www.czasopisma.pan.pl PAN www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 54, No. 4 (2014)

DOI: 10.2478/jppr-2014-0047

Review

Trichoderma spp. – application and prospects for use in organic farming and industry

Lidia Błaszczyk[1], *Marek Siwulski*[2], *Krzysztof Sobieralski*[2], *Jolanta Lisiecka*[2], *Małgorzata Jędryczka*[1]*

[1] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland
[2] University of Life Sciences, Department of Vegetable Crops, Dąbrowskiego 159, 60-594 Poznań, Poland

Received: August 29, 2014
Accepted: October 19, 2014

Abstract: Fungi of the genus *Trichoderma* are a very large group of microorganisms that play a significant role in the environment. They use a variety of mechanisms to colonise various ecological niches. Several *Trichoderma* spp. positively affect plants by stimulating plant growth, and protecting plants from fungal and bacterial pathogens. They are used in biological plant protection as biofungicides as well as in bioremediation. Members of the genus *Trichoderma* are also utilised in various industry branches – mainly in the production of enzymes, antibiotics, and other metabolites, but also of biofuel. Moreover, the genus *Trichoderma* comprises edible and medicinal mushrooms, but also the pathogens of humans. Currently, *Trichoderma* has entered the genomic era and parts of genome sequences are publicly available. This is why, *Trichoderma* fungi have the potential to be used for human needs to an even greater extent than before. Nevertheless, further studies are needed to increase the efficiency and safety of the application of these fungi.

Key words: biological plant protection, cell wall degrading enzymes, green mould

Introduction

Fungi from the genus *Trichoderma* are commonly found in all climatic zones. The most typical habitats of these fungi include soil and rotting wood (Samuels 1996; Druzhinina *et al*. 2006). These fungi may be found on sclerotia and other propagating forms of fungi in the soil environment. They colonise the grain, leaves, and roots of plants (Harman *et al*. 2004). They were also isolated from such unusual sources as marine bivalves, shellfish, and termites. Fungal species from the genus *Trichoderma* are characterised by rapid growth and abundant production of conidial spores as well as the capacity to produce sclerotia. These species produce several pigments, ranging from a greenish-yellow up to a reddish tinge, although some colourless specimens are also present. The conidia may also have diverse colouration, ranging from colourless to different hues of green or even grey or brown tinges (Fig. 1).

In addition to the industrial importance of the genus, certain *Trichoderma* species have the ability to antagonise plant pathogens (Howell 2003; Benitez *et al*. 2004; Schuster and Schmoll 2010). On the other hand, some representatives of the genus have been reported to be emerging opportunistic pathogens of humans (Kredics *et al*. 2003; Druzhinina *et al*. 2008) and as causative agents of green mould, a disease which causes great losses in the production of cultivated mushrooms (Samules *et al*. 2002; Hatvani *et al*. 2007; Komon-Zelazowska *et al*. 2007). In general, relevant studies cover a wide range – from the molecular level to field and pre-industrial applications (Kubicek *et al*. 2001).

Systematics

The genus *Trichoderma* belong to: the phylum Ascomycetes, class Sordariomycetes, order Hypocreales, family Hypocreaceae. The systematics and taxonomy of these fungi have evolved since 1794 when Persoon (1794) introduced the name *Trichoderma*. In 1865, the Tulasne brothers showed that *Hypocrea rufa* is the teleomorph of *Trichoderma viride* Pers. (Tulasne and Tulasne 1860). Up to 1969 it was reported that within the genus *Trichoderma* there is only one species, namely *T. viride* (Bisby 1939). Then in 1969, on the basis of an analysis of morphological characteristics, Rifai distinguished nine "aggregate species": *T. harzianum* Rifai, *T. viride*, *T. hamatum* (Bonord.) Bainier, *T. koningii* (Oudem.) Duché & R. Heim, *T. polysporum* (Link) Rifai, *T. piluliferum* J. Webster & Rifai, *T. aureoviride* Rifai, *T. longibrachiatum* Rifai, and *T. pseudokoningii* Rifai. In the early 1990's, Bissett (1991a-c) identified five sections and 27 biological species within the genus *Trichoderma.* The introduction of tools like restriction fragment length polymorphism markers (RFLP),

