

# Diversity, Biosynthesis, and Role of Phenazines in Cell Physiology, Pathogenesis and Symbiosis

Dmitri Mavrodi

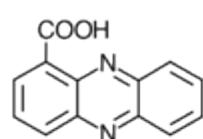


*Department of Plant Pathology, Washington State University  
USDA-ARS Root Disease & Biological Control Research Unit*

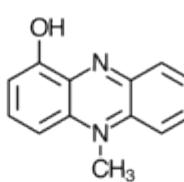


# What are phenazines?

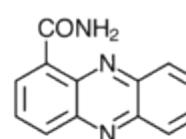
- ◆ Colored biologically active secondary metabolites produced by *Pseudomonas*, *Streptomyces* and a few other bacterial genera



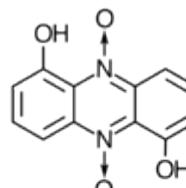
Phenazine-1-carboxylic acid (PCA)



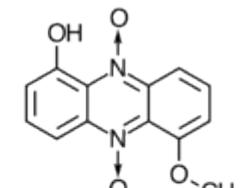
Pyocyanin



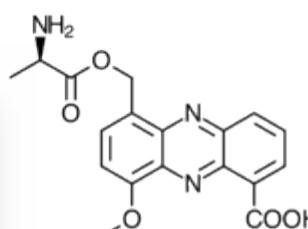
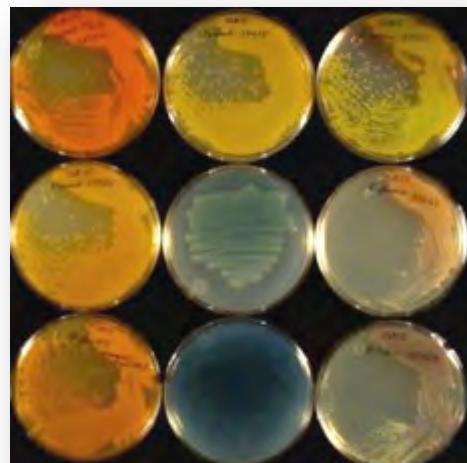
Phenazine-1-carboxamide (Chlororaphin)



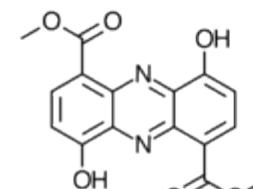
Iodinin



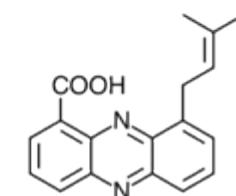
1-Hydro-6-methoxyphenazine -5,10-di-N-oxide (Myxin)



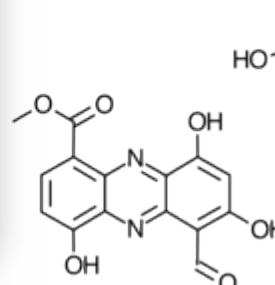
D-Alanyl-griseoluteic acid (AGA)



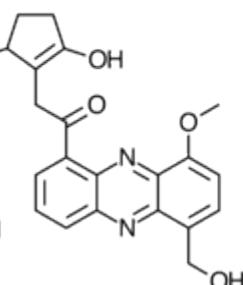
4,9-Dihydrophenazine-1,6-di carboxylic acid dimethylester



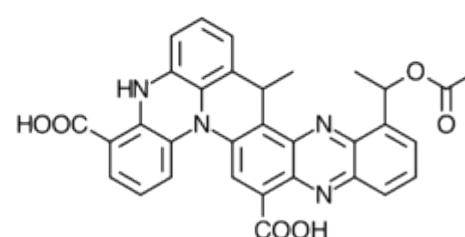
Endophenazine A



Lomofungin



Sendomycin A



Esmeraldin A

# Pyocyanin (Greek “pus” and “blue”)

- ◆ Isolated in 1859 by chloroform extraction from purulent wound dressings by French pharmacist Mathurin-Joseph Fordos
- ◆ In 1882 French military pharmacist Carle Gessard demonstrated that the blue coloration in pus was due to the presence of a microorganism that he called “*Bacillus pyocyanus*” = *Pseudomonas aeruginosa*

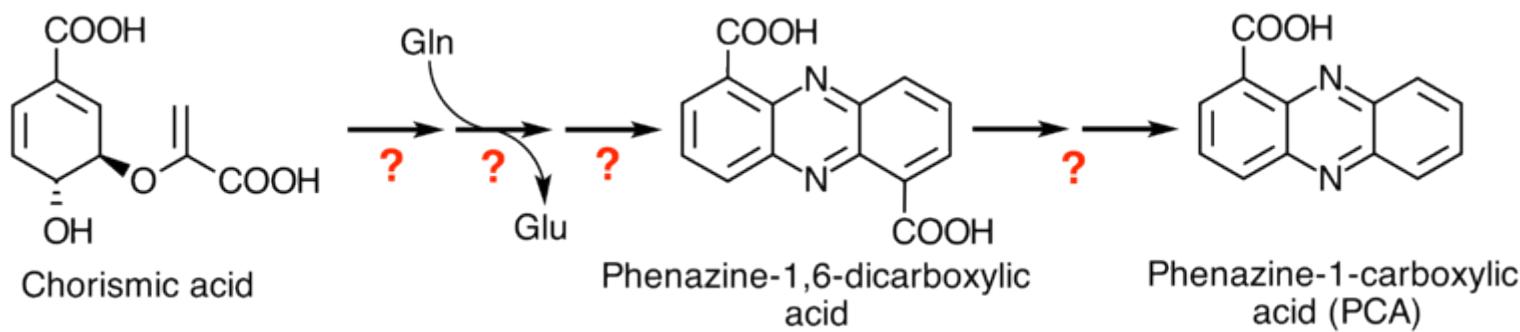


Carle Gessard (1850-1925)



# Early understanding of phenazine ring assembly

- ◆ Phenazines were linked to shikimic acid pathway
- ◆ Chorismic acid was identified as a branchpoint leading from the shikimic acid to phenazines
- ◆ Glutamine was identified as nitrogen source
- ◆ It was established that phenazine core is formed by a symmetrical condensation of two chorismic acid molecules



# Contribution of phenazines to biocontrol properties of *P. fluorescens* 2-79

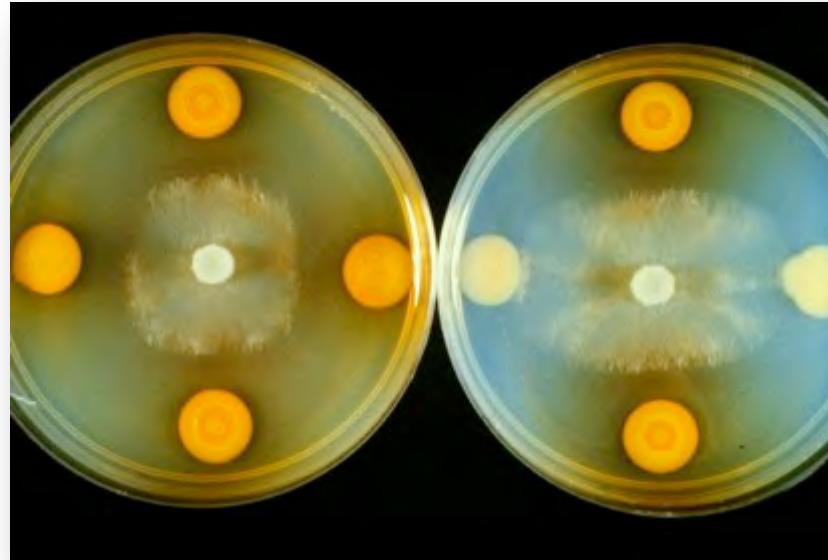
JOURNAL OF BACTERIOLOGY, Aug. 1988, p. 3499-3508  
0021-9193/88/083499-10\$02.00/0  
Copyright © 1988, American Society for Microbiology

Vol. 170, No. 8

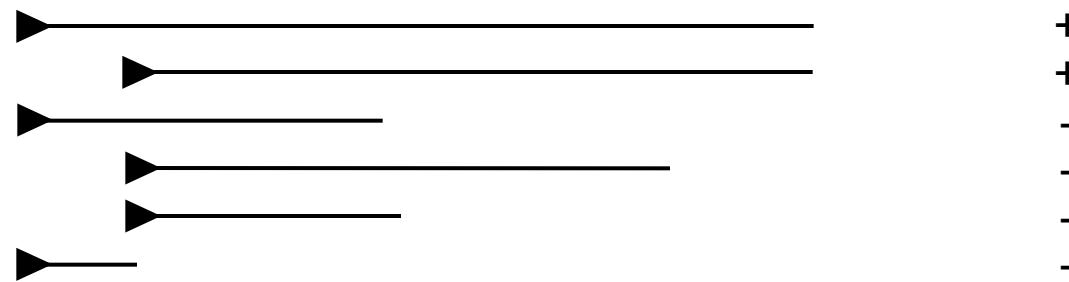
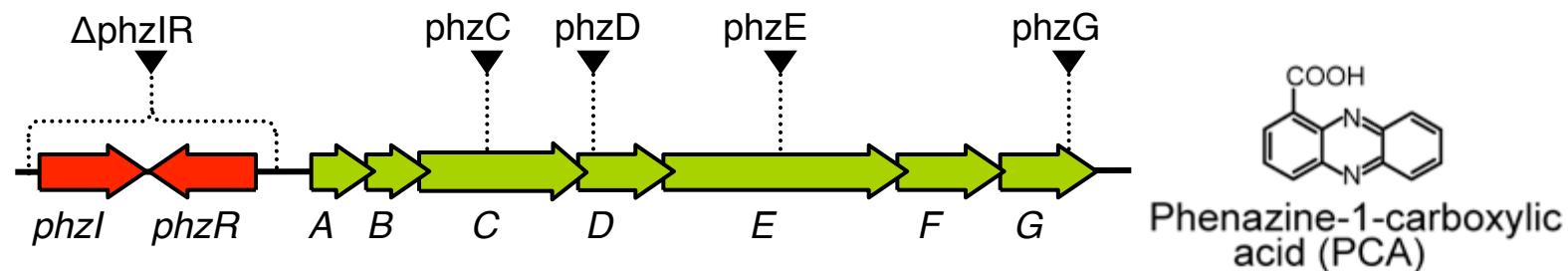
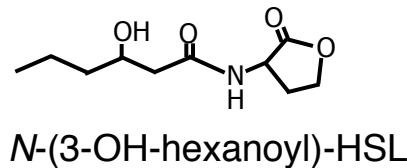
## Role of a Phenazine Antibiotic from *Pseudomonas fluorescens* in Biological Control of *Gaeumannomyces graminis* var. *tritici*

LINDA S. THOMASHOW\* AND DAVID M. WELLER

Agricultural Research Service, U.S. Department of Agriculture, Root Disease and Biological Control Research Unit,  
Washington State University, Pullman, Washington 99164-6430



# Phenazine biosynthesis genes of *Pseudomonas fluorescens* 2-79



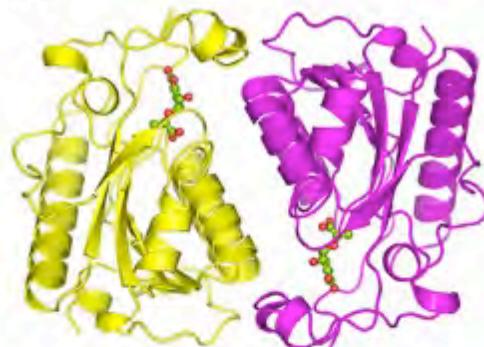
■ - synthesis of phenazine core  
■ - regulatory proteins

Mavrodi et al (1997) J Bacteriol 180: 2541  
Khan et al (2005) J Bacteriol 187: 6517

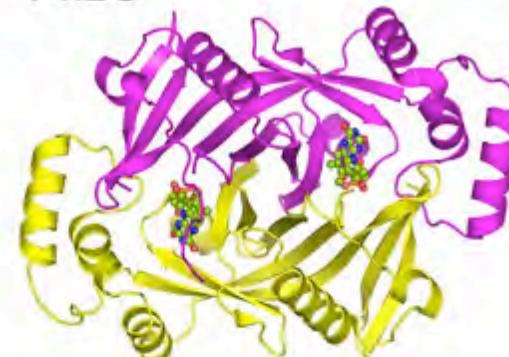
# Biosynthesis of phenazine core in *Pseudomonas*

