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PRODUCTION, PURIFICATION, AND PROPERTIES OF AN α-L-ARABINOFURANOSIDASE FROM *Dichomitus squalens*

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ABSTRACT

A white-rot fungus *Dichomitus squalens*, when grown on 1% wheat-straw glucuronoarabinoxylan under aerated submerged conditions, secreted an α-L-arabinofuranosidase (4.3 nkat/mL). The enzyme was purified 70-fold by ammonium sulfate precipitation, chromatofocusing on PBE 94, gel filtration on Ultrogel AcA 54, rechromatofocusing on PBE 94, and lectin affinity chromatography on Concanavalin A-Ultrogel. The enzyme is a glycoprotein having a molecular weight of 60,000 and a pI of 5.1. The enzyme exhibited maximal activity at pH 3.5 and at 60°, and was fully inactivated within 30 min at 70°. The K_m value for *p*-nitrophenyl α-L-arabinofuranoside was 1.64mM. The α-L-arabinofuranosidase liberated arabinose from sugar-beet arabinan, wheat-straw and oat-spelt arabinoxylans, and wheat-bran heteroxylan, and was inactive towards gum arabic.

INTRODUCTION

Plant arabinose-containing polysaccharides are arabinoxylans, neutral pectic substances (namely, type I arabinogalactans and arabinans), and type II arabinogalactans[1] (including exudate gums). Arabinose, generally in the α-L-furanose form, constitutes monomeric and/or oligomeric side-chains in arabinoxylans and both types of arabinogalactans, and the core of (1→5)-α-arabinans. Arabinose has also been said to play a role in the lignin–xylan covalent interaction[2,3].

Enzymes which cleave α-L-arabinofuranosidic linkages occurring in these polymers are arabinosidases[4,5]: α-L-arabinofuranosidases (EC 3.2.1.55), endo-(1→5)-α-L-arabinanases (EC 3.2.1.99), and a new type of exo-(1→5)-α-L-arabinanase[4].

We have obtained[6] an endo-(1→4)-β-D-xylanase from *Irpex lacteus*, which is

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used for the specific degradation of arabinoxylans from agricultural lignocellulosic waste-materials. However, the lack of the complementary debranching α-L-arabinofuranosidase impedes complete determination of structure.

Arabinofuranosidases have been purified from fungi[7–10], yeast[11], and bacteria[12–14], and are generally highly active on *p*-nitrophenyl α-L-arabinofuranoside with a lower activity on arabinans and arabinoxylans. However, Komae *et al.*[15] have purified an α-L-arabinofuranosidase from the actinomycetes *Streptomyces purpurascens*, which acts exclusively on *p*-nitrophenyl α-L-arabinofuranoside and α-L-arabino-oligosaccharides.

We have been studying the metabolism and secretion of enzymes[16,17] of *Dichomitus squalens* L., a white-rot fungus (basidiomycetes). This fungus is of particular interest since it exhibits ligninolytic activity which could improve the nutritive value of lignocellulosic materials (straws, hulls, *etc.*). *D. squalens*, when grown on arabinoxylan, secretes an arabinofuranosidase, a β-D-xylosidase, and xylanases. We now describe the kinetics of production of an extracellular α-L-arabinofuranosidase by *D. squalens* growing on wheat-straw glucuronoarabinoxylan, and its purification and properties.

RESULTS AND DISCUSSION

Production of enzyme. — The production of the arabinofuranosidase is shown in Fig. 1. The enzyme appeared in the cultivation medium in ~1 day, with massive liberation occurring after 4 days. Maximum activity (4.3 nkat/mL), reached after 6 days, was comparable to that produced by *Sclerotinia sclerotiorum*[10] when

