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Study On: Rheological Characteristics of Exopolysaccharide Obtained From Rhizobium Mayense

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ABSTRACT

Exopolysaccharides (EPS) are high molecular weight polymers with long chain composed of sugar residues and secreted by microorganisms into the surrounding environment. Bacterial EPS has a complex mixture of macro molecular poly electrolytes including carbohydrates, proteins and sometimes nucleic acids. Stem and root nodulating isolate of *Aeschynomene indica* plant was identified by 16S rRNA sequencing as *Rhizobium mayense*. Efficiency of *Rhizobium mayense* for EPS production was studied using Yeast Extract Agar as a basal medium with different carbon and nitrogen sources and the incubation was done at 30^0C for 48 hours. Surface topology of EPS was found smooth by Scanning Electron Microscopy. Alcohol precipitated EPS was further purified using Sephadex G200 and Sephadex G100 gels. The rheological properties of purified EPS were studied by Ostwald's Viscometry and BrookField's Viscometry.

Introduction

Exopolysaccharides are the organic macromolecules often found in the surrounding as the outer most structures of both prokaryotic and eukaryotic cells. They may be closely associated with the cell in the form of discrete capsules or else excreted as a slime layer unattached to the cell surface (1). Bacterial exopolymers are important in the interaction between bacteria and their environment and are chemically diverse (2).

Rhizobium species can secrete outside their cell wall the large amount of long chain, high molecular weight exopolysaccharides (EPS). It is well known that acidic EPS provides the bacteria with hydrophilic and negatively charged coating or network surrounding the cells which in turn provides the protection and mechanical stability to vegetative cells against attacks, lethal drying and other adverse environmental conditions and they are believed to provide self protection against anti microbial substances (3.) , starvation conditions and extreme pH and temperature (4.).

The production of EPS is dependent on the temperature and pH of the medium as well as composition of the culture medium in terms of carbon and nitrogen source, minerals content and fermentation conditions (5).The carbon source used for growth determines both quality and quantity of polysaccharide formation (6, 7) The composition and structure of EPS is varied and consisting of either homopolymers (1,2-beta –glucans or cellulose) or heteropolymers.

The microbial EPS due to their health benefits have been treated as highly potential molecules (8,9) Many interesting physical and chemical properties (e.g. stabilizing, suspending, thickening, gelling, coagulating capability) have found wide range of applications in the fields of textiles, adhesives, paper, paint, food, oil recovery, mining industry. Bacterial growth is often accomplished by production of EPS,that have relevant ecological and physiological functions. The nutrient status and growth phase of surface associated bacteria may influence the quality and the composition of the EPS produced (10).

Materials and Methods

Isolation and Identification

Isolation and identification of the AIRS-I:Rhizobium mayense is the root and stem nodulating bacterium isolated from Aeschynomene indica plant using Yeast Extract Mannitol Agar and was identified by 16S rRNA sequencing.

Surface properties

Topology that is surface properties of the Rhizobiums mayense grown on Yeast extract mannitol agar and Minimal nitrate medium was done by Scanning Electron Microscope (Model Philips XL 30 SEM) at 12-15 KV with a tilt angle of 45^0. The Rhizobium mayense cells were mounted on a stub, coated with gold using sputter coater. Using suitable magnification, micrographs were recorded and surface topography was studied.

Purification of EPS

The column was loaded with Swollen Sephadex G200 gel prepared in 0.01M phosphate buffer pH to form packed bed column. The gel was equilibrated with 0.01M phosphate buffer pH 7.0. The partially ethanol purified EPS fraction was layered on the top of Sephadex G200 column. The column was eluted with same buffer at a flow rate of 0.1 ml/min. The purification of EPS fraction was done using Sephadex G 100 column (Gel Filtration Chromatographic Technique). The polysaccharides purification process was performed with some modification until purified EPS obtained. Purified fractions were collected and again analyzed for carbohydrate and protein contents and stored in freeze-dried conditions.

