Biocontrol of economical important diseases of canola

by using a bacterium and compost

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Prem D. Kharbanda
and Jian Yang

Alberta Research Council
Vegreville, Alberta
T9C 1T4, Canada

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Abstract

A bacterium strain *Paenibacillus polymyxa* PKB1 had inhibitory effect against several fungal pathogens of canola, including *Leptosphaeria maculans, Rhizoctonia solani, Sclerotinia sclerotiorum* and other pathogenic fungi. Research was conducted to evaluate the effect of seed treatments with the bacterium to control seed-borne diseases of canola under controlled and field conditions, and to determine whether compost amended with the bacterium would control blackleg, Sclerotinia stem rot, and Rhizoctonia root rot under controlled and field environments. Studies also evaluated the suppressive effect of composts made from different raw materials on soil-borne diseases.

Canola seeds were coated with *P. polymyxa* PKB1 spores by using polymers and peat moss methods. Bacterial coated seeds had lower infection and higher plant survival inoculated with *L. maculans* in growth chamber tests. Seed coating with the bacterium had no significant effect on the control of Rhizoctonia seedling blight under field conditions. The bacterium was viable over 12 months in peat moss and on seeds coated with polymer Gum Xanthum, but not on seeds coated with polymer Guar product. Further work is needed to optimize the bacterial activity when used as a seed coating.

Compost made from cattle manure and wood chips amended with 5% soybean meal was inoculated with this bacterium and evaluated for disease control under field conditions. The compost significantly inhibited germination of sclerotia of *S. sclerotiorum* in both controlled and natural conditions but did not significantly reduce blackleg seedling infection and Rhizoctonia damping-off under field conditions. Environmental factors were major limits in the biocontrol study.

Composts made from different starting materials, including municipal wastes, pulp sludge, wood chips and cattle manure, were evaluated for their chemical and biological properties to understand the mechanisms involved in disease suppression with compost. All composts had similar microbial activity and chemical composition, except that the pulp sludge had lower microbial activity, wood chips had high C/N ratio (65.37) and municipal solid waste had high lead concentration (2689 $\mu g \ g^{-1}$). Cattle manure compost had higher bacterial populations whereas wood chips had higher fungal populations. Microbes isolated from the composts showing inhibitory effect against pathogenic fungi were identified as *Bacillus licheniformis*, *B. subtilis*, *Trichoderma* spp., *Gliocladium* sp. and *Penicillium* spp. Compost inoculated with PKB1 (cattle manure-based) had larger populations of disease-suppressive bacteria than un-inoculated compost. Results indicate that the disease suppressiveness of composts could be enhanced, and composts with consistent population of disease-suppressive bacteria may be produced, by supplementing composts with known biocontrol agents.

Introduction

Blackleg, Rhizoctonia seedling blight and Sclerotinia stem rot are economically important diseases of canola. These diseases have caused millions of dollars of loss in western Canada. Tolerant cultivars have been used to control blackleg but no resistant cultivar is available for Sclerotinia and Rhizoctonia diseases. Investigation on alternative strategies for disease control is necessary.

Paenibacillus polymyxa (syn. *Bacillus polymyxa*) PKB1, isolated from canola root is effective against fungal pathogens of canola, including *Leptosphaeria maculans, Rhizoctonia solani, Sclerotinia sclerotiorum,* and other pathogenic fungi. Laboratory, greenhouse and field experiments were previously conducted to evaluate the efficacy of *P. polymyxa* PKB1 to suppress inoculum development of *L. maculans, R. solani, S. sclerotiorum* (Kharbanda and Yang, 1998). However, these three pathogens are soil-, or stubble-borne, therefore, it is possible that soil application of the biocontrol agent might inhibit the growth of fungi in the soil. We proposed to evaluate an effective technique for applying the biocontrol agent to the seeds and to soil.

Cultural and biological controls are two alternatives to synthetic chemical pesticides in disease management. Cultural control includes all aspects of crop husbandry, which influence disease development such as crop rotation, tillage and nutrient management. Seed coating methods have been used to deliver chemical pesticides as well as biocontrol agents (Halverson et al., 1993) to control seed-borne pathogens. Organic matter or compost amendment to agricultural soil has been successfully used to control soil-borne diseases (Hoitink and Fahy, 1986; Tuitert et al., 1998).

Organic material soil amendment provides suitable nutrients to the crops and is increasingly used in agriculture and horticulture. We proposed to use compost as a carrier of the biocontrol agent *P. polymyxa* PKB1 and to produce disease suppressive compost. The compost could then be mixed with growth media in the greenhouse and with soil in the field, and could reduce severity of soil-borne diseases.

In previous experiments, compost without inoculation of *P. polymyxa* PKB1 also showed biocontrol effect of inhibiting *L. maculans*. Therefore, research was undertaken to determine the effectiveness of compost, produced with different starting materials, in controlling soil-borne diseases of canola, including *R. solani, S. sclerotiorum* and *L. maculans* in the growth chamber and the field.

Objectives

(1) To evaluate the effect of seed treatment with *P. polymyxa* PKB1 to control seed-borne diseases of canola under controlled environment.

(2) To determine the effect of compost amended with *P. polymyxa* PKB1 to control blackleg, Sclerotinia stem rot, and Rhizoctonia root rot under controlled and field environments.

(3) To compare the disease suppressiveness of different composts on soil-borne diseases under controlled conditions, and to compare the chemical and biological properties of different composts.

Materials and Methods

1. Effect of bacterial seed treatments to control seed-borne diseases of canola under controlled environment

Two seed treatment methods were used:

1.1. Peat moss method:

Bacterium culture (10 mL, $1x10^7$ cells/mL) was added to 490 mL water and then inoculated into a special peat moss product supplied by MicroBio RhizoGen Corporation, Saskatoon. The mixture was incubated at room temperature for one week. The bacterial concentration reached to $1x10^9$ colony forming units (cfu) g^{-1}(wet). Canola seeds were coated with this peat moss containing *P. polymyxa* PKB1 and seeded in Rhizoctonia-infested ProMix in pots. Seedling emergence and root rot were recorded 1 and 3 weeks after seeding, respectively. Bacterial population and survival in peat moss was checked through plate counts every month for 6 months.

1.2. Polymer method:

Five different polymer coatings were used as stickers in seed coating. Canola seeds were coated with freeze-dried bacterial spores and a total of 21 different concentration combinations of bacterial spores and polymer were used (**Table 1**). Bioassay was conducted against *R. solani* on potato dextrose agar (PDA), water agar (WA) and nutrient agar (NA). Coated seeds were evaluated against *Rhizoctonia* in a growth chamber using the method mentioned above.

Survival of *P. polymyxa* PKB1 on coated seeds was checked every 6 months for 18 months. Twenty-five coated canola seeds were placed on a PDA plate and incubated at room temperature. Four plates were used for each treatment. The number of seeds

yielded bacterial colonies were counted one week after plating and used to calculate percent survival of *P. polymyxa* PKB1 on the seeds.

2. Use of compost inoculated with PKB1 to control Sclerotinia stem rot, blackleg and Rhizoctonia root rot under controlled conditions

2.1. Preparation of compost:

Compost used in these experiments was prepared at the Composting Technology Centre, Olds, AB. Ten cubic meters of cattle manure and wood chips, which had been composting for three months, were intensively turned to enhance the oxygen concentration until the material was nearing the maturing phase. One cubic meter near mature compost (41.7% moisture, 539.8 kg m^{-3} bulk density and pH 7.5) was steamed for 5 hours at 80°C and cooled to below 45°C. To half of this material, 13.5 kg of soybean meal (5%, w/v) was added and well mixed. A spore suspension of *P. polymyxa* PKB1 was prepared in distilled water (pH 7.4) from freeze-dried spores. A total of 18.91 g of freeze-dried *P. polymyxa* PKB1-rif^r spores ($4.7x10^{10}$ cfu mg^{-1}) were dispersed in 1 L of distilled water, and the suspension was diluted to 3.785 L and sprayed evenly over half of the pasteurized compost and mixed thoroughly. The resultant inoculum concentration of *P. polymyxa* PKB1 was $3.3x10^9$ cfu g^{-1} dry mass of compost. The inoculated compost was then incubated in two 0.25 m^3 composting chambers (labelled as S1 and S2) with forced aeration for 45 days. Another half of the compost was sprayed with water, incubated in two other 0.25 m^3 composting chambers (labelled as C1 and C2) under the same conditions and served as a control. Temperature and moisture in each composting chamber were measured daily.

Assessment of *P. polymyxa* PKB1 in composts:

At bi-weekly intervals during the composting period, samples from each compost chamber (inoculated with *P. polymyxa* PKB1 or without inoculation) were taken and assessed for bacterial concentration and inhibitory effect against *L. maculans*. One gram (wet weight) of compost sample was suspended in 10 mL sterile distilled water and then heated to 80°C in a hot-water bath for 30 min to kill any vegetative cells of non-spore-forming bacteria and fungi. Dilution series (10 fold) were made, and then 0.1 mL of each dilution was plated onto PDA plates. Bacterial colonies were counted after 48h of incubation at 22°C. The replicas of bacterial colonies on PDA plates were made on PDA + rifampicin (100 mg L^{-1}) plates and incubated at 25°C for 48 h, and the final counts of colony forming units (cfu) were determined per gram wet weight of the compost.

The inhibitory effect of *P. polymyxa* PKB1 re-isolated from compost was confirmed by using two methods. In the first method, a loopful of the bacterium was placed at four places on a PDA plate, around an agar plug of *L. maculans* culture. Inhibition zones in the culture around the bacterial colonies were checked after 10 days incubation at 22°C. In the second method, 0.1 mL of the diluted compost suspension and 0.9 mL of *L. maculans* spore suspension were mixed and then spread onto four PDA plates. Inhibition of fungal growth was observed after one week of incubation at room temperature.

2.2. Sclerotinia stem rot in growth chamber experiments:

Preparation of sclerotia: An isolate of *S. sclerotiorum* was cultured on PDA plates under light at room temperature until many sclerotia were formed. The plates were incubated at 10°C in the dark for 5 weeks. The sclerotia were harvested and stored at 4°C for later use.

Inhibition effect of the bacterium and compost on the sclerotia germination: The sclerotia were buried in compost inoculated with *P. polymyxa* PKB1 in pots, at the rate of 10 sclerotia/pot.

Compost without inoculation of the bacterium was also tested. All pots were kept moist throughout the duration of the experiment. Four replications were arranged in a completely randomized design in a growth chamber at 20°C constantly, 12 h light and 12 h dark. The number of sclerotia germinated, and the number of apothecia formed and died were recorded. The experiment was repeated once.

