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SCREENING OF BASIDIOMYCETES FOR THE PRODUCTION OF EXOPOLYSACCHARIDE AND BIOMASS IN SUBMERGED CULTURE

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ABSTRACT

Fifty-six strains of Basidiomycetes, including native Brazilian fungi isolated from different ecosystems and edible mushrooms, were screened for production of exopolysaccharides and biomass in submerged culture. *Agaricus* sp. (CCB 280) and *Oudemansiella canarii* (Jungh.) Hohn (CCB 179) were the highest exopolysaccharide producers (6.01 and 3.54 g dry w./l respectively) after 7 days of incubation. The best producer of biomass was *Schizophyllum commune* Fr.:Fr. (CCB 473) with 16.68 g dry w./l in 14 days of incubation. When the culture filtrate was submitted to freezing prior to polysaccharide precipitation, a gelatinous fraction was formed.

Key words: Basidiomycetes, exopolysaccharide, biomass, submerged culture

INTRODUCTION

Following previous work regarding collection, identification and isolation of native Brazilian Basidiomycetes in pure culture, investigations were carried out concerning their utilization in biotechnological processes such as lignin and recalcitrant substances degradation, soil bioremediation, edible fungal biomass and metabolites production.

Basidiomycetes have been studied extensively for their capacity of degradation. The so-called white rot fungi, which degrade lignin, have this peculiar capacity that leads to research on degradation of xenobiotics. In addition to enzymes, there is evidence that the extracellular polysaccharides produced by these lignocellulolitic fungi play an important role in the process (6, 16). These exopolysaccharides can immobilize the exocellular enzymes. According to Catley (3), the gel formed by these biopolymers prevents the hyphal dehydration, permits cell adherence to other cells or to surfaces and could possibly select molecules from the environment.

A practical aspect of the study and characterization of fungal exopolysaccharide is the availability of data for the investigation of its physiological and ecological importance. In addition, this biopolymer may have potential industrial applications. An example is the exopolysaccharide known as schizophyllan that is produced by the Basidiomycete *Schizophyllum commune*. This polymer is a β- (1→3), (1→6)-glucan, soluble in water, that forms a viscous solution with high thermal stability. It is already used in commercial areas.

Another possible application of these biopolymers is in human health. There is intensive research on fungal polysaccharides as antitumor agents (8, 9).

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The fungal biomass can have various uses, which is an advantage as far as the fermentation is concerned because the process residue is reduced (14). Possible uses for this biomass are food or feed in the form of protein supplement or source of lipids. It can also be used for the extraction of flavours (10) and other metabolites, such as enzymes and polysaccharides. The most recent utilization of fungal biomass is for wound healing. According to Hamlyn and Schmidt (7), chitin, that has a healing capacity, is already in the fibrous form when extracted from the fungal cell wall. This might facilitate its manipulation.

The aim of this work was to screen 56 strains of Basidiomycetes for exopolysaccharide and biomass production in submerged culture contributing to the study of the potentiality of the Brazilian mycobiota.

MATERIALS AND METHODS

Microorganisms. 48 strains of native Brazilian Basidiomycetes and 8 strains of commercial edible mushrooms, corresponding to 51 different species belonging to 42 genera, were screened. The pure cultures came from the Culture Collection of Basidiomycetes (CCB) of the Instituto de Botânica - São Paulo - Brazil, and are shown in Table 1.

Liquid culture medium (g/l): Peptone 1.0; yeast extract 2.0; K_2HPO_4 1.0; $MgSO_4.7H_2O$ 0.2; $(NH_4)_2SO_4$ 5.0; glucose 39.0; pH 6.0. This medium was selected in preliminary studies as adequate for exopolysaccharide production by Basidiomycetes (4).

Erlenmeyer flasks containing 100 ml of sterilized culture medium were inoculated with the suspension in sterile water of fungal mycelium grown on two potato dextrose agar slants. Incubation was done at 25°C on shaker at 150 rpm.

