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Comparison of xylanase production by fungal pathogens of barley with special reference to *Bipolaris sorokiniana*

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The production of endo-1,4-β-xylanase by fungal pathogens of barley infecting leaves (*Drechslera teres, D. graminea, Rhynchosporium secalis*), roots and stems (*Rhizoctonia solani, Fusarium culmorum, Gaeumannomyces graminis*), and leaves, roots and stems (*Bipolaris sorokiniana*) was followed during liquid culture using xylan from birchwood or a cell wall preparation of barley leaves as carbon sources. The rate and extent of xylanase activity measured differed among fungi and was influenced by the carbon source. *B. sorokiniana* and *R. solani* produced the most xylanase on cell wall preparation medium, but the most pathologically aggressive isolate of *B. sorokiniana* did not produce the highest xylanase activity in culture. *D. teres* and *D. graminea* were the only pathogens which produced considerable amounts of xylanase on both carbon sources. *R. secalis, F. culmorum* and *G. graminis* produced little or no xylanase on either carbon source. *B. sorokiniana, D. teres* and *D. graminea* had 30 kDa xylanases that were related serologically.

Extracellular enzymes of fungi have been studied largely for taxonomic purposes. For example, pectic enzymes have been used to separate anastomosis groups within *Rhizoctonia* species along with morphological features, number of nuclei per cell, teleomorphs and pathogenicity (Sweetingham, Cruickshank & Wong, 1986). On the basis of differences in pectinolytic activity, pectinase isozymes have also been used as markers to distinguish between intersterility groups of *Heterobasidion annosum* (Fr.) Bref. (Karlsson & Stenlid, 1991). Rapid differentiation and classification of pathogenic races of *Fusarium oxysporum* Schltdl. were achieved based on pectic enzyme patterns (Fernandez, Patino & Vazquez, 1993).

With regard to plant pathogens, absence of sexual reproduction, environmental homogeneity and host specificity have all been associated with low enzymic variability, whereas high variability has often been associated with plant pathogens with wide host ranges and geographic diversity (Chen, Schneider & Hoy, 1992). Enzyme polymorphisms have been observed, e.g. between the intersterility groups of *H. annosum* in which the P-group has a wider host range and higher pectinase activity than the S-group. Similarly, the S-group was mainly characterized by exopectinolytic activity, while the P-group showed endopectinase activity leading to a more rapid degradation of pectin (Johansson, 1988). Pathogens of monocotyledonous and dicotyledonous hosts can produce different type and abundance of plant cell wall degrading enzymes. According to Ried & Collmer (1986) all strains of *Erwinia* isolated from dicotyledonous hosts possessed exopolygalacturonase activity, but strains from monocotyledons did not. In addition, strains of *Erwinia chrysanthemi* (Sabet) Victoria, Arboleda & Munoz, that infect maize stems, produced more xylanase than strains isolated from dicotyledonous hosts (Braun & Kelman, 1987). Furthermore, fungal pathogens from graminaceous monocotyledons have often tended to produce xylanases and cellulases rather than pectinases (Anderson, 1978; Cooper *et al.*, 1988; Lehtinen, 1993).

The predominant polymers of the plant cell wall matrix play a significant role in the induction of enzyme activities of pathogens both *in vitro* and *in vivo* (Howell, 1975; Jarvis, Threlfall & Friend, 1981). Therefore, the significant role of pectic enzymes in facultative parasitism could in part result from most studies on cell wall degrading enzymes having been done on pathogens of dicotyledons, in which rhamnogalacturon predominates in the cell wall (Cooper, 1983; Cooper *et al.*, 1988). In monocotyledons, e.g. in the Gramineae, the cell walls contain less than 10% of the pectic polysaccharide content of dicotyledon cell walls, but up to 40% of β-1,4-xylans (Labavitch & Ray, 1978). Xylanases may account significantly for the success of fungi as cereal pathogens because the enzymes are more effective in degrading cell walls of monocotyledons than of dicotyledons. The delayed and reduced production of xylanase by most pathogens of dicotyledons may be due to less accessibility to xylan in the secondary walls of dicotyledons than to that in the primary walls of cereals (Baker, Whalen & Bateman, 1977; Cooper *et al.*, 1988). Furthermore, xylanase synthesis occurs early during infection of graminaceous plants, which also suggests their key role in infection of cereals (Cooper *et al.*, 1988).

Although the production of plant cell wall degrading enzymes by fungal plant pathogens is important in cell penetration and invasion during pathogenesis, the relationship between enzyme production and various parasitic abilities is not well understood. Attempts to correlate enzyme production *in vitro* with pathogenicity *in vivo* have frequently produced contradictory or inconclusive results (Fernandez *et al.*, 1993).

