Research Article

Enhancement of Production of Exopolysaccharides from *Bacillus* Species

Rajendrabhai Daulatbhai Vasait*, Ahire Vanita Laxman, Sonawane Pooja Hemant, Deoghare Neha Subhash, Borse Archana Ashok

Department of Microbiology, KAANMS ASC College, Satana, Maharashtra, INDIA.

Submission Date: 11-07-2023; Revision Date: 12-08-2023; Accepted Date: 15-09-2023.

ABSTRACT

It has been shown that spontaneously synthesized Exopolysaccharides (EPS) are employed for a variety of commercial uses. The kind of EPS generated by bacteria to defend themselves from unsafe circumstances. Food additives as a natural supply of carbohydrates and proteins, bio-emulsifiers, stabilizers, biosorbents, cosmetic preparations, anticancer agents, antioxidants, and biologically active antimicrobials are all examples of how EPS is employed. Because EPS generated by bacteria is at a significant commercial level, the current study focused on screening and isolating EPS-producing organisms from the rhizosphere soil of various plants. Four EPS-producing bacteria were isolated and investigated in this study. A medium to enhance EPS production from a *Bacillus* species, soil isolate, H, was optimized. In order to identify functional groups, FTIR spectrophotometry was employed. Furthermore, the presence of carbohydrates and proteins in EPS was detected subjectively as well as quantitatively. Higher carbohydrate and protein concentrations, such as 23.66 and 15.19 mg/dL, were found in the EPS produced from isolates F and O, respectively. The total protein content of isolate H, EPS was found to be 13.05 g/L. Finally, in presence of optimum conditions, isolate H produced 13.11 g/L of EPS.

Keywords: Optimization, Exopolysaccharide, *Bacillus species*; Exopolymer.

Correspondence:
Dr. Rajendrabhai Daulatbhai Vasait,
Department of Microbiology, KAANMS ASC College Satana, Satana-423301, Maharashtra, INDIA.

Email: rd73vas@rediffmail.com

INTRODUCTION

At the industrial level, micro-organisms are used to produce a variety of commercially relevant products. Exopolysaccharides (EPS), which include high-molecular-weight polymers and sugar residues, are among the diverse polysaccharides that micro-organisms form. These include intracellular and structural polysaccharides.[1,2] Micro-organisms produce EPS in their surrounding environment.[3] It was discovered that bacterial EPS is not utilised as an energy source by the generating bacteria, but rather is produced to protect the producer organisms from starvation as well as severe pH, temperature, heavy metals, and antimicrobial compounds.[4,5] Exopolysaccharides are often made up of carbohydrates as well as certain non-carbohydrate components.[6] In most cases, monosaccharides such as glucose, galactose, and rhamnose are present. N-acetylglucosamine, mannose, fructose, glucuronic acid, and noncarbohydrate substituents such as phosphate, acetyl, and glycerol are also found in a few situations.[7-9] EPS are physiologically active, naturally occurring polysaccharides with a unique mix of functional and environmentally favourable qualities. Polysaccharides found in nature have a unique combination of functional and environmentally favourable qualities. Microbial EPSs are classified into two types: homopolysaccharides and heteropolysaccharides.[10] They are naturally renewable, harmless, and biodegradable. EPS occurs in a variety of different and frequently complicated chemical structures, and it is believed that they provide self-protection against antimicrobial chemicals.[4] Because they serve as thickeners, stabilisers, viscosifiers, emulsifiers, or gelling agents, high molecular weight polysaccharides are utilised as additives in the preparation of a wide range

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2023.12.48

of food items.[4,6,11,12] They have also been shown to have positive impacts on human health, such as cholesterol-reducing and immunomodulating properties, as well as antibacterial, anticancer, and antioxidant properties.[12-16] *Bacillus* spp. may be isolated from the environment in vast numbers, with each producing a varied amount and level of polymer.[2] The concentration and type of EPS generated by bacteria are affected by carbon supply, nutritional status, growth phase, temperature, and other variables.[15] Because of the numerous uses and industrial relevance of EPS, the present work was done to isolate and screen EPS-producing bacteria from the soil as well as optimize process parameters for its enhancement. Furthermore, EPS and EPS-producing isolates were characterised at a later stage of the investigation.

MATERIALS AND METHODS

Materials

Nutrient Broth, Minimal Broth, and Agar Powder were procured from Hi-media Laboratories, India; Chemicals Solvents and reagents were purchased from Merk, Sigma Aldric, Fisher Scientific, and Fine Chem Industry, India. All chemicals, reagents and solvents were of analytical, assay grade as per standards.

Sample collection

For the isolation of EPS-producing organisms, rhizosphere soils from various crop fields in Satana (Maharashtra), such as sugarcane, pomegranate, and maize, were collected in sterile containers and stored at a low temperature.

Screening and isolation of EPS-producing organisms

1 gm of the collected soil was mixed with 99 mL of saline and vertexed in order to isolate EPS producers from rhizosphere soil. 1 mL of the suspension from the flask was taken and serially diluted up to 10^{-5} to 10^{-6} using sterile saline blanks. Then 0.1 mL of suspension was plated from a 10^{-6} dilution and spread over sterile nutrient agar plates. The inoculated plates were incubated at 26°C for 24 hr. The colonies of various morphology were obtained after 24 hr. A mucoid and fluffy appearance of the colonies was selected for further study. Mucoid, fluffy colonies were screened out and restreaked on other sterile nutrient agar plates and slants for further use.

Maintenance of culture

The isolated organisms were streaked on sterile nutrient agar slopes and, after incubation, these were kept in the refrigerator at 4°C till use. The cultures were sub-cultured periodically.