Screening. For the screening, the incubation times were 7 and 14 days. The culture was filtered to separate fungal biomass, which was washed twice with distilled water and quantified as dry weight (105ºC to constant weight). Isopropanol was added to the culture filtrate (1:1 v/v) and after 24 h at 4°C the precipitated biopolymer was separated by centrifugation (8,000 rpm for 10 minutes) and also quantified as dry weight.

Glucose assay. The residual glucose content of the culture filtrate was determined with a colorimetric method (17).

Chemicals used were produced by: E. Merck GmbH, Darmstadt, Germany; BDH Chemicals Ltd, Poole, England; Boehringer Manheim GmbH; Fluka Chemie AG., Buchs, Switzerland; DIFCO Laboratories, Detroit, U.S.A. and A. Constantino & C. s.p.a., Favria, Italy.

Table 1 - Results of the screening for the production of exopolysaccharide (P_p) and biomass (P_x), with the conversion yield of glucose in polymer ($Y_{p/s}$), in biomass ($Y_{x/s}$) and the specific yield (Y_e).

CCB	STRAIN	DAY	P_x g dry w./l	$Y_{x/s}$	P_p g dry w./l	$Y_{p/s}$	Y_e
041	*Agaricus xanthodermus*	7	0.88	0.098	1.61	.0179	1.830
	*	14	0.92	0.137	1.39	0.207	1.511
280	*Agaricus* sp.	7	1.64	0.208	6.01	0.761	3.665
		14	3.09	0.322	1.36	0.142	0.440
211	*Agrocybe platensis*	7	8.37	0.406	1.00	0.048	0.120
		14	10.18	0.318	1.33	0.042	0.131
392	*Antrodiella ginestae*	7	6.28	0.262	0.49	0.020	0.078
		14	7.15	0.248	0.64	0.022	0.090
045	*Auricularia fuscosuccinea*	7	1.20	0.200	0.54	0.090	0.450
	*	14	3.62	0.266	1.10	0.081	0.304
173	*Calvatia cyathiformis*	7	1.10	0.157	2.05	0.293	1.864
	*	14	0.70	0.082	0.72	0.085	1.029
191	*Climacodon pulcherrimus*	7	0.34	0.046	0.75	0.101	2.206
		14	0.63	0.066	1.03	0.107	1.635

(continuação...)

CCB	STRAIN	DAY	P_x g dry w./l	$Y_{x/s}$	P_p g dry w./l	$Y_{p/s}$	Y_e
111	*Coprinus comatus*	7	5.18	0.395	1.11	0.085	0.214
		14	7.56	0.450	1.53	0.091	0.202
513	*Flammulina velutipes*	7	4.86	0.273	1.05	0.059	0.216
		14	8.28	0.213	1.74	0.045	0.210
214	*Fomitopsis spraguei*	7	2.91	0.239	0.21	0.017	0.072
		14	2.38	0.165	0.28	0.019	0.118
168	*Ganoderma australe*	7	14.75	0.382	2.64	0.068	0.179
		14	15.02	0.387	2.04	0.053	0.136
323	*Ganoderma lipsiensis*	7	7.29	0.361	0.98	0.049	0.134
	*	14	13.24	0.404	2.75	0.084	0.208
177	*Gloeophyllum striatum*	7	5.70	0.533	0.20	0.019	0.035
		14	8.60	0.422	0.53	0.026	0.062
188	*Gloeophyllum striatum*	7	4.56	0.356	0.19	0.015	0.042
		14	8.06	0.593	0.15	0.011	0.019
249	*Gymnopilus sp.*	7	2.98	0.339	0.41	0.047	0.138
		14	7.00	0.432	1.08	0.067	0.154
289	*Hydnopolyporus fimbriatus*	7	2.40	0.189	0.26	0.020	0.108
		14	3.71	0.277	0.51	0.038	0.137
160	*Hypochnicium sp.*	7	11.24	0.420	1.50	0.056	0.133
		14	10.30	0.380	1.20	0.044	0.116
207	*Inonotus ludovicianus*	7	0.99	0.116	0.64	0.075	0.646
		14	2.56	0.194	1.32	0.100	0.515
196	*Irpex lacteus*	7	12.46	0.398	2.49	0.080	0.200
	*	14	15.65	0.404	2.01	0.052	0.128
157	*Lachnocladium sp.*	7	12.86	0.331	1.83	0.047	0.142
	*	14	12.74	0.328	2.12	0.055	0.166
072	*Lentinula edodes*	7	2.24	0.311	0.49	0.068	0.219
	*	14	4.70	0.331	1.18	0.083	0.250
162	*Lentinus strigosus*	7	4.74	0.373	0.11	0.009	0.023
		14	6.26	0.252	0.51	0.021	0.082
268	*Lentinus velutinus*	7	6.37	0.344	1.46	0.079	0.229
	*	14	9.30	0.332	1.76	0.063	0.189
110	*Lepista sp.*	7	5.82	0.485	2.68	0.223	0.460
	*	14	13.48	0.709	2.09	0.110	0.155
279	*Macrolepiota procera*	7	1.93	0.179	0.52	0.048	0.269
		14	3.94	0.281	0.88	0.063	0.223
361	*Marasmius cladophyllus*	7	9.40	0.490	0.70	0.036	0.074
	*	14	9.91	0.312	0.56	0.018	0.056