*Corresponding address:
mjed@igr.poznan.pl

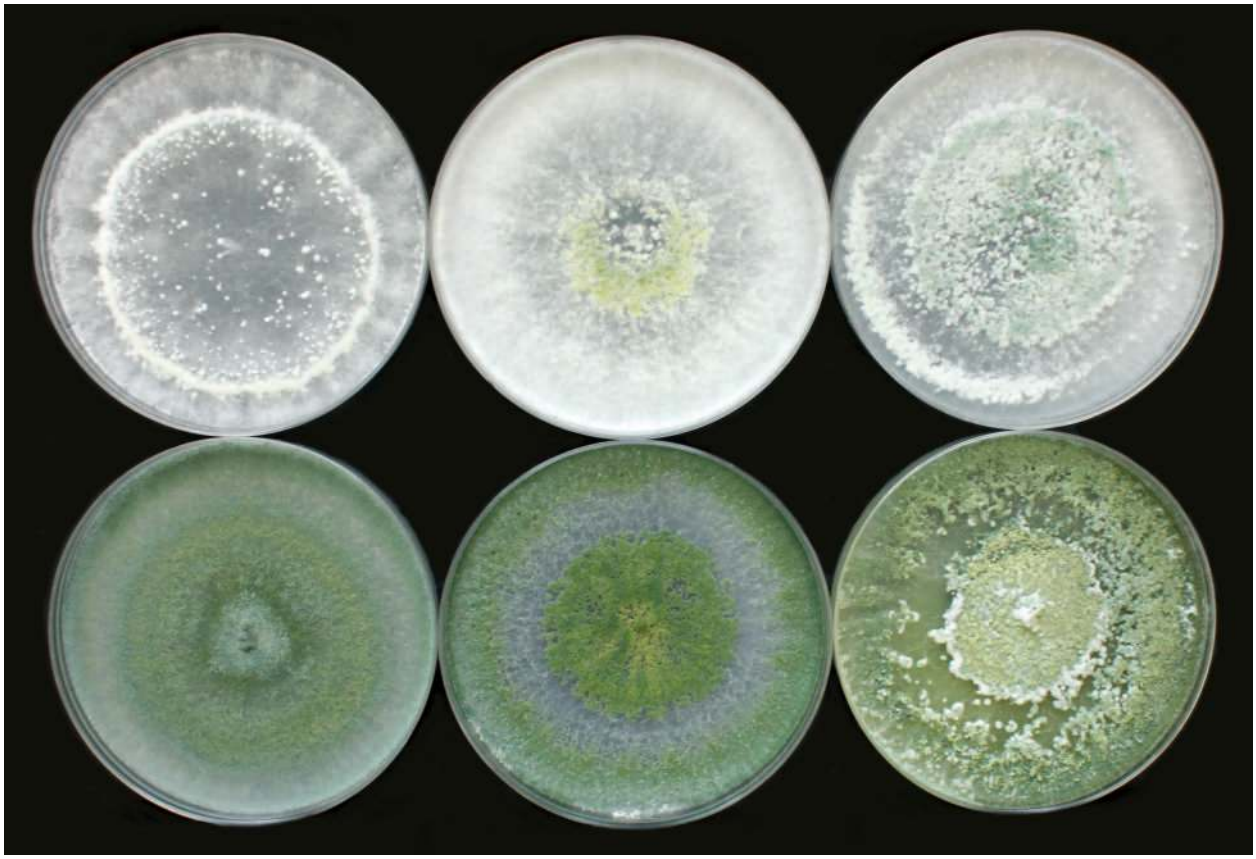

Fig. 1. The variation of colours and morphology of the fungal colonies of *Trichoderma* spp. are shown on Potato Dextrose Agar (PDA) medium. Upper row, then lower row (from left) are: *T. cremeum* (strain AN 392), *T. longipile* (AN 414), *T. viride* (AN 253), *T. harzianum* (AN 261), *T. atroviride* (AN 287), and *T. citrinoviride* (AN 262)

random amplified polymorphic DNA markers (RAPD) and coding sequence variation phylogenetic markers for molecular species identification, had a considerable influence on the development of taxonomy at that time. From the late 1990's up to the year 2002, the number of *Trichoderma* species increased to 47 (Kullnig-Gradinger *et al*. 2002). The verification of the taxonomy of the entire genus was started by Kindermann *et al*. (1998) who analysed the internal transcribed region 1 (ITS1) sequence of the rRNA coding region. Further development of molecular methods, including the presentation of the first fungal oligonucleotide barcode for the identification of *Hypocrea* and *Trichoderma* species – *TrichO*Key version 1.0 (Druzhinina *et al*. 2005), has contributed to the doubling of the number of newly described species. At present, the International Subcommission on *Trichoderma/Hypocrea* (http://www.isth.info/biodiversity//index.php) lists 104 species established on the basis of phylogenetic analyses. According to Jaklitsch (2009), 75 species have been identified in Central Europe. However, it should be stressed that the number of the so-called morphological species has not increased dramatically and at present it amounts to 1/3 of the species described on the basis of molecular analyses.

Interaction of *Trichoderma* with other microorganisms and plants

Fungi from the genus *Trichoderma*, due to their colonisation of different environments, are forced to compete for nutrients and space with many other organisms. The mechanisms facilitating colonisation of different ecological niches are well-developed and highly diverse in *Trichoderma* spp. (Herrera-Estrella and Chet 2004; Harman 2006; Vinale *et al*. 2008). *Trichoderma* interacts with other microorganisms, but mainly with pathogenic fungi. These interactions include hyperparasitism, competition, and antibiosis.

Hyperparasitism is connected with the direct contact of an antagonist with a pathogen and is composed of such stages as: pathogen recognition, attack, gradual penetration of the pathogen cells and death (Vinale *et al*. 2008). In this process a considerable role is played by CWDE (Cell Wall Degrading Enzymes) lytic enzymes, synthesised by *Trichoderma* species that facilitate hydrolytic degradation of pathogen cell walls, composed of chitin and glucan polysaccharides. *Trichoderma* species are also capable of producing cell wall degrading enzymes such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease (Strakowska *et al*. 2014) as well as many volatile metabolites, such as 6-n-pentyl-2H-pyran-2-one (6-PAP) (Jeleń *et al*. 2013). Chitinases are the most important lytic enzymes playing a key role in the degradation of cell walls of other plant pathogenic fungi. Chitinolytic enzymes identified in *Trichoderma* species (*T. harzianum*, *T. atroviride* P. Karst. and *T. asperellum* Samuels, Lieckf. & Nirenberg) include β-N-acetylglucosamidase, endochitinase, and chitobiosidase (Haran *et al*. 1996). Other enzymes determining the capacity of *Trichoderma* fungi for hyperparasitism, mainly in relation to fungus-like organisms, i.e. *Phytophthora* sp.

and *Pythium* sp. are β-1,3- and β-1,6-glucanases (Lorito *et al*. 1994; Thrane *et al*. 1997). An important role is also attributed to proteolytic enzymes (endo- an exoproteases), which in *Trichoderma* are responsible for the control of exocellular enzyme secretion. The proteolytic enzymes influence the activity and stability of exocellular enzymes and participate in post-secretion modifications of cellulases (Viterbo *et al*. 2004). Proteolytic enzymes also affect enzymatic activity of pathogenic fungi such as *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium culmorum*, and nematode *Meloidogyne javanica* (Sharon *et al*. 2001). Cellulases form yet another group of enzymes produced by the *Trichoderma* species. These enzymes are capable of hydrolysing lignocellulose biomass and comprise three types of enzymes which act synergistically. The three types are exo-β-1,4-glucanases, endo-β-1,4-glucanases, and β-glucosidases (Enari and Niku-Paavola 1987).

As often reported in scientific research articles, species from the genus *Trichoderma* compete with pathogens for nutrients, ecological niches or infection sites on plant roots. Certain *Trichoderma* strains are capable of producing siderophores, which are iron chelating compounds. By trapping iron, ions from the environment certain *Trichoderma* strains inhibit the growth of other fungi, e.g. *B. cinerea*, which is strongly affected by a nutrient deficit. *Trichoderma* fungi may also acidify the environment, producing adverse conditions for the development of other fungi (Benitez *et al*. 2004). Furthermore, *Trichoderma* fungi are capable of intensive colonisation of the plant root zone. Benitez *et al*. (2004) claimed that the mechanisms involved in this process are similar to those in mycorrhizal fungi. In the *T. harzianum* strain, an enhanced activity of the so-called hydrophobins was shown during plant root colonisation, causing attachment of mycelial filaments to hydrophobic root surfaces. In addition, *Trichoderma* strains are resistant to toxic compounds produced by plants in response to infection, e.g. phytoalexins, flavonoids, terpenoids, and phenols, which prevent root colonisation.

