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Microbial Exopolysaccharides by *Bacillus Subtilis* as Pharmaceuticals

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Abstract: The aim of the present work was to grow and produce exopolysaccharides by *Bacillus subtilis* and study the applications of exopolysaccharides in pharmaceuticals. The promising outcomes can be seemed as preliminary steps toward the utilization and modification of exopolysaccharides as destiny reasonably-priced resources for production of valuable tablets with antioxidant and anticancer. Microbial exopolysaccharides are extraordinary polymers which can be produced via residing organisms and guard them towards environmental elements. These polymers are industrially recovered from the medium subculture after appearing a fermentative method. Those materials are biocompatible and biodegradable, owning precise and beneficial for biomedical drug delivery structures and they can have a self-assembling phenomenon. We produced and studied the capability use of exopolysaccharides as carriers for drug transport systems, masking their versatility and their substantial opportunities to supply particles, fibers, scaffolds, hydrogels, and aerogels with distinctive strategies and methodologies. Furthermore, the principle of exopolysaccharides are explained, offering information to attain a carrier selection depending on the final application.

Keywords: Exopolysaccharides, Biomaterials, Drug Delivery Systems and *Bacillus subtilis.*

Introduction

Exopolysaccharides (EPS) constitute a collection of polysaccharides synthesized and secreted to the outside environment or synthesized extracellularly by means of cellular wall-associated enzymes of many Gram (+ve) and Gram (-ve) micro organism. It includes repeated devices of sugar monomers and some non-carbohydrate substituent's like phosphates, acetyls, succinate, glycerol, or pyruvate. these slime polymers own a heterogeneous shape, which determines their particular properties and features, noticeably depending on the peculiar situations of niches inhabited through their host [1]. EPS produced with the aid of bacteria can be intestinal health, changing microbes composition enhancing immune system, and improving blood flow. The sources of Exopolymers generating micro organism which can be labeled into cellular wall polysaccharides, Intracellular polysaccharides, and saline habitats, consisting of inland slatterns, marine slatterns and Extracellular polysaccharides.[2] EPS producing bacteria are Acetobacter, Agrobacterium, Bacillus, Brenneria, Geobacillus, Gluconacetobacter, Halomonas, Lactobacillus, Rhizobium, Saccharomyces, Sarcina, Streptococcus, Xanthomonas, and Zymomonas. seasoned-biotic micro organism (E.g.Lactobacillus, Leuconostoc, Lactococcus, Bifidobacterium, Streptococcus, and Enterococcus) have been used predominantly to synthesis EPSs for numerous applications.[3] This is because they are taken into consideration secure and capable of continue to exist amongst gastric juices, bile, and occasional pH and colonize in the gastrointestinal tract's epithelial layer. Since the EPSs is growing because of features which includes their biocompatibility, biodegradability, and non-toxicity, new EPSs are being formed by means of blending them with other natural and synthetic polymers, as a consequence motivating researchers stood is cover novel applications in numerous areas for future use in distinctive international locations as shown in figure 1.

