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ORIGINAL PAPER

Screening and Identification of *Trichoderma* Strains Isolated from Natural Habitats with Potential to Cellulose and Xylan Degrading Enzymes Production

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Abstract

A total of 123 *Trichoderma* strains were isolated from different habitats and tested for their ability to degrade cellulose and xylan by simple plate screening method. Among strains, more than 34 and 45% respectively, exhibited higher cellulolytic and xylanolytic activity, compared to the reference strain *T. reesei* QM 9414. For strains efficiently degrading cellulose, a highest enzyme activity was confirmed using filter paper test, and it resulted in a range from 1.01 to 7.15 FPU/ml. Based on morphological and molecular analysis, the isolates were identified as *Trichoderma*. The most frequently identified strains belonged to *Trichoderma harzianum* species. Among all strains, the most effective in degradation of cellulose and xylose was *T. harzianum* and *T. virens*, especially those isolated from forest wood, forest soil or garden and mushroom compost. The results of this work confirmed that numerous strains from the *Trichoderma* species have high cellulose and xylan degradation potential and could be useful for lignocellulose biomass conversion *e.g.* for biofuel production.

Key words: microorganisms screening, *Trichoderma* species, lignocellulose biomass, cellulolytic activity, xylanolytic activity

Introduction

Lignocellulose is among the most important components of plant biomass. It represents more than half of the globally produced organic matter during photosynthesis. In spite of its high abundance and energetic potential, this resource has not been fully utilized (Piotrowska-Cyplik, Czarnecki, 2003; Sanchez, 2009; Marecik *et al.*, 2012). One of the reasons is a complex structure of plants biomass components, which mainly comprises polymeric compounds, such as cellulose, hemicelluloses, lignin and pectin (Sun and Cheng, 2002; Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009; Sarkar *et al.*, 2012). Regrettably, the presence of compounds with such a high degree of polymerization restricts their use as a carbon or energy source for animals and typical fermentation microorganisms. Finding a cheap, and safe for environment method of lignocellulosic biomass degradation would allow increasing feed digestibility and improve effectiveness of livestock production or simple conversion of plant biomass to biofuels (Harris and Ramalingam, 2010; Marecik *et al.*, 2015; Chakdar *et al.*, 2016).

Efficient use of the lignocellulosic resource as a source of renewable energy requires the employment of processes, which lead to the release of monosaccharides. This allows for obtaining substrates, which are easily assimilated by microorganisms and bioconverted to liquid or gaseous fuels, such as ethanol, methanol, hydrogen, methane and others (Saxena *et al.*, 2009). A wide variety of methods can be employed for the degradation of the lignocellulosic complex, including physical, chemical or biochemical treatment. Especially, combined physical and chemical methods allow for rapid and efficient depolymerization of lignocelluloses; however, considerable energy expenditure is required possessing a notable threat to the environment (Kumar *et al.*, 2009; Park and Kim, 2012).

The development of biotechnological hydrolyzation methods for the lignocellulosic complex is considered to be promising. These methods utilize unique properties of microorganisms to degrade different organic and

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inorganic or even xenobiotic substances to the simpler or nontoxic ones (Cyplik *et al.*, 2012; Pęziak *et al.*, 2013; Lisiecki *et al.*, 2014). The use of such methods is based on the introduction of specific microorganisms or commercially available enzymatic preparations to the lignocellulosic biomass, what causes release of smaller pentose or hexose components. Enzymatic preparations employed for the decomposition of cellulose or hemicellulose are acquired from the cultivation of selected microbial strains (Aehle, 2007). The complete degradation of cellulose requires cellobiose dehydrogenases (CDHs) enzymes complex containing: endo- and exoglucanases and β-glucosidases. Depending on the producers, CDHs are classified into two classes: class I for CDHs produced by basidiomycetes and class II for CDHs from ascomycetes. Cellobiose dehydrogenases are flavocytochromes and belong to oxidoreductase class of enzymes. The efficient degradation of crystalline cellulose or hemicellulose is strongly related to copper-dependent lytic polysaccharide monooxygenases (LPMOs) (Harreither *et al.*, 2011; Tanx *et al.*, 2015). The preparations used for hemicelluloses hydrolysis are verycomplex, since they usually consists of a mixture of eight enzymes, such as endo-1,4-β-D-xylanase, exo-1,4-β-D-xylanase, α-L-arabinofuranosidase, endo-1,4-β-D-mannase, β-mannosidase, acetyl xylan esterase, α-glucuronidase and α-galactosidase (Clarke, 1997; Jorgensen *et al.*, 2003; Banerjee *et al.*, 2010). However, many different species of microorganisms capable of cellulolytic and hemicellulolytic enzymes synthesis have been discovered, including bacteria and fungi. It is important to note that the efficiency of lignocellulose decomposition is still unsatisfactory (Sun and Cheng, 2002).