Production of EPS

The method for EPS production utilized was previously described.[6] The production of EPS was carried in 50 mL of the medium. The medium was used which consisted of 10.0 g/L of peptone, 3.00 g/L of yeast extract, 5.00 g/L of NaCl, and a pH of 7±2. After sterilization, the production medium was inoculated with isolated organisms O, F, G, and H in their respective flasks. The flasks were incubated at 26°C for 48 hr on an orbital shaker at a speed of 110 rpm.[16]

Extraction and purification of EPS

Centrifugation was used to separate the cells from the production medium for 30 min at 5000 rpm. The supernatant was then mixed in 1:2 with chilled ethanol, which was then kept at 4°C for 14 hr.[2,17] Precipitated material was separated by centrifugation at 4000 rpm for 20 min. The pellet from the tubes was dried at 50°C for 14 hr in a hot air oven.[12] The weight of the pellet was measured from each tube after drying. The higher EPS-producing organisms were screened out on the basis of their EPS production.

Identification and characterization

The morphological characteristics of each EPS-producing organism were studied and recorded. The characterization of each isolate was done by Gram's staining; further organisms were characterized by capsule and endospore staining.

Biochemical characterization of isolates

The biochemical characterization of each isolate was carried out.[18] The tests for hydrolysis of starch and gelatine were conducted.[19] The IMViC test, and other biochemical tests were performed.[18]

Characterization of EPS

For the characterization of EPS, total carbohydrates, total proteins, and other components were determined.[20] Total carbohydrates and proteins were determined as follows:

- Estimation of total carbohydrate

- The presence of total carbohydrates was estimated by the Anthrone method.[21]
- Estimation of total protein
- The total proteins present were estimated by the Folin-Lowery method.[21]

Thin Layer Chromatography (TLC)

The TLC for sugar detection was used as previously reported[16] with some modifications. TLC was performed using Merck Silica Gel 60 F_{254} sheets. The solvent system containing n-butanol:propanol:water:acetic acid (7:5:4:2) was used for sugars. The sugars were visualised by spraying the solution of DPA:orthophosphoric acid, and aniline. The TLC plates were dried in a hot air oven for 4 min at 150° C. The sugars glucose, mannitol, maltose, xylose, arabinose, and fructose were used as standard sugars. For amino acids, TLC was performed using solvent phase butanol:water:acetic acid (50:40:10). The amino acids were detected by spraying of ninhydrin reagent. They were then dried in a hot air oven for 4 min at 150° C. The valine, proline, histidine, cysteine, glutamic acid, and serine were used as standard amino acids.

FTIR Spectroscopy analysis of EPS

The EPS was analyzed for the detection of functional groups by Fourier transform infrared spectroscopy. According to the reports, dry EPS was ground with KBr powder and formed into pellets for FTIR spectroscopy between 400 and 4000 cm^{-1}.[16]

Optimization of exopolysaccharide production

A minimal broth containing dipotassium phosphate (K_2HPO_4) - 7.0 g/L, monopotassium phosphate (KH_2PO_4) - 2.0 g/L, sodium citrate - 0.50 g/L, magnesium sulphate - 0.10 g/L, ammonium sulphate - 1.0 g/L, pH 7.0±0.2 was used for the optimization study. The effect of the carbon and nitrogen sources was studied.

Effect of carbon source on EPS production

To study the effect of carbon sources on EPS production, different sugars were selected, such as glucose, sucrose, lactose, maltose, and fructose, and included as sole carbon sources in minimal broth (without carbon source) at a concentration of 20 g/L, while the concentration of nitrogen source, *i.e.*, ammonium sulfate, was 10 g/L. Isolates were inoculated into 150 mL Erlenmeyer flasks containing 50 mL of production medium. The flasks containing different carbon sources were inoculated with 4% of inoculum. The flasks were initially incubated at 26°C on an orbital shaker for 48 hr. After incubation, the extraction and purification of EPS were carried out from each flask as stated above.

Effect of organic nitrogen sources on EPS production

To study the effect of nitrogen sources on EPS production, different nitrogen sources were selected, such as yeast extract, peptone, casein digest, and beef extract, with a concentration of 10 g/L, while the amount of the carbon source, *i.e.*, glucose, 20 g/L was included. Isolates were inoculated as stated in the previous section in the production medium. The flasks containing different nitrogen sources were inoculated with 4% of inoculum. The flasks were incubated at 26°C on an orbital shaker for 48 hr. After incubation, the extraction and purification of EPS were carried out from each flask as stated above.

Effect of concentration carbon source on EPS production

The best carbon source, glucose, was used at various levels including 10, 20, 30, and 40 g/L to study the impact of carbon source quantity on EPS synthesis, while the best nitrogen source, peptone, was held constant at 10 g/L. Isolates were inoculated into 50 mL of production medium as above. The flasks were inoculated with 4 vol.% of inoculums. The flasks were incubated for 48 hr at 26°C over an orbital shaker. After incubation, the extraction and purification of EPS were carried out from each flask as stated above.

Effect of organic nitrogen concentration on EPS production

The best nitrogen source, peptone, was used at various amounts to study the impact of nitrogen source level on EPS synthesis, while the best carbon source amount was held constant at 20 g/L. Isolates were inoculated into the production medium as mentioned previously. The flasks containing different concentrations of lactose were inoculated with 4 vol.% of inoculums. The flasks were incubated at 26°C on a rotary shaker for 48 hr. After incubation, the extraction and purification of EPS were carried out from each flask as stated above.

Effect of temperature on EPS production

By first determining a temperature range, the impact of temperature on the formation of EPS was examined. The EPS production was conducted at 26, 30, 37 and 40°C. The impact of temperature was evaluated by using optimal levels of the best carbon source, glucose, and nitrogen source, peptone, i.e., 20 and 10 g/L, respectively. 4% v/v inoculum was used to inoculate the production medium. The flasks were incubated for

48 hr at 26, 30, 37 and 40°C, respectively, on an orbital shaker. After incubation, the extraction and purification of EPS were carried out from each flask as stated above.

