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Interaction between the soil yeast *Rhodotorula mucilaginosa* and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*

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Abstract

The effect of the soil yeast, *Rhodotorula mucilaginosa* LBA, on *Glomus mosseae* (BEG n°12) and *Gigaspora rosea* (BEG n°9) was studied in vitro and in greenhouse trials. Hyphal length of *G. mosseae* and *G. rosea* spores increased significantly in the presence of *R. mucilaginosa*. Exudates from *R. mucilaginosa* stimulated hyphal growth of *G. mosseae* and *G. rosea* spores. Increase in hyphal length of *G. mosseae* coincided with an increase in *R. mucilaginosa* exudates. No stimulation of *G. rosea* hyphal growth was detected when 0.3 and 0.5 ml per petri dish of yeast exudates was applied. Percentage root length colonization by *G. mosseae* in soybean (*Glycine max* L. Merill) and by *G. rosea* in red clover (*Trifolium pratense* L. cv. Huia) was increased only when the soil yeast was inoculated before *G. mosseae* or *G. rosea* was introduced. Beneficial effects of *R. mucilaginosa* on arbuscular mycorrhizal (AM) colonization were found when the soil yeast was inoculated either as a thin agar slice or as a volume of 5 and 10 ml of an aqueous solution. *R. mucilaginosa* exudates (20 ml per pots) applied to soil increased significantly the percentage of AM colonization of soybean and red clover.

Keywords: Arbuscular mycorrhiza; *Gigaspora rosea*; *Glomus mosseae*; *Rhodotorula mucilaginosa*; Soil yeast

1. Introduction

An increasing number of reports support the concept that establishment and functioning of the arbuscular mycorrhizal (AM) symbioses are affected by a range of soil microorganisms that may act either beneficially or detrimentally (Andrade et al., 1997; Filion et al., 1999). The importance of microbial interactions between nonpathogenic rhizosphere-inhabiting microorganisms and AM fungi is now beyond all doubt (Andrade et al., 1997). Most studies to date have dealt with interactions between selected bacteria or saprophytic fungi in relation to AM colonization enhancement, whereas no studies have investigated interactions with soil yeasts (Bagyaraj, 1984; Fitter and Garbaye, 1994; Fracchia et al., 2000; García-Romera et al., 1998). Only studies dealing with the effect of the commercial yeast, *Saccharomyces cerevisiae*, on AM fungi have been carried out (Larsen and Jackobsen, 1996; Singh et al., 1991).

Soils are known to contain yeasts with the greatest number of yeasts occurring in orchard soil, at a maximum of 245,000 per gram of dry soil. However, the role of yeasts in soil microbial communities has been the subject of controversy (Lund, 1957). Some researchers consider soil more as reservoir for yeasts than as specific habitat, while others consider yeasts as true-soil-inhabitants with high competitive ability against other soil microorganisms (Phaff and Starmer, 1987; Vishniac, 1995). Relatively little is known about the ecology of yeasts and the role that they play in mineral cycling, and less is known of their interaction with other soil microorganisms (Falih and Wainwright, 1995; Vishniac, 1995).

It has been observed that during their presymbiotic phase, while growing from the propagule toward the root, mycorrhizal fungi can be influenced positively by some plants and rhizosphere microorganisms through their

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exudates (Fitter and Garbaye, 1994). Exudates from plants are important in the AM colonization process and they have been well studied; however, few studies have focussed on the effects of microbial exudates on germination of AM spores and AM colonization of roots.

The purpose of this study was to determine the relationships between the yeast *Rhodotorula mucilaginosa* LBA and the AM fungi *Glomus mosseae* and *Gigaspora rosea*, and to examine some of the possible mechanisms involved in such interactions.

2. Material and methods

2.1. Isolation of R. mucilaginosa

The yeast present in the rhizosphere soil and roots of maize cultivated in the Province of Buenos Aires (Argentina) was 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, *R. mucilaginosa* LBA was selected and transferred to tubes of PDA and 2% malt extract agar (MEA) and stored at 4 °C. *R. mucilaginosa* was identified by the Colección Española de Cultivos Tipo (CECT).

