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# Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada

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**Abstract:** The use of beneficial soil microorganisms as agricultural inputs for improved crop production requires selection of rhizosphere-competent microorganisms with plant growth-promoting attributes. A collection of 563 bacteria originating from the roots of pea, lentil, and chickpea grown in Saskatchewan was screened for several plant growth-promoting traits, for suppression of legume fungal pathogens, and for plant growth promotion. Siderophore production was detected in 427 isolates (76%), amino-cyclopropane-1-carboxylic acid (ACC) deaminase activity in 29 isolates (5%), and indole production in 38 isolates (7%). Twenty-six isolates (5%) suppressed the growth of *Pythium* sp. strain p88-p3, 40 isolates (7%) suppressed the growth of *Fusarium avenaceum*, and 53 isolates (9%) suppressed the growth of *Rhizoctonia solani* CKP7. Seventeen isolates (3%) promoted canola root elongation in a growth pouch assay, and of these, 4 isolates promoted the growth of lentil and one isolate promoted the growth of pea. Fatty acid profile analysis and 16S rRNA sequencing of smaller subsets of the isolates that were positive for the plant growth-promotion traits tested showed that 39%–42% were members of the *Pseudomonadaceae* and 36%–42% of the *Enterobacteriaceae* families. Several of these isolates may have potential for development as biofertilizers or biopesticides for western Canadian legume crops.

*Key words:* rhizosphere, bacteria, plant growth promotion, *Pseudomonadaceae*, *Enterobacteriaceae*.

**Résumé :** L'utilisation de microorganismes bénéfiques vivant dans le sol comme apport agricole pour améliorer des récoltes requiert la sélection de microorganismes habitant la rhizosphère et possédant des propriétés de promotion de la croissance des plantes. Une collection de 563 bactéries provenant de la racine du pois, de la lentille et du pois chiche cultivés en Saskatchewan a été criblée quant à la présence de plusieurs traits reliés à la promotion de la croissance des plantes, à la suppression des pathogènes fongiques des légumineuses ainsi qu'à la promotion de facto de la croissance des plantes. La production de sidérophores a été détectée chez 427 isolats (76 %), l'activité de l'acide amino-cyclopropane-1-carboxilique (ACC) désaminase chez 29 isolats (5 %) et la production d'indole, chez 38 isolats (7 %). Vingt-six isolats (5 %) supprimaient la croissance de *Pythium* sp. souche p88-p3, 40 isolats (7 %) supprimaient la croissance de *Fusarium avenaceum* et 53 isolats (9 %) supprimaient la crissance de *Rhizoctonia solani* CKP7. Dix-sept isolats (3 %) favorisaient l'élongation de la racine de canola dans un essai de croissance en pousse et parmi ceux-ci, 4 isolats favorisaient la croissance de la lentille et un isolat favorisait la croissance du pois. L'analyse du profil en acides gras et le séquençage de l'ARNr 16S d'un sous-groupe plus petit d'isolats qui présentaient des traits reliés à la promotion de la croissance ont démontré que 39 % – 42 % de ces isolats étaient membres de la famille des *Pseudomonadaceae* et que 36 % – 42 % étaient membres de la famille des *Enterobacteriaceae*. Plusieurs de ces isolats pourraient avoir du potentiel dans le développement de fertilisants biologiques et de pesticides biologiques pour la récolte de légumineuses au Canada.

*Mots-clés :* Rhizosphère, bactéries, promotion de la croissance des plantes, *Pseudomonadaceae*, *Enterobacteriaceae*.

[Traduit par la Rédaction]

## Introduction

The rhizosphere, the zone of soil in close proximity to roots, supports the metabolic activities of a diverse group of soil microorganisms (Young 1998; Bais et al. 2006). These microorganisms may have beneficial, neutral, or detrimental effects on plant growth and productivity. Microorganisms that are beneficial to plant growth are collectively referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1989) and are of interest agriculturally as potential biofertilizers and (or) biopesticides. The development of a database of isolates characterized for plant growth-promoting physiological traits can assist in the selection of the most promising microorganisms for further advancement as biofertilizers or biopesticides.

Biofertilizers include rhizosphere bacteria that directly promote plant growth by increasing the availability of nutrients such as phosphorus and iron, by producing phytohormones such as indole-3-acetic acid (IAA) and cytokinins,

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and by producing amino-cyclopropane-1-carboxylic acid (ACC) deaminase, which decreases the production of ethylene, an inhibitor of plant growth (Glick 1995; Vessey 2003). Biopesticides include rhizosphere bacteria that indirectly promote plant growth by suppressing the activity of phytopathogens via the production of antagonistic metabolites such as antibiotics, through competition for essential nutrients, and via induction of the plant's systemic and localized defence systems (Glick 1995).

In greenhouse and field studies, inoculation of lentil with *Rhizobium leguminosarum* plus PGPR promoted plant emergence, increased root mass, increased nodule number per root, and increased nitrogen fixation ($C_2H_2$ reduction) (Chanway et al. 1989). Biofertilizers containing symbiotic $N_2$-fixing *R. leguminosarum* and phosphate-solubilizing microorganisms, such as Nodulator® (Becker Underwood Inc., Saskatoon, Saskatchewan) and Tag Team® (Philom Bios Inc., Saskatoon, Saskatchewan), are recognized as beneficial inputs by Western Canadian pea and lentil growers. Xi et al. (1996) reported that several fluorescent pseudomonads and *Pseudomonas* spp. strains PRA25 and AMMD were antagonistic to the pea root rot pathogens *Pythium ultimum* and *Rhizoctonia solani* in field trials in Saskatchewan. However, biopesticides that suppress the activity of soil-borne legume phytopathogens are not yet available in Canada.

The objective of this study was to generate a collection of soil bacteria isolated from the rhizosphere of western Canadian pulse crops and to characterize them for direct and indirect plant growth-promoting traits. Isolates that demonstrate plant growth promotion and suppression of legume pathogens may be suitable candidates for inoculant development.

## Materials and methods

### Isolation of rhizosphere bacteria

Three healthy pea, lentil or chickpea plants with soil adhering to the roots were collected at 6 sites in Saskatchewan (Table 1). Samples were placed in large plastic bags, placed on ice for transport to the laboratory, and stored at 4 °C until processing within 48 h. Plants were collected from all sites on the same day. The bulk of the soil was carefully removed from the roots by a spatula leaving a 2–5 mm thick zone of rhizosphere soil adhering to the roots. Roots with adhering rhizosphere soil were detached from shoots and placed into containers with 0.1 mol $MgSO_4 \cdot 7H_2O \cdot L^{-1}$ and then shaken on a rotary shaker at 150 $r \cdot min^{-1}$ for 30 min prior to dilution in 0.1 mol $MgSO_4 \cdot 7H_2O \cdot L^{-1}$. One hundred microlitre aliquots from several dilutions were spread onto one-tenth-strength tryptic soy agar (TSA) (Difco Laboratories, Detroit, Michigan, USA), and the agar plates were incubated at 22 °C for up to 5 days. Six hundred and ninety-three colonies were selected at random and purified on one-tenth-strength TSA. Isolates were frozen at −70 °C in 20% glycerol – 80% half-strength tryptic soy broth (TSB) (Difco Laboratories, Detroit, Michigan, USA).