Antibiosis is a specific mechanism of antagonistic interactions between *Trichoderma* fungi and other pathogenic fungi. Antibiosis is based on the generation of secondary metabolites, which exhibit an inhibitory or lethal effect on a parasitic fungus. In fungal species from the genus *Trichoderma*, over 180 secondary metabolites have been characterised up to date, representing different classes of chemical compounds (Gams and Bisset 1998; Reino *et al*. 2008). These compounds may be divided into volatile antibiotics, water soluble compounds and peptaibols. *T. viride*, *T. harzianum*, and *T. koningii* are able to produce 6PAP (6-pentyl-α-pyrone) that belongs to the group of volatile antibiotics playing a role in biocontrol of such species as *B. cinerea*, *R. solani*, and *Fusarium oxysporum*. Peptaibols are polypeptide antibiotics of 500–2200 Da, rich in non-proteinogenic amino acids, in particular alfa-aminoisobutyric acid, and their characteristic attributes include the presence of N-acetylated ends and C-end amino alcohols (Degenkolb *et al*. 2003). Peptaibols exhibit potent activity against a number of fungi and Gram-positive bacteria as well as against some viruses. Compounds belonging to this class include trichotoxins A and B, trichodecenins, trichorovins, and trichocellins synthesised by *T. viride*; trichorzianins A and B, trichorzins HA and MA found in the cultures of *T. harzianum*; tricholongins BI and BII, and longibrachins produced by *T. longibrachiatum*; trichokonins originating in cultures of *T. koningii*, as well as atroviridins A-C and neoatroviridins A-D isolated from cultures of *T. atroviride*. Other compounds with antibacterial and fungicidal properties (*R. solani, F. oxysporum, Botrytis alli, Penicilium expansum, Aspergillus niger, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Bacterioides fragilis*) include koningins, viridin, dermadin, trichoviridin, lignoren, and koningic acid, produced by *T. koningii*, *T. harzianum*, *T. aureoviride*, *T. viride*, *T. virens* (J.H. Mill., Giddens & A.A. Foster) Arx, *T. hamatum*, and *T. lignorum* (Tode) Harz (Reino *et al*. 2008). When discussing secondary metabolites produced by fungi from the genus *Trichoderma*, it should be noted that literature sources have reported examples of isolates that were able to synthesise compounds considered toxic to humans and animals. Gliotoxin was the first toxin detected in those species. Production of this mycotoxin was observed in certain isolates belonging to such species as *T. lignorum*, *T. virens* (*G. virens*), *T. viride*, and *T. hamatum* (Reino *et al*. 2008). Corley *et al*. (1994) identified strains of *T. harzianum* capable of synthesising harzianum A toxin, then 10 years later Gallo *et al*. (2004) isolated from the same species a gene (*tri5*) homologous to the gene encoding trichodiene sythetase in *Fusarium* spp.

In *Trichoderma* fungi, the activity of antibiotics is frequently combined with the activity of lytic enzymes. Their joint action provides a higher level of antagonism compared to the activity of either enzymes or antibiotics alone (Monte 2001). According to Howell (2003), preliminary degradation of the cell walls in *B. cinerea* and *F. oxysporum* by lytic enzymes facilitated easier penetration of antibiotics to pathogen cells.

When considering the interactions of *Trichoderma* fungi with plants, it was found that these fungi have an advantageous effect on plants. Stimulation of plant growth and yield takes place thanks to this interaction and the advantageous effects are seen in the production of vitamins, the increased availability of biogenic elements (nitrogen, phosphorus), the mobilisation of nutrients from the soil and from organic matter, and the enhanced intensity of mineral uptake and transport.

Furthermore, *Trichoderma* fungi are capable of producing zeaxanthin and gibberellin, i.e. compounds accelerating seed germination. Many *Trichoderma* strains produce acids, e.g. gluconic, citric, and coumaric acids, causing the release of phosphorus ions and microelements, which subsequently become available to plants (Harman *et al*. 2004). Yedidia *et al*. (2001), after applying *T. harzianum* to growing cucumber, reported an increase in root mass and aboveground mass of plants as well as higher microelement contents. Whereas, in the study by Porras and colleagues (2007), fungi from the genus *Trichoderma* had a positive effect on strawberry yields.

Numerous authors (Howell 2003; Khan *et al*. 2004; Brunner *et al*. 2005; Woo and Lorito 2007) have reported that *Trichoderma* fungi stimulate systemic resistance in plants. When colonising the rhizosphere of plants, *Trichoderma* acts against fungal, bacterial, and viral diseases af-

fecting the aboveground parts (Benitez *et al*. 2004). Based on numerous studies, a conclusion can be drawn that various groups of metabolites may play the role of elicitors or so-called resistance inducers in the interactions of *Trichoderma* with plants (Harmann *et al*. 2004). These metabolites include proteins exhibiting enzymatic activity (e.g. xylanases, chitinases), gene products resembling proteins encoded by avirulent genes, and low molecular compounds released from cell walls of fungi or plants by fungal hydrolytic enzymes. In plants, an increase is observed in the concentration of enzymes and metabolites responsible for the induction of resistance, e.g. phenylalamine ammonia lyase (PAL), phytoalexins, chitinases, and glucanases (Shoresh *et al*. 2005). Yedidia *et al*. (1999) stated that inoculation of cucumber roots with spores of *T. harzianum* induced resistance reactions in the whole plants, which was connected with the increased activity of peroxidase and chitinase. As it was shown above, *Trichoderma* species are mostly opportunistic plant symbionts, with recognisable traits of hyperparasitism and antibiosis towards many pathogenic fungi. *Trichoderma* species are capable of producing enzymes and numerous secondary metabolites, mainly antibiotics, which make them important microorganisms from the point of view of ecology, agriculture, and industry.

Application of *Trichoderma* in biological plant protection

Trichoderma fungi are microorganisms that are most frequently tested and applied in biological plant protection. The use of *Trichoderma* fungi may cause a considerable limitation in the use of chemical fungicides in agriculture (Akhtar and Siddiqui 2008). It is estimated that 90% of all antagonistic fungi used in plant protection belong to the genus *Trichoderma* (Benitez *et al*. 2004). Many scientific reports focus on the positive effect of these fungi on plants (Hermosa *et al*. 2012). These reports focus on the efficacy of these fungi when it comes to disease control in the cultivation of numerous agricultural and horticultural crops, ornamental plants, and in fruit farming (Harman 2000; Howell 2003; Benitez *et al*. 2004; Smolinska *et al*. 2007). For example, recent studies showed the potential of *Trichoderma* spp. to control stem canker of brassicas, caused by *Leptosphaeria maculans* and *L. biglobosa* (Kaczmarek and Jedryczka 2011), with disease severity greatly depending on avirulence genes in fungi *vs.* resistance genes in plants (Kaczmarek *et al*. 2014). The species of *Trichoderma* significantly differed in their hyperparasitic effects towards *Leptosphaeria* spp. The highest decrease in fungal growth rate was caused by *T. atroviride* (Dawidziuk *et al*. 2013).

Disease control treatments using *Trichoderma* spp. are also used in storage practice, e.g. for strawberry fruits (Hjeljord *et al*. 2000) and apples (Batta 2004). The effectiveness of *Trichoderma* fungi has been confirmed in the control of many fungal pests of plants (Lewis and Lumsden 2001; Dolatabadi *et al*. 2011). *Trichoderma* spp. are also used in the control of bacterial and virus pests of crops (Hanson and Howell 2002). Moreover, studies have shown the usefulness of the species in weed control (Heraux *et al*. 2005).