Effect of pH on EPS production

The effect of pH on EPS production was evaluated by selecting different pH. EPS production was conducted at pH 6, 7, 8. The effect of pH was examined by including optimized levels of the best carbon source, glucose, and nitrogen source, peptone, *i.e.*, 20 and 10 g/L, respectively. By taking the same quantity of production medium as stated previously and incubating on a rotary shaker at 26°C for 48 hr. The extraction and purification of exopolysaccharide was carried out from each flask.

EPS production at optimized parameters

Finally, an experimentation was carried out with all optimal conditions to evaluate the potential of EPS production by isolating H.

RESULTS

Screening and isolation of EPS-producing bacteria

As per reports, different crop fields were selected for the collection of soil samples in the regions of Satana, Nashik, India. Four EPS-producing bacteria were isolated.

Characterization of EPS-producing isolates

The morphological characteristics of all four isolates were studied. The isolates O and F were isolated from the rhizosphere soil of pomegranate, and the organisms G and H were isolated from the rhizosphere soil of sugarcane and maize, respectively. All isolates form colonies that are about 5 mm in size. Isolate G formed circular-shaped colonies, while others had irregular shapes. The colonies had a sticky and smooth nature, with a milky white color and raised elevation. The colonies having a mucoid and fluffy appearance were selected for the present study. The colony characters and microscopic observations of isolates were studied (Figure 1**)** and identified by studying their morphological and biochemical characteristics. All four isolates, O, F, G, and H, were Gram-positive, motile, endospore-forming, and capsulated. All isolated organisms were unable to ferment sugars such as glucose, mannitol, and arabinose, while all of them were detected as having the ability to hydrolyze starch and gelatin. All Isolates O, F, G, and H were grown in the presence of 6.5% NaCl and found to be capable of producing enzymes oxidase and catalase but not nitrate reductase. According to the morphological and biochemical characterization the isolates O, G, H, and F belong to the genus *Bacillus*. Further identification of isolates is awaited.

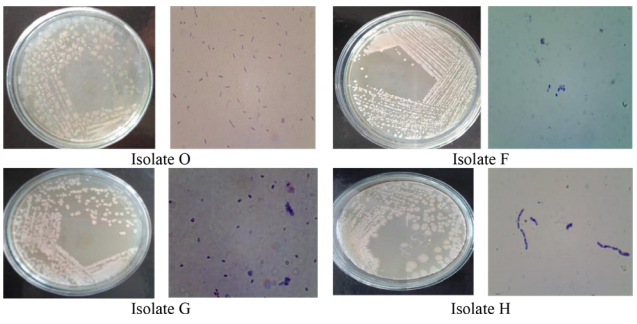

Figure 1: Colony morphology and Gram's staining of EPS producing isolates.

Production of EPS

The comparative account of EPS produced by isolates O, F, G, and H is depicted in Figure 2. All isolates were tested for EPS production primarily where isolate namely O, F, G, and H produced 4, 4, 3 and 8.6 g/L of EPS.

Characterization of exopolysaccharide

Estimation of total carbohydrates in EPS

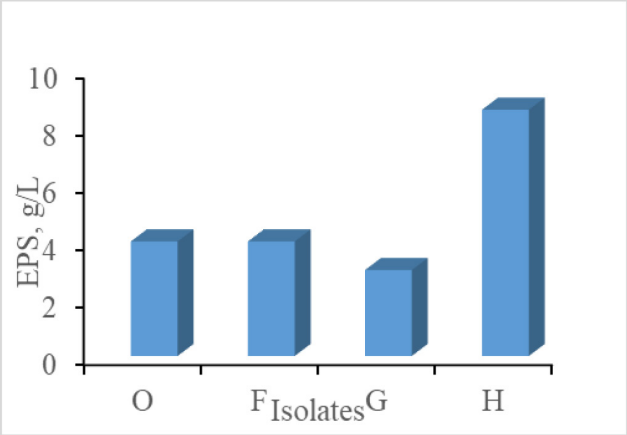

Figure 2: Comparative analysis EPS production by the isolates.

Table 1: Comparative analysis of total carbohydrates detected in EPS.

Isolate	Amount of carbohydrates in EPS, mg/100 mL
O	15.33
F	23.66
G	12.0
H	12.66

Table 2: Comparative analysis of total proteins detected in EPS.

Isolate	Amount of proteins in EPS, mg/100 mL
O	15.19
F	13.76
G	12.35
H	13.05

The amount of total carbohydrates in EPS produced by isolated organisms were determined by the anthrone method which is depicted in Table 1.

Estimation of total proteins in EPS

The number of total proteins in EPS produced by isolated organisms were determined by the Folin-Lowrey method which is summarized in Table 2. Among the four isolates, a higher amount of total carbohydrates, *i.e.*, 23.66 mg/100 mL, was detected in the EPS produced by organism F. The EPS produced by organism F was also found to contain proteins 13.76 mg/100 mL. In the EPS produced by organism O, we detected a higher content of proteins, *i.e.*, 15.19 mg/100 mL, as compared to other isolates F, G, and H.

Thin Layer Chromatography (TLC)

A qualitative analysis by TLC of the EPS produced by isolates was conducted. EPS produced by isolates exhibited the presence of comparable spots with reference amino acids and sugars.

