

RHIZOSPHERE COMPETENT *MESORHIZOBIUM LOTI* MP6 INDUCES ROOT HAIR CURLING, INHIBITS *SCLEROTINIA SCLEROTIURUM* AND ENHANCES GROWTH OF INDIAN MUSTARD (*BRASSICA CAMPESTRIS*)

Shikha Chandra; Kamlesh Choure; Ramesh C. Dubey; Dinesh K. Maheshwari*

Department of Botany and Microbiology, Gurukul Kangri University, Haridwar-249404, India

Submitted: December 03, 2005; Returned to authors for corrections: September 21, 2006; Approved: January 18, 2007

ABSTRACT

The bacterial strain *Mesorhizobium loti* MP6, isolated from root nodules of *Mimosa pudica* induced growth and yield of *Brassica campestris*. The isolate MP6 secreted hydroxamate type siderophore in Chrom-Azuril Siderophore (CAS) agar medium. Production of hydrocyanic acid (HCN), indole acetic acid (IAA) and phosphate solubilizing ability was also recorded under normal growth conditions. Root hair curling was observed through simple glass-slide technique. *In vitro* study showed a significant increase in population of *M. loti* MP6 in rhizosphere due to root exudates of *B. campestris*. In dual culture technique the strain showed a strong antagonistic effect against *Sclerotinia sclerotiorum*, a white rot pathogen of *Brassica campestris*. The growth of *S. sclerotiorum* was inhibited by 75% after prolonged incubation. Efficient root colonization of mustard seedlings was confirmed by using a streptomycin-resistant marker *M. loti* MP6^{strep+}. The *M. loti* MP6 coated seeds proved enhanced seed germination, early vegetative growth and grain yield as compared to control. Also, a drastic decline (99%) in the incidence of white rot was observed due to application of *M. loti* MP6.

Key-words: Biocontrol, Plant growth promoting rhizobacteria, Seed bacterization, white rot, *Mesorhizobium loti*, *Brassica campestris*

INTRODUCTION

Rhizobia form root nodules by infecting a wide range of legumes (35) and limited number of non-legumes in mutual interaction (36). Attraction and attachment of rhizobia to the tips of root hairs resulting in curling or deformation are the first step of infection leading to symbiosis. Recently, rhizobia have been reported as plant growth-promoting rhizobacteria (PGPR) both with legumes and non-legumes. PGPR, directly and indirectly, promote plant growth by production of phytohormones, biocontrol of phytopathogens and/or improvement of nutritional status of plant (14).

Curling of root hairs induced by rhizobia in non-leguminous plants is a rare phenomenon. Usually curling occurs only when infection is caused by compatible rhizobia, which are correlated with initial steps of root nodule formation (30). Scanning of literature indicates that symbiotic root nodule bacteria multiply

and survive in the rhizosphere of non-legumes also under field conditions (19) colonizing their rhizosphere (2,5). Effect of phytohormones on root hairs of non-legumes becomes unavoidable. Trinick and Habdobas (34) documented the inability of rhizobial strains to colonize roots of some plants, such as Brassicaceae and clover, due releasing of toxic compounds (glucosinolates, isothiocyanates, etc.) present in root exudates. Besides, direct growth promotion of different non-legumes by rhizobia has earlier been reported (19). Chabot *et al.* (8) reported the significant increase in dry matter yield of shoot and total phosphorus content by using a phosphate solubilizing strain of *R. leguminosarum* bv. *phaseoli* in field trials with maize and lettuce. Such enhancement of yield cannot be ruled out due to the production of indole acetic acid (IAA) and/or biocontrol of plant pathogens (11) involving the rhizobacteria-mediated characteristics of antibiotics, hydrocyanic acid (HCN) and siderophores production.

*Corresponding Author. Mailing address: Department of Botany and Microbiology, Gurukul Kangri University, Haridwar 249404 ((Uttaranchal) - India. Tel.: (91) 1334-246767. E-mail: maheshwari@indiatimes.com

The present study was aimed at to investigate the effect of plant growth promoting strain of *Mesorhizobium loti* MP6 on root hair curling, enhancement of plant growth and yield of Indian mustard (*Brassica campestris*) and its role as a potential biocontrol agent against a fungal pathogen, *Sclerotinia sclerotiorum*.

