SPECIES COMPOSITION OF *Trichoderma* ISOLATES FROM THE RHIZOSPHERE OF VEGETABLES GROWN IN HUNGARIAN SOILS

Péter Körmöczi[1], Gordana Danilović[2], László Manczinger[1], Ljubinko Jovanović[3], Dejana Panković[2], Csaba Vágvölgyi[1] and László Kredics[1,*]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[2]Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia
[3]Faculty of Ecological Agriculture, Educons University, Sremska Kamenica, Serbia

ABSTRACT

The species composition of *Trichoderma* isolates from the rhizosphere of different vegetables collected at different locations in Hungary was examined during this study. *Trichoderma* strains were isolated from the rhizosphere samples on dichloran-rose bengal medium. After purification of genomic DNA, the PCR amplification of the internal transcribed spacer (ITS1-5.8S rDNA-ITS2) region and its sequence analysis were used for the identification of the isolates at the species level. Altogether, 45 *Trichoderma* isolates were identified from the examined samples. The detected *Trichoderma* species were *T. asperellum, T. atroviride, T. citrinoviride, T. gamsii, T. hamatum, T. harzianum*, *T. koningiopsis/T. ovalisporum, T. longibrachiatum/H. orientalis*, *T. pleuroticola* and *T. virens.* Besides species known as opportunistic pathogens of humans (*T. longibrachiatum / H. orientalis*, *T. citrinoviride*) or as causative agents of mushroom green mould disease (*T. pleuroticola*), beneficial taxa (*T. harzianum*, *T. virens*, *T. atroviride*) widely used for the biological control of plant pathogenic fungi could also be identified in the examined samples, suggesting that the rhizosphere of vegetables may be a rich source of potential biocontrol agents. *In vitro* antagonism was examined in dual culture tests and the Biocontrol Index (BCI) values were determined for the particular isolates. Certain *T. asperellum*, *T. virens* and *T. atroviride* isolates proved to possess good *in vitro* antagonistic activities against plant pathogenic *Fusarium solani* and *F. oxysporum* strains, suggesting that they might be promising for the development of *Trichoderma*-based biocontrol strategies for the suppression of plant pathogenic fungi in the rhizosphere of vegetables produced in organic farmland soils.

KEYWORDS: *Trichoderma*; vegetables; rhizosphere; species composition; biocontrol

* Corresponding author

1 INTRODUCTION

Plant diseases, which are the main limitation of agricultural production, are traditionally controlled by chemical pesticides. This favours the development of resistant pathogens and leads to soil pollution on longer time scale. One approach that overcomes these disadvantages is the use of biological control in plant protection. Although *Trichoderma* species have been recognized for their antifungal abilities long time ago, the search for new species and biotypes in unusual habitats, with new biocontrol traits is still up-to-date. Species of the filamentous fungal genus *Trichoderma* are belonging to the *Hypocreales* order of the *Ascomycota* division. The genus involves representatives with excellent antagonistic abilities against a series of plant pathogenic fungi, being therefore promising candidates for the biological control of fungal pests in agriculture. Modes of action with proposed roles in biocontrol capabilities of *Trichoderma* strains include mycoparasitism, antibiosis by the production of antifungal metabolites, competition for nutrients and space, induction of defence responses in the plant as well as plant growth promotion [1].

The species composition of the genus *Trichoderma* has been examined by molecular methods in a series of natural ecosystems, including a mid-European, primeval floodplain-forest [2], the Danube floodplain [3], Sardinia [4], soils from Russia, Nepal, Northern India [5], south-east Asia [6], China [7], North-Africa [8] and South America [9]. These studies reported about a series of new genotypes as well as new phylogenetic species of *Trichoderma*. On the other hand, only a few studies were focusing on agricultural environments [8, 10-12]. However, the results of these studies demonstrated that – besides the natural ecosystems – the investigation of agricultural soils also reveals important data about *Trichoderma* biodiversity. The practical impact of such studies is that the rhizosphere of agricultural soils may be an ideal source of beneficial strains with biocontrol potential. This study was aimed at the assessment of *Trichoderma* species composition in samples derived from vegetable rhizosphere at different locations of Hungary.

