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Isolation and identification of endophytic diazotrophs from lodgepole pine trees growing at unreclaimed gravel mining pits in central interior British Columbia, Canada

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1 Isolation and identification of endophytic diazotrophs from lodgepole pine trees
2 growing at unreclaimed gravel mining pits in central interior British Columbia,
3 Canada

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Draft

8 Abstract

9 Unreclaimed gravel mining pits located in the central interior of British Columbia have very
10 limited soil N-levels due to gravelly-textured soils with weak profile development, no organic
11 forest floor, and low atmospheric nitrogen (N) inputs through precipitation. However, lodgepole
12 pine (*Pinus contorta* var. *latifolia*) trees can be found growing well at these pits with tissue N-
13 content and growth rate unaffected by extremely low soil N-levels, indicating that pine trees are
14 able to meet their N-requirements from an unknown source. We hypothesized that biological N-
15 fixation by bacteria living in the tree tissues (known as endophytic diazotrophs) could be a
16 potential N source for these trees. To test this hypothesis, we isolated 77 potential endophytic
17 diazotrophs from needle, stem and root tissues of pine trees on N-free culture media. Of these, 32
18 bacteria showed positive N-fixing ability when tested for nitrogenase enzyme activity using the
19 acetylene reduction assay. These endophytic N-fixing bacteria were identified as mainly belonging
20 to the genera *Pseudomonas*, *Bacillus*, *Paenibacillus*, and *Rhizobium*, which are well-known for
21 their plant-beneficial traits including N-fixation. Therefore, it can be concluded that pine trees
22 growing at these severely N-limited gravel pits naturally harbour endophytic diazotrophs, which
23 could be involved in sustaining their vigorous growth, possibly through biological nitrogen
24 fixation.

25 **Keywords:** Endophytic diazotroph; lodgepole pine; biological nitrogen fixation; *Pinus*; gravel
26 mining pits

27 **Introduction**

28 The glacial and geological history of North America has resulted in abundant deposits of gravel
29 near the soil surface throughout Canada. Gravel is a highly sought-after material that is widely
30 used in many construction activities, making its mining economically important (LeMay 1999).
31 However, restoring gravel mining pits back to forests is difficult because gravel mining operations
32 typically leave large disturbed areas that lack natural cover and medium for plant growth (i.e.
33 topsoil). In other words, the bare gravel substrate mimics primary succession conditions (LeMay
34 1999). In 1995, it was reported that there were about 6000 gravel mining pits in British Columbia
35 (BC), a third of which were under active operations (Massey and Bobrowsky 1995). As reported
36 by Chapman and Paul (2012), several unreclaimed gravel pits with severely nutrient-poor
37 conditions for tree growth exist in the Sub-Boreal Pine-Spruce (SBPS) biogeoclimatic zone of BC.
38 The SBPS is a montane zone located in the central interior of BC, characterized by cold, dry
39 winters and cool, dry summers (Steen and Coupé 1997). Such harsh climatic conditions combined
40 with glacial till parent material dictate a very long time period to produce an adequate topsoil layer
41 at gravel pits in this zone.

42 Chapman and Paul (2012) studied six unreclaimed gravel pits in the SBPS zone and
43 reported that soils at these pits are gravelly in texture with weak profile development and no
44 organic forest floor. Precipitation-borne nitrogen (N) inputs at these pits are also extremely low.
45 In addition, the soil N levels at these pits were found to be six-fold lower than nearby sites with
46 intact topsoil. Despite these severely N-limited conditions, lodgepole pine (*Pinus contorta* var.
47 *latifolia*) trees can be found growing well at these pits. Chapman and Paul (2012) also reported
48 that the tissue N content and growth rate of lodgepole pine trees growing at these pits is similar to

the lodgepole pine trees growing in intact topsoil. This is curious since there is a well-known relationship between soil, plant tissue N levels and growth rate for non-N-fixing forest trees (Hobbie et al. 2000). In addition, the $\delta^{15}\text{N}$ content in tissues of lodgepole pine trees at gravel pits was 1.5 times lower than intact topsoil. When soil N supply is limited, plants take up N in higher isotopic forms (^{15}N), which was observed for intact topsoil trees. However, if plants have access to another N source, they won't take up N in higher isotopic form, which was observed for pit trees (Högberg 1997). Results of this study provide a strong evidence that lodgepole pine trees growing at these gravel pits are able to meet their N requirements from an unknown source (Chapman and Paul 2012). Some possible explanations could be associative or symbiotic N fixation. In the past, lodgepole pine trees growing at a forest site in the SBPS zone have been reported to harbour N-fixing bacteria in their internal tissues (Bal et al. 2012) and one of the isolated bacteria was reported to fix significant amounts of N (79%) and promote lodgepole pine seedling growth (Anand et al. 2013). Therefore, we hypothesized that a possible source of N for lodgepole pine trees at gravel pits could be biological N fixation (BNF) by endophytic diazotrophs (bacteria living in their internal tissues).

Endophytic diazotrophs have been reported to fix N and significantly promote plant growth of agricultural crops like rice (*Oryza sativa*) (Baldani et al. 2000), corn (*Zea mays*) (Puri et al. 2015), canola (*Brassica napus*) (Puri et al. 2016a) and tomato (*Solanum lycopersicum*) (Padma et al. 2016a), and forest trees like lodgepole pine (Tang et al. 2017), limber pine (*Pinus flexilis*) (Moyes et al. 2016), and western red cedar (*Thuja plicata*) (Anand and Chanway 2013). Endophytic diazotrophs have also been isolated from black cottonwood (*Populus trichocarpa*) and willow (*Salix sitchensis*) trees growing at gravel sites in Western Washington, USA (Doty et al. 2005, 2009). Nitrogenase enzyme activity and the presence of the *nifH* gene confirmed that several

of these isolates have the ability to fix N and support tree growth at these N-limited gravel sites (Doty et al. 2005, 2009). In addition, Knoth et al. (2014) confirmed their N-fixing ability in polar by using ^{15}N isotope dilution technique. These reports further support our aforementioned hypothesis.

The main objectives of this study were to: (i) isolate endophytic diazotrophs from internal tissues of lodgepole pine trees growing at N-limited unreclaimed gravel pits in the central interior of BC; (ii) examine the N-fixing ability of the isolated bacteria; and (ii) identify the bacteria that showed positive N-fixing ability. The specific hypotheses of this study were: (i) endophytic diazotrophs inhabit the internal tissues of lodgepole pine trees growing at these N-limited gravel pits; and (ii) these endophytic diazotrophs have the ability to fix atmospheric N that could potentially support the growth of lodgepole pine trees at these pits.