FTIR analysis of EPS produced by isolates

FTIR spectroscopy between frequency ranges of 400-4000 cm^{-1} was analyzed as previously described in a research report.[16] The detection of functional groups in EPS was conducted by studying the FTIR spectrum as follows:

Isolate G

The IR spectrum of isolate G (Figure 3) showed the broad peak at region 1061.86 and 1128.41 cm^{-1}, which is the region of alcohol group stretching hence, the EPS sample may contain sugar group in pyranose form components containing OH group.[22] Absorption at 1244.14 cm^{-1} suggests the presence of ester groups. The absorption peak at 1458.25 cm^{-1} indicates C= C of arene group stretching. The sharp band at 1536.37 cm^{-1} indicates there may be a presence of nitro (N-O) group stretching. The peak region at 1686.82 cm^{-1} strongly suggests the presence of carbonyl and amide (C=O) groups. The peak at 2933.85 cm^{-1} indicates vibration of

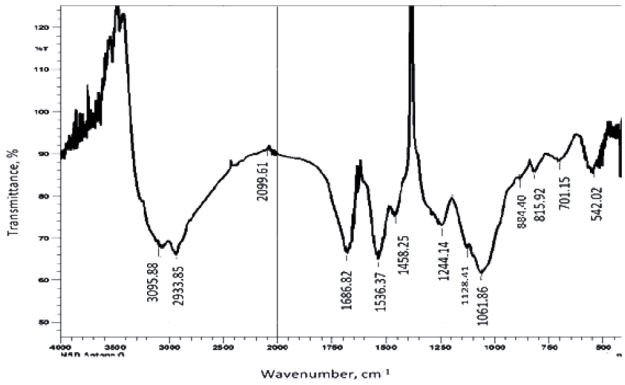

Figure 3: FTIR Spectrum of EPS produced by Isolate G.

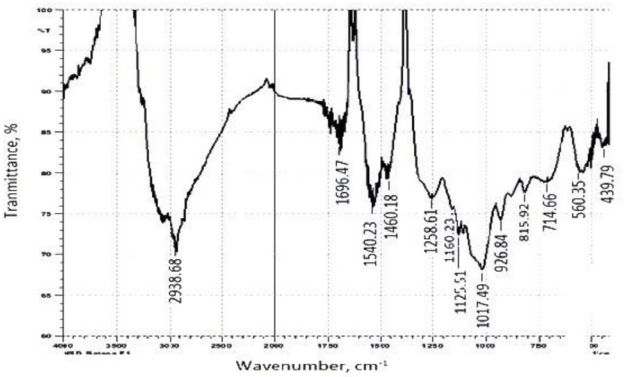

Figure 4: FTIR Spectrum of EPS produced by Isolate F.

the functional group alkane (C-H) group.[16] The peak at region 3095.86 cm^{-1} suggests an alkene (=C-H) group.

Isolate F

A peak region at 1125.51 cm^{-1} was visible in the spectra of isolate F (Figure 4), which denotes the existence of alcohol group C-O stretching. A possible amine (C-N) group is indicated by the peak wavelength of 1160.23 cm^{-1}. A possible acid (C-O) group may be present, as shown by the peak region at 1258.61 cm^{-1}. The arene (C=C) group is three times more abundant, according to the absorption peak at 1460.18 cm^{-1}. Nitro (NO_2) group members are represented by the peak region at 1540.23 cm^{-1}. Aldehyde and ketone (C=O) group occurrence is suggested by the peak region at 1696.47 cm^{-1}.

Isolate H

The IR spectra of isolate H (Figure 5) revealed a peak at 1061.86 and 1104.290 cm^{-1}, indicating the presence of alcohol (C-O group). The peak regions at 1244.14 cm^{-1} and 1321.30 cm^{-1}, respectively, clearly indicated the existence of amine (C-N) groups. The peak at 1461.14 cm^{-1} and 1544.08 cm^{-1} shows that the arene group has undergone C=C stretching. The presence of

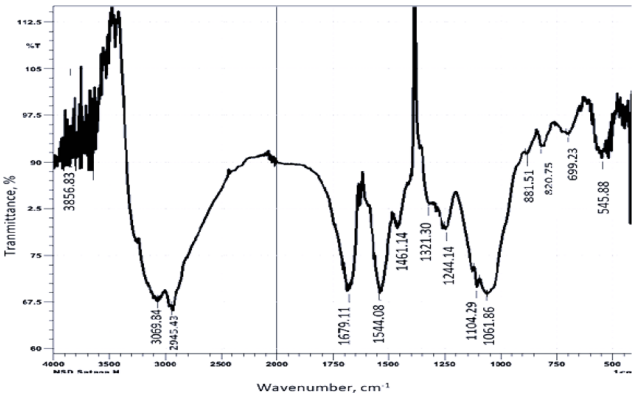

Figure 5: FTIR Spectrum of Exopolysaccharide produced by Isolate H.

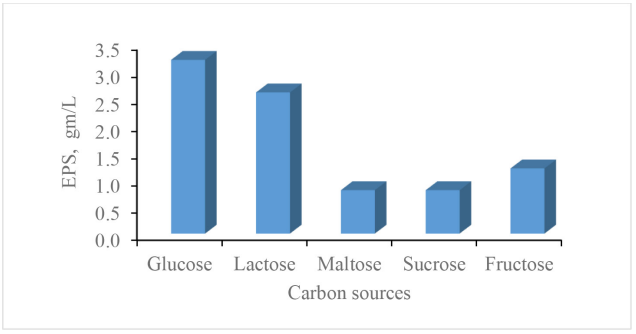

Figure 6: Production of EPS using different carbon sources.

ester (C=O) groups was evident in the peak region at 1679.11 cm^{-1}. The signal at 2945.43 cm^{-1} suggests that an alkane (C-H) group may be present.[16] The aromatic group's C-H stretching is indicated by the peak region at 3069.84 cm^{-1}.

