Journal of Biological Sciences 11 (8): 492-495, 2011
ISSN 1727-3048 / DOI: 10.3923/jbs.2011.492.495

In vitro Interactions and Fungal Populations Isolated from Maize Rhizosphere

[1]Cigdem Kucuk and [2]Merih Kyvanc
[1]Department of Biology, Faculty of Arts and Science, Harran University,
63300 Sanlyurfa, Turkey
[2]Department of Biology, Faculty of Science, Anadolu University,
26470 Eskisehir, Turkey

Abstract: The purpose of the present study was to establish the species composition of fungi living on rhizosphere soils of maize and to establish the antagonistic activity of these fungi. In this study, 15 fungal species have been identified. *Fusarium verticillioides*, *Fusarium oxysporum*, *Fusarium lateritium*, *Fusarium flocciferum*, *Penicillium bilaiae*, *Penicillium solitum*, *Aspergillus versicolor*, *Aspergillus ustus*, *Trichoderma harzianum*, *Cladosporium herbarum*, *Trichoderma viride*, *Gliocladium virens*, *Penicillum verrucosum*, *Fusarium* sp. and *Curvularia lunata* are the fungal species. Interactions between the fungi were also investigated. *A. versicolor, Aspergillus ustus* and *Gliocladium viride* were able to dominant almost all species tested

Key words: Maize, rhizosphere, fungi, antagonistic activity

INTRODUCTION

Soil is perhaps the most complex and heterogenous of natural systems, being composed of a solid, liquid and gaseous phase of changing proportions in time and space. Soil is also a medium for plant growth and a habitat for myriads of microorganisms (Sorensen, 1997). Concerning fungi, the mycelial networks of soil are extensive, several hundred meters per gram of soil (Thorn, 1997; Gomes *et al*., 2003).

Although the total biomass represents only about 0.02-0.1% of the soil mass, the mycelial Networks of soil have an important function in binding the soil particles together, helping to maintain a good soil structure for plant growth (Graystone *et al*., 1998; Soonthornpoct *et al*., 2001).

Fungi play an important role in nutrient cycling and plant health and development (Chiarini *et al*., 1998; El-Mehalawy *et al*., 2004; Sorensen, 1997). While some fungi are well known to cause a range of plant diseases and in some cases to devastate agricultural crops (Rheeder *et al*., 1990; Palumbo *et al*., 2008), others are known to antagonist plant pathogens (Whipps, 2001; Reyes *et al*., 2002). Maize (*Zea mays* L.) is one of major cereal in Turkey (Sahin, 2001).

The aim of this observation was to study fungi distribution in maize rhizospheres and to determine interactions and enzyme activities of isolated cultures.

MATERIALS AND METHODS

Soil: Soil were sampled from agricultural site of Eskiþehir, Turkey. Eskiþehir is located in central part of Anatolia. The characteristics of the soil are shown in Table 1.

Isolation: Fungi were isolated from the rhizosphere of 120 maize plants grown in field site of the Eskiþehir region in Turkey. Soil samples were diluted (Bateman, 1993). An aliquot of 1 mL of each suspension was pipeted into each of five replicate petri dishes containing Creatine Sucrose Agar (KCl, 5 g; $MgSO_4$, 5 g; $FeSO_4.7H_2O$, 0.1 g; $ZnSO_4.7H_2O$, 0.1 g; $CuSO_4.5H_2O$, 0.05 g; agar, 15 g; distilled water, 100 mL), 25 % Glycerol Nitrate agar (K_2HPO_4, 1 g; Chapex concentration, 7.5 g; yeast extract, 3.7 g; glycerol 250 g; adar, 12 g; distilled water 750 mL), Neutral creatine sucrose agar (Chapex concentration 10 mL; sucrose, 10 g; creatine, 5 g; KH_2PO_4, 1 g; bromocresol purple, 0.06 g; agar, 15 g; distilled water, 1000 mL), Malt Extract Agar (MEA, MERCK), Chapex Yeast Agar (K_2HPO_4, 1 g; Chapex concentration 10 mL; yeast extract, 5 g; sucrose, 200 g; agar, 15 g; distilled water 1000 mL) and Potato Dextrose Agar (PDA, MERCK).

