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RESEARCH ARTICLE

ANTIBACTERIAL POTENTIAL OF MICROBIAL EXOPOLYSACCHARIDE FROM *GANODERMA LUCIDUM* AND LYSINIBACILLUS *FUSIFORMIS*

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ABSTRACT

Microbial exopolysaccharide are produced by various genera of bacteria and fungi. The maximum EPS production from *G. lucidum* and *L. fusiformis* from both medium were estimation at 89.33 ± 4.04 g/100ml and 84.33 ± 3.58 g/100ml at dry weight in EPS basal medium. The carbohydrate concentration was higher in EPS malt medium for *G. lucidum* 1.63± 0.11mg/ml and *L. fusiformis*1.60± 0.27mg/ml. The protein concentration was higher in EPS basal medium for *G. lucidum* 1.84± 0.23mg/ml and *L. fusiformis*1.27± 0.45mg/ml. The sulfate concentration was higher in EPS malt medium for *G. lucidum* 0.95± 0.05mg/ml and *L. fusiformis* 0.82± 0.12mg/ml. The chemical compositions of EPS from both basal and malt medium. The percentage of elements carbon, hydrogen, nitrogen and sulfur content of EPS from *G. lucidum* and *L. fusiformis*. This study suggested that the EPS from *G. lucidum* and *L. fusiformis* could potentially be used as antibacterial activity.

INTRODUCTION

Exopolysaccharide (EPS) generally contains high molecular weight compounds with charged functional groups and possess both adsorptive and adhesive properties. Due to the presence of charged moieties EPS ideally serves as a natural ligand source by providing binding sites for other charged particles, molecules including metals. The metal binding properties of microbial EPS is well studied and EPS is widely employed in bioremediation of heavy metals including Pb, Ni, Cd, etc. (Dong *et al*., 2000). EPS can successfully be used as food additives to enhance texture. This produces good impact on the development of normal food products with improved appearance, stability and the rheological properties. The EPS have been proved to have good emulsifying activity which is an important property to be exploited in food formulations (Dunne, 2002).

A number of polysaccharides or exopolysaccharides (EPS) from the fruiting body or the culture filtrate of mushrooms, such as *Ganoderma applanatum, Cordyceps* sp, *Lentinus edodes* and *Grifola frondosa* have been reported with some potential pharmaceutical applications. Some kinds of mushroom polysaccharides such as lentinan, schizophyllan, krestin and grifron-D have now commercial applications. Mushrooms in submerged culture are used to produce EPS gradually substitute for solid culture in recent years. It takes several months for the solid culture mushrooms to grow into the fruiting bodies on solid substrates. Submerged culture gave rise to many potential advantages of higher mycelial biomass or EPS production in a compact space and shorter time with less chances of contamination. In fact food manufactures have directly employed EPS of mushrooms by fermentation to prepare drinks and capsules for sale. Recently some studies showed that the compositions of the growth medium can affect the specific rate of EPS synthesis (Lee *et al*., 2004). Kuo *et al*. (2006) *G. lucidum* mycelia stimulated moderate levels of TNF, IL-6 and IFN- release in human whole blood and moderately stimulate cytokine production without potentiating oxide nitric release. The ineffectiveness in inducing oxide nitric release by *G. lucidum* mycelia indicates that the compositions and structures of glucan in mycelia and fruiting body may be different, and this might result in enhancing innate immune response through different receptors or pathways. Zhu *et al*. (2007) *G. lucidum* polysaccharides enhanced the activity of immunological effectors cells in immune suppressed mice and promoted phagocytises and cytotoxicity of macrophages. The above beneficial effects induced by the low-dose of polysaccharide treatment did not result in any side effects. Cao and Lin, (2006) reported that the most attractive property of *G. lucidum* is its antitumor effect, which has been demonstrated to be mainly associated with its polysaccharides, or protein/peptide-bound polysaccharide fraction. *G. lucidum* polysaccharides can suppress the activity of colon cancer cells and they seem to act as a potent chemo preventive agent for colon carcinogenesis (Wu *et al*., 2006). Francis *et al*. (2009) three bacterial strains *Bacillus subtilis* NCIM 2063, *Pseudomonas aeruginosa* NCIM 2862 and *Streptococcus mutans* MTCC 1943 were examined for their exopolysaccharide (EPS) producing ability at the laboratory level. Basal salts solution (BSS), minimal salts medium (MSM), nitrogen free medium (NFM), chemically defined medium (CDM), milk medium (MLM) and sewage from

