Pol. J. Environ. Stud. Vol. 22, No. 4 (2013), 1083-1090

Original Research

Application of Lignocellulosic Waste Materials for the Production and Stabilization of *Trichoderma* Biomass

Anna Kancelista[1]*, Urszula Tril[2], Regina Stempniewicz[1], Michał Piegza[1], Magdalena Szczech[3], Danuta Witkowska[1]

[1]Department of Biotechnology and Food Microbiology,
Wrocław University of Environmental and Life Sciences, Chełmońskiego 37/41, 51-630 Wrocław, Poland
[2]Department of Animal Products Technology and Quality Management,
Wrocław University of Environmental and Life Sciences, Chełmońskiego 37/41, 51-630 Wrocław, Poland
[3]Department of Vegetable Plant Protection, Research Institute of Horticulture in Skierniewice,
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Received: 18 December 2012
Accepted: 5 March 2013

Abstract

Our study investigated the growth and sporulation of *Trichoderma* strains in solid-state cultures on lignocellulosic materials and determined the survival and growth dynamics in bio-preparations after biomass drying at 30ºC. All waste materials used in the study turned out to be good media for the production of *Trichoderma* fungal biomass and the highest amount of colony-forming units per gram of dry matter (CFU/g DM), and a number of conidia per gram of dry matter (conidia/g DM) were produced on wheat bran and sugar beet pulp. The drying of biomass had no significant influence on the dynamics of fungal growth. Despite the prolonging of the lag phase and reduction in the maximum specific growth rate, the biomass yield of the analyzed strains was similar to the respective value noted in cultures before drying.

Keywords: lignocellulosic waste materials, solid-state cultures, biomass, *Trichoderma*, Bioscreen C system

Introduction

The fungi of the genus *Trichoderma* are commonly found in the environment in all types of soil. They colonize root systems of different cultivable and wild plants and grow on wood [1].

Widespread distribution of *Trichoderma* results from its capability to utilize diverse sources of carbon and nitrogen, whereas the formation of thick-walled chlamydospores enables some species (*T. hamatum*, *T. harzianum*, *T. viride*, *T. virens*) to survive under extreme conditions [2]. *Trichoderma* species compete with other microorganisms for nutrients and may inhibit their growth by secreting antibiotics and toxic compounds. These fungi may also parasitize other fungal species, including phytopathogenic ones, which leads to an effective reduction in the population of pathogenic species due to the production of a wide spectrum of lytic enzymes. This phenomenon is called mycoparasitism [3].

Trichoderma strains are known for their ability to colonize the rhizosphere systems of plants. Application of *Trichoderma* conidia on fruits, flowers, or leaves of plants allows for an effective control and reduces the incidence of diseases induced by phytopathogens. If conidia or fungal biomass are introduced into the soil, they colonize the surface of roots and the external layer of cortex. These zones

*e-mail: anna.kancelista@up.wroc.pl

then become the site of interaction where *Trichoderma prevails* and releases bioactive compounds. These molecules include mainly substances that stimulate the resistance mechanism in plants, i.e. the so-called elicitors: homologues of specific avirulence proteins (AVR) as well as enzymes and proteins with different functions. When leaves are invaded by pathogens, a plant immediately responds by producing enzymes and antimicrobial compounds [1, 3, 4].

Due to the above-mentioned characteristics, certain *Trichoderma* species are potential biological control agents (BCAs), i.e. organisms that are actively involved in biocontrol processes [5, 6]. The term "biocontrol" denotes the use of natural non-pathogenic microorganisms capable of suppressing plant pathogens and the progress of diseases. Owing to these properties, *Trichoderma* spp. are active constituents of numerous commercial biopreparations applied in the biocontrol of plant diseases [7].

Biological control of plant pathologies with antagonistic microorganisms is a natural and environmentally friendly alternative to chemical compounds that contaminate and accumulate as toxic substances in the environment. The production of biopreparations is a straightforward and cost-efficient process and is widely accepted by society [8].

Production of natural plant-protective preparations that contain an active agent in the form of conidiospores and mycelium of *Trichoderma* spp. on conventional, synthetic media with glucose, cellulose, soluble starch, and molasses requires a substantial financial input, which impedes commercial success and the planning of cost-efficient production on a mass scale [7]. A solution to this problem may be the application of cheap waste materials, for instance from cereal (such as cereal bran), starch, and fruit processing industries [9]. The process of production supplemented with the above-mentioned substrates is called solid-state fermentation (SSF). It is based on culturing microorganisms on solid carriers in order to generate a high concentration of cells.

The objective of this study was therefore to produce spores of four fungal species, i.e. *Trichoderma harzianum*, *T. atroviride*, *T. virens*, and *T. citrinoviridein* solid-state cultures on waste materials derived from processing of plant mass and to stabilize the biomass through drying.

