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Bacteria from the rhizosphere and roots of *Brassica napus* influence its root growth promotion by *Phyllobacterium brassicacearum*

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Abstract.- *Phyllobacterium brassicacearum* is a plant growth promoting bacterium (PGPB) that stimulates *Brassica napus* root morphogenesis in absence of bacterial competition. We evaluated in which extent indigenous bacteria associated with *B. napus* roots and rhizosphere may interfere with the plant growth promoting ability of *P. brassicacearum.* Thus, bacteria from *B. napus* roots and rhizosphere were isolated and co-inoculated with the PGPB at different proportions. The root growth promoting ability of *P. brassicacearum* was impaired only when it was co-inoculated in low proportion together with the indigenous bacteria. This was not linked to a decrease in its population size on roots. Hence, this study highlights that the efficacy of a PGPB is not primarily dependent on the extent of its root colonization but also depends on its initial size compared to the indigenous bacteria.

Key words **:** *Brassica napus* - *Phyllobacterium brassicacearum* - plant growth promoting bacteria (PGPB).

Résumé.- *Phyllobacterium brassicacearum* est une bactérie favorisant la croissance des plantes (BFCP) qui stimule la morphogenèse racinaire chez *Brassica napus* en absence de compétition bactérienne. Nous avons évalué l'impact des bactéries indigènes associées aux racines et à la rhizosphère de *B. napus* sur la capacité de *P. brassicacearum* à promouvoir la croissance des plantes. Pour cela, des bactéries associées aux racines et à la rhizosphère de *B. napus* ont été isolées et co-inoculées avec la BFCP à différentes proportions. La capacité de *P. brassicacearum* à favoriser la croissance racinaire a été altérée seulement lorsqu'il a été inoculé en proportion minoritaire avec les bactéries indigènes. Cela n'était pas lié à une diminution de sa population sur les racines. Ainsi, cette étude illustre que l'efficacité d'une PGPB ne repose pas seulement sur son potentiel de colonisation racinaire, mais dépend aussi de sa représentativité par rapport aux bactéries indigènes.

Mots clés **:** *Brassica napus* - *Phyllobacterium brassicacearum* - bactéries favorisant la croissance des plantes (BFCP).

I. INTRODUCTION

Plant growth promoting bacteria (PGPB) used for crop inoculation hold great promise to reduce chemical fertilizers in agriculture (Bashan & Holguin, 1998; Glick, 1995). Living in the rhizosphere, on or inside roots, the former stimulate plant growth through direct mechanisms that can affect root and shoot growth in a positive way (Kloepper & Beauchamp, 1992; Ryu *et al*., 2003). Thus, PGPB may release key hormones for plant development such as indole acetic acid (IAA), gibberellins and cytokinins (Bottini *et al*., 1989; Costacurta & Vanderleyden, 1995; Kuklinsky-Sobral *et al*., 2004; Taller & Wong, 1989; Timmusk *et al*., 1999) or reduce the level of the growth-limiting hormone ethylene (Wang *et al*., 2000). PGPB may also improve mineral nutrition through direct stimulation of the ionic transport or the mineral availability (Bertrand *et al*., 2000; Boddey & Döbereiner, 1995; Ferreira *et al.*, 1987; Goldstein *et al.*, 1999; Mantelin & Touraine, 2004).

Despite successful experiments in laboratories, the performance of PGPB under field conditions is unpredictable and often variable (Bashan, 1998; Bashan & Levanony, 1990; Chanway & Holl, 1993; Kloepper *et al*., 1989). Many factors can explain the inconsistency of the response of amended crops to bacterial inoculants including climatic conditions, soil characteristics, nutrient stress and especially the presence of indigenous microorganisms. Indeed, the rhizosphere is a zone of intensive bacterial competition because the release of organic material from roots provides the energy for the development of active microbial populations (Whipps, 2001). By contrast, the root interior is generally considered as an advantageous ecological niche compared to the rhizosphere (Whipps, 2001). Inoculation failures are therefore often explained by the poor survival of PGPB on plant roots due to the inhibition of their growth by rhizosphere and root associated microorganisms (Bashan, 1998; Kloepper *et al*., 1989; Nautiyal, 1997). However, few studies have examined the response of PGPB to competing bacteria (Bent & Chanway, 1998; Bent *et al*., 2001). Understanding to which extent indigenous bacteria interfere with root colonization and growth promotion is of great significance if PGPB are to be effectively used under field conditions. In particular, we need to determine whether the niche colonized and the inoculum concentration are relevant factors in the PGPB competitive ability.