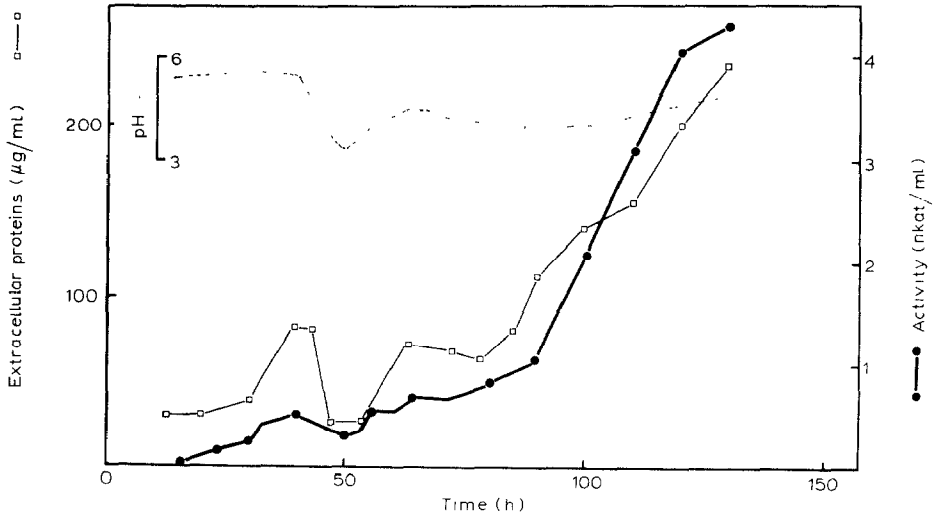

Fig. 1. Production of α-L-arabinofuranosidase by *Dichomitus squalens* as a function of time: pH of the medium (·····), proteins (—□—), α-L-arabinofuranosidase activity (—●—).

TABLE I

PURIFICATION OF ARABINOFURANOSIDASE FROM *Dichomitus squalens*

Step	*Volume (mL)*	*Activity (nkat.mL^{-1})*	*Protein (mg.mL^{-1})*	*Specific activity (nkat. mg^{-1})*	*Total activity (nkat)*	*Yield (%)*	*Purification (fold)*
Crude enzyme	1071	4.3	0.23	18.7	4618	100	—
Ammonium sulfate precipitation	100	42.6	1.90	22.4	4256	92.2	1.2
Chromatofocusing on PBE 94 (pH 7.4–3.7)	48	54.8	0.36	154	2630	57.2	8.2
Gel filtration on Ultrogel AcA 54	13	212.0	0.36	595	2756	59.7	32
Chromatofocusing on PBE 94 (pH 5.8–4.2)	30	66.0	0.08	858	1981	42.9	46
Affinity chromatography on Concanavalin A–Ultrogel AcA 22	23	40.0	0.03	1316	921	19.9	70

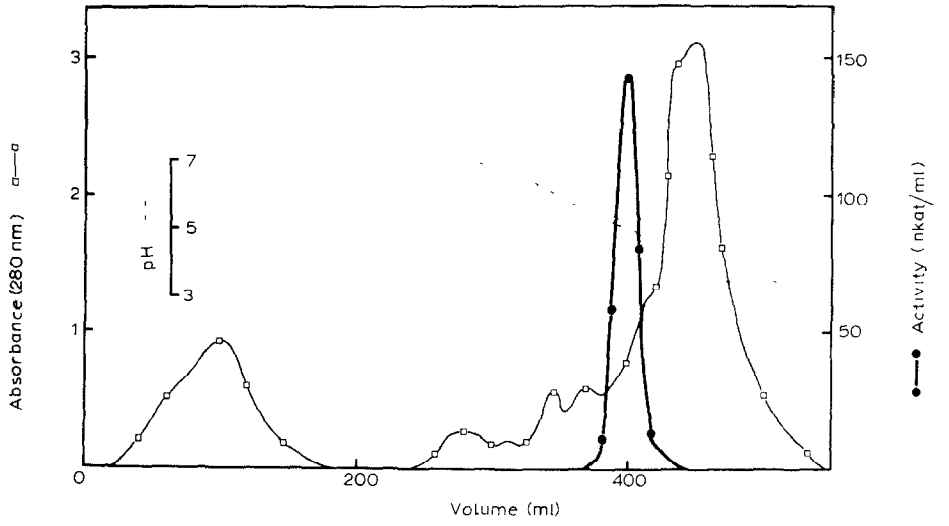

Fig. 2. First chromatofocusing of the *Dichomitus squalens* α-L-arabinofuranosidase on PBE 94 (See Experimental). Fractions (2.8 mL) were assayed for protein at 280 nm (—□—) and arabinofuranosidase activity (—●—).

grown on arabinose and 20-fold when compared to that from *Aspergillus fumigatus* growing on larch xylan[18]. On the other hand, the yeast *Rhodotorula flava*[11] produced 100-times more activity than *D. squalens* when cultivated on beet arabinan. Secretion of the enzyme paralleled that of extracellular soluble proteins, yielding a final specific activity of 19 nkat.mg^{-1}.

Purification (see Table I). — Addition of ammonium sulfate at 90% saturation level to the culture supernatant from *D. squalens* afforded a brown precipitate containing ~92% of the initial α-L-arabinofuranosidase activity and ~77% of the original soluble proteins (1.2-fold purification).