In many countries biocontrol products are based on fungi from the genus *Trichoderma.* These products reduce the development of diseases, stimulate plant growth, enhance their stress resistance, and accelerate composting (Harman 2000). Bettiol and Morandi (2008) reported that cultivation costs are considerably reduced when using *Trichoderma.* Howell (2003) claimed that most effective strains of active microorganisms need to be collected from the regions in which they will be applied. Furthermore, as it was observed by Monte (2001), the application of *Trichoderma* with reduced fungicide doses in integrated farming, results in enhanced plant health – comparable with the level of protection provided by the application of full fungicide doses. This fact makes it possible to reduce cultivation costs and has a positive effect on the environment (Dubey *et al*. 2007). The literature describes numerous success stories of the use of biopreparations based on *Trichoderma*. Sometimes the comparisons of results obtained in different countries are not possible, due to the use of different strains, the ways of their growth and preparation, various ways of formulation and commercial names and doses.

Other applications of *Trichoderma*

The multi-sided activity of fungi from the genus *Trichoderma* provides a potential for their extensive applications in different branches of economy. Several species are frequently used in the degradation of organic pollutants. Bioremediation is often provided e.g. by *T. harzianum*, which detoxifies phenols, cyanides, and nitrates (Lynch and Moffat 2005). The capacity of *Trichoderma* fungi to produce lytic enzymes is used in animal feed, and wine making and brewery industries. Cellulases, hemicellulases, and pectinases produced by *Trichoderma* fungi are used in the partial hydrolysis of plant cell walls in feeds, enhancing the digestibility of the feed and increasing the nutritive value of the feed. This leads to increased body weight gains in animals and higher milk yields (Galante *et al*. 1998b). Enzymes obtained from *Trichoderma* may also be used as food additives, e.g. to increase raw material maceration in the production of fruit and vegetable juices. The enzymes are also used to improve wine flavour and enhance fermentation, filtration, and quality of beer (Blumenthal 2004).

Cellulases produced by *Trichoderma* are applied in the textile industry to soften and condition the textiles as well as to produce high quality washing powders. Enzymes obtained from *Trichoderma* are also used in the pulp and paper industry to modify fibre properties and to reduce lignin contents (Galante *et al*. 1998a). In turn, mutanase obtained from *T. harzianum* may be added in the production of toothpaste to help prevent the formation of plaque (Wiater *et al*. 2005).

Cellulases and hemicellulases produced by *T. reesei* Simmons are used in the production of bioethanol from farm waste. These enzymes catalyse degradation of substrates to simple sugars, which are next subjected to fermentation by the yeasts *Saccharomyces cerevisiae* (Arthe *et al*. 2008). In the food industry, applications have been found not only for enzymes, but also

for other metabolites of *Trichoderma* spp. An example here may be provided by the compound of nut aroma, exhibiting antibiotic properties, initially obtained from *T. viride* and subsequently from *T. atroviride* (Oda *et al*. 2009).

Prospects for the application of *Trichoderma*

Increased ecological awareness of whole societies, and growing interest in alternative sources of energy, make it possible to use fungi from the genus *Trichoderma* in the production of the so-called second generation biofuels. These fuels are obtained from the agricultural waste by adding cellulases or hemicellulases produced by *Trichoderma* species, e.g. *T. reesei* (Schuster and Schmoll 2010). The development of an adequately high efficiency of this process to ensure its economic viability poses a serious challenge for researchers. Fungi from the genus *Trichoderma* may also be applied in modern plant cultivation technologies, in which considerable emphasis is placed on the environmental impact. The introduction of biocontrol products is preceded by research, including modern technologies, such as the creation of Green Fluorescent Protein (GFP) fusants with enhanced expression of chitinase activity (Kowsari *et al*. 2014). At present, the development of *Trichoderma*-based biocontrol products takes place in numerous enterprises to obtain comprehensive protection of vegetables, such as onion, carrot, parsley, red beet, dill, radish (Sadowski *et al*. 2006a, 2007), and to a smaller extent – agricultural crops (Dawidziuk *et al*. 2013; El-Hassan *et al*. 2013). Studies also concern the development of production and formulation technologies for such products (Sadowski *et al*. 2006b; Kancelista *et al*. 2013; Smolińska *et al*. 2014). Another potential use of *Trichoderma* spp. is related to the industrial production of enzymes, antibiotics and other valuable metabolites (Thrane *et al*. 1997; Dengkolb *et al*. 2003; Osbourn 2010).

Trichoderma as a pathogen of humans

Apart from the beneficial species used for human needs, the genus *Trichoderma* also comprises species which are highly dangerous to human health (Druzhinina *et al*. 2008). The pathogenic species include *Hypocrea orientalis*, genetically close but clonal *Trichoderma citrinoviride* Bissett as well as *T. harzianum*, and *T. longibrachiatum*, with the prevalence of the first two closely related species. They constitute a lethal hazard for individuals with reduced resistance, including patients with leukemia, HIV-positive or having transplants (Kredics *et al*. 2003). Infections caused by *Trichoderma* are typically diagnosed late and are difficult to treat, as these fungi exhibit a low sensitivity to commonly applied antifungal drugs (Kratzer *et al*. 2006) and combined treatment is frequently necessary (Alanio *et al*. 2008). Larsen *et al*. (1998) reported that *Trichoderma* spp. may cause diseases of the respiratory tract in humans due to the volatile metabolites they produce.

Trichoderma as a pathogen of cultivated mushrooms

At present, fungi of *Trichoderma* spp. seriously threaten the cultivation of the champignon, button mushroom, oyster mushroom, and shiitake. Up to the 1980's, infestation of mushroom cultures by fungi from that genus, particularly *T. viride* and *T. koningii*, were reported only occasionally. However, in the years 1985–1986, and then again in 1990–1991, *Trichoderma* very seriously affected the button mushroom production in Great Britain, and then, in 1994 in the Netherlands. In the early 1990's, this problem was observed in North America and in the years 1996–1997, in France and Spain. In the last decade, green moulds appeared in button mushroom farms in Hungary, Poland, Croatia, Mexico, and Australia (Hatvani *et al*. 2007; Szczech *et al*. 2008; Kredics *et al*. 2010; Sobieralski *et al*. 2012b; Górski *et al*. 2014). Samuels *et al*. (2002) reported that initially four biotypes were identified (Th1, Th2, Th3, Th4), connected with the occurrence of green moulds classified to *T. harzianum*. However, it turned out that considerable losses in the garden mushroom farms are caused by two of them; Th2 and Th4. Further studies led to the isolation of a *T. aggressivum* Samuels & W. Gams species. Crop infestation in Europe is mainly caused by a *T. aggressivum* f. *europaeum* species, while in Canada, USA, and Mexico it is primarily caused by *T. aggressivum* f. *aggressivum* (Samuels *et al*. 2002; Williams *et al*. 2003).

