Paper # N-181

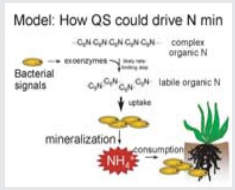
Quorum sensing as a control point in rhizosphere nitrogen transformations

Kristen M. DeAngelis[1], Mary K. Firestone[2] and Steven E. Lindow[1]
University of California, Berkeley

[1]Plant & Microbial Biology, Grad Grp Microbiology
[2]Environmental Science, Policy, & Management
University of California, Berkeley
Berkeley, CA 94720
email: kristen@nature.berkeley.edu
http://nature.berkeley.edu/~kristen

Abstract

Rhizosphere bacteria play an important role in the soil N cycle and plant N nutrition through the release of extracellular enzymes. Temperate terrestrial plants are generally N-limited because most soil N is organic: chitin, proteins, lignoproteins and nucleotides. These compounds require digestion by microbial extracellular enzymes, likely the rate-limiting step in N mineralization. Proteobacteria in particular dominate rhizosphere bacterial communities, suggesting their importance in soil community enzyme activity. There is also evidence of specific interactions between soil bacteria and plants via quorum sensing (QS): root exudates of many plant species can disrupt bacterial QS and impact QS-controlled behaviors, like extracellular enzyme production.



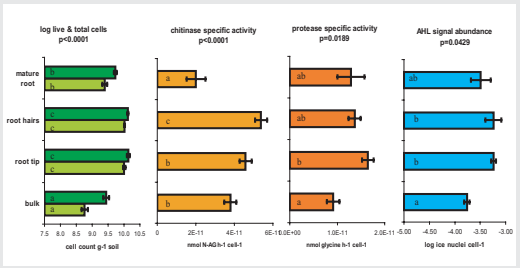
Hypotheses

(1) Bacterial QS is an important control point in rhizosphere N mineralization.
(2) Density-dependent responses by specific populations are primarily responsible for much of the conversion of organic N to inorganic, mineralized N.

Microcosm experiments:

What is the prevalence of enzyme activity and QS signal availability in the rhizosphere comparedto bulk soil?

Soil for microcosms was collected from California annual grassland, sieved to 2mm, homogenzied and planted with *Avena barbata* (wild oat). Microcosms soil was sampled from three root zones and bulk soil, then analyzed.



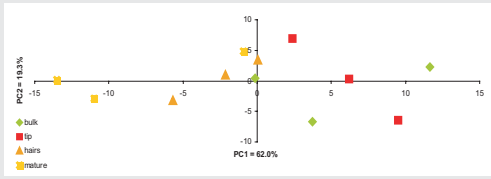
Rhizosphere microbial activity and cell counts are increased in the rhizosphere compared to bulk soil.

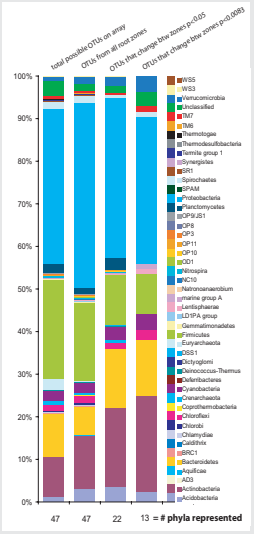
Bacterial cell numbers are direct counts for live and total cells. Soil chitinase activity was assayed with a fluorescently-labeled substrate (N-acetyl-glucosamine-MUB), and protease was assayed using the ninhydrin assay with casein. AHL signal abundance was measured using a whole-cell biosensor in *Agrobacterium tumefaciens* with an ice-nucleation protein behind a trabox promoter.

Microbial community composition changes only slightly between rhizospehre and bulk soil.

T-RFLP revealed no overall change in microbial community composition (data not shown), with 9/132 OTUs p<0.05. Microarray analysis using 16S array scored 2595 hits out of 8675 possible OTUs. Paired t-tests revealed little change in diversity between root zones: 428 OTUs changed significantly for p<0.05, or 84 for p<0.0083. The affected phyla are shown in the bar graph at right.

Principle components analysis of these 84 (below) reveals a pattern to the change in this subset of 84 OTUs .

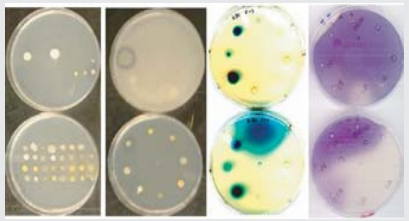




Rhizosphere isolates experiments

What is the prevalence and diversity of QS and exoenzyme producers in the rhizosphere?

Large plugs of annual grassland plants and soil were collected to 10 cm depth into pots and moved to the greenhouse for sampling. Serial dilutions of soil were plated to nonspecific defined and undefined media. Bacteria were isolated by repeated streaking of single colonies, then tested.

