The Journal of Microbiology (2012) Vol. 50, No. 5, pp. 849–854
DOI 10.1007/s12275-012-2001-0

NOTE

Antifungal Activity of Extracellular Hydrolases Produced by Autolysing *Aspergillus nidulans* Cultures

Melinda Szilágyi[1], Fruzsina Anton[1], Katalin Forgács[1], Jae-Hyuk Yu[2], István Pócsi[1], and Tamás Emri[1]*

[1]*Department of Microbial Biotechnology and Cell Biology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary*
[2]*Departments of Bacteriology and Genetics, University of Wisconsin, Madison, WI, USA*

(Received January 2, 2012 / Accepted May 14, 2012)

Carbon-starving *Aspergillus nidulans* cultures produce high activities of versatile hydrolytic enzymes and, among these, ChiB endochitinase and EngA β-1,3-endoglucanase showed significant antifungal activity against various fungal species. Double deletion of *engA* and *chiB* diminished the antifungal activity of the fermentation broths and increased conidiogenesis and long-term viability of *A. nidulans*, but decreased the growth rate on culture media containing weak carbon sources. Production of ChiB and EngA can influence fungal communities either directly due to their antifungal properties or indirectly through their effects on vegetative growth. Our data suggest saprophytic fungi as promising future candidates to develop novel biocontrol technologies.

Keywords: antifungal activity, *A. nidulans*, chitinase, β-1,3-glucanase, proteinase

Plant diseases caused by fungi are among the major concerns in agricultural crop production. Fungi can destroy both growing plants in the fields and stored plant-based foods and feeds. Chemical fungicides used to overcome this problem cause adverse environmental effects and are hazardous to non-target organisms including humans. A promising alternative strategy is the biological control of fungi (Fravel *et al.*, 2003; Harman *et al.*, 2004; Haas and Défago, 2005; Gohel *et al.*, 2006; Sharma *et al.*, 2011). Apathogen *Trichoderma* strains are well-known biocontrol agents against fungal pathogens in pre- and post-harvest periods (Benitez *et al.*, 2004; Harman *et al.*, 2004; Schuster and Schmoll, 2010). Extracellular chitinase, β-1,3-glucanase and proteinase productions are key parameters which determine the biocontrol activity of strains (Benitez *et al.*, 2004). *Trichoderma* chitinases and β-1,3-glucanases are also used to develop fungus-resistant transgenic plants (Markovich and Kononova, 2003; Saiprasad *et al.*, 2009).

Strains of *Aspergillus nidulans*, a soil-borne saprophytic fungus, produce high activities of extracellular hydrolases in autolysing, carbon-starving cultures (Emri *et al.*, 2008). Autolysis can be defined as an active self-digestion process which involves the degradation of intracellular compounds most likely *via* macroautophagy (Kim *et al.*, 2011) and the degradation of hyphae by extracellular enzymes (autolytic cell wall degradation; Emri *et al.*, 2008). Among the autolytic hydrolases produced, ChiB endochitinase accounted for more than 90% of the total extracellular chitinase activity (Pócsi *et al.*, 2009); meanwhile EngA β-1,3-endoglucanase and PrtA and PepJ proteinases were produced concomitantly with other extracellular glucanases and proteinases (Szilágyi *et al.*, 2010, 2011). Both ChiB and EngA (but not PrtA and PepJ) were necessary for the efficient degradation of cell walls during autolysis, and the transcription of all four genes (*chiB*, *engA*, *prtA*, *pepJ*) was induced by carbon starvation *via* FluG-BrlA signaling (Pócsi *et al.*, 2009; Szilágyi *et al.*, 2010, 2011), which also initiates conidiogenesis in surface cultures (Yu, 2010). Here we demonstrate that ChiB and EngA though not extracellular proteinases have considerable antifungal activity against the producer organism itself and several other species as well. We also show that, whereas the production of these hydrolases can influence negatively long-term survival and conidiogenesis, it can be advantageous for the fungus to maintain growth under nutrient-poor conditions or in a competitive environment.

Strains used in this study are listed in Table 1. *A. nidulans* strains were maintained on a minimal nitrate medium (Barratt *et al.*, 1965). All other strains were cultivated on a complex medium containing 20 g/L glucose, 10 g/L malt extract, 5 g/L yeast extract, and 20 g/L agar. Cultures were incubated at either 37°C (*A. nidulans* and *A. fumigatus* strains) or at 24°C (all other strains) for 7 d and only freshly-made conidia were used in the experiments.