(continuação...)

CCB	STRAIN	DAY	P_x g dry w./l	$Y_{x/s}$	P_p g dry w./l	$Y_{p/s}$	Y_e
184	*Melanoporia nigra*	7	6.44	0.467	1.42	0.103	0.220
	*	14	12.16	0.316	1.90	0.049	0.156
216	*Nothopanus hygrophanus*	7	16.16	0.444	1.78	0.049	0.110
	*	14	13.56	0.361	2.20	0.058	0.162
164	*Oligoporus sp.*	7	4.54	0.286	2.71	0.170	0.597
	*	14	12.15	0.334	2.92	0.080	0.240
179	*Oudemansiella canarii*	7	13.40	0.496	3.54	0.131	0.264
	*	14	15.37	0.430	1.70	0.048	0.111
187	*Panaeolus papilionaceus*	7	7.86	0.624	1.38	0.110	0.176
		14	5.91	0.296	1.70	0.085	0.288
204	*Peniophora cinerea*	7	13.22	0.472	2.58	0.092	0.195
	*	14	13.72	0.352	3.04	0.078	0.222
379	*Perenniporia piperis*	7	6.86	0.408	1.18	0.070	0.172
	*	14	12.06	0.520	1.90	0.082	0.158
190	*Phellinus gilvus*	7	8.30	0.506	0.61	0.037	0.074
	*	14	10.32	0.266	1.23	0.032	0.119
078	*Pholiota nameko*	7	2.96	0.302	1.64	0.167	0.554
	*	14	5.18	0.454	0.66	0.058	0.127
394	*Pleurotus flabellatus*	7	5.94	0.381	0.81	0.052	0.136
	*	14	8.50	0.362	2.00	0.085	0.235
004	*Pleurotus ostreatus*	7	4.06	0.366	0.57	0.051	0.140
	*	14	4.50	-	0.32	-	0.071
016	*Pleurotus ostreatoroseus*	7	8.56	0.408	2.20	0.105	0.257
	*	14	9.00	0.280	2.38	0.074	0.264
017	*Pleurotus sajor-caju*	7	11.47	0.484	1.85	0.078	0.161
	*	14	10.39	0.299	1.72	0.049	0.166
001	*Pleurotus* sp. “florida”	7	11.02	0.510	2.85	0.132	0.259
	*	14	11.72	0.480	1.36	0.056	0.116
259	*Psilocybe castanella*	7	8.96	0.498	1.18	0.066	0.132
	*	14	9.80	0.315	1.52	0.049	0.155
224	*Psilocybe subcubensis*	7	2.92	0.243	0.58	0.048	0.199
		14	4.96	0.359	0.59	0.043	0.119
113	*Pycnoporus sanguineus*	7	6.10	0.295	1.04	0.050	0.170
		14	7.83	0.201	0.87	0.022	0.111
277	*Pycnoporus sanguineus*	7	0.36	0.047	0.74	0.096	2.056
		14	0.43	0.090	0.82	0.171	1.907
334	*Rigidoporus microporus*	7	4.70	0.402	0.81	0.069	0.172
	*	14	16.04	0.685	0.83	0.035	0.052

(continuação...)