2.2. Effect of R. mucilaginosa LBA on development of G. mosseae and G. rosea

The effect of *R. mucilaginosa* LBA on hyphal length of *G. mosseae* (BEG n°12) and *G. rosea* (BEG n°9) was tested in two different experiments conducted in 9 cm diameter plastic petri dishes. In the first experiment the effect of *R. mucilaginosa* on mycelial length in vitro was tested. Sporocarps of *G. mosseae* and spores of *G. rosea* were isolated by wet sieving the soil (Gerdemann, 1955) from alfalfa plant pot cultures and were stored in water at 4 °C. The spores of *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe, obtained by dissecting the sporocarps and spores of *G. rosea*, 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, OH, USA). A thin streak of *R. mucilaginosa* was inoculated opposite and at least 7 cm away from the sterilized spores.

The second experiment tested the effect of exudates from *R. mucilaginosa* LBA on hyphal length of *G. mosseae* and *G. rosea* in vitro. Exudates were obtained by growing the soil yeast in a 250 ml flask containing 125 ml of sterile liquid medium B in a shaker at 28 °C. The standard medium B consisted of: $MgSO_4 \cdot 7H_2O$, 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 and sterilized twice by filtration through a 0.45 μm Millipore membrane. The different concentrations of exudates, 0.01, 0.025, 0.05, 0.1, 0.3 and 0.5 ml, were added to a petri dish with 10 ml of 4% Gel-Gro (ICN Biochemicals, Aurora, OH, USA) in 10 mM MES buffer (pH 7). Ten spores of *G. mosseae* and *G. rosea* were placed in the dish. In the control treatment the same volume of sterile liquid medium B was substituted for the exudates.

In each of the two experiments, five replicates of each soil yeast treatment and controls (plates with spores of AM fungi without yeast) were used. The plates were incubated at 25 °C in the dark for 15 days, and were sealed to reduce dehydration and contamination. Hyphal length of the germinated *G. mosseae* and *G. rosea* spores was determined under a binocular microscope at 40 × magnification at the end of the experiment using the gridline intersect method (Marsh, 1971). All the fungal mycelia were measured.

2.3. Interaction between R. mucilaginosa LBA and its exudates and G. mosseae or G. rosea in the rhizosphere of soybean and red clover grown in soil pots

Plants were grown in 100 ml pots of soil collected from the province of Granada, Spain and from Buenos Aires, Argentina. The Granada soil was a calcixerollic xerochrept type, pH 7.6 (García-Romera and Ocampo, 1988) and the Argentina soil was an argiudol type, pH 7.1 (Fracchia et al., 1998). They were steam-sterilized and mixed 1:1 (v/v) with perlite. Soybean (*Glycine max* L. Merill) and red clover (*Trifolium pratense* L. cv. Huia) were used as test plants for the *G. mosseae* and *G. rosea* assay, respectively. Seeds were sterilized 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 μE m^{-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 fed with 10 ml of a nutrient solution per week (Hewitt, 1952).

Inocula of the AM fungi used were *G. mosseae* (BEG n°12) and *G. rosea* (BEG n°9). Both mycorrhizal inocula consisted of soil, spores, mycelium and infected root fragments from an open pot culture of *Medicago sativa* plant. Five grams of inoculum with similar characteristic (an average of 30 spores, mycelium, and infected root) of one of the two isolates was added to each pot at sowing just below the seeds. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal plants was added to the uninoculated treatment. The filtrate contained common soil microorganisms, including bacteria and fungi, but no propagules of AM fungi. *R. mucilaginosa* LBA was obtained from the rhizosphere and rhizoplane of maize plants as described before.

Four experiments were designed to test the interaction between *R. mucilaginosa* and AM colonization of soybean and red clover inoculated with *G. mosseae* and *G. rosea*,

respectively. Four treatments were used in all experiments, (1) control of soybean plants inoculated with *G. mosseae*, (2) control of red clover plants inoculated with *G. rosea*, (3) soybean plants inoculated with both *G. mosseae* and *R. mucilaginosa* (soil yeast and/or exudates), and (4) red clover plants inoculated with both *G. rosea* and *R. mucilaginosa* (soil yeast and/or exudates).

Experiment 1. The first experiment was designed to test the effect of yeast inoculation time. The soil yeast was inoculated as a suspension of *R. mucilaginosa* grown on medium B for 48 h at 28 °C at the dose of 1×10^5 cells g^{-1}, 2 weeks before, at the same time as, or 2 weeks after the arbuscular fungi. The factors used in this experiment were: four treatments and three inoculation time. Five replicate per treatment were made a total of 60 pots.

