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Optimization of Submerged Culture Conditions for Exo-Polysaccharides Production by Streptomyces Nasri-UV 135 in Bioreactor

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Abstract

Production of Exopolysaccharides "EPS" and microbial biomass by *Streptomyces nasri* were influenced by the type of carbon source (glycerol, xylose, fructose, galactose, glucose, mannose, sucrose, lactose, maltose, dextrin, soluble starch, corn starch and potato starch) and the nitrogen source (glycine, aspartic acid, glutamic acid, proline, ammonium sulphate, sodium nitrate and beef extract) used in the medium.

Xylose and glycine were the most suitable sources of carbon and nitrogen, respectively, for both production of EPS and mycelial growth. The highest EPS production was obtained in a medium containing (g/l) 30 xylose, 2.7 glycine, 4.0 NaCl, 0.5 $MgSO_4$, 1.0 K_2HPO_4, and 1.0 $CaCO_3$. Exopolysaccharides production and mycelial growth in the above suggested medium were significantly increased in a 3-l stirred tank bioreactor, where the maximum EPS concentration was 8.73 g/l, which was an approximately 1.6 time higher than that in shake culture.

Keywords: Exopolysaccharides; Streptomyces nasri; Medium composition; Stirred tank bioreactor

Introduction

During the past several decades, much interest has been generated in the subject of polysaccharides produced by microorganisms, due to their various biological and pharmacological activities, including immuno-stimulating and anti-tumour activities (Kuo et al., 1996; Lee and Kang, 1996). Many microorganisms synthesis exopolysaccharides (EPS) that either attached to the cell surface or are found in the extracellular medium in the form of amorphous slime. Microbial polysaccharides are water soluble polymers and may be ionic or non-ionic. The repeating units of these exopolysaccharides are very regular, branched or unbranched, and are connected by glycosidic linkages. *Streptomyces*, a Gram-positive bacterium, is well known as an important industrial microorganism for its production of natural derived antibiotics. However, EPS production in *Streptomyces* has only been reported very recently despite the fast progress at a molecular level in other bacterial species such as lactic acid bacteria (Welman and Maddox, 2003). In our laboratory, *S. nasri*-UV 135 has been identified to produce EPS, which has antimicrobial activity against a wide range of microorganisms. The produced EPS was also tested for cytotoxic activity against human brain tumor cell line using SRB assay (Gohar et al., 2006).

Usually, culture medium is important to the yield of any fermentation products, and carbon and nitrogen sources generally play a significant role because these nutrients are directly linked with cell proliferation and metabolite biosynthesis (Kim et al., 2003; Casas et al., 2003). Also, the nature and concentration of the carbon source can regulate secondary metabolism through phenomena such as catabolic repression. Various statistical experimental design strategies were applied to the optimization of fermentation media. But, as far as we know, there is limited knowledge about the nutritional requirement for EPS production by *Streptomyces nasri*-UV 135, and there have been no reports on medium optimization to improve EPS production. In this work, the effects of carbon and nitrogen sources were focused in order to improve the EPS production by submerged cultivation of *S. nasri-UV 135*. The information obtained is considered fundamental and useful to the development of *S. nasri-UV 135* cultivation process for efficient production of EPS on a laboratory scale stirred tank bioreactor.

Material and Methods

Microorganism and media

Streptomyces nasri was isolated from the desert of Kuwait by Hashem and Diab, (1973). An UV-mutant of the parent *Streptomyces* strain, *S. nasri*-UV 135 was found to produce an antibiotic active against Gram-positive bacteria and is able to produce bioactive compounds like proteopolysacchrides (Gohar et al., 2006). The initial basal medium used contains (g/l): glucose 30, $NaNO_3$ 3.0, yeast extracts 5.0, NaCl 4.0, $MgSO_4$ 0.5, K_2HPO_4 1.0, and $CaCO_3$ 1.0, pH 7.