### Determination of siderophore production

The ability of the isolates to produce siderophores was assessed as described by Schwyn and Neiland (1987), with several modifications. The test was carried out in 96-well microtitre plates and utilized the ternary complex Chrome Azurol S as an indicator. A change in the colour of the dye from blue to orange indicated siderophore production. The isolates were inoculated into Rhizosphere medium containing 853 mL of $dH_2O$, 0.75 g of $Ca(NO_3)_2 \cdot 4H_2O$, 0.246 g of $MgSO_4 \cdot 7H_2O$, 18.22 g of 2[(2-amino-2-oxyethyl)-amino]-ethanesulfonic acid, 2.0 g of NaOH, 1 mL of 1 mol $K_2HPO_4 \cdot L^{-1}$ (pH 7), 1 mL of 0.7 mmol $ZnSO_4 \cdot 7H_2O \cdot L^{-1}$, 1 mL of 0.9 mmol $MnSO_4 \cdot 4H_2O \cdot L^{-1}$, 1 mL of biotin (1 $mg \cdot L^{-1}$), 1 mL of thiamine HCl (20 $mg \cdot L^{-1}$), 100 mL of 10% casamino acids, and 33.3 mL of 30% sucrose (Buyer et al. 1989) and were incubated at 20 °C for 48 h. The following were added to the wells in microtitre plates: 75 µL of a 48 h culture in Rhizosphere medium, 75 µL of Chrome Azurol S and 30 µL of sterile Rhizosphere medium. The microtitre plates were incubated at 20 °C for 48 h, and the appearance of an orange colour in the plates was scored positive for siderophore production. *Pseudomonas syringae* R55 was included in each assay as a positive control (Roy 2003).

**Table 1.** Cropping history and location of sample sites from which the rhizosphere bacteria were isolated.

| Sample sites[a] | Crop | Previous crop |
|---|---|---|
| 1 | Chickpea 'Stanford' | Durum wheat |
| 2 | Lentil 'Crimson' | Durum wheat |
| 3 | Pea 'Grande' | Lentil |
| 4 | Pea 'Grande' | Canola |
| 5 | Pea, green | Hard red spring wheat |
| 6 | Pea, yellow | Hard red spring wheat |

[a] Sampling site locations: 1–4, Rosetown, Saskatchewan; 5 and 6, North Battleford, Saskatchewan.

### Determination of indole production

The ability of the isolates to produce indoles, including IAA, was assayed based on the method of Patten and Glick (2002) using Salkowski's reagent (Gordon and Weber 1951). The isolates were inoculated into half-strength TSB, incubated at 20 °C for 48 h, and streaked onto half-strength TSA for isolation of single colonies. Three identical colonies were suspended into each of 2 media, DF salts minimal medium (Dworkin and Foster 1958) and DF minimal medium supplemented with 200 $\mu g \cdot mL^{-1}$ L-tryptophan, and were incubated at room temperature on a gyratory shaker at 150 $r \cdot min^{-1}$. After 7 days, 1 mL of each culture was centrifuged at 9300*g* for 15 min, and 50 µL of the supernatant placed in the wells of a 96-well plate. A standard curve (0–10 µg of IAA) was prepared in each well, using a stock IAA solution (1 $mg \cdot mL^{-1}$ in methanol) and the minimal medium to a total volume of 50 µL. Following addition of 200 µL of Salkowski's reagent, the plate was gently shaken and incubated at room temperature for 20 min. The resultant red colour, indicating the presence of indoles, was determined by measuring the optical density at $A_{600}$ and comparing with the standards. Assays were repeated for isolates that scored positive for indole production in initial tests.

### Determination of ACC deaminase activity

The ability of the isolates to utilize ACC as a sole N source was assayed as described by Glick et al. (1995) with some modifications. The isolates were grown for 48 h in half-strength TSB, diluted 10-fold in 0.1 mol $MgSO_4{\cdot}7H_2O{\cdot}L^{-1}$, centrifuged at 9300*g* for 15 min and resuspended in 0.1 mol $MgSO_4{\cdot}7H_2O{\cdot}L^{-1}$. An aliquot of 150 µL of DF minimal salts medium was placed in each well of a 96-well plate. Twenty microlitres of filter-sterilized 3 mmol $ACC{\cdot}L^{-1}$, 10 mmol $(NH_4)_2SO_4{\cdot}L^{-1}$, or 100 mmol $MgSO_4{\cdot}7H_2O{\cdot}L^{-1}$ was added to the multi-well plates, followed by a 30 µL aliquot of each isolate. The plates were incubated at 20 °C and growth of the isolates was determined by measuring the optical density at 405 nm at 0, 24, 48, 72, and 96 h. Strain GR12-2, previously shown to utilize ACC as sole N source (Glick et al. 1995), served as the positive control. Assays were repeated for isolates that scored positive for ACC deaminase activity in initial tests.

### Determination of antifungal metabolite production

Fungal pathogens isolated from diseased lentil (*Fusarium avenaceum*), chickpea (*Rhizoctonia solani* CKP7), and pea (*Pythium* sp. strain p88-p3) were kindly provided by Dr. S.-F. Hwang, Alberta Agriculture, Vegreville, Alberta. An in vitro assay was used to examine growth inhibition of *F. avenaceum*, *R. solani*, and *Pythium* sp. by the rhizosphere isolates. A 6 mm plug of the fungus was placed in the centre of one-fifth-strength potato dextrose agar medium. Growth of the fungus was challenged by inoculating a single 2 cm long streak of the soil isolate on the potato dextrose agar plate 3 cm from the fungal plug. Suppression of fungal growth was measured after 48 and 72 h of incubation and compared with that of the control (i.e., without the bacterial isolate). Assays were repeated for isolates that scored positive for antifungal activity toward any of the phytopathogens in initial tests.