Experiment 2. The second experiment was performed to select the most appropriate soil yeast inoculation methods. Plants were inoculated 2 weeks before AM fungi with *R. mucilaginosa* as: (1) a thin agar slice of MEA (1×1 cm^2) containing cells of *R. mucilaginosa*, or (2) a suspension of *R. mucilaginosa* grown on medium B as described before. The factors used in this experiment were: four treatments and two inoculation types. Five replicate per treatment were made a total of 40 pots.

Experiment 3. The third experiment select the most appropriate volume of soil yeast inoculation. An aqueous suspension of *R. mucilaginosa* grown on medium B as described before, was added 2 weeks before AM fungi into the soil pots at the doses of 1×10^5, 2×10^5 and 4×10^5 cells g^{-1} soil. The factor used in this experiment were: four treatments and three dosages. Five replicate per treatment were made a total of 60 pots.

Experiments 4. In the fourth experiment the effect of exudates from the soil yeast on AM colonization was tested. *R. mucilaginosa* exudates obtained as described before was inoculated at the same time of the AM fungi at the doses of 10 and 20 ml per pots. The factors used in this experiment were: four treatments and two exudates dosages. Five replicate per treatment were made a total of 40 pots.

To evaluate the population of *R. mucilaginosa* LBA during the experiments, about 5 g of soil:perlite was removed after 0 and 5 weeks from each of the five replicate pots. Ten fold aqueous dilution series (from $10^{-1}$to 10^{-4}) were prepared for each sample and 1 ml of each solution was plated on PDA. Numbers of colony forming units (CFUs) in suitable dilutions of such sample, taken from the five replicate pots of each treatment, were counted. Soil was dried at 105 °C and weighed. The number of CFUs was expressed per gram of dry soil.

At harvest (5 weeks after planting), the root system was separated from the shoot, and the dry weight of shoot was recorded after drying at 70 °C. Part of the root system at random was cleared and stained (Phillips and Hayman, 1970). The percentage of root colonization was estimated by observing 200 intersection points per sample under a dissecting microscope at 40× magnification, using the gridline-intersect method using hyphae, arbuscules and vesicles present in roots as AM fungal identification (Giovannetti and Mosse, 1980).

2.4. Statistical treatments

Two AM fungi, *G. rosea* and *G. mosseae*, were used. With each of the AM fungi, independent experiments were made. The data for hyphal length of *G. mosseae* or *G. rosea* were subjected to a two-way analysis of variance, one factor was the presence of *R. mucilaginosa* and the other factor was the concentration of exudates of the yeast applied to the AM growth medium. The data for soybean and clover were subjected to a two-way analysis of variance. One factor was the inoculation with *R. mucilaginosa* and the other factor is the inoculation times of *R. mucilaginosa* to soil pots (Experiment 1), the inoculation carriers (Experiment 2), the inoculum volume of *R. mucilaginosa* applied to soil pots (Experiment 3), the different amount of *R. mucilaginosa* exudates applied to soil pots (Experiment 4). The data for each experiment were analysed by two-way analysis of variance. Comparisons of means were made by the Duncan's multiple range test ($P = 0.05$*).

3. Results

Hyphal length of *G. mosseae* (BEG n°12) and *G. rosea* (BEG n°9) increased significantly in the presence of *R. mucilaginosa* LBA (Table 1).

The exudates of *R. mucilaginosa* LBA applied to the Gel-Gro significantly increased the hyphal length of *G. mosseae* and *G. rosea* spores at the doses of 0.01, 0.025, 0.05 and 0.1 ml (Table 2). However, when 0.3 and 0.5 ml of exudates of the soil yeast were applied to Gel-Gro, an increase in the hyphal length of *G. mosseae*, but not in that of *G. rosea*, was found. The exudates of *R. mucilaginosa* increased the hyphal length of *G. mosseae* and *G. rosea* throughout the assay but the highest increase was observed at the doses of 0.3 and 0.5 ml for *G. mosseae* and 0.05 for *G. rosea* spores (Table 2).

The plant dry matter and percentage root length colonization of *G. mosseae* in soybean and of *G. rosea* in

Table 1
Hyphal length of *G. mosseae* (BEG n°12) and *G. rosea* (BEG n°9) in the presence of *R. mucilaginosa* LBA

Treatment	Hyphal length (mm)	
	G. mosseae (BEG n°12)	*G. rosea* (BEG n°9)
Control	10.19 a	10.93 r
R. mucilaginosa	24.33 b	19.73 s

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 ($P = 0.05$*).

