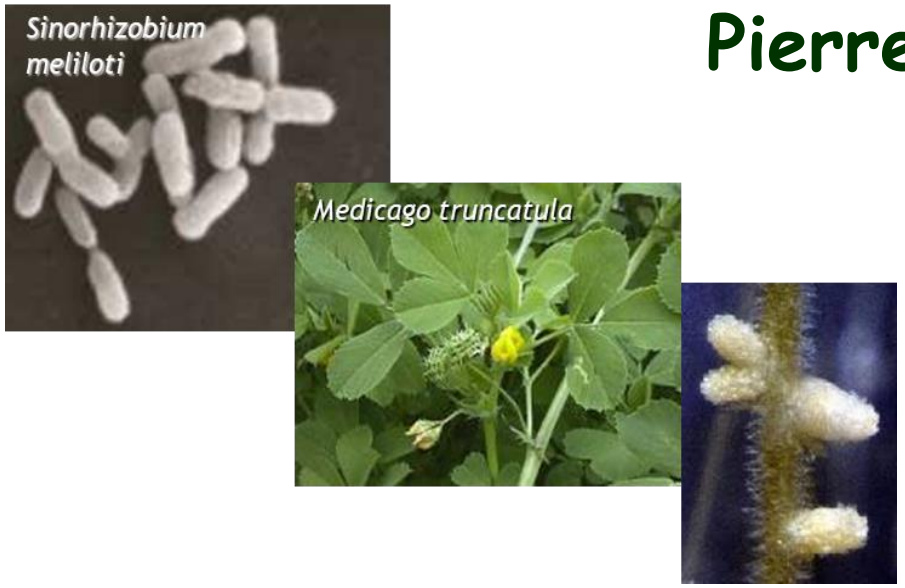
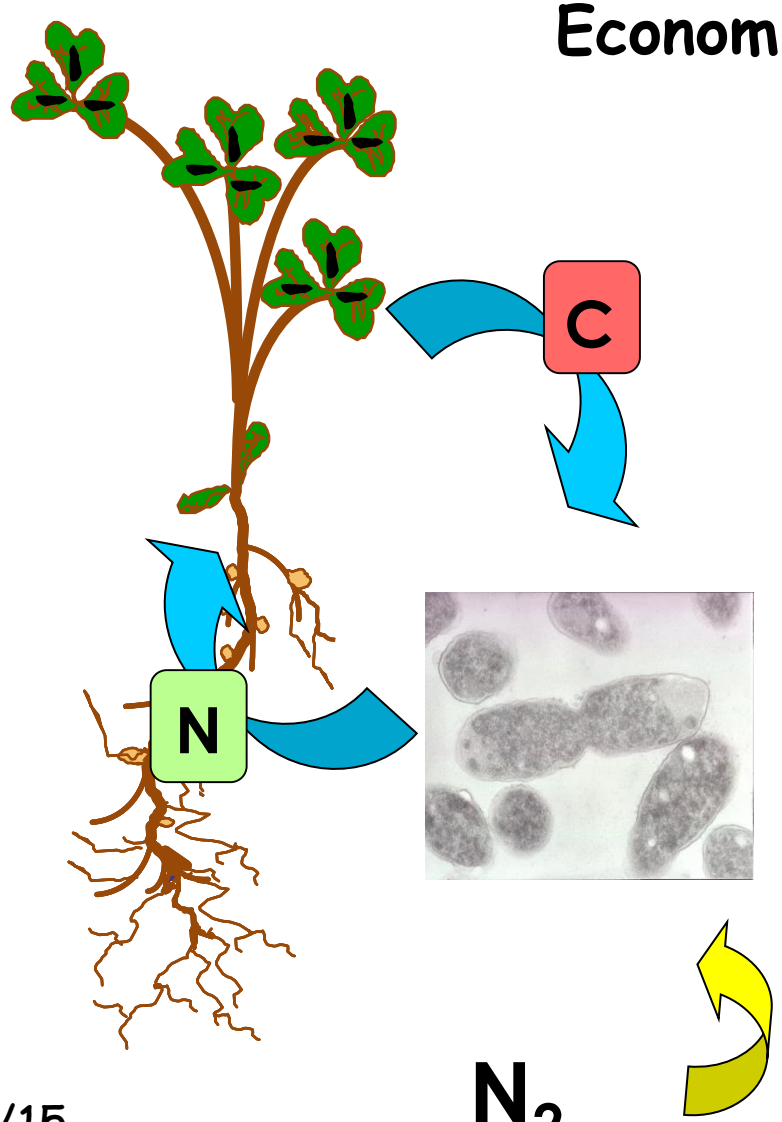
Redox-sensitive fluorescent biosensors detect the symbiotic bacteria *Sinorhizobium meliloti* intracellular redox changes under free-living and symbiotic lifestyles

