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Biochemistry

Production of Exopolysaccharide (EPS) and its Application by New Fungal Isolates SGMP[1] and SGMP[2]

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Abstract

Exopolysaccharide producing fungal cultures were screened from the soil samples collected from New Vallabh Vidyanagar, Dist. Anand, Gujarat (India). The isolates designated as SGMP1 and SGMP2 were found to be significant producers of exopolysaccharide (EPS). The present study shows optimization, characterization and certain applications of EPS produced by selected isolates. The isolates SGMP1 and SGMP2 showed a maximum production of EPS 20.5 ± 0.85 g/l and 7.5 ± 0.4 g/l respectively with supplementation of 2% glucose and starch. Yeast extract was used as a nitrogen source at a concentration of 2% (w/v). The maximum production of EPS 7.5 g/l was obtained for SGMP2. The FTIR analysis of EPS showed the presence of polysaccharides, carboxylic acids and lactone. The fungal EPS showed antimicrobial and antioxidant properties. The EPS produced by the fungal isolate showed 99 % flocculating activity and could also act as an emulsifier. Furthermore, the fungal isolates SGMP1 and SGMP2 were able to remove Al^{+3} and Fe^{+3} up to the 600 mg/l concentration which suffices the role of EPS in bioremediation of heavy metals.

Highlights

- Using optimized media, maximum EPS production 27.5 ± 0.5 g/l and 7.5 g/l was observed for SGMP1 and SGMP2 fungal isolates respectively.
- The FTIR analysis of fungal origin EPS determined the presence of sugars as functional groups.
- The fungal EPS showed high water absorbing capacity which can be served as water absorbing agent.
- The fungal EPS of both the isolates gave antimicrobial activity, antioxidant activity, flocculating activity and also act as emulsifier.
- The SGMP1 and SGMP2 isolates were also able to remove Al^{+3} and Fe^{+3} up to the 600 mg/l concentration.

Keywords: Exopolysaccharide, fungal isolates, antioxidant

The increased demand of natural polymers for various industrial applications in recent years has led to a renewed interest in exopolysaccharide production by microbes. Many microorganisms have an ability to produce exopolysaccharide extracellularly either as soluble or insoluble polymers. Recently many polysaccharides and polysaccharide protein complexes have been isolated from marine algae, lichens and plants. Furthermore, most of them which have various physiological activities originated from fungi, especially from mushrooms (Mahoumad *et al.,* 2004). EPS have ability to fulfill different tasks during the growth on natural substrates, such as adhesion to surfaces, immobilization of secreted enzymes, and prevention of hyphae from dehydration and increased residence time of nutrients inside the mucilage (Bolla *et al.,* 2010).

Many of them contain α-(Pullulan) or β-linked (e.g. Scleroglucan, Schizophyllan) glucose units. The branched β-glucans are biologically active and consequently are used in medicine and biotechnology, as well as additives in food and cosmetics. Cultivation of mushrooms requires several weeks to complete fruiting body formation and thus delaying the production of the required compounds. However, submerged cultivation of fungi offers an advantage for the production of mycelial biomass and bioactive polysaccharides (Zhong and Tang, 2004). The compounds of fungal origin have attracted special attentions from researchers due to their various pharmacological and biological activities, such as antitumor, antidiabetic, antimicrobial and immune-stimulatory. Among these compounds, bioactive polysaccharides isolated from higher basidiomycota are the best known and appear to have strongest anticancer activity among mushroom-derived substances.

The white rot fungi belong to the *Basidiomycetes* family. They are mainly grown on decaying wood material. Wood presents a nutritionally challenging substrate for fungal growth due to its low nitrogen content and the complexity of the bulk carbon present (Bridžiuvienė and Raudonienė, 2013).

EPS production rate and productivity were found varying with environmental conditions and nutrient medium.

Thus, we undertook the present study with following objectives:

- ❒ Screening and isolation of exopolysaccharide producing fungi from natural habitats.
- ❒ To optimize various parameters for high yield of exopolysaccharide.
- ❒ To characterize physico-chemical properties of EPS.
- ❒ To determine various applications of exopolysaccharide produced by newly isolated fungi.

2. Materials and Methods

Media and Chemicals

Malt extract broth and agar were procured from Hi-media Labs. (Mumbai, India).

Basal Medium: (g/l): Glucose 0.5 Yeast Extract 0.5 $NaNO_3$ 2.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_20$ 0.05, KCl 0.5 and pH 5.3.

ABTS (2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid was procured from Sigma Pvt. Ltd. All the other chemicals used were of analytical grade with highest purity.

Micro-organisms and culture conditions

The white rot fungal samples were collected from garden and surrounding area of ADIT Campus, New Vallabh Vidyanagar, Gujarat. The collected fungi were transferred on malt extract agar plate amended with streptomycin (25 µg/ml) and incubated at 28°C for 6-7 days. Potential isolates were sub-cultured and maintained at 4°C on malt extract agar slants.

Culture process and EPS production

Erlenmeyer flasks (250 ml) containing 100 ml of basal culture medium were inoculated with 8 agar plugs of size 7 mm diameter punched from the edge of pre-grown fungal culture on ME plates and incubated on a rotatory incubator shaker (140 rpm) at 28°C for 7 days.

