Sensitivity of *Rhizoctonia* Isolates to Phenazine-1-Carboxylic Acid and Biological Control by Phenazine-Producing *Pseudomonas* spp.

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ABSTRACT

Rhizoctonia solani anastomosis groups (AG)-8 and AG-2-1 and *R. oryzae* are ubiquitous in cereal-based cropping systems of the Columbia Plateau of the Inland Pacific Northwest and commonly infect wheat. AG-8 and *R. oryzae*, causal agents of *Rhizoctonia* root rot and bare patch, are most commonly found in fields in the low-precipitation zone, whereas *R. solani* AG-2-1 is much less virulent on wheat and is distributed in fields throughout the low-, intermediate-, and high-precipitation zones. Fluorescent *Pseudomonas* spp. that produce the antibiotic phenazine-1-carboxylic acid (PCA) also are abundant in the rhizosphere of crops grown in the low-precipitation zone but their broader geographic distribution and effect on populations of *Rhizoctonia* is unknown. To address these questions, we surveyed the distribution of PCA producers (Phz⁺) in 59 fields in cereal-based cropping systems throughout the Columbia Plateau. Phz⁺ *Pseudomonas* spp. were detected in 37 of 59 samples and comprised from 0 to 12.5% of the total culturable heterotrophic aerobic rhizosphere bacteria. The frequency with which individual plants were colonized by Phz⁺ pseudomonads ranged from 0 to 100%. High and moderate colonization frequencies of Phz⁺ pseudomonads were associated with roots from fields

located in the driest areas whereas only moderate and low colonization frequencies were associated with crops where higher annual precipitation occurs. Thus, the geographic distribution of Phz⁺ pseudomonads overlaps closely with the distribution of *R. solani* AG-8 but not with that of *R. oryzae* or *R. solani* AG-2-1. Moreover, linear regression analysis demonstrated a highly significant inverse relationship between annual precipitation and the frequency of rhizospheres colonized by Phz⁺ pseudomonads. Phz⁺ pseudomonads representative of the four major indigenous species (*P. aridus*, *P. cerealis*, *P. orientalis*, and *P. synxantha*) suppressed *Rhizoctonia* root rot of wheat when applied as seed treatments. In vitro, mean 50% effective dose values for isolates of AG-8 and AG-2-1 from fields with high and low frequencies of phenazine producers did not differ significantly, nor was there a correlation between virulence of an isolate and sensitivity to PCA, resulting in rejection of the hypothesis that tolerance in *Rhizoctonia* spp. to PCA develops in nature upon exposure to Phz⁺ pseudomonads.

Additional keyword: barley.

Wheat (*Triticum aestivum* L.) is grown throughout the Pacific Northwest (PNW) in the United States, an area east of the Cascade Mountains of some 62,160 km² divided into three annual precipitation zones: low (<300 mm precipitation), intermediate (300–450 mm), and high (450 to 600 mm) (Schillinger et al. 2008). In the low-precipitation zone, directly east of the mountains and within their rain shadow, wheat has been grown for over 125 years under dryland conditions in an alternate winter wheat–summer fallow rotation (Schillinger and Papendick 2008). During the year of fallow, water accumulates in the soil profile to support a wheat crop the following year. Precipitation increases significantly with increasing distance to the east of the low-precipitation zone. In the easternmost part of Washington (high-precipitation zone known as the Palouse), winter wheat is often grown in a 3-year rotation with spring wheat or spring barley (*Hordeum vulgare* L.) and lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), or canola (*Brassica napus* L.). In the intermediate zone, cropping practices

transition and a winter wheat–spring wheat or barley–summer fallow rotation is often used.

A wide variety of soilborne fungal pathogens occur in cereal-based production systems throughout the Columbia Plateau of the Inland PNW. Four of the most important soilborne fungal diseases of cereals are *Rhizoctonia* root rot and bare patch caused by *Rhizoctonia solani* Kühn anastomosis group (AG)-8 and *R. oryzae* Ryker & Gooch (Paulitz et al. 2002; Weller et al. 1986); take-all caused by *Gaeumannomyces graminis* var. *tritici* J. Walker (Paulitz et al. 2002; Weller et al. 2007); *Pythium* root rot caused by a complex of *Pythium* spp., with *Pythium irregulare* groups I and IV and *P. ultimum* being the two most pathogenic species (Higginbotham et al. 2004; Paulitz and Adams 2003); and crown and root rot caused by *Fusarium pseudograminearum*, *F. culmorum*, and *Bipolaris sorokiniana* (Paulitz et al. 2002, 2009). The severity of take-all, *Rhizoctonia* root rot and bare patch, and *Pythium* root rot is exacerbated by reduced tillage (Paulitz et al. 2002), and *Rhizoctonia* root rot has become the most important disease in no-till (direct-seeded) cereal production. *R. solani* AG-2-1, the dominant *Rhizoctonia* sp. across the region, causes only mild root rot of cereals but is an important pathogen of canola, a rotation crop (Jaaffar et al. 2016; Paulitz et al. 2006).

R. solani AG-8 and *R. oryzae* are ubiquitous in wheat fields throughout the Columbia Plateau of the Inland PNW, yet the severity of root rot and bare patch differs throughout the region. Root rot is most severe in the low-precipitation zone (150 to 300 mm) when wheat is grown with reduced tillage and, under these conditions, classic “bare patches” appear in the field (Paulitz et al. 2009;

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Schillinger and Paulitz 2006). Patches may vary in size from only a few centimeters to tens of meters in diameter, and sometimes an entire field becomes a single patch. In contrast, in the high-precipitation zones such as the Palouse, patches rarely occur and Rhizoctonia root rot generally appears as unevenness in plant height throughout the field (Paulitz et al. 2002, 2009).

Mavrodi et al. (2012a) reported that large populations (greater than 10^5 CFU g^{-1} of root) of fluorescent *Pseudomonas* spp. producing the broad-spectrum antibiotic phenazine-1-carboxylic acid (PCA) colonized the roots and rhizosphere of wheat and barley grown in dryland (nonirrigated) fields in the low-precipitation zone. In addition, they found that the frequency of root systems colonized by PCA-producing (Phz⁺) pseudomonads was inversely related to annual precipitation but not to differences in soil temperature or composition (O. Mavrodi, D. Mavrodi, A. Panchenko, K. Cooper, D. Weller, and L. Thomashow, unpublished data), suggesting that these bacteria thrive in dry soils. PCA also was detected at nanomolar concentrations in the rhizosphere and there was a direct relationship between the amount of PCA extracted from the roots and the population density of Phz⁺ *Pseudomonas* spp. The *Pseudomonas* populations included at least 31 genotypes that clustered as *Pseudomonas synxantha*, *P. orientalis*, and the provisional species *P. aridus* and *P. cerealis* of the *P. fluorescens* complex, all of which carried the phenazine biosynthesis gene *phzF* and synthesized PCA (Parejko et al. 2012, 2013). Phz⁺ pseudomonads were highly inhibitory to *R. solani* AG-8 in vitro, suggesting that they might contribute to the protection of wheat and barley against Rhizoctonia root rot (Mavrodi et al. 2012a,b).

The role of phenazines in biocontrol of plant pathogens was first reported by Thomashow and Weller (1988), who demonstrated that mutants of *P. synxantha* (formerly *P. fluorescens*) 2-79 deficient in PCA synthesis were less suppressive of take-all of wheat than the parental strain. Pierson and Thomashow (1992) then showed that phenazine-deficient mutants of *P. chlororaphis* 30-84 also lost the ability to suppress take-all. Thomashow et al. (1990) recovered phenazines from the roots of wheat seedlings colonized by strains 2-79 or 30-84 but not from roots colonized by the phenazine-deficient mutants. Since those classic studies, there have been numerous reports of introduced phenazine-producing *Pseudomonas* spp. providing biocontrol of diverse soilborne pathogens (Chin-A-Woeng et al. 2003; Mavrodi et al. 2006; Pierson and Pierson 2010). However, few studies have evaluated the effectiveness of phenazine producers in the biocontrol of *Rhizoctonia* spp. For example D'ae et al. (2011) tested *Pseudomonas* strain CMR12a, which produces PCA and phenazine-1-carboxamide as well as cyclic lipopeptide (CLP) surfactants, for ability to suppress AG-2-2 and AG-4 HGI on bean. Mutants deficient in either phenazine or CLP production provided significantly less control of Rhizoctonia root rot than did the parental strain. A double mutant deficient in production of both compounds completely lost biocontrol activity, indicating that the CLP and phenazines function together in the control of *Rhizoctonia* spp. (D'ae et al. 2011).

Phenazine-producing *Pseudomonas* spp. with biocontrol activity have been isolated from numerous crops worldwide (Chin-A-Woeng et al. 2003; Mavrodi et al. 2006, 2010; Yang et al. 2011) and they also contribute to the suppressiveness of the soils in the Châteaurenard region of France to Fusarium wilt by working in concert with nonpathogenic *F. oxysporum* (Mazurier et al. 2009).

