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 plant 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...
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 very complex, 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, a-glucuronidase and a-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 cel lulases 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; Wojtikowiak-Gbarowski, 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 Blaszczzyk 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; Golecin 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 Blaszczzyk 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 rDNA region and a fragment of the translation-elongation factor 1-alpha (tef1) gene. Mycelium for DNA extraction was obtained as described previously (Blaszczzyk 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 TEF1LErev (Jaklitsch et al., 2005). PCR amplification, DNA sequencing and sequence analysis was carried out under the conditions described by Blaszczyk 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 cellulolytic or hemicellulolytic 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: NaNO₃ – 3 g/l, K₂HPO₄ – 1 g/l, MgSO₄ – 0.5 g/l, KCl – 0.5 g/l, FeSO₄ – 7H₂O – 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 hemicellulolytic 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. viridecens – 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 Blaszczyk 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 Accession No. | Cellulolytic activity (the plate method) | Xylanolytic activity (the plate method) |
|--------------|--------|----------------------------|----------------------------------------|----------------------------------------|
|              |        |                            | Localization | Source | ITS | tef1 |                      |                         |
| QM9414       |        |                            |             |        |     |      | 1.00 | 1.00                  |
| **T. reesei**|        |                            |             |        |     |      |      |                        |
| AN109*, AN176| Central Poland Forest wood | HQ292923 | HQ293010  | 0.45 ± 0.50  | 0.70 ± 0.35  |
| AN141        |        |                            | HQ292922 | HQ293008  | 0.75 ± 0.09 | 1.75 ± 0.27 |
| AN142        |        |                            | HQ292920 | HQ293009  | 2.50 ± 0.31 | 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 |        |                            | JX184122 | JX184099  | 0.46 ± 0.10 | 0.75 ± 0.12 |
| AN250, AN255 |        |                            | JX184121 | JX184098  | 1.03 ± 0.78 | 0.77 ± 0.81 |
| AN248        |        |                            | JX184124 | JX184100  | 0.91 ± 0.17 | 0.92 ± 0.09 |
| AN401        |        |                            | JX184121 | JX184099  | 0.75 ± 0.14 | 0.47 ± 0.21 |
| **T. viride**|        |                            |             |        |     |      |      |                        |
| 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 | 0.73 ± 0.23 |
| AN323, AN334, AN405 |        | JX184127 | JX184103  | 0.78 ± 0.85 | 0.58 ± 0.55 |
| **T. tridescent complex**|        |                            |             |        |     |      |      |                        |
| AN68         | Eastern Poland Garden compost | HQ292943 | –         | 2.09 ± 0.27 | 1.10 ± 0.11 |
| AN69         |        |                            | HQ292944 | –         | 1.60 ± 0.19 | 3.00 ± 0.27 |
| AN70         |        |                            | HQ292947 | –         | 2.10 ± 0.22 | 1.10 ± 0.15 |
| AN73         |        |                            | HQ292945 | –         | 2.75 ± 0.31 | 1.10 ± 0.18 |
| AN74         |        |                            | HQ292946 | –         | 1.25 ± 0.12 | 1.40 ± 0.17 |
| AN75         |        | Grass root                 | HQ292948 | –         | 1.53 ± 0.15 | 1.10 ± 0.13 |
| AN160        |        |                            | HQ292945 | –         | 0.83 ± 0.18 | 0.55 ± 0.18 |
| AN185        | Central Poland Mushroom compost | HQ292947 | –         | 0.95 ± 0.12 | 0.55 ± 0.12 |
| AN186*, AN187*, AN188*, AN204* | Eastern Poland | HQ292946 | –         | 0.82 ± 0.02 | 1.00 ± 0.25 |
| AN267* – ITEM 1357 |        | HQ292948 | –         | 1.35 ± 0.21 | 1.45 ± 0.17 |
| AN268* – ITEM 1591 |        | –                      | 1.25 ± 0.17 | 0.55 ± 0.20 |
| AN269* – ITEM 1594 |        | –                      | 2.50 ± 0.23 | 2.70 ± 0.32 |
| **T. vixens**|        |                            |             |        |     |      |      |                        |
| AN91         | Central Poland graden Kompost | HQ292860 | –         | 0.75 ± 0.15 | 1.15 ± 0.11 |
| AN94         | forest soil                  | HQ292873 | –         | 0.80 ± 0.12 | 3.53 ± 0.27 |
| AN101        | forest wood                  | HQ292868 | –         | 1.60 ± 0.21 | 2.58 ± 0.23 |
| AN108/AN110* |        |                            | HQ292869 | –         | 2.80 ± 1.10 | 1.80 ± 1.40 |
| **T. harzianum complex**|        |                            |             |        |     |      |      |                        |