2 MATERIALS AND METHODS

2.1 Isolation of *Trichoderma* strains from vegetable rhizosphere samples

Soil samples from the rhizosphere of different vegetables (pepper, tomato, carrot, salad, spinach, pumpkin, kohlrabi, parsley, celery and bean) were collected from different locations in Hungary (Szeged-Sziksós, Balástya, Szentes, Veszprém, Hatvan and Ózd, Figure 1). Isolations were performed from soil and root surface on dichloran - Rose Bengal medium [13] (5 g l^{-1} peptone, 1 g l^{-1} KH_2PO_4, 10 g l^{-1} glucose, 0.5 g l^{-1} $MgSO_4 \times 7H_2O$, 0.5 ml l^{-1} 0.2% dichloran-ethanol solution, 0.25 ml l^{-1} 5% Rose Bengal, 20 g l^{-1} agar supplemented with 0.1 g l^{-1}oxytetracyclin, 0.1 g l^{-1} streptomycin and 0.1 g l^{-1} chloramphenicol to inhibit bacteria). The isolated strains were deposited at the Szeged Microbiological Collection (SZMC; Table 1).

2.2 Molecular identification of the isolated *Trichoderma* strains

DNA isolation, PCR amplification of the internal transcribed spacer (ITS: ITS1-5.8S rDNA-ITS2) region of the ribosomal RNA gene cluster and automatic DNA sequencing were performed as described previously [12]. *Trichoderma* isolates were identified based on their ITS sequences with the aid of the barcoding program *TrichO*KEY 2.0 [14] available online at the home page of the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (www.isth.info). In the cases where *TrichO*key 2.0 was not able to identify the isolate at the species level, BLASTN homology searches [15] were performed at the homepage of NCBI (National Center for Biotechnology Information). The validities of the BLASTN hits were checked with *TrichO*key 2.0 and literature searches. Sequences were deposited at the NCBI Genbank database, accession numbers are listed in Table 1.

2.3 Dual confrontation assays for the study of *in vitro* antagonism

In vitro antagonism of selected isolates was examined in dual culture tests in confrontation with the plant pathogenic *Fusarium solani* isolates SZMC 11064F, SZMC 11067F and *F. oxysporum* isolate SZMC 6237J, and the Biocontrol Index (BCI) values were determined for the particular isolates by the image analysis-based method of Szekeres *et al.* [16], which is simple to carry out and provides accurate quantitative values for the evaluation of *in vitro* antagonism.