The PGPB *Phyllobacterium brassicacearum* was originally isolated from the rhizoplane of field-grown *Brassica napus* var. *navajo* (Bertrand *et al*., 2001; Mantelin *et al.*, 2006). It was shown under gnotobiotic conditions to improve root growth by increasing both lateral root density and growth rate of mature lateral roots (Larcher *et al.*, 2003). To define whether indigenous bacteria affected *B. napus* growth promotion mediated by *P. brassicacearum*, we first isolated and identified some bacteria associated with *B. napus* roots and rhizosphere. Then, we compared the effect of variable densities of these indigenous bacteria on *P. brassicacearum*, regarding root colonization and root system architecture. We used an accurate bioassay to measure root system variables in a non destructive manner and to manipulate precisely the bacterial densities in the root medium.

II. MATERIALS AND METHODS

A. Isolation of indigenous bacteria associated with *B. napus* roots and rhizosphere

Three *Brassica napus* var. *navajo* (oilseed rape) plants of 3-month-old with their adhering soil were collected from the same field in Béziers, France. To extract rhizosphere bac-

teria, the loosely adhering soil of each plant was removed by washing the root systems with 0.9% NaCl for 15 min and the obtained mixture was shaken under magnetic agitation for 1 h. Root associated bacteria were recovered by crushing the washed root systems in 0.9% NaCl. Serial dilutions of the rhizosphere and root mixtures of the 3 plants were plated in triplicate on nutrient broth agar supplemented with cycloheximide (50 µg/mL).

After 96 h incubation at 28 °C, the plates containing 30-300 colonies were selected and all the bacterial colonies were thoroughly described and enumerated. All different morphological colony types (defined as morphotypes) were purified in duplicate by subculturing on the isolating medium and stored at -70 °C with 15% (v/v) glycerol. Biochemical characteristics of the isolates were examined by Gram staining and API 20 E and 20 NE tests in order to compare the duplicate isolates and to select the numerically dominant isolates. Thus, a total of 23 *B. napus* indigenous strains were selected, *i.e.* 14 strains from the rhizosphere and 9 strains from roots.

B. Partial 16S ribosomal DNA sequencing

The 23 indigenous bacteria selected were identified by partial 16S ribosomal DNA sequencing. Genomic DNA was extracted from a 10 mL 1-day culture. Cells were washed with 0.9% NaCl and resuspended in 100 µL of Tris-HCl (10 mM, pH 8.3) added with 20 µL proteinase K (1 mg/mL) and incubated 2 h at 55 °C. The preparations were then incubated for 10 min at 100 °C. Almost complete 16S rDNA gene fragments were amplified using the primers FGPS 6 (5'-GGA GAG TTA GAT CTT GGC TCA G-3') and FGPS 1509 (5'-AAG GAG GGG ATC CAG CCG CA-3') (Normand, 1995). PCR products of the expected size were cut out from agarose, purified with a QIAquick gel extraction kit (Qiagen, Courtaboeuf, France) and sequenced on both strands with the 3' terminal primers FGPS 1509 and FGPS 1047-295 (5'-ATG TTG GGT TAA GTC-3'). Sequencing reactions were performed using the ABI Prism BigDye Terminator Cycle sequence Kit (Applied Biosystems Foster City, Calif.) and analyzed on an Applied Biosystem model 310 DNA sequencer. The 16S rDNA partial sequences (approximately 500 bp length) were deposited in the Genbank database under accession numbers DQ102716 to DQ102738.

C. Inoculants

Phyllobacterium brassicacearum strain 29-15 known for its PGPB effect (Bertrand *et al*., 2001; Mantelin *et al*., 2006), and 23 indigenous bacteria associated with *B. napus* roots and rhizosphere (this study) were used. To constitute competing microflora, the selected indigenous bacteria of each niche (roots or rhizosphere) were co-inoculated in their average abundance found over the three samplings (Table I). Each strain was grown in nutrient broth at 28 °C for 36 h, then centrifuged (4000 rev/min, 20 min), washed twice and resuspended in 0.9% NaCl. The optical density (A_{620}) of each suspension was determined in order to adjust the bacterial density to a cell concentration 100 times higher than that required for inoculation. The precise bacterial density was checked by plate counts (colony-forming units, CFU).