Among the microorganisms, which exhibit the ability to produce hemicellulolytic enzymes, the filamentous fungi belonging to the *Trichoderma* genus attract particular attention (Xu *et al.*, 1998). Due to substrate induction, these fungi produce and secrete considerable amounts of enzymes, which belong to cellulases as well as hemicellulases, which is why they are capable of growth under unfavourable environmental conditions (Sandgren *et al.*, 2005). This is a valuable adaptive trait, which allows them to utilize different carbon and energy sources and grow under different temperature regimes, regardless of the presence of light (Polizeli *et al.*, 2005). Due to their various metabolic activity, fungi belonging to the *Trichoderma* genus have found numerous practical applications *e.g.* enzyme producers, used as a biofungicides (Vinale *et al.*, 2006; Wojtkowiak-Gębarowska., 2006; Vinale *et al.*, 2008; Harris and Ramalingam, 2010; Chakdar *et al.*, 2016).

The purpose of this study was to examine the ability of *Trichoderma* fungi isolated from different habitats to production of cellulose and xylan degrading enzyme and determine the activity of those enzymes.

Experimental

Materials and Methods

Fungal collection. The one hundred and twenty-three *Trichoderma* strains, belonging to eleven species or species complex: *Trichoderma atroviride*, *Trichoderma citrinoviride*, *Trichoderma hamatum*, *Trichoderma harzianum, Trichoderma koningii*, *Trichoderma koningiopsis*, *Trichoderma longibrachiatum*, *Trichoderma pseudokoningii*, *Trichoderma viride*, *Trichoderma viridescens* and *Trichoderma virens*, were investigated in this study. The one hundred and seven strains were previously identified by Błaszczyk *et al.* (2011, 2016) and Jeleń *et al.* (2014) and deposited in the collection of the Institute of Plant Genetics, Polish Academy of Science, Poznań, Poland. Ten *Trichoderma* isolates were collected from: wheat kernels (Lublin – AN158 isolate), pieces of decaying wood with white or brown rot (Czerwonak – AN109, AN110 isolates; Golęcin Park, Poznań – AN131 isolate; Strzeszyn Park, Poznań – AN177 isolate; Joniec, Warszawa – AN501 isolate) and mushroom compost used for *Agaricus bisporus* cultivation (Skierniewice – AN186, AN187, AN188 isolates; Poznań – AN204 isolate) in Poland and isolated as described by Błaszczyk *et al.* (2011). Other strains including *T. pseudokoningii* (AN219, ITEM 1416), *T. koningiopsis* (AN222, ITEM2688), *T. harzianum* (AN220, ITEM 1328) and *T. virens* (AN267 – ITEM 1357, AN268 – ITEM 1591, AN269 – ITEM 1594) were kindly supplied by dr. Antonio Logrieco, CNR, ISPA, Bari, Italy. *Trichoderma reesei* QM 9414, sourced from the Czech Collection of Microorganisms (CCM), Brno, Czech Republic was used as the reference strain.

Morphological and molecular analysis. Ten isolates of *Trichoderma* sourced from wheat grains, compost used for mushroom cultivation and pieces of decaying wood collected from the floor of forests and parks in eastern and central Poland were identified morphologically following the procedure described by Gams and Bisset (1998). Colony characteristics were examined from cultures grown on PDA and SNA after 3–7 days at a temperature of 25°C. Microscopic observations were performed from cultures grown on SNA. Molecular species identification was based on the sequencing of two different phylogenetic markers: a fragment of the ITS1-5.8S – ITS2 rRNA region and a fragment of the translation-elongation factor 1-alpha (*tef1*) gene. Mycelium for DNA extraction was obtained as described previously (Błaszczyk *et al.*, 2011). Isolation of total DNA was performed using the CTAB method (Doohan *et al.*, 1998). The ITS1 and ITS2 region of the rDNA gene cluster was amplified using primers ITS4 and ITS5 (White *et al.* 1990). A fragment of the 1.2-kb *tef1* gene was ampli-

fied using primers Ef728M (Carbone and Kohn, 1999) and TEF1LLErev (Jaklitsch *et al.*, 2005). PCR amplification, DNA sequencing and sequence analysis was carried out under the conditions described by Błaszczyk *et al.* (2011). The sequences were identified by BLASTn (http://blast.ncbi.nlm.nih.gov/) as well as TrichOKEY and TrichoBLAST (http://www.isth.info; Druzhinina *et al.*, 2005; Kopchinskiy *et al.*, 2005). The sequences were deposited in the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and listed in Table I.

Cultivation of Trichoderma and induction of enzyme synthesis. For assessing the capability to celululolytic or hemicellululolytic enzyme production, the fungi were cultivated on medium consisting only of carboxymethylcellulose sodium salt (Akzo Nobel Chemicals) or xylan (10 g/l) as a sole source of carbon. Furthermore, the medium contained: $NaNO_3$ – 3 g/l, K_2HPO_4 – 1 g/l, $MgSO_4 \cdot 7H_2O$ – 0.5 g/l, KCl – 0.5 g/l, $FeSO_4 \cdot 7H_2O$ – 0.01 g/l and pH was adjusted to 5.6 ± 0.1. The inducing enzyme synthesis culture was carried out in 300 ml Erlenmeyer flasks, on a rotary shaker (150 rpm) for five days at a temperature of 25 ± 1°C. After the cultivation process the fungal cells were centrifuged (4500 rpm for 10 min) and obtained supernatants containing crude cellulolytic and xylanolytic enzymes were used for determination of the enzymes activity.

