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REVIEW

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Improvement by soil yeasts of arbuscular mycorrhizal symbiosis of soybean (*Glycine max*) colonized by *Glomus mosseae*

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Abstract The effects of the soil yeasts *Rhodotorula mucilaginosa*, *Cryptococcus laurentii* and *Saccharomyces kunashirensis* on the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* (BEG 12) was studied in vitro and in greenhouse trials. The presence of yeasts or their soluble and volatile exudates stimulated the percentage spore germination and hyphal growth of *G. mosseae*. Percentage root length colonized by *G. mosseae* and plant dry matter of soybean (*Glycine max* L. Merill) were increased only when the soil yeasts were inoculated prior to the AM fungus. Higher beneficial effects on AM colonization and plant dry matter were found when the soil yeasts were inoculated as an aqueous solution rather than as a thin agar slice. Although soluble and volatile exudates of yeasts benefited the AM symbiosis, their modes of action were different.

Keywords Arbuscular mycorrhizas · *Crytococcus laurentii* · *Glomus mosseae* · *Rhodotorula mucilaginosa* · *Saccharomyces kunashirensis* · Soil yeasts

Introduction

Interest in applying microorganisms beneficial to plants in the context of "sustainable agriculture" and efforts to avoid environmentally deleterious agro-chemicals explain the increasing number of studies on the management of soil-plant-microorganism systems (Bowen and Rovira 1999).

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Arbuscular mycorrhizal (AM) fungi are known to influence and to be influenced by the activities of microorganisms in the soil (Bagyaraj 1990; Andrade et al. 1997). Mycorrhiza formation can affect the microbial population in the rhizosphere directly or indirectly through changes in root exudation patterns, or through fungal exudates (Linderman 1992). Conversely, numerous soil microorganisms interact with mycorrhizal fungi by producing substances that stimulate plant growth or inhibit root pathogens (Jeffries and Dodd 1996). Soil microorganisms mainly influence AM fungi when these fungi are in the extramatrical phase (Caron et al. 1985; McAllister et al. 1994). Volatile and soluble exudates produced by soil microorganisms are involved in these effects (McAllister et al. 1994; Fortin et al. 2002). Nevertheless, few studies on the influence of exudates from microorganisms on germination of AM spores, and AM colonization of roots have been conducted.

Most studies to date have dealt with interactions between selected bacteria or saprophytic fungi in relation to AM colonization enhancement (Bagyaraj 1984; Fitter and Garbaye 1994; Fracchia et al. 2000; García-Romera et al. 1998). Yeasts belonging to the genera *Rhodotorula*, *Crytococcus* and *Saccharomyces* are common components of the soil rhizosphere (Azeredo et al. 1998; Slavikova and Vadkertiova 2000; Spencer and Gorin 1971), but little information on the effect of inoculation with yeast on rhizosphere microorganisms in general and on AM fungi in particular is available. Increases in nodulation and other symbiotic parameters of forage legumes because of combined inoculation with yeast and specific *Rhizobium* spp., have been reported (Tuladhar and Subba Rao 1985). Only studies about the effect of the commercial yeast *Saccharomyces cerevisiae* on AM fungi have been carried out (Larsen and Jackobsen 1996; Singh et al. 1991).

The aim of this study was to examine the influence of different soil yeast inocula, *Rhodotorula mucilaginosa*, *Crytococcus laurentii*, and *Saccharomyces kunashirensis*, on percentage spore germination and hyphal length of *Glomus mosseae* and on plant dry matter and colonization

of soybean (*Glycine max* M. Merril) roots by this AM fungus.

Materials and methods

Isolation of yeasts

The yeasts present in soils from the Province of Granada (Spain) were isolated by dilution of soil in sterile water. An aliquot (0.1 ml) of this suspension was spread onto potato dextrose agar (PDA) and incubated at 30°C for 3–5 days. From the resulting colonies, *Rhodotorula mucilaginosa* , *Cryptococcus laurentii* and *Saccharomyces kunashirensis* were selected and transferred to tubes of PDA and 2% malt extract agar (MEA) for storage at 4°C. These yeasts were identified by the Colección Española de Cultivos Tipo where they are deposited.

