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IDENTIFICATION AND CHARACTERIZATION OF FILAMENTOUS FUNGI ISOLATED FROM THE SUNFLOWER (*HELIANTHUS ANNUS* L.) RHIZOSPHERE ACCORDING TO THEIR CAPACITY TO HYDROLYSE INULIN

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ABSTRACT

Filamentous fungi able to hydrolyse inulin have been isolated from the rhizosphere of plants whose roots contain this polysaccharide. This study reports results concerning the isolation and identification of filamentous fungi from the soil used for sunflower cultivation and from the sunflower rhizosphere cultivated in field and in greenhouse. Fungi were evaluated according to their capacity to hydrolyse inulin and the variation in the diversity of these fungi during the plant's life cycle was also accessed. Forty-nine species of filamentous fungi were isolated. *Penicillium* and *Aspergillus* were the genera that presented higher number of species, nine and seven, respectively. At the end of the sunflower life cycle, cultivated both in field and in the greenhouse, a lower numbers of species were isolated. One hundred and fifty nine strains of filamentous fungi were isolated from soil and from the sunflower rhizosphere; from these, 79 (49.7%) were able to hydrolyse inulin. There was not significant difference in the proportion of species able to hydrolyse this polysaccharide during the sunflower's life cycle, in plants cultivated in field or in greenhouse. Although the sunflower's rhizosphere is a source of filamentous fungi able to hydrolyse inulin, that might be used in biotechnological processes. This system does not present a higher density of such microorganisms. Species of *Aspergillus, Chaetomium, Cunninghamella, Emericela, Eupenicillium, Fusarium, Myrothecium, Neosartorya, Neocosmospora, Penicillium* and *Thielavia* are being related by first time as inulinase producers.

Key words: Fungi, rhizosphere, soil, inulinase, sunflower.

INTRODUCTION

The fungi coexist with other organisms due to many biotic and abiotic factors in the environment which are favorable to the occupation of a common habitat (47). The term rhizosphere is defined as the soil volume adjacent to the roots and influenced by them (26) and represents an area of intense microbial activity (46), in which the organic nutrients coming from the roots favour the development of microorganisms (25). These nutrients are originated by the descamation of cells and of exudates such as sugars, organic acids, amine compounds as well as other substances released by the roots (6,10). Thus, the rhizosphere of plants that accumulate carbohydrates in the roots, as well as the plant material in decomposition are common source of a microbiota able to produce useful metabolites for industry (20,28,43). Inulinase-producing microorganisms may be selected by plating techniques (18,39,41), from the rhizosphere of plants whose roots contain inulin, like *Taraxacum officinarum* Rupr. (lion tooth) (43), *Helianthus tuberosus* L. (Jerusalem artichoke) (12), *Cichorium intybus* L. (chicory), *Dahlia pinnata* Cav. (dahlia) and *Helianthus annus* L. (sunflower) (19), members of the

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Asteraceae. This study accessed the fungal diversity at the sunflower rhizosphere.

The inulinase (2,1-b-D-fructanohidrolase EC 3.2.1.7) hydrolyses the inulin into pure fructose, being an excellent alternative for the production of fructose syrups. This sugar is used by the food and beverage industries, besides sweeteners, and shows several advantages in comparison to sucrose, being less cariogenic, highly soluble and hygroscopic and, therefore, less prone to form crystals; has low calory content and does not cause arteriosclerosis. Furthermore, fructose may be used by diabetes patients and mask the bitter taste of saccharin (2,5,14,43). Inulinases has also been employed for kidney disease diagnosis (22). With the ever-increasing potential of inulinase, it was necessary either to isolate different types of microorganisms producing higher yields of inulinase (44). This work aimed at the isolation and identification of filamentous fungi from soil used for sunflower cultivation and the sunflower rhizosphere from plants cultivated in field and in greenhouse. It was evaluated the diversity of fungi in the sunflower rhizosphere cultivated in field and in greenhouse during with the plant life cycle. Microorganisms were also characterized according to their capacity to hydrolyse inulin.