Materials and methods

Site description and sampling

Two gravel pits (Anah pit and Skulow pit) located in the central interior BC were selected out of the six gravel pits reported by Chapman and Paul (2012). Anah pit is located about 100 km west of Williams Lake (52°09'42.7" N, 123°10'24.3" W, 1132m a.s.l.) and Skulow pit is located 40 km north-east of Williams Lake (52°18'54.1" N, 121°53'39.3" W, 1064m a.s.l.). Normal annual precipitation for 1981 to 2010 at the Williams Lake Airport (which is central to the study area) is 307.6 mm as rainfall and 176.8 cm as snowfall. The average yearly temperature is 4.5°C. Lodgepole pine trees were predominant at the Skulow pit with Douglas-fir (*Pseudotsuga menziesii*) and hybrid white spruce (*Picea glauca* x *engelmannii*) present around the edges of the

pit. However, lodgepole pine was the only tree species observed at the Anah pit. Lodgepole pine trees were growing on bare gravel with no topsoil or organic forest floor at these pits. To isolate endophytic diazotrophs, 10 young lodgepole pine trees (<5 years old and <30 cm height) were uprooted and collected from each gravel pit. Trees were placed in sterile plastic bags, sealed and immediately transported to the laboratory on dry ice, stored at 4°C and processed within a week from sampling.

Isolation of endophytic diazotrophs

To isolate endophytic bacteria, needle, stem and root tissues (0.5g fresh mass) of trees sampled from both pits were surface-sterilized by immersion in 2.5% (w/v) sodium hypochlorite for 2 min, followed by three 30 s rinses in 10 mM sterile phosphate buffer saline (PBS) (pH 7) (Bal et al. 2012). To check for surface contamination, tissues were imprinted on triplicate plates of both tryptic soy agar (TSA) and N-free combined carbon medium (CCM) agar (Rennie 1981). Both media were supplemented with 100 mg/L cycloheximide to suppress fungal growth. Tissues free of surface contamination were ground in 1 mL PBS using a sterile mortar and pestle, serially diluted with PBS and plated onto CCM agar supplemented with 100 mg/L cycloheximide. CCM agar was used to specifically select for potential N-fixing bacteria. Plates were then incubated at 30°C for 3 days. After 3 days, representative bacterial colonies were selected, based on colony size, shape, morphology, and colour. Selected colonies were purified by streaking onto fresh CCM agar plates. Purified isolates were grown in the CCM broth amended with cycloheximide (100 mg/L) until turbid and stored frozen at -80°C in cryovials containing 2 mL CCM amended with 20% (v/v) glycerol.

114 *Evaluation of nitrogenase activity*

115 The nitrogen-fixing ability of isolated strains was tested using the acetylene reduction assay (ARA)
116 described by Holl et al. (1988) with some modifications. This assay tests the activity of nitrogenase
117 enzyme which is required to perform biological nitrogen fixation. For this assay, isolates were
118 grown in 22mL crimp top vials fitted with a pressure release aluminum seals containing
119 PTFE/Silicone septa. Three replicate vials were used per bacterial isolate. Each vial was filled with
120 9 mL CCM broth amended with cycloheximide, inoculated with bacteria, and incubated at room
121 temperature with shaking until the broths appeared turbid. Acetylene gas (Praxair Canada Inc.,
122 Mississauga, ON, Canada) was injected into each vial to a final volume of 10% of headspace (v/v).
123 Correspondingly, triplicate vials of 3 types of controls were used: CCM broth inoculated with
124 bacteria without acetylene; non-inoculated CCM broth with 10% acetylene; non-inoculated CCM
125 broth without 10% acetylene. After 48 h, a 1 mL sample of gas was removed from each vial, and
126 the ethylene content was measured by using flame ionization gas chromatography at the Analytical
127 Chemistry Services Laboratory, BC Ministry of Environment and Climate Change Strategy,
128 Victoria, BC, Canada. Ethylene production in each vial was quantified using a gas chromatograph
129 (Perkin Elmer Clarus 580, Shelton, CT, USA) equipped with a flame ionization detector and a
130 capillary column (Rt-Alumina BOND/Na₂SO₄, 30-m long, 0.53-mm internal diameter, with a
131 0.01-mm-thick film for affinity separation of elements, flow rate of 80ml/min). The inlet
132 temperature was 200°C with an inlet pressure of 6.1 psi. The injector and detector temperatures
133 were 200°C. The column temperature was 45°C at the time of injection, held for 1 min and then
134 increased to 120°C at the rate of 10°C/min and after that increased to 200°C at the rate of 45°C/min
135 and held for 1.5 min. To quantify ethylene production, the resulting chromatograms were used to
136 integrate the area under the ethylene curve. The amount of acetylene converted to ethylene by each

isolate was obtained by subtracting the ethylene content detected for the three controls from the ethylene content detected for the isolate. The whole nitrogenase assay was repeated to check for consistency of the acetylene reduction activity of isolates and to avoid any one-time experimental artifacts.

Identification of endophytic diazotrophs

Bacterial isolates that showed acetylene reduction activity were identified using the 16S rRNA gene sequencing technique described by Paul et al. (2013). Frozen isolates were thawed and streaked onto CCM agar and then grown in CCM broth for 48 h at 30°C. Bacterial cells were harvested by centrifugation and the genomic DNA of each isolate was extracted using the DNeasy UltraClean Microbial Kit (Qiagen Inc., Valencia, CA, USA) following the steps provided by the manufacturer. The concentration of the extracted DNA was determined using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and the quality of the DNA was checked using 0.8% agarose gel electrophoresis. PCR amplification of the 16S rRNA gene was performed using the primers 16S rRNA For [5'-AGAGTTTGATCCTGGCTCAG-3'] and 16S rRNA Rev [5'-ACGGCTACCTTGTTACGACTT-3'] (Integrated DNA Technologies Inc., Coralville, IA, USA) corresponding to positions 8–27 and 1492–1512, respectively, on the *Escherichia coli rrs* sequence. This resulted in a PCR product of approximately 1500 base pairs. PCR amplification was performed in 25 µL reaction volumes containing 2.5 µL of 10x PCR buffer (without MgCl₂), 2.5 µL of MgCl₂ (50 mM), 0.5 µL of Taq DNA Polymerase (5 U/µl), 1.5 µL of dNTP mix (consisting of four nucleotides: dATP, dCTP, dGTP, dTTP), 0.5 µL of each primer, and 3 µL of template DNA. The final volume was adjusted to 25 µL with nuclease-free water. Amplifications were performed on MJ Mini gradient thermal cycler (Bio-Rad Laboratories Inc.,