Effect of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on the spore germination and hyphal growth of *G. mosseae* BEG 12

The effects of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on spore germination and hyphal length of *G. mosseae* were tested in three different experiments conducted in 9-cm-diameter plastic Petri dishes. In the first experiment, the effects of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on spore germination and mycelial length in vitro were tested. Sporocarps of *G. mosseae* were isolated by wet-sieving (Gerdemann 1955) alfalfa plant pot cultures, and were stored in water at 4°C. The spores of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe obtained by dissecting the sporocarps were surface-sterilized as described by Mosse (1962). Ten surface-sterilized spores per plate were placed 1 cm from the edge of a Petri dish with 10 ml of 10 mM 2-(*N* -morpholin) ethane sulphonic acid (MES) buffer (pH 7) plus 0.04 g of Gel-Gro (ICN Biochemicals, Aurora, Ohio). The substrate was inoculated with a thin streak of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* opposite and at least 7 cm from the spores.

The second experiment tested the effect of exudates from *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on hyphal length of *G. mosseae* in vitro. Exudates were obtained by growing the yeasts in 250-ml flasks containing 125 ml of sterile liquid asparagine medium on a shaker at 28°C. The standard asparagine medium consisted of $MgSO_4.7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; glucose, 1 g; asparagine, 4 g; distilled water to 1 l. After 48 h the culture medium with 2×10^6 cells ml^{-1} was filtered through a disk of filter paper (Whatman no. 1) and then twice through 0.45-μm Millipore membranes. Different concentrations of exudates, 0.01, 0.025, 0.5, 0.1 and 0.3 ml, were added to Petri dishes with 10 ml of 4% Gel-Gro (ICN Biochemical) in 10 mM MES buffer (pH 7). Ten surface-sterilized spores of *G. mosseae* were placed in each dish. In the control treatment, the same volume of sterile liquid asparagine medium was substituted for the exudates.

In the third experiment, the effects of volatile compounds released by *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on hyphal length of *G. mosseae* were tested in divided plastic Petri dishes. The plates contained Gel-Gro in one side and PDA, Gel-Gro or asparagine medium on the other. Five AM spores were placed on the Gel-Gro medium and the yeast was inoculated on the other side on the three different nutrient agars.

In each of the three experiments, five replicates of each yeast treatment and controls (plates with spores of AM fungi without yeast) were used. The plates were incubated at 25°C in the dark, and were sealed to reduce dehydration and contamination. Hyphal lengths of the germinated AM fungus spores were determined periodically under a light microscope for 15 days, at the end of which the experiment was terminated and total hyphal length of the germinated *G. mosseae* spores was assessed by the gridline-intersect method (Marsh 1971).

Interaction between *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis*, their exudates, and *G. mosseae* in the rhizosphere of soybean grown in pots

Plants were grown in 300-ml pots of soil collected from the Province of Granada. The soil was a calcixerollic xerochrept type, pH 7.6 (for full details see García-Romera and Ocampo 1988). It was steam-sterilized and mixed 1:1 (v/v) with perlite. Soybean was used as a test plant. Seeds were sterilised with 10% sodium hypochlorite for 2 min, sown in moistened sand, and after 2 weeks uniform seedlings were transplanted to the pots. Plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cold-white lamps, 400 $\mu Em^{-2} s^{-1}$, 400–700 nm, with a 16/8 h day/night cycle at 25/19°C and 50% relative humidity. Plants were watered from below, and were fed with 10 ml nutrient solution $week^{-1}$ (Hewitt 1952).

The AM fungus inoculum consisted of 5 g rhizosphere soil from an alfalfa plant pot culture of an isolate of *G. mosseae* (BEG 12), which contained spores, mycelia and colonized root fragments. Soil filtrate (Whatman no. 1 filter paper) from the rhizosphere of mycorrhizal plants was added to the non-inoculated treatment. The filtrate contained common soil microorganisms, including bacteria and fungi, but no propagules of AM fungi.

Four experiments were designed to test the interaction between *R. mucilaginosa*, *C. laurentii* and *S. kunahirensis* and AM colonization of soybean inoculated with *G. mosseae*. Four treatments were used in all experiments, (1) inoculated with *G. mosseae*, (2) inoculated with both *G. mosseae* and *R. mucilaginosa* (yeast and exudates), (3) inoculated with both *G. mosseae* and *C. laurentii* (yeast and exudates), and (4) inoculated with both *G. mosseae* and *S. kunashirensis* (yeast and exudates). Five replicate pots per treatment were used.