Determination of exopolysaccharide and mycelial biomass

The samples were harvested at different time interval from the shake flasks. The cell biomass was separated by centrifugation at 6000 rpm for 10 min and resulting supernatant was used to determine EPS. For the determination of the EPS (exopolysaccharide), equal volume of isopropyl alcohol was mixed with the cell free supernatant, stirred vigorously and left overnight at 4ºC for precipitation. The resultant precipitates were separated by centrifugation at 6000 rpm for 10 min. The precipitants were dried to a constant weight at 70ºC. The yield of cell biomass and EPS were expressed as the g/l of the culture medium. The precipitates collected (crude EPS fraction) was washed twice with isopropanol and dried to a constant weight at 70°C. The Separated cell biomass was dried at 70ºC in hot air oven till constant weight. The yield of mycelial biomass and EPS were expressed as gram per liter.

Physico-chemical characterization of EPS

In each experiment, one factor was varied by holding other factors constant. Different carbon sources, nitrogen sources and pH were initially studied by employing single factor experiment. The FTIR spectra were characterized using GX-FTIR model, Perkin Elmer, USA spectrophotometer. The dried EPS was mixed with pure KBr powder (1:99) and pressed into pellet for FTIR spectroscopy at a frequency between 4000-450 cm^{-1} (El-dem, 2004). Protein content was determined by the Lowry method using Bovine Serum Albumin as standard. Total carbohydrate was determined by phenol sulfuric method (Dubois *et al.,* 1956) using glucose as standard.

Antimicrobial activity of EPS

To determine the antimicrobial activity of EPS, EPS samples were solubilized in D/W at concentrations of 50 and 100 mg/ml. The antimicrobial activity of EPS was tested against various pathogenic gram-positive (*Staphylococcus aureus)* and gram-negative (*Salmonella typhi, Pseudomonas aeruginosa*, *Escherichia coli*) bacterial sp. by agar well diffusion assay (AWDA) (Nagarajan *et al.,* 2009).

Evaluation of hydrophilic activity of EPS

Hydrophilic activity of EPS was determined in terms of D/W absorption capacity by tea bag method. The EPS sample is placed in a pre-weighed tea bag and the bag is dipped in an excess amount of water at different time intervals. The bag is removed from the D/W, and excess water is drained by hanging the bag until no water drops (Jamil and Ahmed, 2008).

Analysis of Antioxidant properties of EPS

1. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power of EPS samples were determined by using the potassium ferricyanide-ferric chloride method (Liu *et al.,* 2011). Different concentrations of EPS were added to 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50ºC for 20 min, and reaction was stopped with addition of 2.5 ml trichloroacetic acid (10%). An aliquot of the mixture was taken and 0.5 ml 1% (w/v) $FeCl_3$ was added. Upon 30 min incubation, the absorbance is measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

2. Hydrogen peroxide scavenging assay

The free radical scavenging ability of EPS was determined by hydrogen peroxide assay (Malik *et al.,* 2011). Hydrogen peroxide (10 mM) solution was prepared in phosphate buffered saline (0.1M, pH 7.4). 1 ml of EPS sample at various concentrations was rapidly mixed with 2 ml of hydrogen peroxide solution and incubated for 10-60 min. After incubation of 10, 20, 30, 40 and 60 min, the absorbance of the reaction mixture was measured at 230 nm against blank using UV-Visible spectrophotometer (Shimadzu).

3. ABTS Inhibition assay

The free radical scavenging activity of EPS was determined by ABTS (2, 2 azinobis (3-ethylbenzothiazoline-6-sulphonicacid) diamonium salt) radical cation decolourization assay (Li *et al.,* 2009). The reaction mixture was prepared with 2.45 mM potassium persulphate. The mixture was incubated in the dark at room temperature for 12-16 h before use. The radical cations scavenging activity was assessed by mixing 3.9 ml ABTS solution with 0.1 ml crude exopolysaccharide. The final absorbance was measured at 743 nm with spectrophotometer.

Determination of flocculating activity

In 2 ml of EPS samples having concentration of 2.5 mg/ml, 1 ml of 6.8 mM $CaCl_2$ and 10 ml of 5g/l activated charcoal were added. The whole mixture was mixed thoroughly and vortex for 30 sec. The mixture was incubated at room temperature. Control was prepared without adding EPS solution. The upper layer absorbance was measured at 550 nm. Flocculation activity was calculated according to following formula (Abdel-Aziz *et al.,* 2012).

Flocculating activity= [(B-A)/ B]*100*Dilution Factor

Where B = Turbidity of control, A=Turbidity of experimental

Analysis of EPS as emulsifier

In clean glass test tubes, 10 ml of different oils was added. The tubes were inverted and oil were removed in such a way that the portion of oil should stick to the walls of the tube. 1 ml of EPS sample added drop by drop, very slowly in each tube by holding the tube in horizontal position. The inner surface of the test tube observed carefully for dispersion of the oil layer (Jagtap *et al.,* 2010).