Hercules, CA, USA) using the following program: initial cell lysis and denaturation for 3 min at 95°C; followed by 30 cycles of denaturation (30 s at 94°C), annealing (1 min at 55°C), and extension (2 min at 72°C); and a final extension for 10 min at 72°C. The quantity and quality of PCR products were evaluated on a 1% agarose gel. The PCR amplification products were purified using the QIAquick PCR purification kit (Qiagen Inc., Valencia, California, USA) using the protocol provided by the manufacturer. Purified PCR products were sent to the ‘Sequencing and Bioinformatics Consortium Facility, University of British Columbia, Vancouver, BC, Canada’ for sequencing. Taxonomic identification of isolates was done by comparing the obtained sequences to known sequences in the ‘16S ribosomal RNA sequences (Bacteria and Archaea)’ database of the BLAST search tool (NCBI, Bethesda, MD, USA).

Results

Seventy-seven potential endophytic diazotrophs were isolated from lodgepole pine tree tissues using N-free CCM agar media. Twenty-nine isolates were obtained from the tissues of lodgepole pine trees growing at Anah pit (9 from stem tissues, 8 from needle tissues, and 12 from root tissues). From Skulow pit, 48 strains were isolated from lodgepole pine tissues (18 from stem tissues, 14 from needle tissues, and 16 from root tissues). Of the 77 total isolates, 32 showed positive nitrogenase activity in the ARA, confirming their *in vitro* N-fixing ability (Tables 1 and 2). From Anah pit, 15 of 29 isolates (Table 1) and from Skulow pit, 17 of 48 isolates (Table 2) exhibited positive N-fixing ability. It should be noted that we have only reported those strains that showed positive nitrogenase activity in duplicate assays. The mean amount of ethylene produced in the ARA by strains originating from the Anah pit was 1.4 nmol/mL (ranging between 0.2 – 2.8

nmol/mL). Similar amounts of ethylene levels were detected in the ARA for strains originating from the Skulow pit (mean = 1.3 nmol/mL; ranging between 0.2 – 2.8 nmol/mL).

All isolates showing positive N-fixing ability in ARA were identified using 16S rRNA gene sequencing technique. The obtained sequences were deposited under accession numbers MG561808 to MG561839 to the GenBank database (Tables 1 and 2). Isolates were identified based on their closest match (98-100% sequence identity) in the BLAST search. Bacteria isolated from lodgepole pine growing at either gravel pit belonged to the *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla, with *Proteobacteria* dominating at both sites. This dominance was particularly due to the presence of 15 strains belonging to the *Pseudomonas* genus (7 from Anah pit and 8 from Skulow pit). In addition, strains belonging to *Bacillus*, *Paenibacillus* and *Rhizobium* genera were commonly present at both pits.

Discussion

In this study, our aim was to isolate potential endophytic N-fixing bacteria from lodgepole pine trees growing at extremely N-limited unreclaimed gravel mining pits in the central interior of BC. We successfully isolated several potential N-fixers from stem, needle and root tissues of lodgepole pine trees growing at both the Anah pit and the Skulow pit. This confirms our first hypothesis that endophytic diazotrophs reside in the internal tissues of lodgepole pine trees growing at these pits. The number of isolates obtained on N-free CCM is consistent with those reported by Bal et al. (2012) for lodgepole pine trees and Puri et al. (2018a) for hybrid spruce in the SPBS zone and Doty et al. (2009) for poplar and willow trees growing on bare gravel sites in Washington state, USA. Use of N-free media is regarded as the first screening step in isolating endophytic diazotrophs (Rennie 1981). Since this media contain all the required nutrients except N, ideally,

only those bacteria that can fix their own N should grow on this medium (Doty et al. 2009). Isolation of N-fixing strains from internal tissues of lodgepole pine trees indicates that they have either originated from seeds or have colonized the plant via soil. Other possible routes could be colonization of leaves through stomata after transmission via air, transmission via sap-feeders, or transmission to flowers via pollinators (Frank et al. 2017). More work needs to be done to determine the source of the isolated bacteria.

To evaluate the N-fixing ability of strains isolated from lodgepole pine trees, we used the conventional acetylene reduction assay. This is a widely-used technique to evaluate BNF in several different environments like bacterial cultures, detached nodules, plant parts and even whole plants because it is a highly sensitive assay (Vovides et al. 2011). Many studies have found ARA to be a reliable technique of evaluating N-fixing ability of endophytic bacteria (Bal et al. 2012; Doty et al. 2009; Puri et al. 2018a, b). We found that around 40% of the total isolates that grew on N-free media showed positive acetylene reduction activity (Tables 1 and 2), which is consistent with the results of Doty et al. (2009). This indicates that growth on N-free CCM media cannot be directly correlated to the activity of the nitrogenase enzyme (i.e. the N-fixing ability). Several studies in the literature confirm this finding. Brighnigna et al. (1992) and Ozawa et al. (2003) reported that their endophytic isolates were able to grow on N-free media but failed to show any nitrogenase activity in ARA. It is possible that these bacteria are highly efficient scavengers of a very small amount of fixed nitrogen found in CCM (100mg/L yeast extract), or ammonia in the atmosphere (Hill and Postgate 1969; Wynn-Williams and Rhodes 1974; Anand 2010). In comparison to another study conducted on lodgepole pine trees in the forest stands of SBPS zone (Bal et al. 2012), we were able to isolate a much higher number of endophytic bacteria that showed positive N-fixing ability in ARA. This is consistent with the results of Yang et al. (2016, 2017) that N-fixing activity

and soil N levels have an inverse relation. This could have been the reason for isolation of more endophytic N-fixing bacteria from Anah and Skulow pits since they represent one of the most severely N-limited environments where trees have been observed to grow (Chapman and Paul 2012).

