Optimization of polysaccharide production by *Alternaria alternata*

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Abstract

Six strains of microorganisms were screened for Exopolysaccharide (EPS) production. *Alternaria alternata* was recognized to produce high levels of the EPS. The effects of environmental parameters on polysaccharide production by *Alternaria alternata* were investigated. Results showed the optimum temperature and initial pH for exopolysaccharide (EPS) production in shake flask cultures of *Alternaria alternata* were found to be 30°C and pH 3.0, respectively. Incubation period required for maximum production was 9days. Glucose was found to be the best carbon source to production of EPS, various concentrations of glucose were applied for the screening of the suitability of EPS production. 4% glucose was favorable to production of EPS. Yeast extract gave the highest amount of EPS by *Alternaria alternata,* its maximum concentration for EPS was determined.

Keywords: *Alternaria alternata*, EPS production, physiological conditions

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Citation: El-Shamy, AR., Nehad, EA. (2010): Optimization of polysaccharide production *by Alternaria alternata*. www.Gate2Biotech.com. 1 (9) 1-6

1. Introduction

Fungi are currently of interest because they are a biologically rich source of various active substances. Polysaccharides have emerged as an important class of bioactive substances.

Many types of polysaccharides could be produced by submerged cultures of higher fungi including mushrooms. The polysaccharide have been studied and used for pharmaceutical purposes due to their diverse biological activities (Song *et al.*, 1998; Kim *et al.,* 2005; Cui *et al.,* 2003). These include antitumor and immunomodulating activities (Tokunaka *et al.*, 2000; Bohn and Bemiller, 1995). Their mechanism has also been elucidated (Tsukada *et al.*, 2003; Stanley *et al.*, 2005). Most of the polysaccharides mediating biological activities from mycelia were endopolysaccharides (PPS) or exopolysaccharides (EPS) (Cheung, 1996).

Exopolysaccharides are high molecular weight polymers of monosaccharides (> 20) and are secreted by a microorganism into the surrounding environment. Microorganisms synthesize a wide spectrum of multifunctional polysaccharides including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides or exopolysaccharides (EPS).

Exopolysaccharides have found multifarious applications in various food and pharmaceutical industries. Many microbial EPS provide properties that are almost identical to the gums currently in use. With innovative approaches, efforts are underway to supersede the traditionally used plant and algal gums by their microbial counterparts. Moreover, considerable progress has been made in discovering and developing new microbial EPS that possess novel industrial significance (Suresh and Mody, 2009).

The main purpose of this research point is screening the available local fungal strains for their EPS producing ability and selects the most potent isolate.

Also, to determine the optimum of environmental conditions on the Production of Exopolysaccharide by *Alternaria alternata* in submerged cultures.

2. Materials and methods

2.1 Organism:

Six isolates of fungi namely (*Alternaria alternata, Cladosporium herbarum, Acremonium charticela, Fusarium solani, Penicillium paraphergal and Gliomastic gueg*) were obtained from the Culture Collection unit from Department of Chemistry of Natural and Microbial Products at the National Research Center (NRC). Cultures were maintained on potato dextrose agar slopes. Slants were inoculated and incubated at 30°C for 7 days, and stored at 4°C.

2.2 Shake flask culture:

Shake flask cultures were carried out in 250 ml Erlenmeyer flasks containing 50 ml of medium. The media consisting of the following components (in g/l): glucose, 40; yeast extract, 1.0; peptone 0.5 ; KH_2PO_4 0.5; $MgSO_4.7H_2O$ 0.5. Media were sterilized at 121°C for 20 min . The pH was adjusted to 6.5. The flasks were incubated on a rotary shaker at 28 °C and 150 rpm for 2 weeks.

2.3 Extraction and assay of EPS:

The mycelia biomass was separated from the liquid medium by centrifugation (4000 rpm, 15 min) and the supernatant was filtered through a Whatman filter paper No.1. The supernatant was collected and mixed with 5 volumes of 95% ethanol (v/v) and left overnight at 4°C for polysaccharide precipitation. The precipitate was collected as the crude EPS fraction. The concentration of EPS was determined by Phenol-sulfuric acid method (Chaplin and Kenned, 1986). The absorbance of the sample was measured at 490nm and calibrated to total sugar content using glucose as a standard.