MATERIALS AND METHODS

Bacterial strain

Bacterial strain MP6 was isolated from root nodule of *Mimosa pudica* was characterized morphologically, biochemically and physiologically according to Holt *et al.* (20) and identified as *Mesorhizobium loti* (9). *M. loti* MP6 was found most promising based on its antagonistic activity against *Sclerotinia sclerotiorum*, secondary metabolites production viz., siderophore, indole acetic acid (IAA), hydrocyanic acid (HCN) and phosphate solubilization and intrinsic antibiotic resistance towards certain antibiotics (9). The isolate was maintained on yeast extract mannitol agar (YEMA) (36) at 4°C and deposited in the Departmental Culture Collection, Department of Botany and Microbiology, Gurukul Kangri University, Haridwar (India).

Siderophore assay

Siderophore production by the strain MP6 was estimated qualitatively on a universal medium Chrom-Azuroil Siderophore (CAS) agar medium (30). Isolate MP6 was spotted on CAS agar medium and plates were incubated 28±1°C for 48 h. Type of siderophore was detected following the method of Neilands (27). The bacterial culture was grown in YEM broth for 48 h and contents were centrifuged at 7,100 g for 15 min at 4°C. One ml of culture supernatant was added to 1 ml of 1mM FeCl₃ and siderophore activity was determined by the method of Gibson and Magrath (13).

Indole acetic acid (IAA) production

Exponentially grown culture of bacterial strain of MP6 was inoculated in 5 ml YEM broth and incubated at 28±1°C for 48 h. The broth was taken to centrifugation at 7,100 g for 15 min at 4°C. The supernatant was collected and passed through 0.2 µm Millipore filter membrane. One ml of *O*-phosphoric acid was added to 2 ml of bacterial supernatant and allowed to stand for 1 h to develop the colour. For quantitative measurement of IAA, one ml of cell-free culture filtrate (CF) was mixed vigorously with 4 ml of Salkowsky's reagent and kept at room temperature for 20 min. Optical density was measured spectrophotometrically at 535 nm. The concentration of IAA in each sample was determined from the standard curve of IAA.

Hydrocyanic acid (HCN) production

Production of HCN was determined by modified method of Miller and Higgins (26). Bacterial culture (48 h) was streaked on

YEMA amended with glycine (4.4 gl⁻¹) with simultaneous addition of a filter paper soaked in 0.5% (w/v) picric acid in 1% Na₂CO₃ placed in the upper lid of the Petri plate. After incubation at 28±1°C, changes in color were examined. On the other hand, plates devoid of inoculum served as control.

Phosphate Solubilization

Pikovaskya (PKV) agar plates (28) were spot inoculated with a loopful of culture of isolate MP6. After incubation at 28±1°C for 5 days, formation of a clear zone around the spot was recorded. Quantitative estimation of phosphate solubilization was carried out according to Gupta *et al.* (17).

Plant Responses to *M. loti* MP6 *in vitro*

Utilization of root exudates

Seeds of *Brassica campestris* were washed with the sterile distilled water followed by surface sterilized with 0.1% mercuric chloride (HgCl₂) for 2-3 minutes and washed for at least 6 times with sterile distilled water so as to remove the traces of HgCl₂. Sterility was checked by putting seeds on nutrient agar medium (NAM). Surface sterilized seeds of similar shape and size were inoculated on moistened filter paper in sterile Petri plates and incubated at 24±1°C for 72 h (36). Radicles of *B. campestris* seeds were aseptically placed in culture tubes containing Knop's solution and incubated at 25±1°C for 5 days (tubes were covered with black mesh) to obtain root exudates. Root exudates of contamination-free tube were pooled and mixed in 35 ml YEM broth at 15% (v/v) and inoculated with 0.1ml of exponential phase culture of *M. loti* MP6, incubated at 28± 1°C on gyrotory shaker at 150 rpm for 48 h. Aliquots of serially diluted culture were plated onto YEMA and colonies were enumerated after 48 h incubation at 28± 1°C (15).

Assay of bacterial attachment of plant roots

A drop of 0.2 ml of 0.3-0.4% agar (w/v) was placed on a sterile glass slide (70 mm x 20 mm). Surface sterilized seeds of *B. campestris* with emerging roots (5mm) were placed onto it and inoculated with 0.1 ml of exponential phase culture. Thereafter, it was covered aseptically with cover slip and transferred to culture tubes (150 mm x 25 mm) containing 25 ml of N-free medium. After incubation at 25°C for 6 days, it was observed microscopically (12).

Isolation of *Sclerotinia sclerotiorum*

The fungal pathogen, *Sclerotinia sclerotiorum* causing white rot in *B. campestris* was isolated from infected roots of *B. campestris* following blotter technique (10). Sections of diseased root (tissues) are incubated on moist filter paper at 20°C. After several days hyphae transferred to potato dextrose agar (PDA) and incubated at room temperature. The pathogen was identified in the laboratory by observation of the ascoma which is apothecioid in shape and yellow-brown to tan in color. Sclerotia

formed by this fungus are large, irregular in shape, and dark-brown to black in color. This fungus can be partially identified on plant material by the production of a white fluffy mycelium. The pathogen was maintained on PDA at 4°C for further studies.

