Phytoremediation of Alberta oil sand tailings using native plants and a fungal endophyte species

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Abstract

The Athabasca Oil Sands produce a high volume byproduct called tailing sands (TS). Typically, TS are remediated by planting young trees in large quantities of mulch (from elsewhere) plus mineral fertilizer. This is costly and labour intensive. Fungal endophytes colonize host plants without causing disease. Some endophytes confer plant tolerance to harsh environments. *Trichoderma harzianum* strain TSTh20-1 was isolated from a plant growing on Athabasca oil tailings sand (TS). TS are a high volume waste product from oil sand extraction that the industry is required to remediate. TS are low in organic carbon and mineral nutrients, and are hydrophobic due to residual hydrocarbons.

In greenhouse trials, TSTh20-1 supports growth of tomato seedlings on TS without fertilizer. TSTh20-1 is a promising candidate for economical TS remediation. We tested 23 plant species for seed germination and growth on TS in the presence of TSTh20-1. The four best candidates are currently being used in microcosm-scale growth trials, and for outdoor mesocosm trials this summer.

Potential mechanisms that contribute to endophyte-induced plant growth promotion are also being assessed. TSTh20-1 is nutritionally frugal, which may be characteristic of other plant-fungal endophytes. We are also tagging TSTh20-1 with GFP to follow it in the plant and in the environment.

Introduction

The Athabasca Oil Sands deposit in northeastern Alberta is the 3rd largest proven oil reserve in the world, spanning an area of 141000km^2. As of 2010 over 715km^2 of land had been disturbed by oil sands mining activities (1) (Figure 1). By law, the mining companies are required to reclaim land disturbed in the mining process and return it back to a state where it is "able to support a range of activities similar to its previous use before oil sands development" (1). Even if no additional challenges were faced this would be no small task due to the sheer scale involved. Adding to the difficulty of reclamation is the harsh nature of tailing sands (TS) produced during bitumen extraction.

Bitumen is extracted from oil sand by mixing with hot water and lye. This process forces the bitumen to float to the surface, where is can be extracted and processed, while coarse TS and other material sinks so that a portion of the water can be recycled. The fine sand-water slurry is eventually pumped out to settling ponds where the sand is allowed to dry. Also produced in this process are so-called 'fine tailings' composed of clay and other small particles that present additional technical challenges that will not be discussed here. Coarse TS have been shown to be poor in plant macronutrients (in particular phosphate), to have little or no organic carbon, and to be extremely hydrophobic because of potentially toxic residual hydrocarbons (2). These factors make current reclamation methods difficult to apply: less than 1% of the disturbed land has been certified as reclaimed by the government (1).

Current reclamation methods involve capping TS sites with a layer of soil that is rich in organic matter (often peat) and planting a large number of plants with regular fertilizing, hopefully leading to reforestation. This method is costly (soil must be shipped in for capping, and fertilizer is applied at a high rate) and not always effective.

Over time, some weedy pioneer plants colonize tailing sand. Plants growing in harsh environments have been shown to contain endophytic fungi that allow them to survive (3). Using this knowledge we isolated a fungal endophyte helps plants thrive on TS without fertilizer.

Figure 1. A single oil sand mining site can be much larger than Saskatoon. Left: an aerial view of a single company's mine site. Right: the city of Saskatoon at a similar scale.

Materials and Methods

Isolation and Identification of Endophyte Fungi

Plants were washed free of soil, surface sterilized in 1 % (v/v) NaOCl for 3 min, pressed onto potato dextrose agar (PDA) to test sterility (Rodriguez et al., 2008), then pieces were plated on 10 % PDA for 5-7 d at 28 °C to allow for endophyte growth out of the plant tissue.

Preliminary identification of used sporulation morphology. Cultures were grown across clean coverslips in moist chambers, then imaged with transmitted light microscopy. Full identification used ITS followed by sequence comparisons with calmodulin, actin and Ef1α.

Plant growth and endophyte colonization

Tomato plants (*Solanum lycopersicum* L. cv Rutgers) have a high demand for mineral nutrition and are sensitive to water stress. Seeds were surface-sterilized. Axenic seedlings were grown in double decker Magenta boxes (MBs).

A toothpick coated with mature TSTH20-1 spores was inserted into the soil for inoculation . Plant colonization was verified by removing leaves, surface sterilizing, and plating onto 10% PDA.

Stress Assays

Low nutrition assays used seedlings grown on or transferred to TS and watered with distilled water. Plant size, leaf colour, biomass, and root/shoot ratios were measured at 3 wk. Water use and wilt tolerance used plants grown on potting mix. After seedling establishment and colonization the water in all MBs was filled to an equal level and not refilled again. Once both sets of plants had wilted they were watered to observe the speed of recovery (if any).

Results

Trichoderma harzianum strain TSTh20-1 was isolated from a pioneer plant growing on TS. When tomato seedlings were inoculated with TSTh20-1 they grew on TS with only distilled water. After 2 wk on TS, colonized plants were significantly larger and averaged 2-fold higher dry weight than axenic seedlings ($P<0.001$).

