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Detection of soil sulphur oxidising bacteria on Suncor's reclamation material stockpiles and the reclaimed landscape

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Abstract

Suncor Energy Inc. (Suncor) is currently reclaiming some areas to jackpine a1 and b1 ecosites. These ecotypes require slightly acidic to acidic soils. However, much of the existing soil on the reclaimed landscape and in many of the reclamation stockpiles has a neutral to slightly basic soil reaction. Tests were carried out on reclamation peat/mineral mix stockpiles and on the reclaimed landscape to determine the presence of soil sulphur oxidising bacteria (SOB). These bacteria can be used with additions of elemental sulphur to acidify the neutral to basic reclamation material and provide a substrate for the establishment and development of jackpine vegetative communities. HydroQual Laboratories Ltd. (HydroQual), Golder Associates Ltd. (Golder) and Suncor carried out a study to determine the presence of two SOB bacteria species in 90 soil samples (180 DNA tests in total). The two SOB species were chosen based on previous studies described in the scientific literature. Sixty composite soil samples at ten reclamation peat/mineral mix reclamation material stockpiles were sampled at 0 to 20 cm and 1+ m depths. Thirty composite soils samples were collected from ten reclaimed landscape sites. Three replicate composite samples were collected at each site. Testing was carried out to determine presence/absence of SOB in soil samples through standard DNA detection of SOB by polymerase chain reaction (PCR). Soil samples were also analysed for basic soil parameters such as pH, electrical conductivity (EC), sodium adsorption ratio (SAR), cation exchange capacity (CEC), total nitrogen, organic carbon and particle size analysis. The sampling program allowed for the statistical comparison of soil parameters using Analysis of Variance (ANOVA).

1 Introduction

Operators mining Alberta oil sands are required to reclaim their mine sites to the equivalent capability of their pre-disturbance biological productivity. The overall goal of reclamation is to rebuild sustainable ecosystems that will fall within the natural range and variability of productivity, diversity and multiple uses (Salifu et al., 2009). This reclamation processes includes the use of native vegetation communities and ecosites. Oil sand operators are currently reclaiming some areas to jackpine – Lichen (a1), jackpine-blueberry (b1) and jackpine/black spruce – Labrador tea (c1) ecosites. Jackpine – Lichen (a1) and jackpine-blueberry ecotypes are naturally found on rapidly to well drained, subxeric to submesic, acidic, coarse textured soils in Northeastern Alberta (Beckingham and Archibald, 1996) jackpine/black spruce – Labrador tea ecosites are found on subxeric to subhygric acidic substrate. Optimal establishment of these ecosites on the postmine landscape will require slightly acidic to acidic soils.

Peatlands cover approximately 30% of the Athabasca Oil Sands Region of Alberta (Johnson and Miyanishi, 2008). Most of the existing reclamation material in stockpiles and on the reclaimed landscape consists of peat and the underlying mineral substrate (peat-mineral).

The chemical characteristics of peat-mineral mixes are variable, with soil reactions ranging from slightly acidic to neutral to alkaline with free carbonates due to variable peat types and groundwater chemistry. The neutral to slightly alkaline soil reaction range may pose a constraint on the ability of this material to support some of the targeted jackpine ecosites.

Suncor is investigating the feasibility of acidifying alkaline reclamation material with elemental sulphur (a byproduct of the bitumen upgrading process). It is expected that acidification of reclaimed soils to levels more consistent with natural boreal forest floor soils could enhance the establishment of plant species, such as jackpine and blueberry.