Structures of PhzD, PhzG, PhzF and PhzA enzymes

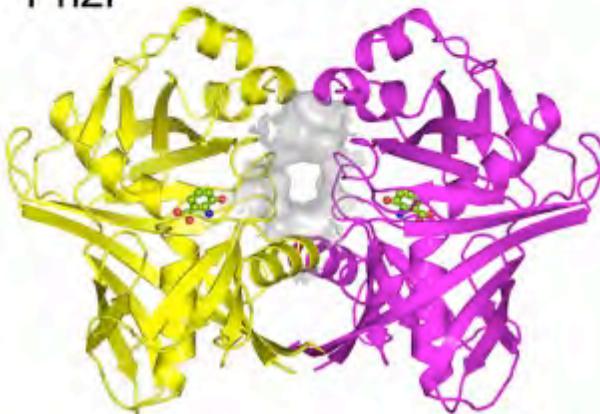
PhzD



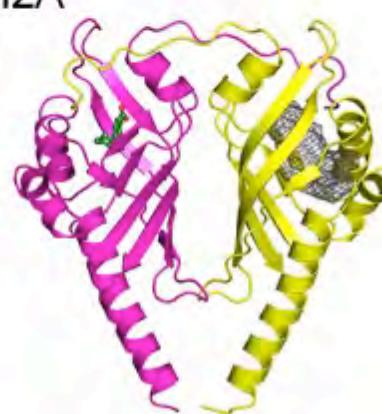
PhzG



PhzF

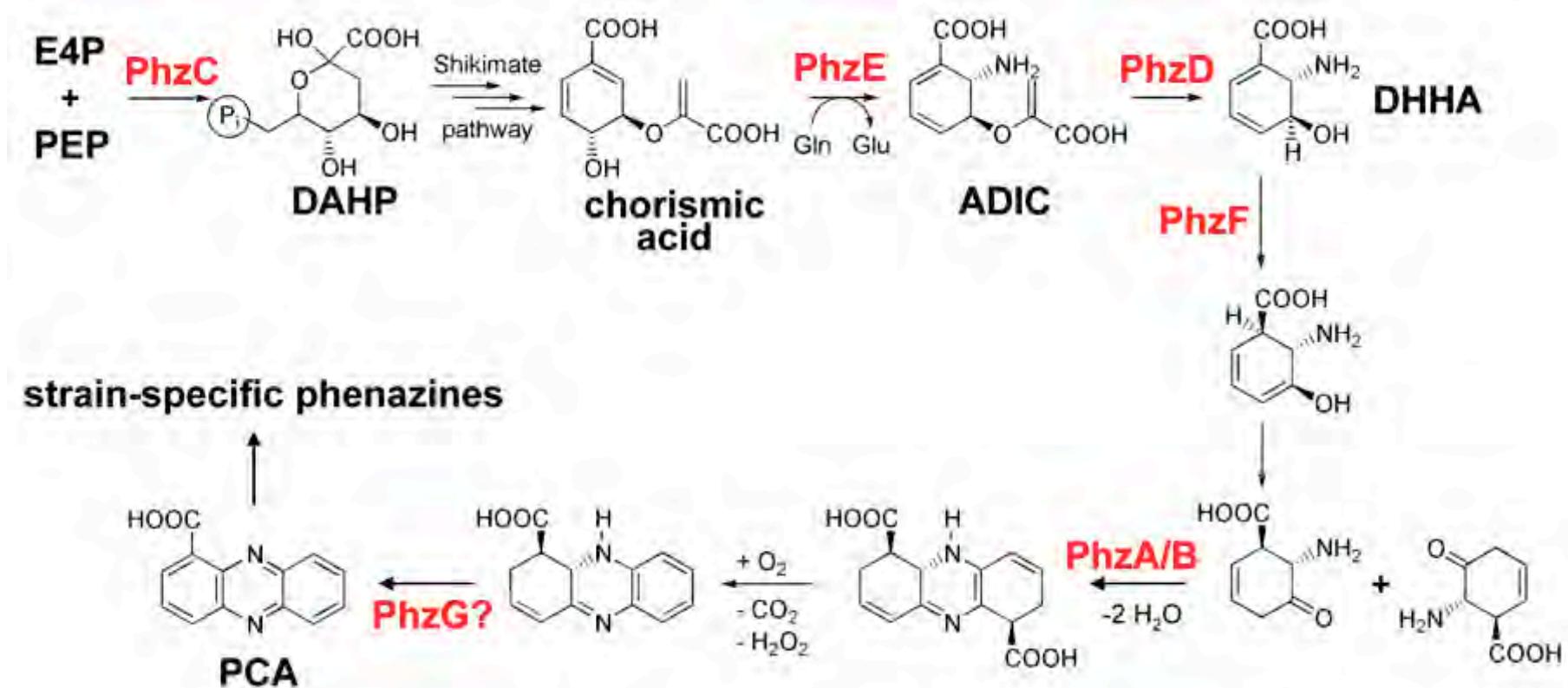


PhzA

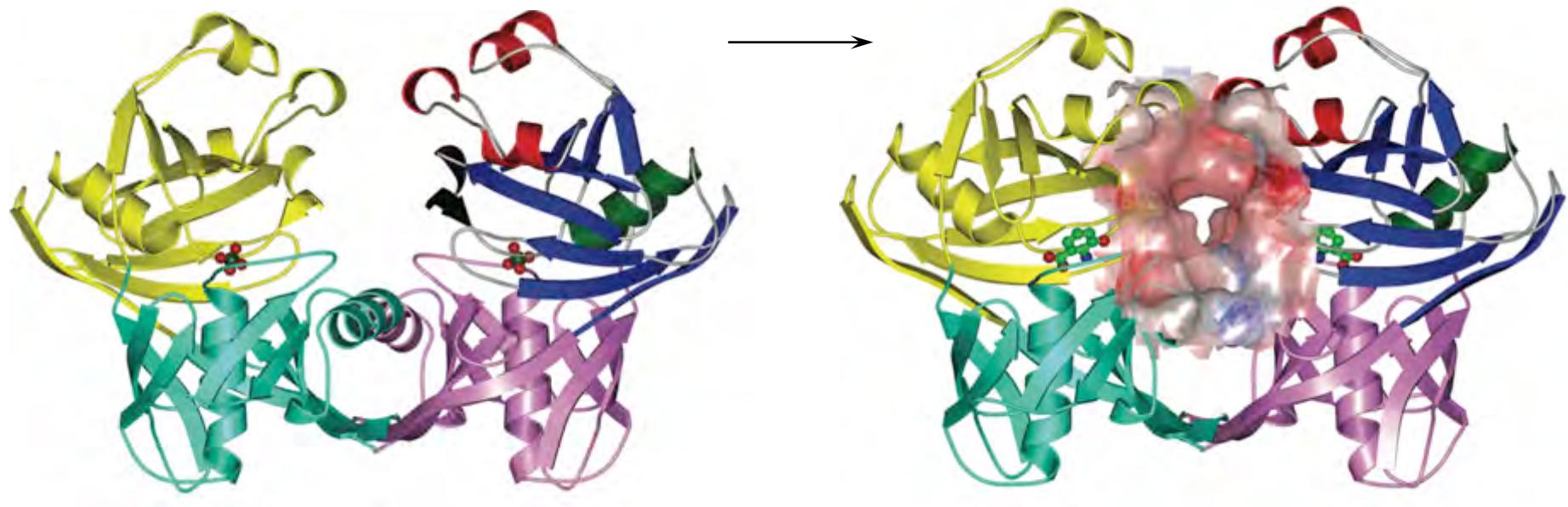


# Biosynthesis of phenazine core in *Pseudomonas*

- ◆ Phenazine core is formed by PhzF and PhzA/B through a condensation of two DHHA molecules



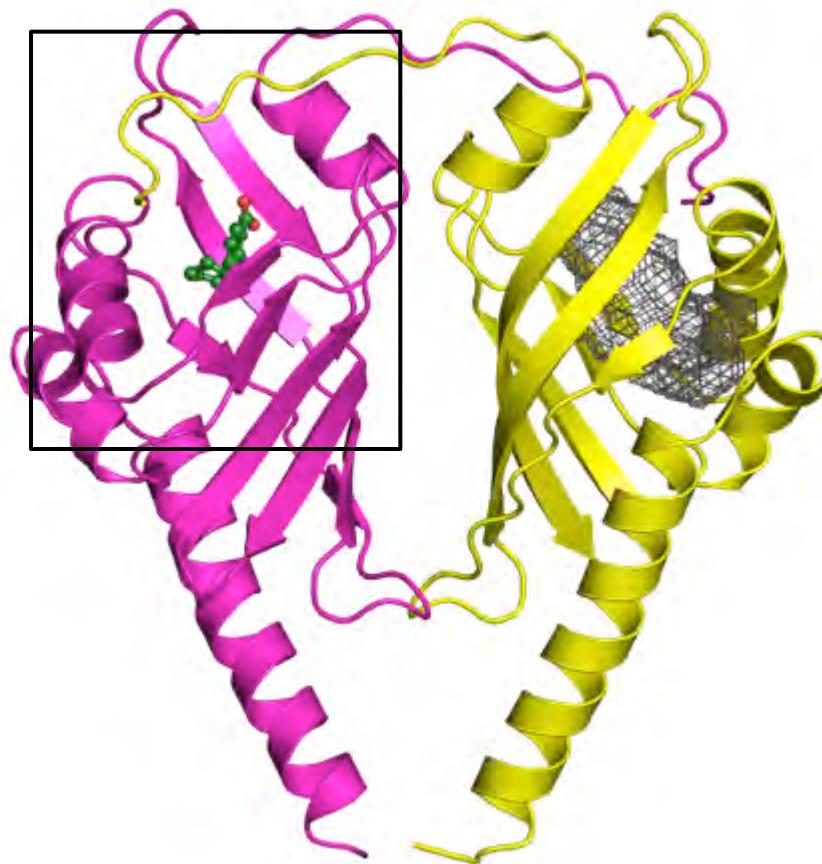
# Structure of PhzF from *P. fluorescens* 2-79



Blankenfeldt et al (2004) PNAS USA 101: 16431

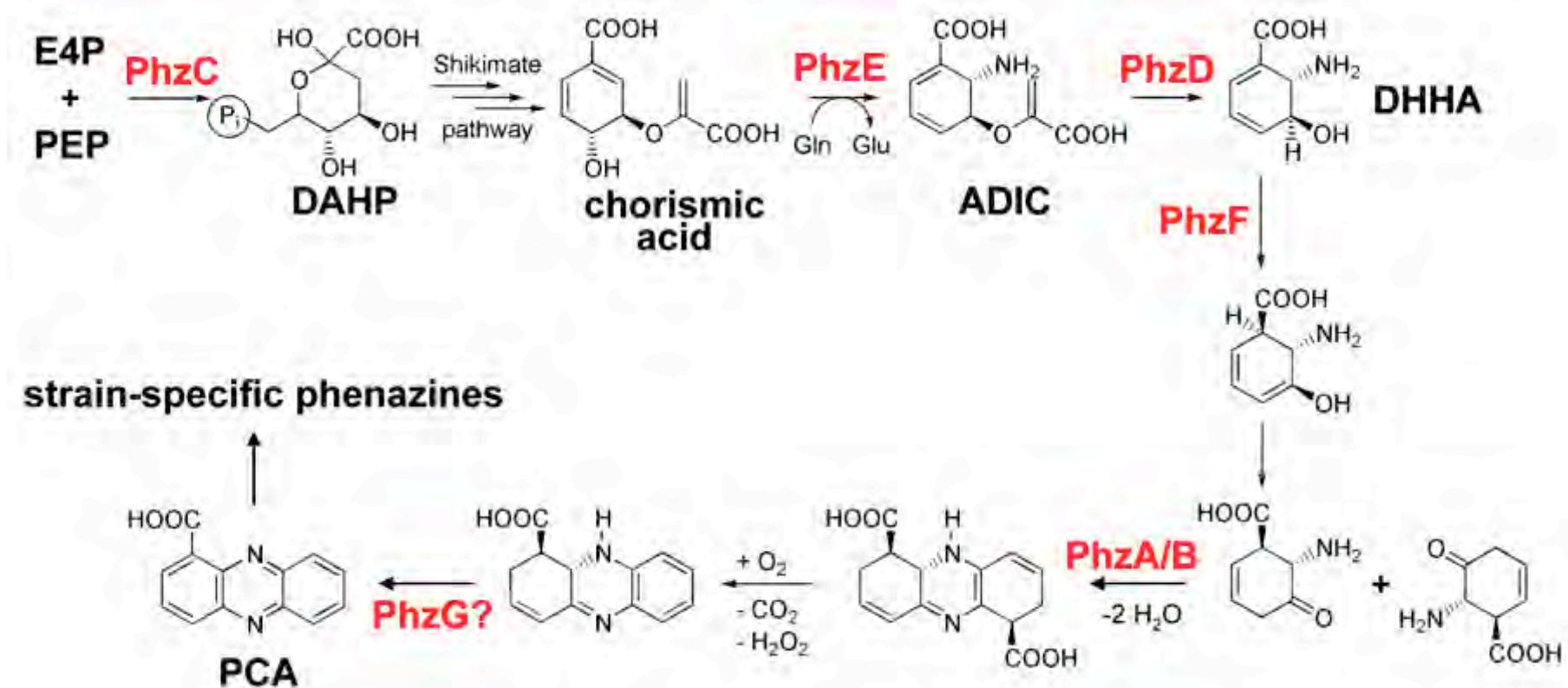
Mentel et al (2009) ChemBioChem Phytopath 10: 1