D. Plant experiments

Brassica napus var. *navajo* seeds were surface-sterilized in 3% calcium hypochlorite (w/v) for 5 min and washed 4 times with sterile water. Seeds were placed for germination in Petri dishes on E' (Larcher *et al*., 2003) semisolid medium diluted 10 times, for 48 h at 20 °C in darkness.

Table I.- Indigenous bacteria isolated from field-grown *B. napus* roots and rhizosphere. [a]Strains were isolated from 3 *B. napus* plants and bacterial relative abundances were calculated for each plant based on phenotypic typing; [b]Strains were identified by partial 16S rDNA sequencing.

Tableau I.- Bactéries indigènes isolées des racines et de la rhizosphère de *B. napus* cultivé au champ.

Strain	Origin	Relative abundance (%)[a]			Taxonomy	Closest phylogenetic species[b]	% identity
		Plant 1	Plant 2	Plant 3			
SR1-1a (1)	Rhizosphere	3.2	-	-	γ-*Proteobacteria*	*Serratia plymuthica*	100
SR1-1a (2)	Rhizosphere	3.2	-	-	β-*Proteobacteria*	*Comamonas terrigena*	99
SR1-2a	Rhizosphere	27.8	15.5	-	γ-*Proteobacteria*	*Stenotrophomonas maltophilia*	99
SR1-4a	Rhizosphere	15.6	19.1	-	*Actinobacteria*	*Agromyces cerinus*	99
SR1-5a	Rhizosphere	15.2	-	0.5	γ-*Proteobacteria*	*Acinetobacter rhizosphaerae*	99
SR1-7a	Rhizosphere	5.4	-	-	β-*Proteobacteria*	*Comamonas terrigena*	99
SR1-16a	Rhizosphere	3	1.6	11.6	γ-*Proteobacteria*	*Stenotrophomonas maltophilia*	100
SR1-19a	Rhizosphere	6.1	-	-	γ-*Proteobacteria*	*Serratia plymuthica*	100
SR2-2a	Rhizosphere	-	5.6	-	*Actinobacteria*	*Microbacterium oxydans*	99
SR2-3a	Rhizosphere	-	-	50.3	*Firmicutes*	*Paenibacillus lautus*	99
SR2-6a	Rhizosphere	-	34.5	-	*Actinobacteria*	*Arthrobacter globiformis*	100
SR3-3a	Rhizosphere	-	-	27.6	β-*Proteobacteria*	*Variovorax paradoxus*	100
SR3-4a	Rhizosphere	-	5.6	-	γ-*Proteobacteria*	*Pseudomonas fluorescens*	100
SR3-5a	Rhizosphere	-	-	6.6	β-*Proteobacteria*	*Variovorax paradoxus*	99
RE1-2a	Roots	21.8	10.1	20.9	γ-*Proteobacteria*	*Serratia plymuthica*	99
RE1-3a	Roots	1.9	9.5	8.6	γ-*Proteobacteria*	*Pseudomonas fluorescens*	99
RE1-4a	Roots	5.4	5	9.3	γ-*Proteobacteria*	*Pseudomonas putida*	99
RE1-6a	Roots	19	10.7	6.5	γ-*Proteobacteria*	*Stenotrophomonas maltophilia*	100
RE1-8a	Roots	0.4	8.8	3.6	*Firmicutes*	*Exiguobacterium undae*	99
RE1-11a(A)	Roots	0.6	4.1	5.8	β-*Proteobacteria*	*Comamonas terrigena*	99
RE1-11a(B)	Roots	0.6	4.1	5.8	γ-*Proteobacteria*	*Pseudomonas fluorescens*	100
RE1-14b	Roots	34.5	14.5	18	*Actinobacteria*	*Microbacterium phyllosphaerae*	99
RE1-17c	Roots	2.7	28.3	17.3	*Actinobacteria*	*Microbacterium keratanolyticum*	99

The bacterial suspensions at the appropriate concentrations (see below) were introduced in the plant medium (0.5 mM $CaSO_4$, 1 mM KH_2PO_4, 2 mM KNO_3, 0.5 mM $MgCl_2$, 50 µM NaFe-ethylenediaminetetraacetic acid (EDTA), 4 µM H_3Bo_3, 6 µM $MnSO_4(H_2O)$, 0.9 µM $ZnSO_4(H_2O)_7$, 1 µM $CuSO_4(H_2O)_5$, 0.1 µM $Na_2MoO_4(H_2O)_2$ and 2.5 mM 2-(*N*-morpholino)ethanesulphonic acid (MES), pH 5.7) with 0.8% noble agar (w/v), added at 50 °C, just prior cooling in 22.8x22.8 cm Petri dishes (200 mL per box, Polylabo, Strasbourg, France). In each Petri dish, four seedlings were placed (in line) onto the solidified medium at 4.5 cm from each other and from the box edges. Each treatment consisted of three Petri dishes. The Petri dishes were stored vertically for eight days in a growth chamber set for a 22 °C/18 °C light/dark thermoperiod and 16 h/8 h light/dark photoperiod at a photon flux density of 130 µmol/m^2/s.