2.4 Optimization of the culture conditions:

To evaluate maximum production of crude EPS produced by fungi, The following criteria were studied.

a. Incubation period: The culture media are incubated for different incubation periods 3, 4, 7, 9, 10 and 14 days at 30°C on an incubator shaker 150 rpm
b. Initial pH: The selected fungal isolate cultivated in fermentation medium at different pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) at 30°C.
c. Temperature: The fungal culture was incubated at 26, 28, 30, 32, and 35°C.
d. Carbon sources: Different carbon sources were examined for the production of EPS. Those carbon sources were glucose, molasses, starch, lactose, fructose, sucrose, xylose and cellulose.
e. Nitrogen sources: Different nitrogen sources were examined for the production of EPS. Those nitrogen sources were yeast extract, peptone, corn steep liquor, malt extract, beef extract, tryptophan urea, casein, soybean and $NaNO_3$ as sole sources of nitrogen.

3. Results and discussion

3.1 Production of Exopolysaccharide (EPS) by different fungal strains:

The purpose of this experiment was to study the best fungal strain which produce high level of EPS, we used six different strains from the culture collection unit of NRC namely *Alternaria alternata, Cladosporium herbarum, Acremonium charticela, Fusarium solani, Penicillium paraphergal and Gliomastic gueg.*

The results in Fig. (1) showed that *Alternenia alternata* was the best Strain for the production of EPS (6.58 mg/ml). Also Yang and Liau, (1998); Gao and Gu, (2007); Leung *et al.*, (2009) produced EPS from *Gonoderma lucidum*, *Aganicus brasiliensis*, *Cordyceps sinensis*, respectively.

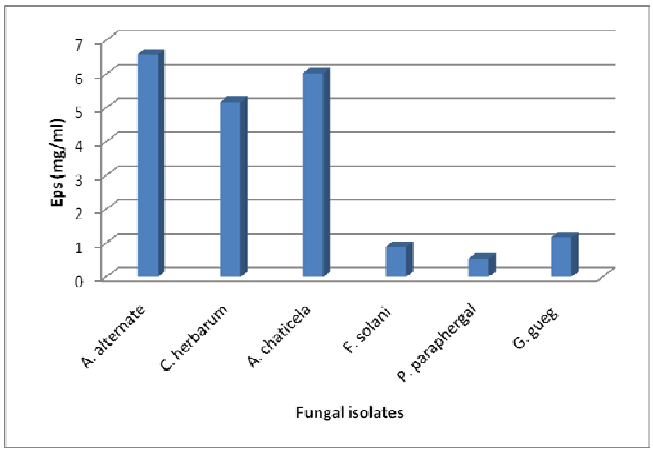

Fig (1): production of Exopolysaccharide (EPS) by different fungal strains.

3.2 Effect of incubation period on (EPS) production by Alternania alternata:

Production of EPS during different incubation periods was studied by *A.alternata* using a culture medium consist of the following components (ing/l): glucose, 40; yeast extract, 1.0; peptone 0.5; KH_2PO_4 0.5; $MgSO_4.7H_2O$ 0.5. The results in Fig. (2) showed the expolysaccharide accumulation by *Alterneria alternata* in the basal medium of shake cultures. We observed a rapid increase in the exopolysaccharide concentration within the first 9 days. The exopolysaccharide concentrations were the highest at day 9 and give a corresponding maximum value 11.96 mg/ml after that this value decreased. Thus we performed all of the following fermentations for 9 days, optimizing the condition in the flask culture. Rong *et al.*, (2010) found that 4 days incubations is the best for exopolysaccharide production with *Hirsutella pat*.