Strains showing N-fixing ability in ARA were identified by sequencing their 16S rRNA gene. It was found that most of these strains belong to plant-associated genera. The most important finding was that 15 of our strains belonged to the *Pseudomonas* genus with the majority of strains identified as *P. mandelii*, *P. frederiksbergensis*, and *P. migulae* (Tables 1 and 2). These *Pseudomonas* species have been previously documented to fix nitrogen and promote plant growth through other mechanisms including phosphate solubilization (Habibi et al. 2014; Zeng et al. 2017; Suyal et al. 2014). *Pseudomonas* strains were also isolated from poplar and willow trees growing at gravel sites in Washington state, USA with the potential to fix N (Doty et al. 2009), which validates the discovery of our strains. *Pseudomonas* strains have been associated with many tree species as potent N-fixers and plant-growth-promoters (reviewed by Puri et al. 2017a). Therefore, it could be postulated that the discovery of several strains of *Pseudomonas* in this study indicates that lodgepole pine trees growing at Anah and Skulow pits could be relying heavily on *Pseudomonas* strains to fulfil their N requirements. However, quantifying the amount of N-fixed by each of these strains *in planta* must be evaluated to determine their significance in providing N to their host plants.

We also identified 3 strains belonging to the *Rhizobium* genera which is renowned for its ability to fix N in the root nodules of legume species (Perret et al. 2000). However, studies also suggest that *Rhizobium* spp. could fix N and promote growth of non-nodulating tree species such

as poplar (Doty et al. 2005) and Douglas-fir (Khan et al. 2015), while living in their internal tissues. Subsequently, these strains *Rhizobium* demonstrated plant growth promotion and N-fixation when inoculated into poplar trees (Knoth et al. 2014). In addition to *Rhizobium* strains, 2 strains each belonging to *Bacillus* and *Paenibacillus* genera were identified. Numerous studies have reported the plant-growth-promoting ability (including N-fixing, phosphate solubilizing, IAA producing, ACC deaminase producing and biocontrol abilities) of *Bacillus* and *Paenibacillus* species (Padda et al. 2017a, b). N-fixing *Bacillus* and *Paenibacillus* species have been isolated from the internal tissues of trees including lodgepole pine (Shishido et al. 1995), hybrid spruce (Shishido and Chanway 2000) and western red cedar (Anand and Chanway 2013) growing in different regions of BC. Endophytic strains of *Paenibacillus* showing N-fixing ability were also isolated from stem tissues of lodgepole pine trees growing in this SBPS zone (Bal et al. 2012). In subsequent studies, one of these strains emerged as an effective endophytic N-fixer of tree species such as lodgepole pine (Bal and Chanway 2012a) and western red cedar (Bal and Chanway 2012b), and agricultural crops including corn (Puri et al. 2016b), canola (Padda et al. 2016b) and tomato (Padda et al. 2016a). Another noteworthy finding of our study is that *Pseudomonas*, *Bacillus*, *Paenibacillus* and *Rhizobium* strains were present at both pits suggesting that trees at these N-limited gravel pits might be recruiting similar endophytic diazotrophs that could be highly effective in fixing N and fulfilling their N requirements (Tables 1 and 2).

In this study, two *Caballeronia* strains were also isolated from the Skulow pit (Table 2). The genus *Caballeronia* is one of the plant-beneficial-environment group recently split from *Burkholderia* genus (Dobritsa and Samadpour 2016). *Burkholderia* spp. are widely recognized for their ability to fix N and promote plant growth in agricultural and forest ecosystems (Puri et al. 2017a, b). Specifically, *Caballeronia sordidicola* strains isolated from spruce forest soils in the

Czech Republic (Lladó et al. 2014) and from root nodules of *Lespedeza* plants (Palaniappan et al. 2010) were recognized as potential plant growth promoters. We also identified strains belonging to the genera *Flavobacterium*, *Staphylococcus*, *Pedobacter*, *Frigoribacterium*, *Herbiconiux*, *Rathayibacter*, *Parviterribacter*, *Rugamonas* (Tables 1 and 2). Endophytic strains belonging to *Flavobacterium* genus isolated from corn and switchgrass (*Panicum virgatum*) have been reported to possess N-fixing ability (*nifH* genes and nitrogenase activity) (Gao et al. 2015; Kämpfer et al. 2015). Similarly, endophytic *Staphylococcus* species have also been reported in poplar, sugarcane (*Saccharum officinarum*) and Ginseng (*Panax ginseng*) with the ability to promote plant growth (Moore et al. 2006; Velázquez et al. 2008; Vendan et al. 2010). In addition, species of *Herbiconiux*, *Pedobacter*, *Frigoribacterium*, *Parviterribacter* and *Rathayibacter* genera have been previously reported in the plant and soil environment (Becker et al. 2008; Gordon et al. 2009; Wang et al. 2015; Foesel et al. 2016; Schroeder et al. 2018).

Gravel mining pits in central-interior BC lack topsoil and essential plant nutrients, particularly N, that would affect normal plant growth (Chapman and Paul 2012). However, lodgepole pine trees have been observed growing well at these pits with no clear source of nitrogen. To the best of our knowledge, this is the first study which has explored the presence of endophytic diazotrophs in trees growing at gravel pits in BC and their possible role in sustaining tree growth through BNF. We are further investigating this possibility by examining the N-fixing ability of these endophytic diazotrophs *in planta* by quantifying the amount of N they can fix biologically from the atmosphere using robust techniques such as the ^{15}N foliar isotope dilution assay (Puri et al. 2018b), along with assessing the overall tree growth promotion. It has been suggested that lodgepole pine and their symbionts have great potential in reclaiming highly disturbed sites including oil sands, mine spills, as well as logged and fire-affected forests (Chapman and Paul

2012). Because lodgepole pine trees have been observed to grow on bare gravel at our sites, they represent an inexpensive alternative of restoring gravel pit back to forests as compared to other costly reclamation procedures such as grading, ripping, replacing topsoil, etc. (LeMay 1999). Therefore, we believe that, if shown to be effective in plant studies, these endophytic diazotrophs in association with lodgepole pine trees could be an environment-friendly and economical way of reclaiming gravel pits. In particular, lodgepole pine trees are part of the natural forest ecosystem and their association with such endophytic diazotrophs could reduce or offset the need to apply chemical fertilizers which is both economical and sustainable option.

