

## Cultivar-specific growth promotion of spring wheat (*Triticum aestivum* L.) by coexistent *Bacillus* species<sup>1</sup>

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Seven bacterial strains belonging to the genus *Bacillus* were isolated from rhizosphere soil of spring wheat (*Triticum aestivum* L.) cv. Katepwa. The plants from which the microorganisms were isolated were collected from a field that had been continuously cropped with spring wheat for the past 27 years. Cultivar Katepwa had been grown for the last 5 years whereas the closely related cv. Neepawa had been cultivated for the 12 previous years. When tested as single strain inoculum in a sterilized sand and Turface (montmorillonite clay) mix, six of seven isolates promoted root growth of cv. Katepwa spring wheat ( $p < 0.05$ ), but none promoted that of cv. Neepawa or of the more distant genetic relative cv. HY320. Shoot height was also increased in cv. Katepwa ( $p < 0.1$ ), but shoot weight of all three cultivars was unaffected by inoculation. Consequently, the root to shoot weight ratio of cv. Katepwa was also greater because of bacterization. These results indicate that beneficial bacteria may be isolated by employing a natural plant cultivar enrichment technique.

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Sept souches bactériennes appartenant au genre *Bacillus* ont été isolées du sol de la rhizosphère de plants de blé de mars (*Triticum aestivum* L.) cv. Katepwa. Ces bactéries ont été isolées à partir de plants récoltés dans un champ qui avait été réservé exclusivement à la culture du blé de mars depuis les 27 dernières années. Le cv. Katepwa y avait été cultivé pendant les derniers 5 ans alors que le cultivar très apparenté Neepawa y avait été cultivé pendant les 12 années précédentes. Chacune des souches isolées a été inoculée séparément dans un mélange stérilisé de sable et Turface (argile de montmorillonite). La croissance des racines du cv. Katepwa a été favorisée par six des sept souches de *Bacillus* ( $p < 0.05$ ), mais aucune souche n'a favorisé la croissance chez le cv. Neepawa ou le cv. HY320 dont la parenté génétique est plus lointaine. La hauteur des tiges est aussi favorisée chez le cv. Katepwa ( $p < 0.1$ ), mais l'inoculation n'a pas fait varier le poids des tiges chez aucun des trois cultivars. En conséquence le rapport de poids racine : tige du cv. Katepwa était encore plus grand à cause de la colonisation bactérienne. Ces résultats montrent bien que des bactéries utiles peuvent être isolées en employant une technique naturelle d'enrichissement du cultivar d'une plante.

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Plant growth-promoting bacteria are a diverse group of soil microbes capable of increasing crop yields through one or more of a variety of mechanisms (Brown 1974; Suslow 1982; Gaskins et al. 1985; Schroth and Weinhold 1986). Most strains investigated belong to the genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, or *Bacillus* and some are members of the *Enterobacteriaceae*.

The strategies that have been employed for isolation of such potentially useful bacteria differ significantly. Strains can be isolated from randomly gathered soil or root samples with subsequent testing on several different species or varieties of plants in the hope of finding useful combinations (Holl et al. 1988). Such trials can be laborious and time consuming as isolates often have no effect on plant growth or cause yield reductions.

Lifshitz et al. (1986) reasoned that plants endemic to regions with cool climates may harbor rhizosphere microbes that enhance performance of plants when grown at lower temperatures. These authors have reported success in isolating

microbes from plants growing in the Canadian Arctic and these isolates showed plant growth-promoting activity on soybean (*Glycine max* (L.) Merrill) (Polonenko et al. 1987) and canola (*Brassica campestris* L.) (Lifshitz et al. 1987); we have seen a growth response in lentil (*Lens esculenta* Moench.) using some of their isolates (unpublished results).