Analysis of cellulolytic and xylanolytic activity of Trichoderma fungi – plate method. The analysis of cellulolytic and hemicellululolytic enzymes activity was carried out using the plate screening method described by Hadkin and Anagnostakis (1977). The method is based on the observation of changes (determination of the size of clearance zones), which occur in the solid medium as a result of enzymatic activity. For determination of the cellulolytic activity, the medium including a 1% solution of carboxymethylcellulose sodium salt and 0.1 g/l of chloramphenicol in 2% solution of agar was used. The media were poured into Petri dishes (diameter of 90 mm) and then, after solidification, the central part was removed using a cork borer to create a well. To evaluate the xylanolytic activity the plates were prepared analogously, however a 1% solution of xylan was used instead of carboxymethylcellulose sodium salt.

The cultures of the *Trichoderma* fungi were centrifuged at 4500 rpm for 15 min, and then 200 µl of supernatants were placed in the wells. The plates were incubated at 37°C for 48 h and rinsed with 5 ml of a 1% Lugol's iodine solution. After 15 minutes, the excess of the Lugol's solution was rinsed with 0.1% solution of NaCl. The areas including non-hydrolyzed carboxymethylcellulose sodium salt or xylan were stained with a deep brown colour, whereas the areas in the direct vicinity of the well were characterized by a visible clearance, due to the enzymatic activity. The size of the clearance in each specific sample reflected the activity of cellulolytic or xylanolytic enzymes. The size of the clearance area, which occurred due to the activity of enzymes secreted by a given strain, was compared with the size of the clearance area obtained for the reference strain with known cellulolytic properties – *T. reesei* QM9414 (Sazci *et al.*, 1986).

Analysis of cellulolytic activity of Trichoderma fungi – a blotting filter paper method. The overall cellulolytic activity (FPU) of selected fungal strains was also determined using the method recommended by Ghose (1987). Blotting filter paper stripes (Whatman No. 1) were placed in test tubes and incubated for 60 minutes at 50°C in the presence of 0.1 mol acetate buffer (pH 4.8) and the post-cultivation medium acquired after cultivation of fungi for 5 days. The amount of reducing sugars released into the supernatant was measured by employing the colorimetric method, using 3,4-dinitrosalicylic acid (DNS) (Miller, 1959). The cellulolytic activity of the post-cultivation medium was expressed as FPU (Filter Paper Unit) according to the International Union of Pure and Applied Chemistry (IUPAC) (Ghose, 1987). The amount of the enzyme, which allowed for the release of 1 µmol of glucose during 1 minute, was adapted as one unit of FPU cellulolytic activity.

Statistical analysis of the results. Each experiment of the enzyme activity analysis was carried out in three replicates. The Levene's test (the homogeneity of variance test) and Turkey's test were carried out in order to conduct a statistical verification of the obtained results. The calculations were carried out using Statistica 6.0 software.

Results and Discussion

Trichoderma species identification. Ten isolates of *Trichoderma* from samples of wheat grains, compost used for mushroom cultivation and decaying wood in Poland were identified at the species level based on morphological as well as ITS1, ITS2 and *tef1* sequencing data. Finally, five species or species complex: were found to be: *T. harzianum* species complexes – 3 strains, *T. virens* – 4 strains, *T. viride* – 1 strain, *T. viridescens* – 1 strain and *T. hamatum* – 1 strain. The identification, origin and NCBI GenBank accession numbers of all *Trichoderma* isolates (both of ten isolates identified in this study and isolates previously recognized by Błaszczyk *et al.* (2011, 2016) and Jeleń *et al.* (2014) originating from the different habitats in Poland are given in Table I.

Cellulolytic activity of the studied fungal strains. The studies regarding the cellulolytic activity based on the plate method described by Hadkin and Anagnostakis (1977) revealed that among the 123 strains belonging to the *Trichoderma* genus more than 34% exhibited higher cellulolytic activity compared to the reference

Table I
List of isolates originating from the different habitats identified as the *Trichoderma* species and analyzed for their cellulolytic and xylanolytic activity.

