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Effect of Spatial Influence on Endophyte Diversity Within Alnus Rubra

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Effect of spatial influence on endophyte diversity within *Alnus rubra*

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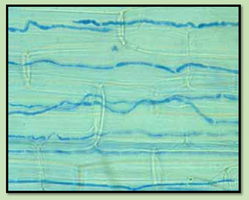
Background

- Red alder (***Alnus rubra***) of the Pacific Northwest are characterized as actinorhizal dicotyledons; these plants possess the ability to form symbiotic relationships with endophytes.



- Endophytes, commonly described as bacterial or fungal organisms that live within the tissues of plants, produce secondary chemicals which tend to enact advantageous (in most cases) or deleterious effects upon the health of the host plant

- The symbiotic relationship established by endophytes and *A. rubra* yields a variety of advantageous benefits, of which increased nitrogen-fixation is of the most understood. The increased capability of *A. rubra* stems from the endophytic bacteria Frankia and its association with the root nodules of *Alnus spp*.

- Although inoculation with *Frankia* bacteria is known to increase symbiotic nitrogen fixation capabilities of actinorhizal plants, whether they could confer the same benefit to other host species is unstudied. Additionally, whether the diversity of endophytic community assemblages vary between host plants of the same species is unknown. As well as the potential to incorporate host-specific findings into agricultural applications.

- The significance of this study is to explore whether differences in foliar (in leaves) and nodular (in root nodes) endophyte community diversity are influenced by the spatial distribution of the host species *A. rubra* of the Tillamook State forest and Portland Metropolitan area.

Objectives

- Explore the potential differences in foliar and nodular endophyte assemblages present within *A. rubra* of the Tillamook State forest and Portland Metropolitan area.

- Compare endophyte population diversity of *A. rubra* population of the NW Oregon coast range to those representing the northern portion of the Willamette Valley

- Determine whether spatial distribution of *A. rubra* influences the diversity of *Frankia* bacteria assemblages .

Hypothesis

1) Nodular Frankia bacterial endophytes sourced from different spatial locations will exhibit similar low population diversity - indicative of strain-host specificity, denoting spatial influence.

2) Foliar fungal endophytes sourced from different spatial locations will exhibit high population diversity - indicative of spatial environmental influence.

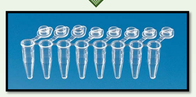
Methodology

Determine study sites
- Three study sites were chosen based upon physical location and representative *A. rubra* populations present.

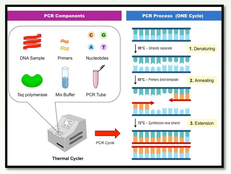
Sample collection
- Sample foliar and nodule specimens from five trees per site (Three leaves and at least one root nodule from each tree)

Foliar Processing
- Surface sterilization
- Isolation foliar endophytes
- Amplification (DNA Extraction & PCR)
- Gel electrophoresis (Amp analysis)
- Sanger sequencing

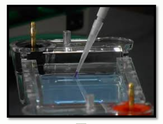
Nodular Processing
- Surface sterilization
- Amplification (DNA extraction & PCR)
- Gel electrophoresis (Amp analysis)
- Sanger sequencing

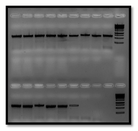


Analysis of results
- One-way ANOVA
- Two sample T-test
- ANOSIM



Conclusion
- Are there differences in (foliar/nodular) endophyte assemblages expressed between the populations of each site?





Study sites

Site A: Tillamook State Forest, near the Summit Trailhead located off Hwy 6 at milepost 33.
GPS: 45.62394,-123.37550

Site B: SE Harney Rd, Portland, located adjacent to Precision Castparts.
GPS: 45.463610, -122.613442

Site C: Portland Raceway, Habitat Regeneration Area, N Portland.
GPS: 45.596819, -122.687319

Ideas to consider

- Increase site range and sample size to allow for an increased representation of the spatial distribution of *A. rubra*.

- Incorporate spore processing methodology, which identifies the difference between non-spore-producing vs producing , as well as the exploration into determining influences associated with “less specific” groups of *Frankia* bacteria as SP- vs Sp+.

- Measuring nitrogen fixation effectiveness
- Nodule allocation

- Potential application of specific endophytes into agriculture through in depth research into host-specific interactions.

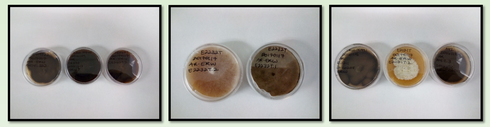
Sequencing!

Expected Results

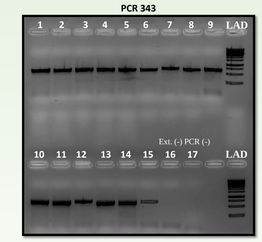
As a study in progress there has been little to no results yielded yet to elaborate upon. Although, based upon relative published studies looking into the diversification of foliar endophytes and nodular *Frankia* bacteria within *Alnus* species, here are a few assumptive results that are thought to result from this study.

- The nodular *Frankia* bacteria will exhibit low population diversity, comparing the diversity of spatially distinct populations to one another, supporting the hypothesis. This result is assumed to be the most prominent because it is supported by a few studies such as in Kennedy et al (2010) produced just that. Where they explored the diversity of *Frankia* present within various *Alnus spp* across the PNW represented by 4 sites encompassing the NW Oregon Coast range, West Cascade range, and West of the Willamette Valley. This study incorporated a sample size of 20 trees per site and at least one nodule per tree.

- Additionally, the diversity of foliar endophytes will express high diversity, especially between sites. *Frankia* bacteria will express similar or greater intra-site diversity, than the foliar endophytes.



Gel of PCR 343			
	Date: 12-Dec-16		Name: NS, SS, ERW
Lane #	DNA #		Primers
1	ERW_00109		ITS4/ITS1F
2	ERW_00115		ITS4/ITS1F
3	ERW_00132		ITS4/ITS1F
4	ERW_00148		ITS4/ITS1F
5	ERW_00231		ITS4/ITS1F
6	ERW_00247		ITS4/ITS1F
7	ERW_00278		ITS4/ITS1F
8	ERW_00324		ITS4/ITS1F
9	ERW_00325		ITS4/ITS1F
10	ERW_00339		ITS4/ITS1F
11	ERW_00369		ITS4/ITS1F
12	ERW_00443		ITS4/ITS1F
13	ERW_00453		ITS4/ITS1F
14	ERW_00479		ITS4/ITS1F
15	ERW_00515		ITS4/ITS1F
		12/12/16	
16	Ext. neg	neg	ITS4/ITS1F
17	PCR neg	PCR H2O	ITS4/ITS1F



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Acknowledgements

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