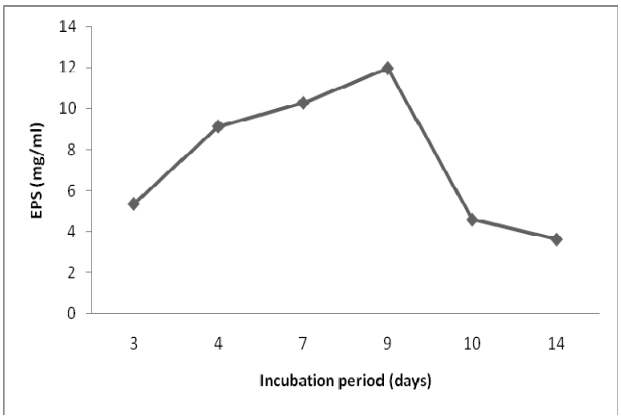

Fig (2): The effect of different incubation periods on Exopolysaccharide (EPS) production by *Alternaria alternata.*

3.3 Effect of intial pH on (EPS) production by Alternania alternata:

The pH of the culture medium is a vital factor that governs mycelial growth and exopolysaccharide production.

The results in Fig. (3) showed that the optimal pH for exopolysaccharide production was 3 and at higher values of pH the exopolysaccharide production declined sharply, other researchers have a reported, optimum pH of 5.5 for other exopolysaccharide synthesizing fungi such as *Pleurotus pulmonarius* (Nour *et al.*, 2004).

Rong *et al.*,(2010) found that the optimum pH for exopolysaccharide by *Hirsultella pat* is 5.5

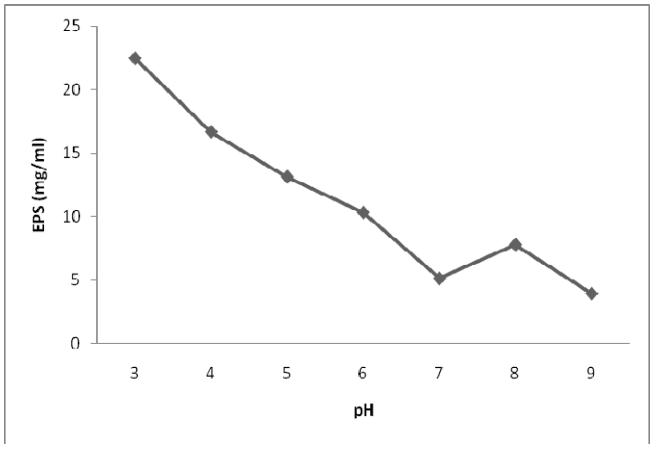

Fig (3): The effect of initial pH value of the medium on Exopolysaccharide (EPS) production by *Alternaria alternata*

3.4. Effect of different temperature on (EPS) production by Alternania alternata:

The results in Fig. (4) shows that the optimal temperature for polysaccharide formation by *Alternaria alternata* was found to be 30°C. The production rate of polysaccharide decreased rapidly above this value.

Yang and Liau (1998) proved that a range between 30°C and 33°C was found to be suitable for EPS production by *Gonoderma lucidum.*

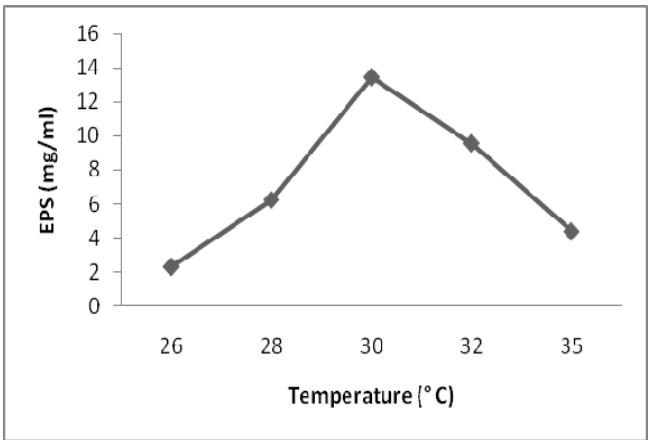

Fig. (4): Effect of temperature on production of Exopolysaccharide (EPS) *by Alternaria alternata.*

3.5 Effect of different carbon source of on EPS production by Alternaria alternata:

Carbohydrates are a major component of the cytoskeleton and an important nutritional requirement for the growth and development of higher fungi (Xiao *et al.*, 2006).

