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DISTRIBUTION OF MONO- AND DISACCHARIDE-RELEASING EXTRACELLULAR ENZYME PRODUCTION ABILITIES WITHIN A *TRICHODERMA* POPULATION FROM HUNGARIAN WINTER WHEAT RHIZOSPHERE

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Abstract

The action of fungal hydrolytic enzymes is playing a crucial role in the biocontrol process of *Trichoderma* strains. In the present study, information was collected about the distribution of mono- and disaccharide-releasing extracellular enzyme production abilities within a Hungarian *Trichoderma* population from winter wheat rhizosphere.

Key Words: *α-glucosidase, β-galactosidase, β-glucosidase,* β-1,4-*N*-acetyl-glucosaminidase, *β-xylosidase, cellobiohydrolase, N-acetyl-β-glucosaminidase,* Trichoderma

Introduction

The efficient control of fungal plant pathogens causing substantial losses in agricultural production is an important issue for all plant cultivation systems. Species belonging to the genus *Trichoderma* are imperfect filamentous fungi of multiple importances. Members of this genus are well known as cellulase producers of biotechnological relevance (Kubicek et al., 1990; Schmoll and Kubicek, 2003). Certain *Trichoderma* species are on the growing list of potential fungal pathogens in immunocompromised hosts (Kredics et al., 2010a), while others are harmful in mushroom cultivation as the causative agents of green mould epidemics (Hatvani et al., 2008; Kredics et al., 2010b). Furthermore, the genus involves promising biocontrol candidates with excellent antagonistic abilities against a number of plant pathogenic fungi. Several modes of action have been proposed to play roles in biocontrol capabilities, including antibiosis by the production of antifungal metabolites (Szekeres, 2005), competition for space and nutrients (Sivan, 1989), plant growth promotion, induction of the defence responses in plants (Harman, 2004) and mycoparasitism (Chet, 1987). These processes are supposed to act synergistically (Schirmböck, 1994; Manczinger, 2002). For the study of this complex synergistic system it is crucial to clear the relative importance of the individual mechanisms in the antagonistic process. Both the competition by colonizing the ecological niche favoured by the pathogen and mycoparasitism by penetration of the host hyphae requires hydrolytic enzyme systems that are playing important roles in the digestion of the available natural substrates in the soil and the polymers constituting the cell-wall and cytoplasm of the target fungi. Based on the involvement in the biocontrol process, the extracellular enzymes secreted by *Trichoderma* species can be

separated into the two major classes of mycoparasitic and competitive enzymes. Certain enzyme systems take part in both mechanisms. The secretion of extracellular enzymes may occur constitutively or inductively. From the aspect of effective biological control it is favourable if the biocontrol strains produce large amounts of enzymes constitutively or as an early response to induction.

Mycoparasitism is based mainly on the action of β-1,3-glucanolytic, chitinolytic and proteolytic cell-wall degrading enzymes. The β-1,4-*N*-acetyl-glucosaminidase enzyme cleaves chitooligomers progressively from the non-reducing end of chitin in an exo-type fashion, releasing β-1,4-*N*-acetyl-glucosamine monomers. Besides endochitinase, this enzyme is another basic component of the chitinolytic enzyme system of *Trichoderma* involved in the mycoparasitic process, therefore it is very important to examine the β-1,4-*N*-acetyl-glucosaminidase production of *Trichoderma* strains planned for biocontrol application (Kubicek, 2001).

Competition for carbon, nitrogen and iron sources aided by extracellular enzyme systems plays an important role in biocontrol (Whipps, 2001). Members of the genus *Trichoderma* are able to produce a series of mono- and disaccharide-releasing enzymes including β-xylosidase, β-glucosidase, α-glucosidase, β-galactosidase and cellobiohydrolase for the utilization of xylanic, cellulosic and lignocellulosic materials and sugars of plant litter as carbon sources.