This has probably been due to the differential production of enzymes by organisms in culture, which has been unrelated to their activities in the host plant. According to Cooper (1983), synthesis of cell wall degrading enzymes *in vitro* is not proof of involvement in disease development, although some indication may be obtained by culture in host cell wall media. This was confirmed by Carder, Hignett & Swinburne (1987) who used host cell wall material as sole carbon source for *in vitro* cultures of *Verticillium albo-atrum* Reinke & Berthold to indicate correlation between enzyme production and pathotype.

In this study production of xylanases was used to compare leaf, root and stem-base pathogens of barley. A more detailed study was carried out with isolates of *Bipolaris sorokiniana*, in which the relationship between infection efficiency and xylanase activity was examined. The molecular weights of xylanases from various fungi were also determined, and antiserum to the purified xylanase of *B. sorokiniana* was used in their analysis by immunoblotting.

MATERIALS AND METHODS

Fungi

The fungal pathogens *Bipolaris sorokiniana* (Sacc.) Shoem., *Drechslera teres* (Sacc.) Shoem., *Drechslera graminea* (Rabenh.) Shoemaker, *Rhynchosporium secalis* (Oudem.) Davis, *Rhizoctonia solani* J. G. Kuhn, *Fusarium culmorum* (W. G. Sm.) Sacc. and *Gaeumannomyces graminis* (Sacc.) Arx & Oliver used in this study were isolated from infected barley (*Hordeum vulgare* L.) leaves, basal stems, roots or grains. From one to seven isolates of each fungus were studied simultaneously. The fungi were grown on potato dextrose agar (*R. solani, F. culmorum, G. graminis*) and on lima bean agar (*B. sorokiniana, D. teres, D. graminea, R. secalis*) in Petri dishes in darkness at room temperature for 10–14 d. *D. teres* and *D. graminea* were grown under nuv light (Philips 40 W lamps) with a dark period of 8 h.

Infection efficiency

A detailed study was undertaken on *B. sorokiniana*, from which the infection efficiencies of three isolates (H83, A90, F90) were examined in field experiments carried out in 1991 and 1993 at the Experimental Farm of the University of Helsinki. Plant material included five barley cultivars; two-rowed Kustaa and Kymppi and six-rowed Loviisa, Pohto and Pokko. The field trials were carried out as a completely randomized block design with two replications. The plot size was 10 m^2 and row spacing 12·5 cm. Sowing density was 650 viable seeds m^{-2} and standard fertilization with 80 kg nitrogen ha^{-1} as ammonium nitrate was used. There was no chemical control of fungal diseases. Plots were separated from each other by extra plots sown to oats (*Avena sativa* L.) to reduce the spread of inocula. Plants were inoculated at growth stage 37 (Zadoks, Chang & Konzak, 1974) with a spore suspension prepared by flooding the Petri dishes with sterile distilled water and adjusting the inoculum density to $1{\cdot}5 \times 10^5$ spores ml^{-1}, determined using a haemocytometer. A few drops of Tween 20 surfactant were added to the inoculum before use. Approximately 100 ml m^{-2} of spore suspension was applied to each plot with a hand-held sprayer. Three upper leaves of 10 main stems per plot were assessed for disease symptoms, expressed as percentage leaf area infected 8, 18 and 28 d after inoculation. The data were subjected to analysis of variance (ANOVA; MSTAT, 1989) and expressed as means. Duncan's multiple range test at $P < 0{\cdot}05$ was used to separate significantly different means.

Culture conditions

The production of xylanases by the fungi was studied in shaking flask cultures using 1% (w/v) xylan from birchwood (Sigma X-0502) or a 2% (w/v) cell wall preparation of barley leaves as carbon sources (Peltonen, Karjalainen & Niku-Paavola, 1994). The fungi were cultured in 300 ml Erlenmeyer flasks containing the carbon source diluted in 100 ml of minimal salt medium (g l^{-1}): $NaNO_3$ (2·0), KCl (0·5), $MgSO_4 . 7H_2O$ (0·5), K_2HPO_4 (1·0), $FeSO_4 . 7H_2O$ (0·01), $ZnSO_4 . 7H_2O$ (0·01) and $CuSO_4 . 5H_2O$ (0·0025). After sterilization (121°, 15 min) thiamine and nicotinic acid (each 0·02 g l^{-1}) were added to the medium through sterile disposable filters. Flasks were inoculated with 12 0·5 cm diam. mycelial plugs taken with a cork borer from cultures grown on agar plates. However, in the experiment which compared infection efficiency and xylanase activity of *B. sorokiniana* isolates, 1 ml of spore suspension ($7{\cdot}0 \times 10^5$ spores ml^{-1}) was used instead to minimize variability between inocula of different isolates. Inoculated flasks were incubated at 28° on a shaker rotating at 180 rpm.