Optimization of Exopolysaccharide Production

Effect of carbon source

Previously, sugars like glucose, galactose, fructose, lactose, sucrose, and starch were employed to test the effect of the 'C' supply on exopolysaccharide production.[23] The higher EPS-producing isolate H was chosen for EPS production parameter optimization. The glucose sugar was selected as the best carbon source among the numerous sugars offered in the production medium, with greater EPS production of 3.2 g/L achieved when compared to other carbon sources (Figure 6). *Bacillus subtilis* produced the most EPS (10 g/L) in the presence of 2% glucose.[24] *Bacillus subtilis* produced the most EPS at 2% sucrose, 2.66 g EPS/L.[12] Sucrose increased EPS production in strain 96CJ10356.[23]

Effect of nitrogen source

The influence of four 'N' sources, including peptone, yeast extract, casein digest, and beef extract, were

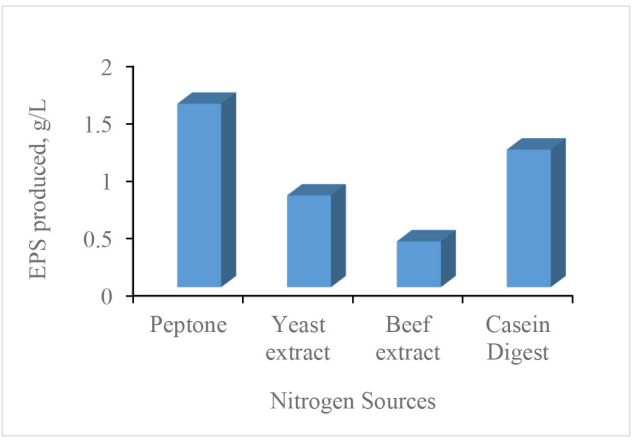

Figure 7: Production of EPS at different nitrogen sources.

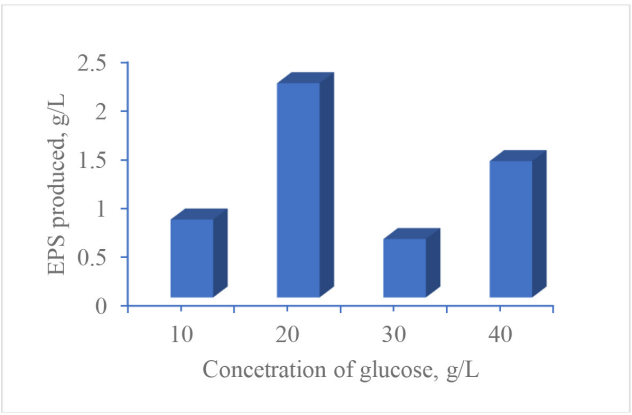

Figure 8: Production of EPS at different concentrations of glucose.

studied for optimization. Among the different nitrogen sources provided in the production medium, peptone was detected as the best where an increased 1.6 g/L of exopolysaccharide was accomplished (Figure 7). EPS synthesis in *Pseudomonas aeruginosa, S. mutans,* and *B. subtilis* is stimulated by nitrogen-free media, according to previous research.[25] Nitrogen source yeast extract at 1% concentration yielded the highest EPS output of 21.3 g/L in *Bacillus amyloliquefaciens* BPRGS.[13]

Influence of carbon concentration of EPS production

The crude EPS concentration was obtained as depicted in Figure 8. At 20 g/L of glucose concentration, the crude EPS concentration was increased significantly. The maximum EPS production was estimated, *i.e.*, 2.2 g/L at glucose concentration of 20 g/L.

Effect of peptone

The crude EPS obtained was represented in Figure 9. At 10 g/L of peptone concentration, the crude EPS

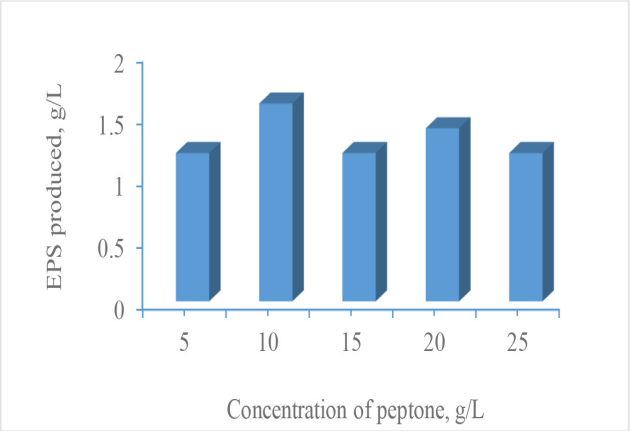

Figure 9: Production of EPS at different concentrations of peptone.

Table 3: Production of EPS at different temperatures

Temperature, °C	EPS produced, g/L
26	0.8
30	0.4
37	0.4
40	0.3

Table 4: Production of EPS at different pH

pH	EPS producing g/L
6	2.2
7	2
8	0.8

concentration was increased significantly. The maximum EPS production was estimated, *i.e.*, 1.6 g/L.

Influence of temperature

The temperatures 26, 30, 37 and 40° C were selected for optimization. In a recent work[23] the best productivity of EPS-R was found at temperatures ranging from 20 to 25° C. The temperature of 30°C was shown to be optimal for the generation of EPS in *Bacillus subtilis*.[24] EPS was produced *i.e.*, 0.8, 0.4, 0.4 and 0.3 g/L at 26, 30, 37 and 40°C respectively (Table 3). A temperature of 26°C detected best for organism H.

Influence of pH

The pH 6, 7, and 8 were selected for optimization. It was discovered that the optimum range of pH for EPS production in *Alcaligenes faecalis* B14 strain 5 to 7.[26] The pH of 7.0 in *Bacillus cereus* KMS3-1 and 7.5 was shown to be optimal for EPS formation.[6,27] The optimized concentrations of "C"-glucose and "N"-peptone, 20 and 10 g/L, respectively, were used to study the impact of pH. At 26°C for 48 hr, the production process was carried out in flasks on a rotary shaker. At pH 6, the high productivity was attained. At pH 7 and pH 8, less EPS was produced. The maximum yield of EPS was 2.2 g/L at pH 6 (Table 4).