A. nidulans fermentation broths possessing autolytic enzyme activities were prepared as described earlier (Szilágyi *et al.* 2010). Fermentation broth specimens were lyophilized, dissolved in 0.1 mol/L K-phosphate buffer (pH 6.8) and dialyzed overnight at 4°C against the same buffer using dialysis tubes (Sigma-Aldrich, USA) retaining only proteins with relative molecular mass M_r>12 kDa. The dialyzed samples were used for the purification of EngA ß-1,3-endogluca-

*For correspondence. E-mail: emri.tamas@science.unideb.hu; Fax: +36-52-512925

Table 1. Strains used in the study

Strain	Genotype	Origin
A. nidulans FGSC26	*biA1 veA1*	FGSC[a]
A. nidulans FGSC744	*pabaA1 yA2 fluG1 veA1*	FGSC
A. nidulans tNJ12	*biA1 argB2 ΔchiB::argB*$^{+}$ *metG1 veA1*	Pócsi *et al.* (2009)
A. nidulans tNJ33.3	*pyrG89 pyroA4 ΔengA::AfupyrG*$^{+}$ *veA*$^{+}$	Szilágyi *et al.* (2010)
A. nidulans tNJ34.8	*pyrG89 ΔchiB::AnpyroA*$^{+}$ *pyroA4 ΔengA::AfupyrG*$^{+}$ *veA*$^{+}$	Szilágyi *et al.* (2010)
A. nidulans tNJ36.1	*pyrG89*; *pyroA4*; *veA*$^{+}$; *AfupyrG*$^{+}$	Szilágyi *et al.* (2010)
A. nidulans tNJ76.7	*pyrG89*; *ΔpepJ::AfupyrG*$^{+}$ *pyroA4 veA*$^{+}$	Szilágyi *et al.* (2011)
A. rugulosus CBS171.71	wild type isolate	CBS[b]
A. niger VG1	wild type isolate	provided by G. Vasas (University of Debrecen, Debrecen, Hungary)
A. fumigatus FGSC1100 (AF293)	wild type	FGSC
Penicillium nalgiovense NCAIM F-001333	wild type	NCAIM[c]
P. chrysogenum NCAIM F-00237	low penicillin producer industrial strain	NCAIM
Fusarium oxysporum VG12	wild type	provided by G. Vasas (University of Debrecen, Debrecen, Hungary)
Trichoderma atroviride T122	wild type	provided by L. Galgóczi (University of Szeged, Szeged, Hungary)

[a] FGSC, Fungal Genetic Stock Centre, University of Missouri, Kansas City, Missouri, USA
[b] CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (Fungal Biodiversity Centre)
[c] NCAIM, National Collection of Agricultural and Industrial Microorganisms, Corvinus University, Budapest, Hungary

nase (Szilágyi *et al.*, 2010), ChiB endochitinase (Erdei *et al.*, 2008), and PepJ metallo-proteinase (Emri *et al.*, 2009). The antifungal activities of dialyzed fermentation broths and purified enzymes were tested in 96-well microtiter plates at 24°C. Each well contained 50 µl twofold concentrated complex medium, 50 µl sample (1×, 2×, 5× or 10× dilution of dialyzed and concentrated fermentation broths or purified enzymes) and were inoculated with 1×10^3 conidia. In control experiments, heat inactivated samples (20 min, 100°C) were used. Antifungal activity was characterized by the inhibition of growth after 2 d. Colony sizes were measured using the CP Atlas software (www.lazarsoftware.com).

Changes in the viability of the *ΔengA*, *ΔchiB* double mutant and the control strain under carbon starvation were tested in liquid submerged cultures as described earlier (Molnár *et al.*, 2006).

Growth and conidia formation of the *ΔengA*, *ΔchiB* double mutant and the control strain were studied on a nitrate agar medium (Barratt *et al.*, 1965) containing either 10 g/L glucose, 5 g/L yeast extract, 4 g/L colloidal chitin (Sigma-Aldrich), 4 g/L laminarin (Sigma-Aldrich), 10 g/L Na-acetate, or 5 g/L yeast extract and 10 g/L Na-acetate. Conidia (1×10^4 suspended in 10 µl sterile water) were spotted onto agar plates and were incubated at 37°C. The diameters of the colonies were measured daily, and conidia produced by selected colonies were washed with 1 v/v % Tween 80 solution and were counted in a Bürker chamber.

