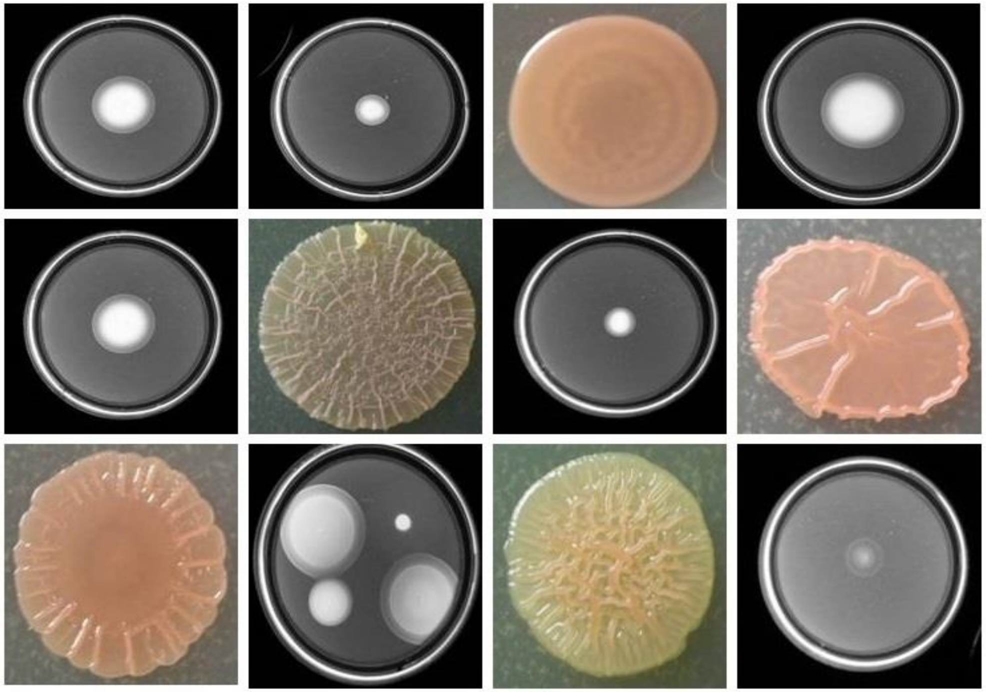


Monitoring plant-microbe associations in the rhizosphere in real time

Gladys Alexandre, Ph.D.

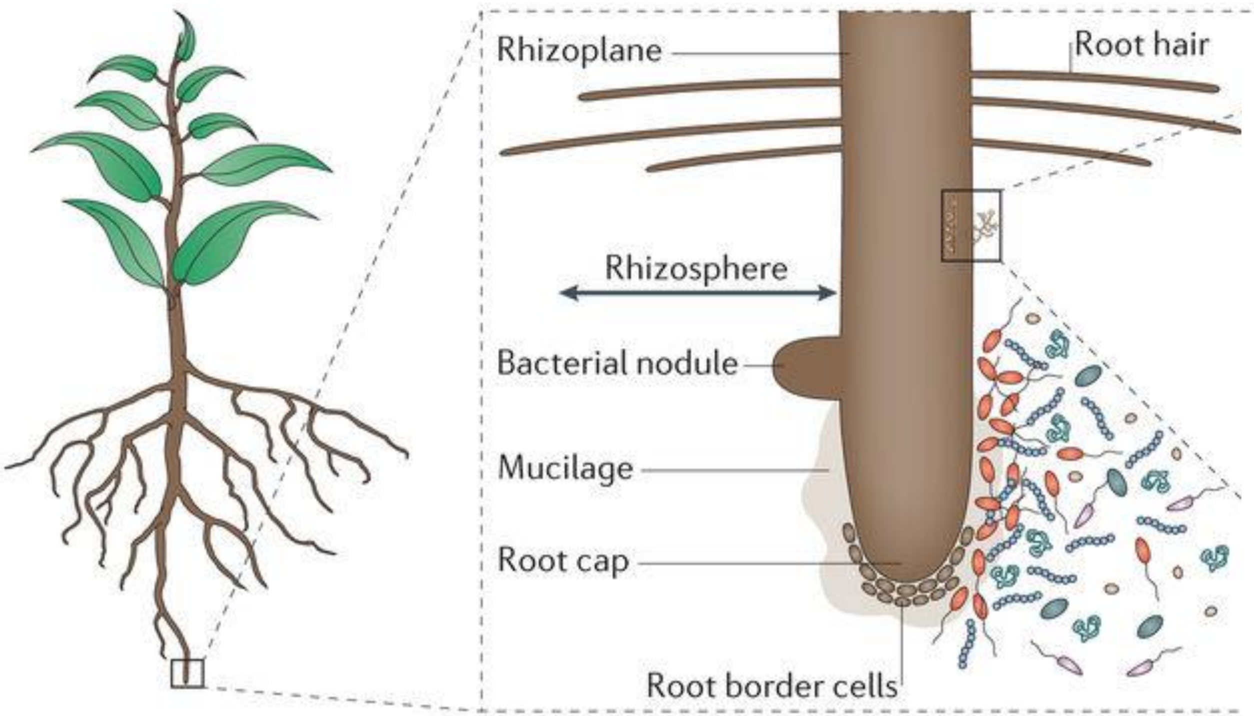
Department of Biochemistry, Molecular & Cellular Biology

University of Tennessee, Knoxville

National Science Foundation

Sustainable agriculture: Feeding 9 billions by 2050

Rhizosphere microbiomes: plant-microbe interactions

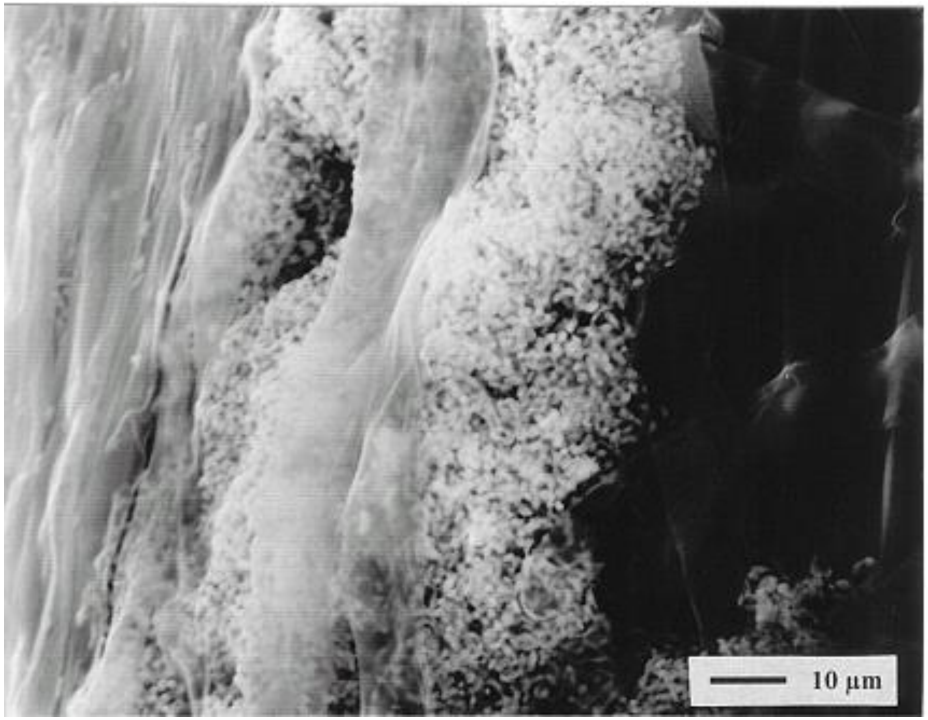


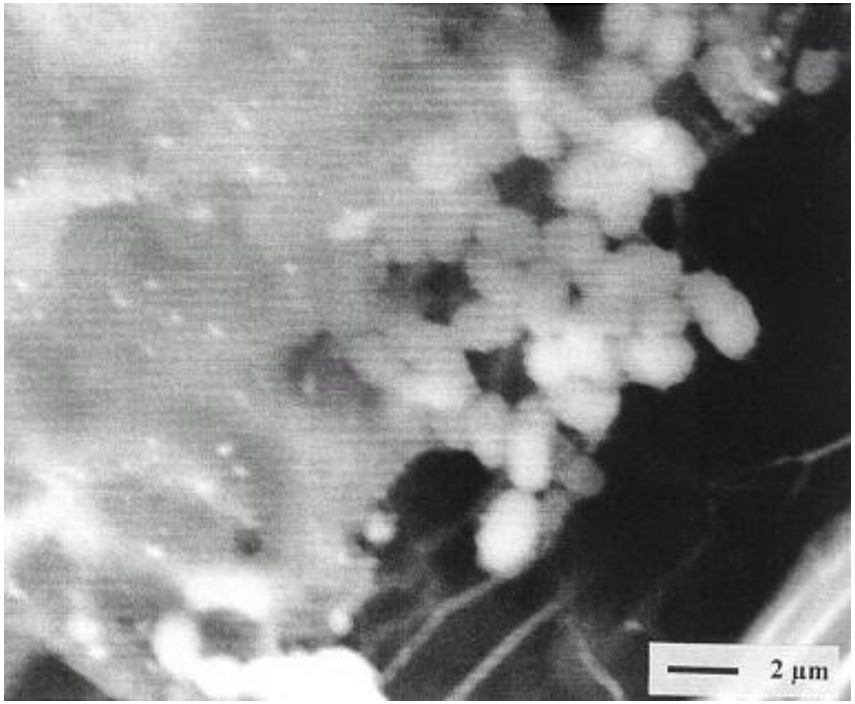
- Pathogens, beneficial, commensal
- Role in rhizosphere productivity
- Manipulation of rhizosphere plant-microbe associations for increased crop yields?

