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Diversity of Exopolysaccharide Producing Fungi from Foot Hills of Shivalik Ranges of Chandigarh Capital Region

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In this investigation, the diversity of exopolysaccharide producing fungi of foot hills of Shivalik ranges of Chandigarh capital region have been studied. The study resulted in isolation of a total of 94 fungal isolates of which 52 isolates belonging to 17 different genera viz., *Agaricus, Alternaria, Aspergillus, Aureobasidium, Candida, Curvullaria, Chaetomium, Fusarium, Ganoderma, Penicillium, Pleurotus, Polyporous, Rhizopus, Saccharomyces, Stemophylium, Termitomyces,* and *Tricholoma* etc. were found to possess the EPS producing potential. These isolates have been categorized into five different groups on the basis of the EPS quantities they produced. These included Group I with production range of : > 1g/l ; Group II with production range of : 1-2 g/l ; Group III with production range of : 2-3 g/l ; Group IV with production range of : 3-4 g/l and Group V with production range of : 4-5 g/l. Out of these 52 EPS producing isolates, 11 were found to be the prominent producers with *Auerobasidium* RYLF 10 as the most potential isolate with EPS concentration of 4.60g/l followed by the species of *Penicillium* RYLF 35, *Aspergillus* RYLF 17 *and Ganoderma* RYMF 15. No correlation between EPS concentration and the biomass yield could be traced.

Keywords: Fungi, Exopolysaccharides, Biomass, Diversity, Submerged Fermentation

The metabolic proficiencies of fungi made this group of organisms capable of producing large number of metabolites of industrial and pharmaceutical significance. The important among these metabolite types are antibiotics, enzymes, organic acids, pigments and exopolysaccharides (EPS). Though the enormous research on these metabolites have been done, the work on exopolysaccharides especially of fungal origin is still very less and require sincere attention. There is need to explore newer isolates from nature especially from rich biodiverse regions of our country, to isolate them into pure culture and to screen out their exopolysaccharide producing efficiencies. The fungal exopolysaccharides represent a wide range of chemical structures and properties, are rich in high molecular weight polysaccharides and mostly have heteropolymeric composition. Owing to this, the fungal exopolysaccharides have found multifarious applications in the food, pharmaceutical and other industries but have not yet given the appreciable significance. Hence, in present investigation one of the important biodiverse region of the country i.e., the lower Shivalik ranges of Himalayas

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occurring in Chandigarh capital region was selected to explore its diversity of fungal organisms capable of producing EPS. The region lie North-West of India with an area of approximately 114 km² and shares its borders with the states of Himachal Pradesh, Haryana and Punjab and are very rich in floral and faunal diversity. The diversity of climatic conditions prevailing in the region made it a natural habitat of large number of fungal flora ranging from lower ascomycetous or deuteromycetous to several higher mushroom forms belonging to class basidiomycetes.

Materials and methods

Study site, isolation of fungi and maintenance

The important biodiverse regions of lower Shivalik ranges of Himalayas belonging to Chandigarh capital region including the Chandigarh itself, adjoining districts of Chandigarh like Mohali in Punjab, Panchkula in Haryana, Shimla and Solan in Himachal Pradesh. These regions were selected for collection of different samples (including soil, litter, roots, leaves, stems, mushroom specimen and other organic materials) to isolate the fungal organisms. The study site included the important lakes, rivers, forest regions, hilly parts, marshy lands etc. The samples were collected as per standard protocol of Hawksworth (1995, 2004) and brought to laboratory for their necessary processing for isolation of fungal organisms. The higher mushroom specimens were collected as per Atri *et al.* (2005). In this method an axe, sharp knife, forceps, measuring tape, hand lens, pens, books, labels, camera, papers and containers were used. An axe was used to incise to a depth sufficient to enable identification of the host, and a sharp knife for collecting sporocarps from soil. Very small sporocarps were collected with the help of forceps using hand lens and the containers were used for collecting the specimens. Isolations from soil samples were made as per Warcup (1950) while the litter fungi and fungi associated with surfaces of plant roots, leaves, and twigs were isolated as per standard method of dilution. The pure culture of different fungal organisms obtained were maintained and preserved on potato dextrose agar slants at –20°C. The successfully isolated fungal cultures were used for screening of their exopolysaccharide producing potential.