Materials and Methods

Microorganisms

The study was carried out on the following strains: *Trichoderma harzianum* TRS 72, *T. virens* TRS 107, *T. atroviride* TRS 9 (Culture Collection of Microbiological Laboratory, Research Institute of Horticulture in Skierniewice), and *T. citrinoviride* C1 (Culture Collection of Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences).

For species identification, sequences of ITS1 and ITS2, tef1alpha, rpb2 and chi18-5, were obtained. A molecular identification based on the sequences of the internal transcribed spacer region (ITS1 and ITS2) were analyzed by TrichOKEY 2. Strain identification was confirmed by sequence similarity search, of the five loci: ITS1 and 2, tef1_int4(large), tef1_int5(short), tef1_exon6(large) and rpb2, against a *Trichoderma* database of vouchered sequences using TrichoBLAST program (Oskiera, unpublished data).

The tested strains were stored on PDA slants (24g/l) at 4ºC.

Lignocellulosic Waste Materials

Three types of lignocellulosic waste materials (sugar beet pulp, wheat bran, and corn cobs) were used in the study; they constituted the main source of carbon and energy to the tested microorganisms in solid-state cultures.

Methods of *Trichoderma* Culturing

Screening cultures (production of conidia) were performed on Petri dishes on the following agar media: Czapek-Dox, Czapek-Dox-W (with 2% addition of sugar beet pulp), Potato Dextrose Agar (PDA) – PDA-12 g/l, and PDA-24 g/l.

Inoculation cultures were carried out on Czapek-Dox-W agar medium with the addition of dried and milled sugar beet pulp (2%) in 1000 ml Roux flasks. Following incubation (25ºC, 7 days), the spores were washed off with 50 ml of 0.1% Tween solution. The suspension was then standardized in a Thoma chamber and used as inoculum in solid-state cultures.

Solid-state cultures. Thirty grams of dried and milled waste materials were placed in 400 ml Kolle flasks and then 60 ml volumes of water were added. After 60 minutes, humidity was measured with a WPS S moisture balance (Radwag) and next the flasks were sterilized (121ºC, 60 minutes). Following sterilization, water activity was determined in each culture medium with a LabMASTER-aw apparatus (Novasina). The prepared media (humidity: 65.6-68.8%) were inoculated with 20 ml of a fungal spore suspension to an initial density of 1.56×10^6-1.72×10^6 conidia/g DM of culture medium and incubated at 25ºC. Each culture was performed in two replications. The content of flasks was manually vortexed every few days, and after 14 days the biomass together with the medium was transferred onto sterile plastic trays and dried at 30ºC for 5 days. After drying, humidity was measured with a WPS S moisture balance (Radwag).

Microcultures of selected *Trichoderma* strains after 7-day cultures on PDA medium slants, 14-day cultures on lignocellulosic waste materials, and after drying were performed with a Bioscreen C microbiological analyzer. The microcultures were carried out on Potato Dextrose Broth (PDB) under the following conditions: incubation temperature 25ºC, incubation time 3 days, constant shaking, and optical density measurement (OD) in visible light (wideband filter) every 20 minutes. The growth of strains was recorded on curves that presented a correlation between optical density and time. All microcultures were performed in five replications. The control tubes contained uninoculated culture medium.

The selected parameters of growth kinetics (duration of the lag phase, maximum specific growth rate μmax and maximum biomass yield ΔOD_{max}) were determined based on the analysis of the growth curves.

Analytical Methods

The determination of viable cell counts (CFU/ml or CFU/g DM) was performed with the plate method by Koch on PDA medium with the addition of Rose bengal (0.035 g/l) from three dilutions at three replications.

The count of fungal spores was measured microscopically in a Thoma chamber.

Germination capacity of fungal spores was determined in 7-day cultures on PDA medium (control), 14-day solid-state cultures on lignocellulosic waste materials, and after drying of biomass on the carriers. The suspension of spores (0.05 ml) prepared by 100-fold dilution of post-culture biomass and drying in 0.1% Tween 80 solution was placed onto microscopic slides on 100 mm^2 surface. The slides with the conidial suspension were then put onto sterile, humid Petri plates and incubated (25ºC, 24 h). Germination capacity of spores was determined under an AXIO Scope.A1 fluorescent microscope (Carl Zeiss) at 400x magnitude. The photographs of germinating spores were taken with AXIO Vision Rel.4.7 software.

Statistical Analyses

The results of biomass yield produced with SSF culture and after drying were statistically processed with two-way analysis of variance (medium type; strain). The significant differences between the means were determined with Duncan test at p=0.05. The calculations were performed with Statistica 10 software.