Enzyme activity assays

The time-course production of endo-1,4-β-xylanase (*EC* 3.2.1.8) in crude culture filtrates was assayed according to Poutanen & Puls (1988) with some modifications (Peltonen *et al.*, 1994) using the dinitrosalicylic acid (DNS) method (Sumner & Somers, 1949). Endopolygalacturonase (*EC* 3.2.1.15) activity in fungal cultures grown in barley cell wall medium was determined by the DNS method of Bailey & Pessa (1990). Enzyme activities were measured daily over 14 d in three experiments each with two replicates. The results were subjected to analysis of variance (ANOVA; MSTAT, 1989) and expressed as means with standard deviations (S.D.).

Electrophoresis

Sodium dodecyl sulphate gel electrophoresis (SDS–PAGE) analyses (Laemmli, 1970) with a 12% running gel were used to estimate the molecular weights of fungal xylanases derived from the crude culture filtrates. The protein content, estimated by the method of Bradford (1976), was adjusted so that 15 μg of each sample was loaded on to the gel. The calibration mixture of Sigma (Dalton Mark VII-L™, Lot 41H9466) served as a molecular weight marker. To reveal the proteins, gels were stained for 40 min with 0·1% Coomassie brilliant blue G-250 (Serva) in methanol/acetic acid/water (40/10/50).

Immunoblotting

Antiserum for the purified *B. sorokiniana* xylanase was prepared according to Leinonen (1985) by subcutaneous inoculation of rabbits at 3 wk intervals with 100 µl of purified enzyme (0·7 mg ml^{-1}) emulsified with an equal volume of Freund's complete adjuvant. The serum was collected after two injections. Detection of protein bands with xylanase antiserum was performed with a horseradish peroxidase immunoblot assay kit (Bio-Rad) according to manufacturer's instructions.

RESULTS AND DISCUSSION

Comparison of xylanase activities

Under the conditions used in this study, the seven isolates of *B. sorokiniana* and *D. teres*, and the two isolates of *D. graminea* and *R. secalis* showed similar timing and production of xylanases within each species, and therefore the results from only one of each are presented. Very low or no endopolygalacturonase activity was detected in all the fungi when grown in barley cell wall medium. This demonstrates that fungal pathogens of cereals tend to secrete xylanases rather than pectinases when grown in host cell wall medium, as noticed by Cooper *et al.* (1988) and Lehtinen (1993).

B. sorokiniana and *R. solani* produced the highest xylanase activities when grown in barley cell wall medium, with maximum activity 7–8 d post-inoculation, respectively (Fig. 1). They also similarly secreted significantly less xylanase in xylan medium than in barley cell wall preparation medium, consistent with observations on *Trichoderma longibrachiatum* Rifai (Royer & Nakas, 1989), *Rhizoctonia* sp. (R. Karjalainen & Kangasniemi, A., unpublished) and *B. sorokiniana* (Peltonen *et al.*, 1994). Maximum xylanase activity of *R. solani* during growth in barley cell wall medium was maintained, whereas that of *B. sorokiniana* was only temporary. These different patterns may reflect differences in regulation of enzyme activity at the transcriptional level, or in enzyme function in catabolite repression (Cooper, 1983).

The highest xylanase activities for *R. secalis* and *D. graminea* occurred on day 5 although the amounts were lower than for *B. sorokiniana*. In the *D. teres* cultures maximum xylanase activity was recorded 6–8 d after inoculation, and xylan from birchwood supported enzyme synthesis at least as well as that from barley cell walls. *D. graminea* produced less xylanase activity when grown in xylan medium than in barley cell wall medium, but in cultures of *R. secalis* grown in xylan medium xylanase activity was barely detectable. Although there are reports of biotrophic pathogens being able to produce plant cell wall degrading enzymes, enzyme production is usually of special importance in penetration of necrotrophic pathogens (Goodman, Kiraly & Wood, 1986). Therefore, the overall low enzyme activity associated with *R. secalis* may in part be due to its perthotrophic nature which includes subcuticular growth without damaging the host cells during the early phases of colonization (Mathre, 1982).

