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Comparative Study of Antagonistic Activity of Scots Pine Root Associated Mycorrhizal Fungus-Bacteria and Wheat Associated Bacteria Against Plant Pathogenic Fungi

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ABSTRACT: The aim of the present work was to test antagonistic activity of Scots pine root associated mycorrhizal fungus-bacteria and wheat associated bacteria against pathogenic fungi *Fusarium culmorum*, *Rhizoctonia solani, Botrytis cinera* and to determine the production of extracellular lytic enzymes by bacterial strains. The higher number of bacterial strains isolated from wheat showed antagonistic activity compared to bacterial strains isolated from mycorrhizal hyphae. The best performing strains from mycorrhizal hyphae were *Arthrobacter ilicis* KNCL24, *Rhodococcus. fascines* HNOL8 and from wheat *Bacillus cohnii* 19, *B. subtilis* 1, and *B. lentus* 28. All isolates presented high level of pectinase activity. Only four strains *A. ilicis* KNCL24, *B. lentus* 17, *B. subtilis* 4, *B. halodurans* 12 were able to produce hydrogen cyanide (HCN). Our results showed that bacterial strains associated with wheat possess more antagonistic activity compared to bacterial strains from mycorrhizal hyphae. The all tested bacterial strains produced one or more cell wall degrading enzymes.

Keywords: Mycorrhiza, wheat, antagonistic activity, lytic enzymes

İskoçya Çam Köküyle İlişkili Mikorizal Mantar-Bakteriler ile Buğdayla İlişkili Bakterilerin Patojenik Mantarlara Karşı Antagonistik Etkilerinin Karşılaştırılması

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ÖZET: Bu çalışmanın amacı, İskoçya çam köküyle ilişkili mikorizal mantar bakterileri ile *Fusarium culmorum*, *Rhizoctonia solani, Botrytis cinera* gibi patojenik mantarlara karşı buğdayla ilişkili bakterilerin antagonistik aktivitesini test etmek, ve bakteri suşları ile ekstrasellüler litik enzimlerinin üretimini belirlemektir. Mikorizal hiflerden izole edilen bakteriyel suşlara nazaran, buğdaydan izole edilen yüksek sayıdaki bakteriyel suşlar antagonistik aktivite göstermiştir. Mikorizal hiflerden elde edilen en iyi performansa sahip suşların, *Arthrobacter ilicis* KNCL24, *Rhodococcus. fascines* HNOL8 ve buğdaylardan ise *Bacillus cohnii* 19, *B. subtilis* 1 ve *B. lentus* 28 olduğu belirlenmiştir. Tüm izolatlar, yüksek düzeyde pektinaz aktivitesi göstermiştir. Sadece 4 suş (*A. ilicis* KNCL24, *B. lentus* 17, *B. subtilis* 4, *B. halodurans* 12) hidrojen siyanür üretebilmiştir. Sonuçlarımız, buğdayla ilişkili bakteriyel suşların, mikorizal hiflerden elde edilen bakteriyel suşlardan daha fazla antagonistik aktiviteye sahip olduğunu göstermiştir. Test edilen tüm bakteriyel suşlar, bir yada daha fazla hücre duvarını parçalayan enzimleri üretmiştir.

Anahtar kelimeler: Mikoriza, buğday, antagonistik aktivite, parçalayıcı enzimler

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INTRODUCTION

The rhizosphere bacteria can be classified according to their effects on plants as plant pathogens or beneficial bacteria (Lugtenberg et al., 2002). The beneficial association of bacteria with a plant root has been related both to their antagonistic activities towards pathogens and to their ability to colonise and produce plant growth promoting compounds within the rhizosphere (Cook et al., 1995).