Determination of frequency distribution

The mean frequency distribution of different fungal genera reported from the region were calculated using the following formula:

$$\text{Mean frequency (\%)} = \frac{\text{Number of fungal genera from the region (CCR)} \times 100}{\text{Total number of fungal isolates reported from the region (CCR)}}$$

Screening for EPS producing capability:

EPS extraction & quantification

The screening of exopolysaccharide producing efficiency of all the successfully isolated fungal cultures were done according to Kim *et al.* (2001); Bae *et al*. (2000) and Lima *et al*. (2008). The submerged fermentation was performed in potato dextrose broth (Himedia) at 150 rpm with pH 6.0 and temperature 30°C for 14 days. The fermentation broth obtained at the end of incubation period was filtered with pre-weighed Whatman filter paper no. 1 to separate the mycelial/cellular biomass while the remanants of mycelial/cellular biomass still in the fermentation broth was removed by centrifuging the broth at 4°C on 10,000 rpm for 20 minutes. The mycelial or cellular pellets thus obtained were mixed with the main mycelial biomass separated earlier on preweighed filter paper and kept in oven at 50°C for 12 h for drying and the dry weight

was taken till the constant weight was obtained and expressed in g/l. The supernatant obtained was mixed with 5% trichloro acetic acid for precipitation of proteins present in the filtrate and kept for 24 h at 4°C. After 24 h the filtrate was centrifuged at 10000 rpm for 20 minutes at 4°C to remove the precipitated proteins from the solution and added with the 4 volume of ethanol (filtrate : ethanol = 1:4 v/v), stirred vigorously and left again for 24 h at 4°C for precipitation of the exopolysaccharides. After 24 h, the precipitated exopolysaccharides in the solution was centrifuged at 10,000 rpm for 20 min at 4°C, the supernatant fluid obtained was discarded and the pellets of precipitated EPS was lyophilized, weighed, expressed in g/l and stored at 4°C.

Identification of fungal isolates

The identification of all the fungal isolates capable of EPS production was done up to genus level on the basis of macroscopic and microscopic studies by consulting relevant literatures including monographs, research papers and books (Hawksworth (1995, 2004); Singer (1986); Arora (1979, 1986); Bigurd Funder (1953); Martinez (1991); Alexopolus (1996); Kirk *et al*. (2001); Klich and Pitt (1988); Lodge (1995); Manoharachary *et al*. (2005); Molina *et al*. (2004); Samson and Pitt 1986 (1985); Michael (2007); Purkayastha (1985); Raper and Fennell (1965).

Results and Discussions

The periodic and extensive survey made for collection of fungal isolates from biodiverse regions of lower Shivalik ranges of Chandigarh capital region including Chandigarh itself, Panchkula, Mohali, Solan and Shimla resulted in isolation of 94 fungal isolates. Of these, 52 isolates were found capable of producing exopolysaccharides in significant amount and therefore, identified up to generic level. On the basis of identification these EPS positive isolates were found to belong to 17 different genera viz., *Agaricus, Alternaria, Aspergillus, Aureobasidium, Candida, Curvullaria, Chaetomium, Fusarium, Ganoderma Penicillium, Pleurotus, Polyporus, Rhizopus, Stemophylium, Termitomyces, Tricholoma,* and *Saccharomyces species.*

Table 1: Frequency distribution of various fungal genera reported from foot hills of Shivalik ranges of Chandigarh capital region

S. No	Fungal Isolates	Lower Shivalik ranges of Chandigarh capital regions					Mean% frequency
		Chandigarh main	Mohali	Panchkula	Shimla	Solan	
1	*Aspergillus*	6	2	3	1	1	25.0
2	*Alternaria*	1	2	-	-	-	5.76
3	*Fusarium*	2	-			-	3.84
4	*Penicillium*	5	-	-	-	-	9.61
5	*Aureobasidium*	1	-	-	-	-	1.92
6	*Rhizopus*	1		-		-	1.92
7	*Candida*	1	-	-	-	-	1.92
8	*Saccharomyces*	1	-	-	-	-	1.92
9	*Stemphylium*	-	-	-	1	-	1.92
10	*Chaetomium*	1	-	-	-	-	1.92
11	*Curvularia*	1	-	-	-	-	1.92
12	*Agaricus*	3	-	-	3	-	11.53
13	*Ganoderma*	2	-	-	1	1	7.69
14	*Pleurotus*	1	-	1	-	-	3.84
15	*Polyporus*	2	-	-	-	1	5.76
16	*Termitomyces*	1	1	-	1	1	7.69
17	*Tricholoma*	1	-	-	-	2	5.76