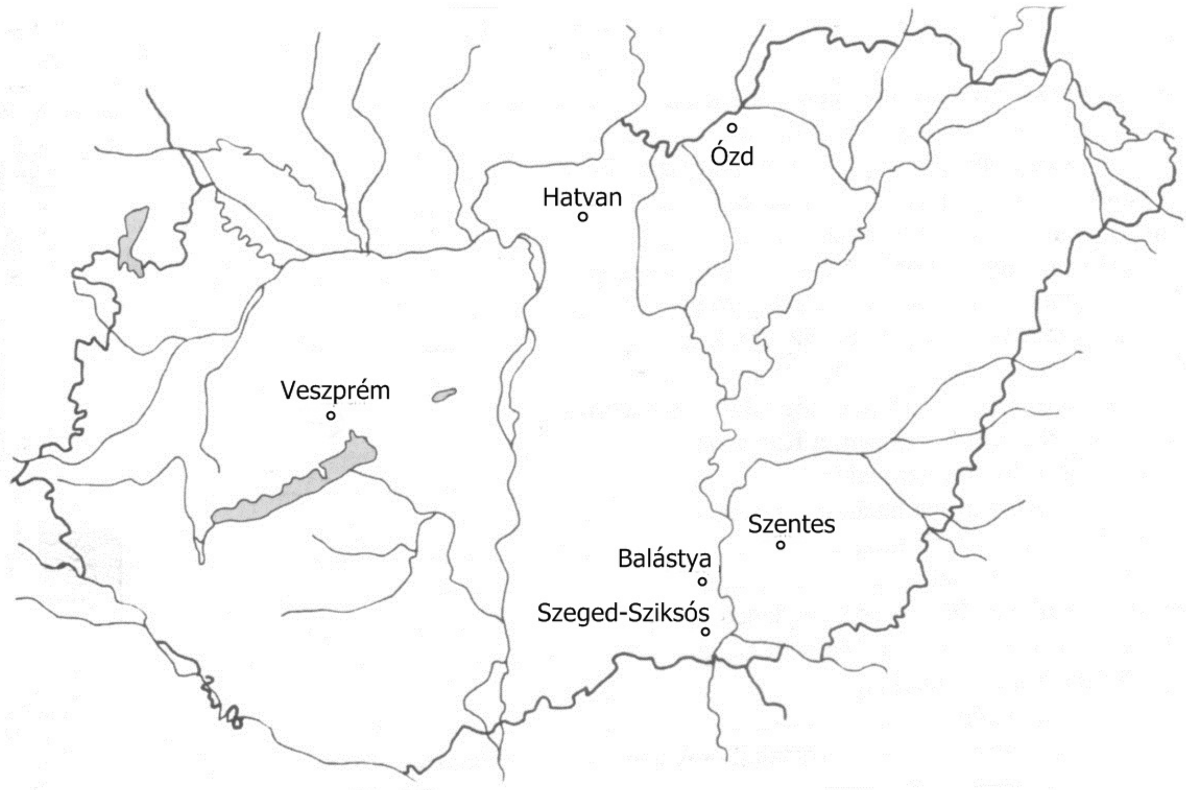

FIGURE 1 - Vegetable rhizosphere sampling locations in Hungary

3 RESULTS AND DISCUSSION

A total of 45 *Trichoderma* strains were isolated from 16 Hungarian soil samples derived from the rhizosphere of different vegetables. Altogether, 10 *Trichoderma* species could be identified in the examined samples (Table 1).

The number of isolated strains was the highest in the case of the tomato rhizosphere sample derived from Szeged-Sziksós (15 isolates from 2 species). The most abundant species was *T. harzianum* (25, 55.6%) which could be found in 7 of the 16 samples. It was the most frequently occurring *Trichoderma* species in five samples including the white kohlrabi rhizosphere sample as well as the tomato rhizosphere samples from Szeged-Sziksós and Veszprém. Interestingly, the second most frequently isolated *Trichoderma* species was *T. pleuroticola* (4, 8.9%), a species known as one of the causative agents of green mould disease in oyster mushroom cultivation [17]. This species could be found both in kohlrabi, tomato and bean rhizosphere. Three-three strains of *T. atroviride* and *T. asperellum* could also be identified from the rhizosphere of tomato and paprika/parsley, respectively. *T. longibrachiatum*/*Hypocrea orientalis* (an exact identification at the species level based on ITS-sequences is not possible for isolates belonging to this species duplet) and *T. citrinoviride* were represented by 2 isolates from salad and spinach rhizosphere and 1 isolate from pumpkin rhizosphere, respectively, both of these species are known as rare opportunistic pathogens of immunocompromised humans [18]. Other species detected were *T. koningiopsis/ T. ovalisporum* (an exact differentiation based on ITS- sequences is not

TABLE 1 - Isolation data and identification details of the examined *Trichoderma* strains

