Population Dynamics of *Bacillus* sp. L324-92R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ in the Rhizosphere of Wheat

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Accepted for publication 7 February 1997.

ABSTRACT

Kim, D.-S., Weller, D. M., and Cook, R. J. 1997. Population dynamics of *Bacillus* sp. L324-92R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ in the rhizosphere of wheat. *Phytopathology* 87:559-564.

Bacillus sp. L324-92 is suppressive to three root diseases of wheat, namely take-all caused by *Gaeumannomyces graminis* var. *tritici*, Rhizoctonia root rot caused by *Rhizoctonia solani* AG8, and Pythium root rot caused by several *Pythium* species. Populations of strain L324-92R₁₂, a rifampicin-resistant mutant of L324-92 applied as a seed treatment, were monitored in the rhizosphere and spermosphere of wheat and compared with populations of *Pseudomonas fluorescens* 2-79RN₁₀, a known, rhizosphere-competent, biocontrol agent. In growth chamber studies, the population sizes of L324-92R₁₂ on roots of wheat were approximately 1,000-fold smaller than those of 2-79RN₁₀ at 5 days after planting, but, thereafter, they increased while those of 2-79RN₁₀ decreased until the two were equal in size at 45 days after planting. In the field with winter wheat, the population sizes of L324-92R₁₂ on roots were at least 10-fold smaller than those of 2-79RN₁₀ during the fall (November 1993) and

early spring (March 1994). Thereafter, the population of L324-92R₁₂ remained constant or increased slightly, while the population of 2-79RN₁₀ decreased until the two were roughly the same at 10⁴ to 10⁵ CFU/plant over the period of 150 days (April 1994) until 285 days (harvest) after planting. In growth chamber studies, strain L324-92R₁₂ remained confined to root sections within 3.5 cm below the seed, whereas 2-79RN₁₀ was recovered from all root sections ranging from 0.5 to 6.5 cm below the seed. In the field on winter wheat, both strains were recovered from root sections down to 5.0 to 6.5 cm below the seed at 75 days after planting (mid December), but only 2-79RN₁₀ was recovered at this depth at 90 days after planting. Both strains were recovered from the seed remnants 6 months after planting in the field. Both strains also were recovered from inside the roots and shoots, but population sizes of strain 2-79RN₁₀ were greater than those of L324-92R₁₂.

Additional keywords: biological control, microbial ecology, plant growth-promoting rhizobacteria, *Triticum aestivum*.

At the present time, the examples of antagonists available commercially for biological control of plant pathogens are either applied directly to the infection courts on the plants (root dip or seed coating) where the protection is needed or they are incorporated into the rooting medium at an inoculum density sufficient to ensure direct contact with the infection courts, primary inoculum of the pathogen(s), or both. The use of antagonists in this way does not depend on ability of the microorganism to spread from an inoculum applied one place, e.g., on the seed, to distant courts of infection elsewhere on the plant, e.g., onto the foliage or into the rhizosphere. These successes represent important technical advances for biological control of plant pathogens, and they will help enormously in gaining acceptance and increasing familiarity within the agribusiness community, and society in general, for this approach to plant disease management. However, many diseases for which biological control is needed as part of the management strategy will require that the antagonist colonize plant parts distant from the point of inoculation as an epiphyte, endophyte, or both,

and provide defense against infections for the entire life of the plant, if necessary.

Recently, we reported on a unique *Bacillus* sp., designated as strain L324-92 (7), that suppressed three root diseases of wheat, namely take-all caused by *Gaeumannomyces graminis* var. *tritici*, Rhizoctonia root rot caused by *Rhizoctonia solani* AG8, and Pythium root rot caused by several *Pythium* species, when applied as a seed inoculation. In addition to ability to suppress these three root diseases under controlled conditions, strain L324-92 was shown to (i) protect wheat against take-all, Rhizoctonia root rot, or both in field trials; (ii) increase yields of wheat in fields in which one or more of the three diseases were yield-limiting factors; (iii) provide in vitro inhibition to all isolates of *G. graminis* var. *tritici*, species and anastomosis groups of *Rhizoctonia*, and species of *Pythium* tested, and (iv) grow at temperatures from 4 to 40°C. However, the ability of this strain to colonize the rhizosphere of wheat was unknown.