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Rheological properties

The rheological properties of the crude and purified EPS were determined by Ostwald's viscometer and Brookfield viscometer (Brookfield Engineering Labs Inc., Stoughton MA, USA), equipped with spindle 1 at 60 rpm was used to determine the apparent viscosities of polysaccharide samples at 30°C ±2^{0}C.The polymeric solutions of different concentrations were exposed to high speed at different temperature. The viscosity and the density of the solutions were measured as a function of temperature and speed. The viscosity was measured by using Brookfield DV-II+Pro Viscometer (Brookfield Engineering Labs Inc. Stoughton, MA, USA), which measured the fluid viscosity at a given shear rate. The viscosity measurements of DV-II+Pro Viscometer is in centipoises. The effect of various rpm, cooling temperature, heating temperature, pH, shear stress and shear rate on viscosity of standard Xanthan and EPS obtained by Rhizobium mayense from newly formulated medium containing water melon juice was studied.

Observations and Results

The stem and root nodulating isolate obtained from Aeschynomene indica plant is identified by 16S rRNA sequencing technique. The DNA sequence of AIRS-I isolate was compared with Gene Bank Data Base using BLAST algorithm available from NCBI (11). It showed a 98.64% query coverage as Rhizobium mayense by 16S rRNA with as Accession number of JX855172 in Gene Bank Data Base. The isolate was named Rhizobium mayense. The Sequences of the primer pair used for amplification -

RPP2 – CCAAGCTTCTAGACGGITACCTTGTTACGACTT
FDD2– CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG

The obtained DNA sequence

CGTGGGTATTTGGCAATGGGCGCTAGCCTGATCC
AGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTG
TAAAGCTCTTTCACCGGAGAAGATAATGACGGTATC
CGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGC
CGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAAT
TACTGGGCGTAAAGCGCACGTAGGCGGATCGATCAG
TCAGGGGTGAAATCCCAGGGCTCAACCCTGGAACTG
CCTTTGATACTGTCGATCTGGAGTATGGAAGAGGTG
AGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGAT
ATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTG
GTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGAATGTTAGCCGTCGGGCAGTATACTGTT
CGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGG
GGAGTACGGTC

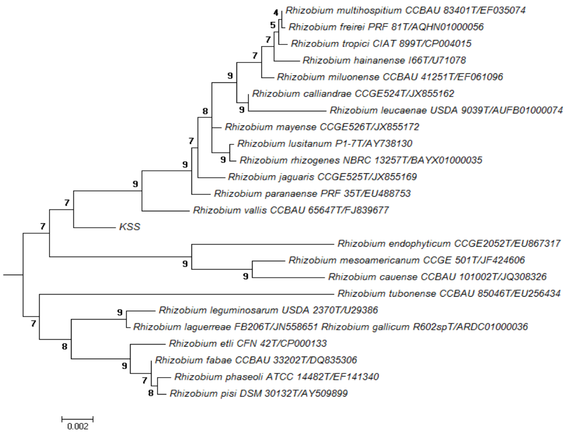

Figure 1. Phylogenetic tree of *Rhizobium mayense* based on 16S rRNA sequence.

Scanning Electron Microscopy was carried out to study the surface topology of a capsular material. The capsular material was with smooth surface topology and compact structure as shown in Figure 2 .Scanning Electron Microscopic images also showed thick capsular material around cells when cells grown on Yeast extract Mannitol Agar and thin capsular material around the cells when grown on Minimal Nitrate medium as shown in Figure 3 and Figure 4 respectively .

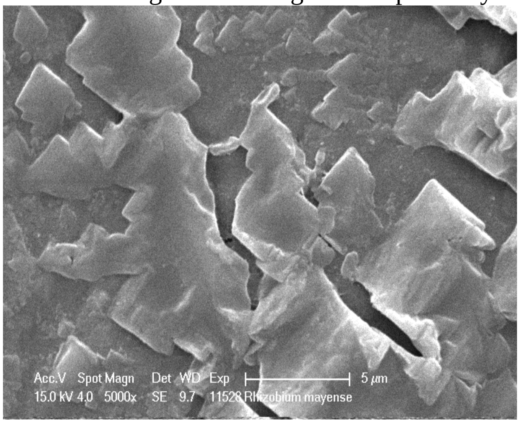

Figure 2. Scanning Electron Micrograph of Smooth Surface topology of EPS.

