Rhizophagy Symbiosis in Cereals: Bacterial Transport of Nutrients to Roots/Oxidative Extraction of Nutrients from Bacteria in Roots

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All plants naturally host endophytes!

Endophytes are everywhere!

Some endophytes are bacteria.

(Plant Eukaryote-Prokaryote symbioses)
Plants control this symbiosis!

‘Cadushy’ cactus: *Subpilocereus repandus* in Bonaire

Seeds

Cadushy seedling

Reactive Oxygen Staining Technique

Assay for bacterial endophytes involves growth on agarose. Then staining with Diaminobenzidine (DAB)/Aniline blue stain overnight.

Reactive oxygen secretion is used by all eukaryotes to kill endoparasitic bacteria. It is part of the innate Defensive system of all Eukaryotes.

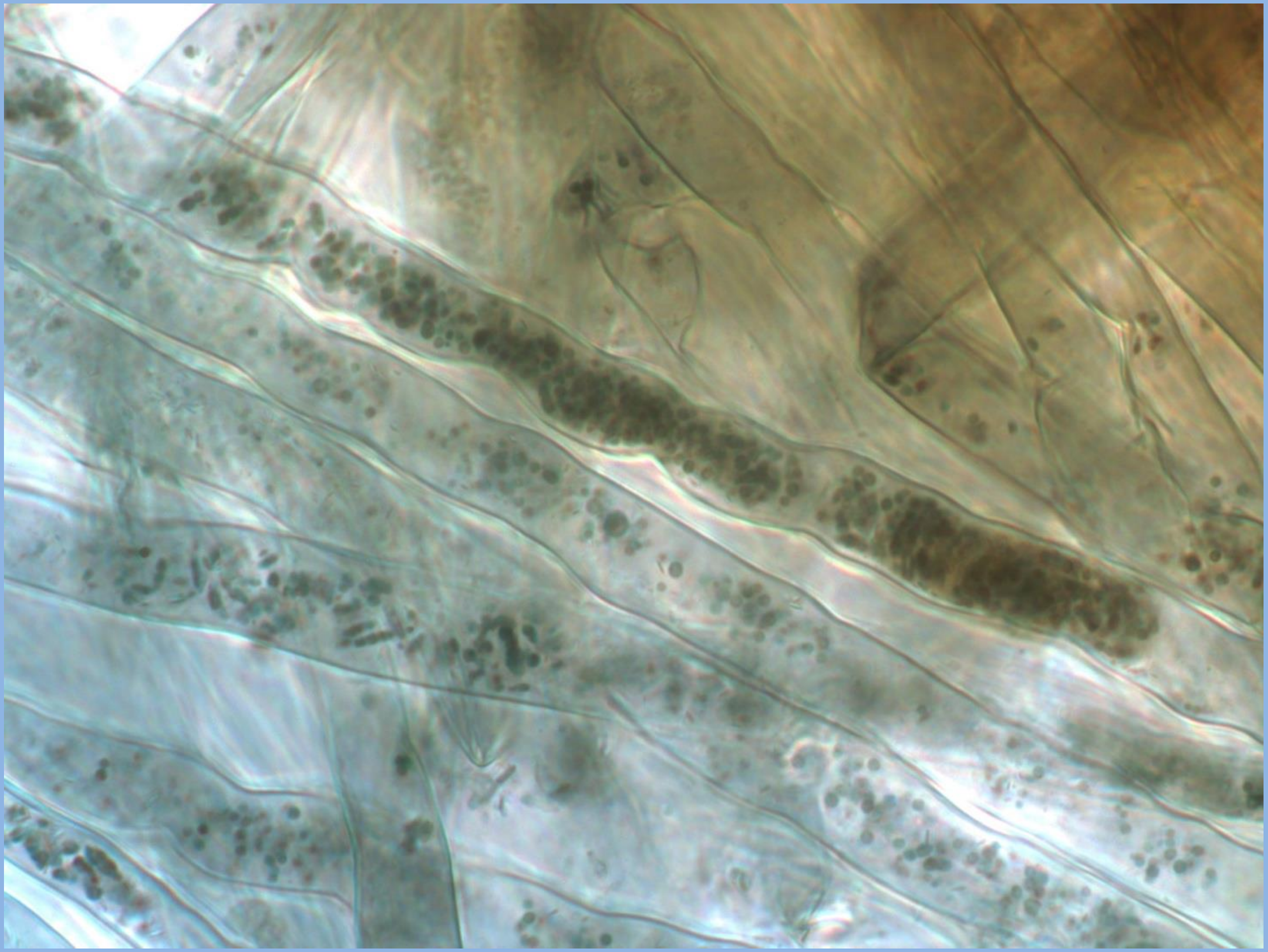
How DAB Works:

1) Plant cells secrete superoxide Onto intracellular bacteria to degrade them.

2) Plant uses superoxide dismutase to transform Superoxide to water and Hydrogen peroxide.

3) DAB reacts with Hydrogen peroxide to form Brown/red coloration

Bacteria in root hairs (Stained in DAB followed by aniline blue).



Bacteria in root hairs showing recently divided pairs

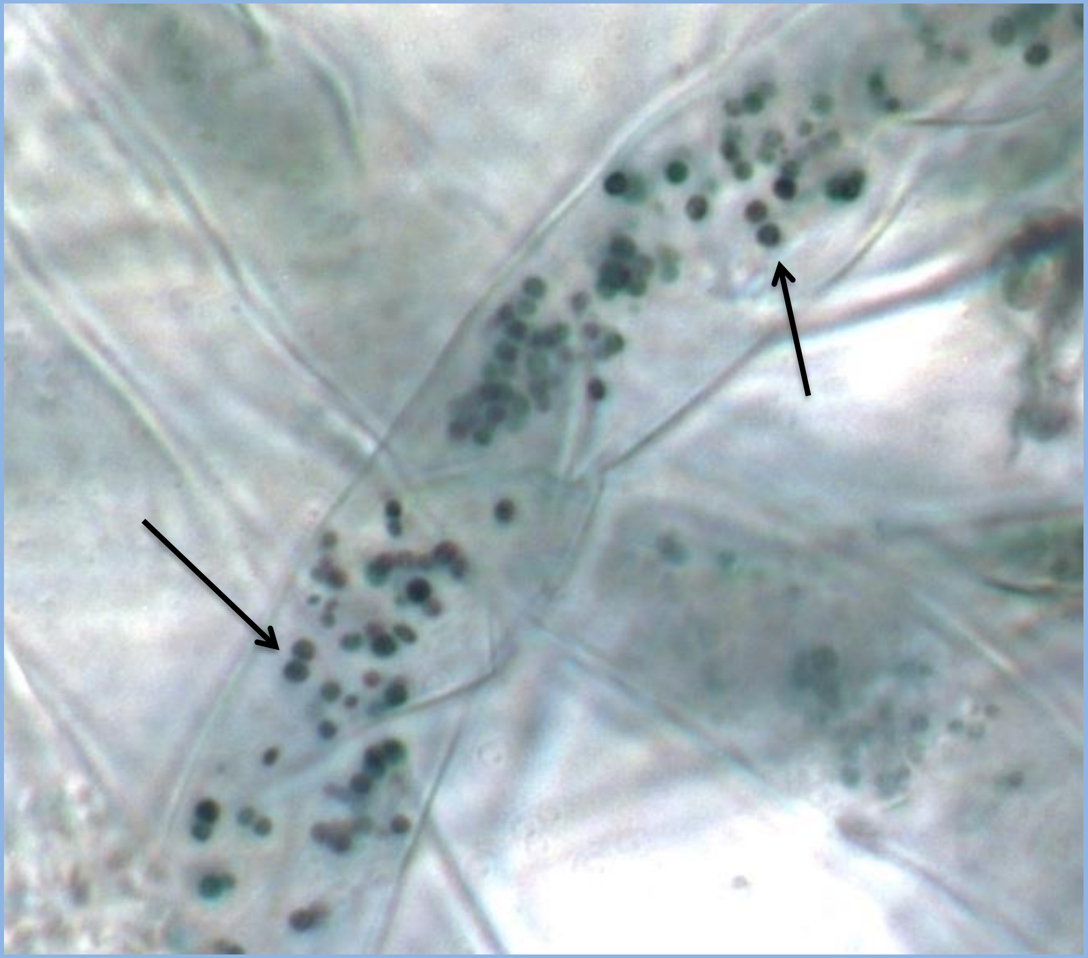
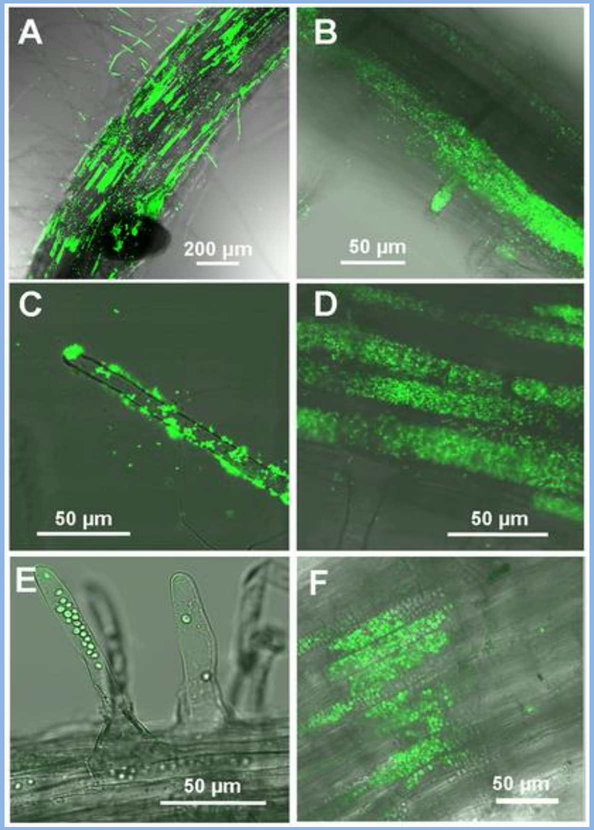


Figure 1. Roots of axenically grown Arabidopsis and tomato were incubated with E coli or yeast expressing green fluorescent protein (GFPE. coli or GFPyeast).

"Rhizophagy"

Do plant roots consume bacteria to obtain nutrients?



'Turning the Table:
Plants Consume Microbes as a Source of Nutrients'

Chany Paungfoo-Lonhienne

Paungfoo-Lonhienne C et al. 2010.
Turning theTable: Plants Consume Microbes as a Source of Nutrients.
PLoS ONE 5(7): e11915, doi:10.1371/journal.pone.0011915

microorganisms

Review

Rhizophagy Cycle: An Oxidative Process in Plants for Nutrient Extraction from Symbiotic Microbes