The pathways of sulphur transformation in ecosystems are complex and involve changes in oxidation state, microbial and enzymatic processes, interaction with inorganic solid phases through adsorption/desorption and trace gas fluxes to the atmosphere (Sanborn et al., 2005). Sulphur oxidising bacteria (SOB) are able to transform elemental sulphur and other sulphur compounds to sulphate. Most sulphur oxidation that occurs in soils is a biochemical process and is carried out by bacteria from the genus Thiobacillus (Brady and Weil, 1999). In addition to the formation of sulphate, which is an essential plant nutrient, sulphur oxidation helps improve alkaline soils through acidification (Vidyalakshnmi et al., 2009). Two species of SOB bacteria were identified as most likely to be present in the area analysed: Acidothiobacillus thiooxidans and Thiobacillus thioparus, which grow under different pH conditions. The optimal pH range for Acidothiobacillus thiooxidans growth is 2.0 to 3.0 (0.5 to 5.5 limit) (Kelly and Wood, 2000), whereas Thiobacillus thioparus optimal pH range from 6.0 to 8.0 (3.5 to 4.5 limit in liquid thiosulphate media) (Holt et al., 2000).

Suncor reclamation soils may or may not have active SOBs in sufficient amounts to facilitate sulphur oxidation. Molecular tools (DNA detection through Polymerase Chain Reaction – PCR) of specific SOBs could help determine if elemental sulphur-oxidising bacteria are present in the peat-mineral mix soil. PCR has been used successfully for the detection of specific microbes in environmental samples, which can be particularly useful when: i) small amounts of DNA are recovered from samples; ii) microorganisms cannot be grown under standard laboratory conditions (it is estimated that less than 1% of soil microbes can be grown in pure cultures in laboratory); or iii) culturing methods for some microbes may be too labour intensive or the microbes may take too long to grow. The 16S ribosomal RNA gene sequence will be used as the target DNA sequence for SOB detection in this study. The 16S rDNA is routinely used in environmental microbiology studies as it is almost identical amongst all bacterial groups, allowing for detection of all representative species in a population. Each bacterial species, however, contains small unique ("signature") sequences that are variable and can be used to detect specific bacterial species (Kirk et al., 2004; Head et al., 1998).

The aim of this study is to determine the presence of the targeted species of SOBs in the existing reclamation material in stockpiles and selected reclaimed areas. Variable degrees of mixing of different peat types and mineral materials are present in peat-mineral stockpiles. Differences are expected in stockpile peat and mineral component composition, chemistry and bacterial populations due to local conditions.

This data will be valuable when assessing if sulphur amendments should be considered to adjust current soil reaction levels.

2 Methodology

2.1 Sample collection and experimental design

Samples were collected from various stockpile and reclaimed areas at the Suncor site in Fort McMurray, Alberta, Canada.

Ninety composite samples were collected from twenty sites. Ten peat mineral mix stockpiles were sampled both at a depth of 0 to 20 cm and at a depth of 1 m. The other ten sites were sampled on reclaimed soils at a depth of 0 to 20 cm; three composite samples were collected at each site/depth, for a total of ninety samples. Samples were labelled as to reclamation polygon, reclamation year, sample depth, salvage year and global positioning system (GPS) coordinates. The sampling program allowed for the statistical comparison of soil parameters and the presence or absence of SOB.

2.2 Soil physico-chemical characterisation

Basic soil characteristics such as pH, electrical conductivity (EC), sodium adsorption ratio (SAR), cation exchange capacity (CEC), total nitrogen, organic carbon, and particle size analyses were completed for each sample. Analyses were performed through standard methodologies by an independent analytical laboratory.

2.3. Statistical analysis

2.3.1 Analysis of variance (ANOVA)

Soil composition between the surface of the stockpiles and reclaimed soil, and the surface and depth area in the stockpiles was analysed by one-way ANOVA on seven soil parameters (pH, Electrical Conductivity, Sodium Adsorption Ratio, Cation Exchange Capacity, Total Nitrogen, Organic Carbon and % Clay).

One-way ANOVA was conducted for testing the null hypothesis that several univariate samples are taken from populations with the same mean. Samples were checked to be normally distributed and have similar variances before proceeding with the analysis of variance.