MATERIALS AND METHODS

Sunflower seeds

Sunflower seeds (variety Embrapa 122-V2000) were supplied by the Centro National de Pesquisa de Soja - Embrapa (Empresa Brasileira de Pesquisa Agropecuária), Londrina/PR.

Sunflower cultivation

Sunflower cultivation in the field was carried out by the Empresa Pernambucana de Pesquisa Agropecuária (IPA), Unidade Experimental de Pesquisa (UEP), in Itapirema/Goiana-PE, using natural soil. One hundred plants were distributed in ten lines containing ten plants each. The distance between the lines was 100 cm and between the plants 50 cm. The soil was kept humid up to the end of the sunflower life cycle of 100 days (1.5 L of water per plant every 4 days). Sunflower cultivation in the greenhouse was performed by the Departamento de Micologia, Centro de Ciências Biológicas (CCB), Universidade Federal de Pernambuco (UFPE). Twenty 5 kg pots were filled up with soil (hydromorphic podzol type, pH 5.4), 6 mg dm^{-3} of P and 0.06, 0.15, 1.85, 0.35 $cmol_c\ dm^{-3}$, respectively of K, Al, Ca and Mg, originated from the Empresa Pernambucana de Pesquisa Agropecuária (IPA), Unidade Experimental de Pesquisa (UEP). The soil used for the sunflower cultivation was analyzed for the presence of filamentous fungi and considered as control of cultivation (T_0). In each pot three seeds were planted and the soil kept humid up to the end of the sunflower life cycle (100 days), using 500 mL of water per plant every four days. After fifteen days, the two less developed plants were taken from each pot.

Samples from the sunflower rhizosphere

Samples from the sunflower rhizosphere (soil adjacent to the roots) were collected in plastic bags within: 25, 50, 75 and 100 days of growth. The field and greenhouse rhizosphere (25 g) of each plant was utilized for the isolation of fungi (five repetitions for each experiment).

Isolation, purification and identification of the fungi

The fungi were isolated according to Warcup (45). The soil or rhizosphere samples (25 g) were suspended in 225 mL of sterilized distilled water (1:10 dilution) and subsequently 10 ml of this suspension was added into 990 mL of sterilized distilled water. Petri dishes containing the Sabouraud Agar medium (23) plus chloranphenicol (100 mg L^{-1}) and Bengal Rose (50 mg L^{-1}) were inoculated with 1 mL of the 1:1000 diluted soil suspension. The plates were kept at room temperature (≅ 28ºC) and the growth of the colonies was accompanied up to 72 h. Fragments of the individual colonies were transferred separately to the same medium containing 50 mg L^{-1} of chloranphenicol and the growth was accompanied for 72 h. The strains were identified after growth on Czapek Agar and Potato Dextrose Agar (PDA) medium (23), by observing its macroscopic characteristics (colour, texture appearance and diameter of the colonies) and microscopic (microstructures), according to Baijal and Mehrotra (1), Bissett (4), Domsch *et al.* (11), Pitt (33), Hammill (17), Raper and Fennell (34), Rifai (35), Samuels *et al.* (36), Schipper (38) and Sutton (42).

Identification of the inulinase producing strains

One hundred fifty nine strains of filamentous fungi isolated from the control soil from the Empresa Pernambucana de Pesquisa Agropecuária (IPA), Unidade Experimental de Pesquisa (UEP) and rhizosphere of sunflower during the plant's life cycle, cultivated in field and in greenhouse were mantained in Czapek agar medium (*Aspergillus* and *Penicillium*) and PDA (other genera). Fragments of seven days old cultures were transferred to the center of a Czapek agar plate, containing 10 g L^{-1} of inulin instead of 10 g L^{-1} sucrose (I medium), 10 g L^{-1} glucose as positive controls (C+ medium) and without carbon source as negative control (C- medium). The plates were incubated at room temperature and the diameter of the colonies measured after seven days to evaluate the cell growth. Results for the I medium, were compared to the C- and C+ media results. The colonies relative growth in the inulin medium (Tx) was calculated by Tx = I x 100/C+, where I represents the diameter of the colony in I and C+ the diameter of the colony in C+ medium according to Cordeiro Neto *et al.* (9). Inulinase producing strains were evaluated by the measurement of the colonies diameters on I medium, their