(-) not found

It was observed that the Chandigarh main harboured the maximum diversity of EPS positive isolates (30 isolates) (Table 1). It was followed by Shimla and Solan with 7 and 6 isolates respectively. The pure culture of all the EPS positive isolates (52) have been obtained, maintained and preserved at 4°C. It was observed that the diversity of higher basidiomycetous fungi especially the mushrooms were more in Shimla and Solan regions. The genus *Aspergillus* was found to occur frequently in all the regions under study and thus possessed the maximum diversity of occurrence (25%). The study revealed no uniformity in the diversity and the distribution pattern of these EPS producing fungi in the region. This may be due to differences in the temperatures and moisture levels, kind of available substrates, their pH and the topography of the hilly and plain regions. These factors in turn affect the quantitative and qualitative properties of the EPS also.

All the 52 fungal isolates capable of producing the EPS were categorized into 5 groups on the basis of their EPS concentration (in g/l) obtained from submerged fermentation (Table 2). These are Group I (*Aspergillus, Fusarium, Alternaria & Penicillium*) with production range of > 1g/l ; Group II (*Ganoderma, Agaricus*) with production range of 1-2 g/l; Group III (*Pleurotus, Termitomyces*) with production range of 2-3 g/l; Group IV (*Ganoderma, Tricholoma)* with production range of 3-4 g/l and Group V (*Aureobasidium)* with production range of 4-5 g/l. Out of these 52 EPS producing isolates, 11 were found to be the prominent producers of EPS whose production ranged between 2-5g/l with *Aureobasidium* RYLF 10 as the best producer followed by *Penicillium* RYLF 35 and *Aspergillius* RYLF17 (Figure 1). It is significant to note that among the genus *Aspergillus,* the isolate number RYLF 17 found to gave the maximum EPS yield which ranged between 3-4g/l while the EPS concentration of other isolates of *Aspergillus* remained in production range of group I and II. In terms of biomass yield, no relation between exopolysaccharide concentration and biomass yield could be traced. Biomass yield of all the isolates ranged from 3.65 to 19.00g/l while the EPS concentration ranged between 0.01 to 4.60 g/l.

Table 2: Profile of exopolysaccharide (EPS) and biomass yield of fifty two fungal isolates reported from foot hills of Shivalik ranges of Chandigarh capital region

S. No.	Isolate No.	Genera	Biomass (g/l)	EPS (g/l)	Specific Yield
1	RYLF 1	*Aspergillus sp.*	6.46±0.10	0.43±0.35	0.066
2	RYLF2	*Aspergillus sp.*	6.60±0.15	1.15±0.34	0.174
3	RYLF3	*Aspergillus sp.*	8.03±0.02	0.35±0.78	0.044
4	RYLF4	*Aspergillus sp.*	6.20±0.20	0.10±0.87	0.016
5	RYLF5	*Penicillium sp.*	3.60±0.41	0.40±0.67	0.111
6	RYLF6	*Penicillium sp.*	7.54±0.09	0.50±0.56	0.066
7	RYLF7	*Alternaria sp.*	10.2±0.12	0.37±0.34	0.036
8	RYLF 59	*Alternaria sp.*	7.04±0.20	0.009±0.45	0.001
9	RYLF8	*Fusarium sp.*	4.90±0.12	1.05±0.45	0.214
10	RYEF1-3	*Alternaria sp.*	9.75±0.20	2.12±0.21	0.217
11	RYLF10	*Aureobasidium sp.*	4.56±0.13	4.60±0.34	1.008
12	RYLF11	*Aspergillus sp.*	6.60±0.30	0.05±0.34	0.008
13	RYLF12	*Aspergillus sp.*	7.10±0.12	0.010±0.42	0.001