2.3.2 Similarity index

The Jaccard similarity index was used to compare the association between sites (stockpiles and reclaimed soil) and depths (0 to 20 cm and 1 m in the stockpiles) in terms of presence or absence of T. thioparus.

Jaccard's index may be expressed as follows:

$$J = C/ (A+B – C) \quad (1)$$

where A is the number of attributes present in the operational taxonomic unit (OTU) a, B is the number of attributes present in OTU b, and C is the number of attributes present in both OTUs a and b.

The associated probability for J was calculated to determine if the value for the index differed from what would be expected at random (Real and Vargas, 1996; Real, 1999), using the below formula. The probabilities associated with Jaccard's index depend on the total number of attributes present in either of the two habitats compared (N). The number of attributes present in either of the OTUs (N) is given by A+B-C (Real, 1999).

2.4. Detection of sulphur oxidising bacteria (SOB) in soil samples

2.4.1 Sulphur oxidising bacterial cultures

Reference strains of Acidothiobacillus thiooxidans American Type Culture Collection ([ATCC]# 19377) and Thiobacillus thioparus (ATCC# 23645) were purchased from the ATCC. Strains were grown in the media prescribed by ATCC (ATTC medium 125 and medium 290 for A. thiooxidans and T. thioparus respectively; http://www.atcc.org/). The A. thiooxidans strain was incubated for 21 to 28 days and T. thioparus for 5 to 7 days at 30oC.

2.4.2 DNA isolation

Testing to determine the presence/absence of SOB in soil samples was conducted through standard DNA techniques. Total genomic DNA was isolated from each soil sample (0.25 g) using the PowerSoil™ Microbial DNA Isolation Kit (MoBio Laboratories Inc.) according to the manufacturer's instructions and subsequently amplified using PCR. Genomic DNA was also isolated from both SOB reference cultures (to be used as positive controls for the PCR reactions) using the MoBio Ultra Clean DNA isolation kit (MoBio Laboratories Inc.). DNA samples were stored at -80°C in Tris-EDTA (TE) to preserve their integrity throughout the study.

2.4.3 Polymerase chain reaction (PCR)

PCR is a standard DNA analysis technique consisting of an enzymatic reaction that creates many copies of a specific DNA fragment in a large DNA sequence. In this study, PCR was used to amplify a section of the 16S ribosomal gene in the genomic DNA from soil samples and genomic DNA from reference strains of the chosen bacteria. The 16S gene sequence is routinely used in environmental microbiology studies as it is almost identical amongst all bacterial groups, allowing for detection of all representative species in a population; yet, each bacterial species contains small unique (“signature”) sequences that are variable and can be used to detect specific bacterial species (Figure 1).

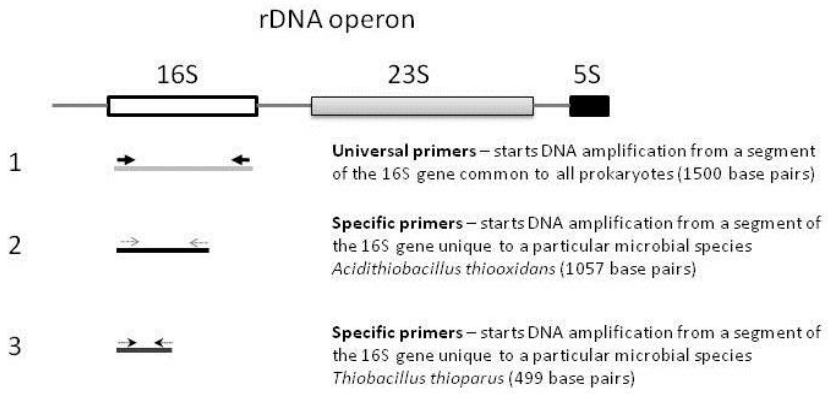

Figure 1 Diagram of PCR primers used in this study

A typical PCR reaction consists of mixing small amounts of the template DNA (genomic and soil DNA in this study); a DNA polymerase enzyme (usually Taq polymerase); small DNA fragments called "primers" that attach to specific targets in the template DNA to allow amplification of sequences of interest; and reagents that enable synthesis of millions of copies of the target DNA sequence. The solution mixes are submitted to 3-step temperature cycles in a thermal cycler (Mullis and Faloona, 1987).