To test interspecific interactions, nitrate agars supplemented

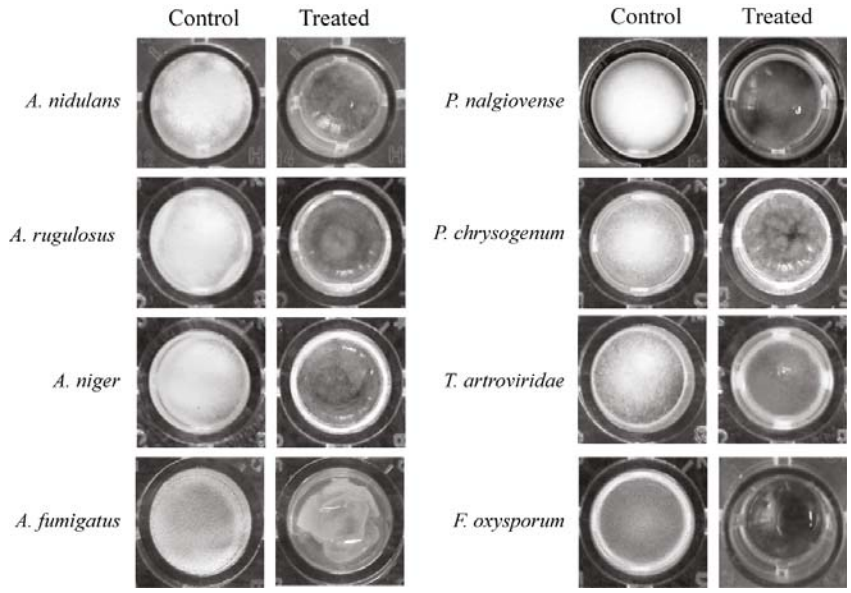

Fig. 1. Antifungal activity of the fermentation broths of autolysing *A. nidulans* FGSC26 cultures. Fermentation broths were lyophilized, dissolved in 0.1 mol/L K-phosphate buffer (pH 6.8) and were dialyzed against the same buffer. After dialysis, the final volume was set to one twentieth of the original volume. Hydrolase activities are presented in Table 2; the samples were heat inactivated in control experiments. All strains were tested in 5 independent experiments, and representative photos are presented here.

Table 2. **ß-1,3-Glucanase, chitinase and proteinase activities of lyophilized fermentation broths and purified hydrolases after dialysis**

Samples	ß-1,3-Glucanase activity[a] (A_{410})	Chitinase activity[a] (A_{550})	Proteinase activity[a] (A_{440})
FGSC26	0.55 ± 0.05	0.09 ± 0.006	1.5 ± 0.2
FGSC26 (growing cultures)	0.1 ± 0.01	< 0.01 ± 0.001	< 0.01 ± 0.001
FGSC744 (*fluG1*)	0.12 ± 0.01	< 0.01 ± 0.001	< 0.01 ± 0.001
tNJ12 (*ΔchiB*)	0.49 ± 0.03	< 0.001 ± 0.002	1.3 ± 0.1
tNJ33.3 (*ΔengA*)	0.41 ± 0.03	0.07 ± 0.003	0.61 ± 0.05
tNJ34.8 (*ΔengAΔchiB*)	0.36 ± 0.02	< 0.002 ± 0.001	0.32 ± 0.02
tNJ76.7 (*ΔpepJ*)	0.5 ± 0.05	0.08 ± 0.008	0.18 ± 0.02
purified EngA	0.32 ± 0.01	-	-
purified ChiB	-	0.1 ± 0.006	-
purified PepJ	-	-	0.5 ± 0.03

[a] Enzyme activities were measured using undiluted samples, and mean±SD values calculated from 4 independent experiments are presented. Note that ChiB is responsible for the majority of the extracellular chitinase activity, while EngA and PepJ are only one of the produced β-1,3-glucanases and proteinases, respectively (Szilágyi *et al.*, 2010).

with 5 g/L yeast extract and with 10 g/L glucose or 10 g/L Na-acetate as carbon sources were used. Conidia of *A. nidulans* strains (1×10^4 suspended in 10 µl sterile water) were spotted onto agar plates and were incubated at 37°C for 3 d. Following that, the agar plates were inoculated with either *A. niger* VG1, *Penicillium chrysogenum* NCAIM F-00237 or *Trichoderma atroviride* T122 conidia (1×10^4 in 10 µ sterile water) and the cultures were incubated further at 24°C.