Various metal removal efficiency determinations using fungal isolates

To investigate the role of EPS in metal removal, the basal medium was supplemented with various metals salts ($K_2Cr_2O_7$, $FeCl_3$, and $AlCl_3$) at the concentration of 100 mg/ml. The flasks were inoculated with actively grown fungal isolate and incubated at 28°C on shaker at 120 rpm for 24-72 hours. The samples were withdrawn at different time intervals. The samples were centrifuged at 6000 rpm for 10 min to separate cell biomass and supernatants were used to determine metal removal efficiency. For estimation of different metals standard methods were used as described in APHA, (1995).

Effect of initial aluminum and iron concentration on metal removal using fungal isolates

The flasks were inoculated with actively grown fungal isolate and incubated at 28^0C on shaker at 120 rpm for 4 days. The flasks containing basal medium were supplemented with variable aluminum and iron concentration (200, 400, 600 mg/ml). The samples were withdrawn and centrifuged at 6000 rpm for 10 min to separate cell biomass and supernatants were used to determine metal removal at different time interval. The supernatant obtained was used to determine aluminum and iron estimation as mentioned earlier.

3. Results and Discussion

Isolation and Screening of EPS producing fungal cultures

Different fungal cultures were screened out for their EPS producing ability. Six fungal isolates (SGMP1, SGMP2, SGMP3, SGMP4, SGMP5 and SGMP6) were obtained among them maximum production was obtained using fungal culture SGMP1 and SGMP2.

Effect of Different Carbon Sources on EPS Production

Carbohydrates are important nutritional requirement for the growth and development. To determine the role of suitable carbon source on EPS production the basal medium supplemented with different carbon sources, among various carbon sources, maximum EPS production

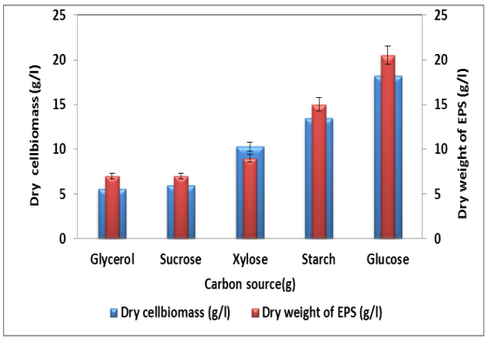

Fig 1(a): Effect of Carbon source on EPS production by SGMP1 fungal isolate

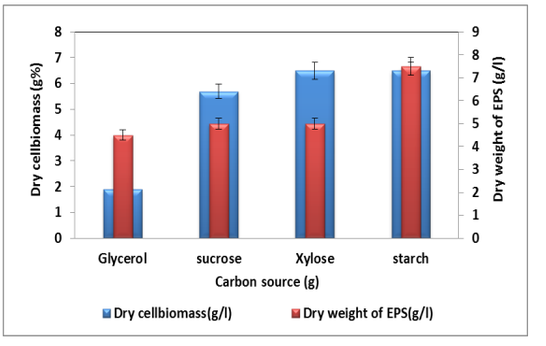

Fig 1(b): Effect of Carbon source on EPS production by SGMP2 fungal isolate

(20.5 g/l) and dry cell biomass (3.1 g/l) were obtained in case of by SGMP1 with glucose as a suitable carbon source as shown in Fig. 1(a). However, SGMP2 isolate showed maximum EPS production and dry cell biomass (7.5 g/l, 6.5g/l) were observed with supplementation of starch (Fig. 1(b)). This indicates supplementation of simple carbon source in one fungal isolate enhances the EPS production while other fugal isolate showed high EPS production with supplementation of complex sugar source. Similarly, Nehad and El-Shamy (2010) reported maximum production (16.3 mg/ml) of EPS using glucose as a carbon source. In contrast significant production of EPS (1.87 g/l) was reported with supplementation of fructose in medicinal fungus *Trametes versicolor* (Bolla *et al.,* 2010).

Furthermore, to determine an effective concentration of carbon source in the flasks were supplemented with variable concentrations. The variation of glucose concentration in nutritional medium showed, 2% of glucose is sufficient to ensure the maximal biomass yield (20.5 g/l) for SGMP1 (Fig. 2(a)). Further increase in glucose/starch concentration leads to decreased EPS production due to feedback inhibition. Jong *et al.,* (2004) reported that using *Sarcodon aspartus* fungal strain showed a maximum EPS production with 2% glucose concentration however further rise in glucose showed decreased EPS production. Whereas as shown Fig. 2(b) shows the fungal isolate SGMP2 gave maximum EPS production 7.9 g/l by using 2% starch as a carbon source. Further increase in starch concentration leads to decreased EPS production, because a high concentration of starch increased medium viscosity. The results obtained in case of fungi SGMP2 are in accordance to the results reported by Hamedi *et al.,* (2007) who described that *Agaricus blazei* utilized 2% starch and gave maximum EPS production.