Carbon and nitrogen source screening

Cultures for screening the carbon and nitrogen sources for ef-

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fects on fermentation performance were conducted in 250 ml shake flasks filled with 50 ml of the medium. The flasks were inoculated with 500 µl of a spore suspension. The flasks were held at 30ºC on a rotary shaker at 200 rpm and harvested on the 7th day.

Fermentation conditions

The fermentation medium was inoculated with 10% (v/v) of the seed culture and then cultivated at 30ºC in a 3-l stirred tank bioreactor (Bioflow III, New Brunswick Scientific Co., New Brunswick, NJ, USA). Unless otherwise specified, fermentation were conducted under the conditions of temperature 30ºC, aeration rate 1.0 vvm, agitation speed 300 min^{-1}, initial pH 7.0 and working volume 2-l.

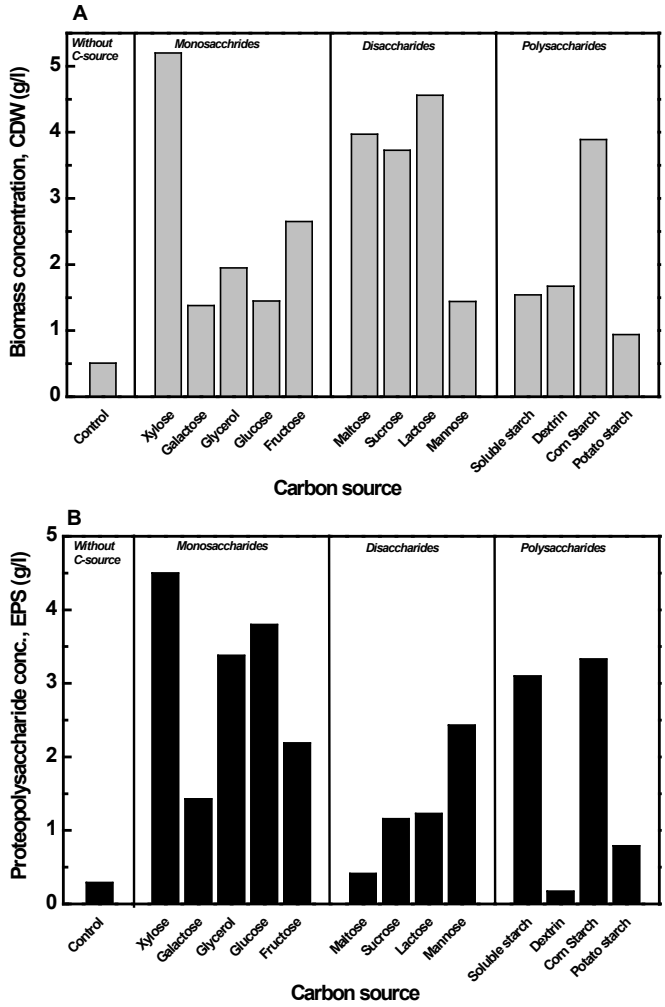

Figure 1: Effect of different carbon sources on cell growth of S. nasri-UV 135 (A) and EPS production (B) after 7 days cultivation.

Analytical methods

All determinations reported here were performed in triplicate and the experiments at least in duplicate, and the results were given as mean values.

Cell biomass determination

Growth was measured as the dry weight per volume by centrifugation (5000 g for 10 min) and then dried to a constant weight in an oven at 60ºC overnight to obtain cell dry weight (CDW).

Extraction and separation of exopolysaccharides (EPS)

Mycelial pellets were separated by centrifugation at 3000 g for 10 min. The supernatant was concentrated to 1/10 its initial volume by a rotary evaporator (Heidolph WB2000, Germany). The concentrates were mixed with equal volume of chilled absolute ethanol to precipitate the exopolysaccharides. To enhance precipitation, these samples were stored at 4ºC for 24 h. The precipitates were recovered by centrifugation at 4000g for 15 min and then dried at 55ºC overnight (van Geel-Schutten et al., 1998).