The fermentation broths of carbon-starving autolytic *A. nidulans* cultures possessed high chitinase, β-1,3-glucanase and proteinase activities (Szilágyi *et al.*, 2010; Table 2) and, not surprisingly, they also had significant antifungal activities against various filamentous fungi including the producer strain itself. The concentrated and dialyzed fermentation broths inhibited efficiently the germination of conidia produced by *A. nidulans* FGSC26, *A. rugulosus* CBS171.71, *A. niger* VG1, *A. fumigatus* AF293, *Penicillium nalgiovense* NCAIM F-001333, *P. chrysogenum* NCAIM F-00237, *Fusarium oxysporum* VG12, and *Trichoderma atroviride* T122 (Fig. 1). The fermentation broths of growing *A. nidulans* FGSC26 cultures were ineffective (data not shown) because these cultures contained only low or negligible extracellular hydrolase activities (Table 2). The best antifungal activities were observed with *P. nalgiovense* NCAIM F-001333 and, therefore, this strain along with the producer *A. nidulans* FGSC26 strain were selected for further experiments.

Both the purified ChiB extracellular endochitinase and EngA extracellular β-1,3-endoglucanase showed antifungal activities against both *A. nidulans* and *P. nalgiovense* (Table 3). The deletion of either the *engA* or the *chiB* genes decreased markedly the antifungal activity of the fermentation broths (Table 3). Furthermore, the fermentation broths of the *ΔengA*, *ΔchiB* double mutant (Szilágyi *et al.*, 2010) and the FGSC744 strain, which produces low extracellular hydrolase activities with no conidia due to the *fluG1* mutation (Adams *et al.*, 1998; Emri *et al.*, 2008), possessed almost no antifungal ac-

Table 3. **Inhibitory effects of lyophilized fermentation broths of different *A. nidulans* strains and purified EngA β-1,3-endoglucanase, ChiB endochitinase, and PepJ proteinase on *P. nalgiovense* NCAIM F-001333 and on *A. nidulans* FGSC26**

Samples	Growth (%)[a]				
	Control	Final dilution[b]			
		2×	4×	10×	20×
FGSC26	100 ± 8 (100 ± 9)	30 ± 5* (38 ± 7*)	52 ± 9* (49 ± 9*)	73 ± 11* (63 ± 10*)	85 ± 10* (85 ± 10*)
FGSC744 (*fluG1*)	100 ± 7 (100 ± 8)	90 ± 9 (92 ± 8)	94 ± 11 (94 ± 7)	95 ± 8 (99 ± 10)	98 ± 10 (101 ± 11)
tNJ12 (*ΔchiB*)	100 ± 9 (100 ± 10)	67 ± 12* (69 ± 8*)	78 ± 8* (78 ± 9*)	84 ± 9* (83 ± 9*)	94 ± 7 (95 ± 11)
tNJ33.3 (*ΔengA*)	100 ± 10 (100 ± 10)	64 ± 11* (53 ± 6*)	81 ± 9* (64 ± 9*)	89 ± 12 (75 ± 8*)	91 ± 11 (88 ± 11)
tNJ34.8 (*ΔengAΔchiB*)	100 ± 8 (100 ± 10)	96 ± 7 (87 ± 9)	92 ± 9 (92 ± 8)	104 ± 12 (99 ± 11)	99 ± 10 (97 ± 10)
tNJ76.7 (*ΔpepJ*)	100 ± 10 (100 ± 11)	42 ± 9* (41 ± 9*)	57 ± 8* (58 ± 7*)	78 ± 7* (74 ± 8*)	87 ± 6* (85 ± 8*)
purified ChiB	100 ± 11 (100 ± 9)	59 ± 12* (43 ± 6*)	66 ± 9* (51 ± 7*)	79 ± 8* (64 ± 9*)	86 ± 9 (87 ± 9)
purified EngA	100 ± 10 (100 ± 8)	48 ± 6* (49 ± 5*)	56 ± 8* (68 ± 7*)	72 ± 10* (89 ± 8)	81 ± 11* (94 ± 9)
purified PepJ	100 ± 9 (100 ± 11)	91 ± 8 (91 ± 9)	91 ± 8 (95 ± 10)	95 ± 10 (99 ± 11)	102 ± 11 (97 ± 10)

[a] The antifungal activity was characterized by the reduced growth of the treated cultures and was given in the percentage of the growth of control (treated with heat inactivated 2× diluted samples). Mean±SD values calculated from 4 independent experiments with *P. nalgiovense* and *A. nidulans* (in parenthesis) as test organisms are presented.
[b] Activities of the undiluted samples are presented in Table 2.
* - Significant ($p<0.05$) difference between the control and the treated wells calculated by Student's t-test.