The dishes were kept in darkness at 20°C for 7 days and then in an illuminated incubator with 16 h length and day/night temperatures 18/13°C for 7 days. Fungi isolated were identified using of Biolog commercial identification systems.

Corresponding Author: Cigdem Kucuk, Department of Biology, Faculty of Arts and Science, Harran University, 63300 Sanlyurfa, Turkey

Interaction: *In vitro* comparisons consisted of removing 5 mm diameter disks from the edge of colonies grown on PDA (Potato Dextrose Agar) in petri dishes. The paired fungi were placed on opposite sides of petri containing PDA. Paired cultures were incubated for 7 days then scored for degree of antagonism on a scale of classes 1-5, 1: antagonist completely overgrew the pathogen and covered the entire medium surface, 2: antagonist overgrew at least two-thirds of the medium surface, 3: antagonist and the pathogen each colonized approximately one-half of the medium surface and either organism appeared to dominate the other, 4: the pathogen colonized at least two-thirds of the medium surface and appeared to withstand encroachment by antagonist, 5: the pathogen completely overgrew the antagonist and occupied the entire medium surface (Bell *et al*., 1982).

Cellulase activity: The plates containing cellulase the medium ($NH_4H_2PO_4$, 1.0 g; $MgSO_4.7H_2O$, 0.2 g; KCl, 0.2 g; $CaCl_2$, 0.2 g; 4% ball milled cellulose 250 mL; agar, 12 g; distilled water, 750 mL) for testing cellulase activity were inoculated either centrally and 25°C incubated. Cellulase activity is then visualized as clear zones around fungal colonies (Paterson and Bridge, 1994).

Chitinase activity: The plates containing synthetic agar medium (glucose, 15 g; $MgSO_4.7H_2O$, 0.2 g; KH_2PO_4, 0.9 g; Kcl, 0.2 g; NH_4NO_3, 1.0 g; Fe^{2+}, 0.002 g; Zn^{2+}, 0.002 g and colloidal chitin, 3 g; agar, 12 g; distilled water 1000 mL) were inoculated and 25°C incubated and then clear zones were observed indicating chitinase activity (Rosato *et al*., 1981).

Metabolite analysis: The tested fungi were inoculated on Yeast Extract Sucrose Agar (YES) (which contained per litre; yeast extract, 20 g; sucrose, 150 g; agar, 20 g and $MgSO_4.7H_2O$, 0.5 g) incubated for 7 days at 25°C. To extract extracellular metabolites remove agar plugs from the colonies grown on YES using a 5 mm cork borer and plugs were extracted with ethylacetate. The extracts (15 μL) were spotted onto TLC plates. The plates were developed with chloroform:methanol (2:1 v/v) and bands were observed under UV light (254 nm) for spots with Rf values (Zelenkova *et al*., 2003).

Table 1: Characteristics of soil used in this study

Soil properties	Values
pH	7.60
$CaCO_3$	1.02
Soil texture	Sandy loam
Organic mater (%)	2.47
P_2O_5 (kg da^{-1})	18.50
K_2O (kg da^{-1})	104.10
EC	0.064

RESULTS AND DISCUSSION

Fungal species from rhizosphere soil of maize were obtained. From 15 fungal species isolated were identified at least to the genus level, however, one of them did not sporulate and could not be identified. The one non-sporulating mycelial fungus as assigned as sterile fungus. Fifteen fungal species were identified from maize rhizosphere soil (Table 2). The genus *Fusarium* seems to be the most abundant genus for representing 78.8% of the total fungal genus. *Curvularia lunata* is considered to be the least abundant organisms for representing 4.2% of the total fungi. Similar results have reported by Chiarini *et al*. (1998), Gomes *et al*. (2003) and Marin *et al*. (1998).