The following treatments were performed: *P. brassicacearum* alone [at 10^6, $4x10^6$, $3x10^7$, $4x10^7$, $1.5x10^8$, $3x10^8$ and $6x10^8$ CFU/mL according to Larcher *et al.* (2003)] or co-inoculated with either the 14 *B. napus* rhizosphere bacteria at ratios of 2:8, 7:3, 9:1 (*P. brassicacearum*: rhizosphere bacteria) or with the 9 *B. napus* root bacteria at ratios of 2:8, 6:4, 9:1 (*P. brassicacearum*: root bacteria). The whole bacterial densities ranged from 0.8 to $1.8x10^8$ CFU/mL. Additional treatments with each mixture of indigenous bacteria at equivalent bacterial densities to those used in co-inoculation were also performed as controls. All treatments were associated to an un-inoculated control.

E. Root system architecture

Plant root development was estimated daily, using a flatbed scanner (Scanjet 5300 C, Hewlett-Packard, Wilmington, DE, USA) at intermediate resolution (450 dpi) as a non destructive method. Pictures were later analyzed according to Freixes *et al.* (2002) using OPTIMAS software (Optimas 6.5; Media Cybernetics, Silver Spring, MD, USA). The seven plants close to the average plant among the twelve plants in each treatment were chosen and root architectural parameters were calculated according to Larcher *et al.* (2003). Only the parameters that accurately described the effect of the bacteria on *B. napus* root system were developed in the results.

F. Root colonization

To quantify root-colonizing bacteria, three segments of 1 cm were extracted in the oldest, intermediate and youngest zones of the primary root in three plants out of seven (see above) at the end of the experiment. Segments were carefully removed from the medium surface, placed in sterile water and crushed. *P. brassicacearum* cells were specifically counted by indirect immunofluorescence test on glass slides using a polyclonal antiserum produced with whole cells of *P. brassicacearum* (Arsac & Cleyet-Marel, 1986; Schmidt *et al*., 1968). The antiserum specificity was ensured by testing all the strains used for competition experiments at different serum and strain dilutions. The 23 strains failed to react with the *P. brassicacearum* antiserum. In co-inoculation experiments, the whole colonizing cells (including *P. brassicacearum* and the *B. napus* indigenous bacteria) were counted by DAPI (4',6-diamidino-2-phenylindole) staining (Assmus *et al*., 1995).

G. Statistical analysis

Data were analyzed by Student's *t*-test (threshold $P = 0.05$) or by one-way analysis of variance and then multiple range Duncan test (threshold $P = 0.05$) using *Supernova* software (Abacus Concepts, Berkeley, CA, USA).

III. RESULTS

A. Isolation and identification of indigenous bacteria from *B. napus* roots and rhizosphere

Culturable bacterial communities were isolated from rhizosphere and roots of three *B. napus* plants grown in field. After colony morphology typing, we selected and identified the numerically dominant morphotypes for each niche (Table I). Thus, both rhizosphere and root indigenous bacteria that we selected were affiliated to the subclasses of the β- and γ-*Proteobacteria*, the *Actinobacteria* and the *Firmicutes* (low-G+C, gram-positive bacteria) divisions. Within this different taxonomic groups, we identified five genera (*i.e. Acinetobacter, Agromyces, Arthrobacter, Paenibacillus* and *Variovorax*) that were associated with the rhizosphere, three genera (*i.e. Exiguobacterium, Microbacterium* and *Pseudomonas*) associated with the roots and four genera (*i.e. Comamonas, Microbacterium, Serratia* and *Stenotrophomonas*) that were retrieved in both roots and rhizosphere (Table I).

B. Effect of *B. napus* indigenous bacteria on *P. brassicacearum* root colonizing ability

To test the hypothesis that indigenous bacteria may interfere with PGPB, we compared root colonization and then plant growth promotion mediated by the PGPB *P. brassicacea-*

rum, when singly inoculated and when co-inoculated with the *B. napus* root and rhizosphere indigenous bacteria we selected.