The following treatments were tested:

1. Compost alone, 25% compost + 75% ProMix
2. Compost alone, 50% compost + 50% ProMix
3. Compost + *P. polymyxa* PKB1, 25% compost + 75% ProMix
4. Compost + *P. polymyxa* PKB1, 50% compost + 50% ProMix
5. Autoclaved ProMix as control
6. *P. polymyxa* PKB1 cell suspension (2 x 10^7 cells/ml), soaking sclerotia in bacterial suspension for 60 min prior to addition to ProMix in pots
7. *P. polymyxa* PKB1 cell suspension (2 x 10^7 cells/ml), mix with ProMix
8. *P. polymyxa* PKB1 spore suspension (2 x 10^6 spores/ml), mix with ProMix

This experiment was repeated twice. All the data were analyzed using SAS GLM procedure.

2.3. Blackleg

Compost containing *P. polymyxa* PKB1 was mixed with autoclaved greenhouse growth medium ProMix (1:4) and put in 5" fibre pots. Canola seeds were placed on the

top of the mixture at 10 seeds per pot. Pycnidiospore suspension of *L. maculans* ($1x10^6$ spores mL^{-1}) was mixed with autoclaved perlite and 100 mL perlite inoculum was used to cover seeds in each pot. Compost without bacterium was included in the test to determine the effect of compost alone on blackleg disease of canola. Ten canola seeds (cv. Westar) were sown into each pot. Plants grown in autoclaved ProMix were used as control. It was a 2-factor factorial experiment with four replications of each treatment and arranged in a completely randomized design in a growth chamber with 20°C, 16 h light and 15°C, 8 h dark. The first factor was seed coating that was tested with five stickers (polyvinyl alcohol, polyacrylamide, Gum Xanthan, Guar product or polyvinyl pyrrolidone), and the second factor was soil amendment with two composts (compost with PKB1 or compost without PKB1 inoculation). Bare seeds and Vitavax coated seeds were also included in the test. Data were collected on emergence count of plants at day 7, and cotyledon/hypocotyl infection and disease rating of seedlings at day 21. This test was repeated twice.

2.4. Rhizoctonia damping-off and root rot

Preparation of Rhizoctonia inoculum: An isolate of *R. solani* AG2-1 was cultured on PDA for 7 days. Barley grains were soaked in water overnight and autoclaved for 1 h. Agar plugs containing *R. solani* mycelia were inoculated into barley grain in bags and incubated at 25°C for 2 weeks. The infested barley grains were air-dried and stored at 4°C.

Direct soil treatment: *P. polymyxa* PKB1 was inoculated into greenhouse growth medium, ProMix, and seeded with canola seeds in pots (25 seeds/pot). Rhizoctonia

solani infested barley grains were mixed with ProMix and 100 mL of the infested ProMix (10 barley grains/100 mL) was added to a pot to cover canola seeds. All pots were incubated in a growth chamber at 18°C, 16 h light and 15°C, 8 h dark. Seedling emergence and root rot data were recorded 1 and 3 weeks after seeding, respectively.

Compost amended with bacterium: Compost containing *P. polymyxa* PKB1 spores were mixed with autoclaved greenhouse growth medium ProMix, filled in 5" fibre pots and twenty-five canola seeds (cv. Westar) were placed on the surface of growth medium in pots. Ten Rhizoctonia infested barley grains were inoculated into each pot using the same method as mentioned above. Compost without bacterium was included in the test to determine the effect of compost alone to Rhizoctonia disease on canola. Plants grow in autoclaved ProMix were used as a control. A fungicide Vitavax RS seed treatment was also included in the test. There were four replications arranged in a completely randomized design in a growth chamber at a temperature/light regime of 18°C, 16 h light and 15°C, 8 h dark. Emergence count of plants at day 7 and survival and healthy plants counts at day 21 were recorded. The test was performed twice.

Treatments included in the experiment were:

1. *P. polymyxa* PKB1+ Vitavax + Compost alone, inoculated with *R. solani* AG 2-1

2. *P. polymyxa* PKB1+ Vitavax + Compost with *P. polymyxa* PKB1, inoculated with *R. solani* AG 2-1

3. *P. polymyxa* PKB1+ Vitavax, no Compost, not inoculated with *R. solani* AG 2-1

4. *P. polymyxa* PKB1+ Compost alone, inoculated with *R. solani* AG2- 1

5. *P. polymyxa* PKB1+ Compost with *P. polymyxa* PKB1, inoculated with *R. solani* AG2-1

6. *P. polymyxa* PKB1, inoculated with *R. solani* AG2-1

7. Vitavax + Compost alone, inoculated with *R. solani* AG2-1

8. Vitavax + Compost with *P. polymyxa* PKB1, inoculated with *R. solani* AG2-1

9. Vitavax, inoculated with *R. solani* AG2-1

10. *R. solani* inoculated

11. Control, no treatments

3. Use of compost inoculated with P. polymyxa PKB1 to control Sclerotinia, blackleg and Rhizoctonia root rot under field conditions

3.1. Sclerotinia stem rot

A mini-plot (1 m^2) method was used to determine the effect of bacterium-amended compost to control Sclerotinia stem rot under natural conditions in 1998-1999. There were four treatments:

1. PKB1-amended compost, inoculated with sclerotia of *S. sclerotiorum* (8L compost + 50 sclerotia)

2. Compost without PKB1 amendment, inoculated with sclerotia of *S. sclerotiorum* (8L

compost + 50 sclerotia)

3. PKB1-amended compost, without inoculation of sclerotia of *S. sclerotiorum*

4. Compost without PKB1 amendment and without inoculation of sclerotia of *S. sclerotiorum*

Sclerotia were prepared as mentioned in section 2.2. The treatments were applied to the field plots in October 1998 at ARC, Vegreville. Eight-litre compost was applied to each plot and mixed with soil. Fifty sclerotia were buried into the soil for each plot (1 cm

deep). The plots were arranged as a randomized complete block with 4 replications. In May 1999, canola seeds were sown to each mini plot. The germinating sclerotia and number of apothecia in each plot were checked and counted from April to Aug. and disease incidence were recorded in middle August. In Nov. 1999, soil from canola filed was mixed with compost (1:1) and filled in 10" plastic pots. Fifty sclerotia were buried in each pot and these pots were buried in 1998-99 field plots with the same treatments, two pots per plot. Pots were removed from field and placed outside near the greenhouse and kept moist by watering. The germinating sclerotia and number of apothecia in each pot were checked and counted from April to Aug. 2000.

3.2. Blackleg

Field experiments were conducted in both 1999 and 2000. Nine treatments were arranged in a randomized complete block design with four replications:

1. Vitavax coated seeds + Compost amendment in soil
2. Vitavax coated seeds + Compost with PKB1 amendment in soil
3. Bare seeds, Compost amendment in soil
4. Bare seeds, Compost with PKB1 amendment in soil
5. Vitavax coated seeds, no compost
6. Bare seeds, no compost
7. PKB1-Vitavax coated seeds + Compost amendment in soil
8. PKB1-Vitavax coated seeds + Compost with PKB1 amendment in soil
9. PKB1-Vitavax coated seeds

In May 1999, two hundred canola seeds (cv. Westar, coated with Vitavax or PKB1 or both) were planted to the 6m x 1m plot at 4 rows per plot at ARC, Vegreville. Composts were applied to the field plot in the seeding time by putting 800 mL screened compost into each seeding cone and seeded with canola seeds together and another 4.8L compost was amended into soil of each plot (a total of 8L per plot). Emergence and plant infection were recorded 3 weeks after seeding. Final disease rating was performed at harvest time. In 2000, the experiment was repeated using canola cultivar Quest (round-up ready) at the same field plots. Compost (8L) was amended into soil of each plot. In addition emergence counts and disease rating, yield data was also collected. Data were analyzied using SAS programs.

3.3. Rhizoctonia seedling blight

Eleven treatments were arranged in a randomized complete block with four replications:

1. *P. polymyxa* PKB1+ Vitavax coated seeds, compost without PKB1, inoculated with *R. solani* AG2-1

2. *P. polymyxa* PKB1+ Vitavax coated seeds, compost with *P. polymyxa* PKB1 amendment, inoculated with *R. solani* AG2-1

3. *P. polymyxa* PKB1+ Vitavax coated seeds, no compost amendment, not inoculated with *R. solani* AG2-1

4. *P. polymyxa* PKB1 coated seeds, compost without PKB1, inoculated with *R. solani*AG2- 1

5. *P. polymyxa* PKB1coated seeds, compost with *P. polymyxa* PKB1, inoculated with *R. solani* AG2-1

6. *P. polymyxa* PKB1 coated seeds, inoculated with *R. solani* AG2-1

7. Vitavax coated seeds, compost alone, inoculated with *R. solani* AG2-1

8. Vitavax coated seeds, compost with *P. polymyxa* PKB1, inoculated with *R. solani* AG2-1

9. Vitavax coated seeds, inoculated with *R. solani* AG2-1

10. Bare seeds, no compost, *R. solani* inoculated

11. Control, no seed coating, no compost amendment, no *Rhizoctonia* inoculation

In May 1999, composts were applied to the field plots at ARC, Vegreville during seeding. Compost was screened and dried. Two hundred canola seeds (cv. Westar) and 800 mL compost plus *Rhizoctonia* inoculum (infested barley grains, 200 grains/row) per row were seeded together by using a seed drill. Another 4.8 L compost was applied to each plot (a total of 8L per plot). Three weeks after seeding, emerged plants were counted and root rot rating was performed at the harvest time. In 2000, the experiment was repeated using canola cultivar Quest (round-up ready) at the same field plots. Compost (8L) was amended into soil of each plot. No *Rhizoctonia* inoculum was added into soil. In addition emergence counts and disease rating, plant stands and yield data were also collected. Data were analyzied using SAS programs.

3.4. Assessment of bacterium survival in the field

Since *P. polymyxa* PKB1-amended compost was applied to the field, it was important to monitor for the presence of *P. polymyxa* PKB1 in the soil. Soil samples were collected

and checked for the bacterial survival. The compost samples were10 fold diluted to 10^4 – 10^9 in potato dextrose broth (PDB) and 0.1 ml of each dilution was plated on antibiotic (100 ppm rifampicin)-amended PDA plates and incubate at 28°C. A set of duplicate plates were made on PDA without antibiotic.

4. Evaluation of disease suppressiveness of different composts

In previous experiments, compost without inoculation of *P. polymyxa* PKB1 showed suppressive effect to *L. maculans* (Kharbanda and Yang, 1998). Therefore, compost samples from different sources were collected from the Composting Centre, Olds College, Alberta and the High River Composting Centre (**Table 2**) and evaluated for their efficacy in disease control. The purpose was to understand the mechanisms involved in the disease suppression by compost, including presence of other disease suppressive microorganisms.

4.1. Observation of microflora in compost:

The microflora in compost were observed by two types of microscopic systems: a Zeiss epifluorescent microscope and a Molecular Dynamics 2001 confocal laser scanning microscope (CSLM). One gram of sample was placed in a test tube containing 10 mL of 0.05% acridine orange solution. The test tube was swirled and inverted. The sample was examined under the fluorescent microscope after 10 min staining. The DNA fragments of microorganisms were stained bright green by acridine orange (Chalmers et al. 1997).

