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Influence of carbon source on the expression of *Cochliobolus carbonum* xylan-degrading enzyme genes

Nyerhovwo J. Tonukari ' *, John S. Scott-Craig and Jonathan D. Walton

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824.

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The expression of four *Cochliobolus carbonum* endo-1,4- -xylanase genes (*XYL1*, *XYL2*, *XYL3*, *XYL4*), and an exo-1,4- -xylosidase gene (*XYP1*) was studied following the growth of the fungus in minimal medium containing glucose, sucrose, xylose, xylan, pectin, or cellulose. The *XYL1* and *XYL2* genes were expressed only when the culture medium contained xylan or cellulose. Both *XYL3* and *XYL4* are induced by xylose and xylan, and *XYP1* expression is induced by xylose, xylan, pectin and cellulose. None of these genes is expressed in glucose or sucrose media. The differential expression of these enzymes may provide means for the fungus to adapt to different conditions.

Key words: Cell wall degrading enzymes, *Cochliobolus carbonum*, xylan, *x*ylanase.

INTRODUCTION

The role of fungal-secreted extracellular enzymes in diseases has been postulated to include penetration of the plant cell walls, release of nutrients that can be assimilated for growth and the elicitation of defense responses (Walton, 1994). *Cochliobolus carbonum* , an ascomycetous pathogen of maize, penetrates into and ramifies through intact leaves and in the process obtains nutrients for growth from the plant cell cytoplasm and walls. *C*. *carbonum* synthesizes numerous extracellular enzymes, such as pectinases, xylanases, glucanases and proteases, which can degrade the polysaccharides of the maize cell wall. The disease caused by this fungus, Northern leaf spot of corn, is characterized by extensive necrotization of susceptible maize tissues.

The full expression of these cell wall degrading enzymes (CWDEs) by fungi depends on mechanisms that are associated with the glucose regulation pathway. In many organisms, glucose represses genes whose products are used to metabolize other carbon sources. Work in yeast and filamentous fungi have revealed a mechanism for glucose repression in eukaryotes that is different from that found in bacteria (Ronne, 1995). We reported previously that the *C*. *carbonum SNF1* gene is required for the expression of many CWDEs (Tonukari et al., 2000). Mutation of *SNF1* in the fungus leads to varying levels of repression of CWDE genes, reduced growth on complex polymers such as xylan and pectin, and also reduced virulence on its host, maize. Here we studied the expression of xylan-degrading genes following the growth of the fungus in minimal medium containing different carbon sources.

Present address: International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya.
*Corresponding author; tel: 254-2-630743, fax: 254-2-631499, e-mail: J.TONUKARI@CGIAR.ORG
Abbreviation: CWDE(s); Cell Wall Degrading Enzyme(s).

RESULTS AND DISCUSSION

Northern hybridization was employed to determine the effect of glucose on the expression of *C. carbonum* CWDE genes. Four endo-1,4- -xylanase genes (*XYL1*, *XYL2*, *XYL3*, *XYL4*) (Apel et al., 1993; Apel-Birkhold and Walton, 1996; J.S. Scott-Craig, Michigan State University**,** unpublished results), and an exo-1,4- -xylosidase gene (*XYP1*) (Ransom and Walton, 1997; Wegener et al., 1999) has been cloned from *C. carbonum*. The expression of these genes was initially studied following growth of the fungus in minimal medium containing glucose or xylan. Expression of all five genes was detected only when the culture medium contained xylan. In glucose medium, the xylan-degrading enzyme genes were repressed (Figure 1A).

The expression of the four endo-1,4- -xylanase and exo-1,4- -xylosidase genes was also studied during growth of the fungus in minimal media containing sucrose, xylose, xylan, pectin, or cellulose as the sole carbon source. Northern hybridization studies show that the *XYL1* and *XYL2* genes were expressed only when the culture medium contained xylan or cellulose. While *XYL3* and *XYL4* expression was detected only when xylose or xylan was in the medium. *XYP1* expression was induced by xylose, xylan, pectin and cellulose. Like glucose, the presence of sucrose in the medium suppressed the expression of all five genes (Figure 1B).