The current study is part of a broader project with the overarching goal of filling gaps in our knowledge about the biogeography of Phz⁺ fluorescent *Pseudomonas* spp. in the Inland PNW; the role of these pseudomonads in the health, productivity, and sustainability of dryland wheat; and their impact on soilborne pathogens and rhizosphere microbial communities in dryland cereal-based cropping systems. We hypothesize that Phz⁺ pseudomonads have a broader role in the control of *Rhizoctonia* spp. across the Columbia Plateau than previously recognized. Here, we have used classical and molecular approaches to assess the biogeographic overlap of PCA producers and *Rhizoctonia* spp. throughout the Columbia Plateau of the Inland PNW to determine whether long-term exposure of *Rhizoctonia* isolates to Phz⁺ fluorescent *Pseudomonas* spp. in the field reduces the sensitivity in *Rhizoctonia* spp. to PCA, and to evaluate the biocontrol activity against *R. solani* AG-8 of various species of Phz⁺ *Pseudomonas* spp. known to exist in cereal-based production systems. We show that, in the Columbia Plateau, the geographic distribution of PCA producers closely overlaps with that of AG-8 isolates, that *Rhizoctonia* AG and species differ in sensitivity to PCA, and that various genotypes of PCA producers found in cereal-based cropping systems are able to suppress Rhizoctonia root rot.

MATERIALS AND METHODS

Bacteria, fungi, and culture conditions. *Rhizoctonia* isolates for this study were collected in the summers of 2010 (117 isolates) and 2011 (180 isolates) by using the toothpick assay of Paulitz and Schroeder (2005), as described previously (Jaaffar et al. 2016). Phenazine-producing (Phz⁺) pseudomonads collected during the field surveys in 2010, as well as previously described strains used in the fungal inhibition analyses and representative of the Phz⁺ species *P. cerealis*, *P. aridus*, *P. orientalis*, and *P. synxantha* (Parejko et al. 2013) (Table 1), were cultured on King's medium B (KMB) broth or agar (King et al. 1954) at 27°C and stored in 40% glycerol at -80°C.

Field sampling. Fields in cereal-based cropping systems in the Inland PNW were surveyed for pseudomonads containing biosynthesis genes for PCA and the capacity to produce phenazine antibiotics. The survey included 59 sites that consisted of commercial fields and Washington State University Cooperative Extension Uniform Cereal Cultivar Testing sites located within the low (150 to 300 mm), intermediate (300 to 450 mm), and high (>450 mm) precipitation zones (Jaaffar et al. 2016) that were sampled from mid-May through mid-July 2010. In addition to winter wheat and spring wheat, samples of spring

TABLE 1. Bacterial strains used in this study

Strain and species	Source and characteristics ^z	Reference
L1-45-08r, <i>Pseudomonas cerealis</i>	Lind commercial winter wheat; genotype 8; Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2012, 2013
4(1):2-10r, <i>P. cerealis</i>	Lind uncultivated soil (thread grass- <i>Stipa comata</i>); Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2013
R1-43-08r, <i>P. aridus</i>	Ritzville commercial winter wheat; genotype 27; Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2012, 2013
S3-6:7-09r, <i>P. aridus</i>	Lind commercial winter wheat; Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2013
L1-3-08r, <i>P. orientalis</i>	Lind commercial winter wheat; genotype 1; Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2012, 2013
R2-66-08Wr, <i>P. orientalis</i>	Ritzville spring wheat rotation; genotype 3; Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2012, 2013
R6-28-08r, <i>P. synxantha</i>	Ritzville irrigated winter wheat; genotype 21; Phz ⁺ , biosurfactant ⁺ , spontaneous Rif ^r	Parejko et al. 2012, 2013
2-79RN10, <i>P. synxantha</i>	Lind spring wheat; genotype 18; Phz ⁺ , Rif ^r Nal ^r ; produces PCA	Thomashow and Weller 1988

^z Phz⁺ = strain produces phenazine-1-carboxylic acid (PCA), Rif^r = rifampicin resistance, Nal^r = nalidixic acid resistance, and biosurfactant⁺ = tests positive in the drop collapse assay for biosurfactant activity.

barley, spring pea, spring chick pea, and spring lentil also were collected. Longitudinal and latitudinal coordinates at each site were recorded using a Garmin global positioning system (GPS) device. At each location, three samples consisting of plants with adhering rhizosphere soil were dug with a shovel to a depth of approximately 20 cm. The first sample was taken approximately 100 m into the field and two additional samples were collected 25 m further into the field in a zig-zag pattern. Each sample in a field served as a replicate and was placed in a separate labeled plastic bag, transported to the laboratory, and stored at 4°C overnight for processing the next day.

From each replicate bag, four plants were assayed separately to determine the population sizes of total culturable heterotrophic aerobic bacteria and *Phz*⁺ pseudomonads, and the frequency with which individual root systems were colonized by *Phz*⁺ pseudomonads.

Enumeration of *Phz*⁺ and total culturable aerobic bacteria.

Bacteria were isolated from the roots of individual plants essentially as described by Mavrodi et al. (2012b). Briefly, each root system with adhering rhizosphere soil was placed in a 50-ml screw-cap centrifuge tube with 20 ml of sterile distilled water. The samples were vortexed vigorously (1 min) and sonicated (1 min) in an ultrasonic cleaner (Branson 521; Branson, Shelton, CT), and bacterial population sizes were determined using the dilution endpoint assay (McSpadden Gardener et al. 2001). An aliquot (100 µl) of the root wash solution was serially diluted (1:3) in a 96-well microtiter plate prefilled with 200 µl of sterile water, and 50 µl of each dilution was transferred to wells of two other microtiter plates containing (i) a semiselective medium for fluorescent *Pseudomonas* spp. consisting of one-third-strength KMB supplemented with cycloheximide (100 µg/ml), ampicillin (25 µg/ml), and chloramphenicol (13 µg/ml) (1/3 KMB⁺⁺⁺); and (ii) one-tenth-strength tryptic soy broth (1/10 TSB) (BD Biosciences, Franklin Lakes, NJ) supplemented with cycloheximide (100 µg/ml) (1/10 TSB⁺). These plates were incubated at room temperature (24 ± 1°C) in the dark for 72 h and then turbidity was assessed spectrophotometrically with a Bio-Rad model 680 microplate reader (Bio-Rad, Hercules, CA). An optical density at 600 nm (OD₆₀₀) > 0.1 was scored as positive for bacterial growth (Mavrodi et al. 2012b; McSpadden Gardener et al. 2001).

Each dilution well with growth in 1/3 KMB⁺⁺⁺ was screened for the presence of *Phz*⁺ bacteria by polymerase chain reaction (PCR) with primers targeting the biosynthesis gene *phzF* (primers Ps_up1 and Ps_low1) (Mavrodi et al. 2010). Strains carrying this marker routinely produce PCA in culture (Parejko et al. 2012). Population densities of *Phz*⁺ pseudomonads were calculated from the final dilution in which a *phzF* signal appeared. The final dilution in which bacterial growth occurred in 1/10 TSB⁺ was used to calculate the population size of total culturable heterotrophic aerobic bacteria.

Effect of pH on the growth of *Rhizoctonia* isolates. In preparation for assessing their sensitivity to PCA, *Rhizoctonia* isolates were first tested to determine the optimum pH for growth in vitro. Two isolates each of *R. solani* groups AG-8, AG-2-1, AG-10, and AG-I-like binucleate *Rhizoctonia* sp. and two isolates each of *R. oryzae* groups II and III were selected for these studies. Growth medium (one-fifth potato dextrose agar [1/5 PDA]) was buffered to six pH values ranging from pH 5.4 to pH 7.6 (pH 5.4, 6.0, 6.4, 6.8, 7.2, and 7.6) with 0.2 M phosphate buffer (Na₂HPO₄ and KH₂PO₄). This range of pH values was chosen because of a previous report by Sherwood (1970), who indicated that isolates of *Rhizoctonia* grew best between pH 5 and pH 7. Agar plugs (4 mm in diameter) were cut with a cork borer from the advancing margin of a 7-day-old culture of an isolate of *Rhizoctonia* grown on 1/5 PDA. The agar plugs were transferred with the mycelium facing down to the center of Petri dishes containing 1/5 PDA adjusted to the various pH values. Three plates were used per treatment and each plate served as a replicate. Radial growth on each plate was measured in four different directions from the edge of the plug to the edge of the mycelium every 12 h after inoculation for 5 days. The rate of growth, expressed as millimeters per day, was calculated based on radial growth at 120 h minus radial growth at 48 h divided by 3 days.

Sensitivity to PCA in vitro. Selected isolates of *R. solani* AG-8, AG-2-1, and AG-10; AG-I-like binucleate *Rhizoctonia*; and *R. oryzae* groups I, II, and III were tested for sensitivity to PCA in agar plate bioassays. In addition, in a separate experiment, 20 isolates each of *R. solani* AG-8 and AG-2-1 collected from regions where cereal roots are normally colonized by either low or high frequencies of PCA producers were tested in the plate bioassay to determine whether tolerance or resistance to PCA can develop in the field. For each assay, 4-mm-diameter agar plugs were cut with a cork borer from the margin of a 1-week-old culture of *Rhizoctonia* spp. grown on 1/5 PDA. The agar plugs were transferred with the mycelium facing downward to the center of 36-mm-diameter wells containing 5 ml of 1/5 PDA buffered to pH 6.0 with 0.01 M 2-(N-morpholino)ethanesulfonic acid (MES) (Sigma Aldrich, St. Louis) in a six-well tissue culture plate (Becton Dickinson Labware, Franklin Lakes, NJ). MES buffer was used to avoid possible effects on growth of the ionic strength of 0.2 M phosphate buffer. Wells in each plate were amended with PCA dissolved in ethyl acetate at 0, 3, 5, 10, 20, and 40 µg/ml. Mycelial growth was measured every 12 h after inoculation from the center of the plug to the edge of the mycelium. Each six-well plate (block) was replicated three times, and each measurement was performed in four different directions per well. Radial growth measurements were converted to percent growth inhibition by comparing growth on medium amended with PCA to that on medium with no PCA (control, ethyl acetate only). Growth measurements at 72 h provided the greatest difference between the control and PCA treatments and, thus, were used for the final analysis of growth inhibition. Growth inhibition data were transformed to a linear regression versus PCA concentration. Points from three replicate plates were pooled (18 points: three replicates × six dosages) to run the regression. The 50% effective dose (ED₅₀) values were calculated from the predicted regression equation.