mycelium characteristics and sporulation. The strains that were unable to grow on I medium or presented a poor growth and reduced sporulation in I medium, in comparison to C- medium were considered unable to degrade inulin. Experiments were performed in duplicate.

Statistical analysis

The Qui-square test was used (48) to evaluate a differential distribution in the number of species of filamentous fungi, and in the frequency of species able or unable inulin degradation during the sunflower 100 days life cycle. The Sorensen Coefficient was used [Ss] (21) to verify the similarity between the fungi species isolated from the rhizosphere of sunflower cultivated in field and in greenhouse.

RESULTS AND DISCUSSION

From the control soil (T_0) used to grow sunflower and from the sunflower rhizosphere cultivated in field and in greenhouse, 49 species were isolated. From the control soil (T_0) 25 species of filamentous fungi were isolated, 21 belong to Deuteromycotina; two belong to Ascomycotina and two to Zygomycotina. From the rhizosphere of sunflower cultivated in field and in greenhouse, 37 and 32, respectively (Table 1). Among the isolated species from the sunflower rhizosphere cultivated in field, 30 belong to Deuteromycotina, five belong to Ascomycotina, and two to Zygomycotina. Among the isolated species from rhizosphere of the greenhouse, 24 belong to Deuteromycotina, six belong to Ascomycotina, and two to Zygomycotina. The lower number of isolated species from the control soil (T_0) may be due to reduced number of these samplings (five), in comparison to the number of isolation procedures performed with each of the other assayed sources: five samplings from T_{25}; T_{50}; T_{75} and T_{100}, respectively from the sunflower rhizosphere, both grown in field and greenhouse (40 samples), instead of five T_0 analyzed samplings. These results agree with those found by Silva *et al.* (40), concerning the prevalence of fungi strains of the Deuteromycotina, isolated from tomato seeds and tomato rhizosphere. Santos *et al.* (37) reported similar results as to the predominance of Deuteromycotina found in sugarcane rhizosphere, by analyzing 142 samples obtained from 22 plantation sites, spread over several regions of the Pernambuco State (Brazil). Cordeiro Neto *et al.* (9), reported similar results on the distribution of 50 species of fungi, isolated from rhizosphere soils of Asteraceae plant, grown in Moji-Guaçu (São Paulo/Brazil). According to Garret (15), the techniques used to count microorganisms by direct observation at the microscope or by serial dilution and plating, usually done to isolate fungi, show distinct predominance of imperfect fungi or Deuteromycotina. As to the isolated genera, was observed a prevalence of *Penicillium* (nine) and *Aspergillus* (seven) species. There has also been a significant difference as to the time elapsed in the experiment, shown by a proportional decrease of isolated species, reaching their lowest number at T_{100}, from field (c^2=13.52; g.L =4; p <0.05) and greenhouse (c^2=10.4; g.L =4; p <0.05) sunflower rhizospheres. According to Cardoso *et al.* (6), Hale and Moore (16) and Westover *et al.* (46), this fact is probably due to the largest exudation by roots of organic compounds like sugars, organic acids and amine compounds, which is higher in the beginning of the plants growth. Cooke (8) pointed out that in the biodegradation process, fungi strains do act separately or in cooperation or ever in antagonism to other organisms. Fungi represent some of the more active organisms of the soil population, and some species may secrete antibiotics or toxins that can eliminate competitors and, besides being killed in some cases. These features may explain the higher number of species found at the beginning of the sunflower's growth stage.