Experiment 1

The first experiment was designed to test the effect of yeast inoculation time. The soil yeasts were inoculated at the rate of 1×10^5 cells per gram as a suspension in asparagine medium, 2 weeks before, at the same time as, or 2 weeks after inoculation with AM fungus.

Experiment 2

The second experiment was performed to select the most appropriate soil yeast inoculation method. Plants were inoculated 2 weeks before AM fungi with *R. mucilaginosa*, *C. laurentii* and *S. kunahirensis* as: (1) a thin agar slice of MEA (1×1 cm) (2) a suspension grown on asparagine medium as described before.

Experiment 3

The third experiment selected the most appropriate volume of soil yeast inoculum. An aqueous suspension of *R. mucilaginosa*, *C. laurentii* or *S. kunahirensis* grown on asparagine medium as described before, was added 2 weeks before AM fungi at the rates of 1×10^5, 2×10^5 and 4×10^5 cells per gram of soil.

Experiment 4

In the fourth experiment the effect of exudates from the soil yeasts on AM colonization were tested. *R. mucilaginosa*, *C. laurentii* and *S. kunahirensis* exudates obtained as described before were applied at the same time as the AM fungus in doses of 5 and 10 ml pot^{-1}.

Plants were harvested 5 weeks after inoculation with the mycorrhizal fungus and the dry matter determined. Samples of 1 g fresh weight were taken from the entire root system at random and were cleared and stained (Phillips and Hayman 1970). The

percentage root colonization was assessed by the gridline-intersect method (Giovannetti and Mosse 1980).

To evaluate the population of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* during the experiments, about 5 g of soil:perlite was taken from each of the five replicate pots. A tenfold aqueous dilution series, from 10^{-1} to 10^{-4}, was prepared for each sample, and 1 ml of each solution was plated on PDA. Numbers of colony forming units (CFUs) in suitable dilutions were counted. Soil was dried at 105°C and weighed. The number of CFUs was expressed per gram of dry soil.

Statistical treatments

Percentages were arcsine transformed. The data obtained for germination and hyphal length of AM spores, plant dry weight, percentage AM colonization and CFU of the yeasts were subjected to ANOVA. The mean values of five replicate pots were compared using Duncan's multiple range test (P =0.05).

Results

Percent germination and hyphal length of *G. mosseae* on Gel-Gro increased significantly in the presence of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* (Table 1). Higher hyphal length of *G. mosseae* spores in the presence of *R. mucilaginosa* or *S. kunashirensis* than in the presence of *C. laurentii* was observed.

The exudates of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* applied to the Gel-Gro significantly increased the hyphal length of germinated *G. mosseae* spores at the doses of 0.05, 0.1 and 0.3 ml per Petri dish (Table 2). However, 0.01 and 0.025 ml of exudates did not affect hyphal length.

Volatile compounds produced by *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* when PDA and Gel-Gro medium were used significantly increased the hyphal length of *G. mosseae* (Table 3). The increase in hyphal length of *G. mosseae* was significantly greater when the soil yeasts were grown on PDA medium. When yeasts were grown on PDA medium, hyphal length was higher in the presence of *R. mucilaginosa* or *S. kunashirensis* than

Table 1 Germination and hyphal length of *Glomus mosseae* in the presence of *Rhodotorula mucilaginosa*, *Cryptococcus laurentii* and *Saccharomyces kunashirensis.* Each value is the mean of five replicates. Column values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05)

Soil yeast	% Germination	Hyphal length (mm)
Control	29 a	10.32 a
R. mucilaginosa	73 b	31.20 c
C. laurentii	66 b	24.16 b
S. kunashirensis	63 b	32.95 c

Table 2 Effect of different concentration of exudates of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on the hyphal length of *G. mosseae*. Each value is the mean of five replicates. Values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05)

Soil yeast	Hyphal length Exudates (ml) per Petri dish				
	0.01	0.025	0.05	0.1	0.3
Control	1.64 a	1.93 a	2.00 a	2.13 a	2.46 a
R. mucilaginosa	12.51 d	2.44 a	3.42 ab	4.32 b	6.10 c
C. laurentii	1.87 a	3.09 ab	3.99 b	5.83 c	10.47 d
S. kunashirensis	2.19 a	3.12 ab	4.28 b	5.99 c	11.83 d