Pierre Frendo



Redox Biology Congress,
August 24 - 26, 2022

The nitrogen-fixing symbiosis between legumes and rhizobia



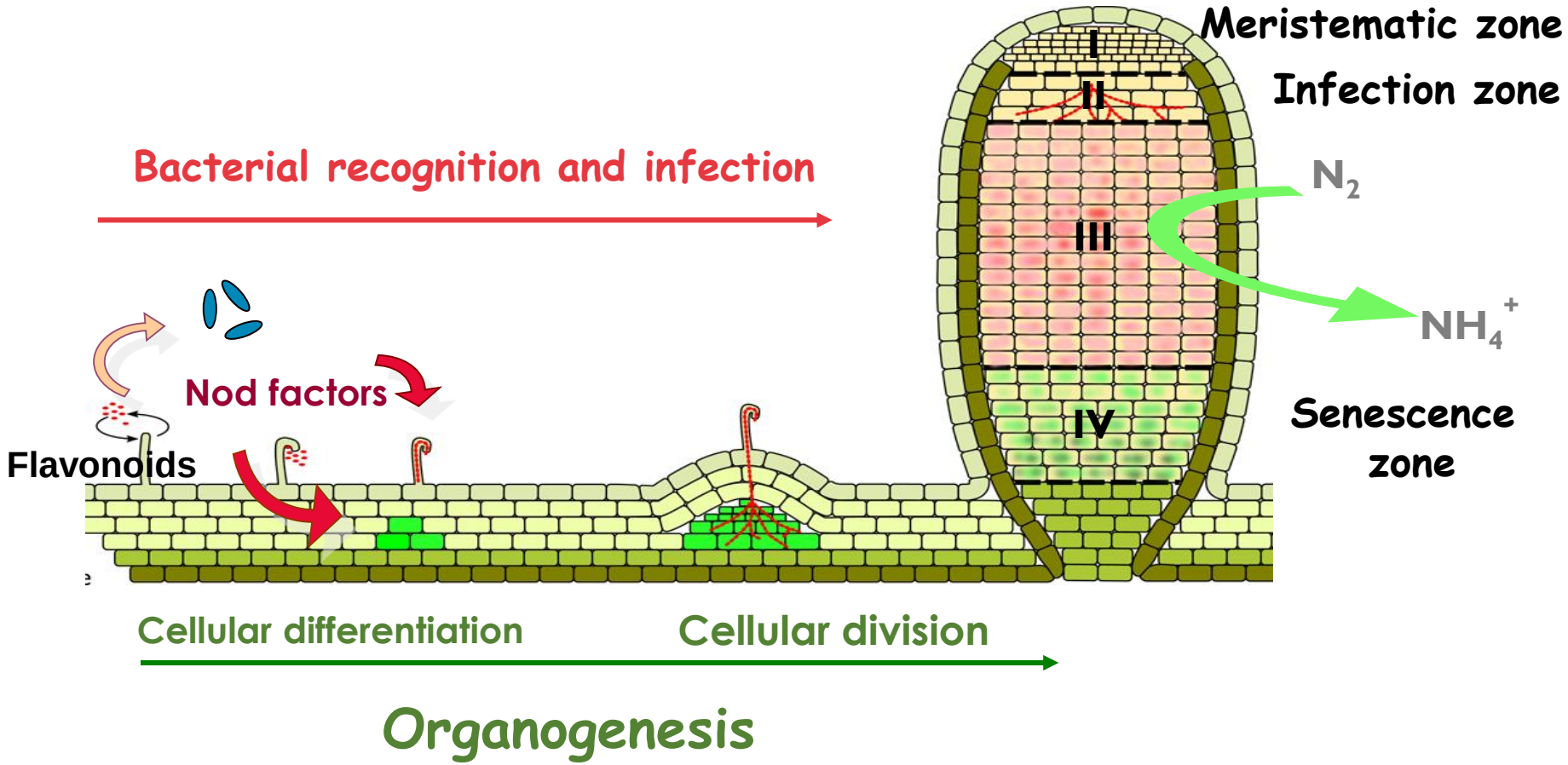
Economical and environmental interests

Economically valuable species (soybean, alfalfa, pea,…)