In this study, the samples from control soil T_0 and from rhizosphere of sunflower cultivated both in field and greenhouse presented several common species: *Acremonium strictum*, *Aspergillus niger*, *A. niveus*, *A. viride-nutans*, *Cunninghamella elegans*, *Eupenicillium javanicum*, *Fusarium oxysporum*, *F. solani*, *Humicola fuscoatra*, *Penicillium citreonigrum*, *P. oxalicum*, *P. vinaceum*, *P. waksmanii* and *Thielavia terricola*. Some species were found only in the control soil T_0 samples as *Penicillium fellutanum* and *Rhizopus microsporus*, whereas the rhizosphere of sunflower cultivated in the field presented *Aspergillus brevipes*, *Curvularia senegalensis*, *Emericela nidulans*, *Fusarium heterosporium*, *F. lateritium*, *Sordaria sclerogenia* and *Phoma leveillei*, and from rhizosphere of sunflower cultivated in the greenhouse, resulted in *Eupenicillium brefeldianum*, *Neucosmospora vasinfecta* and *Penicillium restrictum*.

The similarity found between the fungi species isolated from the control soil T_0, and those isolated from the sunflower rhizosphere during the plant's life cycle was less pronounced at T_{100} growth stage, both in field cultivation (28.6%) as those from cultivation in greenhouse (41.0%). There was also a reduced similarity by comparing the species isolated from sunflower rhizosphere, at T_{100}, both from sunflower cultivated in field and in greenhouse (41.7%) (Table 2). According to Parkinson *et al.* (32) young roots are colonized initially by a diversity of the soil fungi, which after some days, are substituted by a more restricted mycobiota staying the same until the senescence of the roots. On the other hand, the decrease of the number of species isolated, found in the sunflower rhizosphere cultivated in greenhouse was not constant. After 50 days of growth, there was little variation in the number of species, fact that probably occurred due the plants being maintained in pots for the remaining cultivation period, therefore exposing the plants against adverse environmental conditions.

Table 1. Filamentous fungi isolated from the control soil T_0 and from the sunflower rhizosphere with 25, 50, 75 and 100 days of cultivation, in field and in greenhouse.