Analytical electrofocusing of the proteins from the initial supernatant on Agarose IEF showed that most of them had pI <6.0. Therefore, the crude enzyme solution from the ammonium sulfate precipitation was passed through a column of PBE 94 at pH 7.4 (Fig. 2). Some inert proteins remained unbound, indicating pI values higher than 6.5–7.0. Chromatofocusing of the arabinofuranosidase was effected by establishing a pH gradient (7.4→3.7) with Polybuffer 74. α-L-Arabinofuranosidase was eluted as a sharp peak at pI 5.0. Conditions for the separation of the enzyme from other proteins were very favourable since it was well separated from a massive peak that was eluted in the final portion of the gradient (pH 4.6–3.7). Furthermore, most of the initial coloured material remained bound to the top of the column and was only partly removable by M sodium chloride (not shown); ~62% of the arabinolytic activity was recovered (~9% of the injected proteins), giving an 8.2-fold purification.

After freeze-drying, the chromatofocused enzyme was submitted to gel filtration on Ultrogel AcA 54 (Fig. 3). The enzyme was eluted first, followed by a peak

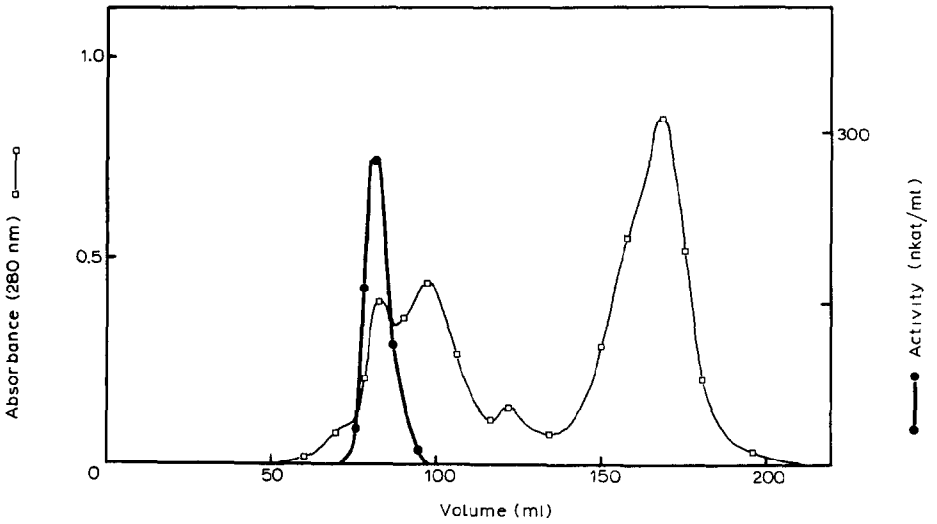

Fig. 3. Gel filtration of the α-L-arabinofuranosidase on Ultrogel AcA 54 (See Experimental). Fractions (2.8 mL) were assayed for protein at 280 nm (—□—) and arabinofuranosidase activity (—●—).

of proteins of lower molecular weight. The yields were ~105% and ~27% for injected activity and proteins, respectively (32-fold purification).

Chromatofocusing (Fig. 2) indicated that narrowing of the pH gradient could improve the purity of the enzyme. Preliminary attempts to bind the arabinosidase to PBE 94 in either 25mM piperazine-HCl (pH 5.0 or 5.4) were unsuccessful. Finally, the enzyme was bound in 25mM histidine-HCl (pH 5.8) (Fig. 4), indicating that at least 0.7 pH-unit between the pI (see Properties) and the pH of eluent is needed for efficient binding of this enzyme. The pH gradient was 5.8→4.2. The enzyme was eluted first at pH 4.75 and was followed by a more acidic (pI 4.5) protein. After the gradient ended, elution with M sodium chloride removed the u.v.-absorbing material. The enzyme was purified 46-fold at this step, with yields of ~72% for injected activity and ~56% for proteins.

Arabinofuranosidase, as for most fungal carbohydrate depolymerases, was thought to be a glycoprotein. Indeed, the enzyme was strongly bound to Concanavalin A–Ultrogel AcA 22 (Fig. 5) and released by methyl α-D-mannopyranoside, as observed for the xylanase[6] from *Irpex lacteus*. This step removed ~70% of the injected proteins, the activity yield being ~46% (70-fold purification). It is noteworthy that, as for the endo-(1→4)-β-D-xylanase from *Irpex lacteus*, half of this arabinofuranosidase activity was lost on Concanavalin A. The presence of isoenzymes, one of which was so firmly bound to the lectin as to resist elution with methyl α-D-mannopyranoside, cannot be invoked as an explanation since α-L-arabinofuranosidase from *D. squalens* seemed to be a single protein (Figs. 2–5). Irreversible inhibition by methyl α-D-mannopyranoside is also excluded since the arabinofuranosidase exhibited full activity in the presence of this compound. Dis-

