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REFERÊNCIA

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PRODUCTION OF XYLAN-DEGRADING ENZYMES BY A *TRICHODERMA HARZIANUM* STRAIN

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SHORT COMMUNICATION

ABSTRACT

Trichoderma harzianum strain 4 produced extracellular xylan-degrading enzymes, namely β -xylanase, β -xylosidase and α -arabinofuranosidase, when grown in liquid medium cultures containing oat spelt xylan as inducer. Cellulase activity was not detected. The pattern of xylan-degrading enzymes induction was influenced by the form of xylan present in the medium. They were detected in different incubation periods. Electrophoretic separation of the proteins from liquid culture filtrates by SDS-PAGE showed a variety of bands with high and low molecular weights.

Key words: xylan, *T. harzianum*, xylanolytic activity

Xylans, the most abundant hemicelluloses, are comprised of D-xylopyranose units connected by β -1,4 linkages which may be linear or branched. Depending on the origin of the wood species, their degree of polymerization varies from 70 to 130 (softwood xylans) and 150 to 200 (hardwood xylans) (15). O-Acetyl-4-O-methylglucuronoxylan and arabino-4-O-methylglucuronoxylan are the main hemicelluloses in hardwood and softwood, respectively. The latter one comprises about one-third of the total hemicelluloses (15). Xylans vary in solubility and may be isolated into water-soluble or -insoluble fractions (8). β -D-Xylanase and β -D-xylosidase are involved in the breaking down of xylan backbone to xylooligosaccharides (1,6). Additional enzymes, such as acetyl xylan esterase and α -L-arabinofuranosidase, are required to remove side-chain substituents that are attached at various points on xylan, creating more sites for subsequent enzymic hydrolysis (2,6).

Selected strains from the soft-rot fungus *Trichoderma* have been shown to be efficient producers of xylan-degrading enzyme activity (13,17). In this work, we report the production of xylan-degrading enzymes obtained from liquid state cultures of *Trichoderma harzianum* strain 4 when grown on medium containing xylans from oat spelt as substrates.

T. harzianum strain 4 was kindly provided by I. S. Melo (National Research Center for Monitoring and Environmental Impact Assessment - Brazil). For xylan-degrading enzyme production, spore suspension from 7 days routine subculture was used to inoculate a liquid medium containing 0.5% xylan supplemented with KH_2PO_4 , 0.7%; K_2HPO_4 , 0.2%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05%; $(\text{NH}_4)_2\text{SO}_4$, 0.1% and yeast extract, 0.06% at pH 7.0. The cultivation was carried out for 5 days in 1000 ml Erlenmeyer flasks at 28°C and 100 rpm. Aliquots were harvested every 6 h during 5 days, and used to estimate xylan-degrading enzyme activities. Soluble and insoluble xylan fractions from oat spelt were prepared as described elsewhere (5). All results represent the mean of three separate experiments. Cellulase, β -xylanase, β -xylosidase and α -arabinofuranosidase activities were determined as reported elsewhere (13). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a 10% acrylamide gel (10). After electrophoresis, protein bands were revealed by silver staining (3). Molecular weight standards from Sigma (USA) were used as size markers.

A comparison was made of the activity of xylan-degrading enzymes from *T. harzianum* strain 4 during growth on different xylan preparations (results not shown). The carbon sources

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included oat spelt xylan and its soluble and insoluble fractions. The fungus did not produce cellulase activity, suggesting that the production of cellulolytic and xylanolytic enzymes is under separate regulatory control (2). The inductive effect of xylan substrates it seems to depend on their chemical composition and structure (12). The induction profile after growth on insoluble fraction of oat spelt xylan showed that the β -xylanase activity increased without a lag and was detected from the first hour to the end of cultivation period. The basal synthesis of β -xylanase suggests the production of a soluble fragment from xylan which penetrated the mycellium and effected induction of xylan-degrading enzymes (9). On the other hand, the time lag for induction of β -xylanase by both oat spelt xylylans and its soluble fraction was about 10 h and thereafter the activity increased steadily and reached the highest value at 106 h of cultivation. Since the degree of substitution in xylan is directly proportional to solubility (8), this result suggest that the arabinose side-chains should be a obstacle for induction of β -xylanase activity. In this case, the synergistic action of α -arabinofuranosidase was required for the removal of arabinosyl groups from xylan (16). A low level of α -arabinofuranosidase activity was found since the early period of growth, reaching a maximum induction at 76 h of cultivation.

The growth profile on all xylan preparations was accompanied by more than two peaks of xylanase activity (results not shown). The multiplicity of forms is commonly described for β -xylanases from fungi and bacteria as result of differential mRNA processing and posttranslational modifications (4,14). Since all xylosidic linkages are not equivalent and equally accessible in xylan molecule, its catalytic cleavage requires the action of multiple forms of xylan-degrading enzyme systems (4). β -Xylosidase activity was only expressed at 52 h of cultivation in all xylan-containing media. Xylan was probably converted into small xylooligosaccharides by β -xylanases which were then further hydrolysed by β -xylosidase into xylose units (7).