Determination of virulence and pathogenicity. Twenty selected isolates each of *R. solani* AG-8 and AG-2-1 collected from fields with high or low frequencies of *Phz*⁺ rhizobacteria were tested for pathogenicity and virulence on wheat (*T. aestivum* ‘Louise’) by using a modification of the tube assay previously described by Jaaffar et al. (2016). Briefly, the assays were conducted in conical plastic tubes (cone-tainers, 2.5 cm wide at the top by 16.5 cm in height; Stewer and Sons, Tangent, OR) plugged with a cotton ball and hung in plastic racks (200 tubes/rack). Each tube was filled with a 10-cm column of sterilized vermiculite and then a 2-cm-layer of Quincy virgin (Shano sandy loam) pasteurized soil (60°C, 30 min.). Three plugs (5 mm in diameter) cut from a plate of PDA colonized by a fungal isolate were added to the top of the soil layer and then covered with a 1-cm layer of vermiculite. Controls consisted of tubes with plugs of PDA without mycelium. Three spring wheat seeds (Louise) were placed on the vermiculite and covered with another 1-cm layer of vermiculite. Each tube received 10 ml of water containing metalaxyl (wetttable powder at 0.075 g liter⁻¹ of tap water) (Syngenta, Wilmington, DE) to suppress indigenous *Pythium* spp. that can cause damping-off and interfere with the disease ratings. Each replicate of a treatment (isolate) consisted of five separate tubes; treatments were replicated three times and arranged in a randomized complete block design. The racks of tubes were covered with transparent plastic and incubated at 24°C for 2 days before being moved into a growth room at 16°C with a 12-h photoperiod (Jaaffar et al. 2016). After emergence, the plastic was removed and plants were irrigated twice a week with 12 ml of water and once with one-third-strength Hoagland’s solution (macroelements only) (Jaaffar et al. 2016). Four weeks after planting, seedlings were removed and washed under a stream of water. To determine the impact of an isolate on plant growth, shoot length was measured and root disease severity was rated on a scale of 0 to 8 (Huang et al. 2004), where 0 = healthy or uninfected (disease not evident) and 8 = plant dead or nearly so. This scale considers both root disease and the health of the foliage.

Preparation of oat kernel inoculum. Inoculum of *R. solani* AG-8 strain C-1 used in biocontrol assays was prepared essentially as described by Kwak et al. (2009). Briefly, 250 ml of whole oat grains and 300 ml of water were mixed in a 1-liter Erlenmeyer flask and autoclaved for 90 min on two consecutive days. Small pieces of agar cut from a 7-day-old culture of *R. solani* AG-8 isolate C-1 were added and the flasks were then incubated for 3 to 4 weeks at room temperature. Colonized oat kernels were dried for 2 days under a stream of sterile air and stored at 4°C.

Bacterial treatment of wheat seed. For biological control studies, bacterial strains were grown on KMB for 48 h at 27°C. Two to three loopfuls of each isolate were suspended in 1.0 ml of sterile water and washed twice by centrifugation for 3 min at 14,000 rpm in an Eppendorf centrifuge, and the suspension was adjusted to an OD₆₀₀ of 1. For each 4.68 g of seed, 173 µl of the bacterial suspension, 107 µl of water, and 280 µl of sterile 2% methylcellulose (MC) (Sigma-Aldrich) were mixed in a test tube, vortexed for 30 s, shaken by hand for 3 min, and then dried under sterile air in a laminar flow hood for at least an hour. Once dry, the inoculated seed were stored at 4°C until sown. The final density of the bacteria was approximately 10⁷ CFU seed⁻¹.

Rhizoctonia root rot suppression assay. A modification of the tube assay described above was used to determine the biocontrol activity of Phz⁺ bacteria against *R. solani*. Each tube was filled with a 10-cm column of sterilized vermiculite topped with a 2-cm-layer of air-dried and sieved pasteurized or nontreated Quincy virgin soil (Shano sandy loam). The soil was mixed with 0.7% (wt/wt) oat kernel inoculum of *R. solani* AG-8 strain C-1 that had been ground and sieved to obtain particle sizes of 0.25 to 0.5 mm. Three wheat seeds (Louise) coated with Phz⁺ wild-type bacteria in 1% MC were placed on the soil surface and covered with a 2-cm-thick layer of sterilized vermiculite. Controls consisted of tubes (i) with pathogen inoculum plus seed coated with 1% MC and (ii) with pathogen inoculum plus noncoated seed. There were five tubes per treatment and each treatment was replicated five times. Treatments were arranged in a randomized complete block design. The tubes were watered and incubated and disease was evaluated as described above.

Statistical analysis. To plot the geographic distribution and frequency of phenazine-producing *Pseudomonas* spp., maps were constructed with GPS coordinates of sampled fields by using ArcGIS 9.3.1 software (ESRI, Redlands, CA). Data from biological control experiments were analyzed by using STATISTIX 8.0 software (Analytical Software, St. Paul, MN). Bacterial population data were converted to log CFU g⁻¹ fresh weight of root. Differences in root disease (disease ratings) and plant height among treatments were determined by standard analysis of variance and mean comparisons among treatments were performed by using Fisher's protected least significant difference test ($P = 0.05$) or by the Kruskal-Wallis test ($P = 0.05$).

RESULTS

Population size and colonization frequency of Phz⁺ *Pseudomonas* spp. in the rhizosphere of dryland crops. Phz⁺ *Pseudomonas* spp. were detected in 37 of 59 samples of winter and spring wheat and spring barley collected from 59 fields in 2010 (Table 2). Population sizes ranged from below the limit of detection (log 3.2 CFU g⁻¹) to log 7.4 CFU g⁻¹ (fresh weight) of roots. Population sizes of total culturable heterotrophic aerobic bacteria in all 59 samples ranged from log 7.4 to log 8.9 CFU g⁻¹ (fresh weight) of root (Table 2). Phz⁺ pseudomonads comprised from 0 to 12.5% of the total culturable heterotrophic aerobic bacteria present in the rhizosphere microbiome.

The frequency with which individual plants within a sample were colonized by Phz⁺ pseudomonads ranged from 0 to 100% (Table 2). To simplify visualization of the distribution of Phz⁺ pseudomonads in the Columbia Plateau of the Inland PNW, colonization frequencies were combined into three frequency groups: low (0 to 0.32), medium (0.33 to 0.66), and high (0.67 to 1). High and medium colonization

frequencies were associated with wheat or barley roots from fields located in the driest areas of the Columbia Plateau (agronomic zones 4 and 5) (Fig. 1). In our study, Phz⁺ pseudomonads were not sampled from cereals grown in agronomic zone 6 (Fig. 1) but they are known to be present in high frequencies on wheat grown there (Mavrodi et al. 2012a). In contrast, only moderate and low colonization frequencies of Phz⁺ pseudomonads were associated with cereals in agronomic zones 1, 2, and 3, where higher annual precipitation occurs (Fig. 1). Linear regression analysis (Fig. 2) demonstrated a low but highly significant ($r^2 = 0.38$, $P = 0.005$) inverse relationship between annual precipitation (averaged for the sampled sites for the 30-year period from 1971 to 2000) and the frequency of rhizospheres of plants colonized by Phz⁺ pseudomonads.

Samples of pea ($n = 5$), lentil ($n = 3$), and chickpea ($n = 2$) collected from 10 separate fields in agronomic zones 1 and 2 all had low frequencies of Phz⁺ pseudomonads; in fact, all samples but two had no detectable colonization (Table 2).

Spatial relationship between Phz⁺ pseudomonads and *R. solani* AG-8 and 2-1. *R. solani* AG-8 is cosmopolitan in the Columbia Plateau but is most frequently isolated from fields located in arid areas (Jaaffar et al. 2016; Okubara et al. 2014). Figure 1, originally published in Jaaffar et al. (2016), has been modified here to show the overlap of the frequency (low, moderate, and high) of rhizospheres colonized by PCA⁺ pseudomonads and the number of AG-8 isolates in a given agronomic zone. The highest frequencies of Phz⁺ pseudomonads occurred in areas where AG-8 isolates also were most frequently isolated (agronomic zones 4 and 5). In agronomic zones 1, 2, and 3, both Phz⁺ pseudomonads and AG-8 isolates were isolated much less often. Similarly, Figure 3, also from Jaaffar et al. (2016), shows the overlap in frequency (low, moderate, and high) of rhizospheres colonized by PCA⁺ pseudomonads and AG-2-1 isolates in a given agronomic zone. In contrast to AG-8 isolates, AG-2-1 isolates did not overlap with Phz⁺ pseudomonads. AG-2-1 isolates are more evenly distributed throughout the Columbia Plateau of the Inland PNW compared with AG-8 isolates (Jaaffar et al. 2016).