Table 4. Comparison of the growth rates and conidia productions of *A. nidulans* tNJ36.1 (control) and tNJ34.8 (*ΔengA ΔchiB*) strains

Media	Growth rate[b] (mm/d)		Conidia formation[b,c] (million conidia/cm[b])	
	tNJ34.8 (*ΔengAΔchiB*)	tNJ36.1 (control)	tNJ34.8 (*ΔengAΔchiB*)	tNJ36.1 (control)
10 g/L glucose	4.8 ± 0.4	5.0 ± 0.5	54 ± 8*	42 ± 7
10 g/L Na-acetate	5.0 ± 0.4*	6.2 ± 0.5	3.8 ± 0.4*	2.6 ± 0.4
4 g/L laminarin	6.1 ± 0.5	6.6 ± 0.7	2.7 ± 0.3*	1.5 ± 0.3
4 g/L chitin[a]	-	-	-	-
5 g/L yeast extract	9 ± 1*	11 ± 1	17 ± 3*	9 ± 2
10 g/L Na-acetate 5 g/L yeast extract	3.6 ± 0.3*	4.7 ± 0.4	46 ± 8*	29 ± 6

[a] No significant growth was observed on media containing 4 g/L chitin as a sole carbon source.
[b] Mean±SD values calculated from 5 independent experiments are presented.
[c] Conidia were counted at 7 d incubation times.
* Significant ($p<0.05$) differences between the control and the double mutant strains were calculated by Student's t-test.

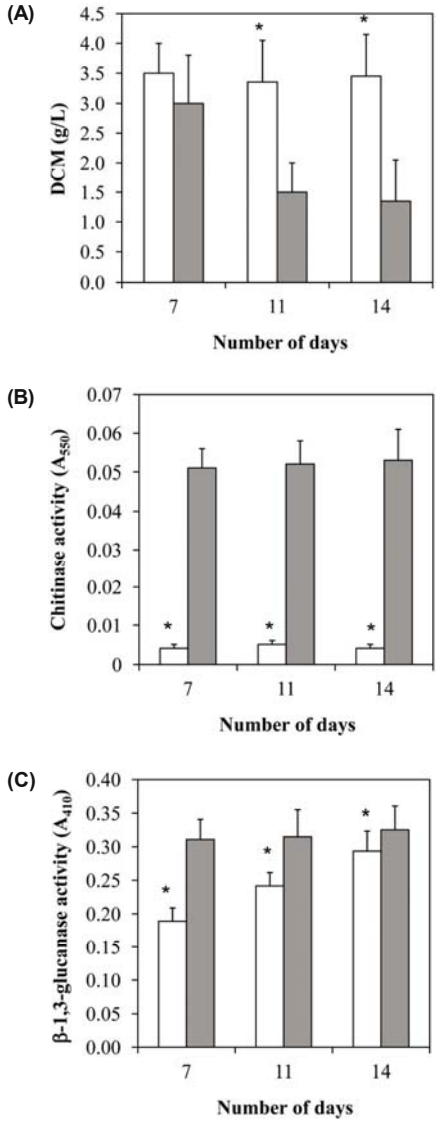

Fig. 2. Changes in the viability (A) as well as in the extracellular chitinase (B) and extracellular β-1,3-glucanase (C) activities of carbon-starving *A. nidulans* cultures. Viability was tested by transferring samples, taken at 7 d, 10 d and 14 d, into fresh media and measuring gains in dry cell mass (DCM). Mean±SD calculated from 4 independent experiments are presented. * - Significant ($p<0.05$) difference between the control and the double mutant strain calculated by Student's t-test.

tivities even at low dilutions (Table 3). The purified PepJ extracellular proteinase had no significant antifungal activity while the deletion of the *pepJ* gene had no effect on the antifungal properties of the fermentation broths either (Table 3). All these data demonstrate that EngA β-1,3-endoglucanase and ChiB endochitinase were responsible for the great majority of the observed antifungal effects. The antifungal potentials of bacterial, fungal and plant β-1,3-glucanases and chitinases have been demonstrated in a number of studies (Nielsen and Sorensen, 1997; El-Katatny *et al.*, 2001; Hong and Meng, 2003; Karasuda *et al.*, 2003; Liu *et al.*, 2009; Sharma *et al.*, 2011). Proteinases are likely to have a role in inactivating enzymes produced by other microorganisms (Elad, 2000) and, in contrast to *A. nidulans* (Table 3), the antifungal effect of an 18.8 kDa proteinase has been demonstrated in *T. harzianum* by De Marco and Felix (2002).