Antagonism *in vitro*

Antagonistic properties of *M. loti* MP6 against *S. sclerotiorum* was tested on YEMA plates using a dual culture technique (32). Five day old mycelial discs (5 mm diameter) were placed in four corners of the modified YEMA (addition of 2% sucrose) plates. Exponentially grown culture of MP6 was spotted 2 cm juxtaposed from the fungal disc and incubated at 28±1°C for 5 days. Growth inhibition was calculated by measuring the distance between the edge of bacterial and fungal colonies as compared to control (without bacterium). The zone of inhibition was recorded by formula: Inhibition (%) = (C) - (T) / (C) × 100, where, C is radial growth in dual culture and T is radial growth in dual culture.

Preparation of *S. sclerotiorum* inoculum

Inoculum of *S. sclerotiorum* was prepared by growing the pathogen on sterile oat (*Avena sativa*) grains *in vitro*. The oat grains containing mycelial fragments plus sclerotia served as inoculum and were added to the sandy loam soil to get 10⁴ fungal propagules g⁻¹ before seed sowing.

Seed bacterization and field experiment

Seeds of *B. campestris* were bacterized according to Weller and Cook (38). Liquid culture of MP6 was grown on YEM broth at 120 rpm at 28°C for 48 h and centrifuged at 7,100 g at 4°C for 15 min to get the pellet. The culture supernatant was discarded and pellets were washed with sterile distilled water and resuspended in distilled water to obtain a population density of 2.1×10⁸ CFU ml⁻¹. This suspension was mixed with 1% carboxymethylcellulose (CMC) to form slurry which was coated on to presterilized seeds of *B. campestris* and dried overnight aseptically for curing. Care was taken to avoid clumping of the seeds. Bacterized and non-bacterized seeds were sown in sandy loam soil (62.3% sand, 13.9% silt, 14.6% clay, 0.349% total organic matter, pH 6.3 and 34% water holding capacity) in four sets of treatments: (i) soil sowed with bacterized seeds, (ii) soil infested with *S. sclerotiorum* and with sowed MP6-bacterized seeds (iii) soil infested with *S. sclerotiorum* (5 g oat seeds mixed properly), and (iv) soil sowed with non-bacterized seeds (control).

The experiment was conducted using a randomized plot design with three replicates of each treatment. Each plot size was 16 m² with 12 rows and row to row distance of 30 cm. 20 seeds were sown in each row of every plot. For evaluation of disease incidence (%) following formula was used: Disease incidence (%) = (T_N) / (T_E) × 100, where, T_N is total numbers of diseased seedlings and T_E is total numbers of emerged

seedlings. The plots were irrigated routinely. Seed germination (%) was noted on the 15th day after sowing (DAS). After 120 days of sowing, plant growth and grain yield were recorded. The data were analyzed statistically by using analysis of variance (ANOVA) to find out significance at 1% and 5% level of LSD (least significant difference).

Rhizosphere colonization

For root colonization, antibiotic marker strain resistance toward streptomycin (100 µg ml⁻¹) of *M. loti*^{Strep+} MP6 was used. *B. campestris* plants, bacterized with strains were sampled after 30, 60, 90 and 120 DAS and bacterial population on the roots were measured. The endophytic bacterial populations on the roots were analyzed. The roots were cut into 1cm long segments and one g of root segment was dipped in 99 ml of sterile 10 mM potassium phosphate buffer (pH 7.0) and vortex 4-5 times to release the rhizosphere bacteria. A dilution of the above suspension was poured into Petri plates containing YEM agar amended with streptomycin (100 mg ml⁻¹) to screen out the population of *M. loti*^{Strep+}. After 24 h of incubation at 28±1°C, cfu g⁻¹ root segment was counted. Population dynamics of *M. loti*^{Strep+} was recorded after 30, 60, 90 and 120 DAS. Aerobic bacteria population was measured without amendment of streptomycin into medium.

RESULTS

Screening for siderophore production

Culture filtrate of MP6 showed a major peak at 400 nm which revealed production of a hydroxamate type siderophore. The maximum production of siderophore (32 µg ml⁻¹) was recorded after 48 h of incubation.