# Structure of PhzA from *Burkholderia lata*

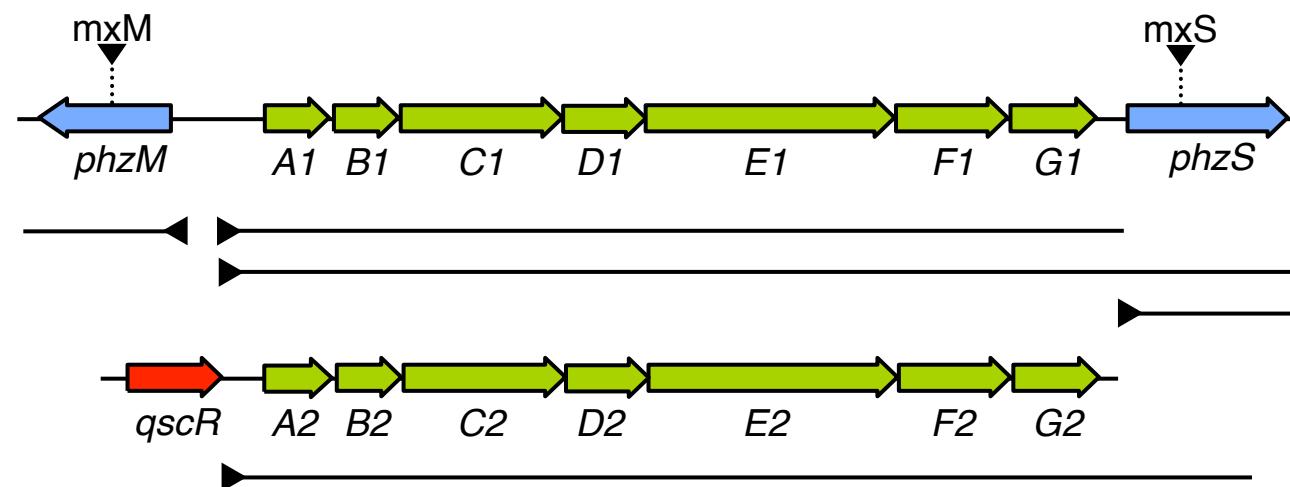
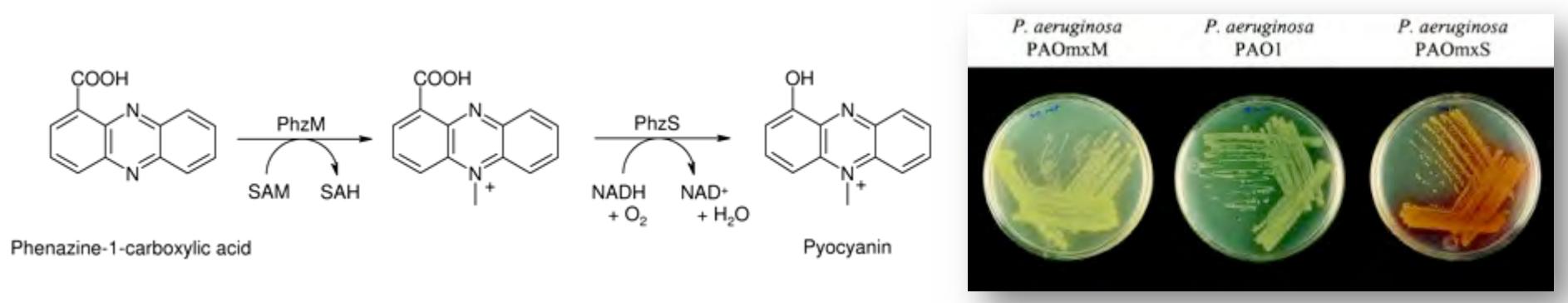


# Biosynthesis of phenazine core in *Pseudomonas*

- ◆ Phenazine core is formed by PhzF and PhzA/B through a condensation of two DHHA molecules



# Biosynthesis of pyocyanin in *P. aeruginosa*

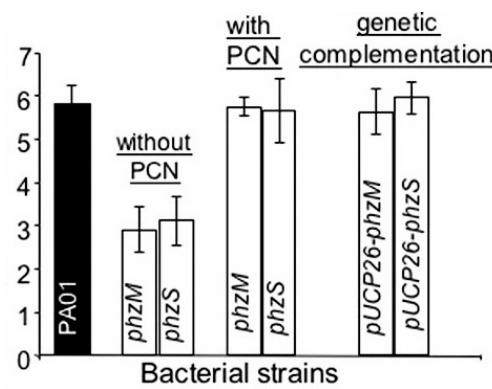


- - synthesis of phenazine core
- - modifying enzymes
- - regulatory proteins

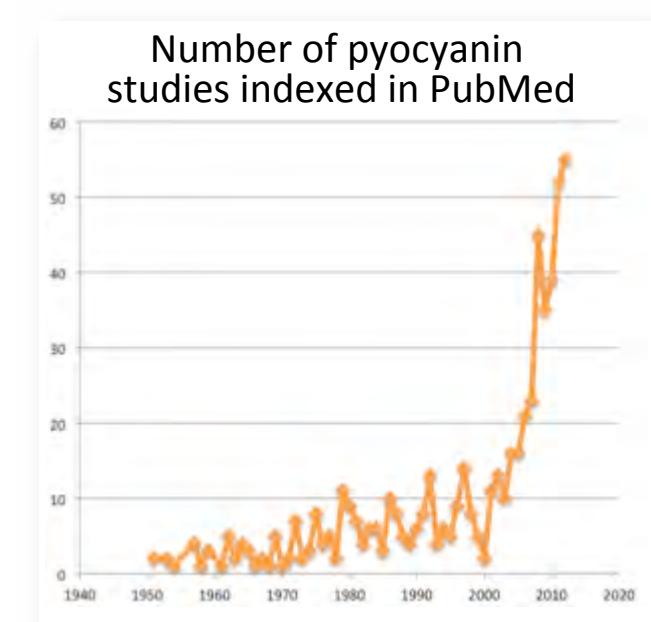
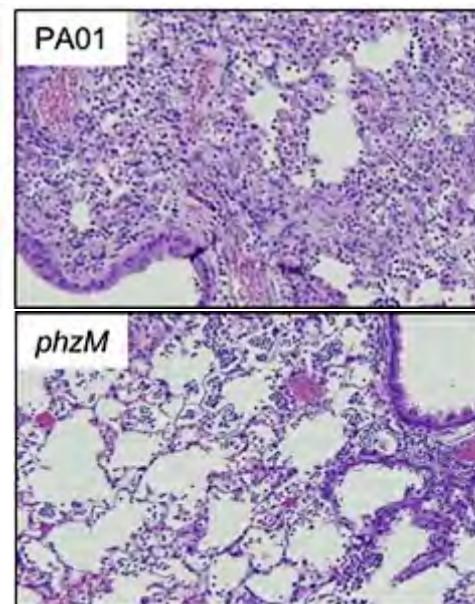
Mavrodi et al (2001) J Bacteriol 183: 6454  
 Gohain et al (2006) Acta Crystallogr F62: 887  
 Gohain et al (2006) Acta Crystallogr F62: 989

# Pyocyanin is a major toxin in *P. aeruginosa*

- ◆ Pyocyanin is required for full virulence in several models of *P. aeruginosa* infection
- ◆ Promotes inflammatory responses by imposing oxidative stress on host cells
- ◆ Pyocyanin levels were correlated with declining lung function in cystic fibrosis



Lau et al (2004) Infection Immun 72: 4275  
Caldwell et al (2009) Am J Pathol 175: 2473

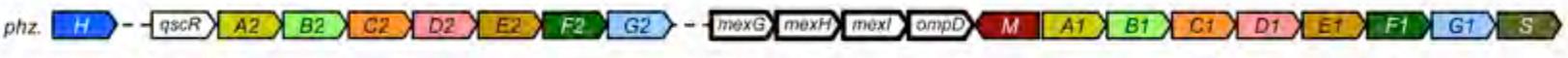


# Phylogeny of phenazine genes

Gammaproteobacteria



*Pseudomonas aeruginosa*



*Pectobacterium atrosepticum*



*Pantoea agglomerans* ehp: R → A → B → C → D → E → F → G → H → I → J → K → L → M → N → O

Betaproteobacteria



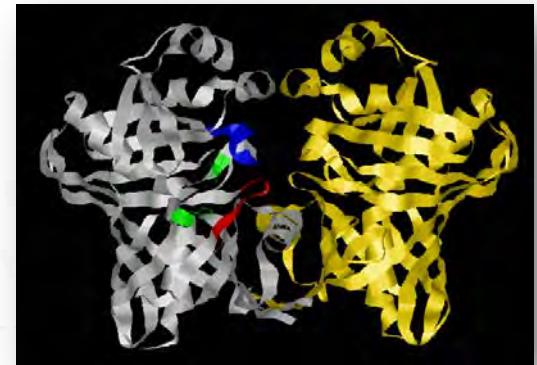
Actinobacteria



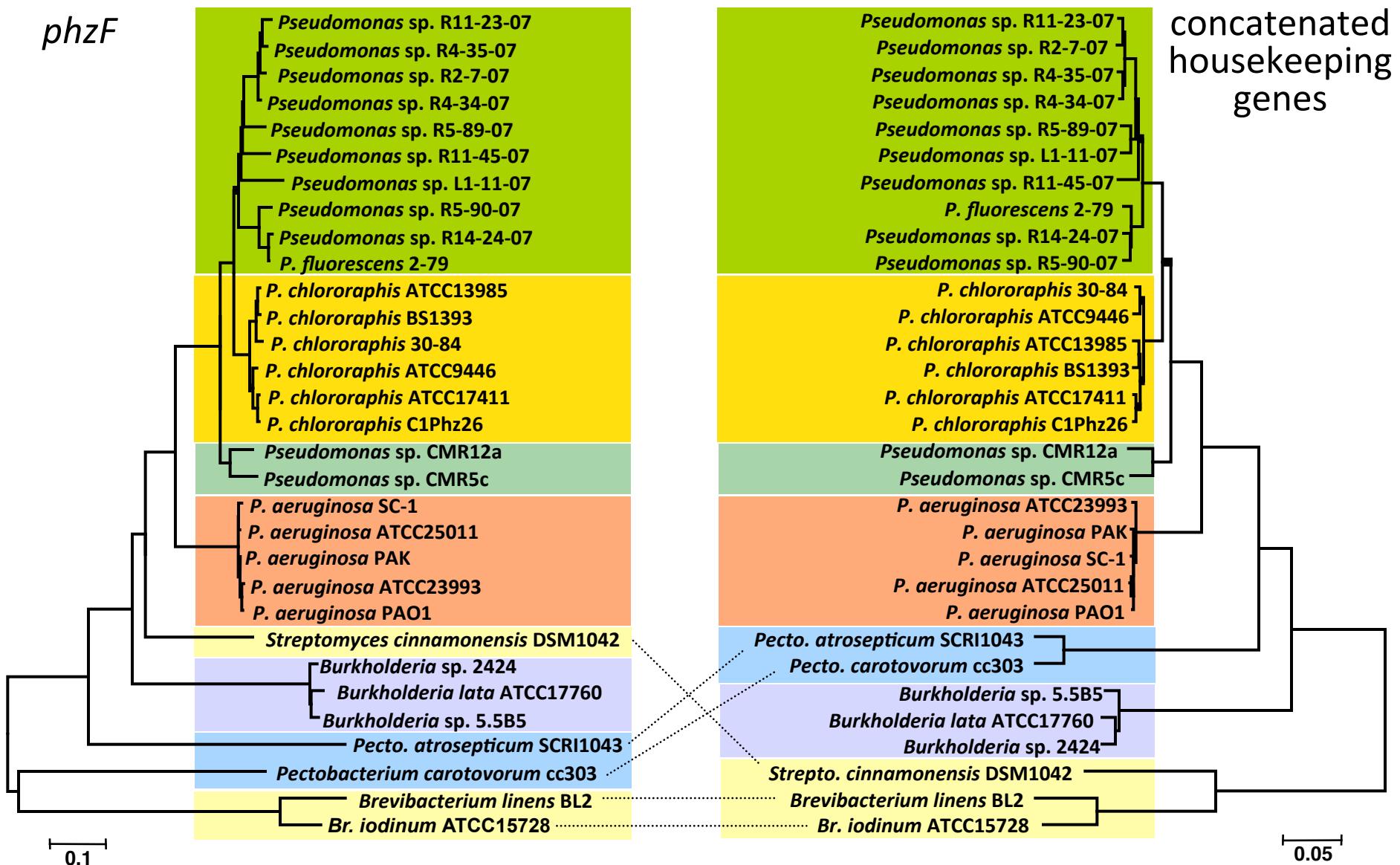
NdasDRAFT\_10... 102 → 101 → 100 → 99 → 98 → 97 → 96 → 95 → 94 → 93 → 92 → 91 Nocardiopsis dassonvillei

ephz: B → C → D → E → F → G → A → -- → G2 → F → G2 → A2 → M Streptomyces cinnamonensis

*Streptomyces anulatus* ppz: B → C → D → E → F → G → A → M → V → U → T → hmgS → hmgR → ippl → pmk → mdpd → mik → P