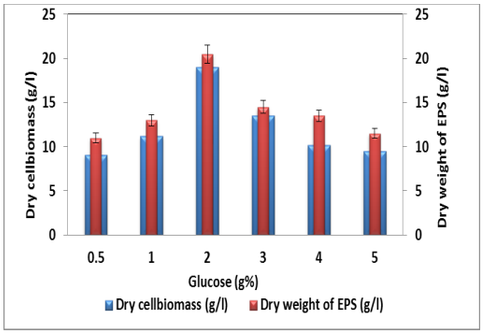

Fig 2(a): Effect of Initial glucose concentrations on EPS production by SGMP1 fungal isolate

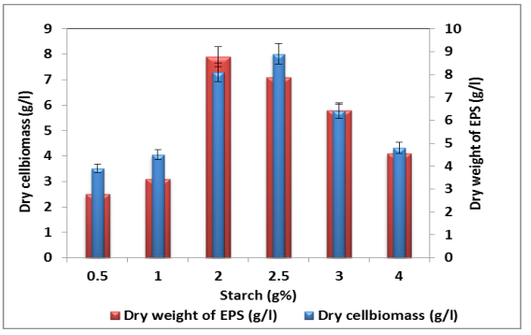

Fig 2(b). Effect of Initial starch concentrations on EPS production by SGMP2 fungal isolate

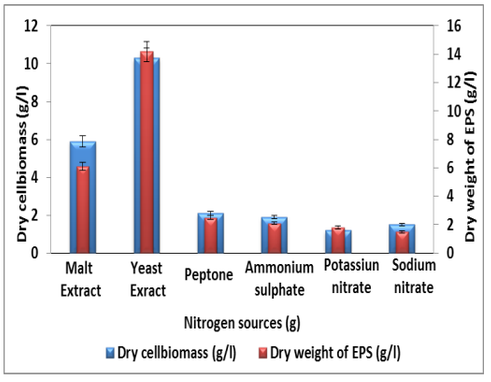

Fig 3(a): Effect of nitrogen source on EPS production by SGMP1 fungal isolate

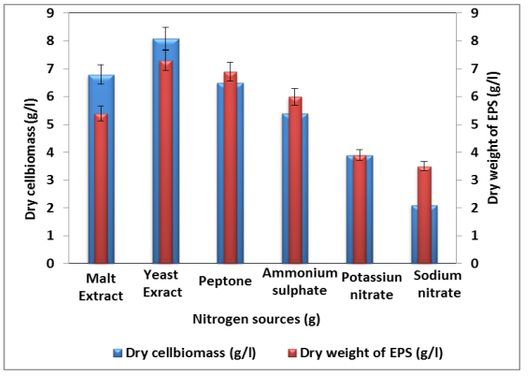

Fig 3(b). Effect of nitrogen source on EPS production by SGMP2 fungal isolate

Effect of Different Nitrogen Sources on EPS Production

Nitrogen is one of the key nutrients for fungus in the synthesis of enzymes involved in the production of both primary and secondary metabolites. To investigate the effect of nitrogen source, both organic and inorganic nitrogen sources were examined. Amongst various nitrogen sources used, yeast extract (organic nitrogen source) was found to be the most significant in influencing mycelial growth and EPS production, while the presence of inorganic nitrogen sources were less effective and showed poor biomass and EPS production (Banerjee *et al.,* 2009). As shown in Fig. 3 (a) and 3(b), the maximum EPS production 21.5 g/l and 7.5 g/l were observed for fungal isolates SGMP1 and SGMP2, respectively. The results obtained are in agreement with those of Huang *et al.,* (2007) who studied the addition of yeast extract in the basal medium showed maximum EPS production 4 g/l in case of *Hericium erinaceus*.

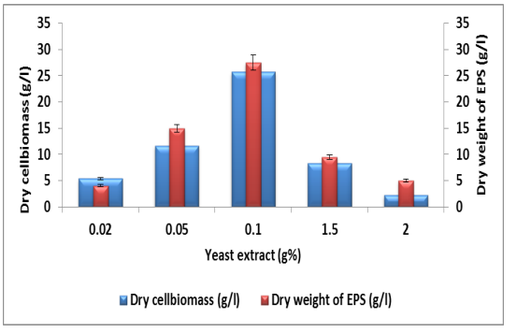

Fig 4(a). Effect of Yeast Extract concentrations on EPS production by SGMP1 fungal isolate

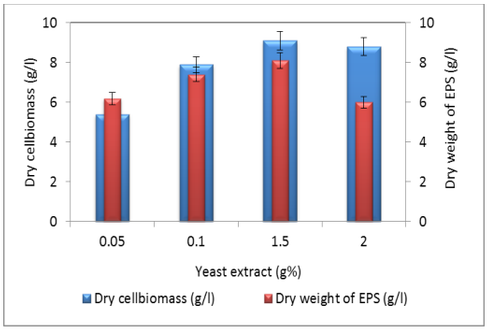

Fig 4(b). Effect of Yeast Extract concentrations on EPS production by SGMP2 fungal isolate

Furthermore, to optimize the yeast extract concentration, medium contained 0.1% of yeast extract showed maximum EPS production as shown in Fig. 4(a) and 4(b). Nehad and El-Shamy (2010) had also described significant EPS production (4.5 g/l) at the concentration of 2 % yeast concentration using fungal culture *A. alternate.*