4.2. Microbial activity in different composts:

The carbon and nitrogen ratio of various compost samples was determined by the Soil Laboratory at Alberta Research Council, Vegreville. The concentration of trace elements in the composts was measured at Trace Analytic Laboratory, ARC, Vegreville.

The total microbial activity was determined by the fluorescein diacetate (3', 6'-diacetylfluorescein (FDA) method (Schnurer and Rosswall, 1982). FDA is hydrolyzed by a number of different enzymes such as protease, lipases, and esterases. The product of this enzymatic conversion is fluorescein, which can be seen within cells by fluorescent microscopy and can also be quantified by fluorometry or spectrophotometry. The production of fluorescein is proportional to the microbial activity.

FDA (Sigma Chemical Co.) was dissolved in acetone (analytical grade, Sigma Chemical Co.) and stored as a stock solution (2 mg mL^{-1}) at -20°C. For all determination of FDA hydrolysis activity, the FDA final concentration was 10 μg mL^{-1}. A compost sample (1g) was dispersed in 50 mL of sterile potassium phosphate buffer (60 mM, pH 7.6) and then amended with FDA. The suspension was incubated at 25°C on a rotary

shaker (120 rpm) for 3 hours, and then 50mL acetone was added to stop the reaction. A 30 mL suspension was removed from the flask and centrifuged at 6,000 rpm for 5 min, and the amount of fluorescein was measured as absorbance at 490 nm with a spectrophotometer (SmartSpec™ 3000, Bio-Rad Laboratories Inc.). FDA added to the buffer was used as a blank. A standard curve of fluorescein was prepared from a stock solution containing 200 mg of fluorescein (Sigma Chemical Co.) in 20 mL of hot ethanol. A serial dilution was made to yield final concentrations of 0.625, 1.25, 2.5, 5, and 10 μg fluorescein mL^{-1}.

4.3. Total bacterial population:

The total bacterial population was determined by serial dilution and plating method. Compost samples were air-dried, and a 1 g sample was suspended in 10 mL distilled water. The well-mixed suspension was divided into two sets of 5 mL. One set of samples was treated in a hot water bath at 80°C for 30 min to kill non-spore-forming bacteria and fungi. A 10-fold serial dilution was made to 10^8 dilution, and 100 µL suspension of each dilution was spread on PDA plates. These plates were incubated at 28°C for 4 days, and the bacterial and fungal colonies were counted.

4.4. Bioassay and identification of bacteria:

Representative bacterial and fungal isolates were selected for the bioassay by using agar plate method. *Trichoderma* spp. and bacterial isolates from composts were selected and tested for their inhibitory effect on *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* A plug of fungal culture was placed on PDA in a plate and 6 different bacterial colonies

were placed on the plate around the fungal plug, 4 cm apart. Bacterial isolates showing inhibitory effect to *R. solani* were transferred to a new PDA plate one week after and later stored in 20% glycerol at -80°C for further study. Three strains were sent to the Microbiology Lab in ARC, Vegreville, Alberta for identification.

4.5. Comparison of beneficial bacteria population between inoculated and non-inoculated composts:

To determine if the introduction of *P. polymyxa* PKB1 into compost could increase the percentage of PKB1 in the compost, bacteria showing inhibitory effect from total bacteria growing on the plates containing compost with or without inoculation of *P. polymyxa* PKB1 were examined. A total of 100 single bacterial colonies from plates containing compost with inoculation of *P. polymyxa* PKB1 were randomly selected and tested for their inhibitory effect against *R. solani* AG 2-1 on PDA plates. The same number of bacterial colonies was selected from plates containing compost without inoculation of *P. polymyxa* PKB1 as a comparison. Bacterial isolates showing an inhibitory effect against *R. solani* AG 2-1 were counted, sub-cultured, and stored in a 20% glycerol solution for further identification.

All bacterial isolates showing inhibitory effect against fungal pathogens were transferred, purified and detected for *P. polymyxa* by dot-blotting method using a Dig-labelled DNA probe P1-7 (Yang, 2001).

4.6. Suppression of plant diseases by composts:

The ability of various composts to suppress Rhizoctonia disease was tested in a growth chamber experiment. Different composts (**Table 2**) were mixed with greenhouse growth medium ProMix (1:3, v:v) and incubated for two days. The mixture was used to fill 5-inch fibre pots, and 25 canola seeds (cv. Quest) were seeded on the surface of the ProMix-compost mixture. Five barley grains infested with *Rhizoctonia solani* AG 2-1 were placed in each pot, and the seeds were covered with the ProMix-compost mixture. There were four pots for each treatment, and all the pots were incubated and completely randomized in a growth chamber. Emergence was recorded 7 days after seeding and survival and disease severity were recorded 21 days after seeding. Data were analyzed by using the SAS GLM procedure.

Results

1. Effect of bacterial seed treatments to control seed-borne diseases of canola under controlled environment

1.1. Peat moss method

P. polymyxa PKB1 inoculated into the peat moss and stored at 4°C could survive for long time. In a six-month period, the population of PKB1 dropped from 10^{10} to 10^{9} cfu/g after 60 days storage but remained at the same level for the next 4 months (**Figure 1**). Canola seeds coated with peat moss inoculated with PKB1 and other bacteria were tested in the growth chamber. Coating canola seeds with PKB1 did not significantly prevent the seedlings from pre-emergence damping-off compared with the bare seed treatment (**Table 3**).

1.2. Polymer method

Various seed treatments containing PKB1 inhibited *S. sclerotiorum* (**Figure 2**) on different culture media but did not increase seed germination in *R. solani* plates. The polymers were not inhibitory to seed germination.

Table 4 shows the results of a growth chamber test on bacterial coating of canola seeds to control seedling disease caused by *R. solani*. The germination of canola seeds in pots inoculated with *R. solani* was not significantly enhanced by treatment with PKB1. It is possible that the activity of PKB1 was low and could not compete against the amount of fungus present. Further tests will be conducted after increasing the concentration of the bacteria in the polymers and in peat moss.

Bacterial survival on canola seeds stored at 4°C was high (81%) when Gum Xanthum was used as a sticker whereas PKB1 did not survive in guar product treated seeds 12 months after the treatment (**Table 5**). These results suggest that continued work with Gum Xanthum as a coating material should be pursued.

2. Use of compost inoculated with PKB1 to control Sclerotinia stem rot, blackleg and Rhizoctonia root rot under controlled conditions

2.1. Effect of compost-bacterium on *Sclerotinia sclerotiorum* in growth chamber tests:

Paenibacillus polymyxa PKB1 concentration in inoculated compost was 4.93 x 10^6 cfu/g. Compost + *P. polymyxa* PKB1 amended with 5% soybean meal significantly

inhibited germination of sclerotia of *S. sclerotiorum* three weeks after inoculation compared to the check (**Figure 3**). Also, the number of apothecia was the lowest compared with other composts of different composition (**Figure 4**).

Compost or compost + *P. polymyxa* PKB1 mixed with ProMix (at the ratio of 1: 1 or 1: 3) significantly inhibited the germination of sclerotia. In the control (sclerotia buried in ProMix), 80% of sclerotia germinated and produced an average of 15 apothecia per pot. Bacterial cell or spore suspension mixed with ProMix also significantly suppressed the germination of the sclerotia (Data not shown).

2.2. Effect of compost-bacterium on blackleg in the growth chamber

In a growth chamber test, compost inoculated with *P. polymyxa* PKB1 (4.93×10^6 cfu g^{-1}) showed no significant effect on the percentage of blackleg disease infection on canola seedlings but had a significant effect on the percent emergence and mean disease severity (**Table 6**). Seed-coating plus soil-amendment with compost and PKB1 significantly reduced the disease severity and had an effect similar to the fungicide Vitavax. **Figure 5** shows differences among the treatments. Plants of compost-, compost + PKB1- and Vitavax RS-treated seeds had reduced disease severity, although 40 percent of the plants became infected in compost- and compost + PKB1-treated pots. More plants in Vitavax and compost treated pots survived compared with those in the untreated control pot.

2.3. Effect of compost-bacterium on *Rhizoctonia solani* in the growth chamber

In a growth chamber test, compost inoculated with *P. polymyxa* PKB1 or compost alone showed no significant effect on the percent emergence and survival of canola seedlings but compost amendment in the soil combined with Vitavax seed coating had a significant effect on the percent emergence and mean survival (**Table 7**). Results indicate that Vitavax was effective in reducing the Rhizoctonia pre-emergence damping-off whereas PKB1 alone had little effect on the disease control under the growth chamber conditions. Compost without PKB1 inoculation did not reduce disease infection by *R. solani*.

3. Use of compost inoculated with PKB1 to control Sclerotinia, blackleg and Rhizoctonia root rot under field conditions

3.1. Sclerotinia stem rot

Under natural conditions, no sclerotial germination was found in all the treatments in the field test in 1998-99. This might be due to dry soil condition in the spring. In the 2000 test, compost, and compost+PKB1 treatments had significantly lower apothecia production (**Figure 6**). Both compost alone and compost with PKB1 significantly reduced germination and number of apothecia of *S. sclerotiorum* under natural conditions.

3.2. Blackleg

No significant effect of *P. polymyxa* PKB1 plus compost on disease severity of blackleg was found among different treatments in field experiments in both 1999 (**Table 8**) and 2000 (**Table 9**). In 2000, there were also no significant differences on percent

emergence and yield between treated plots and controls (**Table 9**). Vitavax treated seeds had higher germination in the growth chamber tests but the results were not consistent from a treatment to another under field conditions. Compost treated plots had higher yield than plots without compost amendment.

3.3. Rhizoctonia seedling blight

No significant effect of *P. polymyxa* PKB1 plus compost was observed on emergence and Rhizoctonia damping-off in field experiments in both 1999 (**Table 10**) and 2000 (**Table 11**). However, PKB1 combined with Vitavax seed coating plus compost soil amendment significantly increased canola emergence and survival in 1999 (**Table 10**) but not in 2000 (**Table 11**). Vitavax seed coating significantly increased canola yield compared with bare seeds (inoculated with *R. solani*) in 2000.

The effect of PKB1, compost and fungicide Vitavax on Rhizoctonia damping-off and blackleg of canola varied from year to year. Rhizoctonia greatly reduced emergence of canola plants but plants in each treatment had twice as much yield as those in the blackleg experiments (**Table 9 and 11**).

3.4. Assessment of bacterial population in the soil

Bacterial population in the compost and soil was enumerated. The cfu value of total bacteria able to grow on PDA was 6.5×10^4 cfu mL^{-1} in inoculated compost and 1.7×10^4 cfu mL^{-1} value in compost without inoculation. **Table 12** shows the enumeration data from the soil and plant samples collected in the Vegreville field plots in 2000. The population of PKB1-like bacterium in soil from compost treated plots as those from

untreated plots was similar. The number of PKB1-like colonies did not vary between the control soil samples and the compost-amended soil samples.

4. Evaluation of disease suppressiveness of different composts

4.1. Image of compost particle:

A confocal microscope image of compost particles showed that the microbial colonies were present in the compost based on the green fluorescent area on the compost particle (**Figure 7**). Quantitative data was not collected.