Results

Apart from efficacy in eradicating plant pathogens and enhancing natural immunity against diseases induced by these agents, the cost of manufacturing is also important in the production of biopreparations. Waste materials originating from industries that process raw plant material seem a good and cheap source of energy and nutrients for microorganisms to be used in biocontrol. Furthermore, their utilization supports the protection of the environment polluted with an excessive amount of unmanaged industrial waste materials.

Trichoderma Cultures

In order to choose the most effective medium for the production of spores, screening cultures of selected *Trichoderma* strains, i.e. *T. harzianum* TRS 72, *T. virens* TRS 107, *T. atroviride* TRS 9, and *T. citrinoviride* C1, were run on four different agar media. The Czapek-Dox-W medium enriched with 2% of dried sugar beet pulp was found to be the best medium for producing conidia of the tested strains (1.19×10^8-1.74×10^8 conidia/ml) and was later used to prepare an inoculum for solid-state cultures. On the other media (PDA – 12g/l, PDA – 24g/l, Czapek-Dox), the tested fungal strains germinated weaker and their count was by 0.5-1 log lower than on the Czapek-Dox-W medium with sugar beet pulp (Fig. 1).

Production of *Trichoderma* Biomass on lignocellulosic Waste Materials

In the presented studies waste materials were used as a medium for biomass production in solid-state cultures. It was demonstrated that the growth of strains was differentiated depending on the type of lignocellulosic carrier applied. In all combinations of media, the number of conidia per g DM of medium was higher than the count of CFU/g DM of medium, which proved that not all of the spores were active and that a part of them could be dormant (Tables 1 and 2).

Two examined strains: *T. atroviride* TRS 9 and *T. citrinoviride* C1, grew most effectively and produced conidia on wheat bran, which yielded the CFU count between 4.37×10^8 and 6.90×10^8 per g DM of medium and the number of conidia at 1.48×10^9-3.13×10^9 per g DM of medium. In turn, the strains *T. virens* TRS 107 and *T. harzianum* TRS 72 produced the highest volume of biomass on sugar beet pulp, yielding the count of CFU at 2.35×10^8 and 4.92×10^8 per g DM of medium, respectively (Tables 1 and 2). However, the differences in biomass yield were insignificant depending on the type of lignocellulosic medium and the inquired *Trichoderma* strain (Table 3).

Preservation of *Trichoderma* Biomass on Lignocellulosic Carriers

The fungal biomass produced on lignocellulosic waste materials was stabilized by drying on plastic trays at 30ºC for 5 days with humidity being reduced to 5.6-9.5%. The count of CFU/g DM in the preparations of the tested strains was higher than in the cultures before drying, which indicated that the fungi were still growing during biomass stabilization.

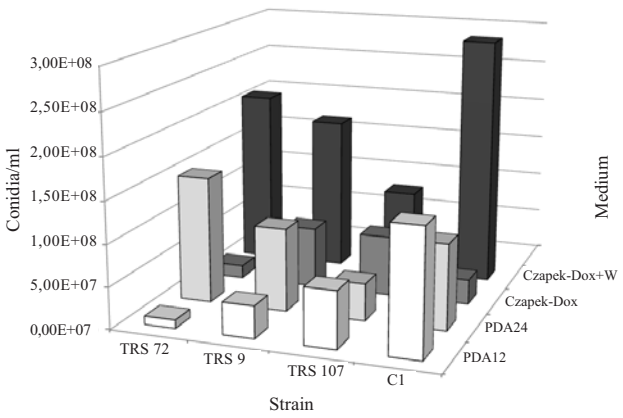

Fig. 1. Intensity of fungal sporulation in *Trichoderma* strains in 14-day plate cultures.

Table 1. Conidial counts per g DM in *Trichoderma* strains cultured on different waste materials.

Medium	Strain	Water activity (a_w)	Number of conidia/g DM		
			Initial state	After culturing	After drying
Corn cobs	*T. harzianum* TRS 72	0.940	1.67×10^{6}	3.94×10^{8}	8.98×10^{8}
	T. atroviride TRS 9	0.904	1.66×10^{6}	5.73×10^{8}	6.30×10^{8}
	T. virens TRS 107	0.961	1.64×10^{6}	1.85×10^{8}	2.26×10^{8}
	T. citrinoviride C1	0.921	1.71×10^{6}	1.23×10^{9}	6.76×10^{8}
Sugar beet pulp	*T. harzianum* TRS 72	0.908	1.66×10^{6}	2.04×10^{9}	3.89×10^{8}
	T. atroviride TRS 9	0.904	1.65×10^{6}	4.94×10^{8}	4.55×10^{8}
	T. virens TRS 107	0.981	1.57×10^{6}	3.08×10^{8}	7.47×10^{8}
	T. citrinoviride C1	0.929	1.72×10^{6}	1.53×10^{9}	9.74×10^{8}
Wheat bran	*T. harzianum* TRS 72	0.941	1.62×10^{6}	1.19×10^{9}	4.92×10^{8}
	T. atroviride TRS 9	0.922	1.61×10^{6}	1.48×10^{9}	-
	T. virens TRS 107	0.979	1.56×10^{6}	3.87×10^{7}	6.56×10^{7}
	T. citrinoviride C1	0.977	1.72×10^{6}	3.13×10^{9}	9.53×10^{8}

Table 2. CFU counts per g DM in *Trichoderma* strains cultured on different waste materials.