Indole acetic acid (IAA) and HCN production

IAA production was observed as evidenced by the production of a pink coloured product. Maximum production of IAA (24 µg ml⁻¹) was recorded after 48 h incubation. Strong HCN production was recorded by the bacterial strain *M. loti* MP6 as evidenced by change in colour of filter paper from yellow to reddish-brown after 2-3 days of inoculation.

Phosphate Solubilization

Formation of clear zone around bacterial colony on Pikovaskya agar medium showed phosphate solubilization by *M. loti* MP6 strain and maximum activity (45 µg ml⁻¹) was recorded after 48 h incubation.

Antagonism *in vitro*

M. loti MP6 strongly inhibited the growth of *S. sclerotiorum* on PDA plates at 28±1°C. The growth inhibition (~75%) of *S. sclerotiorum* was recorded maximally after 120 days incubation (Fig. 1).

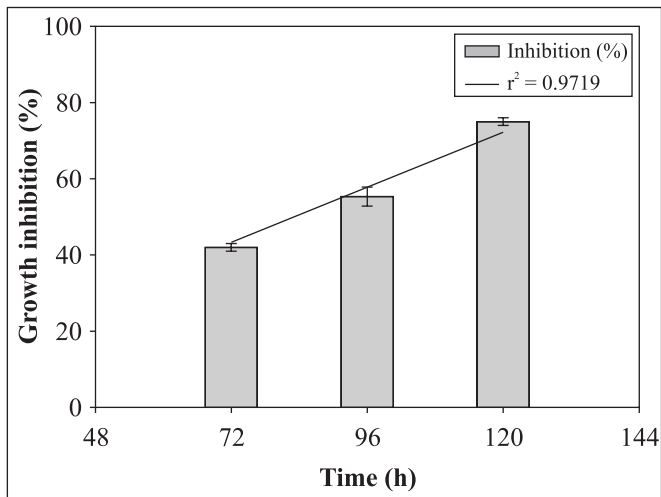


Figure 1. *In vitro* growth inhibition of *Sclerotinia sclerotiorum* by *M. loti* MP6.

Utilization of root exudates and assay of bacterial attachment to roots

A significant increase in colony forming units (CFU) of *M. loti* MP6 was recorded in the YEMA medium supplemented with root exudates (Table 1). The abundant population in root zone was observed which proved utilization of root exudates by *M. loti* MP6. Bacterial attachment to plant roots demonstrated root hair curling of *B. campestris* (Fig. 2) and enhanced early plant growth parameters and nitrogen content of plant (Table 2).

Rhizosphere colonization

The antibiotic marker strain *M. loti* MP6^{Strep+} showed positive root colonization (Table 3). The bacterial population in the root zone was increased tremendously after 30 DAS, thereafter, it maintained more or less a stationary population (Table 3). Strong

Table 1. Growth of *Mesorhizobium loti* MP6 in yeast extract mannitol broth containing root exudates of *Brassica campestris*.

Medium	Log colony forming units after incubation at 28±1°C	
	0 h	48 h
Yeast extract mannitol broth	1.4±0.3	5.4 ^a ±0.5
Root exudates	1.4±0.8	4.8 ^a ±0.7
Yeast extract mannitol broth + Root exudates	1.4±0.4	5.8 ^b ±0.2

Values in each column are the mean of triplets; ±, standard deviation. Means followed by letters (a, b) are significantly different (P<0.05) according to Fischer-test.

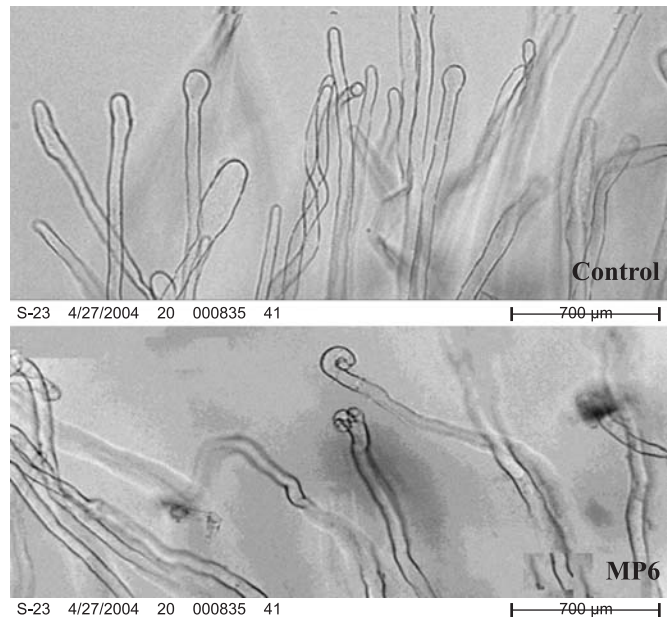


Figure 2. Light photomicrograph of curling of root hairs of *Brassica campestris* after exposure to *Mesorhizobium loti* MP6 in comparison to control (un-inoculated), where no such curling in root hairs was observed.