4.2. Determination of chemical composition and microbial activity in composts:

The chemical compositions of various composts are listed in **Table 13**. Wood chips-based compost had a very high C/N ratio, and the municipal solid waste-based compost had a very high concentration of lead. Most of the composts met the standard requirements for compost in Canada (Compost Council of Canada).

Comparisons of the capacity of microbial communities to hydrolyze FDA indicated differences in activity among various composts (**Table 14**). Pulp sludge-based compost had the lowest fluorecein production (lowest microbial activity) while municipal yard waste-based compost had the highest.

Figure 8 shows the microbial activity of compost inoculated with *P. polymyxa* PKB1 and compost without inoculation. There was no difference between the total microbial activities in the two composts after 25 hrs inoculation.

4.3. Total bacterial population in composts:

The total bacterial population in different composts was very similar except in screened compost from High River which had the lowest microbial population (**Table 14**). Microbes in the composts consisted of various bacteria, actinomycetes and fungi, including *Penicillium* spp., *Trichoderma* spp., *Gliocladium* sp., *Rhizopus* sp., *Fusarium* spp., *Pythium* sp., *Aspergillus* sp. and some unidentified fungi. After heat treatment, only thermophilic microorganisms survived. Screened compost from High River.

4.4. Bioassay and identification of bacteria:

Representative isolates of bacteria from compost samples were selected and tested for their inhibitory effect on pathogenic fungi *Sclerotinia sclerotiorum* and *Rhizoctonia solani* AG2-1. Most bacteria showed various degrees of inhibition against these fungi (**Figure 9**). Most inhibitory bacteria were characterized by white and pinkish colony colours. Three representative isolates were identified by Dr. R. Coleman at the Microbiology lab, ARC, Vegreville. One of the isolates was identified as *Bacillus subtilis* and two were *B. licheniformis.* More bacterial strains have been isolated, and will be identified later. Municipal waste and cattle manure had more bacteria showing inhibitory effect against pathogenic fungi whereas wood chip-based compost had more fungi such as *Trichoderma* spp. Bacteria found in heat-treated wood chip-based compost did not show inhibitory effect against pathogenic fungi. Screened compost from High River had a lower population of bacteria but higher fungal population.

4.5. Comparison of beneficial bacteria population between inoculated and non-inoculated composts:

Out of 100 randomly selected bacterial isolates from *P. polymyxa* PKB1-inoculated compost, 76% showed an inhibitory effect against *R. solani,* whereas 62% of isolates from control compost (without inoculation) had various degrees of inhibition (**Table 15**). Within these isolates, 85% isolates from inoculated compost were identified as *P. polymyxa* whereas only 6% bacterial isolates from non-inoculated compost were *P. polymyxa* (**Table 15**) according to dot-blotting method (**Figure 10**).

4.6. Disease suppression of different composts:

Composts made with different materials showed different disease suppression on Rhizoctonia damping-off of canola (**Table 16**). Cattle manure compost inoculated with bacterium *P. polymyxa* PKB1 and 1999 screened compost significantly increased the percent of emergence and reduced the mean disease severity. Wood chip-based compost performed the worst compared with other composts and the control, greenhouse growth medium without any compost amendment.

Discussion

Seed treatments

Many antagonistic endospore-forming *Bacillus* spp. have a potential as seed inoculants for control of soil-borne pathogens. Seed treatment with a biological control agent Kodiak, a *B. subtilis* strain GB03, (Gustafson, Inc., Plano, TX, USA) has been the first large-scale application of a biological control agent for suppression of seedling diseases and long-term chronic diseases of the rhizosphere of cotton (Brannen and

Kenney, 1997) and sugar beets (Fukui et al., 1994) in the United States. Since *P. polymyxa* and *B. subtilis* both are spore-forming organisms, they are extremely tolerant of environmental stresses and have many advantages over other biocontrol agents, such as *Pseudomonas* spp., for large-scale application. In the present study, the effect of seed treatment with PKB1 was not significant for controlling *R. solani* on canola. The reason is probably due to that the inoculated bacterial spores may remain dormant without germination in the soil, and therefore do not suppress disease. Another avenue needing exploration is the ability of PKB1 to successfully compete with other microbial populations in the soil. Further research needs to be conducted to promote germination and activity of PKB1 applied to the seeds.

Effect of compost inoculated with PKB1 in disease control

This study showed that the bacterial population remained very high in compost after 45 days incubation when soy meal was added to the compost. PKB1 inoculated in compost without soy meal addition did not increase during incubation. It is possible that the addition of food source, such as soy meal, may help or stimulate endospore germination and growth in compost.

Compost alone and compost inoculated with *P. polymyxa* PKB1 inhibited sclerotia germination in both controlled and natural conditions but did not significantly control Rhizoctonia root rot and blackleg (seedling infection) in most growth chamber and field experiments. We suspect that the environmental conditions were not suitable for the bacterial growth and activity. More work needs to be done to determine and optimize the conditions for the biocontrol agent.

In field trails, amending the soil with compost did not cause total bacterial numbers to increase, and addition of PKB1 to the compost did not result in increases in the total population size. The values of PKB1-like bacteria in soil samples were unchanged in the presence or absence of compost. PKB1-like cfu values were the same in the field soil samples as in the compost used to amend the field. Since the *in situ* population of spore-forming bacteria was low as indicated by the control sample, conditions were ideal for successful establishment of a high PKB1 population with the addition of the compost sample inoculated with PKPB1. However, PKB1 did not flourish as noted by the unchanged cfu values for PKB1-like colonies in the amended samples. Either concentrations of PKB1 in the compost were too low to take advantage of the reduced size of the *in situ* microorganism populations or the condition(s) in the soil did not stimulate growth of *P. polymyxa* PKB1.

General effect of compost in disease suppression

Compost alone had an inhibitory effect on apothecia and pseudothecia formation of *S. sclerotiorum* and *L. maculans,* respectively. This effect was due to the presence of naturally-occurring inhibitory microbes in the compost. In this study, various composts were found to contain some *Bacillus* spp., *Trichoderma* spp., *Gliocladium* sp. and *Penicillium* spp. The microbial population and activity were high in all the composts, except wood chip-based compost, tested by plating and FDA methods. General disease suppression was achieved through the presence of beneficial microbes in the composts.

Kharbanda et al. (2000) observed that the cfu values were much higher for the samples collected from the roots of individual canola plants. The soil collected is

considered rhizosphere soil and PKB1 is a rhizosphere-colonizing bacteria therefore one would expect that their numbers would be higher in this area. Indeed, both total bacteria counts and PKB1-like colonies were visibly higher in these samples. However, compost-amendment had no effect on bacterial counts.

The disease-suppression effect might have also been caused by chemical and biological or other factors in compost. It has been reported that some composts, particularly those prepared from tree barks, release inhibitors of plant pathogens and induce systemic acquired resistance in plants to some bacterial pathogens (Zhang et al., 1998). Out of 100 bacterial isolates from *P. polymyxa* PKB1-inoculated compost, 76% showed an inhibitory effect against *R. solani,* whereas only 62% of isolates from control compost (without inoculation of *P. polymyxa* PKB1) had various degrees of inhibition. Of these isolates, 85% from inoculated compost were identified as *P. polymyxa* whereas only 6% of the bacterial isolates from non-inoculated compost were *P. polymyxa* (**Table 15**) according to dot-blotting method (**Figure 9**). This result indicates that *P. polymyxa* PKB1 re-colonized the inoculated compost very quickly and became dominant in the compost while other microbes became dominant in un-inoculated compost with less beneficial bacteria in it. Certain soils and composts are well-known for disease suppression and represent shifts in the microbial population. However, the suppression is sometimes variable due to random re-colonization of compost by effective biocontrol agents during composting. Introduction of beneficial bacteria into the compost could enhance its disease-suppressive effect and obtain consistent results.

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Table 1. Seed coating with different combinations of five polymers and *Paenibacillus polymyxa* PKB1

Number	Treatment (polymer*)	Bacterium Amount (g)	Polymer Amount (g)	Seed (g)
1	Polyvinyl alcohol	2.486	1.08	25
2	Polyvinyl alcohol	1.043	5.066	25
3	Polyvinyl alcohol	5.062	3.099	25
4	Polyvinyl alcohol	1.435	1.04	25
5	Polyvinyl pyrrolidone	1.434	3.02	25
6	Polyvinyl pyrrolidone	5.042	3.017	25
7	Polyvinyl pyrrolidone	1.061	1.019	25
8	Polyvinyl pyrrolidone	5.013	1.003	25
9	Polyacrylamide	1.005	3.012	25
10	Polyacrylamide	5.006	3.011	25
11	Polyacrylamide	1.021	1.008	25
12	Polyacrylamide	5.007	1.025	25
13	Guar product	1.032	3.001	25
14	Guar product	4.295	3.023	25
15	Guar product	1.042	1.003	25
16	Guar product	4.315	1.041	25
17	Guar product	1.076	3.028	25
	Gum Xanthan		1.004	
18	Gum Xanthan	4.279	3.055	25
	Guar product		3.04	
19	Gum Xanthan	1.086	1.031	25
	Guar product		1.022	
20	Gum Xanthan	4.284	1.031	25
21	Gum Xanthan	0.876	3.003	25

* Note: *P. polymyxa* PKB1 concentration = 9.86×10^7 spores g^{-1} water
Polyvinyl alcohol (87-89%, MW = 13k to 23 k), 1.029 g in 50 ml water
Polyvivyl pyrrolidone (MW = 45,000), 1.354 g in 50 ml water
Polyacrylamide A137 PWG, 0.053 g in 50 ml water
Guar Product, 0.116 g in 100 ml water
Gum Xanthan, 0.099 g in 100 ml water

Table 2. Compost samples tested for detection of disease suppression and isolation of beneficial microorganisms

Number	Compost	Source
1	Municipal yard waste	Olds
2	Cattle manure	Olds
3	Pulp sludge	Olds
4	Wood chips	Olds
5	Screened municipal solid waste	Olds
6	Screened compost	High River
7	1998 cattle manure compost	High River
8	1999 unscreened compost	High River
9	Cattle manure + PKB1	High River
10	Cattle manure	High River
11	Cattle manure + soy meal + PKB1, compost bin	Olds
12	Cattle manure + soy meal, compost bin	Olds

Table 3. Effect of seed coating (peat moss method) with *P. polymyxa* PKB1 on canola seeds to prevent Rhizoctonia damping-off in a growth chamber test.

Treatment	Germination (%)	Survival (%)	Healthy Plant
Bare seeds, no Rhizoctonia	81 a *	81 a	20.00 a
Vitavax	75 a	39 b	0.75 b
PKB1	54 bc	37 b	0.00 b
Control (peat moss coating)	46 cd	26 b	0.75 b
Bare seed, + Rhizoctonia	26 d	15 b	0.50 b

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

Table 4. Effect of seed coating with *P. polymyxa* PKB1 on canola seeds to prevent Rhizoctonia damping-off in a growth chamber test.

