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Mycosphaerella graminicola produces a range of cell wall-degrading enzyme activities *in vitro* that vary with the carbon source

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Abstract

In this study, two *Mycosphaerella graminicola* isolates produced a range of cell wall-degrading enzymes (CWDE) *in vitro* that can potentially degrade wheat cell walls. The influence of three carbon sources on CWDE *in vitro* production was tested: 1) 1 % galactose (w/v), 2) 1% wheat cell walls (w/v) and 3) a mixture of 1% galactose (w/v) and 1% wheat cell walls (w/v). Six major activities produced by both isolates were detected: xylanase, β-1,3-glucanase, polygalacturonase, cellulase, β-xylosidase and β-galactosidase. Time-course experiments showed that different levels of enzyme activities were obtained with isolates 323 and 94269. These activities levels varied also with the type of carbon source used. Principal Component Analysis showed that the enzyme activities are gathered into two groups. None of the activities of the first group was correlated to the activities of the second group. It also showed that the optimal medium that allowed the production of most of the major activities contained both galactose and wheat cell walls.

Abbreviations: CWDE – cell wall-degrading enzymes; G – galactose; S – synthetic medium; W – wheat cell wall material

Introduction

The septoria disease complex of wheat is mainly caused by the two species *Mycosphaerella graminicola* (anamorph *Septoria tritici*) and *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*). *Mycosphaerella graminicola* is the most important wheat pathogen causing septoria leaf blotch disease. The increasing interest in this fungus has been stimulated by the prevalence of this pathogen in the major wheat production areas (Eyal and Levy, 1987; Eyal, 1999). Despite its strong economic significance (Scharen, 1999), the molecular and biochemical basis of its pathogenicity are still poorly understood (Palmer and Skinner, 2002).

Plant fungal pathogens produce a wide range of cell wall-degrading enzymes (CWDE) which are thought to degrade the plant cell wall and allow access to the tissues. CWDE, as demonstrated for other phytopathogens, are determinants of the fungal pathogenicity complex (Bateman and

Basham, 1976; Kapat et al., 1998; Kang and Bauchenauer, 2000; Wanyoike et al., 2002). Cell wall degradation may be important to fungi not only for penetration and hyphal branching inside the plant tissue, but also for releasing nutrients from the wall polysaccharides that are necessary for growth (De Lorenzo et al., 1997). The efforts to increase resistance in the host plant may benefit from new information on important factors involved in pathogenicity. Some of these degradation products elicit defence responses in plants (Esquerre-Tugaye et al., 2000). However, little is known about the degree to which the chemical composition of plant cell wall polysaccharides influences the outcome of the plant-pathogen interaction (Vorwerk et al., 2004).

For *P. nodorum*, previous experiments have been performed on CWDE produced by this pathogen. Magro (1984), Lehtinen (1993) and Lalaoui et al. (2000) reported the production of xylanase, β-1,3-glucanase, polygalacturonase and β-xylosidase by this species *in vitro*. However, for *M. graminicola*, only pathogenesis histology has been investigated (Cohen and Eyal, 1993; Kema et al., 1996; Dancer et al., 1999; Duncan and Howard, 2000; Rohel et al., 2001; Shetty et al., 2003). The presence of soluble compounds produced *in vivo* by *M. graminicola*, that are associated with its pathogenicity have been reported by Zelikovitch and Eyal (1989), Levy et al. (1992) and Kema et al. (1996). As suggested by these authors, these soluble compounds could be toxins. Alternatively, they could also be CWDE released by *M. graminicola* and involved in cell wall degradation.

A number of genes coding for CWDEs such as proteases, xylanases, glucanases and cutinases have been isolated from *M. graminicola* expressed sequence tags (ESTs). These enzymes could also be involved in the acquisition of nutrients from the plant (Palmer and Skinner, 2002). Despite this molecular progress, nothing is known about the biochemical events that are associated with the cell wall degradation prerequisite to leaf tissue colonization by *M. graminicola* (Keon et al., 2005). The first aim of this study was to investigate the extent of CWDE production by this fungus. In addition, we tested the effect of carbon source on CWDE production on three growth media by comparing enzyme activities of two isolates of *M. graminicola*. This study is the first report of CWDE production in *M. graminicola*.

