Enhancement of Pulse Crops: Influence of Novel Rhizobacteria on the Interaction of Pea, Lentil, and Pathogenic Fungi

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Abstract

In Saskatchewan, land area used to grow pulse crops (pea, lentil, bean, and chickpea) is increasing every year; between 2000 and 2001, an increase of 20% was recorded. Maintenance of plant health is imperative to produce an economically viable resource. The objective of this study is to characterize novel rhizobacteria for plant growth promoting properties: enhancing the development of pea and lentil plants, and suppressing the growth and disease severity caused by phytopathogenic fungi. Rhizobacterial isolates were selected from a preliminary group of 580 based on the ability to suppress *Pythium*, *Rhizoctonia*, and *Fusarium* spp. In *in vitro* assays, no one isolate, except for isolate 5-6, had shown the ability to strongly suppress all three pathogenic fungi. Additionally, the presence of pea or lentil seeds affected the ability of the strains to suppress the fungi. Bacterial cell-free filtrate suppressed the growth of *Pythium* and *Rhizoctonia* spp., thus fungal suppression is mediated by antifungal metabolites. Furthermore, suppression of *Rhizoctonia* sp. is correlated to the production of proteolytic enzymes by the rhizobacteria. Plant growth promoting rhizobacteria (PGPR) are important in food production by increasing crop productivity, and reducing crop losses caused by soil-borne phytopathogens.

Introduction

The soil immediately surrounding plant roots is defined as the rhizosphere. This environment contains organisms that can be either beneficial or deleterious to plant health and growth. Enhancement of crop yield by beneficial bacteria is mediated by direct and indirect mechanisms, (Glick, 1995). These mechanisms include increasing availability of nutrients, minerals, production of growth-promoting substances and suppression of fungal pathogens.

The objectives of this study are to identify and characterize novel rhizobacteria to enhance the growth of legume crops. These will be characterized as follows:

- Ability to suppress the growth of *Fusarium, Pythium,* and *Rhizoctonia* spp.
- Ability to produce fungal lytic enzymes, such as, chitinases and proteases
- Ability to produce antifungal metabolites
- Ability to solubilize inorganic phosphate, to increase nutrient availability

Materials and Methods

Rhizobacterial Isolates: Sixteen rhizobacterial strains isolated by Hynes and Nelson (2001) from 6 fields in Saskatchewan were characterized. These isolates were stored in 20% glycerol at -70 ℃. Isolates were grown in one-half strength Tryptic Soy Broth (1/2 TSB) for 2 days on a gyratory

shaker (150 rpm) at room temperature. *Pseudomonas chlororaphis* 63-28 (EcoSoil Inc., San Diego, CA) was included as a positive control. Pathogenic fungi included *Pythium, Rhizoctonia,* and *Fusarium* spp.

Assays

Suppression of fungal growth: Antibiosis assays were conducted on 1/5-strength Potato Dextrose Agar (1/5 PDA) plates, with a fungal plug placed in the centre, and bacteria streaked 3 cm perpendicular to the plug. In the presence of pea or lentil seed, six seeds were aligned along the middle of 1/5 PDA plates, and fungal plugs placed 3 cm perpendicular to the seeds. Assays were incubated at room temperature for 2 days (*Pythium*), 3 days (*Rhizoctonia*), or >7 days (*Fusarium*). Fungal suppression was measured as the zone of inhibited growth: the distance between the bacterial isolate and the fungal growth front.

Antifungal metabolite production: Rhizobacterial isolates were grown in ½TSB for 2 days, and the cells removed by centrifugation. The supernatant was filtered through 0.45µm filter paper *in vacuo*. The filtrate was added to 1/5 PDA at a ratio of 1:4, 1:10, and 1:100 (v/v). Fungal plugs were placed in the centre, and the growth was compared to fungi grown on media without the bacterial filtrate.

Production of lytic enzymes: Protease was detected by the clearing of Skim Milk medium surrounding the rhizobacterial isolate. Chitinase was detected by a zone of clearing on minimal medium supplemented with colloidal chitin (Dunne et al., 1997).

Effect of decreasing O_2 availability: Antibiosis plates were placed in an airtight container, along with a candle. The extinction of the flame is indicative of increased carbon dioxide, and decreased oxygen.