Effect of pH on growth in vitro of *Rhizoctonia* AG and species. As a prelude to determining the sensitivity of *Rhizoctonia* AG and species to PCA, the pH at which growth is optimal was first determined in 1/5 PDA buffered to different pH values. The growth rate of the various *Rhizoctonia* isolates, expressed in millimeters per day, was modulated by the pH of the medium (Table 3; Supplementary Fig. S1). Among the isolates of AG-8, AG-2-1, AG-10, AG-I-like binucleate, and *R. oryzae* groups II and III, AG-10 had the fastest growth rate at pH values of 5.4 to 6.4 but AG-I-like binucleate *Rhizoctonia* sp. had the fastest rate among the isolates at pH 7.2 to 7.6. *R. oryzae* group III had the slowest growth rate of all isolates at all pH levels tested (Table 3). In general, all *Rhizoctonia* isolates grew at a slower rate at pH 6.4 and above as compared with at pH 5.4 and 6.0. Especially striking was the difference in growth of the isolates of *R. oryzae* groups II and III. On this basis, pH 6 was selected for studies of the in vitro inhibition of *Rhizoctonia* isolates by PCA.

Sensitivity of *Rhizoctonia* isolates to PCA. Isolates of AG-8, AG-2-1, AG-10, AG-I-like binucleate, and *R. oryzae* groups I, II, and III were tested for their sensitivity to PCA in PDA buffered to pH 6.0. ED₅₀ values varied among *Rhizoctonia* isolates (5 to 20 µg/ml), and isolates of different AG showed differential sensitivity to PCA. For example, AG-8 isolates were least sensitive to PCA but were not significantly different in their sensitivity from isolates of AG-2-1 and *R. oryzae* groups II and III. AG-8 isolates were significantly less sensitive to PCA than isolates of AG-10, AG-I-like binucleate *Rhizoctonia* sp., and *R. oryzae* group I. *R. oryzae* group II isolates were significantly less sensitive to PCA than group I isolates (Table 4). It is well known that long-term exposure of human, animal, or plant pathogens to antibiotics or fungicides can result in the development of resistance to the compound in their populations. We tested the hypothesis that tolerance to naturally occurring antibiotics can occur in nature. Twenty isolates each of AG-8 and AG-2-1 collected from fields where either high or low frequencies of Phz⁺ pseudomonads occurred

on the roots of cereal or grain legume crops were tested for sensitivity to PCA (Table 5). The ED₅₀ of isolates of AG-8 collected from fields with high and low frequencies of Phz⁺ pseudomonads ranged from 5 to 13 and 6 to 12 µg/ml, respectively, and AG-2-1 isolates from high- and

low-frequency Phz⁺ fields ranged from 8 to 16 and 5 to 12 µg/ml, respectively. The mean ED₅₀ values for all isolates of AG-8 from high and low Phz⁺ frequency fields were 10.6 and 9.2 µg/ml, respectively. For AG-2-1, the values were 10.8 and 9.1 µg/ml. Mean ED₅₀ values for

TABLE 2. Population sizes of phenazine-1-carboxylic acid-producing (Phz⁺) and total aerobic culturable heterotrophic bacteria and plant colonization frequencies on crops from fields in the Inland Pacific Northwest collected in 2010

Date ^r	Location ^s	Crop ^t	GPS coordinates ^u	Precip (mm) ^v	Total (CFU ± SD) ^w	Phz ⁺ (CFU ± SD) ^w	Freq (%) ^x	Cult (%) ^y
14 May	Dusty ^z	WW	N: 46° 51.902', W: 117° 42.162'	470	8.0 ± 0.4	ND	0/12 (0)	0
14 May	La Crosse	SW	N: 46° 48.578', W: 117° 53.124'	371	8.3 ± 0.6	6.3 ± 0.9	11/12 (91.7)	1
14 May	Hooper	WW	N: 46° 47.468', W: 118° 10.148'	320	8.1 ± 0.3	6.3 ± 0.5	12/12 (100)	1.6
14 May	Hooper	SW	N: 46° 47.468', W: 118° 10.148'	320	8.3 ± 0.6	6.7 ± 0.5	12/12 (100)	2.5
14 May	Cunningham	WW	N: 46° 49.378', W: 118° 40.283'	231	8.4 ± 0.5	6.4 ± 0.9	12/12 (100)	1
14 May	Lind ^z	WW	N: 47° 0.320', W: 118° 34.280'	252	7.7 ± 0.6	6.4 ± 0.8	12/12 (100)	5
14 May	Lind	SW	N: 47° 0.320', W: 118° 34.280'	252	7.7 ± 0.3	5.3 ± 1.1	12/12 (100)	0.4
14 May	Ritzville ^z	WW	N: 47° 8.198', W: 118° 53.035'	303	7.7 ± 0.5	6.3 ± 0.5	8/12 (66.7)	4
14 May	Marcellus	WW	N: 47° 12.346', W: 118° 23.138'	320	7.8 ± 0.4	5.7 ± 0.9	8/12 (66.7)	0.8
14 May	Odessa	WW	N: 47° 12.410', W: 118° 41.273'	358	7.8 ± 0.5	5.9 ± 1.1	12/12 (100)	1.3
14 May	Scharg	WW	N: 47° 07.185', W: 118° 54.971'	413	7.9 ± 0.5	5.9 ± 0.9	12/12 (100)	1
14 May	Lind	WW	N: 47° 6.041', W: 118° 53.035'	252	7.7 ± 0.4	6.1 ± 1.3	11/12 (91.7)	2.4
14 May	Connell ^z	WW	N: 46° 37.267', W: 118° 46.320'	396	7.4 ± 0.7	5.9 ± 1.0	11/12 (91.7)	3.2
14 May	Kahlotus	WW	N: 46° 38.635', W: 118° 33.487'	321	7.9 ± 0.6	5.1 ± 0.8	11/12 (91.7)	0.2
20 May	Chambers	SC	N: 46° 40.342', W: 117° 11.802'	574	8.2 ± 0.5	ND	0/12 (0)	0
20 May	Wawawai	SP	N: 46° 36.166', W: 117° 9.993'	484	8.9 ± 0.5	ND	0/12 (0)	0
20 May	Uniontown	WW	N: 46° 30.591', W: 117° 4.896'	603	8.2 ± 0.4	5.4 ± 0.2	3/12 (25)	0.2
20 May	Chard	WW	N: 46° 32.398', W: 117° 51.797'	411	8.3 ± 0.4	5.6 ± 0.8	12/12 (100)	0.2
20 May	Whetstone	SW	N: 46° 23.809', W: 117° 55.944'	307	8.1 ± 0.4	6.8 ± 0.7	8/12 (66.7)	5
20 May	Ronan	SP	N: 46° 22.036', W: 117° 55.726'	413	8.0 ± 0.7	7.1 ± 0	1/12 (8.3)	12.5
20 May	Waitsburg ^z	WW	N: 46° 16.347', W: 118° 7.675'	485	8.5 ± 0.5	5.1 ± 0.5	6/12 (50)	0.04
20 May	Coppei	SC	N: 46° 13.800', W: 118° 8.261'	428	8.2 ± 0.4	ND	0/12 (0)	0
20 May	Gillian	WW	N: 46° 7.832', W: 118° 11.055'	612	8.2 ± 0.4	5.9 ± 0.6	7/12 (58.3)	0.5
20 May	Lowden	WW	N: 46° 3.416', W: 118° 35.558'	273	8.4 ± 0.4	5.6 ± 1.3	8/12 (66.7)	0.2
20 May	Hadley	WW	N: 46° 6.009', W: 118° 22.363'	452	8.4 ± 0.4	6.6 ± 1.1	7/12 (58.3)	1.6
20 May	Berryman	WW	N: 46° 11.730', W: 118° 22.902'	343	8.6 ± 0.4	6.4 ± 1.2	4/12 (33.3)	0.6
20 May	Bolles	WW	N: 46° 17.082', W: 118° 14.572'	436	8.2 ± 0.5	ND	0/12 (0)	0
20 May	Colfax	SB	N: 46° 52.432', W: 117° 26.043'	498	8.6 ± 0.3	ND	0/12 (0)	0
7 June	St. John ^z	SW	N: 47° 4.845', W: 117° 31.218'	546	8.7 ± 0.4	ND	0/12 (0)	0
7 June	Lamont	SW	N: 47° 9.452', W: 117° 52.266'	413	8.5 ± 0.4	ND	0/12 (0)	0
7 June	Sprague	WW	N: 47° 20.318', W: 118° 0.215'	400	8.1 ± 0.6	4.3 ± 0.8	8/12 (66.7)	0.02
7 June	Harrington	SW	N: 47° 23.613', W: 118° 8.886'	358	8.9 ± 0.5	ND	0/12 (0)	0
7 June	West of Edwall	SB	N: 47° 30.057', W: 117° 57.360'	434	8.7 ± 0.4	4.7 ± 1.2	9/12 (75)	0.01
7 June	Edwall	WW	N: 47° 30.846', W: 117° 56.418'	423	7.9 ± 0.5	4.4 ± 0.7	4/12 (33.3)	0.03
7 June	Edwall	SW	N: 47° 30.846', W: 117° 56.418'	423	8.1 ± 0.5	ND	0/12 (0)	0
7 June	Reardon	WW	N: 47° 40.114', W: 118° 2.026'	320	8.1 ± 0.4	ND	0/12 (0)	0
7 June	Creston	WW	N: 47° 49.867', W: 118° 32.022'	323	8.1 ± 0.4	4.0 ± 0.6	4/12 (33.3)	0.01
7 June	Almira ^z	SW	N: 47° 48.975', W: 118° 51.725'	354	8.0 ± 0.5	ND	0/12 (0)	0
7 June	Brewster	SW	N: 48° 8.290', W: 119° 44.461'	326	8.4 ± 0.3	4.8 ± 1.9	9/12 (75)	0.03
7 June	Dyer	SW	N: 48° 0.463', W: 119° 45.366'	336	8.7 ± 0.3	7.4 ± 0.4	4/12 (33.3)	5
7 June	Waterville	WW	N: 47° 39.390', W: 120° 4.326'	314	ND	6.9 ± 0.6	5/12 (41.7)	0
7 June	Moses Lake ^z	WW	N: 47° 3.449', W: 119° 12.864'	244	8.3 ± 0.5	6.5 ± 0.7	2/12 (16.7)	1.5
11 June	Othello	WW	N: 46° 47.685', W: 118° 58.406'	227	8.2 ± 0.6	6.1 ± 1.1	12/12 (100)	0.8
11 June	Pullman	SC	N: 46° 48.467', W: 117° 49.654'	560	8.5 ± 0.6	ND	0/12 (0)	0
11 June	Palouse	SL	N: 46° 52.878', W: 117° 7.511'	535	8.1 ± 0.7	4.8 ± 0	1/12 (8.3)	0.05
11 June	Palouse	SB	N: 46° 53.694', W: 117° 6.355'	535	8.2 ± 0.4	ND	0/12 (0)	0
11 June	Grinnell	WW	N: 46° 57.819', W: 117° 5.480'	474	8.3 ± 0.5	3.2 ± 0	1/12 (8.3)	0.0008
11 June	Garfield	SW	N: 47° 0.037', W: 117° 7.868'	610	8.4 ± 0.4	ND	0/12 (0)	0
11 June	Garfield	SL	N: 47° 2.823', W: 117° 9.545'	610	7.8 ± 0.5	ND	0/12 (0)	0
11 June	Belmont	SP	N: 47° 6.321', W: 117° 11.030'	567	7.7 ± 0.4	ND	0/12 (0)	0
11 June	Tekoa	SB	N: 47° 11.629', W: 117° 8.700'	516	8.0 ± 0.3	ND	0/12 (0)	0
11 June	Latah	SL	N: 47° 18.089', W: 117° 9.829'	476	8.0 ± 0.4	6.6 ± 0.6	2/12 (16.7)	3.9
11 June	North Pine	SB	N: 47° 16.697', W: 117° 21.695'	404	8.8 ± 0.3	6.9 ± 0	1/12 (8.3)	1.25
11 June	Thornton	SW	N: 47° 8.250', W: 117° 23.449'	466	8.0 ± 0.8	ND	0/12 (0)	0
11 June	Steptoe	SP	N: 47° 3.480', W: 117° 22.909'	514	8.6 ± 0.4	ND	0/12 (0)	0
11 June	Steptoe ^z	SW	N: 46° 58.051', W: 117° 17.972'	514	8.5 ± 0.3	ND	0/12 (0)	0
11 June	Steptoe	SP	N: 46° 58.051', W: 117° 17.972'	514	8.5 ± 0.5	ND	0/12 (0)	0
11 June	Colfax	SW	N: 46° 53.872', W: 117° 11.304'	498	8.2 ± 0.4	5.2 ± 1.2	1/12 (8.3)	0.1