Table 3 Effect of volatile compounds produced by *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* grown in potato dextrose agar (*PDA*), Gel-Gro and asparagine medium on the hyphal length of *Glomus mosseae*. Each value is the mean of five replicates. Values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05)

Soil yeast	Hyphal length (mm)		
	PDA	Gel-Gro	Asparagine
Control	1.95 a	1.42 a	1.25 a
R. mucilaginosa	6.28 d	3.83 b	1.55 a
C. laurentii	4.88 c	3.49 b	1.25 a
S. kunashirensis	6.32 d	2.79 b	1.25 a

Table 4 Shoot and root dry weights (mg) and percentage root length colonized of soybean (*Glycine max*. L. Merill) in the presence of *Glomus mosseae* inoculated or not with *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* at different times. Each value is the mean of five replicates. Column values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05). *AM* Arbuscular mycorrhizal

Inoculation time	Inoculation treatment	Shoot dry weight	Root dry weight	% Mycorrhizal colonization
Inoculated 2 weeks before AM fungi	Control	168.1 a	145.3 a	15.6 a
	R. mucilaginosa	240.3 c	208.2 c	57.3 c
	C. laurentii	200.4 b	174.5 b	32.9 b
	S. kunashirensis	245.7 c	203.2 c	51.6 c
Inoculated at the same time as AM fungi	Control	171.2 a	148.2 a	17.9 a
	R. mucilaginosa	181.5 a	157.4 a	22.4 a
	C. laurentii	175.4 a	151.9 a	22.7 a
	S. kunashirensis	187.6 a	163.2 a	21.3 a
Inoculated 2 weeks after AM fungi	Control	180.3 a	157.6 a	18.1 a
	R. mucilaginosa	172.1 a	154.3 a	19.2 a
	C. laurentii	183.7 a	157.5 a	20.9 a
	S. kunashirensis	177.1 a	153.6 a	18.7 a

Table 5 Shoot and root dry weights (mg) and percentage root length colonized of soybean in the presence of *G. mosseae* inoculated or not with different carriers of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis.* Each value is the mean of five replicates. Column values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05)

Inoculum carrier	Inoculation treatment	Shoot dry weight	Root dry weight	% Mycorrhizal colonization
Agar slice	Control	161.2 a	121.6 a	18.1 a
	R. mucilaginosa	165.3 a	110.2 a	23.6 a
	C. laurentii	155.8 a	127.3 a	21.6 a
	S. kunashirensis	150.7 a	118.9 a	27.1 a
Suspension	Control	167.1 a	118.5 a	17.9 a
	R. mucilaginosa	282.5 c	221.4 c	59.3 c
	C. laurentii	225.9 b	180.3 b	30.3 b
	S. kunashirensis	278.4 c	200.1 c	56.7 c

Table 6 Shoot and root dry weights (mg) and percentage root length colonized of soybean in the presence of *G. mosseae* inoculated or not with different amounts of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis.* Each value is the mean of five replicates. Column values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05)

Amount of yeast ($\times 10^5$ g^{-1} soil)	Inoculation treatment	Shoot dry weight	Root dry weight	% Mycorrhizal colonization
Control		170.1 a	126.3 a	16.6 a
1	*R. mucilaginosa*	230.3 b	156.2 b	30.3 b
	C. laurentii	225.4 b	148.5 b	26.9 b
	S. kunashirensis	222.7 b	150.2 b	31.6 b
Control		180.2 a	121.2 a	18.2 a
2	*R. mucilaginosa*	274.5 c	209.4 c	61.4 d
	C. laurentii	240.4 b	187.9 b	40.7 c
	S. kunashirensis	272.6 c	210.2 c	62.3 d
Control		187.3 a	111.6 a	16.1 a
4	*R. mucilaginosa*	192.1 a	118.3 a	20.2 a
	C. laurentii	200.7 a	115.5 a	21.9 a
	S. kunashirensis	189.1 a	117.6 a	17.7 a