Nature Reviews | Microbiology

How do motile soil bacteria find the roots of plants?

Plant-microbe associations: how do soil bacteria detect the host?





- Sensing chemicals released by the plant
- Active motility (chemotaxis)
- Attachment

Chemotaxis genes in metagenomes from different environments

CheA= chemotaxis

Oceans

2% with 2 CheA/genome

Human Gut

10% with 2 CheA/genome

Soil

Over 99% soil genomes have > 2 CheA/genome

Buchan et al. (2010) Environ. Microbiol. 12: 3171-3184

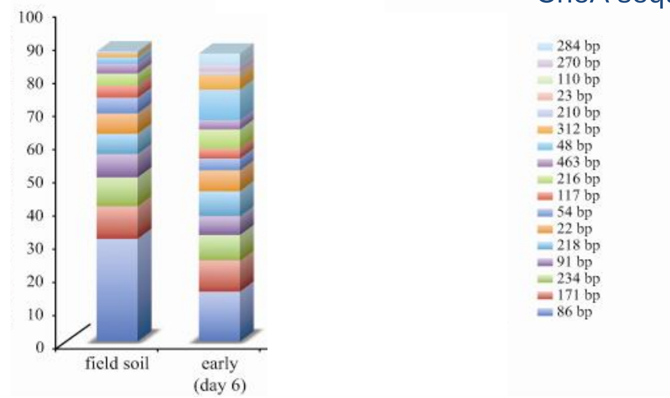
Plant-microbe interactions: the rhizosphere "hot spot"

Rhizosphere

CheA= chemotaxis

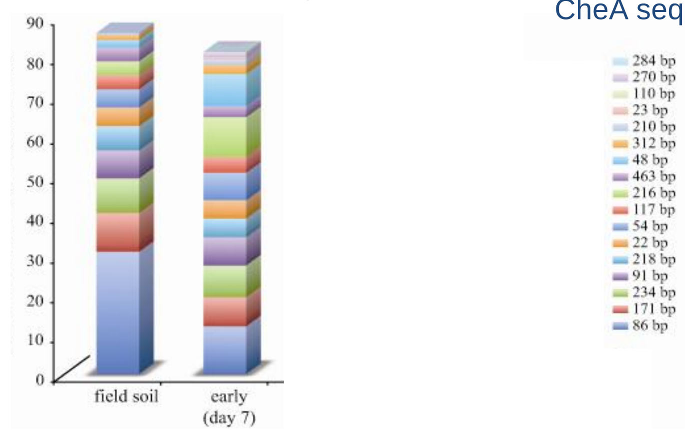
Wheat

CheA sequences



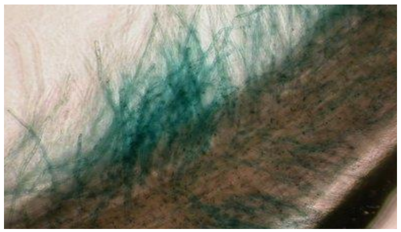
Cowpea

CheA sequences

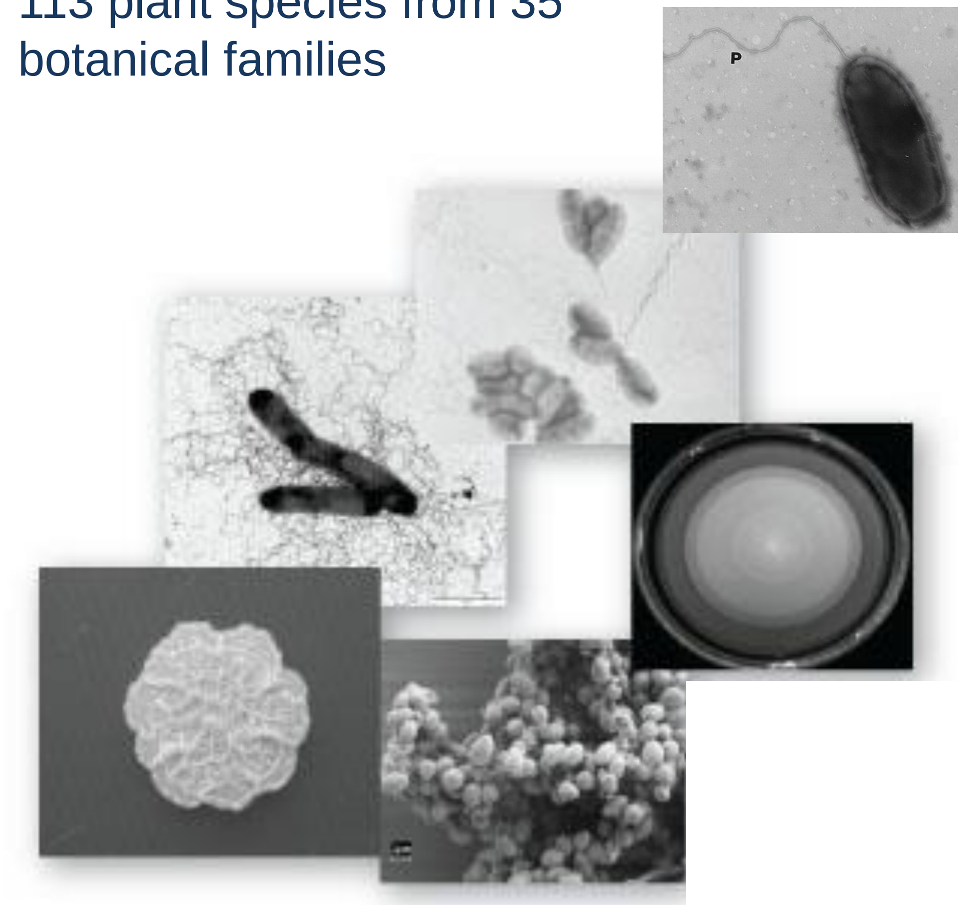


Buchan et al. (2010) Environ. Microbiol. 12: 3171-3184

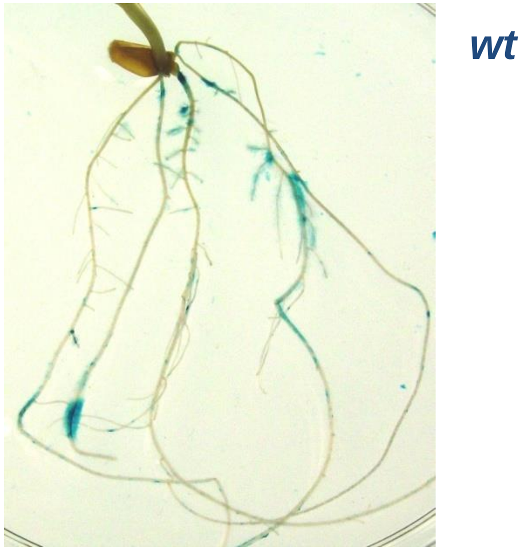
Azospirillum brasilense, a commensal plant-associated bacterium

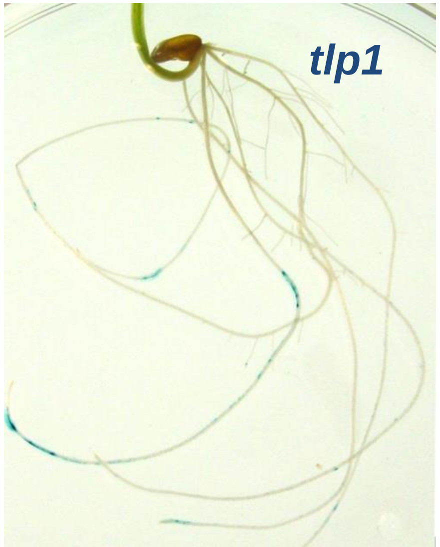


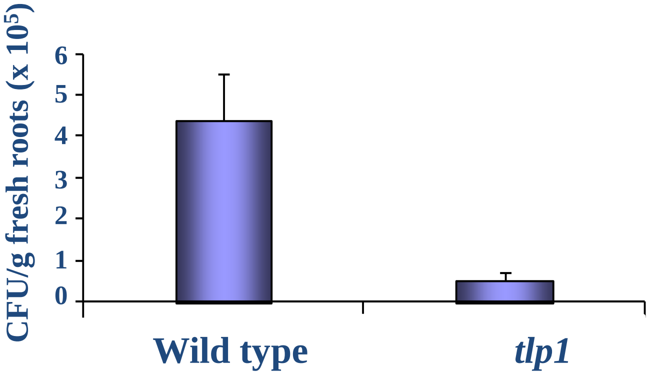
Promotes the growth of 113 plant species from 35 botanical families



Chemotaxis promotes root colonization in *Azospirillum brasilense*







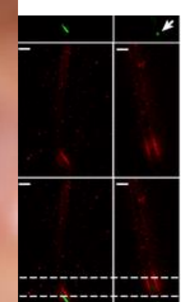
Greer-Phillips SE, Stephens BB, and Alexandre G. (2004) *J Bacteriol.* 186:6595-6604

Tracking plant-microbe association

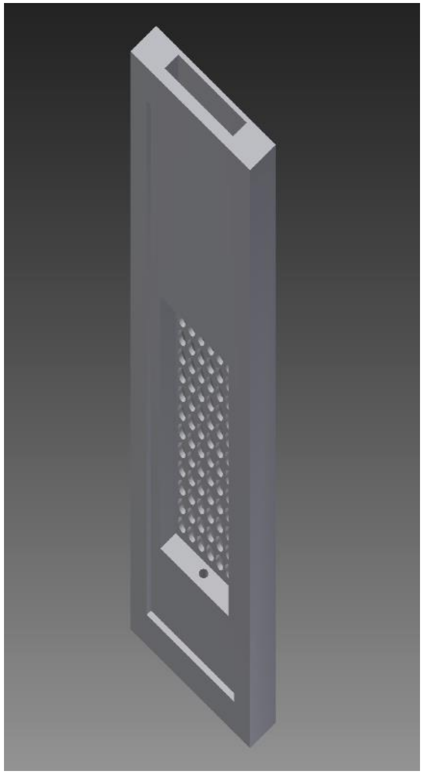
Limitations: Temporal and spatial resolution