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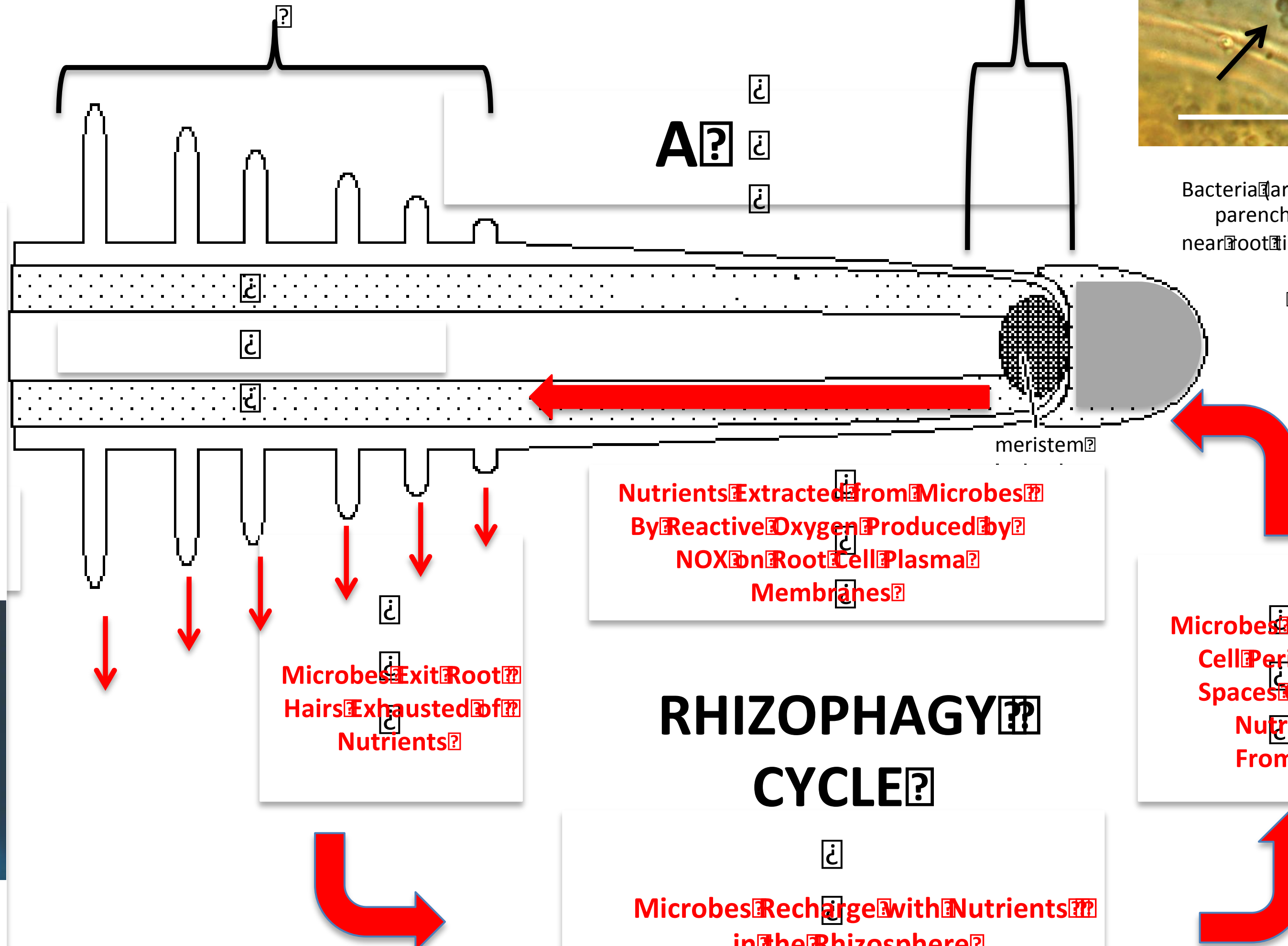

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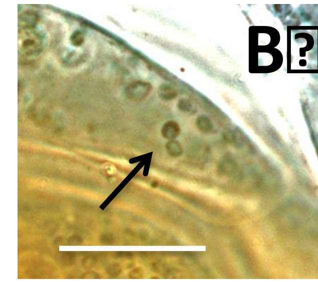
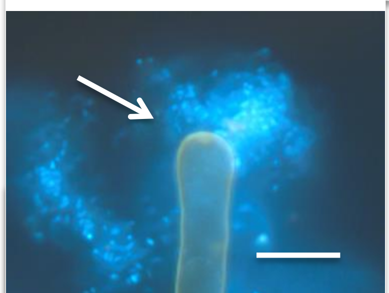


Bacteria (arrow) in root parenchyma cell near root tip meristem.

C

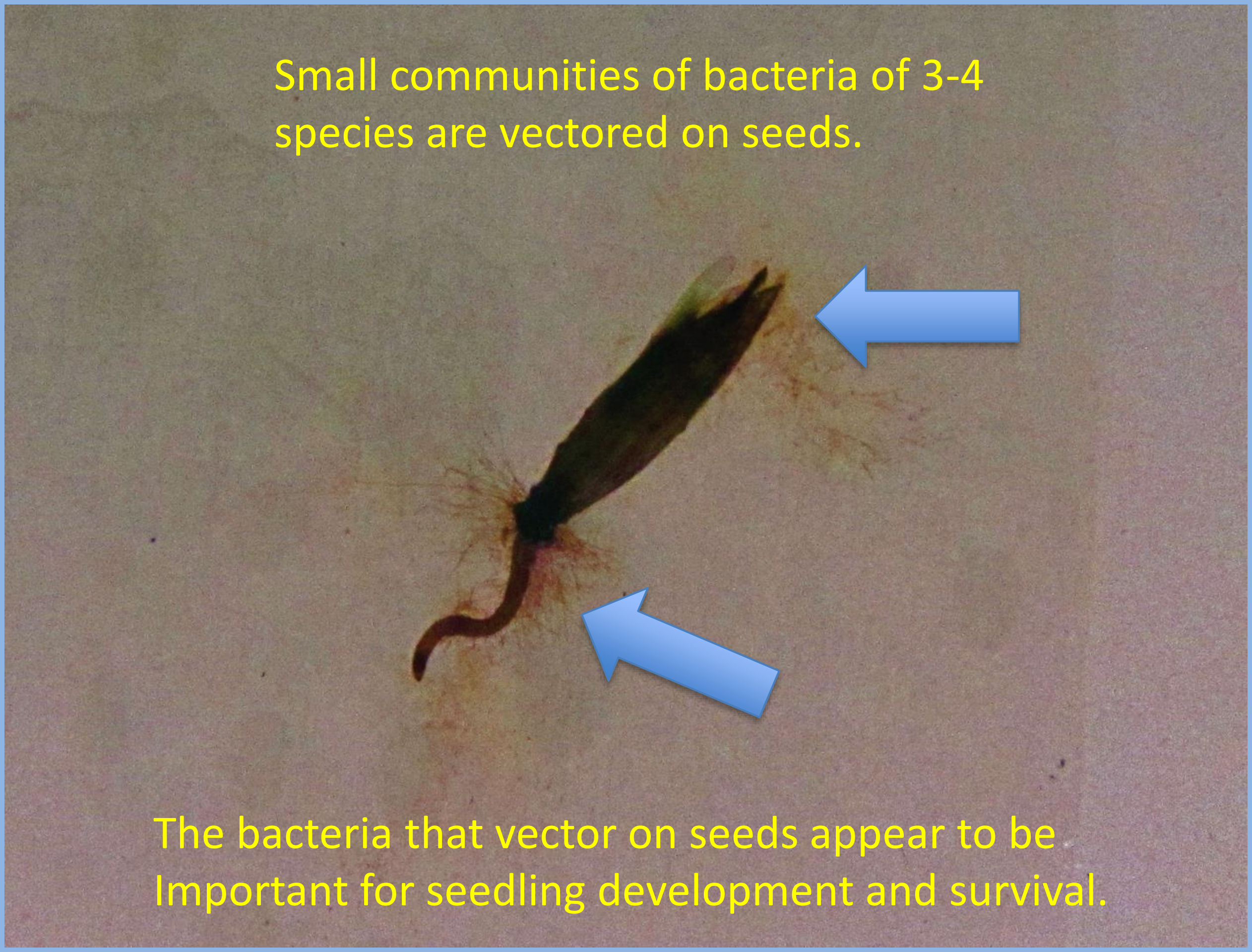


Bacteria (arrow) emerging from root hair tip of millet seedling.

Cereal crops carry symbiotic microbes in seed-associated tissues: Lemmas and Paleas on seed surfaces.

In wheat microbes are vectored in the caryopsis invagination.

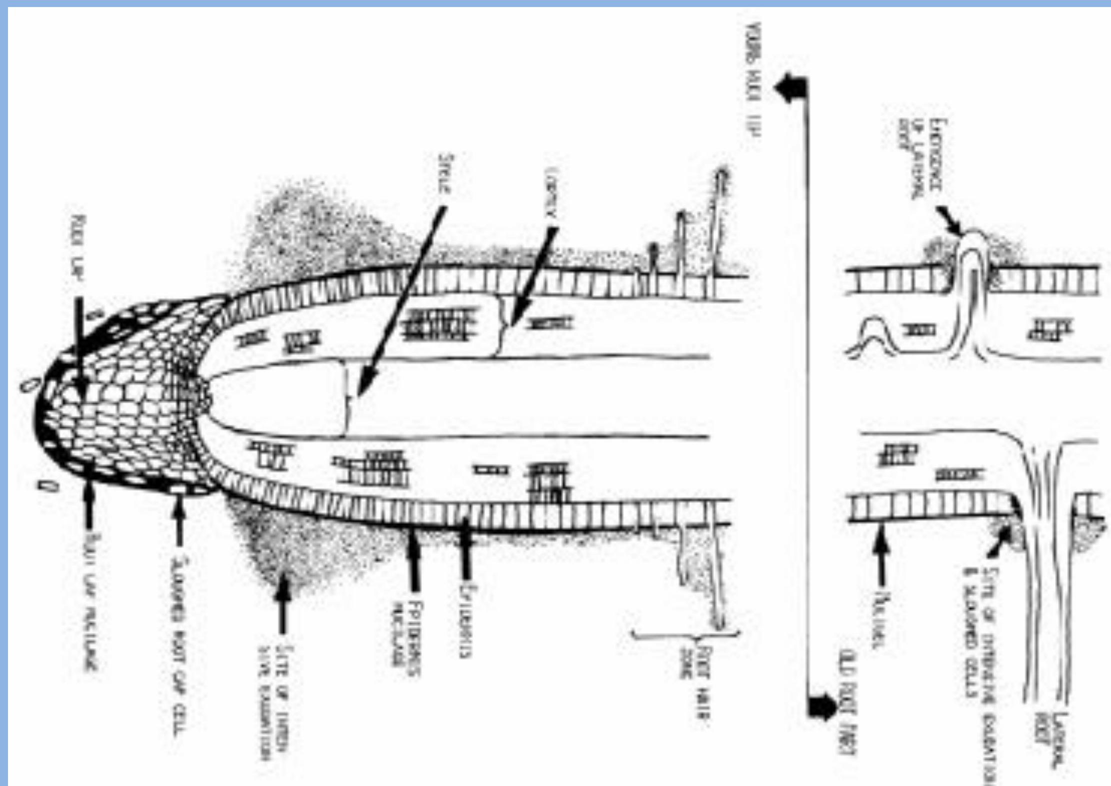
Bacterial symbiosis: germinating tall fescue seed showing seed-transmitted bacteria (*Pantoea agglomerans* and *Pseudomonas* sp.; both gamma-Proteobacteria)



*Bacteria are ‘Proteobacteria’; Perhaps mostly gamma- and beta-Proteobacteria.

Root exudation zones determined by 14C experiments.

Plants manipulate bacteria by cultivating bacteria in
The root exudate zone near tip of root.

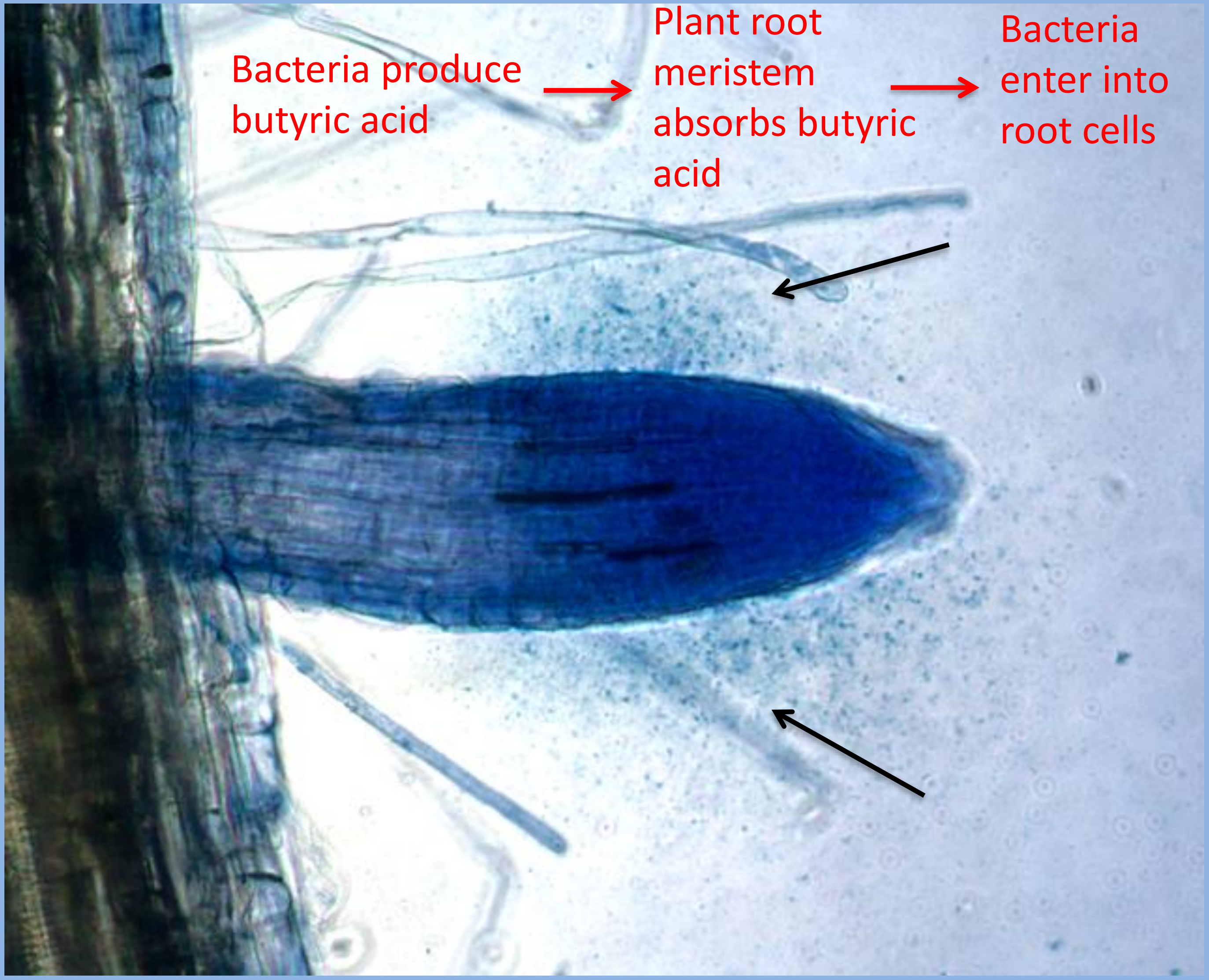


Secretion of exudates in a zone proximal to root tip meristems facilitates
Their entry into cells of the meristem.