In recent years the presence of green moulds has also been detected on the oyster mushroom farms. It was found that oyster mushroom farms were infested mainly by two closely-genetically-related species of fungi from the genus *Trichoderma*; *T. pleuroticola* S.H. Yu & M.S. Park, and *T. pleurotum* S.H. Yu & M.S. Park (Komon-Zelazowska *et al*. 2007). These two species have been reported both in Europe and in many countries worldwide (Szekeres *et al*. 2005; Kredics *et al*. 2006; Park *et al*. 2006; Hatvani *et al*. 2007; Gea 2009; Sobieralski *et al*. 2012a; Błaszczyk *et al*. 2013).

Genomes of *Trichoderma* spp.

Contemporary techniques allow one to sequence and compare whole genomes of different organisms, including fungi. In recent years three species of *Trichoderma* (*Hypocrea*); *T. reesei* (*H. jecorina*), *T. atroviride* (*H. atroviridis*), and *T. virens* (*H. virens*) have been sequenced, and the results are publically available. The smallest genome size (34 Mb) was found in the weakly mycoparasitic *T. reesei*. The largest genome (38.8 Mb) was one of the highly parasitic *T. virens* (Mukherjee 2011). The genome of *T. atroviride* was of intermediate size (36.1 Mb). Similarly to some fungi, such as *Neurospora crassa* (Irelan *et al*. 1994) or *L. maculans* (Fudal *et al*. 2009), the genomes of *Trichoderma* contain fragmented transposable elements, called Repeat Induced Point (RIP) mutations. However, the comparative genomics have also revealed great differences between the genomes of *Trichoderma* and even closely related fungi, such as *Giberella zeae*. These are differences in respect to the decreased number of repetitive DNA sequences and numerous unique genes or gene families.

During evolution, the species of *Trichoderma* have evolved many special mechanisms allowing the *Trichoderma* species to compete with other fungal species (Monte 2001). Genes responsible for these mechanisms are involved in mycoparasitism, antibiosis, inactivation of enzymes produced by pathogens or they help *Trichoderma* to solubilise inorganic nutrients and to compete with other microorganisms for nutrients and space. These parasitic activities require additional storage of genomic information and are the main reason why highly parasitic species, such as *T. virens* or *T. atroviride* have larger genomes and contain about 3,000 more genes than the mild mycoparasite *T. reesei* (Kubicek *et al*. 2011). Sequencing of the latter species unexpectedly showed a relatively small number of genes coding for celulases and hemicelulases, as it is a well-known cell-wall degrading species, commercially used for the production of these enzymes (Schuster and Schmoll 2010). Genomes of highly parasitic *Trichoderma* species contain numerous genes encoding the production of chitinolytic and glucanolytic enzymes as well as a large array of other compounds necessary in the attack against other microbes (Druzhinina *et al*. 2011). Many of these genes are involved in the biosynthesis of small molecules (SMs), such as non-ribosomal peptides, poliketides, terpenoids, and pyrones (Mukherjee *et al*. 2012). Small molecule genes are often found in clusters (Evans *et al*. 2011), which most likely prevents the accumulation of toxic intermediates (Slot and Rokas 2011). The number of genes coding for polyketide synthases and non-ribosomal peptide synthetases is doubled in more parasitic species of *Trichoderma*. Their genomes also contain genes for aegerolysins and the insecticidal Tc toxins, not present in the genome of *T. reesei* (Mukherjee 2011). The amount and diversity of biosynthetic pathways utilised by parasitic *Trichoderma* species explain the size and complexity of their genomes.

Studies on the expression of some genes produced by *Trichoderma* have proved difficult, as their activity may be connected solely with defence against other microbes or multicellular organisms (Osbourn 2010). Brakhage and Schroeckh (2011) suggested some strategies to activate silent gene clusters by cultivating fungi in conditions that simulate competition and allow the usual biosynthetic pathways to be initiated. A detailed metabolomic-genomic study is suggested for elucidating the roles of the numerous gene products of *Trichoderma* (Mukherjee *et al*. 2012). Such a study will allow for the discovery of many compounds and unravel the role of the compounds, before their application as biocontrol agents. In this way,the use of strains that are potentially harmful to plants or toxic to people are avoided. These studies need the cooperation of mycologists, geneticists, biochemists, plant physiologists, toxicologists, and numerous researchers from other fields.

Acknowledgements

The authors thank to Dr. Marta Wrzosek from the Department of Systematics and Plant Geography, University of Warsaw for her valuable comments on the manuscript and thank to Judyta Strakowska M.Sc. from the Institute of Plant Genetics, Polish Academy of Sciences in Poznań for sharing with her photograph of *Trichoderma* cultures.

References

Akhtar M.S., Siddiqui Z.A. 2008. Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp., and *Pseudomonas straita*. Crop Prot. 27 (3–5): 410–417.

Alanio A., Brethon B., Feuihade de Chauvin M., Kerviler E., Leblanc T., Lacroix C., Baruchel A., Menotti J. 2008. Invasive pulmonary infection due to *Trichoderma longibrachiatum* mimicking invasive Aspergillosis in a neutropenic patient successfully treated with voriconazole combined with caspofungin. Clin. Infect. Dis. 46 (10): 116–118.

Arthe R., Rajesh R., Rajesh E.M., Rajendran R., Jeyachandran S. 2008. Production of bio-ethanol from cellulosic cotton waste through microbial extracellular enzymatic hydrolysis and fermentation. Electron. J. Environ., Agric. Food Chem. 7 (6): 2984–2992.

Batta Y.A. 2004. Effect of treatment with *Trichoderma harzianum* Rifai formulated in invert emulsion on postharvest decay of apple blue mold. Int. J. Food Microbiol. 96 (3): 281–288.

Benitez T., Rincon A.M., Limon M.C., Codon A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7 (4): 249–260.

Bettiol W., Morandi M.A.B. 2008. *Trichoderma* in Brazil, history, research, commercialization and perspectives. p. 235–237. In: "Molecular Tools for Understanding and Improving Biocontrol" (B. Duffy, M. Maurhoffer, C. Keel, C. Gessler, Y. Elad, S. Klewnick, eds.). 10th meeting of the working group "Biological Control of Fungal and Bacterial Plant Pathogens", Interlaken, Switzerland, 9–12 September 2008, 389 pp.

Bisby G.R. 1939. *Trichoderma viride* Pers. ex Fries and notes on *Hypocrea*. Trans. Br. Mycol. Soc. 23 (2): 149–168.

Bissett J. 1991a. A revision of the genus *Trichoderma*. II. Infrageneric classification. Can J. Bot. 69 (11): 2357–2372.

Bissett J. 1991b. A revision of the genus *Trichoderma*. III. Sect. Pachybasium. Can. J. Bot. 69 (11): 2373–2417.