### Determination of plant growth-promotion activity by bacterial isolates

The rhizobacterial collection was screened for plant growth-promotion activity using the plant growth pouch assay of Lifshitz et al. (1987) with canola as the model plant. Canola seed, *Brassica napus* '45A71', was surface sterilized by immersion for 10 s in 95% ethanol, followed by 10 min in 20% *v/v* diluted commercial bleach, five 5 min washes and rinses in sterile distilled water, and air-drying in a laminar flow hood. To check for sterility, 20 or more seeds were placed on half-strength TSA and incubated for 48 h at room temperature. To prepare the noninoculated control treatments, 7 seeds were placed in each sterilized plant growth pouch (Mega International, Minneapolis, Minnesota, USA), containing 10 mL of sterile half-strength Hoagland's nutrient solution (Hoagland and Boyer 1936). To prepare inoculated seed treatments, bacterial isolates were grown 48–72 h at 20 °C in half-strength TSB (to stationary phase), centrifuged, resuspended, and diluted 100-fold in 0.1 mol $MgSO_4{\cdot}7H_2O{\cdot}L^{-1}$. The inoculant population densities of isolates 6–8 and 2–20 were $\log_{10}$ 8.3 and 8.5 $cfu{\cdot}mL^{-1}$, whereas those of the other isolates ranged from $\log_{10}$ 7.0 to 7.9 $cfu{\cdot}mL^{-1}$. Surface-sterilized seed was gently agitated in the diluted bacterial suspension for 10 min before transfer to the plant growth pouches. There were 7 $seeds{\cdot}pouch^{-1}$ and 5 replicate $pouches{\cdot}treatment^{-1}$. The growth pouches were wrapped with plastic wrap to prevent evaporation and were incubated at 18 °C with a 16 h : 8 h light:dark cycle in a growth cabinet (Certomat CS-1, Bethelem, Pennsylvania) for 7 days prior to measurement of root length, number of lateral roots, and root dry mass.

Isolates that promoted canola growth were tested for the ability to promote pea and lentil growth. Pea cultivar CDC Mozart and lentil cultivar Milestone seed were sterilized by rinsing with 95% ethanol for 30 s, followed by 20 min on a gyratory shaker with gentle shaking in 20% *v/v* diluted commercial bleach, and then rinsed 5–7 times with sterile distilled $H_2O$. The seeds were then placed in sterile Petri dishes and allowed to dry in a laminar flow hood overnight. A sterility check was performed for both pea and lentil by testing 10 or 25 random seeds as described above. Plant growth promotion of lentil and pea by rhizobacteria was carried out in Tinus Rootainers (Spencer-Lemaire Ind. Ltd., Edmonton, Alberta). Rootainers (4 $cells{\cdot}rootainer^{-1}$, 2 $rootainers{\cdot}treatment^{-1}$) were filled with a mixture of Redi-Earth – sand – perlite (75:20:5), seeded with lentil or pea (8 $seeds{\cdot}rootainer^{-1}$) inoculated with 1 × $10^6$ $bacteria{\cdot}seed^{-1}$ as above, and incubated in a plant growth cabinet maintained at 8 °C with a 16 h : 8 h light:dark cycle. Two rootainers per treatment were harvested 27 days later and root length, lateral root formation, and root dry mass were determined.

### Identification of the rhizosphere bacteria by fatty acid methyl ester analysis

Fatty acid methyl ester (FAME) analysis was used to identify a subset of 116 bacteria that possessed ACC deaminase activity, produced indole, suppressed the growth of one or more of the fungal pathogens, promoted canola root elongation, or enhanced the growth of pea or lentil. FAME analysis was conducted as described by Cavigelli et al. (1995) and Siciliano et al. (1998), and the FAME profile of each isolate was matched with known profiles in the MIDI database (Sherlock TSBA Library version 3.80; Microbial ID, Inc., Newark, Delaware, USA).

### Identification and phylogenetic analysis of rhizosphere bacteria by 16S rRNA sequencing

Full-length 16S rRNA sequences were determined for 38 isolates comprising a subset of the rhizobacterial isolates identified by FAME analysis. PCR amplifications of the 16S rRNA gene were conducted using whole-cell lysates from pure cultures. Amplification reactions were conducted in 50 µL volumes containing 45 µL of Platinum® PCR SuperMix High Fidelity (Invitrogen), 1.0 µL of a 20 $\mu mol{\cdot}L^{-1}$ primer mix, and 3.0 µL of the whole-cell lysate supernatant. The PCR cycle used to amplify the 16S rRNA gene was as follows: 4 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 42 °C, 1 min at 72 °C, and a final extension of 10 min at 72 °C. The primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 531R (5′-ACGCTTGCACCCTCCGTATT-3′) were used to amplify the first 544 bp of the 16S rRNA gene and the primers 515F (5′-TGCCAGCAGCCGCGTAA-3′) and 1542R (5′-GGCTACCTTGTTACGACTT-3′) were used to amplify the remainder of the 16S rRNA gene. PCR products were purified with the Wizard® PCR Preps DNA purification system

Promega) and then sequenced. DNA sequencing was conducted by the Fragment Analysis DNA Sequencing Services at the University of British Columbia Okanagan.

Each 16S gene sequence was queried for similarities with BLAST (Altschul et al. 1990) and with the Ribosomal Database Project Similarity Rank and Chimera Check programs (Maidak et al. 2000). The nucleotide sequences were aligned using the CLUSTAL X program (Thompson et al. 1994) applying default parameters. Phylogenetic trees were constructed using programs from the PHYLIP package (Felsenstein 1989), including SEQBOOT (100 replicates), DNAPARS, DNADIST (Jukes–Cantor model), NEIGHBOR (neighbor-joining model), and CONSENSE.

## Results

### Isolation and characterization of rhizosphere bacteria

The bacterial isolates selected from the rhizosphere of pea, lentil, and chickpea were from commercial fields with a history of legume crop production. Pea or lentil had been grown in rotation with wheat or canola, usually every 3 years, for the past 25 years (Table 1). Six hundred and ninety-three bacterial isolates were initially selected and purified, and 563 of these isolates (81%) survived cold storage at $-70$ °C for over 5 years. All 563 isolates were assayed for siderophore production; ACC deaminase activity (as determined by the ability to use ACC as a sole N source); indole production; in vitro suppression of growth of *F. avenaceum*, *R. solani* CKP7, and *Pythium* sp. strain p88-p3; and canola root elongation. A subset of these, 116 isolates demonstrating ACC deaminase activity, indole production, in vitro suppression of fungal pathogens, or canola root elongation, is described in this paper (Table 2).