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306 **References**

- 307 Anand, R. 2010. Endophytic colonization and nitrogen fixation by *Paenibacillus polymyxa* in
308 association with lodgepole pine and western red cedar. Ph.D. thesis, Department of Soil Science,
309 The University of British Columbia, Vancouver, Canada. doi:10.14288/1.0071481.
- 310 Anand, R., and Chanway, C. 2013. N₂-fixation and growth promotion in cedar colonized by an
311 endophytic strain of *Paenibacillus polymyxa*. Biol. Fertil. Soils, **49**(2): 235–239.
312 doi:10.1007/s00374-012-0735-9.
- 313 Anand, R., Grayston, S., and Chanway, C.P. 2013. N₂-fixation and seedling growth promotion of
314 lodgepole pine by endophytic *Paenibacillus polymyxa*. Microb. Ecol. **66**(2): 369–374.
315 doi:10.1007/s00248-013-0196-1.
- 316 Bal, A., and Chanway, C.P. 2012a. Evidence of nitrogen fixation in lodgepole pine inoculated
317 with diazotrophic *Paenibacillus polymyxa*. Botany, **90**(9): 891–896. doi:10.1139/b2012-044.
- 318 Bal, A., and Chanway, C.P. 2012b. ¹⁵N foliar dilution of western red cedar in response to seed
319 inoculation with diazotrophic *Paenibacillus polymyxa*. Biol. Fertil. Soils, **48**(8): 967–971.
320 doi:10.1007/s00374-012-0699-9.
- 321 Bal, A., Anand, R., Berge, O., and Chanway, C.P. 2012. Isolation and identification of
322 diazotrophic bacteria from internal tissues of *Pinus contorta* and *Thuja plicata*. Can. J. For. Res.
323 **42**(4): 807–813. doi:10.1139/x2012-023.
- 324 Baldani, V.L.D., Baldani, J.I., and Döbereiner, J. 2000. Inoculation of rice plants with the
325 endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol. Fertil. Soils,
326 **30**(5-6): 485–491. doi:10.1007/s003740050027.
- 327 Becker, R., Behrendt, U., Hommel, B., Kropf, S., and Ulrich, A. 2008. Effects of transgenic
328 fructan-producing potatoes on the community structure of rhizosphere and phyllosphere bacteria.
329 FEMS Microbiol. Ecol. **66**(2): 411–425. doi:10.1111/j.1574-6941.2008.00562.x.
- 330 Brighigna, L., Montaini, P., Favilli, F., and Carabez-Trejo, A. 1992. Role of the nitrogen-fixing
331 bacterial microflora in the epiphytism of *Tillandsia* (Bromeliaceae). Am. J. Bot. **79**(7): 723–727.
332 doi:10.1002/j.1537-2197.1992.tb13646.x.
- 333 Chapman, W.K., and Paul, L. 2012. Evidence that northern pioneering pines with tuberculate
334 mycorrhizae are unaffected by varying soil nitrogen levels. Microb. Ecol. **64**(4): 964–972.
335 doi:10.1007/s00248-012-0076-0.
- 336 Dobritsa, A.P., and Samadpour, M. 2016. Transfer of eleven *Burkholderia* species to the genus
337 *Paraburkholderia* and proposal of *Caballeronia* gen. nov., a new genus to accommodate twelve
338 species of *Burkholderia* and *Paraburkholderia*. Int. J. Syst. Evol. Microbiol. **66**(8): 2836–2846.
339 doi:10.1099/ijsem.0.001065.

- Doty, S.L., Doshier, M.R., Singleton, G.L., Moore, A.L., Van Aken, B., Stettler, R.F., Strand, S.E., and Gordon, M.P. 2005. Identification of an endophytic *Rhizobium* in stems of *Populus*. *Symbiosis*, **39**: 27-35.
- Doty, S.L., Oakley, B., Xin, G., Kang, J.W., Singleton, G., Khan, Z., Vajzovic, A., and Staley, J.T. 2009. Diazotrophic endophytes of native black cottonwood and willow. *Symbiosis*, **47**(1): 23–33. doi:10.1007/BF03179967.
- Foesel, B.U., Geppert, A., Rohde, M., and Overmann, J. 2016. *Parviterribacter kavangonensis* gen. nov., sp. nov. and *Parviterribacter multiflagellatus* sp. nov., novel members of *Parviterribacteraceae* fam. nov. within the order *Solirubrobacterales*, and emended descriptions of the classes *Thermoleophilia* and *Rubrobacteria* and their orders and families. *Int. J. Syst. Evol. Microbiol.* **66**(2): 652-665. doi:10.1099/ijsem.0.000770.
- Frank, A., Saldierna Guzmán, J., and Shay, J. 2017. Transmission of bacterial endophytes. *Microorganisms*, **5**(4): 70. doi:10.3390/microorganisms5040070.
- Gao, J.L., Lv, F.Y., Wang, X.M., Yuan, M., Li, J.W., Wu, Q.Y., and Sun, J.G. 2015. *Flavobacterium endophyticum* sp. nov., a *nifH* gene-harboring endophytic bacterium isolated from maize root. *Int. J. Syst. Evol. Microbiol.* **65**(11): 3900-3904. doi:10.1099/ijsem.0.000513.
- Gordon, N.S., Valenzuela, A., Adams, S.M., Ramsey, P.W., Pollock, J.L., Holben, W.E., and Gannon, J.E. 2009. *Pedobacter nyackensis* sp. nov., *Pedobacter alluvionis* sp. nov. and *Pedobacter borealis* sp. nov., isolated from Montana flood-plain sediment and forest soil. *Int. J. Syst. Evol. Microbiol.* **59**(7): 1720-1726. doi:10.1099/ijms.0.000158-0.
- Habibi, S., Djedidi, S., Prongjunthuek, K., Mortuza, M.F., Ohkama-Ohtsu, N., Sekimoto, H., and Yokoyama, T. 2014. Physiological and genetic characterization of rice nitrogen fixer PGPR isolated from rhizosphere soils of different crops. *Plant Soil*, **379**(1-2): 51-66. doi:10.1007/s11104-014-2035-7.
- Hill, S., and Postgate, J.R. 1969. Failure of putative nitrogen-fixing bacteria to fix nitrogen. *J. Gen. Microbiol.* **58**(2): 277-285. doi:10.1099/00221287-58-2-277.
- Hobbie, E.A., Macko, S.A., and Williams, M. 2000. Correlations between foliar $\delta^{15}\text{N}$ and nitrogen concentrations may indicate plant mycorrhizal interactions. *Oecologia*, **122**(2): 273–283. doi:10.1007/PL00008856.
- Högberg, P. 1997. Tansley Review No. 95 ^{15}N natural abundance in soil–plant systems. *New Phytol.* **137**(2): 179–203. doi:10.1046/j.1469-8137.1997.00808.x.
- Holl, F.B., Chanway, C.P., Turkington, R., and Radley, R.A. 1988. Response of crested wheatgrass (*Agropyron cristatum* L.), perennial ryegrass (*Lolium perenne* L.), and white clover (*Trifolium repens* L.) to inoculation with *Bacillus polymyxa*. *Soil Biol. Biochem.* **20**(1): 19–24. doi:10.1016/0038-0717(88)90121-6.