In this study, DNA primer sequences specific for the detection of T. thioparus were identified for a region in the 16S rDNA gene (499 base pairs in length) (Barbosa et al., 2006), whereas specific A. thiooxidans primers covered a 1,057 base pair region of their 16S rDNA sequence (Wulf-Durand et al., 1997). Universal primers (i.e. primers able to detect the 16S gene of all bacterial groups) consisted of 27f and 1528r (Lane, 1991). Primers were ordered from Eurofins MWG Operon (Huntsville, AL, USA). PCR amplification was performed in a BioRad – MiniOpticon™ real-time PCR/Thermal Cycler detection system and PCR products visualised in agarose gels using a BioRad Gel Doc™ XR imaging system. The molecular size standard used in all PCR product evaluations consisted of BioRad 100 bp PCR Molecular Ruler DNA standard (100 to 3,000 base pairs, 30 bands in 100 bp increments).

2.4.4 Nested PCR

Nested PCR is a two-step process consisting of the use of two pairs of primers to amplify a specific DNA fragment. The universal primers 27f/1525r (Lane, 1991) were used to amplify the 16S rRNA fragment from mixed bacterial species present in the soil samples. A second PCR reaction was set, using the product of the first PCR reaction (16S rRNA fragment, 1,500 base pairs) as DNA template and a different pair of primers (which are "nested" within the 16S rRNA sequence and specific for either T. thioparus or A. thiooxidans) were used for detection of SOBs.

2.4.5 Experimental controls

A. thiooxidans and T. thioparus specific primers were verified for specificity in detecting the correct DNA fragments of each respective reference strain through both comparison of molecule size of PCR products against known standards in an agarose gel and through DNA sequencing (specific PCR detection positive control). The negative control, where no PCR product was expected to be generated, consisted of a PCR reaction without addition of any template DNA.

3 Results

3.1 Statistical analysis of soil physico-chemical parameters

To analyse soil composition between sites (stockpiles and reclaimed), and depths (surface and 1+ m depth in stockpiles); seven basic soil parameters were compared. The results presented in Table 1 show significant differences between sites in 3 parameters (electrical conductivity, total nitrogen, and organic carbon) with higher values in the stockpiles than in the reclaimed soil.

No significant difference was detected in the seven soil parameters between 0 to 20 cm and 1 m depths in the peat mineral stockpiles.

Table 1 Statistical analysis of soil physico-chemical parameters

Soil Parameter	Mean Stockpile (min-max)	Mean Reclaimed (min-max)	ANOVA	Mean Stockpile (min-max)	Mean Stockpile (min-max)	ANOVA
	Depth 0 to 20 cm			Depth 0 to 20 cm	Depth 1 m	
pH (units)	6.71 (4.62–7.60)	7.02 (6.13–7.81)	0.076 (NS)	6.71 (4.62–7.60)	6.75 (4.08–7.83)	0.856 (NS)
EC (dS m^{-1})	1.05 (0.21–3.11)	0.72 (0.33–1.4)	0.028 *	1.05 (0.21–3.11)	1.33 (0.15–5.83)	0.317 (NS)
SAR (units)	0.61 (0.09–4.09)	0.78 (0.11–3.19)	0.380 (NS)	0.61 (0.09–4.09)	0.66 (0.13–2.74)	0.785 (NS)
CEC (meq/100g)	37.8 (7.13–107)	29.4 (3.89–120)	0.136 (NS)	37.8 (7.13–107)	44.3 (4.59–88.1)	0.261 (NS)
TKN (%)	0.41 (0.08–0.97)	0.28 (0.08–0.85)	0.022 *	0.41 (0.08–0.97)	0.48 (0.063–1.06)	0.299 (NS)
TOC (%)	11.02 (1.98–28.3)	7.96 (1.93–25.5)	0.034 *	11.02 (1.98–28.3)	12.52 (2.81–25.3)	0.332 (NS)
% clay	14.89 (2.02–26)	16.64 (0.55–32.9)	0.418 (NS)	14.89 (2.02–26)	19.29 (1.56–48.4)	0.052 (NS)