Table 2
Effect of different concentration of exudates of *R. mucilaginosa* LBA on the hyphal length of *G. mosseae* (BEG n°12) and *G. rosea* (BEG n°9) after 15 days growth

AM endophyte	Inoculation treatment	Exudates (ml) per petri dish					
		0.01	0.025	0.05	0.1	0.3	0.5
G. mosseae	Control	1.45 a	1.83 ab	2.40 bc	2.92 c	3.11 c	3.22 c
	R. mucilaginosa	1.82 ab	2.63 c	5.67 d	13.21 e	23.13 f	24.01 f
G. rosea	Control	21.82 r	23.09 rs	19.36 r	23.92 rs	26.55 rs	26.72 rs
	R. mucilaginosa	29.86 s	39.85 t	59.67 v	42.89 u	20.62 r	21.32 r

Each value is the mean of five replicates. Values for each AM endophyte in each inoculation treatment followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$*).

red clover was increased significantly when *R. mucilaginosa* LBA was inoculated 2 weeks before the AM fungi, but was unaffected when the yeast was inoculated at the same time or 2 weeks after *G. mosseae* or *G. rosea* (Table 3).

Higher shoot, root dry weight and AM colonization of soybean and red clover inoculated by *G. mosseae* and *G. rosea*, respectively, were obtained when *R. mucilaginosa* LBA was inoculated as suspension. However, the inoculation of *R. mucilaginosa* as on agar slice and increased of dry matter and AM colonization of soybean but not of clover was observed (Table 4).

Table 5 shows that AM colonization of soybean by *G. mosseae* and red clover by *G. rosea* in the presence of 1×10^5 and 2×10^5 of *R. mucilaginosa* LBA cells g^{-1} soil was higher than that of an uninoculated plant. However, inoculation with 4×10^5 cells g^{-1} soil did not affect significantly the plant dry matter and the percentage of AM colonization of plants (Table 5).

Table 3
Shoot and root dry weights (mg) and percentage root length colonization of soybean (*Glycine max* L. Merrill) and red clover (*T. pratense* L. cv. Huia) by *G. mosseae* and *G. rosea*, respectively, and inoculated or not with *R. mucilaginosa* LBA at different times

Inoculation time for *R. mucilaginosa*	Inoculation treatment	Soybean + *G. mosseae*			Clover + *G. rosea*		
		Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)	Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)
Inoculated 2 weeks before AM fungi	Control	182.1 a	97.3 a	21.3 a	85.3 r	41.2 r	15.1 r
	R. mucilaginosa	280.3 b	170.2 b	51.6 b	174.3 s	122.2 s	32.1 s
Inoculated at the same time as AM fungi	Control	185.2 a	98.2 a	22.4 a	87.5 r	45.6 r	17.7 r
	R. mucilaginosa	190.5 a	110.4 a	25.0 a	90.3 r	52.3 r	21.3 r
Inoculated 2 weeks after AM fungi	Control	187.3 a	107.6 a	22.7 a	84.6 r	48.5 r	14.1 r
	R. mucilaginosa	192.1 a	99.3 a	25.7 a	93.1 r	56.5 r	22.1 r

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 ($P = 0.05$*).

Table 4
Shoot and root dry weights (mg) and percentage of root length of soybean (*Glycine max* L. Merrill) and red clover (*T. pratense* L. cv. Huia) colonized by *G. mosseae* and *G. rosea*, respectively, with or without different *R. mucilaginosa* LBA carriers

Inoculation carrier for *R. mucilaginosa*	Inoculation treatment	Soybean + *G. mosseae*			Clover + *G. rosea*		
		Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)	Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)
Agar slice	Control	191.2 a	111.6 a	23.4 a	91.4 r	52.3 r	20.0 r
	R. mucilaginosa	315.3 b	210.2 b	58.8 b	115.2 rs	67.2 rs	26.5 rs
Suspension	Control	187.1 a	108.5 a	22.1 a	89.5 r	54.5 r	19.0 r
	R. mucilaginosa	312.5 b	221.4 b	63.8 b	140.3 s	85.4 s	34.7 s

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 ($P = 0.05$*).