The ability of *P. brassicacearum* to colonize *B. napus* primary root according to a decreasing gradient from the base to the tip was confirmed with a higher range of bacterial densities than previously described (Larcher *et al*., 2003). Thus, after eight days and whatever the inoculum density assessed, the oldest zones of the primary root were 10-fold more intensively colonized than the youngest zones (Fig. 1A and 1C, lines). Furthermore, the population size of *P. brassicacearum* recovered throughout the primary root was positively correlated with the inoculum density (R^2 = 0.96, 0.91 and 0.95 in the base, intermediate zone and tip, respectively).

When co-inoculated in different proportions with *B. napus* rhizosphere bacteria (Fig. 1A to 1C, rounds), the population size of *P. brassicacearum* throughout the primary root was similar to that observed in plants inoculated with *P. brassicacearum* alone. Likewise, when co-inoculated with *B. napus* root bacteria (Fig. 1A to 1C, triangles), the density of *P. brassicacearum* was similar or slightly increased compared to singly inoculation. As a consequence, co-inoculation with *B. napus* indigenous bacteria did not reduce levels of *P. brassicacearum* whereas they more intensively colonized the primary root than the PGPB (data not shown).

C. Effect of *B. napus* indigenous bacteria on *P. brassicacearum* root growth promoting ability

The PGPB *P. brassicacearum* singly inoculated, was shown to promote *B. napus* total root length by increasing both lateral root density throughout the primary root and growth rate of mature lateral roots (Larcher *et al.*, 2003). This promoting effect was dose-dependent. Thus, the maximum efficiency (nearly 50%) was obtained for bacterial densities around 10^8 CFU/mL while growth promotion was already significant around 2-3x10^7 CFU/mL (Fig. 2).

By contrast, indigenous bacteria from *B. napus* root and rhizosphere had no significant effect on *B. napus* root growth. Thus, at day 7, the total root length of inoculated plants was not significantly different from the un-inoculated control, whatever the bacterial density used (Fig. 3A and 3B).

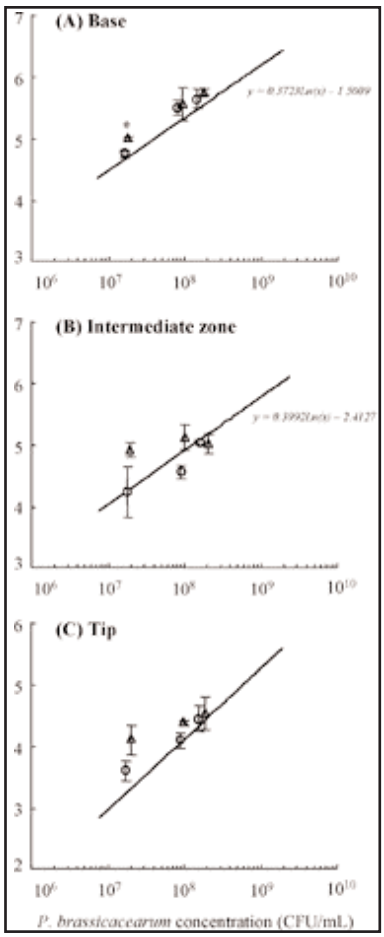

Fig. 1.- *P. brassicacearum* cell counts in the (A) basal, (B) intermediate and (C) tip zones of *B. napus* primary root. *P. brassicacearum* was singly inoculated (—) and co-inoculated with either *B. napus* rhizosphere bacteria (o) at ratios of 2:8; 7:3; 9:1 (from the left to the right on the graph) or with *B. napus* root bacteria (Δ) at ratios of 2:8; 6:4; 9:1 (from the left to the right on the graph). Data are means ± SE (n = 3) after 8 days. Points with an asterisk are significantly different from the regression (Student's t-test, P = 0.05).

Fig. 1.- Concentration cellulaire de *P. brassicacearum* dans (A) la base, (B) le segment central et (C) l'apex de la racine primaire de *B. napus*.

When *P. brassicacearum* was co-inoculated with either root or rhizosphere bacteria at a ratio of 2:8 (*P. brassicacearum*:indigenous bacteria), *B. napus* total root length was similar to the un-inoculated control after 7-day growth (Fig. 4A and B). This indicated that the indigenous bacteria at a population size superior to that of *P. brassicacearum* inhibited the plant growth promoting ability.

