Evaluation of bacterial strains for pathogen suppression and enhancement of survival and growth of conifer seedlings

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Summary. *Fungal root pathogens are widespread and cause seedling losses in conifer nurseries. Furthermore, poor seedling survival and growth on reforestation sites results in reduced forest regeneration. Use of microbial agents for disease control and plant growth promotion has become an important, environmentally-conscious endeavour. A microbial culture collection of approximately 500 strains was assessed for biological control of fungal root pathogens and/or plant growth promotion of conifer seedlings. Strains evaluated included rhizosphere bacteria isolated from conifer seedlings growing in diverse nursery and reforestation environments and effective beneficial strains from other plant systems. Seven of these strains, all pseudomonads, significantly reduced* Fusarium oxysporum *disease/or non-emerged seedlings on Douglas-fir by 24-43% in repeated growth room assays. Strains 17-29 and 64-3 significantly increased healthy stand of white Spruce seedlings inoculated with* F. oxysporum *and* Pythium ultimum *in a conifer nursery. Four strains (64-3, Ral-3, 62-24 and AC4-39) significantly increased survival of white spruce seedlings planted on a reforestation site by 22-33%. Three bacterial strains (17-29, Ral-3 and 17-114) significantly increased new root growth in upper root regions of Engelmann spruce seedlings. Overall, strains 64-3 and 17-29 yielded positive results in both biocontrol and reforestation trials. This study has provided candidate beneficial strains which offer promise for development of microbial inoculants for the forestry industry.*

Introduction

Seven hundred million conifer seedlings are planted annually in Canada making forestry an extremely important industry. It is imperative that seedling quality be high to allow for successful reforestation of harvested land. Seedling losses occur in conifer nurseries as well as on reforestation sites. Fungal pathogens inciting seed and/or root diseases in nurseries include *Fusarium, Cylindrocarpon, Cylindrocladium* and *Pythium.* An integrated approach to disease prevention and management, including cultural and biological control, would be favoured in the forest industry.

Losses in forest productivity also include poor seedling survival and establishment on reforestation sites. Root growth is often limited which contributes to poor seedling growth of outplanted seedlings. The economic impact due to seedling losses on reforestation sites, regardless of the cause, is substantial since the approximate cost to plant a seedling on a reforestation site is $1.00. Beneficial microorganisms specifically selected for forestry are being evaluated for: 1. Biological control of fungal root pathogens in conifer nurseries and 2. Enhancement of conifer seedling growth and survival on reforestation sites.

Materials and methods

Biological control

Approximately 500 microbial strains isolated from conifer seedling rhizospheres, mycelial baits, disease escape plants and beneficial strains from other plant/pathogen systems were screened for suppression of *Fusarium oxysporum* disease on Douglas-fir seedlings. Seed was soaked in bacterial cell suspensions in phosphate buffer (A600 = 0.20). Seed was then planted in 225mL Magenta GA7 jars containing peat/vermiculite planting mix fertilized with a liquid nursery solution to 79% moisture (planting mix pH 5.2). The planting mix surface was inoculated with *F. oxysporum* conidia in 1.0 mL spots under seeds (log 5-6 cfu mL^{-1}). Seeds were covered with granite grit and jars were capped with lids, randomized on a shelf in a plant growth room and incubated for 4 weeks under an 18 h photoperiod using a moderate heat stress (28°C-30°C) to enhance disease development (8 seeds jar^{-1}, 8 jars $treatment^{-1}$; *F. oxysporum* control treatment utilized 24 jars). Seedling emergence and root and shoot disease symptoms were then assessed. All strains which showed a reduction in disease were tested two to four times. Statistical analyses included one way analysis of variance of arcsine-transformed data and treatment means were compared to the *Fusarium* control treatment using Dunnett's test.

Plant growth promotion reforestation trials

Selected bacterial strains were tested for enhancement of seedling growth and survival on field sites in British Columbia and Saskatchewan. The roots of one year old spruce seedlings were soaked in a bacterial suspension in phosphate buffer (log 7-8 cfu mL^{-1}) for one hour and planted in reforestation field trials in a randomized block design. Control seedlings were treated with phosphate buffer. The British Columbia trial assessed 40 seedlings per treatment for seedling growth enhancement. The Saskatchewan trial assessed 200 seedlings per treatment for seedling survival. Trials are in progress.

Results and Discussion

Biological control

Forty-one bacterial strains significantly reduced *Fusarium* disease and/or non-emerged seedlings in one or more biocontrol assays. Variability in disease control results were observed for many strains. Six of the bacterial strains (D31-3A, D31-8B,

Table 1. Candidate bacterial strains

Strain	Identification	Source of isolation
17-114	*Pseudomonas putida*	Alert soil
AC4-39	*Pseudomonas spp.*	Verginia Polytechnic Inst.
17-29	*Pseudomonas putida*	Alert soil
64-3	*Pseudomonas fluorescens*	Canola rhizosphere
D31-3A	*Pseudomonas chlororaphis*	Forestry soil
D32-4D	*Pseudomonas spp.*	Forestry soil
D31-8B	*Pseudomonas chlororaphis*	Forestry soil
D30-1A	*Pseudomonas spp.*	Forestry soil
Ral-3	*Pseudomonas cepacia*	Virginia Polytechnic Inst.