CCB	STRAIN	DAY	P_x g dry w./l	$Y_{x/s}$	P_p g dry w./l	$Y_{p/s}$	Y_e
467	*Ripartitella* cf. *brasiliensis*	7	6.15	0.521	0.86	0.073	0.140
	*	14	11.80	0.371	1.50	0.047	0.127
368	*Schizophyllum commune*	7	7.22	0.185	1.85	0.047	0.256
		14	6.02	0.154	0.91	0.023	0.151
473	*Schizophyllum commune*	7	13.84	0.416	1.32	0.040	0.095
		14	16.68	0.429	1.76	0.045	0.106
474	*Schizophyllum commune*	7	10.84	0.386	1.01	0.036	0.093
		14	16.10	0.415	1.97	0.051	0.122
202	*Trametes versicolor*	7	10.20	0.313	1.51	0.046	0.148
	*	14	7.43	0.191	2.34	0.060	0.315
165	*Trametes villosa*	7	6.86	0.279	1.73	0.070	0.252
	*	14	10.07	0.259	2.65	0.068	0.263
213	*Trametes villosa*	7	7.24	0.234	1.99	0.064	0.275
	*	14	9.02	0.232	2.12	0.055	0.235
203	*Trichaptum byssogenum*	7	9.00	0.232	1.35	0.035	0.150
	*	14	7.86	0.203	1.48	0.038	0.188
082	*Tricholoma crassum*	7	11.15	0.791	2.20	0.156	0.197
	*	14	15.90	0.646	3.23	0.131	0.203
390	*Trogia buccinalis*	7	5.74	0.279	1.03	0.050	0.179
	*	14	7.86	0.273	1.60	0.056	0.204
193	*Tyromyces pseudolacteus*	7	8.34	0.323	1.19	0.046	0.143
		14	6.50	0.227	0.77	0.027	0.118

* Formation of gel due to the freezing of the culture filtrate

P_x = g dry weight biomass/ l culture
$Y_{x/s}$ = g dry weight biomass/ g consumed glucose
P_p = g dry weight biopolymer/ l culture
$Y_{p/s}$= g dry weight biopolymer/ g consumed glucose
Y_e= specific yield