By contrast, when *P. brassicacearum* was co-inoculated at ratios of 7:3 and 9:1 with the rhizosphere bacteria, *B. napus* total root length was significantly increased by 21 and 33% (compared to control), respectively (Fig. 4A). These promoting effects were however inferior to those observed with *P. brassicacearum* in singly inoculations (about 40 to 47%). When *P. brassicacearum* was co-inoculated at a ratio of 6:4 with the root bacteria (Fig. 4B), *B. napus* total root length was significantly increased by 29% compared to control but was slightly lower than with *P. brassicacearum* alone (42% more root length compared to control). Interestingly, at a ratio of 9:1 (Fig. 4B), the increase was higher (51% more root length compared to control) and similar to that observed with *P. brassicacearum* singly inoculated.

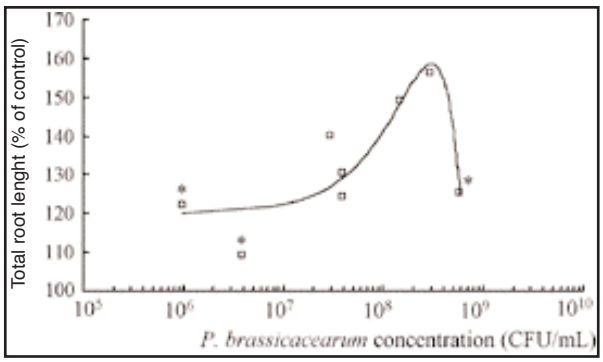

Fig. 2.- Effect of *P. brassicacearum* on total root system length of *B. napus* after 7 days. Seedlings were inoculated with *P. brassicacearum* at initial concentrations of 10^6, $4x10^6$, $3x10^7$, $4x10^7$, $1.5x10^8$, $3x10^8$ and $6x10^8$ CFU/mL. Data are means (n = 7) compared to the un-inoculated control (control = 100%). Points with an asterisk are not significantly different from the un-inoculated control (Student's t-test, P = 0.05).

Fig. 2.- Effet de *P. brassicacearum* sur la longueur totale du système racinaire de *B. napus* après 7 jours.

Our kinetic approach of the root system architecture underscored that when *P. brassicacearum* was co-inoculated in higher proportions with either *B. napus* root or rhizosphe-

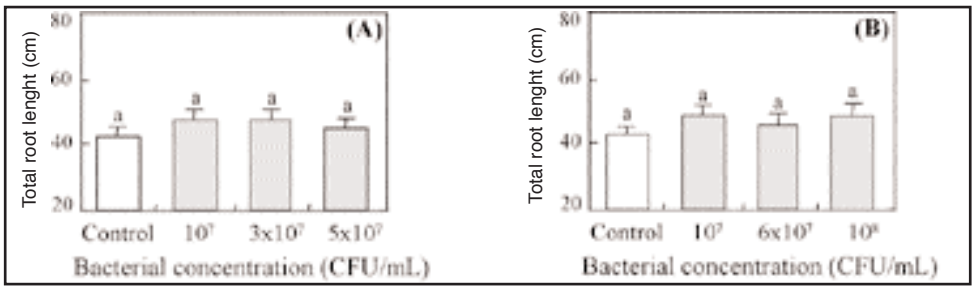

Fig. 3.- Effect of *B. napus* indigenous bacteria on total root system length of *B. napus* after 7 days. Seedlings were inoculated (A) with *B. napus* rhizosphere bacteria at initial concentrations of 10^7, $3x10^7$ and $5x10^7$ CFU/mL or (B) with *B. napus* root bacteria at 10^7, $6x10^7$ and 10^8 CFU/mL. These bacterial densities correspond to those used in co-inoculation experiments with *P. brassicacearum* (Fig. 4 and 5). Each experiment includes an un-inoculated control. Data are means ± SE (n = 7) and bars followed by the same letter are not significantly different (Duncan's test, P = 0,05).

Fig. 3.- Effet de bactéries indigènes associées à *B. napus* sur la longueur totale du système racinaire de *B. napus* après 7 jours.