^r Date of sampling.

^s Isolates are from commercial fields and experimental plots near these Washington cities.

^t Crop grown at time of collection: WW = winter wheat, SW = spring wheat, SB = spring barley, SP = spring pea, SC = spring chickpea, and SL = spring lentil.

^u Global positioning system (GPS) coordinates at each location were recorded using a Garmin device.

^v Average annual precipitation at the location (average over 30-year period).

^w Total bacterial population (Total) and Phz⁺ population (Phz⁺) (mean log CFU g⁻¹ of root ± standard deviation [SD]). Phz⁺ bacteria determined by the terminal dilution endpoint assay with Phz⁺ primers; total culturable aerobic heterotrophic bacterial population was determined in one-tenth-strength tryptic soy broth supplemented with cycloheximide.

^x Frequency of 12 individual rhizospheres colonized by Phz⁺ bacteria.

^y Phz⁺ population as a percentage of the total culturable aerobic heterotrophic bacterial population.

^z Washington State University variety trial.

Fig. 1. Distribution of *Rhizoctonia solani* AG-8 isolates collected in 2010 and 2011. Original map is from Jaaffar et al. (2016), and frequency of phenazine-1-carboxylic acid-producing (Phz⁺) pseudomonad data were overlaid on the map. Low frequency of Phz⁺ = 0 to 32% of rhizospheres colonized, moderate frequency = 33 to 66% of rhizospheres colonized, and high frequency = 67 to 100% of rhizospheres colonized.

Fig. 2. Relationship between annual precipitation and frequency of plant colonization by indigenous phenazine-1-carboxylic acid-producing (Phz⁺) pseudomonads in the Inland Pacific Northwest. For each location sampled (black dot), the mean colonization frequency was determined by screening 12 individual wheat rhizospheres for the presence of Phz⁺ pseudomonads. Mean annual precipitation values were based on records for the years 1971 to 2000.

each AG did not differ significantly between isolates from fields with low and high frequencies, and the hypothesis that these isolates developed tolerance to natural antibiotics in the field was rejected.

Pathogenicity and virulence of *R. solani* AG-8 and AG-2-1. The tube assay was used to test 20 field isolates each of *R. solani* AG-8 and AG-2-1 for their ability to cause root disease on wheat. AG-2-1 produced only minor root damage, manifested as browning of the roots, whereas isolates of AG-8 produced serious symptoms, including spear tips, browning, and cortical collapse. All AG-8 isolates were more virulent than AG-2-1 isolates (Table 5).

Linear regression analysis of the relationship between virulence of an isolate (as determined in the tube assay) and sensitivity to PCA (Table 5) showed no significant correlation for AG-8 isolates ($P = 0.9$) (Supplementary Fig. S2). The relationship between virulence and sensitivity to PCA of AG-2-1 isolates, although statistically significant ($P = 0.02$), was determined to be not biologically significant due to the low r^2 value of the analysis (Supplementary Fig. S3). Therefore, the sensitivity of an isolate to PCA had no impact on its virulence on wheat.

Biological control of *R. solani* AG-8 by Phz⁺ fluorescent *Pseudomonas* spp. Seven fluorescent *Pseudomonas* spp., including two isolates each of *P. aridus*, *P. cerealis*, *P. orientalis*, and *P. synxantha*, were tested for ability to suppress Rhizoctonia root rot

caused by AG-8 isolate C-1 in both raw and pasteurized Quincy virgin soil. Root disease ratings and plant height data for two separate experiments in raw and pasteurized soil are shown in Tables 6 and 7, respectively. All isolates suppressed AG-8 isolate C-1 in both raw and pasteurized soil on the basis of disease ratings. In experiment 1 in raw soil, strain R6-28-08 gave significantly more protection than strain 4(1):2-10 but, when the experiment was repeated (experiment 2), there was no significant difference. The reduction in root disease caused by the eight bacterial strains did not result in a consistent increase in plant height when the bacterial treatments were compared with the two inoculated controls (CK + MC and CK). Thus, there were no consistent differences among Phz⁺ pseudomonads in their biocontrol activity.

On the basis of disease ratings in pasteurized soil, there was no difference in the ability of the eight Phz⁺ pseudomonads to suppress AG-8 isolate C-1. However, isolate S3-6:7-09 consistently showed an increase in plant height as compared with the two inoculated controls.

DISCUSSION

R. solani AG-8 and *R. oryzae*, causal agents of Rhizoctonia root rot and bare patch, are ubiquitous in cereal-based cropping systems of the Columbia Plateau of the Inland PNW, yet the severity of this

Fig. 3. Distribution of *Rhizoctonia solani* AG-2-1 isolates collected in 2010 and 2011, and frequency of phenazine-1-carboxylic acid-producing (Phz⁺) pseudomonads on the roots of wheat recovered in 2010 in eastern Washington State. Original map is from Jaaffar et al. (2016) and data for frequency of Phz⁺ pseudomonads were overlaid on the map. Low frequency of Phz⁺ = 0 to 32% of 12 individual rhizospheres colonized, moderate frequency = 33 to 66% rhizospheres colonized, and high frequency = 67 to 100% of rhizospheres colonized.

disease occurs differentially throughout the region (Jaaffar et al. 2016). *R. solani* AG-8 is most commonly found in the low-precipitation zone, which encompasses agronomic zones 4, 5, and 6 (Fig. 1), and root rot and bare patch is most severe in that same region. In contrast, AG-2-1, causal agent of damping-off and root rot of canola, was more evenly distributed throughout all six agronomic zones of the Columbia Plateau (Fig. 3) (Jaaffar et al. 2016). The distribution of these and other *Rhizoctonia* AG and species in the Inland PNW has been described in depth by both classical and molecular approaches (Jaaffar et al. 2016; Okubara et al. 2008, 2014). Phz⁺ fluorescent *Pseudomonas* spp. also were shown to be ubiquitous on crops throughout the Columbia Plateau of the Inland PNW (Mavrodi et al. 2012a). We show here that the geographic distribution of Phz⁺ pseudomonads overlaps closely with the distribution of *R. solani* AG-8 but not with other *Rhizoctonia* AG and species.

