

Bioactive Compounds from *Streptomyces nasri* and its Mutants with Special Reference to Proteopolysaccharides

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Abstract

The use of microbial exopolysaccharides (EPS) in the food, pharmaceutical, and chemical industries has steadily increased during the past decade. A bioactive EPS producing microorganism, *Streptomyces nasri* was isolated from Kuwait tropical soil and the proteopolysaccharide was tested for its antimicrobial activity. The isolate was subjected to ultraviolet (UV) radiation and acridine orange (AO) treatment to select for superior proteopolysaccharide producers. Among eight (five derived from UV exposure and three from AO exposure) morphological variants of *Streptomyces nasri*, two mutants showed increased EPS production, from 1.8 g/l to 2.3 g/l. The SDS-PAGE profiles of exopolysaccharides were determined. The molecular weight of the proteopolysaccharide ranged from 18 to 200 kDa. Mutants derived from UV exposure produced polysaccharides with higher molecular weight than those derived from acridine orange exposure. Acridine orange derived mutants produced lower molecular weight polysaccharides. Culture supernatants have been partially characterized and they show antimicrobial activity against a wide range of microorganisms. The structure of the exopolysaccharide was determined using NMR spectroscopy. The polysaccharide was also tested for cytotoxic activity against human brain tumor cell line using SRB assay.

Key words: antimicrobial activity, bioactive compound, microbial polysaccharides, *Streptomyces* sp. mutations

Introduction

More than 70 years have passed since Sir Arthur Fleming's outstanding discovery of a *Penicillium* colony, which inhibited the growth of a *Staphylococcus* culture. Today, these two features, the production of bioactive metabolites by microorganisms and their antibiotic effect against pathogenic microbes are continuing to attract scientific and public interest. Though almost 20 000 microbial metabolites and approximately 10 000 plant products have been described so far (Grabley and Thiericke, 1999). Secondary metabolism appears as an inexhaustible source of new antimicrobials, antivirals, antitumour drugs, agricultural and pharmacological agents. Therefore, continuous efforts are being made in the research on microbial secondary metabolites, which have potential applications, such as proteopolysaccharides.

Recent reviews on microbial proteopolysaccharides confirm that there is potentially a huge market (Sutherland, 2001). Moreover, the importance of various proteopolysaccharides for pharmaceutical purposes such as baker's yeast glycans, eg. Scleroglucan and Krestin has a long historical background and increased considerably during the last decades (Franz, 1989). This is not only the case for polysaccharides

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used as exceptions essential for specific drug formulations but also for a series of carbohydrates polymers which have proven to be most useful as physiological active drugs like branched glucans of *Dictyophora* sp. and *Symphitum fructans* (Franz, 1989). Many interesting areas have been opened, which include their role in cancer therapy (Schizophyllan) and prevention of bacterial and viral diseases (Lentinan). It has been known that human malignant growths sometimes undergo regression following an acute bacterial infection. Polysaccharides complexes from *Staphylococcus aureus*, *Acetobactor xylinum*, different species of *Streptomyces* and *Pseudomonas* and some other bacteria were reported to be active against solid tumors.

Many microorganisms synthesize exopolysaccharides (EPS) that either remain 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. Some microbial polysaccharides are commercially accepted products and others are at various stages of development. Currently a small number of biopolymers are produced commercially on a large scale. However, these limited groups of products exhibit an extensive range of physical properties (Sutherland, 1990; 1998).

Actinomycetes are saprophytic bacteria that secrete important hydrolytic enzymes, antibiotics and medicinally important secondary metabolites. Between 1988 and 1992 more than hundred different new molecules from actinomycetes were discovered. Approximately 75% of these originated from *Streptomyces* genus (Sanglier *et al.*, 1993; Sacramento *et al.*, 2004). Therefore and because of their ability to secrete valuable proteins, *Streptomyces* have been considered as an alternative host organism for producing recombinant proteins (Dela Cruz *et al.*, 1992). Induction of mutation and selection to improve the productivity of cultures has been strongly established for over fifty years and is still recognized as a valuable tool for many antibiotics (Venkateswarlu *et al.*, 2000). The mutant strains can be achieved by inducing genetic variation in the natural strain with increased productivity.