Phosphate solubilization: Bacterial strains were inoculated onto medium that contained freshly precipitated calcium phosphate (Katznelson and Bose, 1959). Solubilization was measured by the zone of clearing.

Results and Discussion

Suppression of fungal growth: Isolate 5-6 was able to suppress the growth of all three fungi. However, no other isolates inhibited all three fungal pathogens (Fig 1 and 2): 47.1%, 35.3% and 35.3% of the isolates showed weak or no suppression of *Pythium, Rhizoctonia,* and *Fusarium* spp., respectively.

In the presence of pea seeds, the percentage of strains that suppressed the growth of *Pythium* decreased to 20%, while those that suppressed the growth of *Rhizoctonia* and *Fusarium* increased to 50% and 70%, respectively (Fig 3). Fifty percent of isolates inoculated onto lentil seeds suppressed the growth of *Pythium,* and only 20% inhibited *Fusarium* growth; the presence of the lentil seed did not affect suppression of *Rhizoctonia* growth (Fig 4).

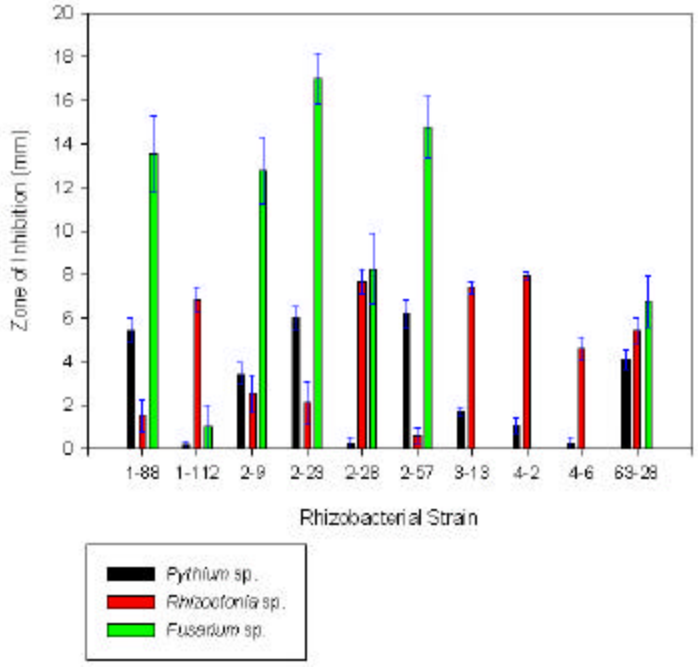

Fig 1. Suppression of growth of fungal pathogens by rhizobacterial isolates

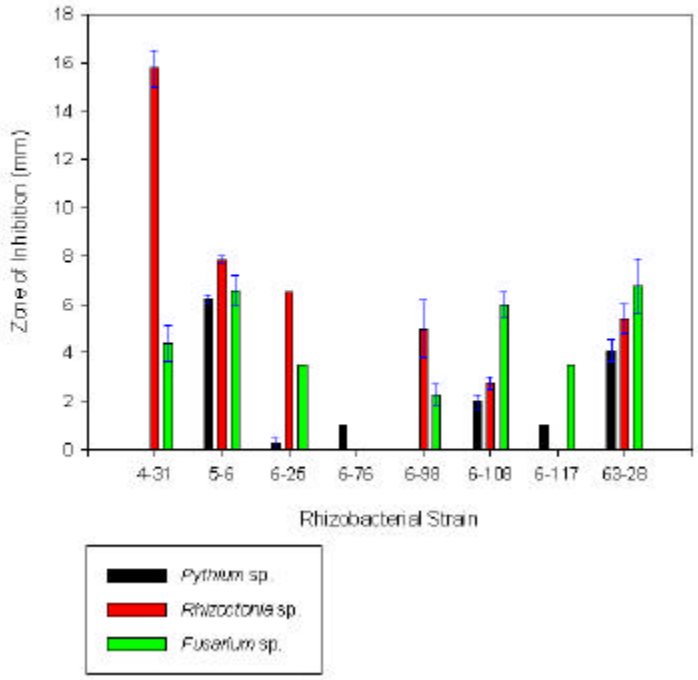

Fig 2. Suppression of growth of fungal pathogens by rhizobacterial isolates

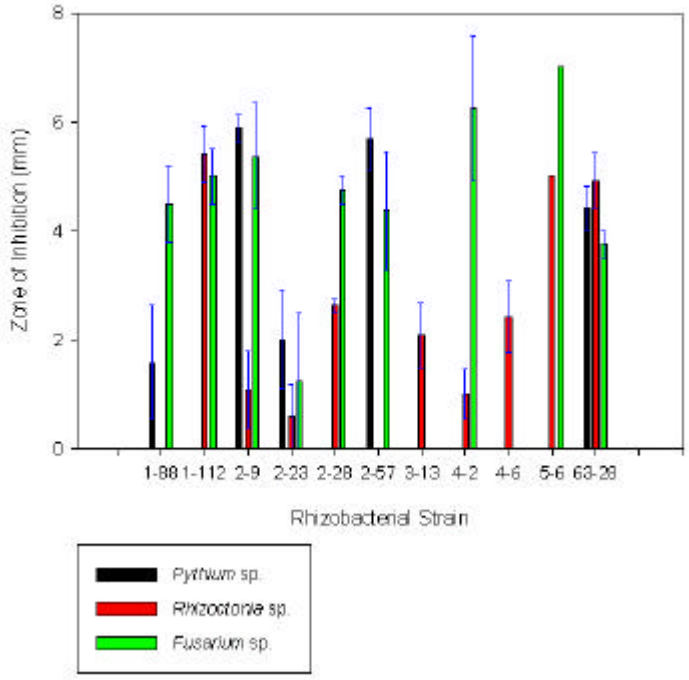

Fig 3. Suppression of growth of fungal pathogens by rhizobacterial isolates inoculated onto pea seed

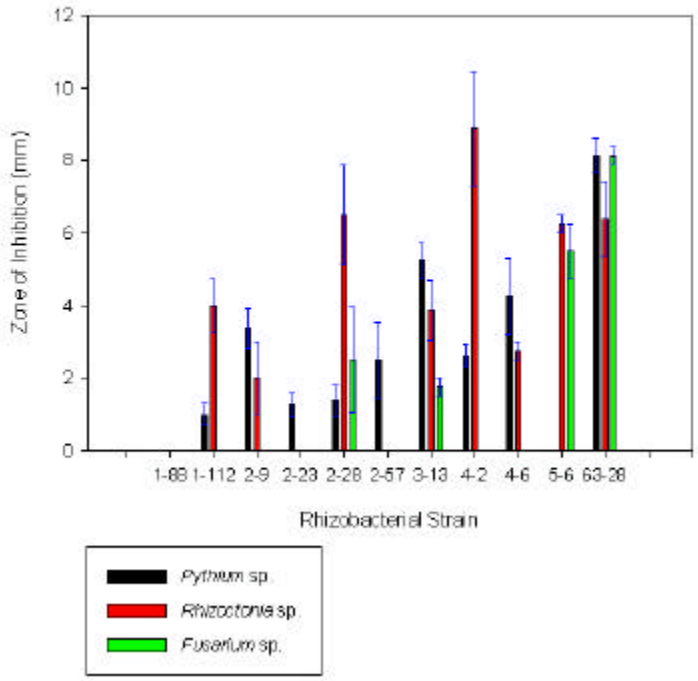

Fig 4. Suppression of growth of fungal pathogens by rhizobacterial isolates inoculated onto lentil seed

Effect of bacterial filtrate: Cell-free filtrate of isolates 3-13 and 63-28 inhibited the growth of *Pythium* by 78.75% to 88.75%, and of *Rhizoctonia* by 58.62% to 84.49%, respectively. When these isolates were grown together, inhibition increased to 100%. This suggests that fungal suppression may be mediated by metabolites excreted into the medium (Table 1).