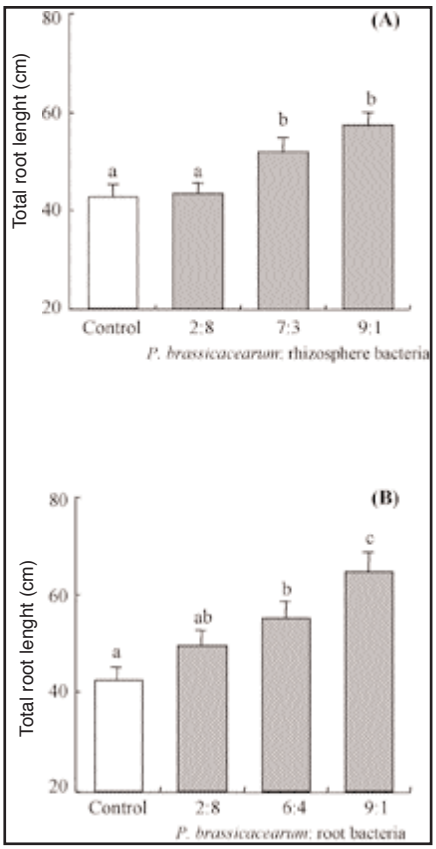

Fig. 4.- Effect of *P. brassicacearum* co-inoculated with *B. napus* bacteria on total root system length of *B. napus* after 7 days. Seedlings were inoculated (A) with *P. brassicacearum* and the rhizosphere bacteria at ratios of 2:8; 7:3; 9:1 or (B) with *P. brassicacearum* and the root bacteria at ratios of 2:8; 6:4; 9:1. Each experiment includes an un-inoculated control. Data are means ± SE (n = 7) and bars followed by the same letter are not significantly different (Duncan's test, P = 0,05).

Fig. 4.- Effet de *P. brassicacearum* co-inoculé avec des bactéries indigènes de *B. napus* sur la longueur totale du système racinaire de *B. napus* après 7 jours.

re bacteria, the increase of total root length was apparent 3 or 4 days after transplanting (Fig. 5A and B). At this early stage of development, plantlet root systems were constituted of a primary root and emerging lateral roots. Regarding elementary variables of the root system, we observed that at day 7 the primary root length was unaffected by inoculation contrary to lateral roots (data not shown). The increase of lateral root length was due to an increase in both number and elongation rate of lateral roots but these architectural variables considered separately were not statistically significant compared to the control.

IV. DISCUSSION

Evaluating competitiveness of PGPB should be a prerequisite for successful inoculation under field conditions. Indigenous microorganisms are the best candidates to achieve this evaluation, because they have been subjected to a natural selection process by the plant (Benizri *et al*., 2001; Kuske *et al*., 2002; Smalla *et al*., 2001). In the experiments reported here, some indigenous bacteria associated with *B. napus* roots and rhizosphere were isolated and characterized in order to determine to which extent they interfered with the PGPB

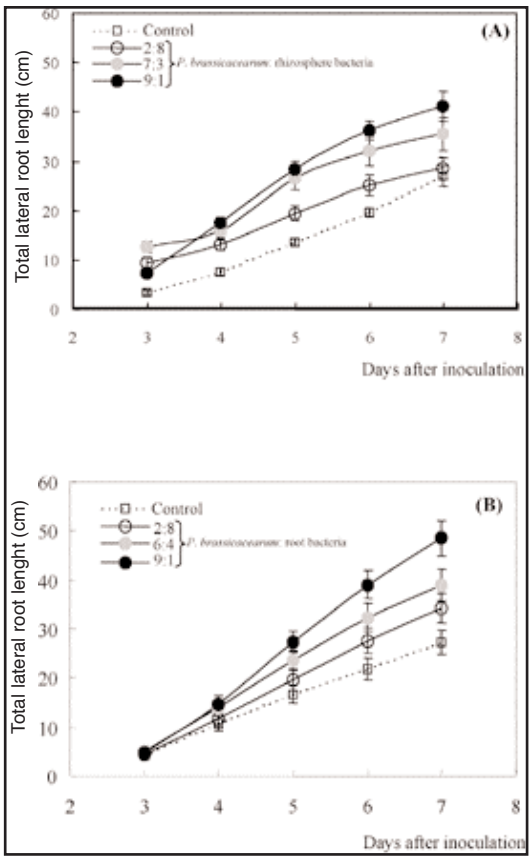

Fig. 5.- Effect of *P. brassicacearum* co-inoculated with *B. napus* bacteria on total lateral root length of *B. napus* during 7 days. Seedlings were inoculated (A) with *P. brassicacearum* and the rhizosphere bacteria at ratios of 2:8; 7:3; 9:1 or (B) with *P. brassicacearum* and the root bacteria at ratios of 2:8; 6:4; 9:1. Each experiment includes an un-inoculated control. Data are means ± SE (n = 7).

Fig. 5.- Effet de *P. brassicacearum* co-inoculé avec des bactéries indigènes de *B. napus* sur la longueur totale du système racinaire de *B. napus* durant 7 jours.