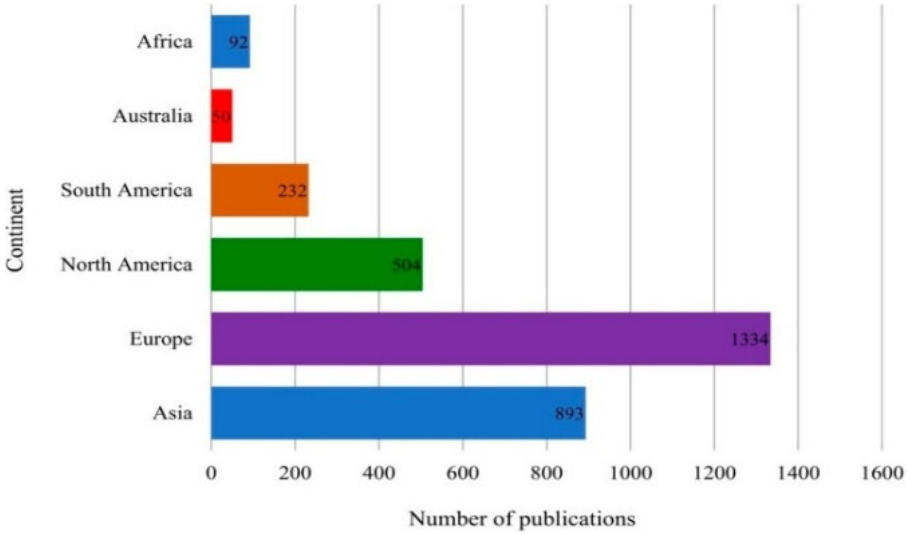


Fig 1: Research trends of microbial exopolysaccharides published in Scopus based on continents.

Biosynthesis of Exopolysaccharides EPSs are synthesized intracellularly either for the duration of growth or throughout late logarithmic or desk bound phase. EPS production also relies upon on stresses, namely, nutrient imbalance, salt, temperature, pH, and so on. Figure 2 represents the manufacturing of biosynthesis of microbial exopolysaccharide.[4-6]

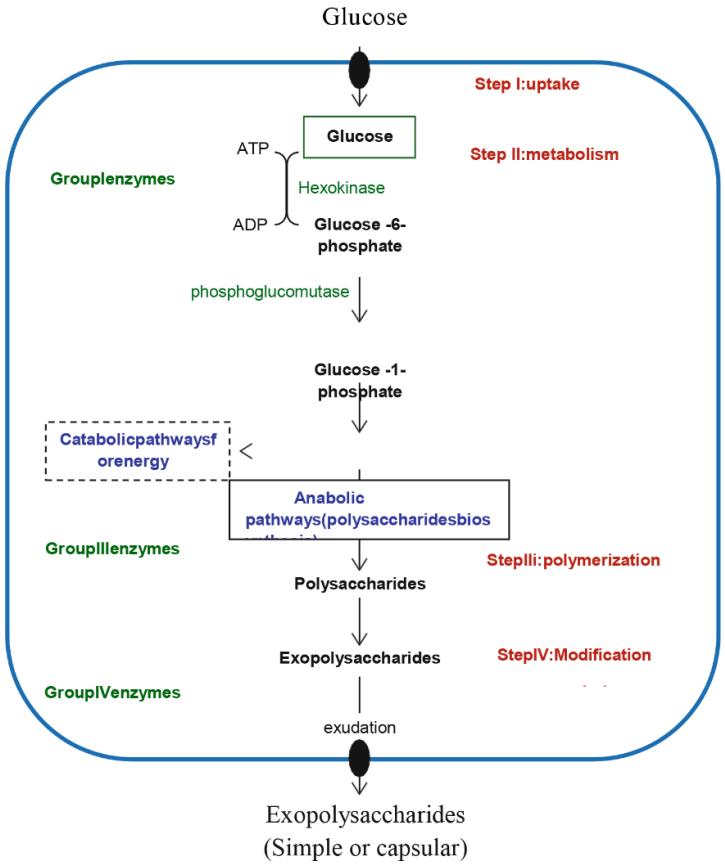


Fig 2: Schematic representation of microbial exopolysaccharide biosynthesis.

Based on the various Microbial polysaccharides are procedure in two forms (i) Capsular polysaccharides[5] (CPS), (ii) Exopolysaccharides (EPS) and The Bacterial Exopolysaccharides are classified into two types i.e., (i) Homopolysaccharides (ii) Heteropolysaccharides. Figure 3 represents the classification of Bacterial Exopolysaccharides.