Results and Discussion

Effect of carbon sources

The following set of experiments examined the effects of various types of carbon sources on cell growth of *S. nasri*-UV 135 and the production of EPS. In all cases, nitrogen was provided as yeast extract at the same concentration used in basal medium, where each carbon source was added to the basal medium at 30 g/l instead of glucose. Figure 1 shows the time profile of biomass concentration (A) and EPS production (B) for cultures carried out with all carbon sources tested and without any carbon source as control. The results in shake-flask experiments revealed that, the change of the carbon source employed affected both the amount of biomass produced and the EPS production. Although

Carbon sources	Dry cell weight (g/l)	EPS (g/l)	EPS/CDW	Final pH
Control [1]	0.51	0.29	0.568	7.36
Glycerol	1.95	3.38	1.733	6.13
Xylose	5.20	4.50	0.865	5.94
Fructose	2.65	2.90	1.094	6.28
Galactose	1.38	1.43	1.036	6.77
Glucose	1.45	3.80	2.621	5.80
Mannose	1.44	2.43	1.687	7.81
Sucrose	3.73	1.16	0.310	6.69
Lactose	4.56	1.23	0.269	7.36
Maltose	3.97	0.41	0.104	6.50
Dextrin	1.67	0.17	0.102	7.82
Soluble starch	1.54	3.10	2.013	9.43
Corn starch	3.89	3.33	0.856	8.58
Potato starch	0.94	0.79	0.840	8.92

[1]Control culture without any carbon source

Table 1: Effects of different carbon sources on the growth of *Streptomyces nasri-UV 135* and its extracellular polysaccharide (EPS) production during cultivation in shake-flasks for 7 days at 30ºC.

maltose, sucrose, and lactose also gave good mycelial growth, they led to low EPS yields. The preferred carbon sources, xylose and glucose, generally gave the highest EPS production of 4.5 and 3.8 g/l, respectively as shown in Table 1. These results are in agreement with the general concept expressed in early studies (Kornmann et al., 2003; Bueno et al., 2006). They mentioned that fructose, sucrose and glucose have been reported to provide the highest exopolysaccharides yield. Recently, Celik et al., (2008) studied the production of exopolysaccharides by growing strains of *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 in medium containing various carbon sources such as glucose, mannose, fructose and xylose. They found that the highest EPS production of the two strains was found in the xylose containing medium.