- 375 Kämpfer, P., Busse, H.J., McInroy, J.A., Xu, J., and Glaeser, S.P. 2015. *Flavobacterium*
376 *nitrogenifigens* sp. nov., isolated from switchgrass (*Panicum virgatum*). Int. J. Syst. Evol.
377 Microbiol. **65**(9): 2803-2809. doi:10.1099/ij.s.0.000330.
- 378 Khan, Z., Kandel, S., Ramos, D., Ettl, G.J., Kim, S-H., and Doty, S.L. 2015. Increased biomass
379 of nursery-grown Douglas-fir seedlings upon inoculation with diazotrophic endophytic consortia.
380 Forests, **6**(10): 3582-3593. doi:10.3390/f6103582.
- 381 LeMay, C. 1999. Forest restoration and reclamation of gravel pits in Canada. University of
382 Minnesota, Department of Horticultural Science. Restoration and Reclamation Review, **4**(5):1-5.
383 Available from <http://hdl.handle.net/11299/59318> [accessed 19 September 2018].
- 384 Lladó, S., Xu, Z., Sørensen, S.J., and Baldrian, P. 2014. Draft genome sequence of *Burkholderia*
385 *sordidicola* S170, a potential plant growth promoter isolated from coniferous forest soil in the
386 Czech Republic. Genome Announc. **2**(4): e00810-14. doi:10.1128/genomeA.00810-14.
- 387 Massey, N., and Bobrowsky, P. 1996. Aggregate Forum Report. BC Ministry of Energy, Mines
388 and Petroleum Resources, Victoria, Canada. Available from
389 [http://www.empr.gov.bc.ca/Mining/Geoscience/SurficialGeology/AggregateProject/AggregateFo](http://www.empr.gov.bc.ca/Mining/Geoscience/SurficialGeology/AggregateProject/AggregateForum95/Pages/OverviewReport.aspx)
390 [rum95/Pages/OverviewReport.aspx](http://www.empr.gov.bc.ca/Mining/Geoscience/SurficialGeology/AggregateProject/AggregateForum95/Pages/OverviewReport.aspx) [accessed 19 September 2018].
- 391 Moore, F.P., Barac, T., Borremans, B., Oeyen, L., Vangrons-veld, J., van der Lelie, D.,
392 Campbell, C.D., and Moore, E.R. 2006. Endophytic bacterial diversity in poplar trees growing
393 on a BTEX-contaminated site: the characterisation of isolates with potential to enhance
394 phytoremediation. Syst. Appl. Microbiol. **29**(7): 539-556. doi:10.1016/j.syapm.2005.11.012.
- 395 Moyes, A.B., Kueppers, L.M., Pett-Ridge, J., Carper, D.L., Vandehey, N., O'Neil, J., and Frank,
396 A.C. 2016. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine
397 conifer. New Phytol. **210**(2): 657-668. doi:10.1111/nph.13850.
- 398 Ozawa, T., Ohwaki, A., and Okumura, K. 2003. Isolation and characterization of diazotrophic
399 bacteria from the surface-sterilized roots of some legumes. Sci. Rep. Grad. Sch. Agric. Biol. Sci.
400 **55**: 29-36. Available from <http://hdl.handle.net/10466/1058> [accessed 19 September 2018].
- 401 Padda, K.P., Puri, A., and Chanway, C.P. 2016a. Effect of GFP tagging of *Paenibacillus*
402 *polymyxa* P2b-2R on its ability to promote growth of canola and tomato seedlings. Biol. Fertil.
403 Soils, **52**(3): 377-387. doi:10.1007/s00374-015-1083-3.
- 404 Padda, K.P., Puri, A., and Chanway, C.P. 2016b. Plant growth promotion and nitrogen fixation
405 in canola by an endophytic strain of *Paenibacillus polymyxa* and its GFP-tagged derivative in a
406 long-term study. Botany, **94**(12): 1209-1217. doi:10.1139/cjb-2016-0075.
- 407 Padda, K.P., Puri, A., Zeng, Q., Chanway, C.P., and Wu, X. 2017a. Effect of GFP-tagging on
408 nitrogen fixation and plant growth promotion of an endophytic diazotrophic strain of
409 *Paenibacillus polymyxa*. Botany, **95**(9): 933-942. doi:10.1139/cjb-2017-0056.