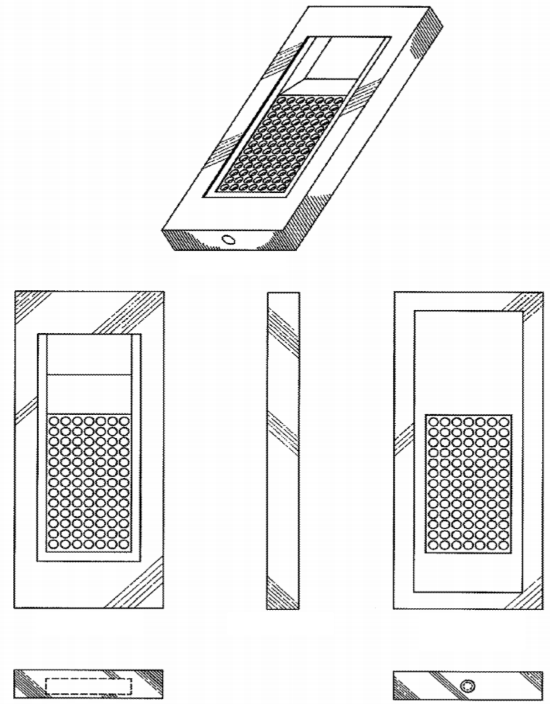
TEM, Confocal microscopy
Mutant libraries

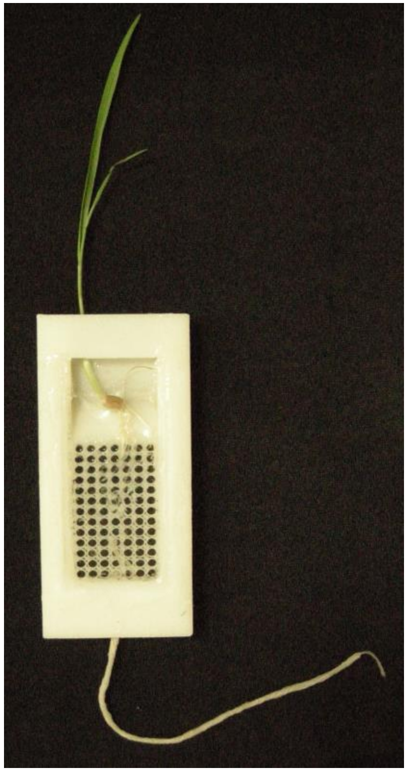
Limitations: *Arabidopsis* seeds and seedlings are extremely small



Tracking plant-microbe associations in real time with any plant: the slide-in microscope chamber



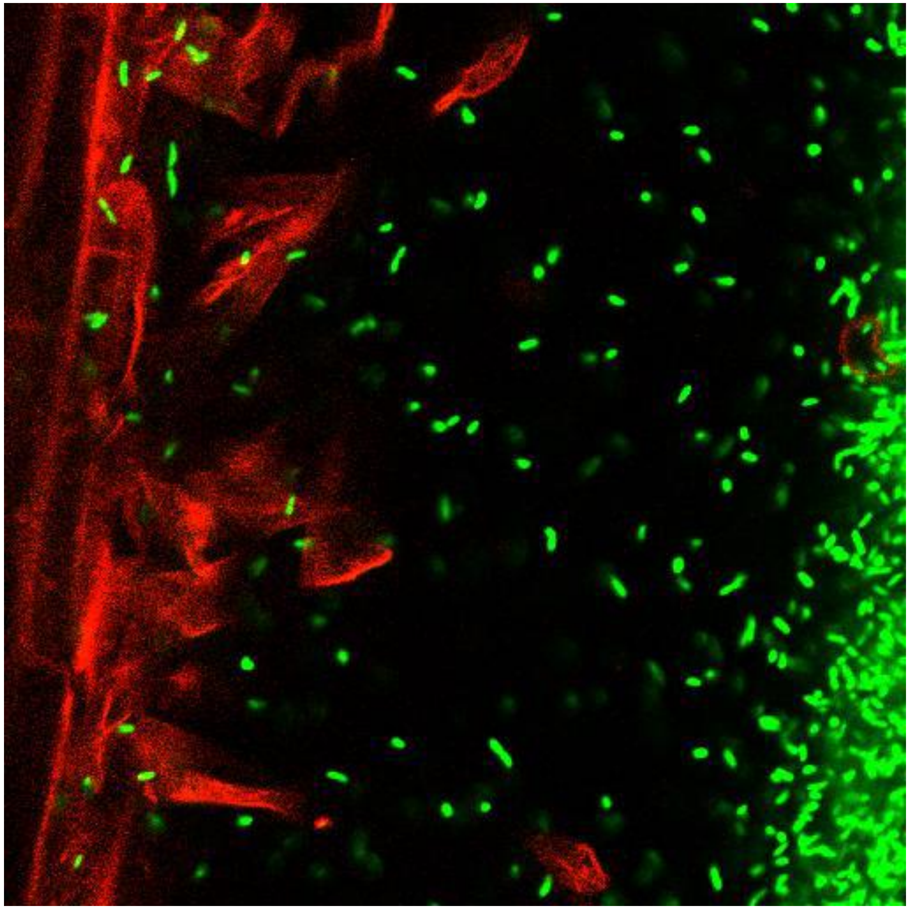




Enhanced spatial and temporal resolution

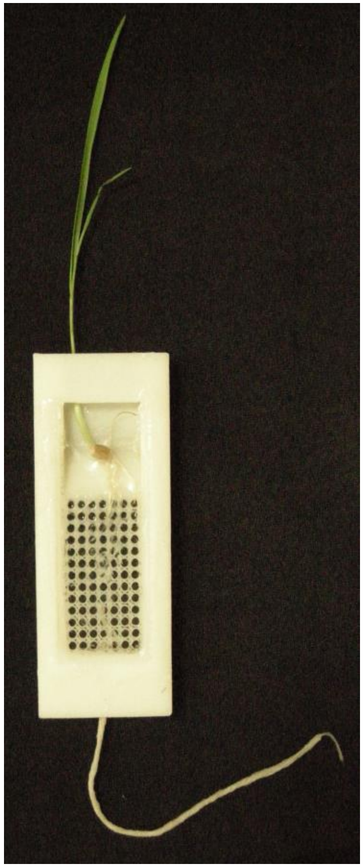
Patent US D754,871 S

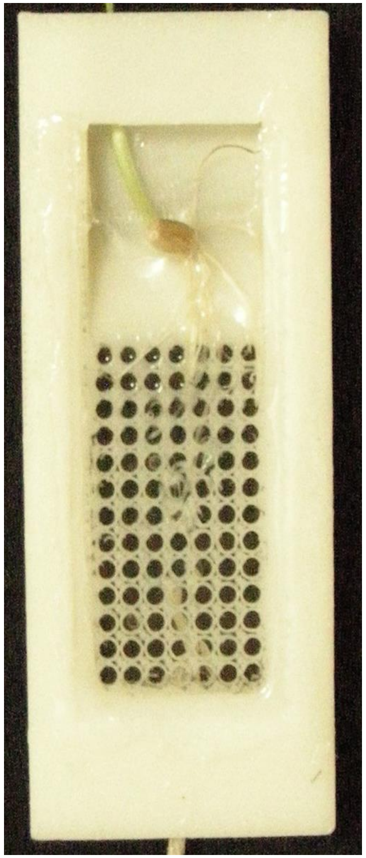
Free swimming behavior of motile bacteria in the rhizosphere

