In Vitro Selection of Boreal Ectomycorrhizal Fungi for Use in Reclamation of Saline-Alkaline Habitats

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Abstract

To identify appropriate species of ectomycorrhizal fungi for use in the reclamation of saline-alkaline sites, such as the composite tailings (alkaline, with high sodium, sulfate, and calcium) produced by the Canadian tar sands industry, pure cultures of nine fungal species indigenous to the Canadian boreal forest were grown on media containing different levels of $CaCl_2$, $CaSO_4$, NaCl, or Na_2SO_4, as well as on medium containing composite tailings (CT) release water, and on media at four different pH levels. Members of the Boletales (*Suillus brevipes*, *Rhizopogon rubescens*, and *Paxillus involutus*) and *Amphinema byssoides* (Aphyllophorales) were sensitive to alkalinity, and their growth was completely inhibited by CT release water. *Laccaria* and *Hebeloma* spp. (Agaricales) as well as *Wilcoxina mikolae* (Pezizales) were tolerant to alkalinity and survived on the medium containing CT release water. Calcium chloride proved to be the most toxic of the salts tested. Growth of seven isolates of *Laccaria bicolor* and three isolates of *Hebeloma crustuliniforme* on media containing $CaCl_2$ and release water showed low intraspecific variation. A combination of fungal species, each with its own beneficial characteristics, is recommended for the inoculation of seedlings to be outplanted onto composite tailings.

Key words: Mycorrhizae, salt tolerance, oil sands, revegetation.

Introduction

The colonization of plant roots by mycorrhizal fungi augments water (Duddridge et al. 1980) and nutrient (Smith & Read 1997) uptake capacities and enhances resistance to disease (Sinclair et al. 1982; Duchesne et al. 1988) and extreme temperatures (Marx et al. 1970; Paradis et al. 1995). Mycorrhizal fungi can also improve plant growth in soils toxified by heavy metals (Colpaert & Van Assche 1987; Jones & Hutchinson 1988) and salts (Pond et al. 1984; Azcón & El-Atrash 1997). All of these benefits make mycorrhizal symbiosis an important consideration in phytorestoration. The benefits of naturally occurring or introduced mycorrhizal fungi may not be fully realized, however, in soils with contaminant levels high enough to elicit fungitoxic effects (Colpaert & Van Assche 1987), such as in saline-alkaline environments, where high levels of salts (Tresner & Hayes 1971; Dixon et al. 1993) and high pH (Hung & Trappe 1983) can inhibit fungal growth.

Saline-alkaline conditions can occur naturally, as in saline-alkali soils (Richards 1954), or can be created anthropogenically, such as in the byproducts of the oil sands industry in northeastern Alberta. In the latter case, once the bitumen has been extracted, tailings slurry is pumped into settling ponds where gypsum ($CaSO_4 \cdot 2H_2O$) is added to increase flocculation of silts and clays (Haas & Wong 1997). The resulting "composite tails" (CT) are high in calcium and sodium salts and have a pH of 8.8 (Li & Fung 1998; Zwiazek et al. 1998). Currently, the oil sands industry is emphasizing the production of these CTs as a method of dealing with the enormous quantity of tailings produced; 350,000,000 m^3 by 1998, and an estimated 1,000,000,000 m^3 by 2025 (Li & Fung 1998). Because composite tails represent a challenging environment for both plants and their fungal symbionts, our approach to the reclamation of these sites is to select and combine tolerant ectomycorrhizal fungi and tolerant plant species and genotypes (Zwiazek et al. 1998; Khasa et al., unpublished).

Among terrestrial fungi, the basidiomycetes (many of the ectomycorrhizal fungi) are considered to be rela-

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tively salt intolerant (Tresner & Hayes 1971), although some coastal species can tolerate up to 2.5 M sodium chloride (Castillo & Demoulin 1997).

Tresner and Hayes (1971) surveyed the NaCl tolerance of a wide range of terrestrial fungi, and Hutchison (1990) used NaCl tolerance as a taxonomic character for ectomycorrhizal species. In both cases tolerance varied among fungal groups. The upper limits of salt tolerance for ectomycorrhizal fungi have not been determined, but Dixon et al. (1993) found various levels of inhibition of growth, protein production, and colonization potential in selected species at 200mM concentrations of various sodium salts.

To assess the relative tolerance of ectomycorrhizal fungi to the saline-alkaline conditions found in composite tailings, we cultured nine species of ectomycorrhizal fungi found in the boreal forest on artificial media containing two levels of NaCl, Na_2SO_4, $CaSO_4$ or $CaCl_2$, as well as release water from the composite tails, and on media at four different pH levels. Seven isolates of *Laccaria bicolor* and three isolates of *Hebeloma crustuliniforme* were also cultured on media containing $CaCl_2$ and CT release water to assess the level of variation in saline-alkaline tolerance within those species.

The selection of saline-alkaline tolerant ectomycorrhizal fungi is part of a larger research program on the potential of these fungi to improve the growth and survival of host plants such as *Picea glauca*, *P. mariana*, and *Pinus banksiana* outplanted into either composite tails or other saline-alkaline environments.