Siderophore production was detected in 427 isolates (76%) (Table 2). There was no correlation between siderophore production by the isolates and site or crop of origin (data not presented). Twenty-nine isolates (5%) were able to grow in a medium with ACC as the sole nitrogen source, implying the presence of ACC deaminase (Table 2), and with the exception of isolate 2-52, *Rhodococcus fascians*, all other isolates positive for ACC deaminase activity were also siderophore producers. The majority of isolates with ACC deaminase activity were members of the *Pseudomonadaceae* (22 isolates) followed by members of the *Nocardiaceae* (3 isolates) and *Bacillaceae* (2 isolates) families (Table 3). Indole production varied from 0.6 to 12 μg·mL$^{-1}$ and was identified in 38 isolates (7%) (Table 2). Eight of the indole-producing isolates utilized ACC as a sole N source and 31 of the isolates also produced siderophores (Table 2). Members of the *Enterobacteriaceae* (19 isolates) were the predominant indole producers, followed by the *Pseudomonadaceae* family (9 isolates) (Table 3). Twenty-six isolates (5%) suppressed the growth of *Pythium* sp. strain p88-p3, 40 isolates (7%) suppressed the growth of *F. avenaceum*, and 53 isolates (9%) suppressed the growth of *R. solani* CKP7 (Table 2), as determined by an in vitro assay. Of these, only 10 isolates, 3 *Pseudomonas*, 3 *Serratia*, 2 *Rahnella*, 1 *Arthrobacter* species, and 1 isolate (not identifiable by FAME) displayed broad spectrum suppression by inhibiting the growth of all 3 phytopathogens. The majority of bacterial isolates capable of suppressing fungal growth were members of the *Enterobacteriaceae* and *Pseudomonadaceae* families (Table 3).

### Plant growth promotion by rhizobacteria

Seventeen isolates (3%) promoted canola root elongation in plant growth pouches (Table 2). All isolates that promoted canola root elongation also produced siderophores and were positive for ACC deaminase, except for isolate 3-31 (*Bacillus pumilus*), which lacked ACC deaminase activity. Four canola root growth-promoting isolates also produced indoles and 6 isolates inhibited growth of at least one of the fungal pathogens. The majority of isolates capable of promoting canola root elongation were *Pseudomonas* spp. (12 isolates) (Table 3).

The 17 canola growth promoters were tested for their ability to enhance lentil and pea growth. Four isolates, *P. syringae* 2-28, *Pseudomonas veronii* 3-10 and 3-67, and *Bacillus pumilus* 3-31, promoted the growth of lentil (Fig. 1), and one isolate, *P. veronii* 5-1, promoted the growth of pea compared with the noninoculated control (data not shown). *Pseudomonas syringae* 2-28 significantly promoted lentil root length, increased the number of lateral roots and root dry mass, while *B. pumilus* 3-31 significantly promoted lentil root length and root dry mass (Fig. 1). *Pseudomonas veronii* 3-10 and 3-67 significantly increased the number of lateral roots and root dry mass of lentil (Fig. 1), and *P. veronii* 5-1 significantly increased pea root dry mass by 29.8% (data not shown).

### Isolate identification

Of the 111 isolates identified by FAME, 39% and 36% were members of the families *Pseudomonadaceae* and *Enterobacteriaceae*, respectively (Table 3). The remaining families represented from 1% to 6% each of the total identified (Table 3). There was no correlation between the location or crop from which the isolates were collected and the distribution of bacterial families. Thirty-eight isolates identified by rRNA sequencing were selected based on their ability to promote the growth of lentil, pea, or canola; their ability to inhibit the growth of *Pythium*, *Rhizoctonia*, and *Fusarium*; and their ability to produce indoles and ACC deaminase activity (Fig. 2). Among the 38 rRNA sequences analyzed, not one was found to be chimeric (data not shown). Similarity searches in the Ribosomal Database Project and GenBank databases revealed that 42% (16 isolates) and 42% (16 isolates) of the examined isolates were members of the families *Pseudomonaceae* and *Enterobacteriaceae*, respectively. The remaining 16% (6 isolates) were members of the *Microbacteriaceae*, *Bacillaceae*, *Nocardiaceae*, and *Micrococcaceae* families. A comparison of the FAME identifications with the 16S rRNA identifications of the 38 isolates sequences indicated that both methods agreed on the family identity of 97% (37) of the isolates, but at the genus level the FAME identification agreed with the 16S identification for only 68% (26) and at the species level for only 21% (8) of the isolates.

## Discussion

The first step in biofertilizer and biopesticide development is the identification of candidate microorganisms with

**Table 2.** Plant growth-promoting traits detected for 116 rhizobacteria isolated from the roots of pea, lentil, and chickpea grown in Saskatchewan soil.

