"Cross talk" Between Bacteria Associated With the Roots of Canola and Wheat

A.L. Misko[1] and J.J. Germida[1]

[1]Saskatchewan Centre for Soil Research, University of Saskatchewan, Saskatoon, SK, S7N 5A8

Keywords: Cross talk, *N*-acyl-homoserine lactone, phenazine, take-all, *Pseudomonas aureofaciens* 30-84

Abstract

Phenazine antibiotics are produced by some soil bacteria and suppress growth of many fungi that cause plant diseases. *N*-acetyl-homoserine lactone (AHL) is a type of signal molecule that can activate the production of phenazine in bacteria. This response is referred to as "cross talk." In this study, bacteria from the rhizosphere and root interior of canola and wheat were screened for AHL production. Our results show that approximately 4% of the isolates produced AHL. *Pseudomonas corrugata* and *P. savastanoi* were the most common bacteria associated with canola that produced AHL, whereas *Enterobacter agglomerans* and *P. corrugata* were the most common in wheat. This study shows that there is a small community of AHL-producing bacteria associated with the roots of both canola and wheat, suggesting that "cross-talking" between bacteria in roots is possible.

Introduction

Some microorganisms can secrete chemical signals that cause a response, such as antibiotic production, in other microorganisms in their community. This phenomenon, referred to as "cross talk", is considered a mechanism of communication between bacteria, providing a competitive advantage for the "cross-talking" population. *N*-acyl-homoserine lactones (AHL) are a class of signal molecules that are reported to activate synthesis of antibiotic genes in a number of bacteria (Wood *et al*., 1997). Studies have shown that a number of soil bacteria produce AHLs that activate the production of the antibiotic phenazine, which is inhibitory to take-all in wheat (Thomashow and Weller, 1988), in *Pseudomonas aureofaciens* strain 30-84 (Pierson *et al*., 1998). Mutant strains of *P. aureofaciens* 30-84 can be used to assay for AHL production, and thus as an indicator of "cross talking" between bacteria in a community.

Objectives

- To assess the potential of rhizosphere and endophytic bacteria, isolated from the roots of field-grown canola and wheat, to produce AHL signals.

- To determine if these bacteria differ by plant cultivar, habitat location and field site.

Materials and Methods

Plant varieties: Canola (Excel, Parkland, Quest varieties) grown in Denholm, Saskatchewan, and wheat (PI 167549, Red Fife, CDC Teal varieties) grown in Kernen and Saskatoon, Saskatchewan.

Bacteria isolates: Bacteria were isolated from the rhizosphere soil and from the root interior of canola and wheat plants according to Germida *et al*. (1998). Isolates were identified using fatty acid methyl ester (FAME) analysis and the Microbial Identification System (MIDI Inc., Newark, Del), and grown in 1/10 tryptone soy broth (TSA) slants at 28ºC for 48h.

Cross talk assay: Mutant strains of *Pseudomonas aureofaciens* 30-84 (L.S. Pierson III, University of Arizona) were used to identify potential AHL-producing bacterial isolates. *P. aureofaciens* 30-84I (produces phenazine with an outside source of AHL) (100 uL) was spread onto a PPMD agar plate (Wood et al., 1997). Bacterial isolates (5 uL) were spotted onto the inoculated PPMD plate and incubated at 28ºC for 48h. Similarly, as controls bacterial isolates and *P. aureofaciens* 30-84I were spotted onto a PPMD plate inoculated with *P. aureofaciens* 30-84Ice (Phz$^-$). The presence of an orange halo around the test isolate was an indicator of phenazine production by 30-84I in response to AHL produced by the isolate.

Results

Out of 2835 bacterial isolates from canola and wheat, 111 (4%) produced AHL. There were 925 screened bacterial isolates from canola assayed for cross talk, and 40 (4%) were found to produce AHL. The majority of these isolates were identified as *Pseudomonas corrugata* or *P. savastonoi* (Tables 1 & 2).

Table 1. AHL-producing bacteria isolated from the rhizosphere of canola plants.

Cultivar	Species	Abundance
Excel	*Bacillus*	1
	Pseudomonas	9
	Identity unknown	2
Parkland	*Pseudomonas*	9
	Identity unknown	1
Quest	*Pseudomonas*	3

Table 2. AHL-producing bacteria isolated from the root-interior of canola plants.

Cultivar	Species	Abundance
Excel	*Micrococcus*	1
	Variovorax	1
	Xanthomonas	1
	Identity unknown	1
Parkland	Identity unknown	2
Quest	*Curtobacterium*	1
	Pseudomonas	6
	Identity unknown	1

Of the 1910 screened bacterial isolates from wheat assayed for cross talk, 71 (4%) produced AHL. The majority of these isolates were identified as *Enterobacter agglomerans* (Tables 3 & 4).

Table 3. AHL-producing bacteria isolated from the rhizosphere of wheat plants.