Location	*Rhizosphere sample*	*Strain number*	*GenBank accession number of ITS*	*TrichOkey 2.0 diagnosis*	*Closest valid NCBI BLAST hit*
Szeged-Sziksós	kohlrabi (white)	SZMC 20852	JX173840	*T. pleuroticola*	
		SZMC 20853	JX173841	*T. harzianum*	
		SZMC 20854	JX173842	*T. harzianum*	
		SZMC 20855	JX173843	*T. harzianum*	
		SZMC 20856	JX173844	*T. harzianum*	
	kohlrabi (red)	SZMC 20857	JX173855	*T. harzianum*	
		SZMC 20774	JX173849	*T. pleuroticola*	
	tomato	SZMC 20858	JX173851	*T. harzianum*	
		SZMC 20859	JX173852	*T. harzianum*	
		SZMC 20758	JX173850	*T. harzianum*	
		SZMC 20759	JX173853	*T. harzianum*	
		SZMC 20760	JX173854	*T. harzianum*	
		SZMC 20761	JX173832	*T. harzianum*	
		SZMC 20762	JX173833	*T. harzianum*	
		SZMC 20777	JX173864	*T. harzianum*	
		SZMC 20763	JX173834	*T. koningiopsis / T. ovalisporum*	
		SZMC 20764	JX173835	*T. harzianum*	
		SZMC 20765	JX173836	*T. harzianum*	
		SZMC 20778	JX173865	*T. koningiopsis / T. ovalisporum*	
		SZMC 20766	JX173837	*T. koningiopsis / T. ovalisporum*	
		SZMC 20767	JX173838	*T. harzianum*	
		SZMC 20768	JX173839	*T. harzianum*	
	bean	SZMC 20863	JX173845	*T. harzianum*	
		SZMC 20864	JX173846	*T. pleuroticola*	
	paprika	SZMC 20769	JX173847	*T. harzianum*	
		SZMC 20779	JX173848	*T. virens*	
Veszprém	parsley	SZMC 20865	JX173861	incomplete sequence	*T. harzianum*
		SZMC 20866	JX173862	*T. asperellum*	
	tomato	SZMC 20770	JX173856	*T. harzianum*	
		SZMC 20771	JX173857	*T. harzianum*	
		SZMC 20772	JX173859	*T. harzianum*	
		SZMC 20773	JX173858	*T. harzianum*	
		SZMC 20780	JX173860	unidentified species of *Trichoderma*	*T. atroviride*
	spinach	SZMC 20867	JX173863	*T. longibrachiatum/ H. orientalis*	
Szentes	tomato	SZMC 20783	JX173876	unidentified species of *Trichoderma*	*T. gamsii*
Balástya	tomato	SZMC 20776	JX173875	*T. pleuroticola*	
	pumpkin	SZMC 20868	JX173874	*T. citrinoviride*	
Ózd	carrot	SZMC 20784	JX173868	*T. hamatum*	
	tomato	SZMC 20781	JX173866	*T. atroviride*	
		SZMC 20782	JX173867	*T. atroviride*	
Hatvan	spice paprika	SZMC 20786	JX173869	*T. asperellum*	
		SZMC 20787	JX173870	*T. asperellum*	
	salad	SZMC 20785	JX173871	*T. hamatum*	
		SZMC 20788	JX173872	*T. longibrachiatum*	
	celery	SZMC 20869	JX173873	*T. harzianum*	