The Δ*engA*, *ΔchiB* double mutant is unable to produce EngA and ChiB hydrolases and, as a consequence, its carbon-starving cultures can be characterized with the lack of autolytic cell wall degradation (Szilágy *et al.*, 2010). In our experiments, the *ΔengA*, *ΔchiB* double mutant produced more conidia on surface cultures (Table 4) and the viability of the double mutant hardly decreased during submerged cultivation when it was compared to that of the control strain producing both EngA and ChiB hydrolases (Fig. 2). These data are in good accordance with the antifungal activity of these enzymes and do not support the hypothesis that these enzymes would supply conidiogenesis or the survival of non-autolysing fungal cells with nutrients released from autolysing cell walls (Emri *et al.*, 2008). More recently, Shin *et al.* (2009) demonstrated that deletion of *nagA* (encoding an extracellular β-*N*-acetylhexosaminidase) resulted in reduced cell death in *A. nidulans*.

A. nidulans was unable to grow on chitin as a sole carbon source. In contrast, *A. nidulans* could utilize laminarin (a β-1,3-glucan) to maintain its growth but the deletion of the *engA* gene had no negative effect on either growth or sporulation (Table 4). It is worth mentioning that while ChiB is the predominant extracellular chitinase of *A. nidulans* (Pócsi *et al.*, 2009), EngA is only one of the several extracellular β-1,3-glucanases produced by the fungus (Szilágyi *et al.*, 2010). Importantly, although ChiB can efficiently hydrolyze the chitin content of the cell wall (Pócsi *et al.*, 2009), it was not sufficient to maintain growth on exogenous chitin as a sole carbon source. On the other hand, EngA was essential

in the decomposition of the β-1,3-glucan biopolymers in the cell wall (Szilágyi *et al*., 2010), but it was dispensable when cells utilized exogenous β-1,3-glucan as a carbon source.

Both the double mutant and the control strains grew equally well on glucose containing medium (Table 4). In contrast, the double mutant grew significantly more slowly on media containing Na-acetate, yeast extract, or yeast extract and Na-acetate instead of glucose (Table 4). This means that EngA and ChiB can support vegetative growth with nutrients under carbon-starvation/limitation – a typical environmental condition in soil – by degradation of macromolecules in older or distant regions of hyphae to supply apical regions with monosaccharides (Bainbridge *et al*., 1971; McIntyre *et al*., 1999, 2000). This was the most obvious with Na-acetate as a sole carbon source where no exogenous chitin or β-1,3-glucan was available. Higher growth rates can also be beneficial in a competitive environment. As can be seen in Fig. 3, the control *A. nidulans* strain with EngA and ChiB productions inhibited the growth of *A. niger* more efficiently than the double mutant. Similar results were gained when *P. chrysogenum* was used instead of *A. niger* (data not shown). Considering the antifungal potentials of EngA and ChiB (Fig. 1 and Table 3), the direct growth inhibitory effects of these enzymes on interacting species cannot be excluded. Nevertheless, the long distances between the interacting colonies (Figs. 3A and 3B) suggested that this inhibitory effect was not attributable solely to the production of extracellular hydrolases. Instead, the faster growth most likely was also accompanied by the production of more secondary metabolites or small antifungal proteins diffusing over longer distances in agar plates than enzymes and this elevated production could be responsible for the growth inhibitory effects of *A. nidulans* against *A. niger* and *P. chrysogenum*. The same hydrolytic enzymes may also play an important role in the defense against mycoparasites because *T. artroviridae* invaded more efficiently the colonies of the Δ*engA*, Δ*chiB* double mutant than those of the control wild-type strain (Figs. 3C and 3D).