*(a)Values expressed as mean ± standard error.*
Table I. Continued

| Culture code | Origin | Source | NCBI GenBank Accession No. | Cellulolytic activity (the plate method) | Xylanolytic activity (the plate method) |
|--------------|--------|--------|----------------------------|------------------------------------------|-----------------------------------------|
|              |        |        |                            |                                           |                                         |
| AN131*       | Central Poland | forest wood | 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 | 1.70 ± 0.21 |
| 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 | 2.04 ± 0.18 |
| AN137        |        |        | HQ292877  - | 1.60 ± 0.13 | 1.03 ± 0.12 |
| AN138        |        |        | HQ292861  - | 1.00 ± 0.10 | 1.41 ± 0.16 |
| AN139        |        |        | HQ292878  - | 0.87 ± 0.08 | 1.67 ± 0.16 |
| AN177*       |        |        | HQ292883  - | 1.25 ± 0.15 | 1.40 ± 0.11 |
| AN181        |        |        | HQ292875  - | 1.66 ± 0.12 | 2.25 ± 0.22 |
| AN203        | mushroom compost |        | HQ292879  - | 1.66 ± 0.13 | 1.00 ± 0.18 |
| AN205        |        |        | HQ292880  - | 1.36 ± 0.17 | 2.45 ± 0.25 |
| AN207        |        |        | HQ292881  - | 1.50 ± 0.13 | 1.00 ± 0.17 |
| AN211        |        |        | HQ292882  - | 1.10 ± 0.11 | 0.20 ± 0.11 |
| AN223        |        |        | HQ292902  - | 1.25 ± 0.10 | 1.05 ± 0.20 |
| AN225, AN238 | Forest soil |        | HQ292944  - | 1.10 ± 0.18 | 1.28 ± 0.11 |
|              |        |        | HQ292995  - | 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±1.70/1.70 | 1.27/1.40/1.75 |
| AN220 – ITEM 1328 | Bari, Italy |        |              | 0.25 ± 0.16 | 1.60 ± 0.13 |

**T. hamatum** (E)

| Culture code | Origin | Source | NCBI GenBank Accession No. | Cellulolytic activity (the plate method) | Xylanolytic activity (the plate method) |
|--------------|--------|--------|----------------------------|------------------------------------------|-----------------------------------------|
| AN118        | Central Poland | Forest wood | HQ292854 - | 0.66 ± 0.21 | 0.61 ± 0.19 |
| AN155        | Eastern Poland | Ryder Rhizosphere | HQ292851 - | 0.82 ± 0.18 | 0.51 ± 0.11 |
| AN175        | Central Poland | Forest wood | HQ292854 - | 0.53 ± 0.12 | 0.28 ± 0.10 |
| AN227, AN279, AN501*| Forest soil | HQ292853 - | 0.41 ± 0.21 | 0.84 ± 0.29 |
| AN521        | Northern Poland | Forest wood | HQ292856 - | 0.50/0.18/0.41 | 2.30/0.72/0.61 |

**T. atroviride** (F)

| Culture code | Origin | Source | NCBI GenBank Accession No. | Cellulolytic activity (the plate method) | Xylanolytic activity (the plate method) |
|--------------|--------|--------|----------------------------|------------------------------------------|-----------------------------------------|
| AN19         | Central Poland | Forest soil | HQ292786 HQ292963 | 0.67 ± 0.13 | 1.04 ± 0.16 |
| AN35         |        |        | HQ292787 HQ292953 | 0.35 ± 0.09 | 0.80 ± 0.19 |
| AN90         |        |        | HQ292788 HQ292954 | 0.49 ± 0.12 | 0.35 ± 0.12 |
| AN95         |        |        | HQ292789 HQ292955 | 0.90 ± 0.17 | 1.30 ± 0.18 |
| AN96         |        |        | HQ292790 HQ292956 | 0.95 ± 0.15 | 0.70 ± 0.14 |
| AN111        |        |        | HQ292791 HQ292964 | 0.37 ± 0.13 | 0.75 ± 0.21 |
strain *T. reesei* and that these differences were statistically significant (p ≤ 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* 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

| Culture code | Origin | NCBI GenBank Accession No. | Cellulolytic activity (the plate method) | Xylanolytic activity (the plate method) |
|--------------|--------|----------------------------|------------------------------------------|-----------------------------------------|
| AN152        | Central Poland | HQ292972, HQ292957 | 0.59 ± 0.19 | 1.85 ± 0.30 |
| AN155        | Central Poland | 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 | HQ292799, HQ292970 | 0.36 ± 0.09 | 0.58 ± 0.13 |
| AN240        | Mushroom compost | JX184119, JX184096 | 0.23 ± 0.19 | 0.57 ± 0.17 |
| AN287        | Central Poland | HQ292798 | 0.41 ± 0.11 | 0.58 ± 0.12 |

*T. longibrachiatum* (G)

| AN197        | Eastern Poland | Mushroom factory | HQ292780 | 0.77 ± 0.19 | 1.40 ± 0.22 |
| AN213        | Central Poland | Mushroom compost | HQ292781 | 0.30 ± 0.16 | 2.70 ± 0.31 |

*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 |
| AN201        | HQ292849 | 1.10 ± 0.21 | 2.08 ± 0.29 |

| AN262, AN303, AN393, AN500 | Southern Poland | Forest wood | JX184109 | 2.26 ± 0.85/1.75 ± 0.90 | 2.88 ± 0.61/0.63 ± 0.80 |

*T. pseudokoningii* (I)

| AN219 – ITEM1416 | Rari, Italy | – | – | 1.42 ± 0.19 | 2.10 ± 0.22 |

*T. koningii* (I)

| AN100        | Central Poland | Forest wood | HQ292803, HQ292975 | 0.35 ± 0.17 | 0.40 ± 0.17 |
| AN105        | HQ292905, HQ292977 | 0.17 ± 0.08 | 0.58 ± 0.14 |
| AN106        | HQ292906 | 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 – ITEM2688 | Rari, Italy | – | – | 0.20 ± 0.10 | 0.30 ± 0.12 |

*a* The isolates identified in this study by a combination of morphological and molecular analyses

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.
Screening and identification of Trichoderma 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

| Strains of Trichoderma | T. reesei | T. virens | T. atroviride |
|------------------------|-----------|-----------|---------------|
| Cellulolytic activity (FPU/ml) | QM 9414 | AN 68 | AN 69 | AN 73 | AN 108 | 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 |

* The value corresponding to difference in clearing zone diameter between analyzed strains
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 analyzed, 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 carcinogetic substances like mycotoxins (Jae-Hyuk and Keller, 2005; Błaszczyk et al., 2011, 2013, 2016) 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 (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, 2010). 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 cellulytic 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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