Materials and Methods

Preparation of Growth Media

Modified Melin-Norkrans medium (MMN) (Marx 1969) (modified by Hutchison [1991]) was prepared with 15 g/L agar and either used as a control medium or amended with NaCl, Na_2SO_4, $CaCl_2$ or $CaSO_4$ at 100mM or 200mM prior to autoclaving (Table 1). Due to incomplete solidification of MMN containing $CaCl_2$, these treatments were prepared using 45 g/L potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). MMN was also prepared in which CT release water (pH 8.26, EC 6170 μS/cm, Ca 2.76mM, Mg 2.76mM, Na 52mM, K 1.78mM, Fe<0.0015mM, Mn<0.0025mM, SO^{3-} 96.1mM, Cl^- 43.18mM, hardness 275 mg/L, TDS 3950 mg/L) was substituted for distilled water.

The pH of the salt amended media ranged from 4.1 in the 200mM $CaCl_2$ to 7.8 in the CT release water medium. In general addition of the calcium salts resulted in relatively low pH values (4.1–4.3), whereas addition of the sodium salts resulted in higher pH values (6.4–6.7) (Table 1). For testing growth at different pH levels, PDA was adjusted to pH 4.4, 5.0, 6.0, or 8.0 with either 1N HCl or 1N NaOH, for final pH values of 4.1, 4.9, 5.8, and 6.8 after autoclaving (Table 1).

Table 1. Treatment media and pH levels before and after autoclaving. MMN = Modified Melin-Norkrans; PDA = Potato Dextrose Agar.

	PH	
Treatment	*Before*	*After*
MMN Control	7.3	6.2
PDA Control	7.5	5.3
MMN + 100mmol NaCl	7.3	6.7
MMN + 200mmol NaCl	7.2	6.5
MMN + 100mmol Na_2SO_4	7.3	6.6
MMN + 200mmol Na_2SO_4	7.3	6.4
MMN + 100mmol $CaSO_4$	6.9	4.2
MMN + 200mmol $CaSO_4$	6.5	4.3
PDA + 100mmol $CaCl_2$	4.4	4.2
PDA + 200mmol $CaCl_2$	4.3	4.1
MMN + CT release water	7.9	7.8
pH adjusted PDA	4.4	4.1
pH adjusted PDA	5.0	4.9
pH adjusted PDA	6.0	5.8
pH adjusted PDA	8.0	6.8

All media were autoclaved for 20 minutes at 121°C, and poured into 8.5-cm petri plates. Media were stirred constantly while pouring to ensure homogeneity. pH was measured with a glass electrode before autoclaving (at room temperature) and after autoclaving, prior to solidification (Table 1).

Isolates Tested

Seventeen isolates of ectomycorrhizal fungi belonging to nine species indigenous to the Canadian boreal forest (Table 2) were obtained from the University of Alberta Microfungus Collection and Herbarium (Sigler & Flis 1998). Species were chosen on the basis of their ability to grow well in culture and on their tolerance to NaCl (Hutchison 1990). A sterile 0.5-cm cork borer was used to aseptically transfer a plug of mycelium from each original isolate to three replicate plates of each treatment medium. For experiments in which single isolates were tested, UAMH 8232 and UAMH 5247 were used for *Laccaria bicolor* and *Hebeloma crustuliniforme*, respectively. To test intraspecific variation in tolerance, an additional six *Laccaria bicolor* and two *Hebeloma crustuliniforme* isolates (Table 2) were plated onto PDA acting as a control or containing either 200mM $CaCl_2$ or CT release water (the two most fungitoxic treatments).

After inoculation, plates were sealed with parafilm and incubated for six weeks at 20°C in the dark. Growth of fungal and bacterial contaminants was monitored weekly.

Table 2. Ectomycorrhizal fungi tested, their University of Alberta Microfungus Herbarium accession numbers, and place of isolation and habitat information.

Species	*UAMH Number*	*Origin (Place of Isolation)*	*Associated Plants (Habitat)*
Amphinema byssoides	9077	Bragg Creek, Alberta	*Picea, Populus*
Hebeloma crustuliniforme	5247	Lac La Biche, Alberta	*Picea glauca*
H. crustuliniforme	5455	Rocky-Clearwater Forest, Alberta	mixed boreal forest
H. crustuliniforme	6246	Bow-Crow Forest, Alberta	*Pinus contorta, Picea*
H. longicaudum	9317	Uppsala, Sweden	*Picea abies*
Laccaria bicolor	5313	Corvallis, Oregon	*Larix* seedlings
L. bicolor	5476	Rocky-Clearwater Forest, Alberta	*Pinus contorta*
L. bicolor	6061	Burt Lake, Ontario	*Picea glauca*
L. bicolor	8231	Burt Lake, Ontario	*Abies, Alnus*
L. bicolor	8233	Abitibi, Quebec	*Pinus* (plantation)
L. bicolor	9438	Madley Lake, British Columbia	*Pseudotsuga, Tsuga*
L. bicolor	8232	Abitibi, Quebec	*Pinus banksiana*
L. proxima	6062	Japan	not available
Paxillus involutus	8235	Grand Portage, Quebec	*Picea glauca*
Rhizopogon rubescens	6183	Bow-Crow Forest, Alberta	*Pinus contorta*
Suillus brevipes	6229	Rocky-Clearwater Forest, Alberta	*P. contorta*
Wilcoxina mikolae	6695	Syracuse, New York	*P. resinosa* seedlings

