African Journal of Biotechnology Vol. 6 (5), pp. 504-508, 5 March, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Studies of microbial development on mycorrhizosphere and rhizosphere soils of potted maize plants and the inhibitory effect of rhizobacteria isolates on two fungi

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Accepted 21 August, 2006

Extracts from maize rhizosphere and mycorrhizosphere soil (both sterilized and unsterilized) were studied to assess the compatibility of the arbuscular mycorrhizae (AM) fungus (*Glomus mosseae*) with certain soil fungi, bacteria and rhizobacteria followed by assessment of the inhibitory effect of the identified rhizobacteria on growth in culture of some selected fungi. The treatments were "non sterile soil without mycorrhizal inoculation (S^-M^-), non sterile soil with mycorrhizal inoculation (S^-M+), sterile soil without mycorrhizal inoculation ($S+M^-$) and sterile soil with mycorrhizal inoculation(S+M+)". The five rhizobacteria species isolated from the rhizosphere and mycorhizosphere of both sterile and non-sterile soil were *Rhizobium leguminsorum* from S+M-, *Rhizobium japonicum* from S+M+, *Pseudomonas* sp. from S-M+ and *Pseudomonas fluorescens* and *Rhizobium melotti* from S-M-. All the three selected rhizobacteria species (*Pseudomonas* sp., *P. fluorescens* and *R. japonicum*) inhibited growth of the target fungi. Both *P. fluorescens* and *R. japonicum* had the most favourable sustained inhibitory effect on *Aspergillus flavus* while *P. fluorescens* had the best sustained inhibitory effect on *Rhizopus oligosporus*.

Key words: Glomus mosseae,maize, mycorrhizosphere, percentage inhibition, Pseudomonas fluorescens, rhizosphere.

INTRODUCTION

In natural ecosystems, much of the root system can be colonized by mycorrhizal fungi, which are restricted to the root cortex and do not enter the vascular cylinder (Kendricks and Berch, 1985). Mycorrhizal fungi occur in nearly all soils on earth and form a symbiotic relationship with roots of most terrestrial plants. The mycorrhizal morphology is the basis for grouping them into two major groups: ectomycorrhizae and endomycorrhizae; with the latter dominated by the ubiquitous and large group of vesicular-arbuscular mycorrhizae (VAM) (Bagyaraj, 1991) now known as AM fungi (Morton and Bentivega, 1994).The term 'rhizosphere'is employed in describing the immediate environment of the root of a plant. It is appropriate therefore to refer to the rhizosphere around mycorrhizae as "mycorrhizosphere" (Rambelli, 1973; Linderman, 1988). Maize (*Zea mays*) is one of the most important food crops in the world. It has a remarkable productive potential along with other members of the family Poaceae (or gramminae) such as wheat andd rice (Kling and Edmeades, 1997). The crop is also known as corn, and it is ranked second only to wheat in the world production of cereal crops. Maize was introduced to West Africa by Portuguese traders and had been an important crop in the forest region for some 500 years (Nbanasor and Obi, 2001). But the open nature of the grassland savanna favoured maize production better than the forest. As a result, cultivation has moved northwards to the savanna area, progressively replacing the traditional grain crops like millet and sorghum (Treharms and Greenland, 1977).

Arbuscular mycorrhizal fungi interface directly with the surrounding soil by means of hyphae or hyphal strands that extend into the soil and increase the nutrient and

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water absorption potential of the root system, which contributes greatly to the improvement of soil texture for better aeration and water percolation. When root becomes mycorrhizal, their physiology significantly changes. For example, there are changes in concentrations of growth regulating compounds such as auxin, cytokinin, gibberellins, and ethylene (Barea and Azcon-Aguilar, 1982). The nutritional composition of the host tissue changes in response to altered uptake of minerals from the soil. The altered nutrient composition in turn can change the structural and biochemical aspects of the root cells, which can alter membrane permeability and thus the quality and quantity of root exudation. When a mycorrhizal fungus forms a symbiotic association with plants roots, they may interact directly with other organisms in the soil, or they may influence those organisms indirectly by their effects on the host plant physiology that could change root morphology, physiology, and thus patterns of exudation into the mycorrhizosphere. Similarly, other root symbionts, such as nodule forming bacteria, can influence the behaviour of mycorrhizal fungi in addition to changes in the host physiology (Meyer and Linderman, 1986).