Notes: EC, Electrical Conductivity; SAR, sodium adsorption ratio; CEC, cation exchange capacity; TKN, total Kjeldah nitrogen; TOC, total organic carbon. The ANOVA column refers to the statistical significance of a one-way ANOVA across each row. *Indicate statistically significant differences at $p < 0.05$. NS = non significant.

3.2 Statistical analysis of presence/absence of T. thioparus

According to the Jaccard's similarity index, the calculated value of T. thioparus habitat A= Stockpiles 0 to 20 cm was 29, habitat B= Reclaimed soil 0 to 20 cm was 30, and the shared habitat 29. The Jaccard's similarity index value is J= 0.97.

Using the same approach, the calculated value of T. thioparus habitat A= Stockpiles 0 to 20 cm was 29, habitat B= Stockpile 1 m was 28, and the shared habitat 27. The Jaccard's similarity index value is J= 0.90.

In both cases, according to Jaccard's Table 1 (Real, 1999), for N = 30, this similarity is higher than is expected to occur at random (with an associated probability of $p<0.05$). Therefore, there is no significant difference between the populations of T. thioparus between stockpiles and reclaimed soil, and between depths within stockpiles.

3.3 Detection of sulphur oxidising bacteria (SOBs) in soil samples

3.3.1 Thiobacillus thioparus

DNA was isolated from all soil samples and pure cultures of A. thiooxidans and T. thioparus reference strains. Several attempts to PCR-amplify the soil-extracted DNA were initially unsuccessful. PCR amplification of pure genomic DNA of reference cultures worked as expected, indicating that there were no problems with the primers or the PCR reactions themselves. Further test work established that the DNA extracted from the soil samples was not being successfully amplified due to co-extraction of PCR inhibitors present in the soils (i.e. polyphenols, humic acids (Baar et al., 2011). After dilution of the soil DNA (and

consequent dilution of inhibitors), PCR amplification was successful (Figure 2). All controls worked as expected. The positive controls (pure culture genomic DNA of A. thiooxidans, T. thioparus and bacterial control) generated a 1,500 base pairs band when amplified with universal primers 27f/1525r (Figure 2A, lanes 2 to 4), whereas the negative control (no template DNA) produced no band (Figure 2A, lane 5). PCR of most of the 90 soil DNA samples with the same universal primers generated a visible 1,500 bp DNA band (Figure 2A, lanes 6 to 9; 12 to 19)

The next step was the amplification of diluted samples of soil with T. thioparus specific primers (Figure 2B). Both positive controls, pure culture genomic DNA of T. thioparus and PCR-amplified 16S DNA fragment from T. thioparus pure culture genomic DNA with 27f/1525r universal primers (Figure 2B, lanes 2 and 3), generated the expected 499 bp DNA fragment. The negative control – no DNA template – shows no band, indicating no issues with contamination of PCR reaction. However, there was no amplification of genomic DNA extracted from soil samples when specific primers were used (Figure 2B, lanes 5 to 15).

The data indicated that a nested PCR approach could potentially work in detecting SOBs present in the soil samples. Nested-PCR is an effective method for detecting microbes, especially when the detection of low numbers of bacterial cells in complex environmental samples is required (conditions that seem to apply to the present study).