Fungi are of biotechnological interest due to their potential use as guetic vectors (Paterson and Bridge, 1994) as a source of secondary metabolites (Herbert, 1989; Miller, 1995) and as biological control agents (Magan and Lacey, 1985). The release of organic substances by plant roots has an interesting ecological aspect, since it influences the nutrient availability in the rhizosphere and indirectly acts on the soil microorganisms that in turn influence plant growth (Graystone *et al*., 1998). It is clear that some of the most isolated form maize rhizosphere soil may benefit the host and further studies will be carried out to evaluate the potential use of endophytes in biological control. In our work, a large number of species could be isolated from maize but only very few species were present in significant amount as cited by McLean and Berjak (1987).

In this study, the interactions of the between fungi were categorized according to their behaviour on the dual assay plates as described in Table 3. For example, interactions between *Fusarium* species were mutually antagonistic upon contact. This results is similar to that by Marin *et al*. (1998). *A. versicolor, Aspergillus ustus* and *Gliocladium viride* were able to dominant almost all species tested (Table 3). *Aspergillus ustus*, *Gliocladium*

Table 2: Fungi isolated from soil and their percentage of occurrence and Rf of metabolites producted by fungi

Fungi	%	Rf x 100
Aspergillus ustus	27.5	36
Aspergillus versicolor	7.3	19
Cladosporium herbarum	4.2	72
Curvularia lunata	2.0	21
Fusarium flocciferum	9.6	25
Fusarium lateritium	9.6	68
Fusarium oxysporum	25.2	30
Fusarium sp.	2.0	72
Fusarium verticillioides	32.4	27
Gliocladium virens	8.7	44
Penicillium bilaiae	12.4	35
Penicillium solitum	7.3	42
Penicillum verrucosum	5.8	25
Sterile fungus	2.0	18
Trichoderma harzianum	18.0	38
Trichoderma viride	12.6	38

Table 3: Interaction types between isolated fungal strains

	Vers	Ust	Vert	Soli	Viri	Bil	Oxy	Lat	Lun	Har	Floc	Vire	Verr	Herb	Fus.	Sf
Vers	-	1	2	1	1	2	2	2	2	2	2	0	4	4	4	3
Ust	1	-	2	2	2	0	2	2	4	2	2	2	2	1	1	3
Vert	2	4	-	2	4	4	2	2	4	3	2	4	2	4	2	3
Soli	1	4	2	-	4	4	4	3	4	3	2	4	4	4	4	2
Viri	1	4	2	0	-	2	4	3	4	0	2	2	2	2	2	2
Bil	0	2	2	0	4	-	2	2	4	0	2	4	2	2	2	2
Oxy	2	2	2	0	4	2	-	2	2	2	2	4	4	4	4	4
Lat	4	4	2	0	2	2	0	-	3	0	4	4	2	0	2	4
Lun	4	4	2	0	2	3	0	1	-	2	3	4	2	2	2	4
Har	2	4	4	0	2	3	3	2	2	-	3	4	2	2	2	4
Floc	2	4	2	0	4	3	2	2	2	4	-	4	3	2	2	1
Vire	2	4	4	0	4	2	0	2	2	4	3	-	3	2	0	1
Verr	0	4	4	0	4	2	0	2	2	2	3	0	-	2	0	1
Herb	0	2	4	0	4	2	0	2	3	2	2	2	2	-	2	1
Fus.	1	2	4	0	3	2	2	2	3	2	2	3	2	2	-	1
Sf	3	3	3	2	2	2	4	4	4	4	3	3	2	2		-

Vers: *A. versicolor*, ust: *A. ustus*, vert: *F. verticillioides*, soli: *P. solitum*, viri: *G. viride*, bil: *P. bilaiae*, oxy: *F. oxysporum*, lat: *F. lateritium*, lun: *C. lunata*, har: *T. harzianum*, floc: *F. flocciferum*, vire: *T. vireus*, verr: *P. verrucosum*, herb: *Cladosporium herbarum*, Fus: *Fusarium* sp., Sf: Sterile fungus. Scores are applied at left interacting with those listed above

viride, *Trichoderma harzianum* and *T.virens* were able to grow faster than the other isolated fungi. *Fusarium verticillioides*, *F. lateritium*, *F. flocciferum*, *Penicillium bilaiae* and Sterile fungus were mutually antagonistic either on contact or at a distance (Table 3).