These plant beneficial microorganisms are known to antagonize phytopathogens through competition for niches or nutrients (e.g. iron through siderophores synthesis); parasitism that may involve production of hydrolytic enzymes, for example, chitinase, glucanase, protease and cellulase that can lyse pathogen cell walls; inhibition of the pathogens by anti-microbial compounds (antibiosis); induction of systemic resistance in host plants (Nielson and Sorensen, 1999). The effectiveness of plant growth promoting rhizobacteria for the biocontrol of phytopathogens has been proved in others studies (Berg et al., 2005; Egamberdieva et al., 2010). Occurrence of antagonistic *Pseudomonas* species associated with crops grown in soils that were naturally suppressive to different plant pathogens, including *Gaeumannomyces graminis* var. *tritici, Fusarium oxysporum*, *Rhizoctonia solani* (Bergsma-Vlami et al., 2005). *Bacillus* species are also showed antagonistic activity against plant pathogenic fungi (Jiang et al., 2001). The mycorrhizal fungi are also interacting with various bacterial species and it occurs in the zone of surrounding the roots and fungal hyphae, mycorrhizosphere (Garbaye, 1994). The bacteria directly influence the physiology of the plants, and together with AM fungi may create a more indirect synergism that supports plant growth, including nutrient acquisition and inhibition of plant pathogenic fungi (Barea et al., 2002; Gamalero et al., 2004). However, origin of bacterial strains effect on their antagonistic activity. The objectives of the present study were to test antagonistic activities and production of extracellular lytic enzymes by Scots pine root associated mycorrhizal fungus-bacteria and wheat associated bacteria.

MATERIAL AND METHODS

Strains which were isolated from the roots of wheat plant growing in loamy sand Germany (Egamberdiyeva and Hoflich, 2002; Egamberdiyeva and Hoflich, 2003) is from the culture collection of Institute of Landscape Matter Dynamics, Muencheberg, Germany. Strains which were isolated from mycorrhizal hyphae associated with Silver birch grown in sandy clay loam of Finland are from the culture collection of Helsinki University of Finland.

Hydrogen cyanide production was detected using cyanide indicator paper (Castric, 1975). Lipase activity was detected using the Tween lipase indicator assay (Howe and Ward, 1976), pectinase activity of bacterial strains was determined as described by Smibert and Krieg (Smibert and Krieg, 1994), ß-glucanase activity using the glucan substrate lichenan in top agar plates (Walsh et al., 1995) and cellulase activity was tested using the substrate carboxymethylcellulose in top agar plates.

The bacterial isolates were tested *in vitro* on antagonistic activity against the plant pathogenic fungi *F. culmorum*, *R. solani* and *B. cinera* using a plate bioassay with Potato Dextrose Agar (PDA). Fungal strains were grown on agar plates at 28^{o}C for 5 days. Disks containing a fresh culture of the fungus (approximately 5 mm in diameter) were cut out of the edge of the fungal growth and placed in the centre of a 9 cm diameter Petri dish. Bacteria grown on solid LC medium (containing per liter demineralised water: tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g and agar-agar, 18 g) were streaked on the test plates perpendicular to the fungus. Plates were incubated at 28^{o}C for 7 days, until the fungi had covered the control plates without bacteria. Antifungal activity was recorded as the width of the zone of growth inhibition between the fungus and the test bacterium.

RESULTS AND DISCUSSION

In vitro antagonism test showed the antagonistic effect exerted on the phytopathogens *F. culmorum*, *R. solani* and *B. cinera* by the isolated strains (Table 1), although not all of them displayed the same competence. The higher number of bacteria isolated from wheat showed antagonistic activity compared to bacterial strains isolated from mycorrhizal hyphae. The best performing strains from mycorrhizal hyphae were *A. ilicis* KNCL24, *R. fascines* HNOL8 and from wheat *B. cohnii* 19, *B. subtilis* 1 and *B. lentus* 28. Several authors have reported on the involvement of antibiosis in biocontrol

of plant pathogens (Raaijmaker et al., 2002; Chin-A-Woeng et al., 2001). Antagonists invade pathogens by excretion of extracellular enzymes that can lyse pathogen cell walls or cause degradation of chlamydospores, conidia, etc.. Such extracellular enzymes include chitinases, cellulases, proteases and ß-1,3-glucanases (Adams, 1990). The production of hydrogen cyanide and different enzymes such lipase, cellulase, pectinase and ß-glucanase by bacterial strains are shown in Table 1. All isolates presented high level of pectinase activity. Nielson and Sorensen (1999) demonstrated that isolates of *P. fluorescens* antagonistic to *R. solani* and *Pythium ultimum*, produced lytic enzymes. Abd Rahman (2005) reported that among studied pseudomonades the *P. aureginosa* produced several proteases that have implicated in its pathogenicity.