In this study an actinomycetes strain (*Streptomyces* sp.), previously isolated from Kuwait tropical desert (Hashem and Diab, 1973), was chosen due its marked stability and tested for its double capacity to produce antimicrobial agents active against some pathogenic with special focus on the cytotoxic activity against brain human tumor cell line. The possibility of producing mutants from the parent strain has been explored. Direct cytotoxic activity against brain cancer cell line has been investigated. An attempt to determine the molecular weight and the structure of the isolated proteopolysaccharides was undertaken.

Experimental

Materials and Methods

Organism and culture media. *Streptomyces nasri* was isolated from the desert of Kuwait by Hashem and Diab (1973). This strain was found to produce an antibiotic active against Gram-positive bacteria. A loop-full of spore stock was spread on ISP-agar plate and incubated for 6–8 days at 30°C. 500 µl spore suspension was then used as an inoculum for each 250-ml Erlenmeyer flask containing 50 ml of the particular medium (M1, M2, M3) and incubated on a rotary shaker (Infors AG, CH-4103 Bottmingen) at 30°C for 7 days at 200 rpm. This culture was used for various mutagenic experiments and production of proteopolysaccharides. Three different media have been used throughout this work, namely M1, M2 and M3. Medium No 1 (M1) contains (g/l): glucose 30, NaNO₃ 3, yeast extracts 5, NaCl 4, MgSO₄ 0.5, K₂HPO₄ 1, CaCO₃ 1, pH 7.0. Medium No 2 (M2) contains (g/l): glucose 10, yeast extracts 3, peptone 5, KH₂PO₄ 1, K₂HPO₄ 1, pH 7.0. Medium No 3 (M3-ISP) contains (g/l): malt extract 10, yeast extract 4, glucose 4, pH 7.2.

Mutagenesis. In *Streptomyces nasri* two classical methods have been successfully used to produce mutants. This includes the use of UV light and acridine orange (AO).

Ultraviolet (UV) treatment. Ultraviolet (UV) mutagenesis was carried out according to Zhang *et al.* (1993). Photoreactivation was avoided by keeping the isolated colonies overnight in the dark by wrapping the plates in aluminum foil (Held and Kutzner, 1991) and a survival rate of not more than 1% was considered.

Acridine orange (AO) treatment. Two ml heavy spore suspension (10⁶ spores/ml) of the parent type *Streptomyces nasri* were subjected to a final concentration of 10 µg/ml acridine orange for 90 min to cause 99% killing. Spores were washed several times using LB medium by repeated centrifugation at 4000 rpm for 10 min and decantation. Washed spore pellet was finally suspended in 2 ml LB medium, vortexed, spread onto LB-agar plates, incubated at 30°C. Macroscopically different colonies were selected.

Cell growth. Samples were taken at the end of cultivation (7 days) and various analyses were performed. 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 60°C overnight to obtain cell dry weight (CDW).

Extraction and separation of proteopolysaccharides (EPS). Mycelial balls 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 proteopolysaccharides. To enhance precipitation, these samples were stored at 4°C for 24 h. The precipitates were recovered by centrifugation at 4000×g for 15 min and then dried at 55°C overnight (van Geel-Schutten *et al.*, 1998).

Proton NMR spectrometry. Proteopolysaccharide (EPS) sample (50 mg) was dissolved in 1 ml dimethyl sulphoxide by ultrasonication for 10 minutes. The solution was introduced into a precision ground tube (5 mm diameter, depth 2–3 cm) then subjected to measurement by an NMR-spectrophotometer (JEOL, Japan).

SDS-PAGE electrophoresis. Protein samples were analyzed by electrophoresis in a 12% (w/v) sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) as described by Laemmli (1970).

Measurement of cytotoxicity by SRB assay. Potential cytotoxicity of proteopolysaccharides was tested using the method of Skehan *et al.* (1990). Briefly, U-251 brain cancer cell line was plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the proteopolysaccharides to adhere cells. Different concentrations of EPS dissolved in DMSO (0, 1, 2.5, 5, 6.5, 8.5 and 10 mg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C in 5% CO₂ atmosphere. After 48 h, cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between the number of viable cells and EPS concentration was plotted to get the survival curve of tumor cell line after the specified compound.

