



'The Latest on Turfgrass Endophytes: Current and Future Research'

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What are endophytes?

(Botany): Endophytic/endosymbiotic non-pathogenic microbes (fungi, bacteria or algae) present asymptotically for all or part of their life cycles in tissues of plants.



(Medical definition): A tumor that grows like a parasite into other tissues.

Fungal hyphae of endophyte in stem tissue of tall fescue grass.

All plants naturally have endophytes!



*Endophytes
are
everywhere!*

Every plant contains multiple endophytes!

Microbial Endophytes

- Fungi and bacteria
- Common in all plants
- Intracellular and intercellular
- Improve plant stress tolerance
- Suppress plant pathogenic fungi
- Modulate root development
- Improve nutrient absorption

‘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 tetrahydrochloride (DAB) overnight.

Reactive oxygen secretion is used by all eukaryotes to kill endoparasitic bacteria.

It is part of the innate Defensive system of all Eukaryotes.

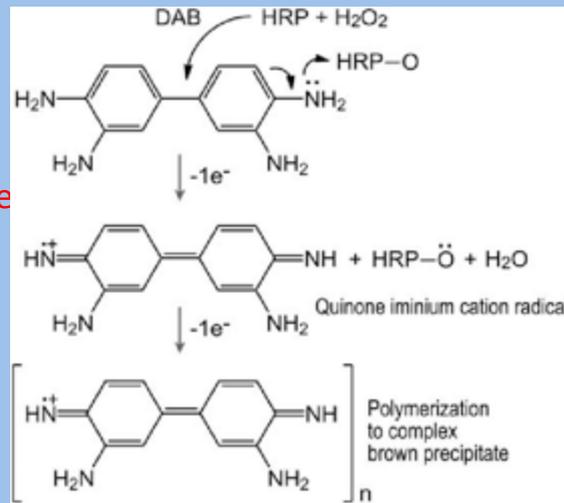
How DAB Works:

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

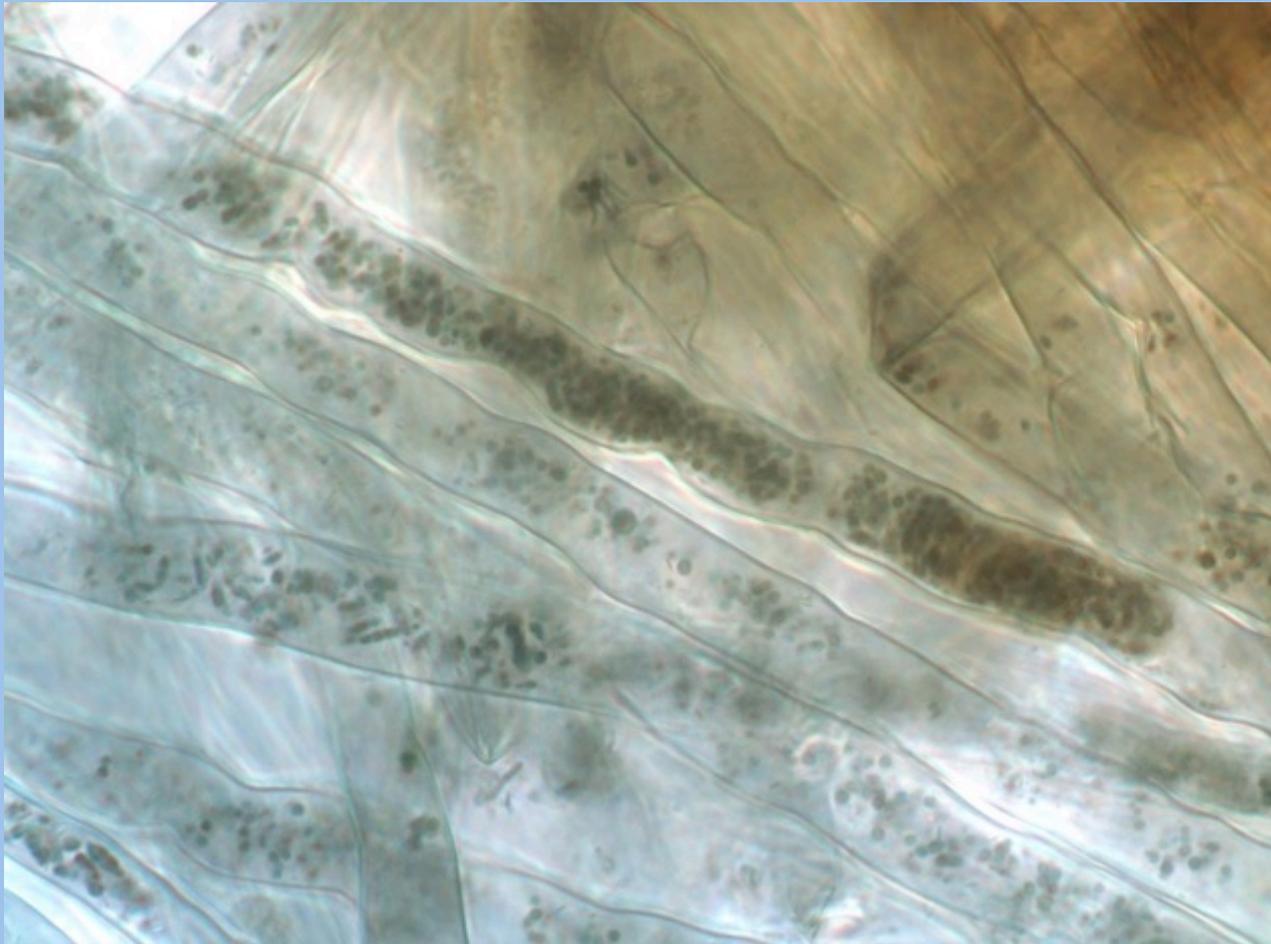
2) Plant uses superoxide dismutase to transform Superoxide to water and Hydrogen peroxide.

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

HRP is peroxidase



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



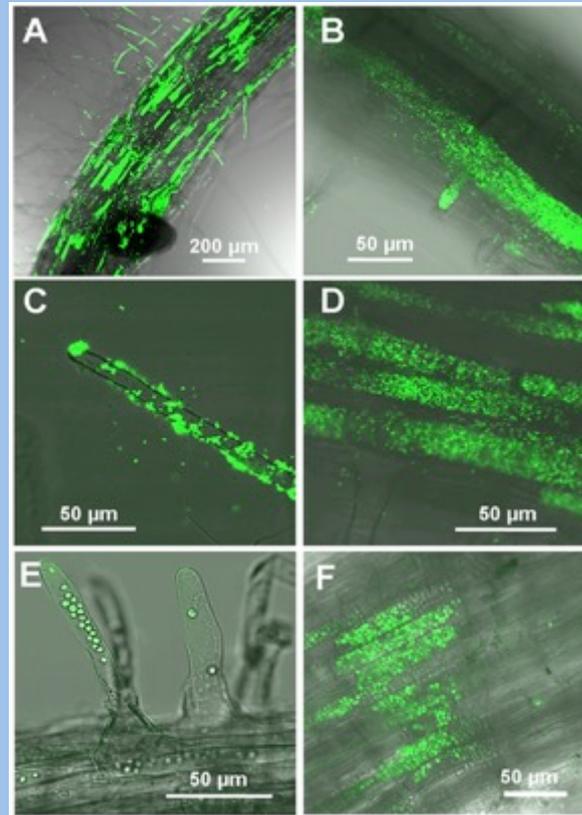
Bacteria in root hairs showing recently divided pairs



Figure 1. Roots of axenically grown *Arabidopsis* and tomato were incubated with *E. coli* or yeast expressing green fluorescent protein (GFP). *E. 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 the Table: Plants Consume Microbes as a Source of Nutrients.
PLoS ONE 5(7): e11915, doi:10.1371/journal.pone.0011915

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Rhizophagy Cycle: An Oxidative Process in Plants for Nutrient Extraction from Symbiotic Microbes. *Microorganisms* **2018**, *6*, 95; <https://doi.org/10.3390/microorganisms6030095>



microorganisms



Review

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

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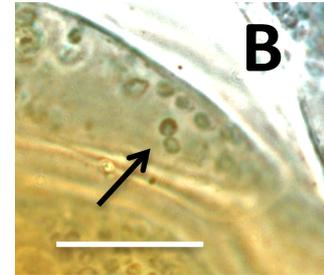
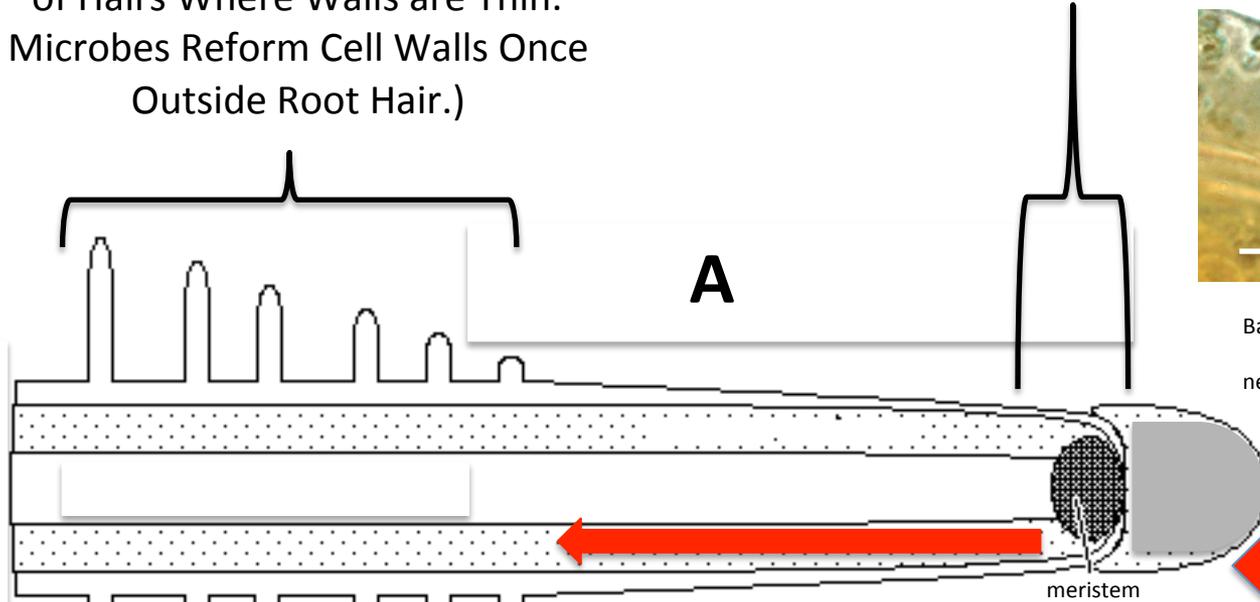
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Microbe Exit Zone

(Microbes Stimulate Elongation of Root Hairs and Exit at the Tips of Hairs Where Walls are Thin. Microbes Reform Cell Walls Once Outside Root Hair.)

Plant Cell Entry Zone

(Microbes Become Intracellular in Meristem Cells as Wall-less Protoplasts.)



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

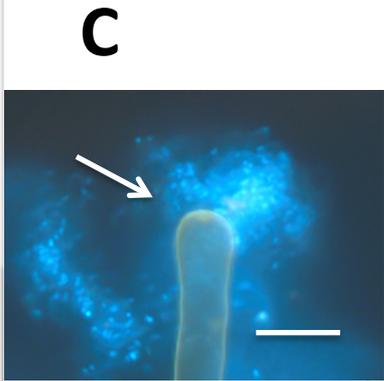
Nutrients Extracted from Microbes By Reactive Oxygen Produced by NOX on Root Cell Plasma Membranes

Microbes Enter Root Cell Periplasmic Spaces Carrying Nutrients From Soil

RHIZOPHAGY CYCLE

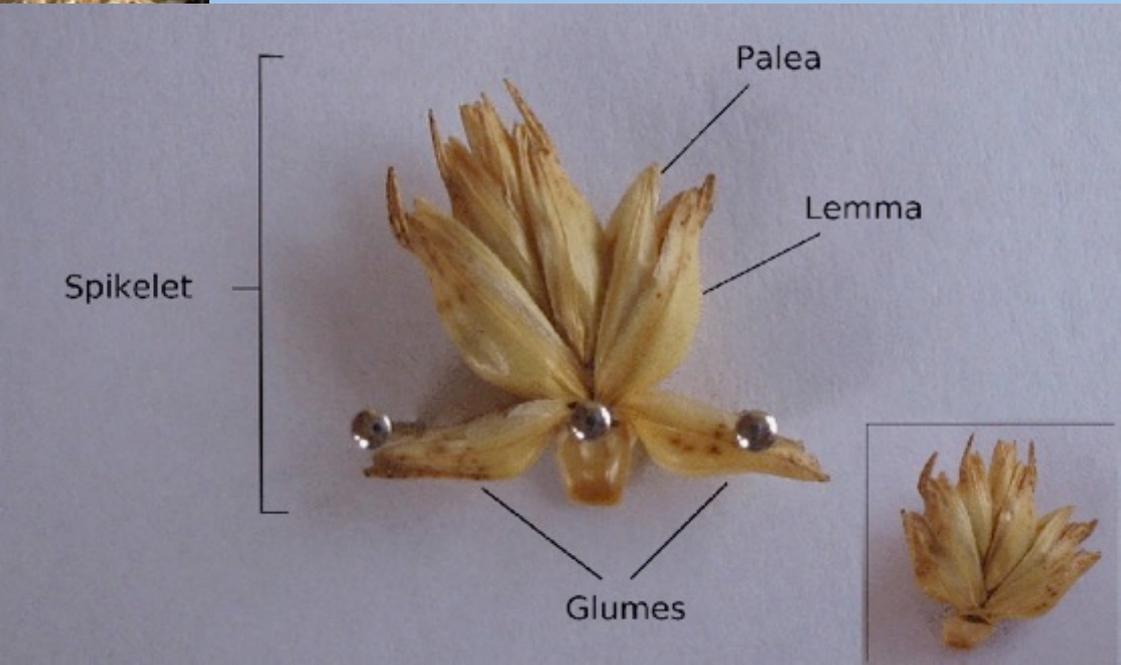
Microbes Recharge with Nutrients in the Rhizosphere

Microbes Exit Root Hairs Exhausted of Nutrients

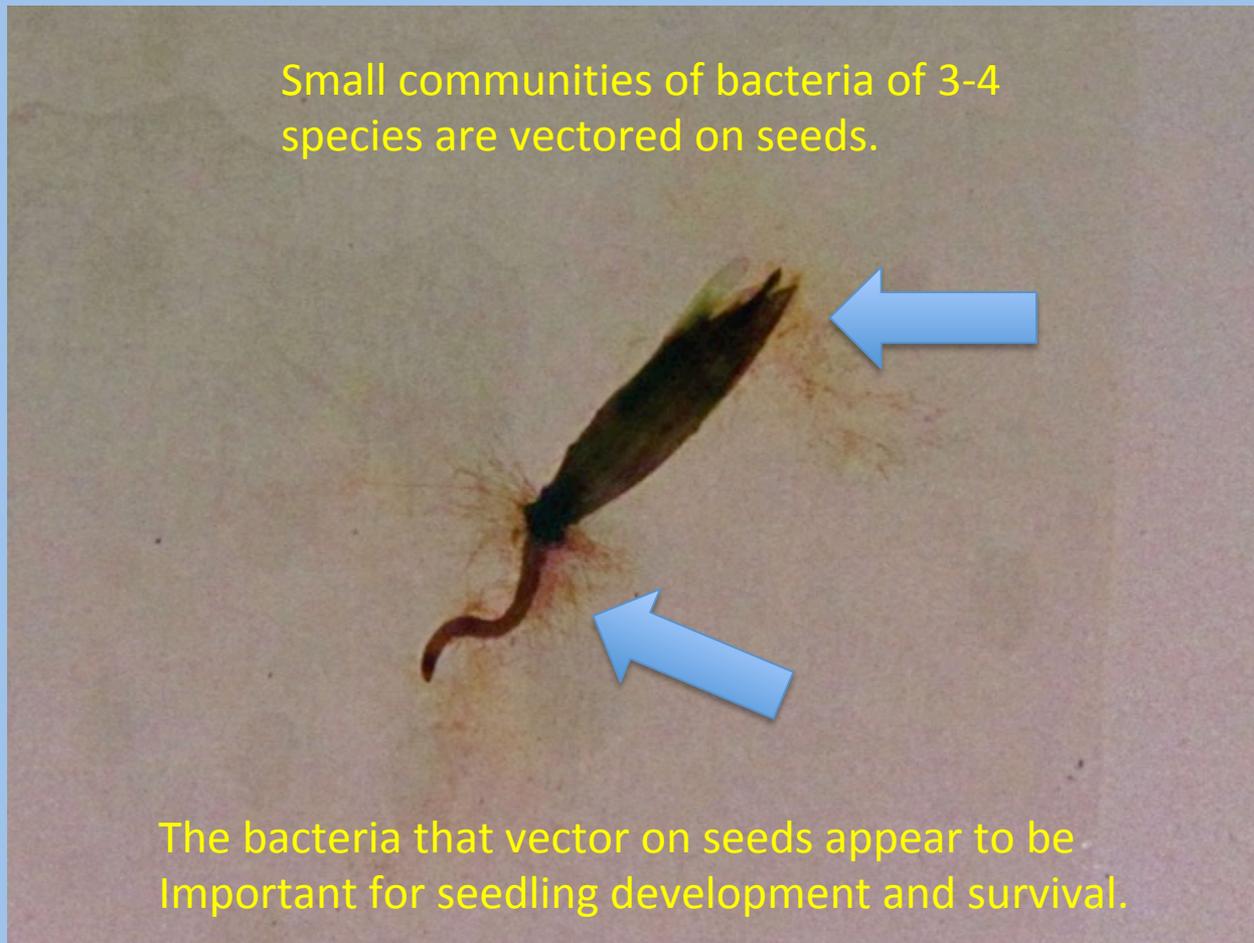


Bacteria (arrow) emerging from root hair tip of millet seedling.

Grasses vector symbiotic microbes on paleas and lemmas



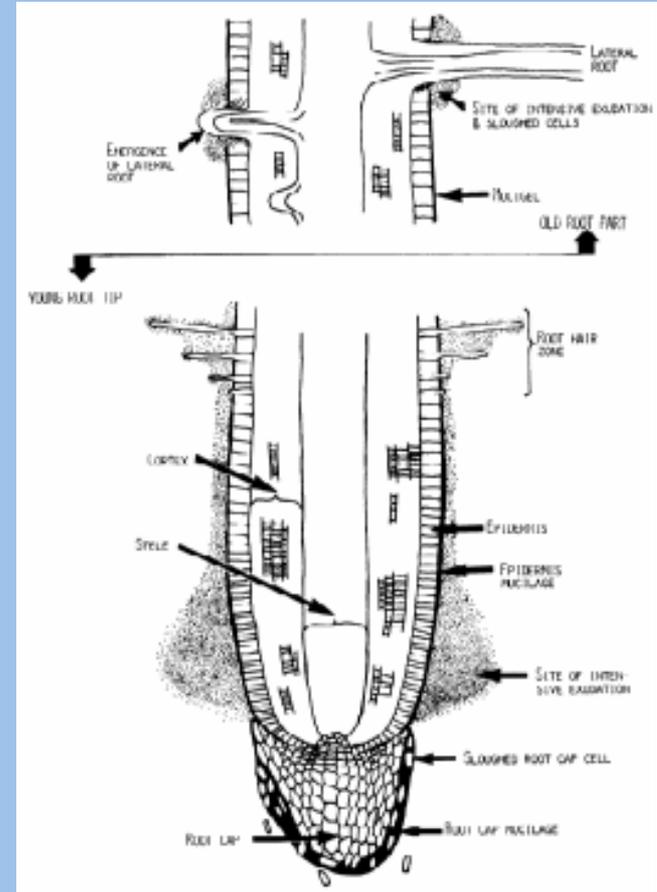
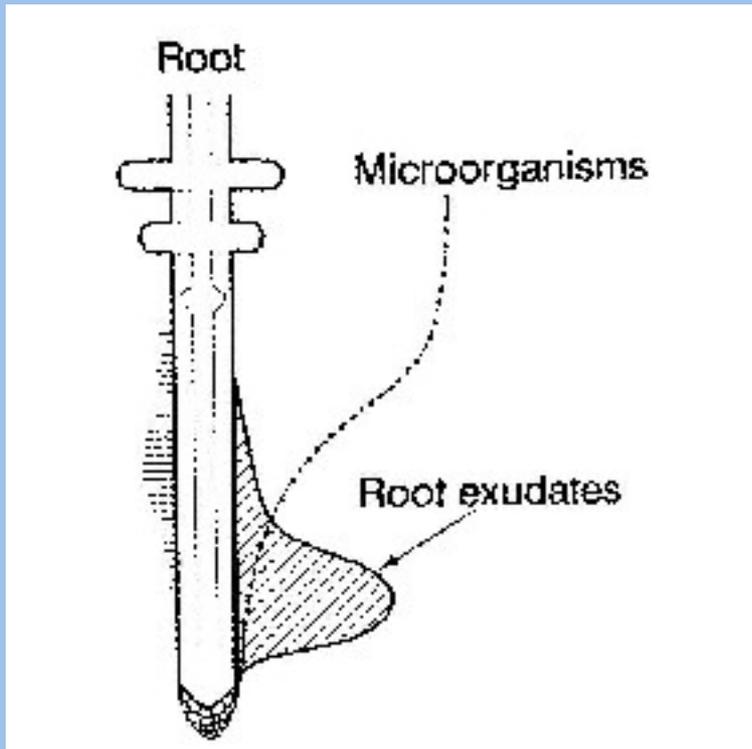
Bacterial symbiosis: germinating tall fescue seed showing seed-transmitted bacteria (*Pantoea agglomerans* and *Pseudomonas* sp.; both gamma-Proteobacteria)



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

Root exudation zones determined by ^{14}C experiments.

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

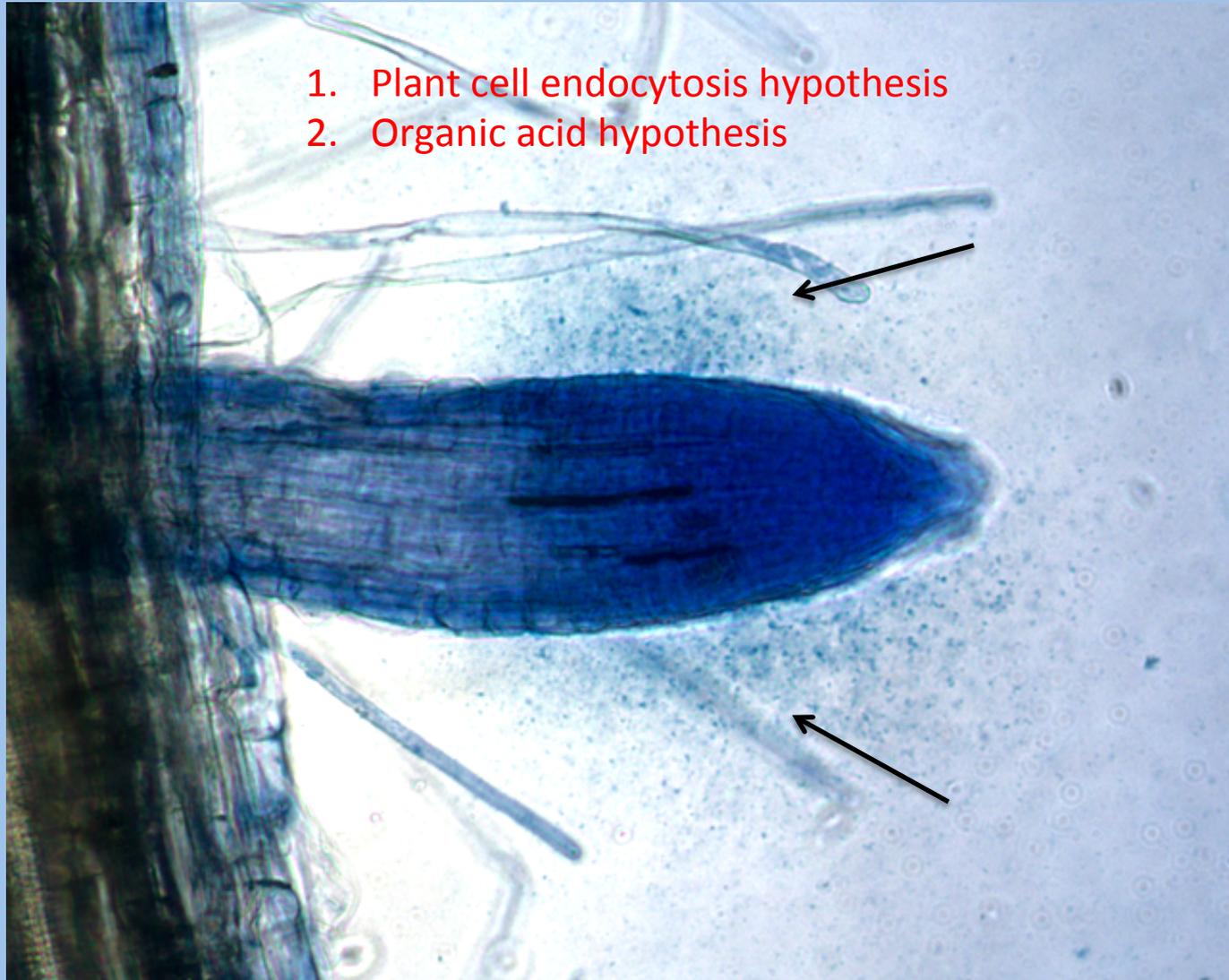


Secretion of exudates in a zone proximal to root tip meristems facilitates
microbe entry into cells of the plant meristem.