P. brassicacearum. Those *B. napus* indigenous bacteria can be potential competitors that the PGPB *P. brassicacearum*, originally isolated from *B. napus* rhizoplane would encounter under field conditions during the rhizosphere and rhizoplane colonization process.

Half of the bacterial genera that we identified were already isolated from *B. napus* roots or rhizosphere (Germida *et al*., 1998; Graner *et al*., 2003; Kaiser *et al*., 2001; Kalbe *et al*., 1996; Misko & Germida, 2002; Siciliano & Germida, 1999), while the others were commonly found in soil or in association with plants. In particular, the genus *Serratia* isolated from *B. napus* rhizosphere (Kalbe *et al*., 1996) was shown to possess antifungal properties and to secrete the plant growth hormone indole acetic acid that can directly promote root growth. Besides, pseudomonads known as aggressive colonizers of the root surface are major members of the *B. napus* root and rhizosphere associated microbial communities (Germida *et al*., 1998; Kaiser *et al*., 2001; Misko & Germida, 2002; Siciliano & Germida, 1999).

We used an accurate bioassay in Petri dishes to measure root system variables in a non destructive manner and to manipulate precisely the bacterial densities in the root medium (Larcher *et al*., 2003). It is important to precise that this gnotobiotic bioassay does not reflect field conditions and soil physicochemical properties.

Surprisingly, the indigenous bacteria singly inoculated to *B. napus* had not significant effect on total root growth after seven days while they intensively colonized the root system. This may be explained by the absence of beneficial or deleterious strains to plant growth in both indigenous mixtures even if they contained bacterial genera with known PGPB properties. We cannot also exclude that in the indigenous bacteria, some competing isolates could have inhibited or compensated the activity of non neutral strains.

Co-inoculating *P. brassicacearum* in various proportions with either *B. napus* root or rhizosphere bacteria did not reduce its colonizing ability compared to singly inoculation. This underscores that *P. brassicacearum* is a competitive colonizer of *B. napus* root system probably through the colonization of specific microsites. By contrast, the indigenous bacteria could alter the plant growth promoting ability of *P. brassicacearum*, depending on their initial proportion. Hence, *P. brassicacearum* promoted root growth only when it was co-inoculated in higher proportions compared to the indigenous bacteria. These PGPB effects were however slightly decreased compared to singly inoculations, except with the root bacteria at the ratio 9:1. When growth promotion occurred, the effects measured on root system architecture were close to that of *P. brassicacearum* alone (Larcher *et al*., 2003). These effects started since lateral root emergence, indicating that *P. brassicacearum* expressed its PGPB ability very early.

Interestingly, the extent of plant growth promotion observed in the competition experiments was not correlated with the extent of root colonization. While it is generally assumed that inoculation failures are due to the poor survival of the PGPB on roots, our results confirm the previous findings that alteration in plant growth promotion could occur without affecting root colonization intensity (Bent & Chanway, 1998; Bent *et al.*, 2001). To explain this, we can first assume that the colonization of accurate sites on the root is more determining than the quantity of colonizing cells. Moreover, it is also conceivable that the inhibition occurred at a later stage than root colonization, on the plant growth promoting mechanisms themselves. For instance, *P. brassicacearum* may have secreted phytoregulators that were entirely consumed by the indigenous bacteria when they were predominant compared to the PGPB. Further work should be carried out to evaluate this hypothesis.

Besides, our results underscore that the initial population size of the PGPB is of great interest to avoid inoculation failure. We can suppose that this critical bacterial size may depend on the PGPB concentration required for a maximum efficiency, on the PGPB competitiveness and also on the population size of the indigenous bacteria. It should also be noted that *P. brassicacearum* was more sensitive to the rhizosphere than the root indigenous bacteria. This supports the hypothesis that the root interior is an advantageous ecological niche compared to the rhizosphere.

PGPB are promising agent for a sustainable agriculture and particularly for a widespread crop as *B. napus.* However, the current study highlights that they are subjected to complex interactions with the indigenous bacteria. Thus, the efficacy of a PGPB, even if it is a good colonizer, may be reduced by the bacterial competition depending on the initial size of the PGPB compared to the indigenous bacteria. Investigating the PGPB competitiveness is therefore essential in order to secure successful inoculation. Further investigations might be conducted considering the whole indigenous bacteria as competitors, *i.e.* culturable and non culturable bacteria. The latter could be identified through molecular approaches as 16S ribosomal DNA cloning and sequencing analysis.

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