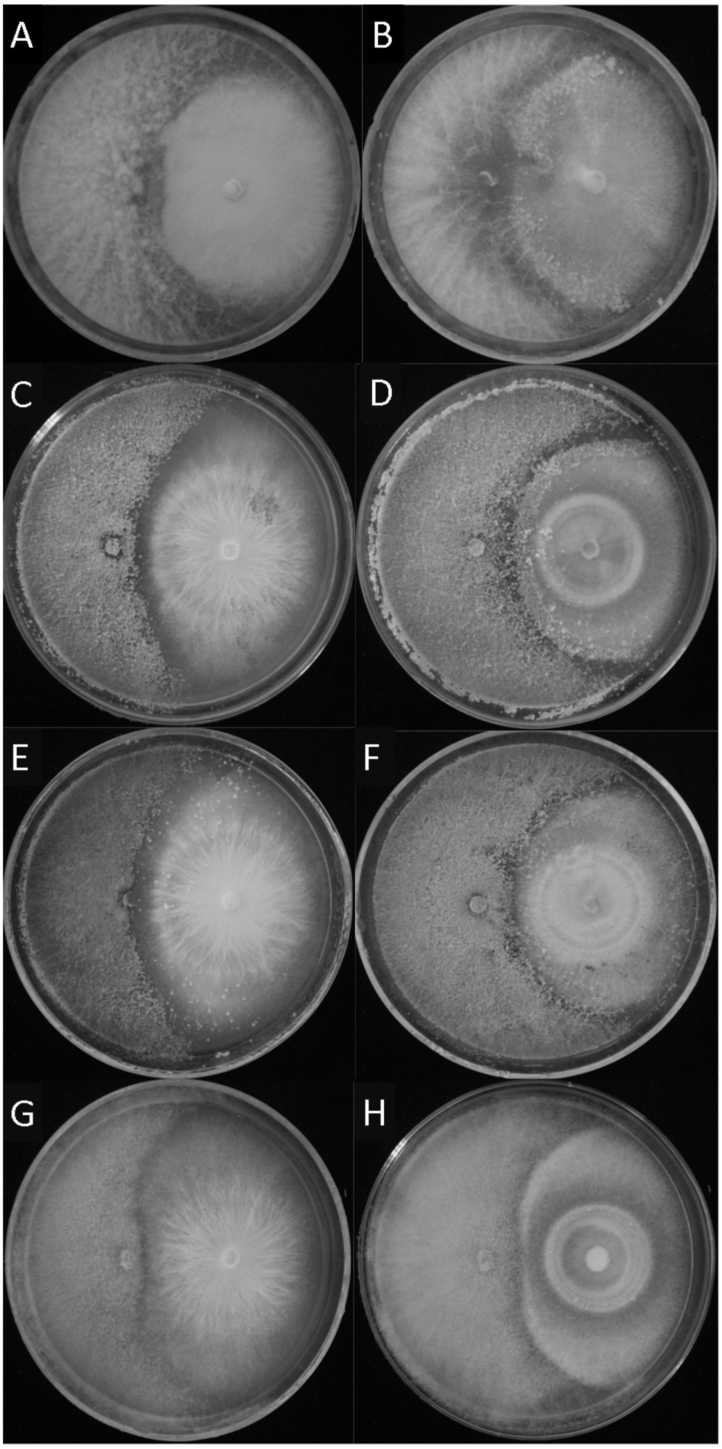

FIGURE 2 - *In vitro* antagonism of *Trichoderma* isolates (left side of the plates) derived from vegetable rhizosphere samples (*T. atroviride* SZMC 20780: A, B; *T. asperellum* SZMC 20866: C, D and SZMC 20786: E, F; *T. virens* SZMC 20779: G, H) against plant pathogenic isolates of *Fusarium oxysporum* (SZMC 6237J: A, C, E, G) and *F. solani* (SZMC 11064F: B; SZMC 11067F: D, F, H) (right side of the plates). Calculated BCI values were A: 66.98, B: 73.88, C: 48.22, D: 71,30, E: 48.66, F: 88.84, G: 41.37, H: 53.44

possible) and *T. hamatum* from the rhizospheres of tomato and carrot/salad, respectively, as well as *T. virens* and *T. gamsii* with single isolates from paprika and tomato rhizosphere, respectively.

Three different *Trichoderma* species known to be applicable for biocontrol purposes, *T. asperellum, T. virens* and *T. atroviride* were also examined in *in vitro* confrontation tests, where they proved to be able to overgrow the tested plant pathogenic *F. solani* and *F. oxysporum* isolates (Fig. 2). The Biocontrol Index (BCI) values calculated based on the images proved to be higher for *Fusarium solani* than for *F. oxysporum* in the case of these three examined *Trichoderma* species (Fig. 2). We found the highest BCI value in the case of *T. asperellum* SZMC 20786 against *F. solani* SZMC 11067F (88.84), while the lowest biocontrol index was determined for *T. virens* SZMC 20779 against *F. oxysporum* SZMC 6237J (41.37). Our results showed that *T. virens* has lower capability to overgrow the tested plant pathogenic fungi.

4 CONCLUSIONS

Besides the clinically relevant opportunistic pathogens *T. longibrachiatum* and *T. citrinoviride* and the mushroom green mould agent *T. pleuroticola*, species known as promising biocontrol agents (*T. harzianum, T. virens, T. atroviride, T. gamsii* and *T. asperellum*) could also be detected in the examined vegetable rhizosphere samples. The results of the recent study suggest that the rhizosphere of vegetables may be a rich source of potential biocontrol agents for environment-friendly, organic agricultural production. Strains belonging to the biocontrol species *T. asperellum*, *T. virens* and *T. atroviride* that are possessing good *in vitro* antagonistic activities against plant pathogenic *Fusarium* species might be promising for the development of fungal-based products that are able to suppress plant pathogenic fungi in the rhizosphere of organic farmland soils.

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CORRESPONDING AUTHOR

László Kredics
Department of Microbiology
Faculty of Science and Informatics
University of Szeged
Közép fasor 52.
6726 Szeged
HUNGARY

Phone: +36 62 544 516
Fax: +36 62 544 823
E-mail: kredics@bio.u-szeged.hu

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