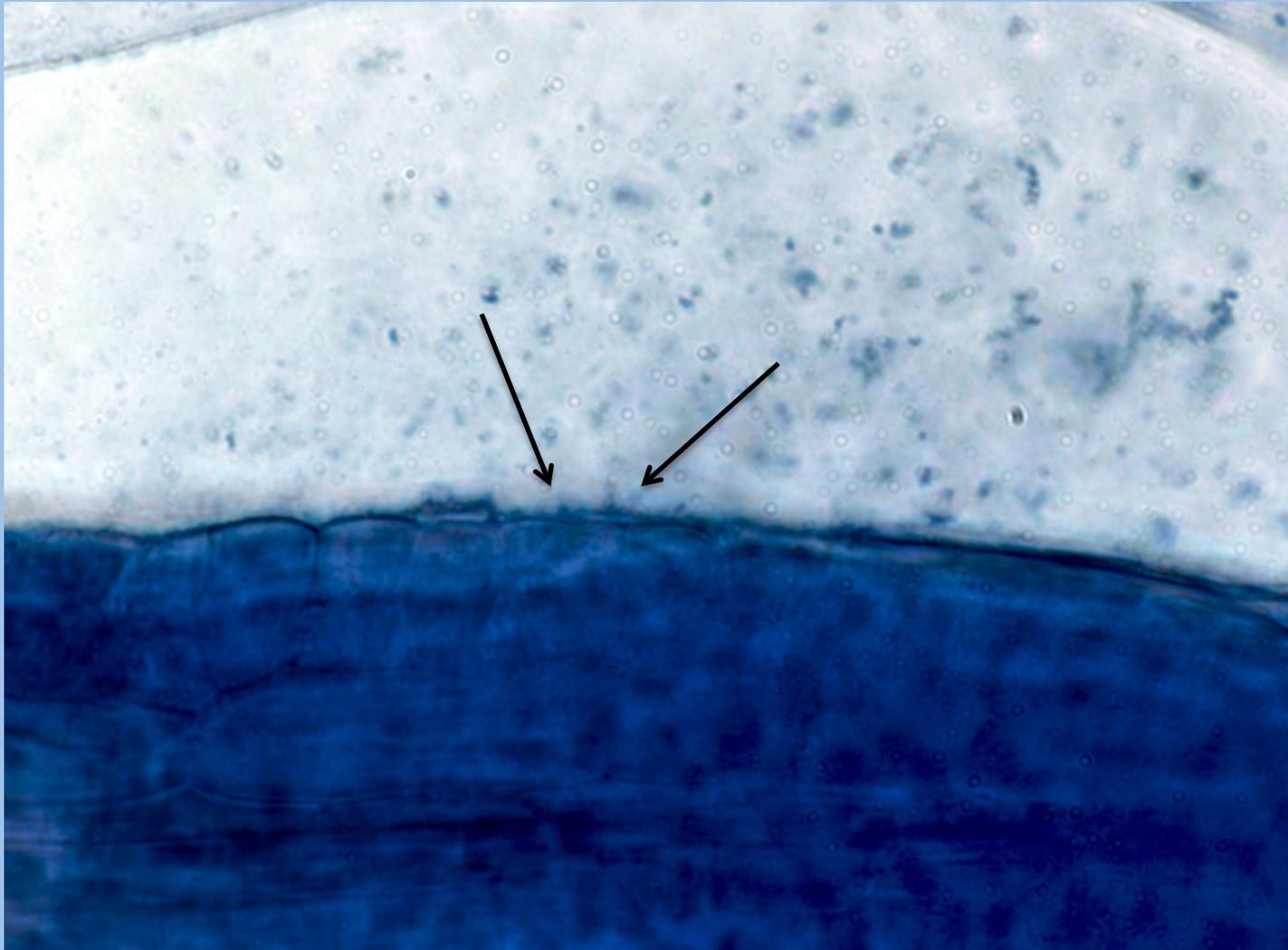
Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd edn., Academic Press, London.

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.



Bacteria (arrows) colonizing the epidermal cells in the zone of intracellular colonization. Bacteria will enter cells as walled bacteria—but soon lose cell walls after exposure to reactive oxygen (superoxide produced on root cell plasma membranes).



BERMUDA GRASS SEEDLING ROOT TIP IN AGAROSE WITHOUT MICROBES

CONSTITUTIVE SECRETION OF REACTIVE OXYGEN IN ROOT TIPS TRIGGER INFECTING MICROBES TO LOSE CELL WALLS.



The brown coloration is due to presence of reactive oxygen. This tissue was stained For 13 hours in diaminobenzidine tetrachloride (DAB)

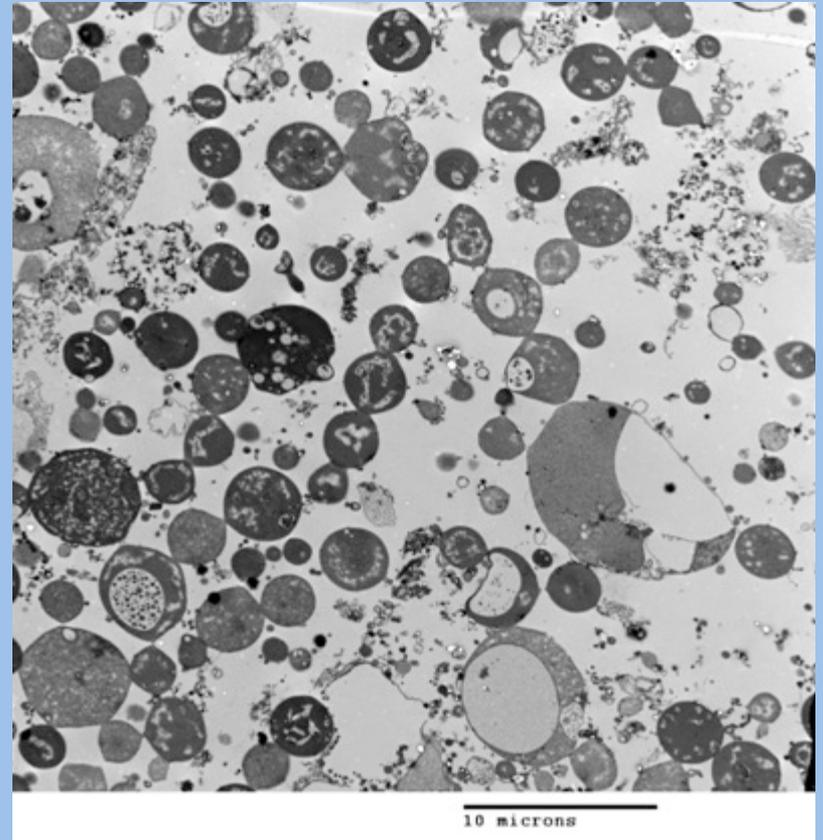
TEM of *Bacillus subtilis* L-forms

Photo by Mark Leaver, New Castle University, UK



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

L-forms = Protoplasts

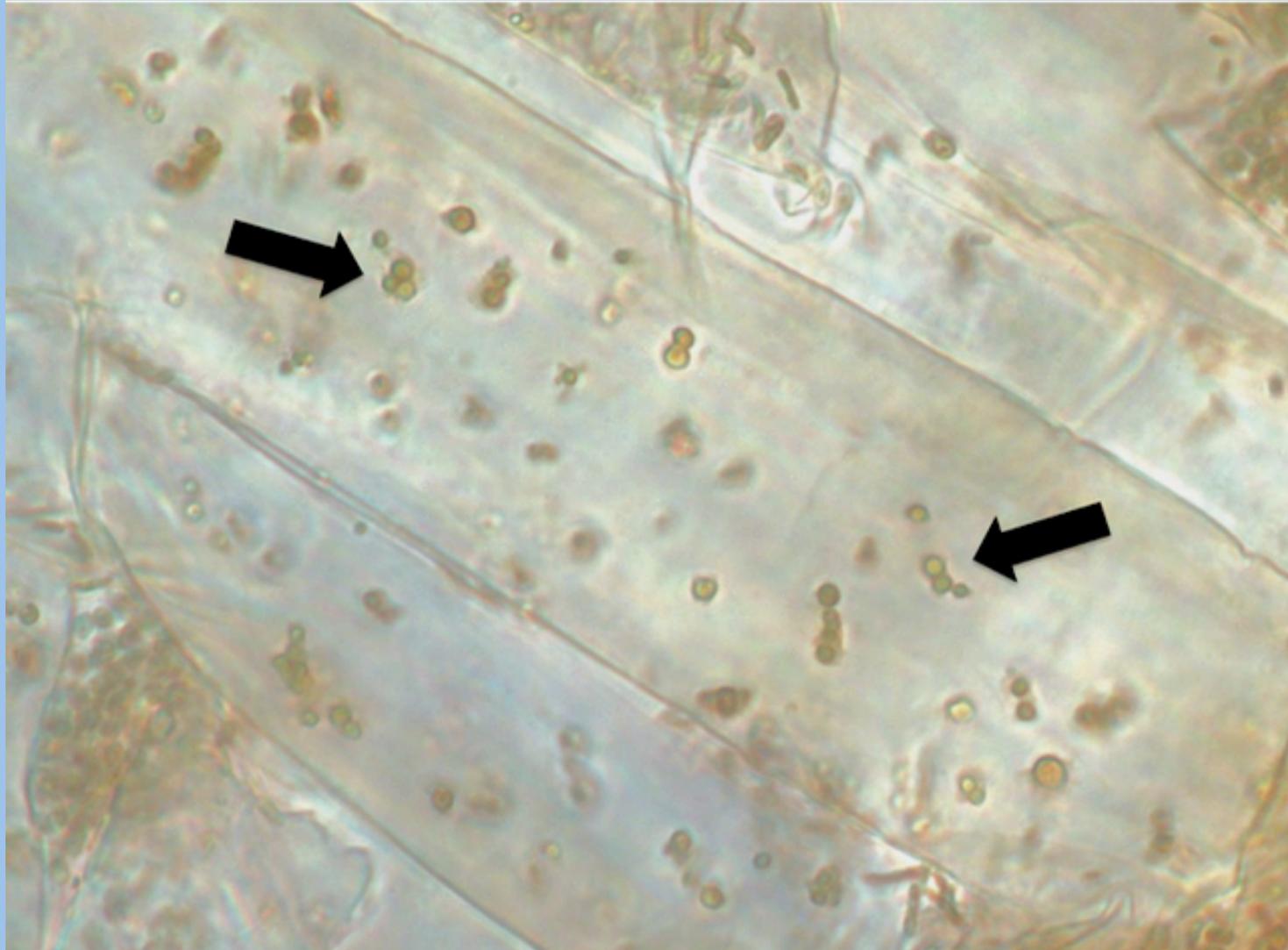


L-forms are bacterial cells that do not form cell walls (also called 'cell wall deficient bacteria'). L-forms typically are seen inside eukaryotic cells. They are thought to be a mechanism to evade host defense response. L-form bacteria are typically variable in size.

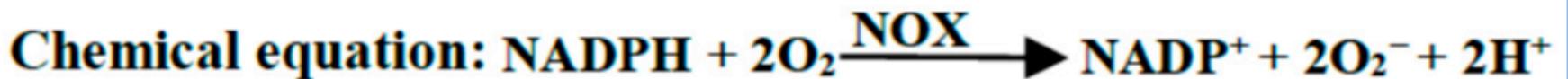
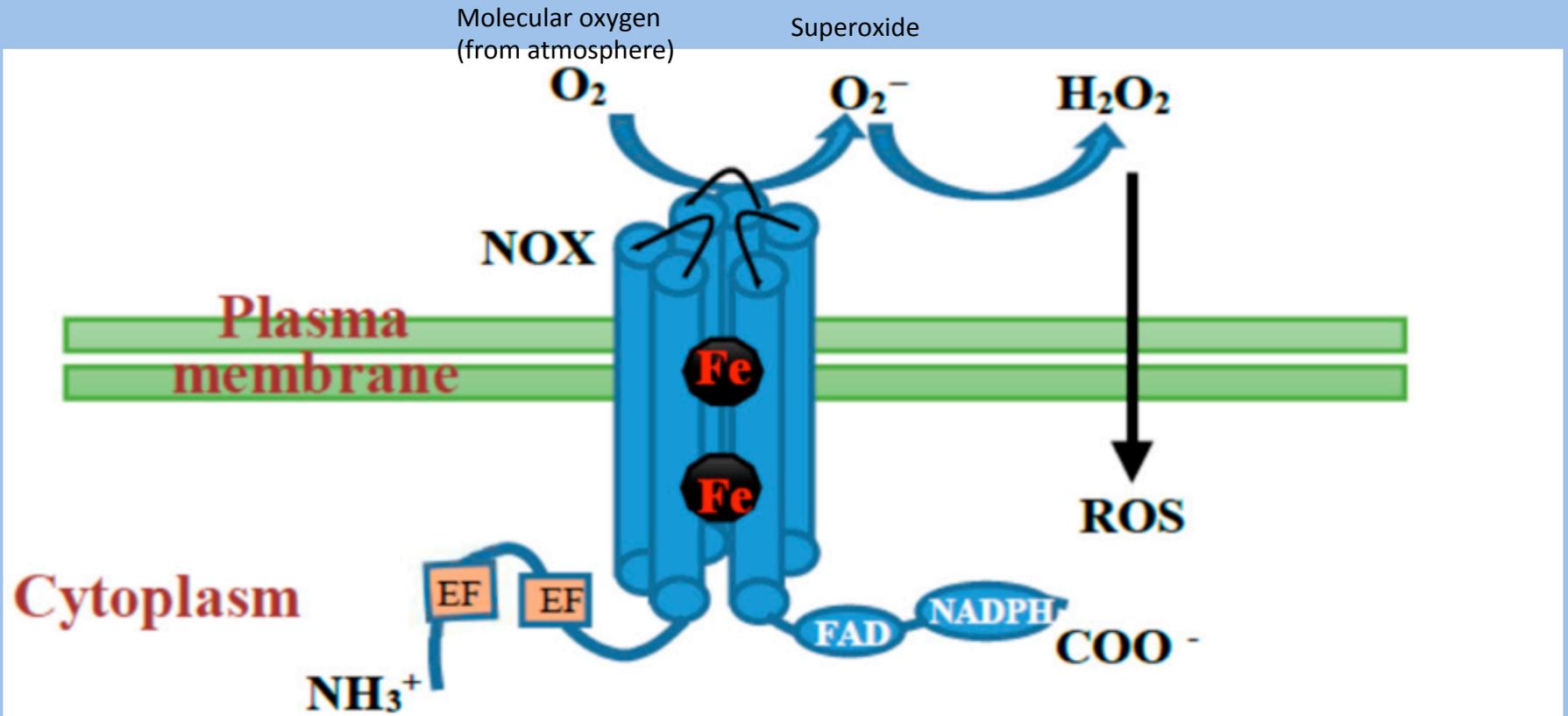
Giant Reed Grass (*Phragmites australis*)



Phragmites root stained with diaminobenzidine DAB to visualize reactive oxygen around bacterial protoplasts (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*.

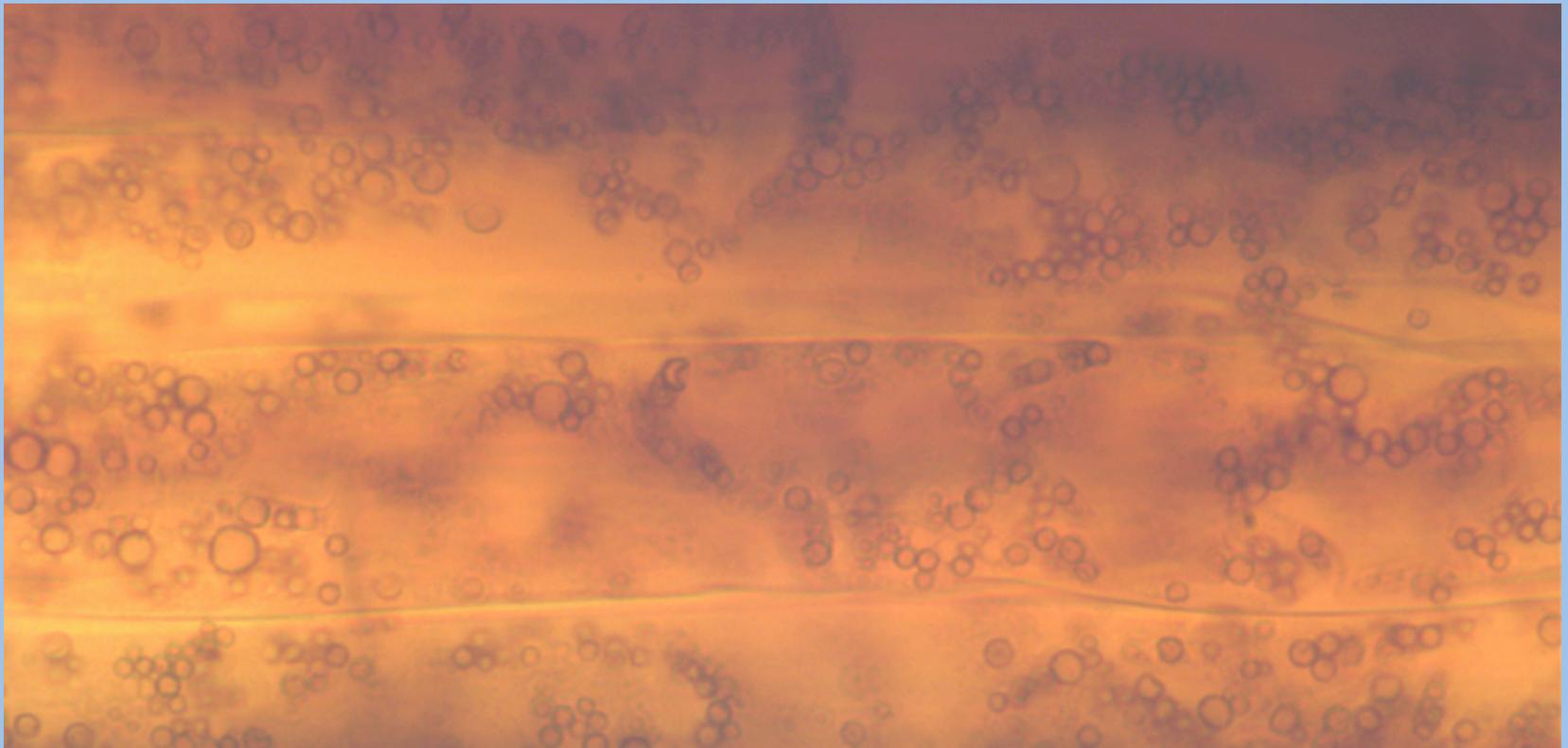


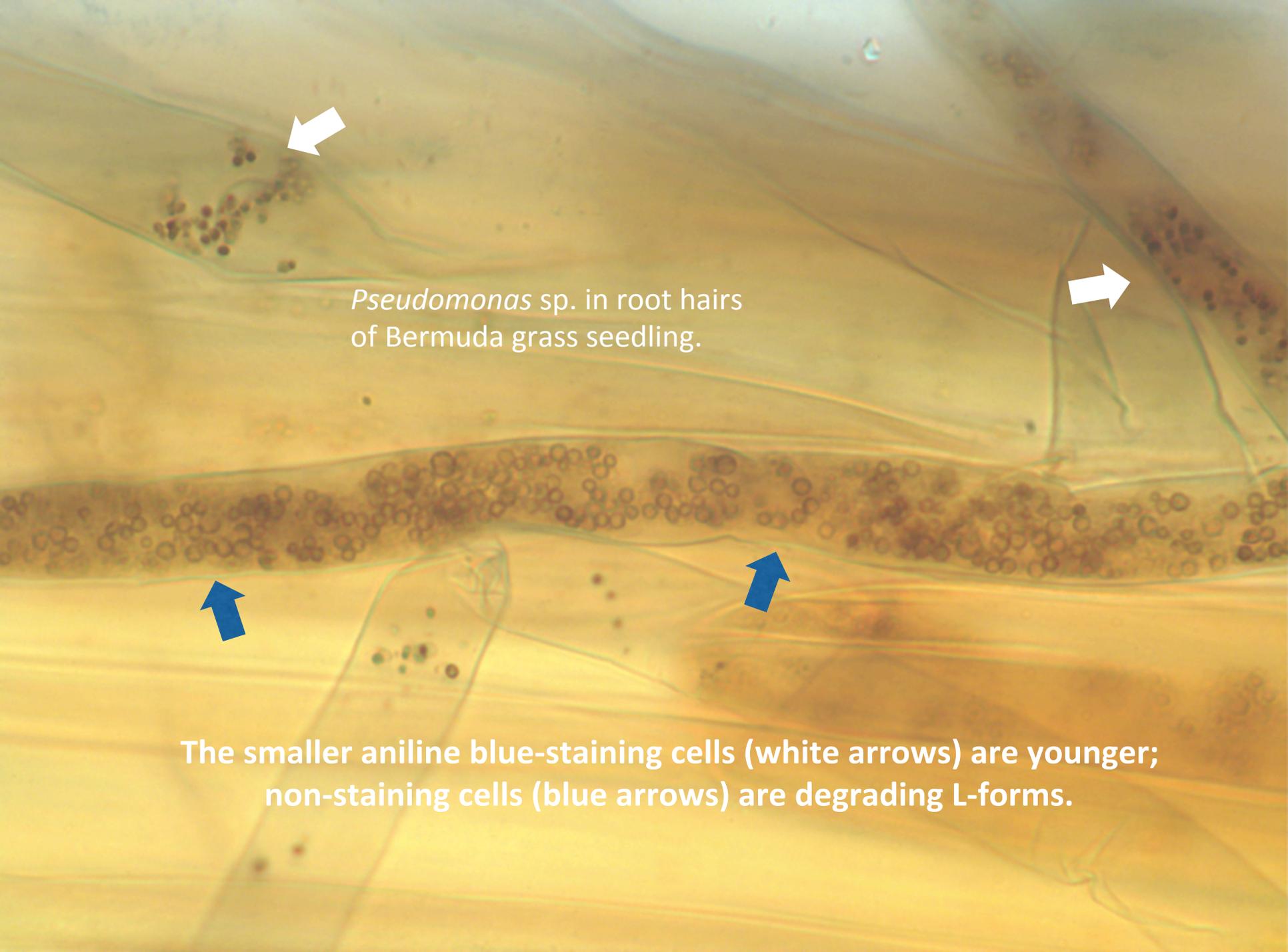
REACTIVE OXYGEN DEFENSE RESPONSE OF THE HOST CELL INVOLVES MEMBRANE-BOUND NADPH OXIDASES (NOX)



How reactive oxygen affects intracellular microbes

1. NADPH oxidase on plant cell plasma membrane converts O_2 to superoxide $O_2^{\cdot -}$.
2. Superoxide breaks down the microbial cell walls.
3. Superoxide damages proteins in the microbe plasma membrane.
4. Superoxide causes leakage in the microbe plasma membrane.
5. Superoxide enters microbe cells and damages proteins and nucleic acids.
6. Microbe protoplasts swell and lose internal proteins as contents are oxidized.





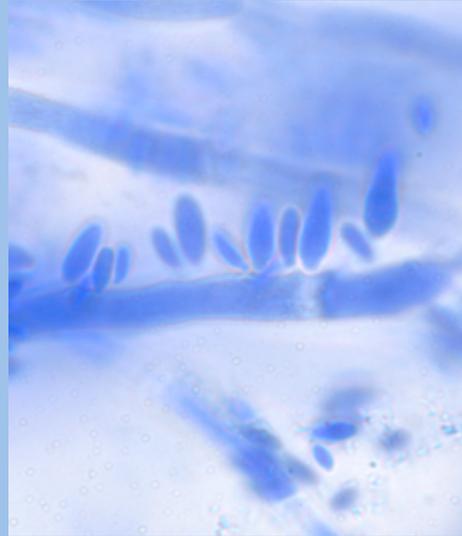
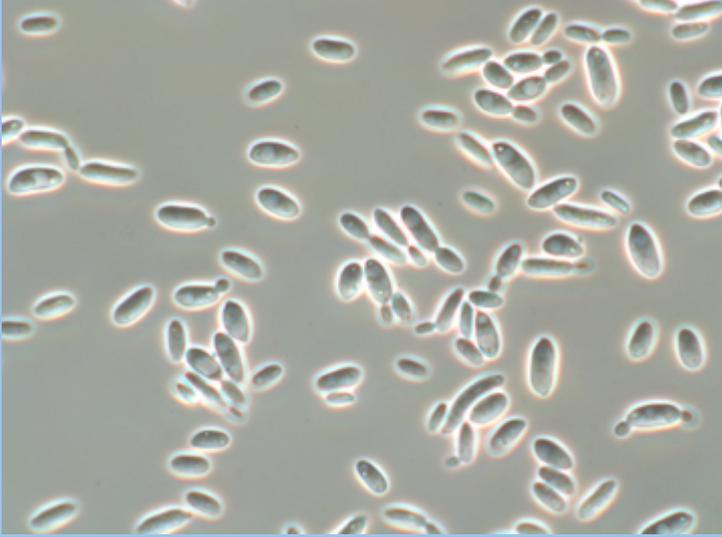
Pseudomonas sp. in root hairs
of Bermuda grass seedling.

The smaller aniline blue-staining cells (white arrows) are younger;
non-staining cells (blue arrows) are degrading L-forms.

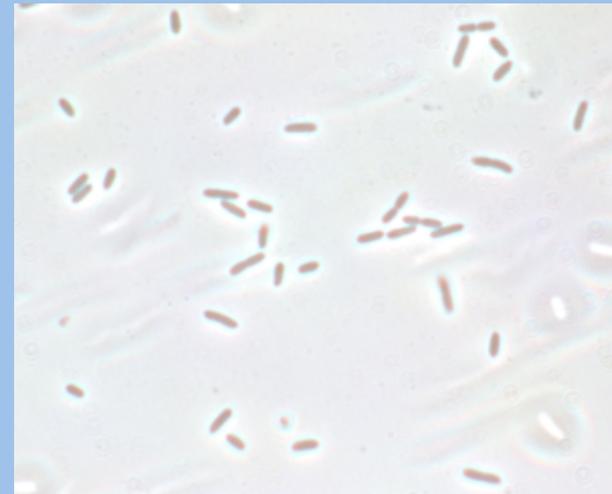
Snakecotton (*Froelichia gracilis*) is an invasive weed in many parts of North America; seeds were sourced from wild populations in New Jersey.