Measurement and Data Analysis

At the end of six weeks, the diameter of each colony was measured twice, the second measurement at 90° to the first, and the values averaged to account for non-symmetry. Proportional growth of the nine species on the various salt treatments was obtained by dividing the diameter of each isolate on each salt amended medium by its average growth on control media. Normality and homogeneity of variance were tested graphically. The resulting data were analyzed by two-way analysis of variance with three replicates of each treatment using the SAS statistical package (SAS 1988). Multiple comparisons of the treatment means were conducted using LS MEANS at $\alpha = 0.05$.

Growth at the four different pH levels was analyzed similarly, except that the data were expressed as absolute growth, rather than as growth proportional to the controls. Data on the seven isolates of *L. bicolor* and three isolates of *H. crustuliniforme* on 200mM $CaCl_2$, CT release water, or control media were analyzed using one-way analyses of variance on proportional growth values.

Results

For growth of the nine species on the various salt amended media, the two-way analysis of variance revealed significant species ($F = 9.81$, $p = 0.0001$) and treatment ($F = 35.92$, $p = 0.0001$) effects, as well as significant interaction between the two ($F = 6.97$, $p = 0.0001$).

Laccaria proxima grew well on all media except for those containing 200mM $CaCl_2$ and CT release water, which significantly reduced growth (Fig. 1a). Growth of *Laccaria bicolor* (Fig. 1b) was generally similar to that of *L. proxima*, except for a lower tolerance to $CaCl_2$ and a greater tolerance to CT release water. *Hebeloma crustuliniforme* was inhibited by 200mM Na_2SO_4 and both levels of $CaCl_2$ (Fig. 1c). *Hebeloma longicaudum* was inhibited by both levels of $CaSO_4$, 200mM Na_2SO_4, 200mM $CaCl_2$, and CT release water (Fig. 1d). *Wilcoxina mikolae* was inhibited by 200mM $CaSO_4$, both levels of Na_2SO_4, both levels of NaCl, and 200mM $CaCl_2$ (Fig. 1e). *Amphinema byssoides* was inhibited by all treatments except $CaSO_4$, and suppression by 200mM Na_2SO_4 and by CT release water was complete (Fig. 1f). *Paxillus involutus* was strongly inhibited by both levels of Na_2SO_4, and to a lesser extent by both levels of NaCl and $CaCl_2$, and was completely inhibited by CT release water (Fig. 1g). Growth of *Rhizopogon rubescens* was diminished by 100mM Na_2SO_4 and 200mM $CaCl_2$ and was completely suppressed by 200mM Na_2SO_4, both levels of NaCl, and CT release water (Fig. 1h). Growth of *Suillus brevipes* was enhanced by the addition of 100mM $CaSO_4$ (200mM $CaSO_4$ had no effect) and inhibited by all other treatments. In the case of 200mM Na_2SO_4, 200mM $CaCl_2$, and CT release water, the suppression of *S. brevipes* was complete (Fig. 1i).

Overall tolerance to the various salts, obtained by averaging across all treatments for each species, follows the hierarchy, from greatest to least: *L. proxima*, *H. crustuliniforme*, *L. bicolor*, *H. longicaudum*, *W. mikolae*, *S. brevipes*, *R. rubescens*, *P. involutus*, *A. byssoides.*

With respect to the effect of the different treatments on all species combined (treatment averages), the overall hierarchy of inhibition of fungal growth is, from greatest to least: 200mM $CaCl_2$, CT release water, 200mM Na_2SO_4, 200mM NaCl, 100mM $CaCl_2$, 100mM