Mavrodi et al. (2012a) hypothesized that Phz⁺ pseudomonads play a role in the natural suppression of *Rhizoctonia* root rot and bare patch known to occur in fields in the Columbia Plateau of the Inland PNW (Lucas et al. 1993; Paulitz et al. 2009; Schillinger and Paulitz 2006, 2014). *Rhizoctonia* decline was first described in Australia (MacNish 1988; Roget et al. 1996) and disease suppressiveness develops in the soil with continual cropping of hosts susceptible to AG-8 during reduced tillage or no-till practices. *Rhizoctonia* decline is usually

associated with wheat and barley production. The fact that Phz⁺ *Pseudomonas* spp. and AG-8 thrive under similar environmental and cropping conditions indicates that these two microorganisms may have overlapping niches on cereal roots, thus making Phz⁺ bacteria ideally positioned to interact with *R. solani* AG-8. In addition, the fact that PCA occurs at greater than nanomolar quantities on roots of wheat and barley grown in the low-precipitation zone (agronomic zones 4, 5, and 6) (Mavrodi et al. 2012a), that PCA is highly inhibitory to *Rhizoctonia* spp. (Table 5), and that Phz⁺ *Pseudomonas* spp. are highly suppressive to *Rhizoctonia* root rot (Tables 6 and 7) provides further support for the role of PCA producers in the natural suppression of *Rhizoctonia* root rot. The Phz⁺ pseudomonads used in our biocontrol studies (Tables 6 and 7) were isolated from near Lind and Ritzville, WA, in the heart of the low-precipitation zone, where high populations of Phz⁺ bacteria are present on nearly every wheat plant. These strains were selected for testing because they are representative of the four species indigenous to fields of the Columbia Plateau (Parejko et al. 2012). All of these strains provided similar levels of biocontrol but it is not known if they differ in rhizosphere competence and ecological fitness. A potential role for Phz⁺ pseudomonads in natural disease suppression is not unprecedented. Phenazine-producing *Pseudomonas* spp. working in concert with nonpathogenic *F. oxysporum* had a major role in the natural suppression of flax wilt caused by *F. oxysporum* f. sp. *lini* in the Châteaurenard Fusarium wilt-suppressive soil in France (Mazurier et al. 2009).

Yin et al. (2013) examined the natural decline or suppression of *Rhizoctonia* patches at a location near Ritzville, WA, in the same sampling region as that studied in this article, using next-generation sequencing (454 pyrosequencing). They implicated *Chysoebacterium soldanellicola* in the phylum *Bacteroides* as one of the bacteria involved in patch decline. They identified many bacterial groups, including Sphingobacteriales and *Pseudomonas* spp., which were significantly higher in the rhizosphere of diseased plants in patches as compared with healthy wheat. However, the technology they used did not discriminate at finer taxonomic resolutions because it was based only on a 200- to 300-bp region of the 16S RNA. The large operational taxonomic unit identified as *Pseudomonas* undoubtedly contains a diversity of species, including those that produce phenazines.

Mavrodi et al. (2012a) reported that the frequency with which the rhizosphere of wheat was colonized with Phz⁺ pseudomonads was inversely correlated with annual precipitation. They went on to hypothesize that Phz⁺ pseudomonads are “uniquely adapted to growing and surviving in the rhizosphere under conditions of water stress and that soil moisture represents a major abiotic factor that

TABLE 3. Growth rate of *Rhizoctonia* anastomosis groups (AG) and species in one-fifth-strength potato dextrose agar buffered to different pH values^x

AG or species ^z	Growth rate (mm/day) ^y					
	pH 5.4	pH 6	pH 6.4	pH 6.8	pH 7.2	pH 7.6
AG-8	6.9 c	6.7 c	6.3 a	3.8 b	3.2 a	2.2 b
AG-8	6.7 cd	6.7 c	5.4 abc	3.9 b	3.3 a	2.0 b
AG-2-1	6.4 d	6.0 d	5.9 a	3.7 b	1.4 c	0.2 de
AG-2-1	7.5 b	7.4 b	4.4 c	2.9 c	1.3 c	0.7 c
AG-I	6.0 e	7.2 b	5.8 ab	5.1 a	3.6 a	3.2 a
AG-10	8.6 a	8.5 a	6.5 a	4.6 a	2.3 b	2.0 b
Ro GII	4.6 f	7.0 bc	4.5 bc	1.0 d	0.7 d	0.3 d
Ro GIII	1.8 g	2.7 e	1.0 d	0 e	0 e	0 e

^x Growth rates of AG and species were compared at the indicated pH values.

^y One-fifth strength potato dextrose agar was buffered to six different pH values ranging from pH 5.4 to 7.6 with 0.2 M phosphate buffer. Means in the same column followed by the same letter are not significantly different at *P* = 0.05 according to Fisher's protected least significant difference test.

^z *Rhizoctonia solani* isolates from different AG (AG-8, AG-2-1, and AG-10), AG-I-like binucleate *Rhizoctonia* (AG-I), and *R. oryzae* groups II and III (Ro GII and Ro GIII).

TABLE 4. Sensitivity to phenazine-1-carboxylic acid of isolates from different anastomosis groups (AG) of *Rhizoctonia solani*, binucleate *Rhizoctonia* AG-I, and *R. oryzae*

Isolate ^u	Source of isolate ^v	Crop ^w	<i>Rhizoctonia</i> spp. ^x	ED ₅₀ (μg/ml) ^y	Mean ED ₅₀ (μg/ml) ^z
AG-8 C1	Clyde	B	<i>Rhizoctonia solani</i> AG-8	16	16 a
T11S2-RS11	Odessa	WW	<i>R. solani</i> AG-8	16	...
Rh 060609-145	Ritzville	WW	<i>R. solani</i> AG-I	9	9.0 bc
Rh 100702-63	Ritzville	SW	<i>R. solani</i> AG-I	9	...
Rh 080741-181	Pullman	SW	<i>R. solani</i> AG-10	9	9.0 bc
Rh 080322-154	Garfield	SW	<i>R. solani</i> AG-10	9	...
T67S1-RS11	Pullman	WW	<i>R. solani</i> AG-2-1	10	9.5 abc
T5S1-RS10	Marcellus	WW	<i>R. solani</i> AG-2-1	9	...
T87S1-RO10	Colfax	SW	<i>R. oryzae</i> group I	5	5.5 c
T70S1-RO11	Grinnell	F	<i>R. oryzae</i> group I	6	...
T21S6-RO11	Chard	CF	<i>R. oryzae</i> group II	10	15 ab
T87S2-RO10	Colfax	SW	<i>R. oryzae</i> group II	20	...
T79S3-RO11	North Pine	SB	<i>R. oryzae</i> group III	12	11 abc
T39S3-RO10	St. John	SW	<i>R. oryzae</i> group III	10	...

^u Isolates were collected by using the toothpick assay as described in Jaaffar et al. (2016).

^v Isolates were from commercial fields and experimental plots near these Washington cities.

^w Crop at time of collection. B = barley, WW = winter wheat, SW = spring wheat, F = fallow, CF = chemical fallow, and SB = spring barley.

^x Isolates of *Rhizoctonia* from different AG groups and species.

^y ED₅₀ = 50% effective dose.

^z Mean ED₅₀ of two representative isolates from each group of *Rhizoctonia* spp. Means in the same column followed by the same letter are not significantly different at *P* = 0.05 according to Fisher's protected least significant difference test.

drives the development of the indigenous phenazine-producing microbial community.” Our study both confirmed and substantially extended those results by showing a similar significant inverse correlation ($r^2 = 0.38$, $P = 0.005$) between annual precipitation and colonization frequency by Phz⁺ pseudomonads (Fig. 2). The strength of this correlation is especially surprising given the great diversity of fields sampled (different management practices, fall and spring sown crops, and varieties) and that the fields were located over distances of hundreds of kilometers throughout the Columbia Plateau of the Inland PNW, which contains the largest contiguous production system in the western United States (Schillinger and Papendick 2008). The current findings fill in major gaps in our understanding of Phz⁺ producers in the high-precipitation zone (>450 mm), which corresponds to agronomic zones 1 and 2. In the

study by Mavrodi et al. (2012a), there were minimal samples of Phz⁺ pseudomonads from those zones. In the current study, the frequency of colonization of wheat and barley by Phz⁺ pseudomonads often became undetectable at higher precipitation, which was not seen by Mavrodi et al. (2012a). Support for the conclusion of a precipitous drop in colonization frequency of Phz⁺ pseudomonads in the high-precipitation zone is also seen in the 10 samples of pea, lentil, and chickpea: 8 of the 10 samples had no Phz⁺ pseudomonads and the other 2 samples had colonization frequencies of 8.3 and 16.7%. The frequency data from the grain legumes were omitted from the regression analysis. The finding of poor Phz⁺ colonization in the high-precipitation zone strongly supports another report by Mavrodi et al. (2012b) that showed very low colonization frequencies of Phz⁺ pseudomonads in irrigated fields located

TABLE 5. Virulence on wheat and sensitivity to phenazine-1-carboxylic acid (PCA) of selected isolates of *Rhizoctonia solani* anastomosis group (AG)-8 and AG-2-1 from fields with low or high frequencies of PCA-producing (Phz⁺) isolates