Materials and methods

Fungal strains and inoculum production

Two fungal strains with opposite mating types were used: 323 and 94269 (Kema and Van Silfhout, 1997). Pycnidiospores for inoculation were produced by growing the fungus on potato dextrose agar medium at 15 °C under white light (12 h light/cycle) for 12 days.

Liquid culture conditions for enzyme production

In vitro culture was carried out using the liquid synthetic (S) medium as described by Halama and Lacoste (1992) supplemented with galactose (Lalaoui et al., 1996) and/or cell walls (Lehtinen, 1993). The carbon sources were added to the synthetic medium as follows: (a) 1% (w/v) galactose (S + G medium), (b) 1% (w/v) wheat cell wall material (S + W medium) or (c) 1% (w/v) galactose supplemented by 1% (w/v) wheat cell wall material (S + W + G medium). The wheat cell wall material was prepared from two week-old wheat seedlings cv. Soissons. The frozen leaves were blended and then suspended in 0.1 M potassium phosphate buffer (pH 7) for 1 h (Cooper et al., 1981). They were then filtered and suspended in 95% ethanol for 6 h and the process was repeated twice, followed by suspension in acetone and then in ether. Finally, they were filtered, dried and stored (Lehtinen, 1993). Cell walls prepared in this way were added to the culture either as a sole carbon source or mixed with galactose. Medium (100 ml) in a 250 ml Erlenmeyer flask was inoculated with 1 ml of a 10^6 spores ml^{-1}suspension and the culture kept at 25 °C in the dark under constant agitation (140 rpm) for 20 days. Every second day, from day 2 until day 20, fungal mycelia were removed by suction filtration. Filtrates were then assayed for enzyme activities: three culture flasks were used as replicates.

Enzyme assays

Preliminary experiments were performed to determine the optimum reaction pH and temperature of the enzyme activities secreted by *M. graminicola*. Endoglycosidase activities (cellulase,

β-1,3-glucanase, polygalacturonase and xylanase) were assayed by the 3,5-dinitrosalicylic acid (DNS) modified method (Miller, 1959; Lalaoui et al., 2000). Cellulase activity was measured using carboxymethylcellulose (0.2% w/v) as substrate at pH 5.0 and 60 °C. Laminarin (0.5% w/v) from *Laminaria digitata* (Sigma Aldrich) was used as the substrate for β-1,3-glucanase activity at pH 5.0 and 50 °C. For both enzymes, the reducing sugar control was glucose. Polygalacturonase activity was determined at pH 5.2 using the polygalacturonic acid (0.1% w/v) as substrate at 50 °C. The corresponding reducing sugar control was galacturonic acid. Xylanase activity was measured using xylan (1% w/v) from oat spelts (Sigma Aldrich) as a substrate at pH 4.8 and 45 °C. The corresponding reducing sugar control was xylose. The reaction mixture for all endoglycosidase activity assays was incubated for 30 min. Absorbance was measured at 540 nm.

Exoglycosidase activities (β-galactosidase, β-xylosidase and α-arabinosidase) were measured by determining the rate of p-nitrophenol released from the appropriate nitrophenyl derivative (Sigma Aldrich) by the modified method of Conchie (1954). 5 mM of p-nitrophenyl-β-D-galactopyranoside, 5 mM of p-nitrophenyl-β-D-xylopyranoside and 5 mM p-nitrophenyl-α-L-arabinopyranoside were used as substrates for measuring the β-galactosidase (pH 4.5, 45 °C), β-xylosidase (pH 4.0, 60 °C) and α-arabinosidase (pH 4.0, 45 °C) activities respectively. The reaction mixtures for all exoglycosidase activity assays were incubated for 20 min and the absorbance was measured at 420 nm.

Appropriate enzyme and substrate controls were included in all assays. Activities expressed in mU-ml were calculated from the linear portion of the activity curves. One mU-ml is the quantity of enzyme necessary to release one micromole of reducing sugar/p-nitrophenol min^{-1} and ml^{-1} of enzyme solution under corresponding pH and temperature conditions. For each activity, three parameters were assessed to differentiate and evaluate enzyme production: (i) area under the enzyme curve (AUEC) was calculated as the area under the graph of observed enzyme level plotted against time, from the first to the last day of the assay; (ii) the level of maximum enzyme activity during the time-course experiment; (iii) the date of this maximum activity.