In an earlier study (Cooper *et al.*, 1988), *Rhizoctonia cerealis* E.P. Hoeven and *F. culmorum* produced a similar sequence of cell wall degrading enzymes, including xylanases, when grown on wheat seedling cell wall medium. In the present study, the level of xylanase activity in cultures of *F. culmorum* was, however, much lower than in *R. solani*. *F. culmorum* also differed from *R. solani* by secreting more xylanase in xylan medium than in barley cell wall medium. *G. graminis* did not produce xylanase in barley cell wall medium, possibly because it primarily colonizes roots and cannot utilize cell walls from barley leaves. In a study of α-galactosidase from *Colletotrichum lindemuthianum* (Sacc. & Magnus) Scribn., cell walls from roots, which were resistant to the fungus, induced considerably lower enzyme activity than cell walls from leaves susceptible to infection (English, Jurale & Albersheim, 1971). *G. graminis*, however, produced xylanase in xylan medium, as observed by Southerton *et al.* (1993).

The only consistent pattern of xylanase induction was that maximum activity occurred a few days earlier for leaf pathogens than for stem base or root pathogens. *B. sorokiniana* showed higher xylanase activity than the principal leaf pathogens of barley, but the pattern of enzyme induction, with a clear activity peak, resembled that of leaf pathogens. However, in the expression of xylanase activity in barley cell wall medium *B. sorokiniana* was most similar to *R. solani*, but comparison of enzyme production *in vitro* by different fungi should be interpreted cautiously because composition of the medium has a large effect on the range of cell wall degrading enzymes produced. It has been observed that cell walls extracted from various plant species can cause a differential rate and extent of synthesis of cell wall degrading enzymes by pathogens (Cooper, Wardman & Skelton, 1981). Also, the age of the leaves used for cell wall preparation may alter the ability of fungi to degrade cell wall polymers (Bateman *et al.*, 1969). Furthermore, the concentration of carbon source used can determine the final level of enzyme activity in culture (English *et al.*, 1971). Adjustment of optimal growing conditions for each fungus is, therefore, required before a meaningful interpretation of enzyme activity levels can be made. This makes comparisons between various fungi difficult.

Relationship between infection efficiency and xylanase activity of B. sorokiniana *isolates*

Due to the onset of senescence of the third leaf from top, which might have given aberrant symptoms, only the results from the two uppermost leaves are presented. The rank of cultivar reactions to *B. sorokiniana* isolates was similar at 8, 18 and 28 d post-inoculation when symptoms were detected, and only the 18 d results are shown (Table 1). *B. sorokiniana* did not cause spot blotch in barley leaves as a natural infection during the years of field experiments in this study.

The isolates of *B. sorokiniana* were significantly different in their infection efficiencies of barley leaves. Isolate H83 caused the most severe symptoms in all cultivars studied while isolates A90 and F90 were only moderately virulent. There were significant differences in cultivar reactions to H83, but not to the other fungal isolates. Cultivars Kymppi, Loviisa and Pohto, which were generally more susceptible to *B. sorokiniana* in this experiment than the other cultivars, seemed to be especially susceptible to H83. However, in spite of the greater aggressiveness of H83, its ability to produce xylanase *in vitro* was less than that of A90 or F90. Therefore, it was indicated