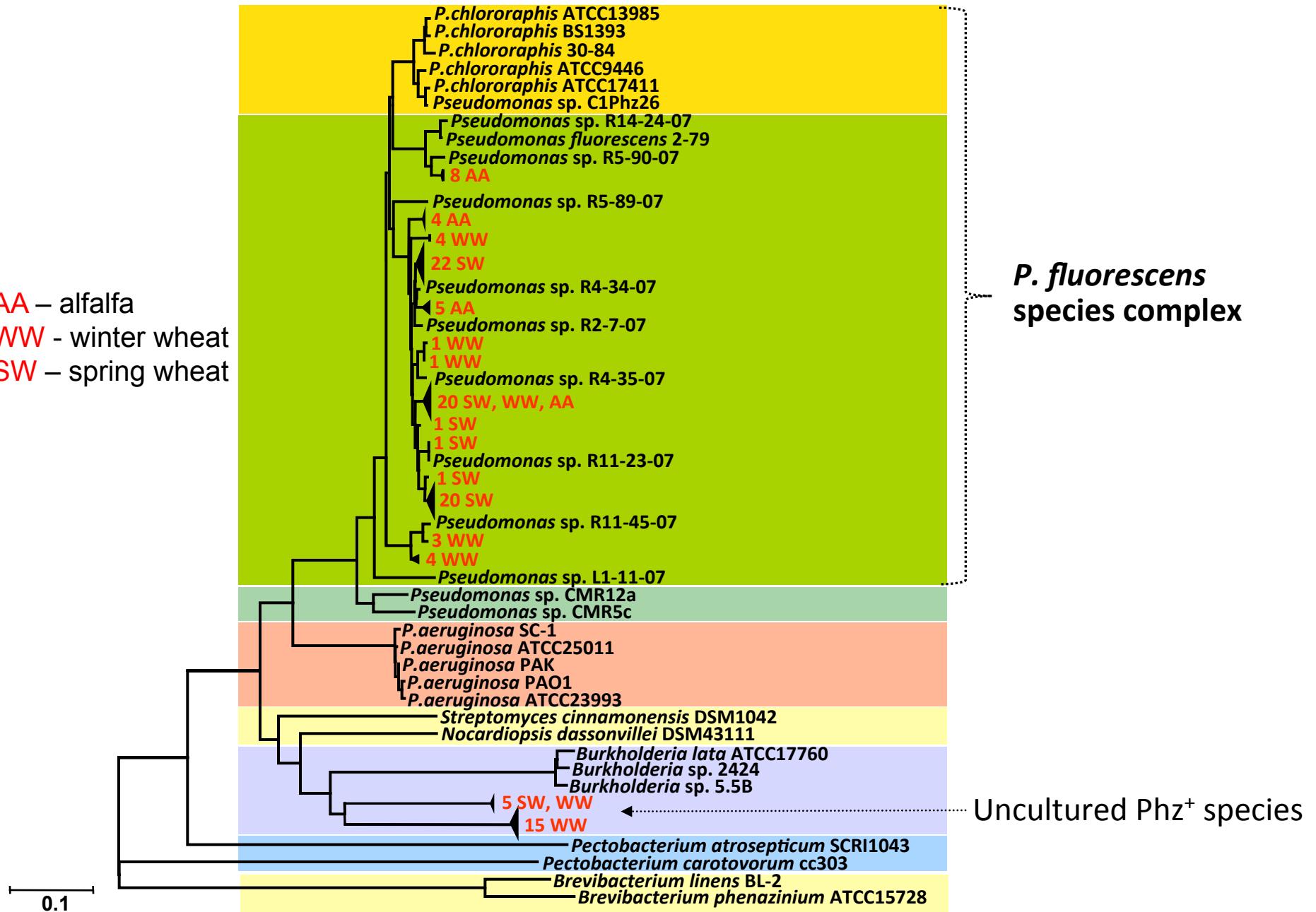


# Phylogeny of phenazine genes



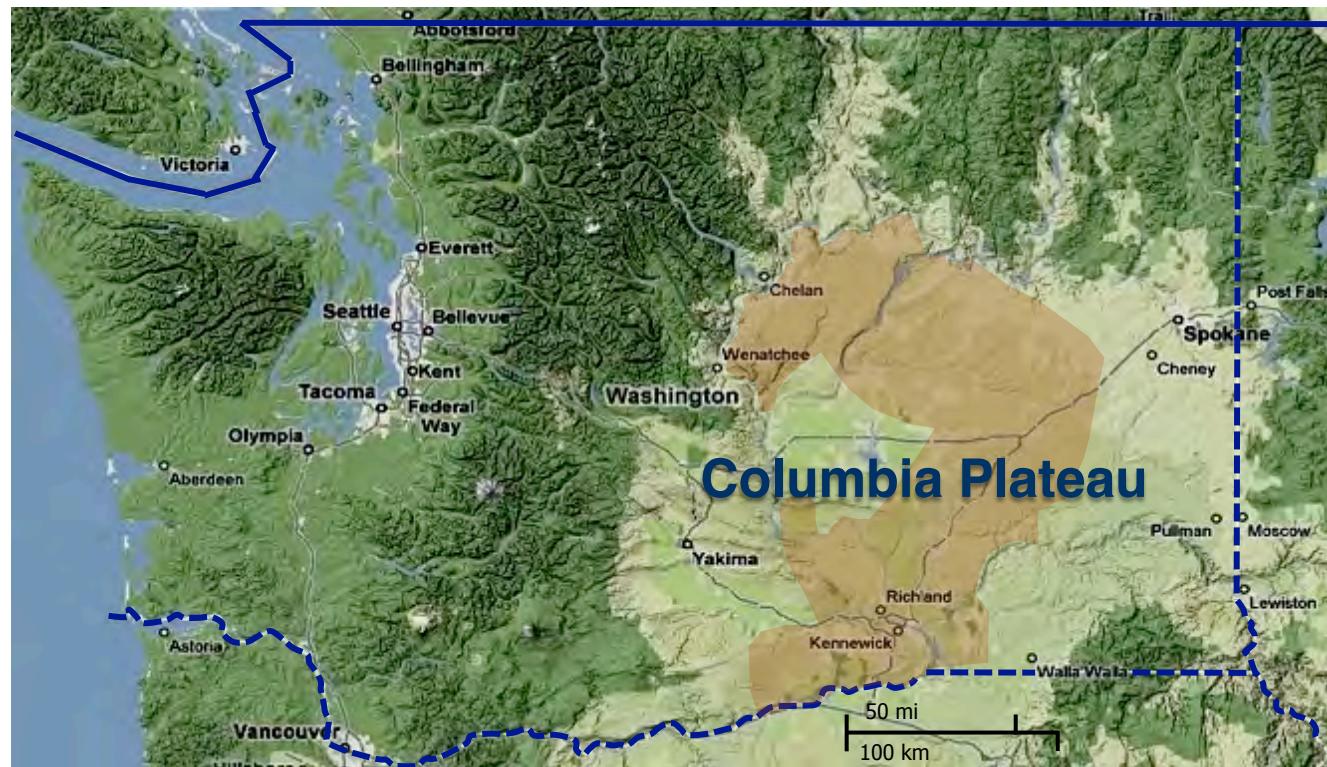
# Diversity of *phzF* alleles from Ritzville soil

AA – alfalfa  
WW - winter wheat  
SW – spring wheat



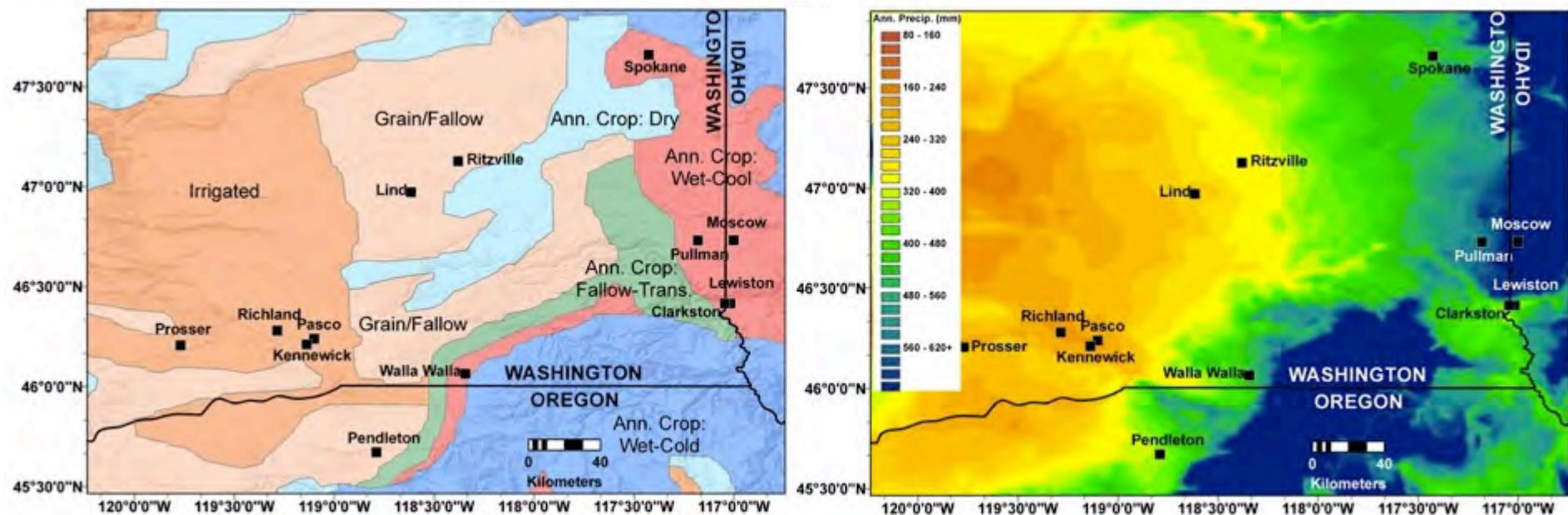
# Inland Pacific Northwest

- ◆ Large flood basalt plateau east of the Cascade Range
- ◆ Top soil is silt loam formed by postglacial sediments and ancient volcanic eruptions
- ◆ Major agricultural area: covers 1.56M cropland hectares



# The Inland Pacific Northwest farming

- ◆ The area is situated in the rain shadow of the Cascades
- ◆ A gradient of annual precipitation: from 150-300 mm (west) to 450-600 mm (east)
- ◆ The annual precipitation and growing-degree days have strong impact on the regional agriculture



# Dryland agroecosystem

- ◆ Winter wheat is dominant, but further east rotation crops are grown (barley, lentils, chickpeas, canola & other brassicas)

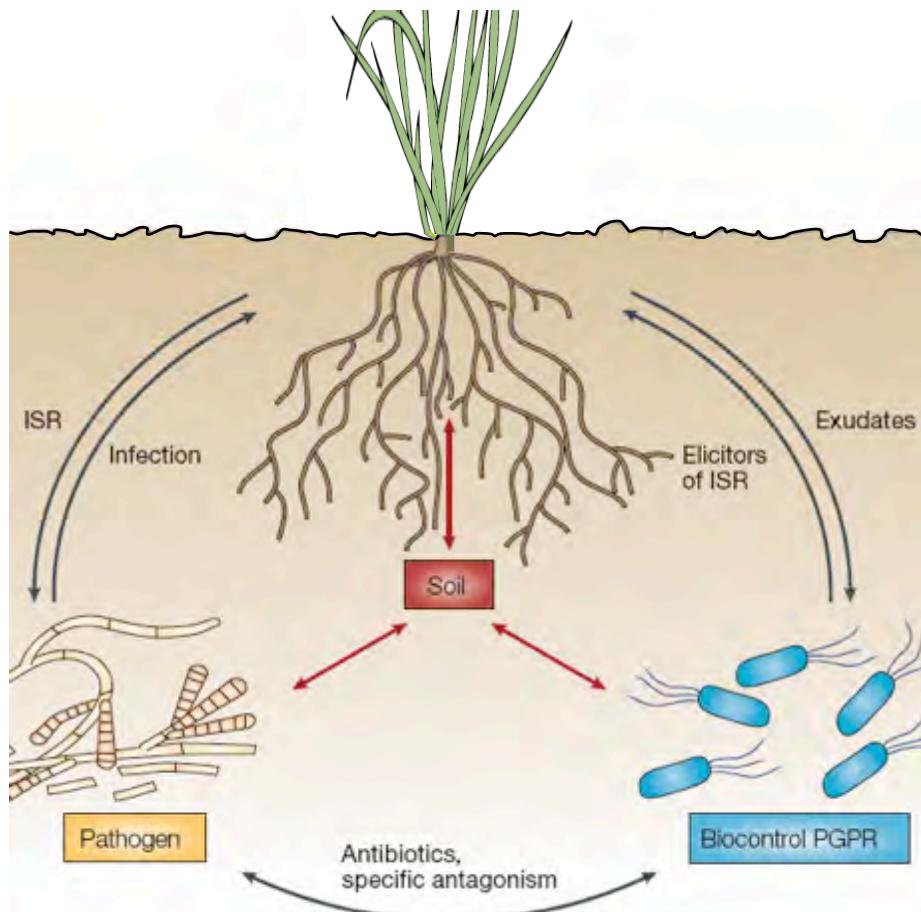


# Soilborne diseases of wheat

- ◆ Root and crown rots caused by *Gaeumannomyces graminis* var. *tritici*, *Fusarium* and *Rhizoctonia* are economically important
- ◆ *Rhizoctonia solani* AG-8 can be particularly destructive and cause bare patches, where the wheat is severely stunted and yields no grain