Aspergillus flavus is a common fungal contaminant of food and stored grains and it produces a characteristic toxin, a mycotoxin called afflatoxin which is toxic to humans and their domestic animals (Pitt, 1989). *Rhizopus oligosporus* on the other hand is a relatively harmless fungus often employed as food modifiers (in fermented food products) in some part of the world.

It has been established that certain microorganisms enhance mycorrhization and this organisms also have an antagonistic effect towards soil borne fungal pathogens. Such association include that of a gram positive bacterium with antagonistic activity towards soil borne fungal pathogens from the mycorrhizosphere of *Sorghum bicolor* inoculated with *Glomus mosseae* reported by Budi et al. (1999). Similar findings have been reported for a host of mycorrhizae/rhizobacteria/plant combination in resistance to fungal pathogens (Dehne, 1982; Linderman, 1988; Paulitz and Linderman, 1991). Andrade et al. (1998), established a plant mediated interaction between *Pseudomonas flourescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizal fungi. However, not much work has been reported on the antifungal activities of plant growth promoting rhizobacteria (PGPR) on animal/human fungal pathogens.

The objective of this research work is the isolation, characterization and identification of plant growth promoting rhizobacterium from potted maize rhizosphere and mycorrhizosphere that may be antagonistic to selected fungi as a prelude to finding one that can extend their activity as a biological control agent against soil borne fungal pathogens that are causal agents for some plant diseases. The possibility of improving bacterial-fungal inoculation for ensuring the production of high value maize plants in systems compatible with the environment will also be investigated.

MATERIALS AND METHODS

Soil collections and preparation

Garden soil was collected from an area on the campus of the Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. Forty clean, pierced plastic pots were sterilized with 90% ethanol. Twenty out of the forty pots were filled with the garden soil. The remaining plastic pots were then re-sterilized, filled with sterile soils (soils sterilized in the oven at 85°C over night inside a steel container), and immediately covered with muslin cloth. All pots were transported to the experimental site and placed on a raised platform. The maize seeds (DMR ESR: Yellow maize seeds) were obtained from International Institute of Agricultural Training (IAR&T), Moor plantation, Ibadan, Oyo State, Nigeria. Certified mycorrhizal inoculum (for *Glomus mosseae*} was obtained from the stock kept and maintained in the biology Laboratory at Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. The inoculum used consists of soil containing spores (with spore population of between 800-1000/100 g dry soil), hyphal fragments, and fine roots of maize infected with *G. mosseae.* The treatments were “non sterile soil without mycorrhizal inoculation (S^-M^-), non sterile soil with mycorrhizal inoculation (S^-M+), sterile soil without mycorrhizal inoculation ($S+M^-$) and sterile soil with mycorrhizal inoculation(S+M+)”.On the site, seeds of the maize plant were sown (two per hole) in each pot. Twenty out of the forty pots (ten each of sterilized and unsterilized soils) were inoculated with arbuscular mycorrhizal fungus *G. mosseae.* The maize seeds were placed into holes 3-5 cm deep that had been partly filled with inoculum (in the case of inoculated soils) and watered twice daily for the period of eight weeks. All the seeds germinated.

Microbial analysis

By the fifth week after emergence (WAE), soil samples were taken from four replicates of each treatment and another set taken during the seventh. All the soil samples were taken in sterile sampling bag for microbial analysis. By the seventh week after emergence (WAE), the experiment was terminated. Soil samples collected at fifth WAE and seventh WAE was serially diluted and two dilutions were aseptically plated out using nutrient agar in order to isolate the bacterial components of the soil biota and in potato dextrose agar in order to isolate the fungal components of the soil biota. The plates were incubated at 37°C for 24 h at room temperature (28°C) for 48 h, respectively, for bacteria and fungi. Seven different distinct colonies were isolated from the above plates, characterized and identified. King’s medium (King et al., 1954), was used for the isolation of rhizobacteria in the soil samples. The four soil samples were serially diluted and plated out. The organisms isolated from the four treatments were characterized and identified. Three isolates of rhizobacteria were assayed for their antagonistic activities towards two selected fungi, *Aspergillus flavus* and *Rhizopus oligosporus*, using percentage inhibition method.