Species	Soil (T_0)	Field Time (days)				Greenhouse Time (days)			
		25	50	75	100	25	50	75	100
Acremonium strictum W. Gams	X	-	X	-	-	X	-	-	-
Alternaria tenuissima (Kunze ex Pers.) Wiltishire	-	-	-	-	X	-	-	-	-
Aspergillus brevipes Smith	-	X	-	-	-	-	-	-	-
A. fumigatus Fresenius	X	-	-	-	-	-	X	X	-
A. niger van Tieghem	X	X	X	X	X	X	X	X	X
A. niveus Blochwitz	X	X	X	X	-	X	X	X	-
A. sydowi Bain. and Sart. Thom and Church	X	-	-	-	-	-	-	-	-
A. terreus Thom	-	-	X	-	-	-	-	-	X
A. viride-nutans Ducker and Thrower	X	X	X	X	X	X	X	X	-
Chaetomium cupreum Ames	-	X	X	X	-	X	X	X	-
Cladosporium cladosporioides (Fresen.) de Vries	-	X	X	X	X	-	-	X	-
Cunninghamella elegans Lendner	X	-	X	-	X	X	-	-	-
Curvularia eragrostidis (P. Henn.) J. A. Meyer	-	-	-	-	X	-	X	X	X
C. lunata var. *aerea* (Batista, Lima & Vasconcelos) M.B.Ellis	-	-	-	-	-	X	-	-	-
C. pallescens Boedijn	X	-	-	-	-	X	-	-	-
C. senegalensis (Speg.) Subram.	-	-	X	-	-	-	-	-	-
Emericela nidulans (Eidam) Vuillemin	-	-	-	X	-	-	-	-	-
Eupenicillium brefeldianum (Dodge) Stolk & Scott	-	-	-	-	-	-	-	X	-
E. javanicum (van Beyma) Stolk & Stolk	X	X	X	X	-	X	X	-	-
Fusarium heterosporium Nees ex Fr.	-	X	-	-	-	-	-	-	-
F. lateritium Nees	-	-	-	-	X	-	-	-	-
F. oxysporum Schlecht. Emend Snyd. & Hans.	X	-	X	X	-	X	X	X	X
F. oxysporum var. *redolens* (Wollenus) Gordon	X	-	-	-	-	-	-	-	X
F. solani (Mart.) Appel & Wollenw. Emend Snyd. & Hans.	X	X	X	-	-	X	X	-	-
Humicola fuscoatra Traaen	X	X	X	-	-	X	-	X	X
Myrothecium roridum Tode ex Steudel	X	X	-	-	-	-	-	-	-
Neosartorya fisheri (Wehmer) Malloch & Cain	-	-	-	-	-	X	-	-	-
Neocosmospora vasinfecta E. F. Sm.	-	-	-	-	-	-	-	-	X
Penicillium citreonigrum Dierckx	X	X	X	-	-	X	-	-	-
P. fellutanum Biourge	X	-	-	-	-	-	-	-	-
P. janthinelum Biourge	X	X	-	-	-	-	-	-	-
P. oxalicum Currie and Thom	X	X	X	-	X	X	-	X	X
P. restrictum Gilman & Abbott	-	-	-	-	-	X	X	-	-
P. variabile Sopp	X	-	-	-	-	X	-	-	-
P. verruculosum Peyronel	-	X	X	X	-	X	X	X	X
P. vinaceum Gilman & Abbott	X	-	-	X	-	-	X	X	X
P. waksmanii Zaleski	X	-	X	-	X	-	X	X	X
Pestalotiopsis guepinii (Desm.) Stey.	X	X	X	X	-	-	-	-	-
Phoma leveillei Boerema & Bollen	-	X	X	X	-	-	-	-	-
Rhizopus microporus van Tieghem	X	-	-	-	-	-	-	-	-
R. oryzae Went & Prinsen Geerligs	-	X	X	X	X	X	X	X	X
Robillarda sessilis (Saccardo) Saccardo	-	-	X	-	-	-	-	-	-
Sordaria sclerogenia Fields & Grear	-	X	-	-	-	-	-	-	-
Thielavia terricola (Gilman & Abbott) Emmons	X	X	-	-	-	X	-	X	X
Torula caligans (Batista & Upadhyay) M. B. Ellis	-	X	-	-	-	-	-	-	-
Trichoderma aureoviride Rifai	X	-	X	-	-	-	-	-	-
T. harzianum Rifai	-	X	-	-	-	X	X	X	X
T. saturnisporum Hammill	-	-	-	X	-	X	-	-	-
T. virens (Miller, Giddens & Foster) Von Arx	-	X	-	-	-	-	X	X	-
Total	25	23	22	14	10	22	16	18	14

X, Isolated species.

Table 2. Sorensen Coefficient Similarity (%) between the species of filamentous fungi isolated from the control soil (T_0) and from the sunflower rhizosphere with 25, 50, 75 and 100 days, cultivated in field and in greenhouse.