Table 1. Ability of Exudates From Rhizobacterial Isolates, Individually or Combined, to Suppress Growth of Pathogenic Fungi

Fungi / Bacterial Isolate filtrate	% Inhibition[a]	
	Pythium[b]	*Rhizoctonia*[c]
Control (no bacterial filtrate)	0.0	0.0
3-13	78.75(1.25)	58.62(3.45)
63-28	88.75(1.25)	84.49(1.73)
3-13 and 63-28	100.0(0.00)	100.0(0.00)

[a] Values represent the mean of two replicates.
[b] Bacterial filtrate was diluted 1:10 (v/v)
[c] Bacterial filtrate was diluted 1:100 (v/v)

Production of lytic enzymes: Protease and chitinase were detected in 87.5% and 43.8% of the isolates (Table 2).

Phosphate solubilization: None of the 16 rhizobacterial strains was able to solubilize inorganic phosphate (Table 2).

Table 2. Production of Fungal Lytic Enzymes, and the Ability to Solubilize Inorganic Phosphate

	% Production[a]		
Isolate	Protease	Chitinase	Phosphate Solubilization
Rhizobacterial strains[b]	87.5	43.8	0.0
63-28	Yes	No	No

[a] Values represent three replicates
[b] 16 isolates tested

Effect of decreasing O_2 availability: The candle-jar assay decreased oxygen and increased carbon dioxide concentrations. Under these conditions suppression of *Pythium* by the rhizobacterial strains was increased and suppression of *Rhizoctonia* was decreased (Fig 5). *Pythium* and *Rhizoctonia* control plates grew normally, suggesting that the environmental conditions may have influenced the production of antifungal metabolites by the isolates.

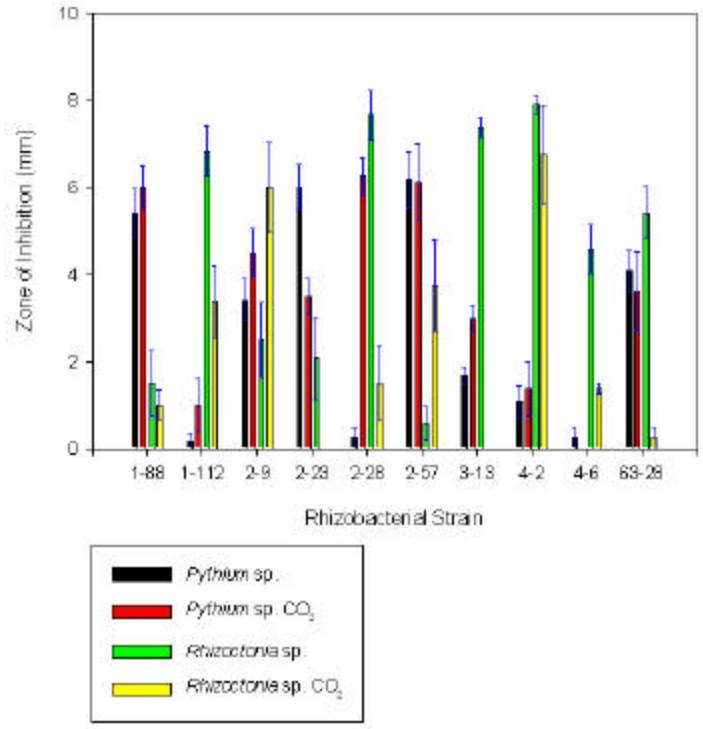

Fig 5. Suppression of growth of fungal pathogens by rhizobacterial isolates under conditions of decreased O_2 availability

Conclusions

Sixteen rhizobacterial isolates were characterized for plant growth promoting properties. Weak or no suppression of growth of *Pythium*, *Rhizoctonia*, and *Fusarium* spp. by rhizobacterial isolates was seen in 47.1%, 35.3%, and 35.3%, respectively. Isolate 5-6 was the only strain to inhibit the growth of all three fungi, with suppression comparable to control strain 63-28. The presence of pea or lentil seeds affected the ability of the isolates to suppress the growth of fungal pathogens. None of the isolates was able to solubilize inorganic phosphates, while 87.5% produced proteases, and 43.8% produced chitinases. Cell-free filtrate extracted from strains 3-13 and 63-28 suppressed the growth of fungal pathogens, indicating that the mode of action may be by secondary metabolites excreted into the culture medium. Two of the most promising isolates will be studied for the ability to enhance plant growth *in plantae*. Characterization of the differing crop-enhancing properties produced by the PGPR will aid in the successful development of a commercial inoculant.

Acknowledgements

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