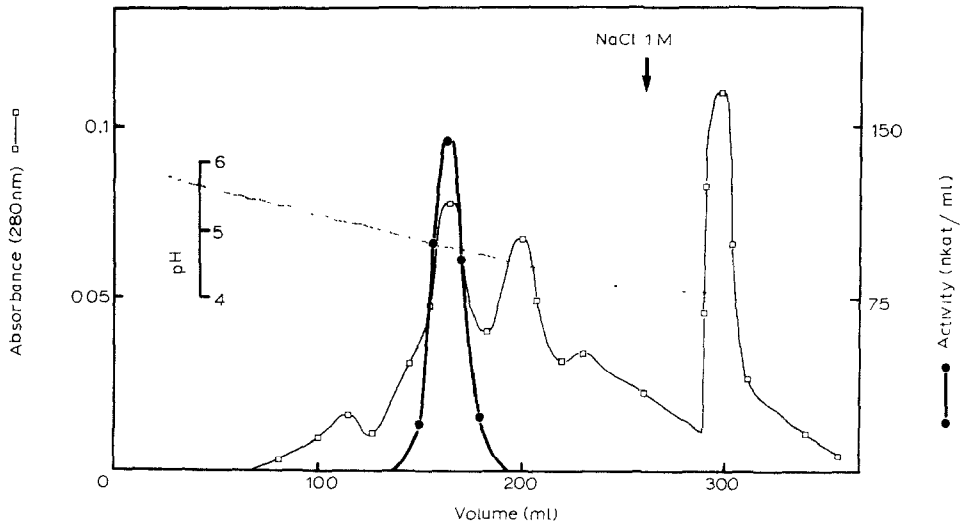

Fig. 4. Second chromatofocusing of the α-L-arabinofuranosidase on PBE 94 (See Experimental). Fractions (2.8 mL) were assayed for protein at 280 nm (—□—) and arabinofuranosidase activity (—●—)

sociation of active complexes linked through sugar–lectin affinity[19] on Concanavalin A could be responsible for these losses.

The purified α-L-arabinofuranosidase had ~20% of the initial activity (70-fold purification), a specific activity of 1316 nkat. mg^{-1} towards *p*-nitrophenyl α-L-arabinofuranoside, and gave a single sharp band on Agarose analytical isoelectric

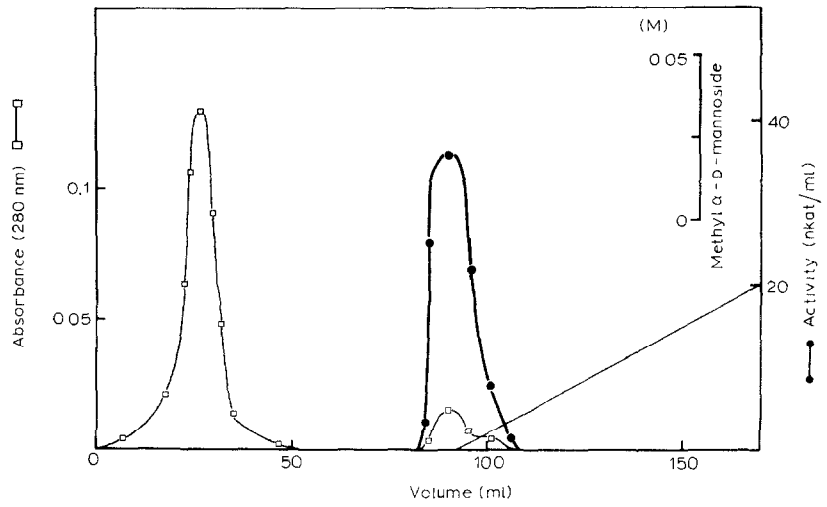

Fig. 5. Affinity chromatography of the α-L-arabinofuranosidase on Concanavalin A–Ultrogel AcA 22 (See Experimental). Fractions (2.2 mL) were assayed for protein at 280 nm (—□—) and arabinofuranosidase activity (—●—).

focusing. It had no activity against *p*-nitrophenyl α-D- and β-D-galactopyranoside, *p*-nitrophenyl β-D-glucopyranoside, and *o*-nitrophenyl β-D-xylopyranoside, or on plant polysaccharides devoid of arabinose.