14	RYLF13	*Aspergillus sp.*	6.68±0.21	0.55±0.30	0.082
15	RYLF14	*Aspergillus sp.*	7.35±0.21	0.40±0.21	0.054
16	RYLF15	*Aspergillus sp.*	14.97±0.40	1.05±0.21	0.097
17	RYLF16	*Fusarium sp.*	5.35±0.30	0.45±0.32	0.084
18	RYLF17	*Aspergillus sp.*	6.12±0.45	3.66±0.54	0.598
19	RYLF18	*Rhizopus sp.*	5.85±0.23	0.74±0.78	0.126
20	RYMF19	*Ganoderma sp.*	5.00±0.12	0.26±0.35	0.052
21	RYLF20	*Candida sp.*	9.10±0.56	0.23±0.21	0.025
22	RYLF21	*Saccharomyces sp.*	8.30±0.22	0.19±0.45	0.022
23	RYLF22	*Penicillum sp.*	8.10±0.24	0.10±0.34	0.012
24	RYMF23	*Termitomyces sp.*	7.20±0.45	0.40±0.32	0.055
25	RYLF24	*Aspergillus sp.*	6.00±0.65	0.10±0.34	0.016
26	RYLF 60	*Aspergillus sp.*	4.80±0.32	0.008±0.21	0.001
27	RYLF30	*Aspergillus sp.*	3.65±0.45	0.47±0.56	0.129
28	RYMF33	*Ganoderma sp.*	5.40±0.21	0.35±0.34	0.065
29	RYLF35	*Penicillium sp.*	4.05±0.12	3.86±0.32	0.953
30	RYLF58	*Penicillium sp.*	4.30±0.1	1.05±0.21	0.224
31	RYMF1	*Polyporus sp.*	4.80±0.12	0.08±0.43	0.016
32	RYMF14	*Polyporus sp.*	8.29±0.31	0.8±0.34	0.097
33	RYMF12	*Polyporus sp.*	3.60±0.30	0.93±0.34	0.258
34	RYMF7	*Termitomyces sp.*	4.60±0.30	2.31±0.21	0.502
35	RYMF9	*Tricholoma sp.*	4.01±0.25	0.05±0.81	0.012
36	RYMF5	*Agaricus sp.*	5.30±0.56	1.53±0.68	0.288
37	RYMF11	*Termitomyces sp.*	6.00±0.81	0.6±0.21	0.100
38	RYMF17	*Termitomyces sp.*	4.00±0.27	0.03±0.12	0.007
39	RYMF16	*Pleurotus sp.*	8.09±0.29	2.45±0.42	0.302
40	RYMF3	*Agaricus sp.*	8.05±0.21	0.96±0.20	0.119
41	RYMF4	*Ganoderma sp.*	7.69±0.25	1.2±0.20	0.156
42	RYMF2	*Agaricus sp.*	5.90±0.76	0.5±0.20	0.085
43	RYMF15	*Ganoderma sp.*	19.00±0.21	3.59±0.21	0.188
44	RYMF6	*Agaricus sp.*	2.08±0.21	0.02±0.43	0.001
45	RYMF8	*Agaricus sp.*	5.02±0.32	0.04±0.23	0.008
46	RYMF10	*Agaricus sp.*	5.01±0.21	0.03±0.63	0.006
47	RYMF13	*Tricholoma sp.*	7.02±0.23	3.47±0.56	0.494
48	RYMF19	*Pleurotus sp.*	5.08±0.45	0.04±0.45	0.008
49	RYMF111	*Tricholoma sp.*	9.75±0.32	2.12±0.34	0.217
50	RYEF1-5	*Stemphylium sp.*	10.33±0.43	2.08±0.21	0.201
51	RYEF2	*Chaetomium sp.*	4.15±0.21	2.47±0.23	0.595
52	RYEF 6-1	*Curvullaria sp.*	3.20±0.21	2.10±0.31	0.006

Each value represents the mean ± SD (n=3)/SD standard deviation; Production medium: Potato dextrose broth; Temperature: 28±1^{o}C; pH : 5.6; Shaking Intensity: 150 rpm; Incubation period: 14 days