Statistical analysis

Multiple comparisons of means were performed by the Tukey test at a significance level of $P = 0.05$ using R software, version 1.9.1 for Windows XP (R Development Core Team, 2004) to differentiate the enzyme levels during the time-course experiment. The enzyme activities were also subjected to normed principal component analysis (PCA) using R software. PCA was carried out on the whole dataset obtained. The first two principal components were plotted to visualize the correlation between enzyme activities (correlation circle) and the activity production ability of each isolate on each medium (individual representation).

Results

Comparison of maximum activity levels between strains and media

The secreted enzyme activities were determined every other day throughout the growth period (20 days). The maximum values of enzyme activities, the corresponding day and the AUEC are represented in Table 1. Based on the comparison of maximum activity levels, we observed that on the S + G medium, isolate 323 produced, in order of decreasing endoglycosidase activity levels: β-1,3-glucanase, xylanase, cellulase and polygalacturonase, whereas isolate 94269 produced cellulase, xylanase, β-1,3-glucanase and polygalacturonase. For exoglycosidase activities, isolate 323 produced on S + G medium more β-xylosidase than β-galactosidase. Alpha-arabinosidase was found in low amounts in the medium. Isolate 94269 produced, on S + G medium, β-galactosidase and β-xylosidase. Again, α-arabinosidase was found in small amounts (Table 1).

On S + W medium, both isolates showed the same rank of endoglycosidase production: xylanase, β-1,3-glucanase, polygalacturonase and cellulase. On this medium, isolate 323 produced in order of decreasing exoglycosidase activities: β-xylosidase, β-galactosidase and α-arabinosidase. On the other hand, isolate 94269 produced β-xylosidase, α-arabinosidase and β-galactosidase (Table 1).

The two isolates had the same decreasing order of endoglycosidase activities on S + W + G

Table 1. Maximum activity levels, corresponding day and Area Under the Enzyme Curve (AUEC) of enzymes produced by *M. graminicola* isolates 323 and 94269 grown on the three media differing in carbon sources

Enzyme activity	Isolate	S + G[a] medium			S + W[b] medium			S + W + G[c] medium		
		Maximum level	Corresponding day	AUEC	Maximum level	Corresponding day	AUEC	Maximum level	Corresponding day	AUEC
Xylanase	323	66.81 a	20	791	219.65 b	10	2891	224.8 b	20	1529
	94269	86.40 a	16	924	246.67 c	18	4300	279.64 d	14	2263
β-xylosidase	**323**	**50.04 ab**	**18**	**307**	**64.32 b**	**12**	**1400**	**14.49 a**	**18**	**109**
	94269	**8.51 a**	**20**	**42**	**202.89 c**	**18**	**2182**	**21.50 ab**	**18**	**124**
α-arabinosidase	**323**	**1.57 ab**	**20**	**12**	**7.52 c**	**16**	**115**	**1.50 ab**	**20**	**8**
	94269	**0.31 a**	**18**	**1.3**	**13.46 d**	**18**	**162**	**2.24 b**	**18**	**12**
Cellulase	323	35.21 d	8	184	6.08 b	20	47	36.92 d	2	283
	94269	116.14 e	12	514	4.24 a	18	26	26.98 c	4	118
β-1,3-glucanase	323	264.33 e	20	956	125.30 c	10	2132	89.25 b	20	476
	94269	41.74 a	18	332	204.73 d	18	2145	123.16 c	18	943
Polygalacturonase	323	32.63 c	6	169	16.07 b	16	123	27.91 c	10	239
	94269	32.05 c	6	192	4.63 a	14	40	18.42 b	10	146
β-galactosidase	**323**	**16.11 d**	**18**	**138**	**22.98 e**	**18**	**151**	**4.65 a**	**20**	**28**
	94269	**9.94 c**	**20**	**30**	**6.88 b**	**18**	**77**	**4.14 a**	**18**	**36**

The maximum-corresponding day is the day when maximum enzyme activity (mU-ml) was detected during the time-course experiment. The exoglycosidase activities are represented in bold characters.