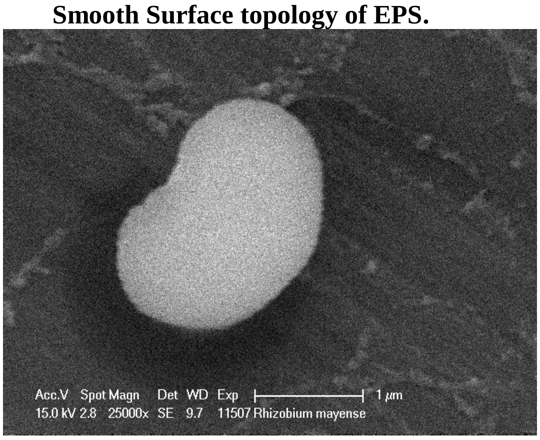

Figure 3. Scanning Electron Micrograph.

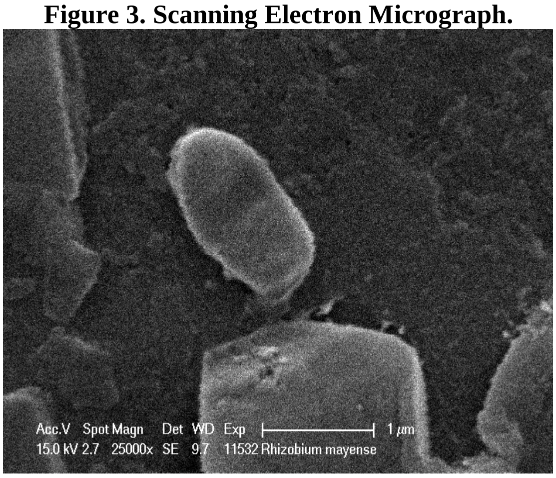

Figure 4. Scanning Electron Micrograph.

Purification of EPS

The elution profile of the EPS purification is depicted in Figure 5. On purification, the purified EPS was free of any protein and nucleic acid contamination. The yield of purified polysaccharides was 20% (w/w) with around 80% polysaccharides purity (1.31% fold purity expressed as glucose equivalence).The molecular weight (Mw) of purified EPS was found to be 1,14,235 Da.

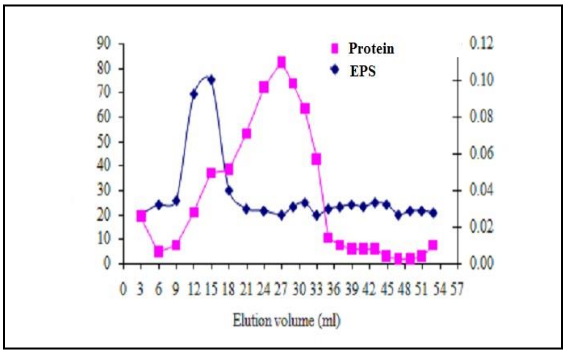

Figure 5. Elution profile of the EPS purification

Effect of modified synthetic media w.r.t. carbon source onviscosity of EPS

Different carbonaceous compounds have been used for the EPS production by *Rhizobium mayense* . Among the various carbon sources screened and each carbon source employed supported the EPS production. The carbon supplement in the medium influences the EPS production. Sugar nucleiotides play an important role in EPS synthesis as activated precursors.The modification with respect to 2.0% carbon source in Yeast Extract Broth was done to determine the viscosity of EPS. Maximum viscosity was 10.7 cps found and was found in Yeast Extract Mannitol Medium as described in Table 1.

Table 1. Effect of Carbon source.

Sr. No.	Carbon source used in YEMB	Viscosity (cps)
1	Mannitol	10.7 cps
2	Glucose	9.9 cps
3	Inositol	8.8 cps
4	Xylose	6.8 cps
5	Sucrose	6.5 cps
6	Arabinose	5.4 cps
7	Sorbitol	4.9 cps

The Brookfield Viscometer (Brookfield Engineering Labs Inc., Stoughton MA, USA), was used with spindle 1 at 60 rpm to determine the apparent viscosities of polysaccharide samples at 30°C.

Effect of Nitrogen source on EPS production and viscosity by Rhizobium mayense

Among the various nitrogen sources screened each organic and inorganic nitrogen source employed supported the EPS production. However maximum EPS production was observed with Yeast Extract followed by Beef extract, Tryptone, Peptone, KNO_2, $NaNO_2$, KNO_3, $NaNO_3$, Ammonium Sulfate. This indicates that Yeast Extract is the best suited nitrogen source for EPS production. The viscosity of EPS was determined by Ostwald's Viscometer as shown in Table 2

Table 2. Effect of Nitrogen source.