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different areas were used as nutrient source individually to assess EPS production by the above mentioned bacterial strains. As *Ganoderma lucidum* is very rare in nature, the amount of wild mushroom is not sufficient for commercial exploitation. The potential EPS producing bacteria *Lysinibacillus fusiformis* were isolated, characterized by 16S rRNA sequencing analyses. The main goals of this research work were EPS production in basal and malt medium using *G. lucidum* and *L. fusiformis* in submerged cultivation. To evaluate the potential antibacterial activities of exopolysaccharides (EPS) produced by *Ganoderma lucidum* and *Lysinibacillus fusiformis*.

MATERIALS AND METHODS

Maintenance of the fungus

The fungus mushroom was obtained from Ayya Nadar Janaki Ammal college culture collection bank. The fungal isolation was done by tissue culture method. The mushroom piece was surface sterilized with 0.2% $HgCl_2$ for 5 minutes and then it was plated on potato dextrose agar (PDA) media and it was kept for incubation for 14 days. It was then sub cultured and maintained on potato dextrose agar medium plate and slants stored at 4°C and sub cultured every four weeks.

EPS production from fungal

The fungal culture was used to inoculate in 100 ml liquid media were done using 250 ml flask each containing 100 ml of basal and malt medium. The basal medium contains Glucose-10 gm, yeast extract-3 gm, malt extracts-3 gm, peptone-5 gm, $MgSo_4.7\ H_2O$-1 gm, KH_2PO_4-0.3 gm and 10 mg of vitamin B1 with initial pH 5.2 in 1000 ml distilled water. Malt medium, malt extract-40 gm, peptone-5 gm, distilled water 1000 ml and pH 5.2. The flask was incubated at 25°C on a shaker incubator at 110 rpm for 14 days (Mahendran *et al.,* 2012).

Fungal EPS quantification

After 14 days of incubation both basal and malt medium sample were centrifuged at 5000 rpm for 20 minutes. The EPS was then precipitated from the supernatant by addition of equal volume of methanol. The mixture were agitated with addition of methanol to prevent local high concentration of the precipitate and left over night at 4ºC before centrifuged at 7000 rpm for 20 minutes. After centrifugation the precipitate was collected in Petri plate and dried at 60ºC.

Culture conditions and EPS production from bacteria

Exopolysaccharide producing bacteria was obtained from Ayya Nadar Janaki Ammal College culture collection bank. Bacterial culture was maintained on nutrient agar plates. It was sub cultured and slants were inoculated and maintained at 28ºC for 24 hours. Experiments were done using 250 ml flask each containing 100 ml of basal and malt medium inoculated with the bacterial culture. The basal medium contains Glucose-10 gm, yeast extract-3 gm, malt extracts-3 gm, peptone-5 gm, $MgSo_4.7\ H_2O$-1 gm, KH_2PO_4-0.3 gm and 10 mg of vitamin B1 with initial pH 7 in 1000 ml distilled water. Malt medium, malt extract-40 gm, peptone-5 gm, distilled water 1000 ml and pH 7. The flask was incubated at 28°C on an orbital shaker incubator at 110 rpm for 72 hours (Banerjee *et al.,* 2009).

Bacterial EPS quantification

After 72 hours of incubation basal and malt medium samples were centrifuged at 5000 rpm for 20 minutes (Titus *et al.,* 1995). The EPS was then precipitated from the supernatant by addition of equal volume of methanol. The mixture were agitated with addition of methanol to prevent local high concentration of the precipitate and left over night at 4ºC and centrifuged at 7000 rpm for 20 minutes. After centrifugation the precipitate was collected in a Petri plate and dried at 60ºC. EPS was extracted according to the method followed by Ohno *et al*. (2000).