Seed-vectored endophytes from snakecotton seedlings include:



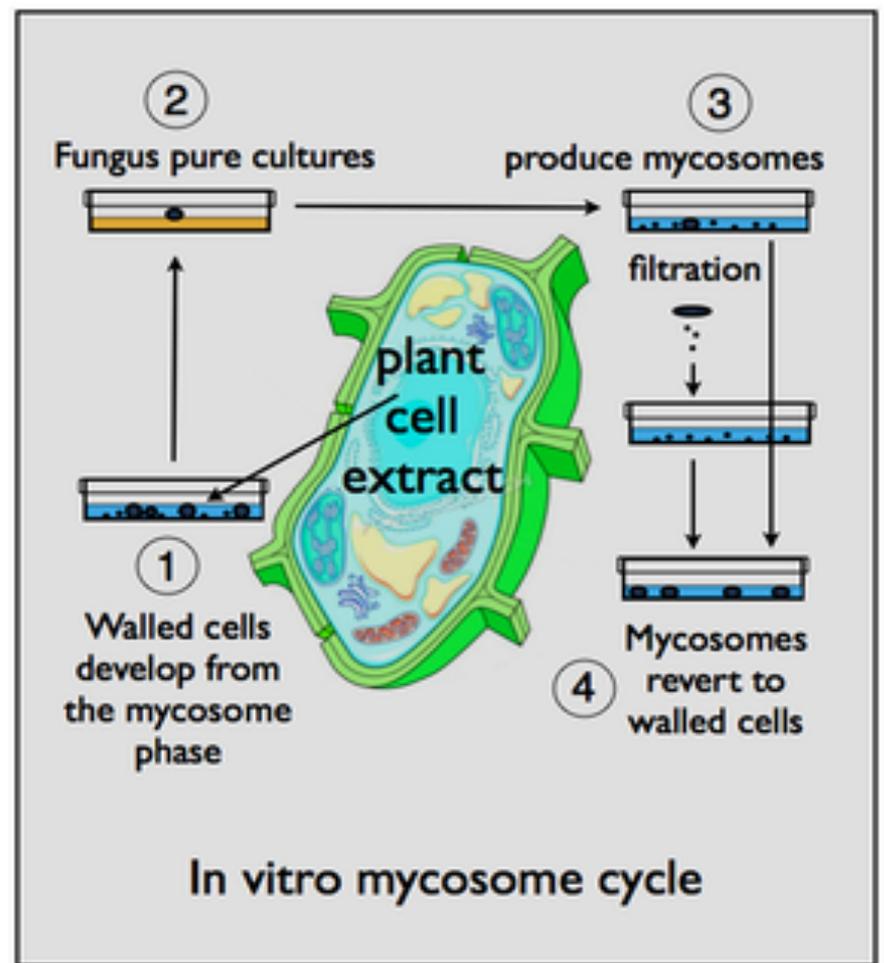
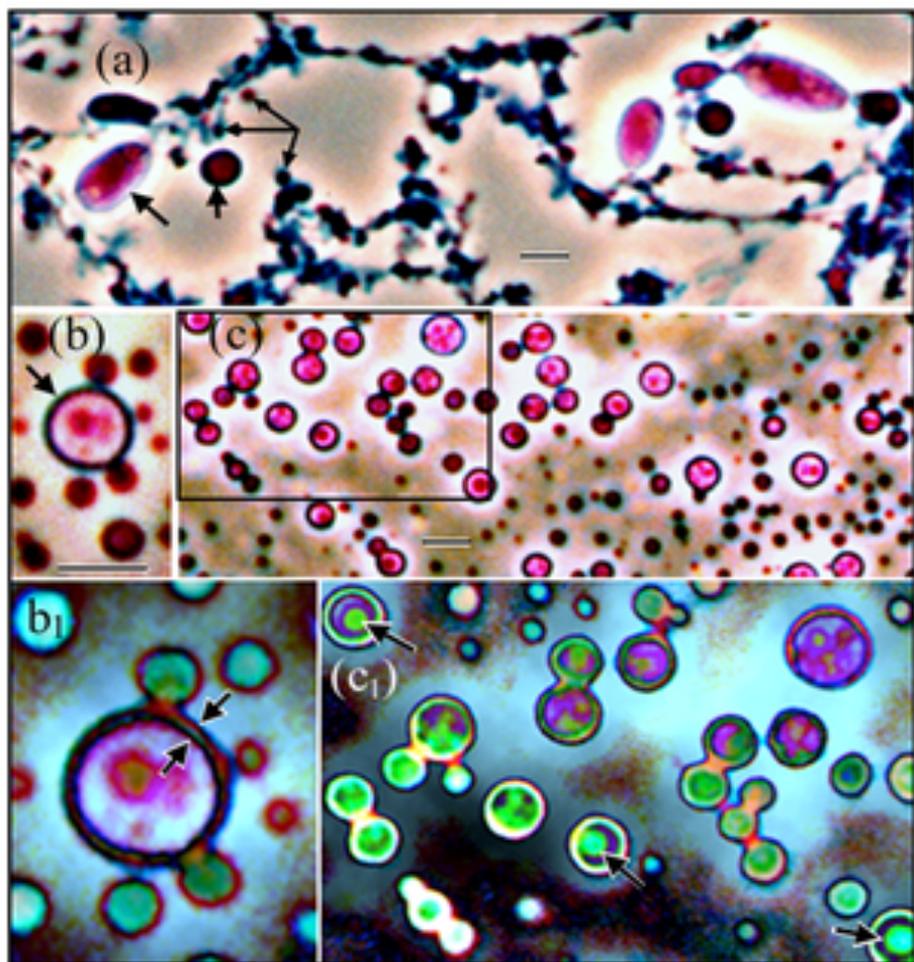
Yeast: *Aureobasidium pullulans* (Froelichia # 2)



Bacterium: *Curtobacterium* sp. (Froelichia #4)

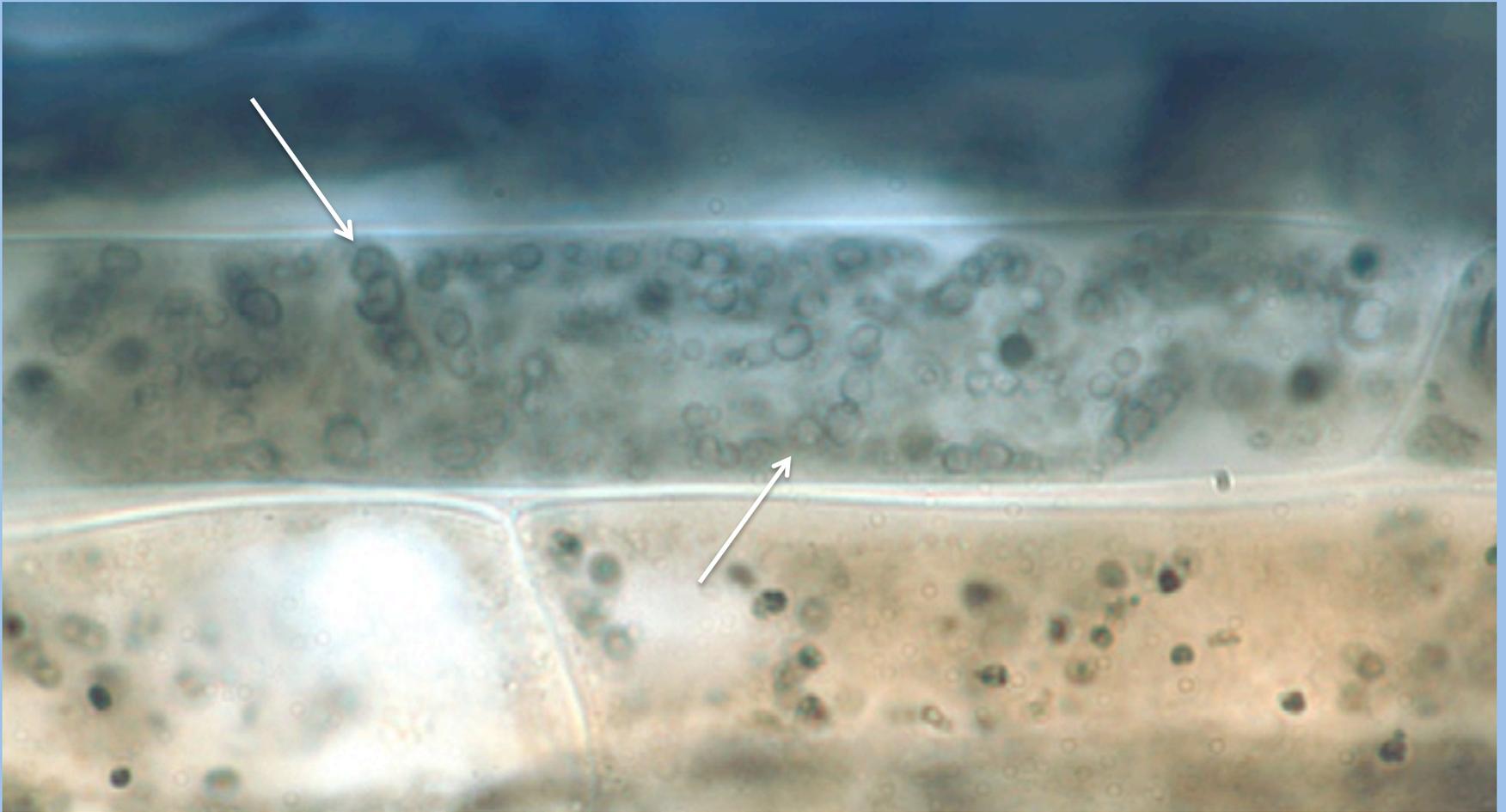
Identifications of bacteria made using Sequences of rDNA and blast to NCBI database. *Aureobasidium pullulans* identified using morphological features in culture.

Intracellular phases of fungi may form protoplasts called **'mycosomes'**.



Atsatt PR, Whiteside MD (2014) Novel Symbiotic Protoplasts Formed by Endophytic Fungi Explain Their Hidden Existence, Lifestyle Switching, and Diversity within the Plant Kingdom. PLOS ONE 9(4): e95266. <https://doi.org/10.1371/journal.pone.0095266>
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0095266>

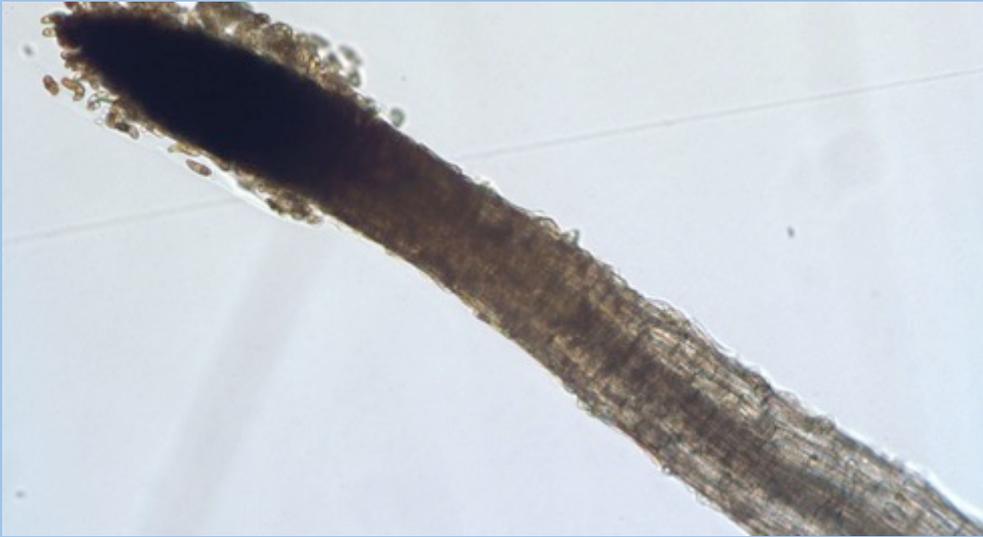
Mycosomes (arrows) in snakecotton seedling root parenchyma.



Rhizophagy cycle microbes modulate development of seedlings

- Microbes trigger the gravitropic response in roots
- Microbes trigger root hair elongation
- Microbes increase root branching
- Microbes increase root and shoot elongation

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



Root tip

More developed region of seedling root

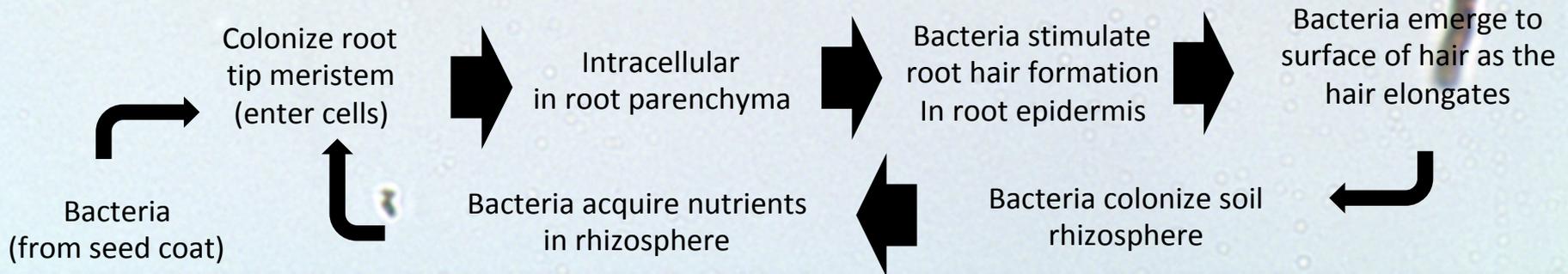


Bermuda grass root containing *Pseudomonas* (bacterium)

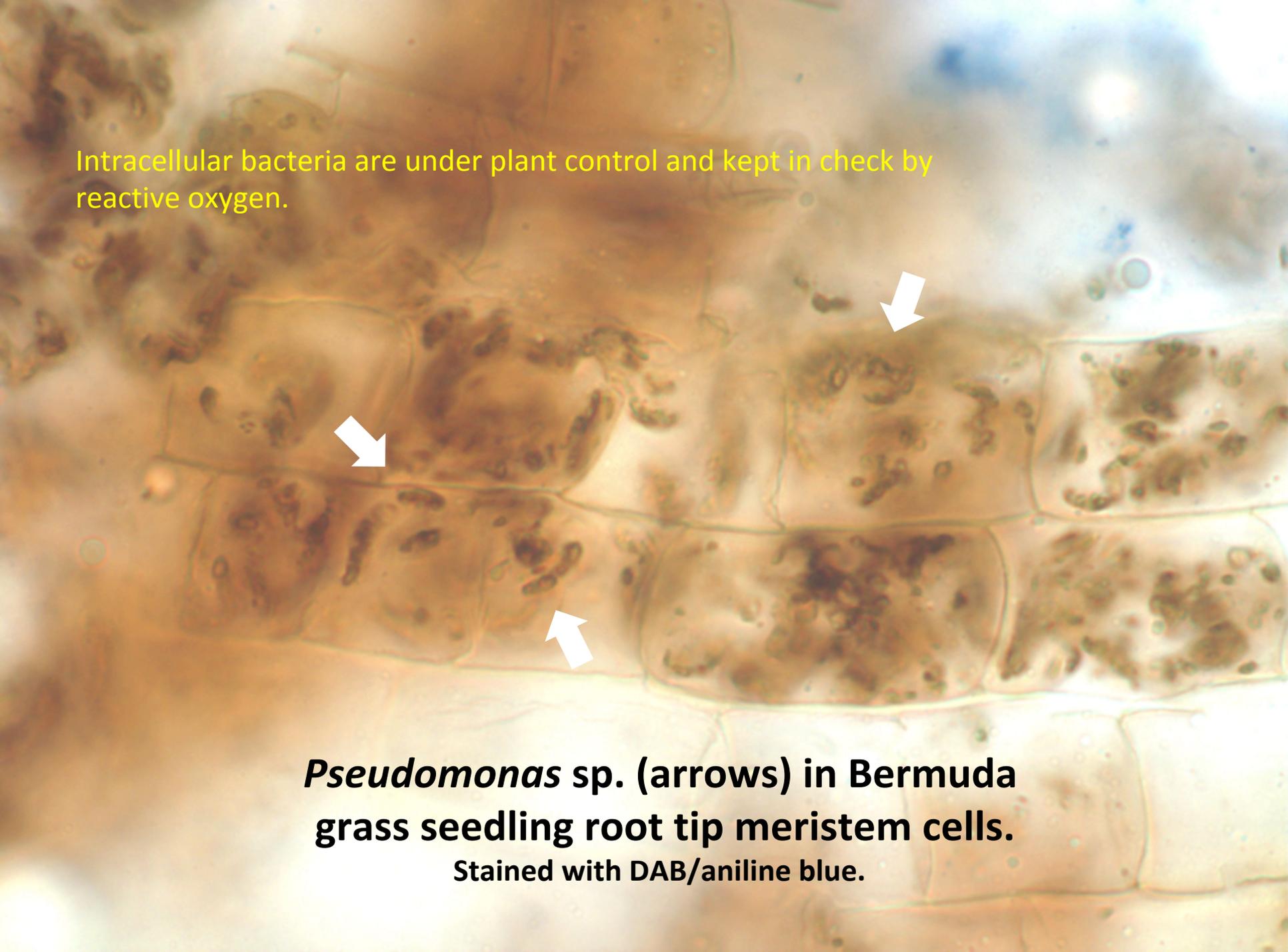
Proposed route of endophyte colonization of root and reentry to rhizosphere from root hairs



RHIZOPHAGY CYCLE



Intracellular bacteria are under plant control and kept in check by reactive oxygen.

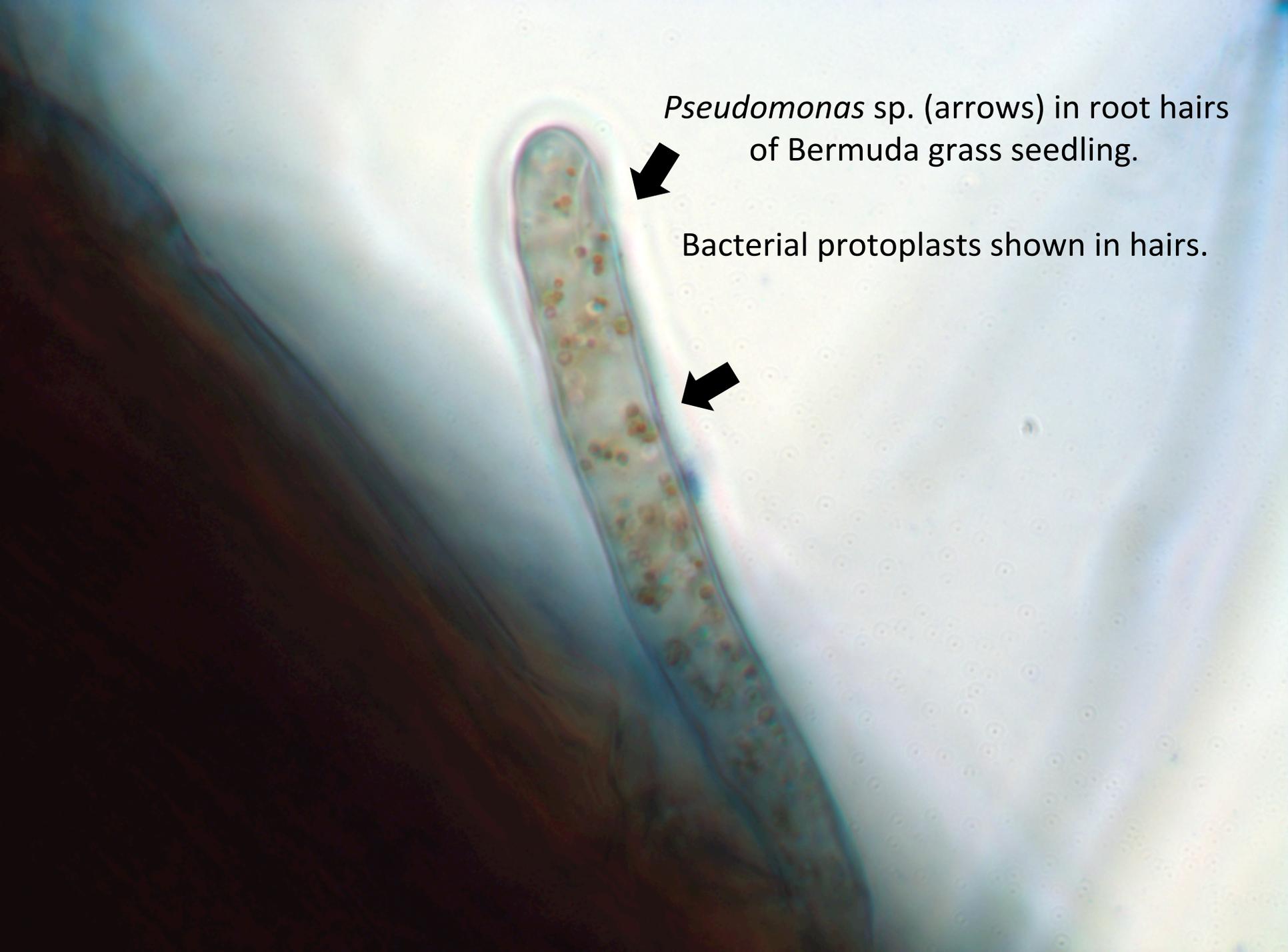


***Pseudomonas* sp. (arrows) in Bermuda grass seedling root tip meristem cells. Stained with DAB/aniline blue.**

**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.

Bacterial protoplasts shown in hairs.



Lara Brindisi

Tomatoes harbor endophytic microbes that stimulate growth of roots and root hairs in tomato seedlings. *Micrococcus luteus* is one of the tomato endophytes.



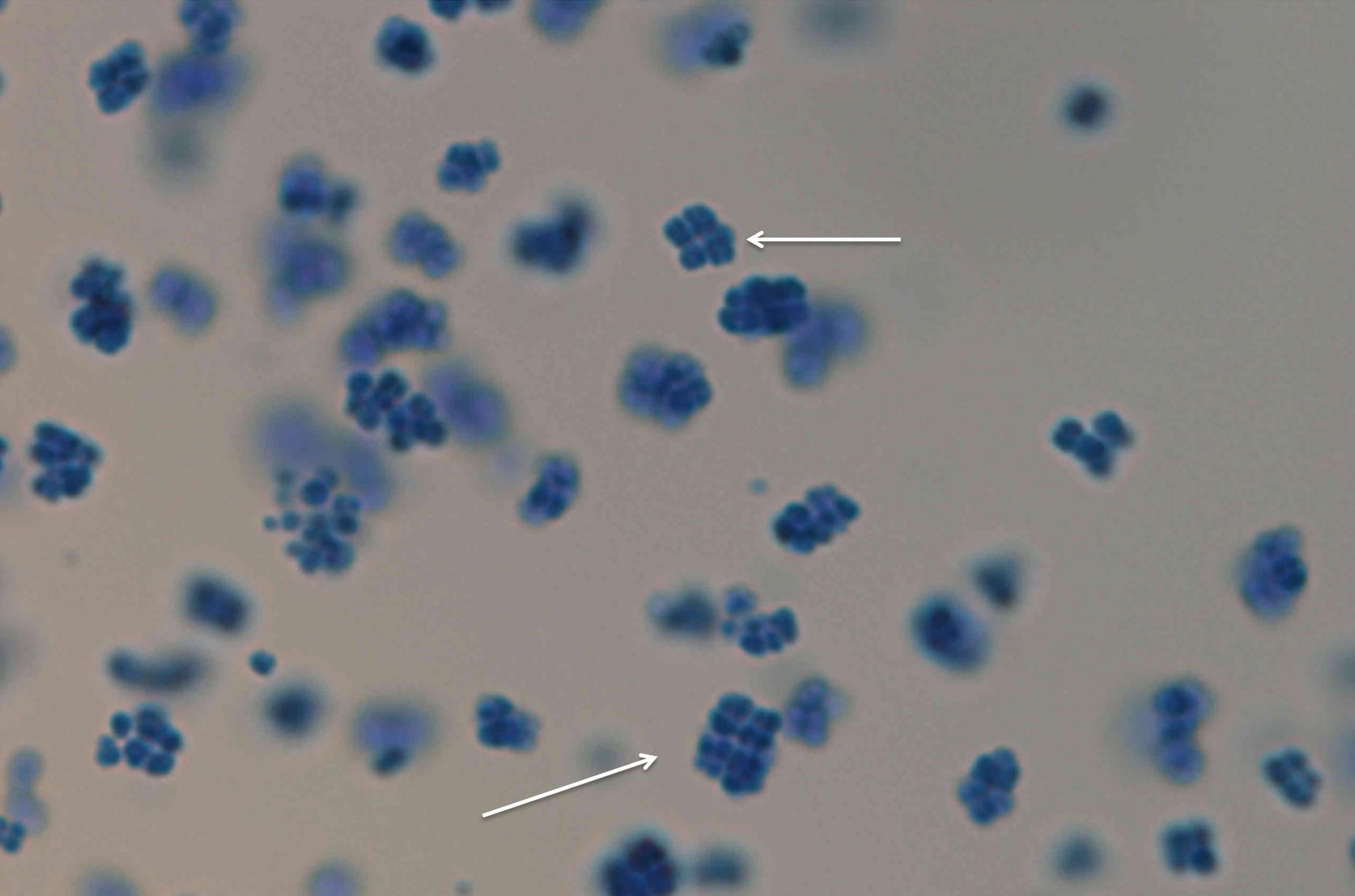
Satish K. Verma



Endophyte is visible intracellularly as singlets to packets of cells (arrow)



