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Acetyl esterase production by *Termitomyces clypeatus*

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Production of acetyl esterase by *Termitomyces clypeatus* was stimulated by xylan, cellulose, arabinose and arabinose-containing polysaccharides in the growth medium. The culture filtrate was equally active with *p*-nitrophenyl acetate and acetyl xylan. Acetyl xylan was completely deacetylated by the enzyme. Activity was optimum at pH 6.5 and at 50°C. The Km values for *p*-nitrophenyl acetate and acetyl xylan were 0.83 mM and 0.38% (w/v) with Vm of 48 and 55 mmole acetate produced/min.mg protein, respectively.

Introduction

Acetyl (xylan) esterase (EC 3.1.1.6) deacetylates acetylated xylose residues present in hardwood xylan. Christov and Prior (1993) suggested that acetyl esterases possessing aryl esterase activity produced by different fungi might not be identical with the acetyl esterase activity that deacetylates acetyl ester bonds in xylan. We report here that *T. clypeatus*, which simultaneously produces α-arabinofuranosidase with xylanase (Sinha and Sengupta, 1995), also produces acetyl esterase with these enzymes and the enzyme was equally active on *p*-nitrophenyl acetate and acetyl xylan.

Materials and methods

Xylan (birchwood), *p*-nitrophenyl acetate, α-naphthyl acetate, Fast Corinth V salt, arabinogalactan were obtained from Sigma Chemical Company, USA. Acetyl xylan (10% acetate content) was prepared from xylan (Jones *et al.*, 1961). Arabinan was obtained from beet pulp (Tagawa and Kaji, 1988). Growth conditions and composition of synthetic growth medium of *T. clypeatus* were the same as reported earlier (Sinha and Sengupta, 1995). Growth was carried out in a shake flask with the same medium containing 0.5% (w/v) yeast extract. Xylanase activity was assayed at pH 5.0 by dinitrosalicylate reagent using 2% (w/v) carboxymethyl xylan (Sengupta *et al.*, 1987) as substrate. Activities of culture filtrate on *p*-nitrophenyl acetate and α-naphthyl acetate were assayed at pH 6.5 using 3 mM *p*-nitrophenyl acetate and 1 mM α-naphthyl acetate as substrate (Christov and Prior, 1993). Acetyl xylan esterase activity was assayed at pH 6.5 using 2% (w/v) acetyl xylan as substrate. All the reactions were carried out at 50°C for 10 minutes. Acetic acid liberated from acetyl xylan was measured by HPLC using μ-Bondapak C-18 column and isocratic mobile phase of 50 mM potassium phosphate buffer, pH 3.7, containing 5% (v/v) each of acetonitrile and methanol (Ghosh *et al.*, 1992). Under the assay conditions, unit of xylanase activity was expressed as the μmoles of xylose equivalent liberated/min while those of acetyl xylan esterase and aryl esterase were represented respectively as the μmoles of acetic acid and *p*-nitrophenol or α-naphthol liberated/min. Acetyl esterase activity of the culture filtrate was represented by the activity of the same on *p*-nitrophenyl acetate.

Results and discussion

Extracellular acetyl esterase was produced by *T. clypeatus* grown with different carbon sources which supported high (xylan, cellulose, arabinogalactan), low (xylose, arabinose, arabinan) and no (glucose, starch) xylanase production in the culture filtrate. A basal activity of acetyl esterase was present in the media where xylanase activity was absent (Fig. 1). Acetyl esterase activity was also not changed simultaneously with xylanase activity in the media supporting low xylanase production (Fig. 1, Table 1). Xylose did not stimulate acetyl esterase production but arabinose and arabinan stimulated esterase production without increasing xylanase production. Production of acetyl esterase might not be only linked with that of xylanase. The effects of those carbon sources on acetyl esterase and xylanase production were similar to that of the carbon sources on the production of α-arabinofuranosidase and xylanase by *Aspergillus niger* (v.d. Veen *et al.*, 1993), *Aspergillus nidulans* (Fernandez-Espinar *et al.*, 1994) and *T. clypeatus* (Sinha and Sengupta, 1995). The production of acetyl esterase appeared to be more closely linked with that of α-arabinofuranosidase than that of xylanase. However, cellulose, xylan, arabinogalactan and a cellulose-xylan combination, all stimulated productions of both acetyl

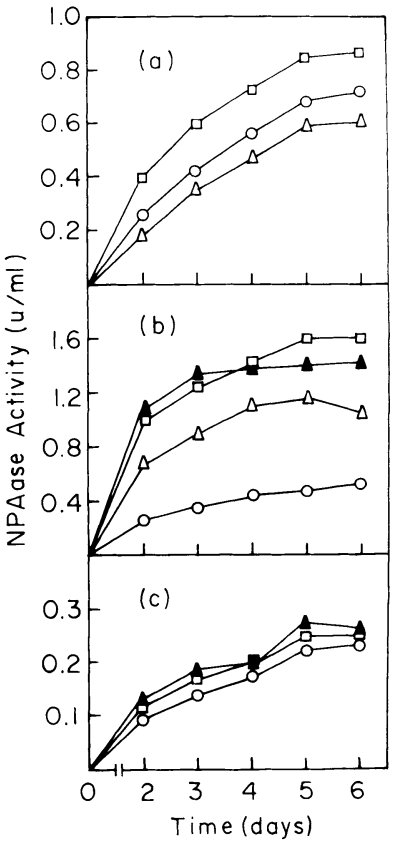

Figure 1 Influence of carbon sources (1%, w/v) on the production of acetyl esterase (NPAase) by *T. clypeatus*.
a. –□– Arabinose, –○– Arabinan, –△– Arabino-galactan.
b. –▲– Cellulose+Xylan, –□– Xylan + Cellulose acetate, –△– Xylan, –○– Cellulose.
c. –▲– Xylose, –□– Glucose, –○– Starch.