Sr. No.	Nitrogen source used in YEMB	Viscosity (cps)
1	Yeast Extract	13.532 cps
2	Beef extract	13.370 cps
3	Tryptone	13.166 cps
4	Peptone	10.638 cps
5	KNO_2	10.077 cps
6	$NaNO_2$	9.151 cps
7	KNO_3	8.816 cps
8	$NaNO_3$	7.784 cps
9	$(NH_4)_2SO_4$	4.625 cps

Rheological studies of EPS

He evaluation of rheological properties was done using Brookfield Viscometer and by measuring the viscosity of 0.5% (w/v) aqueous solution of EPS over a range of different shear rates.

Table 3. Effect of various RPM on Viscosity of 0.5% aqueous Xanthan and 0.5% aqueous EPS.

Sr. No.	RPM	Xanthan (0.5%)			EPS (0.5%)		
		Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)
1	1.0	13600	16592	2156960	100	122	122
2	2.5	7040	8588.8	6046515.2	20	24.4	4.88
3	5.0	4000	4880	195200	10	12.2	1.22
4	10	2300	2806	53084	8.5	10.37	0.88
5	20	1380	1683.6	23233.68	7.0	8.54	0.59
6	30	628	766.16	4811.48	4.3	5.246	0.22

From the above observation table it is indicated that there was reduction in the viscosity of standard water solutions of Xanthan and EPS with increase in RPM. However at varied rpm values 0.5% EPS showed lower viscosity than the standard 0.5% Xanthan. The results of the temperature with respect to heating of EPS solution (0.5%) showed that there was gradual decrease in the viscosity (15.2cps, 9.7 cps, 7.05 cps, 5.4 cps, 3.50 cps, 3.53 cps, 3.01 cps) on exposure to temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C and 90°C).

Table 4. The effect of Heating temperature on viscosity.

Sr. No.	Temperature (°C)	Xanthan (0.5%)			EPS (0.5%)		
		Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)
1	30°C	628	766.16	4811.481	15.2	18.544	2.8186
2	40°C	608	741.76	4509.900	9.7	11.834	1.1478
3	50°C	288	351.36	1011.916	7.05	8.601	0.6063
4	60°C	164	200.08	328.131	5.4	6.588	0.3557
5	70°C	76	92.72	70.467	3.53	4.306	0.1520
6	80°C	72	87.84	63.244	3.50	4.270	0.1494
7	90°C	68	82.96	56.4128	3.01	3.672	0.1105

The results of the temperature with respect to cooling of EPS solution (0.5%) showed that there was gradual increase in the viscosity (3.5 cps, 7.9 cps, 8.5 cps, 9.7 cps, 15.2 cps) on exposure to temperatures (70ºC, 60ºC, 50ºC, 40ºC, 30ºC) as indicated in Table 4.14.3. Increase in the viscosity of EPS started from 70ºC to 30ºC. At 90ºC, 80ºC, the viscosity remained constant at 2.8 cps.

Table 5. The effect of Cooling temperature on viscosity.

Sr. No.	**Temperature (^{0}C)**	**Xanthan (0.5%)**			**EPS (0.5%)**		
		Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)
1	90^{0}C	68	82.96	56.4128	2.8	3.416	0.09564
2	80^{0}C	70	85.4	59.7800	2.8	3.416	0.09564
3	70^{0}C	75	91.5	68.625	3.5	4.27	0.14945
4	60^{0}C	175	231.5	373.625	7.9	9.638	0.76140
5	50^{0}C	280	341.6	956.48	8.5	10.37	0.88145
6	40^{0}C	574	700.28	4019.6072	9.7	11.834	1.14789
7	30^{0}C	598	729.56	4362.7688	15.2	18.544	2.81868

The effect of various pH values on viscosity of EPS solution (0.5%) was done and it was observed that there was increase in viscosity (37.5 cps. 46.5 cps) form pH values 4.0 to 6.0. and at pH 7.4 the viscosity was maximum (52.9 cps) and at pH 8.0 to 9.0 there was decrease in the viscosity (47.7cps, 45.5cps respectively) . This showed that at neutral pH the viscosity of EPS was higher than that of acidic and alkaline pH values .

Table 6. The effect of pH on viscosity.