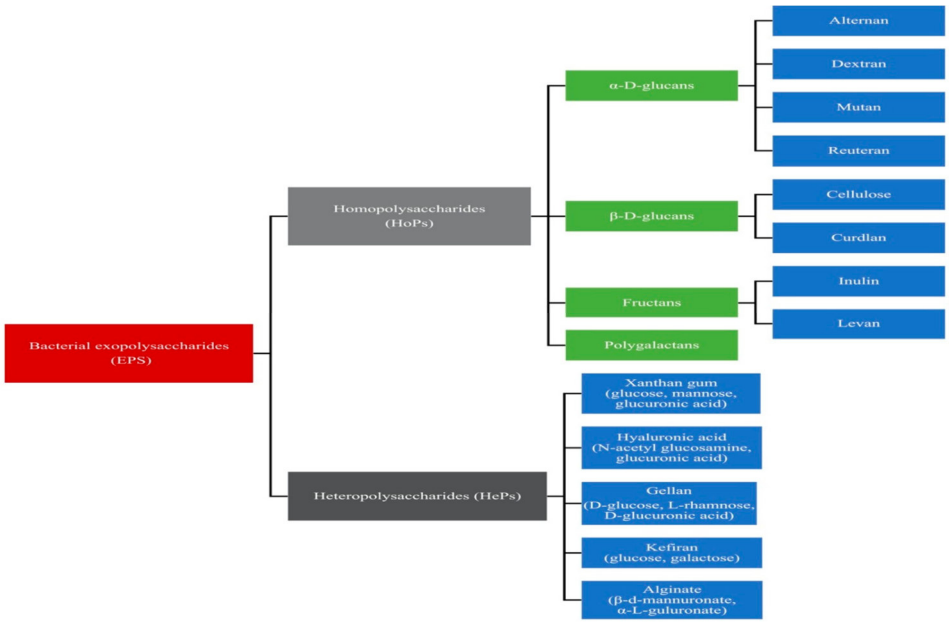


Fig 3: Classifications of Bacterial Exopolysaccharides

Applications [7]

Pharmaceutical industry

Food processing

Drug detoxification

Bioremediation

Cosmetics

Bioflocculants

Bio-absorbents

Heavy metal removal agents

Drug delivery agents

Uses in the food industry [8]

In the food industry microbial EPSS can be used in

Thickeners

Suspending agents

Low calories food products

Dietary fibers

Films and coating agents

Salad dressings

Frozen food icing

Moisturizing agents

Materials and Methods

Bacterial strain *(Bacillus Subtilis)* and Excipients Dextrose Peptone Agar Glucose Cysteine and Basal media was provided by the Geethanjali College of Pharmacy.

Preparation of Exopolysaccharides [9-11]

The production of exopolysaccharides is a significant energy cost to bacteria, and yet direct observation of bacterial cells in a wide variety of natural and industrial environments show, unequivocally, that all such cells are surrounded by structured exopolysaccharides and that many produce very large amounts of extracellular glycocalyx material.

Isolated species of bacterium were identified using growth characteristics and various physiological and biochemical activities according to Bergey's Manual of Systematic Bacteriology and the Procaryotes.

Bacterial strains were maintained and routinely cultured on nutrient agar medium that has the following composition as beef extract-3 gms., peptone-5 gms, agar-20 gms, distilled water- 1L & pH was adjusted to 7.It was inoculated at 30C for24 hrs.

Single spore cultures of the bacterial strains were grown on nutrient agar slopes at 30°C for 24hours, and suspension of the investigated bacteria were used to inoculate flasks containing exopolysaccharide liquid basal medium (50) ml. The culture was incubated at 30 ± 1°C at 150rev.min-1 on a rotary incubator shaker for 24 hours. Production flasks were then inoculated using6% inoculum (24-hseedculture).The bacterial growth was measured using a turbidity meter. The bacteria were routinely grown in conical flask (250ml) containing liquid basal exopolysaccharide medium that has sucrose as carbon source (20 g l-1). The pH of the liquid medium was initially adjusted to 7.0 before autoclaving. After inoculation, cultures were incubated at 30°C with shaking at 150 rev.min-1 for3 days.

