (g/L)
Effect of deletion of single media components		
Tube 1	peptone, beef extract, yeast extract, ammonium citrate	100
Tube 2	peptone, yeast extract, ammonium citrate	85
Tube 3	peptone, beef extract, ammonium citrate	90
*Tube 4	peptone, beef extract, and yeast extract	110
Tube 5 (control 1)	MRS + 2% glucose	14
Tube 6 (control 2)	MRS + 2% sucrose	104.5
Effect of deletion of two media components		
Tube 1	beef extract, yeast extract	80
*Tube 2	beef extract, peptone	115
Tube 3	beef extract, ammonium citrate	7
Tube 4	yeast extract, peptone	80
Tube 5	yeast extract, ammonium citrate	100
Tube 6	ammonium citrate, peptone	75
Tube 7 (control 1)	MRS + 2% glucose	13.5
Tube 8 (control 2)	MRS + 2% sucrose	105
Effect of varying nutrient concentrations		
Tube 1	peptone (0.5g) and beef extract (0.5g)	80
Tube 2	peptone (0.5g) and beef extract (1g)	108
Tube 3	peptone (1g) and beef extract (0.5g)	100
*Tube 4	peptone (1g) and beef extract (1g)	114
Tube 5 (control 1)	MRS + 2% glucose	14
Tube 6 (control 2)	MRS + 2% sucrose	103

*Tubes showing highest EPS yield

3.3.3 Optimization of physiochemical and growth conditions

Besides media composition, the physicochemical parameters are important factors affecting EPS production. Factors such as pH, temperature, and incubation time affect the metabolic activity of microorganisms, and hence can affect secretion of secondary metabolites and structural components such as EPS (Prete et al., 2021). The effect of varying physiochemical and growth conditions on EPS production by *W. confusa* is demonstrated in Fig. 6. The optimum EPS yield was obtained in MRS media adjusted to pH 6, containing 2% inoculum, and on incubation at room temperature under static conditions. Generally, LAB strains produce EPS at neutral or near neutral pH and temperatures between 35°C- 40°C (ideal growth temperatures). Under optimized physicochemical conditions, EPS yield by *L. rhamnosus* ZFM216 increased by 76.70% from 281.07 ± 5.90mg/L to 496.64 ± 3.15mg/L. The optimum conditions included 1% inoculum size, 37°C, pH 6.5 and 20h incubation period (Chen et al., 2022). *L. plantarum* R301 showed optimum yield of 97.85 g/L EPS in presence of 0.01% $MgSO_4$ and 6.4% inoculum size at pH 7.4 (Wang et al., 2023). *L. casei* AL.15 produced 606.03 mg/L EPS in media with 10% glucose on incubation for 48h at 42°C (Pinaria et al., 2023).