Isolate ^o	Year ^p	Source ^q	Crop ^r	Phz ⁺ (CFU $\pm$ SD) ^s	Phz ⁺ freq (%) ^t	AG-8 ^u	AG-2-1 ^u	Rating ^v	ED ₅₀ (μ g/ml) ^w	Mean ED ₅₀ (μ g/ml) ^x
T2S2-RS10	2010	La Crosse	SW	6.3 $\pm$ 0.9	11/12 (91.7)	+	...	4.4	5	...
T8S2-RS10	2010	Lind	SW	5.3 $\pm$ 1.1	12/12 (100)	+	...	4.7	13	...
T15S2-RS10	2010	Kahlotus	WW	5.1 $\pm$ 0.8	11/12 (91.7)	+	...	2.9	12	...
T3S4-RS11	2011	Hooper	SW	6.3 $\pm$ 0.5	12/12 (100)	+	...	4.8	12	...
T6S3-RS11	2011	Cunningham	WW	6.4 $\pm$ 0.9	12/12 (100)	+	...	5.2	10	10.6 a
T7S4-RS11	2011	Lind ^y	WW	6.4 $\pm$ 0.8	12/12 (100)	+	...	5.4	12	...
T8S11-RS11	2011	Lind	SW	5.3 $\pm$ 1.1	12/12 (100)	+	...	5.0	12	...
T9S1-RS11	2011	Ritzville ^y	WW	6.3 $\pm$ 0.5	8/12 (67)	+	...	5.9	12	...
T11S6-RS11	2011	Odessa	WW	5.9 $\pm$ 1.1	12/12 (100)	+	...	4.9	8	...
T21S3-RS11	2011	Chard	CF	5.6 $\pm$ 0.8	12/12 (100)	+	...	4.8	10	...
T87S2-RS10	2010	Colfax	SW	5.2 $\pm$ 1.2	1/12 (8.3)	+	...	4.3	10	...
T76S1-RS11	2011	Latah	WW	6.6 $\pm$ 0.6	2/12 (16.7)	+	...	1.7	10	...
T25S1-RS11	2011	Coppei	WW	ND	0/12 (0)	+	...	5.3	10	...
T23S1-RS11	2011	Ronan	SP	7.1 $\pm$ 0	1/12 (8.3)	+	...	5.6	12	...
T79S2-RS11	2011	North Pine	SB	6.9 $\pm$ 0	1/12 (8.3)	+	...	5.0	6	9.2 a
T5S3-RS11	2011	Marcellus	WW	ND	0/12 (0)	+	...	5.6	10	...
T69S4-RS11	2011	Palouse	SL	ND	0/12 (0)	+	...	4.7	6	...
T73S1-RS11	2011	Belmont	WW	ND	0/12 (0)	+	...	4.8	10	...
T74S2-RS10	2010	Tekoa	SB	ND	0/12 (0)	+	...	2.5	12	...
T32S3-RS10	2010	Bolles	WW	ND	0/12 (0)	+	...	4.7	6	...
T3S2-RS10	2010	Hooper	WW	6.3 $\pm$ 0.5	12/12 (100)	...	+	0.8	16	...
T6S2-RS10	2010	Cunningham	WW	6.4 $\pm$ 0.9	12/12 (100)	...	+	0.6	10	...
T7S3-RS10	2010	Lind ^y	WW	6.4 $\pm$ 0.8	12/12 (100)	...	+	0.3	12	...
T12S1-RS10	2010	Scharg	WW	5.9 $\pm$ 0.9	12/12 (100)	...	+	0.2	12	...
T14S2-RS10	2010	Connell ^y	WW	5.9 $\pm$ 1.0	11/12 (91.7)	...	+	0.5	10	...
T21S1-RS10	2010	Chard	WW	5.6 $\pm$ 0.8	12/12 (100)	...	+	0.3	8	10.8 a
T8S6-RS11	2011	Lind	SW	5.3 $\pm$ 1.1	12/12 (100)	...	+	0.9	10	...
T21S2-RS11	2011	Chard	CF	5.6 $\pm$ 0.8	12/12 (100)	...	+	0.9	10	...
T7S1-RS11	2011	Lind ^y	WW	6.4 $\pm$ 0.8	12/12 (100)	...	+	0.8	10	...
T9S6-RS11	2011	Ritzville ^y	WW	6.3 $\pm$ 0.5	8/12 (67)	...	+	0.6	10	...
T49S3-RS10	2010	Edwall	SW	ND	0/12 (0)	...	+	0.6	10	...
T36S2-RS10	2010	Colfax (M) ^z	SB	ND	0/12 (0)	...	+	0.6	10	...
T68S2-RS10	2010	Palouse	SL	4.8 $\pm$ 0	1/12 (8.3)	...	+	0.2	10	...
T79S2-RS10	2010	North Pine	SB	6.9 $\pm$ 0	1/12 (8.3)	...	+	0.3	8	...
T87S1-RS10	2010	Colfax	SW	5.2 $\pm$ 1.2	1/12 (8.3)	...	+	0.5	12	9.1 a
T23S2-RS11	2011	Ronan	SP	7.1 $\pm$ 0	1/12 (8.3)	...	+	0.8	10	...
T70S1-RS11	2011	Grinnell	F	3.2 $\pm$ 0	1/12 (8.3)	...	+	1.2	5	...
T70S2-RS11	2011	Grinnell	F	3.2 $\pm$ 0	1/12 (8.3)	...	+	1.0	10	...
T79S1-RS11	2011	North Pine	SB	6.9 $\pm$ 0	1/12 (8.3)	...	+	0.8	8	...
T87S2-RS11	2011	Colfax	WW	5.2 $\pm$ 1.2	1/12 (8.3)	...	+	0.6	8	...

^o Isolates were collected by using the toothpick assay as described in Jaaffar et al. (2016).

^p Year collected.

^q Isolates were from commercial fields and experimental plots near these Washington cities.

^r Crop at the time of collection. WW = winter wheat, SW = spring wheat, CF = chemical fallow, SP = spring pea, SB = spring barley, SL = spring lentil, and F = fallow.

^s Phz⁺ population size (log CFU/g⁻¹ of root $\pm$ standard deviation [SD]) as determined by the terminal dilution endpoint assay with Phz⁺ primers. ND = not detected.

^t Frequency of 12 individual rhizospheres colonized by Phz⁺ bacteria.

^u Isolates of *Rhizoctonia solani* AG-8 and AG-2-1 from fields with low or high frequencies of root colonization by Phz⁺ bacteria.

^v Root rot disease rating on wheat, where 0 = no disease and 8 = dead or nearly dead at 4 weeks after planting.

^w ED₅₀ = 50% effective dose of PCA for each isolate.

^x Mean ED₅₀ for 10 isolates of AG-8 or AG-2-1 from fields with low or high frequencies of Phz⁺ bacteria. Means in the same column followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least difference.

^y Washington State University variety trial site.

^z Colfax, McGregor Farm.

adjacent to dryland wheat supporting high Phz⁺ populations in the low-precipitation zone. Irrigated wheat in the low-precipitation zone may receive as much as 23 cm of water between April and June, making the precipitation in an irrigated field as great as or greater than precipitation in the high-precipitation zone.

Historically, it has been thought that fluorescent pseudomonads, which are highly adapted to growing and surviving on roots and in the rhizosphere, thrive under high soil moisture conditions that are at or near field capacity (Howie et al. 1987; Mavrodi et al. 2012b; Weller 2007). In fact, the issue of inconsistent colonization by introduced pseudomonads as biocontrol agents of soilborne pathogens has been attributed, in part, to insufficient soil moisture for colonization (Weller 1988, 2007). The findings of the current study and those of Mavrodi et al. (2012a,b) completely alter accepted principles about fluorescent pseudomonads that provide biocontrol and the environmental conditions required for biocontrol. The reason why Phz⁺ pseudomonads thrive and produce PCA in such an arid agroecosystem as the low-precipitation zone remains unclear and is a major topic of ongoing research. Mavrodi et al. (2012b) suggested that soil moisture levels change the amount or composition of root exudates which, in turn, affects the growth and competitiveness of indigenous pseudomonads. Thus, Phz⁺ pseudomonads would be ideally adapted to utilizing the quantity and quality of exudates produced by wheat and barley grown under water stress conditions in the low-precipitation zone. Second, they suggested that other fluorescent pseudomonads—for example, those that produce the antibiotic 2,4-diacetylphloroglucinol (DAPG)—are more competitive at higher soil moisture and displace Phz⁺ pseudomonads in the rhizosphere. Mavrodi et al. (2012b) identified a striking decline in

populations of Phz⁺ pseudomonads in the rhizosphere of wheat growing in an irrigated field when DAPG-producing pseudomonads also colonized the roots. A third possibility (Mavrodi et al. 2012b) is that Phz⁺ pseudomonads are much better adapted for survival under conditions of extended moisture stress than other pseudomonads because of the production of PCA. Pseudomonads, like other bacteria on root surfaces and in the rhizosphere, form structurally complex colonies enmeshed in mucilage called biofilms (Bloemberg et al. 2000; Chin-A-Woeng et al. 1998). The formation of biofilms is one of the physiological and molecular mechanisms underpinning the protection of bacteria against chemical and physical environmental stresses and adaption to survival in low-moisture habitats (Bloemberg et al. 2000; Harris 1980). Phenazines have been directly linked to the formation and architecture of biofilms (Maddula et al. 2006, 2008; Mavrodi et al. 2006), and Price-Whelan et al. (2006) hypothesized that, because the rate of diffusion of oxygen through biofilms is low, phenazines help to maintain the redox homeostasis of cells in the biofilm by serving as electron acceptors for the reoxidation of NADH.