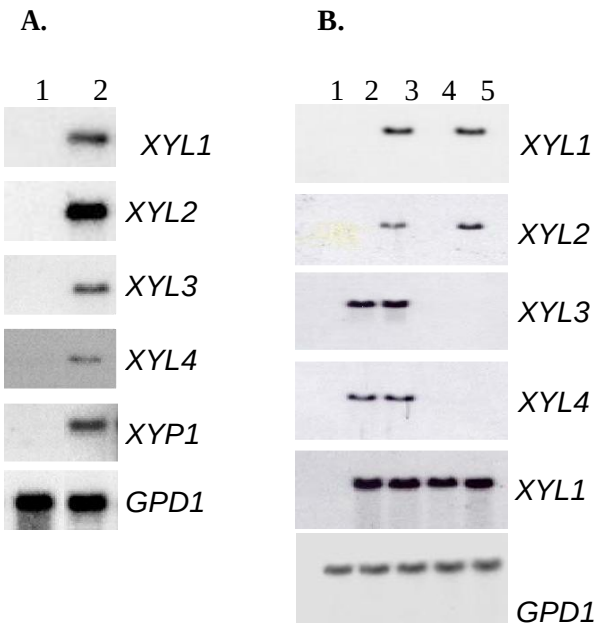

Figure 1. Expression *of C. carbonum* xylan-degrading enzymes. The wild-type race 1 strain of *C. carbonum* (367-2A) was grown in liquid media containing mineral salts, 0.2 % yeast extract, and trace elements (Van Hoof et al. 1991) with 2 % carbon source. Four fungal agar plugs (each about 5 mm^2) were inoculated into a 1 L Erlenmeyer flask containing 125 ml of medium and grown in still culture for seven days at room temperature. Mycelial mats were washed briefly in distilled water, frozen, and lyophilized. Total RNA was extracted from lyophilized mats (Pitkin et al. 1996; Apel et al. 1993) and northern blot analysis conducted as described by Apel-Birkhold and Walton 1996. **A.** Effect of glucose on *C. carbonum* xylan-degrading enzymes: glucose (lane 1) or xylan (lane 2) were used as sole carbon source. **B.** Effect of other carbon sources on *C. carbonum* xylan- degrading enzymes: sucrose (lane 1), xylose (lane 2), xylan (lane 3), pectin (lane 4), or cellulose (lane 5) were used for fungal growth. The *Cochliobolus heterostophus GPD1* gene, encoding glyceraldehyde- 3-phosphate dehydrogenase (Van Wert and Yoder 1992), was used as a reference.

The repression of xylanases and other CWDEs by preferred carbon sources such as glucose (Asymeric et al., 1988) is an efficient energy-conserving mechanism because the activity of enzymes that degrade xylan and other polysaccharides may not be required when glucose is abundant in the growth medium. However, genes that are important for promoting host colonization by pathogens must be expressed at some stage during infection (Hensel and Holden, 1996). Many of these genes are only activated in the appropriate environment, often in response to signals from the host, and therefore may only expressed when required. This study revealed that the expression of the various *C. carbonum* xylan-degrading enzyme genes is substrate-induced, though not necessarily in the same manner *in vitro*.

The biosynthesis of some xylanases may also be induced in the presence of other polysaccharides such as pectin and cellulose, in addition to xylan, as was observed in our experiments. In addition, the cell wall degradation product from one xylanase or other CWDEs may induce expression of other xylanase as well as other CWDE genes. We found that xylose, a product of xylan degradation, can also induce the expression of three of the five xylan-degrading enzyme genes (*XYL 3*, *XYL4* and *XYP1*) in this investigation. Polygalacturonase, pectate lyase and pectin lyase activities are also induced in media supplemented with galactose or galacturonic acid (Crotti et al., 1998; Scott-Craig et al., 1990, 1998). Kolattukudy et al. (1995) also reported that cutinase carried by spores of virulent pathogens, upon contact with plant surface, release small amounts of cutin monomers that trigger cutinase gene expression. Furthermore, *Trichoderma reesei XYN2* (xylanase) gene is induced in the presence of xylose (in addition to xylan), and like the xylanases in this study, it is virtually silenced in the presence of glucose (Zeilinger et al., 1996; Mach et al. 1996). The differential expression of these functionally redundant xylanases may serve to ensure that enzymes capable of degrading xylan are present under the various conditions encountered by the fungus during growth in its host plant. This may provide means to adapt to different conditions, and is indicative of processes with vital importance to an organism.

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