RESULTS AND DISCUSSION

Characterization and identification of soil isolates of soil extract from sterilized and un-sterilized maize rhizosphere and mycorrhizophere soil revealed the presence of *Streptococcus pyogenes* in S^+M^+, *Bacillus subtilis* in S^+M^+, *Pseudomonas aureginosa* in S^+M^+ and *Micrococcus* sp. in S^-M^- (Table 1). Characterisation of PDA isolat-

Table 1. Microbial isolates from nutrient agar cultures of both sterile and non-sterile maize rhizophere and mycorrhizosphere soils.

Treatment	Gram Stain	Shape	Motility	Catalase	Oxidase	Urease	Coagulase	Indole	Methyl Red	Voges – Proskauer	Starch Hydrolysis	Casein Hydrolysis	Citrate Utilization	Glucose	Lactose	Sucrose	Arabinose	Maltose	Sorbitol	Mannitol	Probable identification
S^+M^+	+	S	-	-	-	-	-	+	-	-	+	+	-	A	A	A	A	A	-	-	*S. pyogenes*
S^+M^-	+	R	+	+	-	-	-	-	-	-	+	+	-	A	A	A	A	A	-	A	*B. subtilis*
S^-M^+	-	R	+	+	+	+	-	-	-	-	-	-	+	A	-	-	A	A	-	A	*P. aureginosa*
S^-M^-	+	C	+	+	-	-	-	-	-	-	+	+	-	A	A	-	-	A	A	A	*Micrococcus* sp.

S. pyogenes = *Streptococcus pyogenes*
B. subtilis = *Bacillus subtilis*
P. aureginosa = *Pseudomonas aureginosa*
A = Acid production; d = doubtful; R = rod; + = positive; - = Negative.
S^-M^+ = Unsterilized inoculated; S^+M^- = sterilized uninoculated; S^+M^+ = sterilized inoculated; and S^-M^- = unsterilized uninoculated.

Table 2. Description and identification of isolates from PDA cultures taken from both sterile and non-sterile maize rhizophere and mycorrhizosphere soils.

Sterilization/ inoculation	Description of isolates	Probable Identification
S^-M^+	The texture is flococcose and it is creanish –yellow in obverse. Its reverse is scarlet to deep burgundy while its polyphialides is like a palm. The chlamydospores are abundant, in chains or cluster, rough and golden brown.	*Fusarium chlamdosporum*
S^+M^-	It is pink in colour, oval, smooth, round and large Colony. It is gram positive, urease positive and does not Ferment sugars like Arabinose. Lactose, Sucrose, Glucose, Fructose, Maltose and galactose.	*Rhodosporum sp.*
S^+M^+	The texture is floccose and whitish-cream in colour. Chlamydospores are abundant and usually single on hyphae. The reverse is pale to bluish-violet in colour.	*Fusarium oxysporum*

S^-M^+, unsterilized inoculated; S^+M^-, sterilized uninoculated; S^+M^+, sterilized inoculated; and S^-M^-, unsterilized uninoculated.

es from maize rhizosphere and mycorrhizosphere soils revealed the presence of *Fusarium chlamydosporum* in S^-M^+, *Rhodosporum* sp. in S^+M^- and *Fusarium oxysporum* in S^+M^+ (Table 2). Characterisation of PDA isolates from maize rhizosphere and mycorrhizosphere soils cultured on selective (Kings) medium revealed the presence various species of rhizobacteria such as *Rhizobium leguminsorum* from S^+M^-, *Rhizobium japonicum* from S^+M^+, *Pseudomonas* sp. from S^-M^+ and *Pseudomonas fluorescens* and *Rhizobium melotti* from S^-M^- (Table 3).