***Micrococcus luteus* from culture showing tetrads (arrows).**



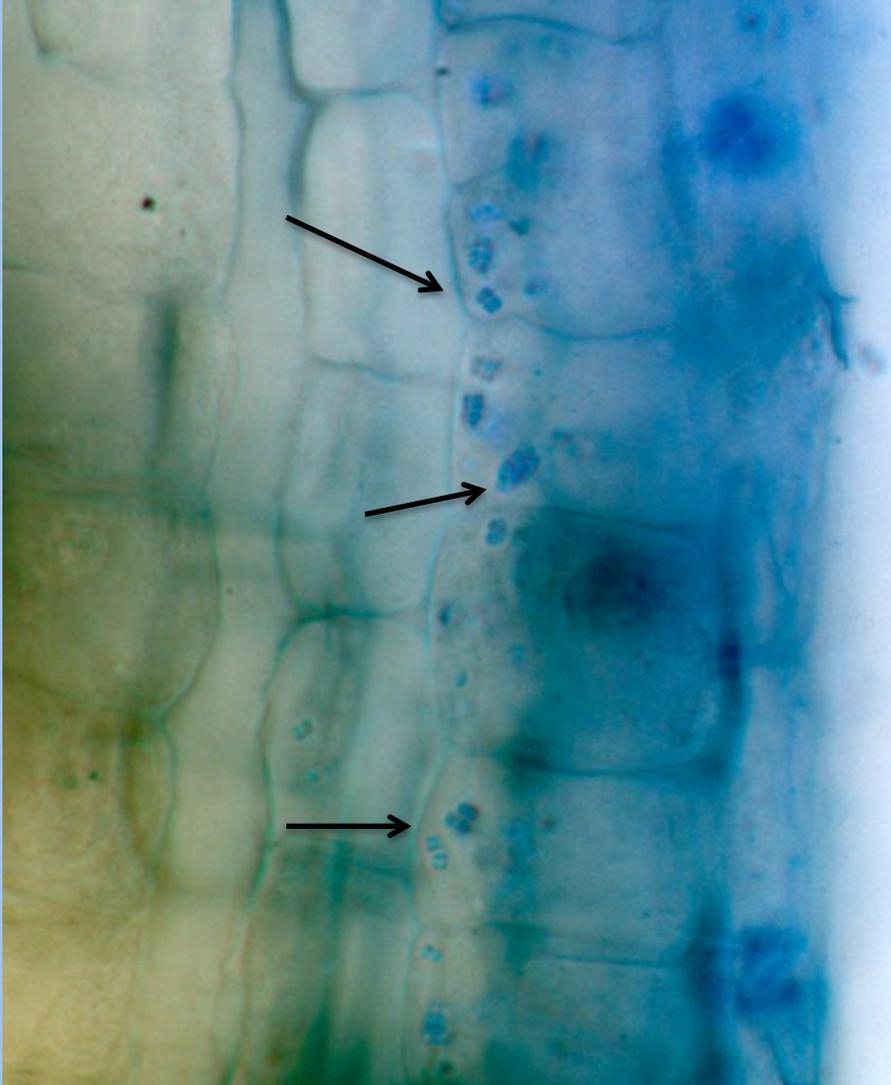
Curley dock (*Rumex crispus*; Polygonaceae) endophyte colonization experiment

Procedure:

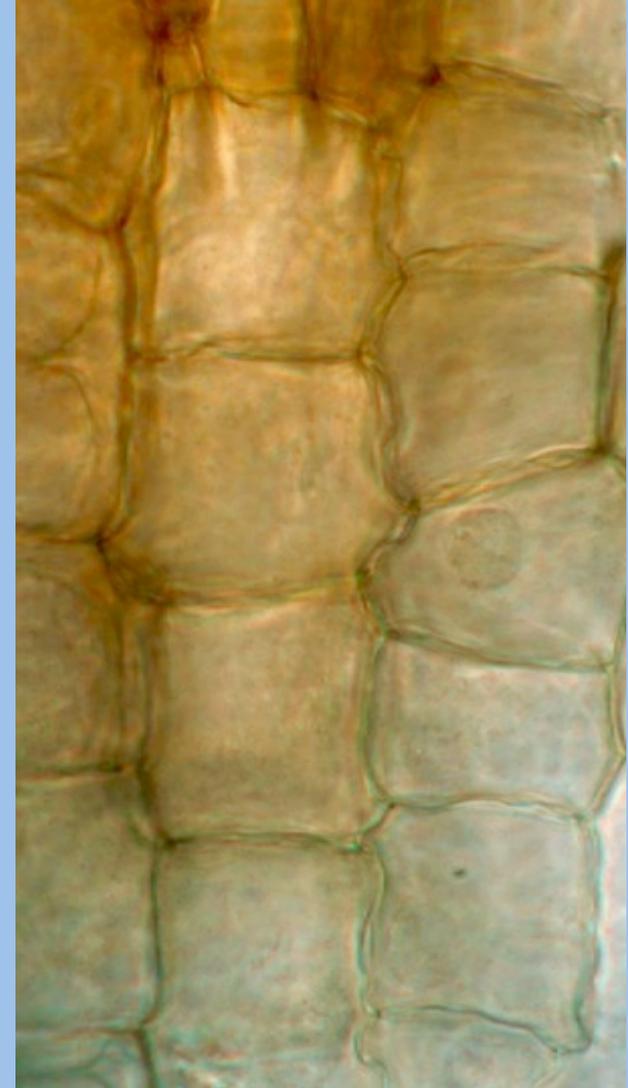
1. Seeds of *Rumex crispus* were surface disinfected to remove native bacteria.
2. Seeds placed onto agarose.
3. ½ seeds inoculated with suspension of *Micrococcus luteus* in water.
4. Seeds incubated for 7 days.
5. Seedlings stained in diaminobenzidine tetrachloride for 15 hours.
6. Slides prepared using aniline blue and examined microscopically.
7. All evidence of bacteria in seedling tissues photographed.

***Micrococcus luteus* in *Rumex crispus*: Path of bacteria in the rhizophagy cycle**

Micrococcus enters the outer two cell layers of the root tip meristem as walled tetrads (left photo, arrows). The bacterium is present only in the periplasmic space between cell wall and plasma membrane. The image to the right shows root meristematic cells of a seedling that was not inoculated with *Micrococcus luteus*. All tissues were stained with DAB followed by aniline blue.

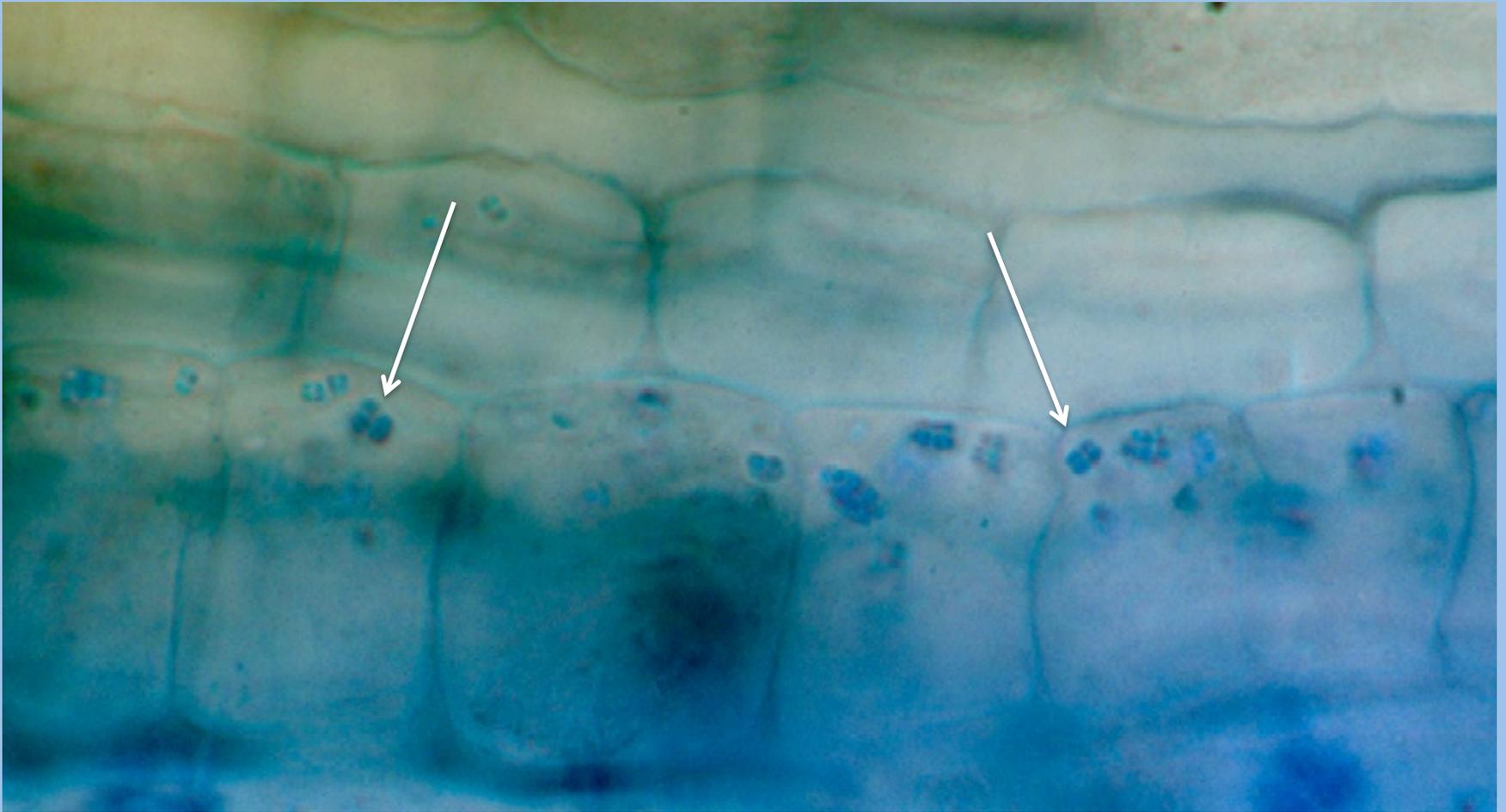


Seedling inoculated with *Micrococcus luteus*

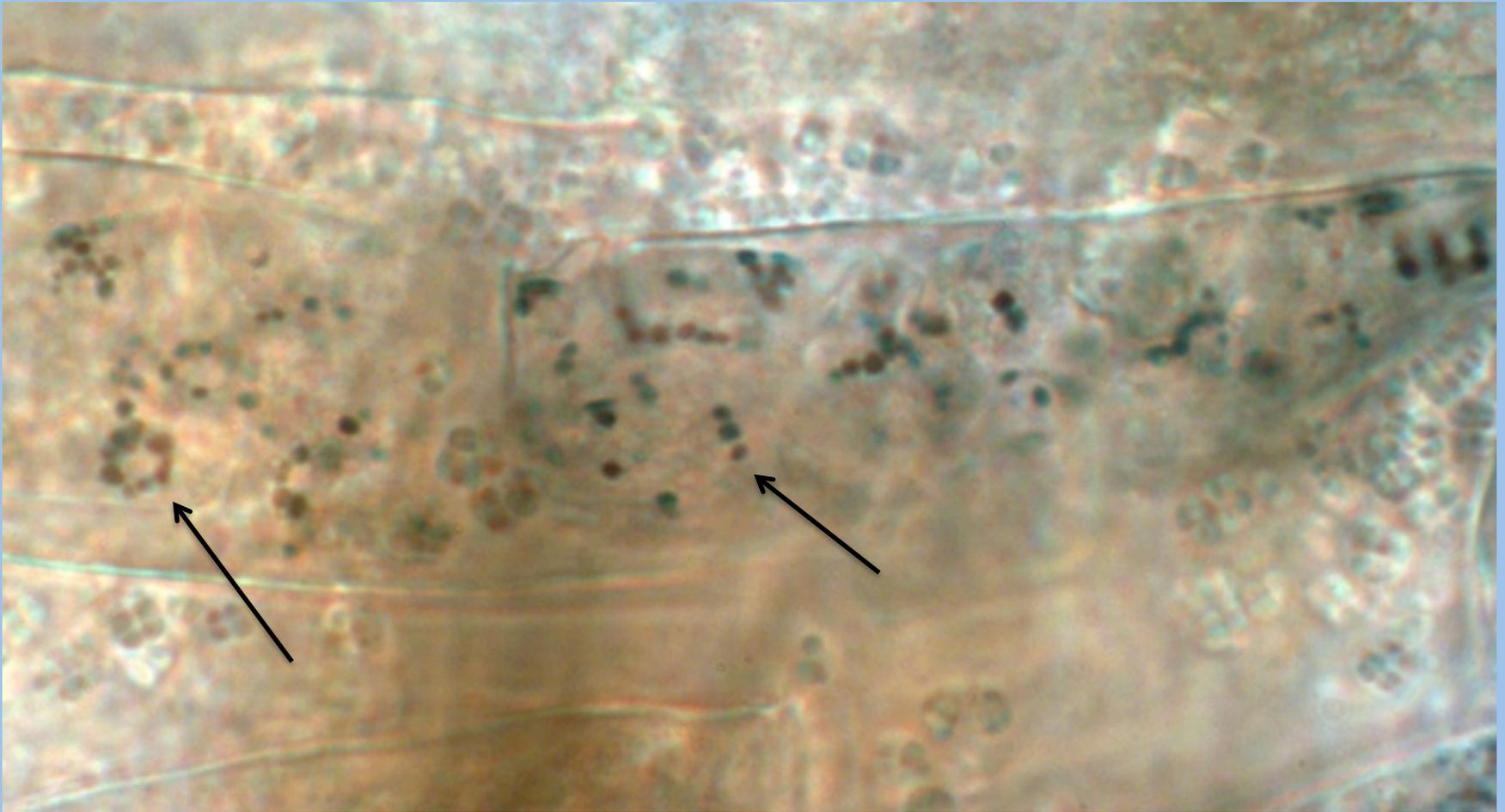


Seedling not inoculated

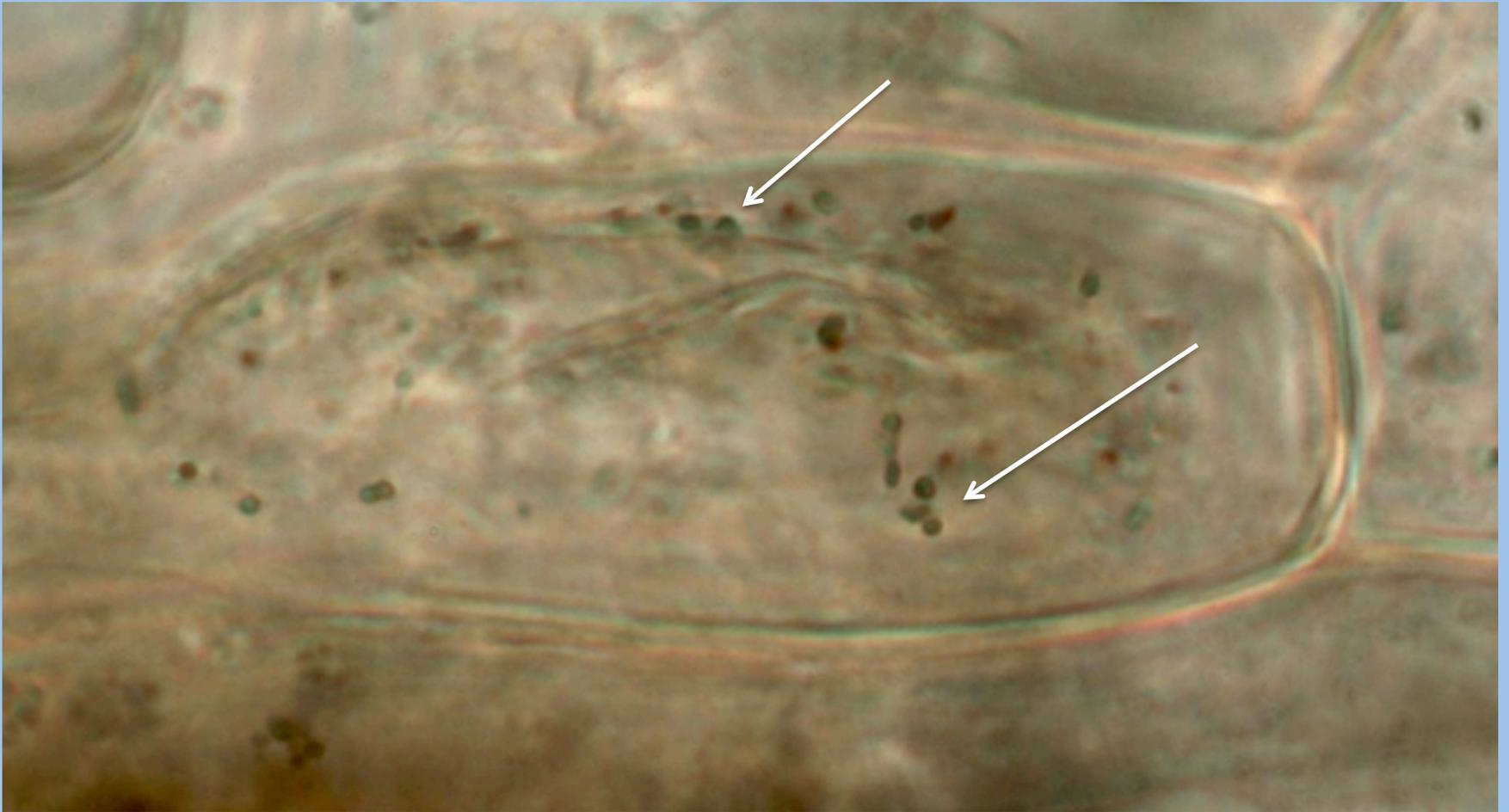
Close-up of *Micrococcus* tetrads (arrows) in periplasmic space of root meristematic cells (stained with DAB and aniline blue).



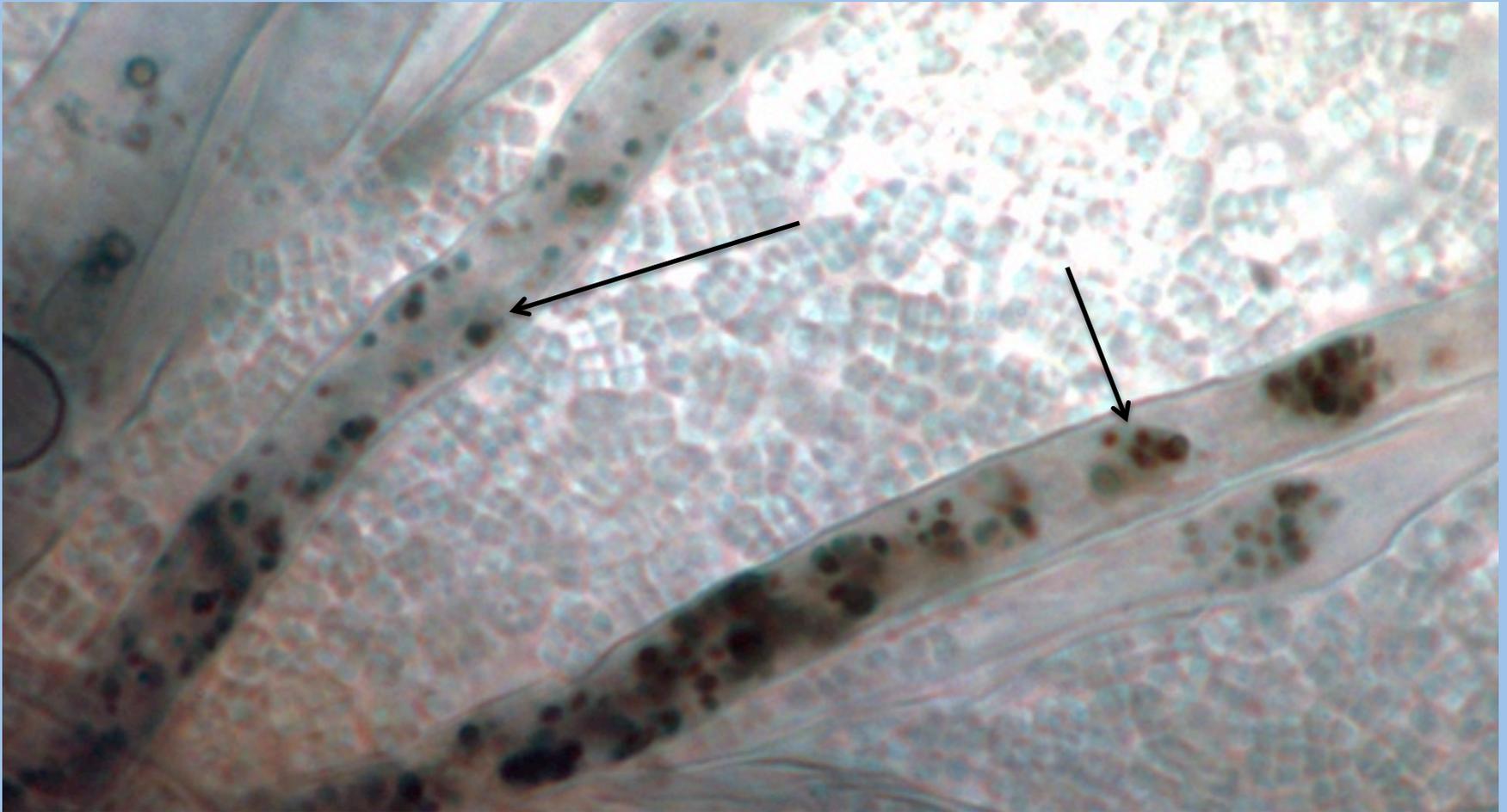
In older cells tetrads lose clustered form convert into smaller spherical cells (arrows). These are staining with aniline blue. These are likely wall-less protoplasts.



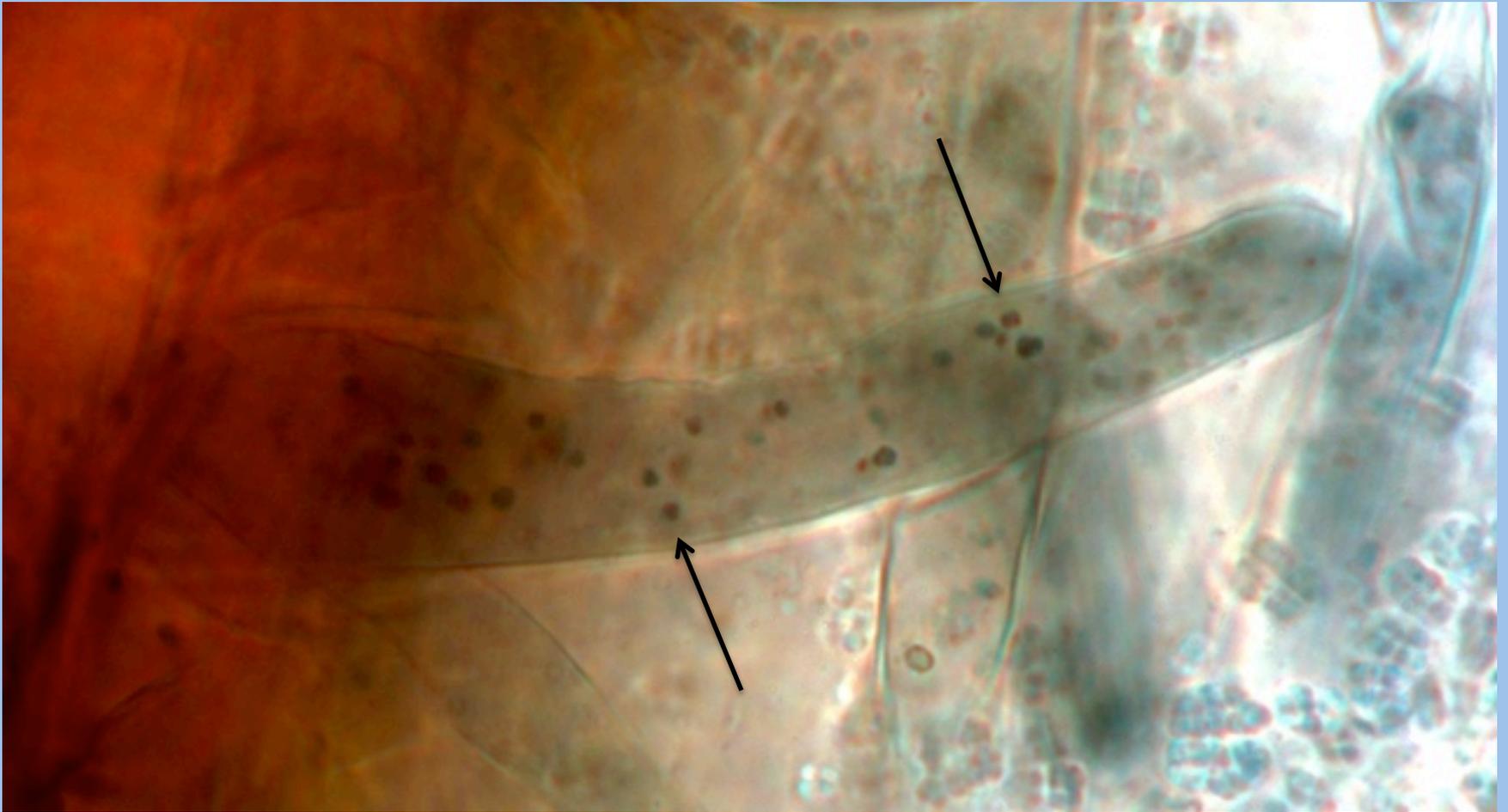
Spherical L-forms (arrows) in the periplasmic space of root parenchyma.