The aim of this work was to examine the production of β-1,4-*N*-acetyl-glucosaminidase, α-glucosidase, β-glucosidase, β-galactosidase, β-xylosidase and cellobiohydrolase activities in the case of *Trichoderma* isolates derived from Hungarian winter wheat rhizosphere samples and to study the distribution of their production within the examined population.

Materials and methods

Strains

The 94 *Trichoderma* strains examined in this study are representing the taxa *T. atroviride, T. brevicompactum, T. gamsii, T. harzianum, T. koningiopsis/T. ovalisporum, T. longibrachiatum/H. orientalis, T. pleuroticola, T. rossicum, T. spirale, T. tomentosum/T. cerinum* and *T. virens*. The strains were isolated from chopped roots of wheat and identified by the sequence analysis of the ITS region in a previous study (Kredics et al., 2011).

Culture conditions and measurement of mono- and disaccharide releasing extracellular enzyme activities

Extracellular enzyme production of the isolated *Trichoderma* strains was examined in liquid yeast extract glucose (YEG) and minimal (2 g l^{-1} glucose, 1 g l^{-1} KH_2PO_4, 1 g l^{-1} $MgSO_4 \times 7H_2O$, 1 g l^{-1} $NaNO_3$ in distilled water) media. In 50 ml Erlenmeyer flasks, these media were inoculated with conidial suspensions of the *Trichoderma* strains to a final concentration of 10^5 conidia ml^{-1} and incubated on a shaker at 200 rpm and 25 °C. Samples were collected from the crude culture supernatants after 6 days of incubation. The activities of extracellular enzymes were measured with the

chromogenic substrates (Sigma-Aldrich Hungary) listed in Table 1. The substrates were dissolved in small aliquots of dimethyl-sulfoxide and then diluted to 2 mg ml^{-1} in Serensen phosphate buffer (pH 6). One hundred μl substrate solution was added to 100 μl aliquots of the samples in the wells of microtiter plates, resulting in a substrate end concentration of 1 mg ml^{-1}. Control mixtures were prepared by adding 100 μl phosphate buffer to the samples. The optical densities of the reaction mixtures were determined at 405 nm with an Uniskan II microtiter plate spectrophotometer (Labsystems, Helsinki, Finland) after 2 h of incubation at 35°C. Activities were expressed in unit (U), 1 U is defined as the activity that releases 1 nmol *p*-nitrophenol ml^{-1} in 1 min at 35 °C. All experiments were carried out in three replicates and the SD values were determined.

Table 1. List of the measured extracellular enzymes and the corresponding substrates

Enzyme	E.C. Number	Abbr.	Substrate
β-1,4-*N*-acetyl-glucosaminidase	EC 3.2.1.52	NAG	*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide
α-glucosidase	EC 3.2.1.20	αGLU	*p*-nitrophenyl-α-D-glucoside
β-glucosidase	EC 3.2.1.21	βGLU	*p*-nitrophenyl-β-D-glucopyranoside
β-galactosidase	EC 3.2.1.23	βGAL	*p*-nitrophenyl-β-D-galactopyranoside
β-xylosidase	EC 3.2.1.37	βXYL	*p*-nitrophenyl-β-D-xylopyranoside
cellobiohydrolase	EC 3.2.1.150	CBH	*p*-nitrophenyl-β-D-cellobioside

Results and discussion

Quantities of extracellular enzymes on minimal medium

The amounts of certain examined enzymes showed high variability within the isolates in minimal medium. Constitutive secretion at a moderate level proved to be common among the isolates for enzymes known to play important roles in competition and the utilization of nutrients, like αGLU, βGLU, βGAL, βXYL and CBH. Relative high enzyme activities were observed among the members of the isolated population for NAG, an activity supposed to play determining role in the high biocontrol abilities (Kubicek, 2001). The distribution of NAG, αGLU, βGLU, βGAL, βXYL and CBH enzyme activities was exponential-like in the examined population (Figure 1). When examining the presence of certain strains in the corresponding bins of the histograms for different extracellular enzyme activities, notable deviations were found.