Treatment	Germination	Survival	Healthy Plant
T1[a]	46 b[b]	32 b	1 c
T2	37 b	24 b	0 c
T3	32 b	28 b	0 c
T4	32 b	25 b	0 c
T5	44 b	31 b	2 c
T6	49 b	39 b	0 c
T7	57 b	46 b	0 c
T8	39 b	34 b	0 c
T9	38 b	30 b	0 c
T10	52 b	41 b	7 b
T11	38 b	30 b	0 c
T12	47 b	34 b	0 c
T13	43 b	33 b	0 c
T14	46 b	25 b	1 c
T15	-	-	-
T16	37 b	25 b	1 c
T17	43 b	24 b	1 c
T18	41 b	40 b	4 bc
T19	47 b	37 b	0 c
T20	38 b	23 b	1 c
T21	42 b	32 b	1 c
T22 (CK1[c])	91 a	92 a	92 a
T23 (CK2[d])	45 b	33 b	0 c

[a] T1- T21 were seed treatment listed in Table 1.
[b] Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).
[c] CK1 = Bare seeds, no *Rhizoctonia* inoculum.
[d] CK2 = Bare seeds, *Rhizoctonia* inoculated.

Table 5. Bacterial survival on canola seeds (cv. Westar) with different polymers as the sticker.

	Survival of PKB1 (%)	
Treatment	Within 1 month	12 months
Polyvinyl alcohol	100	76
Polyvinyl pyrrolidone	100	69
Polyacrylamide	100	78
Guar product	100	0*
Gum Xanthum + Guar product	100	12
Gum Xanthum	100	81
CK (bare seed)	100	0

* Seeds were infected by *Penicillium* sp.

Table 6. Effect of compost and compost + PKB1 as soil amendment and PKB1 and Vitavax RS as seed treatment on blackleg disease of canola seedlings in a growth chamber test*

Treatment		Emergence (%)	Infection (%)	Mean Disease Severity
Seed Treatment	Soil Amendment			
Polyvinyl alcohol +PKB1	Compost	92.5 ab	76.1 abc	3.84 ab
Polyacrylamide+ PKB1	Compost	75.0 bcd	53.6 cd	2.89 bc
Gum Xanthan +PKB1	Compost	-	-	-
Polyvinyl pyrrolidone +PKB1	Compost	67.5 cd	52.7 cd	3.71 ab
Guar product +PKB1	Compost	85.0 abc	90.9 ab	3.52 ab
Polyvinyl alcohol +PKB1	Compost+PKB1	90.0 ab	70.9 abc	3.69 ab
Polyacrylamide+ PKB1	Compost+PKB1	67.5 cd	62.3 bc	3.90 ab
Gum Xanthan +PKB1	Compost+PKB1	77.5 abcd	82.5 abc	4.44 a
Polyvinyl pyrrolidone +PKB1	Compost+PKB1	75.0 bcd	68.7 abc	2.78 bc
Guar product +PKB1	Compost+PKB1	62.5 d	59.6 c	2.89 bc
Bare seeds	Compost	82.5 abcd	63.2 bc	2.96 bc
Bare seeds	Compost+PKB1	95.0 ab	73.6 abc	3.53 ab
Bare seeds	No compost	82.5 abcd	94.4 a	4.75 a
Bare seeds, no BLA**	No compost,	95.0 ab	0.0 e	0.00 d
Vitavax, no BLA	No compost	85.0 abc	0.0 e	0.00 d
Vitavax	No compost	97.5 a	29.9 d	2.20 c

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

** BLA = a virulent isolate of *Leptosphaeria maculans*

Table 7. Effect of compost and compost + PKB1 as soil amendment and PKB1 and Vitavax RS as seed treatment on Rhizoctonia damping-off of canola in a growth chamber test*

Treatment		Emergence (%)	Survival (%)	Healthy Plants (%)
Seed Treatment	Soil Amendment			
PKB1 and Vitavax	Compost+PKB1	58.0 b	46.0 bc	8.0 b
Vitavax	Compost+PKB1	52.0 bc	52.0 bc	7.0 b
PKB1	Compost+PKB1	24.0 e	15.0 ef	5.0 b
PKB1 and Vitavax	Compost	86.0 a	36.0 cd	0.0 b
Vitavax	Compost	75.0 a	24.0 de	0.0 b
PKB1	Compost	43.0 bcd	15.0 ef	0.0 b
Bare seeds	Compost+PKB1	26.0 de	20.0 def	3.0 b
Bare seeds	Compost	22.0 e	5.0 f	0.0 b
Vitavax	No compost	82.0 a	55.0 b	4.0 b
PKB1	No compost	38.0 dce	26.0 de	1.0 b
PKB1 and Vitavax	No compost	86.0 a	60.0 b	7.0 b
Bare seeds	No compost	42.0 bcd	34.0 cde	3.0 b
Bare seeds, no Rhizoctonia	No compost	85.0 a	88.0 a	88.0 a

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

** BLA = a virulent isolate of *Leptosphaeria maculans*

Table 8. Effect of compost, compost + PKB1 and Vitavax RS on blackleg disease of canola in field tests in 1999

Treatment	Disease Severity
Vitavax + Compost	3.3475 a*
Vitavax + CompostPKB1	3.4675 a
Compost	3.3975 a
CompostPKB1	3.5375 a
Vitavax	3.3450 a
Bare Seeds	3.5775 a
PKB1-Vitavax coating + Compost	3.3400 a
PKB1-Vitavax coating + CompostPKB1	3.5075 a
PKB1-Vitavax coating	3.4450 a

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

Table 9. Effect of compost, compost + PKB1 and Vitavax RS on blackleg disease of canola in field tests in 2000

Treatment	Emergence	Disease Severity	Yield (g)
Vitavax + Compost	244 a*	1.54 a	457 a*
Vitavax + CompostPKB1	213 ab	1.70 a	488 a
Compost	210 ab	1.28 a	459 a
CompostPKB1	202 ab	1.43 a	491 a
Vitavax	239 a	1.74 a	375 a
Bare Seeds	222 ab	1.47 a	357 a
PKB1-Vitavax coating + Compost	158 b	1.49 a	478 a
PKB1-Vitavax coating + CompostPKB1	184 ab	1.26 a	460 a
PKB1-Vitavax coating	198 ab	1.55 a	385 a

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

Table 10. Effect of compost, compost + PKB1 and Vitavax RS on Rhizoctonia root rot of canola in field tests in 1999

Treatment	Emergence	Survival	Disease Severity
PKB1-Vitavax coating + Compost	84 cde*	73 cd	0.7900 ab
PKB1-Vitavax coating + CompostPKB1	111 bcd	111 bc	0.5300 b
PKB1-Vitavax coating	199 a	185 a	0.6800 ab
PKB1 coating + Compost	73 cde	77 bcd	0.5800 b
PKB1 coating + CompostPKB1	46 de	47 d	0.7500 ab
PKB1-coating	25 e	36 d	0.3900 b
Vitavax coating + Compost	170 ab	133 ab	1.1025 a
Vitavax coating + CompostPKB1	168 ab	132 abc	0.6300 b
Vitavax coating	140 abc	119 bc	0.7900 ab
Bare Seeds, Rhizoctonia	84 cde	91 bcd	0.6900 ab
Bare Seeds, No Rhizoctonia	214 a	176 a	0.7600 ab

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

Table 11. Effect of compost, compost + PKB1 and Vitavax RS on Rhizoctonia root rot of canola in field tests in 2000

Treatment	Emergence	Disease Severity	Yield (g)
PKB1-Vitavax coating + Compost	131 c*	1.6975 a	857 ab
PKB1-Vitavax coating + CompostPKB1	143 abc	1.7350 a	889 ab
PKB1-Vitavax coating	179 abc	1.8900 a	712 bc
PKB1 coating + Compost	149 abc	1.5475 a	863 ab
PKB1 coating + CompostPKB1	134 bc	1.8300 a	809 ab
PKB1- coating	139 bc	1.5250 a	690 c
Vitavax coating + Compost	182 abc	1.9800 a	837 ab
Vitavax coating + CompostPKB1	188 abc	1.7175 a	956 a
Vitavax coating	149 abc	1.2525 a	754 bc
Bare Seeds, No Rhizoctonia	216 a	1.2900 a	723 bc
Bare Seeds, Rhizoctonia	205 ab	1.2000 a	686 c

* Means in a column followed by the same letter are not significantly different determined by Duncan's Multiple Range Test (P=0.05).

Table 12. Bacterial population in soil samples collected from canola experimental plot on October 11, 2000 at Vegreville Site

Treatment	Moisture Content (%)	Bacteria* CFU/g dry weight	PKB1-like** CFU/g dry weight
Soil sample			
Control	23	6.6E+04	7.1E+03
Compost	23	1.3E+05	4.9E+03
Canola Roots			
Control		1.2E+07	1.9E+06
Compost		5.1E+07	3.2E+06

* Total colonies grown on PDA plates after sample was heat treated were presented as total bacteria counts.
** Colonies which looked like PKB1 were designated "PKB1-like".

Table 13. Carbon and nitrogen ratio and micronutrient concentration of various composts

Compost	C/N Ratio	Concentration of Trace Elements ($\mu g\ g^{-1}$)										
		As	Cd	Cr	Co	Cu	Pb	Hg	Mo	Ni	Se	Zn
Municipal yard waste	7.23	5.0	0.58	26.0	5.7	24.2	18.3	0.19	1.54	15.3	1.0	116.0
Cattle manure (Olds)	7.18	5.2	0.34	22.0	4.85	36.9	9.9	0.30	2.59	13.6	1.3	160.0
Pulp sludge	18.62	0.47	1.80	9.1	1.25	53.3	4.82	0.88	2.63	4.38	0.8	187.0
Wood chips	65.37	0.94	0.82	5.9	1.07	5.69	1.69	0.70	0.39	3.03	0.2	115.0
Screened municipal solid waste	10.20	5.62	0.57	30.9	6.0	42.7	2689.0	0.27	2.09	17.4	1.0	133.0
1999 screened compost	7.79	3.72	0.345	20.2	5.65	48.2	6.24	0.32	2.53	10.7	1.5	220.0
1998 compost	11.27	3.91	0.39	30.5	6.46	50.3	6.89	0.20	2.41	12.6	1.5	223.0
1999 unscreened compost	11.48	1.87	0.286	10.7	3.94	52.3	3.68	0.35	3.35	5.5	2.0	250.0
Cattle manure + PKB1 + soy meal	7.69	2.81	0.29	15.2	3.08	27.7	5.52	0.90	3.74	11.2	1.4	136.0
Cattle manure + soybean meal	7.58	3.7	0.387	17.2	3.33	27.3	6.20	0.86	2.90	12.0	1.3	127.0
Cattle manure+PKB1	12.99	5.52	0.41	31.1	6.93	44.9	8.23	0.22	2.39	15.9	1.9	200.0
Cattle manure (High River)	12.47	5.36	0.44	27.1	6.8	46.0	7.93	0.12	2.45	14.7	1.9	206.0

Table 14. Total microbial activity determined by fluorescein diacetate hydrolysis assay and total bacterial population able to grow on PDA in different composts

Compost	Source	Microbial Activity Fluorecein ($\mu g\ g^{-1}$)	Total Bacterial Population (CFU g^{-1})
Municipal yard waste	Olds	12.735	3.28E+08
Cattle manure	Olds	10.493	2.60E+08
Pulp sludge	Olds	4.852	2.51E+08
Wood chips	Olds	10.051	5.46E+08
Screened municipal solid waste	Olds	9.602	3.00E+08
1999 screened compost	High River	10.657	1.41E+04
1998 compost	High River	8.079	2.82E+08
1999 unscreened compost	High River	8.111	1.45E+08
Cattle manure + PKB1 + soy meal	Olds	8.509	5.52E+12
Cattle manure + soybean meal	Olds	11.813	2.60E+11
Cattle manure+PKB1,	High River	10.120	1.70E+10
Cattle manure (CK)	High River	7.056	1.12E+10

Table 15. Results of bioassay of bacterial isolates from compost with or without inoculation of *P. polymyxa* PKB1 and DNA detection, 100 colonies were tested for each compost.