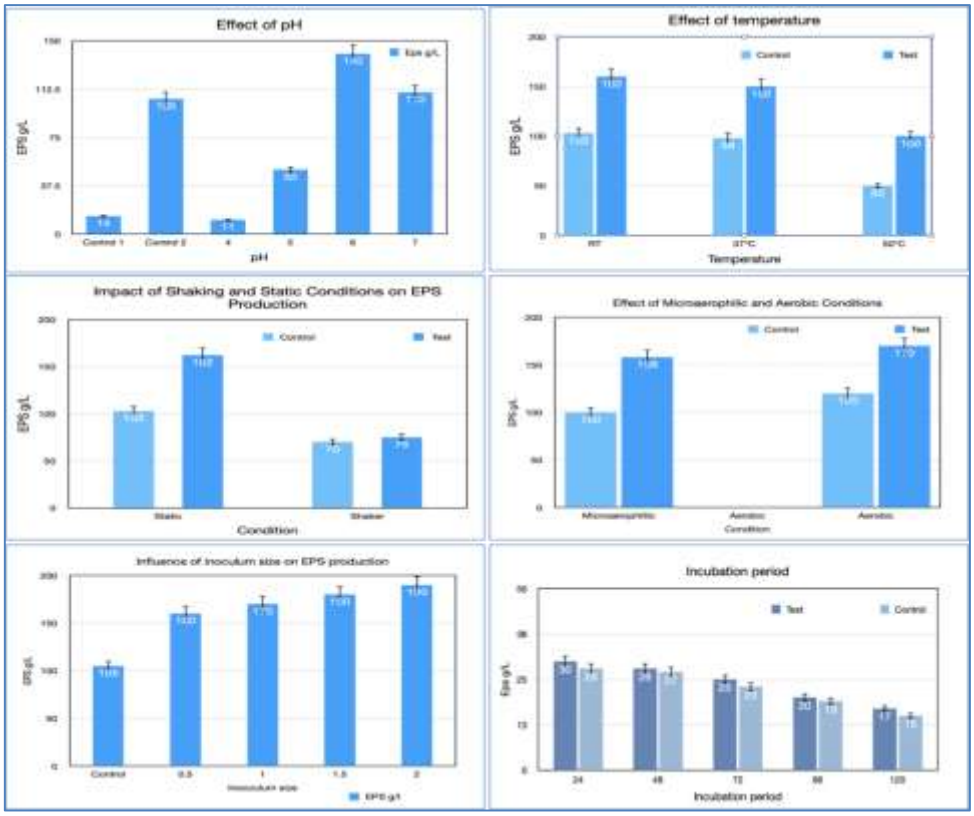

Fig. 6: Optimization of physiochemical parameters for EPS production by *Weissella confusa*

3.4 Purification and TLC analysis of EPS

The EPS produced by *W. confusa* was successfully purified (Fig. 7), and the total yield after optimization and purification steps was 165 g/L. The purified EPS exhibited enhanced solubility and reduced contamination with proteins, amino acids and nucleic acids, as demonstrated by Biuret and Ninhydrin tests, respectively. The concentration of carbohydrate was found to be 5,600µg/mL and 5,500µg/mL based on phenol-sulfuric acid and anthrone methods, respectively. The phenol-sulfuric acid method detects a broad range of carbohydrates, including pentoses, hexoses, and polysaccharides, making it more inclusive. On the other hand, the anthrone method primarily reacts with hexoses and polysaccharides, which may result in slightly lower carbohydrate estimations (Yadav et al., 2024; Madhuri and Prabhakar, 2014). This explains the minor difference observed in the EPS quantification using the two methods.

Monosaccharide composition analysis indicated that the EPS was primarily composed of glucose units. This finding is consistent with homopolysaccharide EPS structures reported for many Weisella strains (Adesulu-Dahunsi et al., 2018; Zhou et al., 2024; Özpınar et al., 2024; Malik et al., 2015). Among other LAB strains, the EPS of *Lacticaseibacillus rhamnosus* ZFM216 mainly comprised glucose and guluronic acid, with an average molecular weight of 19.9 kDa (Chen et al., 2022). *Leuconostoc mesenteroides* SJC113, isolated from cheese curd, produced large amounts of a mucoid EPS. Characterization studies revealed the glucan nature of this EPS with 84.5% (1→6)-linked α-d-glucose units and 5.6% (1,3→6)-linked α-d-glucose units as branching points (Juraskova et al., 2024).