Culture code	Origin		NCBI GenBank Assession No.		Cellulolytic activity (the plate method)	Xylanolytic activity (the plate method)
	Localization	Source	ITS	*tef1*		
T. reesei						
QM9414					1.00	1.00
***T. viride* (A)**						
AN109*, AN176[1]	Central Poland	Forest wood[2]	HQ292923	HQ293010	0.45/0.50	0.70/0.35
AN141			HQ292922	HQ293008	0.75 ± 0.09	1.75 ± 0.27[a]
AN142			HQ292920	HQ293009	2.50 ± 0.31[a]	0.67 ± 0.15
AN179			HQ292924	HQ293011	0.56 ± 0.15	0.55 ± 0.18
AN 235		Forest soil	HQ292921	HQ293013	0.45 ± 0.15	0.75 ± 0.12
AN242	Southern Poland	Forest wood	JX184121	JX184098	0.55 ± 0.18	0.58 ± 0.15
AN244, AN249[1]			JX184122	JX184099	0.46/1.40	0.75/1.42
AN250, AN255[1]			JX184121	JX184098	1.03/0.78	0.77/0.81
AN371			JX184124	JX184100	0.91 ± 0.17	0.92 ± 0.09
AN401			JX184121	JX184099	0.75 ± 0.14	0.47 ± 0.21
***T. iridescent* complex (B)**						
AN93	Central Poland	Forest wood	HQ292927	HQ292995	0.85 ± 0.21	0.73 ± 0.18
AN122			HQ292928	HQ292994	0.79 ± 0.12	0.57 ± 0.19
AN145			HQ292930	HQ292996	0.85 ± 0.17	0.3 ± 0.33
AN148			HQ292933	HQ292999	0.79 ± 0.23	0.44 ± 0.11
AN149			HQ292934	HQ293000	0.67 ± 0.20	0.48 ± 0.14
AN158*	Eastern Poland	Wheat kernels	JX184127	JX184103	0.55 ± 0.12	0.76 ± 0.19
AN227	Central Poland	Forest wood	HQ292936	HQ293001	0.67 ± 0.21	0.57 ± 0.13
AN229			HQ292937	HQ293002	0.83 ± 0.16	0.19 ± 0.08
AN231			HQ292938	HQ293003	0.50 ± 0.23	0.58 ± 0.13
AN245	Southern Poland	Forest wood	JX184127	JX184103	0.50 ± 0.10	0.56 ± 0.16
AN248			JX184128	JX184104	0.63 ± 0.15	0.75 ± 0.23
AN323, AN334, AN405[1]			JX184127	JX184103	0.78/0.85/0.10	0.58/0.58/0.55
***T. vixens* (C)**						
AN68	Eastern Poland	Garden compost	HQ292943	–	2.09 ± 0.27[a]	1.10 ± 0.11
AN69			HQ292944	–	1.60 ± 0.19	3.00 ± 0.27[a]
AN70			HQ292947	–	2.10 ± 0.22[a]	1.10 ± 0.15
AN73			HQ292945	–	2.75 ± 0.31[a]	1.10 ± 0.18
AN74			HQ292946	–	1.25 ± 0.12	1.40 ± 0.17
AN75			HQ292948	–	1.53 ± 0.15	1.10 ± 0.13
AN160		Grass root	HQ292945	–	0.83 ± 0.18	0.55 ± 0.18
AN185	Central Poland	Mushroom compost[3]	HQ292947	–	0.95 ± 0.12	0.55 ± 0.12
AN186*, AN187*,	Eastern Poland		HQ292946	–	0.8/2.6/2.0	1.00/2.25[a]/1.15
AN188* AN204*[1]			HQ292948	–	1.35 ± 0.21	1.45 ± 0.17
AN267 – ITEM 1357	Bari. Italy		–	–	1.25 ± 0.17	0.55 ± 0.20
AN268 – ITEM 1591			–	–	2.50 ± 0.23[a]	2.70 ± 0.32[a]
AN269 – ITEM 1594			–	–	1.6 ± 0.19	1.10 ± 0.12
***T. harzianum* complex (D)**						
AN91	Central Poland	graden Kompost	HQ292860	–	0.75 ± 0.15	1.15 ± 0.11
AN94		forest soil	HQ292873	–	0.80 ± 0.12	3.55 ± 0.27[a]
AN101		forest wood[2]	HQ292868	–	1.60 ± 0.21	2.58 ± 0.23[a]
AN108/AN110*			HQ292869	–	2.80[a]/1.10	1.80[a]/1.40