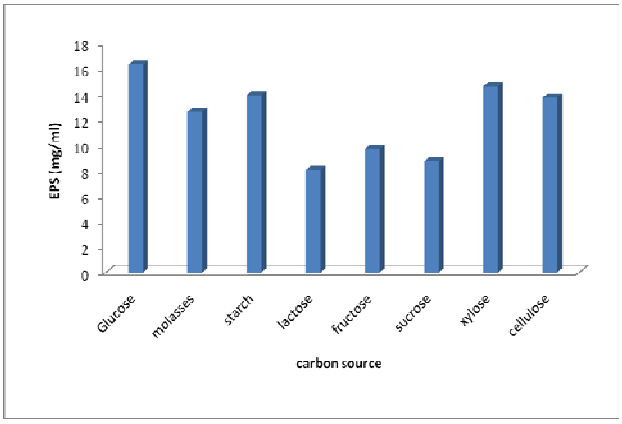

Fig (5): Effect of different carbon sources on EPS production by *Alternaria alternata.*

The effects of carbon sources on the production of EPS were studied in the fermentation medium containing various carbon sources. The results in Fig. (5) showed that glucose is the best carbon source for ESP production using *Alternaria alternata* giving 16.3mg/ml followed by Xylose which gave 14.6mg/ml. it was reported that sucrose the most suitable carbon source for mycelium growth production in *Cordyceps militaris* (Park *et al.*, 2001), while glucose was selected for *Phellinus gilures* (Hwang *et al.*, 2003; Xu *et al.*, 2003). Maltose was also known as an efficient carbon source for EPS production by mushrooms (Bae *et al.*, 2000; Wu *et al.*, 2003). Starch was chosen as carbon source for *Pleurotus tuberregium* production. Various concentration of glucose (1-5%) were applied for the screening of the suitability of polysaccharide production. 4% glucose was significantly desirable at achieving maximum production of EPS (Fig. 6).

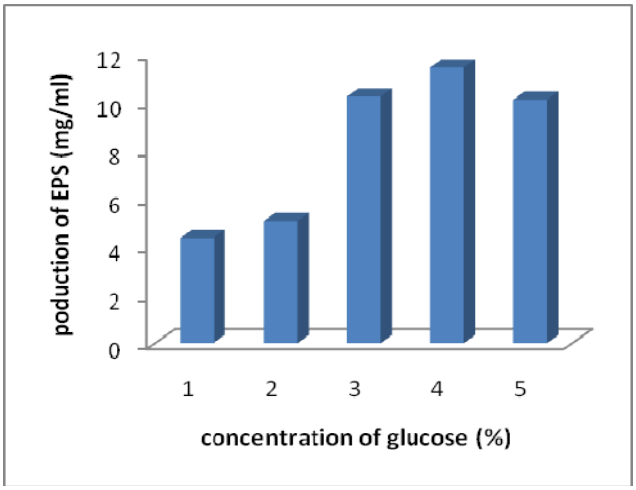

Fig (6): Effect of different concentration of glucose on EPS production by *Alternaria alternata.*

3.6 Effect of different nitrogen sources on EPS production by Alternaria alternata.

As illustrated in Fig. (7) the effects of nitrogen sources on the production of EPS were studied in the fermentation medium containing various nitrogen sources. Yeast extract followed by $NaNO_3$ were the best sources of nitrogen for EPS production giving 25.3 and 22.13 mg/ml respectively, the stimulatory effect of yeast extract is due to its protein, amino acid, and vitamin content (Bolton and Blain, 1982). The highest levels of EPS were obtained when peptone was used as a sole nitrogen source by *Hirsutella* sp. (Rong *et al.*, 2010).