Quantities of extracellular enzymes in yeast extract medium

The constitutive extracellular enzyme activities among the examined *Trichoderma* isolates were usually higher in YEG medium than in minimal medium with the exception of NAG, βGLU and βXYL. Unlike in minimal medium, the activity of NAG decreased significantly. The distributions of the enzyme activities were likewise exponential-like for NAG, αGLU, βGLU, βGAL, βXYL and CBH as in minimal medium, however, the frequency values of falling into certain groups were different in

all of the cases (Figure 2). On minimal medium the αGLU, βGLU, βGAL, βXYL and CBH activities were low in the whole population, which list completed with NAG on YEG medium. Some researchers also found that trace quantities of some chitinases (e.g. the 102-kDa NAG, the 42-kDa endochitinase and the 33 kDa endochitinase) are produced constitutively (Garcia et al., 1994; Haran et al., 1995; Inbar and Chet, 1995; Margolles-Clark et al., 1996; Carsolio et al., 1999).

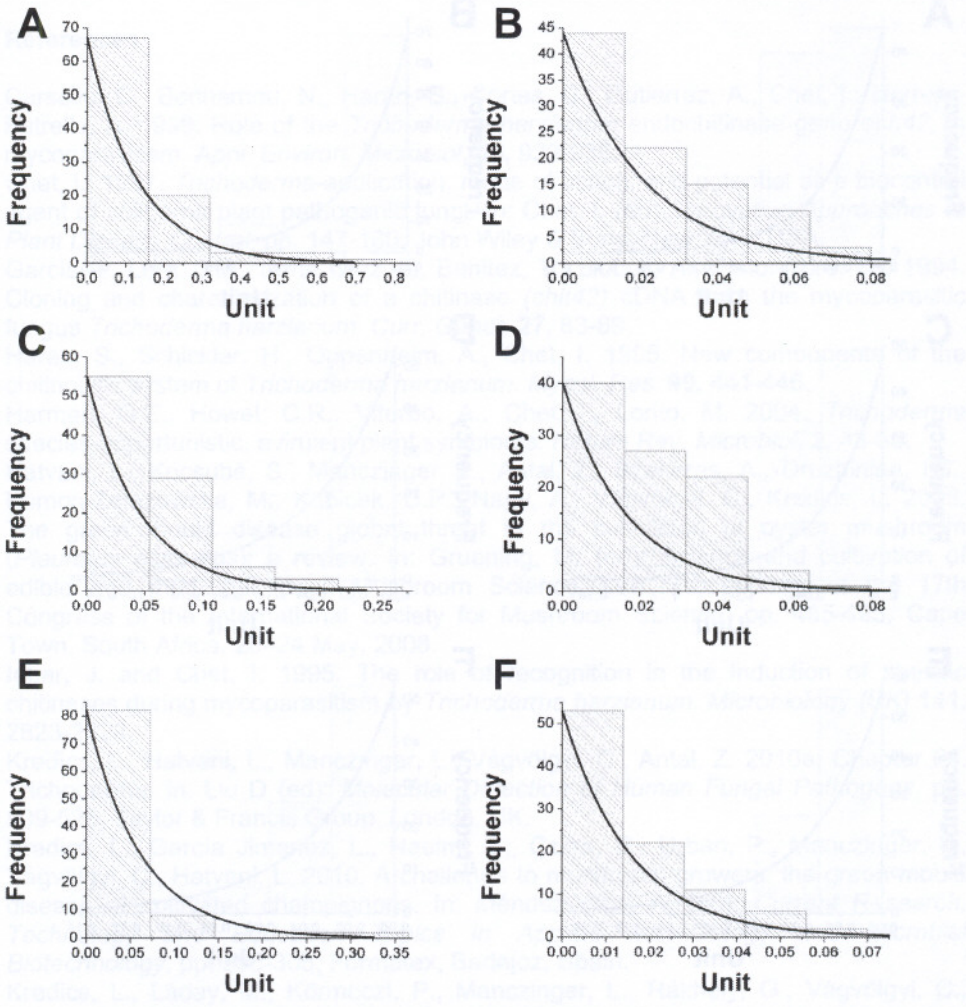

Figure 1. Distribution of extracellular activities of NAG (A), αGLU (B), βGLU (C), βGAL (D), βXYL (E) and CBH (F) within the examined *Trichoderma* population on minimal medium

