Enhancement of faba bean nodulation, nitrogen fixation and growth by different microorganisms

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Abstract

The effect of four bacterial and six fungal species on nodulation and growth of *Vicia faba* cv. Giza 3 inoculated with *Rhizobium leguminosarum* biovar *viceae* strain RCR 1001 were assessed in a pot experiment. *Bacillus cereus*, *Pseudomonas fluorescens*, *Aspergillus niger* and *A. quadriliniatus* either alive cells or sterile filtrate significantly promoted nodulation, growth and nitrogen accumulation. Heat-killed cells had no effect.

Introduction

In recent years there has been a renewed interest in the use of soil microflora which, when applied to seeds, roots or tubers, is able to colonize plant roots and stimulate plant growth and crop yield (Kloepper *et al.* 1980). The mechanism by which these bacteria and fungi stimulate plant growth are not well understood but it has been suggested that production of plant hormones, enhancement of plant nutrient uptake, suppression of pathogens in the plant rhizosphere, solubilization of phosphorus, rhizospheric nitrogen fixation or conversion of materials to a form useful to the plant may be involved (Brown 1974, Barber and Lynch 1977, Kloepper and Schroth 1981, Okon and Kaplink 1986, Windham *et al.* 1986).

Plant growth-promoting microorganisms are not universally effective on crop plants and genotypes (Rennie and Larson 1979, Chanway *et al.* 1988 a,b, Kumar and Dube 1992). One way how inoculant microbes could stimulate performance of leguminous species is by affecting the process of symbiotic nitrogen fixation. Inoculation with root colonizing microflora and *Rhizobium* has been demonstrated to affect symbiotic nitrogen fixation by enhancing root nodule number or mass (Angle *et al.* 1981,Yahalom *et al.* 1987, Halverson and Handelsman 1991) and by increasing nitrogenase activity (Iruthayathas *et al.* 1983).

Faba bean (*Vicia faba* L.) is becoming important protein food and feed constituents in many countries. Our objectives were to evaluate the effect of different bacteria and fungi on nodulation, nitrogenase activity and growth of faba bean.

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Materials and methods

Four bacterial and six fungal species were utilized for this experiment. Bacteria used in this work were *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Serratia marcescens* and *Rizobium leguminosarum* biovar *viceae* strain RCR 1001. Six fungal species were also used, *Aspergillus niger*, *A. terreus*, *A. quadriliniatus*, *Penicillium funiculosum*, *P. waksmanii* and *Phoma glomerata*. All bacteria except *Rhizobium* were grown for 2 d in nutrient broth and *Rhizobium* for 4 d in yeast extract mannitol. Also, fungi were mantained on Czapek's agar slant for 7 d prior utilization.

Plant growth: Symbiotic faba bean (*Vicia faba* L. cv. Giza 3) plants were planted in November, 1992 and grown in a wire proof greenhouse under natural light. Temperatures during the experiment averaged 22/6 °C (day/night). The pots (17 cm diameter, 22 cm depth) were filled with 4 kg sterilized clay soil (the sterilization was carried out by autoclaving the sand at 121 °C for 3 h). Five surface-sterilized seeds were planted into each pot. Pots were inoculated with 5 cm^3 of *Rhizobium leguminosarum* biovar *viceae* strain RCR 1001 in combination with 10 cm^3 of nutrient solution containing about 10^8 cells cm^{-3}, heat-killed cells or membrane sterilized culture filtrate of tested organisms, each was added separately. Pots used as controls were inoculated with 10 cm^3 of *Rhizobium* and equivalent volume of sterilized growth media. 7 d after emergence, seedlings were thinned to two per pot. Treatments were arranged in a randomized block design with three replicates for each. Plants were harvested 35 d after planting.

Analyses: Nitrogenase activity was determined on a detached root system as described previously (Abd-Alla 1992) using method of LaRue and Kurz (1973). Afterwards nodules of each individual root were counted and nodule fresh mass were measured. Dry masses of shoots and roots were estimated and N content of plant tissue were determined by Kjeldahl method.

Statistical analysis: Experimental data were subjected to analysis of variance.

Results and discussion

Two of four strains of tested viable bacteria or sterile culture filtrate and two of six species of fungi inoculated with *R. leguminosarum* biovar *viceae* strain RCR 1001 significantly increased nodules number and mass as well as absolute nitrogenase activity (Table 1). However, heat-killed microorganisms had no effect on nodule formation and nitrogenase activity. These differences in nodulation and nitrogenase activity resulted in significantly higher dry matter production and nitrogen accumulation (Table 2).