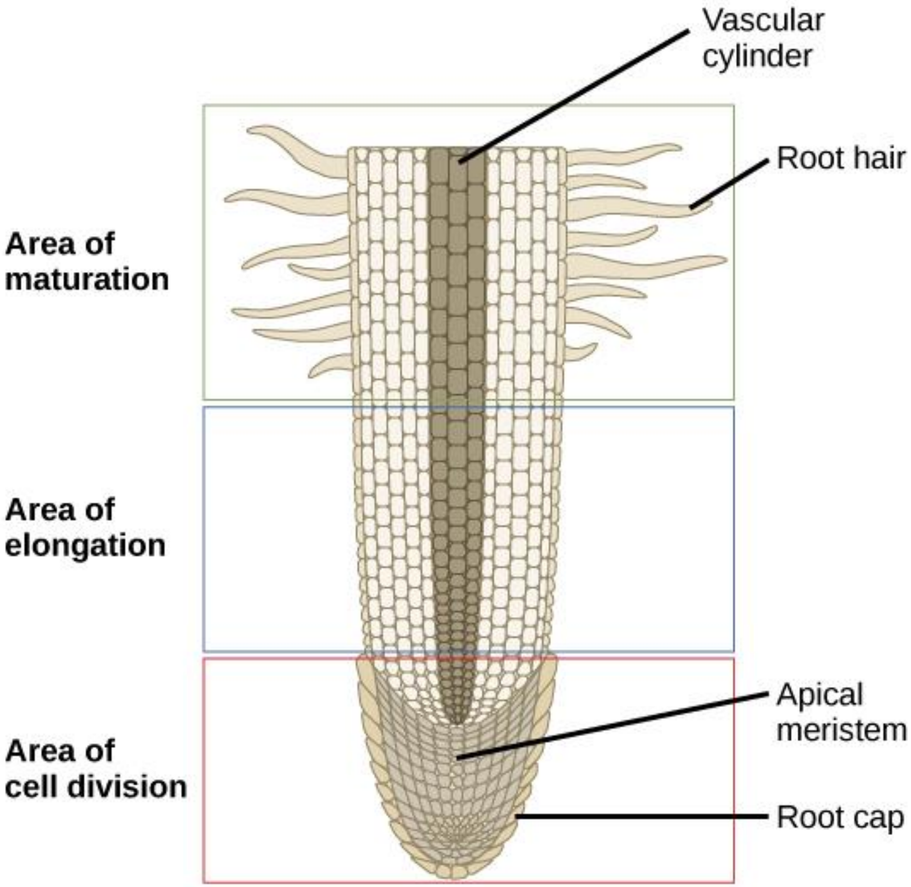


A.brasilense- green
Roots- red

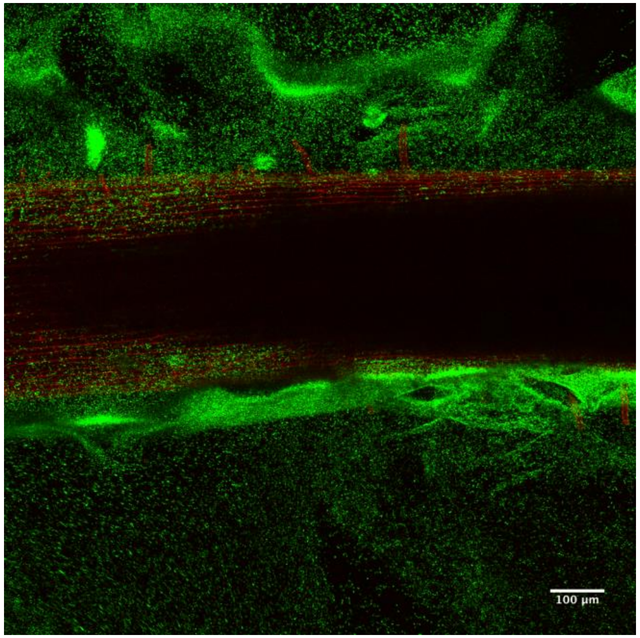
Real time chemotaxis in the rhizosphere: the slide-in-chamber

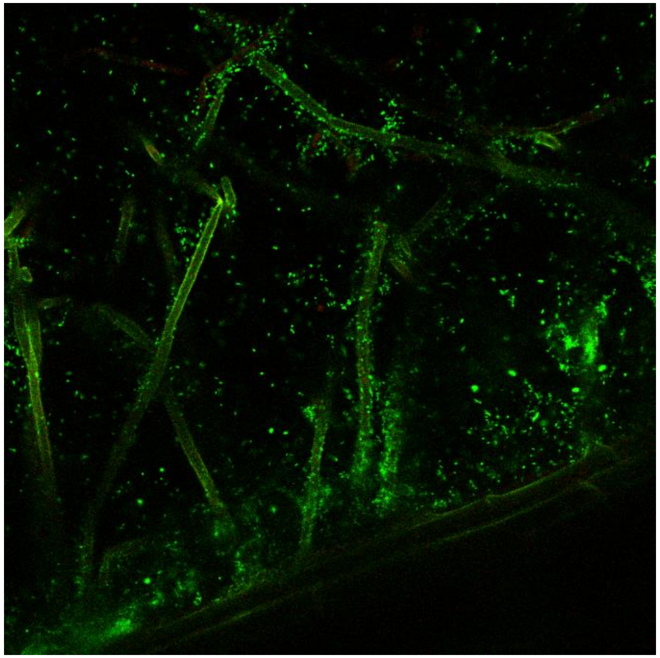






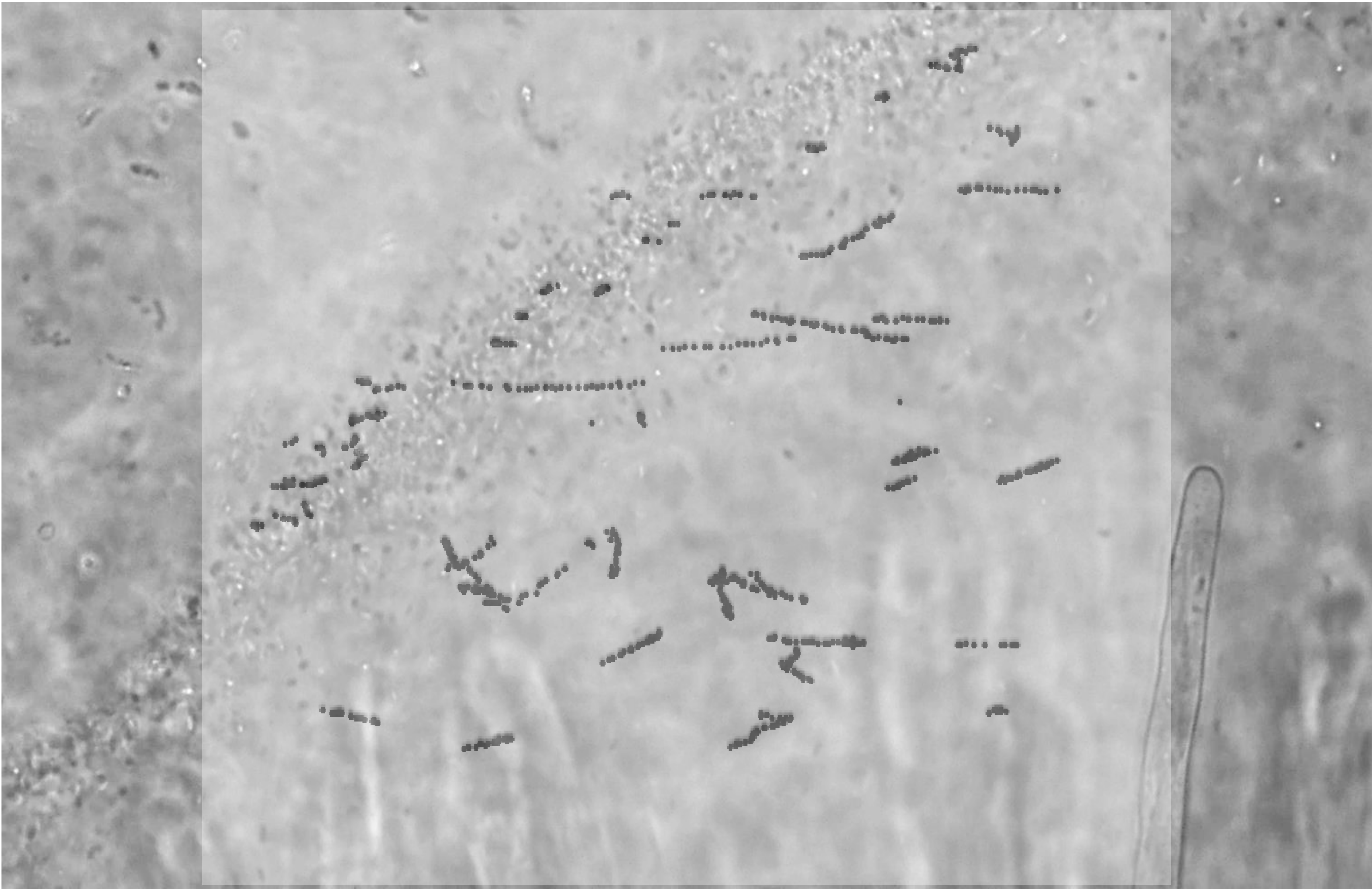
Real time plant-bacteria interactions





O'Neal, et al. in prep.

Motility patterns around specific root zones: root hairs



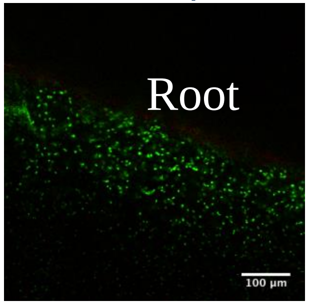
Bacterial accumulation in different root zones precedes colonization in wheat

WT

Wheat

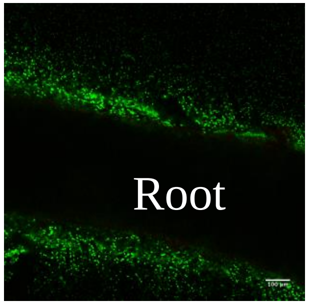
24 hpi

Root hair

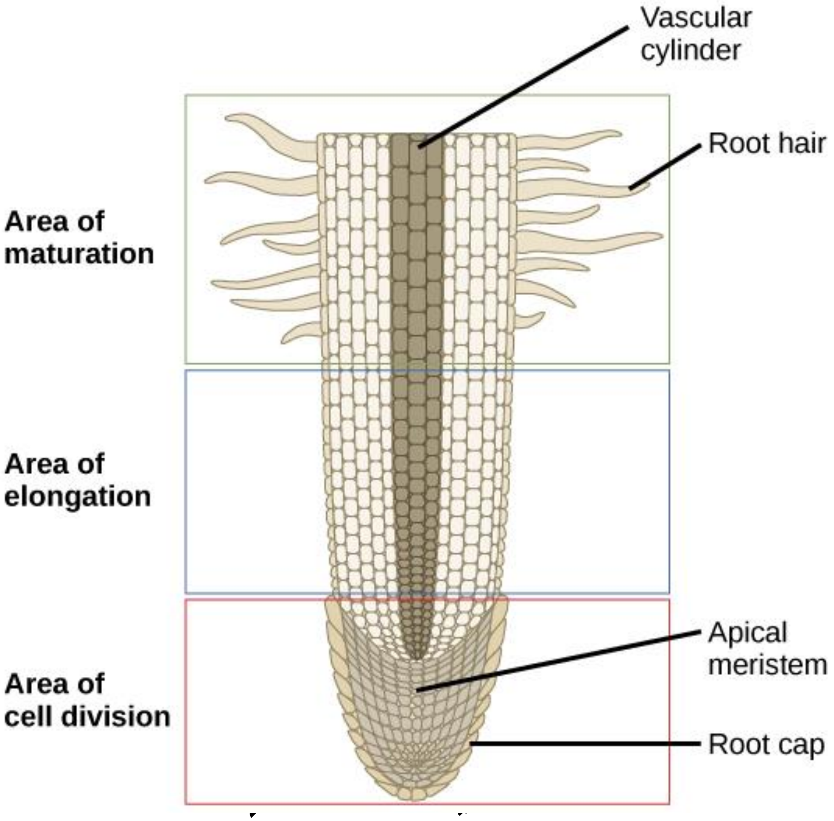


150 ± 50 µm

Elongation



110 ± 47 µm



GFP-labeled A. brasilense (WT)