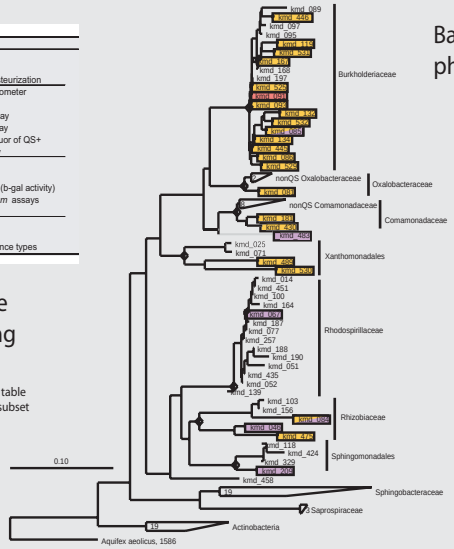


pasteurization 10min 80C | exoenzyme chitin overlays | *A. tumefaciens* pAHL-bgal assay | *C. violaceum* QS signal assay

screen	count	%	
round 1 - pasteurization to select for non-sporulators			
	533	-	... subjected to screen
	347	65.1	... that succumbed to the pasteurization
round 2 - exo-enzyme activities by plate &/or spectrofluorometer			
	347	-	
	87	25.1	... exo-protease by plate assay
	14	4.0	... exo-chitinase by plate assay
	63	18.2	... exo-chitinase by spectrofluor of QS+
	136	39.2	... either exo-enzyme activity
round 3 - QS signal of exoenzyme producers			
	128	-	
	22	17.2	... AHL signal by QS overlay (b-gal activity)
	9	7.0	... AHL signal by *C. violaceum* assays
	28	21.9	... any AHL signal
round 4 - genotype signal & exoenzyme producers			
	28	-	
	14	50.0	... different 16S rRNA sequence types

QS-signal producers are well-represented among bacterial isolates

A summary of all isolates is shown in the table above. Each round of screens includes a subset of the previous round of screens.



Bacterial isolates are diverse phylogenetically and phenotypically

The phylogenetic tree of all exoenzyme producers (left) shows that the signal-producers (shaded colored boxes) are all alpha-, beta- and gamma-proteobacteria.

The table (below) is closer look at the phenotypic variation in all isolates that were identified by 16S rDNA sequence as uncultured *Burkholderia* sp. AKIW931. The isolates fall into roughly five phenotypic groups. Isolates with a single nucleotide change in 16S sequence fall into different phenotypic groups, though the majority were the same.

Characteristics of all Uncultured Burkholderia AKIW931

group		isolation media	exo-protease	exo-chitinase	AHL signal by tra(ocoC8)	AHL signal by C.viol short	AHL signal by C.viol long
1	86	VL55 xylan	+	+	++	-	-
	93	VL55 xylan	+	+	+	-	-
	119	VL55 casein	+	+	++	-	-
	132	VL55 casein	+	+	++	-	-
	134	VL55 casein	+	+	++	-	-
2	445	VL55 xylan	-	+	+	-	-
	446	VL55 xylan	-	+	+	-	-
	525	VL55 casein	-	+	++	-	-
	529	VL55 xylan	-	+	+	-	-
	531	VL55 xylan	-	+	+	-	-
	532	VL55 xylan	-	+	+	-	-
3	91	VL55 xylan	+	++	-	-	+
4	167	SEA	+	-	-	+	-
5	85	VL55 xylan	+	-	++	+	-

Conclusions & Discussion

Microbial exoenzyme activity and QS signal are increased in the rhizosphere compared to bulk soil.

This activity is increased per gram soil and per cell, indicating that it is this increased enzyme activity that is responsible for converting high molecular weight organic N to more labile N, the rate limiting step in N mineralization.

Microbial community composition does not change dramatically with the root compared to bulk soil.

Repeated tests of community composition reveal little overall change in community composition. The most sensitive method, 16S rRNA microbial community microarry, indicated that there is change in a few OTUs. This suggests that a small subset of the community is responsible for large changes in activity.

Exoenzyme activity and quorum sensing is common among cultured rhizosphere isolates.

Many species of bacteria have been shown to have their extracellular enzymes under the control of quorum sensing, but this has been largely studied in the context of pathogenesis. This study suggests that quorum sensing may be important in ecosystem processes such as rhizosphere nitrogen mineralization.

Rhizosphere isolates are more diverse phenotypically than they are phylogenetically.

Proteobacteria make up the bulk of culturable gram-negative exoenzyme producers , followed by actinobacteria and bacteriodetes. There is a high coincidence of QS-signal production that accompanies the exo-enzyme producing phenotype.

Thanks to the Environmental Protection Agency Science to Achieve Results (STAR) Graduate Research Fellowship as well as the National Science Foundation Doctoral Dissertation Improvement Grant Program (NSF DDIG).