Table 7 Shoot and root dry weights (mg) and percentage root length colonized of soybean in the presence of *G. mosseae* inoculated or not with different volumes of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* exudates. Each value is the mean of five replicates. Column values followed by the *same letter* are not significantly different according to Duncan's multiple range test (P =0.05)

Amount of exudates (ml)	Inoculation treatment	Shoot dry weight	Root dry weight	% Mycorrhizal colonization
Control		145.1a	116.3a	20.6a
5	*R. mucilaginosa*	200.3b	146.2b	34.3b
	C. laurentii	210.4b	137.5b	36.9b
	S. kunashirensis	215.7b	142.2b	39.6b
Control		182.2a	111.2a	25.2a
10	*R. mucilaginosa*	278.5c	199.4c	60.4c
	C. laurentii	266.4c	187.9c	56.7c
	S. kunashirensis	270.6c	209.2c	54.3c

in the presence of *C. laurentii*. When asparagine growth medium was used, the volatile compounds produced by the soil yeast did not affect hyphal length.

Plant dry matter and percentage root length colonized by *G. mosseae* in soybean were increased significantly when *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* were inoculated 2 weeks before the AM fungus, but were not affected when the yeasts were inoculated at the same time or 2 weeks after *G. mosseae* (Table 4). When yeasts were inoculated 2 weeks before the AM fungus, plant dry matter and percentage root length colonized were higher in the presence of *R. mucilaginosa* or *S. kunashirensis* than in the presence of *C. laurentii*.

As Table 5 shows, higher plant dry matter and AM colonization of soybean were obtained when *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* were inoculated in a suspension than when they were inoculated as agar slices. When the yeasts were inoculated in a suspension,

plant dry matter and percentage root length colonized were higher in the presence of *R. mucilaginosa* or *S. kunashirensis* than in the presence of *C. laurentii*.

Table 6 shows that plant dry matter and AM colonization in the presence of 1×10^5 and 2×10^5 *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* cells per gram soil were higher than those of non-inoculated plants. However, inoculation with 4×10^5 cells g^{-1} soil did not significantly affect plant dry matter or percentage AM colonization (Table 6). When 2×10^5 yeast cells g^{-1} soil was applied, plant dry matter and percentage root length colonized were higher in the presence of *R. mucilaginosa* or *S. kunashirensis* than in the presence of *C. laurentii*.

Plant dry matter and percentage AM colonization were increased significantly when 5 or 10 ml of yeast exudates was applied to soil (Table 7).

The number of CFUs yeast g^{-1} rhizosphere soil decreased throughout the experiments. Populations of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* in the rhizosphere of soybean were not affected by the presence of *G. mosseae* (data not shown).

Discussion

The interaction between soil microorganisms and AM fungi is important for plant growth (Linderman 1992). That the percentage of spore germination and hyphal length of *G. mosseae* clamydospores were stimulated by *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* indicates that some of the beneficial effects of these yeasts on AM symbiosis, as happens with other soil microorganisms, seem to take place at the initial phase of AM fungus development (Caron et al. 1985; McAllister et al. 1994). At least some compounds responsible for the stimulation of AM fungus development were soluble and volatile because hyphal length increased in their presence. Some substances are considered germination “modulators”, stimulating or inhibiting hyphal length depending on their concentrations (Becard and Piche 1989; Fortin et al. 2002). The different results observed with volatile substances when the yeasts grew in rich or poor culture media, or with different concentrations of soluble exudates, suggest that the stimulation of hyphal length of *G. mosseae* is attributable to the effects of these modulators.

Our results show that dual inoculation with *G. mosseae* and yeasts increases plant dry matter and AM colonization of soybean. A similar type of observation on the increase in AM root colonization with various legumes in the presence of *S. cerevisiae* has been recorded (Singh and Kapoor 1989). Interactions between various groups of soil bacteria and AM fungi have often been observed but the mechanism of interaction is still not completely understood. There are some reports of a stimulatory effect of bacteria and fractionated bacterial cultures that produce plant growth regulators (Azcón et al. 1978; Gyndler and Vosatka 1996). We found that percentage AM colonization increased only when the soil yeasts were inoculated before *G. mosseae* was introduced. This finding also indicates that the yeasts stimulated the development of the fungus in the presymbiotic stage. Similar beneficial effects have been proposed for other microorganisms (Fracchia et al. 2000; García-Romera et al. 1998; McAllister et al. 1994).