Wheat roots

Accumulation of cells depends on chemotaxis

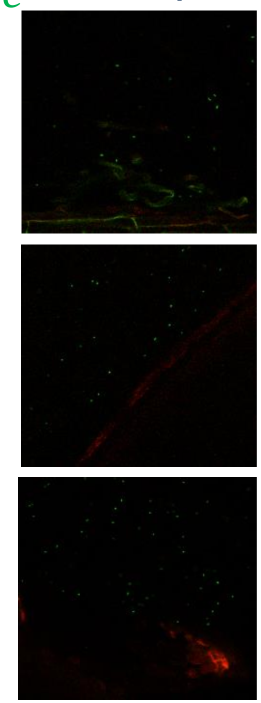
nonchemotactic

24 hpi

Root hair

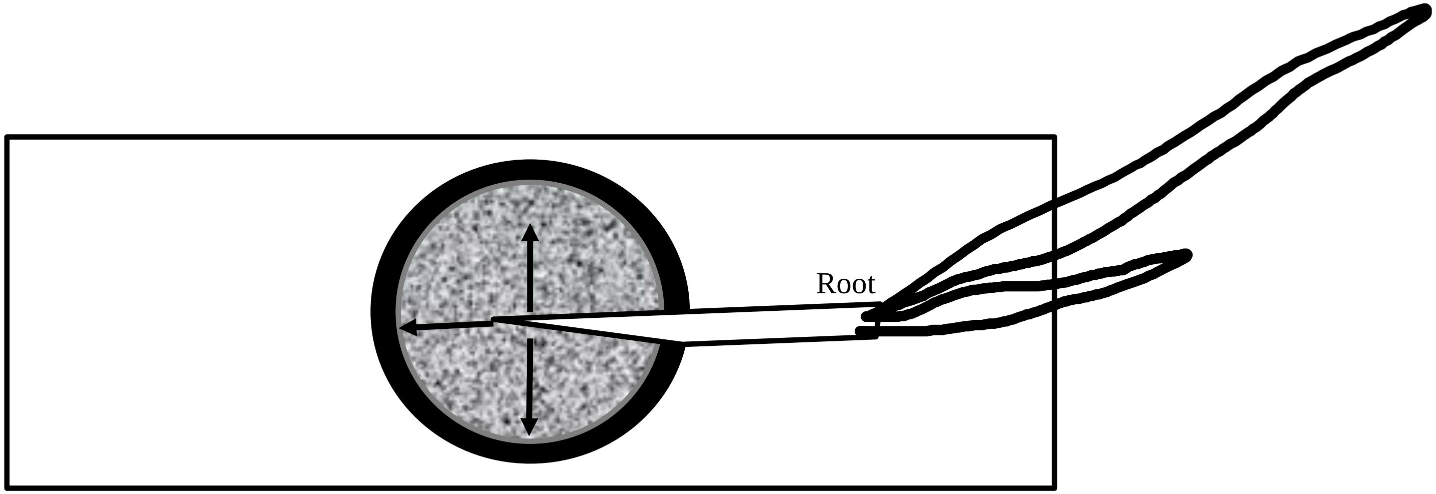
Elongation

Root tip



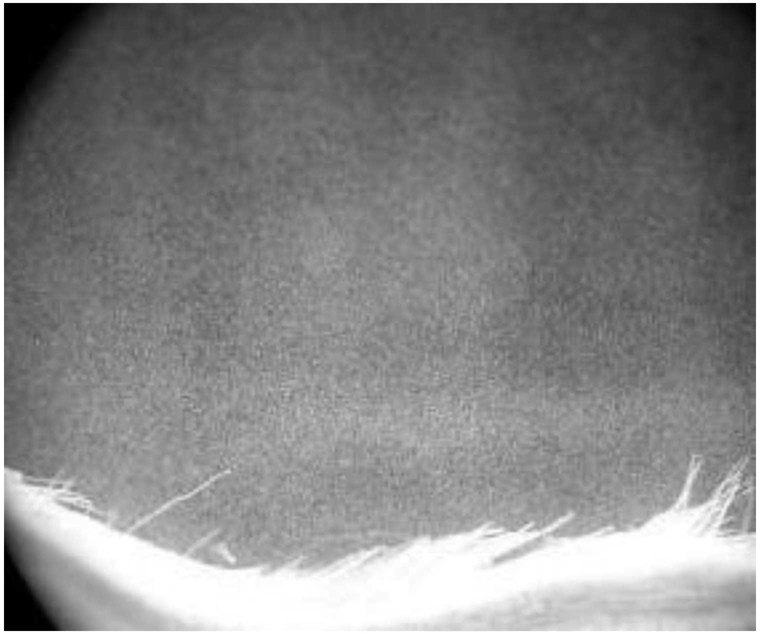
If this behavior depends on chemotaxis, then a similar pattern should be observed in a short term assay

Root-in-pool assay measures short term (> 5 minutes) motile bacterial accumulation around the roots of plants



Root in pool assay to measure real time chemotaxis to roots

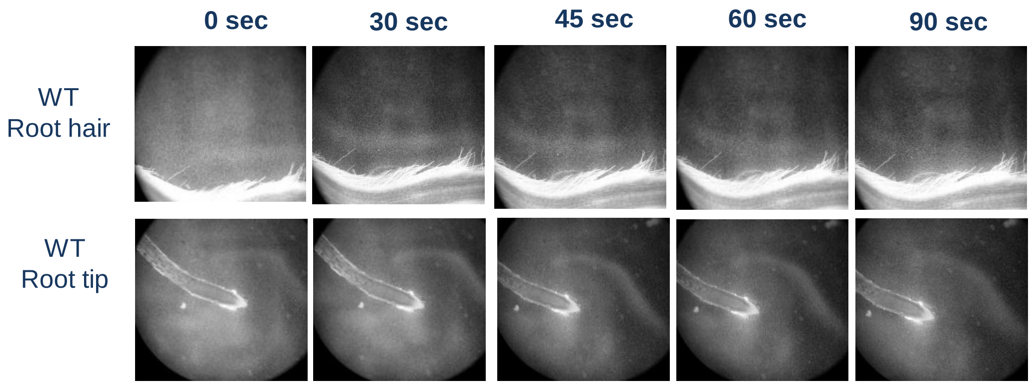
WT accumulates as a band in the root hair zone (0 sec) and then moves closer to root surface (75 sec)



WT forms a band away from the root tip (22 sec)

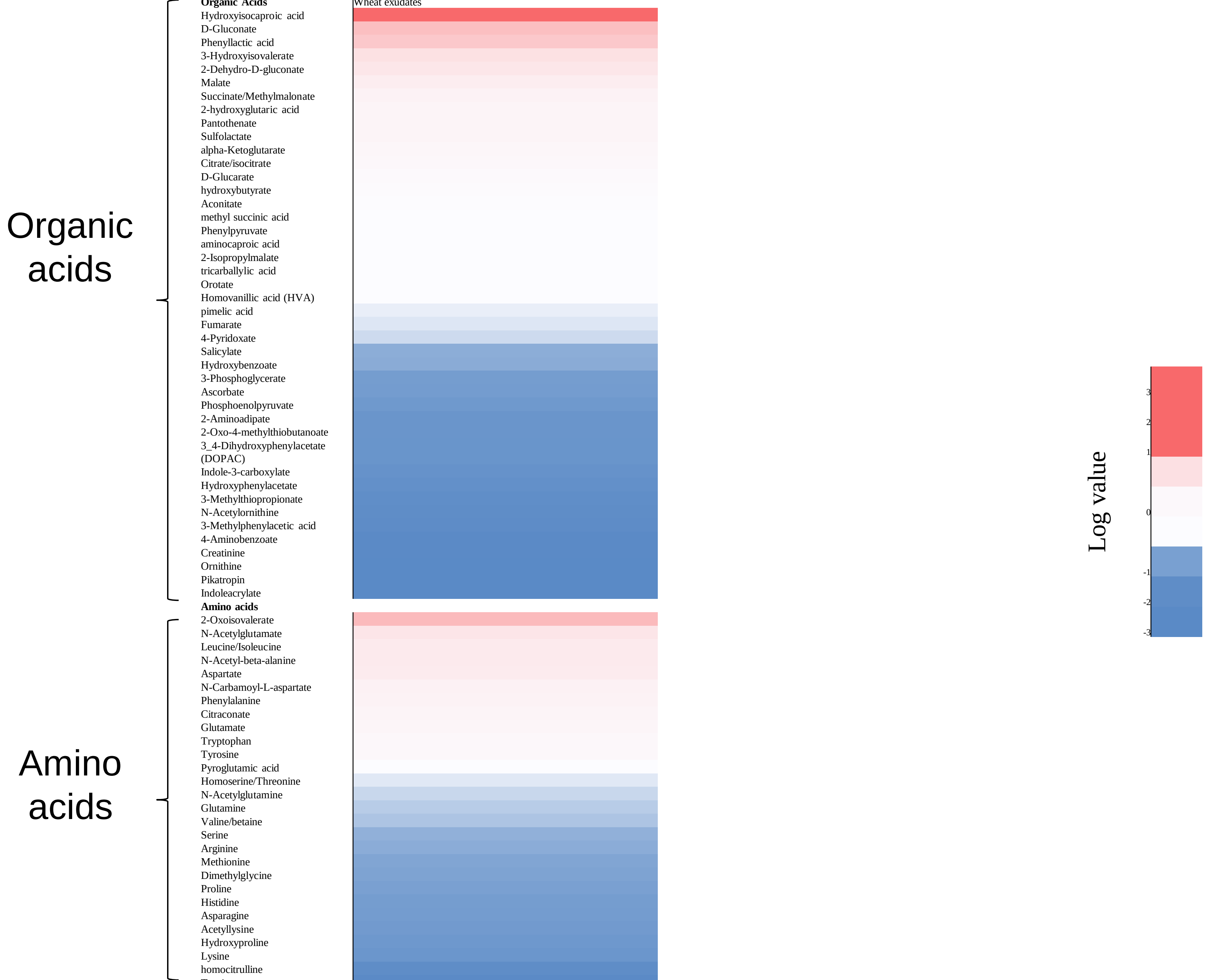
O'Neal et al, in prep.