It is well known that application of antibiotics in human therapy, animal production, and plant protection contributes to the emergence of antibiotic resistance in pathogens (Martínez 2008; McManus et al. 2002; Wang and Schaffner 2011). The majority of antibiotics currently used to treat human, animal, and plant diseases caused by bacteria have an environmental origin, and are most commonly isolated from soil organisms (Martínez 2008). Although a tremendous amount is known about antibiotic resistance genes and their transfer through pathogen populations, virtually nothing is known about the development of resistance in microorganisms in natural habitats to naturally occurring antibiotics.

TABLE 6. Biological control of *Rhizoctonia solani* AG- 8 isolate C-1 in natural soil by phenazine-1-carboxylic acid-producing *Pseudomonas* spp.^x

Treatment ^y	Source ^z	Disease rating (0-to-8 scale)		Plant height (mm) (mean ± SD)	
		Exp 1	Exp 2	Exp 1	Exp 2
L1-45-08, <i>Pseudomonas cerealis</i>	Lind	3.05 bc	2.61 b	22.09 ± 3.83 bcd	22.83 ± 3.62 abcd
4(1):2-10, <i>P. cerealis</i>	Lind	2.94 cd	2.70 b	21.39 ± 4.17 cd	22.34 ± 3.75 bcde
R1-43-08, <i>P. aridus</i>	Ritzville	2.76 cde	2.33 b	21.94 ± 4.06 bcd	22.21 ± 3.36 bcde
S3-6:7-09, <i>P. aridus</i>	Lind	2.60 cde	2.23 b	21.89 ± 3.30 bcd	22.02 ± 3.09 de
L1-3-08, <i>P. orientalis</i>	Lind	2.72 cde	2.47 b	22.36 ± 2.74 bc	23.19 ± 3.49 ab
R2-66-08W, <i>P. orientalis</i>	Ritzville	2.81 cde	2.51 b	21.32 ± 4.15 cd	22.63 ± 3.08 abcd
R6-28-08, <i>P. synxantha</i>	Ritzville	2.24 e	2.36 b	21.94 ± 3.38 bcd	23.12 ± 3.00 abc
2-79RN10, <i>P. synxantha</i>	Lind	2.32 de	2.33 b	21.79 ± 3.39 bcd	22.92 ± 3.25 abcd
CK + MC	...	3.57 ab	3.42 a	20.96 ± 3.04 d	21.24 ± 3.23 e
CK	...	3.89 a	3.50 a	21.68 ± 3.70 bcd	22.49 ± 3.33 abcd

^x Experiments (Exp) were conducted in Quincy virgin soil. Means in the same column followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference (for plant height) or Kruskal-Wallis all-pairwise comparison (for disease rating). Disease ratings and plant height measurements were taken at 4 weeks. SD = standard deviation.

^y Bacteria were applied to the seed with methyl cellulose (MC) at a dose of 10^7 seed⁻¹; CK + MC = seed treated with 1% MC only and CK = no seed treatment.

^z Isolates are from commercial fields in the low-precipitation zone of the Columbia Plateau of the Inland Pacific Northwest.

TABLE 7. Biological control of *Rhizoctonia solani* AG-8 isolate C-1 in pasteurized soil by phenazine-1-carboxylic acid-producing *Pseudomonas* spp.^x

Treatment ^y	Source ^z	Disease rating (0-to-8 scale)		Plant height (mm) (mean ± SD)	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
L1-45-08, <i>Pseudomonas cerealis</i>	Lind	2.44 ± 0.76 bc	2.30 ± 0.67 b	21.58 ± 2.81 bcd	25.13 ± 3.50 abc
4(1):2-10, <i>P. cerealis</i>	Lind	2.43 ± 0.66 bc	2.34 ± 0.56 b	21.49 ± 2.46 bcd	25.44 ± 2.64 ab
R1-43-08, <i>P. aridus</i>	Ritzville	2.22 ± 0.54 c	2.37 ± 0.63 b	22.24 ± 2.37 b	24.51 ± 3.31 bcd
S3-6:7-09, <i>P. aridus</i>	Lind	2.46 ± 0.69 bc	2.37 ± 0.54 b	21.99 ± 3.00 b	25.97 ± 2.10 a
L1-3-08, <i>P. orientalis</i>	Lind	2.45 ± 0.56 bc	2.32 ± 0.53 b	21.72 ± 2.38 bcd	25.27 ± 2.88 ab
R2-66-08W, <i>P. orientalis</i>	Ritzville	2.26 ± 0.72 bc	2.47 ± 0.87 b	21.78 ± 2.62 bcd	24.00 ± 3.65 cde
R6-28-08, <i>P. synxantha</i>	Ritzville	2.47 ± 0.60 bc	2.47 ± 0.76 b	21.98 ± 2.93 bc	25.44 ± 3.43 ab
2-79, <i>P. synxantha</i>	Lind	2.34 ± 0.59 bc	2.35 ± 0.67 b	20.99 ± 2.73 d	25.61 ± 2.58 a
CK + MC	...	3.50 ± 0.67 a	3.50 ± 0.89 a	20.92 ± 3.11 d	23.71 ± 3.92 de
CK	...	3.68 ± 0.64 a	3.70 ± 0.80 a	21.13 ± 2.29 cd	23.16 ± 4.59 e

^x Experiments were conducted in pasteurized Quincy virgin soil (60°C, 30 min). Means in the same column followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference (for plant height) or Kruskal-Wallis all-pairwise comparison (for disease rating). Disease ratings and plant height measurements were taken at 4 weeks. SD = standard deviation.

^y Bacteria were applied to the seed with methylcellulose (MC) at a dose of 10^7 seed⁻¹. CK + MC = seed treated with MC only and CK = no seed treatment.

^z Isolates are from commercial fields in the low-precipitation zone of the Columbia Plateau of the Inland Pacific Northwest.

An important question of this study was: Can a pathogen develop resistance to an antibiotic produced by a biocontrol agent in a natural habitat such as the rhizosphere? The finding of high populations of Phz⁺ fluorescent pseudomonads and accumulation of greater than nanomolar quantities of PCA on the roots of dryland wheat in the low-precipitation zone (Mavrodi et al. 2012a) and the finding that *R. solani* AG-8 and AG-2-1 are ubiquitous in fields in the Columbia Plateau of the Inland PNW (Jaaffar et al. 2016; Okubara et al. 2014) indicated that this agroecosystem provided an ideal field laboratory for testing this question. Therefore, isolates of AG-8 and AG-2-1 were selected from fields in the low- and high-precipitation zones where Phz⁺ pseudomonads occurred at either high or low frequency on the roots of wheat. Because the amount of PCA on a cereal root system was directly related to the population size of Phz⁺ pseudomonads colonizing the roots (Mavrodi et al. 2012a), it was assumed that *Rhizoctonia* isolates from fields with a high frequency of Phz⁺ pseudomonads would, over decades, be exposed more frequently to inhibitory concentrations of PCA than isolates from fields with a low frequency of Phz⁺ pseudomonads. In the low-precipitation zone, wheat has been grown for over 125 years (Mavrodi et al. 2012a).

In the in vitro assay used to test the sensitivity of *Rhizoctonia* isolates to PCA, the medium was buffered to pH 6 because it was shown to be optimal for growth of all of the AG and species tested, and it is also a pH at which PCA is active (Ownley et al. 1992). The activity of PCA previously was shown to be related to the concentration of protonated PCA, and inhibition of *G. graminis* var. *tritici* by PCA and strain 2-79 in vitro declined as alkalinity increased (Brisbane et al. 1987). Isolates of *R. solani* AG-8 and AG-2-1 from the high- and low-precipitation zones showed no significant difference in sensitivity to PCA as determined by ED₅₀ values (Table 6), indicating that long-term exposure of *Rhizoctonia* spp. to PCA did not result in the development of resistance or tolerance in the pathogen to the antibiotic. The current findings are similar to those of Kwak et al. (2009), who showed that long-term exposure of *G. graminis* var. *tritici* to DAPG in take-all decline fields with high populations of DAPG-producing pseudomonads did not result in the take-all pathogen becoming less sensitive to the antibiotic. Using a *Saccharomyces cerevisiae* genome-wide mutant screen, Kwak et al. (2011) went on to show that resistance to DAPG probably does not develop because the antibiotic affects multiple basic cellular processes, including membrane function, reactive oxygen regulation, and cell homeostasis. Similar studies have not been conducted with PCA to determine its mechanisms of action but it is likely that this antibiotic also attacks multiple cellular processes, making it unlikely for resistance to develop. A study with pyocyanin, a derivative of PCA produced by *P. aeruginosa*, showed that this phenazine acts on major cellular pathways involved in the cell cycle, electron transport and respiration, protein sorting, vesicle transport, and the vacuolar ATPase (Ran et al. 2003).

In conclusion, an overarching question that should continue to drive research on Phz⁺ pseudomonads in the Columbia Plateau of the Inland PNW is: What role do Phz⁺ pseudomonads play in the natural suppression of *Rhizoctonia* root rot and in the sustainability of wheat production in the Columbia Plateau?

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