Field (%)	Greenhouse (%)				
Time (Days)	0	25	50	75	100
0	100.0	59.6	43.9	46.5	41.0
25	50.0	57.8	51.3	58.5	37.8
50	59.6	63.6	52.6	55.0	44.4
75	35.9	50.0	60.0	56.2	35.7
100	28.6	31.2	38.5	50.0	41.7

Enzymatic characterization of fungi strains according to their capacity to hydrolyse inulin

From the 159 assayed fungi strains, 79 (49.7%) were able to hydrolyse inulin: 12 from control soil T_0 followed by 31 and 36 from the sunflower rhizosphere cultivated in field and in greenhouse, respectively (Table 3). During the sunflower's life cycle the strains able to hydrolyse inulin were distributed as follows: from the isolated strains of the field, 8 (57.1%) strains from T_{75}, followed by 11 (50.0%) in T_{50}, 9 (33.1%) in T_{25} and 3 (30.0%) in T_{100}. From the isolated strains of the greenhouse, 10 (66.7%) in T_{50}, followed by 8 (61.5%) in T_{100}, 8 (53.3%) in T_{75} and 10 (45.4%) in T_{25} (Table 3). There were no distinct differences related to the proportion of species characterized as able to hydrolyse inulin during the sunflower's life cycle, for both the field (c^2=4.127; g.L =4; $p < 0.05$) and greenhouse (c^2=6.895; g.L =4; $p < 0.05$). Nevertheless, the results show a higher percentage of fungi strains characterized as able to hydrolyse inulin obtained from 50 and 75 days of cultivation of the sunflower in field and from 25 and 100 days of cultivation of the sunflower in greenhouse. According to Cardoso *et al.* (6) the liberation of exudates occur when the roots suffer an injury through abrasion of rough soil particles. This may also release root fragments that represent potential substrates to fungi, being therefore, a way of these microorganisms obtaining inulin as a carbon source in the rhizosphere. Furthermore, filamentous fungi can use different substrates beyond those derived from plants, as arthropod shells, nematodes, among other biological materials. The presence of fungi strains that do not hydrolyse inulin in the sunflower rhizosphere may be explained by the diversity of substrates from biological origin that exist next to the roots, and that can be used by them.

Aspergillus niger, A. niveus, A. terreus, Chaetomium cupreum, Cladosporium cladosporioides, Cunninghamella elegans, Eupenicillium javanicum, Fusarium heterosporium, Fusarium oxysporum, F. oxysporum var. *redolens, F. solani, Humicola fuscoatra, Myrothecium roridum, Neosartorya fischeri, Neocosmospora vasinfecta, Penicillium citreonigrum, P. janthinelum, P. restrictum, P. variabile, P. verruculosum, P. vinaceum* and *Thielavia terricola* presented better development in the I medium (growth rate = 100%), thus considered as capable to hydrolyse inulin. Several *Aspergillus* and *Penicillium* species are known as inulinase producer (3,9,13,27,29,30,43,44). *C. elegans, P. verruculosum, Pestalotiopsis guepinii* and *Rhizopus oryzae* isolated from the *Viguiera* aff. *robust* (Asteraceae) rhizosphere by Cordeiro Neto *et al.* (9), showed a different behavior from those species described in this study, isolated from the sunflower rhizosphere. However, *Aspergillus fumigatus, Penicillium oxalicum* and *Trichoderma aureoviride* isolated from the sunflower rhizosphere were not able to hydrolyse inulin, according to data obtained by Cordeiro Neto *et al.* (9). *Cladosporium cladosporioides* presented a 100% growth rate on medium containing inulin, thus able to degrade inulin. This species was used by Lacerda Filho (24) to verify the capacity of inulinase production in medium prepared with extract of sunflower roots, obtaining a percent of 71% of enzyme production. Some species presented different behaviors during the sunflower's life cycle, as to the inulinase producing capacity. This behavior suggests that the genetical and physiological characteristics differ among different strains of the same fungi species. Some data on *Rhizopus microsporus* and *R. oryzae* strains disclosed that they were not able to grow on the media used for the enzymatic characterization, suggesting that the composition of the medium didn't supply the physiological needs of these species. This study provided the first data on the inulinase producing capacity of *Aspergillus niveus, A. terreus, Chaetomium cupreum, Cunninghamella elegans, Emericela nidulans, Eupenicillium javanicum, Fusarium heterosporium, F. oxysporum* var. *redolens, Myrothecium roridum, Neosartorya fischeri, Neocosmospora vasinfecta, Penicillium verruculosum, P. vinaceum* and *Thielavia terricola*.