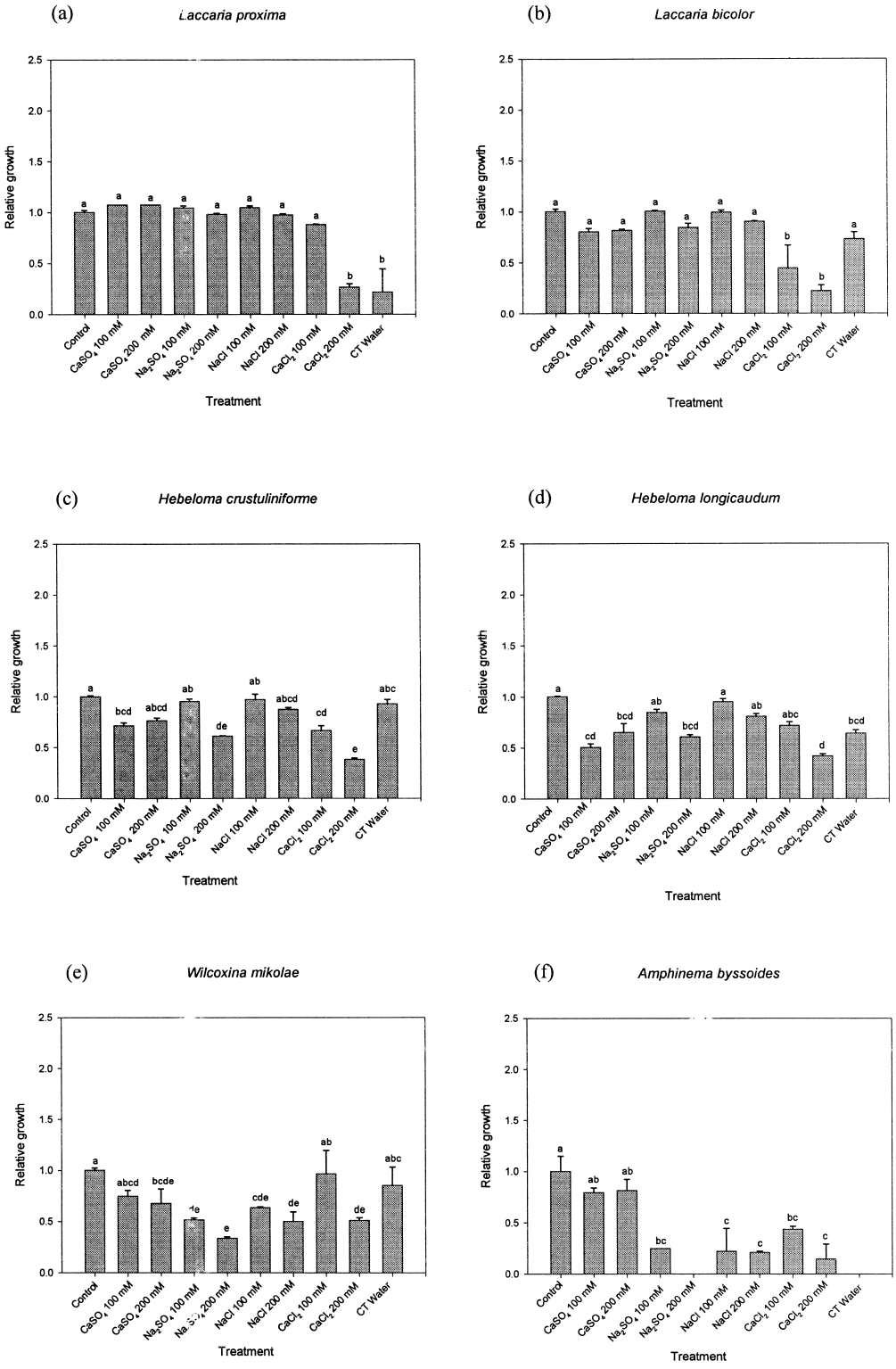

Figure 1. Effects of two levels of four salts, CT release water, and two control media on the growth (diameter in cm ± SE) of each of nine fungal species as a proportion growth on control media after six weeks. (a) *Laccaria proxima*; (b) *Laccaria bicolor*; (c) *Hebeloma crustuliniforme*; (d) *Hebeloma longicaudum*; (e) *Wilcoxina mikolae*; (f) *Amphinema byssoides*; (g) *Paxillus involutus*; (h) *Rhizopogon rubescens*; (i) *Suillus brevipes*. Values are averages of three replicates. Treatments with common letters are not significantly different (α = 0.05). Control bar represents both Modified Melin Norkrans (MMN) and Potato Dextrose Agar (PDA) controls.

NaCl, 100mM Na_2SO_4, 200mM $CaSO_4$, 100mM $CaSO_4$, although the inhibitory effect of CT release water was highly species specific.

The species exhibiting the least inhibition at the 200mM level of each salt were: *S. brevipes*, *R. rubescens*, *L. proxima*, and *P. involutus* for $CaSO_4$; *L. proxima* and *L. bicolor* for Na_2SO_4; *L. proxima*, *L. bicolor*, *H. crustuliniforme*, and *H. longicaudum* for NaCl, and *R. rubescens*, *Wilcoxina mikolae*, *H. longicaudum*, *H. crustuliniforme*, and *L. proxima* for $CaCl_2$. In the case of the medium