| Isolate | Isolate identification by FAME analysis | Siderophore production[a] | ACC deaminase activity[b] | Indole production[c] (μg·mL$^{-1}$) | % Inhibition of:[d] *Pythium* | *Rhizoctonia* | *Fusarium* | Canola root elongation[c] (cm) |
|---|---|---|---|---|---|---|---|---|
| 1-8 | *Pseudomonas chlororaphis* | + (1.64) | 1.2 | – | – | – | – | – |
| 1-18 | *Klebseilla pneumoniae* | + (1.41) | – | 0.6 | 7 | 14 | – | – |
| 1-20 | *Pseudomonas putida* | + (1.56) | – | – | 3 | – | – | – |
| 1-29 | *Bacillus pumilus* | + (1.36) | – | – | – | 7 | – | – |
| 1-39 | *Pseudomonas corrugata* | + (1.77) | 0.71 | 1.2 | – | – | – | – |
| 1-44 | *Pseudomonas putida* | + (0.96) | 2 | – | – | – | – | – |
| 1-51 | *Klebseilla pneumoniae* | + (1.54) | – | – | 7 | – | 30 | – |
| 1-72 | *Pantoea agglomerans* | + (1.72) | – | 3.94 | – | – | – | – |
| 1-73 | *Pseudomonas syringae* | + (1.73) | 0.48 | – | – | – | – | – |
| 1-74 | *Rahnella aquatilis* | + (1.45) | – | – | 10 | – | 43 | – |
| 1-80 | No match | + (1.73) | – | – | 7 | – | 40 | – |
| 1-88 | *Serratia grimesii* | + (1.58) | – | 3.47 | 17 | 10 | 47 | – |
| 1-89 | *Paenibacillus gordonae* | – (0.49) | – | 4.18 | – | – | – | – |
| 1-90 | *Rahnella aquatilis* | + (1.41) | – | – | 17 | 10 | – | – |
| 1-93 | *Kocuria kristinae* | – (0.45) | – | – | 14 | – | – | – |
| 1-94 | *Nocardioides luteus* | – (0.52) | – | – | – | – | 23 | – |
| 1-101 | *Pseudomonas fluorescens* | + (1.55) | 0.54 | – | – | – | – | – |
| 1-107 | *Pseudomonas putida* | + (1.7) | – | – | – | 7 | – | – |
| 1-112 | *Pseudomonas veronii* | + (0.8) | – | – | 20 | 23 | 14 | – |
| 1-114 | *Pseudomonas corrugate* | + (1.28) | 0.81 | – | – | – | – | – |
| 1-132 | *Pseudomonas syringae* | + (0.89) | 0.81 | – | – | – | – | – |
| 1-134 | *Pseudomonas syringae* | + (1.18) | 0.85 | – | – | – | – | 7.26 |
| 1-135 | *Serratia grimesii* | + (1.46) | – | 1.12 | – | 17 | 33 | – |
| 2-9 | *Rahnella aquatilis* | + (1.26) | – | – | 17 | 14 | 20 | – |
| 2-12 | *Nocardia brasiliensis* | + (1.38) | 0.8 | – | – | – | – | 6.83 |
| 2-13 | *Pseudomonas putida* | + (1.75) | – | – | – | 4 | 23 | – |
| 2-18 | *Pseudomonas putida* | + (1.49) | – | – | – | 4 | 33 | – |
| 2-20 | *Exiguobacterium acetylicum* | + (0.74) | 1.33 | – | 7 | – | – | 7.59 |
| 2-21 | *Enterococcus faecium* | – (0.73) | – | 2.8 |  |  | – |  |
| 2-23 | *Serratia grimesii* | + (1.39) | – | 1.59 | 14 | 14 | 17 | – |
| 2-27 | *Pseudomonas syringae* | + (1.36) | – | – | – | 14 |  | – |
| 2-28 | *Pseudomonas syringae* | + (1.07) | 0.5 | – | – | 10 | 14 | 9.19 |
| 2-32 | *Erwinia persicina* | + (1.36) | – | 8.06 | – | – | – | – |
| 2-39 | *Pseudomonas chlororaphis* | + (1.22) | – | – | – | – | – | – |
| 2-45 | *Brevibacterium linens* | + (0.76) | – | – | – | 14 | 17 | – |
| 2-47 | *Bacillus licheniformis* | + (0.82) | – | – | – | 4 | – | – |
| 2-52 | *Rhodococcus fascians* | – (0.76) | 1.23 | – | – | – | – | – |
| 2-54 | *Erwinia persicina* | + (1.04) |  | 11.86 |  | 10 |  |  |
| 2-57 | *Rahnella aquatilis* | + (1.04) | – | 1.25 | 17 | 10 | 17 | – |
| 2-60 | *Sphingomonas macrogoltabidus* | – (0.88) | – | 5.88 | – | – | – | – |
| 2-63 | *Flavobacterium johnsoniae* | + (0.92) | – | – | – | – | – | – |
| 2-64 | *Flavobacterium johnsoniae* | + (0.9) | – | – | – | 4 | – | – |
| 2-68 | *Enterobacter agglomerans* | + (1.1) | – | 1.75 | – | 10 | – | – |
| 2-70 | *Enterobacter agglomerans* | + (1.1) | – | 2.42 | – | 10 | – | – |

**Table 2** (*continued*).

**Table 2 (continued).**

| Isolate | Isolate identification by FAME analysis | Siderophore production[a] | ACC deaminase activity[b] | Indole production[c] (μg·mL$^{-1}$) | % Inhibition of:[d] | | | Canola root elongation[e] (cm) |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Pythium* | *Rhizoctonia* | *Fusarium* | |
| 2-96 | *Pseudomonas putida* | + (1.32) | | | | 10 | | |
| 2-106 | *Pseudomonas putida* | + (1.3) | – | – | 10 | – | 20 | – |
| 3-10 | *Pseudomonas veronii* | + (1.45) | 0.5 | 1.87 | – | – | – | 5.93 |
| 3-13 | *Arthrobacter polychromogenes* | + (1.49) | – | – | 20 | 17 | 10 | – |
| 3-19 | *Bacillus licheniformis* | + (1.07) | – | – | – | 7 | – | – |
| 3-31 | *Bacillus pumilus* | + (1.23) | – | – | – | – | – | 5.75 |
| 3-32 | *Pseudomonas corrugata* | + (1.02) | 0.67 | 1.67 | – | – | – | – |
| 3-67 | *Pseudomonas veronii* | + (1.15) | 0.63 | 1.67 | – | – | 10 | 5.58 |
| 3-76 | *Pseudomonas syringae* | + (1.04) | 1.52 | 1.71 | – | – | – | 5.69 |
| 3-89 | *Nocardia asteroides* | + (0.64) | – | – | – | – | 14 | – |
| 3-106 | *Bacillus firmus* | + (1.19) | 0.69 | – | – | – | 20 | 5.53 |
| 3-109 | *Pseudomonas corrugate* | + (1.22) | 0.63 | – | – | – | – | – |
| 3-117 | *Erwinia persicina* | + (1.31) | | 7.85 | | | | |
| 4-2 | *Pseudomonas veronii* | + (1.13) | – | – | 17 | 23 | 17 | – |
| 4-6 | *Pseudomonas veronii* | + (1.31) | – | – | 7 | 17 | 23 | – |
| 4-8 | *Pantoea agglomerans* | + (1.26) | – | 2.02 | – | – | – | – |
| 4-9 | *Hafnia alvei* | + (1.05) | – | – | – | 10 | 50 | – |
| 4-15 | *Pseudomonas veronii* | + (0.95) | – | – | 10 | – | – | – |
| 4-17 | No match | + (0.76) | – | – | – | – | – | – |
| 4-19 | *Erwinia persicina* | + (1.32) | – | – | 7 | 10 | – | – |
| 4-20 | *Erwinia persicina* | + (1.27) | – | 9.69 | – | – | – | – |
| 4-31 | *Serratia proteamaculans* | + (1.39) | – | – | – | 37 | 50 | – |
| 4-42 | *Pseudomonas syringae* | + (1.07) | 1.64 | 1.49 | | | | |
| 4-46 | *Pseudomonas corrugate* | + (1.06) | 0.57 | 1.71 | – | – | – | – |
| 4-56 | *Pseudomonas syringae* | + (0.88) | 0.38 | – | – | – | – | 6.74 |
| 4-61 | *Streptoverticillium recticulum* | + (1.0) | – | – | – | – | 10 | – |
| 4-62 | *Flavobacterium johnsoniae* | + (0.93) | – | – | – | – | – | – |
| 4-65 | No match | + (1.43) | – | – | – | 14 | – | – |
| 5-1 | *Pseudomonas veronii* | + (1.31) | 1.27 | – | – | 7 | – | 7.67 |
| 5-3 | *Microbacterium liquefaciens* | + (1.22) | – | – | – | 10 | – | – |
| 5-4 | *Pseudomonas chlororaphis* | + (1,29) | – | – | – | 14 | – | – |
| 5-6 | *Serratia plymuthica* | + (1,32) | – | – | 7 | 37 | 33 | – |
| 5-21 | *Rahnella aquatilis* | + (1.3) | – | 1.52 | 10 | 17 | – | – |
| 5-24 | *Rahnella aquatilis* | + (1,31) | | | 10 | 20 | | |
| 5-28 | *Pseudomonas putida* | + (1.16) | 0.21 | – | – | – | – | 6.17 |
| 5-37 | *Klebsiella terrigena* | + (1.72) | – | 6.42 | – | – | – | – |
| 5-48 | *Enterobacter agglomerans* | + (1.32) | – | – | 10 | – | – | – |
| 5-51 | *Erwinia persicina* | + (1.18) | – | 10.14 | – | – | – | – |
| 5-58 | *Nocardia globerula* | + (1.27) | 0.38 | – | – | – | – | – |
| 5-68 | *Xanthomonas axonopodis* | + (0.74) | – | – | – | – | 7 | – |
| 5-79 | *Klebsiella terrigena* | + (1.[illegible]4) | – | 6.65 | – | – | – | – |
| 5-80 | *Pseudomonas putida* | + (1.5) | 1.31 | – | – | 17 | – | 6.69 |
| 5-105 | *Erwinia persicina* | + (1.5) | – | 11.55 | – | – | – | – |
| 5-109 | *Subtercola pratensis* | – (1.[illegible]4) | – | 2.49 | – | – | – | |
| 6-2 | *Alcaligenes piechaudii* | + (1.[illegible]5) | – | – | 7 | 23 | – | – |