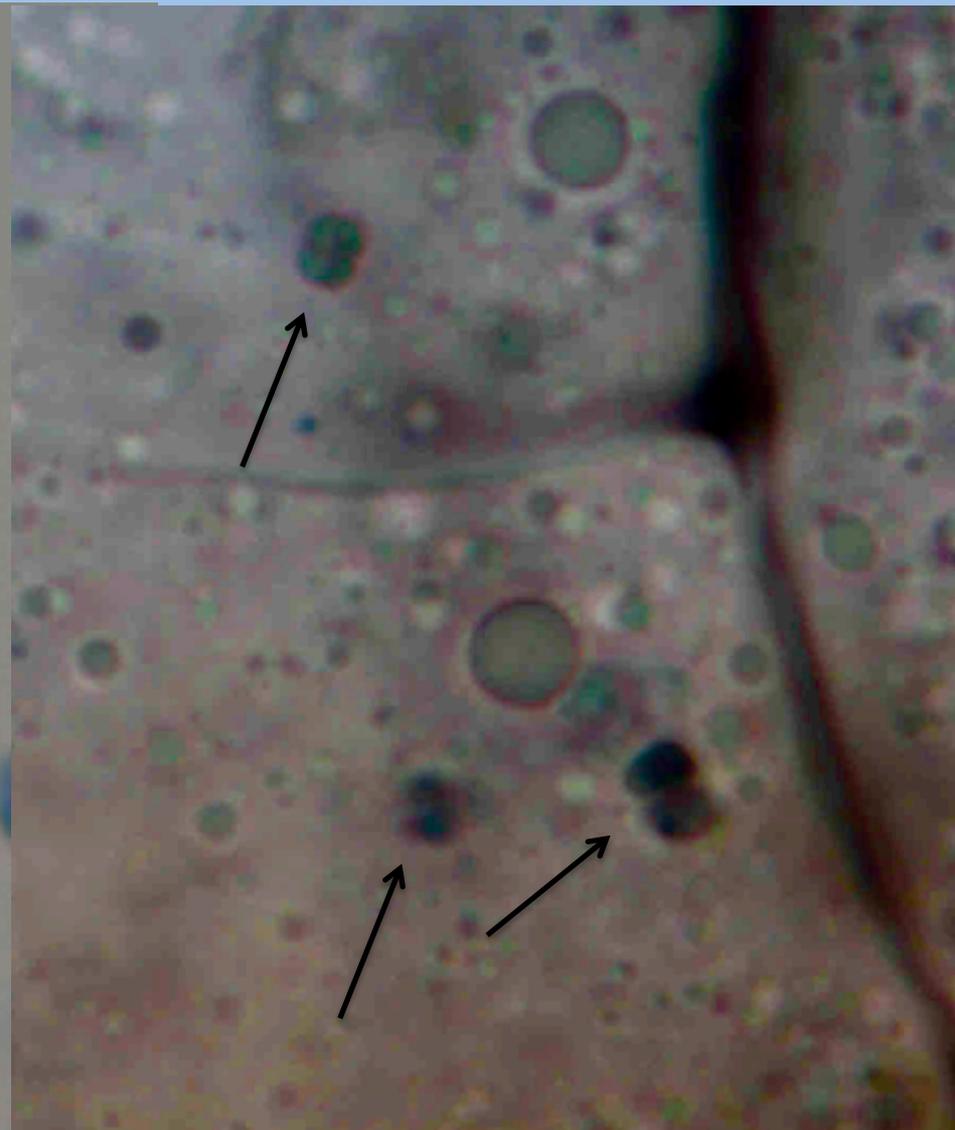
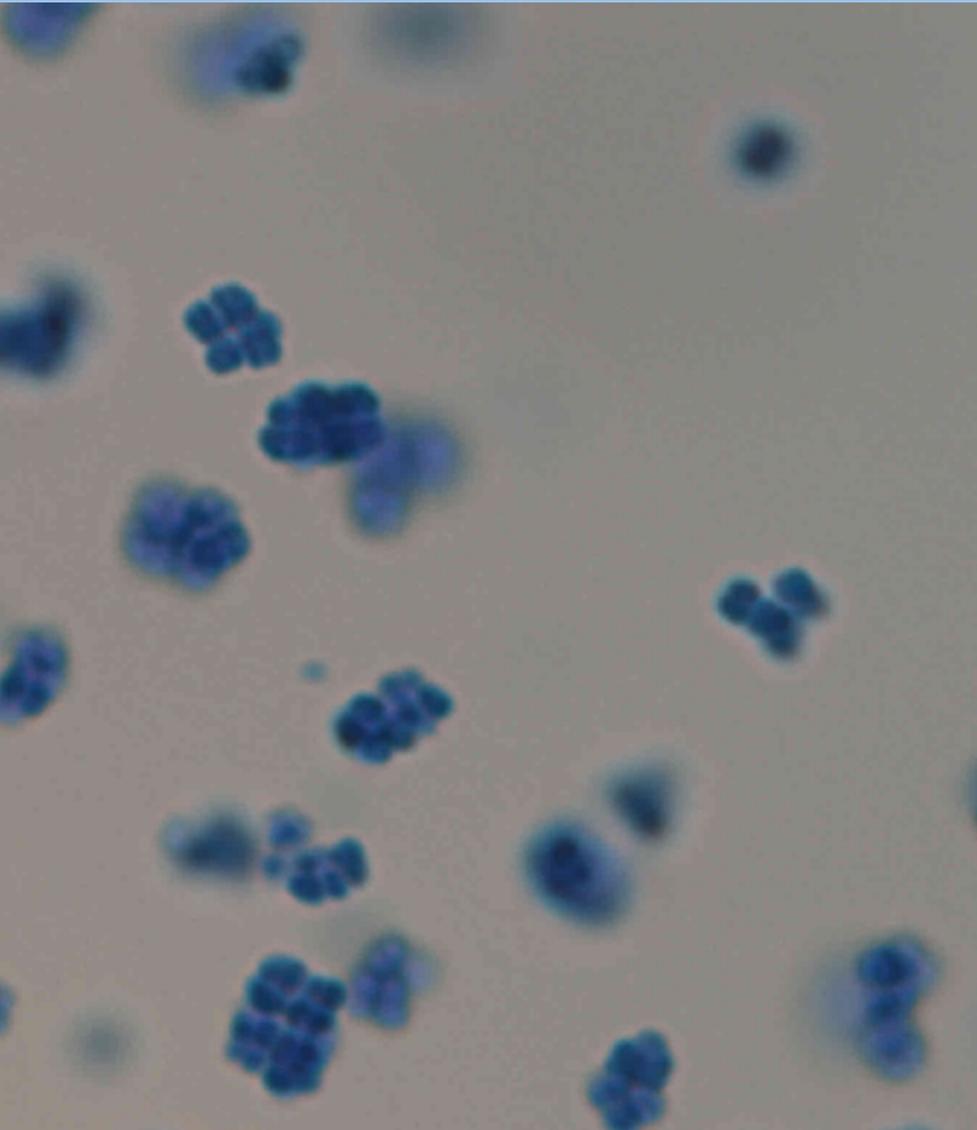
Spherical protoplasts (arrows) are present in root hairs as they form. Reddish brown color is due to reactive oxygen secreted from NADPH oxidases on the plant cell membrane. Blue is from aniline blue stain.



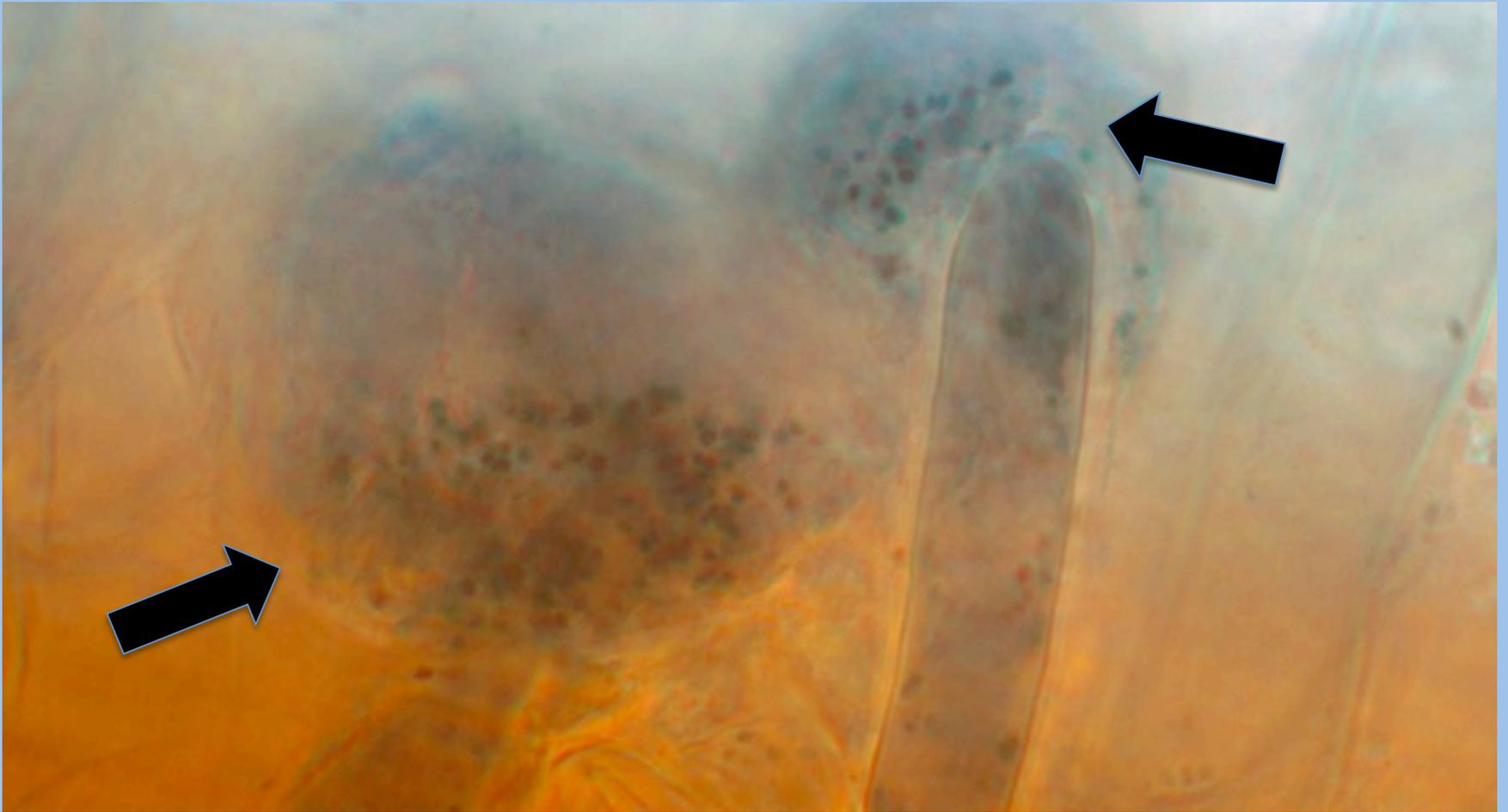
Spherical protoplasts (arrows) of bacterium (*M. luteus*) in developing root hair.



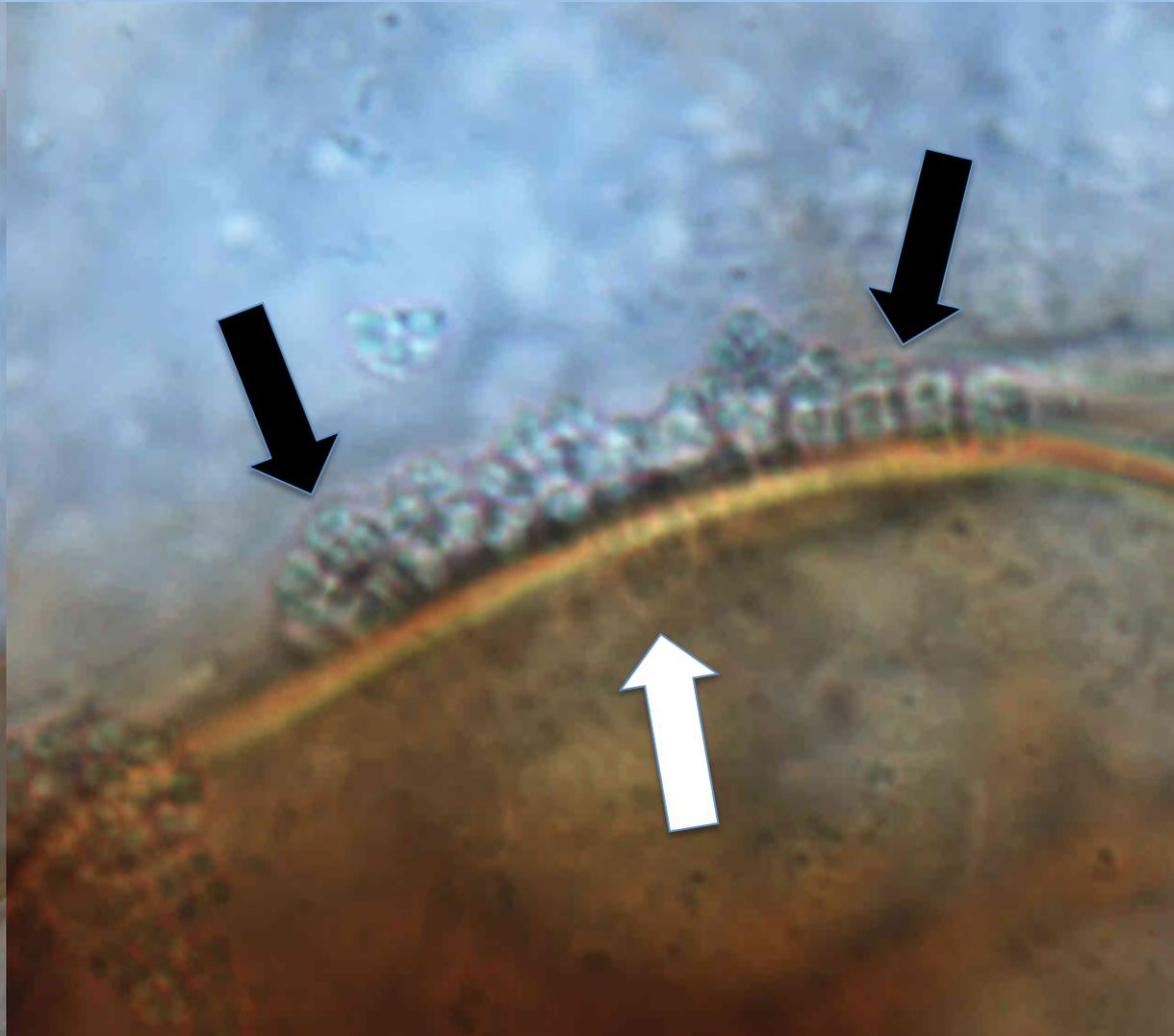
In some cases protoplasts reform tetrads (with walls) in root cells. The image to the left is of *Micrococcus luteus* from culture. The image to the right shows protoplasts and tetrads (arrows) reforming in root parenchyma cells of a dandelion seedling.



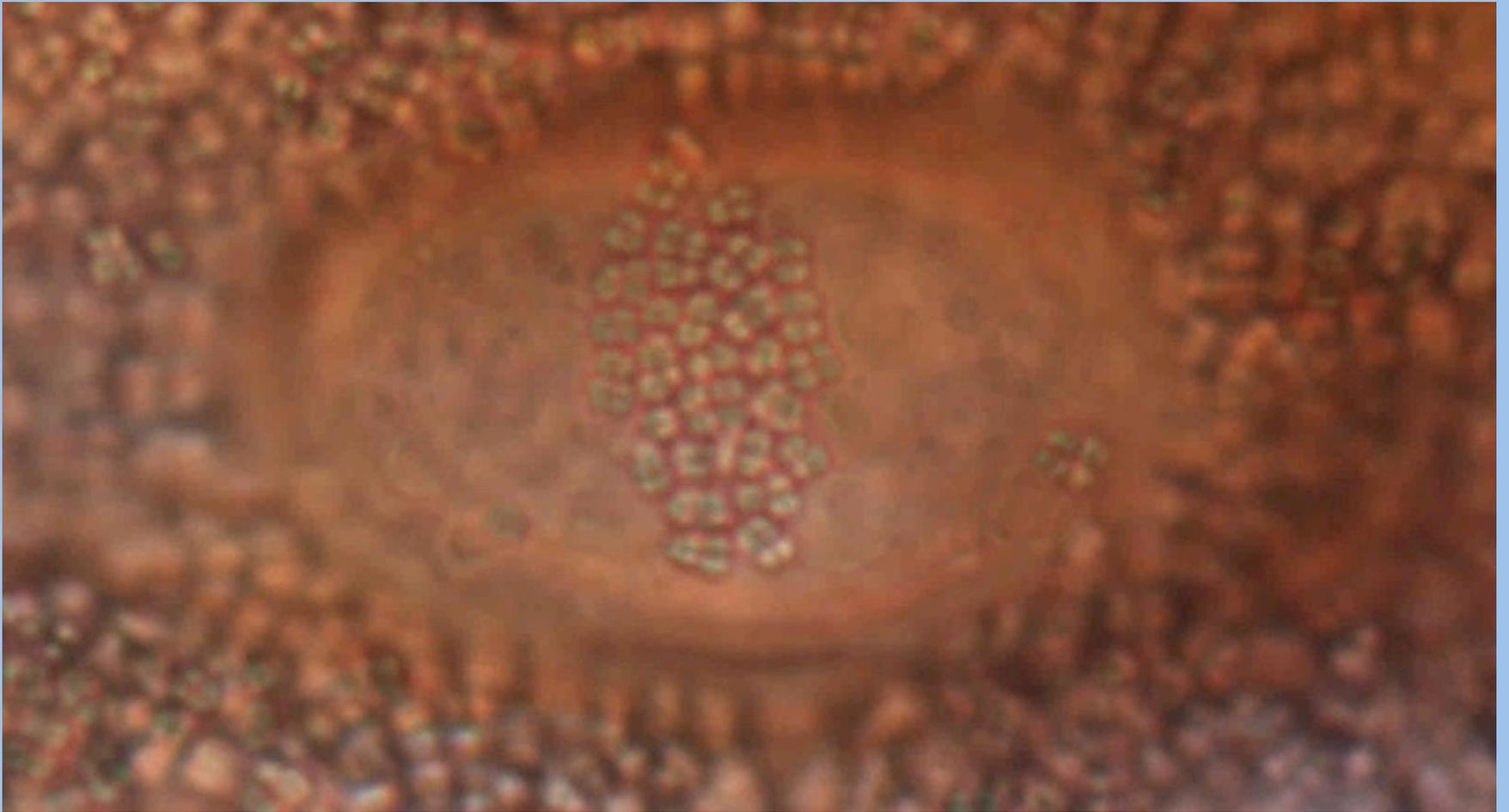
Two root hairs showing masses of bacteria (arrows) emerging from hairs.



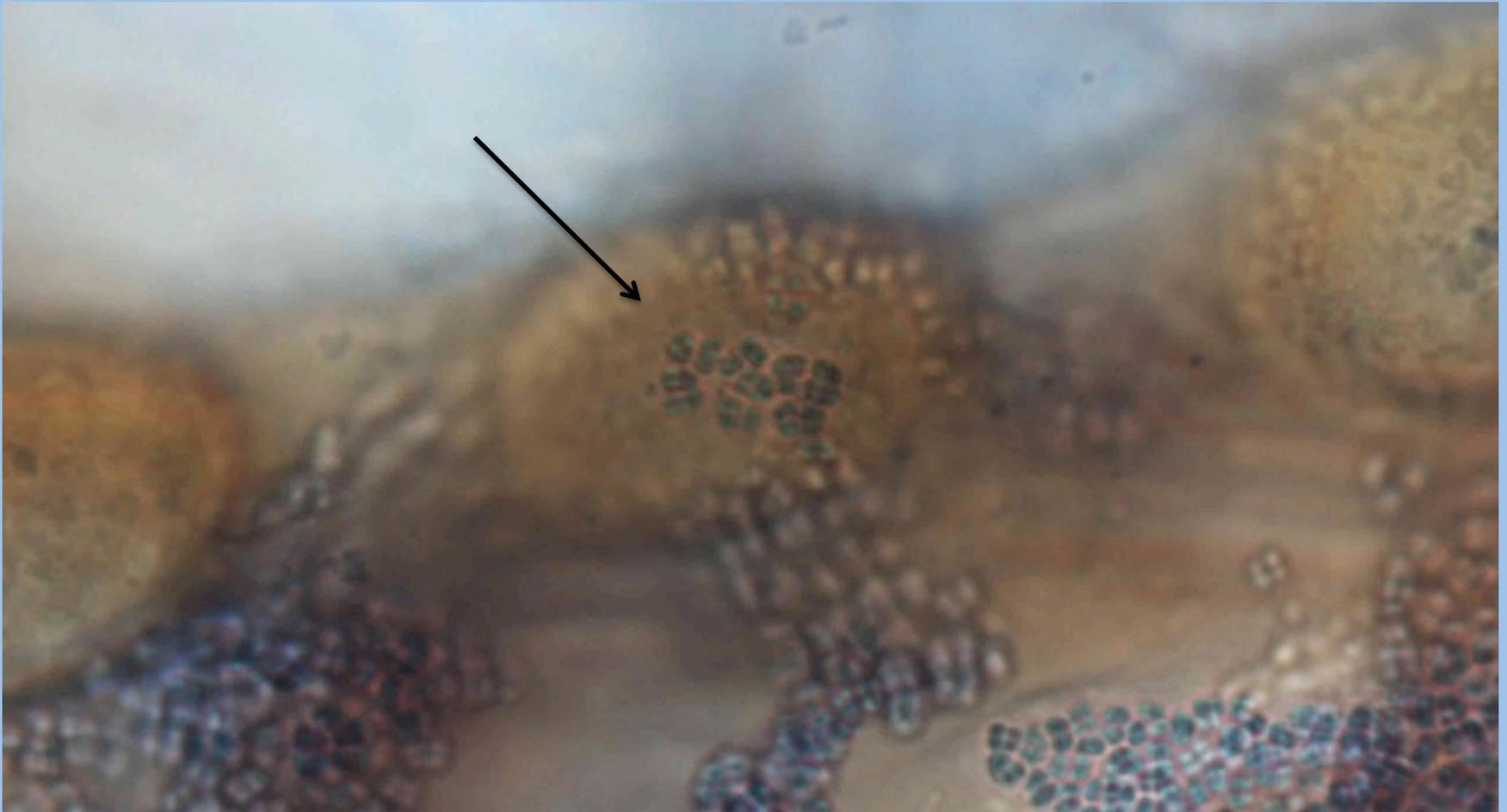
Micrococcus luteus bacteria exiting root hair tips. Spherical L-forms (white arrow) visible within hair beneath plant cell wall, tetrads of cells (black arrows) outside wall, and channels visible through plant cell wall. Host is *Rumex crispus* seedling.



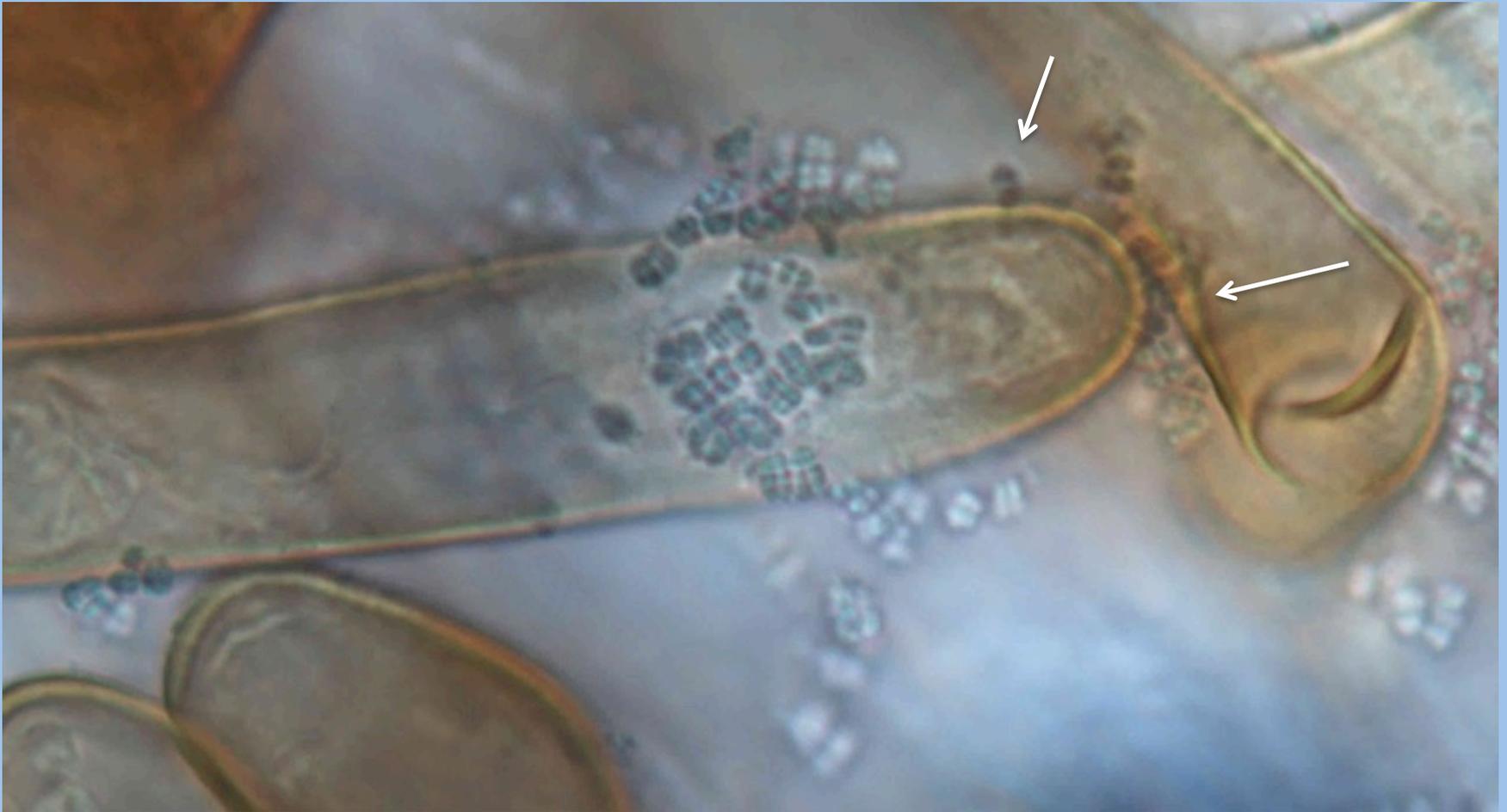
Rumex root hair initial showing emergence of bacteria at apex. Note also brown coloration due to high levels of H₂O₂ in cells.