Extraction of Exopolysaccharides (EPS) From Bacteria [12-14]

After the incubation period, the cultures were harvested by centrifugation at 10000 rpm for 45 min. at 4°C using a micro centrifuge (Denver Instrument Micro centrifuge).

Culture supernatants were used in precipitating exopolysaccharides. The EPS become triggered from the supernatant via addition of four quantity of 95% (v/v) aqueous ethyl alcohol.

The mixture was agitated at some stage in addition of alcohol to prevent local excessive concentration of the precipitate, and the resulting solution become kept at 4 °C in a single day before being centrifuged at 7000 rpm for 20 min.

The precipitate was collected and dried at 80 °C to constant weight.

Screening of Exopolysaccharides Production by bacteria [15-17]

This screening was done in the process as follows on solid media.

The solid basal media which contains glucose or sucrose as a carbon source.

The solid basal media contains the contents as per litre distilled water.

The *bacteria* were grown on agar plates at 30 + 1C for 3 days and the plates were visually inspected for simple production.

Single spore cultures of the *bacterial* strains were grown on nutrient agar slopes at 30°C for 24 hours, and suspension of the investigated bacteria were used to inoculate flasks containing exopolysaccharide liquid basal medium 50ml.

The culture was incubated at 30 ± 1°C at 150 rev.min-1 on rotary incubator shaker for 24 hours.

Production flasks were then inoculated using 6% inoculum (24-hr seed culture).

The *bacterial* growth was measured using Turbidity meter.

The *bacteria* were routinely grown in conical flask (250 ml) containing liquid basal exopolysaccharide medium that has sucrose as carbon source (20 g l-1). The pH of the liquid medium was initially adjusted to 7.0 before autoclaving. After inoculation, cultures were incubated at 30°C with shaking at 150 rev.min-1 for 3 days.

Results and Discussion

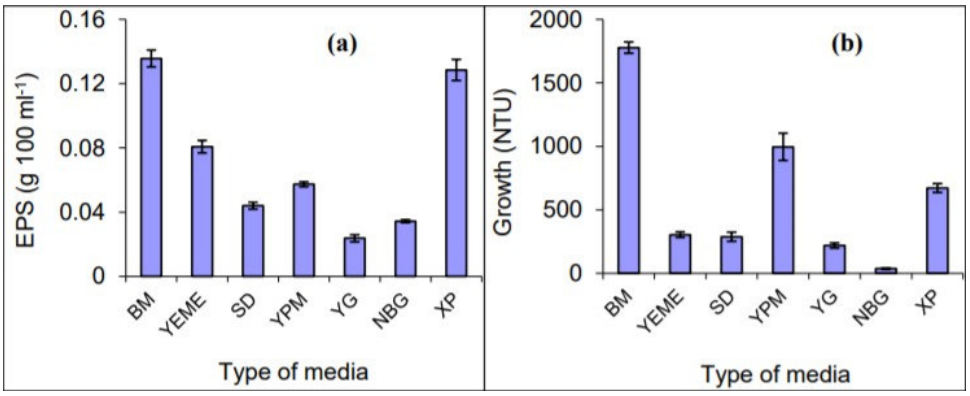
The study of effect of medium type on the production of EPS and growth of *Bacillus subtilis*

Graph 1 represents the different types of media were used for the study of effect of medium type on the production of EPS and growth of *Bacillus subtilis.* The solid basal media was shown the maximum growth of *B.subtilis*. Hence the basal media was found to be selected as the suitable and optimum media for growth and production of EPS by *B.subtilis*. The details of the growth and production *B.subtilis* as shown in table 1.