Reliance on a mass screening program or travel to environmental extremes may be unnecessary for the isolation of agriculturally useful microbes. Lie et al. (1987) have compiled evidence which suggests that legumes and root nodule bacteria have coevolved to form effective symbiotic associations. Similarly, Mytton et al. (1977) have demonstrated that the specific *Rhizobium* strain and legume genotype interaction can be the major determinant of symbiotic effectiveness. Person (1959) has suggested that in an evolutionary context, gene-for-gene relationships between plants and microbial pathogens are likely to be the rule.

If there is pressure for growth-promoting microbes to adapt to host plants, then the probability of observing a positive effect on plant performance due to inoculation with coexistent bacterial strains should be greater than if plants are inoculated with strains to which they have not been previously exposed. Recently, Chanway et al. (1988) provided evidence that growth promotion may occur when a plant genotype is inoculated with soil bacteria with which it had previously

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coexisted. However, because the plant species used in that study were cross-pollinated, it was necessary to use clonal material to maintain genetic uniformity. The practical application of these relationships may be more effectively demonstrated using a self-pollinating, agronomically important crop which has been grown in the same field for some time. Consequently, wheat was chosen for further investigation in the present study as it has been the mainstay of Canadian prairie agriculture for decades.

Spring wheat responds positively to inoculation with *Azospirillum* spp. and *Bacillus* sp. (Rennie and Larson 1979; Kapulnik et al. 1985). Pseudomonads stimulate growth of winter wheat (Elliot and Lynch 1984, 1985), although the majority of the isolates studied by these authors were inhibitory. Because *Azospirillum* is not a common microbial component of field soils in western Canada (Rennie 1980; Germida 1985), isolates belonging to the genus *Bacillus* were chosen for our study. The growth response to these commonly occurring soil microbes may depend on relatively minor differences in the genetic make-up of the host plant (Rennie and Larson 1979). Therefore, the objectives of this study were (i) to determine whether coexistent *Bacillus* isolates could be useful as agricultural inoculants for spring wheat, and (ii) to determine the degree of specificity which may exist between plant and bacterial genotypes with respect to plant growth-promoting activity of the inoculant strains.

Bacteria were isolated from rhizosphere soil samples containing roots of spring wheat (*Triticum aestivum* L.) cv. Katepwa. Plants were collected at the Agriculture Canada Research Station at Scott, Saskatchewan, from a field that has been continuously cropped with three spring wheat varieties in succession for the past 27 years. The varietal history of the field is as follows: cv. Canthatch, years 1-10; cv. Neepawa, years 11-22; and cv. Katepwa, years 23-27.

*Bacillus* species were isolated from rhizosphere soil and assayed for acetylene reducing capability as previously described (Rennie 1981; Holl et al. 1988). Isolates were further tested for their reaction to Gram stain, for endospore formation, and for motility (Gerhardt 1981).

Plants were grown in glass tubes (125 mm × 25 mm in diameter) filled with a sand and Turface (montmorillonite clay; Applied Industrial Materials Corp., Deerfield, IL, U.S.A.) mixture (69% w/w silica sand; 29% w/w Turface; 2% w/w CaCO<sub>3</sub>). A nutrient solution (25 mL) containing (g/L): KH<sub>2</sub>PO<sub>4</sub>, 0.14; KNO<sub>3</sub>, 0.51; Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 1.18; MgSO<sub>4</sub>, 0.49; Sequestrene 330 Fe (CIBA-GEIGY, Mississauga, Ont.), 0.02; H<sub>3</sub>BO<sub>3</sub>, 0.001; MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.001; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.001; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.0001; and Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.001 was added to each tube. Tubes containing the fertilized sand and Turface mixture were autoclaved for 1 h before use in experiments.

Seeds of spring wheat (*T. aestivum* L.) cv. Katepwa, cv. Neepawa, and cv. HY320 were obtained from Dr. W. L. Crowle, Agriculture Canada Research Station Saskatoon, Saskatchewan. Seed was surface sterilized by immersion in 70% (v/v) ethanol for 10 s followed by a 2 min soak in 5% (v/v) H<sub>2</sub>O<sub>2</sub>. After several rinses in sterile distilled water, three seeds were aseptically sown per tube, and were covered with ca. 10 mm sterile silica sand.