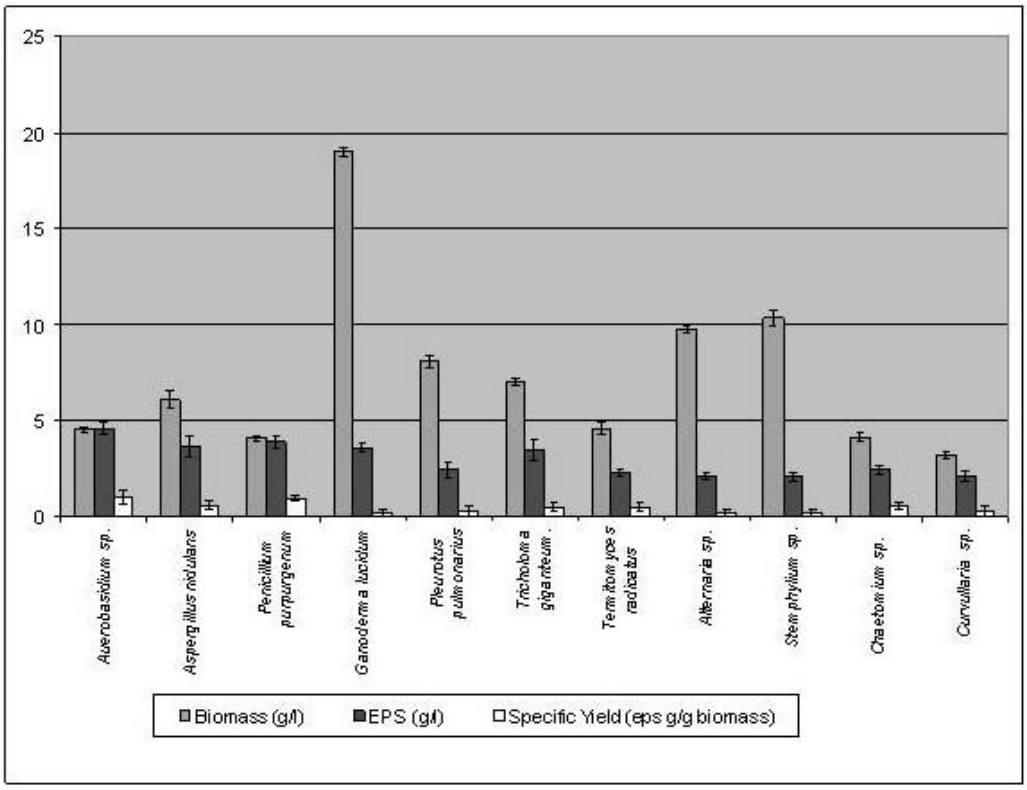

Figure 1: Eleven prominent exopolysaccharide (EPS) producing fungal isolates.

The study also revealed the variations in cultural characteristics by different isolates during EPS fermentation under submerged cultural conditions. Some isolates (*Aspergillus, Pleurotus & Termitomyces*) found to grow in regular, spherical to globose pellet forms while in others the formation of pellets was not observed, but rather a mycelium agglomeration without defined shape and size were observed (*Aureobasidium, Penicillium, Fusarium & Tricholoma* species). Besides, the colour and consistency of the production medium also varied depending on the physiology of different isolates. It usually remained clear, slight transparent during first to 3rd day of incubation but with increase in incubation period the change in colour and consistency were observed in maximum cultures due to breaking up of pellets, depletion of glucose level and presence of excreted metabolites which gave the medium dark, thick, viscous, and turbid appearances.

The study revealed that the fungi belonging to asco- and deuteromycetes are also the good source of exopolysaccharide apart from basidiomycetous isolates and there is need for more exploration and evaluation of fungi belonging to these very groups for their EPS producing efficiencies. The data obtained from this screening are just the indicative for selecting isolates for further investigation on exopolysaccharide production. If production conditions modified and /or optimized their yield can also be increased and the EPS produced can find application in various industrial and medical fields. The present study is an attempt in this regard and can pave the path for the investigators working in the subject.

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References

Arora D. 1979. Mushroom Demystified. IInd edn. Ten Speed Press, Berkeley, California.

Arora D. 1986. Mushroom Demystified: A comprehensive guide to the fleshy fungi. Berkeley: Ten Speed Press.

Alexopoulos, Mims CW, Blackwell M. 1996. Introductory Mycology. New York: John Wiley & Sons.

Atri NS, Kaur A, Kour H. 2005. Wild mushrooms collection and identification. In: Frontiers Mushroom Biotechnology. eds: RD Rai, RC Upadhyay and SR Sharma, pp. 9-26.

Banerjee D, Jana M and Mahapatra S. 2009. Production of exopolysaccharides by endophytic Stemphylium sp. Micologia Aplicada International. 21: 57-62.

Bae JT, Sinha J, Park JP, Song CH, Yun JW. 2000. Optimization of submerged condition for exo-biopolymer production by *Paecilomyces japonica*. *J Microbiol Biotechnol* **10**: 482-487.

Hawksworth, DL. 1995. The fungal dimension of biodiversity: Magnitude, significance and conservation. *Mycol Res.* **95**: 641-655.