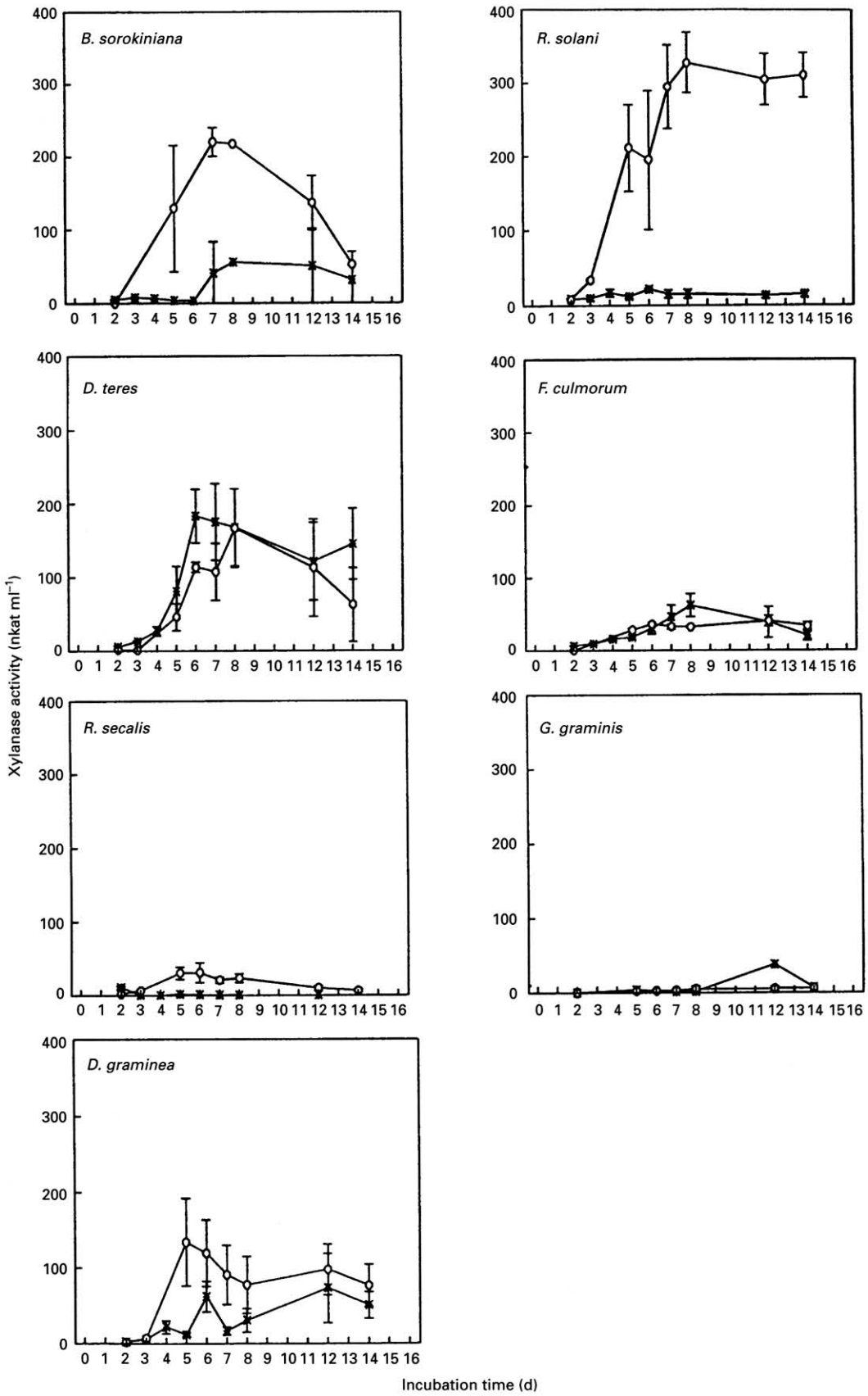

Fig. 1. Production of xylanases by barley pathogens grown in a cell wall preparation of barley leaves (○) and xylan from birchwood (×). Each point is a mean (±S.D.) from three experiments, each with two replicates.

Table 1. Infection efficiencies of *B. sorokiniana* isolates in barley leaves from field experiments and the corresponding xylanase activity in barley cell wall cultures. Symptoms were recorded 18 d after inoculation

Isolate	Cultivar	Symptoms (% leaf area infected)		Maximum xylanase activity, nkat ml^{-1} (day of peak activity)
		Flag leaf	2nd upper leaf	
H83	Kustaa	12·3 b[1]	68·0 ab	
	Kymppi	4·6 b	28·7 d	
	Loviisa	35·2 a	53·2 bc	
	Pohto	36·6 a	71·8 a	
	Pokko	10·7 b	44·1 cd	
	Mean[2]	19·9 a	53·1 a	170·2 b (13)
A90	Kustaa	3·5 a	28·9 ab	
	Kymppi	2·6 a	15·0 b	
	Loviisa	3·5 a	21·5 ab	
	Pohto	9·4 a	34·3 a	
	Pokko	2·9 a	15·6 b	
	Mean	4·3 b	23·0 b	209·8 a (12)
F90	Kustaa	4·5 a	39·6 a	
	Kymppi	2·7 a	10·7 b	
	Loviisa	3·5 a	13·5 b	
	Pohto	7·1 a	21·6 b	
	Pokko	2·3 a	7·5 b	
	Mean	4·0 b	18·6 b	196·2 ab (10)
	Cultivar × isolate	***	ns	

*** Significant interaction at the 0·001 probability level; ns, non-significant interaction.

[1] Within an isolate, means for cultivars followed by the same letter in a column do not differ significantly at $P < 0·05$ (Duncan's multiple range test).

[2] Within a column, means followed by the same letter do not differ significantly at $P < 0·05$ (Duncan's multiple range test).

that xylanase activity *in vitro* could not entirely be related to fungal virulence *in vivo*, even though host cell walls were used for xylanase induction. It is, however, known that despite the ability of pathogens to produce high enzyme activity, penetration of host cell walls may often fail due to modifications which make them more resistant to the approach of a potential parasite (Ride, 1978). It is also possible that a fungal isolate with less efficient enzyme activity might delay elicitation of resistance responses in host plants, thus allowing colonization of the tissue. Similarly, isolates with higher enzyme activity may elicit plant resistance responses rapidly, thereby causing minor damage (Cervone *et al.*, 1987). The many reports of xylanases inducing plant defence reactions (Dean, Gamble & Anderson, 1989; Fuchs *et al.*, 1989; Bailey, Dean & Anderson, 1990; Lotan & Fluhr, 1990) thereby explain the role of xylanases in plant–fungus interactions through potential elicitor activity. It was also recently reported by Apel *et al.* (1993) using cloning and transformation experiments with a xylanase gene (*XYL1*) from a maize pathogen, *Cochliobolus carbonum* R. R. Nelson, that xylanase was not required in the infection process.