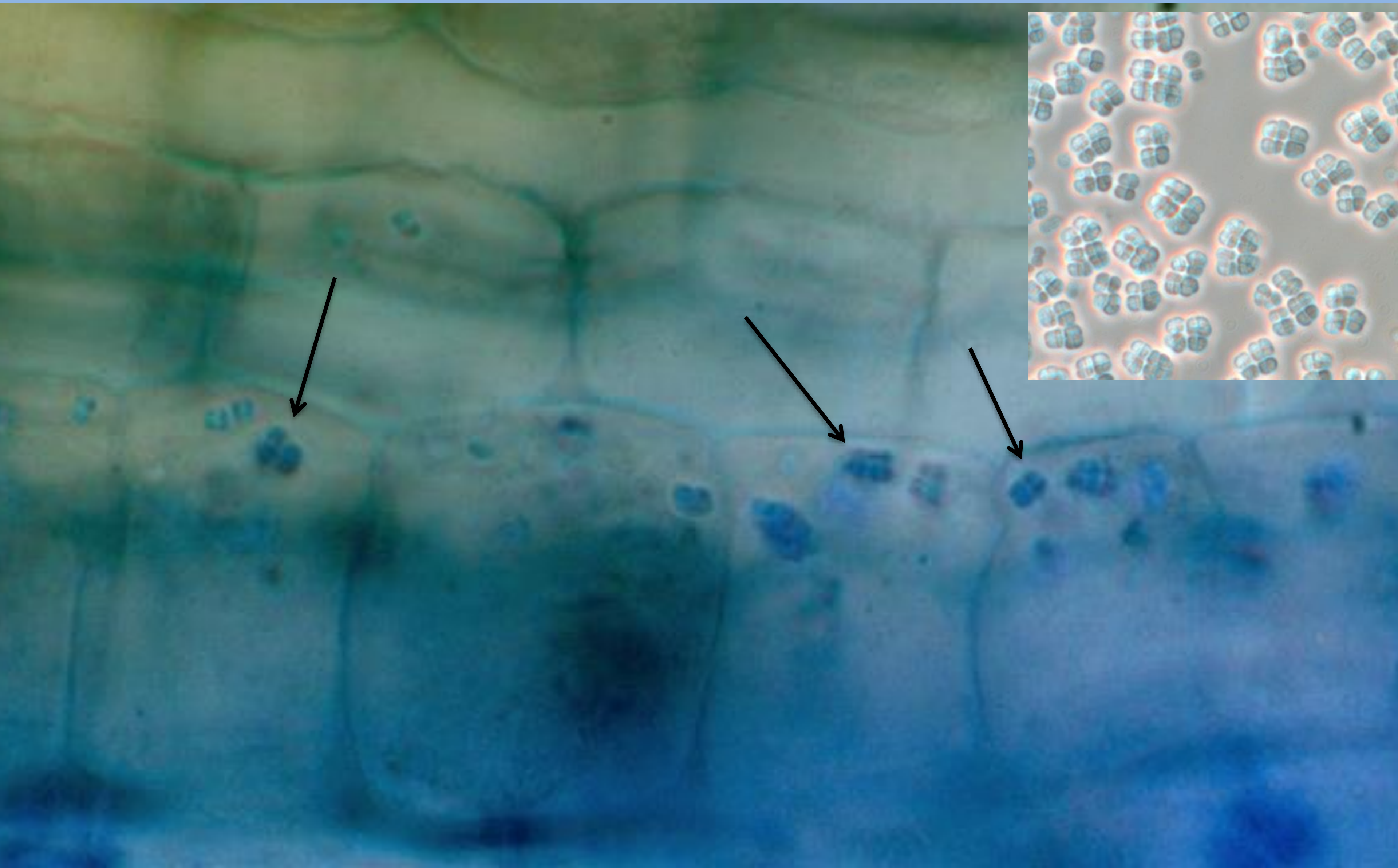
Marschner, H., 1995. Mineral Nutrition
of Higher Plants, 2nd edn., Academic Press, London.

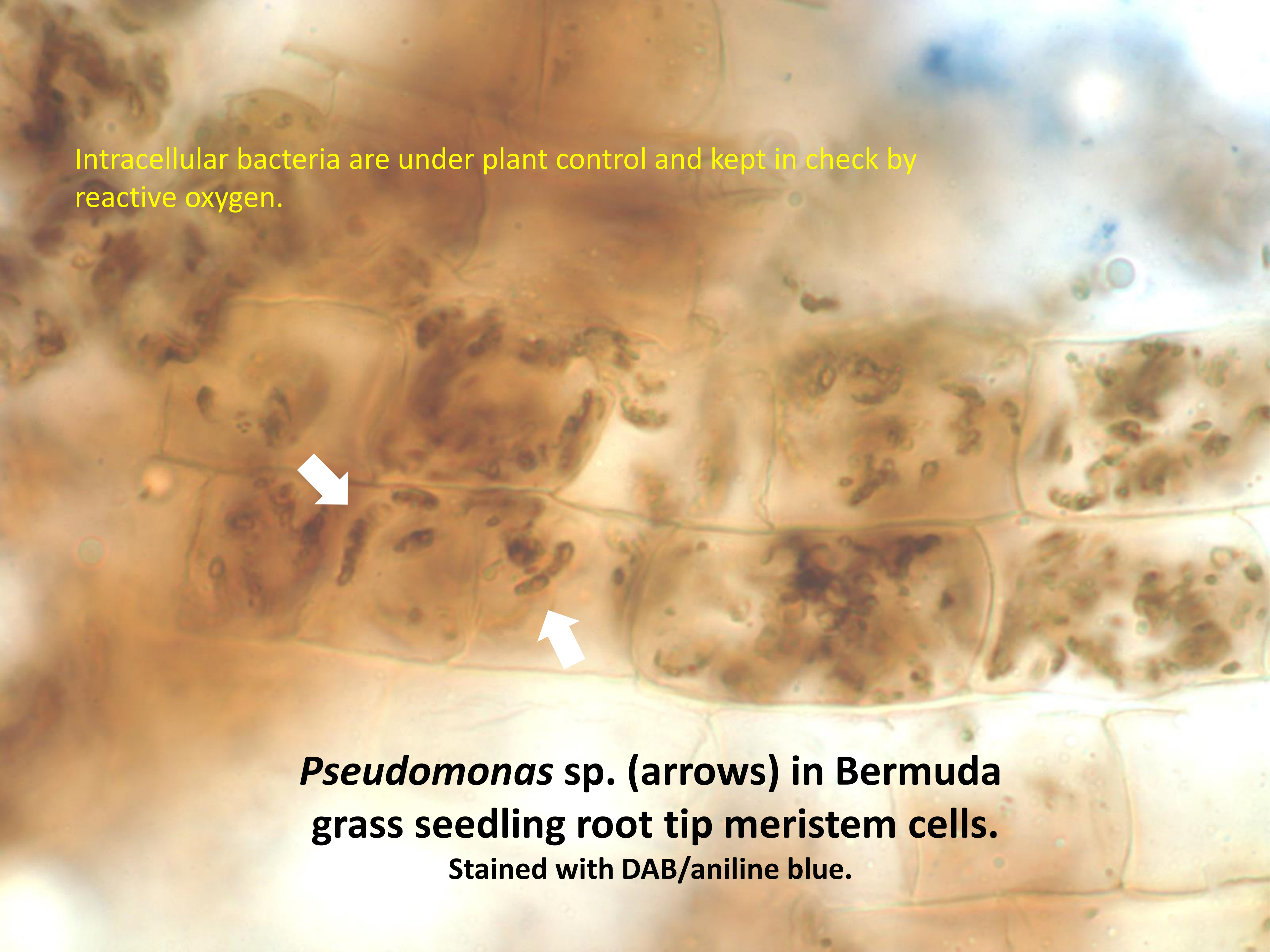
FUNCK-JENSEN, D. & HOCKENHULL, J. 1984. Root exudation, rhizosphere
microorganisms and disease control. Växtskyddsnotiser 48: 3-4, 49-54.

Bacteria entering root epidermal cells in the 'zone on intracellular colonization' at the root tip meristem. A cloud of bacteria (arrows) is seen around the root tip meristem where intracellular colonization is occurring. The blue stain is aniline blue.



Micrococcus luteus tetrads (arrows) in periplasmic space of root meristematic cells of *Rumex crispus*. Insert is *Micrococcus* from agar culture.





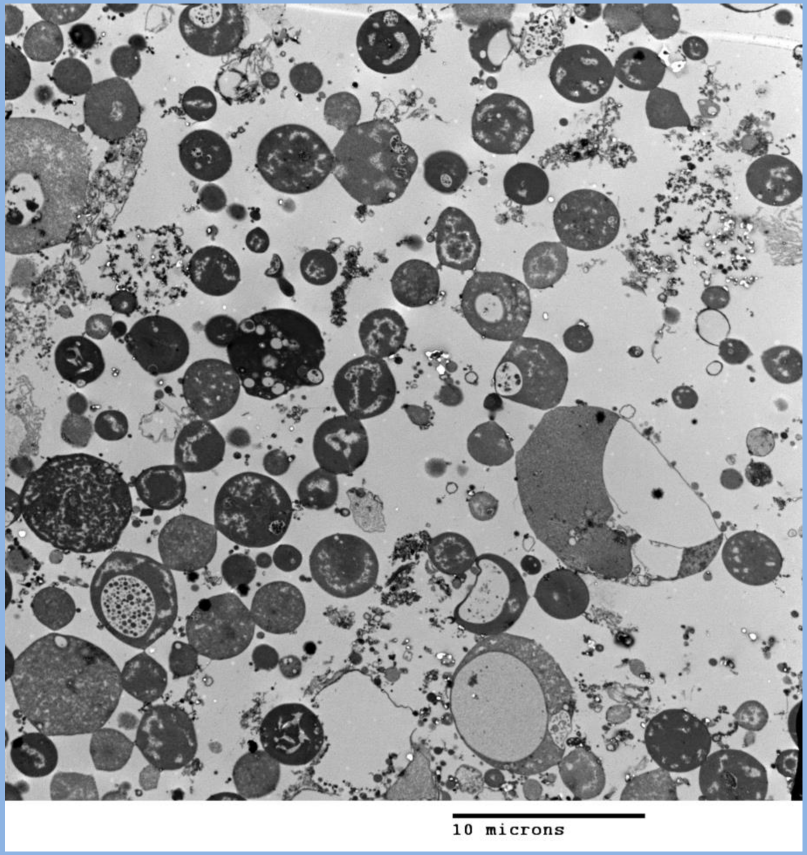
Pseudomonas **sp. (arrows) in Bermuda grass seedling root tip meristem cells.**

Stained with DAB/aniline blue.