Effect of pH on EPS Production

The pH of the culture medium is a vital factor that governs mycelial growth and EPS production. To investigate the effect of pH on the EPS production, the initial pH of the medium was adjusted in the range of 3.5 to 6. The result showed at pH 5.5 maximum EPS production and cell biomass of SGMP1 (25 g/l and 21.9 g/l) and SGMP2 (7.5g/l and 6.5 g/l) were observed (Fig 5 (a) and 5 (b)). It indicates that acidic condition favors the EPS production. At high pH value, the EPS production declines sharply as alkaline pH is not suitable for fungal growth. Nguyen *et al.,* (2012) reported 5.29 mg/ml EPS produced at pH 5.5 in medicinal mushroom *Grifola frondosa*. The high yield of EPS and mycelial growth (10.56 g/l and 0.854 g/l) were also reported between pH 5 and 6 in *Hericium erinaceus* (Kim *et al.,* 2002).

The time course study of EPS production

The incubation period is very crucial factor for the EPS production. The time course study of EPS production shows that with an increase in incubation period there is an increase in EPS production. However, EPS production on 4th, 5th and 6th day almost remained constant. The EPS production of SGMP1 and SGMP2 were 20.5 g/l and 7.5 g/l, respectively on 4th day (Fig. 6(a) and 6(b)). Rong L. *et al,* (2010) suggested that *Hirsutella* sp. gives highest EPS production and mycelial biomass (7.94 g/l, 1.12 g/l) on the 4th day of incubation period. The highest EPS production 11.69 mg/ml was observed in *Alternania alternate* on 9th day by Nehad and El -Shamy (2010).

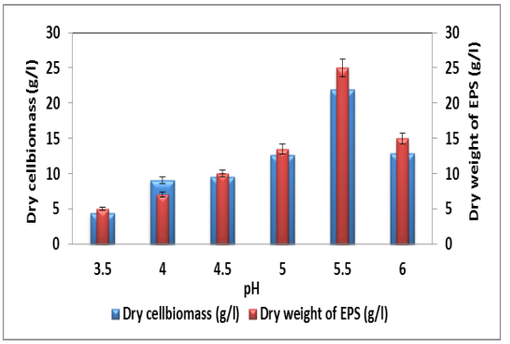

Fig 5 (a): Effect of pH on EPS production by SGMP1 fungal isolate

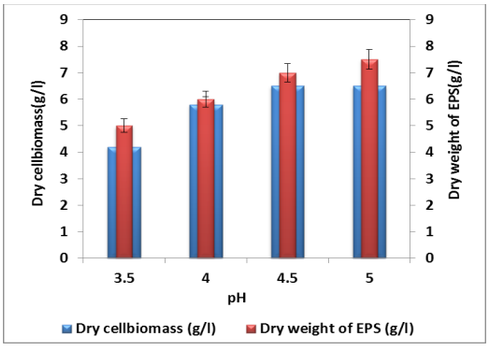

Fig 5 (b): Effect of pH on EPS production by SGMP2 fungal isolate

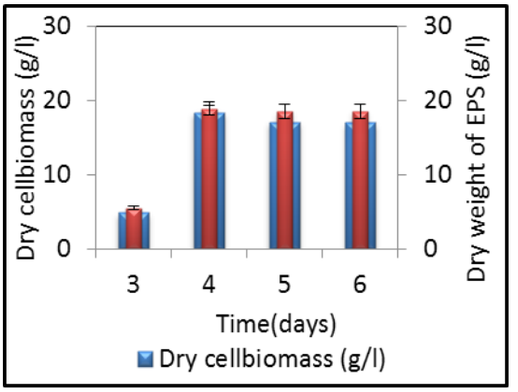

Fig 6 (a): Effect of incubation period on EPS production by SGMP1 fungal isolate

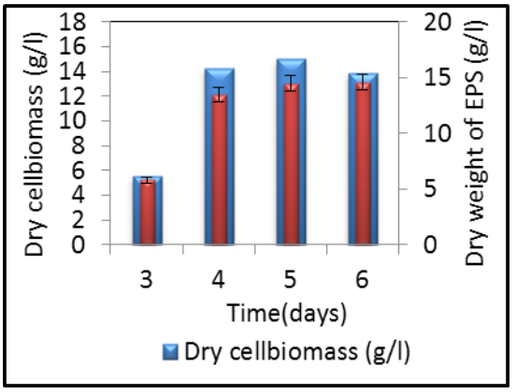

Fig 6 (b): Effect of incubation period on EPS production by SGMP2 fungal isolate

Fourier Transform Infrared Spectrophotometer (FTIR)

IR spectrum of crude EPS obtained from SGMP1 and SGMP2 revealed the characteristic functional groups of EPS (Fig. 7(a) and 7(b)). The sharp peak in the range of 1400-1500 cm^{-1} indicates the presence of polysaccharide, carboxylic acids and lactones which suffices the nature of polysaccharide. In the spectra of SGMP1 EPS, the sharp peak at 1200-1000 cm^{-1} also strongly suggests the presence of phosphorus compounds. In the spectra of SGMP2 EPS, the sharp peak at 1631.45 cm^{-1} strongly suggest presence of vinyl alkenes with C=O stretching.