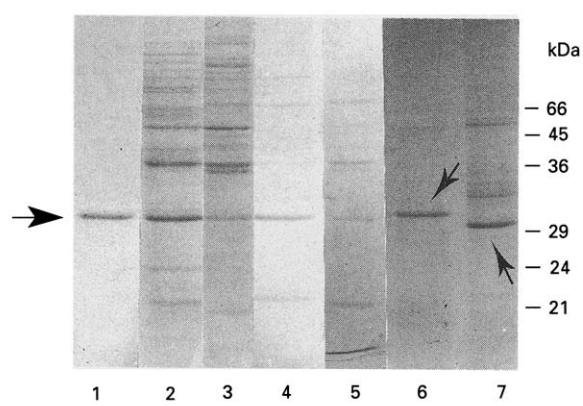

Fig. 2. SDS–PAGE of fungal xylanases. Samples 2–7 derive from the crude culture filtrates with the total protein content of 15 µg. 1, purified xylanase from *B. sorokiniana* (7 µg); 2, *B. sorokiniana* grown in barley cell wall medium; 3, *B. sorokiniana* grown in xylan medium from birchwood; 4, *D. teres* grown in barley cell wall medium; 5, *D. graminea* grown in xylan medium from birchwood; 6, *R. secalis* grown in barley cell wall medium; 7, *R. solani* grown in barley cell wall medium. Arrows indicate the location of xylanases. Protein standards (in kDa) are indicated in the margin.

Molecular weight estimation of xylanases

Mol. wt estimation was based on previous results (Peltonen *et al.*, 1994), and also on the presence of new, or dominant, bands in culture filtrate samples with induced xylanase activity as compared with samples from cultures without xylanase activity. The intensity of the bands was closely correlated with the xylanase activity in culture supernatants. *F. culmorum* and *G. graminis* xylanase activities were so low that no xylanase bands were detected, but the other fungi apparently produced the same xylanases with similar mol. wt in both cell wall and xylan media.

The mol. wt of fungal xylanases usually range from 16 to 38 kDa (Dekker & Richards, 1976), with which the present finding of 29–31 kDa (Fig. 2) agrees. Bands of 30 kDa were found in lanes loaded with samples derived from the culture filtrates of *B. sorokiniana* (Fig. 2, lanes 2 and 3), in accord with purified xylanase from the same fungus (Peltonen *et al.*, 1994). *R. solani* showed a dominant band of 29 kDa (Fig. 2, lane 7), which was not detected in uninduced cultures. Karjalainen & Kangasniemi (1994, unpublished) detected a xylanase of 30 kDa in cultures of a *Rhizoctonia* sp. that was not

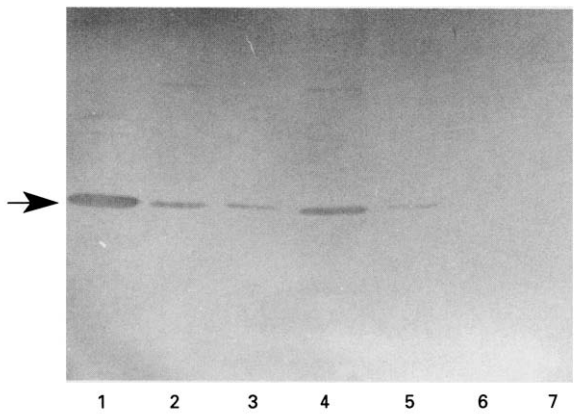

Fig. 3. Immunoblot analysis of fungal xylanases with antiserum prepared from purified *B. sorokiniana* xylanase (arrow). Samples as for Fig. 2.

a pathogen of barley but that infected conifer roots. A dominant 31 kDa band for xylanase was detected in cultures of *R. secalis* grown on barley cell wall medium (Fig. 2, lane 6), even though the activity was quite low, but no band was detected in cultures grown on xylan medium. In cultures of *D. teres*, a strong band with an apparent mol. wt of 30 kDa (Fig. 2, lane 4), corresponded with a faint band in *D. graminea* cultures (Fig. 2, lane 5) and the mol. wt was further confirmed by immunological analysis.