Compost	Inhibitory bacterial isolates (%)	# of *P. polymyxa* isolates	*P. polymyxa* isolates (%)
Inoculated	78	66	85.0
Non-inoculated	61	4	6.5

Table 16. Effect of various composts on Rhizoctonia damping-off of canola in a greenhouse test

Treatment (Compost)	Emergence (%)	Survival (%)	Mean Disease Severity
Municipal yard waste	57.0 de*	35.0 cd	2.75 bc
Cattle manure	72.0 bcd	35.0 cd	3.33 abc
Pulp sludge	70.0 bcd	49.0 c	3.17 abc
Wood chips	40.0 e	10.0 d	4.52 a
Screened municipal solid waste	82.0 ab	40.0 c	3.64 abc
1999 screened compost	58.0 cde	45.0 c	2.34 c
1998 compost	72.0 bcd	46.0 c	2.97 abc
1999 unscreened compost	85.0 ab	46.0 c	3.58 abc
Cattle manure + PKB1 + soy meal	73.0 abcd	38.0 c	3.11 abc
Cattle manure + soybean meal	83.0 ab	54.0 bc	2.95 abc
Cattle manure+PKB1	95.0 a	80.0 ab	2.60 c
Cattle manure, no PKB1 inoculum	82.0 ab	57.0 bc	3.21 abc
Cattle manure, windrow #99	79.0 abc	39.0 c	3.98 ab
Cattle manure, windrow #98	79.0 abc	44.0 c	3.42 abc
Cattle manure, windrow #98, inoculated with *P. polymyxa* PKB1	90.0 ab	51.0 c	3.46 abc
Control-1, Promix, inoculated with *R. solani*	69.0 bcd	43.0 c	3.97 ab
Control-2, Promix, no *R. solani* inoculum	95.0 a	96.0 a	0.00 d

* Means followed by the same letters within a column are not significantly different from each other determined by Duncan's Multiple Range test (P=0.05).

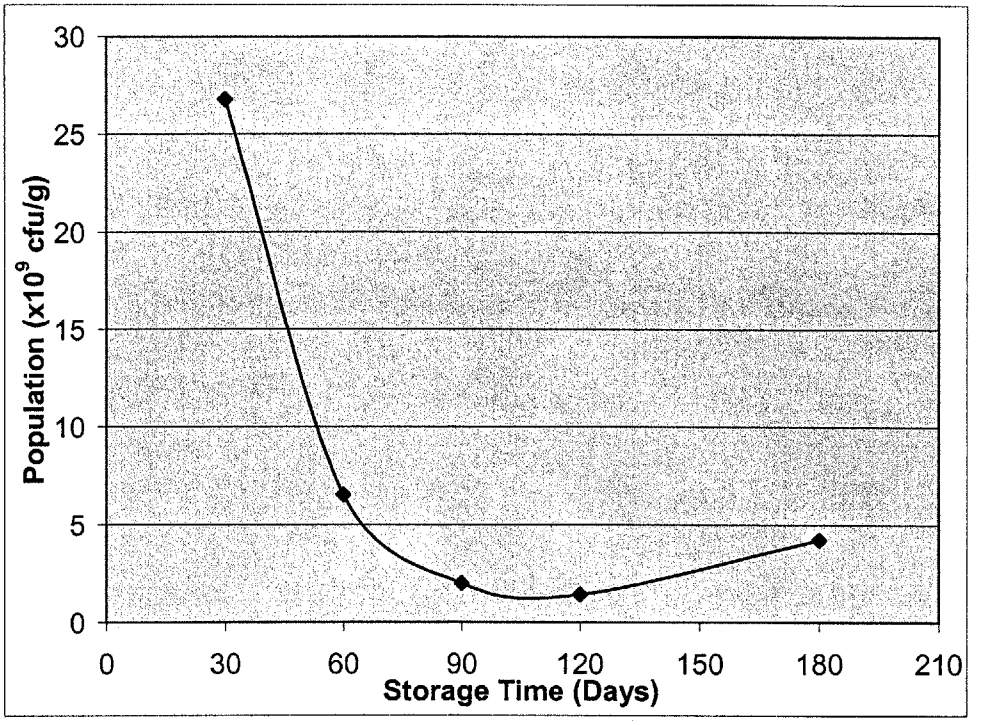

Figure 1. Population of *P. polymyxa* PKB1 in peat moss over 6 months at 4°C.

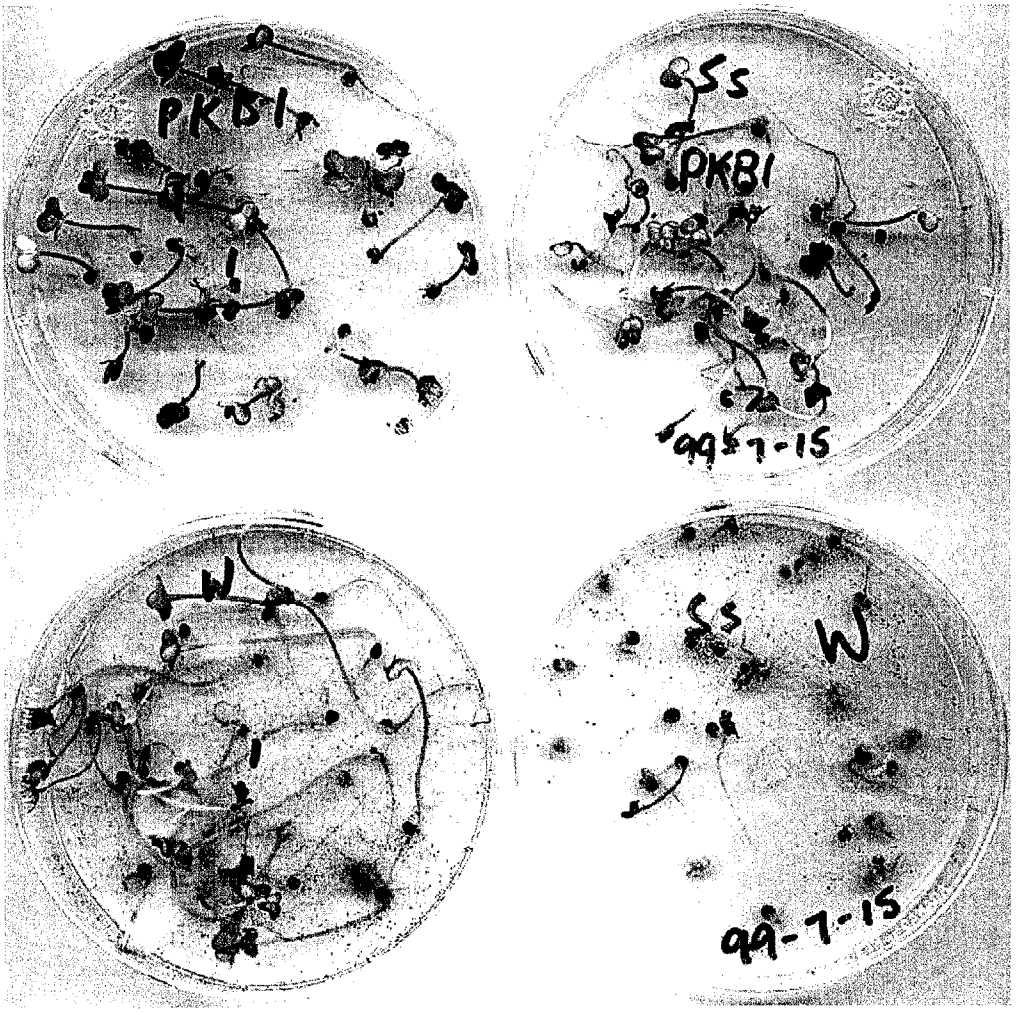

Figure 2. Germination of *P. polymyxa* PKB1 coated canola seeds (top) and un-coated seeds (bottom) in *Sclerotinia* culture plates (right), and no fungus (left).