Table I. Continued

Culture code	Origin		NCBI GenBank Assession No.		Cellulolytic activity (the plate method)	Xylanolytic activity (the plate method)
	Localization	Source	ITS	*tef1*		
AN131*	Central Poland	forest wood[2]	HQ292870	–	1.10 ± 0.16	0.60 ± 0.09
AN132			HQ292867	–	0.78 ± 0.12	0.60 ± 0.07
AN133			HQ292874	–	2.05 ± 0.26[a]	1.70 ± 0.21[a]
AN134			HQ292875	–	1.01 ± 0.11	1.00 ± 0.17
AN135			HQ292876	–	0.81 ± 0.13	1.65 ± 0.14
AN136			HQ292901	–	2.40 ± 0.22[a]	2.04 ± 0.18[a]
AN137			HQ292877	–	1.60 ± 0.13	1.03 ± 0.12
AN138			HQ292861	–	1.00 ± 0.10	1.41 ± 0.16
AN150			HQ292878	–	0.87 ± 0.08	1.67 ± 0.16[a]
AN177*			HQ292883	–	1.25 ± 0.15	1.40 ± 0.11
AN181			HQ292875	–	1.66 ± 0.12[a]	2.25 ± 0.22[a]
AN203		mushroom compost[3]	HQ292879	–	1.66 ± 0.13[a]	1.00 ± 0.18
AN205			HQ292880	–	1.36 ± 0.17	2.45 ± 0.25[a]
AN207			HQ292881	–	1.50 ± 0.13	1.00 ± 0.17
AN211			HQ292882	–	1.10 ± 0.11	0.20 ± 0.11
AN223		Forest soil	HQ292902	–	1.25 ± 0.10	1.05 ± 0.20
AN258		Forest wood	HQ292271	–	0.80 ± 0.08	1.41 ± 0.26
AN260			HQ292884		0.89 ± 0.11	1.20 ± 0.19
AN273			HQ292886	–	1.37 ± 0.19	1.45 ± 0.15
AN275			HQ292888	–	1.20 ± 0.12	1.40 ± 0.11
AN276			HQ292889	–	0.80 ± 0.09	1.05 ± 0.07
AN278			HQ292890	–	0.75 ± 0.10	1.25 ± 0.19
AN282			HQ292891	–	0.30 ± 0.07	1.10 ± 0.12
AN283			HQ292892	–	0.85 ± 0.15	1.27 ± 0.27
AN284			HQ292893	–	1.15 ± 0.20	1.27 ± 0.22
AN285			HQ292894		1.10 ± 0.18	1.28 ± 0.11
AN286			HQ292895	–	1.35 ± 0.29	1.30 ± 0.13
AN349	Southern Poland	Forest wood	JX184111	JX184089	1.05 ± 0.09	1.38 ± 0.24
AN360, AN367, AN373, AN3811			JX184113	JX184090	2.20[a]/1.70[a]/0.70 1.46	1.27/1.40/1.75[a] 1.52
AN220 – ITEM 1328	Bari, Italy		–	–	0.25 ± 0.16	1.60 ± 0.13
***T. hamatum* (E)**						
AN118	Central Poland	Forest wood[2]	HQ292854	–	0.66 ± 0.21	0.61 ± 0.19
AN155	Eastern Poland	Ryder Rhizosphäre	HQ292851	–	0.82 ± 0.18	0.51 ± 0.11
AN175	Central Poland	Forest wood	HQ292854	–	0.53 ± 0.12	0.28 ± 0.10
AN225, AN238		Forest soil	HQ292856	–	0.95 ± 0.08	0.75 ± 0.11
			HQ292853		0.41 ± 0.21	0.84 ± 0.29
AN277, AN279, AN501*[1]		Forest wood	HQ292853	–	0.50/0.18/0.41	2.3[a]/0.72/0.61
AN521	Northern Poland		HQ292856	–	1.05 ± 0.24	0.75 ± 0.15
***T. atroviride* (F)**						
AN19	Central Poland	Forest soil	HQ292786	HQ292963	0.67 ± 0.13	1.04 ± 0.16
AN35		Maize kernels	HQ292787	HQ292953	0.35 ± 0.09	0.80 ± 0.19
AN90		Garden soil	HQ292788	HQ292954	0.49 ± 0.12	0.35 ± 0.12
AN95		Garden compost	HQ292789	HQ292955	0.90 ± 0.17	1.30 ± 0.18
AN96			HQ292790	HQ292956	0.95 ± 0.15	0.70 ± 0.14
AN111		Forest wood	HQ292791	HQ292964	0.37 ± 0.13	0.75 ± 0.21