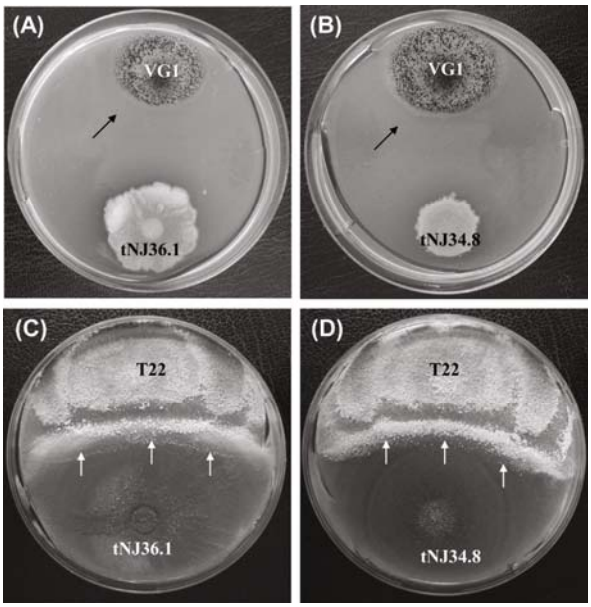

Fig. 3. Detection of interspecies interactions. The following strains were co-cultivated: (A) *A. nidulans* tNJ36.1 (control) and *A. niger* VG1; (B) *A. nidulans* tNJ34.8 (*ΔengA*, *ΔchiB*) and *A. niger* VG1; (C) *A. nidulans* tNJ36.1 (control) and *T. artroviridae* T122; (D) *A. nidulans* tNJ34.8 (*ΔengA ΔchiB*) and *T. artroviridae* T122. (A and B) culture media contained 10 g/L Na-acetate and 5 g/L yeast extract while, in (C and D), fungi were cultivated on media containing 10 g/L glucose and 5 g/L yeast extract. Note that the colony size of *A. niger* VG1 was smaller when co-cultivated with the *A. nidulans* control strain (A) than it was in the presence of the *ΔengA*, *ΔchiB* double mutant (B). Moreover, the mycelial mass of *Trichoderma* on *A. nidulans* colony was less compact with *A. nidulans* tNJ36.1 control (C) than with *A. nidulans* tNJ34.8 (*ΔengA, ΔchiB* double mutant; D). These assays were performed in quintuplets, and representative interspecies interactions are presented.

In summary, productions of the ChiB extracellular endochitinases and EngA extracellular β-1,3-endoglucanase in carbon-starving/carbon-limited cultures are multifunctional in terms of cell physiology. In addition to decomposition of the cell walls of dead or unnecessary cells and the utilization of the released nutrients for the maintenance of vegetative growth, these hydrolases may also influence fungal communities, either directly through their antifungal effect or indirectly *via* their impact on vegetative growth. Overproduction of these enzymes is not beneficial because they inhibit conidiogenesis and decrease the viability of the cultures. As a consequence, the production of ChiB and EngA are under complex regulation in *A. nidulans* (Szilágyi *et al*., 2010). Our experiments suggest that saprophytic fungi or their mutants, which release high activities of autolytic hydrolases, should be promising candidates to develop novel biocontrol technologies in the future and/or to produce highly efficient antifungal enzymes at acceptable prices.

This work was supported by the TÁMOP-4.2.2/B-10/1-2010-0024 project. The project is co-financed by the European Union and the European Social Fund.

References

Adams, T.H., Wieser, J.K., and Yu, J.H. 1998. Asexual sporulation in *Aspergillus nidulans*. *Microbiol. Mol. Biol. Rev.* **62**, 35–54.

Bainbridge, B.W., Bull, A.T., Pirt, S.J., Rowley, B.I., and Trinci, A.P.J. 1971. Biochemical and structural changes in non-growing maintained and autolysing cultures of *Aspergillus nidulans*. *Trans. Br. Mycol. Soc.* **56**, 371–385.

Barratt, R.W., Johnson, G.B., and Ogata, W.N. 1965. Wild-type and mutant stocks of *Aspergillus nidulans*. *Genetics* **52**, 233–246.

Benitez, T., Rincon, A.M., Limon, M.C., and Codon, A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbiol.* **7**, 249–260.

De Marco, J.L. and Felix, C.R. 2002. Characterization of a protease produced by a *Trichoderma harzianum* isolate which controls cocoa plant witches' broom disease. *BMC Biochem*. **3**, 3.

Elad, Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot.* **19**, 709–714.

El-Katatny, M.H., Gudelj, M., Robra, K.H., Elnaghy, M.A., and Gübitz, G.M. 2001. Characterization of a chitinase and an endo-beta-1,3-glucanase from *Trichoderma harzianum* Rifai T24 involved in control of the phytopathogen *Sclerotium rolfsii*. *Appl. Microbiol. Biotechnol.* **56**, 137–143.

Emri, T., Molnár, Zs., Szilágyi, M., and Pócsi, I. 2008. Regulation

of autolysis in *Aspergillus nidulans*. *Appl. Biochem. Biotechnol.* **151**, 211–220.

Emri, T., Szilágyi, M., László, K., Hamvas, M., and Pócsi, I. 2009. PepJ is a new extracellular protease of *Aspergillus nidulans*. *Folia Microbiol.* **54**, 105–109.

Erdei, E., Pusztahelyi, T., Miskei, M., Barna, T., and Pócsi, I. 2008. Characterization and heterologous expression of an age-dependent fungal/bacterial type chitinase of *Aspergillus nidulans*. *Acta Microbiol. Immunol. Hung.* **55**, 351–361.

Fravel, D., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. *New Phytol.* **157**, 493–502.

Gohel, V., Singh, A., Vimal, M., Ashwini, P., and Chhatpar, H.S. 2006. Bioprospecting and antifungal potential of chitinolytic microorganisms. *Afr. J. Biotechnol.* **5**, 54–72.