Root surface colonization pattern results from short term chemotaxis responses



Short term chemotaxis correlates with colonization patterns– Cells are responding to exudates!

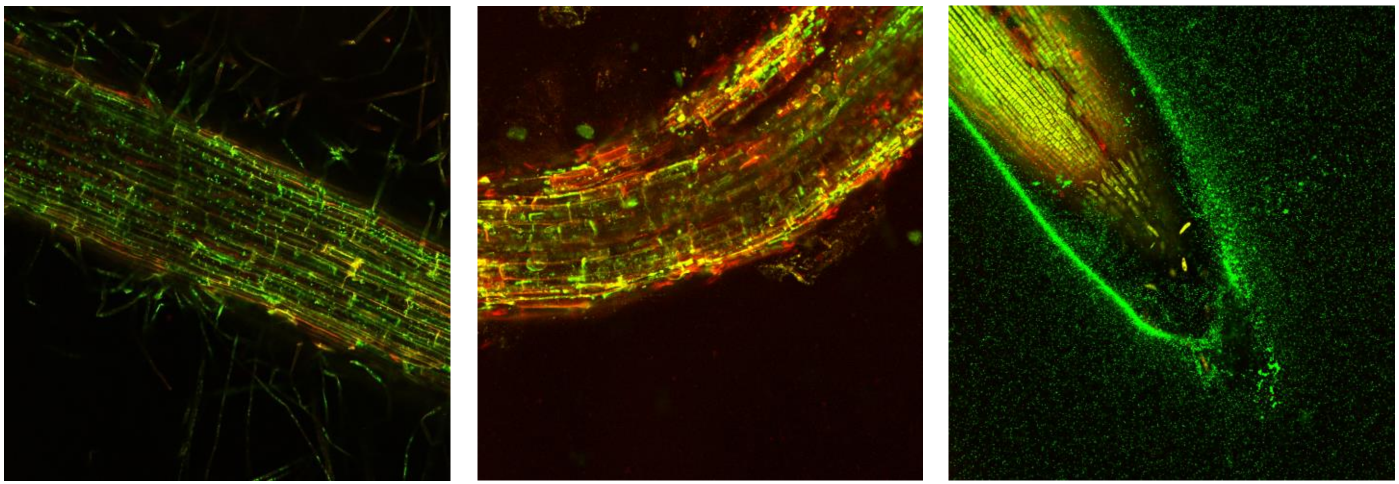
O'Neal et al. in prep


Organic acids
Amino acids
Organic Acids
Wheat exudates
Hydroxyisocaproic acid
D-Gluconate
Phenyllactic acid
3-Hydroxyisovalerate
2-Dehydro-D-gluconate
Malate
Succinate/Methylmalonate
2-hydroxyglutaric acid
Pantothenate
Sulfolactate
alpha-Ketoglutarate
Citrate/isocitrate
D-Glucarate
hydroxybutyrate
Aconitate
methyl succinic acid
Phenylpyruvate
aminocaproic acid
2-Isopropylmalate
tricarballylic acid
Orotate
Homovanillic acid (HVA)
pimelic acid
Fumarate
4-Pyridoxate
Salicylate
Hydroxybenzoate
3-Phosphoglycerate
Ascorbate
Phosphoenolpyruvate
2-Aminoadipate
2-Oxo-4-methylthiobutanoate
3_4-Dihydroxyphenylacetate (DOPAC)
Indole-3-carboxylate
Hydroxyphenylacetate
3-Methylthiopropionate
N-Acetylornithine
3-Methylphenylacetic acid
4-Aminobenzoate
Creatinine
Ornithine
Pikatropin
Indoleacrylate
Amino acids
2-Oxoisovalerate
N-Acetylglutamate
Leucine/Isoleucine
N-Acetyl-beta-alanine
Aspartate
N-Carbamoyl-L-aspartate
Phenylalanine
Citraconate
Glutamate
Tryptophan
Tyrosine
Pyroglutamic acid
Homoserine/Threonine
N-Acetylglutamine
Glutamine
Valine/betaine
Serine
Arginine
Methionine
Dimethylglycine
Proline
Histidine
Asparagine
Acetyllysine
Hydroxyproline
Lysine
homocitrulline
Log value
3
2
1
0
-1
-2
-3

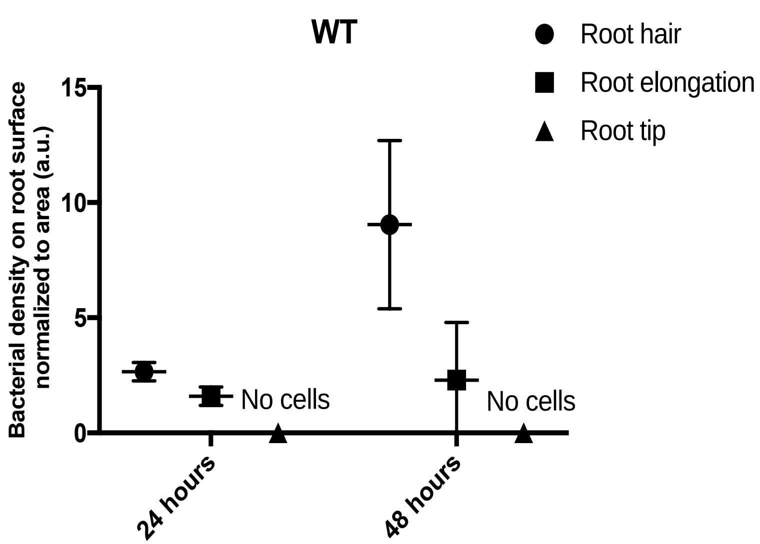
Preferential accumulation pattern depends on (at least) one chemoreceptor

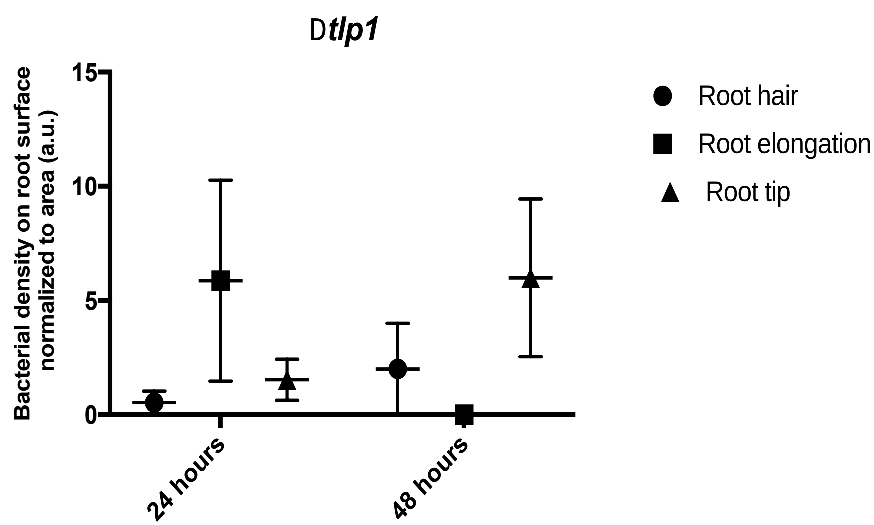
Root hair | **Elongation zone** | **Root tip**