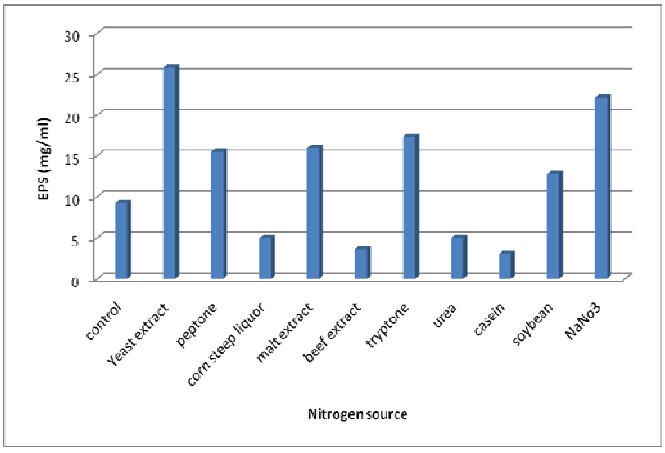

Fig.(7): Effect of different nitrogen sources on EPS production by *Alternaria alternata.*

Various concentrations of yeast extract, 0.5%, 1%, 1.5% and 2% were applied to identify the suitable concentration for polysaccharides production. A yeast extract of 2% concentration stimulate the maximum EPS production (4.5 mg/ml). This results were in agreement with (Pokhrel and Ohga, 2007) who proved that 2% yeast extract was the best for maximum EPS production by *Lyophyllum decastes*, *Pleurotus pulmonarius*.

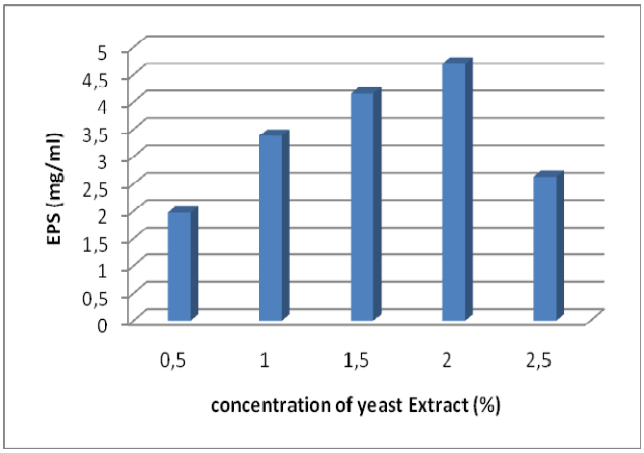

Fig.(8): Effect of different concentration of yeast Extract on EPS production by *Alternaria alternata.*

5. Concolusion

Alternaria alternata was selected from six types of fungi for maximum production of Exopolysaccharides. Our study concentrated on selecting a suitable duration time, nutrient source, their concentrations, initial pH and temperature. the optimum temperature and initial pH for exopolysaccharide (EPS) production in shake flask cultures of *Alternaria alternata* were found to be 30°C and pH 3.0, respectively. Incubation period required for maximum production was 9days. 4% glucose was favorable to production of EPS.

References:

Bae, J.T.; Sinha, J.; Park, J.P.; Song, C.H and Yun, J.W. (2000). Optimization ofsubmerged culture conditions for exo-biopolymer production by *Paecilomyces japonica.* J. Microbiol. Biotechnol. 10: 482-487

Bohn ,J.A.; Bemiller, J.N.(1995). (1–3) d-Glucans as biological response modifiers: a review of structure functional activity relationships. Carbohydrate Polym.; 28:3–14.

Bolton, W. and Blair,R. (1982). Poultry nutrition (Ministry of agriculture, fisheries and food reference book174)(4th ed). London: Her Majesty's Stationery Office (pp.115-121).

Chaplin,M.F. and Kennedy, J.F. (1986). Carbohydrate analysis: Apartical Approach oxford university press, Oxford,UK, pp.1-2.

Cheung ,P.C.K.(1996). The hypocholesterolemic effect of extracellular polysaccharidefrom the submerged fermentation of mushroom. Nutri. Res.;16:1953–1957.

Cui. J.; Chisti, Y. (2003). Polysaccharides of *Coriolus versicolor*: physiological activity, use and production. Biotechnol Adv.; 21:109– 22.

Gao, H. and Gu, W.Y. (2007). Optimization of polysaccharide and ergostrol production from *Agaricus brasiliensis* by fermentation process. Biochemical Engineering Journal, 33:202-210.

