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# Sneaky bacteria? Just maybe in Scotchbroom

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# Sneaky Bacteria? Just Maybe in Scotch Broom Nodules

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## ABSTRACT

Scotch broom (*Cystis scoparius*) is invading the South Puget Sound lowland, presumably with help from the nitrogen-fixing symbiotic bacteria that are found in its root nodules. With this nitrogen source, Scotch broom is not limited by low nitrogen levels in the soils as is characteristic of prairie soils. The goals of our research are to identify the nodule bacteria and to assess their abundance and ecological roles in the soil microbial community in response to broom invasion. Using 16s rRNA sequencing we identified the nodulating bacteria of Scotch broom nodules from the South Puget Sound lowland prairies as predominantly from the genera *Burkholderia* and *Rhizobium*. Moreover, there were two nodules from which both *Burkholderia* and *Rhizobium* were successfully isolated and sequenced. Therefore, it is probable that multiple bacterial species can live within a single Scotch broom nodule. Neither the presence of *Burkholderia* nor multiple nodule occupation has been previously well-documented in a temperate zone plant. With the identity of the bacteria known, we can now explore the population responses and ecological roles of these bacteria in soils of various levels of Scotch broom invasion.

## Introduction

Scotch broom (*Cytisus scoparius*) is an extremely invasive species that is over-running the prairies in the South Puget lowlands (Figure 1). One reason Scotch broom is such an effective invader is because of its symbiosis with bacteria that help fix nitrogen for Scotch broom's use. In exchange for nitrogen, Scotch broom gives these bacteria photosynthetic products, and a protected anaerobic environment in root nodules. While these nodulating bacteria presumably help Scotch broom invade the South Puget lowlands, their identity, their abundance in the soil, their ecological roles in the microbial community, and their population responses to Scotch broom invasion all remain unknown. To begin to explore these questions, we first set out to determine the identity of these nodulating bacteria in preparation for constructing a genetic marker to determine the presence and abundance of these nodulating bacteria in soils with different Scotch broom invasion intensities and histories.

In legume roots, nodulating bacteria typically invade microscopic root hairs to create a nodule. In this mechanism of invasion, the root hair curls and a single bacterial cell enters through an invagination in the root hair cell wall. This founding bacterium multiplies and works its way through several layers of cells and causes the plant to form a nodule. Therefore, a nodule should contain only a single bacterial species.

## Acknowledgements

We would like to thank Mark Martin for his much appreciated guidance and assistance, and the University of Puget Sound for funding.


**Figure 2.** Nodules connected to Scotch broom roots. Nodules can be clustered or single and are usually found on shallow distal roots.

## PCR Amplification of 16s rRNA Gene

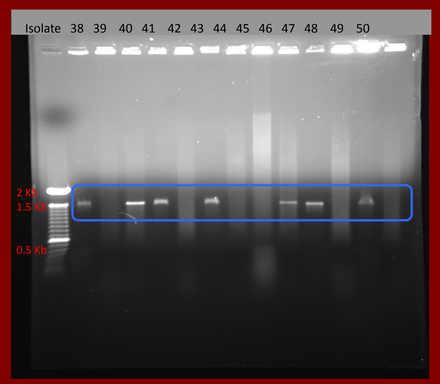


**Figure 4**. After colony PCR amplification of the 16s rRNA gene, a gel was run to confirm a successful PCR reaction. The 16s rRNA amplicon is 1.4 Kb long. Isolates 38, 40, 41, 43, 46, 47, and 49 were successful as shown by the band at approximately 1.4 Kb.

## Morphological Colonies

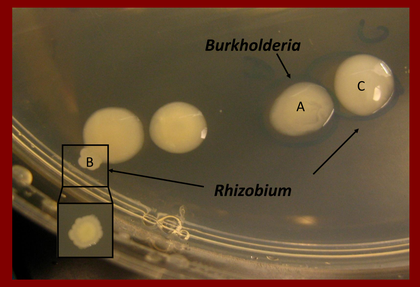


**Figure 3.** Predominant morphological colonies of Scotch broom isolates. After sequencing, A was identified as *Burkholderia* and B and C were identified as *Rhizobium*. A= milky semitransparent mucoid. B= white scalloped edges. C= white mucoid.

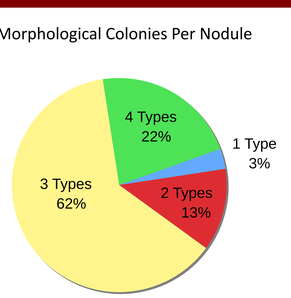


**Figure 5.** Colonies grown from streaked surface-sterilized nodules were separated morphologically. Multiple colony types were found from most nodules, suggesting that multiple types of bacteria are found inside a single nodule.

## General Procedure

- Collected Scotch broom nodules from four plants on each of two different prairies (Figure 2)
- Isolated bacteria from nodules by streaking on mannitol plates, and determined morphological colony types (Figure 3,5).
- Amplified 16s rRNA sequences with colony PCR reaction using 27F and 1492R universal primers. Determined success of colony PCR via gel electrophoresis (Figure 4).
- Sequenced 16s rRNA PCR product (Macrogen)
- Identified bacteria by matching sequences with known bacteria using online databases (BLAST and Classifier) (Figure 6).

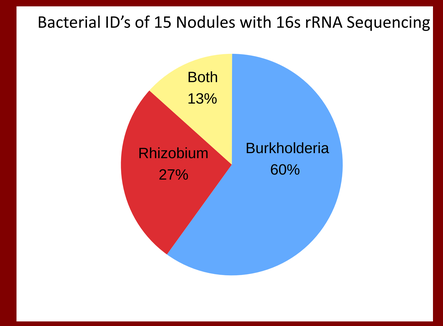


**Figure 6.** After sequencing a portion of the bacterial isolates from the Scotch broom nodules, sequences were identified using online databases BLAST and Classifier. Most of the isolates were 98-100% identical with the bacterial genus *Burkholderia*, and all others were 98-100% identical with the bacterial genus *Rhizobium*. However, there were 2 nodules where both morpho-types of bacteria were successfully isolated and sequenced. These nodules presumably housed both *Burkholderia* and *Rhizobium,* which was unexpected because the presumed nodulation mechanism is initiated by a single bacterium.

**Figure 1.** Scotch broom invaded field.

## Future Research

- Confirm that *Burkholderia* and *Rhizobium* inhabit the same nodule.
- Confirm the mechanism of nodulation. The presumed mechanism of nodulation makes it difficult for multiple bacterial species to enter a single nodule. However, through another nodulation mechanism it is possible for multiple bacteria to "sneak" into nodules.
- Determine presence of nod/nif genes to confirm nodulation capability
- Compare soil microbiology in environments with different intensities of Scotch broom invasion