TEM of *Bacillus subtilis* L-forms

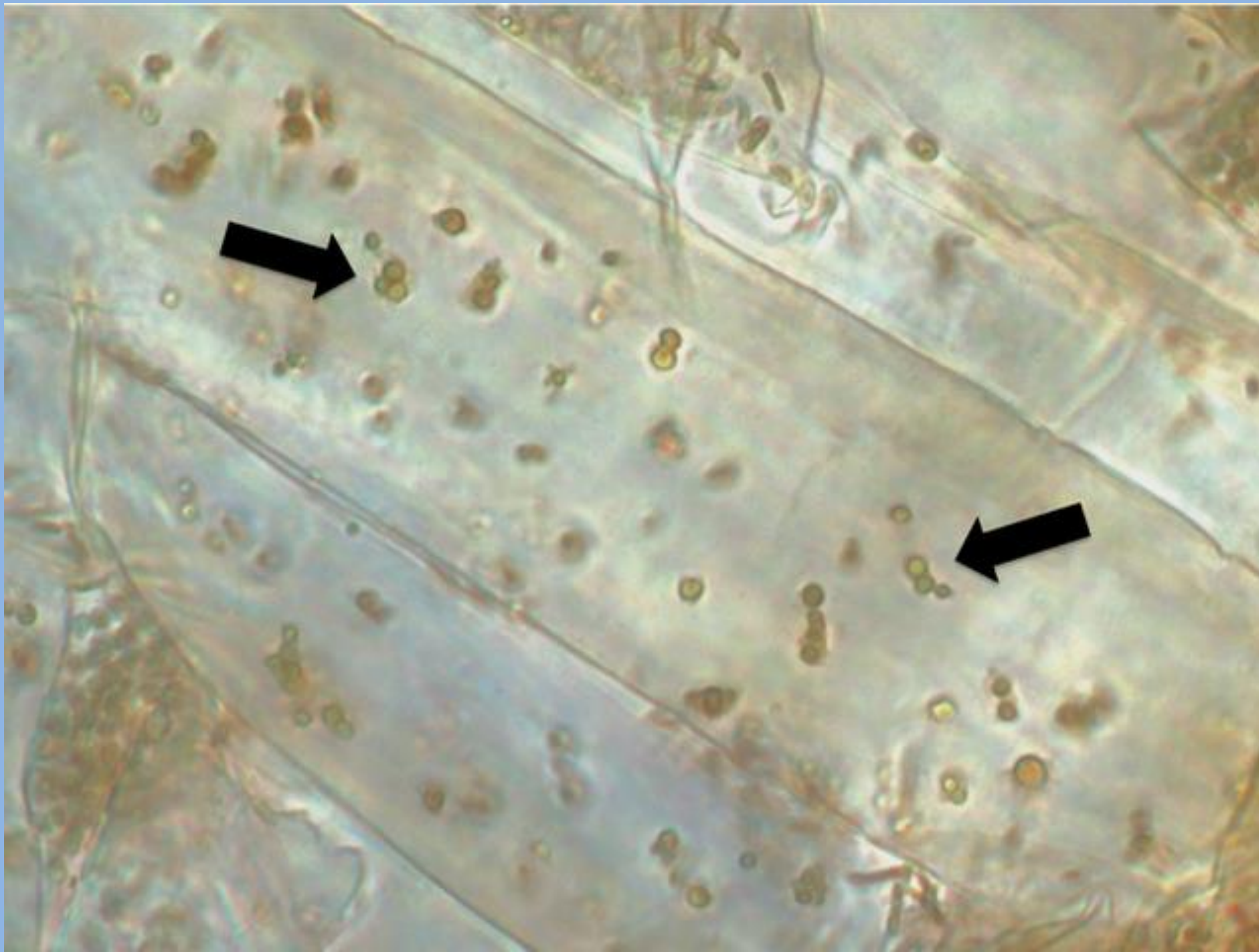
Photo by Mark Leaver, New Castle University, UK

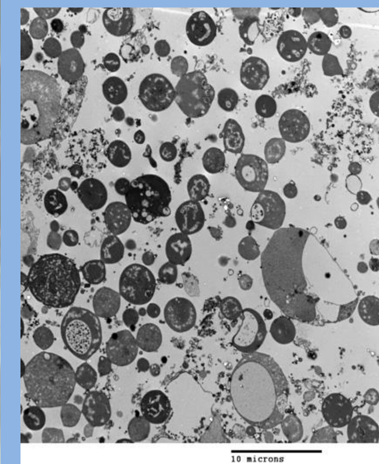
Jeff Errington
New Castle University, UK
L-forms in bacteria



L-forms are bacterial cells that do not form cell walls (also called ‘cell wall deficient bacteria’). L-forms typically are seen inside eukaryotic cells. They are thought to be a mechanism to evade host defense response. L-form bacteria are typically variable in size.

Phragmites root stained with diaminobenzidine DAB to visualize reactive oxygen around bacteria (arrows). Reactive oxygen is visualizable as brown or red coloration around bacteria. The reactive oxygen is the result of superoxide produced by NADPH oxidases on the root cell plasma membranes. The reactive oxygen extracts nutrients from the bacteria (mostly pseudomonads) that are symbiotic with *Phragmites*. Some of the bacteria may be completely degraded/oxidized by reactive oxygen. Other bacteria stimulate root hair elongation and exit the root hair at the elongating tip to reenter soil populations.