Table 5
Shoot and root dry weights (mg) and percentage of root length of soybean (*Glycine max* L. Merrill) and red clover (*T. pratense* L. cv. Huia) colonized by *G. mosseae* and *G. rosea*, respectively, inoculated with different amounts of *R. mucilaginosa* LBA, or left untreated

AM endophyte	Inoculation treatment	5 ml			10 ml			20 ml		
		Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)	Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)	Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)
G. mosseae + soybean	Control	195.3 a	120.5 a	17.3 a	188.3 a	122.4 a	18.7 a	185.5 a	119.1 a	21.2 ab
	R. mucilaginosa	290.4 b	216.4 b	32.7 b	279.2 b	210.5 b	43.0 b	195.1 a	115.3 a	29.8 b
G. rosea + clover	Control	93.2 r	52.2 r	18.0 r	86.5 r	55.6 r	17.6 r	84.2 r	57.4 r	23.5 rs
	R. mucilaginosa	194.5 s	135.5 s	30.3 s	177.6 s	138.1 s	32.3 s	85.3 r	53.2 r	30.3 s

Each value is the mean of five replicates. Values for each AM endophyte followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05^*$).

Table 6
Shoot and root dry weights (mg) and percentage of root length of soybean (*Glycine max* L. Merrill) and red clover (*T. pratense* L. cv. Huia) colonized by *G. mosseae* and *G. rosea*, respectively, inoculated with different volumes of *R. mucilaginosa* LBA exudates or left untreated

AM endophyte	Inoculation treatment	10 ml			20 ml		
		Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)	Shoot dry weight	Root dry weight	Mycorrhizal colonization (%)
G. mosseae + soybean	Control	185.1 a	99.2 a	32.4 a	182.5 a	97.7 a	30.3 a
	R. mucilaginosa	250.3 b	171.5 b	45.1 b	268.1 b	180.1 b	56.4 b
G. rosea + clover	Control	81.5 r	45.6 r	24.6 r	77.4 r	44.2 r	22.3 r
	R. mucilaginosa	75.3 r	47.7 r	22.5 r	182.7 s	122.6 s	44.3 s

Each value is the mean of five replicates. Values of each AM endophyte followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05^*$).

The plant dry matter and the percentage of AM colonization of soybean inoculated with *G. mosseae* was increased significantly when 10 or 20 ml of yeast exudates were applied to soil. However, the shoot and root dry weight and the percentage of AM colonization of red clover by *G. rosea* was increased only when 20 ml of exudates of *R. mucilaginosa* LBA was applied (Table 6).

The number of CFUs of yeast g^{-1} rhizosphere soil decreased throughout the experiments. The population of *R. mucilaginosa* LBA in the rhizosphere of soybean were not affected by the presence of *G. mosseae* or *G. rosea* (Table 7).

4. Discussion

The interaction between saprobe fungi and AM fungi are important for plant growth. However, these interactions are complex and synergistic, antagonistic or neutral relationships between both microorganisms have been found (Linderman, 1992). The main effect of soil microorganisms on AM symbiosis seem to take place at the initial phase of AM symbiotic development (Caron et al., 1985; McAllister et al., 1994).

Hyphal growth of *G. mosseae* clamydospores and *G. rosea* azygospores was stimulated by *R. mucilaginosa* LBA. At least some compounds responsible for the stimulation were water soluble substances since hyphal growth increased in the presence of the soluble exudates produced by *R. mucilaginosa*. Although exudates from *R. mucilaginosa* consistently stimulated hyphal growth of *G. mosseae* and *G. rosea* spores, the degree to which hyphal growth occurred varied between both endophytes according to the quantity of exudates applied. The increase in hyphal length of *G. mosseae* coincided with an increase in *R. mucilaginosa* exudates, except at the higher concentrations of *G. rosea* exudates, where no stimulation of hyphal growth was observed. Some substances are considered germination 'modulators', stimulating or inhibiting hyphal growth depending on their concentrations (Becard and Piche, 1989; Vidal-Dominguez, 1991). The nature of these soluble substances is unknown but, it is known that AM fungi vary in their sensitivity to plant and microbial metabolites (Vierheilig et al., 1998; Tilka et al., 1991).