Properties. — Some of the properties of the α-L-arabinofuranosidase are reported in Table II. The molecular weight, as determined by gel filtration on Ultrogel AcA 54 (molecular weight markers: cytochrome C, 11,700; chymotrypsinogen, 25,000; ovalbumin, 43,000; bovine serum albumin, 67,000) (Fig. 3) was 60,000. The isoelectric point was 5.1, good agreement being observed between agarose analytical electrofocusing and chromatofocusing on PBE 94 (Fig. 2).

TABLE II

PROPERTIES OF ARABINOFURANOSIDASE

Property	*Arabinofuranosidase*
Molecular weight	60,000
pI	5.1
Carbohydrate	+
pH optimum	3.5
θ optimum (°)	60
p-Nitrophenyl α-L-arabinofuranoside	
V_{max} (40°, pH 4.2) (nkat.mg^{-1})	2200
K_m (40°, pH 4.2) (mM)	1.64

The enzyme exhibited maximum activity at pH 3.5 (Fig. 6) and at 60° (Fig. 7) in a 20-min assay. The unusual acidic optimum pH was similar to those of the arabinofuranosidases from *Corticium rolfsii*[9] and *Rhodotorula flava*[11]. On the other hand, the *D. squalens* arabinofuranosidase was unstable in acidic media, the optimum pH range for stability being 5.5–7.5 (Fig. 6). Inactivation occurred after 30 min at 70° (Fig. 7). The enzyme was partially inactivated by Ag^+ and Mn^{2+} (Table III); only Fe^{2+} and Mn^{2+} developed a strong yellow colour in blank tests. No inhibition was found with mM L-arabinose. The K_m for *p*-nitrophenyl α-L-arabinofuranoside was 1.64mM, which was of the same magnitude as that for *Corticium rolfsii* arabinosidase[9], as was the V_{max} (132 μmol/min/mg of protein).

Action of arabinofuranosidase on various arabinose-containing polysaccharides. — Each of the polysaccharides tested (except larchwood xylan) contained arabinofuranosidic linkages (in gum arabic, some β anomer and pyranosidic form are also found). Arabinose always preponderates in the terminal non-reducing position[12,20–22]. In wheat-straw and oat-spelt glucuronoarabinoxylans, arabinose constitutes monomeric side-chains linked to the xylan core; in wheat-bran heteroxylan[21], it forms both monomeric substituents and short side-chains through C-2 and C-3, giving a bristle shape to the molecule. Gum arabic is also heavily branched and sugar-beet arabinan exhibits numerous side-chains[12] attached to the central core through C-3[20].

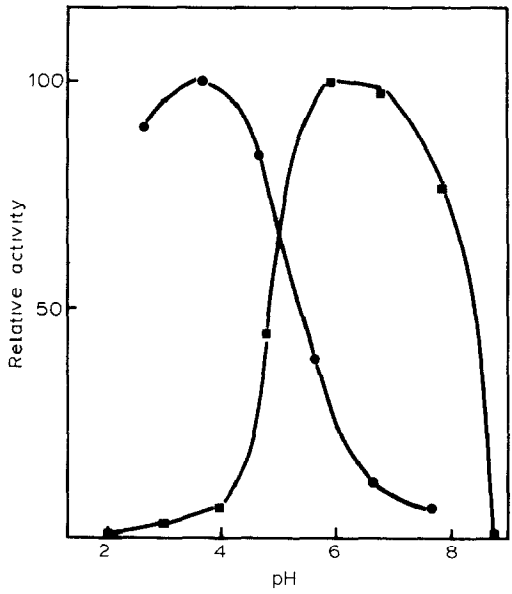

Fig. 6. Effect of pH on enzyme activity (—●—) and on enzyme stability (—■—) (See Experimental).

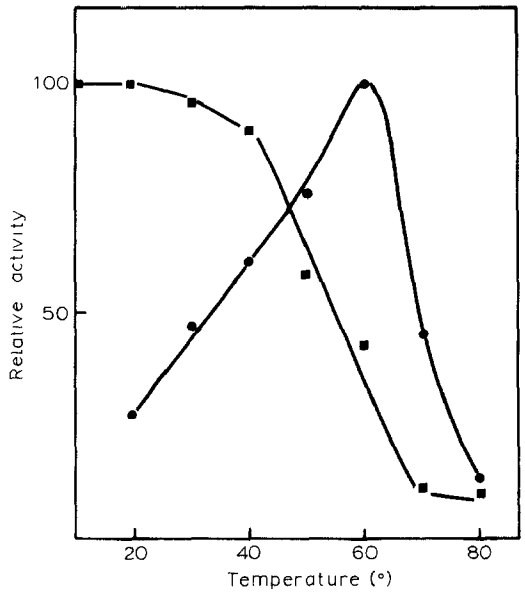

Fig. 7. Effect of temperature on enzyme activity (—●—) and on enzyme stability (—■—) (See Experimental).