Seven isolates possessing characteristics of the genus *Bacillus* (Rennie 1980, 1981; Sneath 1986) were grown in separate nutrient broths for 2 days, harvested by centrifugation (10 000 × g for 10 min) and resuspended in fresh sterile

nutrient broth to a concentration of ca. 10<sup>7</sup> cells per mL (OD<sub>420</sub> = 0.15). Plant tubes were then inoculated with 1 mL of the appropriate bacterial suspension followed by the addition of 1 mL sterile distilled water. Control tubes received sterile nutrient broth or 0.1 M MgSO<sub>4</sub> and water but no bacteria. Five days after sowing, plants were thinned to one plant per tube.

Plants were grown in a growth chamber (Convion, Winnipeg, Man.) with a 15 h photoperiod, a day-night temperature regime of 20:15°C, and photosynthetically active radiation at the canopy level of 500 μE m<sup>-2</sup> s<sup>-1</sup>. After growth for 4 weeks, shoot height from the surface of the sand was recorded and plants were harvested. Roots were separated from shoots and plant material was dried for 2 days (70°C) before weights were recorded.

The effect of seven *Bacillus* isolates on performance of three wheat varieties was studied using a completely random design with eight replicates. Treatment combinations were arranged factorially, resulting in 8 levels of inoculation × 3 varieties of wheat to give 24 treatments. The entire experiment was repeated and all data were pooled and analyzed with experiments as an additional factor. The homogeneity of the variance test was significant under these conditions, but not when inoculation treatments within each variety were tested. Therefore, results from each variety were analyzed separately. Pairwise comparisons of each treatment mean with the uninoculated control were made using Fisher's protected LSD. Comparisons involving the pooled treatment mean and the control were done by using an individual degree of freedom test (orthogonal contrast).

The isolates used in these experiments possessed characteristics that were consistent with those of the genus *Bacillus*. These included the production of endospores, a facultative oxygen requirement, motility, a variable reaction to Gram stain, and the capacity for *in vitro* acetylene reduction (Rennie 1980, 1981; Rennie and Thomas 1987; Sneath 1986).

Plant performance was unaffected by the addition of 1 mL nutrient broth (data not shown). Shoot dry weight of all varieties tested was unaffected by bacterial treatment (Table 1). However, root dry weight was significantly increased by six of the seven isolates when tested on cv. Katepwa (Table 2). The average increase in root weight of this variety due to inoculation was 26% (*P* < 0.05). Root weight of cultivars Neepawa and HY320 were statistically unaffected by inoculation. Due to the magnitude of the root effect, all isolates significantly increased the root to shoot weight ratio of cv. Katepwa spring wheat but not of cultivars Neepawa or HY320 (data not shown).

Shoot height was unaffected by inoculation with any single bacterial isolate, but the composite inoculum mean for cv. Katepwa was 3% (*P* < 0.1) higher than the uninoculated control (Table 3). Again, cultivars Neepawa and HY320 were unaffected by inoculation when this yield variable was measured.

Evidence is accumulating which suggests that a plant genotype rhizosphere bacterial strain interaction is influential in the expression of some agriculturally important traits (Holl 1983; Gerhardson and Clarholm 1986). Much of this evidence has related plant and bacterial genotypic interactions to the effectiveness of nonlegume associative dinitrogen fixation (Dobereiner and Campelo 1971; Baldani and Dobereiner 1979; Rennie and Larson 1979; Saric et al. 1987). Rennie and Thomas (1987) speculated that higher rates of dinitrogen fixa-

TABLE 1. Effect of inoculation with *Bacillus* isolates on shoot dry weight (mg) of three cultivars of spring wheat