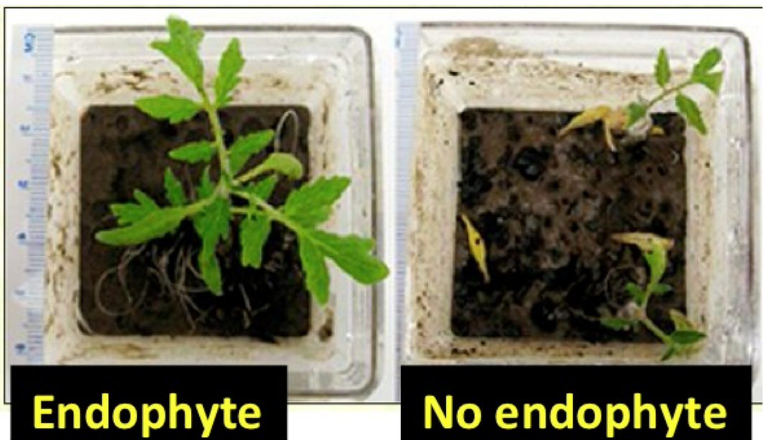

Figure 2. Left: tomato seedlings plants colonized with TSTh20-1, transplanted onto TS for 2 wk. Right: Axenic plants of the same age. Both had ultrapure water without added fertilizer.

Tomato plants colonized with TSTh20-1 had improved water use efficiency and wilt tolerance. Tomatoes grown on potting mix were left completely dry for 48 h, then watered thoroughly. Plants with TSTh20-1 were slower to wilt during the dry down period. This picture was taken 45 min after watering. The plants with endophyte had largely recovered, whereas the ones without endophyte required several hours

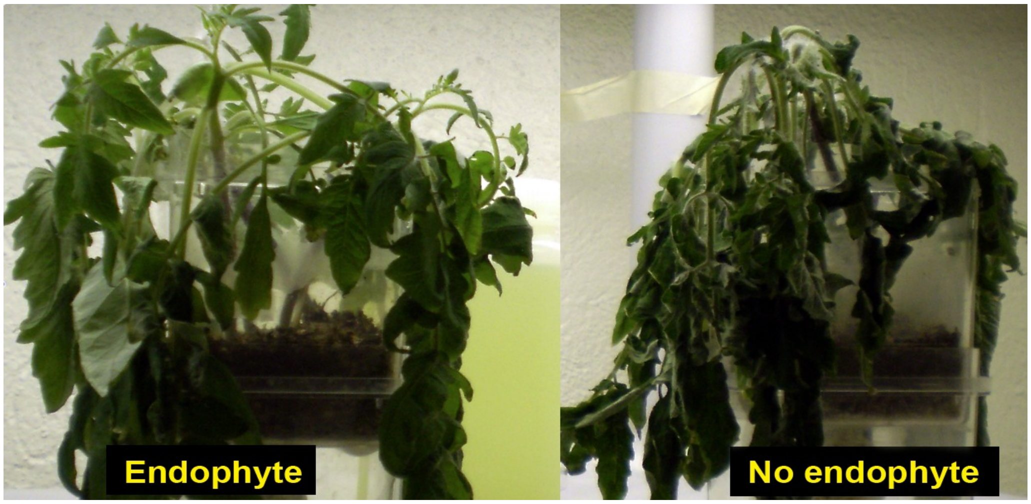

Figure 3. TSTh20-1 leads to increased drought tolerance and recovery. On the left are plants grown without TSTh20-1 and on the right are plants grown with the endophyte.

Four native Prairie plants were selected from 22 commercially-available species for their seeds' ability to germinate on well-watered TS. These are: *Bouteloua gracilis* (Blue Grama), *Elymus trachycaulus* (Slender Wheat Grass), *Trifolium repens* (White Clover), and *Medicago sativa* (Alfalfa).

Discussion & Current Work

TSTh20-1 shows great promise for improving upon current accepted and used reclamation methods. Current methods employ capping of tailing sands with a soil rich in organic matter and regular fertilization to support the growth of trees. While this method does work it is costly, labour intensive, and doesn't always work. TSTh20-1 has been shown to address some of these problems by helping plants survive in a low nutritional status (eg, less fertilizer needed) directly on TS (no capping needed). By doing this the cost of reclamation could be reduced drastically and perhaps be more effective by requiring less fertilizer and labour. TSTh20-1 is also extremely easy to apply and will work with a broad range of species including native grasses, forbs, and legumes.

Currently we are testing 23 native plant species for their ability to grow on TS when inoculated with TSTh20-1 and not fertilized. We have narrowed our search down to 4 species which are compatible with one another and seem to grow well on TS with TSTh20-1. We are now studying these on a microcosm-scale and hope to try a larger scale trial this summer. We are also investigating the mechanism behind TSTh20-1's ability to confer these tolerances and are examining a variety of factors such as: phytohormone production, siderophore production, protection from reactive oxygen species, phosphate solubilization, among others. These results will be published in the future.

References

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