Haas, D. and Défago, G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* **3**, 307–319.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., and Lorito, M. 2004. *Trichoderma* species: opportunistic, avirulent plant symbionts. *Nature Rev. Microbiol.* **2**, 43–56.

Hong, T.Y. and Meng, M. 2003. Biochemical characterization and antifungal activity of an endo-1,3-beta-glucanase of *Paenibacillus* sp. isolated from garden soil. *Appl. Microbiol. Biotechnol.* **61**, 472–478.

Karasuda, S., Tanaka, S., Kajihara, H., Yamamoto, Y., and Koga, D. 2003. Plant chitinase as a possible biocontrol agent for use instead of chemical fungicides. *Biosci. Biotechnol. Biochem.* **67**, 221–224.

Kim, Y., Islam, N., Moss, B.J., Nandakumar, M.P., and Marten, M.R. 2011. Autophagy induced by rapamycin and carbon-starvation have distinct proteome profiles in *Aspergillus nidulans*. *Biotechnol. Bioeng.* **108**, 2705–2715.

Liu, B., Lu, Y., Xin, Z., and Zhang, Z. 2009. Identification and antifungal assay of a wheat beta-1,3-glucanase. *Biotechnol. Lett.* **31**, 1005–1010.

Markovich, N.A. and Kononova, G.L. 2003. Lytic enzymes of *Trichoderma* and their role in plant defense from fungal diseases: A review. *Appl. Biochem. Microbiol.* **39**, 341–351.

McIntyre, M., Berry, D.R., and McNeil, B. 1999. Response of *Penicillium chrysogenum* to oxygen starvation in glucose and nitrogen limited chemostat cultures. *Enzyme Microb. Technol.* **25**, 447–454.

McIntyre, M., Berry, D.R., and McNeil, B. 2000. Role of proteases in autolysis of *Penicillium chrysogenum* chemostat cultures in response to nutrient depletion. *Appl. Microbiol. Biotechnol.* **53**, 235–242.

Molnár, Zs., Emri, T., Zavaczki, E., Pusztehelyi, T., and Pócsi, I. 2006. Effects of mutations in the GanB/RgsA G protein mediated signaling on the autolysis of *Aspergillus nidulans*. *J. Basic Microbiol.* **46**, 495–603.

Nielsen, P. and Sorensen, J. 1997. Multi-target and medium-independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. *FEMS Microbiol. Ecol.* **22**, 183–192.

Pócsi, I., Leiter, É., Kwon, N.J., Shin, K.S., Kwon, G.S., Pusztahelyi, T., Emri, T., Abuknesha, R.A., Price, R.G., and Yu, J.H. 2009. Asexual sporulation signaling regulates autolysis of *Aspergillus nidulans* via modulating the chitinase ChiB production. *J. Appl. Microbiol.* **107**, 514–523.

Saiprasad, G.V.S., Mythili, J.B., Lalitha, A., Suneetha, C., Rashimi, H.J., Naveena, C., and Girija, G. 2009. Development of *Trichoderma harzianum* endochitinase gene construct conferring antifungal activity in transgenic tobacco. *Indian J. Biotechnol.* **8**, 199–206.

Schuster, A. and Schmoll, M. 2010. Biology and biotechnology of *Trichoderma*. *Appl. Microbiol. Biotechnol.* **87**, 787–799.

Sharma, N., Sharma, K.P., Gaur, R.K., and Gupta, V.K. 2011. Role of chitinase in plant defense. *Asian J. Biochem.* **6**, 29–37.

Shin, K.S., Kwon, N.J., Kim, Y.H., Park, H.S., Kwon, G.S., and Yu, J.H. 2009. Differential roles of the ChiB chitinase in autolysis and cell death of *Aspergillus nidulans*. *Eukaryot. Cell.* **8**, 738–746.

Szilágyi, M., Kwon, N.J., Bakti, F., M-Hamvas, M., Jámbrik, K., Park, H.S., Pócsi, I., Yu, J.H., and Emri, T. 2011. Extracellular proteinase formation in carbon starving *Aspergillus nidulans* cultures – physiological function and regulation. *J. Basic Microbiol.* **51**, 625–634.

Szilágyi, M., Kwon, N.J., Dorogi, Cs., Pócsi, I., Yu, J.H., and Emri, T. 2010. The extracellular β-1,3-endoglucanase EngA is involved in autolysis of *Aspergillus nidulans*. *J. Appl. Microbiol.* **109**, 1498–1508.

Yu, J.H. 2010. Regulation of development in *Aspergillus nidulans* and *Aspergillus fumigatus*. *Mycobiology* **38**, 229–237.