Table 7
CFU of *R. mucilaginosa* LBA from the rhizosphere (g^{-1} dry weight soil) of soybean (*Glycine max* L. Merrill) plants inoculated with *G. mosseae* and *G. rosea* at different times or left untreated

AM endophyte	Inoculation treatment	CFU g^{-1} soil after (weeks)	
		0	5
G. mosseae + soybean	Control	35.000 a	2.400 b
	R. mucilaginosa	34.000 a	2.800 b
G. rosea + clover	Control	35.000 r	2.400 s
	R. mucilaginosa	37.000 r	2.500 s

Each value is the mean of five replicates. CFU values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$*)

Our results show that dual inoculation of *G. mosseae* or *G. rosea* and *R. mucilaginosa* LBA increases the plant dry matter and the AM colonization of the roots. We found that percentage of AM colonization increased only when the soil yeast was inoculated before *G. mosseae* or *G. rosea* were introduced. This finding together with our in vitro observations indicate that *R. mucilaginosa* stimulated development of AM fungi in the extramatrical 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 *R. mucilaginosa* LBA on plant dry matter and AM root colonization of soybean and red clover 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 (Van Elsas and Heijnen, 1990; Fracchia et al., 2000). However, the effect of soil yeasts on AM colonization was more remarkable when cells were applied as a suspension. Inoculating yeasts as suspension has been used in other studies of interactions between yeasts and AM fungi, where significantly increased root colonization by AM fungi was observed in the presence of yeasts (Larsen and Jackobsen, 1996; Singh et al., 1991).

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 10×10^6 ml^{-1} cells, a beneficial effect on plant dry matter and AM colonization of roots was observed. However, when the abundance of soil yeasts was increased to 40×10^6 ml^{-1} the beneficial effect disappeared. These results suggest that the number of *R. mucilaginosa* LBA present in the rhizosphere when AM colonization of roots is initiated seems to determine the extent of the beneficial effect of this yeast on the AM symbiosis. In another study, the combined application of some microorganisms and AM fungi was more effective on percentage of AM colonization when the microbial abundance in the soil was low (Godeas et al., 1999).

Interestingly, the exudates of *R. mucilaginosa* LBA increased the AM colonization of soybean by *G. mosseae* and red clover by *G. rosea* to the same extent as they increased hyphal growth of the AM fungi. The capacity of both AM fungi to colonize the plants and to increase their dry matter varied according to the quantity of yeast exudates applied. The AM colonization and plant dry matter of soybean by *G. mosseae* was increased with 10 and 20 ml of *R. mucilaginosa* exudates while 20 ml of yeast exudates was necessary to increase the AM colonization and plant dry matter of red clover by *G. rosea*. Our assay suggests that inoculation with yeast cells rather than yeast exudate stimulates hyphal growth and percent AM root colonization to a greater degree. In fact, the application of 10×10^6 cell ml^{-1} was enough to increase the plant dry weight and AM colonization of red clover by *G. rosea*, whereas the application of 10 ml of yeast exudates obtained from 20×10^6 cell ml^{-1} culture was not able to increase plant dry weight and AM colonization of this plant. It is known that live yeast cells of *S. cerevisiae* are more effective than dead cells in increasing AM root colonization (Singh et al., 1991).

In spite of the stimulatory effect of *R. mucilaginosa* LBA on the plant dry matter and colonization of soybean and red clover roots by AM fungi, no AMF effect on the number of CFUs of *R. mucilaginosa* 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* LBA on the extramatrical phase of the AM fungi towards AM fungal symbiosis seems to be partially due to the exudates produced by this soil yeast. The ability of *R. mucilaginosa* or their exudates to stimulate AM hyphal growth may increase the chance of contacts between fungal hyphae and plant roots, and, consequently, to increase mycorrhizal establishment. The effect of *R. mucilaginosa* or their exudates on AM establishment varied with yeast cell concentration and with the type of AM fungus. The different sensitivities among AM fungi to metabolites produced by soil microorganisms may lead to the selection of different isolates within AM-fungal communities after the inoculation of particular soil microorganism.

On the other hand, *R. mucilaginosa* or its exudates increased the effectiveness of *G. mosseae* or *G. rosea* on dry matter of plants. Therefore *R. mucilaginosa* might be exploited to improve the colonization of some AM inoculated fungi, especially in plant nurseries. However, one of the most important limitations in the use of AM fungi for field crops in agriculture is because of the impossibility of culturing the AM fungus in the absence of plant roots. The beneficial effect of application of the exudates of *R. mucilaginosa* on root colonization and its effect on plant growth indicates the possibility of using this microorganism to increase the effectiveness of soil indigenous AM fungi (Jeffries and Dodd, 1996).

In future studies, the beneficial effect of *R. mucilaginosa* LBA on AM symbiosis will be investigated by determining what stage of the root colonization process, i.e. spore germination, penetration of plant root, and extramatrical hyphal development, are most affected by the soil yeast.

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