Medium	Strain	Water activity (a_w)	CFU/g DM		
			Initial state	After 14-day culture	After drying
Corn cobs	*T. harzianum* TRS 72	0.940	7.87×10^{5}	8.85×10^{7}	8.52×10^{8}
	T. atroviride TRS 9	0.904	4.44×10^{5}	1.69×10^{8}	5.97×10^{8}
	T. virens TRS 107	0.961	4.82×10^{5}	1.25×10^{8}	1.58×10^{8}
	T. citrinoviride C1	0.921	5.54×10^{5}	2.09×10^{8}	4.07×10^{8}
Sugar beet pulp	*T. harzianum* TRS 72	0.908	8.54×10^{5}	4.92×10^{8}	9.11×10^{8}
	T. atroviride TRS 9	0.904	7.44×10^{5}	2.33×10^{8}	3.10×10^{8}
	T. virens TRS 107	0.981	5.18×10^{5}	2.35×10^{8}	3.86×10^{8}
	T. citrinoviride C1	0.929	4.82×10^{5}	2.34×10^{7}	2.06×10^{7}
Wheat bran	*T. harzianum* TRS 72	0.941	7.25×10^{5}	2.44×10^{8}	4.04×10^{8}
	T. atroviride TRS 9	0.922	8.42×10^{5}	4.37×10^{8}	6.42×10^{8}
	T. virens TRS 107	0.979	3.88×10^{5}	1.43×10^{7}	5.78×10^{6}
	T. citrinoviride C1	0.977	5.18×10^{5}	6.90×10^{8}	9.77×10^{8}

Corn cobs were the best carriers; they generated a 0.2 – 10.0-fold higher increase of biomass (1.58×10^{8}-8.52×10^{8} CFU/g DM) as compared to the cultures before drying (8.85×10^{7}-2.09×10^{8} CFU/g DM) (Table 2). Less noticeable changes were reported in the formation of conidia. The temperature of drying did not exert any impact on the count of conidia in *T. atroviride* TRS 9 strain, whereas it had a negative influence on the number of conidia in *T. citrinoviride* C1, reducing it on all tested carriers by 36%-70% (6.76×10^{8}-9.74×10^{8} conidia/g DM) in comparison with their count before drying (1.23×10^{9}-3.13×10^{9} conidia/g DM). The culture of *T. virens* TRS 107 on all combinations of growth media produced a higher number of conidia by 22-142% (6.56×10^{7}-7.47×10^{8} conidia/g DM) as compared to the count before drying (3.87×10^{7}-3.08×10^{8} conidia/g DM) (Table 1). The comparison of the number of CFU/g DM and the count of conidia/g DM, depending on strain and biomass carrier, did not reveal any statistical difference between them (Table 3).

Table 3. Mean biomass yield expressed as the count of CFU/g DM and the count of conidia/g DM after SSF culture and post-drying (p=0.05).

Variability		Conidia/g DM		CFU/g DM	
		After culture	After drying	After culture	After drying
Medium	Sugar beet pulp	1.09×10^{9} a	6.41×10^{8} a	2.46×10^{8} a	4.53×10^{8} a
	Corn cobs	5.96×10^{8} a	6.08×10^{8} a	1.48×10^{8} a	5.04×10^{8} a
	Wheat bran	1.46×10^{9} a	5.04×10^{8} a	3.46×10^{8} a	5.07×10^{8} a
Strain	*T. harzianum* TRS 72	1.21×10^{9} a	5.93×10^{8} a	1.24×10^{8} a	7.22×10^{8} a
	T. atroviride TRS 9	8.49×10^{8} a	5.43×10^{8} a	2.79×10^{8} a	5.16×10^{8} a
	T. virens TRS 107	1.77×10^{8} a	3.46×10^{8} a	1.25×10^{8} a	1.83×10^{8} a
	T. citrinoviride C1	1.96×10^{9} a	8.67×10^{8} a	3.07×10^{8} a	5.30×10^{8} a

Evaluation of Germination of *Trichoderma* Conidia

The high post-preservation density of *Trichoderma* spores, which can be potentially used in the manufacturing of biopreparations, is not necessarily associated with their efficacy in biocontrol. The viability and growth dynamics of fungi are very important parameters. Two strains, i.e. *T. harzianum* TRS 72 and *T. virens* TRS 107, whose conidial count ranged between 2.26×10^{8} and 8.98×10^{8} conidia/g DM in the biopreparations after drying, were selected to determine growth activity and spore germination.