Estimation of carbohydrate, protein and sulfate content on fungal and bacterial EPS

The total carbohydrate content was estimated by phenol sulphuric acid method proposed by Dubois *et al.* (1956). The amount of protein present in both fungal and bacterial in both EPS basal medium and malt medium was estimated by the Lowry's *et al.* (1951). Sulfate content in was determined by barium chloride gelatin method according to the procedure of Lloyd *et al.* (1961).

Elemental analysis of fungal and bacterial EPS

Carbon, hydrogen, nitrogen and sulfur contents were analyzed using elemental analyzer (Elementar vario EL III, Carlo Erba-1180) at sophisticated analytical instrument facility, Central Drug Research Institute (CDRI) Lucknow, India.

Antibacterial activities of fungal and bacterial EPS

Target microorganisms

Antibacterial susceptibility tests were performed using seven common pathogenic strains which include *E. coli* MTCC741, *S. aureus* MTCC96, *Proteus* sp, *Bacillus subtilis* MTCC121, *Pseudomonas aeroginosa* MTCC741, *Klebsiella* sp and *Bacillus cereus*. All the strains were maintained in nutrient agar slants at 4ºC.

Well diffusion method

Muller Hinton ager plates were prepared in a sterile condition and was inoculated with 0.1 ml of 6 hours culture of the bacterial strain and spread properly throughout the solid media in a Petri dish with the help of a spreader. Agar well diffusion method used to analyze the antibacterial activity. Distilled water was used as control and 20 µl (1mg/ml) samples taken to evaluate the bactericidal activity. Erythromycin disc was used as standard antibiotic. The plates were then incubated at 37°C for 24hours. After incubation, in the case of positive antibacterial activity, the zone of inhibition was measured and expressed in millimeter (mm).

RESULT

Culture process of G. lucidum

The fungus mushroom was obtained from Ayya Nadar Janaki Ammal college culture collection bank. The purity of the fungal strain tested on PDA plate (Figure 1).

EPS production from G. lucidum and L. fusiformis

The *G. lucidum* EPS recovered from both basal and malt medium were 89.33 ± 4.04 mg/100 ml and 79.33 ± 4.04 mg/100 ml of dry weight respectively. The *L. fusiformis* EPS recovered from both

basal and malt medium were 84.33 ± 3.58 mg/100 ml and 76.33 ± 3.58 mg/100 ml of dry weight respectively (Figure 2).

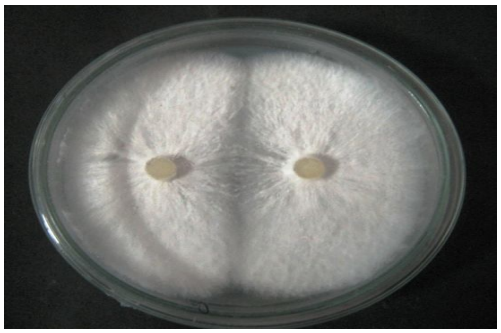

Figure 1 Radial growth of *Ganoderma lucidum* was isolated

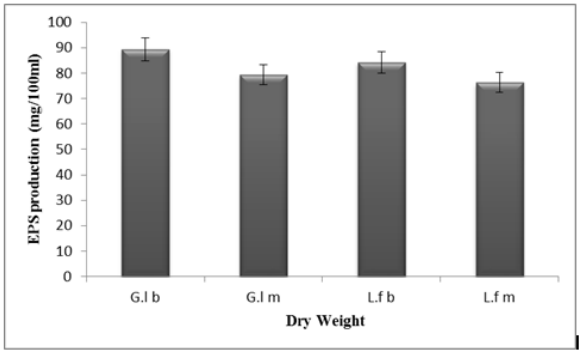

Figure 2 Dry weight of EPS production from *G. lucidum* and *L. fusiformis*

Estimation of chemical constituents of EPS in G. lucidum and L. fusiformis

The carbohydrate concentration of *G. lucidum* was found to be maximum in malt medium (1.63 ± 0.11 mg/ml) and a minimum in (0.25 ± 0.005 mg/ml) basal medium. The carbohydrate concentration of *L. fusiformis* was found to be maximum in malt medium (1.60 ± 0.27 mg/ml) and a minimum in basal medium (0.55 ± 0.04 mg/ml) (Figure 3).