Table 1: Type of media

TYPE OF MEDIA	EPS(gm/100ml)	Growth(NTU)of *B.subtilis*
BM-Basal Media	0.14gm	1800
YEME -Yeast Extract-Malt Extract Media	0.08gm	300
SD - Sabaroud Dextrose Extract Media	0.04gm	300
YPM- Yeast Extract Peptone Glucose Media	0.06gm	1000
YG- Yeast Extract Glucose	0.02gm	200
NBG- Nutrient Broth Glucose Media	0.03gm	50
XP- Xanthenes Production	0.13gm	800

NOTE: Here, NTU (Nephelo metric turbidity units) are the units of turbidity meter



Graph 1: The effect of medium type on the production of EPS and

Growth of Bacillus subtilis

A) Showing the effect of media on the production of Exopolysaccharides.

B) Showing the effect of media on the growth of *Bacillus subtilis*.

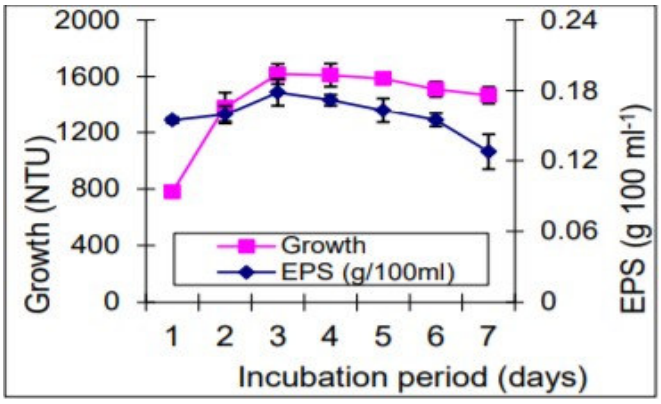
Incubation period

Graph 2 represents the of effect of incubation period on the production of EPS and growth by *Bacillus subtilis*. The increase in EPS production was observed during the growth period of the culture and the maximum production took place up to 72 hours i.e; the 3rd day. After 72 hours, on the 4th day the gradual decline was seen in the production rate. So, the cultures were incubated for 3 days. The details of the incubation period as shown in table No. 2

Table 2: Incubation Period

Incubation Period (Days)	Growth(NTU)	EPS(gm/100ml)
Day1	800	0.15gm
Day2	1300	0.16gm
Day3	1600	0.17gm
Day4	1500	0.16gm
Day5	1400	0.15gm
Day6	1300	0.14gm
Day7	1300	0.12gm

NOTE: Here, NTU (Nephelo metric turbidity units) are the units of turbidity meter



Graph 2: Effect of incubation period on the production of EPS and growth by *Bacillus subtilis*

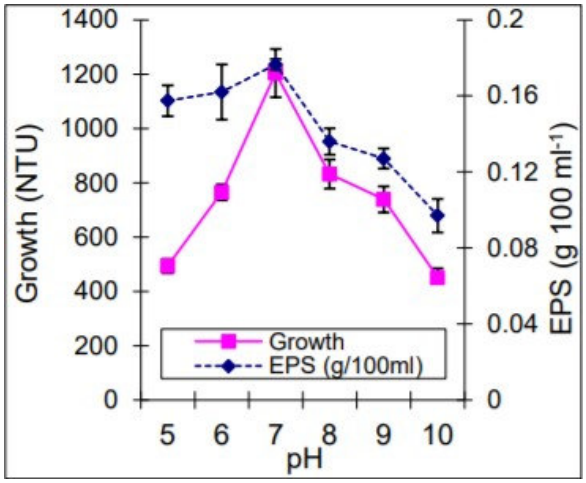
pH Conditions

Graph 3 represents the effect of various pH values on the manufacturing of EPS and and growth by *Bacillus subtilis.* The gradual increase in production of EPS was observed at pH5.0 and 6.0. The maximum growth production of *Bacillus subtilis* was obtained at pH 7.0 and gradually decreased at pH 8.0 to 10.0. Hence the pH 7.0 was observed to be the suitable and optimum pH for the production of EPS. The details of the pH conditions as shown in table No.3