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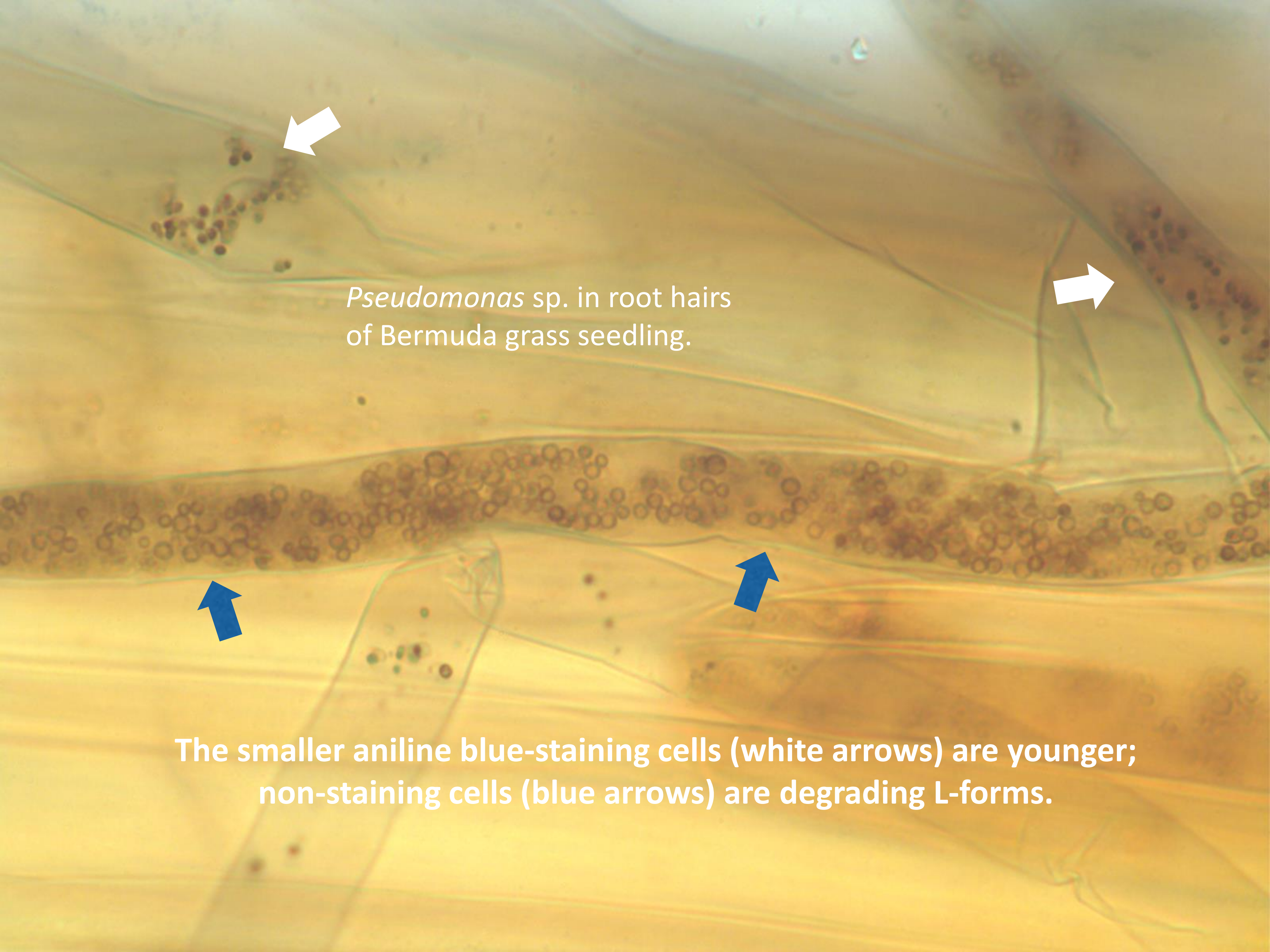
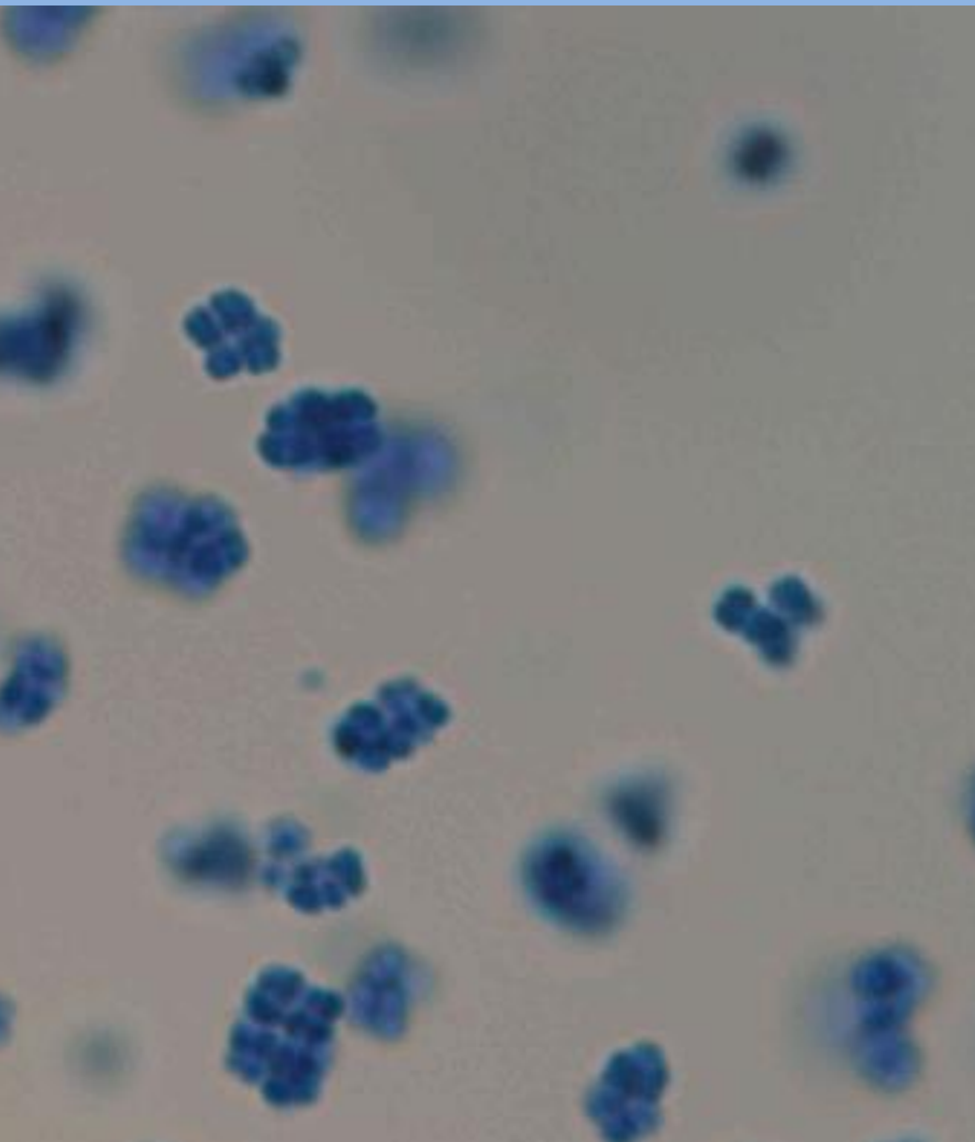
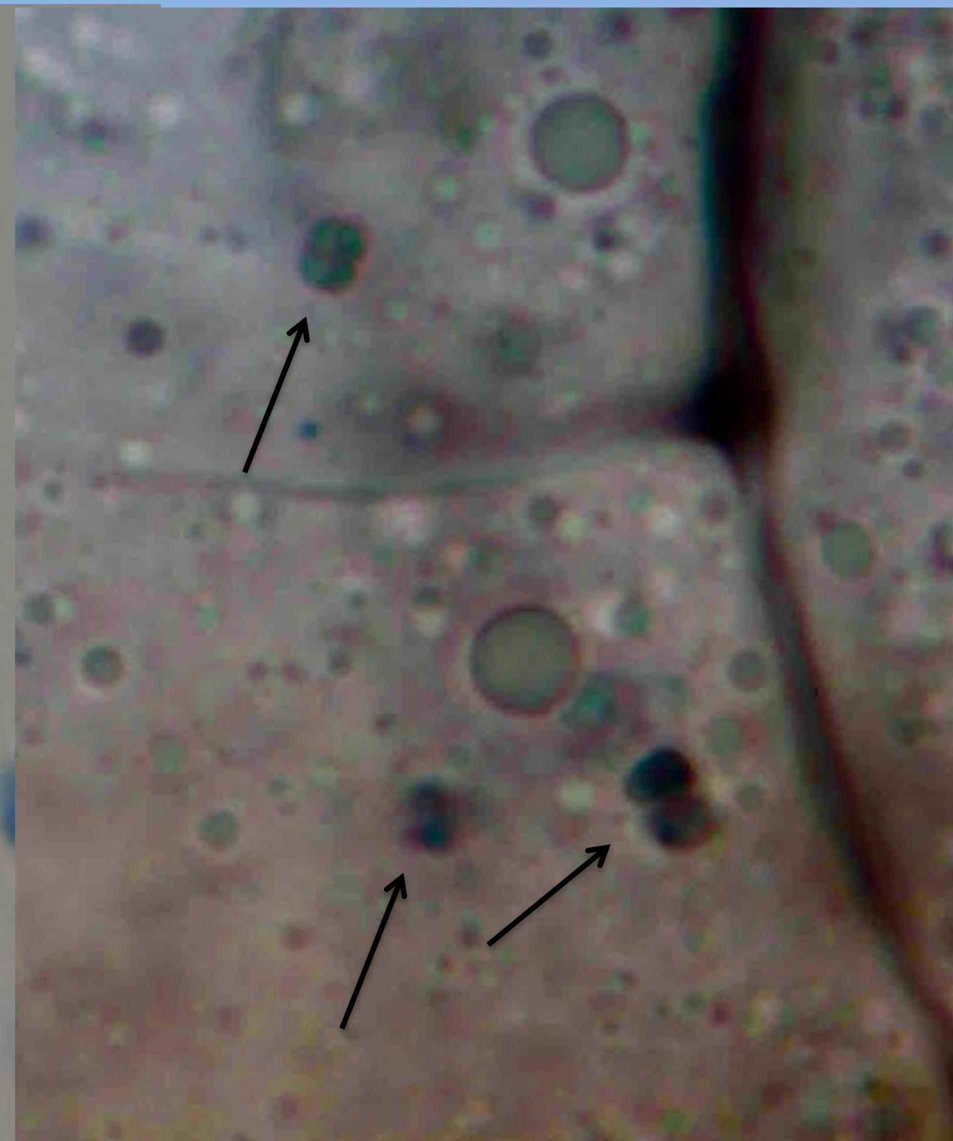
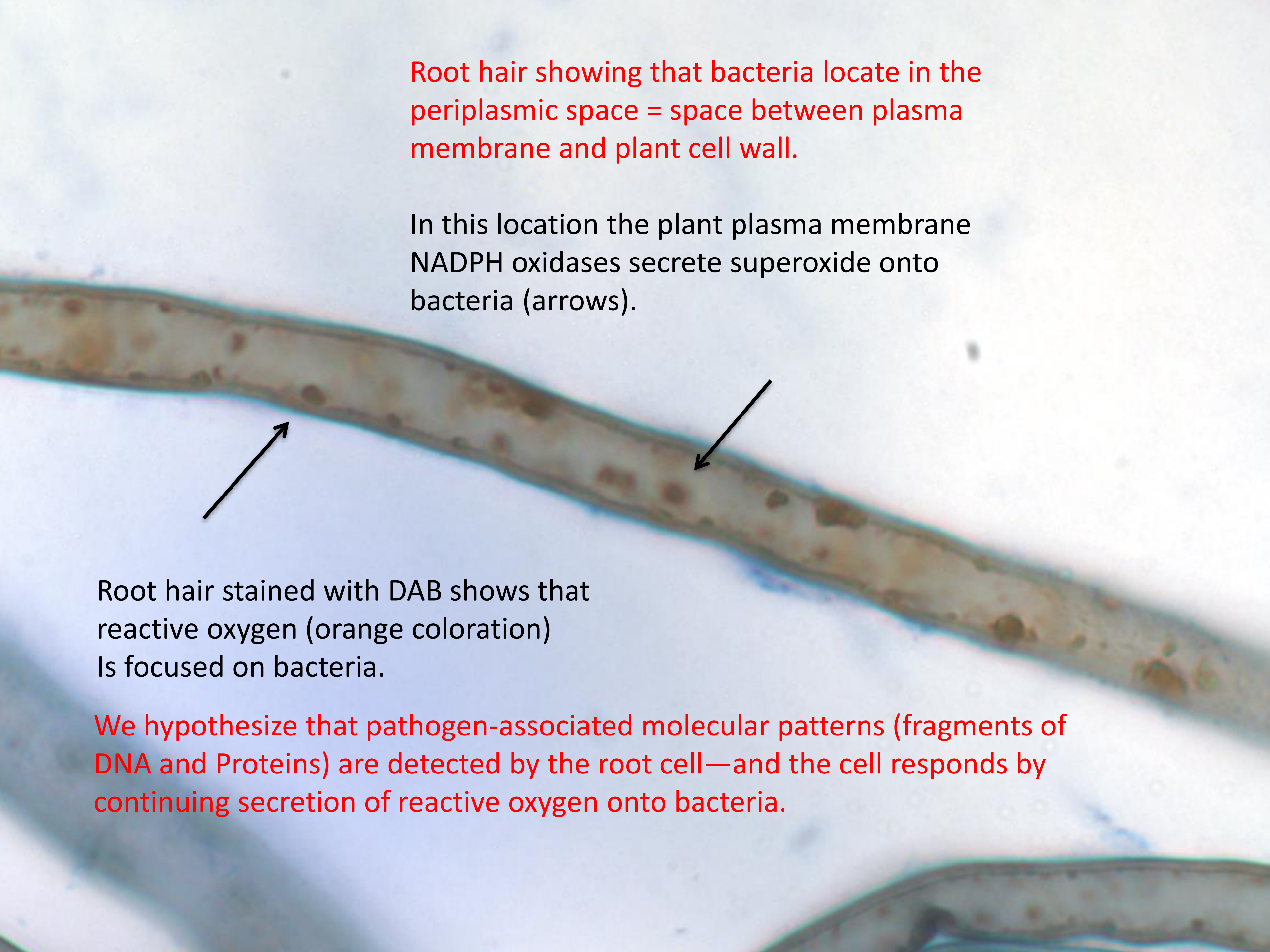

Pseudomonas sp. in root hairs
of Bermuda grass seedling.
The smaller aniline blue-staining cells (white arrows) are younger;
non-staining cells (blue arrows) are degrading L-forms.

Pseudomonas sp. in root parenchyma of Bermuda grass seedling. Bacteria in cells that do not form root hairs are eventually degraded completely.

In some cases L-forms reform tetrads (with walls) in root cells. The image to the left is of *Micrococcus luteus* from culture. The image to the right shows L-forms and tetrads (arrows) reforming in root parenchyma cells of a dandelion seedling.



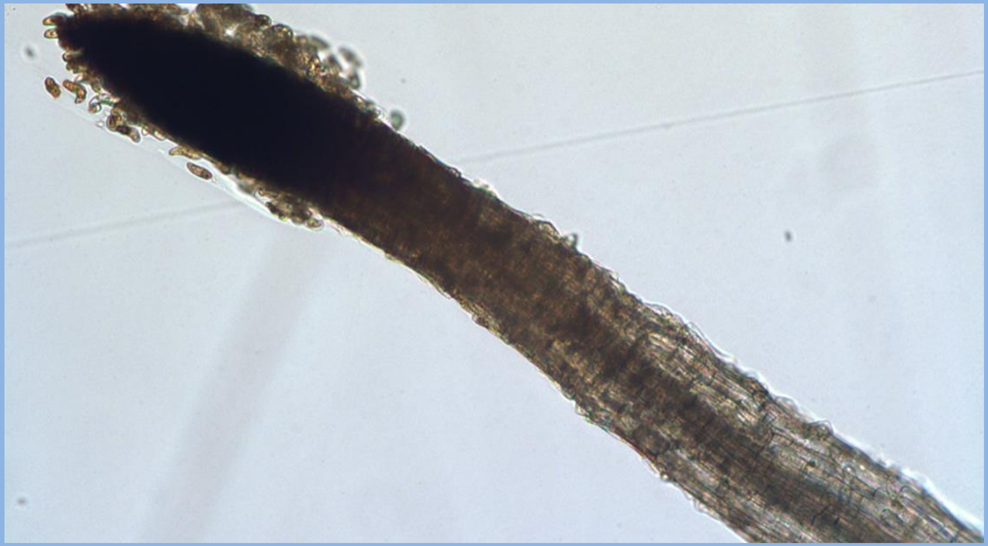



Root hair showing that bacteria locate in the periplasmic space = space between plasma membrane and plant cell wall.
In this location the plant plasma membrane NADPH oxidases secrete superoxide onto bacteria (arrows).
Root hair stained with DAB shows that reactive oxygen (orange coloration) Is focused on bacteria.
We hypothesize that pathogen-associated molecular patterns (fragments of DNA and Proteins) are detected by the root cell—and the cell responds by continuing secretion of reactive oxygen onto bacteria.

Intracellular bacteria modulate development of seedlings

- Bacteria trigger the gravitropic response in roots (function of ACC deaminase reduction of ethylene?)
- Bacteria trigger root hair elongation (function of auxins?)
- Bacteria increase root branching (function of auxins?)
- Bacteria increase root and shoot elongation (function of auxins?)

Bermuda grass seedling root in agarose without bacteria showing absence of root hairs