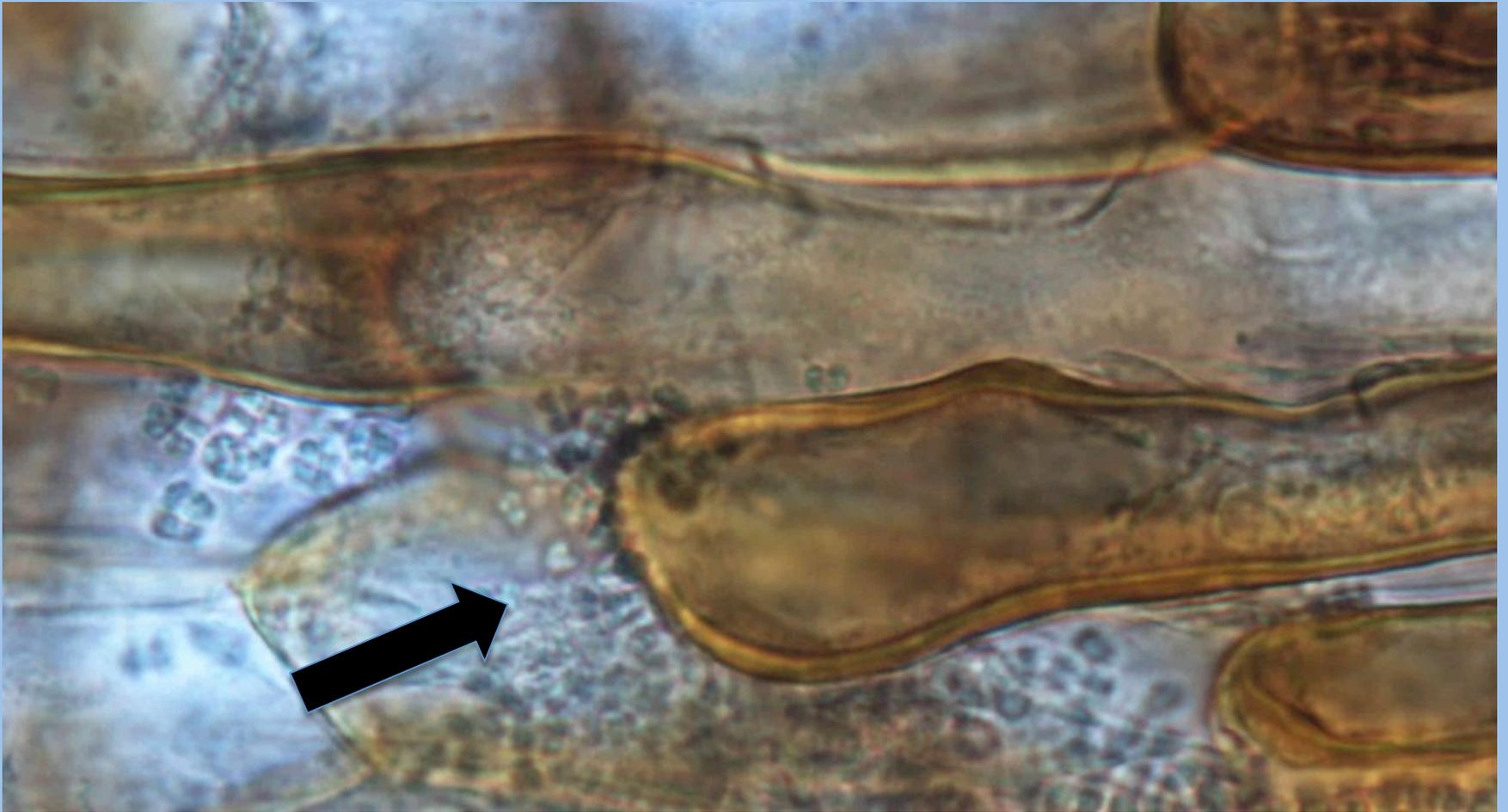
Rumex root hair initial showing bacterial emergence to surface (arrow). Bacterial cells appear to be spilling off of the hairs and accumulating around the hairs. Note that the youngest (smallest and lightest staining) bacterial cells are on the surface of the hair initial.



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



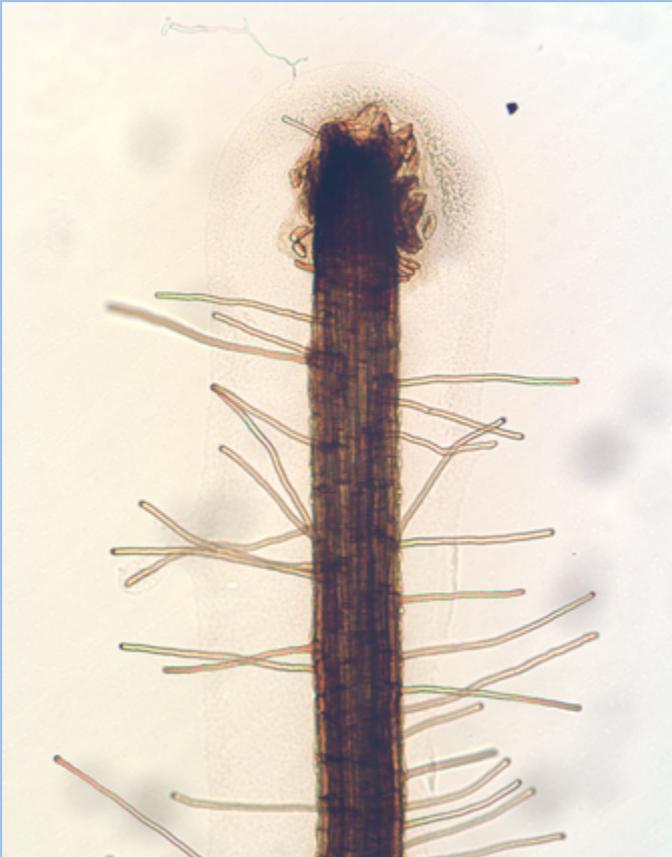
Rumex root hair showing emergence of bacterial cells (*M. luteus*) at tip (arrow).



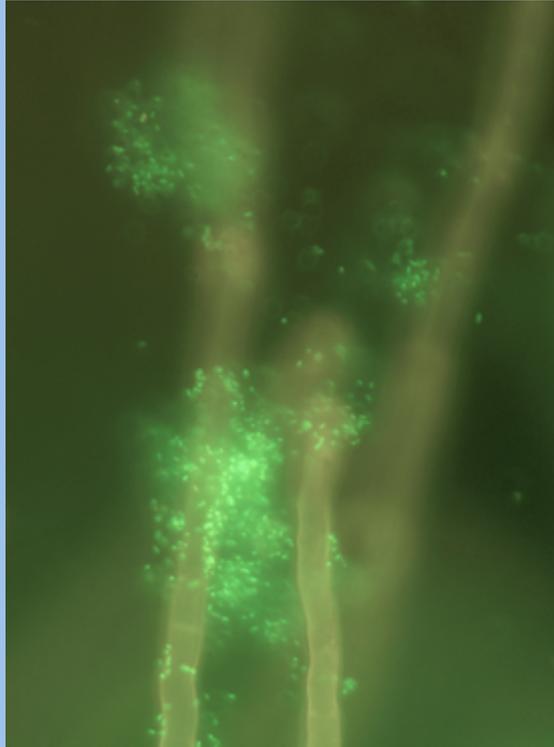
What is the function of root hairs?

Root hairs function to eject rhizophagy microbes out into the soil where they may acquire nutrients.

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.

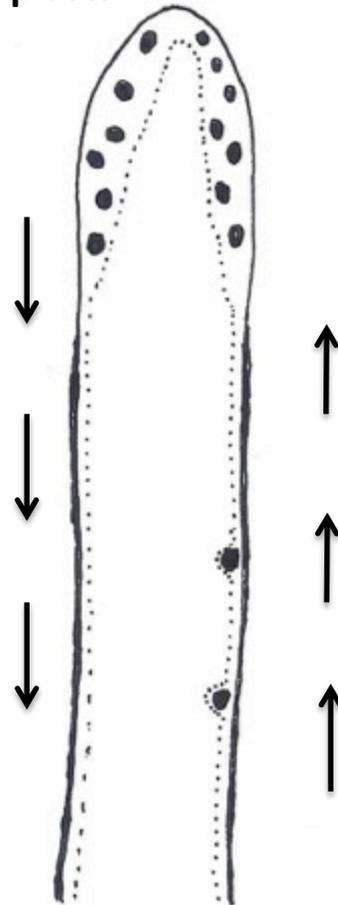


Bacteria emerging from root hair tip. Bacteria in hairs are present as wall-less L-forms. Bacteria reform their walls after exiting from the tip of the hair.



Cyclosis/Expansion Wave Mechanism for Microbe Expulsion from Root Hairs in Rhizophagy Cycle

1. Cyclosis moves microbes to tip and facilitates replication of microbe protoplasts.



Emerging Microbe Protoplasts

Expansion Wave

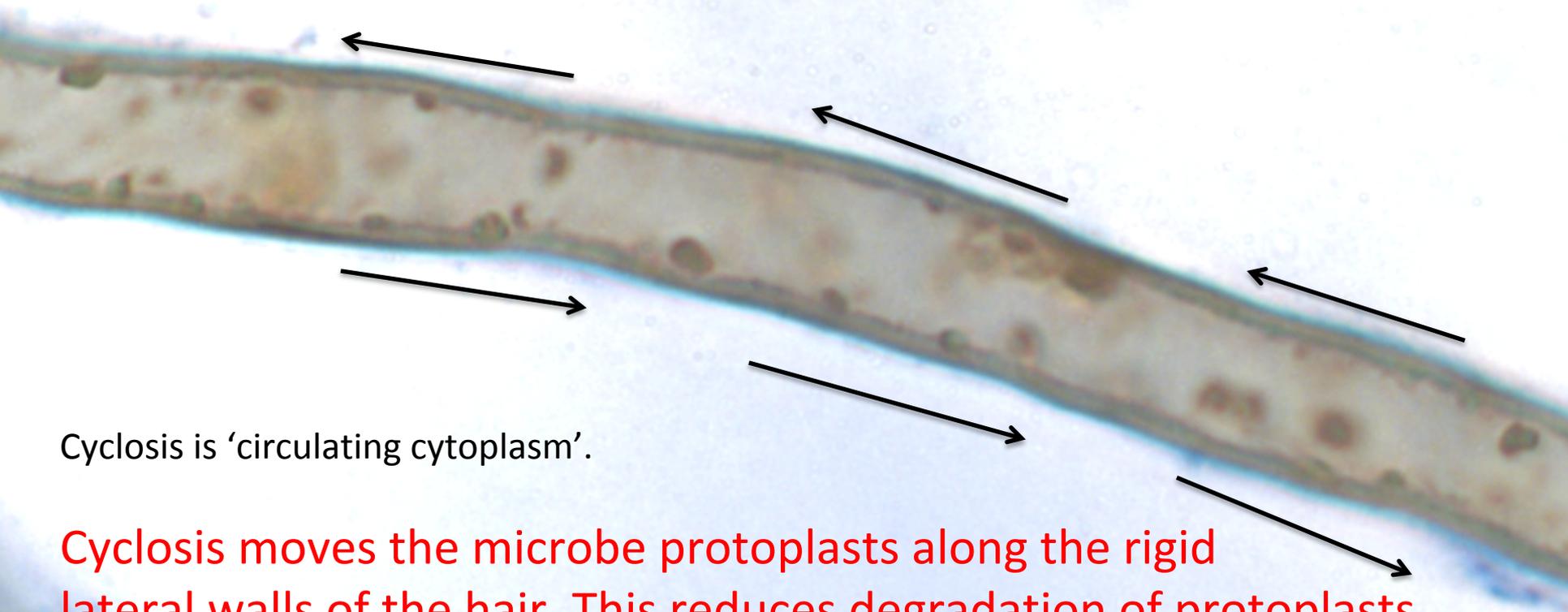
2. A wave of expansion in the hair protoplast begins in the base of the hair and progresses to the tip of the hair. This expansion wave forces microbe protoplasts through pores that form in the thin wall at the hair tip.

Fimbristylis cymosa

Plant grows in pits and crevices of limestone or in sand along high salt Caribbean shore environments.



Root hair of *Fimbristylis cymosa* showing bacterial protoplasts in periplasmic space. Bacteria are seen to be surrounded by red-staining reactive oxygen. Bacteria are transported to the tip of the hair and deposited by the action of unidirectional cyclosis in the root hair cell.



Cyclosis is 'circulating cytoplasm'.

Cyclosis moves the microbe protoplasts along the rigid lateral walls of the hair. This reduces degradation of protoplasts and increases their recovery and replication.

Clusters of replicating bacteria within periplasmic space of root hair of sedge *Fimbristylis cymosa*. The red coloration around clusters of bacterial protoplasts (arrows) is indicative of reactive oxygen secreted by the root cell plasma membrane to induce nutrient leakage from bacteria (stained with DAB/aniline blue).

Plants increase the numbers of microbe protoplasts prior to releasing microbes back into the soil.



Root hair of sedge (*Fimbristylis cymosa*) showing expulsion of bacteria (large arrow) from the soft-walled hair tip. Red-staining bacterial protoplasts are seen in root hair. A wave of expansion of the hair protoplast propagates from base to tip of hair and this wave forces microbes through pores that form in the hair tip.



Root hair of sedge *Fimbristylis cymosa*

Cyclosis was measured to move microbes at a rate of 8-11 micrometers/second in root hairs of the sedge *Fimbristylis cymosa*.

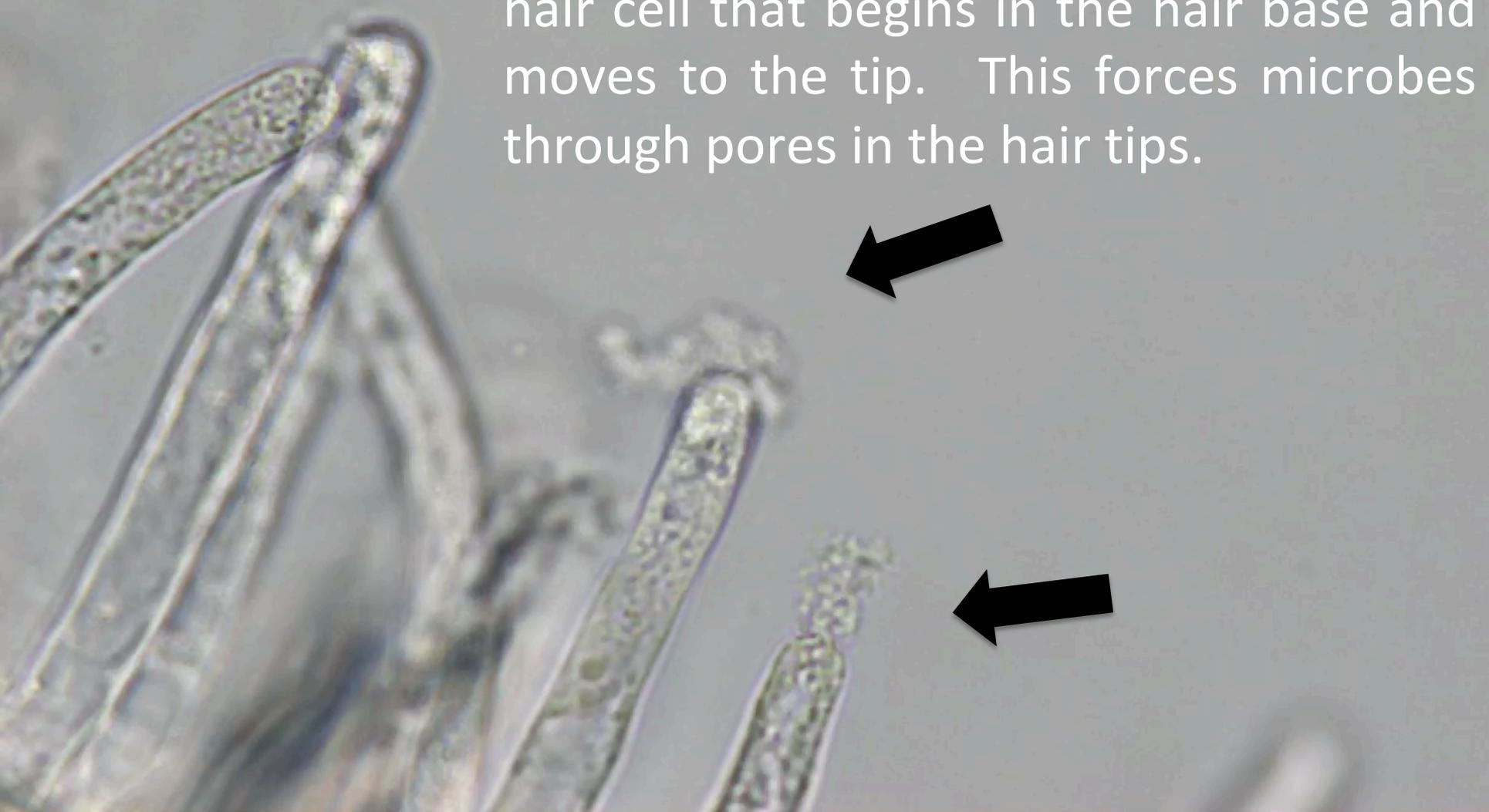


Microbes accumulating in hair tip.

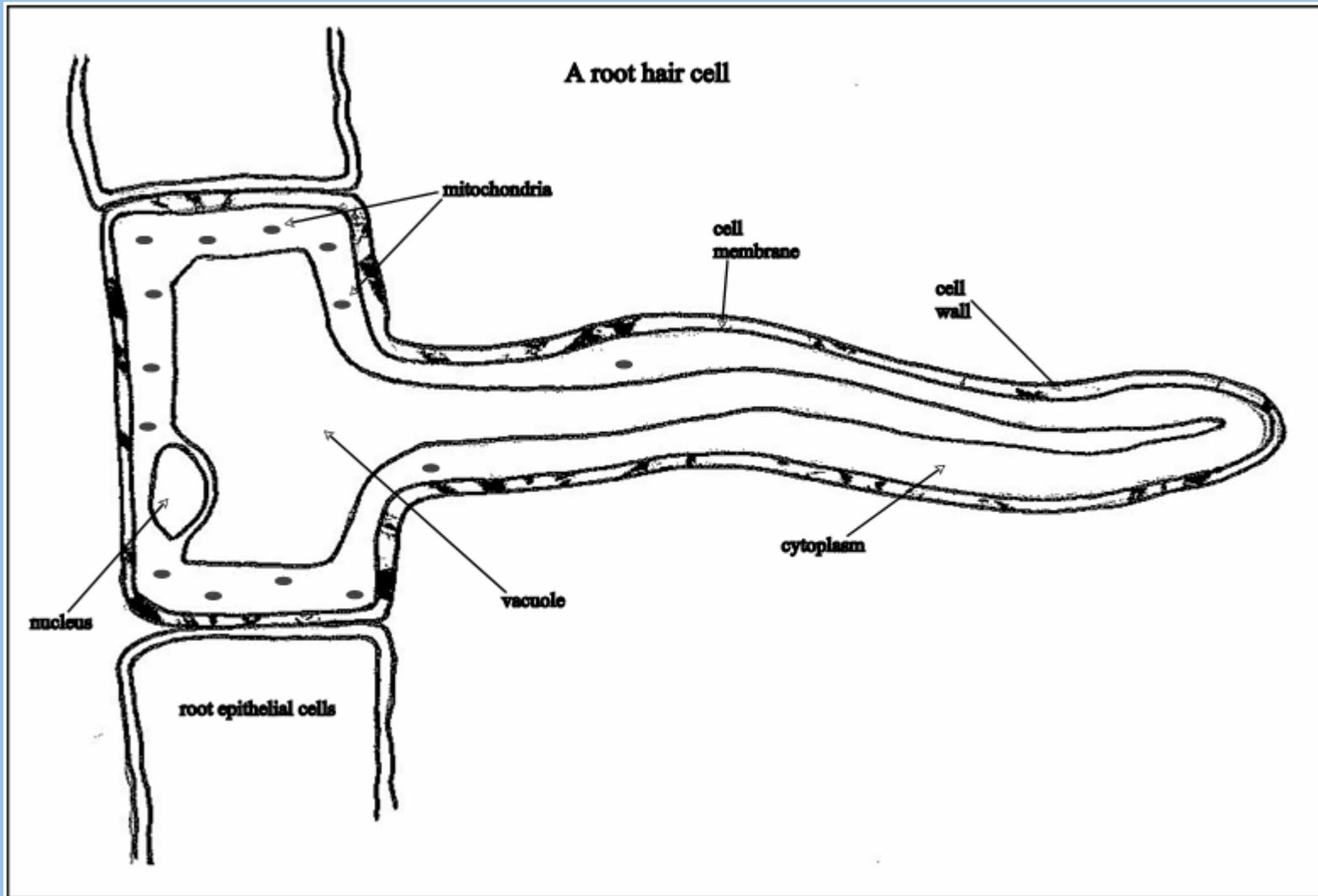
Microbes circulating along length of root hair.

This constant circulation may be a way to induce replication in the microbe protoplasts.

This ejection of microbes (arrows) occurs rapidly with a wave of expansion in the hair cell that begins in the hair base and moves to the tip. This forces microbes through pores in the hair tips.



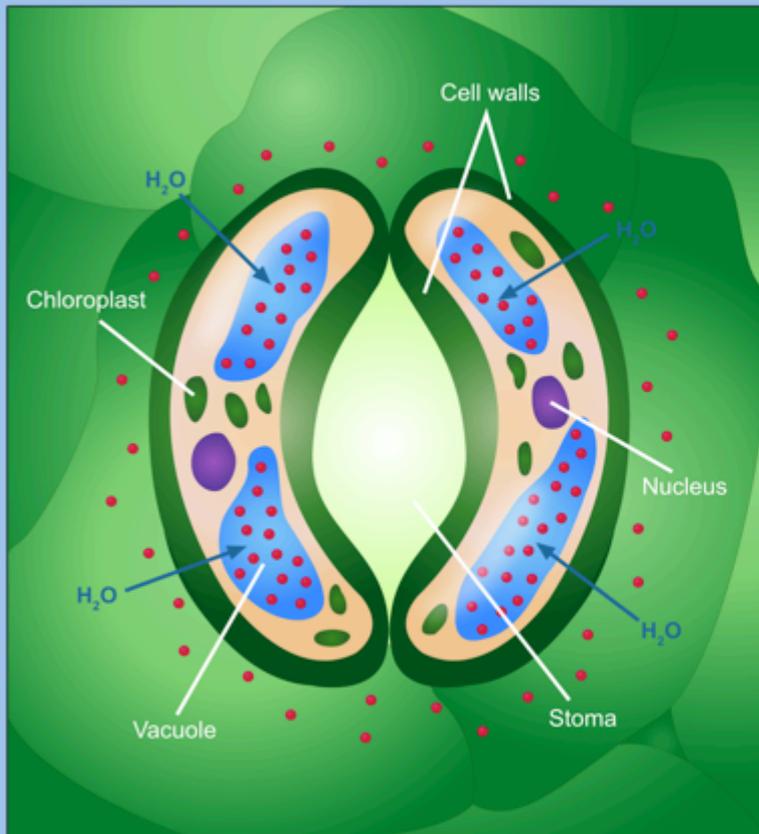
Root hairs possess a central vacuole that runs from the base to the tip.



Could potassium loading into root hair cell vacuole at the hair base result in the expansion wave that ejects microbes?

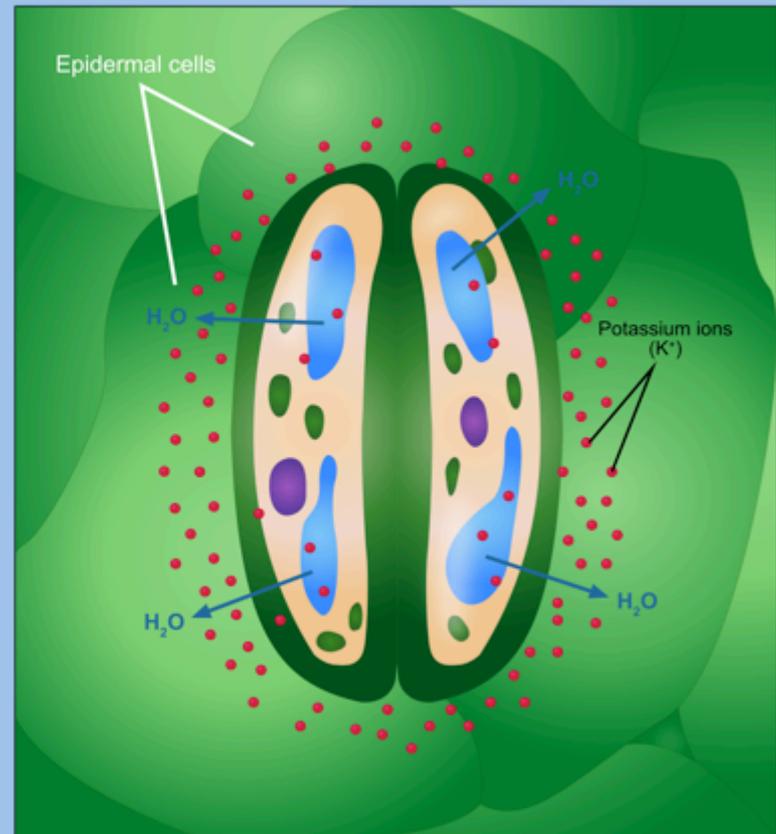
The expansion wave in root hairs may function like expansion in guard cells around plant stomata. In stomata K^+ ions are pumped into the vacuole of the guard cell. This increases the osmotic potential in the guard cell vacuole and water enters resulting in expansion of the guard cell.