RESULTS AND DISCUSSION

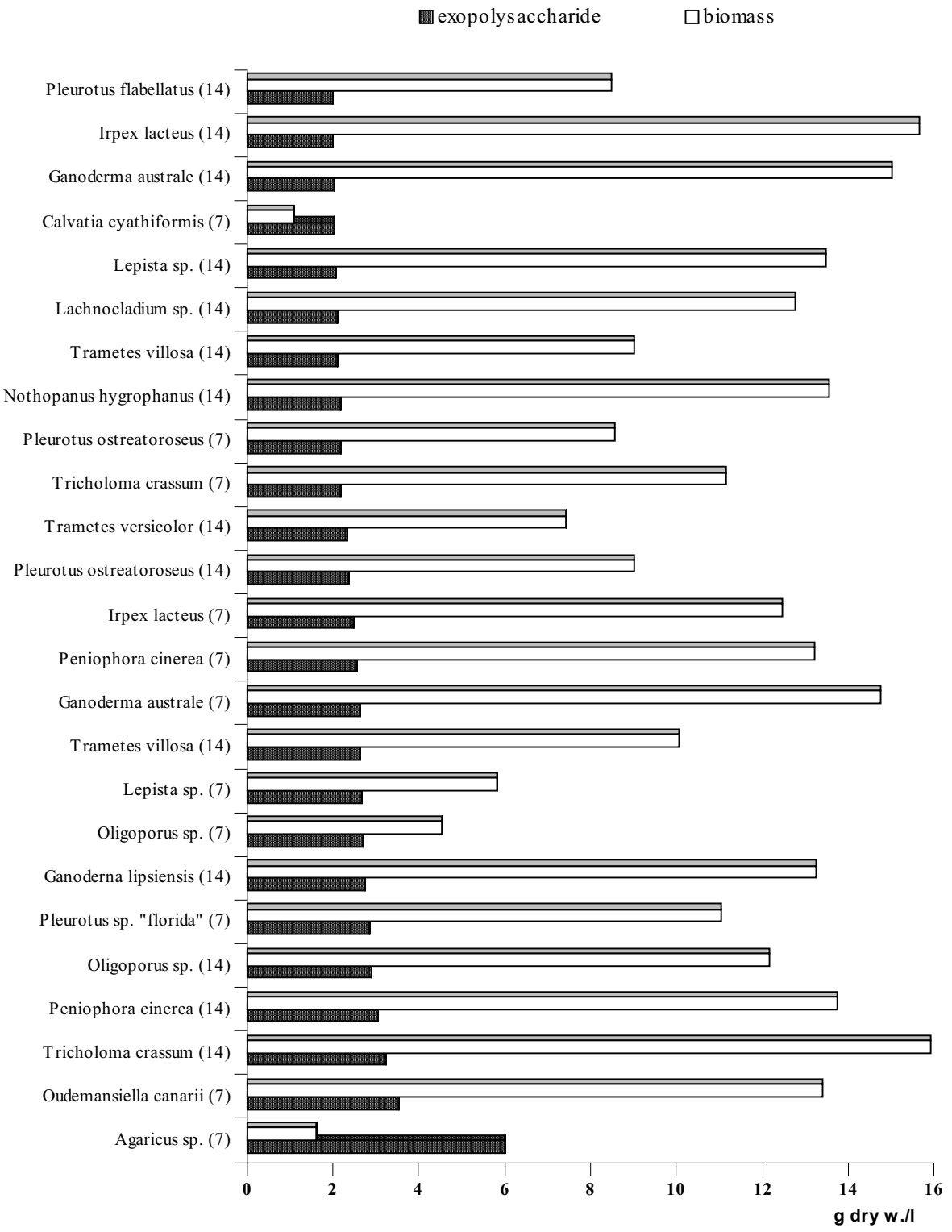
Almost all the strains produced exopolysaccharide in different quantities (Table 1). The best yield was produced by *Agaricus* sp., with 6.01 g dry w./l (conversion yield, $Y_{p/s}$= 0.761) and *Oudemansiella canarii* with 3.54 g dry w./l ($Y_{p/s}$= 0.131) after 7 days of incubation. *Tricholoma crassum* had a similar production (3.23 g dry w./l) with conversion yield of 0.131, but after 14 days of incubation.

About 30% of the strains produced more exopolysaccharide after 7 days incubation; however 70% produced more after 14 days, which indicates that an accurate study of each strain with growth and product kinetic profiles should be carried out if there is a possibility of its utilization for polymer production. The strains that produced more than 2.0 g dry w./l of exopolysaccharide are shown in Fig. 1.

There is no relation between biomass and exopolysaccharide production and in some cases a considerable decrease of biopolymer was observed after 14 days incubation *(Agaricus* sp., *Calvatia cyathiformis*, *Oudemansiella canarii* and *Pleurotus* sp. "florida").

The conversion yield of glucose as polymer varied between 0.020 and 0.100 for 75% of the strains and the best yields were those of *Agaricus* sp. (0.761) and *Calvatia cyathiformis* (0.293).

Figure 1. Strains that produced more than 2.0 g dry w./l of polymer after 7 and 14 days.



Different strains of *Schizophyllum commune*, *Pycnoporus sanguineus* and *Trametes villosa* showed different results not only for biomass, but also for polymer production. These data confirm the diversity of exopolysaccharide production among different strains in submerged culture. Another strain of *Schizophyllum commune* was submitted to a similar screening by Cavazzoni and Adami (4) with the same growing conditions used here. The polymer production was higher (5.3 g dry w./l).

An interesting observation was made concerning the formation of an insoluble gel when the culture filtrate was frozen prior to polysaccharide precipitation. In the Table 1 these strains are marked. This peculiar characteristic could aid polymer separation, since there is no need of an organic solvent such as isopropanol, ethanol or acetone for the precipitation of the polymer, thus increasing the process viability. Moreover, it is important to observe that the product obtained by solvent precipitation cannot be considered pure polysaccharide because proteins and salts present in the medium coprecipitate. The data obtained from this screening are just indicative for selecting strains for further investigations on exopolysaccharide production.