<i>Bacillus</i> isolate	cv. Katepwa	cv. Neepawa	cv. HY320
Control	116 ± 5 <sup>a</sup>	117 ± 5	107 ± 4
1A2	109 ± 4 (-6) <sup>bc</sup>	117 ± 3 ( 0)	106 ± 3 (-1)
1B	118 ± 5 (+2)	119 ± 5 (+2)	108 ± 5 (+1)
3A	113 ± 5 (-3)	116 ± 5 (-1)	104 ± 4 (-3)
5A1	122 ± 6 (+5)	114 ± 4 (-3)	105 ± 4 (-2)
5A2	110 ± 6 (-5)	114 ± 8 (-3)	102 ± 6 (-5)
5B	112 ± 5 (-3)	118 ± 3 (+1)	104 ± 5 (-3)
5C	120 ± 5 (+4)	112 ± 5 (-4)	98 ± 6 (-8)
Composite mean (7 isolates)	115 ± 2 (-1) <sup>d</sup>	116 ± 2 (-1)	104 ± 2 (-3)

<sup>a</sup> Means ± standard errors; *n* = 16.

<sup>b</sup> Values in parentheses represent the percent change from the control due to inoculation within each variety.

<sup>c</sup> Comparisons of individual treatment means with the control utilized Fisher's protected LSD.

<sup>d</sup> Comparisons of the composite treatment mean and the control utilized an individual degree of freedom test (orthogonal contrast).

TABLE 2. Effect of inoculation with *Bacillus* isolates on root dry weight (mg) of three cultivars of spring wheat

<i>Bacillus</i> isolate	cv. Katepwa	cv. Neepawa	cv. HY320
Control	98 ± 8	151 ± 17	109 ± 8
1A2	111 ± 8 (+13)	140 ± 13 (-7)	113 ± 8 (+4)
1B	123 ± 9 (+26)*	130 ± 10 (-14)	110 ± 7 (+1)
3A	122 ± 8 (+25)*	131 ± 14 (-13)	104 ± 5 (-5)
5A1	135 ± 11 (+38)*	158 ± 14 (+5)	97 ± 6 (-11)
5A2	118 ± 10 (+20)*	162 ± 22 (+7)	94 ± 10 (-14)
5B	132 ± 14 (+35)*	163 ± 15 (+8)	99 ± 7 (-9)
5C	120 ± 9 (+22)*	158 ± 21 (+5)	88 ± 9 (-19)
Composite mean (7 isolates)	123 ± 4 (+26)*	149 ± 6 (-1)	101 ± 3 (-8)

NOTE: Values are given as means ± SE; the percent change from the control is listed in parentheses.

\**p* < 0.05

TABLE 3. Effect of inoculation with *Bacillus* isolates on shoot height (cm) in three cultivars of spring wheat

<i>Bacillus</i> isolate	cv. Katepwa	cv. Neepawa	cv. HY320
Control	36.9 ± 0.4	37.8 ± 0.6	28.1 ± 0.3
1A2	37.4 ± 0.5 (+1)	38.2 ± 0.7 (+1)	26.8 ± 0.4 (-5)
1B	37.9 ± 0.5 (+3)	38.9 ± 0.6 (+3)	27.2 ± 0.3 (-3)
3A	37.9 ± 0.4 (+3)	37.5 ± 0.6 (-1)	26.8 ± 0.5 (-5)
5A1	38.4 ± 0.7 (+4)	37.1 ± 0.6 (-2)	27.6 ± 0.5 (-2)
5A2	38.8 ± 0.6 (+5)	37.1 ± 0.6 (-2)	27.9 ± 0.9 (-1)
5B	37.6 ± 0.6 (+2)	37.9 ± 0.4 ( 0)	26.7 ± 0.4 (-5)
5C	38.6 ± 0.6 (+5)	36.9 ± 0.7 (-2)	28.4 ± 0.4 (+1)
Composite mean (7 isolates)	38.1 ± 0.2 (+3)	37.7 ± 0.2 ( 0)	27.3 ± 0.2 (-3)

NOTE: Values are given as means ± SE; the percent change from the control is listed in parentheses.

tion associated with spring wheat cultivars Cadet and Rescue when inoculated with *Bacillus* C-11-25 as compared with *Azospirillum* spp. may be explained in terms of evolving genetic compatibility between plants and microbes. *Azospirillum* spp. are not common in western Canadian soils whereas *Bacillus* C-11-25 was isolated from the rhizosphere

of a disomic chromosome substitution line of cv. Cadet grown in the field in Canada.