Table 2. Dry weight and nitrogen content of *Brassica campestris* grown on nitrogen free media with or without *Mesorhizobium loti* MP6 inoculation.

Treatment	Plant shoot length (mm)	Plant dry weight (mg)	Nitrogen content (N%)
With inoculation	220 ^a ±0.5	3.2 ^a ±0.15	1.8 ^a ±0.07
Without inoculation (Control)	70 ^b ±0.7	1.1 ^b ±0.08	0.5 ^b ±0.09

Means in each column followed by letters (a, b) are significantly different (P<0.05) according to Fischer-test.

colonization ability of MP6 lies in it being the successful efficient colonizer both on the mustard seed surface as well as in the rhizosphere.

Field experiment

Seed bacterization of *B. campestris* with MP6 significantly (5% level of LSD) induced seed germination (Table 4), early vegetative growth and late reproductive growth as compared to *S. sclerotiorum*-infested soil (Table 5). Seeds bacterized with *M. loti* MP6 showed 71% seed germination as compared to *S. sclerotiorum*-infested soil (Table 4). The protection of root

Table 3. Population of *Mesorhizobium loti* MP6^{Strep+} and general aerobacteria (AB) in rhizosphere of *Brassica campestris*.

Treatment	Population of <i>M. loti</i> MP6 in rhizosphere of mustard (CFU/g root segments)							
	30 DAS		60 DAS		90 DAS		120 DAS	
	MP6	AB	MP6	AB	MP6	AB	MP6	AB
<i>M. loti</i> MP6	5.8×10 ^{4*}	4.3×10 ³	6.6×10 ^{4*}	4.1×10 ³	6.5×10 ^{4*}	3.9×10 ³	6.5×10 ^{4*}	3.8×10 ³
<i>M. loti</i> MP6+	5.9×10 ^{4*}	4.2×10 ³	3.8×10 ^{4*}	3.0×10 ³	7.0×10 ^{4*}	3.8×10 ³	6.9×10 ^{4*}	3.7×10 ³
<i>S. sclerotiorum</i>								

Values are the mean of 3 replicates randomly selected in each set.; *, Significant at 5% level of LSD (ANOVA); MP6, Total population of *M. loti* MP6^{Strep+}; AB, Total population of aerobic soil bacteria.

Table 4. Effect of seed bacterization with *Mesorhizobium loti* MP6 on seed germination (%) and disease incidence of white rot (%) of *Brassica campestris*.

Treatment	Seed Germination (15 days after sowing)	White rot (%) 90 days after sowing
<i>M. loti</i> MP6	72*	1*
<i>M. loti</i> MP6+	70*	1.2*
<i>S. sclerotiorum</i>	42 ^{ns}	99 ^{ns}
Control	54	13
Cd at 1%	1.49	
±SEM	0.33	

Values are the mean of 3 replicates randomly selected in each set.; *, significant at 5% level of LSD (ANOVA); CD, Critical difference; SEM, Standard error means.

against *S. sclerotiorum* is an added advantage due to application of rhizosphere competent *M. loti* MP6 and resulting in enhanced yield of mustard (Table 5). In *S. sclerotiorum*-infested soil, plants showed clear white rot symptoms. A drastic decline (99%) in the incidence of white rot was found where the soil was inoculated with MP6-*S. sclerotiorum* followed by MP6 alone

(Table 4). In control, seed germination was 28% higher than *S. sclerotiorum*-infested soil (Table 4). The bacterized seeds in *S. sclerotiorum*-infested soil showed early germination as compared to control. Grain yield also increased by 52% in case of bacterization of seeds, which was significant at 5% level of LSD as compared to control (untreated) (Table 5). *M. loti* MP6-coated seeds resulted in 53% increased grain yield in *S. sclerotiorum*-infested soil as compared to control (untreated) (Table 5).

DISCUSSION

Few rhizobia are known to produce certain metabolites such as antibiotics (7, 21), siderophores (3) and HCN (23) which play a significant role in rhizosphere affecting the growth and activity of the other microbes and plant health as well. Based on results, *M. loti* MP6 produces hydroxamate type of siderophore. Siderophore-mediated competition in reduction of disease incidence results in the exclusion of pathogen in rhizosphere, due to lack of iron required for sclerotia germination and hyphal growth. Due to IAA producing ability of MP6, it is effective for promoting the plant growth. Earlier, 58% symbiotic nitrogen fixing rhizobia were reported to produce IAA (2) that has been implicated in plant growth promotion (17). HCN produced by

Table 5. Effect of seed bacterization with *M. loti* MP6 on growth and grain yield of *Brassica campestris*.