Immunological analysis

Culture filtrate samples from *B. sorokiniana* grow both in barley cell wall and xylan media gave the same positive reaction as purified xylanase (Fig. 3, lanes 1–3), demonstrating production of the same xylanase in both media. The antiserum from *B. sorokiniana* xylanase also reacted with the xylanases of both *D. teres* and *D. graminea* (Fig. 3, lanes 4–5). At the late stages of incubation, other, minor cross-reactive bands appeared, especially in samples derived from barley cell wall culture supernatants, possibly indicating either multiple forms of xylanases or non-specific binding. The relationship between xylanases from *B. sorokiniana*, *D. teres* and *D. graminea* is reasonable because the fungi were originally assigned to *Helminthosporium* and thus were considered to be genetically similar (Alexopoulos & Mims, 1979). Furthermore, these fungi are necrotrophic pathogens and have only graminaceous hosts (Mathre, 1982), which suggests a similar role for xylanases in their infection processes.

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REFERENCES

Alexopoulos, C. J. & Mims, C. W. (1979). *Introductory Mycology*. 3rd edition. John Wiley & Sons: New York.

Anderson, A. J. (1978). Extracellular enzymes produced by *Colletotrichum lindemuthianum* and *Helminthosporium maydis* during growth on isolated bean and corn cell walls. *Phytopathology* **68**, 1585–1589.

Apel, P. C., Panaccione, D. G., Holden, F. R. & Walton, J. D. (1993). Cloning and targeted gene disruption of XYL1, a β-1,4-xylanase gene from the maize pathogen *Cochliobolus carbonum*. *Molecular Plant–Microbe Interactions* **6**, 467–473.

Bailey, B. A., Dean, J. F. D. & Anderson, J. D. (1990). An ethylene biosynthesis-inducing endoxylanase elicits electrolyte leakage and necrosis in *Nicotiana tabacum* cv. *xanthi* leaves. *Plant Physiology* **94**, 1849–1854.

Bailey, M. J. & Pessa, E. (1990). Strain and process for production of polygalacturonase. *Enzyme and Microbial Technology* **12**, 266–271.

Baker, C. J., Whalen, C. H. & Bateman, D. F. (1977). Xylanase from *Trichoderma pseudokoningii*: purification, characterization and effects on isolated plant cell walls. *Phytopathology* **67**, 1250–1258.

Bateman, D. F., Van Etten, H. D., English, P. D., Nevins, D. J. & Albersheim, P. (1969). Susceptibility to enzymatic degradation of cell walls from bean plants resistant and susceptible to *Rhizoctonia solani* Kühn. *Plant Physiology* **44**, 641–648.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Braun, E. J. & Kelman, A. (1987). Production of cell wall-degrading enzymes by corn stalk rot strains of *Erwinia chrysanthemi*. *Proceedings of the 6th International Conference on Plant Pathogenic Bacteria*. College Park, Maryland, 1985, pp. 208–213. Nijhoff/Junk: Dordrecht.

Carder, J. H., Hignett, R. C. & Swinburne, T. R. (1987). Relationship between the virulence of hop isolates of *Verticillium albo-atrum* and their *in vitro* secretion of cell-wall degrading enzymes. *Physiological and Molecular Plant Pathology* **31**, 441–452.

Cervone, F., De Lorenzo, G., Degra, L. & Salvi, G. (1987). Elicitation of necrosis in *Vigna unguiculata* Walp. by homogeneous *Aspergillus niger* endo-polygalacturonase and by alpha-D-galacturonate oligomers. *Plant Physiology* **85**, 626–630.

Chen, W., Schneider, R. W. & Hoy, J. W. (1992). Taxonomic and phylogenetic analyses of ten *Pythium* species using isozyme polymorphisms. *Phytopathology* **82**, 1234–1244.

Cooper, R. M. (1983). The mechanisms and significance of enzymic degradation of host cell walls by parasites. In *Biochemical Plant Pathology* (ed. J. Callow), pp. 101–135. John Wiley & Sons: New York.

Cooper, R. M., Longman, D., Campbell, A., Henry, M. & Lees, P. E. (1988). Enzymic adaptation of cereal pathogens to the monocotyledonous primary wall. *Physiological and Molecular Plant Pathology* **32**, 33–47.

Cooper, R. M., Wardman, P. A. & Skelton, J. E. M. (1981). The influence of cell walls from host and non-host plants on the production and activity of polygalacturonide-degrading enzymes from fungal pathogens. *Physiological Plant Pathology* **18**, 239–255.

Dean, J. F. D., Gamble, H. R. & Anderson, J. D. (1989). The ethylene biosynthesis-inducing xylanase: its induction in *Trichoderma viride* and certain plant pathogens. *Phytopathology* **79**, 1071–1078.

Dekker, R. F. H. & Richards, G. N. (1976). Hemicellulases: their occurrence, purification properties and mode of action. *Advances in Carbohydrate Chemistry and Biochemistry* **32**, 277–352.