TABLE III

EFFECT OF mM METAL IONS ON ARABINOFURANOSIDASE

Ion	*Relative activity*[a]	*Ion*	*Relative activity*[a]
Mn^{2+}	41	Mg^{2+}	96
Ag^{+}	57	K^{+}	99
Ba^{2+}	82	Co^{2+}	107
Ca^{2+}	92	Fe^{2+}	124
Zn^{2+}	96		

[a]To a reference without added ion.

D. squalens α-L-arabinofuranosidase released (t.l.c.) arabinose (Table IV) from each of the polymeric substrates tested except gum arabic. This enzyme can be classified as an exo-enzyme. It is related to most fungal and bacterial arabinosidases which act on both synthetic glycosides and polymers[8–12], and contrasts with the *Streptomyces purpurascens* enzyme[15] which is inactive against arabinans and arabinogalactans.

TABLE IV

RELEASE OF ARABINOSE BY THE ACTION OF ARABINOFURANOSIDASE ON VARIOUS SUBSTRATES

Substrate	*Composition*[a]						*Arabinose released*	
	Ara	*Xyl*	*Gal*	*Glc*	*Rha*	*U*[b]	*μg/h/mg of enzyme*	%[d]
Wheat-straw glucurono-arabinoxylan	1	7.22	0.10	0.16	0.02	0.34	620	42
Oat-spelt arabinoxylan	1	8.08	0.12	0.16	0	—	770	32
Wheat-bran arabinoxylan	1	0.91	0.02	0	0	0.14	356	3.7
Larch arabinogalactan	1	0	2.90	0.03	0	—	44	1.1
Sugar-beet arabinan	1	0	0.08	0.03	0.04	—	3240	22
Sugar-beet cell-walls	1	0.08	0.18	1.07	0.11	0.89	268	7.4
Larchwood glucuronoxylan	0	1	0	0.01	0	0.06	0	—
Gum arabic[c]	1	0	1.05	0	0.24	0.27	0	0

[a]Molar ratios relative to arabinose except for larchwood xylan. [b]Uronic acids. [c]Ref. 22. [d]% of available arabinose released within 16 h.

The best substrate was crude sugar-beet arabinan followed by oat-spelt and wheat-straw xylans; Uesaka *et al.*[11] observed a similar pattern with an arabinofuranosidase from the yeast *Rhodotorula flava*. The observed activities are related to the content of terminal non-reducing arabinose in the substrates, 18% for crude beet arabinan (~70% arabinose)[12] and 9% for the *gramineae* xylans (~9% arabinose). The enzyme liberated arabinose from native parenchyma cell-

walls from sugar-beet root, as did those from *Sclerotinia sclerotiorum*[10] and *Ruminococcus albus*[14] from rice and alfalfa cell-walls, respectively. The enzyme released much more arabinose from the isolated and soluble sugar-beet arabinan, which could reflect limitation of diffusion by steric hindrance in the cell walls.

The enzyme hydrolysed bran heteroxylan at half the rate for more linear xylans, although this substrate contained ~26% of terminal arabinose[21], reflecting steric hindrance due to the great complexity of the arabinose-containing side-chains. In fact, in this molecule, terminal arabinosyl groups are not as exposed as those in straw or spelt xylans, the overall "hairy" character also probably being different. This hypothesis is supported by the lack of activity of the enzyme on gum arabic, a highly branched polymer[22]. Tagawa and Kaji[23] also observed a very limited action of the *Aspergillus niger* α-L-arabinofuranosidase on gum arabic, since only 5% of the available arabinose was released within 42 h using 100 times more activity (10 nkat/mg of polysaccharide) than in our experiments.

EXPERIMENTAL

Organism and cultivation. — The white-rot basidiomycetes *Dichomitus squalens* (Karst) Reid CBS 432.34, obtained from the Centraalbureau voor Schimmelcultures (Baarn, The Netherlands), was grown at 34° in a Biolaffite C5 type fermentor (12 L) under aerated (600 L/h) submerged conditions. The cultivation medium contained (per L) wheat-straw glucuronoarabinoxylan (10 g, see below), $(NH_4)H_2PO_4$ (0.9 g), urea (0.1 g), proteose peptone (1 g), KH_2PO_4 (0.6 g), K_2HPO_4 (0.4 g), $MgSO_4 \cdot 7\ H_2O$ (0.5 g), $CaCl_2 \cdot 2\ H_2O$ (0.074 g), vitamins, and oligoelements according to Vogel[24]. Poly(propylene glycol) 2000 (1 mL/L) was used as an antifoaming agent. Ground mycelium[16] yielding a protein equivalent of 10^7 spores of *Sporotrichum pulverulentum* was inoculated per 50 mL of growing medium.

