Remediation and revegetation of tar sands composite tailings containing naphthenic acids and high salt using alder-*Frankia* symbionts.

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Tailings sands and tailings water

OBJECTIVES

- Develop efficient greenhouse production procedures for alder-*Frankia* symbionts.
- Develop a protocol for studying rhizosphere microflora inside and outside the root system.
- Screen alders and symbionts in greenhouse trials for use on CT soil.
- Evaluate the performance of symbiont alders in a field trial on CT sites.
- Determine the impact of rhizosphere microflora on the degradation of hydrocarbon contaminants.

Alnus crispa

Indigenous alder species

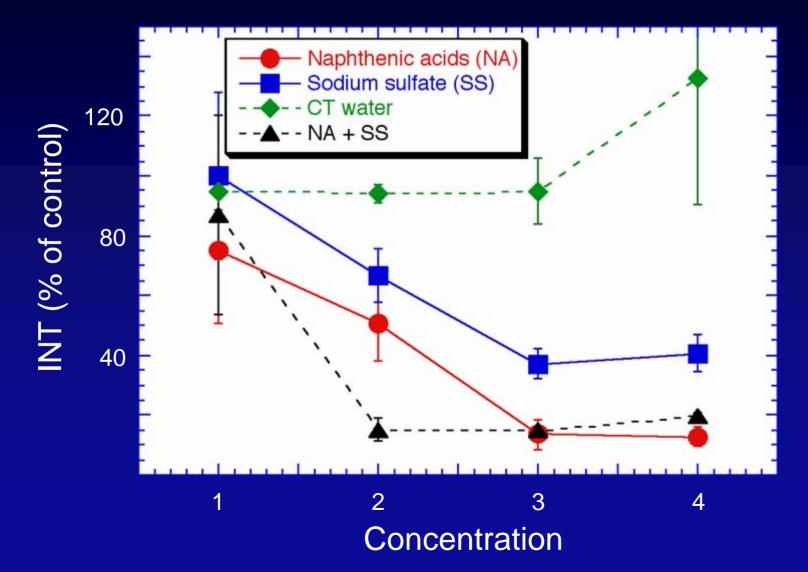


Alnus rugosa

Indigenous alder species



Resistance patterns of *Frankia* in naphthenic acids and sodium sulfate



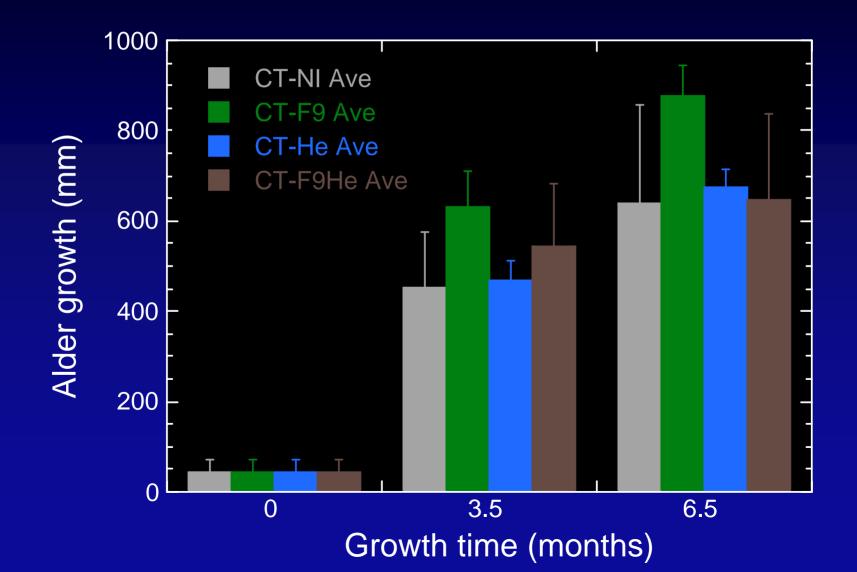
Non-inoculated, 6.5 months

F9-inoculated, 6.5 months

F9/ 602

B_ct

Alder (*A. crispa*) growth in CT sands with and without bacterial and fungal inocula



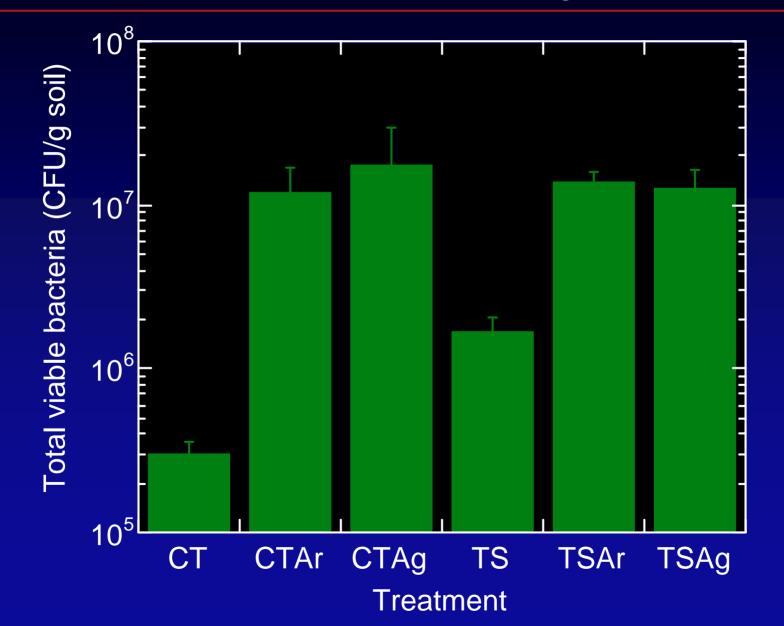
Frankia nodules in inoculated alder roots