[a] S + G corresponds to the synthetic medium supplemented with 1% (w/v) of galactose.

[b] S + W corresponds to the synthetic medium supplemented with 1% (w/v) of wheat cell wall material.

[c] S + W + G corresponds to the synthetic medium supplemented with 1% (w/v) of galactose and 1% (w/v) of wheat cell wall material.

Levels of each enzyme activity tagged with the same letter are not significantly different ($P > 0.05$) using the Tukey test.

medium: xylanase, β-1,3-glucanase, cellulase and polygalacturonase. The exoglycosidase activities exhibited were β-xylosidase and β-galactosidase. Again, α-arabinosidase was released at a low level (Table 1).

Furthermore, the rank of isolate AUEC on each medium corresponded to the rank of enzyme maximum activity, except for the xylanase on S + W and S + W + G media for both isolates, for the β-1,3-glucanase on the three media for isolate 323 only, for the polygalacturonase on S + G and S + W + G media for isolate 323 only, and for β-galactosidase on S + G and S + W media for isolate 94269 only.

Enzymes degrading xylan and its side chains during time course experiments

Data given in Table 1 show that xylanase was the major activity induced by cell walls in S + W and S + W + G media compared to the S + G medium. This activity increased significantly with a maximum 2.9-fold increase on S + W and a 3.25-fold increase on S + W + G medium for 94269 compared to S + G medium. The increase was 3.3-fold on S + W medium and 3.35-fold on S + W + G medium for isolate 323. Isolate 94269 had the highest xylanase activity levels on both S + W and S + W + G media, with maximum levels on S + W + G medium on day 14. The high amount of xylanase exhibited by isolate 323 on the two cell wall-containing media were on day 10 on S + W medium, and on day 20 on S + W + G medium (Table 1).

Enzyme activity from *M. graminicola* involved in xylan side chain cleavage (α-arabinosidase and β-xylosidase) could be detected in S + W medium. Beta-xylosidase and α-arabinosidase activities were produced in small amounts in the media containing galactose and were stimulated by the presence of cell walls when used alone especially for isolate 94269. For β-xylosidase activity, isolate 323 had a 1.2-fold higher amount on S + W medium in addition to an earlier maximum production compared to S + G medium. Isolate 94269 showed a 9 to 25-fold increase of β-xylosidase activity on the S + W medium compared to S + G and S + W + G media respectively. Again, isolate 94269 showed higher levels on this S + W medium. For α-arabinosidase activity,

isolate 94269 had at least a 7-fold increase on S + W medium compared to the two other media and isolate 323 had a 4-fold increase on S + W medium.

Enzymes degrading glucans and cellulose

In contrast to the activities discussed above, cellulase production reached its maximum level in the presence of galactose and in the absence of cell walls except on S + W + G medium for isolate 323 (Table 1). On the S + G medium, isolate 323 attained its maximum level on day 8, earlier to isolate 94269 (day 12) whereas the maximum level of cellulase activity was higher. On the S + W + G medium, isolate 323 retained its early cellulase production on day 2, but isolate 94269 reached its maximum level by day 4.

Beta-1,3-glucanase activity was detected in the three media. For isolate 94269, the activity level was higher on media containing wheat cell walls than on the medium containing galactose. β-1,3-glucanase activity for this isolate was the highest on S + W medium, then on S + W + G medium, followed by S + G medium. This activity remained lower than that of xylanase on wheat cell wall media. In contrast, isolate 323 had significantly higher production of β-1,3-glucanase on S + G medium. On S + W medium, isolate 323 level reached its maximum level on day 10, earlier than isolate 94269 which reached its maximum level of β-1,3-glucanase activity on day 18. Furthermore, on S + W + G medium, isolate 94269 had maximum production on the same date of culture as isolate 323 (Table 1).

