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Short communication

Rhodotorulic acid enhances root colonization of tomato plants by arbuscular mycorrhizal (AM) fungi due to its stimulatory effect on the pre-symbiotic stages of the AM fungi

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ABSTRACT

Exudates of *Rhodotorula mucilaginosa*, a yeast commonly found in the rhizosphere, increased hyphal length of the arbuscular mycorrhizal (AM) fungi *Gigaspora rosea* and *Gigaspora margarita*. Rhodotorulic acid (RA), a siderophore compound obtained from *R. mucilaginosa* exudates, increased hyphal length and branching. Thus, the increase in the number of entry points and the higher AM root colonization of tomato plants in the presence of RA can at least partially be explained by the positive effect of RA on the pre-symbiotic stages of the AM fungi.

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Arbuscular mycorrhizal (AM) fungi are known to interact with other soil microorganisms (Andrade et al., 1997). Yeasts belonging to the genera *Rhodotorula* are common components of the soil rhizosphere (Slavikova and Vadkertiova, 2000). Recently, it has been observed that yeasts, mainly through their exudates, can have a positive effect on AM fungi (Fracchia et al., 2003; Sampedro et al., 2004).

Siderophores are low-molecular weight (<1500 Da) ferric-specific chelates, produced under iron limitation. Iron(III) is solubilized by binding to siderophores and thus, can enter microbial cells via specific siderophores transport systems (Winkelmann, 2007). Siderophores are also involved in microbial interactions in the rhizosphere and in the colonization of roots by microorganisms (Neilands, 1981). There are some indications that siderophores affect phosphorous (P) cycling by releasing P from iron phosphates (Zaid et al., 2003), or by regulating the production of phosphatases (Monds et al., 2006). This can be important in the AM symbiosis as AM fungi apparently are not able to produce siderophores (Caris et al., 1998), but possibly, similar to other microorganisms, interact with siderophores

(Winkelmann, 2007). Rhodotorulic acid (RA) is a hydroxamate siderophore produced by *Rhodotorula mucilaginosa* and found in its exudates (Andersen et al., 2003). No data on its effect on AM fungi are available yet.

In the present work we studied the effect of exudates and RA produced by *R. mucilaginosa* (BAFC 1769) on several stages of the development of the AM fungi, *Gigaspora rosea* (BEG no. 9) and *Gigaspora margarita* (J7). The exudates were obtained by growing the yeast in Y liquid medium (Fracchia et al., 2003). After 6, 12, 18, 24, 32, 40 and 48 h the yeast growth was measured at 600 nm. When OD = 1 was equivalent to 1×10^7 cells ml⁻¹ (Andersen et al., 2003), the culture medium was centrifuged at 3000 rpm and the supernatant was sterilized (0.22 µm Millipore). The effect of yeast exudates on hyphal length of AM spores was tested by adding 1, 2.5, 5, 8, 10, 15, 20 or 40 µl of the liquid culture medium after 6, 12, 18, 24, 32, 40 and 48 h of yeast growth to Petri dishes with 10 ml of 4% Gel-Gro in 10 mM of MES buffer (pH 7) and with 10 surface-sterilized spores (Mosse, 1962). Ten replicates were used for each treatment. The plates were incubated at 25 °C for 15 days and the hyphal length was determined (Marsh, 1971).

The RA was obtained from *R. mucilaginosa* cultured in 500 ml medium (Fracchia et al., 2003) and the greatest production of RA was found after 24 h of culture (Andersen et al., 2003). At this time

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RA was isolated and characterized (Atkin et al., 1970). In our experiments RA purchased from Sigma was used which was identical in form, melting point, MS and ¹H-RMN spectra to the RA obtained from *R. mucilaginosa*. The effect of RA on spore germination, hyphal length, hyphal branches and the clusters of auxiliary cells of the tested AM fungi were determined. RA was dissolved in distilled water, sterilized by filtration through a 0.20 μm Millipore membrane and transferred to 10 ml of Gel-Gro at a final concentration of 0.5, 1, 5, 10 and 30 μM. The Petri dishes with 10 surface sterilized spores and controls without RA were incubated 25 °C for a fortnight. Ten replicates of each treatment were prepared.

In another experiment the effect of RA on AM entry points and on AM root colonization of tomato (*Solanum lycopersicum* L.) was tested, using a monosporic culture technique (Fracchia et al., 2001), which consists of 5-cm diameter Petri dishes with 10 ml of Gel-Gro and 10 ml of vermiculite-perlite and five spores of the tested *Gigaspora* strain. RA was added to the Petri dishes at a final concentration of 0.5, 1, 5, 10 and 30 μM or no RA was added (control). After 2 weeks in the growth chamber, when a first AM hyphal contact with the root was observed, 10 replicates were harvested and the number of entry points was assessed (Ocampo et al., 1980). After 6 weeks another 10 replicates were harvested, the root system was cleared and stained (Phillips and Hayman, 1970) and the root colonization was determined (Giovannetti and Mosse, 1980). Experimental data were analysed by a one-way analysis of variance and Tukey's test (*P* = 0.05).

Rhodotorula mucilaginosa reached a logarithmic growth after 6 h and reaching a maximum of 11 × 10⁷ cells ml⁻¹ at 24 h, and after which stationary growth was observed. RA production (100 mM) was detected after 24 h of yeast growth where 2.5, 5 and 8 μL of exudates of *R. mucilaginosa* (equivalent 3, 6 and 10 μM of RA per Petri dish), increased the hyphal length of *Gi. rosea* and *Gi. margarita* (Table 1). Because of it, the effects of 0.5, 1, 5, 10 and 30 μM of RA on AM fungi were tested.