Cultivar	Species	Abundance
CDC Teal	*Pseudomonas*	1
	Identity unknown	1
PI 167549	*Arthrobacter*	1
	Bacillus	2
	Enterobacter	4
	Pseudomonas	1
	Salmonella	1
	Variovorax	1
	Identity unknown	2
Red fife	*Enterobacter*	2
	Erwinia	1
	Pseudomonas	11
	Identity unknown	6

Table 4. AHL-producing bacteria isolated from the root-interior of wheat plants.

Cultivar	Species	Abundance
CDC Teal	*Clavibacter*	1
	Cytophaga	1
	Enterobacter	6
	Erwinia	1
	Pseudomonas	3
	Identity unknown	3
PI 167549	*Acinetobacter*	1
	Curtobacterium	3
	Enterobacter	2
	Erwinia	1
	Variovorax	1
	Xanthomonas	1
	Identity unknown	6
Red fife	*Cedecea*	2
	Kluyvera	1
	Identity unknown	4

Habitat location and field site had an effect on the number of AHL-producing bacteria. The AHL-positive canola isolates were primarily rhizosphere bacteria (Figure 1), whereas most of the AHL-positive wheat isolates were found in the root interior (Figure 2). Field site also influenced the production of AHL by microorganisms; the Kernen site had almost twice as many AHL-producing isolates (by percentages) as the Saskatoon site (Figure 2).

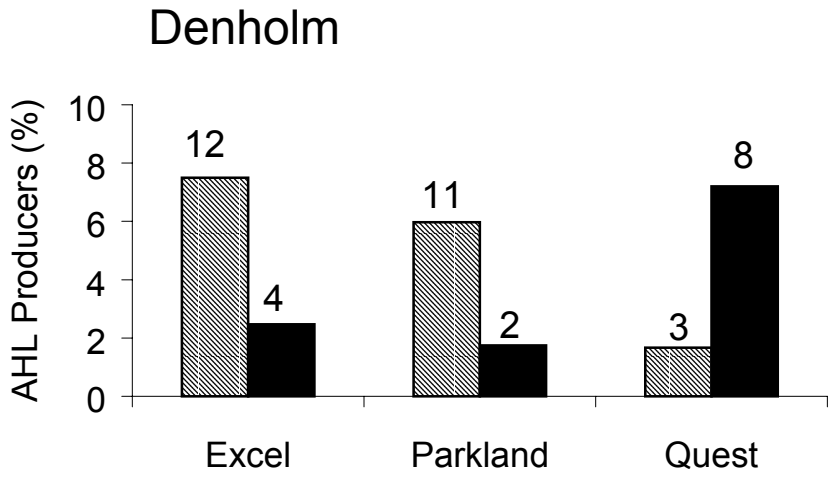

Figure 1. Percentage AHL-producing rhizosphere (thatched bars) and endophytic (solid bars) bacteria, isolated from the roots of field grown canola plants. Field site was located at Denholm, SK. Numbers above the bars refer to the actual number of AHL-positive isolates.