Antimicrobial activity evaluation (Bioassay). Mueller-Hinton agar (MHA) medium was used as an assay medium. The agar medium at 45°C was mixed with 0.1 ml bacterial suspension containing approximately 10^5 cfu/ml. The mixture was poured into 9 cm Petri dish and allowed to solidify. Sterile paper discs (5 mm) were placed on the dried surface of the medium. Each disc received 20 µl of the culture filtrate. Petri dishes were incubated at 37°C for 18 hours. The inhibition zone, if any, was measured in mm diameter (Amade *et al.*, 1994). The test organisms for bioassay were the Gram-positive bacteria *Bacillus subtilis* ATCC 31324, *Micrococcus luteus* ATCC 10240, *Sarcina lutea* ATCC 10773, *Staphylococcus aureus* ATCC 6538 and the Gram-negative bacteria *Bordetella bronchiseptica* ATCC 4617, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 15050, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhi* ATCC 3112.

Results and Discussion

Effect of AO and UV treatment on *Streptomyces nasri*. Five UV and three AO mutants were obtained (Figure 1). They differ in macroscopic colony characteristics including rate of growth, color and density of the spores, reverse colony color and production of soluble pigments. In addition they were all stable on subsequent cultivation in both solidified and liquid media considering their antimicrobial activity and EPS production.

Comparison between *Streptomyces nasri* mutants and parent strain for the production of EPS in shake flasks. Parent strain of *S. nasri* was used as a standard organism for screening and comparing productivity of the isolated mutants. The mutants were grown on a solid ISP-medium. Fermentation experiments in suspension culture were conducted in 250 ml shake-flasks by inoculating the mutant spores in 50 ml of three different media: M-1, M-2 and M-3.

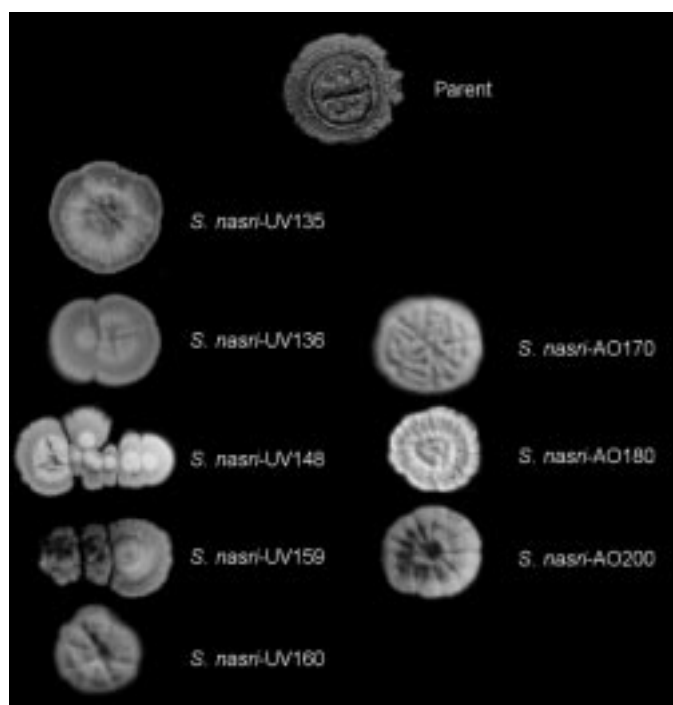


Fig. 1. Colony morphology of *S. nasri* parent type, UV and AO mutants grown on oat-meal agar medium for 10 days at 30°C.

Table I
Cell growth, proteopolysaccharide (EPS) and final pH for the parent strain *S. nasri* and its UV- as well as AO-mutants