The species characterized as able or unable to hydrolyse inulin were deposited in the Collection of Culture Micoteca URM, Departamento de Micologia, Universidade Federal de Pernambuco, Recife-PE, Brazil.

The results attained in this study, conduced by herein described experimental conditions, suggest that the Deuteromycotina (anamorphs fungi) are the fungi with higher occurrence in the soil at the Unidade Experimental de Pesquisa (UEP)/IPA, Goiana-PE and in the sunflower rhizosphere cultivated in field and greenhouse, and that *Aspergillus* and *Penicillium* species are more frequent than species of other genera. Furthermore, the obtained data indicate that the sunflower rhizosphere may interfere with the diversity of fungi, besides at the beginning of the sunflower's life cycle, the rhizosphere manifested a higher species diversity. The sunflower rhizosphere may be a source of species of filamentous fungi able to hydrolyse inulin, intending to find useful species to biotechnological processes. However, the sunflower rhizosphere does not concentrate fungi able to hydrolyse inulin during the entire plant's life cycle.

Table 3. Growth rate (%) regarding capacity to hydrolyse inulin by filamentous fungi strains, isolated from the control soil (T_0) and from the sunflower rhizosphere cultivated in field and in greenhouse during the plant life cycle.

Nº	Species	Soil (T_0)	Growth rate (%)							
			Field (days)				Greenhouse (days)			
			25	50	75	100	25	50	75	100
1	*Acremonium strictum*	0	-	0	-	-	0	-	-	-
2	*Alternaria tenuissima*	-	-	-	-	0	-	-	-	-
3	*Aspergillus brevipes*	-	0	-	-	-	-	-	-	-
4	*A. fumigatus*	0	-	-	-	-	-	67.4	0	-
5	*A. niger*	93.4	90.8	100	92.3	100	100	88.6	100	92.2
6	*A. niveus*	100	100	100	100	-	100	100	100	-
7	*A. sydowi*	0	-	-	-	-	-	-	-	-
8	*A. terreus*	-	-	100	-	-	-	-	-	100
9	*A. viride-nutans*	0	0	0	0	0	0	0	0	-
10	*Chaetomium cupreum*	-	100	0	100	-	100	100	100	-
11	*Cladosporium cladosporioides*	-	100	100	100	100	-	-	100	-
12	*Cunninghamella elegans*	100	-	94.4	-	100	0	-	-	-
13	*Curvularia eragrostidis*	-	-	-	-	0	-	0	0	0
14	*C. lunata* var. *aerea*	-	-	-	-	-	0	-	-	-
15	*C. pallescens*	0	-	-	-	-	0	-	-	-
16	*C. senegalensis*	-	-	0	-	-	-	-	-	-
17	*Emericela nidulans*	-	-	-	100	-	-	-	-	-
18	*Eupenicillium brefeldianum*	-	-	-	-	-	0	-	0	-
19	*Eupenicillium javanicum*	100	0	94.7	0	-	-	100	-	-
20	*Fusarium heterosporium*	-	100	-	-	-	-	-	-	-
21	*Fusarium lateritium*	-	-	-	-	0	-	-	-	-
22	*F. oxysporum*	100	-	100	100	-	100	100	100	100
23	*F. oxysporum* var. *redolens*	100	-	-	-	-	-	-	-	100
24	*Fusarium solani*	100	97.3	100	-	-	85.1	100	-	-
25	*Humicola fuscoatra*	0	100	100	-	-	100	-	-	-
26	*Myrothecium roridum*	100	100	-	-	-	-	-	-	-
27	*Neosartorya fisheri*	-	-	-	-	-	100	-	-	-
28	*Neocosmospora vasinfecta*	-	-	-	-	-	-	-	-	100
29	*Penicillium citreonigrum*	0	0	100	-	-	0	-	-	-
30	*P. fellutanum*	0	-	-	-	-	-	-	-	-
31	*P. janthinelum*	100	0	-	-	-	-	-	-	-
32	*P. oxalicum*	0	0	0	-	0	0	-	0	0
33	*P. restictum*	-	-	-	-	-	0	100	-	-
34	*P. variabile*	98.0	-	-	-	-	100	-	-	-
35	*P. verruculosum*	-	100	100	100	-	100	100	100	100
36	*P. vinaceum*	100	-	-	100	-	-	100	100	100
37	*P. waksmanii*	0	-	0	-	0	-	0	0	0
38	*Pestalotiopsis guepinii*	0	0	0	0	-	-	-	-	-
39	*Phoma leveillei*	-	0	0	0	-	-	-	-	
40	*Rhizopus microporus*	0	-	-	-	-	-	-	-	
41	*R. oryzae*	-	0	0	0	0	0	0	0	0
42	*Robillarda sessilis*	-	-	0	-	-	-	-	-	-
43	*Sordatia sclerogenia*	-	0	-	-	-	-	-	-	-
44	*Thielavia terricola*	85.7	0	-	-	-	100	-	100	90.9
45	*Torula caligans*	-	0	-	-	-	-	-	-	-
46	*Trichoderma aureoviride*	0	-	0	-	-	-	-	-	-
47	*T. harzianum*	-	0	-	-	-	0	0	-	0
48	*T. saturnisporum*	-	-	-	0	-	0	-	-	-
49	*T. virens*	-	0	-	-	-	-	-	-	-
	Total tested strains	25	23	22	14	10	22	15	15	13
	Total positive strains	12	9	11	8	3	10	10	8	8
	% positive strains	48.0	33.1	50.0	57.1	30.0	45.4	66.7	53.3	61.5