**Table 2** (*concluded*).

| Isolate | Isolate identification by FAME analysis | Siderophore production[a] | ACC deaminase activity[b] | Indole production[c] (μg·mL$^{-1}$) | % Inhibition of:[d] *Pythium* | *Rhizoctonia* | *Fusarium* | Canola root elongation[e] (cm) |
|---|---|---|---|---|---|---|---|---|
| 6-4 | *Enterobacter agglomerans* | + (1.42) | – | – | – | 20 | 50 | – |
| 6-5 | *Comamonas acidovorans* | + (1.4) | – | – | – | 17 | 50 | – |
| 6-7 | *Enterobacter intermedius* | + (1.41) | – | – | – | 23 | 37 | – |
| 6-8 | *Pseudomonas veronii* | + (0.98) | 1.76 | | | | | 6.84 |
| 6-9 | *Microbacterium esteraromaticum* | + (1.38) | 1.82 | – | – | – | – | 6.74 |
| 6-18 | *Enterobacter agglomerans* | + (1.37) | – | – | – | 23 | 37 | – |
| 6-20 | *Enterobacter agglomerans* | + (1.37) | – | – | 7 | 23 | 40 | – |
| 6-25 | *Hafnia alvei* | + (1.12) | – | – | – | 37 | 40 | – |
| 6-32 | No match | + (1.0[illegible]) | – | 3.93 | – | – | – | – |
| 6-34 | *Enterobacter agglomerans* | + (1.36) | – | – | – | 23 | 40 | – |
| 6-50 | *Enterobacter agglomerans* | + (1.29) | – | – | – | 23 | 40 | – |
| 6-51 | *Enterobacter intermedius* | + (1.0) | – | – | – | 33 | 33 | – |
| 6-55 | *Pseudomonas putida* | + (0.9[illegible]) | 1.02 | – | – | – | – | 6.52 |
| 6-56 | *Corynebacterium bovis* | + (1.38) | – | 3.41 | – | – | – | – |
| 6-57 | *Erwinia persicina* | + (1.3) | – | 4.31 | – | – | – | – |
| 6-63 | *Pseudomonas putida* | + (0.99) | 1.67 | 1.37 | – | – | – | 5.56 |
| 6-76 | *Enterobacter intermedius* | + (1.22) | – | | | 40 | 43 | |
| 6-87 | *Pseudomonas putida* | + (1.49) | – | | – | 10 | – | – |
| 6-96 | *Xanthomonas axonopodis* | – (0.91) | – | 2.52 | – | – | – | – |
| 6-98 | *Hafnia alvei* | + (1.42) | – | – | – | 37 | 53 | – |
| 6-99 | *Pseudomonas putida* | + (1.46) | – | – | – | 23 | – | – |
| 6-108 | No match | + (0.85) | – | 1.22 | 10 | 20 | 47 | – |
| 6-113 | *Xanthomonas axonopodis* | – (0.82) | – | 2.44 | – | – | – | – |
| 6-114 | *Pseudomonas chlororaphis* | + (1.21) | – | – | – | 17 | 7 | – |
| 6-117 | *Enterobacter agglomerans* | + (1.24) | – | – | – | 57 | 47 | – |
| 6-124 | *Arthrobacter globiformis* | + (1.28) | – | 2.1 | – | – | – | – |
| 6-140 | *Xanthomonas axonopodis* | – (0.87) | – | 3.48 | – | – | – | – |

[a] +, indicates change in colour of medium from blue to orange, positive for siderophore production; –, indicates no change in colour of medium, negative for siderophore production. The number in parentheses indicates isolate growth in siderophore production medium as measured by OD$_{450}$ following 48 h of incubation at 20 °C. The OD$_4$ of siderophore-positive strain *Pseudomonas syringae* was 0.77.

[b] Isolate growth as measured by OD$_{405}$, indicating the ability to use ACC (amino-cyclopropane-1-carboxylic acid) as sole nitrogen source. –, indicates no growth with ACC as sole nitrogen source. The OD$_{405}$ of GR12-2, ACC deaminase-positive strain, was 0.44.

[c] Production of indoles following growth for 7 days in Dworkin and Foster minimal medium supplemented with tryptophan and using Salkowski's reagent and indole acetic acid as a standard. –, indicates no indole production. OD was measured at 600 nm.

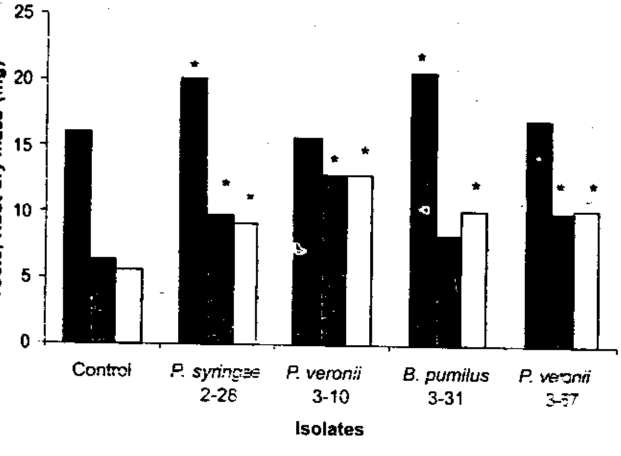
[d] Percent suppression of growth of fungal isolates by the soil isolates on in vitro assays on potato dextrose agar medium.