Germination of spores was evaluated on microscopic slides after 24-hour incubation. The conidia of *T. harzianum* TRS 72 and *T. virens* TRS 107 produced on solid-state cultures on lignocellulosic waste materials showed higher intensity of germination than the conidia generated on the control PDA medium, which was particularly tangible in the cultures on wheat bran. Drying at 30ºC resulted in a reduction of the viability of conidia produced in the cultures of tested strains on wheat bran and sugar beet pulp. The dried conidia produced in the cultures on corn cobs showed a good germination capacity, yet with a lower growth activity, which was indicated by shorter hyphae growth after 24-hour incubation in comparison with the length of hyphae in conidia originating from the cultures before drying (Fig. 2).

Evaluation of Selected Parameters of Growth Dynamics in *Trichoderma* Strains

The dynamics of growth in *T. harzianum* TRS 72 and *T. virens* TRS 107 strains was determined in Bioscreen C on PDB medium with conidia that originated from:

a) 7-day culture on PDA slants
b) 14-day culture on lignocellulosic waste materials
c) stabilized biopreparations

The selected parameters of growth kinetics were determined based on the growth curves (Table 4).

The lag phase in *T. harzianum* TRS 72 strain in solid state cultures was longer by 5 to 10 hours than in the control cultures. The longest lag phase was recorded in the culture on wheat bran (20 hours), whereas the shortest one was on corn cobs (15 hours). The rate of growth of the tested strain was approx. 2 times higher than in the control culture (μ_{max}= 0.160-0.194 h^{-1}). The yield of biomass on lignocellulosic waste materials was also higher by 14-19% than on the control PDA medium (ΔOD_{max}= 1.707-1.786).

In turn, the growth of *T. virens* TRS 107 strain in cultures on waste materials and in control PDA medium was comparable. The lag phase was longer only by 1-2 hours on the lignocellulosic media, and the specific growth rate (μ_{max}= 0.135-0.158 h^{-1}) and biomass yield (ΔOD_{max}= 1.660-1.720) were higher by 8-23% than in the control culture.

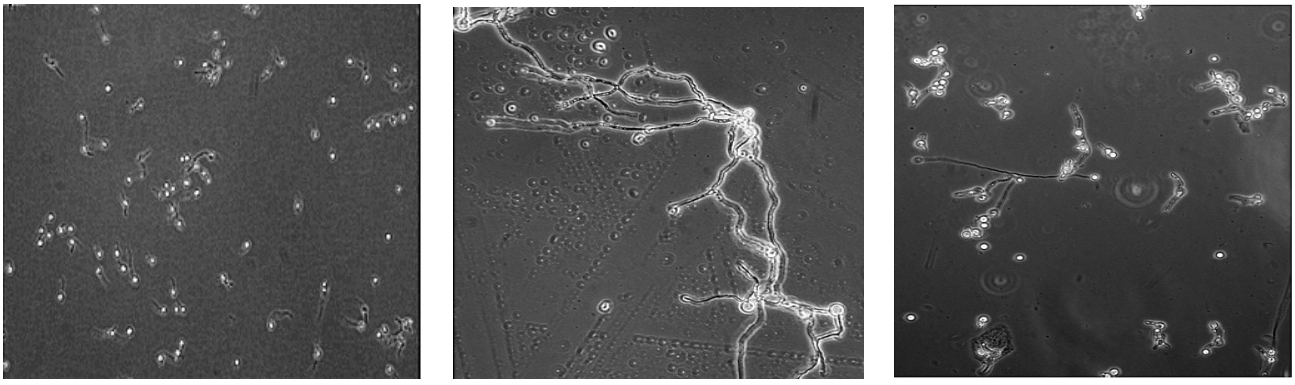

Fig. 2. Germination of *Trichoderma harzianum* TRS 72 conidia after 24-hour incubation on microsposcopic slides (a – 7-day culture on control PDA medium, b – 14-day culture on corn cobs, c – conidia on corn cobs subjected to drying at 30ºC).

Table 4. Selected parameters of growth kinetics in *T. harzianum* TRS 72 and T. virens TRS 107.