# Biological control of soilborne diseases

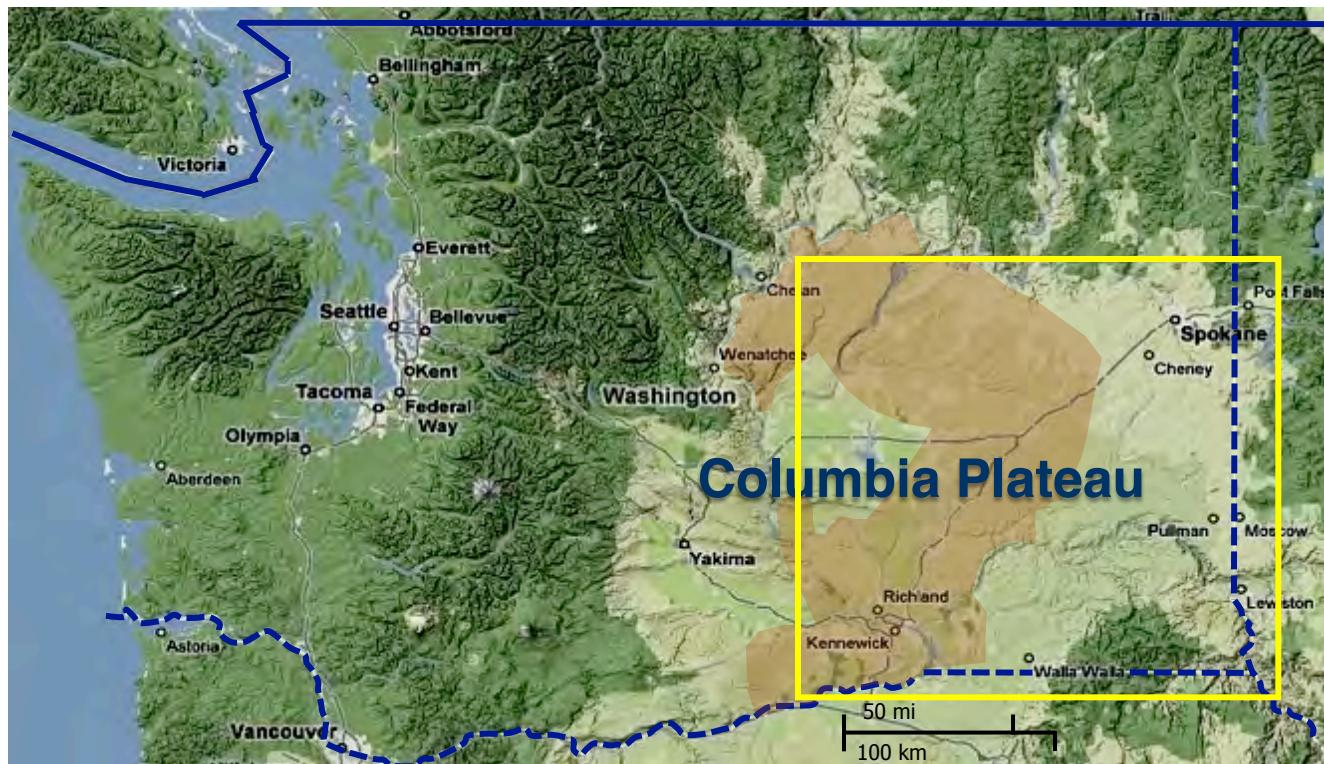


Haas & Defago (2005) Nat Rev Microbiol

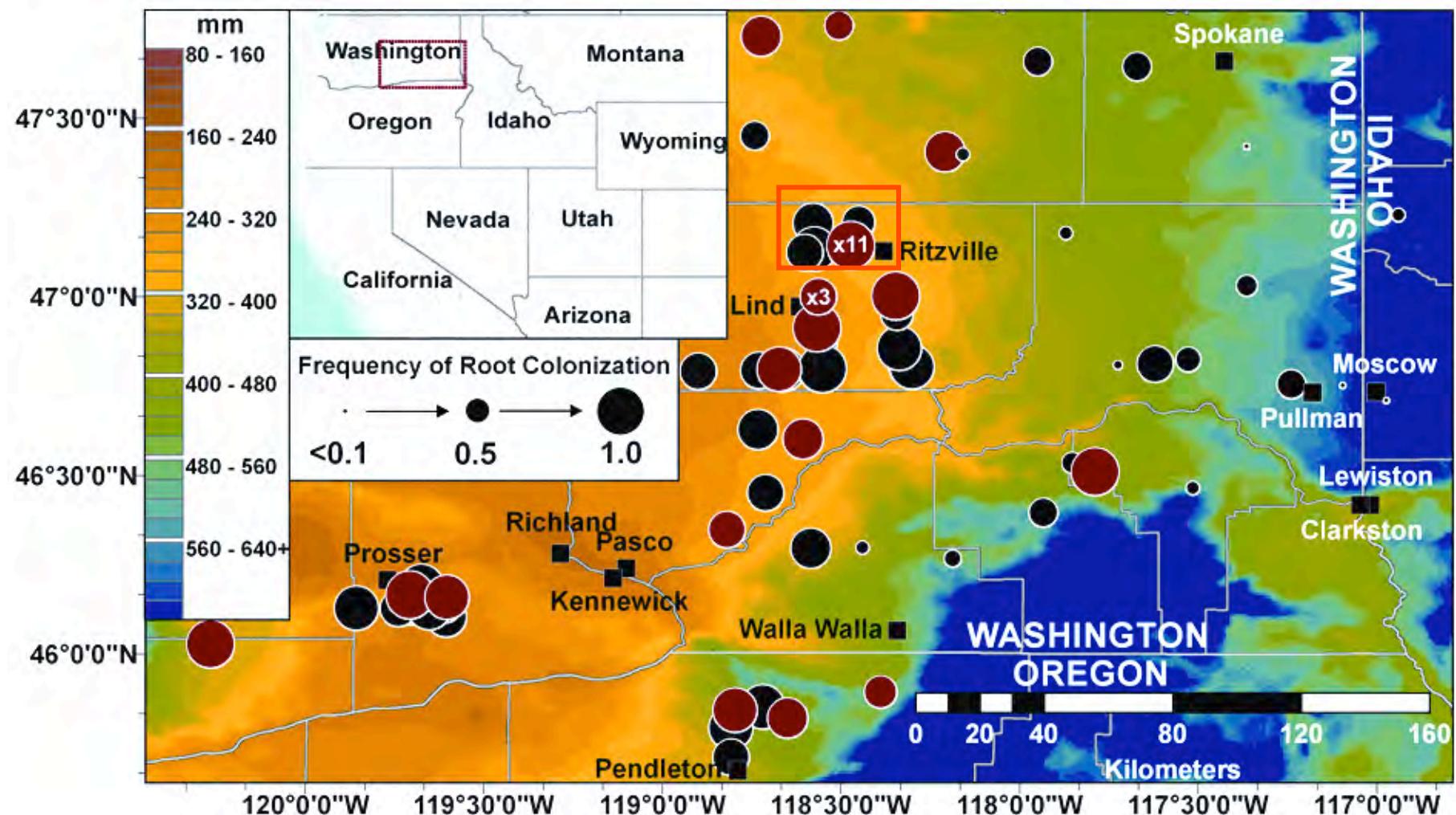
- ◆ Our goal is to develop biocontrol management practices for soilborne diseases of wheat
- ◆ An attractive option due to lack of resistant varieties and chemical treatments
- ◆ Focus on indigenous *Pseudomonas* that suppress soilborne pathogens

# Large-scale ecology of biocontrol Phz<sup>+</sup> *Pseudomonas*

- ◆ In 2008 and 2009 we sampled >80 commercial fields within total area of ~22,000 km<sup>2</sup>
- ◆ Populations of Phz<sup>+</sup> rhizobacteria (dilution plating + PCR) and phenazines in the plant rhizosphere (HPLC-MS)

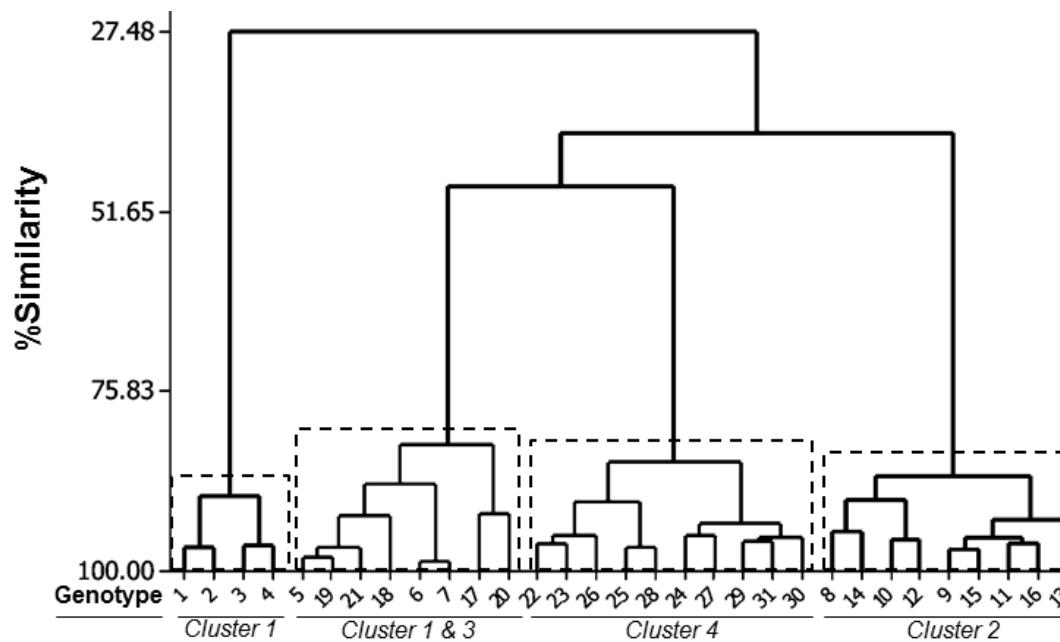


# Distribution of populations of Phz<sup>+</sup> *Pseudomonas* spp. in wheat fields of central and eastern Washington, Oregon and Idaho



# Diversity of Phz<sup>+</sup> species in Washington state soils

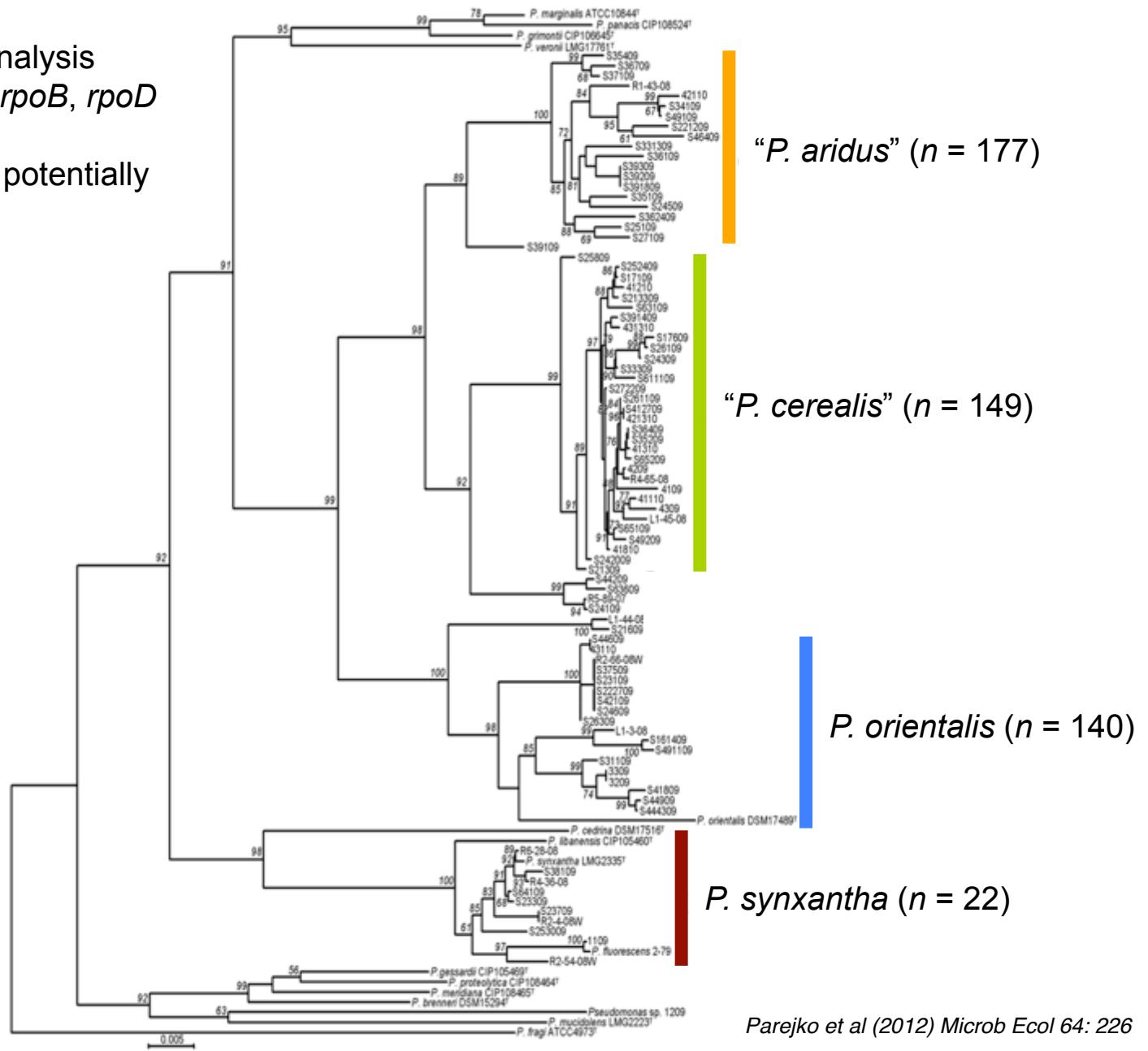
- ◆ A total of 412 Phz<sup>+</sup> isolates were collected from commercial farm fields and profiled using DNA fingerprinting, MLSA (DNA sequence) typing, and phenotyping
- ◆ Four distinct groups of Phz<sup>+</sup> strains were identified



