Divining Rods: *Pseudomonas putida* as a Microbiosensor of Fine-scale Osmotic Potentials in Soil

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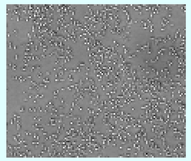
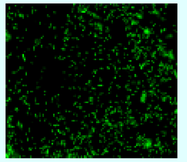




ABSTRACT

The physical distribution and availability of water in soil influences plant growth, the mineralization of organic matter, the diffusion of dissolved nutrients and microbial dynamics. Current tools commonly used to measure water availability in soil, such as psychrometers, tensiometers and time domain reflectometry, integrate water availability on a gross scale but do not provide information at microscopic scales where microbes are operating. We have inserted an osmotically controlled *proU-gfp* transcriptional fusion developed by Axtell and Beattie (2002) into the soil bacterium *Pseudomonas putida* KT2442. The resulting soil microbial biosensor produces green fluorescent protein (GFP) as a function of osmotic potential around the bacterium. Cells can be recovered from the soil with very small sample sizes and analyzed using fluorescent flow cytometry for intensity of green fluorescence. The intensity of green fluorescence in these cells provides fine scale information on an important determinant of water potential in the soil microbial environment. We have successfully used *Pseudomonas putida* KT2442 (pPProGreen) to report on moisture level in sand microcosms and *Pantoea agglomerans* BRT98 (pPProGreen) in both sand and soil microcosms. These microbiosensors promise to provide a novel portrait of rhizosphere water potential dynamics associated with root water uptake.

BACKGROUND:

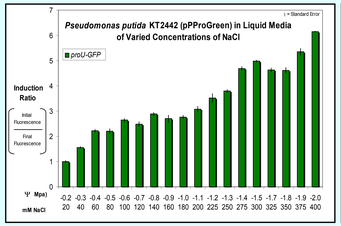
The proU operon from *E. Coli* encodes a transport system for the osmoprotectant glycine-betaine. The activity of the proU operon correlates with osmotic potential as it attempts to adjust internal cellular osmotic potential to that of the environment. By 'tagging' the activity of this promoter with GFP, we get a report on the osmotic environment the bacterium experiences. We inserted the P_{proU}-*gfp* transcriptional fusion into *Pseudomonas putida* using triparental mating, and tested its ability to report on osmotic and water potential in liquid culture and applications in sand and soil.

BIG QUESTION:

Can the P_{proU} -*gfp* biosensor report on osmotic and water potential in soil?

TEST 1: Does *P. putida* KT2442 (pPProGreen) respond proportionally to the strength of osmotic and water potentials in liquid?

TEST 1A: Does Reporter Activity (GFP) Scale with Osmotic Potential?



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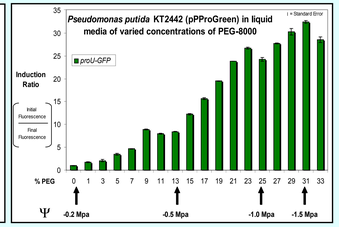
TEST 1B: Does Reporter Activity (GFP) Scale with Water Potential?



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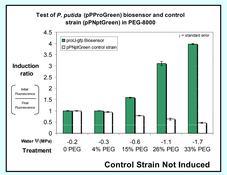
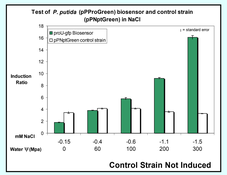
The initial test of the biosensor was to determine if there is a graded response to an increase in osmotic or water potential (Ψ). In other words, does the promoter-gfp activity ramp up with an increase in osmotic potential or Ψ. The results clearly show a graded response to both increases in osmotic potential and Ψ.

METHODS:

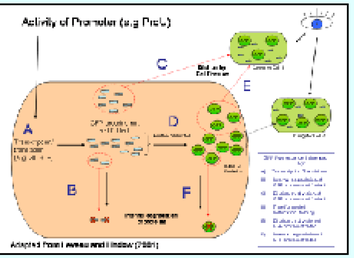
Cultures of *P. putida* KT2442 (pPProGreen) were grown for 36 hours in ½ 21C Media (-0.15 MPa) at 30 °C. Bacteria were resuspended and inoculated in a variety of concentrations of NaCl (Test 1a) and PEG-8000 (Test 1b). Fluorescence was measured after 24 hours of and corrected with O.D. The experiment was repeated with a treatment time of 6 hours, the inclusion of a control strain *P. putida* (pPNptGreen), and analysis on a flow cytometer (FACScalibur, Becton-Dickinson); these results are shown in the small graphs to the right.



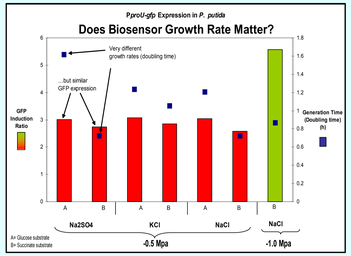


TEST 2: Is Promoter Activity Accurately Portrayed by GFP Intensity?

In biosensors, the correlation between the promoter (e.g. P_{proU}) activity and the expression of the reporter (GFP, *inaZ*, e.g.) is influenced by other cellular processes. Leveau and Lindow (2001) used a single-cell model to describe how the measured fluorescent GFP content is dependent not only upon promoter activity but also upon rates of transcription, protein folding time, degradation of GFP within cells and finally, dilution of the GFP by division of the bacteria. Tests on the influence of these factors on the 'reporter signal' is an integral part of biosensor development.



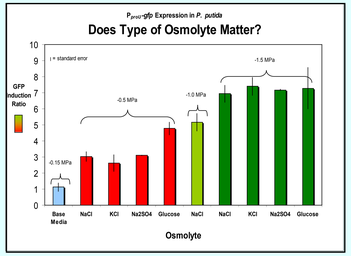
TEST 2A: Is the GFP Report Consistent at Different Growth Rates?



