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Production of extracellular carbohydrases by mushrooms

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Seven different mushrooms, in submerged culture, are capable of utilizing various polysaccharides, i.e., xylan, mannan, cellulose, dextran, inulin, added in the medium as sole source of carbon. But chitin was found to be not utilized by any of them. Xylan is commonly utilized by all the mushrooms tested.

Inducible and constitutive types of hydrolytic enzymes for those polysaccharides have been identified in the fermented broth of the mushrooms. Xylanase was found to be a constitutive enzyme for most of the strains except for *Panaeolus papilionaceus* (Bull. ex Fr.) Fr. for which it is inducible.

INTRODUCTION

Utilization of different polysaccharides by higher fungi has been observed in different laboratories (Styer 1928; Reusser et al. 1958). Treschow (1944) studied the growth of mushrooms on hemicellulose, lignin and xylan in still culture and found that xylan was best utilized. Perlman (1949) obtained higher yields of mushroom mycelia on starch compared to yields on glucose or other monosaccharides as carbon sources. Reusser et al. (1958), Humfeld et Sugihara (1952) also reported the utilization of xylan by mushrooms as a source of carbon in submerged culture. It has also been reported previously (Ghosh, Sengupta 1977, 1978) that most of the mushrooms preferentially use polymers of glucose as compared to the monomer.

Thus it appears that utilization of different types of polysaccharides by the mushrooms may be an interesting problem particularly for the isolation of different specific carbohydrases.

MATERIAL AND METHODS

Organisms. The following mushrooms: *Termitomyces clypeatus* (Heim), *Panaeolus papillionaceus* (Bull. ex Fr.) Quél., *Gymnopilus chrysimyces* (Berk.), *Coprinus lagopus* (Fr.) Fr., *Lentinus squarrosulus* (Mont.), *Volvariella volvacea* and *Agaricus bisporus* (Lange) Sing., were grown under submerged condition in shaken flasks.

Mushroom growth. Conditions for the optimum submerged growth of these mushrooms (Table 1) have already been reported (Ghosh, Sengupta 1977, 1978).

Medium for the production of carbohydrases. Various carbon sources in each were added one at a time to mushroom basal medium instead of the usual carbon source (Table 1). Mannan, xylan, chitin, inulin, dextran and cellulose were used as sole carbon sources at a concentration of 1% w/v. In control experiments basal medium containing the usual carbon source for the given mushroom was used at a concentration of 1% w/v.

Table 1

Carbon and nitrogen sources used for the optimum mushroom growth

Organisms	Initial pH of the medium	N-source		C-source	
		compounds	amount /%, w/v/	compounds	amount /%, w/v/
Termitomyces clypeatus	5.0	$NH_4H_2PO_4$	2.463	dextrin	10
Panaeolus papilionaceus	5.0	$NH_4H_2PO_4$	2.463	soluble starch	9
Gymnopilus chrysimyces	5.0	urea	0.214	"	5
Coprinus lagopus	5.0	urea	0.032	"	6
Lentinus squarrosulus	5.0	NH_4Cl	0.057	dextrin	8
Volvariella volvacea	4.0	KNO_3	0.108	soluble starch	5
Agaricus bisporus	8.0	NH_4Cl	0.382	glucose	6

Micronutrients present in g/100 ml: KH_2PO_4, 0.087; $MgSO_4$, $7H_2O$, 0.05; $FeSO_4$, $7H_2O$, 0.025; $MnCl_2$, $4H_2O$, 0.0036; $NaMoO_4$, $4H_2O$, 0.0032; $ZnSO_4$, $7H_2O$, 0.03; $CaCl_2$, $2H_2O$, 0.037; boric acid, 0.057; $CuSO_4$, $5H_2O$, 0.0039; with the omission of Cu^{2+} for T. clypeatus, C. lagopus and V. volvacea; $NaMoO_4$ for G.chrysimyces and L.squarrosulus; Zn^{2+} for A. bisporus. Omission of all except KH_2PO_4 and $MgSO_4$ was made for P. papilionaceus. The incubation temperature for all the mushrooms was maintained at 30 ± 1°C with the exception of A. bisporus for which it was at 24 ± 1°C

Growth measurement. The mycelial growths 15 days after inoculation were filtered, washed thoroughly and dry mycelial weights were taken after dehydration at 70-80°C for 48 hrs.