Optimized parameters for EPS production

In the optimization study, glucose and peptone were served as the best carbon and nitrogen sources, respectively, for the production of EPS for isolate H. A temperature of 26°C was found to be optimal for organism H. The optimal production of EPS was accomplished at pH 6. The optimization of parameters for the EPS production of organism H was conducted with promising outcomes. Optimized parameters are depicted in Table 5.

EPS production at optimized parameters

Finally, an experiment with all optimized parameters was designed to evaluate the potential of EPS production by isolate H. EPS production was demonstrated by

Table 5: Optimized parameters for EPS production for isolate H

Parameters	Particulars
Carbon source	Glucose
Nitrogen source	Peptone
Concentration of carbon source, g/L	20
Concentration of nitrogen source, g/L	10
Temperature, °C	26
pH	6

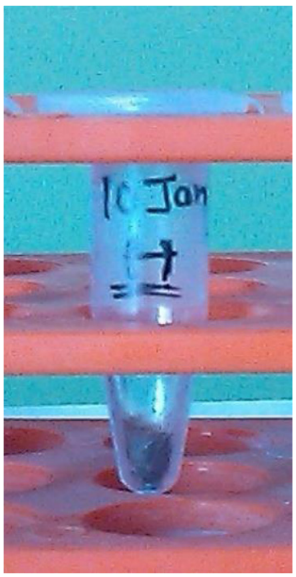

Figure 10: EPS produced by isolate H at optimized parameters.

isolate H during the preliminary stage, *i.e.*, 8.6 g/L. EPS production in isolate H considerably rose from 8.6 to 13.11 g/L (Figure 10**)** after optimization of the parameters mentioned above. The highest total EPS dry weight for *B. licheniformis* 2CS in M3 medium was obtained to be 121 mg.[28] The EPS production exhibited by isolate, H was relatively higher *i.e.*, 13.11 g/L as compared to initial production, which implies the viability of optimization.

DISCUSSION

Micro-organisms fight themselves against adverse environmental conditions in their natural habitat, particularly through their extraordinary metabolic systems. One method by which organisms make their environment wet, encourage food absorption, enhance metabolism, and defend themselves from external factors including temperature, pH, antibiotics, and predators is by the production of EPS.[4] The absorption at 1200-900 cm^{-1}, generated by stretching vibrations of C-C, C-O-C, and CO, may suggest a characteristic of carbohydrates.[17,26] In the EPS of isolates, important functional groups such as C-C, C=C, C-O, C-H, and CO were discovered. The FTIR spectra of the polymer indicated the presence of carboxyl groups, which might operate as divalent cation binding sites.[6,26] Peaks were also observed in FTIR tests for the presence of C-N and C=O groups, indicating the presence of amino acids and polysaccharide bonds.[16] In FTIR investigations, the presence of functional groups such as aromatic carbon, aldehyde, and ketonic groups indicates the presence of sugars. The optimization study was helpful in determining the impact of various parameters on EPS production. In the present investigation, crucial carbon sources, *i.e.*, glucose and its significant concentration of 20 g/L, as well as nitrogen sources, i.e., peptone and its level of 10 g/L, were identified as the best among the different sources used in the study. This indicated the maximum EPS production observed under any of the conditions. At 26°C and pH 6, the highest amount of EPS production was recorded. The optimization analysis demonstrated the maximum amount of EPS production from *Bacillus* species H, with a maximal amount of 13.11 g/L detected as compared to 8.6 g/L during the preliminary stage. The most significant approach one factor at a time was adopted in most of the studies reported for optimization.[6,26,31,32] The presence of 4.97% N was determined by elemental analysis of *Limnothrix redekei* PUPCCC 116 EPS, and the type of EPS generated by *Limnothrix redekei* PUPCCC 116 was disclosed as heteropolysaccharides containing protein moieties in prior work.[29] Gellan generated by *S. paucimobilis* is gaining attention because of its unusual characteristic of creating thermo-reversible gels, and it has significant economic promise in food, medicines, and, most notably, environmental bioremediation.[30] Previously reported that bagasse produced the most EPS (13.17 g/L) after 120 hr in pH 7 media with a 10% inoculum size and 30°C incubation.[31] At 30°C and pH 7, where glucose and yeast extract were found to be the best carbon and nitrogen sources, respectively, for EPS production in the previous report.[26] Soil isolates belonging to the alpha proteobacterium group produced optimal EPS at 7.5 pH and 30°C.[6] In 48 hr of fed-batch cultivation, as much as 54 g/L of EPS was obtained at a yield of 63% (g EPS/g sucrose) at 7.5 pH.[33] In literature report, *Bacillus aerophilus* rk1 produced optimal EPS at pH 7, 30°C, and 72 hr of incubation in the presence of yeast extract and sucrose.[34] Similarly, Glucose was evaluated best carbon source for EPS formation in *Alkalibacillus* sp.[35] Bacterial exopolysaccharides (EPS) are a group of carbohydrate polymers that are secreted into the extracellular environment and are incredibly varied.[36] Polysaccharides produced by microbes have received the most attention because they aid in triggering immunological processes.[37] As noted in earlier sections, EPS is mostly composed of sugars, giving them a clinging look, and they play a key part in a variety of functions. Because of its beneficial qualities, EPS has frequently been employed in the manufacture of textiles as a binding agent with dyes or hydrogels.[5] Understanding the biosynthetic pathways and processes might help optimize EPS synthesis and improve product quality and characteristics.