Aliquots (10–20 mL) were withdrawn (every 6–8 h) under sterile conditions and centrifuged to remove mycelium and residual undegraded xylan. Extracellular soluble proteins, α-L-arabinofuranosidase activity, and pH were measured on supernatant solutions (Fig. 1).

After cultivation for 133 h, the remaining culture medium (7.5 L) was centrifuged (4°), and the supernatant solution was 90% saturated with ammonium sulfate and stored overnight in the cold. The resulting heavy precipitate was collected by centrifugation and washed thrice with 25mM imidazole buffer (pH 7.4) containing ammonium sulfate (90% saturation). The pellet was then resuspended in 700 mL of this medium.

Substrates. — Wheat-straw glucuronoarabinoxylan was prepared according to Adams[25]. Copper complexing was omitted since it does not purify the xylan significantly (Glc/Xyl 0.013, *cf.* 0.035 with the additional purification step) and because of the possible toxicity of residual copper towards the organism. The yield from native straw was ~13%.

Arabinoxylan from oat spelts (Sigma X-0376), larchwood xylan (Sigma X-0375), carboxymethylcellulose sodium salt (Sigma C-4888, medium viscosity), gum arabic (Sigma G-9752), larchwood arabinogalactan (Sigma A-2012), dextran (Sigma D-4751), lichenan from *Cetraria islandica* (Sigma L-8378), laminarin from *Laminaria* spp. (Fluka 61400), and crude sugar-beet arabinan[12] (Koch–Light 52867) were commercial products. The mixed-linked β-D-glucan from barley (from Dr. B. Stone) and mannan from *Phoenix canariensis* (from Dr. F. Percheron) were gifts. Wheat-bran arabinoxylan[21], waxy-maize beta-limit dextrin[6], white-lupin acidic galactan[26], and sugar-beet cell walls (prepared in this department by treatment with pronase[26]) were prepared by literature procedures.

Enzyme assay. — α-L-Arabinofuranosidase activity was determined by incubating 0.1 mL of 4mM *p*-nitrophenyl α-L-arabinofuranoside in 0.1M acetate buffer (pH 4.2) with 0.1 mL of enzyme solution (<0.1 nkat) at 40° for 20 min. *p*-Nitrophenol was determined after the addition of 0.6 mL of M sodium carbonate by measuring the absorbance at 400 nm; 1 nkat of enzyme liberated 1 nmol of *p*-nitrophenol/s.

Contaminating glycosidases were assayed by using *p*-nitrophenyl α-D- and β-D-galactopyranoside, *p*-nitrophenyl β-D-glucopyranoside, and *o*-nitrophenyl β-D-xylopyranoside (λ_{max} 414 nm for *o*-nitrophenyl β-D-xylopyranoside), and 1 nkat of α-L-arabinofuranosidase activity. Glycanases were measured by mixing 0.1 mL of a 1% solution or suspension of substrate in 0.1M acetate buffer (pH 4.2) with 0.1 mL of enzyme solution for 16 h at 40°. Liberated reducing sugars were measured by the Somogyi–Nelson procedure[27].

Protein determination. — Proteins were recorded in column effluents at 280 nm or measured by the Lowry[28] method using bovine serum albumin (Sigma, Fraction V, A-4503) as a standard. Proteins appearing in the medium during growth of *D. squalens* were determined by the Lowry procedure after precipitation with trichloroacetic acid (7% final concentration) and subsequent dissolution in 0.2M sodium hydroxide.

General methods. — Polysaccharides (substrates) were hydrolysed[29] in 2M trifluoroacetic acid for 1.25 h at 120°. Sugars were converted into the alditol acetates[30] and analysed[21] by g.l.c. Uronic acids were measured by the *m*-phenylphenol technique[31], using glucuronic or galacturonic acid as a standard. The arabinose released from various arabinose-containing polysaccharides was separated by t.l.c. on silica gel (Schleicher and Schüll, F 1500), using 1-propanol–ethyl acetate–ethanol–pyridine–acetic acid–water (7:3:3:2:2:1)[17] and detection by spraying with 5% sulphuric acid in ethanol and heating at 80°.