Antimicrobial activity of EPS

EPS samples of both the fungal isolates were investigated for antimicrobial activity against different pathogenic gram positive and gram negative bacterial strains. As shown in Fig. 8, the zone of inhibition against gram-positive *Staphylococcus aureus* and gram-negative *Salmonella typhi, Pseudomonas aeruginosa*, *Escherichia coli* were observed. Saskiawan (2009) also reported the antimicrobial activity of EPS produced by *P. ostreatus* against *E.coli, B. subtilis* and *S. cerevisiae*.

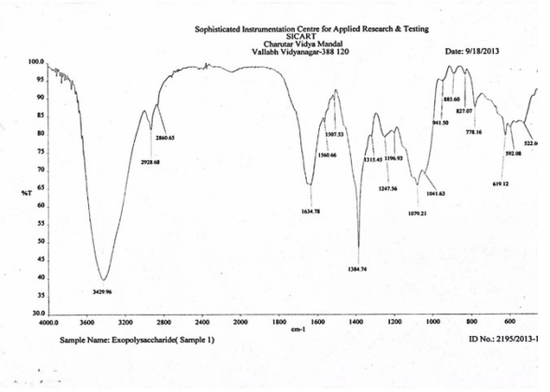

Fig 7 (a): FTIR spectra of EPS produce from SGMP1 fungal culture

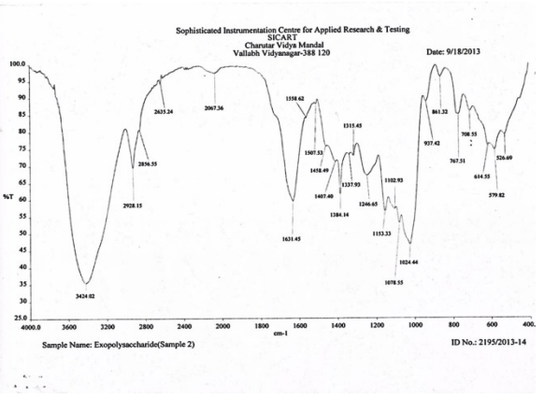

Fig 7(b): FTIR spectra of EPS produce from SGMP2 fungal culture

Fig 8. Antimicrobial activities of EPS against test organism

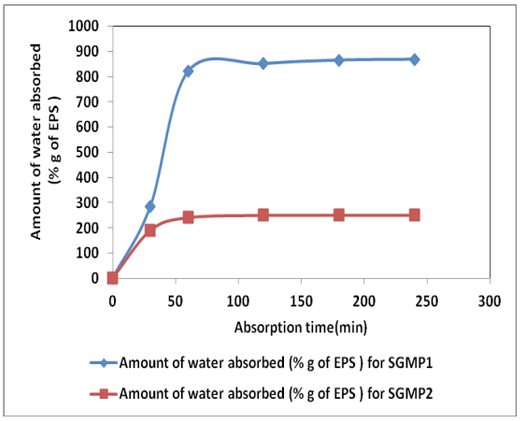

Fig 9. Optimum water absorption capacities of EPS produced by fungal isolates SGMP1 and SGMP2

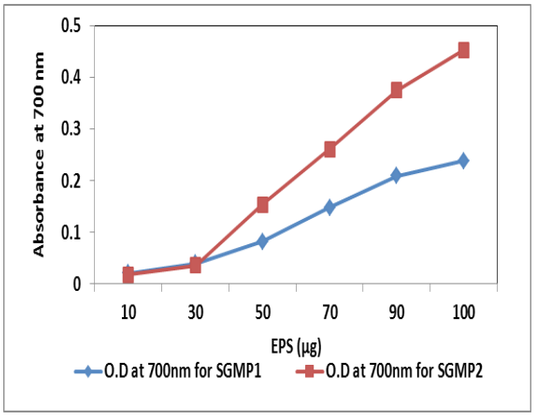

Fig 10(a): Ferric Reducing Antioxidant Power (FRAP) of EPS produce from SGMP1 and SGMP2 fungal culture