CONCLUSION

We tested the ability of four rhizosphere soil isolates to produce EPS. It was evaluated that peptone (1%) and glucose (2%) were the optimal carbon and nitrogen sources for EPS synthesis. The optimal physical conditions for EPS synthesis were 26°C and pH 6. EPS analysis reveals that the exopolysaccharide generated by isolates O, F, G, and H comprises proteins and carbohydrates. Although additional research is needed, FTIR investigations suggest that EPS may contain heteropolysaccharides. Isolate H produced the most EPS of the four isolates, stating the necessity of scale-up studies and would serve for EPS production, which may be applicable for many uses as noted in the study's introduction. The optimization study's importance is highlighted by the fact that isolate H optimized EPS production, which was 13.11 g/L, was significantly higher than initial production.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and Head of the Department of Microbiology, KAANM Sonawane ASC College, Satana, for providing laboratory facilities to carry out the research work and their inspiring help.

Funding

The authors did not receive support from any organization for the submitted work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Financial interests

The authors declare they have no financial interests.

Author Contributions

Rajendrabhai Dualatbhai Vasait contributed to the study's concept, design, and supervision. Ahire Vanita Laxman, Sonawane Pooja Hemant, Deoghare Neha Subhash, and Borse Archana Ashok worked on material preparation, data collecting, and analysis. Rajendrabhai Daulatbhai Vasait wrote the first draught of the manuscript, and all authors read and approved the final manuscript.

Consent for Publication

All authors have consent to publish the paper.

ABBREVIATIONS

hr: hour; **rpm:** revolutions per minute; **min:** minute; **IMViC: I:** Indole test; **M:** Methyle Red test; **V:** Voges-Proskauer test; **C:** Citrate utilization test; **TLC:** Thin layer chromatography; **DPA:** Diphenylamine; **FTIR:** Fourier-transform infrared spectroscopy; **'N':** Nitrogen source; **'C':** Carbon source.

SUMMARY

The EPS production in isolates of *Bacillus species* is summarised in the current work. The optimization study's importance is highlighted by the fact that isolate H optimized EPS production, which was 13.11 g/L, was significantly higher than initial production. It was evaluated that peptone (1%) and glucose (2%) were the optimal carbon and nitrogen sources for EPS production. The optimal paramenters for EPS synthesis were 26° C and pH 6. Despite the fact that more investigation is required, scale-up studies are essential for EPS production and may be useful for a wide range of commercial applications.

REFERENCES

1. Prashant S, Patel N, Rao RM, Shukla J, Verma S, Jha S. Isolation and characterization of polyhydroxyalkanoate and exopolysaccharide producing *Bacillus* sp. PS1 isolated from sugarcane field in Bhilai, India. J Microb Biochem Technol. 2011;3:033-5. doi: 10.4172/1948-5948.1000048.
2. Nagamani P, Mahmood SK. Production of poly (3- hydroxybutyrate-co-3-hydroxyvalerate) by a novel *Bacillus* OU40^{T} from inexpensive carbon sources. Int J Pharm Biol Sci. 2013. 2014;4:182-93.
3. Mazzoli R, Bosco F, Mizrahi I, Bayer EA, Pessione E. Towards lactic acid bacteria-based biorefineries. Biotechnol Adv. 2014;32(7):1216-36. doi: 10.1016/j.biotechadv.2014.07.005, PMID 25087936.
4. Pandit B. Microbial exopolysaccharides and their potential applications. Int J Life Sci. 2022;10:151-60.
5. Sharma S, Panchal R, Prajapati K, Prajapati M, Saraf MS. Bacterial exopolysaccharides: types, its biosynthesis and their application in different fields. Acta Sci. 2022;3:03-11.
6. Pawar ST, Bhosale AA, Gawade TB, Nale TR. Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. J Microbiol Biotechnol Res. 2017;3:24-31.
7. De Vuyst L, Degeest B. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol Rev. 1999;23(2):153-77. doi: 10.1111/j.1574-6976.1999.tb00395.x, PMID 10234843.
8. De Vuyst L, De Vin F, Vaningelgem F, Degeest B. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. Int Dairy J. 2001;11(9):687-707. doi: 10.1016/S0958-6946(01)00114-5.
9. Orsod M, Joseph M, Huyop F. Characterization of exopolysaccharides Produced by Bacillus cereus and *Brachybacterium* sp. Isolated from Asian Sea Bass (*Lates calcarifer*). Malays J Microbiol. 2012;8:170-4. doi: 10.21161/mjm.04412.
10. Mohd Nadzir M, Nurhayati RW, Idris FN, Nguyen MH. Biomedical applications of bacterial exopolysaccharides: A review. Polymers. 2021;13(4):530. doi: 10.3390/polym13040530, PMID 33578978.
11. Horikoshi K. Alkaliphiles: some applications of their products for biotechnology. Microbiol Mol Biol Rev. 1999;63(4):735-50. doi: 10.1128/MMBR.63.4.735-750.1999, PMID 10585964.
12. Razack SA, Velayutham V, Thangavelu V. Medium optimization for the production of exopolysaccharide by *Bacillus subtilis* using synthetic sources and agro wastes. Turk J Biol. 2013;37:280-8. doi: 10.3906/biy-1206-50.
13. Rao PB, Sudharsan K. Characterization of exopolysaccharide from *Bacillus amyloliquefaciens* BPRGS for its bioflocculant activity. Int J Sci Eng Res. 2013;4:1696-704.
14. Kumar CG, Joo H, Choi J, Koo Y, Chang C. Purification and characterization of an extracellular polysaccharide from Haloalkalophilic *Bacillus* sp. I-450. Enzyme Microb Technol. 2004;34(7):673-81. doi: 10.1016/j.enzmictec.2004.03.001.
15. Nichols CAM, Guezennec J, Bowman JP. Bacterial exopolysaccharides from extreme marine environments with special consideration of the Southern Ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol. 2005;7(4):253-71. doi: 10.1007/s10126-004-5118-2, PMID 16075348.
16. Vidhyalakshmi R, Nachiyar CV. Microbial production of exopolysaccharides. J Pharm Res. 2011;4:7.
17. Bramhachari PV, Dubey SK. Isolation and characterization of exopolysaccharide produced by *Vibrio harveyi* strain VB23. Lett Appl Microbiol. 2006;43(5):571-7. doi: 10.1111/j.1472-765X.2006.01967.x, PMID 17032234.
18. Bergey DH, Holt JG. Bergey's manual of determinative bacteriology. 9th ed. Lippincott Williams and Wilkins. ISBN 9780683006032; 2000.
19. Benson HJ; 1990. Microbiological applications: laboratory manual in general microbiology. 7th ed, short version. Available from: https://archive.org/details/microbiologicala0000bens_t6t0/page/n7/mode/2up.
20. Ibrahim HAH, Abou Elhassayeb HE, El-Sayed WMM. Potential functions and applications of diverse microbial exopolysaccharides in marine environments. J Genet Eng Biotechnol. 2022;20(1):151. doi: 10.1186/s43141-022-00432-2, PMID 36318392.
21. Plummer DT. An introduction to practical biochemistry. 3rd ed. 1988;1988.