# Diversity of Phz<sup>+</sup> species in Washington state soils

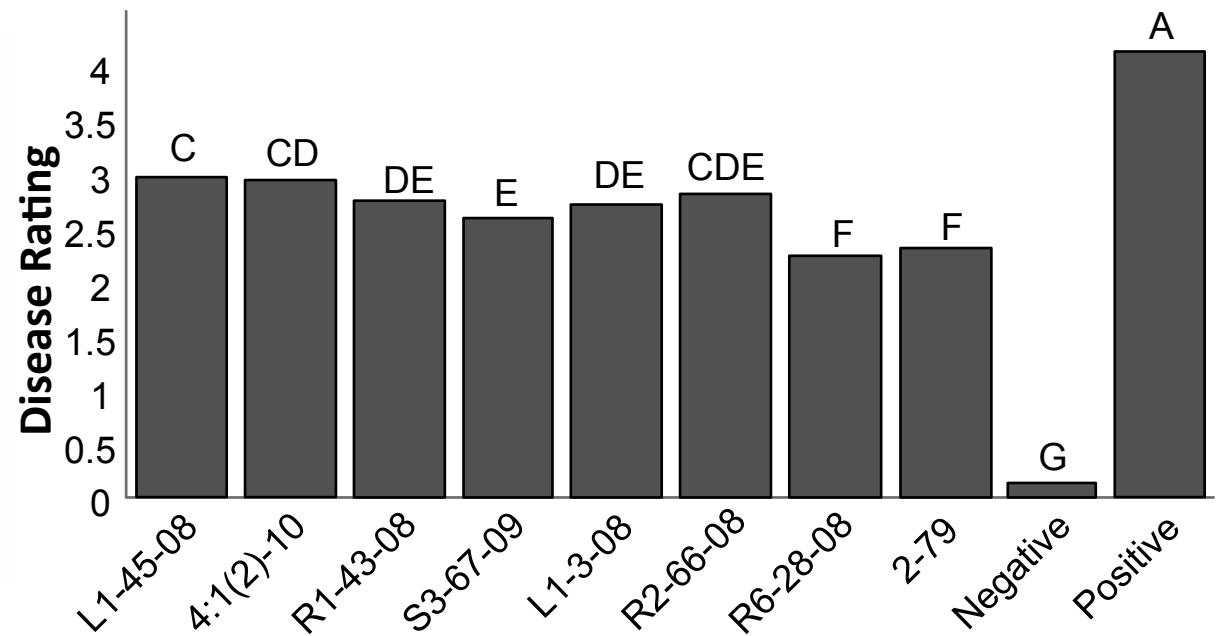
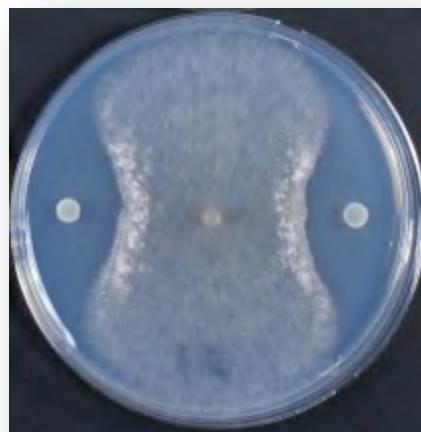
Multi-locus sequence analysis  
using 16S rRNA, *gyrB*, *rpoB*, *rpoD*

Dissimilarity of  $\geq 97\%$  = potentially  
different species



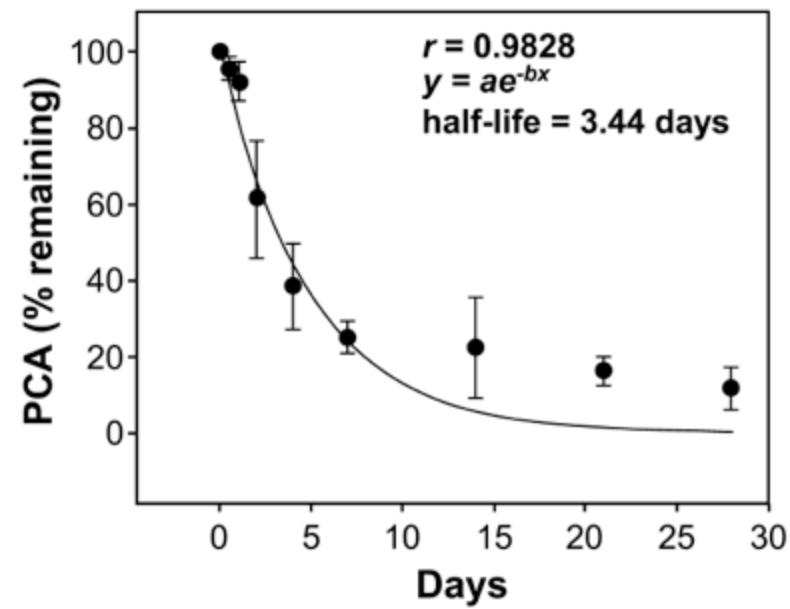
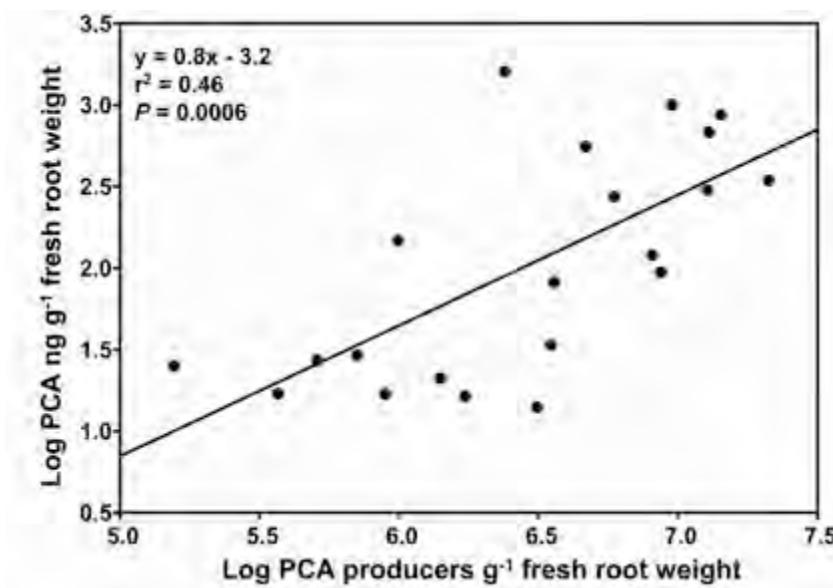
# **Phz<sup>+</sup> *Pseudomonas* control Rhizoctonia root rot**

- ◆ Most Phz<sup>+</sup> strains are strongly inhibitory to *Rhizoctonia*
- ◆ All four groups/species of indigenous Phz<sup>+</sup> *Pseudomonas* increase the shoot length and root weight of wheat grown in soil infested with *R. solani* AG-8



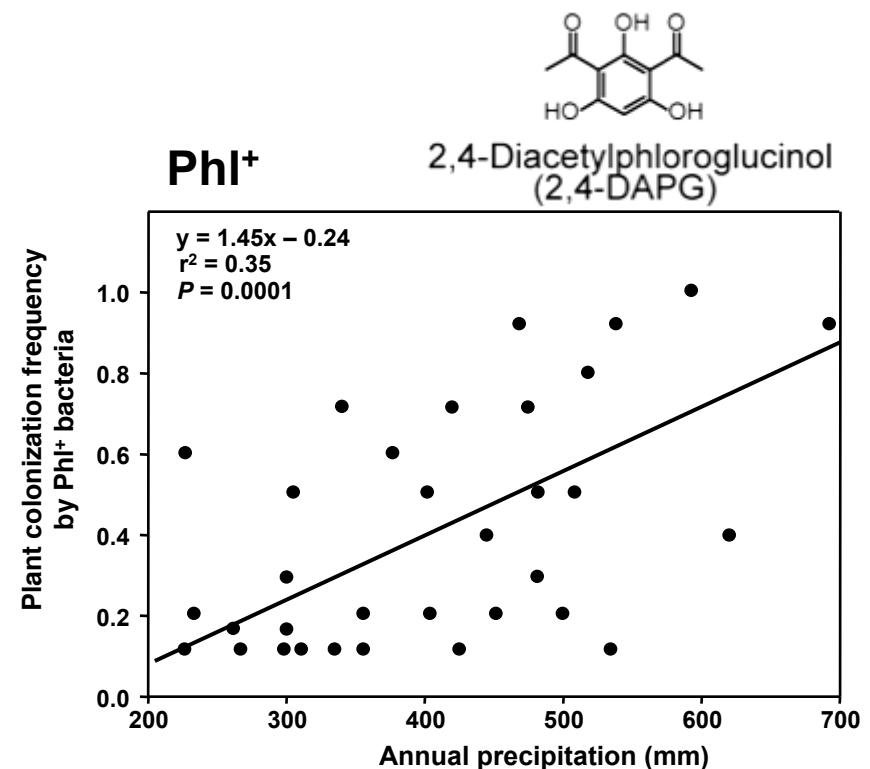
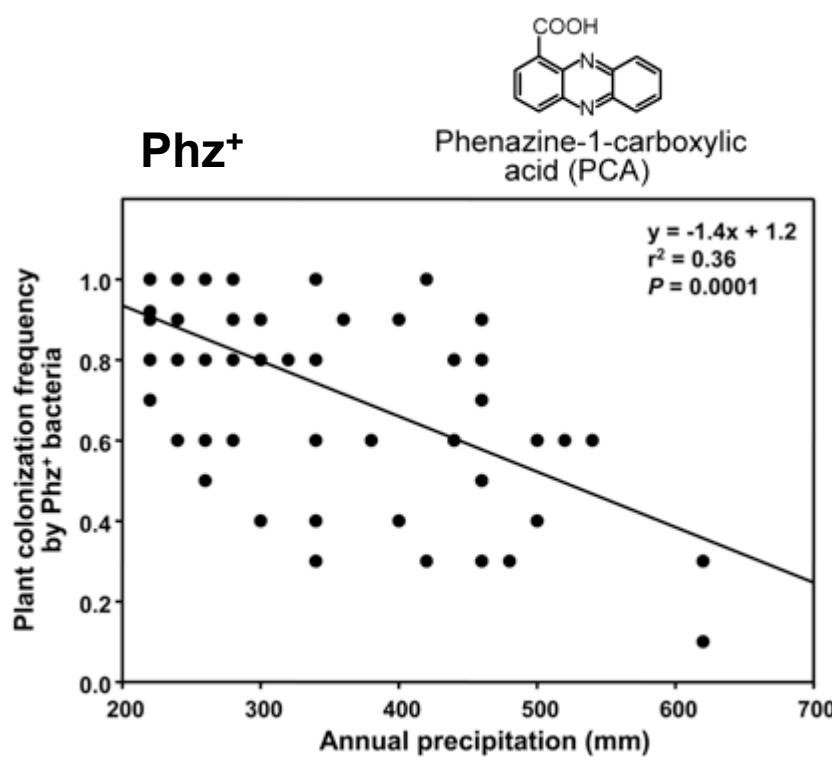
# Phenazines in the rhizosphere of field-grown cereals

- ◆ PCA concentrations up to 1.6 µg (7.1 nmoles)/g of root
- ◆ Bacteria colonize 12-15% of rhizosphere volume (Watt *et al*, 2006) and Phz<sup>+</sup> species comprise 1-10% of the culturable population = localized PCA concentrations approach millimolar levels

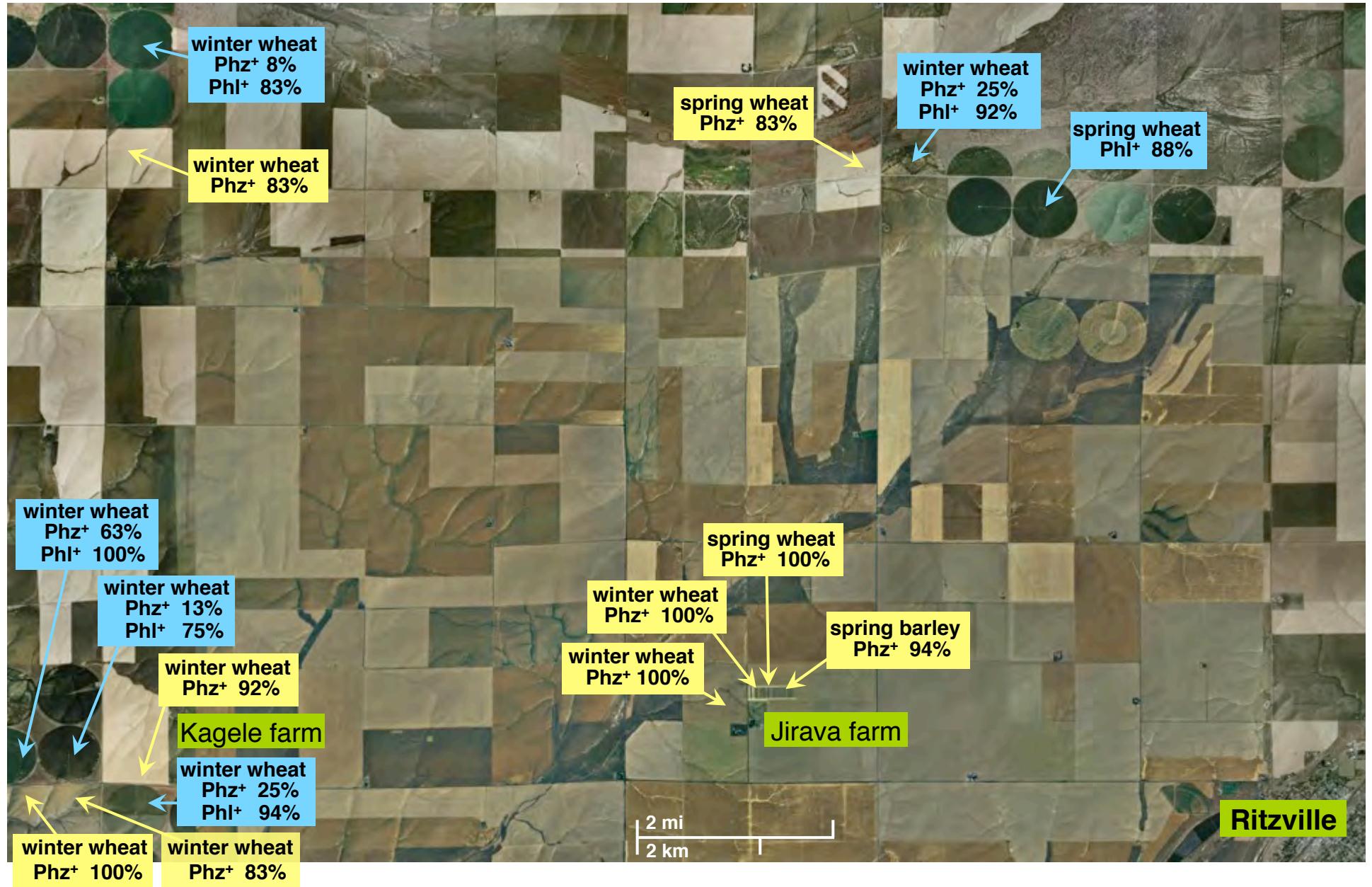


# Phz<sup>+</sup> rhizobacteria and precipitation

- ◆ Phz<sup>+</sup> *Pseudomonas* are adapted to conditions of water stress
- ◆ Soil moisture drives the development of antibiotic-producing microbial communities in the rhizosphere of wheat