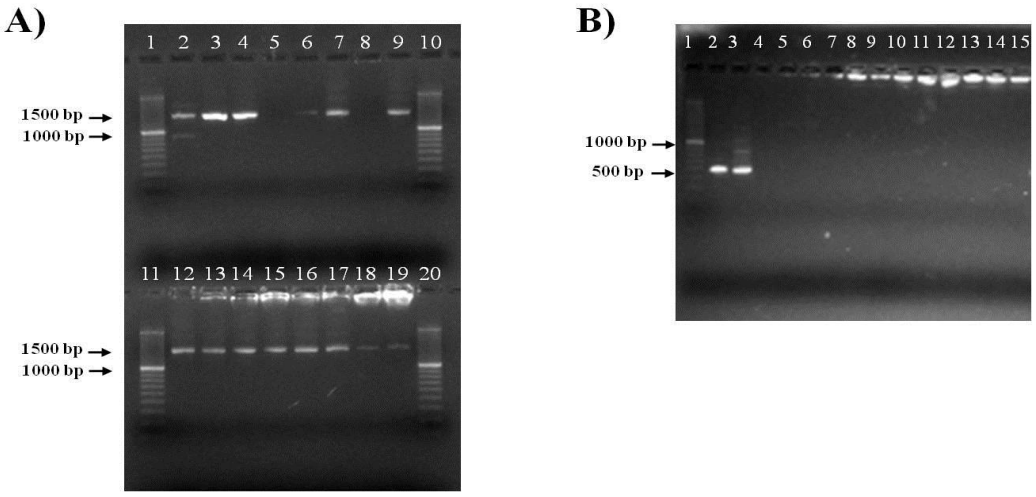

Figure 2 PCR amplification of soil-extracted genomic DNA and bacterial culture genomic DNA with universal primers 27f/1525r (A) and Thiobacillus thioparus specific primers (B). (A) 1, 10, 11, 20 – 100 bp ladder; 2 - T. thioparus pure culture genomic DNA; 3 - A. thiooxidans pure culture genomic DNA; 4 - bacterial control genomic DNA; 5 - No template DNA; 6 to 9, 12 to 19 – genomic DNA extracted from various soil samples. (B) 1 – 100 bp ladder; 2 - T. thioparus pure culture genomic DNA; 3 – PCR-amplified 16S DNA fragment amplified from T. thioparus pure culture genomic with 27f/1525r universal primers; 4 - No template DNA; 5 to 15 – genomic DNA extracted from various soil samples

The presence of T. thipoparus in soil samples representing several areas of the Suncor site in Fort McMurray was finally established by using nested PCR reactions (Figure 3; Table 2). PCR amplification was performed using the 16S DNA PCR-amplified from soil-extracted genomic DNA samples with universal primers (1,500 bp PCR product, Figure 2A) and T. thioparus specific primers. Single DNA fragments of approximately 500 bp were generated for all soil samples tested to date (Figure 3; Table 2).

3.3.2 Acidithiobacillus thiooxidans

The same nested PCR approach was attempted using specific primers for A. thiooxidans, but the amplification did not produce the expected 1,000 bp DNA fragment. Instead, it generated multiple bands, indicating that optimisation steps are needed before the presence or absence of this SOB species in Suncor's soils can be established. Based on minimum pH values, presence of A. thiooxidans might be expected in stockpiles at both 0 to 20 cm and at 1 m depths (pH 4.62 and 4.08, respectively).