22. Abid Y, Casillo A, Gharsallah H, Joulak I, Lanzetta R, Corsaro MM, *et al*. Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. Int J Biol Macromol. 2018;108:719-28. doi: 10.1016/j.ijbiomac.2017.10.155, PMID 29080817.
23. Ko SH, Lee HS, Park SH, Lee HK. Optimal conditions for the production of exopolysaccharide by marine microorganism *Hahella chejuensis*. Biotechnol Bioprocess Eng. 2000;5(3):181-5. doi: 10.1007/BF02936591.
24. Patil SV, Bathe GA, Patil AV, Patil RH, Salunke BK. Production of bioflocculant exopolysaccharide by *Bacillus subtilis*, 8. Advanced Biotech. p. 14-21.
25. Borgio JF, Bency BJ, Ramesh S, Amuthan M. Exopolysaccharide production by *Bacillus subtilis* NCIM 2063, *Pseudomonas aeruginosa* NCIM 2862 and *Streptococcus mutans* MTCC 1943 using batch culture in different media. Afr J Biotechnol. 2009;9:5454-7.
26. Kaur V, Bera MB, Panesar PS, Chopra HK. Production and characterization of exopolysaccharide Produced by *Alcaligenes Faecalis* B14 Isolated from Indigenous Soil. Int J Biotechnol Bioeng Res. 2013;4:365-74.
27. Mathivanan K, Uthaya J, Mathimani CT, Vinothkanna A, Rajaram R, Annadurai G. Optimization, compositional analysis, and characterization of exopolysaccharides produced by multi-metal resistant *Bacillus cereus* KMS3-1*Carbohyd*. Poly. 2020;227:115369.
28. Tuşar FR, Güven K, Matpan Bekler FM, Polat N. Production and optimization of exopolysaccharide from thermophilic bacteria. Int J Pure Appl Sci. 2022;8(2):524-33. doi: 10.29132/ijpas.1142315.
29. Khattar JIS, Singh DP, Jindal N, Kaur N, Singh Y, Rahi P, *et al*. Isolation and characterization of exopolysaccharides produced by the cyanobacterium *Limnothrix redekei* PUPCCC 116. Appl Biochem Biotechnol. 2010;162(5):1327-38. doi: 10.1007/s12010-010-8922-3, PMID 20174886.
30. Bajaj IB, Survase SA, Saudagar PS, Singhal RS. Gellan gum: fermentative production, downstream processing and applications. Food Technol Biotechnol. 2007;45:341-54.
31. Andharia K, Shilpkar P, Kundu S. Production and optimization of exopolysaccharides (eps) using low-cost bagasse as substrate by *Lysinibacillus macroides*. Asian J Biol Life Sci. 2023;12(1):54-9. doi: 10.5530/ajbls.2023.12.8.
32. Oleksy-Sobczak M, Klewicka E. Optimization of media composition to maximize the yield of exopolysaccharides production by *Lactobacillus rhamnosus* strains. Probiotics Antimicrob Proteins. 2020;12(2):774-83. doi: 10.1007/s12602-019-09581-2, PMID 31410767.
33. Lee IY, Seo WT, Kim GJ, Kim MK, Ahn SG, Kwon GS, *et al*. Optimization of fermentation conditions for production of exopolysaccharide by *Bacillus polymyxa*. Bioprocess Eng. 1997;16(2):71-5. doi: 10.1007/s004490050290.
34. Gangalla R, Gattu S, Beduru S, Kasarla S, Rasiravathanahalli KG, Fuad A, *et al*. KT, Optimization and characterization of exopolysaccharide produced by *Bacillus aerophilus* rk1 and its *in vitro* antioxidant activities. J King Saud Univ Sci. 2021;33:101470. doi.org/10.1016/j.jksus.2021.101470.
35. Arayes MA, Mabrouk MEM, Sabry SA, Abdella B. Exopolysaccharide production from *Alkalibacillus* sp. w3: statistical optimization and biological activity Biologia. Biologia. 2023;78(1):229-40. doi: 10.1007/s11756-022-01233-1.
36. Sutherland IW. Bacterial exopolysaccharides. In: Kamerling JP, editor. Comprehensive glycoscience. Elsevier; 1972:143-213. doi: 10.1016/S0065-2911(08)60190-3.
37. Lin MH, Yang YL, Chen YP, Hua KF, Lu CP, Sheu F, *et al*. A novel exopolysaccharide from the biofilm of *Thermus aquaticus* YT–1 induces the immune response through toll–like receptor 2. J Biol Chem. 2011;286(20):17736-45. doi: 10.1074/jbc.M110.200113, PMID 21454596.

Cite this article: Vasait RD, Ahire VL, Sonawane PH, Deoghare NS, Borse AA. Enhancement of Production of Exopolysaccharide from *Bacillus species*. Asian J Bio Life Sci. 2023;12(2):359-68.