# Phz<sup>+</sup> and Phl<sup>+</sup> bacteria in dryland and irrigated wheat fields near Ritzville, WA

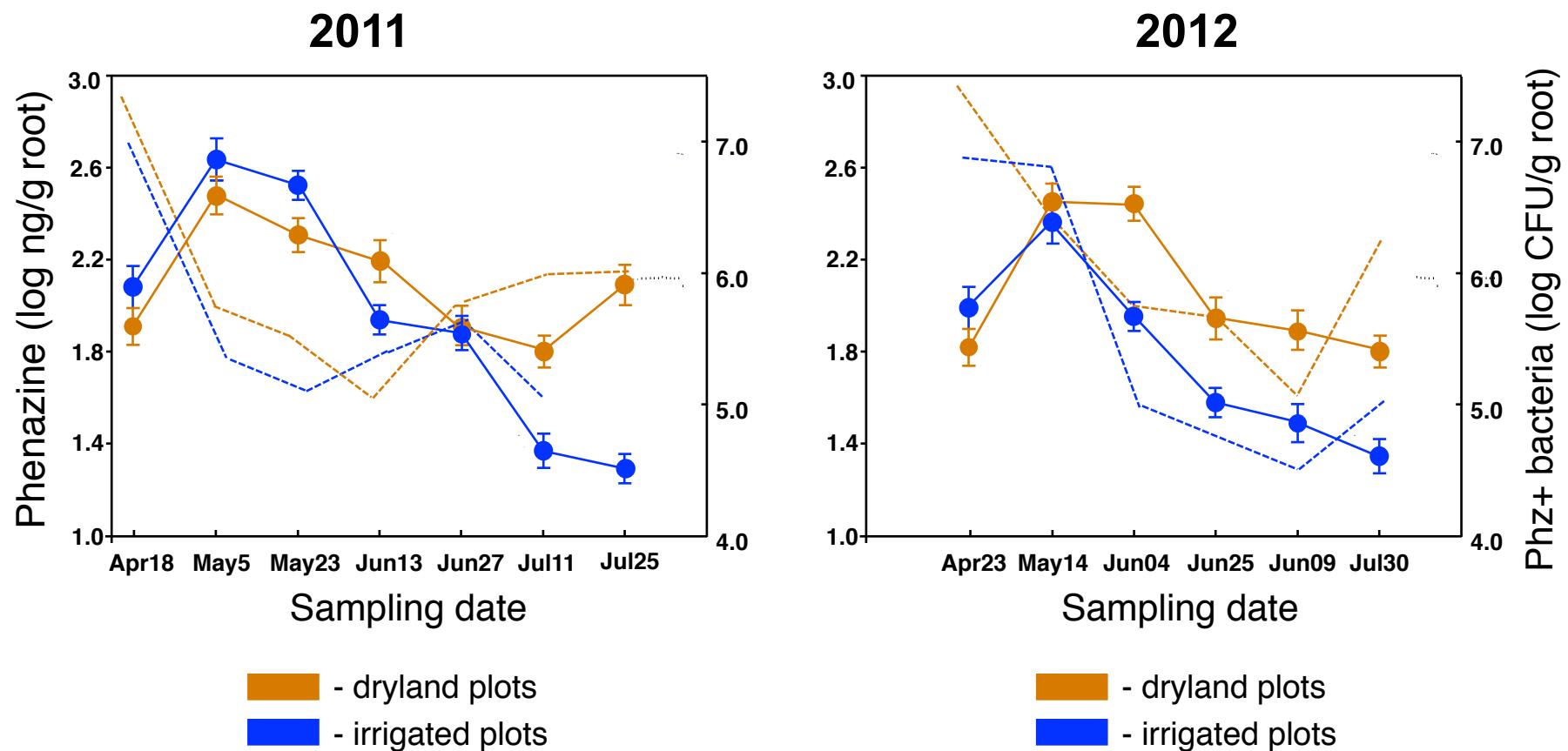


# The impact of water availability on the microbial community in the wheat rhizosphere

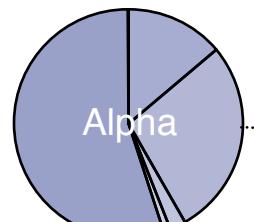
- ◆ A 3-yr field experiment at the WSU Lind Dryland Research Station
- ◆ Objective is to track the dynamics and activity of microbial communities in wheat grown in adjacent irrigated and dryland plots
- ◆ 454 sequencing of 16S (V5-V6) and *phzF* amplicons



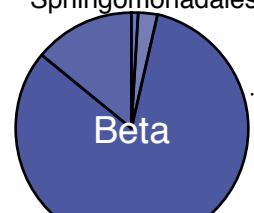
# Dynamics of Phz<sup>+</sup> rhizobacteria and production of antibiotic in the field



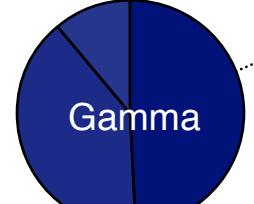
# Relative abundance of bacterial phyla



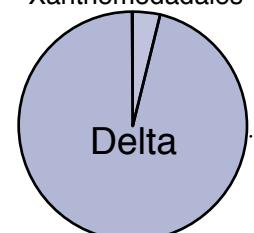
Caulobacterales  
Rhizobiales  
Rhodobacterales  
Rhodospirillales  
Sphingomonadales



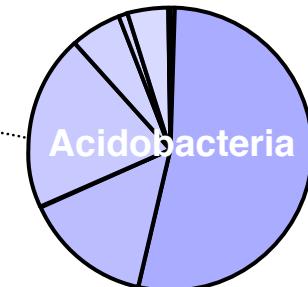
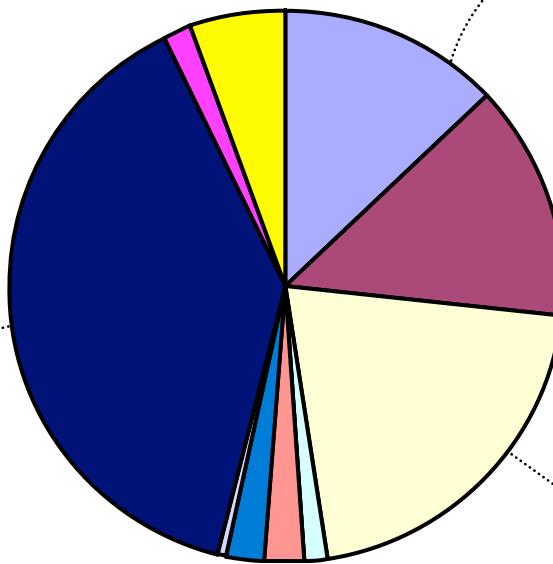
Methylophilales  
Rhodocyclales  
Burkholderiales  
Nitrosomonadales



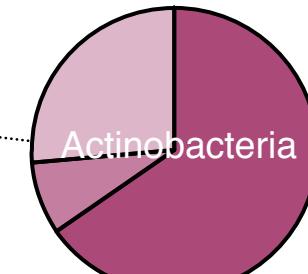
Enterobacteriales  
Pseudomonadales  
Xanthomodadales



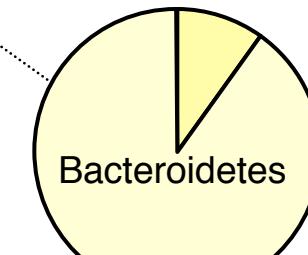
Bdellovibrionales  
Myxoccoccales



Actinomycetales  
Rubrobacteriales  
Solirubrobacteriales



Flavobacteriales  
Sphingobacteriales



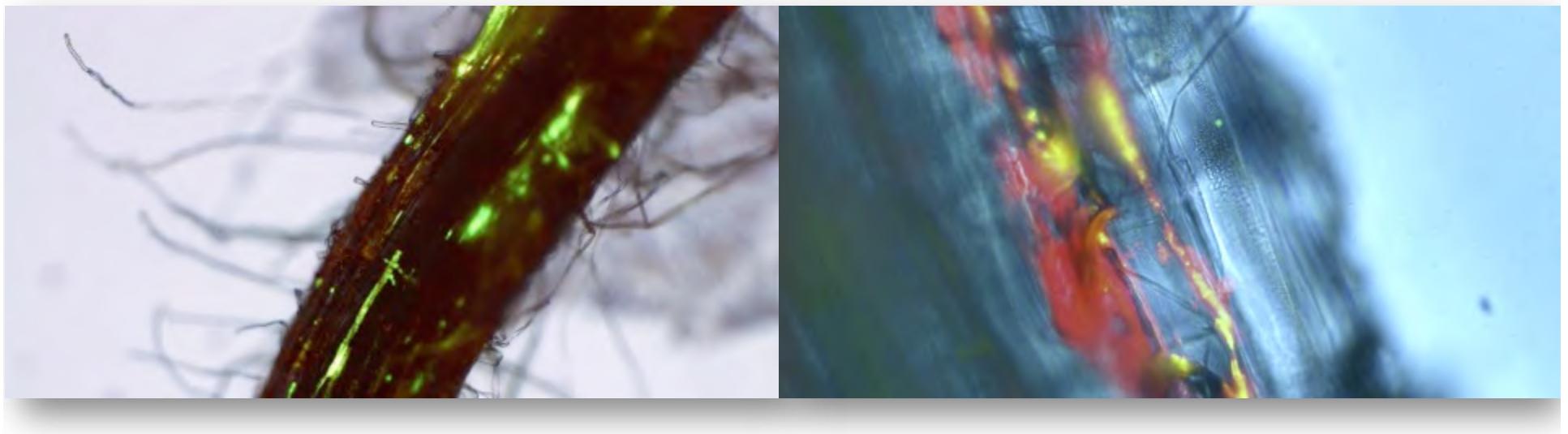
- Acidobacteria
- Actinobacteria
- Bacteroidetes
- Chloroflexi
- Fibrobacteres
- Firmicutes
- Gemmatimonadetes
- Nitrospira
- Proteobacteria
- Verrucomicrobia
- Unclassified

## Preliminary findings

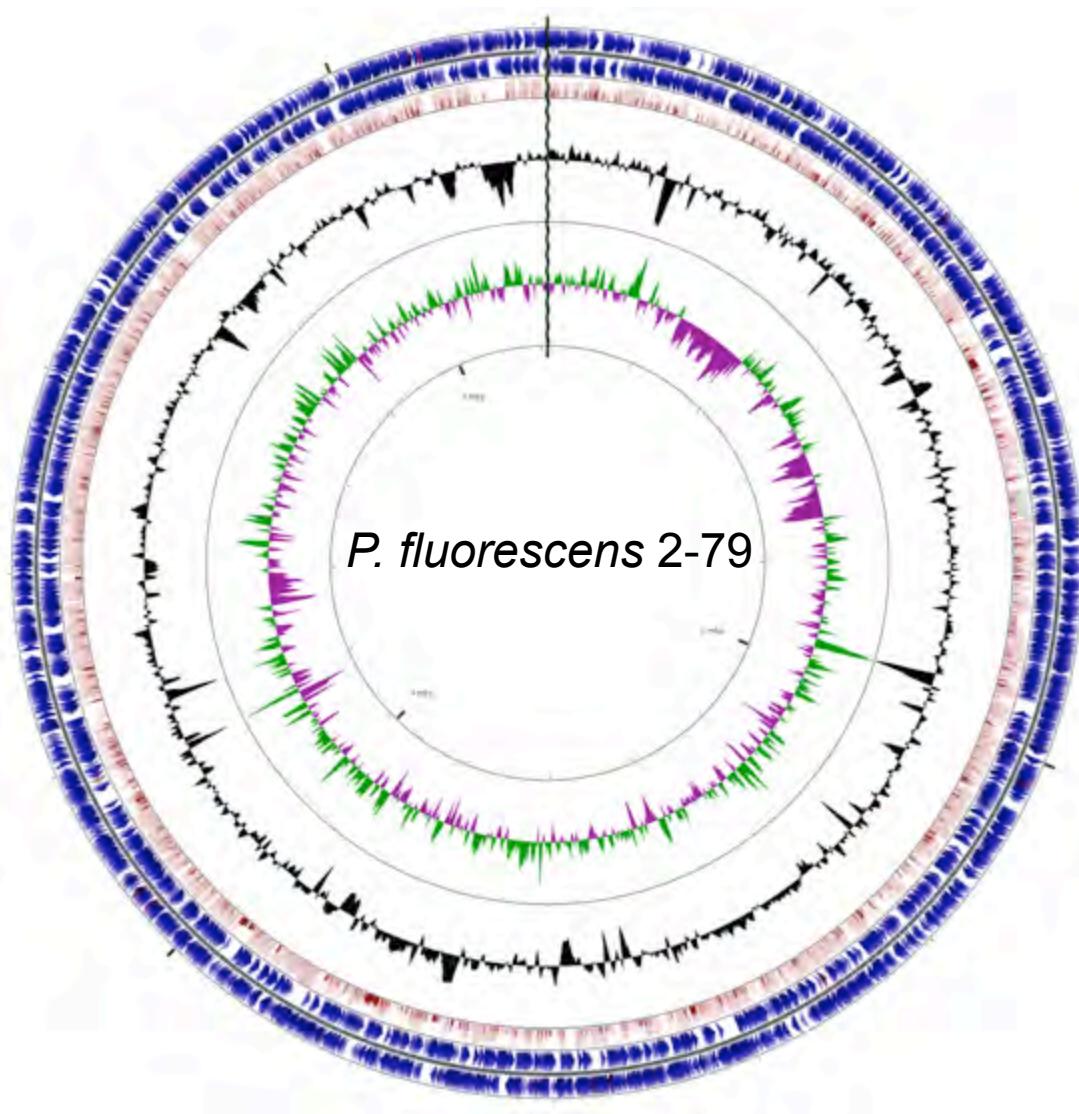
- ◆ Roots of diseased plants are colonized by copiotrophic species such as members of Oxalobacteraceae and Chitinophagaceae (Sphingobacteria)
- ◆ Healthy roots outside of patches are more colonized by oligotrophic unculturable Gemmatimonas, Acidobacteria and Actinobacteria
- ◆ Some evidence that, in addition to Phz<sup>+</sup> *Pseudomonas*, Flavobacteria also contribute to *Rhizoctonia* suppression in the field