RESUMO

Identificação e caracterização de fungos filamentosos isolados de rizosfera de girassol (*Helianthus annus* L.) de acordo com a capacidade de hidrolisar inulina

Fungos filamentosos capazes de hidrolisar inulina tem sido isolados de rizosfera de plantas que acumulam esse polissacarídeo nas raízes. Este estudo compreendeu o isolamento e identificação de fungos filamentosos do solo utilizado para o cultivo do girassol e da rizosfera de girassol cultivado em campo e em casa de vegetação, a fim de verificar se há variação na diversidade destes fungos ao longo do ciclo de vida da planta. O fungos foram também caracterizados quanto a capacidade de hidrolisar inulina. Das quarenta e nove espécies de fungos filamentosos isoladas, *Penicillium* e *Aspergillus* foram os gêneros que apresentaram maior número de espécies, nove e sete, respectivamente. No final do ciclo de vida do girassol, cultivado tanto em campo quanto em casa de vegetação, foi isolado um menor número de espécies. Cento e cinquenta e nove amostras de fungos filamentosos, isoladas do solo e da rizosfera de girassol e destas 79 (49,7%) foram capazes de hidrolisar inulina. Não houve diferença significativa quanto a proporção de espécies capazes ou não de hidrolisar esse polissacarídeo, ao longo do ciclo de vida do girassol, cultivado tanto em campo quanto em casa de vegetação. Embora a rizosfera de girassol seja uma fonte de fungos filamentosos capazes de hidrolisar inulina, que podem ser utilizados em processos biotecnológicos, ela não atua de modo a concentrar fungos com esta característica. Espécies de *Aspergillus, Chaetomium, Cunninghamella, Emericela, Eupenicillium, Fusarium, Myrothecium, Neosartorya, Neocosmospora, Penicillium* and *Thielavia* estão sendo relatados pela primeira vez como produtores de inulinase.

Palavras-chave: Fungos, rizosfera, solo, inulinase, girassol.

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