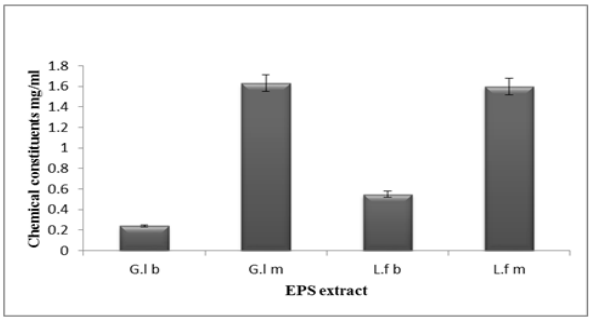

Figure 3 Estimation of carbohydrate content of *G. lucidum* and *L. fusiformis* EPS

The protein concentration of *G. lucidum* was found to be maximum in basal medium (1.84 ± 0.23 mg/ml) and a minimum in (0.24 ± 0.14 mg/ml) in malt medium. The protein concentration of *L. fusiformis* was found to be maximum in basal medium (1.27 ± 0.45 mg/ml) and a minimum in malt medium (0.24 ± 0.11 mg/ml) (Figure 4).

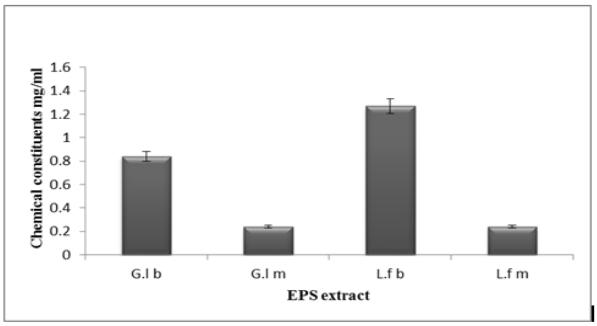

Figure 4 Estimation of protein content of *G. lucidum* and *L. fusiformis* EPS

The sulfate content of *G. lucidum* was found to be maximum in malt medium (0.95 ± 0.05 mg/ml) and a minimum in (0.33 ± 0.011 mg/ml) in basal medium. The sulfate content of *L. fusiformis* was found to be maximum in malt medium (0.82 ± 0.12 mg/ml) and a minimum in basal medium (0.27 ± 0.005 mg/ml) (Figure 5).

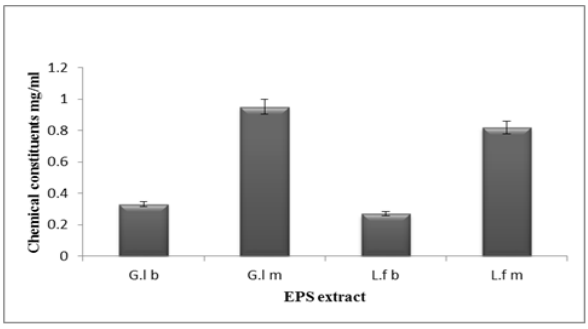

Figure 5 Determination of sulfate content of *G. lucidum* and *L. fusiformis* EPS

Elemental analysis of EPS from G. Lucidum and L. fusiformis

Chemical composition of the EPS from both basal and malt medium were analyzed. The percentage of elemental carbon, hydrogen, nitrogen and sulfur content of EPS from *G. lucidum* and *Lysinibacillus fusiformis* are given in Table 1.