Strain	Medium	Lag phase [h]		μ_{max} [h^{-1}]		ΔOD_{max}	
		After culture	After drying	After culture	After drying	After culture	After drying
T. harzianum TRS 72	PDA*	10.5		0.081		1.497	
	Corn cobs	15.0	23.0	0.160	0.130	1.786	1.418
	Sugar beet pulp	17.5	35.0	0.181	0.161	1.782	1.768
	Wheat bran	20.0	27.0	0.194	0.174	1.707	1.959
T. virens TRS 107	PDA*	18.3		0.136		1.397	
	Corn cobs	19.8	15.7	0.135	0.114	1.668	1.743
	Sugar beet pulp	18.2	17.0	0.153	0.112	1.660	1.748
	Wheat bran	20.2	25.7	0.158	0.045	1.720	1.266

*culture on PDA slants: control

The drying of fungal biomass on carriers resulted in the prolongation of the lag phase by 1.0-17.5 hours (lag phase: 15.7-35.0 hours) and reduction of growth rate by 11-72% (μ_{max}= 0.045-0.174 h^{-1}) in relation to the value of this coefficient in cultures before drying (μ_{max}= 0.135-0.194 h^{-1}). The yield of biomass did not change significantly (ΔOD_{max}= 1.418-1.959) as a result of the stabilization process.

Discussion

Members of the genus *Trichoderma* are widespread in the environment and may be potential components of preparations used in biocontrol of plant diseases owing to numerous distinguishing characteristics such as the capability of producing antibiotics [11] and lytic enzymes involved in mycoparasitism [12], as well as the abilities to colonize root systems and induce natural resistance in plants [13].

In recent years, a number of commercial biopreparations containing different *Trichoderma* strains and other fungal species have been developed in the USA, Israel, the Czech Republic, and New Zealand [4, 14, 15]. In Polish climatic conditions foreign preparations do not produce satisfactory results and therefore a search is underway for local strains of different microorganisms, including *Trichoderma*, with beneficial properties desirable for the production of biopesticides useful in moderate climate. In the manufacturing process an important step is to prepare a large volume of inoculum containing conidia or a mixture of conidia and mycelium for inoculation of production media.

In the presented study, the screening cultures allowed us to select the most effective medium for sporulation, i.e. a 7-day culture on Czapek-Dox-W agar medium with 2% sugar beet pulp that generated 1.19×10^{8}-1.74×10^{8} conidia/ml. Sugar beet pulp enriched the culture medium in additional nutrients (proteins, sugars, calcium, phosphorus), which stimulated fungal growth and spore formation. Watanabe et al. [16] reported slightly lower results, i.e. 6.90×10^{7} conidia/ml in a 7-day culture of *T. asperellum* SKT1 strain on PDA medium. Likewise, Munoz et al. [17] found that the culturing on agar medium was the most effective method to produce high numbers of *T. harzianum* P1 conidia. Conidia produced in this way are more resistant to stress factors such as drying, changes in water activity (a_w), or radiation. They are also characterized by high hydrophobic properties, which facilitate their rapid and effective adhesion to a target surface.

The next step after preparation of inoculum with proper density was the selection of the medium and the appropriate culturing method. Biopreparations are most often produced in solid-state cultures on different carriers such as waste materials and by-products of industries that process plant raw materials which are widely available, acceptable, and inexpensive [7, 18].

In the presented studies, wheat bran, sugar beet pulp and corn cobs were used as lignocellulosic carriers in solid-state cultures of *Trichoderma* strains. These industrial waste materials and by-products contained substances essential for fungal growth, i.e. the source of carbon, nitrogen and mineral salts, and had a pre-inoculation humidity of 65.6-68.8%. Orzua et al. [19] report that the content of water in fungal culture media should range from 30 to 80% depending on the carrier. In the production of biopreparations it is important to know not only the content of water, but also its availability for metabolic processes. The activity of water in the tested media was diverse and ranged between 0.904 and 0.981. Cavalcante et al. [9] indicated that water absorption capacity depended on a number of factors such as structure, surface of carrier, and ability to form hydrogen bonds [20]. They also demonstrated that considerable deviations in water activity in waste materials could be observed due to the inhomogeneity of their structure.

In each of the culture variants with tested *Trichoderma* strains, the yield of biomass was higher on average by 2-3 log in comparison with the amount of introduced inoculum. *T. harzianum* TRS 72 and *T. virens* TRS 107 strains were growing and sporulating most effectively in the cultures on sugar beet pulp, whereas *T. atroviride* TRS 9 and

T. citrinoviride C1 strains – on wheat bran. In contrast, the tested strains grew the least effectively on corn cobs. Similar correlations have been reported by other authors. Tewari and Bhanu [8] also produced a higher volume of *T. harzianum* biomass (3.47×10^8 CFU/g DM) on sugar cane pulp with the chemical composition similar to that of sugar beet pulp than on dried corn cobs (2.57×10^8 CFU/g DM).