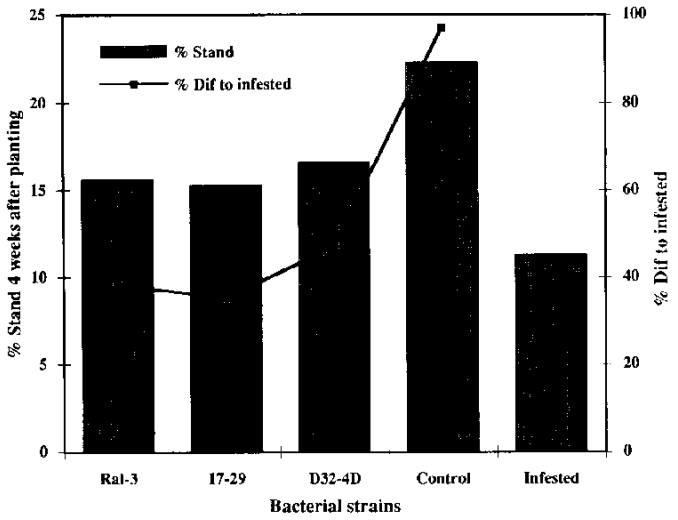

Figure 1. Effect of bacterial strains on healthy stand of White Spruce seedlings planted in pathogen infested greenhouse mix (*Fusarium* and *Pythium*) and grown under commercial greenhouse conditions near Prince Alberta, Saskatchewan. Strains showed are significantly different from pathogen infested control.

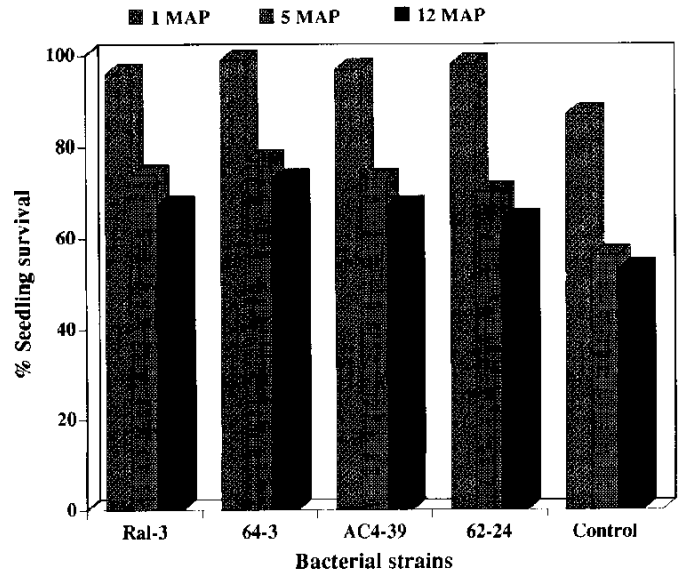

Figure 2. Effect of bacterial inoculation on survival of White Spruce bare-root seedlings planted in reforestation site near Duck Lake, Saskatchewan in 1992. All the strains significantly ($P = 0.05$) increased seedling survival compared to untreated control. Note: MAP = months after planting.

D32-4D, D30-1A, 17-29 and 64-3) significantly reduced diseased and nonemerged seedlings and/or needle disease symptoms (due to *Fusarium*) by 24% to 43% in two of three or two of three assays. Strains 17-29, Ral-3 and D32-4D also significantly increased the healthy stand of white spruce seedlings infested with *P. ultimum* and *F. oxysporum* at a conifer nursery in Saskatchewan, however, seedling emergence was very low for all treatments including uninoculated controls (Figure 1). The pathogens were re-isolated from plants carrying symptoms.

Strains yielding significant reductions in disease were *Pseudomonas* spp. (Table 1). Four strains D30-1A, D31-3A, D31-8B and D32-4D were isolated with baiting methods, either from disease escape plants or from mycelial baits. Three of the strains (17-29, Ral-3 and 64-3) had shown biocontrol or plant growth promotion efficacy in other plant systems.

Plant growth promotion

Three bacterial strains (17-29, Ral-3, and 17-114) significantly increased new root growth in upper root regions of Engelmann spruce seedlings planted in British Columbia. Four bacterial strains (Ral-3, 64-3, 62-24 and AC4-39) significantly increased survival of white spruce seedlings planted on a reforestation site in Saskatchewan by 22% to 33% (Figure 2). Strain Ral-3 showed beneficial effects in field trials in both Saskatchewan and British Columbia. Strains 64-3 and 17-29 yielded positive results in both biocontrol and reforestation trials. Candidate beneficial strains are being tested in 5 conifer nurseries and on 7 reforestation field sites in British Columbia, Saskatchewan and Alberta, Canada.