- 410 Padda, K.P., Puri, A., and Chanway, C.P. 2017b. *Paenibacillus polymyxa* – a prominent
411 biofertilizer and biocontrol agent for sustainable agriculture. In *Agriculturally Important
412 Microbes for Sustainable Agriculture: Applications in Crop Production and Protection. Edited by
413 V.S. Meena, P. Mishra, D. Thakuria, J. Bisht, and A. Pattanayak. Springer, Singapore. pp. 165-
414 191. doi:10.1007/978-981-10-5343-6_6.*
- 415 Palaniappan, P., Chauhan, P.S., Saravanan, V.S., Anandham, R., and Sa, T. 2010. Isolation and
416 characterization of plant growth promoting endophytic bacterial isolates from root nodule of
417 *Lespedeza* sp. *Biol. Fertil. Soils*, **46**(8): 807-816. doi:10.1007/s00374-010-0485-5.
- 418 Paul, L.R., Chapman, W.K., and Chanway, C.P. 2013. Diazotrophic bacteria reside inside *Suillus*
419 *tomentosus*/*Pinus contorta* tuberculate ectomycorrhizae. *Botany*, **91**(1): 48-52. doi:10.1139/cjb-
420 2012-0191.
- 421 Perret, X., Staehelin, C., and Broughton, W.J. 2000. Molecular basis of symbiotic promiscuity.
422 *Microbiol. Mol. Biol. Rev.* **64**(1): 180–201. doi:10.1128/MMBR.64.1.180-201.2000.
- 423 Puri, A., Padda, K.P., and Chanway, C.P. 2015. Can a diazotrophic endophyte originally isolated
424 from lodgepole pine colonize an agricultural crop (corn) and promote its growth? *Soil Biol.
425 Biochem.* **89**: 210-216. doi:10.1016/j.soilbio.2015.07.012.
- 426 Puri, A., Padda, K.P., and Chanway, C.P. 2016a. Evidence of nitrogen fixation and growth
427 promotion in canola (*Brassica napus* L.) by an endophytic diazotroph *Paenibacillus polymyxa*
428 P2b-2R. *Biol. Fertil. Soils*, **52**(1): 119-125. doi:10.1007/s00374-015-1051-y.
- 429 Puri, A., Padda, K.P., and Chanway, C.P. 2016b. Seedling growth promotion and nitrogen
430 fixation by a bacterial endophyte *Paenibacillus polymyxa* P2b-2R and its GFP derivative in corn
431 in a long-term trial. *Symbiosis*, **69**(2): 123-129. doi:10.1007/s13199-016-0385-z.
- 432 Puri, A., Padda, K.P., and Chanway, C.P. 2017a. Beneficial effects of bacterial endophytes on
433 forest tree species. In *Endophytes: Crop Productivity and Protection. Edited by D.K. Maheshwari
434 and K. Annapurna. Springer International Publishing, Switzerland. pp. 111-132.
435 doi:10.1007/978-3-319-66544-3_6.*
- 436 Puri, A., Padda, K.P., and Chanway, C.P. 2017b. Plant growth promotion by endophytic bacteria
437 in nonnative crop hosts. In *Endophytes: Crop Productivity and Protection. Edited by D.K.
438 Maheshwari and K. Annapurna. Springer International Publishing, Switzerland. pp. 11-45.
439 doi:10.1007/978-3-319-66544-3_2.*
- 440 Puri, A., Padda, K.P., and Chanway, C.P. 2018a. Evidence of endophytic diazotrophic bacteria in
441 lodgepole pine and hybrid white spruce trees growing in soils with different nutrient statuses in
442 the West Chilcotin region of British Columbia, Canada. *For. Ecol. Manage.* **430**: 558-565.
443 doi:10.1016/j.foreco.2018.08.049.

- 444 Puri, A., Padda, K.P., and Chanway, C.P. 2018b. Nitrogen-fixation by Endophytic Bacteria in
445 Agricultural Crops: Recent Advances. *In* Nitrogen in Agriculture – Updates. *Edited by* A. Khan
446 and S. Fahad. InTech, Croatia. pp. 73-94. doi:10.5772/intechopen.71988.
- 447 Rennie, R.J. 1981. A single medium for the isolation of acetylene-reducing (dinitrogen-fixing)
448 bacteria from soils. *Can. J. Microbiol.* **27**(1): 8-14. doi:10.1139/m81-002.
- 449 Schroeder, B.K., Schneider, W.L., Luster, D.G., Sechler, A., and Murray, T.D. 2018.
450 *Rathayibacter agropyri* (non O’Gara, 1916) comb. nov., nom. rev., isolated from western
451 wheatgrass (*Pascopyrum smithii*). *Int. J. Syst. Evol. Microbiol.* **68**(5): 1519-1525.
452 doi:10.1099/ijsem.0.002708.
- 453 Shishido, M., and Chanway, C.P. 2000. Colonization and growth of outplanted spruce seedlings
454 pre-inoculated with plant growth-promoting rhizobacteria in the greenhouse. *Can. J. For. Res.*
455 **30**(6): 848-854. doi:10.1139/x00-010.
- 456 Shishido, M., Loeb, B.M., and Chanway, C.P. 1995. External and internal root colonization of
457 lodgepole pine seedlings by two growth-promoting *Bacillus* strains originated from different root
458 microsites. *Can. J. Microbiol.* **41**(8): 707-713. doi:10.1139/m95-097.
- 459 Steen, O.A., and Coupé, R.A. 1997. A field guide to forest site identification and interpretation
460 for the Cariboo forest region. Land management handbook no. 39. BC Ministry of Forests,
461 Lands, Natural Resource Operations and Rural Development, Victoria, Canada. Available from
462 <https://www.for.gov.bc.ca/hfd/pubs/docs/lmh/lmh39.htm> [accessed 19 September 2018].
- 463 Suyal, D.C., Yadav, A., Shouche, Y., and Goel, R. 2014. Differential proteomics in response to
464 low temperature diazotrophy of Himalayan psychrophilic nitrogen fixing *Pseudomonas migulae*
465 S10724 strain. *Curr. Microbiol.* **68**(4): 543-550. doi:10.1007/s00284-013-0508-1.
- 466 Tang, Q., Puri, A., Padda, K.P., and Chanway, C.P. 2017. Biological nitrogen fixation and plant
467 growth promotion of lodgepole pine by an endophytic diazotroph *Paenibacillus polymyxa* and its
468 GFP-tagged derivative. *Botany*, **95**(6): 611-619. doi:10.1139/cjb-2016-0300.
- 469 Velázquez, E., Rojas, M., Lorite, M.J., Rivas, R., Zurdo-Piñeiro, J.L., Heydrich, M., and
470 Bedmar, E.J. 2008. Genetic diversity of endophytic bacteria which could be found in the
471 apoplastic sap of the medullary parenchym of the stem of healthy sugarcane plants. *J. Basic.*
472 *Microbiol.* **48**(2): 118-124. doi:10.1002/jobm.200700161.
- 473 Vendan, R.T., Yu, Y.J., Lee, S.H., and Rhee, Y.H. 2010. Diversity of endophytic bacteria in
474 ginseng and their potential for plant growth promotion. *J. Microbiol.* **48**(5): 559-565.
475 doi:10.1007/s12275-010-0082-1.
- 476 Vovides, A.G., Bashan, Y., López-Portillo, J.A., and Guevara, R. 2011. Nitrogen fixation in
477 preserved, reforested, naturally regenerated and impaired mangroves as an indicator of functional
478 restoration in mangroves in an arid region of Mexico. *Restoration Ecol.* **19**(201): 236-244.
479 doi:10.1111/j.1526-100X.2010.00713.x.