Fig. 7: Purified EPS obtained from *Weissella confusa*

3.5 Applications of EPS

3.5.1 Determination of water retention capacity of EPS- treated cocopeat

The effect of EPS treatment was primarily evaluated for the moisture retention capacity of cocopeat. After six days, the EPS-treated cocopeat retained 11.50g of water, whereas the RO-watered control retained only 9.93g of water, representing an approximately 16% increase in water retention in the presence of EPS. Moreover, interestingly, the visual assessment of cocopeat further revealed pronounced differences in aggregation and physical structure of cocopeat between treatments. Precisely, the RO-watered cocopeat appeared loose, fragmented, and poorly aggregated, indicating limited structural stability. On the other hand, EPS-treated cocopeat formed a cohesive and well-structured mass, suggesting enhanced particle binding and improved aggregation (Fig. 8). The enhanced water retention observed in EPS-treated cocopeat can be attributed to the highly hydrophilic nature of microbial EPS. They contain abundant hydroxyl and carboxyl functional groups that strongly bind water molecules, forming a gel-like matrix that slows water loss through evaporation and drainage. Similar observations have been reported in other studies where microbial EPS significantly improves soil water retention by stabilizing soil microaggregates (Sandhya et al., 2009; Flemming and Wingender, 2010; Chenu, 1993; Costa et al., 2018). Overall, these findings suggest that EPS-based amendments or EPS producing microorganisms could play a significant role in sustainable agriculture, particularly in arid and semi-arid regions or in degraded and sandy soils where water retention and aggregation are limiting factors.

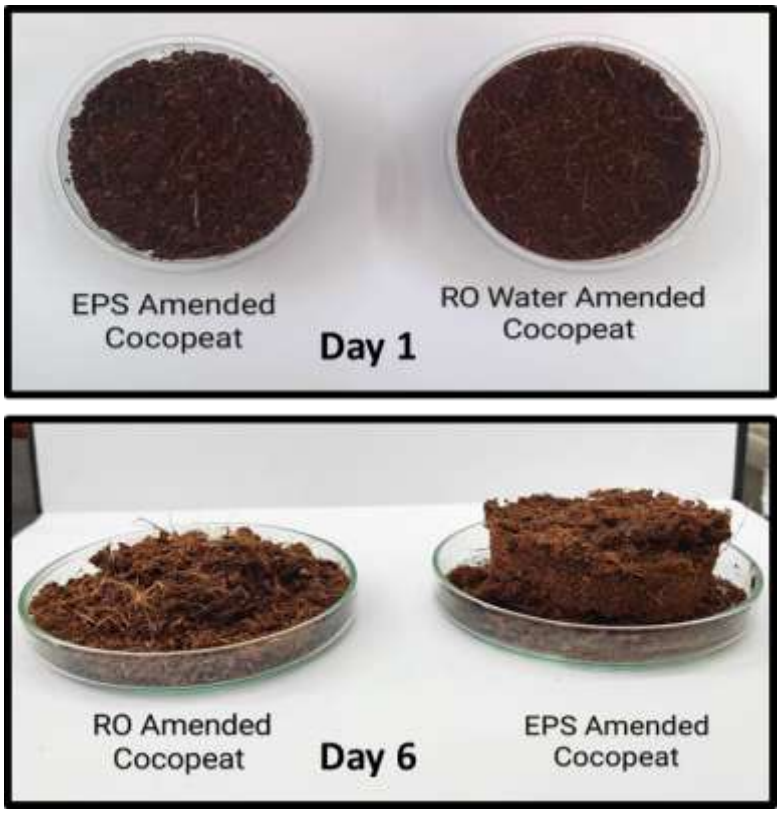

Fig. 8: Water retention and soil aggregation capacity of EPS-treated cocopeat

3.5.2 Determining the effect of EPS treatment on microbial load

Microbial enumeration was carried out using the plate count method to evaluate the effect of EPS treatment on microbial proliferation in cocopeat. The initial microbial load of untreated cocopeat showed detectable growth up to a dilution of 10^{-8}, indicating a moderate baseline microbial population. In the RO-watered control cocopeat, microbial growth was observed up to 10^{-10} dilution on day 6. This indicates that while moisture availability supported microbial survival, it did not significantly stimulate microbial proliferation. In contrast, EPS-treated cocopeat exhibited a pronounced increase in microbial load as observed on day 6. Growth was detected up to 10^{-15} dilutions, with colony counts exceeding 300 CFU/ml up to 10^{-14} dilution and remaining high (256 CFU/ml) even at 10^{-15} dilution. It is well known that EPS is a key structural component of microbial habitats, since it facilitates cell adhesion, biofilm formation, and stabilization of microbial communities on solid surfaces (Roberson and Firestone, 1992). Additionally, the hydrated, gel-like EPS network creates a moisture-rich microenvironment that allows microbial persistence in soil (Costa et al., 2018). Furthermore, EPS can serve as a readily available carbon and energy source for heterotrophic microorganisms (Costa et al., 2018; Flemming and Wingender, 2010). Overall, these findings highlight the efficacy of EPS application in sustainable agriculture and horticulture practices to improve nutrient cycling and soil health.