<i>Streptomyces nasri</i>	Culture media											
	M-1				M-2				M-3			
	CDW (g/l)	EPS (g/l)	EPS/CDW	pH	CDW (g/l)	EPS (g/l)	EPS/CDW	pH	CDW (g/l)	EPS (g/l)	EPS/CDW	pH
Parent <i>S. nasri</i>	0.80	0.47	0.59	6.0	0.63	0.42	0.67	7.1	0.44	0.84	0.37	6.4
<i>S. nasri</i> -UV 135	1.06	3.98	3.77	5.6	0.90	2.02	2.24	6.5	0.27	1.13	0.31	6.3
<i>S. nasri</i> -UV 136	1.00	3.63	3.64	5.6	0.95	1.69	1.78	6.3	0.31	1.17	0.36	6.6
<i>S. nasri</i> -UV 148	1.15	3.61	3.14	5.5	0.85	1.56	1.84	6.0	0.33	1.06	0.35	7.0
<i>S. nasri</i> -UV 159	1.05	3.10	2.95	5.6	0.90	1.40	1.56	6.3	0.35	0.94	0.33	7.2
<i>S. nasri</i> -UV 160	1.26	3.73	2.96	5.0	1.33	1.38	1.04	6.2	0.40	0.79	0.31	7.1
<i>S. nasri</i> -AO 170	2.15	2.58	1.20	4.8	1.10	1.01	0.92	7.2	0.49	0.86	0.42	5.7
<i>S. nasri</i> -AO 180	2.14	2.42	1.13	4.7	1.00	0.89	0.89	7.1	0.42	0.98	0.41	6.5
<i>S. nasri</i> -AO 200	2.15	2.59	1.20	4.7	1.38	0.84	0.61	7.2	0.45	0.53	0.24	7.2
Average AO&UV	1.49	3.21	2.50	5.2	1.05	1.35	1.36	6.6	0.38	0.93	0.34	6.7
Average UV	1.10	3.61	3.29	5.5	0.99	1.61	1.69	6.3	0.33	1.02	0.33	6.8
Average AO	2.14	2.53	1.18	4.7	1.16	0.91	0.80	7.2	0.45	0.79	0.36	6.5
Fold Increase for UV + AO*	1.86	6.82	4.27		1.67	3.21	2.04		0.86	1.11	0.92	
Fold Increase for UV*	1.37	7.68	5.62		1.57	3.83	2.53		0.75	1.21	0.90	
Fold Increase for AO*	2.67	5.38	2.02		1.84	2.17	1.21		1.03	0.94	0.96	

The strains were grown in three different media (M-1, M-2, and M-3; see Methods) for 7 days at 30°C with shaking (200 rpm)

* = Relative to the parent.

CDW: Cell dry weight

Table I shows the biomass as a dry weight and EPS production as well as final pH, in shake-flask cultures of parent *Streptomyces* strain and UV as well as AO mutants cultivated in three different media. All mutants were able to grow in the three tested culture media with distinguishable differences in EPS production. The response of the parent type of *S. nasri* and its UV- as well as AO-mutants to changes in the medium composition considering CDW, EPS, and pH was noticeably different. M-1 could produce a relatively very high record for the CDW (1.49 g/l) considering all mutants. Also the average for any of *S. nasri* UV- or AO-mutants, M-1 was the best (1.10 and 2.14 g/l), respectively. Other media (M-2 and M-3) were inferior to M-1 (only 0.99 and 1.16 g/l) for M-2 and 0.33 and 0.45 g/l with *S. nasri* UV- and AO-mutants, respectively. For the average EPS production by all mutants, M-1 medium was also superior (3.21 g/l) and M-3 inferior (0.93 g/l).

To simplify comparison between the three medium for the validity of EPS production, the EPS/CDW ratio (as a fold increase factor, or an index, for the specific activity for unit mycelial dry weight) was calculated for the three media compared to that of the parent type. EPS-fold increase for all mutants (UV and AO) when grown in M-1 was 6.82 times the parent type, 3.21 times in M-2 and only 1.11 times in M-3. The level of increase was unequally divided between UV- and AO-mutants. For instance, the increase of EPS and specific activity were dominated by the *S. nasri* UV-mutants with the superiority in M-1 (7.68 folds) and inferiority in M-3 (1.21 folds). *S. nasri*-AO mutants were superior in the level of increase of CDW measures but not in EPS production.

According to the previous results, *S. nasri*-UV 135 mutant was chosen to be used in the foregoing investigations. To confirm the obtained results and to get some details about the nature, constitution and molecular weight(s) of the EPS produced by this strain, SDS-PAGE technique was applied. The antimicrobial activity and the cytotoxicity were checked. In addition, the proton-NMR fingerprint of the EPS was also determined.