Bissett J. 1991c. A revision of the genus *Trichoderma*. IV. Additional notes on section *Longibrachiatum*. Can. J. Bot. 69 (11): 2418–2420.

Blumenthal C.Z. 2004. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*, justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Regul. Toxicol. Pharmacol. 39 (2): 214–228.

Błaszczyk L., Siwulski M., Sobieralski K., Frużyńska-Jóźwiak D. 2013. Diversity of *Trichoderma* spp. causing *Pleurotus* green mould diseases in Central Europe. Folia Microbiol. 58 (4): 325–333.

Brakhage A.A., Schroeckh V. 2011. Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet. Biol. 48 (1): 15–22.

Brunner K., Zeilinger S., Ciliento R., Woo S.L., Lorito M., Kubicek C.P., Mach R.L. 2005. Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic resistance. Appl. Environ. Microbiol. 71 (7): 3959–3965.

Corley D.G., Miller-Wideman M., Durley R.C. 1994. Isolation and structure of harzianum A: a new trichothecene from *Trichoderma harzianum*. J. Nat. Prod. 57 (3): 422–425.

Dawidziuk A., Popiel D., Jędryczka M. 2013. The influence of *Trichoderma* species on *Leptosphaeria maculans* and *L. biglobosa* growth on agar media and in oilseed rape plants. In-

tegrated Control in Oilseed Crops IOBC-WPRS Bulletin 92: 119–126.

Dengkolb T., Berg A., Gams W., Schlegel B., Grafe U. 2003. The occurrence of peptaibols and structurally related peptabiotics in fungi and their mass spectrometric identification via diagnostic fragment ions. J. Pept. Sci. 9: 666–678.

Dolatabadi K.H., Goltapeh E.M., Varma A., Rohani N. 2011. *In vitro* evaluation of arbuscular mycorrhizal-like fungi and *Trichoderma* species against soil borne pathogens. J. Agric. Technol. 7: 73–84.

Druzhinina I., Kubicek C.P. 2005. Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters? J. Zhejiang Univ. Sci. 6 (2): 100–112.

Druzhinina I.S., Kopchinsky A.G., Kubicek C.P. 2006. The first 100 *Trichoderma* species characterized by molecular data. Mycoscience 47 (2): 55–64.

Druzhinina I.S., Komon-Zelazowska M., Kredics L., Hatvani L., Antal Z., Belayneh T., Kubicek C.P. 2008. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable to cause invasive mycoses of humans. Microbiology 54 (11): 3447–3459.

Druzhinina I.S., Seidl-Seiboth V., Herrera-Estrella A., Horwitz B.A., Kenerley C.M., Monte E., Mukherjee P.K., Zeilinger S., Grigoriev I.V., Kubicek C.P. 2011. *Trichoderma*: the genomics of opportunistic success. Nature Rev. Microbiol. 9 (10): 749–759.

Dubey S.C., Suresh M., Singh B. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. Biol. Control 40 (1): 118–127.

El-Hassan S.A., Gowen S.R., Pembroke B. 2013. Use of *Trichoderma hamatum* for biocontrol of lentil vascular wilt disease: efficacy, mechanisms of interaction and future prospects. J. Plant Prot. Res. 53 (1): 12–26.

Enari T.M., Niku-Paavola M.L. 1987. Enzymatic hydrolysis of cellulose: Is the current theory of mechanism of the hydrolysis valid? Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. Crit. Rev. Biotechnol. 5: 67–87.

Evans B.S., Robinson S.J., Kelleher N.L. 2011. Surveys of nonribosomal peptide and polyketide assembly lines in fungi and prospects for their analysis *in vitro* and *in vivo*. Fungal Genet. Biol. 48 (1): 49–61.

Fudal I., Ross S., Brun H., Besnard A.L., Ermel M., Kuhn M.L., Balesdent M.H., Rouxel T. 2009. Repeat-induced point mutation (RIP) as an alternative mechanism of evolution towards virulence in *Leptosphaeria maculans*. Mol. Plant-Microbe Interact. 22 (8): 932–941.

Galante Y.M., De Conti A., Monteverdi R. 1998a. Application of *Trichoderma* enzymes in the textile industry. p. 311–326. In: "*Trichoderma* and *Gliocladium*"(G.E. Harman, C.P. Kubicek, eds.), Taylor & Francis, London, UK, 393 pp.

Galante Y.M., De Conti A., Monteverdi R. 1998b. Application of *Trichoderma* enzymes in the food and feed industry. p. 327–342. In: "*Trichoderma* and *Gliocladium*" (G.E. Harman, C.P. Kubicek, eds.). Taylor & Francis, London, UK, 393 pp.

Gallo A., Mule G., Favilla M., Altomare C. 2004. Isolation and characterisation of a trichodiene synthase homologous gene in *Trichoderma harzianum*. Physiol. Molec. Plant Pathol. 65 (1): 11–20.

Gams W., Bisset J. 1998. Morphology and identification of *Trichoderma*. p. 3–34. In: "*Trichoderma* and *Gliocladium*" (G.E. Harman, C.P. Kubicek, eds.). Taylor & Francis, London, UK, 393 pp.

Gea F.J. 2009. First report of *Trichoderma pleurotum* on oyster mushroom crops in Spain. J. Plant. Pathol. 91 (2): 504.

Górski R., Sobieralski K., Siwulski M., Frąszczak B., Sas-Golak I. 2014. The effect of *Trichoderma* isolates, from family mushroom growing farms, on the yield of four *Agaricus bisporus* (Lange) Imbach strains. J. Plant Prot. Res. 54 (1): 102–105.

Hanson L.E., Howell C.R. 2002. Biocontrol efficacy and other characteristics of protoplast fusants between *Trichoderma koningii* and *T. virens*. Mycol. Res. 106 (3): 321–328.

Haran S., Schickler H., Oppenheim A., Chet I. 1996. Differential expression of *Trichoderma harzianum* chitinases during mycoparasitism. Phytopathology 86 (9): 980–985.

Harman G.E. 2000. Myths and dogmas of biocontrol changes in perceptions derived on *Trichoderma harzianum* T-22. Plant Dis. 84 (4): 377–393.

Harman G.E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 96 (2): 190–194.

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M. 2004. *Trichoderma* species – opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2 (1): 43–56.

Hatvani L., Antal L., Manczinger L., Szekeres A., Druzhinina I.S., Kubicek C.P., Nagy E., Vagvolgyi C., Kredics L. 2007. Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderema* species. Phytopathology 97 (4): 532–537.

Heraux F.M.G., Hallett S.G., Ragothama K.G., Weller S.C. 2005. Composted chicken manure as a medium for the production and delivery of *Trichoderma virens* for weed control. HortScience 40 (5): 1394–1397.