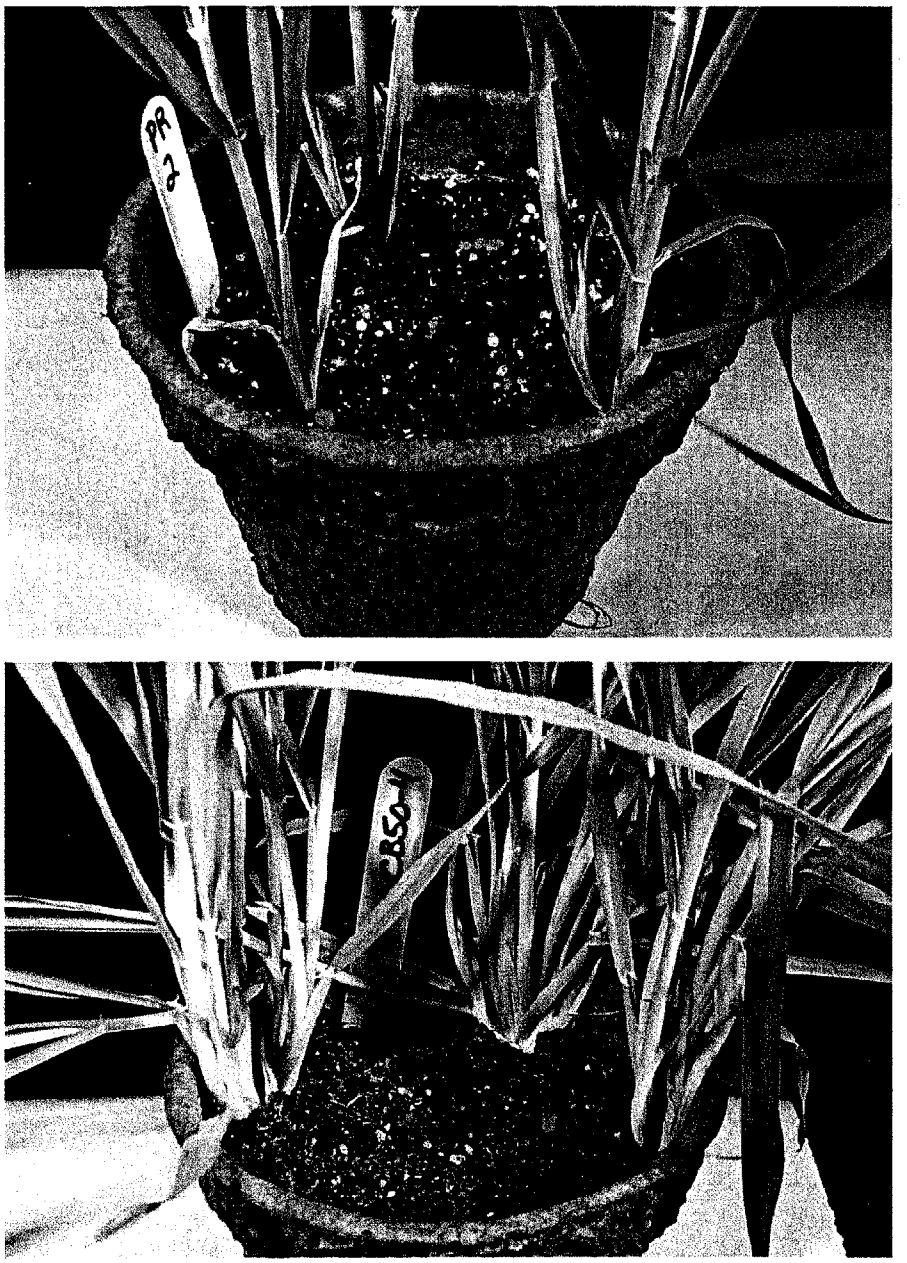

Figure 3. Inhibitory effect of compost on sclerotia germination, left = Sclerotia buried in Promix alone, right = sclerotia buried in compost inoculated with *P. polymyxa* PKB1.

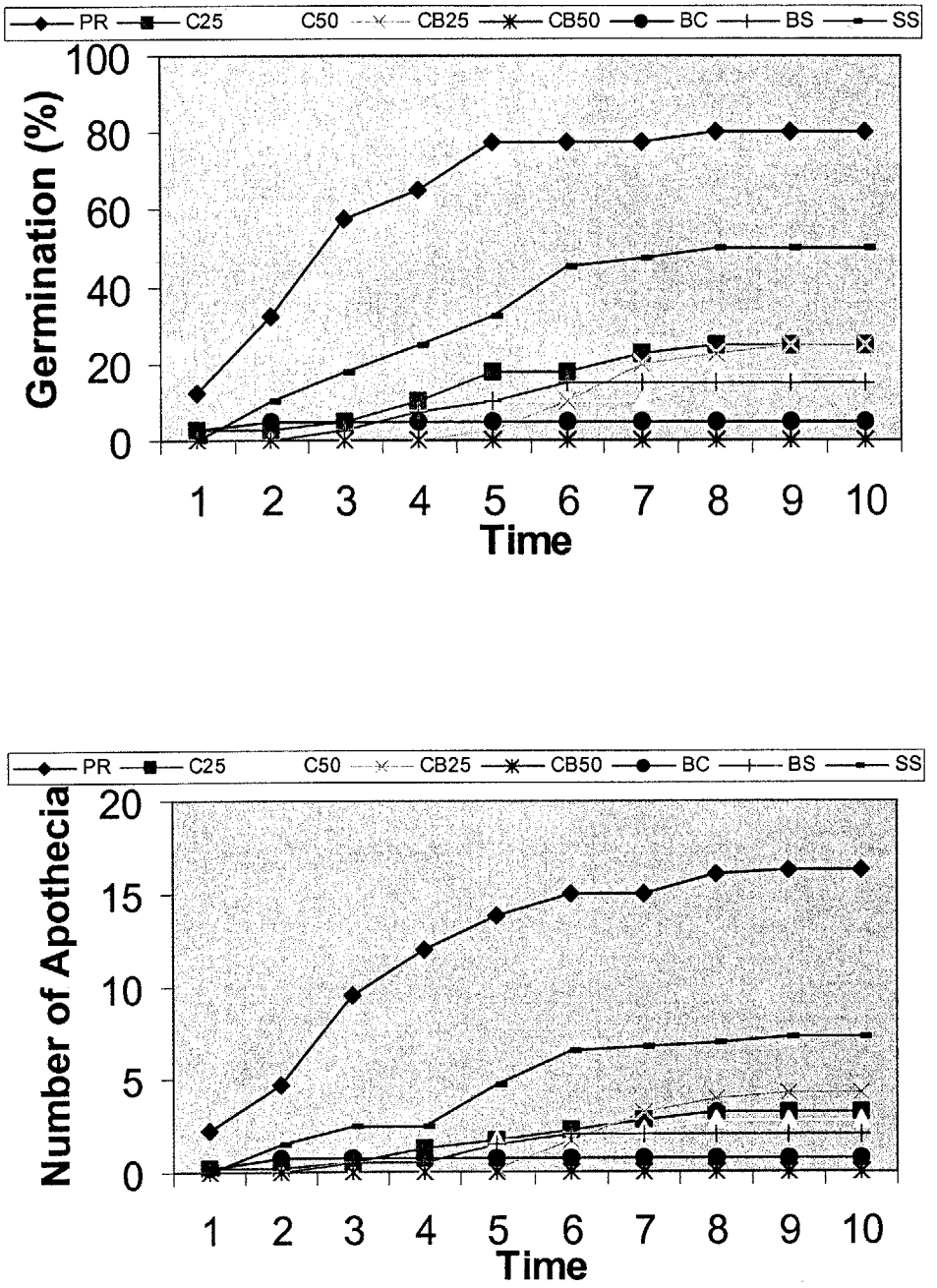

Figure 4. Effect of compost (C), compost+PKB1 (CB), PKB1 cell (BC) or spore (BS) suspension on the germination and number of apothecia formation of sclerotia of *Sclerotinia sclerotiorum* in a growth chamber test. SS = soaking sclerotia in PKB1 cell suspension for 30 min.

Figure 5. Effect of compost amended with *Paenibacillus polymyxa* PKB1 spores in reducing blackleg disease severity of canola in a growth chamber test. CK1 = No compost, no blackleg inoculum; CK2 = Compost, no inoculum; CK3 = No compost, inoculated with *Leptosphaeria maculans*. BLA = a virulent isolate of *L. maculans*.

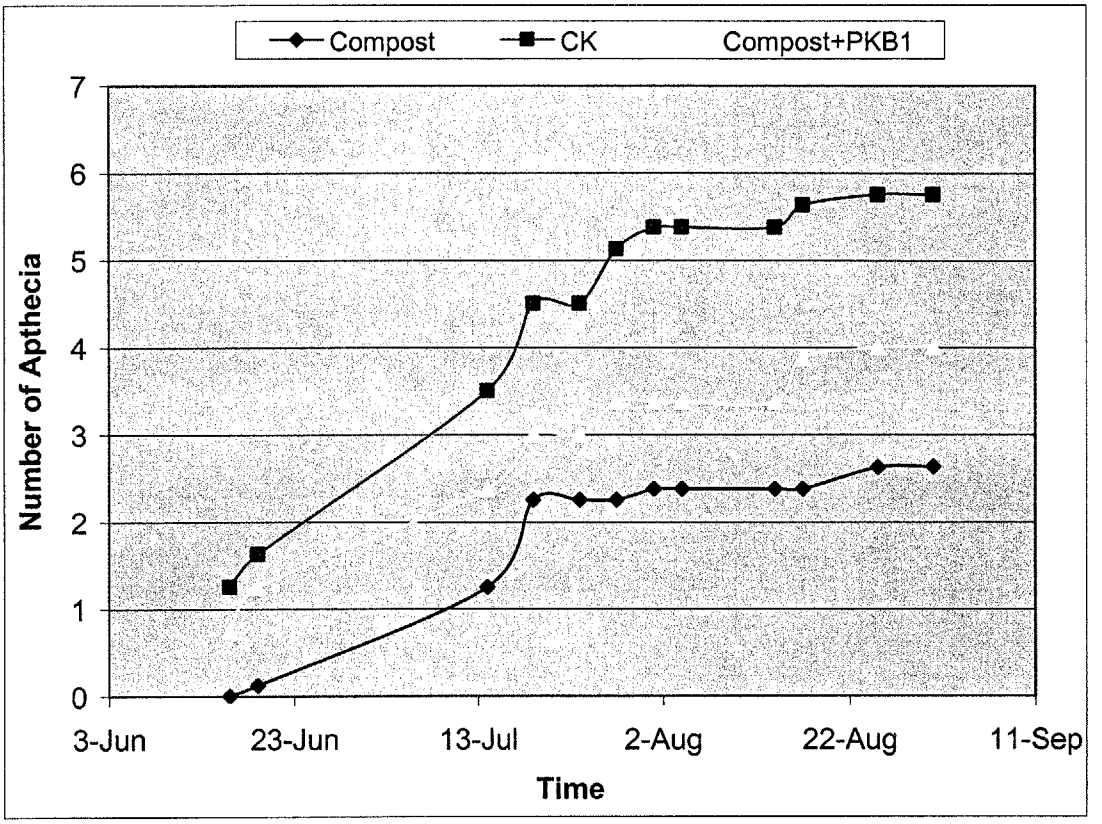

Figure 6. Effect of compost, and compost + *P. polymyxa* PKB1 on apothecia production of sclerotia of *Sclerotinia sclerotiorum*.