SDS-PAGE for the EPS produced by parent strain of *S. nasri*, its UV- and AO-mutants. Figure 2 shows the SDS gel electrophoretic protein profile of the EPS of the parent *S. nasri*, its UV- and AO-mutants. EPS isolated from each strain are a mixture of low and high molecular weight molecules ranging from 18–200 kDa. Generally, the parent strain was found to produce EPSs molecules whose molecular weights ranged from 29–97 kDa. *S. nasri* UV-mutants showed band range relatively wider than that of the parent type (29–200 kDa). In addition, UV-*Streptomyces* mutants produce more protein fragments than the parent

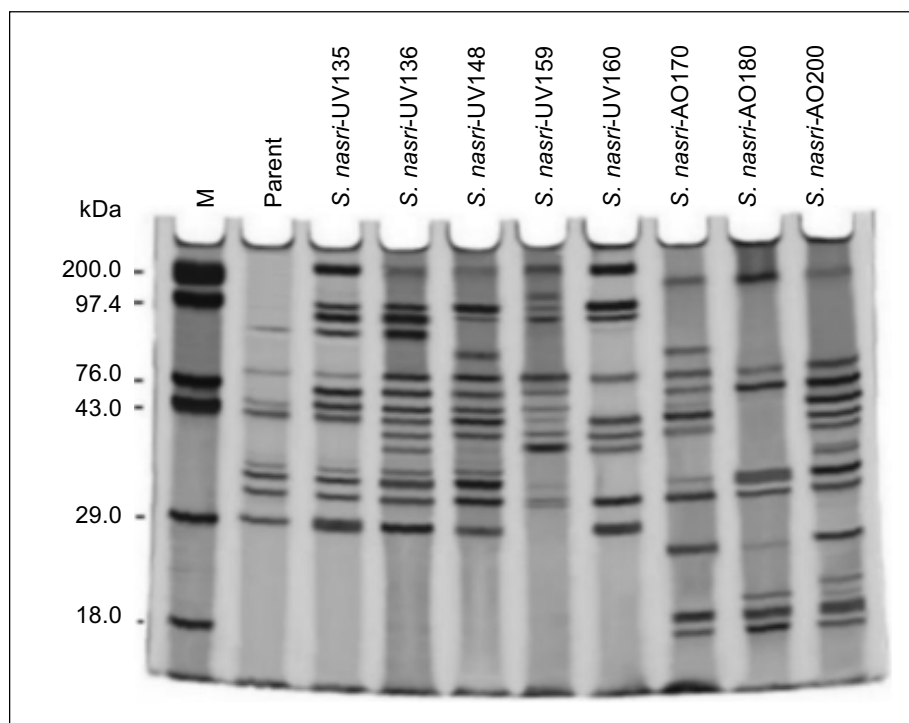


Fig. 2. SDS-PAGE protein profile for the extracellular proteopolysaccharides (EPS) of *S. nasri* parent type, UV and AO mutants grown on M-1-medium for 7 days at 30°C at 200 rpm.

strain. On the other hand, protein bands of *S. nasri*-AO mutants showed a wider range of molecular weight, from relatively low to high MTs (18–200 g/l).

There is a correlation between the molecular weight and the biological activity of proteopolysaccharides. The activity depends on the size of molecules and degree of branching and conformation (Ohno *et al.*, 2001). Adachi *et al.* (1990) reported that while very large molecular weight proteopolysaccharides activate a wide variety of immune functions, after reducing the size of the molecules by heating the range and strength of the activity is proportionally reduced. Small compounds with low molecular weight show only the ability to activate glucose consumption possibly lowering blood glucose and synthesis of lysosomal enzyme (an antibacterial protective function). Mizuno *et al.* (1996) also reported that high molecular weight proteopolysaccharides appear to be more effective than those of low molecular weight.

Antimicrobial activity and its relation with EPS. Culture filtrates of different *S. nasri* UV- and AO-mutants grown on different 3 media, after full extraction of the EPS, were tested for their antimicrobial activity against 4 Gram-positive and 10 Gram-negative bacteria. Neither the parent nor its mutants could exhibit any anti Gram-negative activity. As for anti Gram-positive activity (Table II), M-1 was the best for production of antimicrobial agent(s) followed by M-2 then M-3. M-1 allowed production of broader antimicrobial spectra against the 4 test bacteria with the parent strain, UV-135 and AO-170 *Streptomyces* mutants. *S. nasri* UV-159 exhibited narrow activity against *S. aureus* only, whereas *S. nasri* UV-160 against *M. luteus*. M-2 was suitable for broad anti Gram-positive bacteria with the parent and AO-170 *Streptomyces*. *S. nasri* UV-135 mutant exhibited activity against the 4 tested bacteria whereas UV-160 against *M. luteus* and failed to allow UV-159 to produce any active compounds. M-3 caused none of the producers to exhibit any broad spectrum activity in addition to failure of production with both AO-170 and UV-159. Maximum inhibition zone was recorded for UV-135 when grown in M-1 against *Micrococcus luteu* (28 mm) followed by *S. aureus* (25 mm).