Enzymes degrading pectic polysaccharides and their side chains

The maximum activity of fungal-secreted polygalacturonase was found on galactose medium, where both isolates produced the same levels of polygalacturonase at day 6. On S + W medium, isolate 323 showed higher polygalacturonase activity than 94269 for most of the time period. Furthermore, on S + W + G medium, isolate 323 had significantly higher maximum polygalacturonase levels on day 10.

Beta-galactosidase activity was secreted in all three media, but at lower levels on S + W + G medium. This enzyme was produced late during the time-course experiment. The levels were lower for both isolates in the medium containing both galactose and cell walls. On S + G and S + W media, the maximum level of activities secreted by isolate 323 were significantly higher than those from isolate 94269, whereas on S + W + G medium, maximum activities for both isolates were not significantly different.

Principal component analysis (PCA)

The enzyme correlation circle of PCA shows a projection of the initial variables (enzyme activities) in the factors space (Figure 1). The enzyme activities are grouped into two. Interestingly, xylanase, β-1,3-glucanase, β-xylosidase, β-galactosidase and α-arabinosidase activities occurred on the same side of the first component axis (Factor 1). These enzymes are therefore significantly and positively correlated with each other. On the other hand, the second principal component (Factor 2), is linked positively to cellulase and polygalacturonase. These enzymes are not correlated to the first group of enzymes.

The next graph, which is the ultimate goal of the PCA, enables identification of the enzyme production ability of each isolate on each medium (Figure 2). Each subject (isolate and medium) with a positive coordinate on Factor 1 (Figure 2), has xylanase, β-1,3-glucanase, β-xylosidase, β-galactosidase and α-arabinosidase activities (Figure 1) superior to the mean which corresponds to the central point. Each subject with a positive coordinate on Factor 2 (Figure 2) has cellulase and polygalacturonase activity (Figure 1) superior to the mean. Furthermore, each isolate on each medium is unique and produces a different range of activities: isolate 94269 produced higher levels of cellulase and polygalacturonase on S + G than the mean (Figure 2). Both isolates produced lower levels of xylanase, β-1,3-glucanase, β-xylosidase, β-galactosidase and α-arabinosidase than the mean on S + G medium (Figure 2). On S + W medium, the two isolates shared common characteristics, both producing xylanase, β-1,3-glucanase, β-xylosidase, β-galactosidase and α-arabinosidase (Figure 2). Finally, isolate 323 was characterized by a higher production of cellulase and polygalacturonase than the mean on S + W + G medium (Figure 2). For isolate 94269, the graph illustrates an intermediate

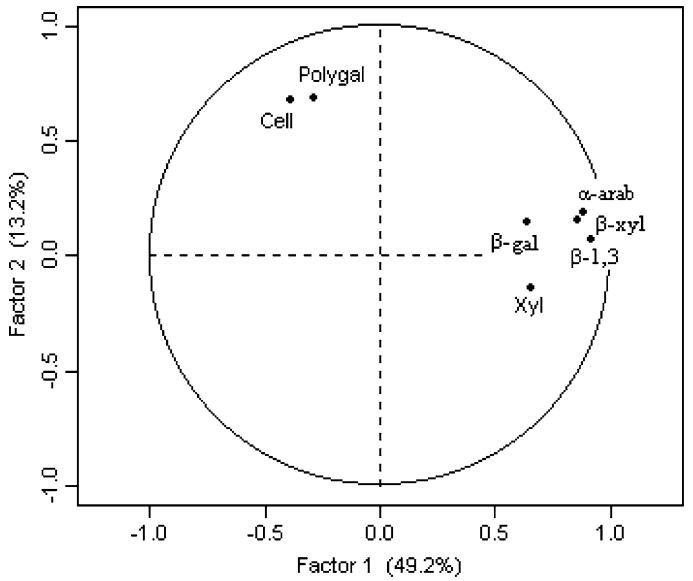

Figure 1. Principal component analysis. Enzymes correlation circle. Components 1 and 2 accounted for 49.23% and 13.25% of the total variation, respectively. Cell: cellulase, Polygal: polygalacturonase, Xyl: xylanase, β-1,3: β-1,3-glucanase, β-gal: β-galactosidase, β-xyl: β-xylosidase, α-arab: α-arabinosidase.

production of xylanase, β-1,3-glucanase, β-xylosidase, β-galactosidase and α-arabinosidase and a lower production than the mean of cellulase and polygalacturonase (Figure 2).