Isoelectric focusing. — Agarose IEF[32] (Pharmacia) was used in the pH range 3–10 and an LKB 2117 Multiphor device. The pI was measured by calibration with a broad pI calibration kit. Proteins bands were stained with Coomassie Brilliant Blue G.

Arabinofuranosidase purification. — An aliquot (100 mL) from the ammonium sulfate suspension was centrifuged, and a solution of the pellet in 100 mL of 25mM

imidazole buffer (pH 7.4) was dialysed overnight against the same buffer. After centrifugation, the solution was redialysed for an additional 4 h.

Chromatography. — (a) On PBE 94. A column (1.0 × 40 cm) of PBE 94 (Pharmacia) was equilibrated with 25mM imidazole buffer (pH 7.4). The above enzyme solution was applied to the column at 50 mL/h. After washing with 10-column vol., proteins were eluted with 400 mL of Polybuffer 74 adjusted to pH 3.7 (dilution 1/8). The chromatogram is shown in Fig. 2.

(b) On Ultrogel AcA 54. Fractions from (*a*) showing arabinofuranosidase activity (eluted at pH ~5.0) were combined (48 mL) and freeze-dried. After dissolution in 25mM imidazole buffer (pH 7.4), the sample (3 mL) was injected on to a column (1.6 × 85 cm) of Ultrogel AcA 54 equilibrated with the same buffer and eluted at 10 mL/h. The resulting chromatogram is shown in Fig. 3.

(c) On PBE 94. Fractions from (*b*) exhibiting arabinofuranosidase activity were combined (13 mL), dialysed overnight against 25mM histidine-HCl buffer (pH 5.8), and injected at 50 mL/h on to a column (1.0 × 40 cm) of PBE 94 equilibrated with the same buffer. Proteins were eluted with 400 mL of Polybuffer 74 adjusted to pH 4.2 (dilution 1/8). When the final pH of the eluant reached 4.2, Polybuffer 74 (pH 4.2) containing M sodium chloride (50 mL) was used. The results are shown in Fig. 4.

(d) On Concanavalin A–Ultrogel AcA 22. Fractions from (*c*) having arabinofuranosidase activity (pH of elution, 4.8) were mixed (30 mL), dialysed extensively against 25mM imidazole buffer (pH 7.4) containing 0.1M sodium chloride and 0.1mM manganese chloride, then added to a column (1.6 × 7.5 cm) of Concanavalin A–Ultrogel AcA 22 equilibrated with the above buffer, and eluted at 45 mL/h with 4-column vol. A linear gradient (0→0.05M) of methyl α-D-mannopyranoside (80 mL) in the starting buffer was then applied. The chromatogram is shown in Fig. 5. The α-L-arabinofuranosidase released was dialysed against 25mM imidazole buffer (pH 7.4) and concentrated by dialysis with Sephadex G 200 to ~0.6 mg/mL concentration.

Properties. — (a) pH optimum. The arabinofuranosidase activity was measured under standard conditions. using citrate–phosphate buffer of various pH values (Fig. 6).

(b) pH stability. Arabinofuranosidase (0.1 nkat) was incubated in universal buffer of various pH values at 30°. After 17 h, the enzyme samples were dialysed against mM phosphate buffer (7.0), and the residual activity was measured under standard conditions (Fig. 6).

(c) Temperature optimum. Activity was measured under standard conditions in the temperature range 20–80° (Fig. 7).

(d) Temperature stability. The enzyme (0.1 nkat) in 0.1M acetate buffer (pH 4.2) was kept at various temperatures (0–80°) for 0.5 h, and the residual activity was measured under standard conditions (Fig. 7).

(e) Kinetic parameters. The activity of the purified arabinofuranosidase was measured under standard conditions with variation of the final concentration of

p-nitrophenyl α-L-arabinofuranoside from 0.25 to 5mM. The data were expressed by the Lineweaver–Burk reciprocal-plots method.

(f) Effect of metal ions. The arabinofuranosidase activity was assayed under standard conditions in the presence of mM metal ions. Blanks without enzyme were used since some ions gave a yellowish colour in sodium carbonate (Table III).

(g) Hydrolysis of arabinose-containing polysaccharides. Solutions or suspensions (200 μL) containing various polysaccharides (1% in 0.1M acetate buffer, pH 4.2) were added with 200 μL of enzyme (0.2 nkat in distilled water) and incubated for 16 h at 40°. Half of the solution was analysed for reducing sugar[26]. To the remainder, 600 μL of ethanol (acetone for beet arabinan) was added, the precipitate was removed by centrifugation, the supernatant was concentrated to dryness, and 40 μL of water were added prior to t.l.c.

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