…Yes

We were concerned that different doubling times of the bacteria would dilute the GFP signal at different rates, leading to divergent reports on the same osmotic potential. Bacteria doubling very slowly would yield a deceptively high GFP report without greater promoter activity. The results above suggest the influence of growth rate on GFP report is small compared to the consistent response to differences in osmotic potential.

TEST 2B: Is the GFP Report Consistent to the Variety of Osmolytes that might be Encountered in soil?



…Yes

The soil environment includes a variety of salts (osmolytes). We needed to determine whether the biosensor responded similarly to the osmotic potential induced by different compounds. Results demonstrate that the influence of osmolyte is small compared to the differences in GFP report generated by osmotic differences.

METHODS:

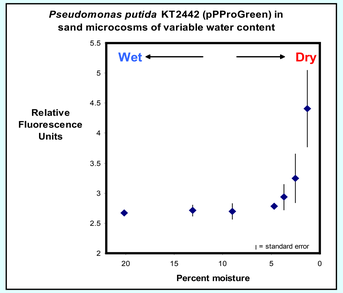
P. putida KT2442 (pPProGreen) was inoculated into 250ml Erlenmeyer flasks containing ½ 21C Media amended with Na_2SO_4, KCl or NaCl to produce the desired osmotic potential. Growth rate was manipulated by providing either of two different carbon substrates, (0.15%), (A) glucose, or (B) succinate. Bacterial cultures were kept in exponential growth throughout the experiment and sampled hourly for a period of 8 hours in the growth rate experiment and 20 hours in the osmolyte experiment. Samples from both Test 2A and 2B were analyzed by flow cytometry.

Applications of the Biosensor In Sand and Soil

TEST 3: Can *P. putida* KT2442 (pPProGreen) report on water availability in sand?

TEST 3:

We tested the biosensor in sand microcosms of varied moisture contents to determine how well the biosensor would respond to the overall availability of water. The highest P_{proU}-GFP activity was reported in the driest treatment (1.3% moisture content) and diminished until reaching 5% moisture, above which, the biosensor did not differentiate moisture content.



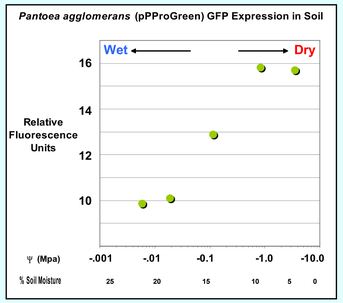
METHODS:

Glass distillation tubes were filled with fine washed sand and brought to known water content (by weight). *P. putida* KT2442 (pPProGreen) in stationary phase was resuspended in ½ 21C media amended with NaCl (-0.4 MPa) and inoculated in four 20 - µl drops below the sand surface. Tubes were sealed and sampled 10 hours later for GFP fluorescence using flow cytometry.

TEST 4: Can *Pantoea agglomerans* (pPProGreen) report on water potential in soil?

TEST 4:

P. agglomerans (pPProGreen) was applied to soil microcosms with known water potentials and allowed to respond for 10 hours. *P. agglomerans* is a bacterium normally associated with plant leaves, but seems to survive in the soil, and offers a very strong P_{proU} - GFP signal. Results demonstrate that as water potential decreases from -0.1 MPa toward -1.0 MPa, a strong upshift in GFP expression is observed. The encouraging results from this initial experiment will be followed with further tests to find the location of response saturation at water potentials below -1.0 MPa.



METHODS:

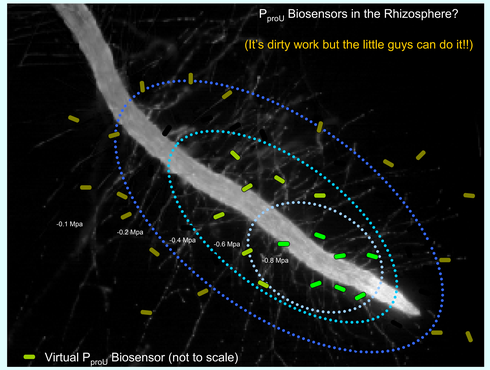
Bacteria were taken from culture in stationary phase, and resuspended in ddH$_2$0. Application of bacteria and analysis was performed exactly as in Test 3, with *P. Putida*.

CONCLUSIONS:

- The *Pseudomonas putida* (pPProGreen) biosensor responds proportionally to osmotic potential, with higher GFP expression at higher potentials. This expression is consistent across osmolyte type and differences in growth rates.
- Initial testing of *P. putida* (pPProGreen) in sand microcosms demonstrates that the biosensor is able to function well outside of liquid culture and provide a measure of water availability in solid media.
- Successful application of *Pantoea agglomerans* (pPProGreen) in both sand and soil microcosms suggests this leaf bacterium may also function as a biosensor in the soil environment.
- Multiple experiments suggest that both *P. putida* and *P. agglomerans* are able to respond not only to osmotic potential but also water potential.

Future Work: Water in the Rhizosphere

We plan to use these biosensors in rhizosphere soil to investigate fine scale dynamics of water availability. This work will inform models that are being developed to explore how hydraulic activities of roots influence microbial processes and nutrient availability in the rhizosphere.



Work Cited:

Axtell, C.A., and G. Beattie. 2002. Construction and characterization of a *proU*-GFP transcriptional fusion that measures water availability in a microbial environment. Appl. Environ. Microbiol. 68: 4604-4612

Leveau JH and SE Lindow 2001. Predictive and interpretive simulation of green fluorescent protein expression in reporter bacteria. J Bacteriol 183 (23): 6752-6762

Acknowledgements:

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