[e] Root length (cm) of canola following inoculation of the seed with soil isolates and growth in gnotobiotic pouches for 7 days. Values are shown only for isolates showing significant root growth promotion and are the means of 5 replicates. LSD = 0.84 ($P$ = 0.05). –, indicates no effect on root length compared with that of the noninoculated control. Root length of the noninoculated control was 4.25 cm.

**Table 3.** Plant growth-promotion traits of the 116 rhizobacterial isolates grouped by family, as determined by fatty acid methyl ester analysis.

| Family | No. of isolates | Siderophore production | ACC deaminase activity | Indole production | Inhibition of: *Pythium* | *Rhizoctonia* | *Fusarium* | Canola root elongation |
|---|---|---|---|---|---|---|---|---|
| *Alcaligenaceae* | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| *Bacillaceae* | 6 | 6 | 2 | 0 | 0 | 3 | 1 | 3 |
| *Brevibacteriaceae* | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| *Comamonadaceae* | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| *Corynebacteriaceae* | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Enterobacteriaceae* | 40 | 39 | 0 | 19 | 13 | 26 | 21 | 0 |
| *Enterococcaceae* | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Flavobacteriaceae* | 3 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| *Microbacteriaceae* | 3 | 2 | 1 | 1 | 0 | 1 | 0 | 1 |
| *Micrococcaceae* | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| *Nocardiaceae* | 4 | 3 | 3 | 0 | 0 | 0 | 1 | 1 |
| *Nocardioidaceae* | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Paenibacillaceae* | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Pseudomonadaceae* | 40 | 40 | 22 | 9 | 9 | 16 | 9 | 12 |
| *Sphingomonadaceae* | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Streptomycetae* | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Xanthomonadaceae* | 4 | 1 | 0 | 3 | 0 | 0 | 1 | 0 |
| No match | 5 | 5 | 0 | 2 | 2 | 3 | 3 | 0 |

**Fig. 1.** The effect of selected rhizobacterial isolates on root length, number of lateral roots, and root dry mass of lentil 'Milestone' following growth for 27 days at 8 °C. Bars are the means of 5 replicates and an asterisk above the bar indicates that the mean was significantly greater than that of the noninoculated control ($P$ = 0.05). Lentil root length (black bar), number of lateral roots per plant (grey bar), and lentil root mass (white bar).



putative traits for plant growth promotion (Vessey 2003; Glick 2005). Five hundred and sixty-three bacteria isolated from the rhizosphere of pea, lentil, and chickpea from commercial fields in Saskatchewan were screened for traits commonly attributed to PGPR. Of these only 3% showed plant growth-promoting ability in a model canola root elongation assay, and 29% of these (or 0.9% of the original 563 isolates) promoted growth of lentil or pea. Thus, the ability to promote legume growth was rare among culturable isolates from these agricultural soils. In a similar study screening for soybean PGPR, Cattelan et al. (1999) reported that 7 of 116 isolates (6%) increased early soybean growth. Khalid et al. (2004) showed that of 30 rhizosphere bacteria isolated from wheat, 4 (13%) that were strong auxin producers in vitro were effective in enhancing the root growth of 2 cultivars of wheat under gnotobiotic conditions. Ashgar et al. (2004) screened 100 rhizobacteria isolated from *Brassica* species for growth-promoting activity of *Brassica napus* L. under gnotobiotic conditions and 58% increased root length.

Production of iron-chelating siderophores was the most common plant growth-promotion trait observed among the isolates, with 76% testing positive. Similar results were reported by Principe et al. (2007) in the characterization of 72 isolates from saline soils in Argentina, and Cattelan et al. (1999) found that 32% of 22 isolates exhibiting PGPR traits were positive for siderophore production.

Microorganisms capable of lowering levels of ACC, a biosynthetic precursor of the phytohormone ethylene, may promote greater root growth (Glick 2005; Saleem et al. 2007); indeed this is observed with *P. fluorescens* CHA0 transformed with the ACC deaminase gene (Wang et al. 2000). Growth on ACC as a sole nitrogen source, implying ACC deaminase production, was much less common then siderophore production among the isolates, with only 5% displaying this trait. This is in agreement with earlier reports that only a minority of soil microorganisms possess ACC deaminase (Honma and Shimomura 1978; Glick et al. 1999). Blaha et al. (2006) demonstrated the presence of the gene for ACC deaminase (*acdS*) in phytopathogenic and opportunistic human pathogenic as well as phytobeneficial *Proteobacteria*. Wang et al. (2001) reported that 70% of 87 biocontrol pseudomonads producing 2,4-diacetylphloroglucinol isolated from around the world possess ACC deaminase. In this study, 59% of the 39 pseudomonad isolates identified possessed ACC deaminase activity.

IAA, a plant growth regulator responsible for cell elongation, division, and plant development, is produced by many plant-associated microorganisms (Arshad and Frankenberger 1993; Vessey 2003). Indole production was detected in 7% of the isolates. In contrast to the isolates that possessed