SDS-PAGE analysis was performed on the crude extracts from inducing media. Fig. 1 shows the results of SDS-PAGE of the crude extract from oat spelt xylan medium. The number of protein bands suggests the effects of the inducers on the synthesis of multiple forms of xylan-degrading enzymes. The zymogram technique (11) will be necessary for identification and preliminary characterization of the multiple forms of xylanase activity. Proteins bands began to be detected at 34 h of cultivation period. A strong protein band with molecular weight value of about 24 kDa was observed at the incubation range of 46-118 h. After 34 h of cultivation was visualized a protein band with high molecular weight size. In the above culture, a xylanase activity peak was found at cultivation period of 10-34 h. The same result was found for the inducing medium containing soluble xylan fraction. In this case, protein bands were detected after 22 h of cultivation. In contrast to the above results, protein bands from crude extract of growth culture containing insoluble xylan fraction as carbon source were detected in the early stage of growth. It is noteworthy that

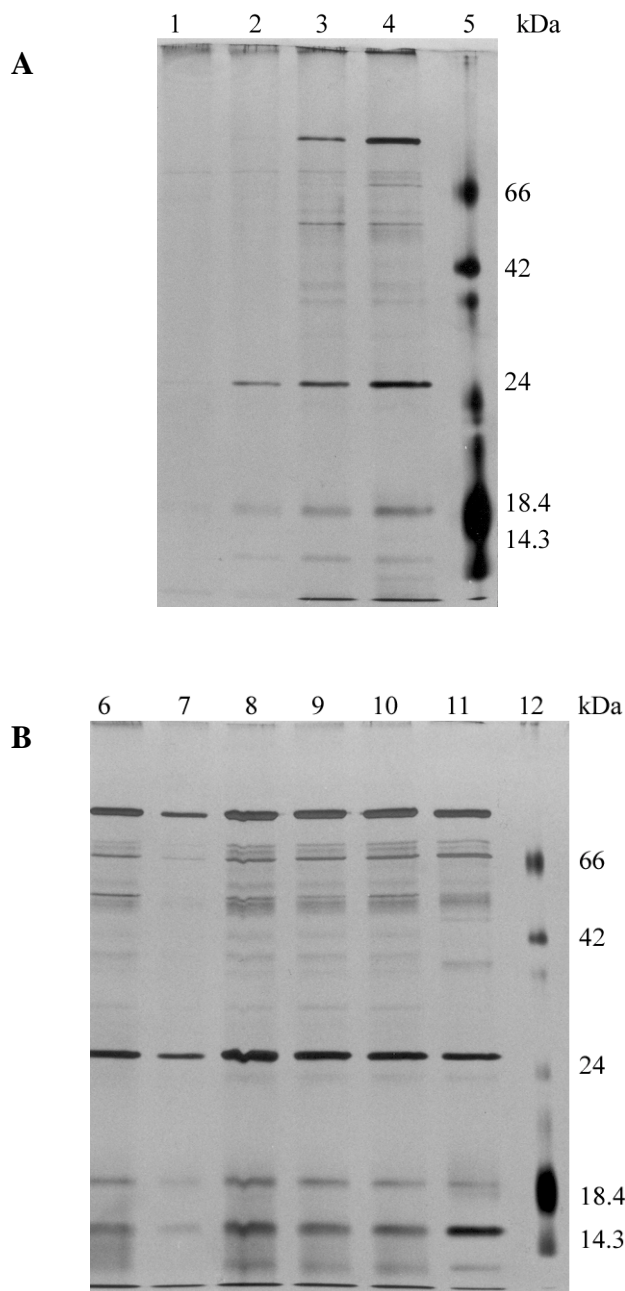


Figure 1. SDS-PAGE of the crude extract from *T. harzianum* strain T4 grown on oat spelt xylan. a) Lanes 1-4, 34, 46, 52 and 70 h cultivation, respectively. Lane 5, molecular weight standards (from the top): bovine serum albumin (66 kDa); ovalbumin (42 kDa); trypsinogen (24 kDa); lactoglobulin (18.4 kDa); lysozyme (14.3 kDa). b) Lanes 6-11, 76, 82, 94, 100, 106 and 118 h cultivation, respectively. Lane 12, molecular weight standards (from the top): bovine serum albumin (66 kDa); ovalbumin (42 kDa); trypsinogen (24 kDa); lactoglobulin (18.4 kDa); lysozyme (14.3 kDa).

the above 24 kDa protein band was also strongly detected at 70 h of cultivation. A protein band with high molecular weight size was also revealed at the same period.

In conclusion, *T. harzianum* strain 4 secretes a very active xylanolytic system with no cellulase activity when grown in liquid media containing different xylan forms as the carbon source. This is advantageous considering exploitation of the above fungus in biopulping and preparation of modified hemicellulose (14). Further work will be required to determine the role of these various forms of xylan-degrading activities on the mechanisms of hydrolysis of the substrate.

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RESUMO

Produção de enzimas xilanolíticas por uma cepa de *Trichoderma harzianum*

O fungo *Trichoderma harzianum* linhagem 4 produziu, enzimas xilanolíticas quando crescido em meios de cultura líquido contendo xilana como indutor. Atividade celulolítica não foi detectada. O tipo de xilana presente no meio de cultura influenciou a indução das enzimas xilanolíticas. As enzimas foram detectadas em diferentes períodos de incubação. SDS-PAGE de amostras de meios de cultura apresentou uma variedade de bandas protéicas com alto e baixo pesos moleculares.

Palavras-chave: xilana, *T. harzianum*, atividade xilanolítica

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