Root tip

More developed region of seedling root

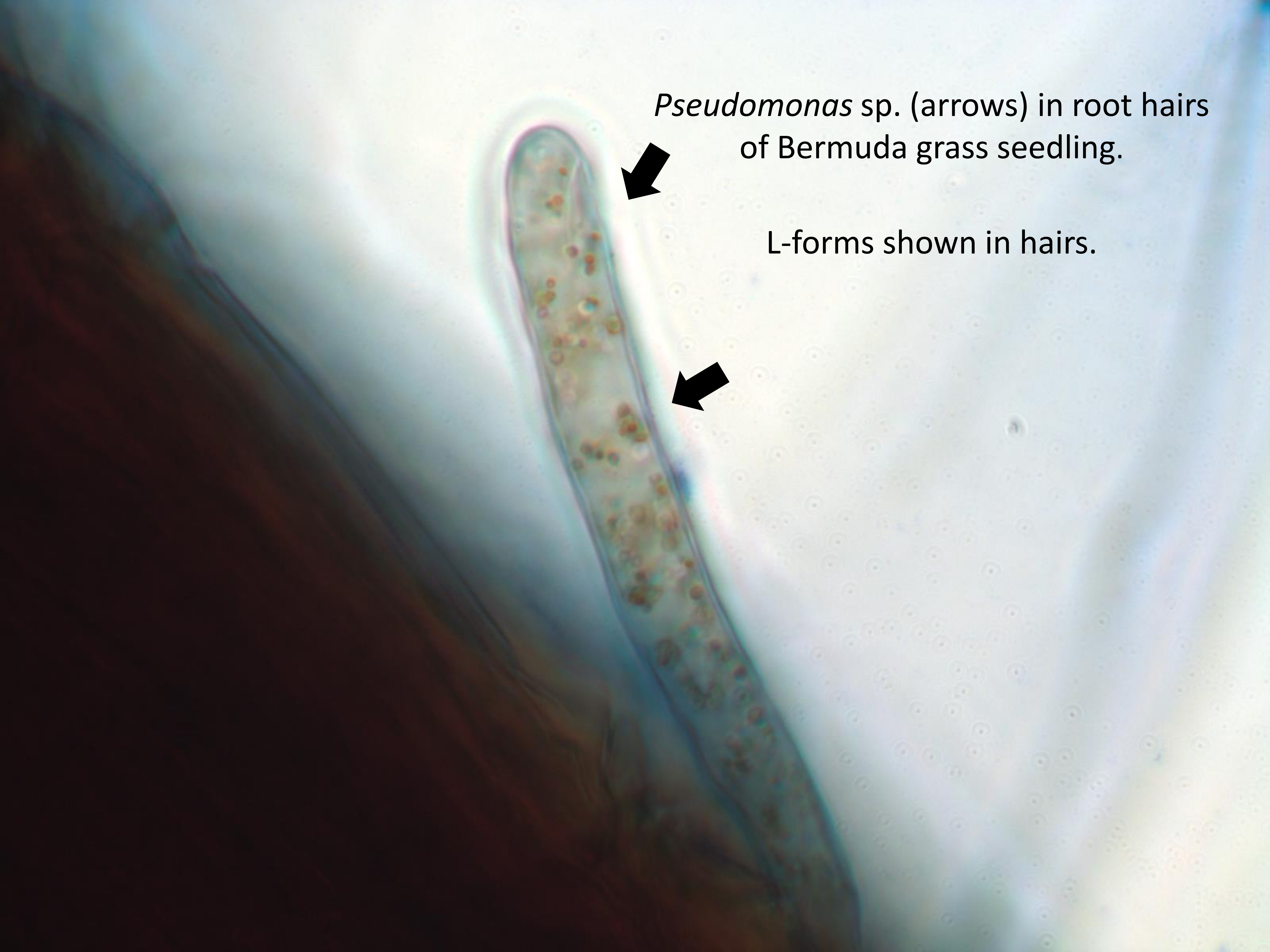
Bermuda grass root containing *Pseudomonas* endophyte

Proposed route of endophyte colonization of root and reentry to rhizosphere from root hairs

RHIZOPHAGY CYCLE

Bacteria (from seed coat) → Colonize root tip meristem (enter cells) → Intracellular in root parenchyma → Bacteria stimulate root hair formation In root epidermis → Bacteria emerge to surface of hair as the hair elongates → Bacteria colonize soil rhizosphere → Bacteria acquire nutrients in rhizosphere → Colonize root tip meristem (enter cells)


Bermuda grass seedling root containing *Pseudomonas* endophyte.
All brown spots in roots are intracellular bacteria.

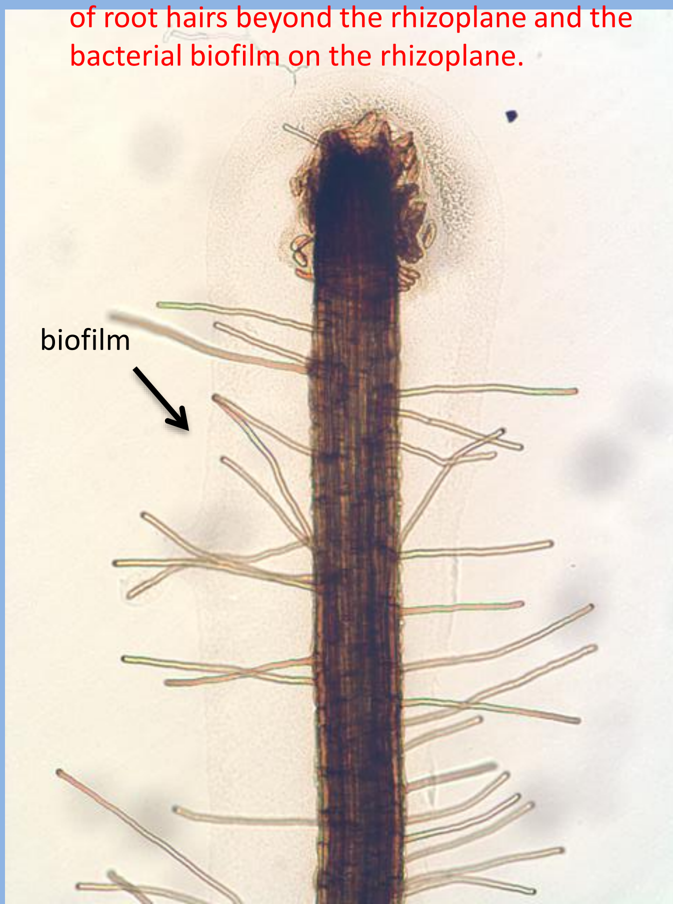

Pseudomonas sp. (arrows) in root hairs
of Bermuda grass seedling.
L-forms shown in hairs.

James White 11/5/2017
Rutgers University

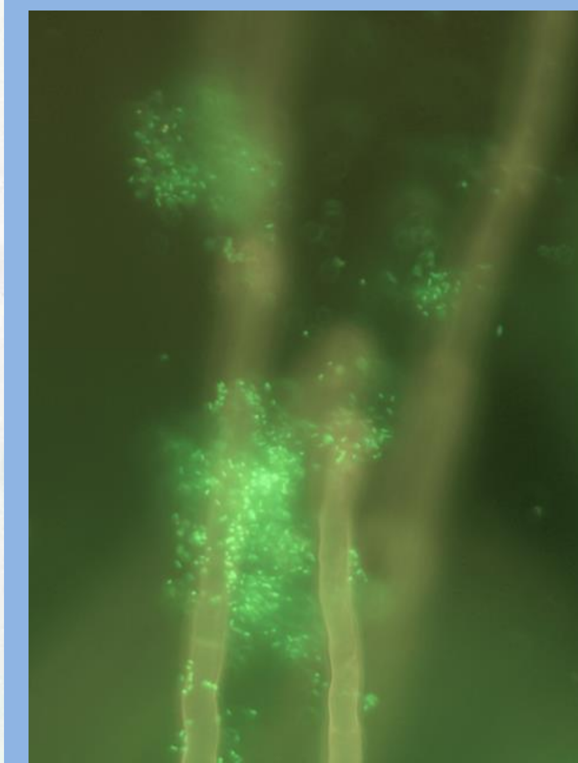
What is the function of root hairs?

The traditional answer is that root hairs function to absorb water and nutrients. Although this function is logical since hairs increase absorption surface area of roots, another function is suggested relative to the rhizophagy cycle. Since intracellular bacteria of the rhizophagy cycle trigger root hair development and exit at the tips of elongating hairs, the hairs effectively function to extend bacteria away from the rhizoplane and into the rhizosphere. This encourages bacteria to rejoin rhizosphere populations and acquire soil nutrients. This optimizes functioning of the rhizophagy cycle and increases nutrients acquired by the plant. Later attraction of these soil bacteria to the exudate zone behind the root tip meristem places them in position to reenter plant cells at the meristem where nutrients can be extracted oxidatively from them. From this perspective root hairs have dual functions that maximize nutritional benefits to plants.

Root growing in agarose showing extension of root hairs beyond the rhizoplane and the bacterial biofilm on the rhizoplane.



Bacteria emerging from tips of elongating root hairs. Stained with nuclear stain Syto 13.

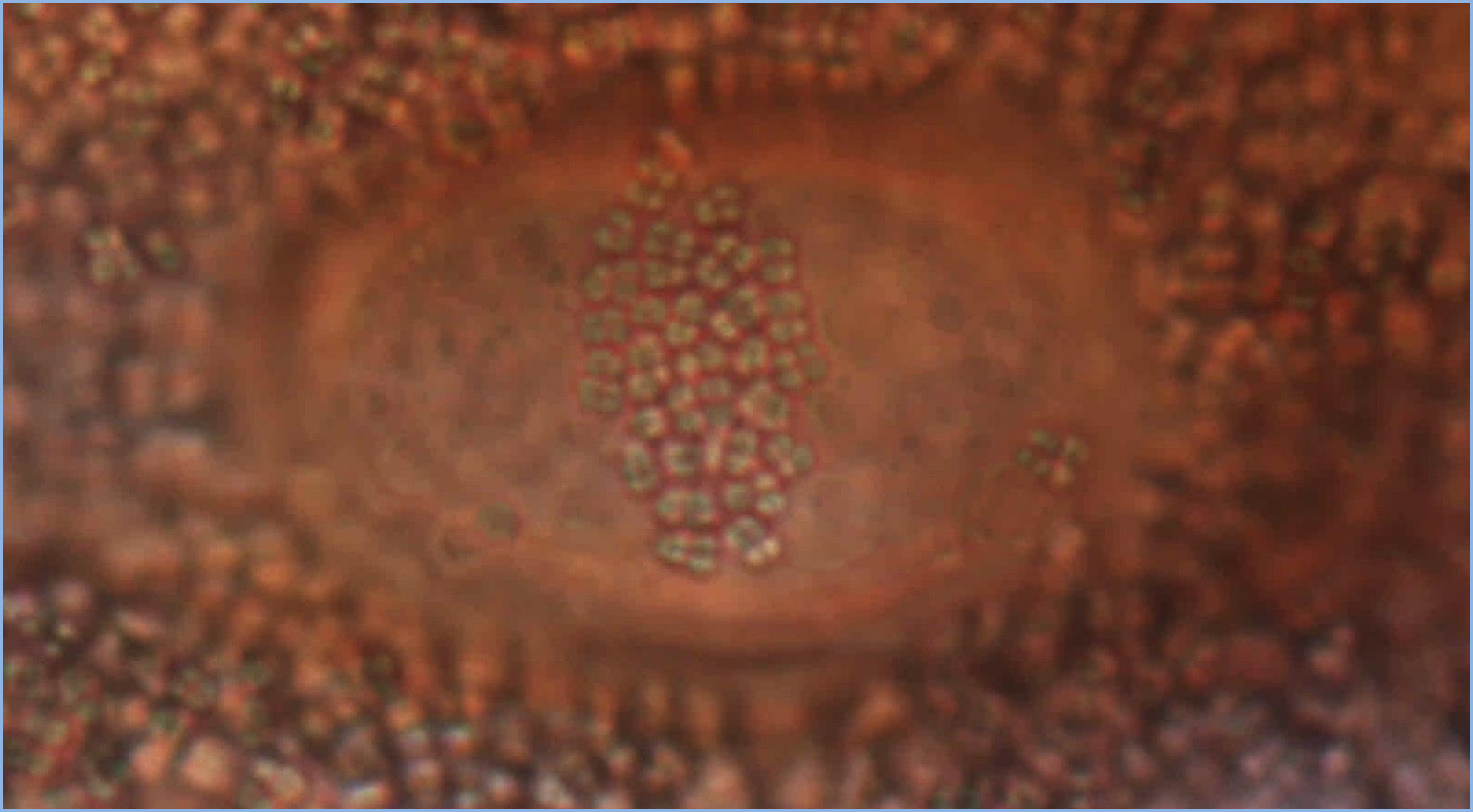


Bacteria emerging from root hair tip. Bacteria in hairs are present as wall-less L-forms. Bacteria reform their walls after exiting from the tip of the hair.