Bacillus species have been considered less rhizosphere-competent compared with *Pseudomonas* species and, perhaps for this reason, most research aimed at development of biological control agents for introduction into the rhizosphere has been with fluorescent *Pseudomonas* species (22). However, considering the genetic diversity within the genus *Bacillus* in soil and the wheat rhizosphere (13), the evidence-come-principle that rhizosphere competence is a characteristic of the strain (genotype) rather than genus or species of soil microorganism, and the reports of wheat rhizosphere colonization by *Bacillus* species (6,11,15), it would seem likely that rhizosphere-competent genotypes occur within the genus *Bacillus*. Studies of Juhnke et al. (6) with spring wheat

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Publication no. P-1997-0321-02R

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in Montana, and Milus and Rothrock (15) with winter wheat in Arkansas both showed that select strains of *Bacillus* species introduced on seed established in the rhizosphere of wheat as well as, or better than, strains of fluorescent *Pseudomonas* species. However, their results do not indicate whether the introduced strains colonized the entire length of root sampled or only the first few centimeters of root below the seed. Maplestone and Campbell (11) reported high populations of *B. mycoides* and *B. pumilus* in the wheat rhizosphere at 20 to 30 cm below the site of inoculation of bacteria in liquid culture at 0.2 ml/seed at the time of planting (equivalent to an in-furrow drench at about 750 liters/ha). They concluded that the bacteria were carried downward either with water infiltration or on the tips of elongating wheat roots. Halverson et al. (3) demonstrated in field studies that *B. cereus* UW85 was the dominant *B. cereus* on roots of soybean at harvest after its introduction on the seed at planting, but they also reported that the population of this biocontrol agent, selected for its ability to protect alfalfa and soybeans against damping-off, remained confined mostly to the top 2 to 3 cm of soybean roots. Liu and Sinclair (9,10) reported that *B. megaterium* B153-2-2 colonized the rhizosphere of soybean under field conditions to a depth of 20 cm in the soil when introduced into the seed furrow at planting as a dry product consisting of bacteria grown on ground soybean stems. However, whether this strain would also colonize the roots of soybean to this depth when introduced as a seed treatment, as likely to be required for commercial use, is not known.

The objective of our study was to determine the ability of *Bacillus* sp. L324-92R₁₂ to colonize the rhizosphere of wheat when applied as a seed inoculation. We included *Pseudomonas fluorescens* 2-79RN₁₀, a well-described biocontrol agent, as a benchmark and an example of a highly rhizosphere-competent strain (1,5,20,21).

MATERIALS AND METHODS

Studies of rhizosphere competence. A spontaneous rifampicin-resistant mutant of *Bacillus* sp. L324-92, designated L324-92R₁₂, was obtained as described by Liu and Sinclair (10). Strain L324-92R₁₂ was identical to the wild type in cultural characteristics and ability to suppress Rhizoctonia root rot in the growth chamber (data not shown). *P. fluorescens* 2-79RN₁₀ is a rifampicin- and nalidixic acid-resistant mutant of strain 2-79 that has been studied extensively because of its ability to suppress take-all of wheat (20,21). For stock cultures, both strains were grown in 5 ml of nutrient broth-yeast extract (NBY) broth (19), harvested by centrifugation, resuspended in NBY broth amended with 40% glycerol, and stored at -80°C. For routine culturing, L324-92R₁₂ was grown on 1/10 tryptic soy agar (TSA) (12) and 2-79RN₁₀ was grown on King's medium B agar (8) at room temperature (21 to 24°C) for 48 h. Strains were not subcultured more than three times to assure viability and genetic purity.

Treatment of wheat seeds with bacteria. Seeds of wheat, *Triticum aestivum* L. cv. Penawawa were disinfected as described by Mazzola and Cook (14) and coated with bacteria by a method similar to that described by Weller et al. (23). Briefly, seeds were immersed in 5.25% sodium hypochlorite for 3 min, rinsed for 30 min under running tap water with a final rinse in sterile water, dried under a stream of sterile air, and stored at 21 to 24°C in a new plastic bag. One milliliter of each test bacterium was spread on plates of NBY and incubated for 48 h at 21 to 24°C. Four milliliters of 0.5% (wt/vol) methylcellulose was added to each plate, and the vegetative cells were scraped into suspension in the methylcellulose. The methylcellulose suspension with each bacterium was mixed with 10 g of the seeds in a petri plate, and the coated seeds were then dried for 30 min under a stream of sterile air. The initial population density of bacteria on the seeds was determined by dilution-plating on 1/10 TSA. Colonies were counted after 2 to 3 days of incubation at 21 to 24°C.