Considering the antibacterial activity of EPS presented by the parent *S. nasri* and its mutants in different culture conditions, the activity of the parent strain was restricted to Gram-positive test bacteria only, without any anti Gram-negative activity (Table III). No active agents were produced against *Klebsiella* and only *S. nasri* UV-160 mutant could exhibit activity against *E. coli* when grown in M-2. Also, *S. nasri* AO-170 mutant failed to develop any activity against any of the tested organisms when grown in either M-2 or M-3, while *S. nasri* UV-135 was active against all Gram-positive and Gram-negative organisms except *E. coli* and *K. pneumoniae* when grown in M-1. Moreover, *S. nasri* UV-135 mutant recorded the widest antimicrobial

Table II
Antimicrobial activity of the culture filtrates of the parent strain *S. nasri* and its mutants grown on different media (M-1, M-2, M-3) after removal of EPS

Me- dium	Organism	Gram-positive bacteria			
		Inhibition zone diameter (mm)			
		<i>Micrococcus luteus</i> ATCC 10240	<i>Sarcina lutea</i> ATCC 10773	<i>Bacillus subtilis</i> ATCC 31324	<i>Staphylococcus aureus</i> ATCC 6538
M-1	Parent <i>S. nasri</i>	13	18	15	14
	<i>S. nasri</i> -AO 170	21	16	22	18
	<i>S. nasri</i> -UV 135	14	28	18	25
	<i>S. nasri</i> -UV 159	0	0	0	21
	<i>S. nasri</i> -UV 160	0	15	0	0
M-2	Parent <i>S. nasri</i>	14	17	20	18
	<i>S. nasri</i> -AO 170	0	0	0	10
	<i>S. nasri</i> -UV 135	17	11	13	11
	<i>S. nasri</i> -UV 159	0	0	0	0
	<i>S. nasri</i> -UV 160	0	16	0	0
M-3	Parent <i>S. nasri</i>	9	0	10	15
	<i>S. nasri</i> -AO 170	0	0	0	0
	<i>S. nasri</i> -UV 135	0	11	9	0
	<i>S. nasri</i> -UV 159	0	0	0	0
	<i>S. nasri</i> -UV 160	0	12	0	0

(0) = No inhibition zone.

Each 5 mm disc received 20 µl culture filtrate after removal of EPS

Table III
Antimicrobial activity of the EPS produced by the parent strain *S. nasri* and its mutants grown on different media (M-1, M-2, M-3)

Me- dium	Mutant No	Inhibition zone diameter (mm)									
		Gram-positive bacteria					Gram-negative bacteria				
		<i>Bacillus subtilis</i> ATCC 31324	<i>Micrococcus luteus</i> ATCC 10240	<i>Sarcina lutea</i> ATCC 10773	<i>Staphylococcus aureus</i> ATCC 6538	<i>Bordetella bronchiseptica</i> ATCC 4617	<i>Escherichia coli</i> ATCC 8739	<i>Klebsiella pneumoniae</i> ATCC 15050	<i>Proteus mirabilis</i> ATCC 12453	<i>Pseudomonas aeruginosa</i> ATCC 9027	<i>Salmonella typhi</i> ATCC 3112
M-1	Parent <i>S. nasri</i>	11	13	10	9	0	0	0	0	0	0
	<i>S. nasri</i> -AO 170	9	11	7	20	9	0	0	15	0	16
	<i>S. nasri</i> -UV 135	16	23	17	13	20	0	0	21	19	23
	<i>S. nasri</i> -UV 159	0	0	0	16	7	0	0	0	0	0
	<i>S. nasri</i> -UV 160	0	10	0	0	8	0	0	22	0	0
M-2	Parent <i>S. nasri</i>	9	12	15	13	0	0	0	0	0	0
	<i>S. nasri</i> -AO 170	0	0	0	0	0	0	0	0	0	0
	<i>S. nasri</i> -UV 135	12	7	8	6	8	0	0	13	0	0
	<i>S. nasri</i> -UV 159	0	0	0	0	0	0	0	0	0	0
	<i>S. nasri</i> -UV 160	0	11	0	0	10	22	0	21	0	0
M-3	Parent <i>S. nasri</i>	0	0	8	10	0	0	0	0	0	0
	<i>S. nasri</i> -AO 170	0	0	0	0	0	0	0	0	0	0
	<i>S. nasri</i> -UV 135	0	6	7	0	6	0	0	0	0	0
	<i>S. nasri</i> -UV 159	0	0	0	0	0	0	0	0	0	0
	<i>S. nasri</i> -UV 160	0	8	0	0	0	0	0	15	0	0