Beneficial effects of yeasts on plant dry matter and AM root colonization varied with the carrier of the yeast inoculum. Agar has been shown to overcome some of the problems associated with survival, stability and ease of application of some microorganisms in soil (Fracchia et al. 2000; Van Elsas and Heijnen 1990). However, the effect of soil yeasts on AM colonization was greater when cells were applied as a suspension. Inoculating microorganisms as a suspension has been used in other studies of interactions between AM fungi and yeasts (Larsen and Jakobsen 1996; Singh et al. 1991) and bacteria (Vosatka and Gryndler 1999) that showed significantly increased root colonization. The microorganisms produce numerous metabolites in the culture such as plant growth regulators and vitamins, which affect the growth of plants and microorganisms present in soil (Prikryl et al. 1985).

The number of yeast cells present in the rhizosphere of plants influences their beneficial effect on AM colonization. When the number of inoculated soil yeasts was 1–2 ± 10^5 cells g^{-1} soil, a beneficial effect on plant dry matter and AM colonization was observed. When the abundance of soil yeasts was increased to 4×10^5 cells g^{-1} soil, however, the beneficial effect disappeared. These results suggest that the number of yeasts present in the rhizosphere when AM colonization of roots is initiated seems to determine the extent of the beneficial effect of these yeasts on the AM symbiosis. The combined application of some microorganisms and AM fungi had greater effects on percentage AM colonization when the microbial abundance in the soil was low (Godeas et al. 1999)

Interestingly, the soluble exudates of yeasts increased AM colonization of soybean by *G. mosseae* to approximately the same extent that they increased hyphal length of the AM fungus. The hyphal length and the capacity of the AM fungus to colonize soybean and to increase its dry matter increased as the quantity of soluble yeast exudates applied was increased. A lower increase in hyphal length in the presence of volatile exudates of *C. laurentii* than in the presence of volatile exudates of *R. mucilaginosa* and *S. kunashirensis* was found. The effect of volatile exudates of yeasts on hyphal length was similar to that in the presence of yeasts on AM colonization and on plant dry matter. These results indicate that volatile and soluble exudates had different natures and effects on the AM symbiosis, and both can be important with respect to the role of yeasts in AM colonization of plants.

In spite of the stimulatory effect of yeasts on the plant dry matter and colonization of soybean roots by *G. mosseae*, no AM effect on the number of CFUs of yeasts was found. This lack of effect has been observed previously for several beneficial saprobe fungi co-inoculated with AM fungi (Fracchia et al. 2000; García-Romera et al. 1998)

In conclusion, the beneficial effect of *R. mucilaginosa*, *C. laurentii* and *S. kunashirensis* on the extramatrical phase of *G. mosseae* seems to be partially because of the exudates produced by these soil yeasts. The ability of yeasts or their exudates to stimulate AM hyphal length may increase the chance of contact between fungal hyphae and plant roots, and consequently may increase mycorrhiza establishment. The capacity of yeasts or their exudates to increase the positive effects of *G. mosseae* on soybean dry matter might be exploited to improve the use and efficiency of this fungus in agriculture.

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References

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71–79

Azcón R, Azcón-G de Aguilar C, Barea JM (1978) Effects of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhiza. New Phytol 80:359–364

Azeredo LA, Gomes EA, Mendonca-Hagler AN (1998) Yeast communities associated with sugarcane in Campos, Rio de Janeiro, Brazil. Int Microbiol 1:205–208

Bagyaraj DJ (1984) Biological interactions with VA mycorrhizal fungi. In: Powell, CLL, Bagyaraj DJ (eds) VA mycorrhiza. CRC, Boca Raton, Fla., pp 131–153

Bagyaraj DJ (1990) Biological interactions between VA-mycorrhizal fungi and other beneficial soil organisms. In: Jalali BL, Chand HJ (eds) Proceedings of the National Conference on Mycorrhiza, 14–16 February 1990, Haryana Agricultural University, Hisar, India, pp 76–77

Becard G, Piche Y (1989) Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. Appl Environ Microbiol 55:2320–2325

Bowen GD, Rovira AD (1999) The rhizosphere and its management to improve plant growth. Adv Agron 66:1-102

Caron M, Fortín JA, Richards C (1985) Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomatoes. Plant Soil 87:233–239