Only four strains *A. ilicis* KNCL24, *B. lentus* 17, *B. subtilis* 4, *B. halodurans* 12 were able to produce hydrogen cyanide (HCN). There are many reports that note the production of biologically active compounds including different enzymes, and also sideraphores by rhizosphere bacteria (Höflich et al., 1994). Siderophores, low molecular weight compounds with high iron affinity, are produced by most biocontrol agents to solubilize and competitively acquire ferric ion under iron-limiting conditions, thereby making iron unavailable to other soil microorganisms which cannot grow for lack of it (Loper and Henkels**,** 1997; Haas and Defago, 2005). De Boer et al. (2003) found that the role of siderophores was associated with the antagonistic properties of *Pseudomonas putida* WCS358 in suppressing fusarium wilt of radish.

Our results showed that bacterial strains associated with wheat possess more antagonistic activity compared bacterial strains from mycorrhizal hyphae. The all tested bacterial strains produced one or more cell wall degrading enzymes.

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Table 1. Antagonistic activity and production of cell wall degrading enzymes by bacterial strains associated with mycorrhizal hyphae and wheat

Bacterial strains	*F. culmorum*	*R. solani*	*B. cinera*	HCN	ß-glucanase	Lipase	Pectinase	Cellulase
Mycorrhizal hyphae								
Arthrobacter citreus KMOL10	-	-	-	-	+	+	+	+
A. ilicis KNCL24	3*	2	3	+	-	+	-	-
A. aurescens HMCNM2	-	-	-	-	-	+	-	+
Nocardia asteroids HNOL10	-	-	-	-	-	-	+	+
N. asteroids KKCL1	-	-	-	-	-	+	+	+
N. globerula HMCM1	-	-	-	-	-	+	+	+
N. globerula KKOM10	-	-	-	-	-	+	+	+
Rhodococcus erythropolis HCL 5	-	-	-	-	-	-	+	+
R. erythopolis KMCNL20	-	-	-	-	-	-	+	+
R. fascines HNOL8	2	3	3	-	-	+	+	-
Wheat								
Bacillus sp. 41/1	-	-	-	-	-	-	+	-
B. cohnii 19	4	4	3	-	-	+	-	+
B. lentus 17	-	-	-	+	-	+	-	+
B. subtilis 4	5	4	3	+	+	+	+	+
B. subtilis 1	4	3	4	-	-	-	+	-
B. halodurans 12	-	-	-	+	+	+	+	-
B. lentus 28	5	5	4	-	+	+	-	-
Cellulomonas sp. 43	-	-	-	-	-	-	+	-
Cellulomonas sp. 22	-	-	-	-	-	-	+	-
Kocuria varians 13	3	3	-	-	-	-	+	-
Microbacterium sp. 44	-	-	-	-	-	-	-	-

*mm

REFERENCES

Abd Rahman R.N.Z., Geok L., Basri M., Abd Rahmen B.S., 2005. Physical factors affecting the production of organic solvent-tolerant protease by Pseudomonas aeruginosa strain K . Bio resource Technology 96: 429-436.

Adams, P.B., 1990. The potential of mycoparasites for biological control of plant diseases. Annual Review of Phytopathology 28: 59–72.

Barea, J.M., Azcon R., Azcon-Aguilar C., 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81: 343–351.

Berg, G., Krechel A., Ditz, M., Sikora, R., Ulrich, A., Hallmann, J., 2005. Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiology Ecology 51: 215-229.

Bergsma-Vlami, M., Prins, M.E., Staatzs, M., Raaijmakers, J.M., 2005. Assessment of genotypic diversity of antibiotic producing Pseudomonas species in the rhizosphere by denaturing gradient gel electrophoresis. Applied and Environmental Microbiology 71: 993-1003.