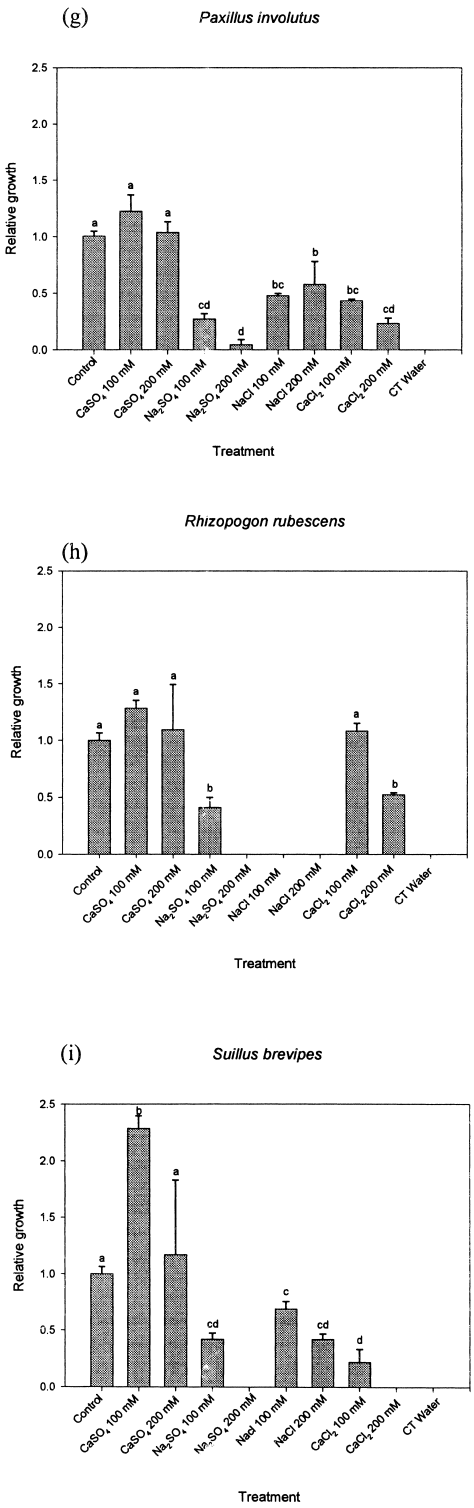

Figure 1. Continued

amended with CT release water, the greatest-to-least hierarchy of tolerance was: *H. crustuliniforme*, *Wilcoxina mikolae*, *L. bicolor*, *H. longicaudum*, *L. proxima*, *A. byssoides* = *P. involutus* = *R. rubescens* = *S. brevipes.*

Growth of the nine species on media adjusted to four different pH levels resulted in two general patterns: (1) relatively slow overall growth, maximal at intermediate pH values (between 4.9 and 5.8), and (2) relatively fast growth, increasing with increasing pH up to 6.8 (Fig. 2). Taxa included in the first group are *A. byssoides*, *P. involutus*, *R. rubescens*, and *S. brevipes*; the second group consisted of *H. crustuliniforme*, *H. longicaudum*, *L. bicolor*, *L. proxima*, and *W. mikolae.* Analysis of variance revealed significant pH effects ($F = 49.13$, $p = 0.0001$), species effects ($F = 65.1$, $p = 0.0001$), and interactions ($F = 12.37$, $p = 0.0001$). The effects of pH on the growth of *S. brevipes* and *L. bicolor* were not statistically significant, however.

Analysis of variance on proportional growth data from the seven isolates of *Laccaria bicolor* and from three isolates of *Hebeloma crustuliniforme* grown on 200mM $CaCl_2$ medium or CT release water medium revealed significant differences among *L. bicolor* isolates grown on the 200mM $CaCl_2$ medium ($F = 15.23$, $p<0.000$) but not among isolates grown on CT release water medium ($F = 2.34$, $p = 0.089$). No significant differences were seen among isolates of *H. crustuliniforme* ($F = 1.23$, $p = 0.355$ on CT water and $F = 1.346$, $p = 0.329$ on 200mM $CaCl_2$), although only three isolates were tested. Although the intraspecific variation in growth on 200mM $CaCl_2$ was significant within *L. bicolor* (Fig. 3), it was less than the interspecific variation seen among the other eight species tested (Fig. 1a–1j).

Discussion

Much of the information on fungal salt tolerance comes from studies on aquatic fungi; marine fungi exist in seawater at 500mM NaCl (Clipson & Jennings 1992), and one species was reported from Great Salt Lake at 12.8 M NaCl (Cronin & Post 1977). The effects of these high salt concentrations are two-fold; organisms living in saline conditions must contend with both high osmotic potentials and toxic metal ions (Munns et al. 1983). In general fungi tolerate these conditions by a combination of vacuolization, compartmentalization, and production of large concentrations of glycerol and mannitol that act as nontoxic osmoregulators (Clipson & Jennings 1992).

Sodium chloride has been shown to inhibit enzymatic activity in plants and fungi at concentrations of 50 to 200mM (Blomberg & Adler 1993). The constituent ions, Na^+ and Cl^-, have been shown to accumulate at equivalent levels in the cytoplasm of the cells of marine fungi (Clipson et al. 1990), where each ion may interact with different enzymes involved in protein synthesis (Serrano 1996). Metal ions can also interfere with transport systems, disrupt membranes, and displace other essential ions (Ochiai 1987). Ca^{2+}, for example, can become