# Genomics of rhizosphere Phz<sup>+</sup> *Pseudomonas*

- ◆ Genomes of four Phz<sup>+</sup> strains from the rhizosphere of wheat (*P. fluorescens* 2-79, *P. cerealis* L1-45-08, *P. aridus* R1-43-08, *P. orientalis* L1-3-08)
- ◆ Identify pathways that are likely to confer biocontrol traits and/or contribute to plant-microbe interactions
- ◆ Use molecular tools to understand how abiotic factors affect performance of biocontrol agents *in situ*



# Genomics of rhizosphere Phz<sup>+</sup> *Pseudomonas*

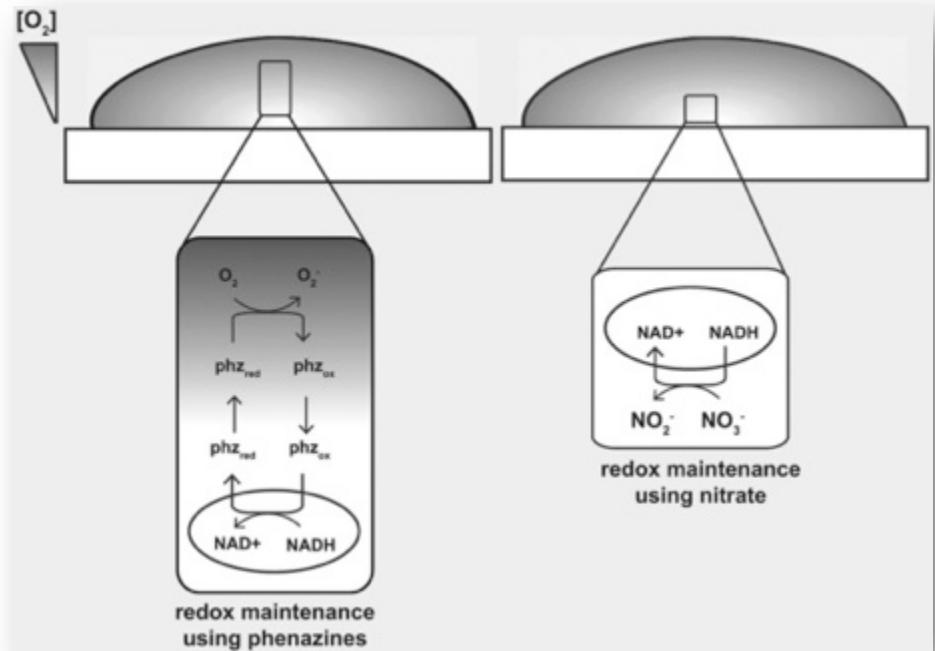
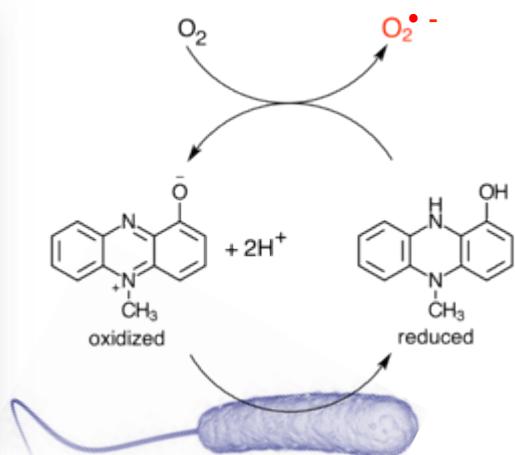


## Genome features of *P. fluorescens* 2-79

Chromosome size (Mbp)	6.46
G+C (%)	59.8
Protein-coding genes	5635
Conserved hypothetical genes	679
Hypothetical genes	322
Average gene length (bp)	984
Coding (%)	87.6
rRNA operons	5
tRNA genes	63
Number of scaffolds	22

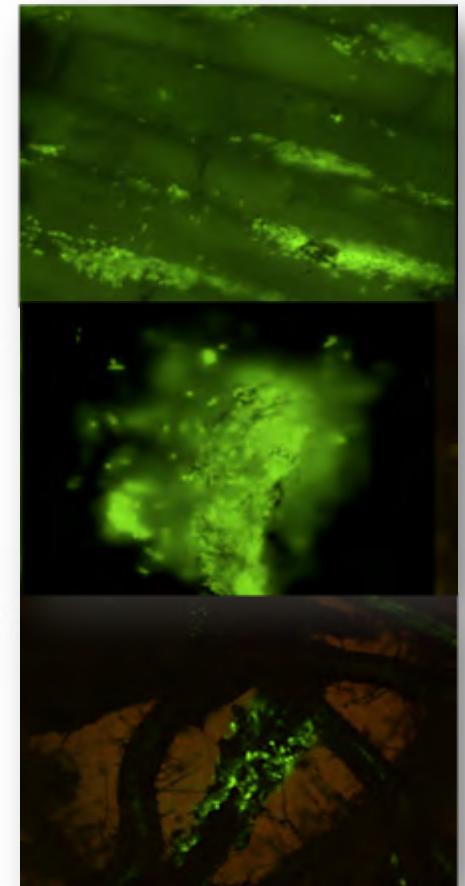
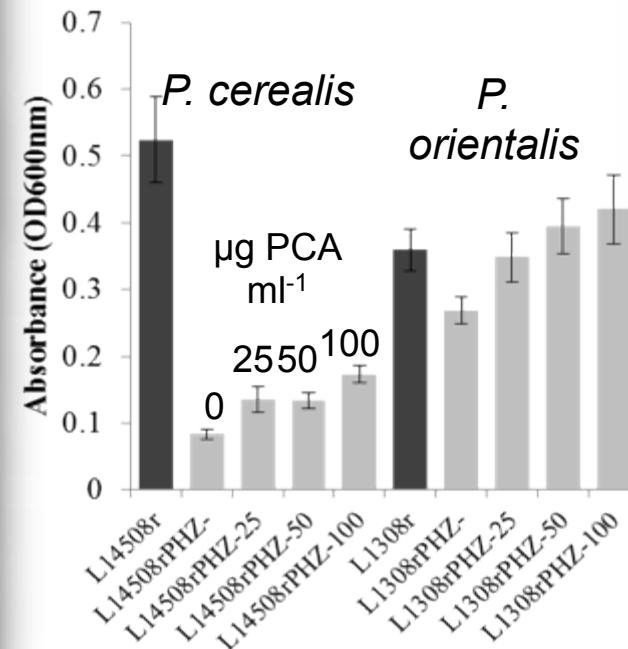
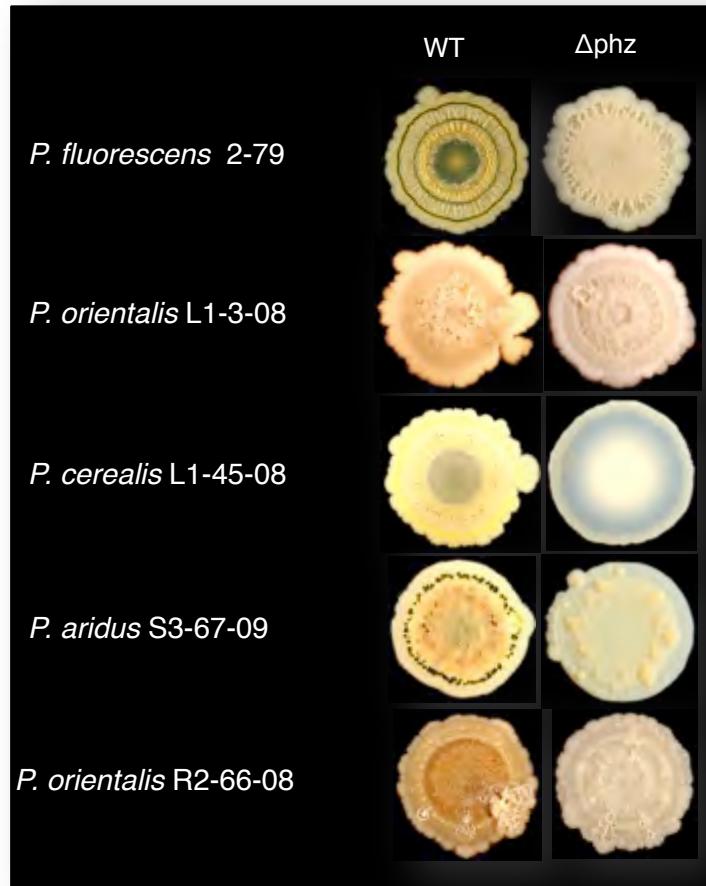
# Biological activity of phenazines

- ◆ Phenazines are redox-active and act as extracellular electron shuttles
- ◆ Under the oxygen-limited conditions (i.e. in biofilms), phenazines may help *Pseudomonas* to generate energy for growth by acting as alternative electron acceptors for re-oxidation of NADH



Source: Dietrich et al (2013) J Bacteriol 195: 1371

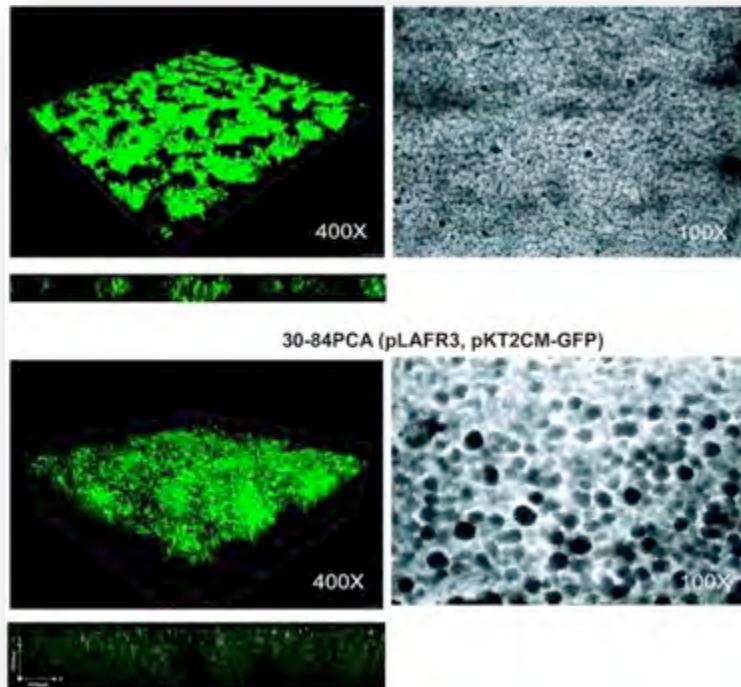
# Possible link between phenazines and biofilms in *P. fluorescens*-like strains



# Phenazines are important for biofilm growth

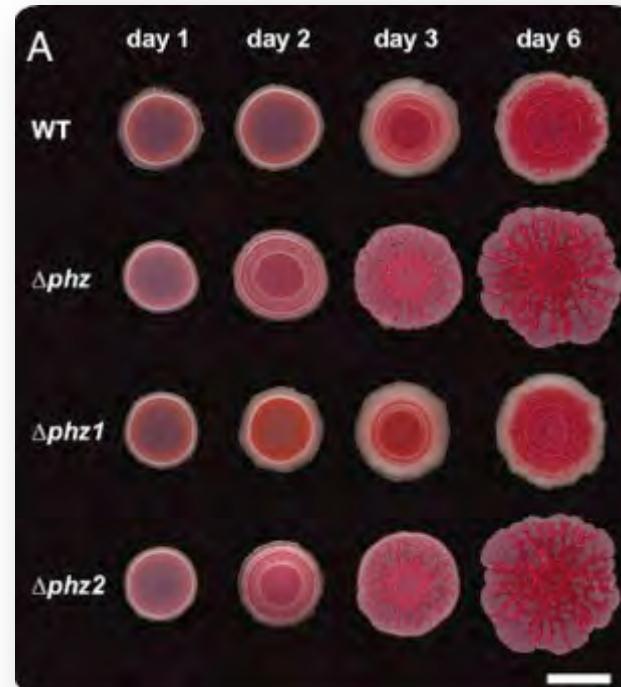
- ◆ Phenazines play an important role in biofilm formation and colony morphology in *Pseudomonas* spp.

*P. chlororaphis*



Maddula et al (2008) J Bacteriol 190: 2759

*P. aeruginosa*



Recinos et al (2012) PNAS USA 109: 19420



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