Test organism	Vegetative parameters				Grain Yield		
	Shot length	root Length	Fresh shoot weight	Fresh root weight	Pods plant ⁻¹	Grain yield plant ⁻¹ (gm)	Grain yield (Kg h ⁻¹)
<i>M. loti</i> MP6	180*	14*	82*	75*	140*	70*	840*
<i>M. loti</i> MP6 + <i>S. sclerotiorum</i>	178*	13*	80*	73*	139*	69.2*	846*
<i>S. sclerotiorum</i>	90	11	39	58	60 ^{ns}	35 ^{ns}	352 ^{ns}
Control	75 ^{ns}	6 ^{ns}	20 ^{ns}	40 ^{ns}	82	50	552

Values are the mean of 3 replicates randomly selected in each set.; *Significant at 5% level of LSD as compared to control. (ANOVA); ns, non-significant at 5% level of LSD. Length (cm), weight (mg), h (hectare) .

MP6 limited the growth of *S. sclerotiorum*. Earlier, Bhatia *et al.* (4) reported that volatile HCN exhibited inhibition of sclerotia germination of *Macrophomina phaseolina*. The mode of action of HCN in disease control is partially understood (18). Our results show that MP6 has potential to solubilize inorganic phosphates. Several rhizobia can solubilize inorganic phosphate (3). Many rhizospheric bacteria have ability to solubilize inorganic phosphate from soil. Such bacteria improved solubilization of unavailable soil phosphate accounting high efficiency of phosphorus use. *In vitro* results of growth inhibition of *S. sclerotiorum* may be attributed to the antagonistic properties shown by MP6. Microscopic examination showed the unfolding, abnormal intercalary swelling, tip deformation, degeneration of cytoplasm and lysis of hyphae of fungal pathogen *S. sclerotiorum* during interaction with rhizobia (11). A similar observation has also been made by Gupta *et al.* (17) by using fluorescent pseudomonads.

Population of MP6 got increased in the rhizosphere which reflects its potentiality to utilize the root exudates as energy source. Root exudates such as free amino acids, proteins, carbohydrates, alcohols, vitamins or hormones are the important sources of nutrients for the microorganisms present in the rhizosphere and participate in colonization process through chemotaxis of soil microorganisms (25). These features of MP6 facilitate to colonize the rhizosphere. A strong correlation between root exudation and ability to reach high growth rates on exudates have also been demonstrated by Kuiper *et al.* (24).

As observed in the present study the root hair curling of *B. campestris*, various workers (31, 33) have also observed the involvement of rhizobia in root hair curling of rice, oat, asparagus and wheat. This supports the hypothesis of attachment of rhizobia to the root hairs and proves the process of root hair curling as non-host specific process (6). No evidence of infection thread or formation of nodules in *B. campestris* was observed. Role of a large amount of toxic gluconasturtiin present inside the plant tissues on thread can not be ruled out (34). *Rhizobium* requires the proper recognition of nutrients present in root exudates that allow rhizobia to colonize the rhizosphere of the non-legume and to curl the root hairs which is the prerequisite of symbiosis. Our data support that non-legumes too react with rhizosphere rhizobia but unlike *Brassica napus*, rice, etc. nodule-like structures on roots were not observed (1, 34).

Results of field experiments have proved that MP6 significantly (5% level of LSD) induced seed germination, early vegetative and late reproductive growth (Table 5). Involvement of rhizobia in enhancement of plant growth and suppression of soil-borne fungi has earlier been reported (22, 16, and 3). Since MP6 has an antagonistic nature, its relative close adherence gives the protection during fungal infection that helps in establishing and resisting against preexisting deleterious microorganisms occupying the microbial niche in the rhizosphere. MP6 being a siderophore producing rhizosphere-

competent bacterial strain, survives in soil, its PGPR traits help the enhanced plant growth and yield of *B. campestris*. It may be concluded that *M. loti* MP6 strain not only controlled *S. sclerotiorum* but also induced plant growth and yield of Indian mustard (*B. campestris*).

ACKNOWLEDGEMENTS

The financial supports from Technology Mission on Oil seeds, Pulses & Maize and Council of Scientific & Industrial Research, New Delhi are gratefully acknowledged.