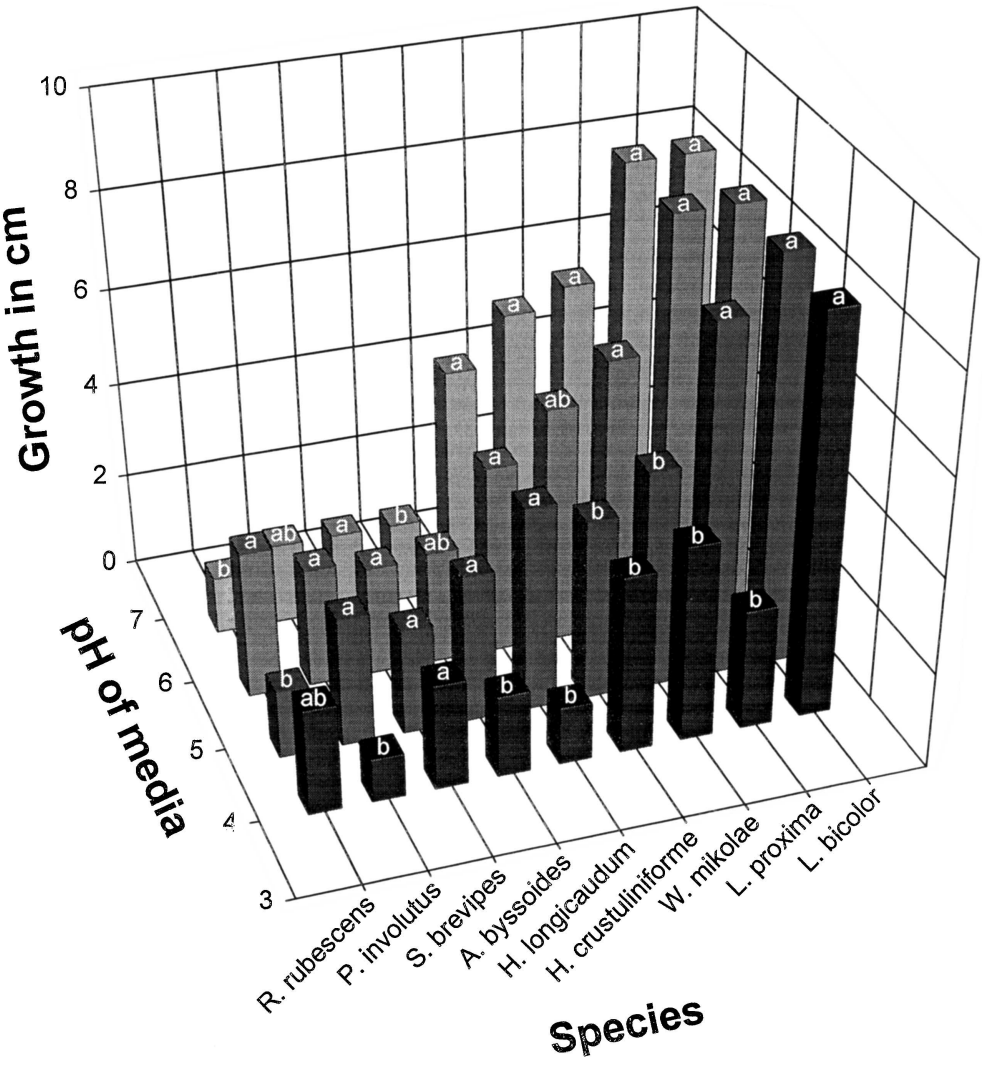

Figure 2. Growth (diameter in cm) of isolates on media at pH 4.1, 4.9, 5.8, and 6.8 after six weeks. Bars with common letters are not significantly different ($\alpha = 0.05$).

toxic at high concentrations due to precipitation of phosphates (Gadd 1993).

The precise mechanisms by which different organisms take up and excrete ions can vary considerably (Gadd 1993) however, and it is the efficiency of these mechanisms that likely governs the observed tolerance in the species tested here. In spite of this variability, certain genera (e.g., *Laccaria* and *Hebeloma*) were relatively tolerant across treatments, whereas other groups (e.g., the Boletales) grew poorly. This is in agreement with Tresner and Hayes (1971) and Hutchison (1990), who found that tolerance to NaCl tends to be uniform within fungal genera and therefore has potential as a taxonomic character. Dixon et al. (1993), however, found *Suillus luteus* to be relatively tolerant to sodium salts, whereas we found *S. brevipes* to be relatively intolerant. Also, in the present study, *Laccaria bicolor* performed relatively well on CT release water, but *L. proxima* did not. It appears, therefore, that the ability to tolerate saline-alkaline conditions can also vary at the species level.

The pH of the substratum must be taken into consideration when selecting ectomycorrhizal fungi for use in reclamation because of the sensitivity of a number of species to this parameter (Hung & Trappe 1983; Ohta 1990). In the present study, the variation in fungal growth on different treatment media appeared related to the pH of the media, with some species showing progressive growth increases from medium containing 200mM $CaCl_2$ (pH 4.1) through to 100mM NaCl (pH 6.5) (Fig. 1; Table 1).

To determine the effect of pH on the fungi tested, we grew all nine species at four different pH levels. Fungi growing on the pH-adjusted media either grew relatively slowly at all pH levels (4.1–6.8), and showed optimal growth at intermediate pH values (4.9–5.8) (*R. rubescens*, *S. brevipes*, *P. involutus*, [the Boletales] and *Amphinema byssoides*), or were more alkaliphilic, with growth in-