Δtlp1



48hpi



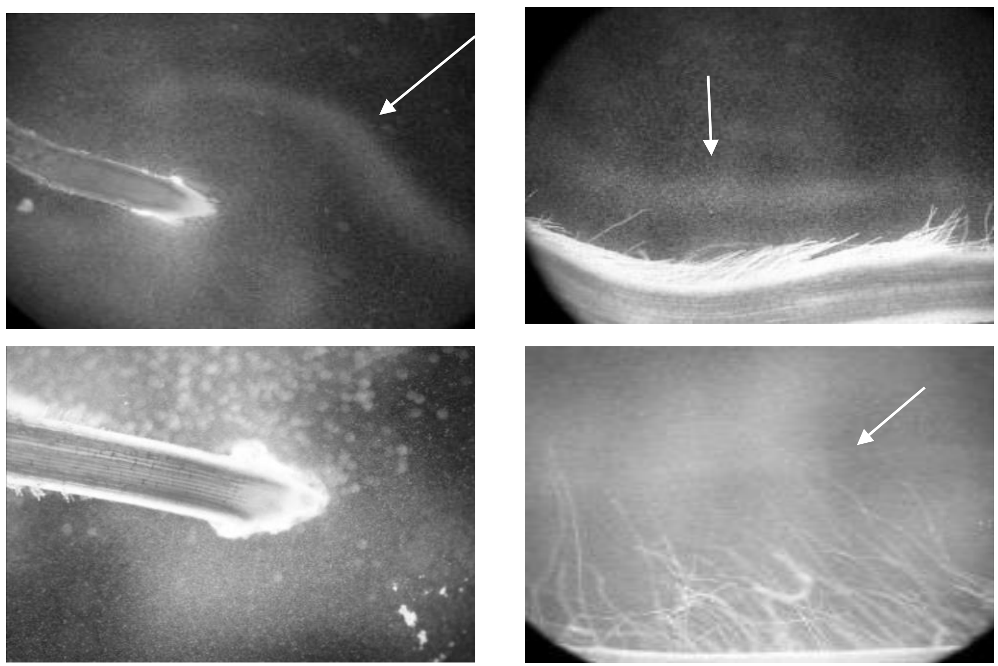


Short term chemotaxis impaired in a strain lacking the Tlp1 chemoreceptor

Root Tip **Root Hair**

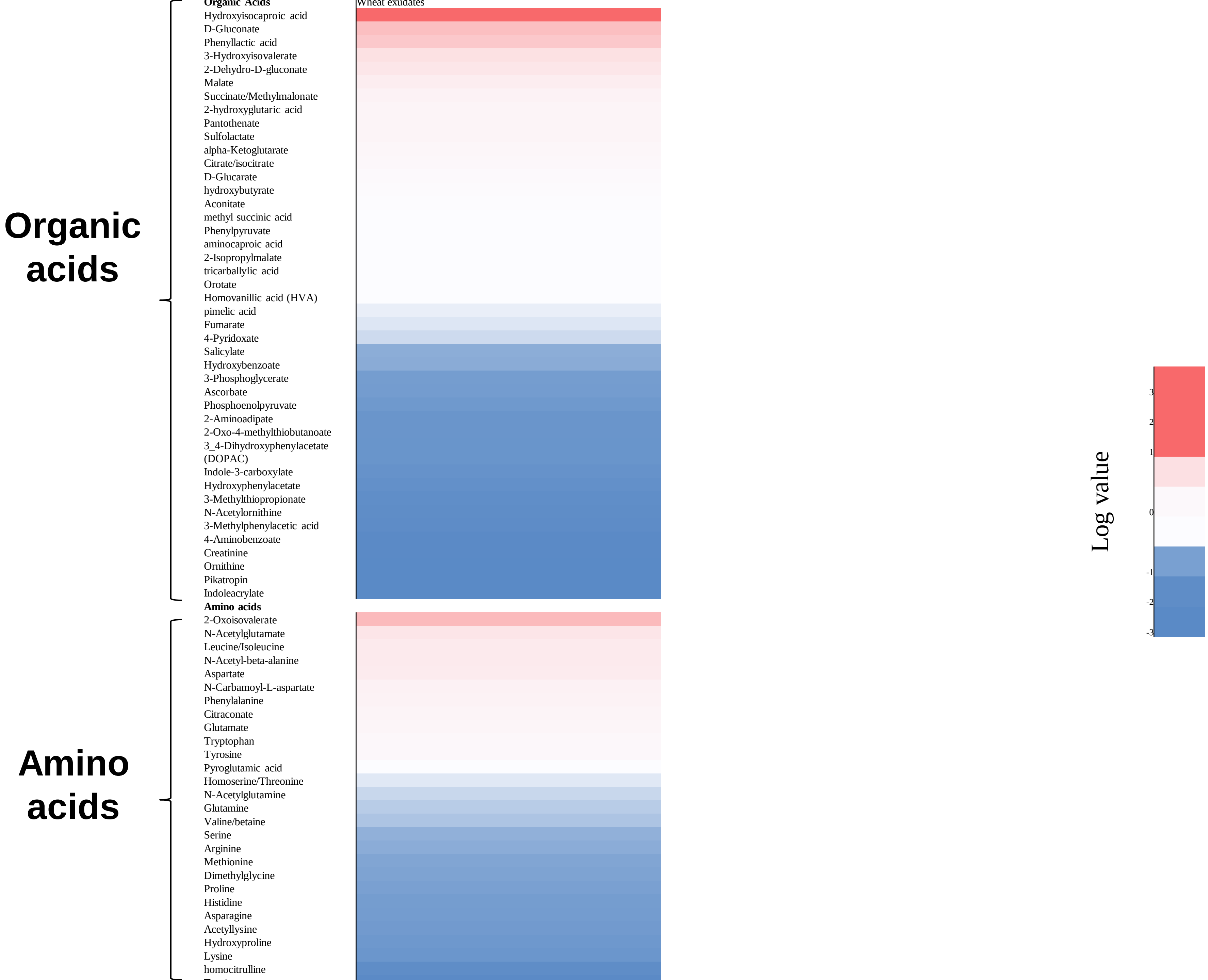
A. brasilense **wild type**

A. brasilense ***Δtlp1***



Faint response, but band does not move over time

Chemotaxis defects in Tlp1 suggest distinct chemical conditions in different regions of the roots



Organic acids
Amino acids
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Wheat exudates
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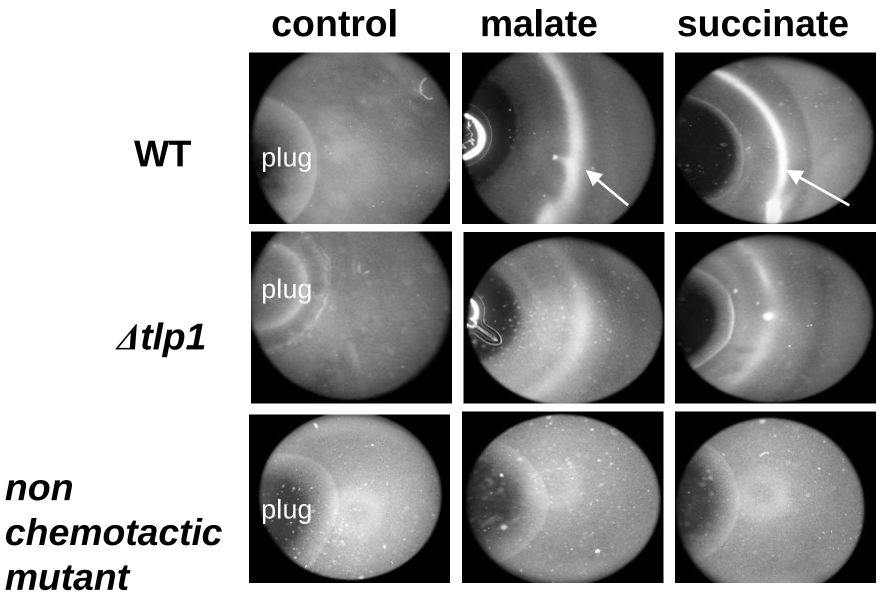
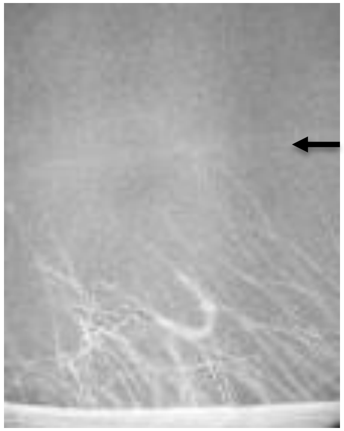
D-Gluconate
Phenyllactic acid
3-Hydroxyisovalerate
2-Dehydro-D-gluconate
Malate
Succinate/Methylmalonate
2-hydroxyglutaric acid
Pantothenate
Sulfolactate
alpha-Ketoglutarate
Citrate/isocitrate
D-Glucarate
hydroxybutyrate
Aconitate
methyl succinic acid
Phenylpyruvate
aminocaproic acid
2-Isopropylmalate
tricarballylic acid
Orotate
Homovanillic acid (HVA)
pimelic acid
Fumarate
4-Pyridoxate
Salicylate
Hydroxybenzoate
3-Phosphoglycerate
Ascorbate
Phosphoenolpyruvate
2-Aminoadipate
2-Oxo-4-methylthiobutanoate
3_4-Dihydroxyphenylacetate (DOPAC)
Indole-3-carboxylate
Hydroxyphenylacetate
3-Methylthiopropionate
N-Acetylornithine
3-Methylphenylacetic acid
4-Aminobenzoate
Creatinine
Ornithine
Pikatropin
Indoleacrylate
Amino acids
2-Oxoisovalerate
N-Acetylglutamate
Leucine/Isoleucine
N-Acetyl-beta-alanine
Aspartate
N-Carbamoyl-L-aspartate
Phenylalanine
Citraconate
Glutamate
Tryptophan
Tyrosine
Pyroglutamic acid
Homoserine/Threonine
N-Acetylglutamine
Glutamine
Valine/betaine
Serine
Arginine
Methionine
Dimethylglycine
Proline
Histidine
Asparagine
Acetyllysine
Hydroxyproline
Lysine
homocitrulline
Log value
3
2
1
0
-1
-2
-3