RESUMO

***Mesorhizobium loti* MP6 rizosférico competente induz encurvamento do pelo da raiz, inibe *Sclerotinia sclerotiorum* e estimula o crescimento de mostarda indiana (*Brassica campestris*)**

A cepa bacteriana *Mesorhizobium loti* MP6 isolada de nódulos de raiz de *Mimosa pudica* induziu o crescimento e o rendimento de *Brassica campestris*. A cepa MP6 secretou sideróforo do tipo hidroxamato em meio sólido Chrom-Azurol Siderophore (CAS). Em condições normais de crescimento, a cepa foi também capaz de produzir de ácido cianídrico (HCN) e ácido indolacético (AIA) e solubilizar fosfato. O encurvamento do pelo da raiz foi observado usando a simples técnica de lâmina e lamínula. Estudos *in vitro* mostraram um aumento significativo na população de *M. loti* MP6 na rizosfera devido aos exsudatos de *B. campestris*. Empregando-se técnica de co-cultura, a cepa mostrou um grande efeito antagônico contra o fungo *Sclerotinia sclerotiorum*, o patógeno da podridão branca de *Brassica campestris*. Após incubação prolongada, o crescimento de *S. sclerotiorum* foi inibido em 75%. Uma eficiente colonização de sementes de mostarda foi confirmada pelo emprego da linhagem *M. loti* MP6strep+ que contém um marcador de resistência à estreptomicina. As sementes cobertas com *M. loti* MP6 apresentaram aumento da sua germinação, crescimento vegetativo rápido e melhor rendimento quando comparadas ao controle. Além disso, foi observado um drástico declínio na incidência da podridão branca decorrente da aplicação de *M. loti* MP6.

Palavras-chave: Biocontrole, rizobactérias promotoras de crescimento, colonização de sementes, podridão branca, *Mesorhizobium loti*, *Brassica campestris*

REFERENCES

1. Al-Mallah, M.K.; Davey, M.R.; Cocking, E.C. (1990). Enzyme treatment, PEG, biotin and nodule stimulation in white clover by *Rhizobium trifolii*. *J. Plant. Physiol.*, 15, 225-258.