Assays of population dynamics under controlled conditions.

Initially, strains L324-92R₁₂ and *P. fluorescens* 2-79RN₁₀ were compared for ability to colonize the spermosphere and rhizosphere of wheat in tube assays in the growth chamber. Plastic tubes (4.0 cm diameter × 20.5 cm long) were plugged in the bottom with cotton and filled with 50 ml of sterile vermiculite followed by 50 g of Thatuna silt loam from the Washington State University (WSU) Plant Pathology Research Farm, Pullman (17). The soil was collected from the top 10 to 15 cm of the soil profile and sifted through a 0.5-cm-mesh screen before use. Pasteurized soil refers to moist soil that was treated at 60°C for 30 min using air mixed with steam. Raw soil refers to the same soil without heat treatment. Soils were prepared 1 week prior to each experiment and air-dried at room temperature on a bench in the greenhouse.

One seed, coated with cells of either L324-92R₁₂ or 2-79RN₁₀, was sown on the soil surface in each tube and then covered with 20 ml of sterile vermiculite. Each tube received 30 ml of tap water at planting and was incubated in the growth chamber under a 12-h photoperiod at 15°C. Each tube received 10 ml of dilute (1/3, vol/vol) Hoagland's solution once each week beginning 10 days after planting. After 45 days, individual plants were removed from the tubes by pushing the column of soil and vermiculite containing the plants upward from the bottom and then gently shaking the seedling to remove excess soil from the roots. The remnant seed was removed for subsequent analysis, and then the root system was excised at a point 0.5 cm below the point of seed attachment to reduce the possibility of contamination from inoculum on the seed. Spermosphere populations were estimated by placing two remnant seeds in 5 ml of phosphate buffer for 30 min, vortexing at the maximum speed for 30 s, and dilution-plating on 1/10 TSA amended with rifampicin and cycloheximide each at 100 µg/ml (TSA_{rc}).

Rhizosphere populations were estimated by placing the roots representing one plant in 50 ml of sterile 0.0125 M phosphate buffer (1.21 g of KH₂PO₄, 280 g of K₂HPO₄, and 1 liter of deionized water; pH 7.1), shaking on a rotary shaker at 300 rpm for 30 min at 21 to 24°C, and dilution-plating on 1/10 TSA_{rc}. Preliminary studies demonstrated that greater than 95 and 90% of the populations of L324-92R₁₂ and 2-79RN₁₀, respectively, were removed from the roots by this method (data not shown). Five plants were assayed at each sampling date.

To study spatial distribution along the root axis, individual roots were sampled at 10, 17, and 24 days after planting, and each root was divided into 1.5-cm-long sections starting 0.5 cm below the seed. Each section was placed individually in 5 ml of phosphate buffer in a test tube for 30 min, vortexed at the maximum speed for 30 s, and aliquots of washings were plated on 1/10 TSA_{rc}. For each strain, three roots from different plants were sectioned and assayed at each sampling date.

To estimate both the external (rhizoplane and rhizosphere combined) and internal population sizes of L324-92R₁₂ and 2-79RN₁₀, four 3-cm-long segments of seminal roots from a single plant, severed at 0.5 and 3.5 cm below the seed, were placed in 5 ml of phosphate buffer for 30 min, vortexed at the maximum speed for 30 s, macerated, and dilution-plated on 1/10 TSA_{rc}. To estimate only the internal population sizes, 3-cm-long root sections were immersed in 5.25% sodium hypochlorite for 2 min, washed twice with sterile water, placed in 5 ml of phosphate buffer for 30 min, and vortexed at the maximum speed for 30 s. Following this treatment, root washings only were dilution-plated on 1/10 TSA_{rc} to check effectiveness of the surface sterilization. Surface-sterilized root fragments were then macerated in a mortar, and the root macerates resuspended in 5 ml of phosphate buffer and dilution-plated on 1/10 TSA_{rc}. The initial root washing, the root washing after surface sterilization, and the root macerates each were dilution-plated in duplicate onto TSA_{rc}. External population sizes were estimated by colony counts from the initial root washing, and internal population sizes were estimated by colony counts from the root ma-

cerates (made from surface-sterilized roots) on TSA_{rc}. Four plants treated with either cells of L324-92R₁₂ or 2-79RN₁₀ were used at each sampling time.