Guard cells (swollen)



Stoma opening

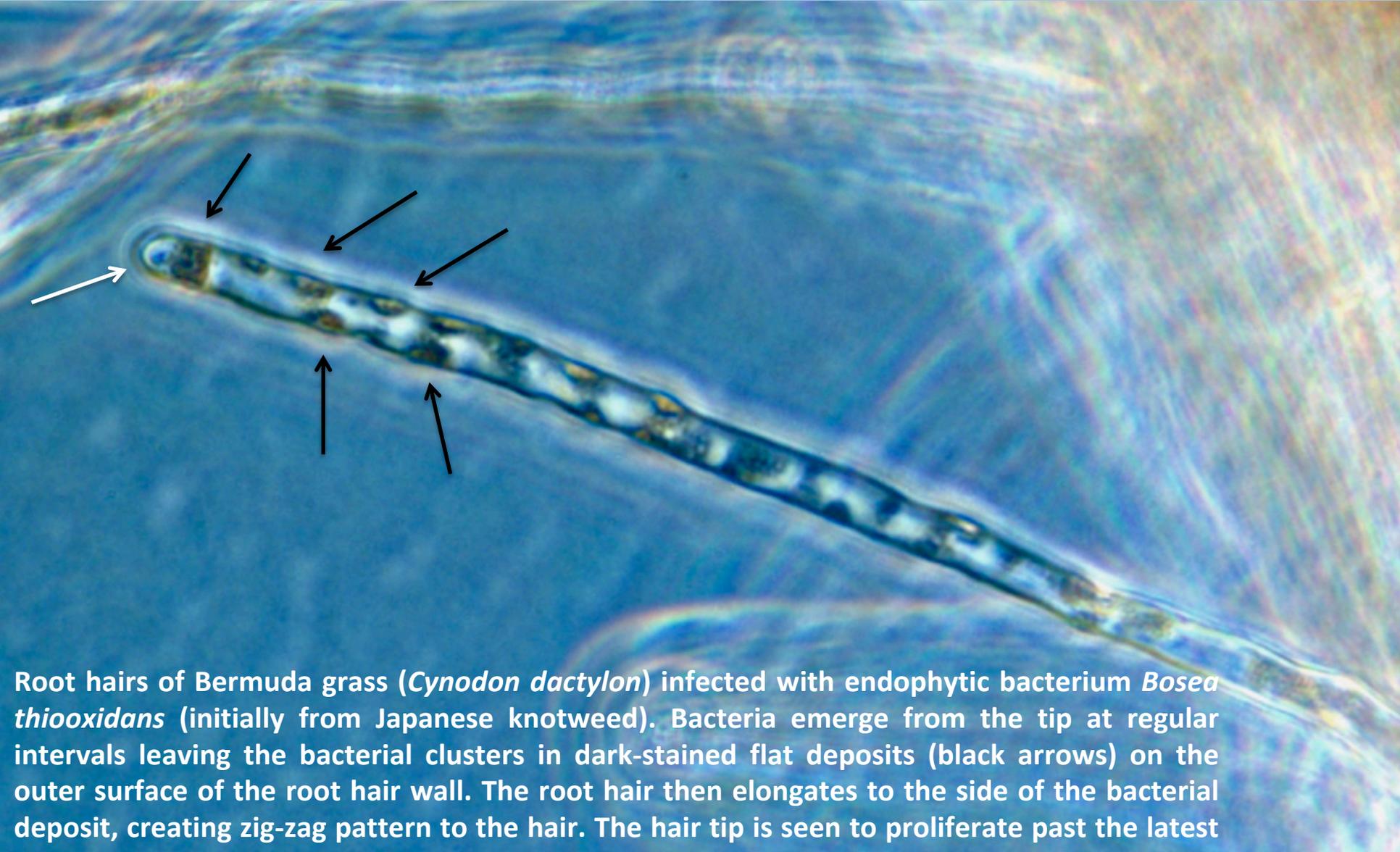
Guard cells (shrunken)



Stoma closing

Microbe ejection from hairs is periodic rather than continuous.

Microbe ejection appears to be periodic rather than continuous. Microbes may be ejected in clusters rather than 1 at a time. It is unknown what causes the periodicity.



Root hairs of Bermuda grass (*Cynodon dactylon*) infected with endophytic bacterium *Bosea thiooxidans* (initially from Japanese knotweed). Bacteria emerge from the tip at regular intervals leaving the bacterial clusters in dark-stained flat deposits (black arrows) on the outer surface of the root hair wall. The root hair then elongates to the side of the bacterial deposit, creating zig-zag pattern to the hair. The hair tip is seen to proliferate past the latest

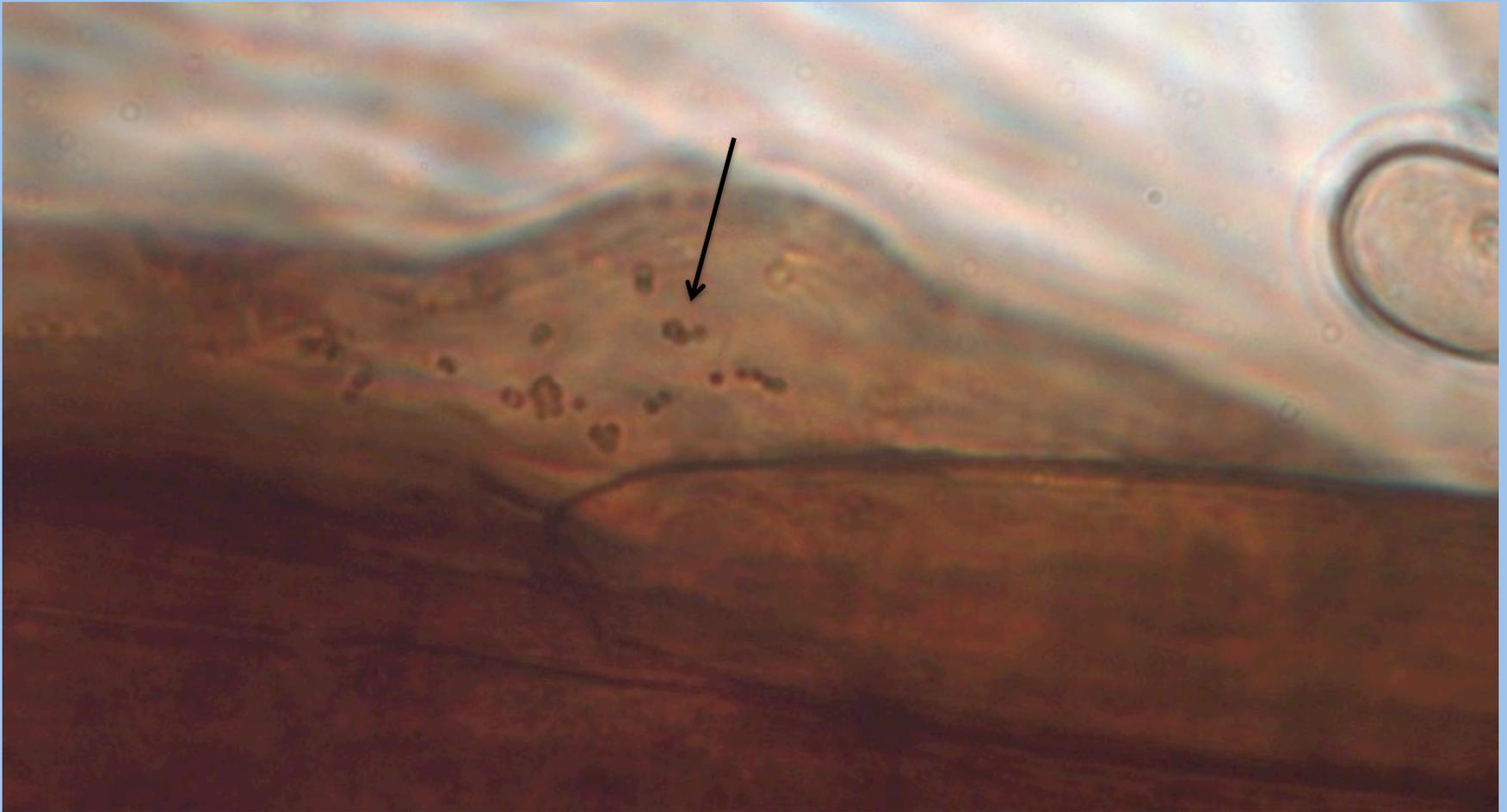
Tomato endophyte *Acinetobacter* sp.



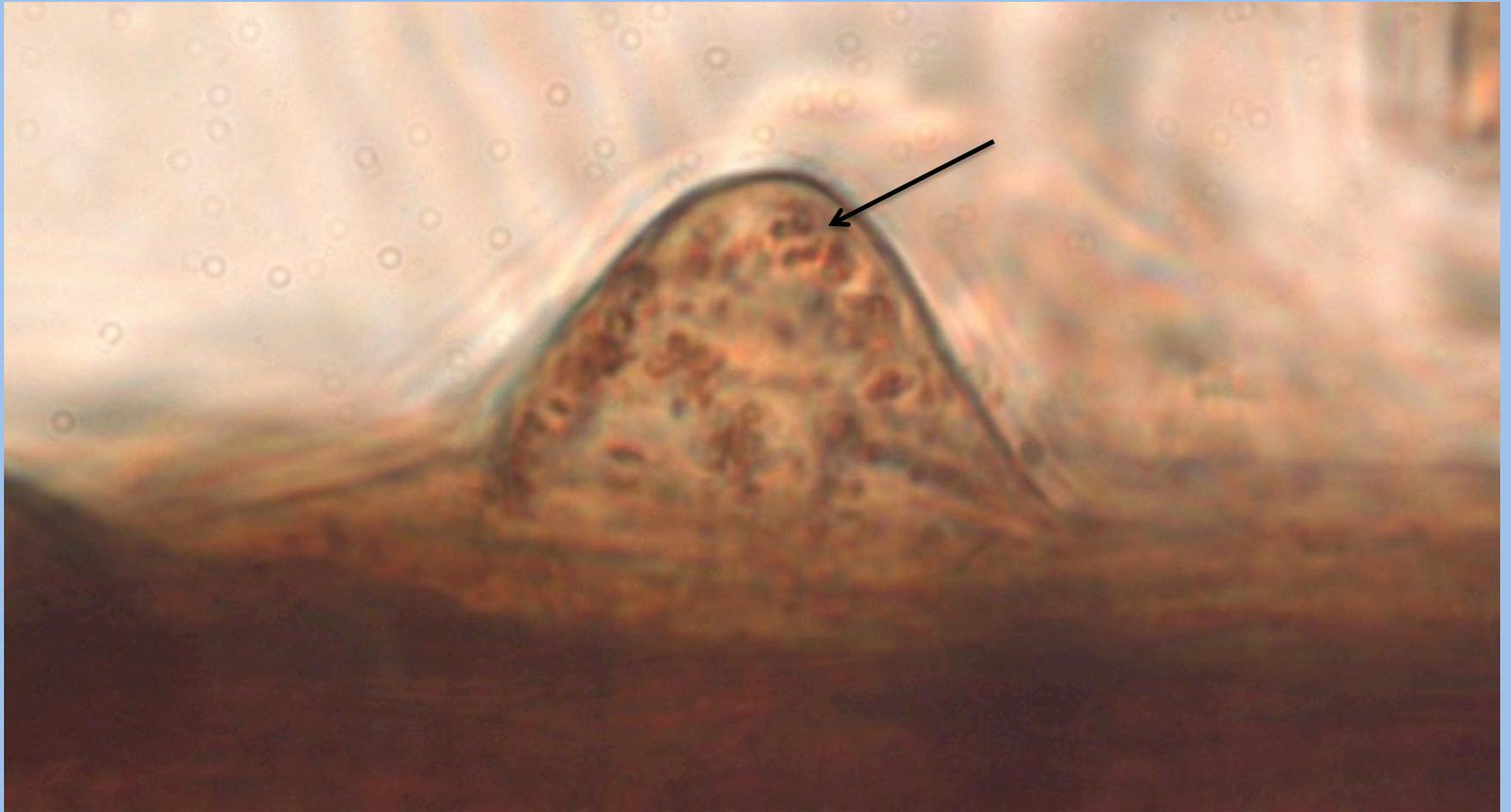
Root epidermis of tomato seedling without microbes. Root hair papillae are present—but hairs do not elongate without bacteria.



Root hair Initials of tomato seedling showing internal bacteria (arrow). The papillae accumulate microbes that continue to replicate. This papilla permits microbes to immobilize and accumulate.



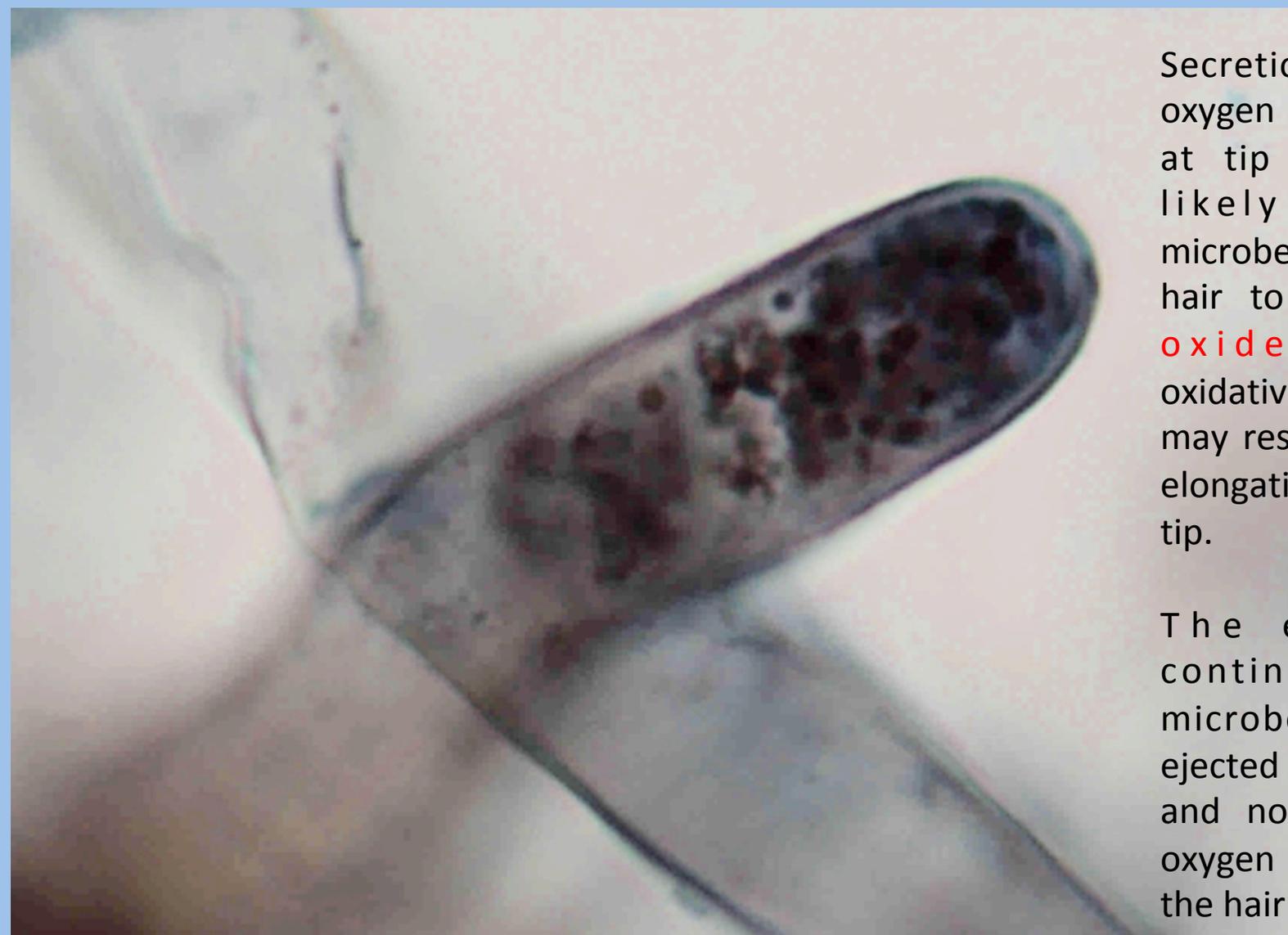
Tomato seedling hair initial with internal replicating bacteria (arrow).



Bacteria (arrow) in tip of young root hair of tomato seedling. Brown color shows where reactive oxygen is concentrating in the cell. This is also the site where most nitric oxide is expected to accumulate.



Bacterial concentration in root hairs appears to increase as hairs elongate. This image is of a tomato seedling root hair (stained with DAB/aniline blue).



Secretion of reactive oxygen around bacteria at tip (brown color) likely causes the microbe and the root hair to produce **nitric oxide** to reduce oxidative damage. This may result in continued elongation at the hair tip.

The elongation continues until all microbes have been ejected from the hair and no more reactive oxygen is produced at the hair tip.

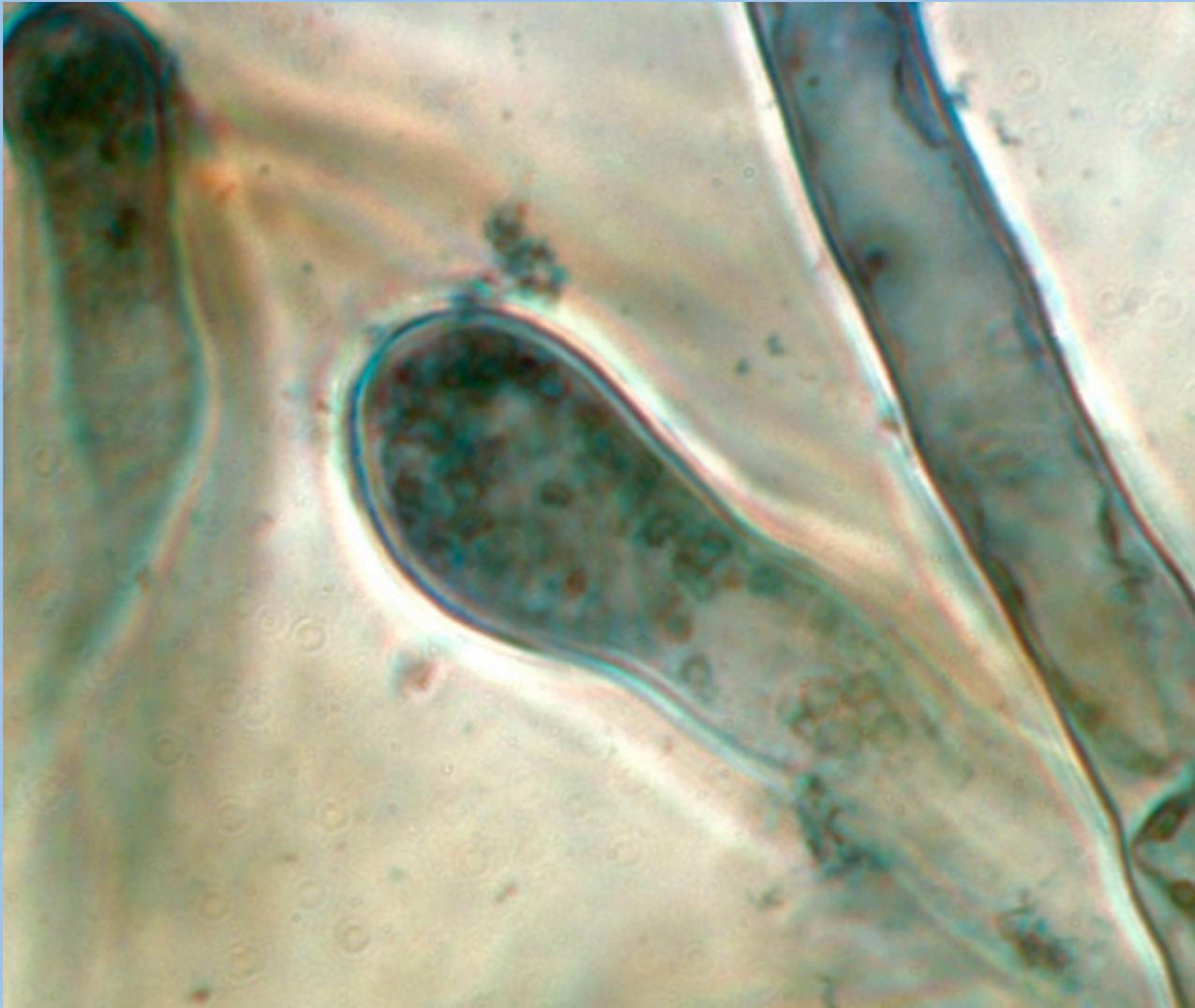
Tomato seedling root hairs containing bacteria showing intense hydrogen peroxide staining (brown) around bacteria. Often root hair tips with internal bacteria appear swollen. This shows the elasticity of the wall at the hair tip. Microbes continue to replicate in the hair.



Tomato seedling root hair showing swollen tip containing microbes.



Tomato root hair showing microbes being ejected from the hair tip.



Tomato root hair showing ejection of bacterial protoplasts (*Acinetobacter* sp.) from tip of hair (arrow).





Bacteria (large arrow) being expelled from the hair tip of tomato seedling. Stained with aniline blue. Bacteria (small arrow) are present beneath tip of the root hair.

Nutrient Flow in Hair is Toward Tip!

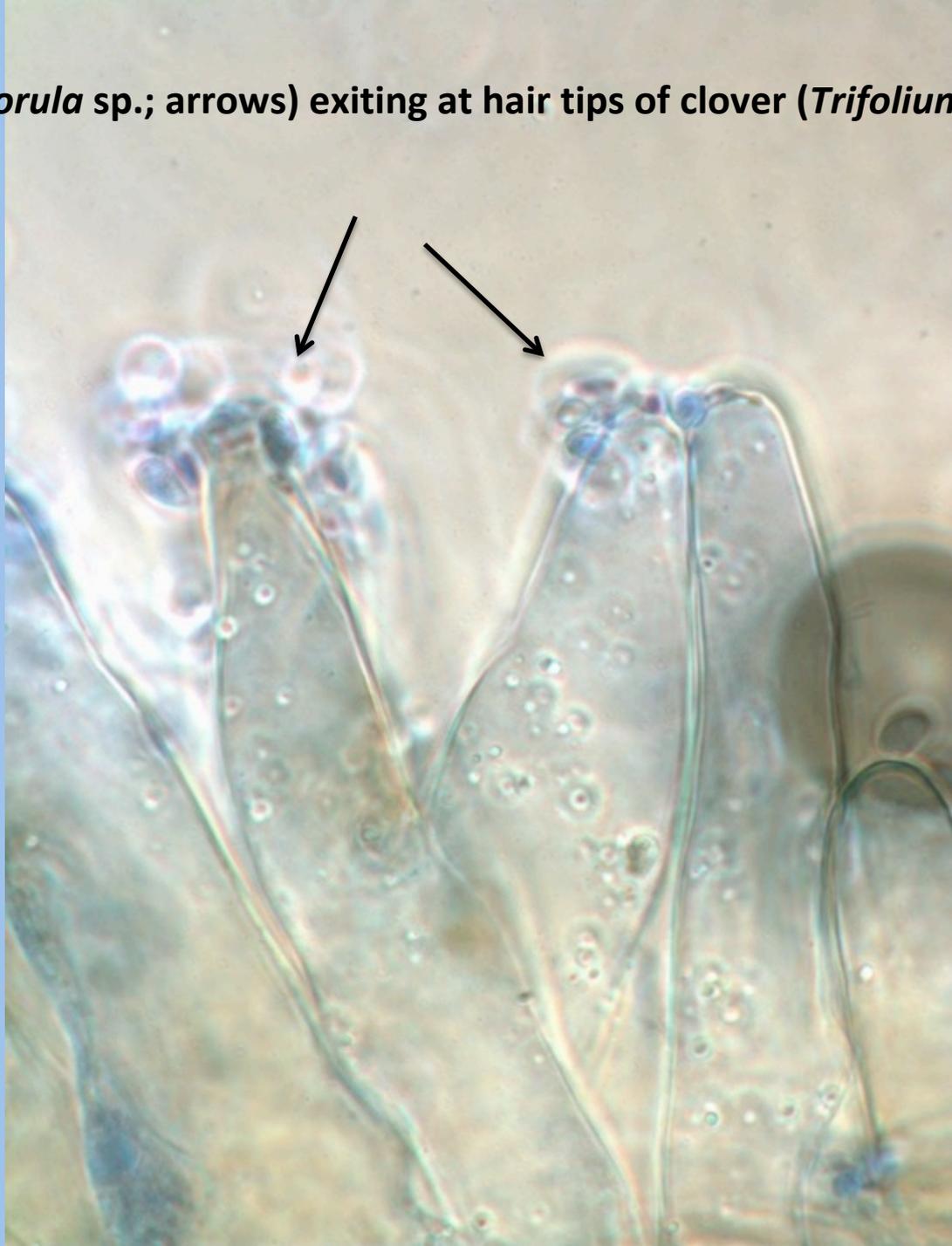
**Nutrients Flow to Hair Tip Providing Nutrients Rebuild
Microbe Cell Walls and Sizes.**

Yeast cells (*Rhodotorula* sp.) reforming cell walls and cell size after extrusion from root hair tip of a clover seedling. Cells are small when first extruded, and they enlarge as the cells age. (1 = youngest; 4 = oldest).



Microbe Protoplasts
In Hairs Are Indicated
By Small Arrows.

Yeast cells (*Rhodotorula* sp.; arrows) exiting at hair tips of clover (*Trifolium repens*) seedlings.