2. Antoun, H.; Beaucham, C.J.; Goussard, D.N.; Chabot, R.; Lalande, R. (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth-promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil*, 204, 57-67.
3. Arora, N.K.; Kang, S.C.; Maheshwari, D.K. (2001). Isolation of siderophore- producing strain of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Curr. Sci.*, 81, 673-677.
4. Bhatia, S.; Bhatia, S.; Dubey, R.C.; Maheshwari, D.K. (2003). Antagonistic effect of fluorescent pseudomonas against *Macrophomina phaseolina* that causes charcoal rot of ground nut. *Ind. J. Exp. Biol.*, 41, 1442-1446.
5. Biswas, J.C.; Ladha, J.K.; Dazzo, F.B. (2000). Rhizobial inoculation improves nutrient uptake and growth of lowland rice. *Sci. Soc. Am. J.*, 64, 1344-1650.
6. Bodenoch-Jones, J.; Flander, D.J.; Rolfe, B.G. (1985). Association of *Rhizobium* strains with roots of *Trifolium repens*. *Appl. Environ. Microbiol.*, 49, 1511-1520.
7. Breil, B.T.; Borneman, J.; Triplett, E.W. (1996). A newly discovered gene *ftuA*, involved in the production of the ribosomally synthesized peptide antibiotic trifoliotoxin. *J. Bacteriol.*, 178, 4150-4157.
8. Chabot, R.; Antoun, H.; Cescas, M.P. (1996). Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. *Plant Soil*, 184, 311-321.
9. Chandra, S. (2004). *Impact of rhizobia and chemical nutrients status on productivity of non-leguminous crop (Brassica campestris* L. var. local). Uttaranchal, India. (Ph.D. Thesis, Gurukul Kangri University, Haridwar).
10. de Tempe, J. (1963). The blotter method for seed health testing. *Proc. Int. Seed. Test. Assoc.*, 28, 1933.
11. Deshwal, V.K.; Pandey, P.; Kang, K.C.; Maheshwari, D.K. (2003). Rhizobia as biological control agent against soil borne plant pathogenic fungi. *Ind. J. Exp. Biol.*, 41, 1160-1164.
12. Fahraeus, G. (1957). The infection of clover root hairs by nodule bacteria studied by a simple glass slide technique. *J. Gen. Microbiol.*, 16, 374-381.
13. Gibson, F.; Magrath, D.E. (1969). The isolation and characterization of hydroxamic acid (aerobactin) formed by *Aerobacter aerogenes*. *Biochem. Biophys. Acta.*, 192, 175-184.
14. Glick, B.R. (1995). The enhancement of plant growth by free living bacteria. *Can. J. Microbiol.*, 47, 109-117.
15. Gu, Y.H.; Mazzola, M. (2001). Impact of carbon starvation on stress resistance, survival in soil habitats and biocontrol ability of *Pseudomonas putida* strain 2C8. *Soil. Biol. Biochem.*, 33, 1155-1162.
16. Gupta, C.P.; Dubey, R.C.; Maheshwari, D.K. (2001). Antibiosis-mediated necrotrophic effect of *Pseudomonas* GRC₂ against two fungal pathogens. *Curr. Sci.*, 81, 90-94.
17. Gupta, C.P.; Dubey, R.C.; Maheshwari, D.K. (2002). Plant growth enhancement and suppression of *Macrophomina phaseolina* causing charcoal rot of peanut by fluorescent *Pseudomonas*. *Biol. Fertil. Soils.*, 35, 399-405.
18. Hass, D.; Befago, G. (2005). Biological control of soil-borne pathogen by fluorescent pseudomonads. *Nat. Rev. Microbiol.*, 10, 1038, 1129-141.
19. Hoflich, G.; Wiehe, W.; Buchholz, C.H. (1995). Rhizosphere colonization of different crops with growth promoting *Pseudomonas* and *Rhizobium* bacteria. *Microbiol. Res.*, 150, 139-147.
20. Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T.; Williams, S.T. (1994). *Bergey's manual of determinative bacteriology*. Williams and Wilkins, London, p.151-157.
21. Kang, J.G.; Shin, S.Y.; Kim, M.J.; Bajpai, V.; Maheshwari, D.K. (2004). Isolation and antifungal activities of 2-hydroxymethylchroman 4-one produced by *Burkholderia* sp. MSSP. *J. antibiot.*, 5, 726-731.
22. Kloepper, J.W.; Schroth, M.N. (1981). Relationship of *in vitro* antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathol.*, 71, 1020-1024.
23. Knowles, C.J. (1976). Microorganisms and Cyanide. *Bacteriol. Rev.*, 40, 652-680.
24. Kuiper, I.; Kravchenko, L.V.; Bloemberg, G.V.; Lutenberg, B.J. (2002). *Pseudomonas putida* strain PCL 1444, selected for efficient root colonization and naphthalene degradation effectively utilizes root exudates components. *Mol. Plant. Microbe. Interact.*, 15, 734-741.
25. Lynch, J.M.; Whipps, J.M. (1990). Substrate flow in the rhizosphere. *Plant Soil*, 129, 1-10.
26. Miller, R.L.; Higgins, V.J. (1970). Association of cyanide with infection of birdfoot trefoil by *Stemphylium loti*. *Phytopathol.*, 60, 104-110.
27. Neilands, J.B. (1981). Microbial iron compounds. *Annul. Rev. Biochem.*, 50: 715-731.
28. Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiol.*, 17, 362-370.
29. Planzinsky, J.; Innes, R.W.; Rolf, B.G. (1985). Expression of *Rhizobium trifolii* early nodulation genes on maize and rice plants. *J. Bacteriol.*, 163, 612-815.
30. Schwyn, B.; Neilands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, 160: 47-56.
31. Shimshick, E.J.; Hebert, R.R. (1979). Binding characteristics of N₂ fixing bacteria to cereal roots. *Appl. Environ. Microbiol.*, 38, 447-453.
32. Skidmore, A.M.; Dickinson, C.H. (1976). Colony interaction and hyphal interference between *Sartoria nodorum* and phylloplane fungi. *Trans. Brit. Mycol. Soc.*, 57-64.
33. Terouchi, N.; Syono, K. (1990). *Rhizobium* attachment and curling in asparagus, rice and oat plants. *Plant. Cell. Physiol.*, 31, 119-127.
34. Trinick, M.J.; Habdobas, P.A. (1995). Formation of nodular structures on the non-legumes *Brassica napus*, *B. campestris*, *B. Juncea* and *Arabidopsis thaliana* with *Bradyrhizobium* and *Rhizobium* isolated from *Parasponia* spp. on legumes grown in tropical soils. *Plant Soil*, 172, 207-219.
35. Vincent, J.M. (1970). A Manual for the Practical Study of the Root Nodule Bacteria. IPB Handbook No. 15, Blackwell Scientific Publication, Oxford.
36. Weller, D.M.; Cook, R.J. (1983). Suppression of take-all the wheat by seed treatment with fluorescent pseudomonads. *Phytopathol.*, 23, 23-54.