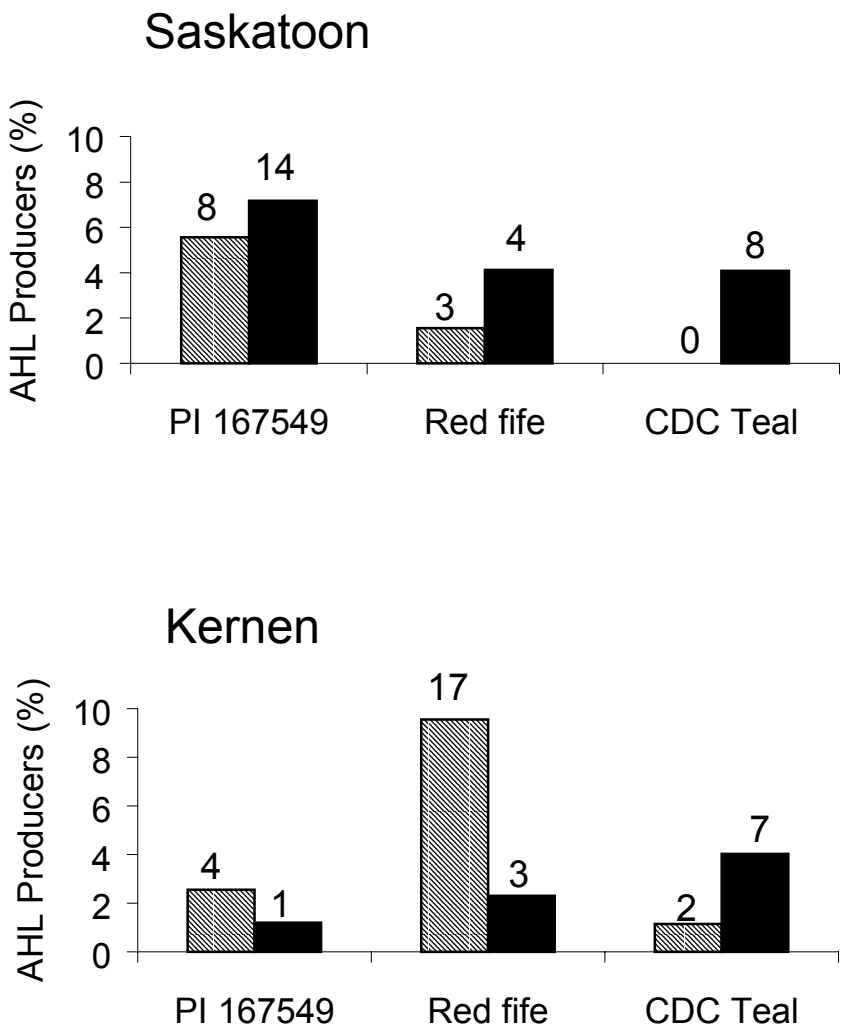

Figure 2. Percentage AHL-producing rhizosphere (thatched bars) and endophytic (solid bars) bacteria, isolated from the roots of field grown wheat plants. Field sites were located at Saskatoon and Kernen, SK. Numbers above the bars refer to the actual number of AHL-positive isolates.

Plant cultivar did not affect the number of AHL-producing bacteria. There were no significant differences in the number of bacterial isolates from each cultivar of canola (Figure 3) and wheat (Figure 4) that could produce AHL.

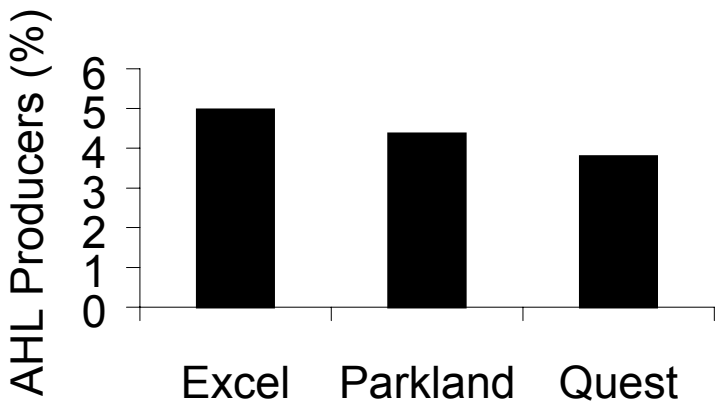

Figure 3. Percentage AHL-producing bacteria isolated from canola. Endophytic and rhizosphere values are combined for each cultivar.

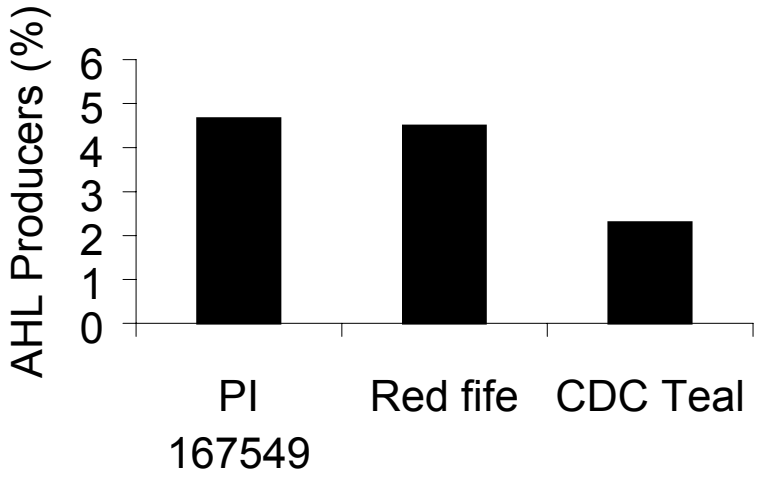

Figure 4. Percentage AHL-producing bacteria isolated from wheat. Endophytic and rhizosphere values are combined for each cultivar.

Conclusions

In this study, a small population of AHL producers (*ca*. 4%) was present in the rhizosphere and root interior of both canola and wheat plants. However, the number and habitat location of AHL producers varied between plant species and field locations. There were, however, no significant differences in AHL-producers between plant cultivars. In a similar study, Pierson *et al*. (1998) found that *ca*. 8% of wheat root-associated bacterial isolates produced AHL signals. Our results indicate that a small community of root-associated bacteria produce AHL signal molecules, suggesting that "cross-talking" between bacteria inside plant roots may be possible.

Phenazine antibiotics have been found to inhibit fungal pathogens (Mazzola et al., 1992), therefore "cross talk" may be a mechanism involved in biocontrol in plant roots. Additional studies are needed to determine whether these AHL-producing isolates could be useful agents for biocontrol in the rhizosphere of canola and wheat plants.

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Acknowledgements

The work was supported by a grant from NSERC.