To estimate external and internal population sizes of bacteria associated with the shoots of wheat, a 3-cm-long fragment of shoot severed at 0.5 and 3.5 cm above the seed was processed as described above. The external shoot population was estimated by colony counts from the initial shoot washings, and the internal population was estimated by colony counts from the surface-sterilized shoot macerates plated on $1/10$ TSA_{rc}. Four shoots were used at each sampling time. The external and internal populations of each strain in either roots or shoots were compared within each strain by the Student *t* test using the Statistical Analysis System (SAS Institute, Cary, NC).

Population dynamics in the field. Colonization of the wheat rhizosphere by strains L324-92R₁₂ and *P. fluorescens* 2-79RN₁₀ was studied in a field plot established on the WSU Plant Pathology Research Farm, Pullman, in 1993. Seeds of the winter wheat cultivar Madsen, treated with either L324-92R₁₂ or 2-79RN₁₀, were hand-sown on 30 October 1993. Rows were 2.44 m long and spaced 1.5 m apart, and seeds were sown 3 cm deep. Each repli-

cated treatment consisted of five rows with 10 g of seed per row. Treatments were replicated six times. Population sizes of strains L324-92R₁₂ and 2-79RN₁₀ in the rhizosphere were estimated starting at 14 days and continuing until 280 days after planting. Because of frozen soil, samples were not collected during December 1993 and January 1994. At each sampling date, three plants were dug at random from the three center rows, and excess soil was removed from the roots by gentle shaking. Plants were placed in a new plastic bag, transferred to the laboratory, and stored at 4°C until processing the next day. Until the tillering stage, the whole subterranean root system including seminal and crown roots was processed. After tillering, only roots on the mainstem (not roots on tillers) were processed. Roots from three plants were pooled and washed in 50 ml (100 ml after tillering) of phosphate buffer in a plastic tube (5.5 cm diameter $\times$ 11.5 cm long) on a rotary shaker at 300 rpm for 30 min. Each root washing was dilution-plated on $1/10$ TSA_{rc} as described above. Remnant seeds were used to estimate the spermosphere population.

To determine the spatial distribution of bacteria along the axis of seminal roots, the longest seminal root available on plants dug at 75 or 90 days after planting was divided into four 1.5-cm-long

Fig. 1. Population sizes of *Bacillus* sp. L324-92R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ in the rhizosphere and spermosphere of wheat seedlings in pasteurized and raw Thatuna silt loam in a tube assay. **A**, Population dynamics in the rhizosphere of wheat planted in pasteurized soil. **B**, Population dynamics in the spermosphere of wheat planted in pasteurized soil. **C**, Population dynamics in the rhizosphere of wheat planted in raw soil. **D**, Population dynamics in the spermosphere of wheat planted in raw soil. The vertical bars indicate standard deviation.

fragments starting 0.5 cm below the seed. Each fragment was placed individually in 5 ml of phosphate buffer in a test tube for 30 min and vortexed for 30 s. The washing then was dilution-plated on $1/10$ TSA as described above. Three roots from different plants treated with either L324-92R₁₂ or 2-79RN₁₀ were used at each sampling time.

RESULTS

Population dynamics of strains L324-92R₁₂ and 2-79RN₁₀ in the spermosphere and rhizosphere of wheat in growth chamber studies. In the tube assay, both *Bacillus* sp. L324-92R₁₂ and *P. fluorescens* 2-79RN₁₀ colonized the roots of wheat in raw and pasteurized Thatuna silt loam. However, L324-92R₁₂ exhibited a different pattern of root colonization than *P. fluorescens* 2-79RN₁₀ (Fig. 1A and B). At 5 days after planting, the rhizosphere population sizes of strain L324-92R₁₂ were 4.06 and 3.59 log CFU/plant in pasteurized and raw soil, respectively. These populations were approximately 3 log CFU smaller than those of strain 2-79RN₁₀. Thereafter, the population of strain L324-92R₁₂ increased, while that of strain 2-79RN₁₀ decreased. At 45 days after planting (five- to six-leaf stage), the population sizes of L324-92R₁₂ were 5.77 and 5.38 log CFU/plant in pasteurized and raw soils, respectively, and those of 2-79RN₁₀ were 6.06 and 5.42 log CFU/plant in pasteurized and raw soils, respectively.