RA showed no effect on spore germination of *Gi. rosea* and *Gi. margarita*. At least one of the RA concentrations increased the hyphal length and the number of hyphal branches of the two *Gigaspora* species. RA did not affect the number of clusters of auxiliary cells of *Gi. margarita* but 10 and 30 μM of RA increased those of *Gi. rosea* (Table 2).

Table 1
Effect of exudates of *R. mucilaginosa* isolates after different times of culture on the hyphal length of *Gi. rosea* and *Gi. margarita*

Exudates doses (μl)	AM hyphal length (mm) in presence of exudates from yeast grown after (h)								
	0	6	12	18	24	30	36	42	48
<i>Gi. rosea</i>									
0	8	7.8	5	10	5	6.6	6	7.2	7.2
1		7.2	6.2	5.6	7.8	6.6	8	18.2	22*
2.5		6.2	6.2	6	18*	20.4*	58.8*	60*	32*
5		8.4	3.6	6.4	55.2*	58.2*	35.8*	15	12
8		6.6	6.4	6.6	27*	24.8*	19.4	15	16
10		6	8.5	7.2	9.4	11.2	7.2	10	5
15		5.5	6	8	6	7.4	7	5	4.5
20		5	5.5	4.6	7.2	7.8	5	4	4
40		6	7	10	8	7	8	6.6	5
<i>Gi. margarita</i>									
0	12	10	11.5	9.5	8.5	11	10.6	8.5	7.8
1		12.2	5.5	5.4	6.6	10.3	10.4	15.2	12
2.5		10.2	10	7.8	19.1*	33*	65*	55*	11
5		11.5	3	8	44.5*	55*	28*	15	12
8		2	5	4	22*	35*	28.5*	12	10
10		10.2	8.2	4	9.5	11.4	6.5	8.5	9
15		9.5	8.5	10.2	11.3	5	7.2	6	6.2
20		8	3	2.5	5	10	11	5	3
40		7	4	3	6	9	10	4	3

*Significantly different as determined by Tukey's test (*P* = 0.05).

Table 2
Effect of rhodotorulic acid on the percentage of germination, hyphal length, hyphal branching, number of clusters of *Gi. rosea* and *Gi. margarita* spores

AM fungus	RA doses (μM)	Percent germination	Hyphal length	Hyphal branching	Cluster number
<i>Gi. rosea</i>					
	0	83a	9.4a	1.2a	0.4a
	0.5	80a	9.8a	1.1a	0.5a
	1	86a	9.7a	3.8a	0.6a
	5	84a	19.2a	3.5a	0.8a
	10	86a	21.8b	7.1c	6.7b
	30	87a	29.3b	7.3c	6.4b
<i>Gi. margarita</i>					
	0	70a	9.5a	3.7a	0.8a
	0.5	70a	8.3a	3.2a	0.9a
	1	75a	8.2a	3.8a	1.2a
	5	78a	22.4b	7.7b	1.1a
	10	80a	8.6a	3.7a	0.7a
	30	81a	7.4a	2.8a	0.9a

Within each AM endophyte, column values with the same letter are not significantly different as determined by Tukey's test (*P* = 0.05).

At least one concentration of RA increased the number of entry points and the percentage of root length of tomato colonized by the *Gigaspora* species (Table 3). The dry weights of tomatoes grown in Petri dishes were similar in all treatments (data not shown).

Exudates of *R. mucilaginosa* increased the hyphal length of *Gi. rosea* and *Gi. margarita* spores except at high doses. Exudates of some saprobe fungi and yeasts are considered germination "modulators", stimulating or inhibiting hyphal growth depending on their concentrations (Fracchia et al., 2004; McAllister et al., 1995). The nature of these soluble substances is unknown but in our study the beneficial effect of exudates from *R. mucilaginosa* on the hyphal length of *Gigaspora* took place after the exponential growth of the yeast. Some of these beneficial substances can be attributed to the siderophore RA produced by the yeast because the beneficial effect of the *R. mucilaginosa* exudates happened when the RA was produced by the yeast. However, the exudates of *R. mucilaginosa* increase the percentage of spore germination (Fracchia et al., 2003), whereas in our experiment RA showed no effect on spore germination. On the other hand, exudates with lower RA concentrations than those of applied RA increased hyphal length of *Gigaspora*. These facts indicate that the beneficial effect of the exudates from *R. mucilaginosa* on AM fungal development was not solely due to the presence of RA.

In a recent study it has been reported that an uptake of siderophores observed in the germ tubes and young mycelia of fungi enhances fungal growth (Winkelmann, 2007). According to Caris et al. (1998) AM fungi are not able to produce siderophores,

Table 3
Effect of rhodotorulic acid (RA) on the percentage of root length colonization and number on entry points of tomato (*Lycopersicum esculentum*) by *Gi. rosea* and *Gi. margarita*

AM fungus	RA doses (μM)	Number of entry points	Percentage of root length colonization
<i>Gi. rosea</i>			
	0	12.4a	10.2a
	0.5	11.7a	9.7a
	1	11.5a	9.3a
	5	12.2a	10.5a
	10	21.1b	27.4b
	30	32.2c	46.8c
	0	10.1a	30.2a
<i>Gi. margarita</i>			
	0.5	9.4a	32.4a
	1	11.2a	28.1a
	5	17.9b	29.4a
	10	8.9a	50.3b
	30	9.1a	31.5a

Within each AM endophyte, column values with the same letter are not significantly different as determined by Tukey's test (*P* = 0.05).

but our data show that *Gigaspora* strains can use the siderophore RA to increase their hyphal length and the number of hyphal branches, pointing towards a similar effect of RA on AM fungi as observed with other fungi. Thus, the increase in the number of entry points and the higher AM root colonization in the presence of RA can be at least partially explained by the positive effect of RA on the pre-symbiotic stages of the AM fungi.

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