Conclusions

Ninety four *Trichoderma* strains isolated from roots of winter wheat were examined for the production of mono- and disaccharide-releasing enzyme activities on two different media. For all extracellular enzymes studied, the distributions of the abilities to produce the particular activities proved to be exponential-like in the examined population.

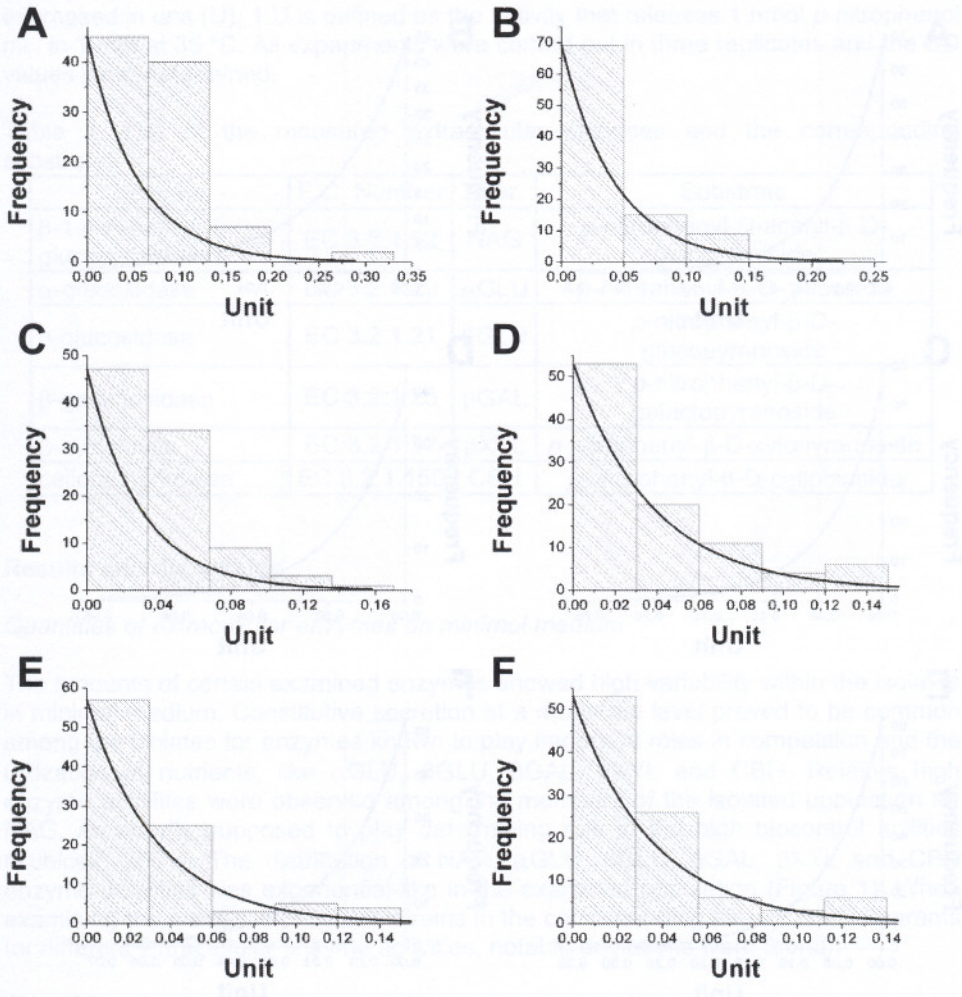

Figure 2. Distribution of extracellular activities of NAG (A), αGLU (B), βGLU (C), βGAL (D), βXYL (E) and CBH (F) within the examined *Trichoderma* population on YEG medium

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