Table I. Continued

Culture code	Origin		NCBI GenBank Assession No.		Cellulolytic activity (the plate method)	Xylanolytic activity (the plate method)
	Localization	Source	ITS	*tef1*		
AN152	Central Poland	Triticale kernel	HQ292792	HQ292957	0.59 ± 0.19	1.85 ± 0.30[a]
AN153			HQ292793	HQ292958	0.58 ± 0.10	0.57 ± 0.12
AN182		Forest wood	HQ292794	HQ292965	0.57 ± 0.12	0.45 ± 0.23
AN206		Mushroom compost	HQ292804	HQ292960	0.40 ± 0.19	1.02 ± 0.11
AN212			HQ292795	HQ292966	0.68 ± 0.21	1.27 ± 0.21
AN215			HQ292796	HQ292967	1.10 ± 0.16	1.19 ± 0.14
AN224	Southern Poland	Forest wood	HQ292799	HQ292970	0.36 ± 0.09	0.58 ± 0.13
AN240			JX184119	JX184096	0.23 ± 0.19	0.57 ± 0.17
AN287	Central Poland		HQ292798	HQ292969	0.41 ± 0.11	0.58 ± 0.12
***T. longibrachiatum* (G)**						
AN197	Eastern Poland	Mushroom factory	HQ292780	–	0.77 ± 0.19	1.40 ± 022
AN213	Central Poland	Mushroom compost[3]	HQ292781	–	0.50 ± 0.16	2.70 ± 0.31[a]
***T. citrinoviride* (H)**						
AN89	Central Poland	Garden soil	HQ292841	–	0.50 ± 0.14	1.25 ± 0.27
AN98		Forest wood	HQ292843	–	1.55 ± 0.13	1.07 ± 0.19
AN198		Mushroom factory	HQ292845		0.57 ± 0.17	0.47 ± 0.13
AN199			HQ292846	–	0.95 ± 0.12	2.07 ± 0.26[a]
AN201			HQ292849	–	1.10 ± 0.21	2.08 ± 0.29[a]
AN262, AN303, AN393, AN500[1]	Southern Poland	Forest wood	JX184109	–	2.26[a]/0.85/1.75[a] 0.90	2.88[a]/0.61/0.63 0.80
***T. pseudokoningii* (I)**						
AN219 – ITEM1416	Bari. Italy		–	–	1.42 ± 0.19	2.10 ± 0.22[a]
***T. koningii* (J)**						
AN100	Central Poland	Forest wood	HQ292903	HQ292975	0.35 ± 0.17	0.40 ± 0.17
AN105			HQ292905	HQ292977	0.17 ± 0.08	0.58 ± 0.14
AN106			HQ292906	HQ292978	1.16 ± 0.19	0.40 ± 0.16
AN121			HQ292913	HQ292985	0.58 ± 0.11	1.41 ± 0.27
AN128			HQ292918	HQ292989	0.31 ± 0.09	0.48 ± 0.12
AN151			HQ292919	HQ292990	0.47 ± 0.11	0.55 ± 0.12
***T. koningiopsis* (K)**						
AN222 – ITEM 2688	Bari, Italy		–	–	0.20 ± 0.10	0.30 ± 0.12

* The isolates identified in this study by a combination of morphological and molecular analyses
a – Indicate the statistically significant higher values in comparison to the reference strain *T. reesei* (QM 9414) at $P \leq 0.05$
1 – The identical accession numbers refer to identical sequences
2 – The pieces of decaying wood collected from the floor of forests/parks
3 – The compost used for *Agaricus bisporus* cultivation
A. B. C. … – corresponds to particular species presented in fig. 1.

strain *T. reesei* and that these differences were statistically significant ($p \leq 0.05$) (Fig. 1A). *T. harzianum* can be included as a species with high cellulolytic activity. Among the representatives of this species up to 21 out of 39 strains displayed a higher activity compared to the reference *T. reesei* QM9414 strain. The highest activity was observed for strains AN108, AN133, AN136, and AN360. The activity of these strains exceeded the activity of the reference strain by 2.4 times on the average. An activity exceeding 50% was noted for strains AN101, AN137, and AN367. All of these efficient *T. harzianum* strains were isolated from different locations of forest wood. Another species, which included very active strains with regard to degradation of cellulose, was *T. virens,* especially isolated from garden or mushroom compost. Among these species, 12 out of 15 strains were more active compared to the reference strain. The activity exceeding that of the reference strain by 2.6 times was observed for strains AN73, AN187, and AN268. Additionally, the degradation of cellulose