The inhibitory effect of the selected rhizobacteria on *R. oligosporus* and *A. flavus* could be due to antibiotic substances produced by them which inhibited the growth of the fungi in culture (Table 4). Andrade et al. (1998) have reported antibiotic-producing *Pseudomonas fluorescens* strain F113 enhanced nodulation. The rhizobacteria also stimulate both mycelial development and spore germination in soil and plant root colonization (Barea et al., 1998). Presence of *G. mosseae* increased the population of *P. fluorescens* in the rhizosphere soil (Edward et al., 1998). Even though the interactions between AM fungi and growth promoting rhizobacteria like *P. flourescens* have always been plant mediated we have not been able to establish whether a precedence of plant-mycorrhiza association is more important than that of rhizobacteria-mycorrhiza since growth promoting rhizobacteria are found in all soils extracts irrespective of soil sterilization treatment. This is unlike what was established by Lindermann et al. (2001) in a previous work where distinctions in population increases and potentials for suppression of soil pathogens were established between rhizobacteria from mycorrhizosphere and rhizosphere of tomato plants.

The development of rhizobacteria in non-mycorhizal maize rhizosphere soils is probably due to the fact that

Table 3. Characterization and identification of isolates taken from cultures of both sterile and non-sterile maize rhizophere and mycorrhizosphere soils using king's medium.

Sterilis/ Inocula	Grain Stain	Shape	Motility	Catalase	Coagulase	Oxidase	Mehyl Red	Voges-prokaeur	Endospore stain	Oxygen relationship	Teamp of inocubation (°C)	Glucose	Lactose	Sucrose	Fructose	Manni	Probable identification
S^+M^-	-	R	+	+	-	-	-	-	-	a	25	A	A	A	A	A	*R. leguminosarum*
S^+M^+	-	R	-	+	-	+	-	-	-	a	25	A	-	-	A	-	*R. japonicum*
S^-M^+	-	R	+	+	-	+	-	-	-	a	37	A	A/G	A/G	D	A	*Pseudomonas* sp.
S^-M^- A_1	-	R	+	+	-	+	+	-	-	a	37	A	A/G	A	A	A	*P. fluorescens*
S^-M^- A_2	-	R	+	+	-	-	-	-	-	a	27	A	A	A	A	A	*R. melotii*

R. leguminosarum = Rhizobium leguminosarum, R. japonicum = Rhizobium japonicum, P. fluorescens = Pseudomonas fluorescens, and R. melotii = Rhizobium melotii.

S^-M^+, unsterilized inoculated; S^+M^-, sterilized uninoculated; S^+M^+, sterilized inoculated; and S^-M^-, unsterilized uninoculated.

A = Acid production, d = doubtful, R = Rod, + = Positive, - = Negative, A_2 = S^-M^- 1st organism, A_1 = S^-M^- 2nd organism.

Table 4. Percentage inhibition of colony growth of *A. flavus* and *R. oligosporus* by three rhizobacteria isolates after 24 and 48 h of incubation.

Rhizobacteria	*A. flavus* (% Inhibition)		*R. oligosporus* (% Inhibition)	
	After 24 h	After 48 h	After 24 h	After 48 h
Pseudomonas fluorescens	40	58.8	66.7	45
Pseudomonas sp	33.3	41.2	52.4	40
Rhizobium japonicum	53.3	52.9	61.9	35

maize is an obligate mycotroph and a trap plant with ability to attract mycorrhizal propagules even when present in low amounts, a likely event in the tropical soils used for the experiment. Similar observations had been made on tomato by Vierheilig et al. (1998). And in the sterilized soils, wind blown propagules could have entered into soil as the experiment progressed. The stimulatory effect of AM fungal inoculation on rhizobacteria and the latter inhibitory effect on soil pathogens could qualify *G. mosseae* and other similarly investigated AM fungi as modulators of plant's immune response to infection by soil pathogens. The inhibitory effect of the selected rhizobacteria on *R. oligosporus* and *A. flavus* therefore extends their antifungal antibiosis beyond the realm of soil pathogens.