Table 3: pH conditions

Here, NTU (Nephelo metric turbidity units) are the units of turbidity meter

pH Condition	Growth(NTU)	EPS(g/100ml)
pH5.0	500	0.15gm
pH6.0	750	0.16gm
pH7.0	1100	0.18gm
pH8.0	800	0.14gm
pH9.0	700	0.13gm
pH10.0	450	0.10gm



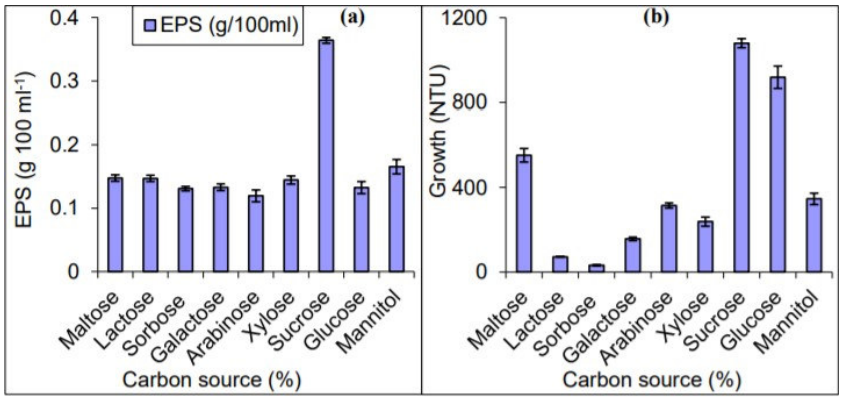
Graph 3: Effect of different pH values on the production of EPS and growth by *Bacillus subtilis.*

Carbon Source

Graph 4 represents the effect of different carbon sources on the (a) EPS production and (b) growth by *Bacillus subtilis.* There is an increase in the production of EPS when sucrose was taken as a carbon source compared to the other carbon sources such as glucose and other. Though it also supports a good production. Out of all the carbon sources sucrose was found to be suitable as it increased the production of EPS. Hence, sucrose is considered as the carbon source. The details of the carbon sources as shown in the table 4

Table 4: Carbon source
Here, NTU (Nephelo metric turbidity units) are the units of turbidity meter

Carbon source (%)	EPS(gm/100ml)	Growth(NTU)
Maltose	0.15gm	500
Lactose	0.15gm	50
Sorbose	0.14gm	20
Galactose	0.14gm	100
Arabinose	0.13gm	300
Xylose	0.16gm	200
Sucrose	0.38gm	1000
Glucose	0.13gm	850
Mannitol	0.17gm	350



Graph 4: Effect of different carbon sources on the (a) EPS production and (b) growth by *B. Subtilis*

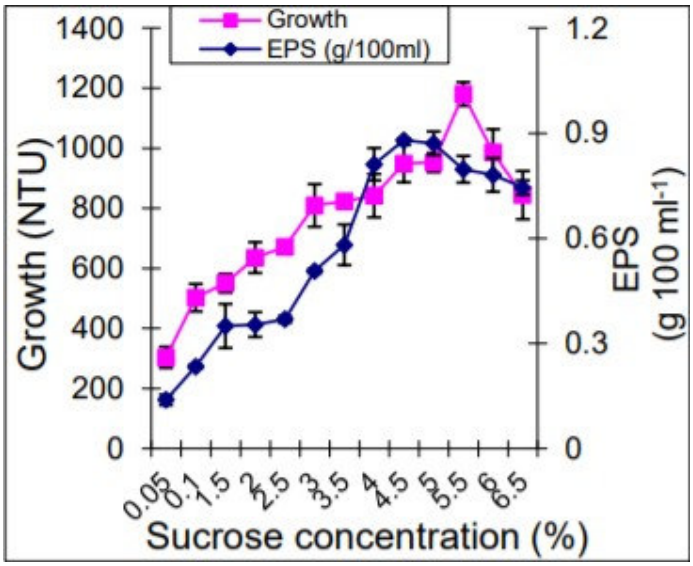
Sucrose Concentration

Graph 5 represents the effect of sucrose concentration on the production of EPS and growth of *B.Subtilis* When the sucrose concentration increased up to 5.5%, the production of EPS also increased and after this concentration, it was observed that the production gradually decreased i.e, after increasing the sucrose concentration more than 5.5%. Hence, the 5.5% concentration of sucrose is considered. The details of the sucrose concentration as shown in the table 5.