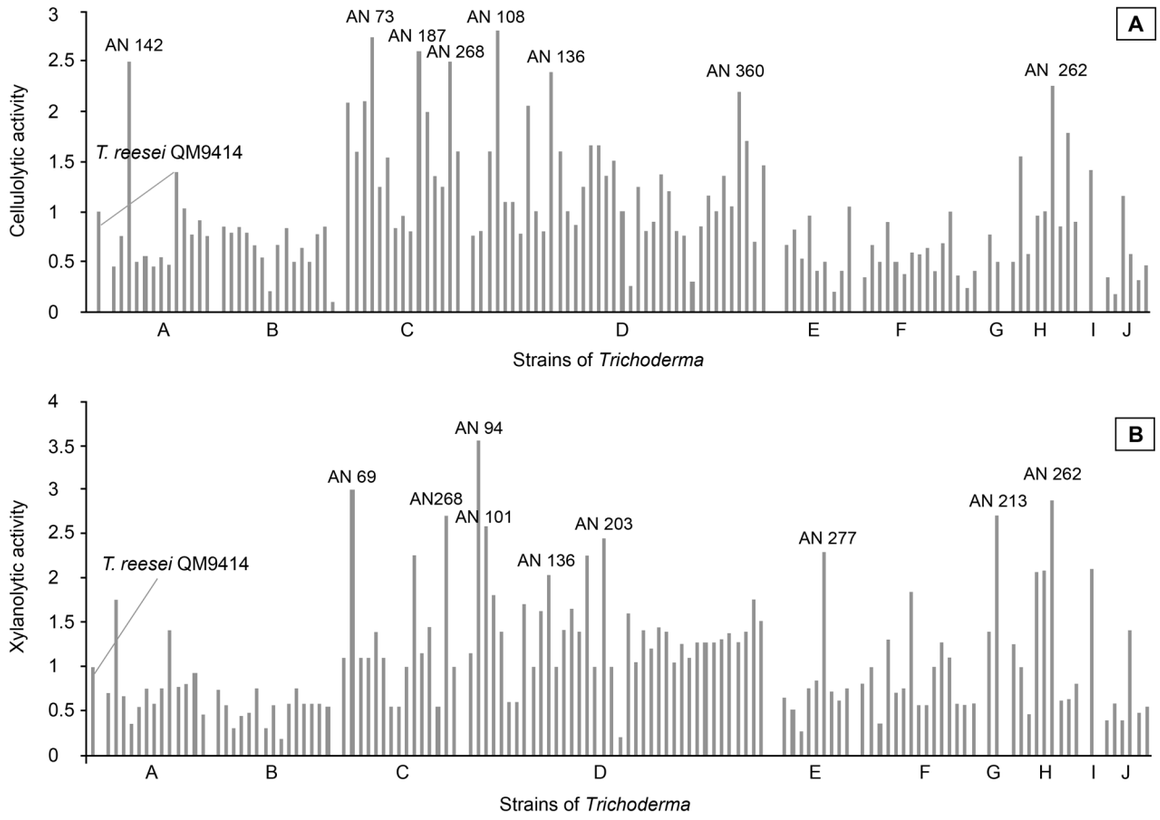

Fig. 1. Cellulolytic (part A) and xylanolytic activity (part B) of the studied fungal strains relative to the reference strain *T. reesei* QM9414 – the plate method analysis. The studied fungal strains belonged to the following species: A – *T. viride*, B – *T. viridescens*, C – *T. virens*, D – *T. harzianum*, E – *T. hamatum*, F – *T. atroviride*, G – *T. longibrachiatum*, H – *T. citrinoviride*, I – *T. pseudokoningii*, J –*T. koningii*.

* The value corresponding to difference in clearing zone diameter between analyzed strains

was approximately twice as efficient for strains AN68, AN70, and AN188. Higher cellulolytic activity compared to the reference *T. reesei* strain was also observed in the case of three strains belonging to the *T. viride* and *T. citrinoviride* species as well as strain from the *T. pseudokoningii* (AN219). Among these species, a particularly high activity was exhibited by AN262 belonging to *T. citrinoviride* species and AN142 belonging to the *T. viride* species, both collected from forest wood. The cellulolytic activity of strains belonging to the remaining species, identified as *T. viridescens*, *T. hamatum*, *T. koningii*, *T. koningopsis* and *T. atroviride* were usually at a much lower level compared to the reference strain. High cellulolytic activity of the selected fungal strains belonging to the *Trichoderma* genus was also confirmed using the blotting filter paper method described by Ghose (1987). The selected strains characterized by the highest cellulolytic activity were presented in Table II.

Table II
Total cellulase activities of selected *Trichoderma* strains measured by the filter paper assay method (FPA).

Cellulolytic activity (FPU/ml)	Strains of *Trichoderma*								
	T. reesei	*T. virens*						*T. harzianum*	
	QM 9414	AN 68	AN 69	AN 73	AN 187	AN 188	AN 268	AN 94	AN 108
	2.11 ± 0.25	4.41 ± 0.31	3.21 ± 0.15	6.05 ± 0.46	5.62 ± 0.41	4.42 ± 0.39	6.36 ± 0.48	1.69 ± 0.09	7.15 ± 0.30

Cellulolytic activity (FPU/ml)	Strains of *Trichoderma*							
	T. harzianum						*T. longibrachiatum*	*T. citrinoviride*
	AN 133	AN 136	AN 181	AN 203	AN 360	AN 367	AN 213	AN 262
	5.31 ± 0.28	6.13 ± 0.55	4.11 ± 0.16	3.33 ± 0.22	5.60 ± 0.25	3.70 ± 0.23	1.01 ± 0.21	4.70 ± 0.34

Xylanolytic activity of the studied fungal strains. The studies regarding the xylanolytic activity of the selected fungal strains belonging to the *Trichoderma* genus revealed that 56 out of 123 studied isolates were characterized by higher activity compared to the reference *T. reesei* strain (Fig. 1B). *T. harzianum* exhibited the highest activity. Up to 31 strains of these species displayed higher activity compared to the reference strain. Among these strains the highest activity was observed for strain AN94 obtained from forest soil, which was capable of degrading xylan over 3.5 times more efficiently compared to the reference strain. A notable xylanolytic activity was also observed in the case of strains AN101 and AN205. These strains exhibited activity, which was over 2.5 times higher compared to the reference strain. *T. citrinoviride* was another species, which included strains with high xylanolytic activity. The strain AN262 that belonged to this species, was capable of degrading xylan over 2 times more efficiently compared to the reference strain. High xylanolytic activity was also noted for AN213, belonging to *T. longibrachiatum* species, AN69 of *T. virens* species and AN277 of *T. hamatum* species.