Chemicals. Mannan (yeast), xylan (larch wood), dextran (60-90 thousand) and chitin (crab shells) were purchased from Sigma Chemical

Comp. U.S.A. Inulin and dextrin were from E. Merck, Germany and cellulose from Centron Research Laboratory, India.

Assay of carbohydrases in the fermented broth. The culture filtrates after 15 days of fermentation were assayed for the diferent carbohydrase activities as follows.

Xylanase. The activity was assayed by measuring the amount of reducing group liberated by the action of enzyme on xylan according to the method of Nelson (1944) as modified by Somogyi (1952). The assay mixture contained 0.1 ml of culture filtrate, 1.0 ml of xylan suspension (10 mg/ml of 0.1 M citrate-phosphate buffer, pH 5.0) and 0.9 ml of same buffer.

The reaction mixture was incubated for 30 min at 40°C and the reaction was terminated by adding 2 ml of alkaline copper reagent. The mixture was then kept for 10 min in boiling water, cooled to room temperature and 1 ml of arseno-molybdate reagent was added. After 15 mins, the mixture was diluted five times. The resulting colour was measured at 500 nm. The intensity of colour was expressed in terms of xylose equivalent as obtained from a standard curve. Activities of the remaining enzymes were also expressed in terms of xylose equivalent. One unit of enzyme activity was expressed as that amount enzyme protein which produce one μ mole of D-xylose or its equivalent per minute under the assay conditions.

Mannanase. The enzyme activity in the culture filtrate was assayed by the above procedure using mannan as substrate in 0.1 M ammonium acetate buffer of pH 5.0 (Kubačková et al. 1976).

Carboxymenthyl cellulase. The CM-cellulose activity was measured in the same procedure using CM-cellulose as substrate in 0.1 M sodium acetate buffer, pH 5.0 and with an incubation period of 10 min only (Kubačková et al. 1976).

Dextranase. The activity of the enzyme in the culture filtrate was assayed in the same way as for xylanase with dextran as substrate in 0.1 M potassium phosphate buffer, pH 5.0 (Kubačková et al. 1976).

Chitinase. The activity of the enzyme was assayed nephelometrically (Jeuniaux 1966). The assay mixture contained 1 ml of culture filtrate as enzyme, 1 ml of chitin solution (1.8 mg/ml) in 0.6 M citrate-phosphate buffer (pH 5.0). The reaction mixture was incubated for 120 min at 37.5°C and change in turbidity was measured after suitable dilution at 660 nm in comparison with a control containing boiled enzyme.

Inulinase. Due to high blank colour inulinase activity could not be measured according to the standerd method (Avigad, Bauer 1966).

RESULTS AND DISCUSSION

It is evident (Table 2) that all the mushroom strains are capable of utilizing a number of polysaccharides other than dextrin or soluble starch. From the relative growth of mushrooms on different carbohydrates, it appears that all the mushrooms can commonly utilise xylan as their sole source of carbon. *T. clypeatus*, *P. papilionaceus* and *V. volvacea* can utilize cellulose, while the other species cannot. Interestingly cellulose and mannan appeared to be the best carbon sources for the growth of *T. clypeatus* and *L. squarrosulus* respectively. Thus it may be the fact that the mushrooms are capable of utilizing at the optimum level, any of the polysaccharides of α or β, 1→4 linked polymer unit of glucose, xylose or mannose. But no β (2→1) or α (1→6) linked polymer is better utilized amongst the polysaccharides tested. Chitin was not utilized by any of the mushrooms. The non-utilization of chitin (β, 1→4, N-acetyl glucosamine polymer) by any of the mushrooms may

Table 2

Growth* of mushrooms on different carbohydrates as sole source of carbon

Mushrooms	Control	Carbohydrates (1 %, w/v)					
		dextran	cellulose	inulin	mannan	xylan	chitin
Termitomyces clypeatus	0.540[a]	0.174	0.563	0.256	0.006	0.397	0.080
Panaeolus papilionaceus	0.980[b]	0.178	0.470	0.377	0.118	0.620	0.010
Gymnopilus chrysimyces	0.395[b]	0.234	0.008	0.154	0.099	0.263	0.005
Coprinus lagopus	0.744[b]	0.045	0.007	0.263	0.035	0.183	0.044
Lentinus squarrosulus	0.330[a]	0.050	0.009	0.040	0.600	0.420	0.015
Volvariella volvacea	0.506[b]	0.162	0.378	0.320	0.040	0.345	0.060
Agaricus bisporus	0.640[c]	0.600	0.012	0.590	0.750	0.860	0.008

*Growth was measured as dry mycelial weight /average of three sets/ in gm per 100 ml of medium at the end of growth period.