Table 5: Sucrose Concentration

Sucrose concentration (%)	Growth(NTU)	EPS(gm/100ml)
0.05 %	300	0.1gm
0.1 %	500	0.2gm
1.5 %	550	0.29gm
2 %	600	0.3gm
2.5 %	610	0.31gm
3 %	790	0.5gm
3.5 %	780	0.6gm
4 %	800	0.7gm
4.5 %	900	0.7gm
5 %	890	0.7gm
5.5 %	1100	1gm
6 %	1000	0.8gm
6.5 %	800	0.7gm

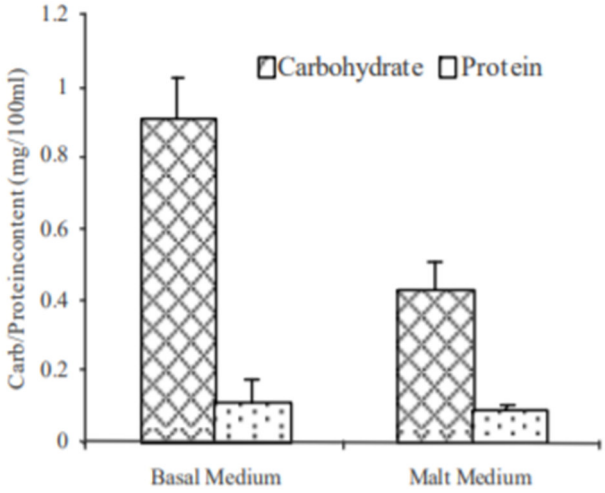
NOTE: Here, NTU (Nephelo metric turbidity units) are the units of turbidity meter



Graph 5: Effect of sucrose concentration on the production of EPS and growth of *B.Subtilis*

Carbohydrate and Protein estimation

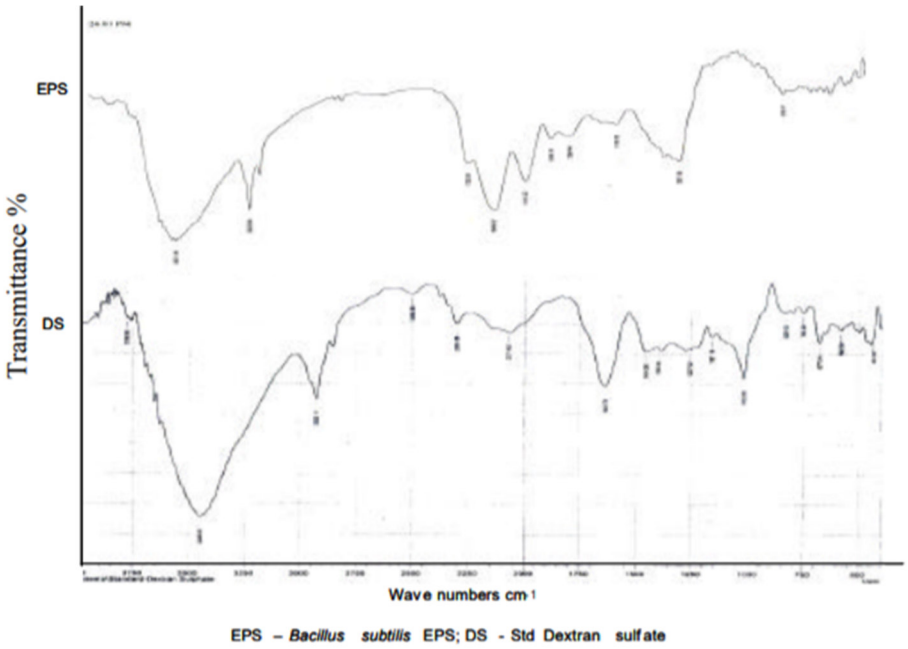
Graph 6 represents the carbohydrate and protein estimation of *B.subtilis* in EPS basal medium and malt medium in which the optical density for carbohydrate was 0.91 ± 0.11 mg/100ml and 0.43 ± 0.08 mg/100ml for basal and malt medium, respectively. Protein was higher for *B. subtilis* in malt medium, where as the optical density was 0.11 ± 0.07 mg/100ml and optical density for *B. subtilis* in malt medium was 0.09 ± 0.02 mg/100ml. Compared to both proteins estimation in bacteria gave higher optical density for the two media.