Sterile fungus and *Cladosporium herbarum* grew more slowly than all the other fungi. The present work gives a general impression of how a range of fungal colonies from maize grown soil interact with each other and their potential competitiveness. There are many reports that note the interaction between fungi (Rheeder *et al*., 1990; Chiarini *et al*., 1998; Hussein *et al*., 2002). The enhanced competitiveness due to metabolite production in agar medium needs to be treated with caution as in grain substrates the production patterns and role may be very different (Magan and Lacey, 1985; Marin *et al*., 1998).

Marin *et al*. (1998) reported some *Aspergillus* species were able to inhibit the growth of some *Fusarium* species. In conrast, the dominance of *Fusarium* and *Trichoderma* species could be predominantly due to their ability to grow rapidly and invasively. Although the *Pencillium* species are known to export antifungal metabolites they did not have high levels under tested conditions and grew significantly more slowly than the *Trichoderma, Fusarium* and *Aspergillus* spp.

Wicklow *et al*. (1988) indicated that common fungal colonists of maize kernels interfere with the ability of *A. flavus* to infect preharvest maize; *F. moniliforme* was particularly effective in inhibiting kernel infection by *A. flavus*. The study of fungal interactions has attracted much interest due to the fact that mycotoxin production by toxigenic species which are present in stored grain can be both enhanced or inhibited by the other species which are colonizing the grain.

Wicklow *et al*. (1988) showed how aflatoxin production by *A. flavus* was stimulated by *Bacillus amyloliquefacins* and *Hyphopichia burtonii* when growing together on irradiated maize and rice grains under certain environmental conditions.

The enzymic activity tests gave more encouraging results in respect of differentiating fungal species. All cultures tested showed different level of cellulase and chitinase activity and only four (*Penicillium solitum*, *Cladosporium herbarum*, *Curvularia lunata* and *Penicillium verrucosum*) showed no reducing ability.

In this study, examination of thin layer chromatograms of fungi showed that several different compounds were being produced by the isolated fungal species (Table 2). Toxins produced by *Fusarium, Penicillium* and *Aspergillus* species have been reported among the general phytotoxins being able to result in leaf chlorosis and could inhibit seed germination and growth (Visconti *et al*., 1992; D'Mello, 1997). If the fungi isolated in this assay were pathogens in latent infection phase, their early detection in the field would influence the recognition of disease spread within the maize crop. On the other hand, these fungi could be either avirulent or hypovirulent strains. These fungi could be adapted to this host and be antagonists of soilborne pathogens and depending on their antagonistic capacity, they would be able to displace reduce, suppress or induce resistance against them.

Conslusively the results shown a strong warning for the toxicant productivities by soil fungi and rhizosphere is a useful potential source for isolation of fungi producers of biological active products. Further work will be carried out to identify those metabolites produced by soil fungi.

REFERENCES

Bateman, G.L., 1993. Development of disease symptoms and fungal pathogens on shoot bases in continuous winter wheat. Plant Pathol., 42: 595-608.

Bell, D.K., H.D. Wells and C.R. Markham, 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72: 379-382.

Chiarini, L., A. Beviviona, C. Dalmastri, C. Nacamulli and S. Tabacchioni, 1998. Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Applied Soil Ecol., 8: 11-18.

D'Mello, J.P.F., 1997. Handbook of Plant and Fungal Toxicants. CRC Pres Inc., Boca Raton, FL., USA., ISBN-13: 9780849385513, Pages: 356.

El-Mehalawy, A.A., N.M. Hassanein, H.M. Khater, E.Z.A.K. El-Din and Y.A. Youssef, 2004. Influence of maize root colonization by the rhizosphere actinomycetes and yeast fungi on plant growth and on the biological control of late wilt disease. Int. J. Agric. Biol., 6: 599-605.