Table 1 Xylanase activity during optimum production of acetyl esterase by T. clypeatus (Fig. 1) in presence of different carbon sources

Carbon sources (% w/v)	*Enzyme activity (U/ml)*	
	Acetyl esterase	*Xylanase*
Glucose (1)	0.25	0
Starch (1)	0.22	0
Xylose (1)	0.28	2.0
Arabinose (1)	0.86	1.5
Arabinan (1)	0.68	0.66
Arabinogalactan (1)	0.60	8.5
Xylan (1)	1.14	20
Cellulose (1)	0.52	10
Cellulose (0.5) + Xylan (0.5)	1.32	23.5
Xylan (1) + Cellulose acetate (0.1)	1.64	25

Growth of *T. clypeatus* was carried out in shake flask (250 ml) with 50 ml medium. Method of enzyme assays were referred in Materials and Methods. Results were mean values of three identical sets of experiments.

esterase and xylanase, xylan being the best carbon source for growth. Similar effects of these carbon sources on the production of α-arabinofuranosidase by the fungus was reported by Sinha and Sengupta (1995). The presence of small amount of cellulose acetate in xylan medium preferentially increased acetyl esterase production (Table 1).

Sugar acetate might have some role on acetyl esterase production, although growth of the fungus was inhibited by cellulose acetate at concentration higher than 0.1% (w/v) in the medium. Coordinated production of acetyl esterase and xylanase by *Trichoderma reesei* and *Schizophyllum commune*, grown on cellulose and xylan was reported by Biely *et al.* (1988). The production of enzymes involved in the biodegradation of complex polysaccharides are coordinately linked but some of the enzymes are more closely linked among themselves. Production of acetyl esterase by *T. clypeatus* was more closely linked with that of α-arabinofuranosidase than with those of xylanase and cellulase. The optimum acetyl esterase activity obtained with *T. clypeatus* was 1.64 units/ml (Table 1). The enzyme activity was optimum at pH 6.5 and at 50°C. The enzyme activity was stable from pH 4.5 to 8 and 35% of the activity was lost after 30 minutes incubation at 50°C. The enzyme activity was insensitive to end-product inhibition by xylose or acetate. Preincubation of the enzyme in presence of 50 mM xylose or acetate did not affect activity of the enzyme on *p*-nitrophenyl acetate. The culture filtrate was equally active on *p*-nitrophenyl acetate and acetyl xylan but it had only 1% activity on α-naphthyl acetate compared to those on other

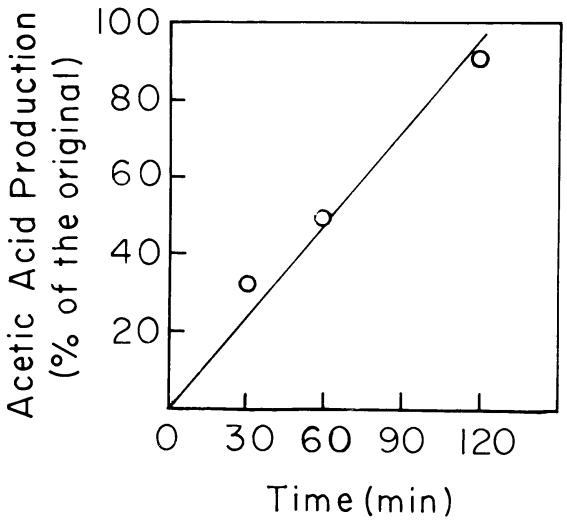

Figure 2 Liberation of acetic acid from acetyl xylan (10% acetate content) by the culture filtrate of *T. clypeatus*.

substrates. The Km for *p*-nitrophenyl acetate and acetyl xylan were 0.83 mM and 0.38% (w/v) with Vm values of 48 and 55 mmol acetate/min.mg of culture filtrate protein, respectively. Fungal esterases, in general, are more active with *p*-nitrophenyl acetate than with acetyl xylan (Christov and Prior, 1993). Thus, acetyl (xylan) esterase activity of *T. clypeatus* might be less contaminated with other esterases not acting on acetyl xylan. The culture filtrate enzyme mostly (> 90%) deacetylated 2% (w/v) acetyl xylan within 2 h at 50°C at 1 unit/ml (Fig. 2). The extracellular acetyl (xylan) esterase of *Thermomonospora fusca* (Christov and Prior, 1993) removed 74% of all acetyl groups present in xylan. Thus, *T. clypeatus* is a potent acetyl xylan esterase producer and the enzyme can readily de-acetylate acetyl xylan.

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