3.5.3 Efficacy of EPS producing LAB in plant growth promotion

The application of EPS significantly influenced germination rate and root and shoot development in wheat and moong plants (Table 2). In both plants, seeds subjected to EPS treatments germinated within 2 days, whereas seeds in the water control required approximately 4-5 days for germination. Observations of shoot and root development in wheat plants indicated maximum efficacy of EPS-coating of seeds and watering with RO water, followed by EPS coating of seeds and watering with EPS solution. Statistical evaluation using one-way ANOVA revealed a highly significant difference among treatments for shoot development ($F = 42.57693$, $p < 0.00001$) as well as root development ($F = 67.1197$, $p = 0.0000$). The high F-value confirms that treatment effects were substantially greater than within-group variability, demonstrating that EPS application significantly enhanced shoot and

elongation in wheat plants. In the moong plant, EPS-coated seeds watered with EPS solution showed the highest shoot elongation, followed by EPS-coated seeds watered with RO water. The longest roots were observed in non-coated seeds watered with EPS solution, followed by EPS-coated seeds watered with RO water. In the moong plant too, the statistical analysis with a high F-value confirmed the positive influence of EPS on shoot ($F = 8.91538$, $p = 0.000033$) and root ($F = 117.0344$, $p < 0.00001$) development.

Table 2: Effect of EPS treatment on plant growth promotion

Plant growth parameters	Water Control	Treatments		
		EPS-Coated and RO Watered	EPS-Coated and EPS Watered	Non-Coated and EPS Watered
Wheat				
Number of seed germinated	2	3	4	5
Number of leaves	4	6	9	10.5
Shoot development	4.69 cm	19.19 cm	17.73 cm	7.25 cm
Root development	9.80 cm	18.10 cm	12.72 cm	11.40 cm
Moong				
Number of seed germinated	3	5	5	5
Number of leaves	6	23.5	26	22.5
Shoot development	2.924 cm	11.12 cm	11.832 cm	10.272 cm
Root development	9.0 cm	12.7cm	12.3 cm	14.0 cm

The accelerated germination observed in EPS-treated seeds suggests a favorable microenvironment during early seedling establishment. EPS improves water retention around the seed surface, ensuring continuous hydration that supports metabolic activation and radicle emergence (Sandhya et al., 2009; Roberson and Firestone, 1992). Enhanced root and shoot elongation in EPS-treated plants can be attributed to improved soil physical properties, including increased moisture retention, better aeration, and enhanced nutrient availability. Overall, the study highlights the potential of EPS as a sustainable, bio-based agricultural tool capable of improving crop establishment and growth, especially under suboptimal environmental conditions.

IV. Conclusion

The present study demonstrates the multifaceted role of microbial exopolysaccharides (EPS) in enhancing the physicochemical properties of cocopeat, microbial activity, and plant growth. EPS produced by *Weissella confusa* significantly improved water retention and substrate aggregation, resulting in a more stable and hydrated growth medium compared to untreated controls. The enhanced structural integrity of the substrate contributed to reduced moisture loss, improved aeration, and increased suitability for microbial colonization. Furthermore, EPS application markedly increased microbial populations in cocopeat, indicating that EPS creates a favorable microenvironment by promoting moisture retention, facilitating surface adhesion, and providing protection against environmental stress. The sustained microbial abundance observed in EPS-treated substrates highlights the role of EPS in stabilizing and enriching microbial communities, which are essential for nutrient cycling and overall soil health. Overall, the study suggests that EPS-based applications hold significant potential for improving crop establishment, enhancing tolerance to water stress, and promoting sustainable agricultural practices, particularly in degraded, sandy, or water-limited environments.

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