(0) = No inhibition zone. Each 5 mm disc received 20 µl of EPS

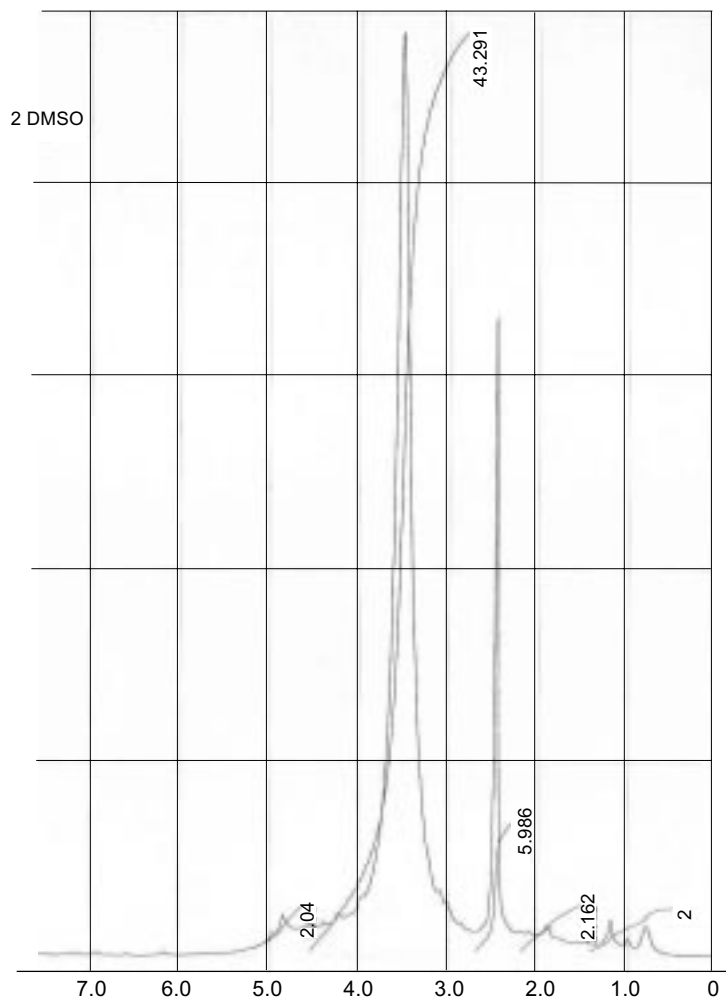


Fig. 3. Proton NMR spectrum of proteopolysaccharides (EPS) produced by *S. nasri* UV-135 mutant grown on M-1 medium at 30°C and 200 rpm for 7 days.

spectrum (against 8 test bacteria) among other producers followed by AO-170 when grown in M-1. Presence of high concentration of glucose, and presence of NaNO_3 , NaCl , MgSO_4 and CaCO_3 in M-1 were advantageous for production of both non-EPS and EPS anti-bacterially active products by *S. nasri* and its mutants.

Proton NMR spectroscopic analysis of separated proteopolysaccharides. Proteopolysaccharides (EPS) produced by *S. nasri*-UV 135 mutant were analyzed by NMR analysis (Figure 3). Although NMR peaks are poorly resolved because of the viscosity, the characteristic signals for glucan (a branched polysaccharide chain with either 1,4 or 1,3 and 1,6 linkage) anomeric protons could be detected.

Cytotoxic activity. The exposure of U-251 brain cancer cells to various concentrations of EPS produced by *S. nasri*-UV 135 mutant (0, 1, 2.5, 5, 6.5, 8.5 and 10 $\mu\text{g/ml}$) exhibited a general slow decrease except at 2.5 $\mu\text{g/ml}$ EPS and continued its decrease to reach 87% of the start number of U-251 brain cancer cells at the highest concentration 10 $\mu\text{g/ml}$ as shown in Fig. 4.