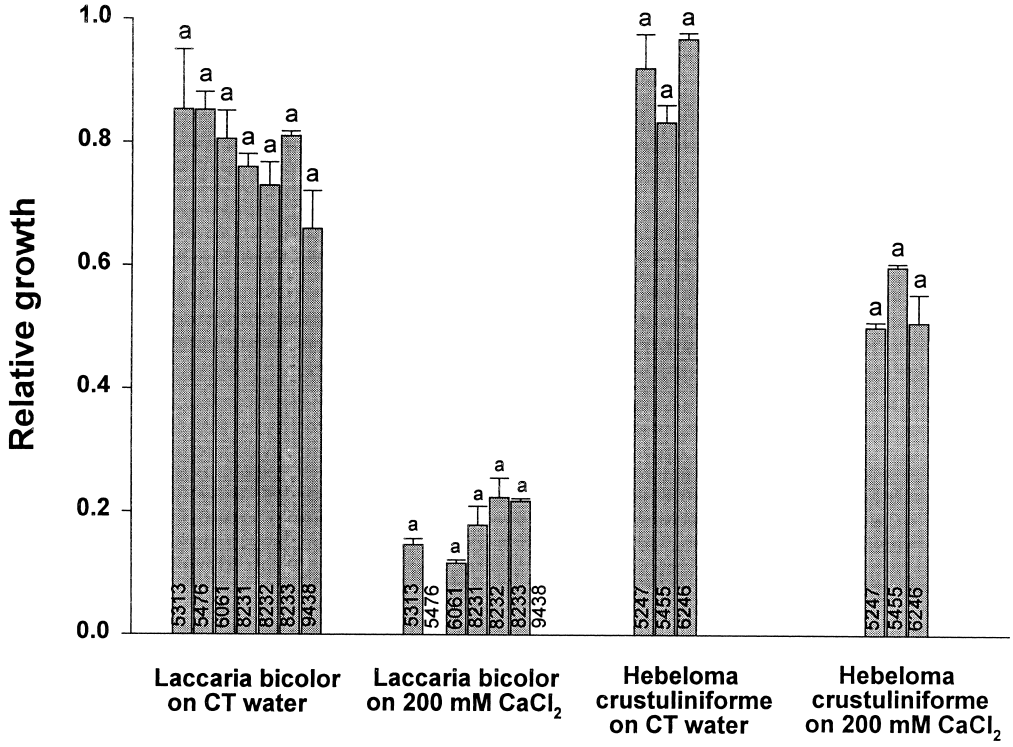

Figure 3. Growth (diameter in cm ± SE) of seven isolates of *Laccaria bicolor* and three isolates of *Hebeloma crustuliniforme* on Potato Dextrose Agar (PDA) control medium, PDA containing CT release water, and PDA containing 200mM $CaCl_2$. Values are averages of three replicates. Bars labeled with common letters are not significantly different (α = 0.05). UAMH accession numbers are printed on each bar.

creasing with increasing pH up to 6.8 (*H. crustuliniforme*, *H. longicaudum*, *L. bicolor*, *L. proxima*, and *W. mikolae*) (Fig. 2). This is congruent with the findings of Hung and Trappe (1983), who studied the optimal pH level for several ectomycorrhizal fungi and found that growth of *Hebeloma crustuliniforme* and some isolates of *Laccaria laccata* showed increased growth with increasing alkalinity up to a pH of 7.0. Ohta (1990) also found a similar trend with four species of ectomycorrhizal fungi at different pH levels. Two members of the Tricholomataceae (which includes *Laccaria*) showed optimal growth between pH 5.0 and 5.4, whereas *Rhizopogon rubescens* and *Suillus bovinus* (Boletales) grew best between pH 4.2 and 4.7, and growth thereafter decreased with increasing pH.

The importance of the pH of the salt-amended media used in the present study is exemplified by fungal growth on the CT release water medium (pH 7.8); those species with intermediate pH optima (the Boletales and *Amphinema byssoides*) were completely inhibited, whereas the relatively alkaliphilic species (*Hebeloma* and *Laccaria* spp. and *Wilcoxina mikolae*) survived. Inhibition of fungal growth in our salt-amended media could therefore be due to suboptimal pH or salt toxicity per se or an interaction between the two.

We can attempt to separate the effects of pH from salt toxicity in two ways. First by comparing fungal growth on each salt treatment to growth on media adjusted to a similar pH, and second by comparing growth at 100mM and 200mM levels of each salt. In the latter case pH changed only slightly with increasing salt concentration (Table 1), so large differences in growth between the two media should indicate a salt effect rather than a pH effect.

Fungal growth on $CaSO_4$ media was generally comparable to that on media adjusted to the same pH (Figs. 1 & 2) and similar between the two concentrations of $CaSO_4$ (Fig. 1), indicating a pH effect. *Suillus brevipes*, *R. rubescens*, and *P. involutus* were noticeable exceptions, however, in that they grew well on $CaSO_4$, in spite of suboptimal pH (Table 1; Fig. 1g, 1h, & 1i). This stimulatory effect might reflect a sulfur deficiency of some members of the Boletales when grown on MMN. The pH of the Na_2SO_4 media and the NaCl media were only slightly higher than that of control media (Table 1), yet the 200mM-level of each resulted in various degrees of inhibition in several species (Fig. 1). This suggests detrimental salt effects rather than pH effects. Calcium chloride amended media was the most fungitoxic treatment tested, inhibiting all species to some extent. Because all the fungi tested were able to grow at pH 4.1, on both the pH-adjusted media (Fig. 2) and on the 200mM $CaSO_4$ medium (Fig. 1), but many were completely inhibited on the $CaCl_2$ media (also pH 4.1), it is clear that $CaCl_2$ has a strong fungitoxic effect. Large differences in growth on 100mM $CaCl_2$ medium (pH 4.2) and 200mM $CaCl_2$ medium (pH 4.1) (Fig. 1) are also indicative of sensitivity to the salt, rather than pH.