Fitter AH, Garbaye J (1994) Interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533

Fortin JA, Bécard G, Declerck S, Dalpé Y, St-Arnaud M, Coughlan AP, Piché Y (2002) Arbuscular mycorrhiza on root-organ cultures. Can J Bot 80:1-20

Fracchia S, García-Romera I, Godeas A, Ocampo JA (2000) Effect of the saprophytic fungus *Fusarium oxysporum* on arbuscular mycorrhizal colonization and growth of plants in greenhouse and field trials. Plant Soil 223:175–184

García-Romera I, Ocampo JA (1988) Effect of the herbicide MCPA on VA mycorrhizal infection and growth of *Pisum sativum*. Z Pflanzenernaerh Bodenkd 151:225–228

García-Romera I, García-Garrido JM, Martín J, Fracchia S, Mujica MT, Godeas A, Ocampo JA (1998) Interactions between saprotrophic *Fusarium* strains and arbuscular mycorrhizas of soybean plants. Symbiosis 24:235–246

Gerdemann JW (1955) Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47:619–632

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Godeas A, Fracchia S, Mujica MT, Ocampo JA (1999) Influence of soil impoverishment on the interaction between *Glomus mosseae* and saprobe fungi. Mycorrhiza 9:185–189

Grynler M, Vosátka M (1996) The response of *Glomus fistulosum*-maize mycorrhiza to treatments with culture fractions from *Pseudomonas putida*. Mycorrhiza 6:207–211

Hewitt EJ (1952) Sand water culture methods used in the study of plant nutrition. Technical communication no. 22. Commonwealth Agriculture Bureau, Farnham Royal, UK, p 547

Jeffries P, Dodd JC (1996) Functional ecology of mycorrhizal fungi in sustainable soil-plant systems. In: Azcon-Aguilar C, Barea JM (eds) Mycorrhizas in integrated systems from genes to plant development. European Commission, Brussels, pp 497–501

Larsen J, Jackobsen I (1996) Interactions between a mycophagous Collembola, dry yeast and the external mycelium of an arbuscular mycorrhizal fungus. Mycorrhiza 6:259–264

Linderman RG (1992) Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Bethlenfalvay GJ, Linderman RG (eds) Mycorrhizae in sustainable agriculture. (ASA special publication) ASA, Madison, Wis., pp 45–70

Marsh BAB (1971) Measurement of length in random arrangements of lines. J Appl Ecol 8:265–270

McAllister CB, García-Romera I, Godeas A, Ocampo JA (1994) Interaction between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: effect on plant growth, arbuscular mycorrhizas and saprohytic population. Soil Biol Biochem 26:1363–1367

Mosse B (1962) The establishment of vesicular arbuscular mycorrhiza under aseptic conditions. J Gen Microbiol 27:509–520

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Prikryl Z, Vancura V, Wurst M (1985) Auxin formation by rhizosphere bacteria as a factor of root growth. Biol. Plant. 27:159–163

Singh CS, Kapoor A (1989) Effect of seed inoculation with yeast on root colonization by native vesicular-arbuscular mycorrhizal (VAM) fungi and symbiotic parameters of legumes under potted soil conditions. Zentralbl Microbiol 144:385–388

Singh GS, Kapoor A, Wange SS (1991) The enhancement of root colonisation of legumes by vesicular-arbuscular mycorrhizal (VAM) fungi through the inoculation of the legume seed with commercial yeast (*Saccharomyces cerevisiae*). Plant Soil 131:129–133

Slavikova E, Vadkertiova R (2000) The occurrence of yeasts in the forest soils. J Basic Microbiol 40:207–212

Spencer JFT, Gorin PAJ (1971) Yeasts isolated from soils of citrus orchards and citrus waste disposal areas in California and Florida. Can J Microbiol 17:871–877

Tuladhar KDY, Subba Rao NS (1985) Interaction of yeasts and some nitrogen fixing bacteria on nodulation of legumes. Plant Soil 84:287–291

Van Elsas JD, Heijnen CE (1990) Methods of introduction of bacteria into soil. A review. Biol Fertil Soils 10:127–133

Vosátka M, Gryndler M (1999) Treatment with culture fraction from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. Appl Soil Ecol 11:245–251