Hwang, H.J.; Kim, S.W.; Xu, C.P.; Choi, J.W and Yun, J.W. (2003). Production and molecular characteristics of four groups of exopolysaccharides from submerged culture of Phellinus gilvus. J. Appl. Microbiol. 94: 708-719

Kim, Y.O.; Han, S.B.; Lee, H.; Ahn, H.J.; Yoon, Y.D.; Jung, J.K.(2005). Immunostimulating effect of the endo-polysaccharide produced by submerged culture of *Lnonotus obliquus*. Life Sci., 77:2438–56.

Leung, P.H.; Zhao,S.; Ho,K.P. and Wu,J.Y.(2009).Chemical properties and antioxidant activity of exopolysaccharide from mycelia culture of *Cordyceps sinensis* fungus CS-HK1. Food Chemistry, 114:1251-1256.

Nour, D.M.; Fallal, A.A.; Shahat, A.T. and Hereher, F.E.(2004). Exopolysaccharides production by *Pleurotus pulmonarius*: factors affecting formation and their structures. Pak. J. Biol. Sci. 7: 1078-1084.

Park, J.P.; Kim, S.W.; Hwang, H.J. and Yun, J.W. (2001). Optimization of submerged culture conditions for the mycelial growth and exobiopolymer production by *Cordyceps militaris*. Lett. Appl. Microbiol. 33: 76-81.

Pokhrel,C.P. and Ohga,S. (2007). Synthetic cultivation of *Lyophyllum decastes* on a combination of livestock compost and corn cob. Mushroom Science and Biotechnology,15.

Rong, L. Xiao, L.J. and Guan, H.S. (2010). Optimization of mycelium biomass and exopolysaccharides production by *Hirsutella sp.* In submerged fermentation and evaluation of exopolysaccharides antibacterial activity. African Journal of Biotechnology Vol. 9 (2), pp. 195-202.

Song, C.H.; Jeon, Y.J.; Ra KS, Kim HI. (1998). Anti-complementary activity of endopolymers produced from submerged mycelial culture of higher fungi with particular reference to *Lentinus edodes*. Biotechnol Letter, 20:741–4.

Stanley. G.; Harvey, K.; Slivova.; Jiang.; Sliva ,D.(2005). *Ganoderma lucidum* suppresses angiogenesis through the inhibition of secretion of VEGF and TGF-1 from prostate cancer cells. Biochem Biophys Res Commun.;330:46–52.

Suresh and Mody (2009). "Microbial Exopolysaccharides: Variety and Potential Applications". *Microbial Production of Biopolymers and Polymer Precursors*. Caister Academic Press. ISBN 978-1-904455-36-3.

Tokunaka, K.; Ohno, N.; Adachi ,Y.; Tanaka, S.; Tamura ,H.; Yadomae T.(2000). Immunopharmacological and immunotoxicological activities of a watersoluble (1–3)d-glucan CSBG from *Candida* spp. Int J. Immunopharm ;22:383–94.

Tsukada C, Yokoyama H, Miyaji C, Ishimoto Y, Kawamura H, Abo T.(2003). Immunopotentiation of interaepithelial lymphocytes in the intestine by oral administrations of glucan. Cell Immunol.; 221:1–5.

Wu, J.Z.; Peter, C.K.; Wong, K.H. and Huang, N.L. (2003). Studies on submerged fermentation of *Pleurotus tuberregium* (Fr.) Singer-Part 1: physical and chemical factors affecting the rate of mycelial growth and bioconversion efficiency. Food Chem. 81: 389-393

Xiao, J.H.; Chen, D.X.; Wan, W.H.; Hi, X.J.; Ying, Q. and Liang, Z.Q. (2006). Enhanced simultaneous production of mycelia and intracellular polysaccharide in submerged cultivation of *Cordyceps jiangxiensis* using desirability functions. Process biochemistry, 41: 1887-1893.

Xu, C.P.; Kim, S.W.; Hwang , H.J.; Choi, J.W. and Yun, J.W. (2003). Optimization of submerged culture conditions for mycelial growth and exo- Biopolymer production by *Paecilomyces tenuipes* C240. Process Biochemistry, 38:1025-1030.

Yang,F.C. and Liau, C.B. (1998). The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. Process Biochemistry, 33:547-553.