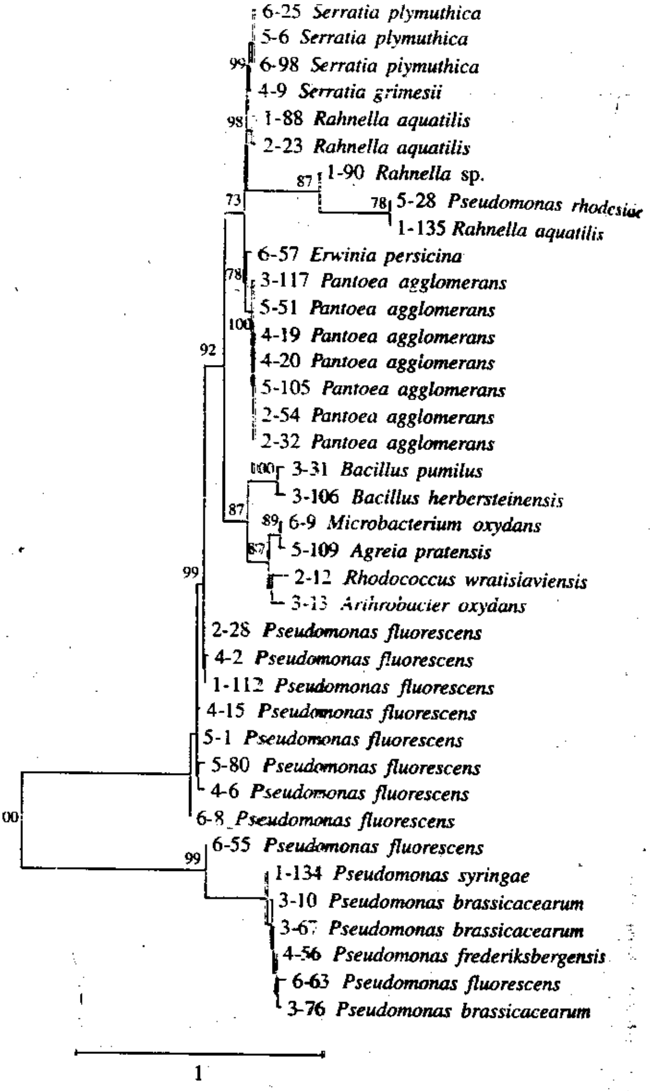
ACC deaminase, indole producers were represented by several of the bacterial families isolated from the rhizosphere of pea, lentil, and chickpea. The *Enterobacteriaceae* were the dominant indole producers followed by *Pseudomonadaceae* and *Xanthomonadaceae*.

Antagonism between rhizobacteria and soil-borne phytopathogens is a basic characteristic of survival in the rhizosphere and is the principle behind biocontrol in the rhizosphere (Whipps 2001; Compant et al. 2005). In this study, 5% of the isolates suppressed the in vitro growth of *Pythium* sp. strain p88-p3, 7% of *F. avenaceum*, and 9% of *R. solani* CKP7. While *Pseudomonas* species have received considerable attention because of their capacity to suppress soil-borne pathogens (Walsh et al. 2001; Haas and Defago 2005), members of the *Enterobacteriaceae* were the predominant fungal antagonists in this study, followed by the *Pseudomonadaceae*. *Enterobacteriaceae* are also commonly reported inhabitants of the rhizosphere and demonstrate antagonistic traits that contribute to their success in this nutrient-rich and highly competitive environment (Berg et al. 2005). *Enterobacter* and *Serratia* spp. have been readily detected in the rhizosphere of many crop and non-crop plant species, e.g., pea (Elvira-Recuenco and van Vuurde 2000), wheat (Kim et al. 2005), corn (Naureen et al. 2005), tomato and reed grass (Holguin and Glick 2001), and strawberry (Berg et al. 2002).

Possession of one or more of these PGPR traits was not a good predictor of the ability of a bacterial isolate from this collection to promote plant growth. For example, 16 of the canola growth promoters possessed ACC deaminase activity; however, 13 other isolates from the collection with ACC deaminase activity did not promote canola root growth. This contrasts with reports of others that ACC deaminase activity can be used as an efficient method for selection of PGPR (Glick et al. 1995; Penrose and Glick 2003). The capacity for indole production in vitro was not predictive of the ability to promote canola root growth, and isolates with the highest production of indoles, 2-54, 5-51, and 5-105 (Table 2) were all *Panotoea agglomerans* (Fig. 2) and none promoted canola root growth. This contrasts with the findings of Khalid et al. (2004) and Ashgar et al. (2004) who reported a strong correlation between in vitro auxin production and plant growth promotion in wheat and canola, respectively. However, further studies of our collection of indole-producing isolates have shown that several of these isolates have the ability to promote plant growth (Sergeeva et al. 2007).

There was some specificity between PGPR and the host plant, as 24% of the canola growth promoters were effective for lentil and only 6% for pea. Of the 4 isolates that increased lentil root growth, *P. syringae* 2-28 displayed the greatest variety of plant growth-promoting traits, including biocontrol activity against 2 of the fungal pathogens, siderophore production, and ACC deaminase activity, whereas *P. veronii* 3-67 showed biocontrol activity against one fungal pathogen, siderophore and indole production, and ACC deaminase activity. The other lentil growth-promoting rhizobacteria, *P. veronii* 3-10 and *Bacillus pumilus* 3-31, showed no antagonism to the fungal pathogens and had 3 and 1 other plant growth-promoting traits, respectively. The only isolate that enhanced pea root growth, *P. veronii* 5-1, produced siderophores, had ACC deaminase activity, and was antagonistic to *R. solani* CKP7. It is interesting that the lentil PGPRs were isolated from soil that had grown lentil in rotation for the past 25 years and the pea PGPR was isolated from soil with a similar history of pea growth.

**Fig. 2.** Phylogenetic comparison among selected rhizobacteria isolated from the roots of pea, lentil, and chickpea based on analysis of their full-length 16S rRNA sequences. The scale bar represents a branch length of 1 nucleotide substitution per site.



FAME identification of 111 of the isolates and 16S rRNA gene sequencing of a subset of 38 of the isolates indicated that the dominant culturable bacterial families isolated from the rhizosphere of pea, lentil, and chickpea in Saskatchewan were members of the *Pseudomonadaceae* and *Enterobacteriaceae*. Four of 5 lentil and pea growth promoters were *Pseudomonas* species. The ability of *Pseudomonas* isolates in this study to produce siderophores, indoles, and ACC deaminase, and to antagonize legume fungal pathogens emphasizes their potential as biofertilizers or as biopesticides. The prevalence of *Pseudomonas* spp. in the rhizosphere has been well documented and reviewed (Espinosa-Urgel 2004; Boer et al. 2005). Their presence in the rhizosphere is attributed to their ability to establish, compete, and survive in the rhiosphere, due in part to their diverse catabolic systems that degrade polymeric molecules from root exudates and to their production of antimicrobial metabolites (Barea et al. 2005; Haas and Defago 2005). The *Enterobacteriaceae* are also frequently detected in the rhizosphere and, like the *Pseudomonadaceae*, promote plant growth by several direct and indirect mechanisms (Benizri et al. 2001; Dobbelaere et al. 2003; Vessey 2003; Berg et al. 2005). However, their potential to act as opportunistic human pathogens (Berg et al. 2005) may limit their development as commercial biopesticides or biofertilizers.

The development of a local collection of rhizosphere bacteria and selection of putative PGPR is the first step in biofertilizer and (or) biopesticide product development for western Canadian pea and lentil crops. Research to optimize fermentation protocols and formulation development that maximizes the efficacy and shelf-life of biofertilizers and biopesticides is required to facilitate effective field studies and to lower the risk associated with the development of these agricultural products by industry (Nelson 2004; Hynes and Boyetchko 2006).

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