Cavalcante et al. [9] were running cultures of different strains: *T. harzianum*, *T. viride*, *T. koningi*, and *T. polysporum* on wheat bran which yielded 1.75×10^8-2.41×10^9 conidia/g DM; these results were comparable with the value recorded in our studies (3.87×10^8-3.13×10^9 conidia/g DM). According to Tewari and Bhanu [8], wheat bran suppresses mycelium growth yet stimulates the production of conidia, which is confirmed by the results of the presented study. The cultures on wheat bran yielded the count of conidia per g DM higher by 0.7 log than CFU/g DM.

The method of stabilization of biopreparations should preserve high viability of fungi and their technological characteristics as well as facilitate their storage. The biomass of the tested fungi produced on lignocellulosic waste materials was dried at 30ºC. The process of biomass drying on carriers resulted in its growth in all culture variants with the highest value, i.e. by 0.2-1.0 log, reported in the cultures on corn cobs compared to the culture before drying. The high viability of fungal biomass produced on corn cobs after drying was probably associated with their loose structure and large surface to volume ratio, which increases fungal growth potential. Ooijkaas et al. [18], in their studies on the use of waste materials as media for fungal growth, emphasize that strains, during their growth, degrade carriers in order to utilize nutrients. Corn cobs are probably raw material that has relatively stable physical parameters, which in turn allow fungi to grow during 30ºC drying. Moreover, a beneficial ratio of carbon-to-nitrogen (C/N) in raw materials may exert a considerable impact on fungal sporulation. In the case of corn cobs, the C/N ratio is high (65.2), which is associated with reduction in nitrogen content. Olsson et al. [21] observed that the decrease in C/N ratio in *Trichoderma reesei* Rut-30 cultures resulted in a reduction in the number of spores.

The analysis of fungal growth dynamics was carried out in Bioscreen C, which is an automated system capable of simultaneous monitoring of 200 cultures. Rossi-Rodrigues et al. [22] demonstrated that it could be successfully used for evaluating an impact of carbon source on the growth potential of different *Trichoderma* strains. In our study, lignocellulosic waste materials were found to be good media for biomass production and maintenance of a high growth capacity of the tested strains. The increase in the growth rate and biomass yield was recorded in comparison with the control cultures on PDA medium. The microorganisms only required a longer period to adapt to the conditions of the experimental medium. In turn, drying had a negative influence on the parameters of growth kinetics. The strains showed a prolonged lag phase, reduced specific growth rate (μmax) and lower biomass yield than before drying. *T. harzianum* TRS 72 was more susceptible to stabilization than *T. virens* TRS 107.

Pedreschi and Aguilera [23] demonstrated that commercial biopreparations stabilized by drying should be characterized by a minimal reduction in viability during 18-month storage, and therefore our studies will continue to evaluate the viability and growth dynamics of stored *Trichoderma* biopreparations.

Conclusions

Lignocellulosic waste materials used as media in solid-state cultures of *Trichoderma* strains turned out to be good media for biomass production and the tested strains showed diversified, yet statistically insignificant growth, depending on the type of medium.

T. harzianum TRS 72 and *T. virens* TRS 107 strains produced the highest biomass yield in the cultures on sugar beet pulp. In the case of *T. atroviride* TRS 9 and *T. citrinoviride* C1 strains, the highest biomass yield was detected on wheat bran. The biomass generated in the cultures on solid substrates with two selected strains, i.e. *T. harzianum* TRS 72 and *T. virens* TRS 107, showed a good germination capacity and a high growth activity expressed in selected parameters of growth kinetics in comparison with the control cultures. The growth rate and biomass yield increased, whereas only the duration of lag phase increased, compared to the control culture on PDA medium.

The process of drying on carriers at 30ºC resulted in the increase in fungal biomass in all culture variants with the highest increase in the cultures on corn cobs. The increment in biomass volume indicated further growth of fungi during drying under long (5 days) influence of temperature being slightly higher than the optimal for fungal growth. Stabilization of biomass had a negative impact on the germination capacity of conidia and duration of the lag phase and specific growth rate (μ_{max}), whereas it did not exert any significant impact on the maximum biomass yield (ΔOD_{max}).

Acknowledgements

Our study was a part of the project "Polish *Trichoderma* strains in plant protection and organic waste management," under Priority 1.3.1, subject area "Bio," co-financed by the European Union through the European Regional Development Fund within the Innovative Economy Operational Program, 2007-2013. Project No. UDA-POIG.01.03.01-00-129/09-05.

References

1. HARMAN G.E., HOWELL C.R., VITERBO A., CHET I., LORITO M. *Trichoderma* species – opportunistic, avirulent plant symbionts, Nat. Rev. Microbiol. **2**, (1), 43, **2004**.
2. KREDICS L., ANTAL Z., MANCZINGER L., SZEKERES A., KEVEI F., NAGY E. Influence of environmental parameters on *Trichoderma* strains with biocontrol potential, Food Technol. Biotechnol. **41**, (1), 37, **2003**.