English, P. D., Jurale, J. B. & Albersheim, P. (1971). Host-pathogen interactions. II. Parameters affecting polysaccharide-degrading enzyme secretion by *Colletotrichum lindemuthianum* grown in culture. *Plant Physiology* **47**, 1–6.

Fernandez, N., Patino, B. & Vazquez, C. (1993). Pectin degrading enzymes secreted by six isolates of *Fusarium oxysporum*. *Mycological Research* **97**, 461–466.

Fuchs, Y., Saxena, A., Gamble, H. R. & Anderson, J. D. (1989). Ethylene biosynthesis-inducing protein from cellulysin is an endoxylanase. *Plant Physiology* **89**, 138–143.

Goodman, R. N., Kiraly, Z. & Wood, K. R. (1986). *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press: Columbia.

Howell, H. E. (1975). Correlation of virulence with secretion *in vitro* of three wall-degrading enzymes in isolates of *Sclerotinia fructigena* obtained after mutagen treatment. *Journal of General Microbiology* **90**, 32–40.

Jarvis, M. C., Threlfall, D. R. & Friend, J. (1981). Potato cell wall polysaccharides: degradation with enzymes from *Phytopthora infestans*. *Journal of Experimental Botany* **32**, 1309–1319.

Johansson, M. (1988). Pectic enzyme activity of spruce (S) and pine (P) strains of *Heterobasidion annosum* (Fr.) Bref. *Physiological and Molecular Plant Pathology* **33**, 333–349.

Karlsson, J.-O. & Stenlid, J. (1991). Pectic isozyme profiles of intersterility groups in *Heterobasidion annosum*. *Mycological Research* **95**, 531–536.

Labavitch, J. M. & Ray, P. M. (1978). Structure of hemicellulosic polysaccharides of *Avena sativa* coleoptile cell walls. *Phytochemistry* **17**, 933–937.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lehtinen, U. (1993). Plant cell wall degrading enzymes of *Septoria nodorum*. *Physiological and Molecular Plant Pathology* **43**, 121–134.

Leinonen, M. (1985). Serological methods for the study of bacterial surface antigens. In *Enterobacterial Surface Antigens: Methods for Molecular Characterization* (ed. T. K. Korhonen, E. A. Dawes & P. H. Mäkelä), pp. 179–206. Elsevier Science Publishers (Biomedical Division).

Lotan, T. & Fluhr, R. (1990). Xylanase, a novel elicitor of pathogenesis-related proteins in tobacco, uses a non-ethylene pathway for induction. *Plant Physiology* **93**, 811–817.

Mathre, D. E. (1982). *Compedium of Barley Diseases*. American Phytopathological Society: St Paul, MN.

MSTAT Development Team (1989). *MSTAT User's Guide. A Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments*. Michigan State University: East Lansing.

Peltonen, S., Karjalainen, R. & Niku-Paavola, M.-L. (1994). Purification and characterization of a xylanase from *Biopolaris sorokiniana*. *Mycological Research* **98**, 67–73.

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Poutanen, K. & Puls, J. (1988). Characteristics of *Trichoderma reesei* β-xylosidase and its use in the hydrolysis of solubilized xylans. *Applied Microbiology* and *Biotechnology* **28**, 425–432.

Ride, J. P. (1978). The role of cell wall alterations in resistance to fungi. *Annals of Applied Biology* **89**, 302–306.

Ried, J. L. & Collmer, A. (1986). Comparison of pectic enzymes produced by *Erwinia chrysanthemi*, *Erwinia carotovora* subsp. *carotovora*, and *Erwinia carotovora* subsp. *atroseptica*. *Applied and Environmental Microbiology* **52**, 305–310.

Royer, J. C. & Nakas, J. P. (1989). Xylanase production by *Trichoderma longibrachiatum*. *Enzyme and Microbial Technology* **11**, 405–410.

Southerton, S. G., Osbourn, A. E., Dow, J. M. & Daniels, M. J. (1993). Two xylanases from *Gaeumannomyces graminis* with identical N-terminal amino acid sequence. *Physiological and Molecular Plant Pathology* **42**, 97–107.

Sumner, J. B. & Somers, G. F. (1949). Dinitrosalicylic method for glucose. In: *Laboratory Experiments in Biological Chemistry*, pp. 38–39. Academic Press: New York.

Sweetingham, M. W., Cruickshank, R. H. & Wong, D. H. (1986). Pectic zymograms and taxonomy and pathogenicity of the Ceratobasidiaceae. *Transactions of the British Mycological Society* **86**, 305–311.

Zadoks, J. C., Chang, T. T. & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421.