These data illustrate enzyme production for each isolate in different carbon sources and allow the choice of an adequate medium for CWDE production by *M. graminicola*. The PCA analysis has shown that enzyme production and diversity on the three growth media differing in their carbon source are grouped into two. S + W medium promotes xylanase, β-1,3-glucanase, β-xylosidase, β-galactosidase and α-arabinosidase activities. In contrast, S + G medium did not promote these enzyme activities; instead it enhanced cellulase and polygalacturonase activities. S + W + G

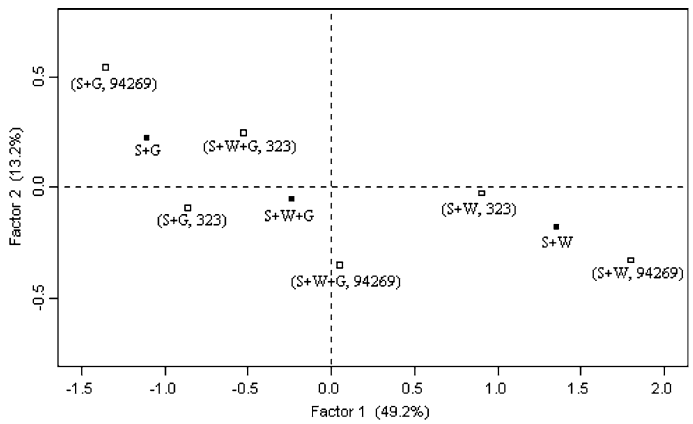

Figure 2. Principal component analysis. Individual (isolates and media) representation. Components 1 and 2 accounted for 49.23% and 13.25% of the total variation, respectively. S + G: S + G medium, S + W: S + W medium, S + W + G: S + W + G medium, (S + G, 323): isolate 323 in S + G medium, (S + G, 94269): isolate 94269 in S + G medium, (S + W, 323): isolate 323 in S + W medium, (S + W, 94269): isolate 94269 in S + W medium, (S + W + G, 323): isolate 323 in S + W + G medium, (S + W + G, 94269): isolate 94269 in S + W + G medium.

medium has a relatively central position, compared to the other media positions on the individual representation chart. Consequently, the seven enzymes were produced at an intermediate level of activity. As a result, S + W + G is the medium that permits the release of the greatest range of CWDE.

Discussion

Experimental confirmation of the production of CWDE by different phytopathogenic fungi species on monocots have been reported (Giesbert et al., 1998; Kang and Bauchenauer, 2000; Kang et al., 2000; Wanyoike et al., 2002; Thygesen et al., 2003). The present article is the first to report and measure CWDE production by *M. graminicola*. Although this species is globally more widespread compared to other wheat pathogens, CWDE produced by *M. graminicola* have not received much attention, contrary to those produced by *P. nodorum*. (Magro, 1984; Lehtinen, 1993; Lalaoui et al., 2000).

In this study, *M. graminicola* was found to produce a broad range of enzyme activities able to hydrolyze cell wall polymers. Our results indicate that changing the carbon source for fungal growth modified the levels and the kinetics of enzyme activities. Xylanase activities were the most abundant. The level of xylanase activity was higher in the media containing cell wall material and decreased in the galactose medium. For *P. nodorum*, Lalaoui et al. (1996) also observed that xylanase activity was produced on the wheat cell wall-containing medium. The relationship between strain aggressiveness and the xylanase level produced *in vitro* strongly suggested a key role for this activity during pathogenesis. *Phaeosphaeria nodorum* was also found to produce xylanase activity in both pure culture and during pathogenesis (Magro, 1984). Furthermore, Cooper et al. (1988) found that xylanase was involved in the pathogenicity of several other cereal pathogens. In particular, it was the first enzyme detected at enhanced levels in developing lesions in *Rhizoctonia cerealis*. Moreover, two xylanase genes from *Claviceps purpurea* were detected *in planta* during the wheat infection process (Giesbert et al., 1998). This activity is therefore stimulated in the presence of the corresponding substrate. Alpha-arabinosidase and β-xylosidase contribute synergistically to cleavage of the side chain of xylans and therefore to xylan degradation (Cooper et al., 1988). Cooper (1983) suggested the involvement of xylanase and α-arabinosidase in infection of maize by *Ustilago maydis*. Although the α-arabinosidase activity was low in all cultivation conditions, this enzyme had the same production kinetics as β-xylosidase where the highest level was observed for isolate 94269 on S + W medium. No activity was present during the initial growth phase, but on the cell wall media, production of the enzyme was eventually observed during later growth (results not shown). The late expression of these enzymes during the time-course of the cultivation remains to be investigated: it could be due either to the presence of a repressor, or more probably to a lack of induction, as other parts of the carbon source might be preferentially used by *M. graminicola*.