Tlp1 senses malate and malate is present in wheat root exudates



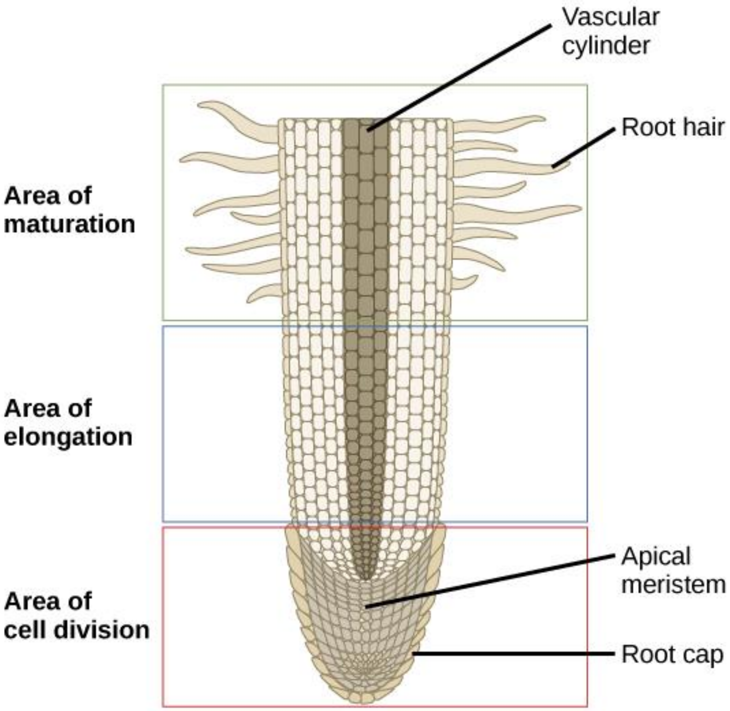
Chemotaxis toward wheat root hairs



Metabolomics of wheat root exudates: abundance of organic acids

A repellent produced by plant roots: ROS

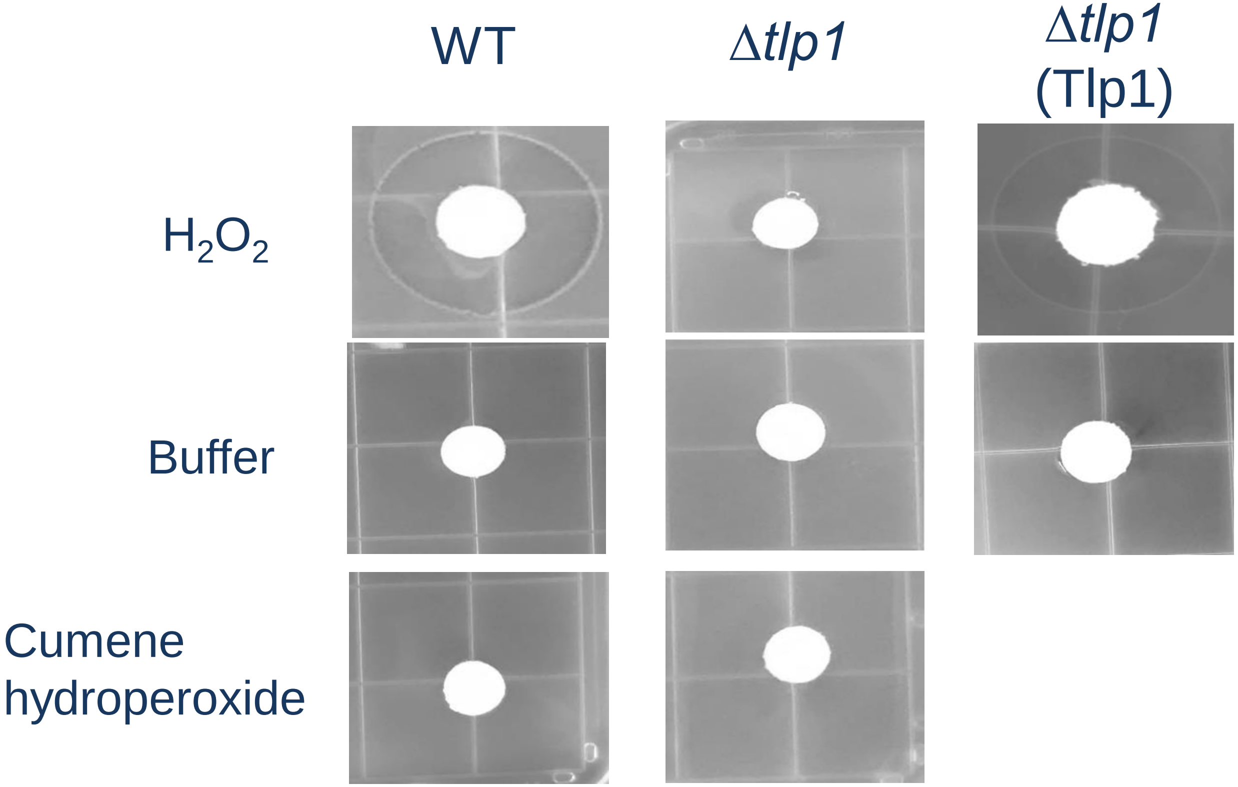
Wheat Root tips generate redox-active compounds (ROS)



Tlp1 is less sensitive than WT to repellent effect of redox active compounds (Greer-Phillips *et al,* 2004)

Méndez-Gómez, et al. (2015). Plant and Soil. 400(1): 55-65
Greer-Phillips, et al. (2004) *J Bacteriol.* 186:6595-6604

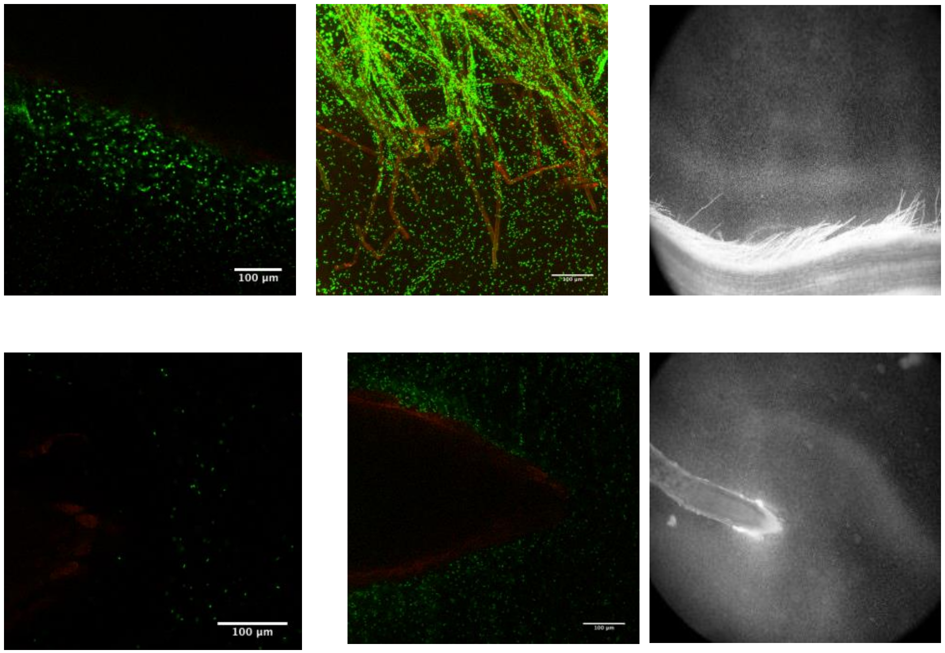
Chemotaxis-dependent response to hydrogen peroxide requires Tlp1



A. brasilense chemotaxis sets colonization preference

Root hair

Root tip

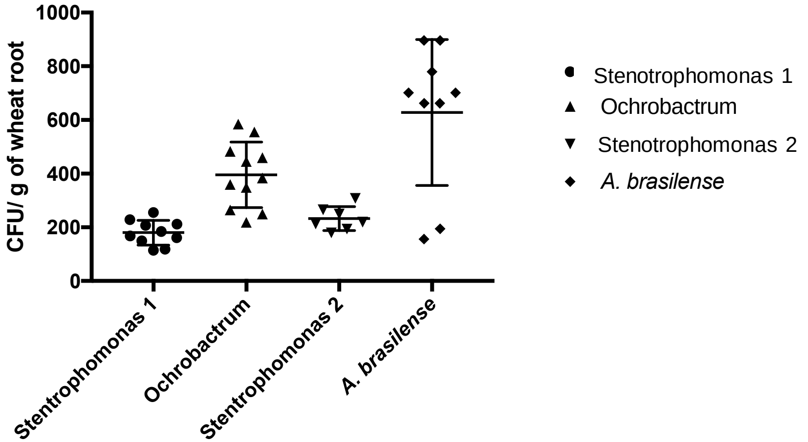


Attractant

Repellent

What about other bacterial species?

Bacterial species with preferential accumulation in the rhizosphere also colonize better the root surface



Stenotrophomonas

Ochrobactrum

Conclusions

- Bacterial chemotaxis drives root colonization patterns: signaling and chemoreceptors

- What are bacteria sensing? How do bacteria integrate sensing of multiple gradients to ultimately colonize root surfaces?

- Dynamics of root surface colonization? Plant-microbe signal exchange?

Thank you

UTK
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Jessica Gullett
Anastasia Askenova
Dr. Elena Ganusova

Kylie Hendricksen
Nathan Burriss
Adam Hubler
Shehroze Akhter
Lam Vo
Zachary Day

UTK- MABE
Andy Sarles

Oak Ridge National Laboratory
Jenny Morell-Falvey
Amber Bible

Overview of exudates analysis

Mass spectrometry-

After collection, exudates are filter-sterilized and lyophilized. The lyophilized exudates are weighted (dry weight) before being resuspended in 1 ml of water and separated by Hydro Reverse Phase HPLC. Samples are then ionized by electrospray in negative mode on an Orbitrap Mass spectrometer. Data are processed and peaks picked via Maven software (Princeton University). Metabolite area counts are normalized to dry sample weight.

Normalization to total C/N in sample possible with additional analysis.

Data processing- statistical analysis, clustering etc.

Value of the analysis depends on experimental design, follow up.

Exudates analysis provides insight into the effect of bio-inoculants/additives on plant growth

- Project A- Imaging by PSC indicated clear effects of an additive on promoting fine roots growth of plants. Exudate analysis confirmed metabolic evidence of active growth → applications: optimized plant nutrition in balanced soils
- Project B- Exudate analysis following comparison of treatments at PSC indicated
  - a treatment provided the cells with sufficient nitrogen nutrition -> applications: reduce nitrogen fertilization in field
  - A different treatment seemed to prime plants for abiotic stress resistance
- Project C- Exudate analysis following inoculation at PSC indicated a treatment caused plants to release sugars in exudates → applications: indication that NPK nutrition is optimum, no need for added fertilization under optimal conditions.