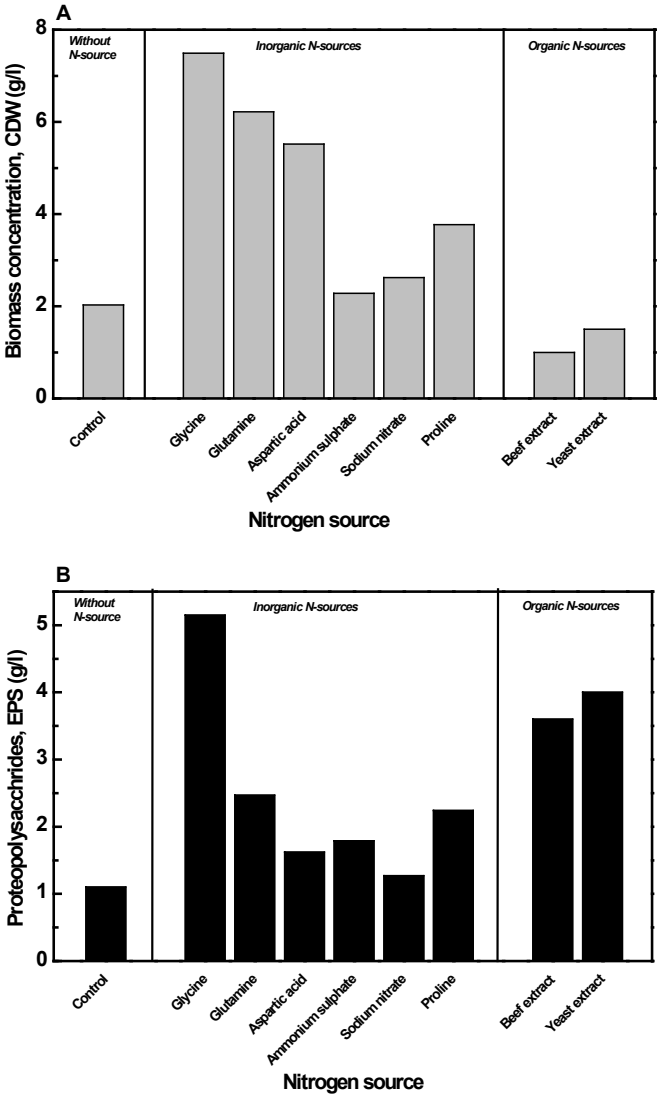

Figure 2: Effect of different nitrogen sources on cell growth of S. nasri-UV 135 (A) and EPS production (B) after 7 days cultivation.

Effect of nitrogen source

To investigate the effect of nitrogen source on the production of EPS and mycelial growth eight different nitrogen sources were examined (Figure 2). These N-sources were added on the basis of an equivalent N-content. Although glutamine and aspartic acid were favorable for the mycelial growth of *Streptomyces nasri*, the maximum EPS production of 5.15 g/l was achieved when glycine (2.7 g/l) was employed (Figure 2B). Improvement in cell growth, EPS production, and EPS/CDW ratio with different nitrogen sources are gathered in Table 2.

Cultivation of S. nasri-UV 135 in shake-flasks with optimized medium

Using all optimized medium components, trials in a series of shake-flasks were carried out using 30 g/l xylose as carbon source and 2.7 g/l glycine as nitrogen source. The time course of cell growth, EPS production and pH values are shown in Figure 3. The maximal EPS production indicated 5.4 g/l after 6.5 days of fermentation, while maximum mycelial yield was 7.25 g/l after 6.5 days. This indicates 36% increase of the EPS production used the optimized medium (5.4 g/l) compared to EPS production using the basal medium (3.98 g/l) as previously described by Gohar et al., (2006). In addition, similar improvement in biomass concentration was achieved using the optimized medium.

Fermentation of S. nasri-UV 135 in stirred tank bioreactor

Although shake flasks are the bioreactors most frequently used in biotechnology for initial process development, very little is known regarding their characteristics from an engineering point of view (Buechs and Zoels, 2001; Buechs, 2001). There are some reports in the literature regarding the scaling-up of processes from shake flasks to stirred tank bioreactors (Muley et al., 1999; Katzer et al., 2001). Therefore, the bioreactor fermentation process was developed on the basis of data obtained from shake flasks cultivation experiments. The production of EPS was studied in a 3 liter bioreactor using the basal and the optimized culture medium.

Figure 4 shows the time courses of mycelial growth and EPS production by *Streptomyces nasri*-UV 135 in a 3-L stirred tank fermenter with the basal medium and the optimized culture medium. In a basal medium, the EPS concentration reached a maximum level of 5.6 g/l after 5.5 d, while maximum mycelial concentration was 3.9 g/l after 4.5 d (Figure 4A). In an optimized

Nitrogen sources	Dry cell weight (g/l)	EPS (g/l)	EPS/CDW	Final pH
Control[1]	2.03	1.10	0.542	5.07
Glycine	7.49	5.15	0.687	5.70
Aspartic acid	5.52	1.62	0.293	5.79
Glutamine	6.22	2.47	0.397	5.27
Proline	3.77	2.24	0.594	4.94
Ammonium sulphate	2.28	1.79	0.785	4.82
Sodium nitrate	2.62	1.27	0.485	5.70
Beef extract	1.00	3.60	3.60	5.31
Yeast extract	1.50	4.00	2.67	5.60

[1]Control culture without any nitrogen source

Table 2: Effects of different nitrogen sources on the growth of *Streptomyces nasri*-UV 135 and its extracellular polysaccharide (EPS) production during cultivation in shake-flasks for 7 days at 30°C.