Although the effects of pH and salt toxicity on fungal growth are obviously confounded, we are able to draw the following conclusions: (1) there was a clear division among the species tested with respect to pH optima; (2) most of the relatively alkaliphilic fungi (those that would be of use in an alkali environment) exhibited some sensitivity to $CaCl_2$, NaCl, and Na_2SO_4 beyond that accounted for by the suboptimal pH produced by their addition to the growing media; and (3) the fungitoxic effect of $CaCl_2$ is relatively similar across all species tested, whereas the effect of the sodium salts is more genus- or species-specific. Variation in fungal pH optima and tolerance to sodium salts would therefore seem to explain the wide range of growth responses to CT release water.

Attributes of Fungi Tested

The *Laccaria* species tested appear to be good candidates for use in saline-alkaline environments. Although *Laccaria proxima* showed the best growth averaged across all treatments, it performed poorly on the CT release water medium. *Laccaria bicolor*, however, performed almost as well on all treatment media and outperformed *L. proxima* on CT release water. *Laccaria bicolor* is common in the boreal forest, has a wide host range, and has been used extensively in in vitro syntheses of ectomycorrhizae (Mueller 1992). Testing of seven isolates of *L. bicolor* from throughout northern North America showed relatively low levels of intraspecific variation with respect to tolerance to $CaCl_2$ and CT release water (Fig. 3), indicating that the selection of particular strains of *L. bicolor* for use in saline-alkaline habitats should not be necessary.

The *Hebeloma* species also performed well, and *H. crustuliniforme* exhibited the highest tolerance to the CT release water, although both species were relatively sensitive to $CaSO_4$ and Na_2SO_4 (Fig. 1c & 1d). *Hebeloma crustuliniforme* is especially common in the boreal forest and has a wide host range (Kernaghan & Currah 1998). *Wilcoxina mikolae* exhibited the best absolute growth on 200mM $CaCl_2$, the most fungitoxic salt tested, although it was relatively sensitive to the sodium salts. A member of a group of ectendomycorrhizal fungi collectively known as "E-strain fungi," it is an early successional stage ascomycete, commonly associated with both coniferous and deciduous trees in nurseries, burned areas, and other disturbed sites (Egger et al. 1991).

Amphinema byssoides is one of the most common ectomycorrhizal fungi associated with spruce in northwestern North America (Kernaghan & Currah 1998), but it grew slowly and, like *Rhizopogon rubescens* and *Suillus brevipes*, performed poorly on many of the treatment media.

Paxillus involutus is also widely distributed and associated with various coniferous and broad-leaved hosts (Laiho 1970), but it grew poorly on $CaCl_2$ and Na_2SO_4. Although it showed improved growth on $CaSO_4$ relative to the controls, this was likely due to a preference for the intermediate pH of the $CaSO_4$-amended media (Fig. 2; Table 1) or to relief from the proposed sulfur deficiency of the MMN medium. The ability to solubilize naturally occurring $CaSO_4$ by the excretion of oxalic acid has recently been demonstrated in certain fungi (Gharieb et al. 1998). Calcium oxalate crystals, formed along fungal hyphae due to the reaction of oxalic acid with excess calcium, have been documented in pure cultures of *Paxillus involutus* grown on calcareous soil-based media (Lapeyrie et al. 1987) (and on the naturally occurring hyphae of some other basidiomycetes [Graustein et al. 1977; Malajczuk & Cromack 1982]). Such crystals may act as an exclusion mechanism protecting fungal cells from toxic levels of calcium (Lapeyrie et al. 1987) and may make *Paxillus involutus* a good candidate for use in reclamation of calcareous habitats with intermediate pH levels.

Several of the species tested here possess unique characteristics that may make them appropriate for use in reclamation of saline-alkaline habitats: (1) *Laccaria bicolor* because of its rapid growth and overall salt tolerance, (2) *Hebeloma crustuliniforme* because of its tolerance to the CT release water, and (3) *Wilcoxina mikolae* because of its tolerance to $CaCl_2$ and preference for disturbed habitats. Inoculation of seedlings with all three of these fungi might enhance plant growth above that which might be expected from inoculation with each species individually (McAfee & Fortin 1988), and could help create a more diverse and stable fungal community, facilitating further succession.

Acknowledgments

This research was supported by funding from the Environmental Science and Technology Alliance Canada (ESTAC) and Syncrude Canada. An National Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant (STPGP 202037-97) provided additional funding. We thank Lynne Sigler and Arlene Flis of the University of Alberta Microfungus Collection and Herbarium (UAMH) for cultures, Norwest Labs for chemical analyses, Emanuel Mapfumo for advice on statistics, and Daniel Archambault and Markus Thormann for their valuable comments.

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