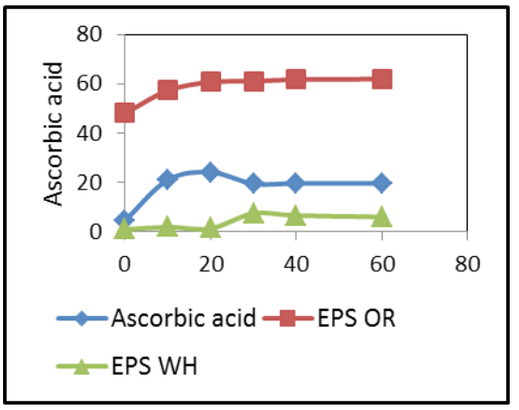

Fig 10(b): Hydrogen peroxide scavenging activity of Ascorbic acid and EPS

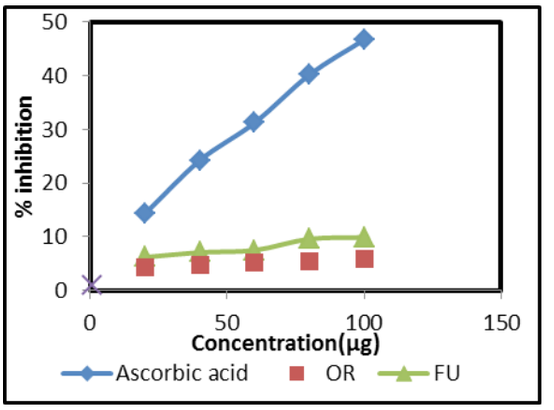

Fig 10(c): ABTS inhibition activity Ascorbic acid and EPS

Evaluation of Hydrophilic activity of EPS

It was observed that EPS of SGMP1 and SGMP2 absorbed 860 and 250 times more water than its own dry weight, respectively. The constant plateau was obtained with further increase in time (Fig. 9). It was showed, that polymer is insoluble compound and can act as a water reservoir. A similar observation was also reported in polymer obtained in *Pseudomonas aeruginosa* from marine sources which showed 400 times water absorption capacity (Jamil and Ahmed, 2008).

Analysis of antioxidant properties of EPS

1. Ferric Reducing Antioxidant Power (FRAP)

The reducing capacities of various concentrations of EPS are compared with standard compound (ascorbic acid). As shown in Fig. 10(a) higher the absorbance, greater the efficiency of reduction. The results obtained by this study revealed that reduction ability of EPS and ascorbic acid were concentration dependent however, reduction ability of EPS was less than standard ascorbic acid. The reducing power of EPS implies that hydroxide groups of

polysaccharides can act as electron donor to react with free radicals to from stable products (Vamanu *et al.,* 2012).

2. Hydrogen peroxide scavenging assay

The hydrogen peroxide inhibition activity of EPS and ascorbic acid was increased with increase in incubation time. The scavenging activity of EPS obtained from fungal isolate SGMP2 was higher than that of the ascorbic acid and the scavenging activity of EPS obtained from fungal isolate SGMP1 was lower than that of the ascorbic acid (Fig.10 (b)). Hydrogen peroxide scavenging activity of EPS obtained from marine filamentous fungi *Keissleriella* sp. YS 4108 was concentration dependent and maximum activity 72 % was observed at a concentration of 133 µg/ml (Li *et al.,* 2012).

3. ABTS Inhibition assay

As shown in Fig.10 (b), the ABTS inhibition activity of EPS and ascorbic acid was increased with increase in concentration of EPS and ascorbic acid. The results indicate that EPS of fungal origin possessed scavenging power for ABTS radicals. Similar type of scavenging power was reported in obtained EPS from edible mushroom *P. ostreatus* (Vamanu *et al.,* 2012) and in thermophilic green alga *Cosmarium* sp. (Challouf *et al.,* 2012).

Determination of flocculating activity

Qin *et al.,* (2007) suggested that EPS could make colloidal and suspended particle in solution which justify the EPS is a good flocculating agent and has a good adsorptive capacity. Particles of charcoal were effectively aggregated and precipitated by the addition of the EPS. With charcoal as the suspended particles, the FA of the crude supernatant reached, approximately, 99% flocculating activity without addition of cations such as Ca^{2+} and Mg^{2+}. However, addition of Ca^{2+} or Mg^{2+} was found to be necessary to achieve maximum FA of fungal EPS produced by *Mucor* sp. (Abdel-Aziz *et al.,* 2012).

Table 1. EPS production of different isolates

Culture	EPS (g/l)	Dry cell Biomass (g/l)
SGMP1	17	7.3
SGMP2	15.25	4.1
SGMP3	4.25	5.4
SGMP4	3.0	1.0

Table 2. Antimicrobial activities of SGMP1 and SGMP2 fungal culture against test organism

Test organism	SGMP1(mm)	SGMP2(mm)
Staphylococcus aureus	3	5
Salmonella typhi	9	5
Escherichia coli	8	7
Pseudomonas aeruginosa	6	8

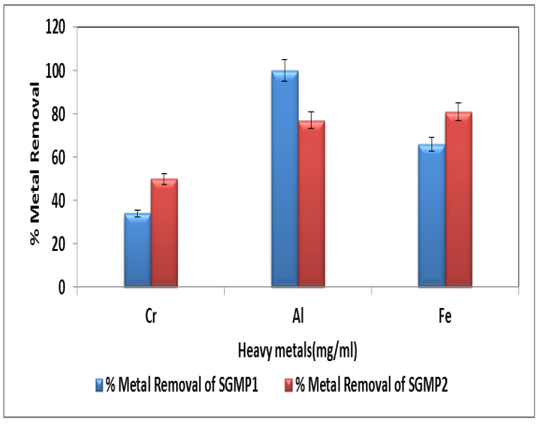

Fig 11(a). various Metal Removal efficiency using fungal isolates SGMP1 and SGMP2