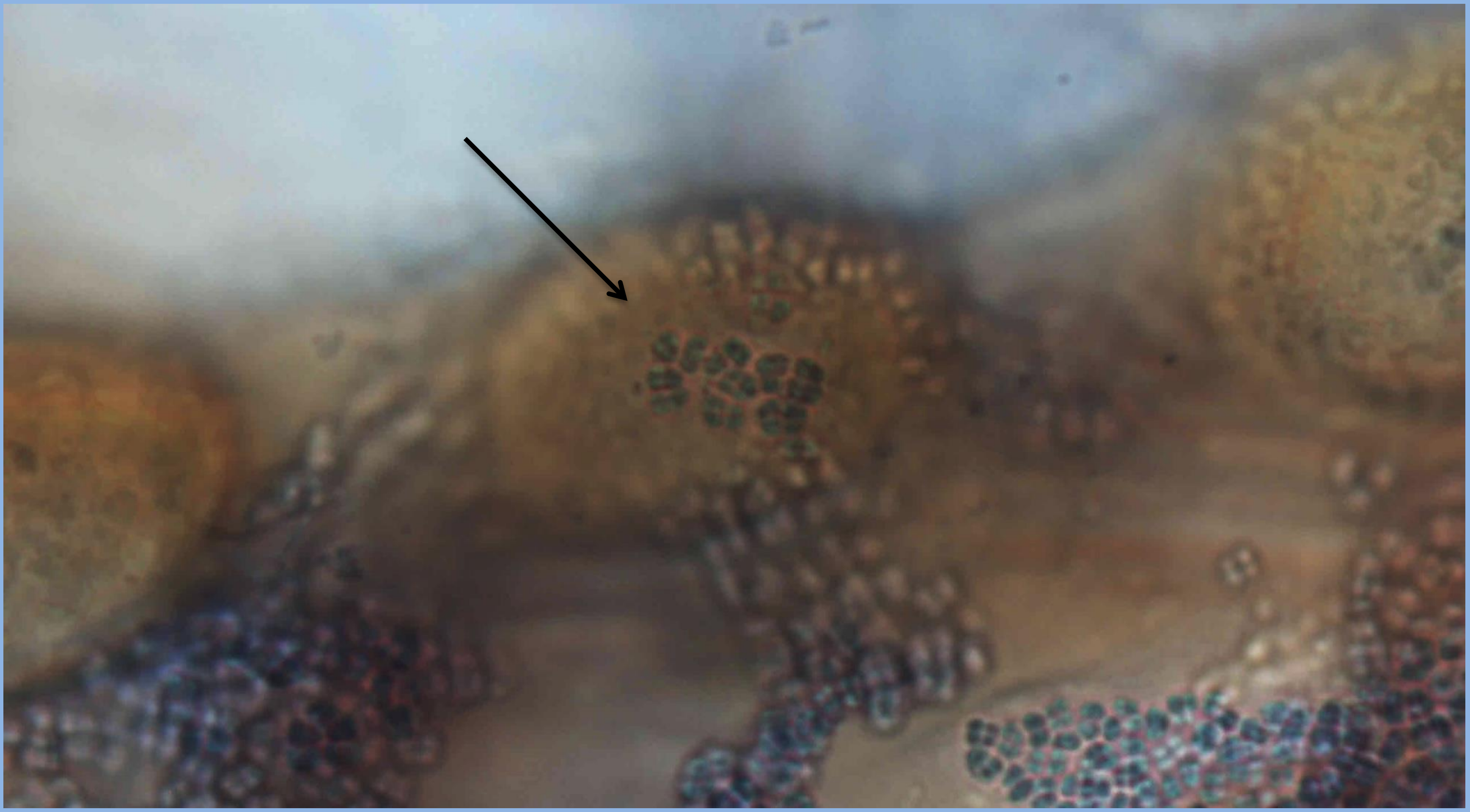
Rumex root hair showing emergence of bacterial cells at tip (arrow).

Rumex root hair showing emergence of bacterial cells at tip (arrow).

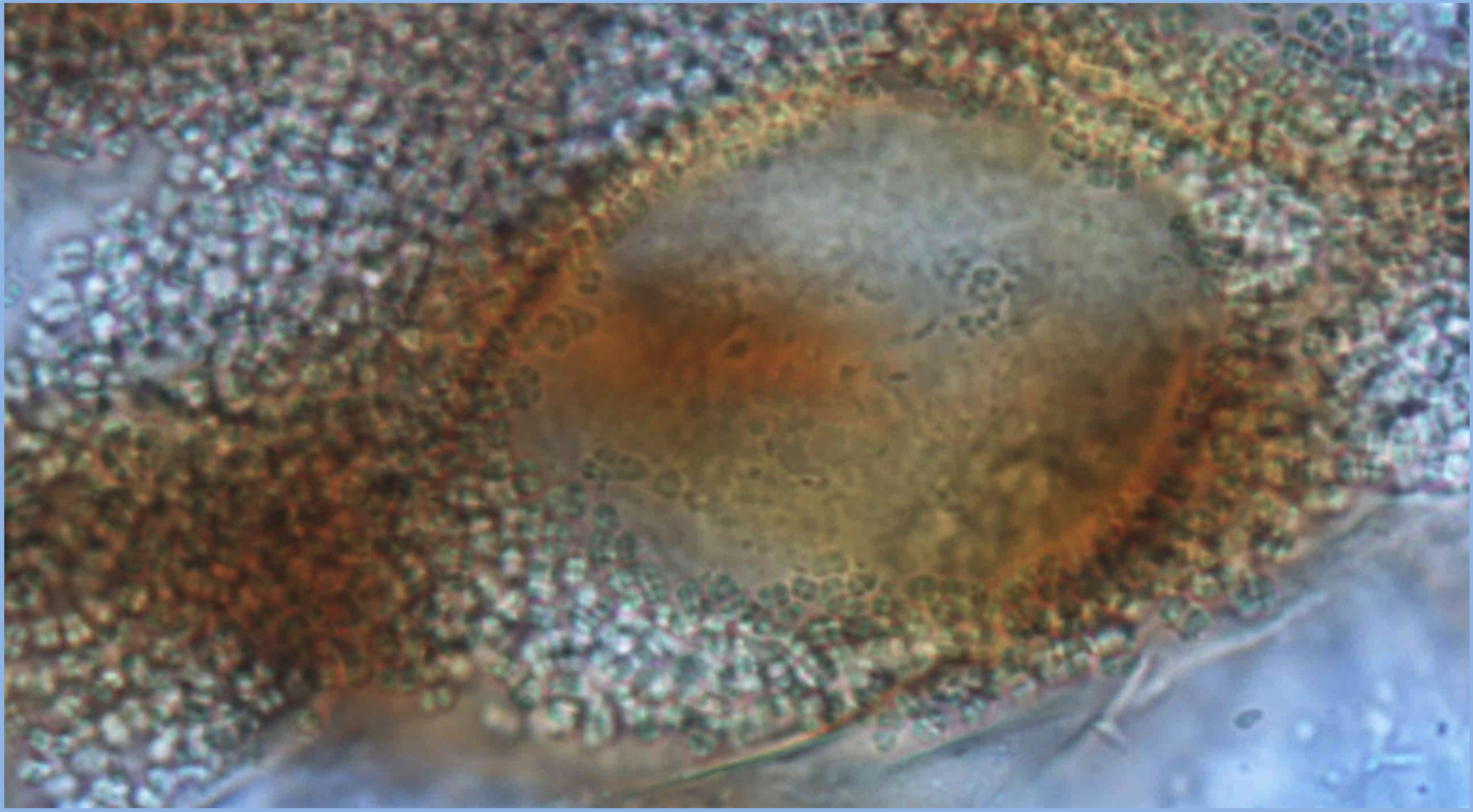
Rumex root hair initial showing emergence of bacteria at apex. Note also brown coloration due to high levels of H_2O_2 in cells.



Rumex root hair initial showing bacterial emergence to surface (arrow). Bacterial cells appear to be spilling off of the hairs and accumulating around the hairs. Note that the youngest (smallest and lightest staining) bacterial cells are on the surface of the hair initial.



Bacterial cells emerging to the surface from root hair initial of *Rumex*.



Rumex root hair initial showing bacterial cells emerging to the surface. Note slightly greenish canals through cell wall. Also note greenish spherical structures beneath cell wall (arrow). These spherical lightly-staining structures may be L-forms of the bacteria.

L-form bacteria appear to exit plant cells from pores formed in the cell walls. Below is a root hair of Bermuda grass showing bacteria (*Bacillus amyloliquefaciens*; arrows) exiting plant cell to reform rods on the root cell surface.

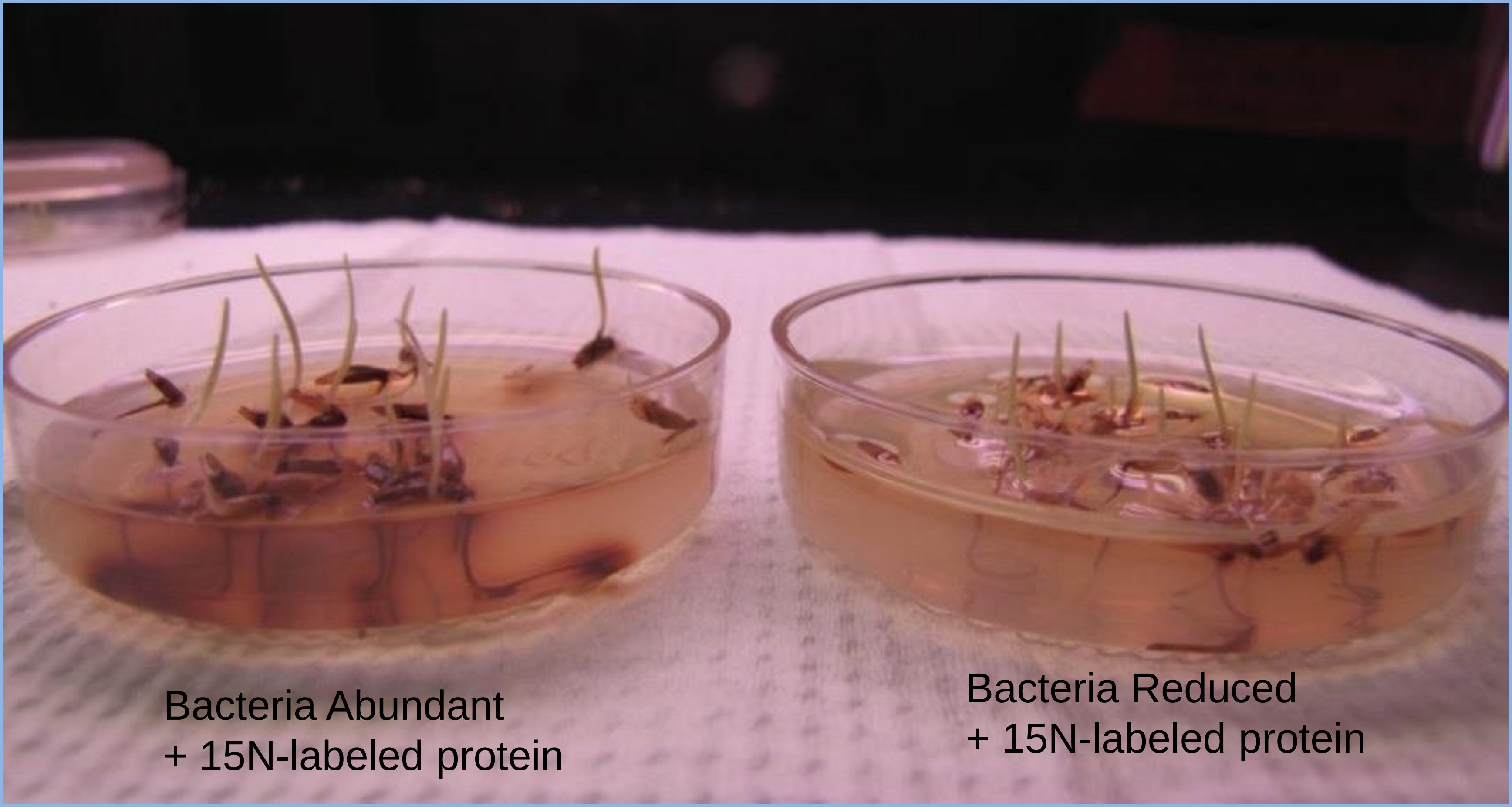
Nutrient Absorption Function of the Rhizophagy Cycle:

1. Isotope tracking experiments.

15N-labeled protein absorption experiment:

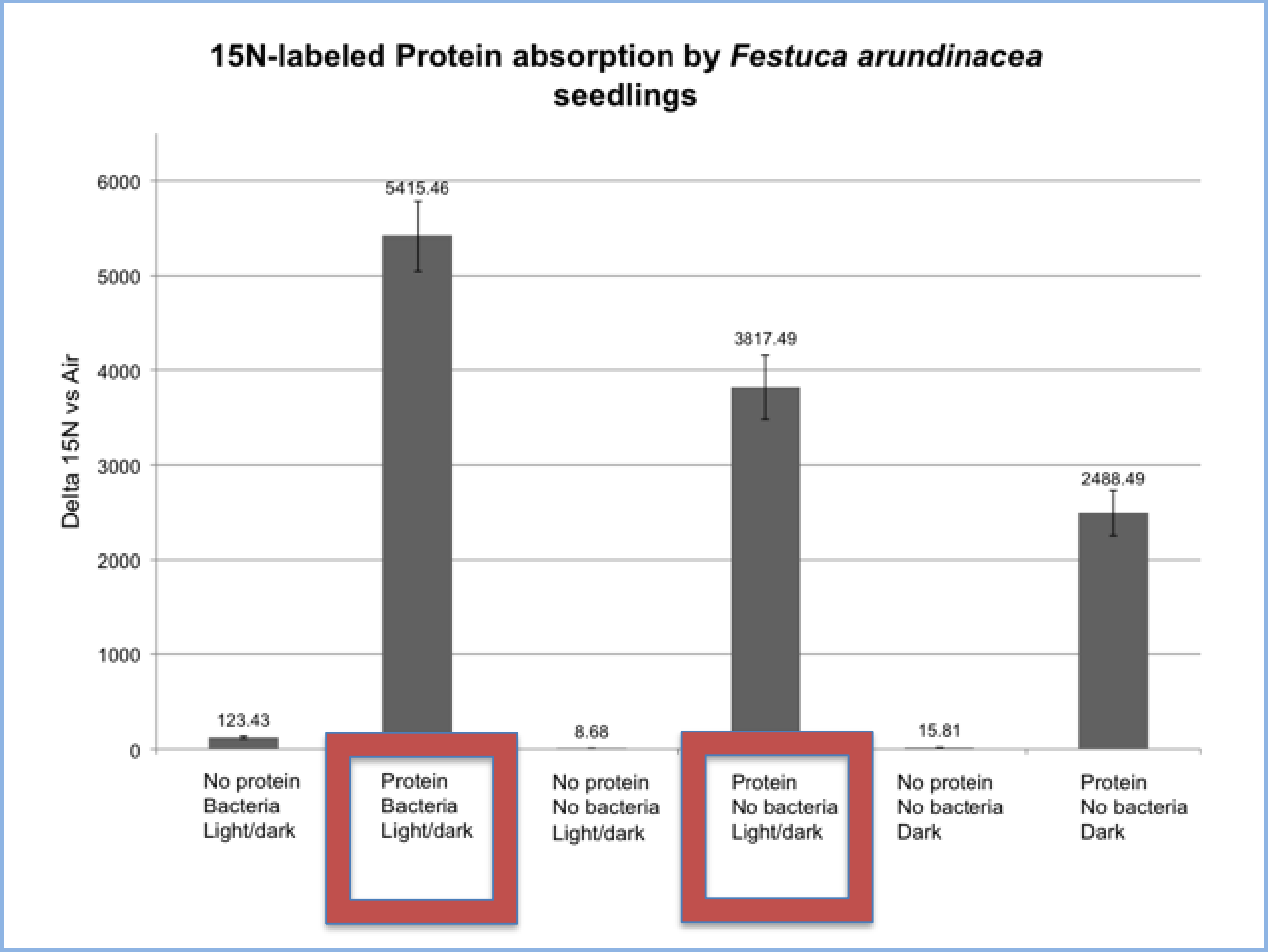
- *Bacillus amyloliquefaciens* grown in 15N-labeled glycine medium
- Total proteins extract from bacterial cells and freeze dried
- Proteins mixed with egg albumin at ration of 1:5
- Proteins (0.05%) incorporated into 0.7% agarose
- Tall fescue seeds with and without bacteria were germinated on the labeled protein media
- Seedling shoots analyzed for incorporation of 15N using Mass Spec Analysis

15N tracking experiment: 15-N labeled protein incorporated into agar.



15N-labeled protein absorption experiment: seed disseminated microbes increase labeled protein acquisition by seedlings.

Seedlings with bacteria absorb 30% more 15N-labeled nitrogen than seedlings that lack bacteria!



What nutrients does the rhizophagy cycle provide?

Examples of nutrients that may be extracted from bacteria oxidatively:

1. Proteins → nitrate
2. Nucleic acids → phosphate
3. Cellular cations of potassium → potassium oxide
4. Cellular ions of calcium → calcium oxide
5. Macro- and micro-nutrients → oxidized forms

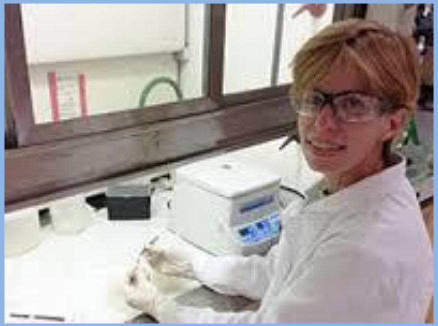
Hill et al. (2013) reported that absorption of N via direct degradation of bacteria was 10 to 100X slower than absorption of mineralized N.

Rhizophagy may be more important for micronutrient acquisition.

Hill, P. W., Marsden, K. A., & Jones, D. L. (2013). How significant to plant N nutrition is the direct consumption of soil microbes by roots? *The New Phytologist*, *199*(4), 948–955. http://doi.org/10.1111/nph.12320

What happens to plants without the rhizophagy cycle?

Satish K. Verma

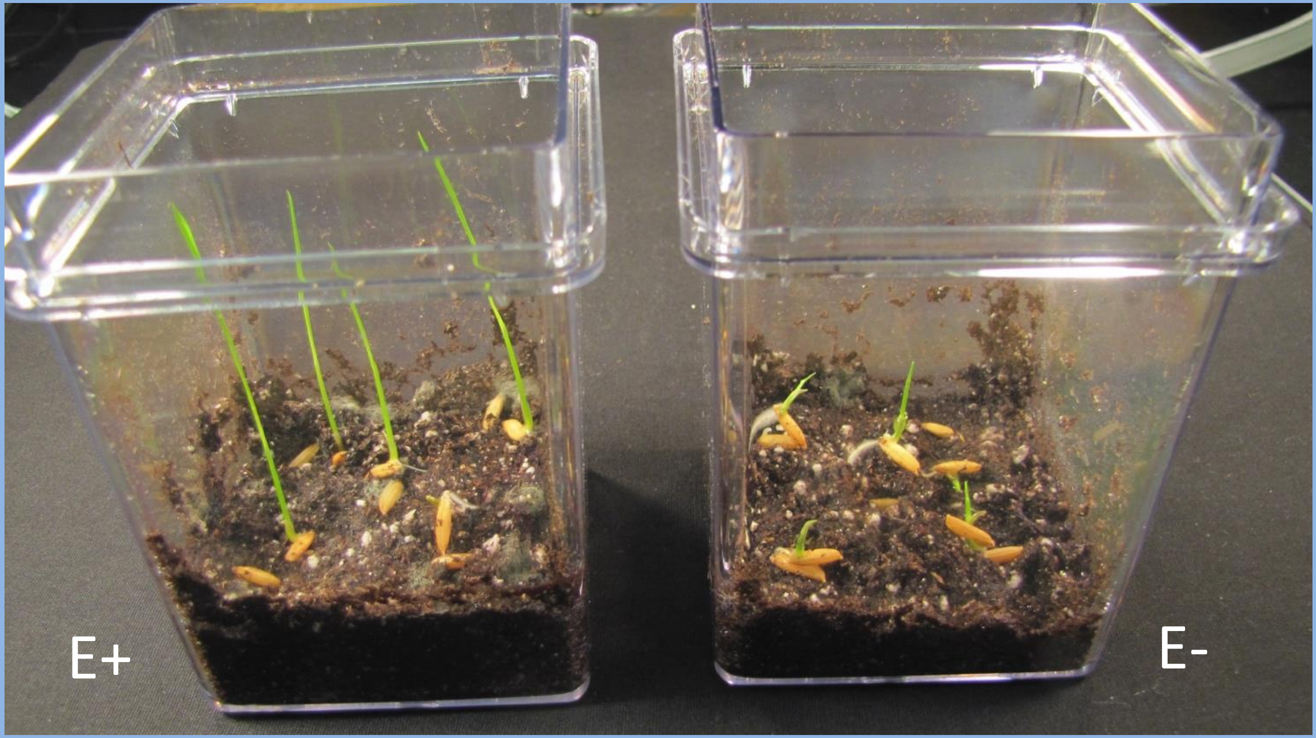


Kate Kingsley

Kurt Kowalski

Rice:

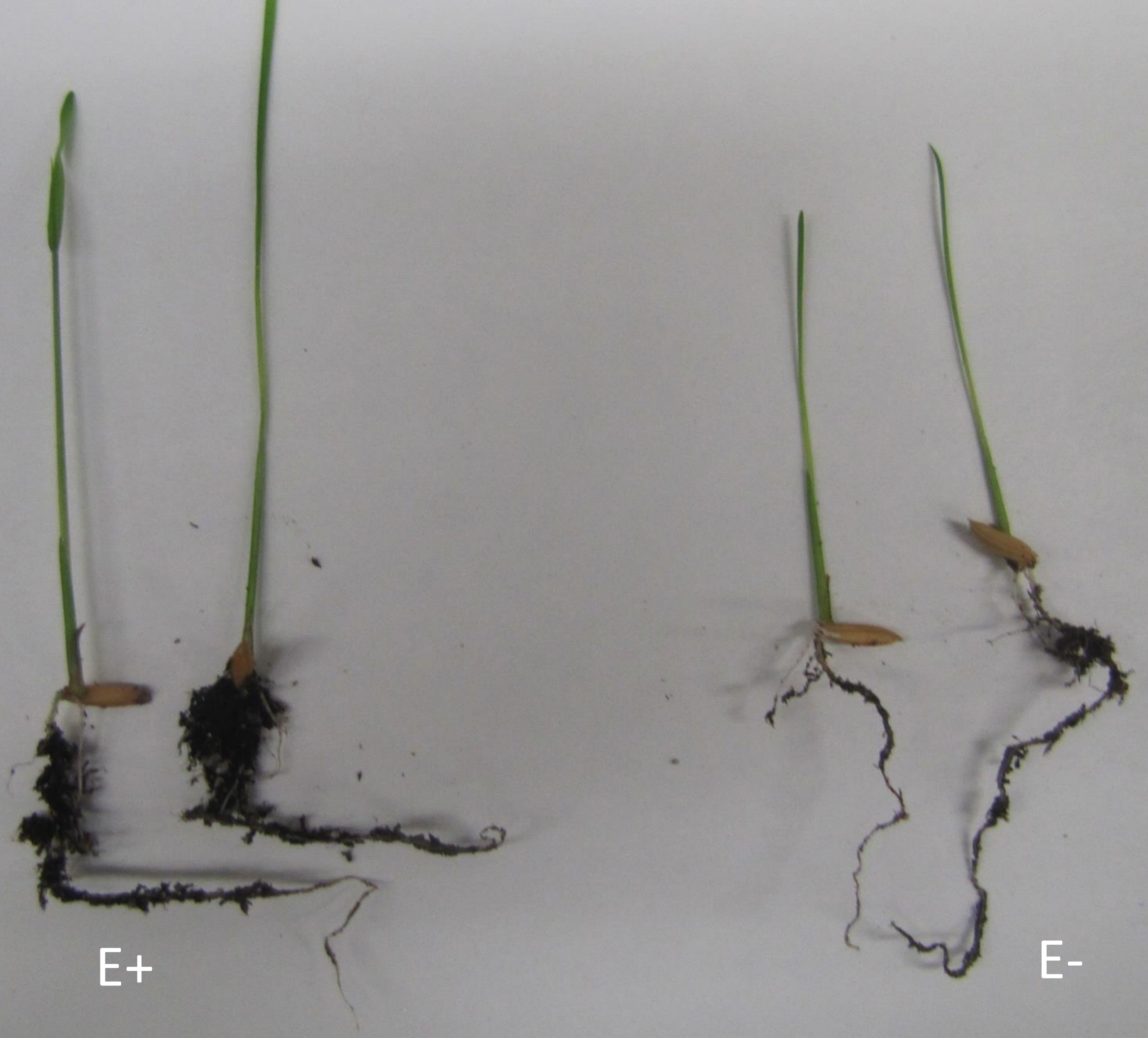
Growth
Promotion!



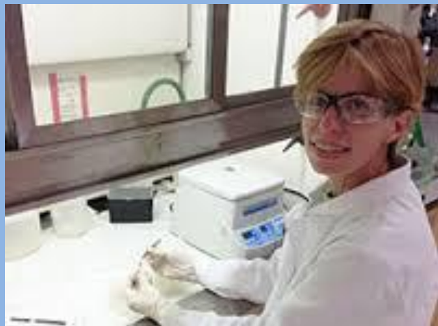
1. Endophytes removed from rice by surface sterilization.
2. Endophytes (*Pseudomonas* spp.) isolated from *Phragmites australis* inoculated onto seeds to restore development.

Rice experiment

Endophyte = *Pseudomonas* sp. (from *Phragmites*)



Acknowledgments







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