Table 2 Presence (+), Absence (-) of T. thioparus in soil/ peat-mineral mix samples collected from stockpile and reclaimed areas at the Suncor site in the athabasca oil sands

Area	Type	Depth	Site Age	Triplicates
Crane Lake	Stockpile	0 to 20 cm	1975–1993	+, +, +
		1 m		+, +, +
MD2	Stockpile	0 to 20 cm	2009	+, +, +
		1 m		+, +, +
NSE	Stockpile	0 to 20 cm	2008–2009	+, +, +
		1 m		+, +, +
SE Dump 2	Stockpile	0 to 20 cm	2004	+, +, +
		1 m		+, +, +
Stockpile 3 - MD9	Stockpile	0 to 20 cm	2009	+, +, +
		1 m		+, +, +
Waste Area 5	Stockpile	0 to 20 cm	1977	+, +, -
		1 m		+, +, +
Exclusion Zone	Stockpile	0 to 20 cm	1993–1997	+, +, +
		1 m		+, -, -
MD9-West	Stockpile	0 to 20 cm	2009	+, +, +
		1 m		+, +, +
MD8-South	Stockpile	0 to 20 cm	2009	+, +, +
		1 m		+, +, +
DDA	Stockpile	0 to 20 cm	2010	+, +, +
		1 m		+, +, +
MD8	Reclaimed	0 to 20 cm	< 3 years old	+, +, +
MD5	Reclaimed	0 to 20 cm	<3 years old	+, +, +
ML24	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
ML 25 - A	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
SB 15	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
SE Dump	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
Site 16	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
Site 34	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
Site 41	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +
Tar Island	Reclaimed	0 to 20 cm	≥ 3 years old	+, +, +

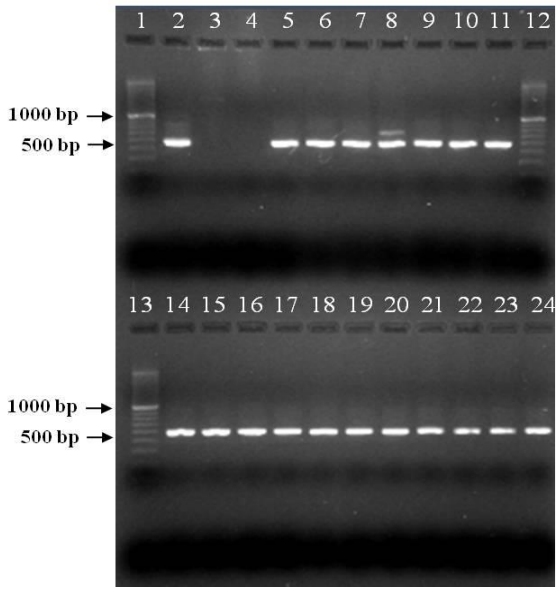

Figure 3 Positive detection of T. thioparus in soil samples. 1, 12 and 13–100 bp ladder; 2 – Positive control - PCR-amplified 16S DNA gene (1,500 bp PCR) as template DNA; 3 – T. thioparus specific primer with A. thiooxidans 16S DNA (no amplification as expected); 4 - no template DNA (negative control); Lanes 5 to 11, 14 to 24 – soil samples showing presence of T. thioparus

4 Conclusions

Suncor's soils from both the reclaimed and stockpile areas are able to support populations of at least one of the SOB species targeted in this study, as T. thioparus was detected in both soil areas.

These results are encouraging, as they indicate that elemental sulphur could potentially be utilised to acidify the soil and support the desired jackpine a1 and b1 ecosites.

Nested PCR was defined as the preferred method for SOB detection in soils, as it is a very sensitive method that allows for detection of very small numbers of bacterial cells in a population. Optimisation of amplification conditions is still required to confirm either presence or absence of A. thiooxidans in Suncor's soil samples.

Though there were statistically significant differences between the stockpile and reclaimed areas for three physiochemical parameters, T. thioparus has been detected in samples representing both types of soils. There was no significant difference between the populations of T. thioparus between stockpiles and reclaimed soil, and between depths (0 to 20 cm and 1 m) in the stockpiles.

5 Recommendations

Once the second SOB has been identified, the quantification of the nominal acid neutralising capacity of the soils could be explored in addition to the quantification of current populations of SOBs to in soil samples through quantitative PCR. It would also be beneficial to track the SOB growth/decline over time upon addition of different amounts of elemental sulphur.