Control medium contained 1 % (w/v) of dextrin in /a/, soluble starch in /b/ and D-glucose in /c/ as the sole source of carbon.

be either due to lack of chitinase enzyme or incapability in utilizing monomer units. But it is evident that the mushrooms are capable of utilizing various polysaccharides. The production of extracellular enzyme for the hydrolysis of polysaccharides by mushrooms grown either presence or in absence of the respective carbohydrates were assayed.

Table 3 represents the extracellular carbohydrates in the culture filtrates of the respective mushrooms. The presence of a carbohydrase in the fermented broth in presence or in absence of its substrate in the medium was considered as evidence for the inducible and constitutive nature of the enzyme. During inulinase assay by Nelson-Somogyi method as proposed for all al fructan hydrolysing enzymes (A v i g a d, B a u e r 1966), it has been found that inulin containing only twenty

Table 3

Extracellular carbohydrase activities of mushrooms grown either in presence or in absence of the respective polysaccharides

Organisms	Xylanase U x 10		Mannanase U x 10		CM-Cellulase U x 10		Dextranase U x 10	
	I	C	I	C	I	C	I	C
T. clypeatus	0.615	2.450	-	-	13.000	3.800	-	-
P. papilionaceus	2.050	-	1.135	-	3.400	3.400	1.265	-
G. chrysimyces	0.300	0.415	-	-	-	-	1.135	-
C. lagopus	0.315	0.335	-	-	-	-	-	-
L. squarrosulus	0.335	0.400	3.650	1.535	-	-	-	-
V. volvacea	0.265	0.385	-	-	-	-	-	-
A. bisporus	2.250	0.665	1.650	1.600	-	-	7.865	-

I Inducible, C - Constitutive enzyme activity per ml of culture filtrate

\- Could not be detected under the experimental conditions

to thirty fructose residues and relatively more reducing groups always gives a high colour in the control tubes. Other methods based on the colorimetric estimation of liberated reducing sugars during enzymatic action were also tried but same difficulty was noted. Hence, due to the lack of any simple specific method for the estimation of liberated free fructose residues, inulinase activity could not be measured.

It is further to be mentioned that although no chitin utilization was observed for any of the mushroom strains, the enzyme activity was assayed on the assumption that the monomer unit of chitin, i.e., N-acetylglucosamine, may not be consumed as a carbon source by the mushrooms though they have the capability to hydrolyse chitin. No inducuble or constitutive chitin hydrolysing activity was found to be present in any of the culture filtrates.

Only inducible dextranase activity was found in the culture filtrates of *Pnaeolus papilionaceus*, *Gymnopilus chrysimyces* and *Agaricus bisporus* where the last may be considered as the highest producer of this enzyme. Constitutive CM-cellulase activity was found only in the culture filtrates of *Termitomyces clypeatus* and *Panaeolus papilionaceus* among which the former can produce a large amount of the enzyme when cellulose was used as a sole source of carbon. Mannanase activity, both inducible and constitutive, was observed for *Lentinus squarrosulus* and *Agaricus bisporus*.

Possession a constitutive xylan hydrolysing enzyme is a common phenomenon observed for all the mushrooms except *P. papilionaceus* for which it is inducible. The highest inducible and constitutive xylanase activities were recorded for *A. bisporus* and *T. clypeatus* respectively. It is further to be mentioned that for most of the constitutive xylanase producers, activity was found to be relatively lower when the strains

were grown in xylan. *A. bisporus*, however, is an exception for which an about three fold increase in activity was observed.

Thus the present investigation indicates that muchrooms have the potentiality in producing different types of extracellular carbohydrases, among which xylanase is found to be most abundant. On the other hand, enzymes hydrolysing dextran, mannan, inulin and cellulose is also produced extracellularly durnig mushroom fermentation.

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