3. MUKERJI K.G., CHAMOLA B.P., UPADHYAY R.K. (EDS). Biotechnological Approaches in Biocontrol of Plant Pathogens, Kluwer/Plenum, New York, **1999**.
4. WOJTKOWIAK-GĘBAROWSKA E. Mechanisms of biological control soil-borne plant pathogen by fungus from genus *Trichoderma*, Post. Mikrobiol. **45**, (4), 261, **2006** [In Polish].
5. HARMAN G.E. Overview of mechanisms and uses of *Trichoderma* spp., Phytopathology, **96**, (2), 190, **2006**.
6. SCHUSTER A., SCHMOLL M. Biology and biotechnology of *Trichoderma*, Appl. Microbiol. Biotechnol. **87**, (3), 787, **2010**.
7. VERMA M., BRAR S.K., TYAGI R.D., SURAMPALLI R.Y., VALERO J.R. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control, Biochem. Eng. J. **37**, (1), 1, **2007**.
8. TEWARI L., BHANU C. Evaluation of agro-industrial wastes for conidia based inoculum production of bio-control agent: *Trichoderma harzianum*, J. Sci. Ind. Res. **63**, 807, **2004**.
9. CAVALCANTE R.S., LIMA H.L.S., PINTO G.A.S., GAVA C.A.T., RODRIGUES S. Effect of moisture on *Trichoderma* conidia production on corn and wheat bran by solid state fermentation, Food Bioprocess Technol. **1**, 100, **2008**.
10. PIONTEK M. Filamentous fungi. Atlas. Printing House of the University of Zielona Góra, **1999** [In Polish].
11. WIEST A., GRZEGORSKI D., XU B.W., GOULARD C., REBUFFAT S., EBBOLE D.J., BODO B., KENERLEY C. Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase, J. Biol. Chem. **277**, (23), 20862, **2002**.
12. KUBICEK C.P., MACH R.L., PETERBAUER C.K., LORITO M. *Trichoderma*: From genes to biocontrol, J. Plant Pathol. **83**, 11, **2001**.
13. HOWELL C.R. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evaluation of current concepts, Plant Dis. **87**, (1), 4, **2003.**
14. KURZAWIŃSKA H., MAZUR S. Usefulness of *Pythium oligandrium* in the protection of potato against some diseases, Progr. Plant Prot. **47**, (4), 185, **2007** [In Polish].
15. CWALINA-AMBROZIAK B., AMAROWICZ R. Effects of biological and fungicidal environmental protection on chemical composition of tomato and red pepper fruits, Pol. J. Environ. Stud. **21**, (4), 831, **2012**.
16. WATANABE S., KATO H., KUMAKURA K., ISHIBASHI E., NAGAYAMA K. Properties and biological control activities of aerial and submerged spores in *Trichoderma asperellum* SKT-1, J. Pestic. Sci. **31**, (4), 375, **2006**.
17. MUÑOZ G.A., AGOSIN E., COTORAS M., SAN MARTIN R., VOLPE D. Comparison of aerial and submerged spore properties for *Trichoderma harzianum*, FEMS Microbiol. Lett. **125**, (1), 63, **1995**.
18. OOIJKAAS L.P., WEBER F.J., BUITELAAR R.M., TRAMPER J., RINZEMA A. Defined media and inert supports: their potential as solid-state fermentation production systems, Trends Biotechnol. **18**, (8), 356, **2000**.
19. ORZUA M.C., MUSSATTO S.I., CONTRERAS-ESQUIVEL J.C., RODRIGUEZ R., de la GARZA H., TEIXEIRA J.A., AGUILAR C.N. Exploitation of agro industrial wastes as immobilization carrier for solid-state fermentation, Ind. Crops Prod. **30**, (1), 24, **2009**.
20. PANDEY A., SOCCOL C.R., MITCHELL D. New developments in solid state fermentation: I-bioprocess and products, Proc. Biochem. **35**, (10), 1153, **2000**.
21. OLSSON L., CHRISTENSEN T., HANSEN K.P., PALMQVIST E.A. Influence of the carbon source on production of cellulases, hemicellulases and pectinases by *Trichoderma reesei* Rut C-30, Enzyme Microb. Technol. **33**, (5), 612, **2003**.
22. ROSSI-RODRIGUES B.C., BROCHETTO-BRAGA M.R., TAUK-TORNISIELO S.M., CARMONA E.C, ARRUDA V.M, NETTO J.C. Comparative growth of *Trichoderma* strains in different nutritional sources, using Bioscreen C automated system, Braz. J. Microbiol. **40**, (2), 404, **2009**.
23. PEDRESCHI F., AGUILERA J.M. Viability of dry *Trichoderma harzianum* spores under storage, Bioproc. Biosyst. Eng. **17**, (3), 177, **1997**.