Once the optimum amount of sulphur has been determined to reach the desired soil pH, bench scale studies could be performed to determine if the soil could support plant growth.

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References

Baar, C., d'Abbadie, M., Vaisman, A., Arana, M.E., Hofreiter, M., Woodgate, R., Kunkel, T.A. and Holliger, P. (2011) Molecular breeding of polymerases for resistance to environmental inhibitors, Nucleic Acid Research, pp. 1–12, doi:10.1093/nar/gkq1360.

Barbosa, V.L., Atkins, S.D., Barbosa, V.P., Burgess, J.E. and Stuetz, R.M. (2006) Characterization of Thiobacillus thioparus isolated from an activated sludge bioreactor used for hydrogen sulfide treatment, Journal of Applied Microbiology, Vol 101, pp. 1269–1281.

Beckingham, J.D. and Archibald, J.H. (1996). Field Guide to Ecosites of Northern Alberta, Canadian Forest Service Northwest Region, Northern Forestry Centre, Special Report #5.

Brady, N.C. and Weil, R.R. (1999) The Nature and Properties of Soils, 12th edition, 532 p.

Head, I.M., Saunders, J.R. and Pickup, R.W. (1998). Microbial Evolution, Diversity, and Ecology: A Decade of Ribosomal RNA Analysis of Uncultivated Microorganisms, Microbial Ecology. Vol. 35, pp. 1–31.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (2000) Bergey's Manual of Determinative Bacteriology, Ninth Edition, Lippincott Williams & Wilkins, Philadelphia, 787 p.

Johnson, E.A. and Miyanishi, K. (2008) Creating new landscapes and ecosystems: the Alberta Oil Sands, Annals of the New York Academy of Science, Vol. 1134, pp.120–145.

Kelly, D.P. and Wood, A.P. (2000) Reclassification of some species of Thiobacillus to the newly designated genera Acidithiobacillus gen. nov., Halothiobacillus gen. nov. and Thermithiobacillus gen. nov., International Journal of Systematic and Evolutionary Microbiology, Vol. 50, pp. 511–516.

Kirk, J.L., Beaudette, L.A, Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., and Trevors, J.T. (2004) Methods of studying soil microbial diversity. Journal of Microbiological Methods, Vol. 58, pp. 169–188.

Lane, D.J. (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds.). Nucleic Acid Techniques in Bacterial Systematics. Academic Press: Chichester, UK, pp. 115–175.

Mullis, K.B. and Faloona, F.A. (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed ain reaction, Methods Enzymology, Vol. 155, pp. 335–350.

Real, R. (1999). Tables of significant values of Jaccard's index of similarity. Miscelania Zoologica, Vol 22.1, pp. 29–40.

Real, R. and Vargar, J.M. (1996) The Probabilistic Basis of Jaccard's Index of Similarity. Systematic Biology, Vol. 45, pp. 380–385.

Salifu, K.F., Acton, C.A., Warner, C. and Anderson, B. (2009) Response of Forest Tree Seedlings and natural Vegetation to Fertilization: Suncor Dyke 5 Year Study, Canadian Land Reclamation Association, Issue 2.

Sanborn, P., Brockley, R., Mayer, B., Yun, M. and Prietzel, J. (2005). Sulphur Fertilizer of Lodgepole Pine: A Stable Isotype Tracer Study (E.P. 886.15) Establishment Report, BC Ministry of Forests, Technical Report #020.

Vidyalakshmi, R., Paranthaman, R. and Bhakyaraj, R. (2009). Sulphur Oxidizing Bacteria and Pulse Nutrition – Review. World Journal of Agricultural Sciences, Vol. 5 No. 3, pp. 270–278.

Wulf-Durand, P., Bryant, L.J. and Sly, L.I. (1997) PCR-Mediated Detection of Acidophilic, Bioleaching-Associated Bacteria, Applied and Environmental Microbiology, Vol. 63, No. 7, pp. 2944–2948.