Gomes, N.C.M., O. Fagbola, R. Costa, N.G. Rumjanek, A. Buchner, L. Mendona-Hagler and K. Smalla, 2003. Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Applied Environ. Microbiol., 69: 3758-3766.

Graystone, S.J., S. Wang, C.D. Campbell and A.C. Edwards, 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem., 30: 369-378.

Herbert, R.B., 1989. The Biosynthesis of Secondary Metabolites. 2nd Edn., Springer, UK., ISBN-13: 9780412277207, Pages: 231.

Hussein, H.M., M.J. Christensen and M. Baxter, 2002. Occurrence and distribution of *Fusarium* species in maize fields in New Zealand. Mycopathologia, 156: 25-30.

Magan, N. and J. Lacey, 1985. Interactions between field and storage fungi on wheat grain. Trans. Br. Mycol. Soc., 85: 29-37.

Marin, S., E. Companys, V. Sanchi, A.J. Ramos and N. Magan, 1998. Effect of aw and temperature on competing abilities of common maize fungi. Mycol. Res., 120: 950-964.

McLean, M. and P. Berjak, 1987. Maize grains and their associated mycoflora-a microecological study. Seed Sci. Technol., 15: 831-850.

Miller, J.D., 1995. Fungi and mycotoxins in grain: Implications for stored product research. J. Stored Prod. Res., 31: 1-16.

Palumbo, J.D., T.L. O?Keeffe and H.K. Abbas, 2008. Microbial interactions with mycotoxigenic fungi and mycotoxins. Toxin Rev., 27: 261-285.

Paterson, R.R.M. and P.D. Bridge, 1994. Biochemical Techniques for Filamentous Fungi. CAB International, UK., ISBN-13: 9780851988993, Pages: 125.

Reyes, I., L. Bernier and H. Antoun, 2002. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. Microbial Ecol., 44: 39-48.

Rheeder, J.P., W.F.O. Marasas and P.S. van Wyk, 1990. Fungal associations in corn kernels and effects on germination. Phytopathology, 80: 131-134.

Rosato, Y.B., C.L. Messias and J.L. Azevedo, 1981. Production of extracellular enzymes by isolates of *Metarhizium anisopliaem*. J. Invertebrate Pathol., 38: 1-3.

Sahin, S., 2001. Turkiye de misir ekilis alanlariný dagilisi ve misir uretimi. Gazi Universitesi, Egitim Fakultesi Dergisi, 21: 73-90.

Soonthornpoct, P., L.E. Trevathan and D. Ingram, 2001. The colonization of maize seedling roots and rhizosphere by *Fusarium* spp. in mississippi in two soil types under conventional tilage and no-tillage systems. Phytoprotection, 81: 97-106.

Sorensen, J., 1997. The Rhizosphere as a Habitat for Soil Microorganisms. In: Modern Soil Microbiology, Van Elsas, J.D., J.T. Trevors and E.M.H. Wellington (Eds.). Marcel Dekker, Inc., New York, USA., pp: 21-45.

Thorn, R.G., 1997. The Fungi in Soil. In: Modern Soil Microbiology, Elsas, J.D., J.T. Trevors and E.M.H. Wellington (Eds.). Marcel Dekker, Inc., New York, pp: 63-127.

Visconti, A., A. Sibillia and C. Sabia, 1992. *Alternaria alternata* from oilseed rape: mycotoxin production and toxicity to *Artemia salina* larvae and rape seedlings. Mycotoxin Res., 8: 9-16.

Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot., 52: 487-511.

Wicklow, D.T., B.W. Horn, O.L. Shotwell, C.W. Hesseltine and R.W. Caldwell, 1988. Fungal interference with *Aspergillus flavus* infection and aflatoxin contamination of maize grown in a controlled environment. Phytopathology, 78: 68-74.

Zelenkova, N.F., N.G. Vinokurova and M.U. Arinbasarav, 2003. Analysis of secondary metabolites of microscopic fungi of the genus *Penicillium* by chromatographic techniques. Applied Biochem. Microbiol., 39: 44-54.