Hermosa R., Viterbo A., Chet I., Monte E. 2012. Plant-beneficial effects of *Trichoderma* and its genes. Microbiology 158 (1): 17–25.

Herrera-Estrella A., Chet I. 2004. The biological control agent *Trichoderma* – from fundamentals to applications. p. 147–156. In: "Fungal Biotechnology in Agricultural, Food and Environmental Applications" (D.K. Arora, M. Dekker, eds.). Vol. 21. CRC Press, New York, USA, 700 pp.

Hjeljord L.G., Stensvand A., Tronsmo A. 2000. Effect of temperature and nutrient stress on the capacity of commercial *Trichoderma* products to control *Botrytis cinerea* and *Mucor piriformis* in greenhouse strawberries. Biol. Control 19 (2): 146–160.

Howell C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases; the history and evolution of current concepts. Plant Dis. 87 (1): 4–10.

Irelan J.T., Hagemann A.T., Selker E.U. 1994. High frequency repeat-induced point mutation (RIP) is not associated with efficient recombination in *Neurospora*. Genetics 138 (4): 1093–1103.

Jaklitsch W.M. 2009. European species of *Hypocrea.* Part I. The green-spored species. Stud. Mycol. 63: 1–91.

Jeleń H., Błaszczyk L., Chełkowski J., Rogowicz K., Strakowska J. 2013. Formation of 6-n-pentyl-2H-pyran-2-one (6-PAP) and other volatiles by different *Trichoderma* species. Mycol. Progress 13 (3): 589–600. DOI: 10.1007/s11557-013-0942-2.

Kaczmarek J., Jędryczka M. 2011. Characterization of two coexisting pathogen populations of *Leptosphaeria* spp., the cause of stem canker of brassicas. Acta Agrobot. 64 (2): 3–14.

Kaczmarek J., Latunde-Dada A.O., Irzykowski W., Cools H.J., Stonard J.F., Jędryczka M. 2014. Molecular screening for avirulence alleles *AvrLm1* and *AvrLm6* in airborne inoculum of *Leptosphaeria maculans* and winter oilseed rape (*Brassica napus*) plants from Poland and the UK. J. Appl. Genet. 55 (4): 529–539. DOI 10.1007/s13353-014-0235-8.

Kancelista A., Tril U., Stempniewicz R., Piegza M., Szczech M., Witkowska D. 2013. Application of lignocellulosic waste materials for the production and stabilization of *Trichoderma* biomass. Pol. J. Environ. Stud. 22 (4): 1083–1090.

Khan J., Ooka J.J., Miller S.A., Madden L.V., Hotitink H.A.J. 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora* crown rot and leaf blight. Plant Dis. 88 (3): 280–286.

Kindermann J., El-Ayouti Y., Samuels G.J., Kubicek C.P. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genet. Biol. 24 (3): 298–309.

Komon-Zelazowska M., Bisset J., Zafari D., Hatvani L., Manczinger L., Woo S., Lorito M., Kredics L., Kubicek C.P., Druzhinina I.S. 2007. Genetically closely related but phenotypically divergent *Trichoderma* species cause green mold disease in oyster mushroom farms worldwide. Appl. Environ. Microbiol. 73 (22): 7415–7426.

Kowsari M., Motallebi M., Zamani R.M. 2014. Construction of new GFP-tagged fusants for *Trichoderma harzianum* with enhanced biocontrol activity. J. Plant Prot. Res. 54 (2): 122–131.

Kratzer C., Tobudic S., Schmoll M., Graninger W., Georgopoulos A. 2006. *In vitro* activity and synergism of amphotericin B, azoles and cationic antimicrobials against the emerging pathogen *Trichoderma* spp. J. Antimicrob. Chemother. 58 (5): 1058–1061.

Kredics L., Antal Z., Doczi I., Manczinger L., Kevei F., Nagy E. 2003. Clinical importance of the genus *Trichoderma*. A review. Acta Microbiol. Immunol. Hung. 50 (2–3): 105–117.

Kredics L., Hatvani L., Antal L., Manczinger L., Druzhinina I.S., Kubicek C.P., Szekeres A., Nagy A., Vagvolgyi C., Nagy E. 2006. Green mold disease of oyster mushroom in Hungary and Transylvania. Acta Microbiol. Immunol. Hung. 53 (3): 306–307.

Kredics L., Garcia Jimenez L., Nagimi S., Czifra D., Urban P., Manczinger L., Vagvolgyi C., Hatvani L. 2010. A challenge to mushroom growers, the green mould disease of cultivated champignons. p. 295–305. In: "Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology" (A. Mendez-Vilas, ed.). Microbiology Book Series 2 (1), Formatex, Badajoz, Spain, 788 pp.

Kubicek C.P., Herrera-Estrella A., Seidl-Seiboth V., Martinez D.A., Druzhinina J.S. 2011. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. Genome Biol. 12 (4): R40. DOI: 10.1186/gb-2011-12-4-r40.

Kubicek C.P., Mach R.L., Peterbauer C.K., Lorito M. 2001. *Trichoderma*: From genes to biocontrol. J. Plant Pathol. 83 (2): 11–23.

Kullnig-Gradinger C.M., Szakacs G., Kubicek C.P. 2002. Phylogeny and evolution of the fungal genus *Trichoderma*: a multigene approach. Mycol. Res. 106 (7): 757–767.

Larsen F.O., Clementsen P., Hansen M., Maltbaek N., Ostenfeldt-Larsen T., Nielsen K.F., Gravesen S., Skov P.S., Non S. 1998. Volatile compounds from the indoor mould *Trichoderma viride* cause histamine release from human bronchoalveolar cells. Inflamm. Res. 47 (1): 55–56.

Lewis J.A., Lumsden R.D. 2001. Biocontrol of damping-off of greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. Crop Prot. 20 (1): 49–56.

Lorito M., Hayes C.K., Di Pietro A., Woo S.L., Harman G.E. 1994. Purification, characterization, and synergistic activity of a glucan 1,3-*b*-glucosidase and an N-acetyl-*b*-glucosaminidase from *Trichoderma harzianum*. Phytopathology 84 (4): 398–405.

Lynch J.M., Moffat A.J. 2005. Bioremediation – prospects for the future application of innovative applied biological research. Ann. Appl. Biol. 146 (2): 217–221.

Monte E. 2001. Understanding *Trichoderma*, between biotechnology and microbial ecology. Int. Microbiol. 4 (1): 1–4.

Mukherjee P.K. 2011. Genomics of biological control – whole genome sequencing of two mycoparasitic *Trichoderma* spp. Curr. Sci. 101 (3): 268.

Mukherjee P.K., Horwitz B.A., Kenerley C.M. 2012. Secondary metabolism in *Trichoderma* – a genomic perspective. Microbiology 158 (1): 35–45.