β -1,3-glucan is widely used as a dietary supplement, with well-established stimulating effects on the immune defense system (Vetvicka, 2001; Ross *et al.*, 1999). A large body of published data supports this use. Browder *et al.* (1990) described strongly decreased septic morbidity with β -1,3-glucan administration. A series of well documented multicenter blind studies demonstrated that β -1,3-glucan-treated patients had significantly lower infection rates (Babineau *et al.*, 1994a; Babineau *et al.*, 1994b). Positive effects were also found in patients after cardiopulmonary bypass (Hamano *et al.*, 1999) and inhibition of antiviral activity has been found in HIV-infected patients (Itoh *et al.*, 1990). Some β -1,3-glucans are routinely used in patients for tumor immunotherapy (Kimura *et al.*, 1994; Nakano *et al.*, 1996).

Within the field of pharmacologically active polymers, the area of immune-stimulating polysaccharides is an attractive field of tumor therapy. The interest in polysaccharides as antitumor substances came from the dissatisfaction with current cancer chemotherapy. Countless numbers of chemical compounds have been

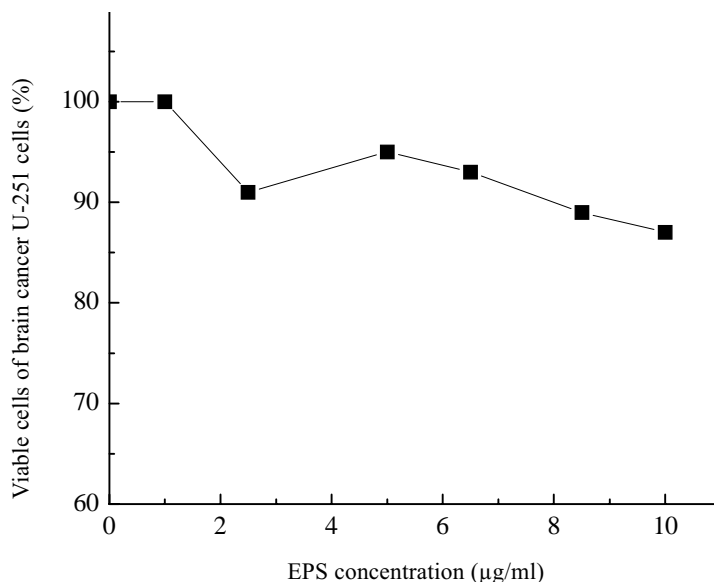


Fig. 4. Cytotoxic activity of different concentrations of proteo-polysaccharides (EPS) on U-251 brain cancer cells.

tested as cytostatic agents with possibly a high specificity for the cancer cell. But unfortunately in many cases, this specificity is rather low, and therefore many chemotherapeutic antitumor substances have considerable side effects. Extensive studies have been carried out on nontoxic antitumor substances and most interestingly, some polysaccharides were shown to be most useful. Several papers have reported that microbial EPS cannot have direct effect on tumour cells *in-vitro* (Chihara *et al.*, 1987; Chihara *et al.*, 1989). It has been known for more than a century that human malignant growths sometimes undergo regression following an acute bacterial infection. The earliest bacterial-derived EPS reported to have antitumour activity was attributed to *Serratia marcescens* infection and became known as Shear's polysaccharide (Ooi and Liu, 2000). This polysaccharide could cause extensive cytotoxic damage to Sarcoma 37 tumours, but as it had serious side effects, clinical trials have not been performed.

It has been the experience of clinicians, as well as of laboratory experimenters, however, that successful total kill of cancer cells is apt to include the host too. Hence the enhancement or potentiation of host resistance emerged as a possible means of inhibiting tumour growth without harming the host. Starting from this point of view, extensive studies have been made on noncytotoxic and host mediated antitumour proteopolysaccharides. These studies are still in progress in many laboratories and the role of proteopolysaccharides as immunopotentiators is being especially debated. The proteopolysaccharides so far examined although may be strongly active in suppressing transplanted tumours, do not seem eligible for clinical trials in human cancer therapy. Therefore, search for new polysaccharide more strongly antitumour active than those thus far discovered stands as a challenge for further studies on antitumour proteopolysaccharides.

Literature

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