The carbon source had an effect upon the rate of cellulase production. When wheat cell walls were used as the carbon source, cellulase production was repressed by their polysaccharides, but appeared to be induced by galactose in both isolates; this is despite the fact that primary wheat cell walls contain 14% cellulose (Burke et al., 1974; Agrios, 1997). Surprisingly, the fungus did not grow well on S medium supplemented with carboxymethylcellulose (1%) (results not shown).

Beta-1,3-glucanase was the second highest enzyme produced *in vitro* by *M. graminicola*. This could be explained by the high concentration of glucan in graminaceous primary cell walls (McNeil et al., 1984). This activity was detected in the rye leaf lesions infected by *Claviceps purpurea* and was found correlated to the pathogenicity of this fungus (Dickerson et al., 1978).

Polygalacturonase maximum activity produced by *M. graminicola* occurred preferentially during the early stages of the fungal culture mainly on S + G medium. The action of polygalacturonase on cell walls appears to be a prerequisite for wall degradation by other CWDE and increases access of other polysaccharidases to substrates that are interspersed in the pectin matrix (Karr and Albersheim, 1970; Cooper, 1983; De Lorenzo et al., 1997). Kang and Buchenauer (2000) showed that *Fusarium culmorum* infects the wheat ovary through the pectin-rich junctions between the epidermal cell walls. In addition, *P. nodorum* was found to produce polygalacturonase activity both

in vitro and *in vivo* (Magro, 1984). However, polygalacturonase activity was undetectable in cultures of *Pseudocercosporella herpotrichoides* on media with wheat cell wall as the sole carbon source (Cooper et al., 1988).

In conclusion, these results showed that several activities are produced at a significant level on a synthetic medium containing galactose and/or wheat cell walls as the carbon source: xylanase, β-1,3-glucanase, polygalacturonase, cellulase and β-xylosidase. Although xylanasic activities were more important on S + W medium than on S + W + G medium, the medium containing both W and G carbon sources allowed the expression of the widest range of enzyme activities at significant levels. Moreover, we tested two different fungal isolates of *M. graminicola* for their ability to produce these activities. For both isolates, the maximum level of activities varied on the three media as well as the day when the activity reached its maximum level. Finally, the PCA analysis revealed that the different CWDE are clustered into two distinct groups when considering the different cultivation parameters as a whole: fungal isolate, carbon source and time-course cultivation. This strongly suggests that the activities from a given group may act in synergy during cell wall degradation. Moreover, the same statistical analysis revealed that the S + W + G medium allowed the release of the majority of the tested activities at a level close to the average obtained in the three media. The synthesis and secretion of enzymes *in vitro* does not necessarily imply production during pathogenesis (Byrde, 1979; Reignault et al., 2000). However, several genetical studies have provided new lines of evidence implicating cell-wall polysaccharides as factors in host-pathogen interactions (Vorwerk et al., 2004). Our knowledge of the role of *M. graminicola* CWDE in pathogenicity has to be completed. We intend to understand and explain the role of polysaccharide-degrading enzymes during pathogenesis more precisely by discerning the relationship of these polysaccharidases activities with the degree of severity of disease. In order to study the diversity among *M. graminicola* population *via* the production of CWDE, the range of enzyme activities needs to be tested with a larger number of isolates differing in pathogenicity. In addition, the use in this study of isolates of opposing mating type enables future crosses between these two isolates to study progeny virulence related to CWDE production.

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