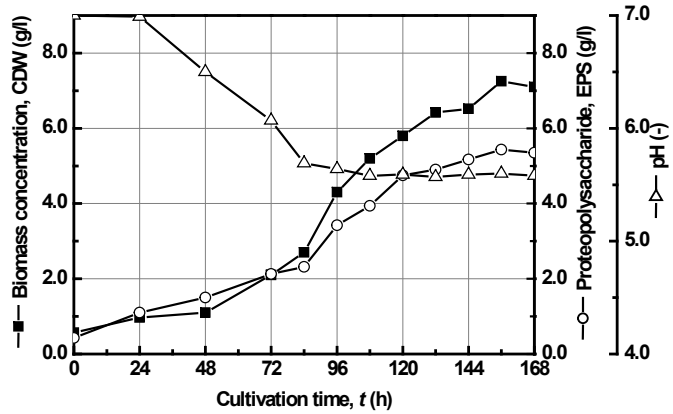

Figure 3: Batch cultivation of S. nasri-UV 135 in shake flasks using the optimized medium.

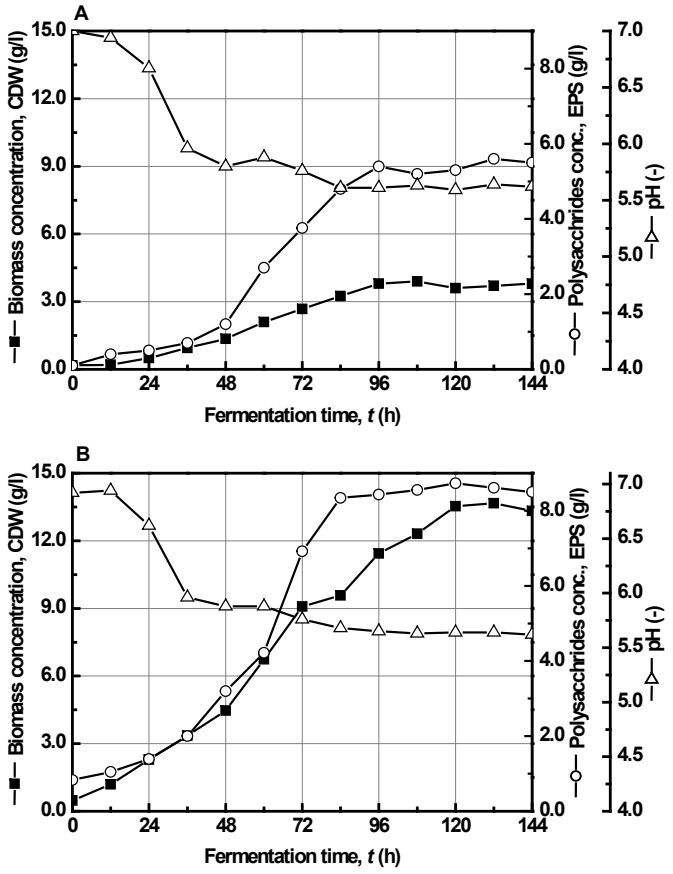

Figure 4: Batch fermentation of S. nasri-UV 135 in stirred tank bioreactor in basal medium (A) and in the optimized medium (B).

culture medium as shown in Figure 4B, the mycelial growth was continuously increased towards the end of fermentation and its final mycelial concentration indicated 13.6 g/l at day 5.5. The initial pH of the fermentation broth slowly decreased from 6.94 to 5.6. The EPS production reached 8.73 g/l after 5 d of fermentation, which were 1.6 times higher than that in fermentation in basal medium. Optimization of operating parameter (e.g. agitation, aeration, and dissolved oxygen tension) in bioreactor fermentation deserves further investigation, which is being carried out in our laboratory.

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