Some of the strains studied here were submitted to a lignin degradation activity test (2). All strains that produced more than 2.0 g dry w./l of exopolysaccharide showed good lignin degradation activity (24.8-65.4%) at 25°C and 60 days of incubation. For *Irpex lacteus* the result was higher at 30°C with 78.4% of substrate lignin degradation. Okino (15) studied some of these strains for laccase and peroxidase production and all of them showed enzyme activity.

Biomass production ranged from 0.34 to 16.68 g dry w./l. Some strains, such as *Agaricus xanthodermus, Calvatia cyathiformis* and *Climacodon pulcherrimus*, had a slow growth rate in these culture conditions. Others, such as *Schyzophyllum commune, Rigidoporus microporus, Oudemansiella canarii, Irpex lacteus* and *Nothopanus hygrophanus* produced more than 15.00 g dry w./l of biomass.

Among the edible strains, those that produced more biomass after 7 days incubation were *Pleurotus sajor-caju* (11.47 g dry w./l), *Pleurotus* sp. "florida" (11.02 g dry w./l), and *Agrocybe platensis* (10.18 g dry w./l). After 14 days incubation, the best biomass producer was *Lepista* sp. (13.48 g dry w./l).

The conditions used for the submerged culture could be considered adequate for biomass production. Data presented in literature (1, 5, 11, 12) showed lower production for *Pleurotus* species with other culture parameters.

During estimation of polymer and biomass produced it is important to consider that exopolysaccharides adherent to the hyphae are also entrapped into the pellets formed during the submerged culture (1), which means that the dry weight of biopolymer which precipitated from the culture filtrate does not correspond to the total exopolysaccharide and that the biomass can be overestimated. To minimize this problem biomass was washed twice with distilled water.

During the screening it was observed that the submerged cultures showed different characteristics according to the fungal species. The pellets formed can be regular or irregular in form and size. The form varies from spherical to cylindrical and the size from 1 to 20 mm. In some cases the formation of pellets was not observed, but rather a mycelial agglomeration without a defined form (13).

The pellets were smooth, hairy (with looser outer zones) or with fringes of aggregated hyphae that give the pellet a star form. The color and consistency were also different, as well as the flavour. In the case of *Auricularia fuscosuccinea* the pellet had a gelatinous consistency. Sometimes the culture filtrate was very clear, other times was turbid and very viscous. In most of the cultures the presence of crystals with different forms was observed, which could indicate, in some cases, the presence of excreted metabolites.

When there is a depletion of glucose in the medium it was observed that pellets begin to become darker and break up. The dead hyphae are decomposed and the resulting substances are reabsorbed by the mycelium.

Results showed that most of the Basidiomycetes strains screened are potential exopolysaccharide producers. The possibility of using these biopolymers for medical application promises a large opportunity to improve the study of such group of fungi. Besides the Brazilian mycobiota has been scarcely investigated although its great potentiality.

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RESUMO

Triagem de basidiomicetos para a produção de exopolissacarídeos e biomassa em cultura líquida

Este trabalho diz respeito à produção de exopolissacarídeos e biomassa por basidiomicetos em cultura líquida. O "screening" foi realizado com 56 linhagens incluindo fungos nativos de diferentes ecossistemas do Brasil e de fungos comestíveis. *Agaricus* sp. (CCB 280) e *Oudemansiella canarii* (Jungh.) Hohn (CCB 179) foram os melhores produtores de exopolissacarídeo (6,01 e 3,54 g peso seco/l respectivamente), em 7 dias de incubação. O melhor produtor de biomassa foi *Schizophyllum commune* Fr.:Fr. (CCB 473) com 16,68 g peso seco/l em 14 dias de incubação. Quando o filtrado cultural foi submetido à congelamento antes da precipitação do polissacarídeo, formou-se uma fração gelatinosa.

Palavras-chave: Basidiomiceto, exopolissacarídeo, biomassa, cultura líquida

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