L324-92R₁₂ was not detected from root sections at distances greater than 3.5 cm below the seed at either 17 or 24 days after planting, whereas 2-79RN₁₀ was detected from all root sections sampled at these two times (Fig. 2).

Fig. 2. Population sizes of *Bacillus* sp. L324-92R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ on roots at the indicated distances below the seed in raw Thatuna silt loam in the tube assay. **A**, Population sizes at 10 days after planting. **B**, Population sizes at 17 days after planting. **C**, Population sizes at 24 days after planting. The vertical bars indicate standard deviation.

In the spermosphere, the initial populations of 7.18 log CFU/seed for L324-92R₁₂ and 8.16 log CFU/seed for 2-79RN₁₀ gradually declined in both pasteurized and raw soils. The introduced bacteria were still detected in the spermosphere at 45 days after planting, when spermosphere populations in pasteurized and raw soil were 5.87 and 5.13 log CFU/seed, respectively, for L324-92R₁₂, and 5.85 and 5.12 log CFU/seed, respectively, for 2-79RN₁₀ (Fig. 1C and D).

Strains L324-92R₁₂ and 2-79RN₁₀ were also recovered from inside both the roots and shoots of wheat (Fig. 3). Population sizes of L324-92R₁₂ in these two internal habitats varied greatly, but generally were smaller than those of 2-79RN₁₀ (Fig. 3).

Population dynamics of *Bacillus* sp. L324-92R₁₂ and *P. fluorescens* 2-79RN₁₀ in the field. Strains L324-92R₁₂ and 2-79RN₁₀ were recovered from the wheat rhizosphere throughout the growing season following application to the seed at the time of planting (Fig. 4). Until 150 days after planting (April 1994), the rhizosphere population sizes of L324-92R₁₂ were at least 1 log CFU smaller than those of 2-79RN₁₀, but, thereafter, they were roughly the same. Populations of both strains in the spermosphere gradually declined. Initial population sizes for L324-92R₁₂ and 2-79RN₁₀ from seed remnants were 6.09 and 6.90 log CFU/seed, respectively, but, at 214 days after planting, population sizes were 3.34 and 5.49 log CFU/seed, respectively. Populations of L324-92R₁₂ in the wheat spermosphere were never larger than those of 2-79RN₁₀ at these two sampling dates.

Strain L324-92R₁₂ was detected from all root sections obtained between 0.5 to 6.5 cm below the seed at 75 days after planting and from root sections ranging from 0.5 to 5.0 cm below the seed at 90

Fig. 3. Population sizes of *Bacillus* sp. L324-92R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ from inside and outside roots and shoots at the indicated positions of wheat grown for 28 days in raw Thatuna silt loam in the tube assay. For a given location (internal or external), means of bacterial number with the same letter in each graph are not significantly different at $P = 0.05$ by the Student t test. **A and B**, Population sizes on roots and shoots, respectively, for experiment 1. **C and D**, Population sizes on roots and shoots, respectively, for experiment 2. The vertical bars indicate standard deviation.

days after planting. In contrast, strain 2-79RN₁₀ was detected from all root sections ranging from 0.5 to 6.5 cm below the seed at both dates (Fig. 5). Populations of 2-79RN₁₀ from each root section were always higher than those of L324-92R₁₂.

DISCUSSION

A direct comparison of the ability of *Bacillus* sp. L324-92R₁₂ and *P. fluorescens* 2-79RN₁₀ to colonize wheat roots when introduced as a seed inoculation was made in an attempt to evaluate the rhizosphere competence of L324-92. Strain 2-79RN₁₀ was selected for comparison because its population dynamics in the rhizosphere of wheat, including long-distance transport on elongating roots of wheat, has been studied extensively (1,5,14, 20,21). The most obvious difference was that L324-92R₁₂ established at lower initial population sizes on seminal roots of wheat seedlings compared with 2-79RN₁₀, but, thereafter, the population of this *Bacillus* sp. stayed constant or gradually increased, while the relatively high initial population of 2-79RN₁₀ gradually declined. The net result was that the population sizes of the two strains were roughly equal by the time the experiments were terminated. A second obvious difference was in the vertical movement of the introduced bacteria along the root axis; the population of L324-92R₁₂ remained confined mainly to

the top 5 cm of roots in both the greenhouse and field studies, whereas 2-79RN₁₀ was recovered from sections 5.0 to 6.5 cm below the seed. Weller (21) showed earlier that 2-79RN₁₀ colonizes the seminal roots of wheat at least as deep as 6.0 to 7.5 cm below the seed under field conditions.