Table 1 Elemental analysis of both *G. lucidum* and *L. fusiformis* EPS

EPS production	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulfur (%)
G. lucidum (Basal)	26.24	6.25	0.152	1.24
G. lucidum (Malt)	29.35	5.72	0.138	1.98
L. fusiformis (Basal)	25.85	5.82	0.124	1.08
L. fusiformis (Malt)	27.12	4.34	0.131	1.83

Antibacterial activity of EPS against pathogens

The antibacterial activity of EPS of *G. lucidum* and *L. fusiformis* was tested against seven bacterial strains such as *E. coli* MTCC741, *S. aureus* MTCC96, *Proteus* sp, *Bacillus subtilis* MTCC121, *Pseudomonas aeroginosa* MTCC741, *Klebsiella* sp and *Bacillus cereus.* Both of the fungal and

bacterial EPS possess potent antibacterial activity against the tested pathogens. *G. lucidum* EPS from both malt and basal medium shows highest activity against the growth of *Bacillus cereus* (23 ± 0.61mm and 18 ± 0.38mm respectively). *L. fusiformis* EPS from both malt and basal medium shows highest activity against the growth of *Klebsiella* sp (18 ± 0.49mm) and *E. coli* MTCC741 (10 ± 0.28mm) respectively. The zone of inhibition was measured in mm and it was given in Table 2.

Table 2 Antibacterial activity of both *G. lucidum* and *L. fusiformis* EPS

Test organisms	Std (mm)	G.l (B) (mm)	G.l (M) (mm)	L.f (B) (mm)	L.f (M) (mm)
E. coli MTCC741	18 ± 0.54	12 ± 0.54	19 ± 0.38	10 ± 0.28	14 ± 0.44
S. aureus MTCC96	22 ± 0.39	8 ± 0.39	12 ± 0.56	5 ± 0.19	10 ± 0.38
Proteus sp	19 ± 0.25	12 ± 0.41	16 ± 0.69	4 ± 0.77	11 ± 0.67
B. subtilis MTCC121	24 ± 0.62	13 ± 0.10	19 ± 0.38	5 ± 0.17	9 ± 0.54
P. aeroginosa MTCC741	18 ± 0.28	7 ± 0.38	10 ± 0.27	3 ± 0.78	6 ± 0.25
Klebsiella sp	16 ± 0.18	8 ± 0.59	9 ± 0.38	6 ± 0.13	18 ± 0.49
B. cereus	20 ± 0.72	18 ± 0.38	23 ± 0.61	7 ± 0.84	15 ± 0.48

Std - Erythromycin; G.l (B)- *G. lucidum* (Basel); G.l (M)- *G. lucidum* (Malt); L.f (B)- *L. fusiformis* (Basel); L.f (M)- *L. fusiformis* (Malt).

DISCUSSION

In present study the recovered EPS from *G. lucidum* from both the medium were estimated at 89.33 ± 4.04 mg/100ml and 79.33 ± 4.04 mg/100ml of dry weight in EPS basal medium and malt medium. In similar studies the production was increased from the two different media of cultivation when maximal production was reached at EPS basal medium fresh weight (8.28 ± 4.38 g/100 ml) and dry weight (6.25 ± 1.45 g/100 ml after that it gradually decreased, the malt medium (6.59 ± 3.29 g/100 ml) fresh weight and (4.35 ± 0.04 g/100 ml) dry weight which is in accordance with the results of Fang and Zhong (2002). Keliang *et al*. (2010) reported the EPS production for two methods for production media and fermentation methods 1.262 g/L and 2.831 g/L respectively. In present study the recovered EPS from *Lysinibacillus fusiformis* both medium were estimation at 84.33 ± 3.5 mg/100ml and 76.33 ± 3.5 mg/100ml of dry weight in EPS basal medium and malt medium. This similar to the result of the EPS in the complex was separated by dissociation of high ionic medium. The isolated EPS form *Bacillus subtilis* MTCC121 both medium were estimated at 2.54 ± 0.23 mg/100ml of fresh weight in EPS basal medium and 1.47 ± 0.17 mg/100ml of fresh weight in malt medium. After dry weight of fractionated product were found to be 1.58 ± 0.13 mg/100ml and 0.82 ± 0.10 mg/100ml in basal and malt medium (Vijayabaskar *et al*., 2011). Vijayabaskar *et al.* (2011) reported the carbohydrate estimation was done *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. In present study the total carbohydrate present in the crude extracts. The carbohydrate concentration of *G. lucidum* was found to be maximum in malt medium 1.63 ± 0.115 mg/ml and a minimum of 0.25 ± 0.005 mg/ml in basal medium. The carbohydrate concentration of *Lysinibacillus fusiformis* was found to be maximum in basal medium 1.6 ± 0.272 mg/ml and a minimum of malt medium 0.55 ± 0.04 mg/ml.