REFERENCES

Andrade G, DeLeij FAAM, Lynch JM (1998). Plant mediated interactions between *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizae on pea. Lett. Appl. Microbiol. 26: 311-316.

Barea JM, Azcon-Aguilar C (1978). Effects of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhizae. New Phytol. 80: 359-364.

Barea JM, Andrade G, Bianciotto V, Dowling D, Lohrke S, Bonfante P, OGara F, AzconAguilar C (1998). Impact on arbuscular mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. Appl. Environ. Microbiol. 64: 2304-2307.

Budi SW, Tuinen DV, Martinotti G Gianinazzi (1999). In Isolation from the sorghum bicolor mycorrhizosphere of a bacterium compatible with arbuscular mycorrhizal development, and antagonistic towards soil pathogen. Appl. Environ. Microbiol. 6(11): 5148-5150.

Dehne HN (1982). Interactions between vesicular arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115-1117.

Edwards SG, Young JPW, Fitter AH (1998). Interactions between *Pseudomonas fluorescens* biocontrol agents and *Glomus mosseae*, an arbuscular mycorrhizal fungus, within the rhizosphere. FEMS Microbiol. Lett. 166(2): 297-30

Kendricks B, Berch S (1985). *Mycorrhizae:* Application in Agriculture and Forestry. In Comprehensive biotechnology, (CW Robinson (ed.) Pergamon press, Oxford). 4: 109-150.

Kling JG, G Edmeades (1997) Morphology and Growth of Maize IITA/CIMMYT. Research Guide 9 Training programme. International Institute of Tropical Agriculture IITA Ibadan. Nigeria 1997. pp: 31.

Linderman RG, JL Marlow, EA Davis (2001). Comparison of theantagonistic potential of rhizobacteria from ,mycorrhizosphere and rhizosphere soils against soil borne fungal pathogens. International Conference On *Mycorrhiza ICOM* 3 Adelaide 2001.

Linderman RG (1988) *Mycorrhizal* interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology. 78: 366-371.

Meyer JR, Linderman RG (1986). Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by Glomous fasciculatum. Soil Biol. Biochem. 18: 191-196.

Morton JB, SP Bentivenga (1994). Levels of diversity in endophytic Mycorrhizal fungi (Glomales, Zygomycetes) and their role in defining Taxonomic and non taxonomic groups. In AD Robson, LK Abott, Malajczuk (Eds). Management of *Mycorrhizas* in Agriculture, Horticulture and Forestry. pp: 47-60. Kluver academic publishers, Netherlands.

Nbanasor JA, IU Obi (2001) Performance evaluation of three cultivators of maize during late seasons in the derived savanna zone of Nigeria: Implications for adoption. Nig Agric. J. pp: 42-51.

Paulitz TC, Linderman R (1991) Mycorrhizal Interactions With Soil Organisms, pp: 77-129. In Arora, D. K., Rai, K. Mukerji G, Knudsen G (eds.) Handbook of Applied Mycology. Vol. 1: Soil and Plants. Marcel Dekker, Inc., New York. (Book Chapter).

Pitt JI (1989). Toxigenic *Aspergillus* and *Penicillium* species. In: Mycotoxin prevention and control in food grains 1989 Eds. RL Semple, AS Frio, PA Hicks, JV Lozare. A collaborative publication of the UNDP/FAO Regional Network- Intercountry cooperation on pre-havest Technology and Quality control of food grains(REGNET and the ASEAN Grain). Bankok. Thailand.

Treharms G, DJ Greenland (1977). Maize Production in lowland tropics: In Sprague G.SF Ed: Corn and Corn Improvement. Academic Press, 1977 New York.

Vierheilig HM, Alt-Hug R, Engel-Streitwolf P, Mader I, Weimken A (1998). Studies on the attractional effect of root exudates on hyphal growth of an arbuscular mycorrhizal fungus in a soil compartment membrane system. Plant and Soil. 203: 137-144.