In fields with wheat seeds planted 3 to 4 cm deep, root colonization 5 cm below the seed would place the introduced bacteria 8 to 9 cm deep in the soil. For seeds planted 5 to 6 cm deep, the bacteria could be 10 to 11 cm deep in the soil. The inoculum of *Pythium* and *Rhizoctonia* species pathogenic to wheat typically is concentrated within the top 10 to 15 cm of soil, especially in direct-drilled fields (2). The inoculum of *G. graminis* var. *tritici* represented by colonized tiller bases likewise is concentrated in the top 10 to 15 cm of soil or less in direct-drilled fields (R. J. Cook, unpublished data), owing to the fact that this residue is left mostly undisturbed on or superficially within the soil surface when fields are direct-drilled. Inoculum of *G. graminis* var. *tritici* represented by colonized root tissues can occur deeper into the soil, but is also shorter lived (4). Ability to establish at least 5.0 cm below the seed would, therefore, be sufficient to provide protection against both *Pythium* and *Rhizoctonia* root rot and protection against at least some inoculum responsible for take-all. On the other hand, failure to establish populations deeper than 5.0 cm below the seed could leave roots unprotected against primary in-

Fig. 4. Population sizes of *Bacillus* sp. L324-R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ in the rhizosphere and spermosphere of a winter wheat planted on October 31, 1993, in the field at the Washington State University Plant Pathology Research Farm, Pullman. **A**, Population dynamics in the rhizosphere. **B**, Population dynamics in the spermosphere. **C**, Dry weight of mainstem roots. The vertical bars indicate standard deviation.

Fig. 5. Population sizes of *Bacillus* sp. L324-92R₁₂ and *Pseudomonas fluorescens* 2-79RN₁₀ on roots at the indicated distances below the seed in the field at the Washington State University Plant Pathology Research Farm, Pullman. **A**, Population sizes at 75 days after planting. **B**, Population sizes at 90 days after planting. The vertical bars indicate standard deviation.

oculum present at these depths and account for the inconsistent results obtained in our earlier field trials (7).

The tendency of L324-R₁₂ to remain confined to the top 5 cm of root is similar to the findings of Halverson et al. (3) for *B. cereus* UW85 in the rhizosphere of soybean. Long-distance transport of introduced rhizobacteria from an inoculum source on a seed or seedpiece occurs by the way of passive carriage on the root apex (5) or with percolating water (16). Strain 2-79RN₁₀ is easily transported by either method (1,21). In contrast, the larger cell size of the *Bacillus* compared with that of *Pseudomonas* is a potential handicap to its vertical movement, whether passively on the root apex or through soil pores with moving water, particularly in the silt loam used in this study. In a more sandy soil, we would expect L324-92R₁₂ to be moved more extensively by percolating water as shown by Maplestone and Campbell (11).

Strains L324-92R₁₂ and 2-79RN₁₀ each were recovered from the interior of both the root and shoots of wheat seedlings. These strains were isolated from the same field plot cropped continuously to irrigated wheat at Lind, WA, with 2-79 isolated in the twelfth and L324-92 in the twenty-fifth consecutive year of irrigated wheat. Strain L324-92 was selected by a process that enriched for endospore formers closely associated with the surface or the inside of the root (7). It is, therefore, not surprising that this strain can establish inside as well as on the surface of roots of wheat, indicated by the populations measured before and after surface sterilization. This is the first report of the ability of 2-79 to establish within both root and shoot tissues of wheat when introduced on the seed.

The rhizosphere competence of L324-92 in the top 10 to 12 cm of soil, along with its ability to produce broad-spectrum antibiotic(s)

and grow at temperatures as low as 4°C, can explain the biocontrol activity of this strain against take-all, *Rhizoctonia* root rot, and *Pythium* root rot of wheat under direct-drilled conditions in which soil temperatures are typically 5 to 10°C or less during the early part of the growing season of either spring or winter wheat (18).

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