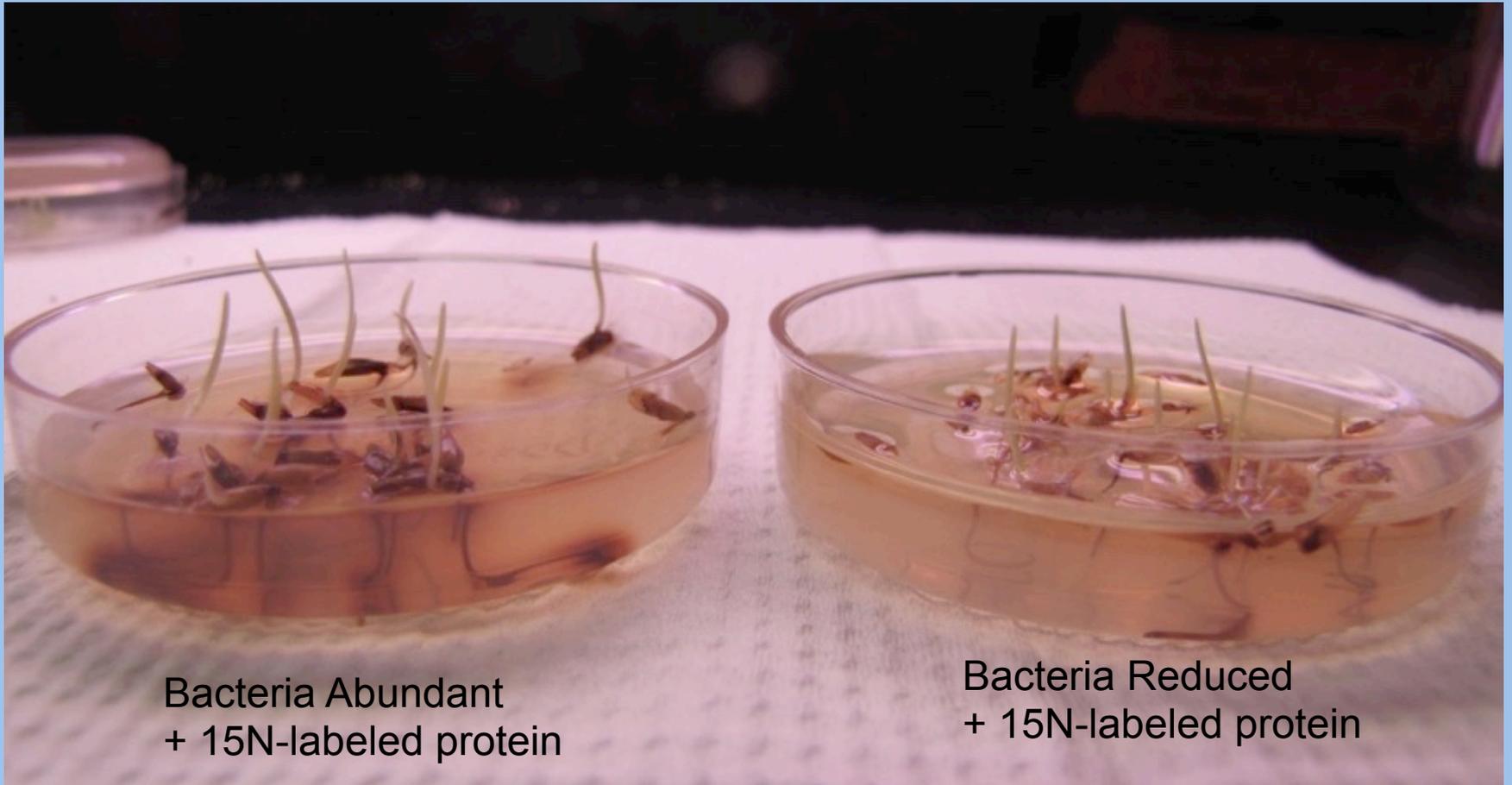
Nutrient Absorption Function of the Rhizophagy Cycle:

1. Isotope tracking experiments.

15N-labeled protein absorption experiment:

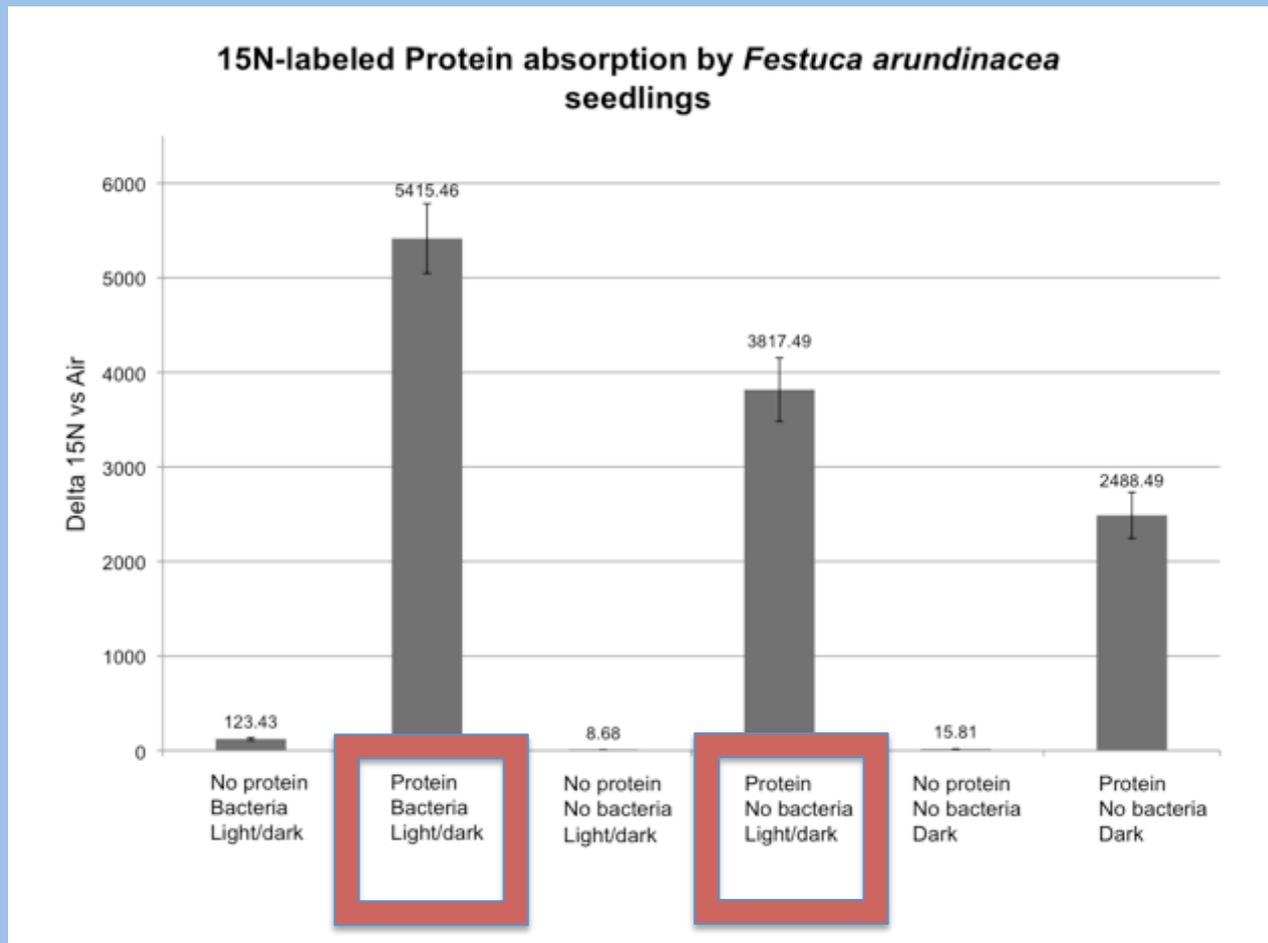
- *Bacillus amyloliquefaciens* grown in 15N-labeled glycine medium
- Total proteins extract from bacterial cells and freeze dried
- Proteins mixed with egg albumin at ration of 1:5
- Proteins (0.05%) incorporated into 0.7% agarose
- Tall fescue seeds with and without bacteria were germinated on the labeled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

1. Proteins → nitrogen

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

2. Micronutrients → iron, zinc, manganese

Microbes contain metals in order of concentration: Mg > Ca > Fe > Zn etc.. (Monowar et al., 2019).

High-affinity zinc and iron binding proteins are common in soil microbes (Hantke, 2005).

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>

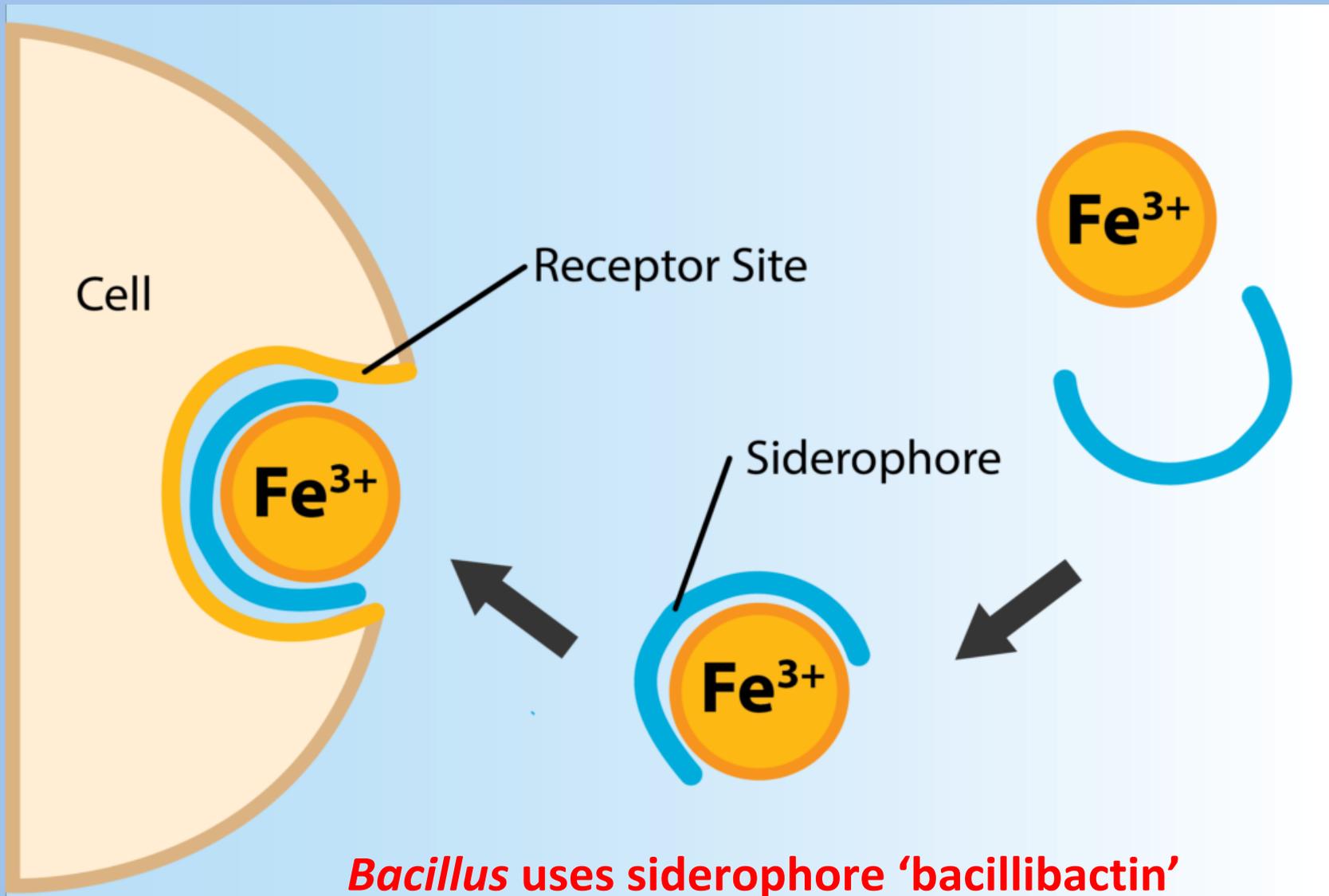
Hantke K. (2005) Bacterial zinc uptake and regulators. *Current Opinion Microbiol.* 8: 196-202.

Tahmina Monowar, Md. Sayedur Rahman, Subhash J. Bhole, Gunasunderi Raju, and Kathiresan V. Sathasivam (2019). “Secondary Metabolites Profiling of *Acinetobacter baumannii* Associated with Chili (*Capsicum annuum* L.) Leaves and Concentration Dependent Antioxidant and Prooxidant Properties,” *BioMed Research International*, vol. 2019, Article ID 6951927, 13 pages, 2019. <https://doi.org/10.1155/2019/6951927>.

How do microbes acquire nutrients in rhizosphere?

1. Siderophores
2. Hemophores
3. Adsorption of metals to microbial cell walls
4. Direct absorption using metal transporters (e.g., iron permease)
5. Absorption of organic acid—metal complexes using Cit-M Transporters
6. Use of 'biosurfactants' (hemolysins) to lyse or cause nutrient leakage in other bacteria

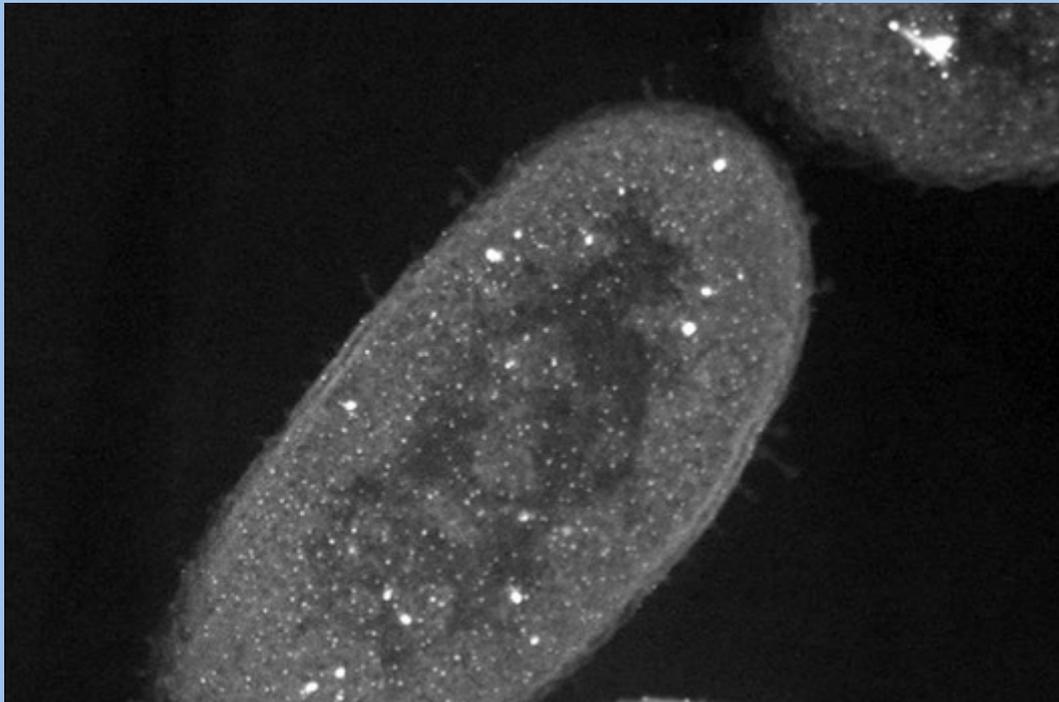
How siderophores work



Microbe cell walls carry nutrients that plants require for basic functions (e.g., photosynthesis)

The cell wall of Gram + bacteria is a polyelectrolyte due to content of peptidoglycans and teichoic acids that give a strong negative charge. Metals (Ca^{2+} , Mg^{2+} , Zn^{2+} , Mn^{2+}) bind tightly to the negatively-charged wall surface. The image below shows silver (white particles) adhering to a microbe cell wall.

Stripping away the cell walls of microbes delivers key nutrients to plants!



Microbes (e.g. *Bacillus* spp.) and plants collaborate in scavenging soils for nutrients using organic acids!

Higher levels of organic acids secreted into rhizospheres of plants under nutrient limitation and nutrient toxicity (e.g., Al toxicity)

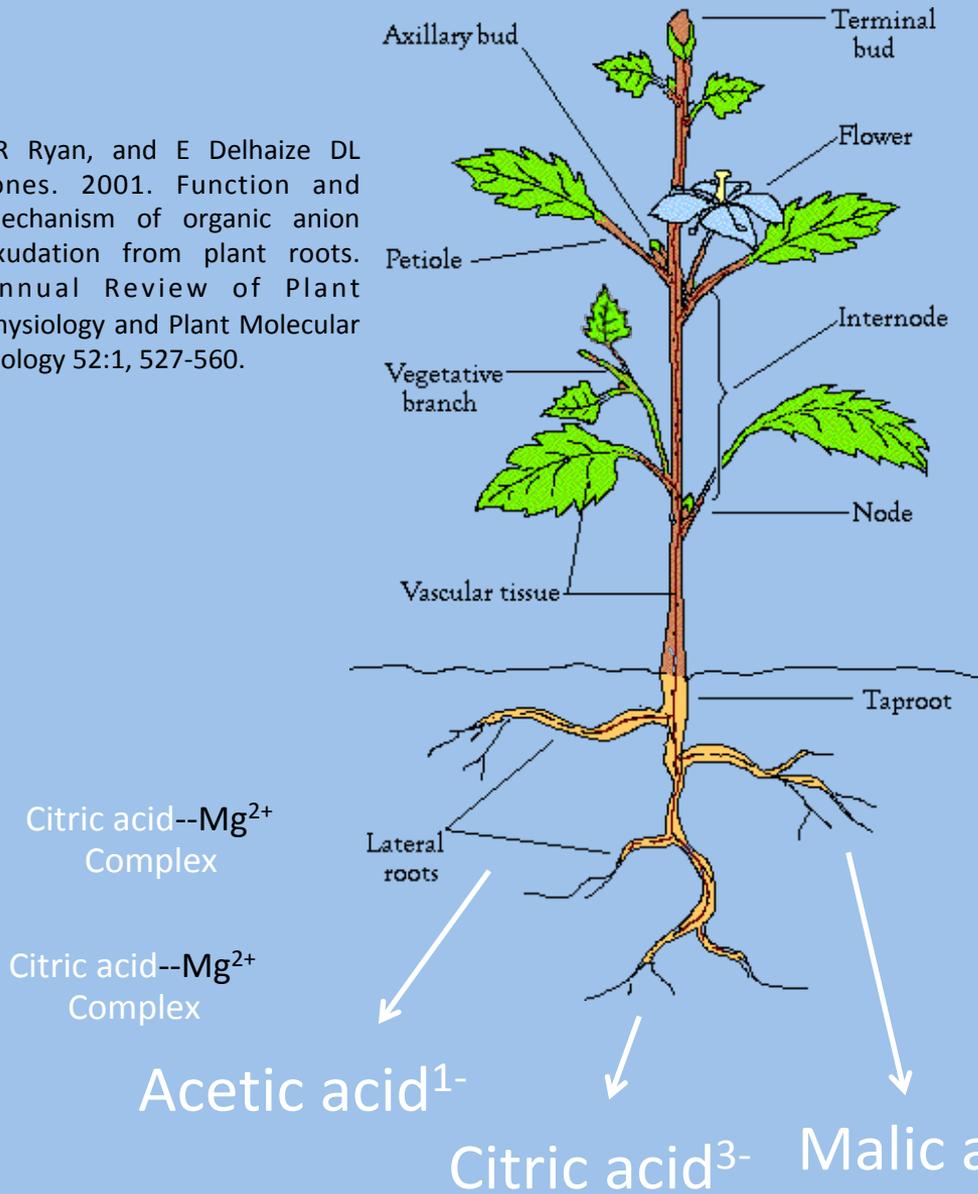
Binding capacity of organic acids

Citrate³⁻ > Malate²⁻ > Acetate¹⁻

OAs bind to Fe³⁺, Mg²⁺, Co²⁺, Mn²⁺, Cu²⁺, Ni²⁺

1) Plants secrete organic acids

2) *Bacillus* transports Citrate-metal complex into bacterial cell using Cit-M Transporter.



Citric acid--Fe³⁺
Complex

Citric acid--Fe³⁺
Complex

Fe³⁺

Fe³⁺

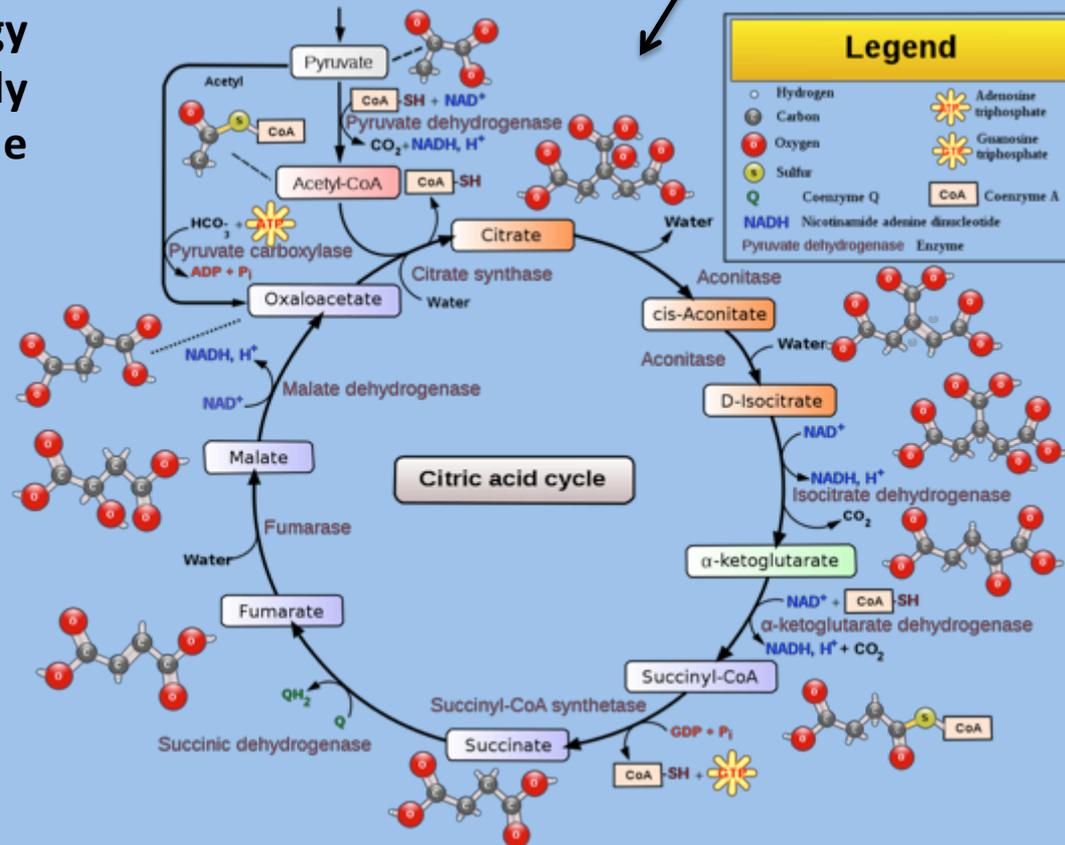
Fe³⁺

Citrate—Metal Complex (in soil)

CitM Transporter
(in *Bacillus* plasma membrane)

Citrate + Metal

In *Bacillus* citrate is used as an energy source by directly entering into the Krebs cycle.



Warner JB, Lolkema JS. 2002. Growth of *Bacillus subtilis* on citrate and isocitrate is supported by the Mg^{2+} -citrate transporter CitM. *Microbiology* (2002), 148, 3405–3412.

Rhizophagy microbes scavenge
nutrients from other soil microbes!!

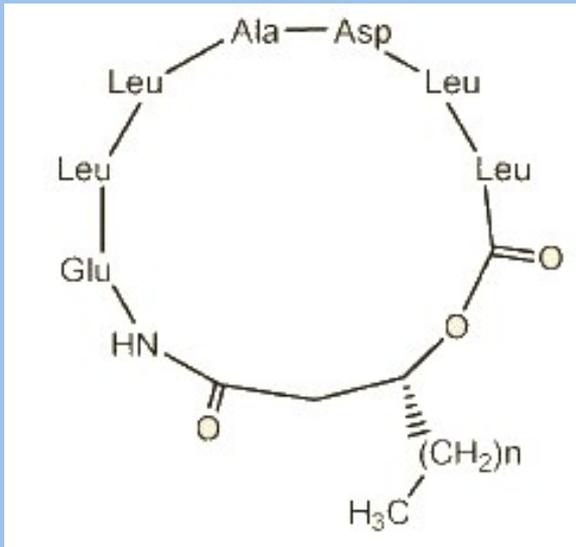
Bacillus may acquire iron from other microbes using 'hemolysins'!

Hemolysins produce leakage in plasma membranes.

Bacillus secretes biosurfactins (lipopeptides) that go into bacterial membranes and cause poration and nutrient leakage.

Bacillus also produces powerful secreted proteases to
Degrade proteins of other microbes.

Nutrient acquisition arsenal of *Bacillus* endophytes: surfactin lipopeptides



Cyclic peptide head
+
Fatty acid tail

Direct antibiosis effects on fungi and bacteria

What happens to plants without the rhizophagy cycle?



Satish K. Verma

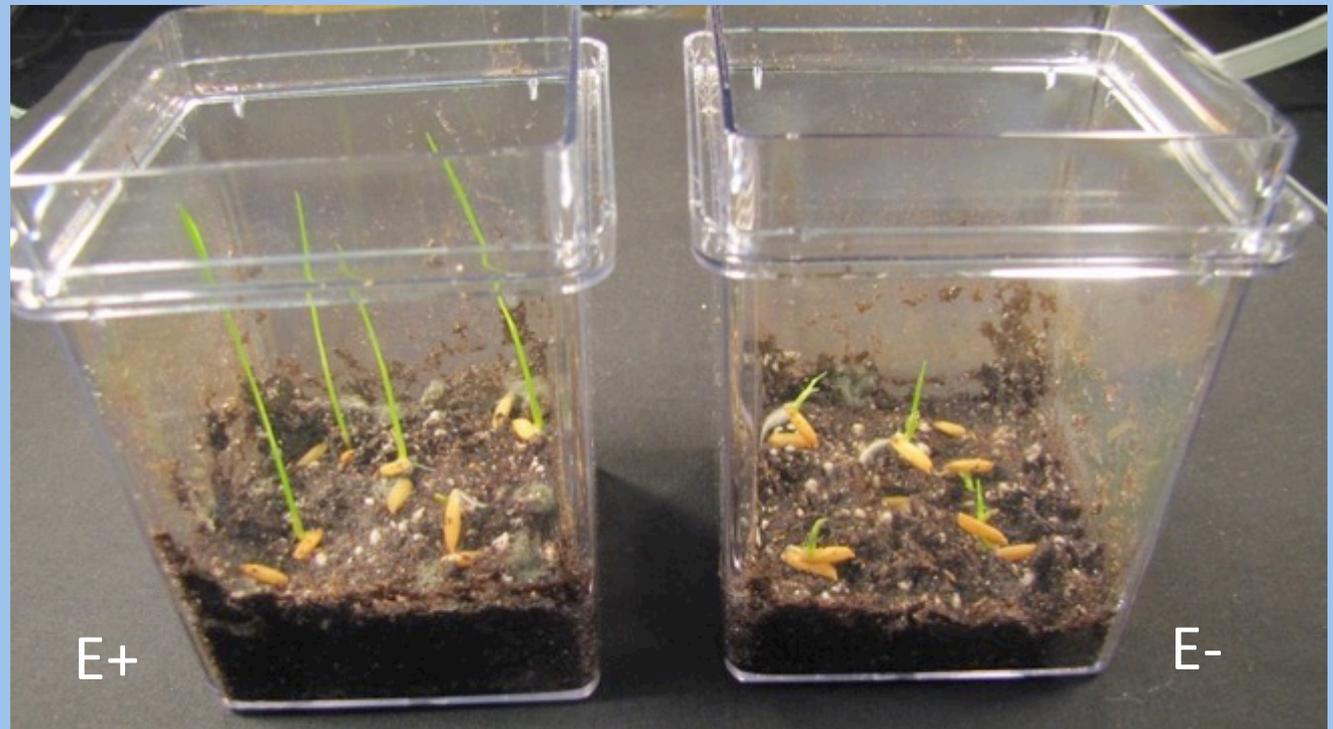


Kate Kingsley



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*)



Rhizophagy Cycle Implications for Sustainable Plant Cultivation

- Preserve native microbes on seeds (i.e., no antimicrobials on seeds, and no removal of seed tissues (de-husking)).
- Use organic amendments in soil to build up the soil microbial community.
- No-till is a good practice to keep the topsoil microbial community intact.
- Applications of inorganic fertilizers to plants and soil may reduce benefit of nutrient acquisition through the rhizophagy cycle (e.g., nitrate applications to soil inhibits nitrogen fixation in soil).

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