For both activities analysed, no direct dependence between particular source of fungi strains and their degradative potential was observed; however, the strains isolated from forest wood, forest soil and compost were the most effective.

Filamentous fungi exhibit a broad spectrum of secondary metabolic activity representing important for the people – enzymes or antibiotics production, but also secretion of some dangerous, toxic or cancerogenic substances like mycotoxins (Jae-Hyuk and Keller, 2005; Błaszczyk *et al.*, 2013; Błaszczyk *et al.*, 2016). This is the effect of excellent adaptation ability to different extreme environment condition and the reason why these fungi are very interesting as a source of novel bioactive substances (Altinok, 2009; Chavez, 2015). The strains of *Trichoderma* used in this study were isolated from different habitats: decaying wood, forest soil, garden and mushroom compost, wheat and maize kernels. These habitats are a reach in carbon source but available only for microorganisms able to degrade lignocellulose compounds. This feature is widespread among the different fungi, including *Trichoderma* species (Druzhinina *et al.*, 2010; Amore *et al.*, 2013; Kubicek, 2013). *Trichoderma* genus is very common, diverse and occurs in a wide geographic distribution likewise north regions of Europe; however, the most of *T. harzianum* species identified in this study are uncommon, known mainly from Europe and North America (Jaklitsch *et al.*, 2011; Chavez, 2015; Qin and Zhuang, 2016). The differences in the *Trichoderma* occurrence, related to habitats and geographic regions in Poland, were described also in previous studies (Błaszczyk *et al.*, 2011, 2013, 2016)

Participation of cellulases and hemicellulases in global enzymes market has been increasing year to year. It is the effect of the expanding possibilities of their application in industrial practice (Beg *et al.*, 2000). They may be used as a supplement in animal feeding as well as food or wood industry (Harris and Ramalingam, 2010). Developing biofuel industry (biogas, bioethanol) is also the area of cellulose and hemicellulose enzymes application to increase of the fermentation efficiency (Taherzadeh and Karimi, 2008; Ziemniński *et al.*, 2012; Chakdar *et al.*, 2016). These are the reasons that new and more effective sources of these enzymes are still studied. Many of the microorganisms are saprotrophs and contribute to the decay of organic matter exhibiting the possibility to cellulose and hemicellulose enzymes production (Crowther *et al.*, 2012). However, despite that different microorganisms like bacteria, actinomycetes, yeast or even algae or insects are able to secrete these enzymes, filamentous fungi are especially worth of attention (Polizeli *et al.*, 2005). The genus *Aspergillus* and *Trichoderma* secrete these enzymes directly into the environment at the remarkably higher than other microorganisms efficiency. The ability of different fungi strains belonging to the *Trichoderma* to produce cellulolytic and hemicellulolytic enzymes was extensively studied (Clarke, 1997; Xu *et al.*, 1998; Sandgren *et al.*, 2005; Banerjee *et al.*, 2010). Such enzymes are obtained on industrial scale by aerobic cultivation of fungi, such as *T. reesei* and *Humicola insolens* or from recombinant strains (Liming and Xueliang, 2004; Wilson, 2009). The strains of filamentous fungi isolated from soil, decaying wood and sawdust were analyzed by Inuwa Ja'afaru (2013). Up to 42.6% of the 110 identified isolates belonged to the *Trichoderma* genus. The highest xylanolytic activity was exhibited by *T. viride* Fd18 strain, whereas the highest cellylolytic activity was observed for *Trichoderma* sp. F4 strain. The high potential of fungi belonging to the *Trichoderma* to produce cellulolytic and hemicellulolytic enzymes was confirmed in further studies (Wen *et al.*, 2005; Chandel *et al.*, 2013). Additionally, 23 out of 36 fungal isolates originating from compost also displayed cellulolytic activity. The isolates were identified as *Trichoderma*, *Aspergillus*, *Rhizopus* and *Penicillium* species (Chandel *et al.*, 2013). The ability to synthesize cellulolytic enzymes by the modified *T. reesei* RUT-C30 strain QM 9414 with the use of cow manure as a substrate was confirmed by Wen *et al.* (2005). This strain was characterized by a higher production of cellulose compared to the reference *T. reesei* QM 9414 strain.

In summary, the results obtained in our study confirmed that numerous strains from the *Trichoderma* species are characterized by high lignocellulose degradation potential. The studies performed on forest soil, decaying wood or different kind of compost indicate

a source of effective degraders of cellulose and hemicellulose. Due to potentially benefits related to the production of cellulolytic and hemicellulolytic enzymes and a relatively good growth rate, which is a characteristic trait of such microorganisms; these fungi may be helpful in the industrial practice. For this reason the screening of new producers and study of molecular mechanisms of metabolite secretion regulation should be continued.

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