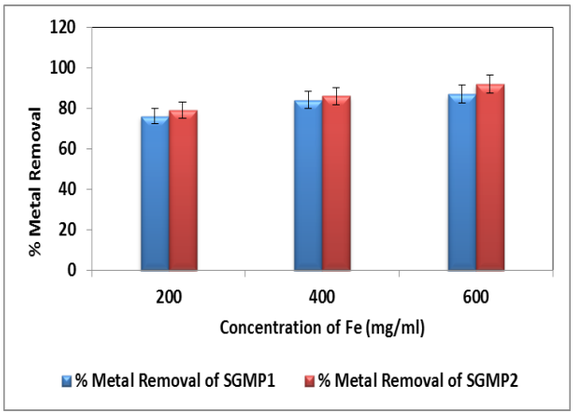

Fig 11(b): Effect of initial Fe^{+3} on Fe removal efficiency using fungal isolates SGMP1 and SGMP2

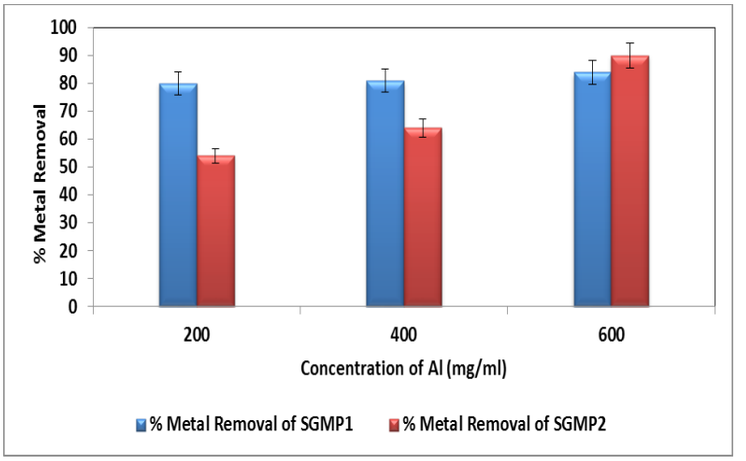

Fig 11(c). Fig 11(c): Effect of initial Al^{+2} on Al removal efficiency using fungal isolates SGMP1 and SGMP2

Analysis of EPS as emulsifier

The emulsifying activity of EPS is determined by its strength of retaining the emulsion of the hydrocarbon in water (Mathur *et al.,* 2010; Jagtap *et al.,* 2010). EPS produce from SGMP1 and SGMP2 fungal culture showed emulsifying activity against coconut oil, sesum oil and cotton oil.

Various metal removals efficiency determination using fungal isolates

The metal removal efficiency was analyzed using different metal salts ($K_2Cr_2O_7$, $AlCl_2$ and $FeSO_4$). The Cr removal efficiency was 34 % and 50 % at the 100 mg/ml concentration of Cr^{+6} for the fungal isolates SGMP1 and SGMP2, respectively. The fungal isolates SGMP1 and SGMP2 were removed 100 % and 77% of Al^{+3}. However, both the isolates remove 66 % and 81 % of the Fe^{+3} at the same concentration (Fig. 11(a)).

Bishnoi *et al.,* (2007) reported that Cr^{+4} removal of *Trichoderma viride* was 16.06 mg/l. Toxic metals bind to biofilm exopolymers, and facilitating metal transport and ameliorating metal toxicity. Thus, binding of heavy metals by EPS is thought to be an important mechanism in the natural detoxification of heavy metal contaminated sites (Salehizadeh and Shojaosadati, 2003).

Effect of initial Fe^{+3}/Al^{+3} on Fe/Al removal using fungal isolates

The maximum Fe removal 87% and 92% were found at high concentration 600 mg/l for SGMP1 and SGMP2 fungal isolates, respectively (Fig. 11(b)). 82.24% and 84.84% of Fe^{+3} removal was reported in *Plerotus sajor-raju* and *Plerotus floridianus* respectively (Prasad and Raleganker, 2013). As shown in (Fig. 11(c)), the maximum Al removal 84 % and 90 % were found at 600 mg/l concentration of Al^{+3} for SGMP1 and SGMP2 fungal isolates, respectively. *Agaricus campester* has been most effective in aluminum removal. Omeike *et al.,* (2013) reported that *Aspergillus oryzae* was removed 45 % of Al at 10-20 mg/l concentration of Al^{+3}.

Conclusions

Mushrooms are known to be a potential producer of EPS. The OFAT method was carried out to determine the effect of carbon source, nitrogen source pH and incubation period for mycelial biomass and EPS production. The FTIR analysis of EPS showed the presence of polysaccharides, carboxylic acids and lactones group. The fungal EPS gave antimicrobial and antioxidant activities. The EPS produced by the fungal isolate showed flocculating activity and could also act as emulsifier. The fungal isolate SGMP1 and SGMP2 were able to remove Al^{+3} and Fe^{+3} which shows that EPS use

in bioremediation of heavy metals. On the basis of above experiments, SGMP1 and SGMP2 can be considered as a significant EPS producer and their potential capabilities can be explored further.

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