EPS also had protein content in it. The protein concentration of *G. lucidum* was found to be maximum in basal medium 1.84 ± 0.23 mg/ml and a minimum of 0.24 ± 0.14 mg/ml in malt medium. The protein concentration of *Lysinibacillus fusiformis* was found to be maximum in basal medium 1.27 ± 0.45 mg/ml and a minimum of malt medium 0.24 ± 0.11 mg/ml and sulfate content of *G. lucidum* was found to be maximum in malt medium (0.95 ± 0.05 mg/ml) and a minimum in (0.33 ± 0.011 mg/ml) in basal medium. The sulfate content of *Lysinibacillus fusiformis* was found to be maximum in malt medium (0.82 ± 0.12 mg/ml) and a minimum in basal medium (0.27 ± 0.005 mg/ml). Vijayabaskar *et al.* (2011) reported the 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. Compared to both proteins estimation in bacteria gave higher optical density for the two media.

Polysaccharides with high concentrations of charged components such as uronic acids, sulphates and phosphates often form gels in the presence of metal ions and have great potential for removing toxic metals from polluted environments and wastewater as an alternative to other more aggressive physical and chemical methods (Vincenzini and De, 1998). In present study the chemical compositions of the EPS from both basal and malt medium were studied. The *G. lucidum* basal medium percentage of elements carbon 29.35%, hydrogen 6.25%, nitrogen 0.152% and sulfur 1.24% content of EPS. The *Lysinibacillus fusiformis* basal medium percentage of elements carbon 27.85%, hydrogen 5.82%, nitrogen 0.124% and sulfur 1.08% content of EPS. *Ganoderma* exhibits a broad spectrum of antibacterial and antiviral activities, whereas data in human beings are scanty. Antibacterial activities showed by *G. lucidum* shown in may be due to the presence of bioactive substances in these fungi and the solubility of the active compounds in the solvent used (absolute acetone). Different theories have been put forward to explain EPS antimicrobial mode of action especially molecular weight. The first extraction was made through methanol by using the soxhlet apparatus (Russell and Paterson, 2006). The methanolic extract from *G. licudium* was found to possess efficient antibacterial activity Resistant *Staphylococcus aureus* (MRSA). The second organic extraction was made by using the 100 ml each of petroleum ether (20-80˚C) and 80% acetone for 6 hours using the soxhlet apparatus. This is the first investigation reporting the antibacterial activity of the petroleum ether extract from the fruiting bodies of *Ganoderma*. The petroleum ether extracts from *G. licudium* were found to possess some antibacterial activity against basal and malt medium EPS products. In the present study antibacterial activity of EPS from basal medium and malt medium against some bacterial species as in which the maximum zone was obtained for EPS from *G. lucidum* EPS from both malt and basal medium shows highest activity against the growth of *Bacillus cereus* (23 ± 0.61mm and 18 ± 0.38mm) respectively. *L. fusiformis* EPS from both malt and basal medium shows highest activity against the growth of *Klebsiella* sp (18 ± 0.49mm) and *E. coli* MTCC741 (10 ± 0.28mm) respectively.

CONCLUSIONS

In this work, extraction of EPS from *Ganoderma lucidum* and *Lysinibacillus fusiformis* both EPS revealed similar estimation of carbohydrate, protein, sulfate content and antibacterial

activity. Further study in more detail about the structure of the EPS and other biological activities such as antioxidant activities are of interest

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