Castric, P.A., 1975. Hydrogen cyanide, a secondary metabolite of Pseudomonas aeruginosa. Can J Microbiol 21: 613–618.

Chin-A-Woeng, T.F.C., Thomas-Oates, J.E., Lugtenberg B.J.J., Bloemberg, G.V., 2001. Introduction of the phzH gene of Pseudomonas chlororaphis PCL1391 extends the range of biocontrol ability of phenazine-1-carboxylic acid-producing Pseudomonas spp. strains. Molecular Plant–Microbe Interactions 14: 1006–1015.

Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Banger, G., Kim, D.S., 1995. Molecular mechanisms of defense by rhizobacteria against root diseases. Proceedings of National Academy of Sciences, USA 92: 4197-4201.

De Boer, M., Bom, P., Kindt, F., Keurentjes, J.J.B., Van Der Sluis, I., Van Loon, L.C., Bakker, P.A.H.M. 2003. Control of Fusarium wilt of radish by combining Pseudomonas putida strains that have different disease-suppressive mechanisms. Phytopathology 93: 626-632.

Egamberdieva, D., Kucharova, Z., Davranov, K., Berg, G., Makarova, N., Azarova, T., Chebotar, V., Tikhonovich, I., Kamilova, F., Validov, Sh. Lugtenberg, B., 2010. Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils. Biology Fertility of Soils, 47:197-205.

Egamberdiyeva, D., Hoflich, G., 2002. Root colonization and growth promotion of winter wheat and pea by Cellulomonas spp. at different temperatures. Journal of Plant Growth Regulation 38: 219-224.

Egamberdiyeva, D., Hoflich, G., 2003. The effect of associative bacteria from different climates on plant growth of pea at different soils and temperatures. Archive Agronomy and Soil Science 49: 203-213.

Gamalero, E., Martinotti, M.G., Trotta, A., Lemanceau, P., Berta, G., 2004. Morphogenetic modifications induced by Pseudomonas fluorescens A6RI and Glomus mosseae BEG12 in the root system of tomato differ according to plant growth conditions. New Phytol 155: 293–300.

Garbaye, J., 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128: 197–210.

Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology 3: 307-319.

Howe, T.G., Ward, J.M., 1976. The utilization of Tween 80 as carbon source by Pseudomonas. J Gen Microbiol 92: 234-235.

Höflich, G., Wiehe, W., Kühn, G. 1994. Plant growth stimulation with symbiotic and associative rhizosphere microorganisms. Experientia 50: 897-905.

Jiang, Y.M., Zhu, X.R., Li, Y.B., 2001. Postharvest control of litchi fruit rot by Bacillus subtilis. Lebensm. Wiss. U. Technol., 34: 430-436.

Lugtenberg, B.J.J, Chin-A-Woeng, T. F.C., Bleomberg, G.V., 2002. Microbe-plant interactions: principles and mechanisms. Antonie van Leeuwenhoek 81: 373-383.

Loper, J.E., Henkels, M.D., 1997. Availability of iron to Pseudomonas fluorescens in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. Applied and Environmental Microbiology 63: 99–105.

Nielson, M.N., Sorensen, J., 1999. Chitinolytic activity of Pseudomonas fluorescens isolates from barley and sugar beet rhizosphere. FEMS Microbiology, Ecology 30: 217–227.

Raaijmakers, J.M., Vlami, M., De Souza, J.T., 2002. Antibiotic production by bacterial biocontrol agents. Antonie van Leeuwenhoek 81: 537–547.

Smibert, R.M., Krieg, N.R., 1994. Phenotypic characterization. In: Methods for General and Molecular Bacteriology eds. P. Gerhardt et al. Washington, D.C.: American Society for Microbiology. pp. 607-654.

Walsh, G.A., Murphy, R.A., Killeen, G.F., Headon, D.R., Power, R.F., 1995. Technical note: Detection and quantification of supplemental fungal b-glucanase activity in animal feed. J Anim Sci 73: 1074-1076.