Graph 6: Carbohydrate and protein estimation

Fourier Transform Infrared Spectrometer

Graph 7 represents the IR spectroscopy of intact basal medium exopolysaccharides (EPS) showed the presence of hydrogen bonded compound, possible acid or amine salt. The bacterial EPS extracts revealed characteristic absorption bands of EPS was observed in the reference compound dextran sulphate sample showed the band at 1000-1500 cmG1 which is characteristic to glucan. The list of the bands at 400-950 cmG1 interval is present. In addition, the spectra showed bands around 1000, 1200, 1400, 1500 and 1600 cmG1 revealed the (1,3) - glucan linkages in addition to the bands in the region of 2900 and 3400 cmG1 chemical bands were presented. Polysaccharides C-O-C and C-O-P was at 1037 cmG1, absorption at 1000 cmG1 was typical for glucose in pyranose form. In the anomeric region (1000-1600 cmG1) the polysaccharides exhibited the obvious characteristic absorption at 1037 cmG1



Graph 7: FTIR Spectrum of bound EPS in *B.subtilis*

Conclusion

Exopolysaccharides produced by microorganisms are secreted from the cell to form a layer over the surface of the organism, and have applications in food, pharmaceutical, and medical fields including vaccines.

Exopolysaccharides (EPSs) play an extensive role as biopolymers in the environment by replacing synthetic polymers as they are degradable, nontoxic, and produced by microorganisms.

The promising results can be regarded as initial steps towards the utilization and modification of exopolysaccharides as future cheap sources for production of valuable drugs with antioxidant and anticancer properties.

As the solid basal media found to be the suitable and optimum media for growth and production of EPS by *Bacillus subtilis.*

The maximum growth was seen up to 3 days and gradual decrease observed from the 4th day. So, the cultures were incubated for 3 days.

In order to choose the suitable pH condition of the growth medium, it is adjusted between 0.5 to 10.0 pH and found that pH 7.0 is suitable and optimum for the EPS production. Hence, pH 7.0 was chosen.

When sucrose concentration increased up to 5.5%, the production of EPS also increased and after this concentration, it is observed that the production gradually decreased i.e, after increasing the sucrose concentration more than 5.5%.

Hence, the 5.5% concentration of sucrose is considered.

Optical density for carbohydrate was 0.91 ± 0.11 mg/100ml and 0.43 ± 0.08 mg/100ml for basal and malt medium, respectively.

Protein was higher for *B. subtilis* in malt medium, whereas the optical density was 0.11 ± 0.07 mg/100ml and optical density for *B. subtilis* in malt medium was 0.09 ± 0.02 mg/100ml.

Polysaccharides C-O-C and C-O-P was at 1037 cmG1, absorption at 1000 cmG1 was typical for glucose in pyranose form. In the anomeric region (1000-1600 cmG1) the polysaccharides exhibited the obvious characteristic absorption at 1037 cmG1.

We studied and produced exopolysaccharides by *Bacillus subtilis* and applications of exopolysaccharides in pharmaceuticals and future studies are required to analyse and access exopolysacharides from *Bacillus subtilis*

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