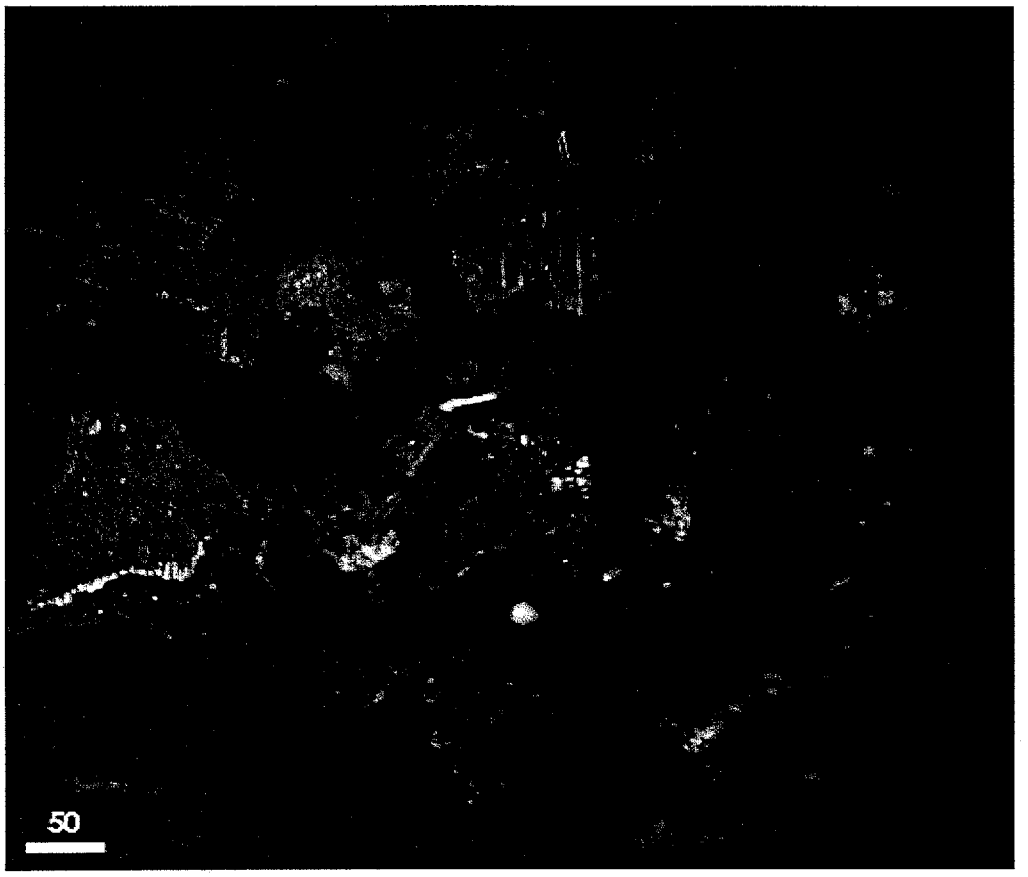

Figure 7. Confocal laser scanning microscope image of a compost particle.

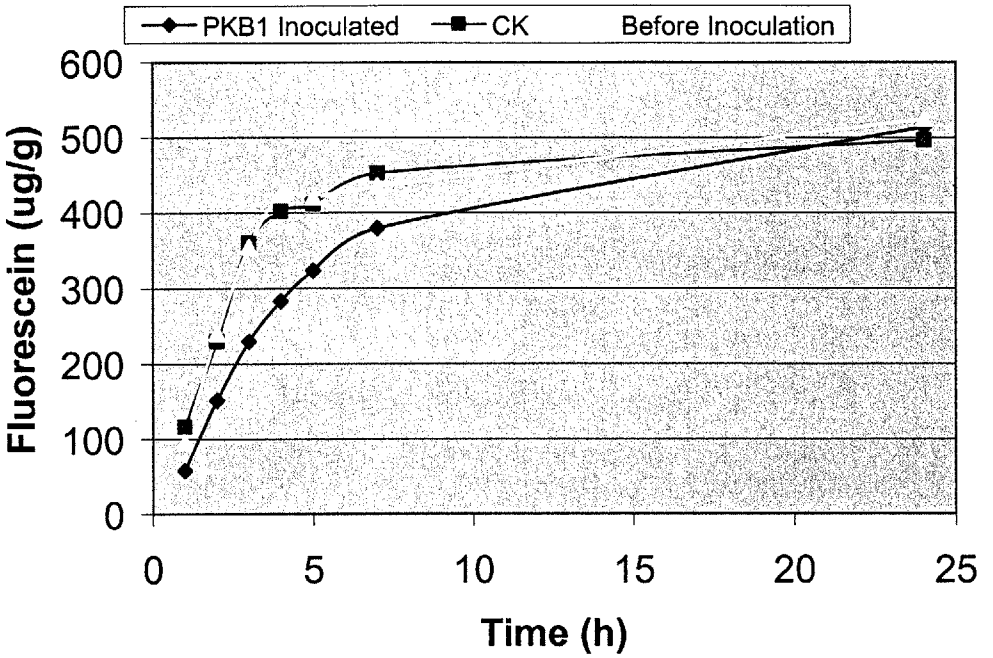

Figure 8. Microbial activity in compost over time as measured by fluorescein diacetate (FDA) hydrolysis (mean of three samples). Three compost samples include control compost, compost from windrow prior to inoculation and compost after inoculation with PKB1.

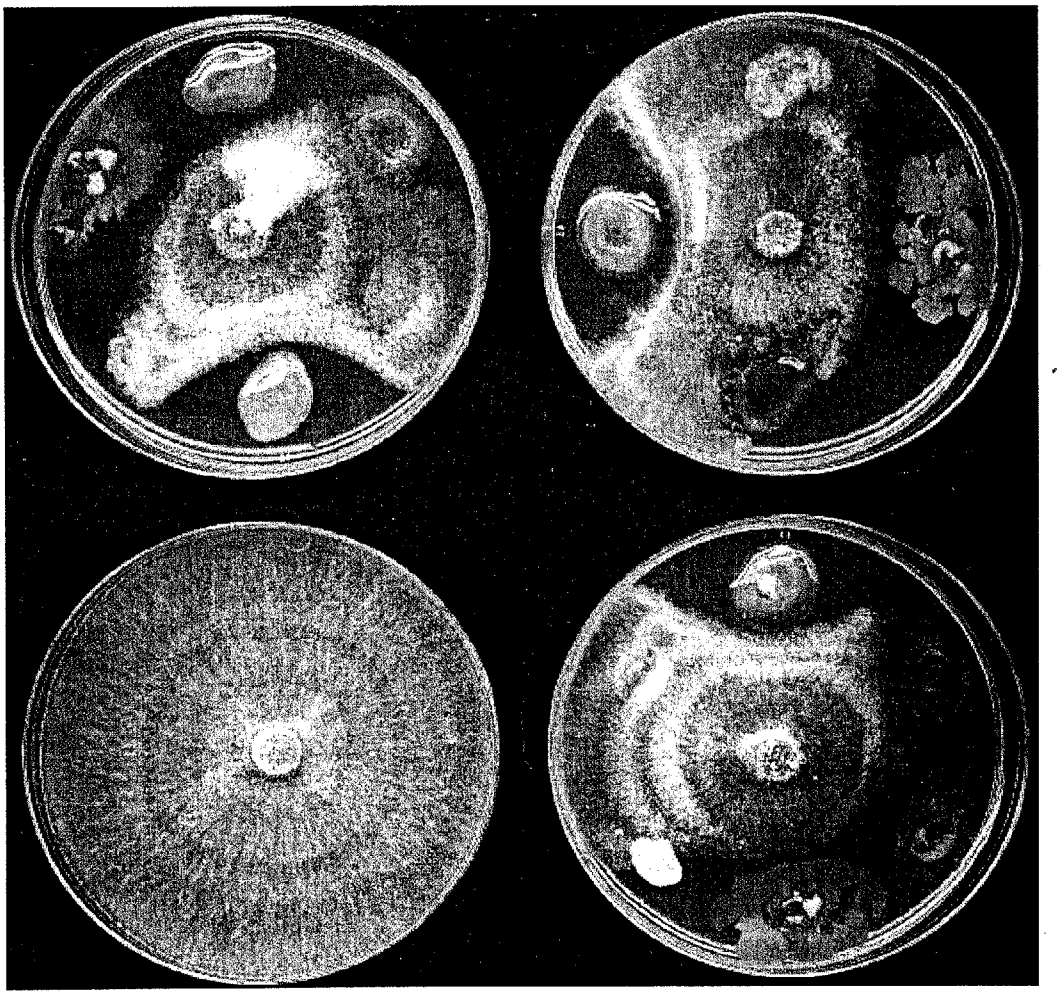

Figure 9. Bioassay of bacteria isolates from compost against *Rhizoctonia solani* AG 2-1 on potato dextrose agar plates. Bottom left plate is the control plate.

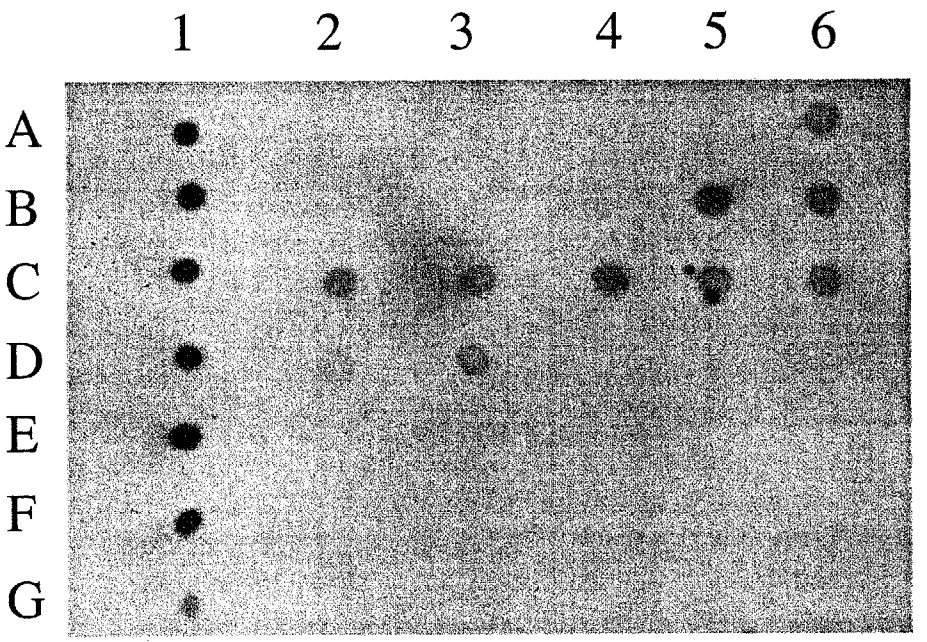

Figure 10. Dot-blotting of bacterial isolates from compost with or without inoculation of *P. polymyxa* PKB1 using Dig-labeled DNA probe P1-7. Seven dots in column 1 are positive control (PKB1) and G2 – G6 are negative controls (extraction buffer).

ALBERTA RESEARCH COUNCIL

Vegreville
Mailing
PO Bag 4000
Vegreville, Alberta
Canada T9C 1T4
Street
Hwy 16A & 75 Street
Vegreville, Alberta
Tel (780) 632-8211
Fax (780) 632-8379

Calgary
Mailing/Street
3608 – 33 St. N.W.
Calgary, Alberta
Canada T2L 2A6
Tel (403) 210-5222
Fax (403) 210-5380

Devon
Mailing/Street
1 Oil Patch Drive
Suite A129
Devon, Alberta
Canada T9G 1A8
Tel (780) 450-5111
Fax (780) 987-5280

Edmonton
Mailing/Street
250 Karl Clark Road
Edmonton, Alberta
Canada T6N 1E4
Tel (780) 450-5111
Fax (780) 450-5333

File No.: 9306010

August 28, 2001

Ms. Lisa Gruener
Manager, Research & Technical Services
Canola Council of Canada
400 – 167 Lombard Avenue
WINNIPEG, MB R3B 0T6

Dear Ms. Gruener:

Please find enclosed the progress report for Canola Agronomic Research Program Project #AG99-16 "Biocontrol of Economical Important Diseases of Canola by Using a Bacterium and Compost".

Sincerely,

Jian Yang
Plant Pathologist (Biocontrol)
Crop & Plant Management

JY/ly
CPM2002.073.LTR

Enclosure