Recent work suggests that the relationship between plants and rhizobacteria which promote growth through mechanisms other than associative dinitrogen fixation may be similar (Chanway et al. 1988). In that study, it was

observed that plant growth promotion of white clover (*Trifolium repens* L.) by *Bacillus polymyxa* was improved when the inoculant strain had previously coexisted with the plant genotype used in the tests. Plant material used in those experiments was collected from a pasture that was sown in 1939 and has since been managed only by adjustment of the timing and intensity of grazing by cattle (Aarssen and Turkington 1985).

Our hypothesis was that growth of cv. Katepwa and (or) cv. Neepawa would be enhanced by inoculation with at least some of the *Bacillus* isolates collected from a field currently cropped with cv. Katepwa and having a recent history of exposure to cv. Neepawa. These two cultivars have similar parentage and are virtually indistinguishable in the field (W. L. Crowle, personal communication). However, root weight of cv. Katepwa was substantially lower than that of cv. Neepawa in uninoculated treatments which indicates that genetic differences between the two varieties do exist (Table 2). Cultivar HY320 was utilized as an inoculation response control because it is genetically more removed from cv. Katepwa and has no known history of coexistence with these microbes. Data from the present study support the hypothesis that bacteria may adapt to a plant genotype as coexistent *Bacillus* strains promoted root growth and shoot height of cv. Katepwa but not of cultivars Neepawa or HY320 (Tables 2 and 3).

From an ecological perspective, these data suggest that bacterial adaptation to plants may occur within 5 years, which corresponds to the length of time that cv. Katepwa has been grown in this field. The fact that all bacterial isolates tested promoted growth of cv. Katepwa emphasizes the potential influence that plants may exert on rhizosphere microflora.

Neal et al. (1970, 1973) showed that chromosome substitution in lines of spring wheat affects rhizosphere microflora qualitatively and quantitatively. While it would be premature to hypothesize that a gene-for-gene relationship similar to that of host-pathogen pairs (Flor 1955) operates between plants and growth-promoting microbes, it appears that growth promotion depends on relatively minor genetic differences between hosts (Rennie and Larson 1979; Holl 1983; Chanway et al. 1988).

Although the mechanism by which the growth response occurs was not investigated in this study, root-associated dinitrogen fixation can almost certainly be excluded as a major contributor to the observed effect. Plants were grown in a concentration of nitrate (5 mM KNO<sub>3</sub> and 5 mM Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O) that should diminish or completely suppress nitrogenase activity (Munns 1977; Rigaud 1981; Gibson and Jordan 1983). Furthermore, enhanced nitrogen nutrition should favor shoot development over that of the root system, but shoot weight was unaffected by inoculation whereas root weight increased by an average of 26%.

Increased availability of nutrients remains a possible explanation, but two lines of evidence suggest that phytohormone production by *Bacillus* may be the cause of the growth response. These include the observations that (i) plant height was increased by inoculation (Table 3,  $P < 0.1$ ) without a corresponding change in shoot weight, and (ii) root growth was clearly favored over shoot growth, resulting in significantly higher root to shoot weight ratios.

Plants were grown under conditions which should have restricted biotic interactions to those involving only the plants and inoculum bacteria. It remains to be seen whether or not the plant and microbe genotypic interaction is strong enough

to be manifest in the field and under what conditions a shoot or grain yield advantage may be realized. Work is continuing to further characterize the nature of the growth response and to determine the mechanism(s) underlying it.

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