- 480 Wang, H.F., Zhang, Y.G., Chen, J.Y., Guo, J.W., Li, L., Hozzein, W., Zhang, Y.M., Wadaan,
481 M., and Li, W.J. 2015. *Frigoribacterium endophyticum* sp. nov., an endophytic actinobacterium
482 isolated from the root of *Anabasis elatior* (CA Mey.) Schischk. Int. J. Syst. Evol. Microbiol.
483 **65**(4): 1207-1212. doi:10.1099/ijs.0.000081.
- 484 Wynn-Williams, D.D., and Rhodes, M.E. 1974. Nitrogen fixation in seawater. J. Appl.
485 Microbiol. **37**(2): 203-216. doi:10.1111/j.1365-2672.1974.tb00432.x.
- 486 Yang, H., Puri, A., Padda, K.P., and Chanway, C.P. 2016. Effects of *Paenibacillus polymyxa*
487 inoculation and different soil nitrogen treatments on lodgepole pine seedling growth. Can. J. For.
488 Res. **46**(6): 816-821. doi:10.1139/cjfr-2015-0456.
- 489 Yang, H., Puri, A., Padda, K.P., and Chanway, C.P. 2017. Substrate utilization by endophytic
490 *Paenibacillus polymyxa* that may facilitate bacterial entrance and survival inside various host
491 plants. FACETS, **2**: 120-130. doi:10.1139/facets-2016-0031.
- 492 Zeng, Q., Wu, X., and Wen, X. 2017. Identification and characterization of the rhizosphere
493 phosphate-solubilizing bacterium *Pseudomonas frederiksbergensis* JW-SD2 and its plant
494 growth-promoting effects on poplar seedlings. Ann. Microbiol. **67**(3): 219-230.
495 doi:10.1007/s13213-016-1237-z.

Table 1 List of endophytic diazotrophs isolated from lodgepole pine trees growing at **Anah pit** with their taxonomic identity and the amount of ethylene produced by each strain in acetylene reduction assay

Strain	Taxonomic Identity	Acetylene reduction activity (nmol C ₂ H ₄ /mL) ^a	GenBank accession no.
AS1	<i>Pseudomonas mandelii</i>	2.7 ± 0.24 ^b	MG561808
AS2	<i>Rhizobium leguminosarum</i>	2.0 ± 0.32	MG561809
AS3	<i>Rugamonas rubra</i>	0.4 ± 0.02	MG561810
AS4	<i>Staphylococcus auricularis</i>	0.4 ± 0.04	MG561811
AS5	<i>Paenibacillus urinalis</i>	1.3 ± 0.09	MG561812
AN1	<i>Pseudomonas graminis</i>	1.9 ± 0.10	MG561813
AN2	<i>Rathayibacter tanacetii</i>	2.0 ± 0.31	MG561814
AN3	<i>Frigoribacterium endophyticum</i>	0.2 ± 0.04	MG561815
AN4	<i>Pseudomonas rhizosphaerae</i>	1.0 ± 0.12	MG561816
AN5	<i>Pseudomonas frederiksbergensis</i>	1.0 ± 0.08	MG561817
AN6	<i>Bacillus simplex</i>	0.6 ± 0.03	MG561818
AR1	<i>Pseudomonas migulae</i>	2.8 ± 0.35	MG561819
AR2	<i>Pseudomonas migulae</i>	2.8 ± 0.19	MG561820
AR3	<i>Pseudomonas mandelii</i>	0.8 ± 0.07	MG561821
AR4	<i>Pedobacter suwonensis</i>	0.7 ± 0.02	MG561822

^a nanomoles of ethylene produced per mL of culture vial headspace

^b mean ± standard error; duplicate assays with n = 3 per assay

Note: Strain names were given based on the sampling pit and tissue type from which they were isolated (e.g. AS1: Anah pit pine stem strain 1, AN1: Anah pit pine needle strain 1, AR1: Anah pit pine root strain 1)

Table 2 List of endophytic diazotrophs isolated from lodgepole pine trees growing at **Skulow pit** with their taxonomic identity and the amount of ethylene produced by each strain in acetylene reduction assay

Strain	Taxonomic Identity	Acetylene reduction activity (nmol C ₂ H ₄ /mL) ^a	GenBank accession no.
SS1	<i>Pseudomonas mandelii</i>	2.8 ± 0.17 ^b	MG561823
SS2	<i>Pseudomonas frederiksborgensis</i>	1.7 ± 0.02	MG561824
SS3	<i>Herbiconiux solani</i>	0.4 ± 0.10	MG561825
SS4	<i>Caballeronia udeis</i>	0.8 ± 0.06	MG561826
SS5	<i>Bacillus circulans</i>	1.6 ± 0.19	MG561827
SS6	<i>Parviterribacter sp.</i>	1.8 ± 0.42	MG561828
SS7	<i>Rhizobium rosettiformans</i>	0.2 ± 0.09	MG561829
SS8	<i>Paenibacillus urinalis</i>	1.8 ± 0.04	MG561830
SN1	<i>Pseudomonas lini</i>	0.3 ± 0.04	MG561831
SN2	<i>Flavobacterium aquidurens</i>	2.8 ± 0.30	MG561832
SN3	<i>Pseudomonas frederiksborgensis</i>	0.2 ± 0.01	MG561833
SN4	<i>Pseudomonas mandelii</i>	0.4 ± 0.03	MG561834
SR1	<i>Caballeronia sordidicola</i>	2.4 ± 0.01	MG561835
SR2	<i>Rhizobium herbae</i>	0.4 ± 0.06	MG561836
SR3	<i>Pseudomonas brassicacearum</i> subsp. <i>neaurantiaca</i>	0.9 ± 0.01	MG561837
SR4	<i>Pseudomonas frederiksborgensis</i>	2.5 ± 0.25	MG561838
SR5	<i>Pseudomonas frederiksborgensis</i>	0.4 ± 0.01	MG561839

^a nanomoles of ethylene produced per mL of culture vial headspace

^b mean ± standard error; duplicate assays with n = 3 per assay

Note: Strain names were given based on the sampling pit and tissue type from which they were isolated (e.g. SS1: Skulow pit pine stem strain 1, SN1: Skulow pit pine needle strain 1, SR1: Skulow pit pine root strain 1)