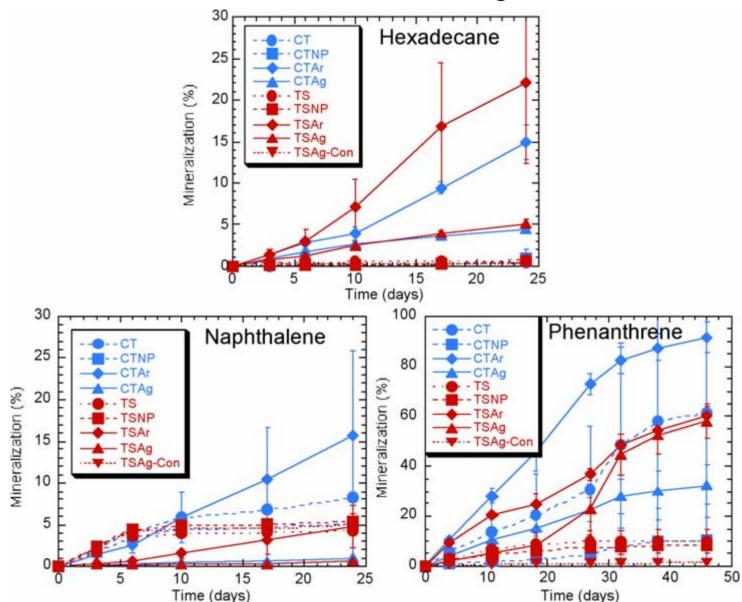
Alder roots penetrating CT sand



Viable bacteria in tailings sands before and after alder growth



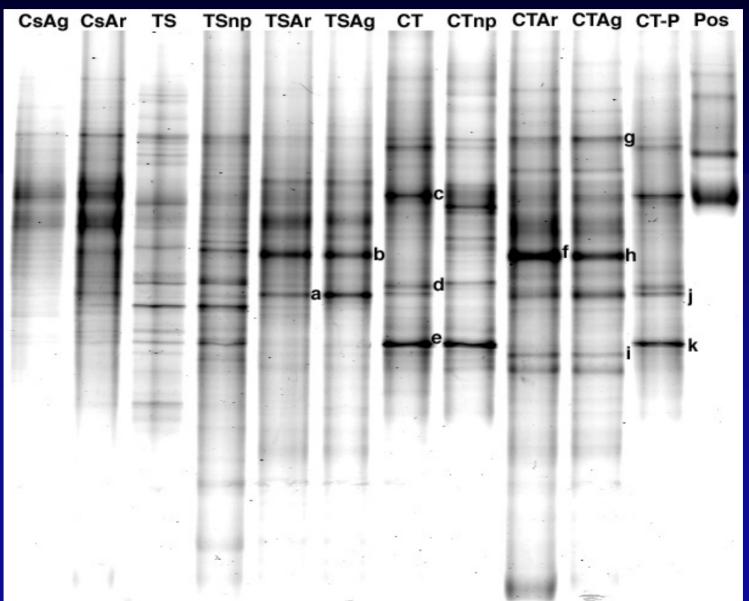
Hydrocarbon mineralization in TS and CT before and after alder growth



Detection of *alkB* in TS and CT before and after alder growth



Denaturing Gradient Gel Electrophoresis (DGGE) of tailings sands with and without alder growth



Detection of *Frankia* F9 in alder endophyte community DNA after alder growth in CT and control soil BE CE DE EE GE HE F9 F6 F4 - M • Germination trials of various alder seedlots identified *Alnus glutinosa* and *A. rugosa*, two species indigenous to northern Alberta, to be among the best.

• Screening of several *Frankia* species for tolerance/resistance to naphthenic acids (NA) and salts identified *Frankia* Avcl1 (strain F9) as the best for inocula production.

• Large-scale production of *Frankia* has been performed, including studies on chitosan encapsulation to increase the consistency and shelf-life of the produced inocula.

Summary of Results (cont'd)

• Characterization of microbial community structure and degradation activity in TS and CT sands before and after growth of alders, demonstrated that the alders had a significant positive effect on the microbial population size, composition and degradation activity.

• Alders (*Alnus rugosa*) inoculated with *Frankia*, an ectomycorrhizal fungus (*Hebeloma*) or both, have been examined in CT sand in greenhouse trials with similar results to the above, and are now being used in field trials on CT sand in Alberta.

Frankia - inoculated alder field trial



• A molecular method was developed to specifically detect *Frankia* F9, the inoculated strain, in soil and plant material. The method will allow us to perform environmental monitoring for the survival and mobility of the inoculated strain.

• The use of alder-*Frankia* symbionts for the remediation and revegetation of tailings sands from the tar sands shows considerable promise. The alders were able to successfully grow in TS and CT sand, and the matrix showed signs of improvement, based on changes in the microbial community structure and activity.