Oda S., Isshiki K., Ohashi S. 2009. Production of 6-pentyl-a-pyrone with *Trichoderma atroviride* and its mutant in a novel extractive liquid-surface immobilization (Ext-LSi) system. Process Biochem. 44 (6): 625–630.

Osbourn A. 2010. Secondary metabolic gene clusters: evolutionary toolkits for chemical innovation. Trends Genet. 26 (10): 449–457.

Park M.S., Bae K.S., Yu S.H. 2006. Two new species of *Trichoderma* associated with green mold of oyster mushroom cultivation in Korea. Mycobiology 34 (3): 111–113.

Persoon C.H. 1794. Disposita methodica fungorum. Romer's Neues Mag. Bot. 1: 81–128.

Porras M., Barrau C., Romero F. 2007. Effects of soil solarization and *Trichoderma* on strawberry production. Crop Prot. 26 (5): 782–787.

Reino J.L., Guerrero R.F., Hernandez-Galan R., Collado I.G. 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem. Rev. 7 (1): 89–123.

Rifai M.A. 1969. A revision of the genus *Trichoderma*. Mycol. Pap. 116: 1–56.

Sadowski C., Lenc L., Domoradzki M., Korpal W., Weiner W., Lukanowski A. 2006a. Studies on plant health in organic production of vegetable seeds. p. 410–411. In: Proc. European Joint Organic Congress, Odense, Denmark, 30–31 May 2006, 713 pp.

Sadowski C., Lenc L., Korpal W. 2006b. Z badań nad otoczkowaniem nasion warzyw z wykorzystaniem *Trichoderma viride* i zdrowotnością roślin w uprawie ekologicznej. [Out of investigations of vegetable seed coating with *Trichoderma viride* and plant health in organic system]. J. Res. Appl. Agric. Eng. 51 (2): 150–153.

Sadowski C., Lenc L., Korpal W. 2007. Badania nad możliwością ochrony buraka ćwikłowego przed chorobami grzybowymi w uprawie ekologicznej. [Investigation on the pos-

sibility of protection of organically grown red beet against fungal diseases]. J. Res. Appl. Agric. Eng. 52 (4): 38–44.

Samuels G.J. 1996. *Trichoderma*: a review of biology and systematics of the genus. Mycol. Res. 100 (8): 923–935.

Samuels G.J., Dodd S.L., Gams W., Castelbury L.A., Petrini O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 94 (1): 146–170.

Schuster A., Schmoll M. 2010. Biology and biotechnology of *Trichoderma*. Appl. Microbiol. Biotechnol. 87 (3): 789–799.

Sharon E., Bar-Eyal M., Chet I., Herrera-Estrella A., Kleifeld O., Spiegel Y. 2001. Biological control of root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology 91 (7): 687–693.

Shoresh M., Yedidia I., Chet I. 2005. Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. Phytopathology 95 (1): 76–84.

Slot J.C., Rokas A. 2011. Horizontal transfer of a large and highly toxic secondary metabolic gene cluster between fungi. Curr. Biol. 21 (2): 134–139.

Smolińska U., Kowalska B., Kowalczyk W., Szczech M. 2014. The use of agro-industrial wastes as carriers of *Trichoderma* fungi in the parsley cultivation. Sci. Hortic. 179: 1–8.

Smolińska U., Kowalska B., Oskiera M. 2007. The effectivity of *Trichoderma* strains in the protection of cucumber and lettuce against *Rhizoctonia solani*. Veg. Crops Res. Bull. 67 (1): 81–93.

Sobieralski K., Siwulski M., Błaszczyk L., Frąszczak B., Sas-Golak I. 2012a. Impact of infections with *Trichoderma pleurotum* and *Trichoderma pleuroticola* isolates on yielding of wild strains of *Pleurotus ostreatus* (Fr.) Kumm. obtained from natural sites. Acta Sci. Pol. – Hortorum Cultus 11 (1): 239–249.

Sobieralski K., Siwulski M., Błaszczyk L., Frużyńska-Jóźwiak D., Lisiecka J. 2012b. The effect of infestation with isolates of *Trichoderma* sp. on mycelium growth and yielding in single-spore heterokaryotic cultures of *Agaricus bisporus* (Lange) Imbach. Acta Sci. Pol. – Hortorum Cultus 11 (1): 47–57.

Strakowska J., Błaszczyk L., Chełkowski J. 2014. The significance of cellulolytic enzymes produced by *Trichoderma* in opportunistic lifestyle of this fungus. J. Basic Microb. 54 (Suppl. 1): S2–13. DOI: 10.1002/jobm.201300821.

Szczech M., Staniaszek M., Habdes H., Ulinski Z., Szymanski J. 2008. *Trichoderma* spp. – the cause of green mold on Polish farms. Veg. Crops Res. Bull. 69 (1): 105–114.

Szekeres A., Kredics L., Antal L., Hatvani L., Manczinger L., Vagvolgyi C. 2005. Genetic diversity of *Trichoderma* strains isolated from winter wheat rhizosphere in Hungary. Acta Microbiol. Immunol. Hung. 52: 156.

Thrane C., Tronsmo A., Jensen D.F. 1997. Endo-1,3-β-glucanase and cellulose from *Trichoderma harzianum*: purification and partial characterization, induction of and biological activity against plant pathogenic *Pythium* spp. Europ. J. Plant Pathol. 103 (4): 331–344.

Tulasne L., Tulasne R. 1860. De quelques Sphéries fungicoles, à propos d'un mémoire de M. Antoine de Bary sur les *Nyctalis*. Ann. Sci. Nat. Bot. 13: 5–19.

Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L., Lorito M. 2008. *Trichoderma* – plant – pathogen interactions. Soil Biol. Biochem. 40 (1): 1–10.

Viterbo A., Harel M., Chet I. 2004. Isolation of two aspartyl proteases from *Trichoderma asperellum* expressed during colonization of cucumber roots. FEMS Microbiol. Letters 238 (1): 151–158.

Wiater A., Szczodrak J., Pleszczyńska M. 2005. Optimization of conditions for the efficient production of mutant in streptococcal cultures and post-culture liquids. Acta Biol. Hung. 56 (12): 137–150.

Williams J., Clarkson J.M., Mils P.R., Cooper R.M. 2003. Saprotrophic and mycoparasitic components of aggressiveness of *Trichoderma harzianum* groups toward the commercial mushroom *Agaricus bisporus*. Appl. Environ. Microbiol. 69 (7): 4192–4199.

Woo S.L., Lorito M. 2007. Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. p. 107–130. In: "Novel Biotechnologies for Biocontrol Agent Enhancement and Management" (M. Vurro, J. Gressel, eds.). Springer Press, Amsterdam, The Netherlands, 362 pp.

Yedidia I., Benhamou N., Chet I. 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65 (3): 1061–1070.

Yedidia I., Srivastra A.K., Kapulnik Y., Chet I. 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil 235 (2): 235–242.