Other microorganisms have been shown to enhance emergence and early vigour of plants (Kloepper *et al.* 1988, Halverson and Handelsman 1991, Kumar and Dube 1992). One possible mechanism by which microflora may influence legume

Table 1. Stimulatory effect of viable or sterile culture filtrate on nodulation and nitrogenase activity (per plant) of *Vicia faba* inoculated with *R. leguminosarum* biovar *viceae* strain RCR 1001.

Species	Viable culture			Sterile culture filtrate			Heat-killed cells		
	Nodule/plant		Nitrogenase activity [nmol(C_2H_4)h^{-1}]	Nodule/plant		Nitrogenase activity [nmol(C_2H_4)h^{-1}]	Nodule/plant		Nitrogenase activity [nmol(C_2H_4)h^{-1}]
	Number	Fresh mass [g]		Number	Fresh mass [g]		Number	Fresh mass [g]	
Control	41	1.60	8.9	41	1.60	8.3	41	1.60	8.3
B. cereus	95	2.50	12.7	68	2.10	10.9	42	1.60	7.9
P. fluorescens	78	2.10	10.7	83	2.40	12.5	44	1.70	8.4
A. quadriliniatus	83	2.30	11.8	48	1.80	9.2	39	1.60	8.1
A. niger	115	2.95	15.3	95	2.50	13.1	43	1.60	8.5
L.S.D.									
P = 5 %	29	0.75	2.9	16	0.60	3.1	NS	NS	NS

NS = No significant difference

perfomance is by affecting the process of symbiotic nitrogen fixation (Burns *et al.* 1981, Poloneko *et al.* 1987).

Faba bean plants responded to alive inoculum and sterile culture filtrate but not heat-killed cells. Rovira (1963) reported an increase in wheat yield due to inoculation with alive or heat-killed *Bacillus*, but effects due to the latter treatment were not statistically significant. Autoclaved microorganisms did not elicit a response in our experiment which suggests that microbal metabolism is required for the growth response to occur. Alternatively, if a growth promoting compound is associated with microbial cells, it may be heat-labile and destroyed by autoclaving.

Table 2. Stimulatory effect of viable or sterile culture filtrate on dry mass and total nitrogen accumulation of *Vicia faba.*

Species	Viable culture				Sterile culture filtrate			
	Dry mass [g plant^{-1}]		Total nitrogen [mg plant^{-1}]		Dry mass [g plant^{-1}]		Total nitrogen [mg plant^{-1}]	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	2.80	0.50	97.3	12.3	2.8	0.50	97.2	12.3
B. cereus	3.60	0.90	126.2	26.1	3.2	0.50	107.5	15.3
P. fluorescens	3.10	0.90	113.1	25.6	4.1	0.70	154.8	18.3
A. quadriliniatus	3.70	1.10	142.9	36.4	4.1	0.60	168.9	15.2
A. niger	3.80	0.90	136.6	30.0	6.0	1.10	285.3	33.2
L.S.D. P = 5 %	0.65	0.20	19.0	8.2	1.3	0.26	28.0	9.1

The positive effect of sterile culture filtrate in these experiments indicated that microbial production of plant-growth regulating substance(s) may be responsible for the observed effects. Several workers have demonstrated the production of phytohormones by rhizosphere microflora including *Bacillus* (Barbieri *et al.* 1986, Holl *et al.* 1988). The suppression of rhizosphere deleterious microbes (Suslow and Schroth 1982) is also an unlikely explanation of our results because growth promotion was observed under sterile conditions. Many investigators have emphasized the fact that plant growth-promoting microbes must be vigorous root colonizers in order to elicit a growth response (Schroth and Weinhold 1986). Reddy and Rahe (1989) reported that the growth promotion of onion by *Bacillus subtilis* was not correlated with inoculum survival in the rhizosphere. Their data suggest that bacterial activity in the rhizosphere may be more important than population size in affecting plant growth promotion.

The growth response of faba bean may be attributed to phytohormonal or nutritional relationships between these microorganisms and plants. These relationships led to a healthier plant with more sites for nodulation and nitrogen fixation.

There is a scope for screening potential plant growth promoting microbes would be of value in the development of agricultural inoculants.

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