Sr. No.	**pH**	**Xanthan (0.5%)**			**EPS (0.5%)**		
		Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Viscosity (cps)	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)
1	4.0	280	341.60	956.48	37.5	45.75	17.1562
2	6.0	252	307.44	774.748	46.5	56.73	26.3794
3	7.4	140	170.40	238.56	52.9	64.538	34.14060
4	8.0	96	117.12	112.43	47.7	58.194	27.7585
5	9.0	92	112.24	103.26	45.5	55.51	25.2570

The viscosities were measured at various rpm, heating temperatures, cooling temperatures, pH values and the effect of shear rate on the viscosity was determined. This difference in the rheological behaviour can have several reasons, for example : 1. Different molecular weight and different molecular shape, that is chain length and branching; 2. Solvent polysaccharide interactions; 3. Electrostatic interactions, since all polysaccharides are poly-electrolytes.

Conclusion

The stem and root nodulating isolate obtained from Aeschemomene indica plant is identified as Rhizobium mayense. The capsular material of Rhizobium mayense was with smooth surface topology and compact structure .This indicates that the EPS has a potential of viscosifying agent, a thickner as well as a stabilizing agent for novel food products. The carbon source used for the growth determines both the quality and quantity of polysaccharide formation . The nature of nitrogen supplement in the medium influences the EPS production. EPS production was found to be maximum with organic nitrogenous compounds as compared to inorganic ones.The viscosities difference for various rpm, heating temperatures, cooling temperatures, pH values for between EPS solutions and standard Xanthan increases at higher shear rates. The relationship between shear stress and shear rate of EPS solutions showed there was an increase in shear stress of EPS solutions with increase in shear rate. This indicated the pseudoplastic nature of EPS which was similar to Xanthan.

References

1.P. Vijaybaskar, S. Babinasterlin, T. Shankar, T. Sivakumar and K.T.K. (2011),"Quantification and characterization of Exopolysaccharide from Bacillus subtilis (MTCC 121)" ,Advances in Biological Research 5 (2),Page No. 71-76

2.Nielson, P.H. and Jahn, A., (1999), " Extraction of EPS", In Microbial Extracellular Polymeric Substance:
Characterization, Structure and Function ed. Wingender, J., Neu., T.R. and Flemming,H.C. pp. 50-72, Berlin, Germeny: Springer-VerlagNaurani, G.L., Blondeau, K and Simonet, J.M., (1998), " Influence extraction and purification of EPS from Klebsiella pneumoniae", J. Microbiol Methods 9,211-220

3.Kumon,H., K. Tomoshika, T. Matunaga, M.Ogawa and H.A. Ohmori, (1994), " Sandwich cup method for the penetration assay of antimicrobial agents through Pseudomonas exopolysaccharides" , Microbiol. Immunol., 38:615-619

4. Kim, H.O., J.M. Lim Joo, S.W.Hwang, J.W. Chio and J.W. Yun, (2000), " Optimization of submerged culture condition for the production of mycelia biomass and exopolysaccharides by Agrocybe cylindracea" Bioresource Technol., 96: 1175-1182

5.Gorret,A.U., Maubois, N., Engasser, J.L., Ghoul, J.M., (2001), " Study of the effects of temperature, pH and yeast extract on the growth and exopolysaccharide production by Propionibacterium acidipropionici on milk microfiltrate using a response surface methodology", J. Appl. Microbiol, 90: pg no. 788-796

6.Cerning, J., Renard, C.M.G.C. and Thibault, J.F., (1994), "Carbon source requirements for EPS production by Lactobacillus casei CG11 and partial structure analysis of the polymer", Appli. Environ. Microbiol 60, 3914-3919

7.Naurani, G.L., Blondeau, K and Simonet, J.M., (1998), " Influence extraction and purification of EPS from Klebsiella pneumoniae", J. Microbiol Methods 9,211-220

8.P. Ruass-Madiedo and C.G. de los Reyes-Gavilan " Invited review: Methods for the screening, isolation and characterization of Exopolysaccharide produced by Lactic Acid Bacteria"

9. De Vuyst, L., Degeest, B., (1999), " Heteropolysaccharides produced from Lactic Acid Bacteria",
FEMS Microbiology Reviews:23: 153-177

10.Decho, A.W., (1990), " Microbial Exopolymer secretions in ocean environments; and their role(s) in food webs and marine processes", Oceanography Marine Biology, Annual Review,28:73-153

11.www.ncbi.nlm.nih.gov