Hawksworth DL**.** 2004. Fungal diversity and its Implifications for Genetic Resource collections. *Stud Mycol* 50: 19.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DM. 1995. Ainsworth & Bisby's dictionary of the fungi. 8th ed. Egham: CAB International, pp. 616.

Hwang HJ, Kim SW, Xu CP, Choi JW and Yun JW. 2004. Morhological and rheological properties of the three different species of Phellinus in submerged cultures. J. App. Microbiol. 96: 1296-1305.

Kirk PM, Cannon PF, David JC, Stalpers J. 2001. Ainsworth and Bisby's Dictionary of the Fungi. 9th ed. CAB International, Wallingford, UK.

Kim SW, Hwang HJ, Park JP, Cho SP, Das S, Yun, Song CH. 2001. Production of a hypoglycemic extracellular poly-saccharides from submerged culture of the mushroom, *Phellinus lintteus*. *Biotechnol lett.* **23**: 513-517.

Klich MA, Pitt JI. 1988. A laboratory guide to common *Aspergillus* species and their Teleomorphs. North Ryde, NSW, CSIRO, Div. of Food Processing.

Lima LF O, Habu S, Gern JC, Nascimento, Parada JL, Noseda MD, Goncalves AG, Nisha VR, Pandey A, Soccol VT, Soccol CR. 2008. Production and characteri-zation of exopolysaccharides produced by *Agaricus brasiliensis* in submerged fermentation. *Appl Biochem and Biotechnol.* **151**: 283-294.

Lodge DJ**.** 1995. A Survey of patterns of Diversity in Non-Lichinized Fungi. *Mitt. Eidgenoss. Forsch. Anst. Wald Schnee Landsch.* **70**: 157-173.

Manoharachary C, Sridhar K, Singh R, Adholeya, Suryanarayanan TS, Rawat S and Johri BN. 2005. Fungal Biodiversity: Distribution, Conservation and Prospecting of Fungi from India *Curr Sci.* **89**: 58-71.

Martinez AT, Barrasa JM, Prieto A and Blanco MN. 1991. Fatty acid composition and taxonomic status of *Ganoderma austral* from Southern Chile. *Mycol Res.* **95**: 782-784.

Mahmoud Yehia AG, Mahmoud Eman HFA and Abd Elzaher EHF. 2007. Response of the higher basidiomycetic *Ganoderma resinaceum* to sodium chloride stress. Mycobiol. 35: 124-128.

Michael Kuo. 2007. 100 edible mushroom. The university of Michigan press, p 329.

Molina RO, Dell T, Luoma D, Amaranthus Mueller MG, Lodge DJ, Ammirati JF and O'Dell TE. 2004. Collecting and Describing Macrofungi. *Biodiversity of fungi-inventory and monitoring methods.* 128-158.

Margaritis A, Pace GW. 1985. Microbial polysaccharides. In: Blanch HW, Drew S, Wang DIC, eds. *Comprehensive Biotechnology*, Vol. 3. Oxford, UK: Pergamon Press, pp. 1005–1044.

Purkaryastha RP. 1985. International Bioscience monographs vol 16: Manual of Indian edible Mushroom.

Rau U, Kuenez, A, Wray V, Nimtz M, Wrenger J and Cicek H. 2009. Production and structural analysis of the polysaccharide secreted by *Trametes (Coriolus) versicolor* ATCC 200801. Appl Microbiol Biotechnol 81: 827-837.

Raper KB and Fennell DI. 1965. The genus *Aspergillus*. Williams and Wilkins, Baltimore, Maryland.

Singer R. 1986. Agaricales in Mordern taxonomy. 4th edn. Koeltz scientific Books, Konigatein.

Sutherland IW. 1996. Extacellular polysaccharides.In: Biotechnology, vol. 6. eds, H.J. Rhem and G. Reed. VCH, Weinheim, pp. 615-657.

Samson RA and Pitt JJ. 1985. Advances in *Penicillium* and *Aspergillus* Systematics. Plenum Press, New York.

Samson RA and Pitt JJ. 1990. Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New york.

Sutherland IW. 1997. Microbial exopoly-saccharides – structural subtleties and their consequences. *Pure Appl. Chem.* **69**: 1911– 1917.

Sutherland IW. 1998. Novel and established applications of microbial poly-saccharides. *Trends Biotechnol.* **16**: 41–46.

Warcup JH. 1950. The soil plate method for isolation of fungi from soil. *Nature* **178**:1477.