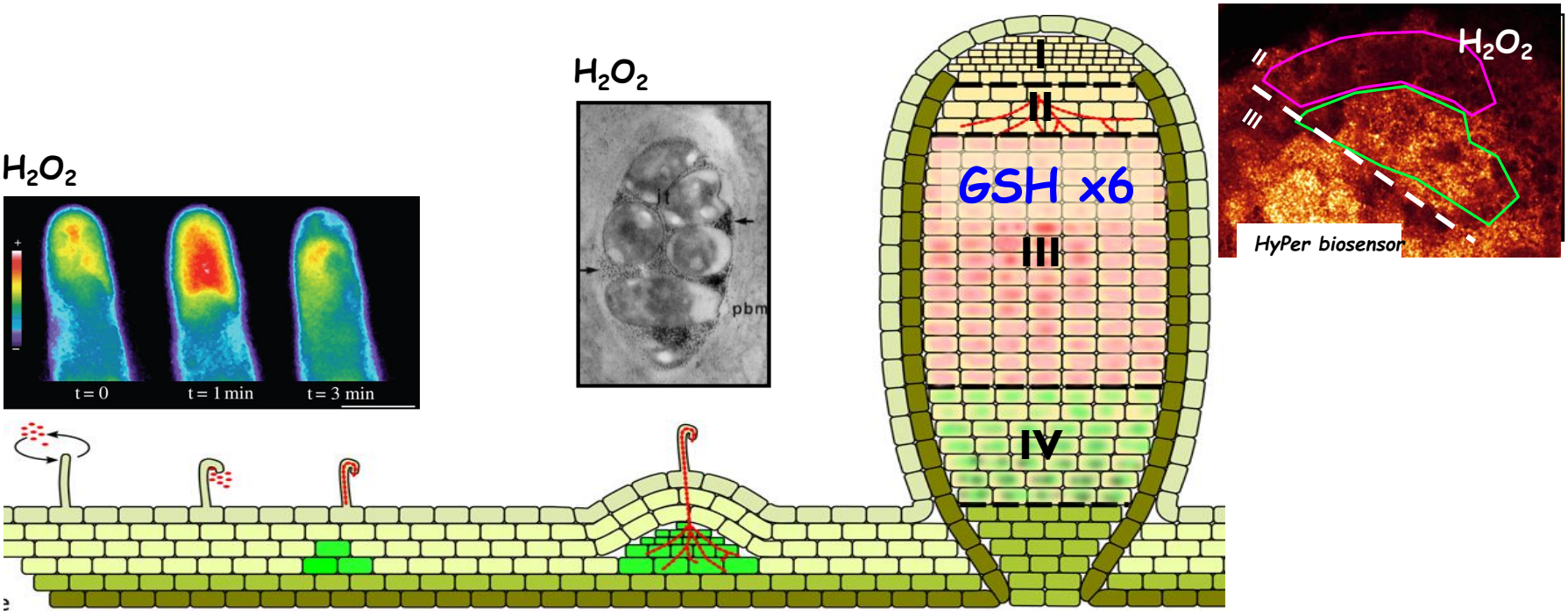
Interesting nutritional quality for food and feed (20 to 40% protein)

Energy saving and reduction of water pollution (reduced use of nitrogen fertilizers)

The nitrogen-fixing symbiosis



Accumulation of ROS in the plant partner during symbiosis



The components of the redox balance are modified in the plant partner during symbiosis.

Importance of bacterial redox regulation in symbiosis

MPMI Vol. 16, No. 3, 2003, pp. 217–225. Publication no. M-2003-0109-01R. © 2003 The American Phytopathological Society

Expression of the Bacterial Catalase Genes During *Sinorhizobium meliloti–Medicago sativa* Symbiosis and Their Crucial Role During the Infection Process

Alexandre Jamet, Samuel Sigaud, Ghislaine Van de Sype, Alain Puppo, and Didier Hérouart

Catalase mutants are impaired in cell infection and in biological nitrogen fixation.

frontiers in Plant Science

ORIGINAL RESEARCH
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Glutathione Deficiency in *Sinorhizobium meliloti* Does Not Impair Bacteroid Differentiation But Induces Early Senescence in the Interaction With *Medicago truncatula*

OPEN ACCESS

Edited by:
Benjamin Gourion, Laboratoire Interactions Plantes-Microorganismes, France

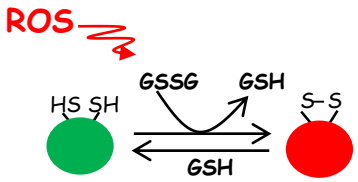
Reviewed by:

Li Yang[1†], Sarra El Msehli[2†], Sofiane Benyamina[1], Annie Lambert[1], Julie Hopkins[1], Julie Cazareth[3], Olivier Pierre[1], Didier Hérouart[1], Samira Achi-Smiti[2], Eric Boncompagni[1] and Pierre Frendo[1]*

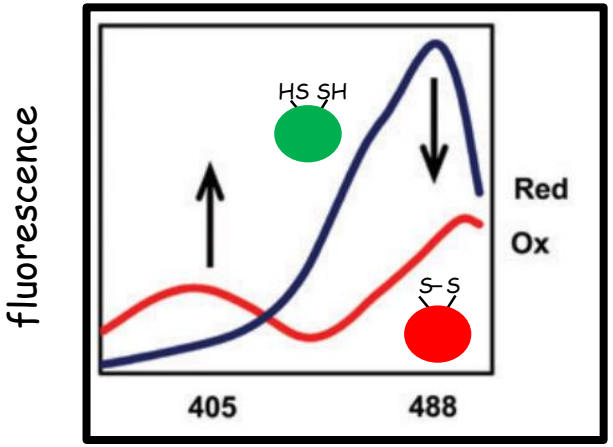
Glutathione is crucial for an efficient symbiotic nitrogen fixation.

What is the redox state of the bacteria ?

The redox-sensitive GFPs (roGFP) biosensors



roGFP2 (GSH specific, low rate)



from Schwarzländer et al., 2015

- Ratiometric measurements = independent of GFP expression level
- ↗ 405/488 ⇔ ↗ oxidation

Measure of E_{GSH}

Grx1-roGFP2
(GSH specific, higher rate)

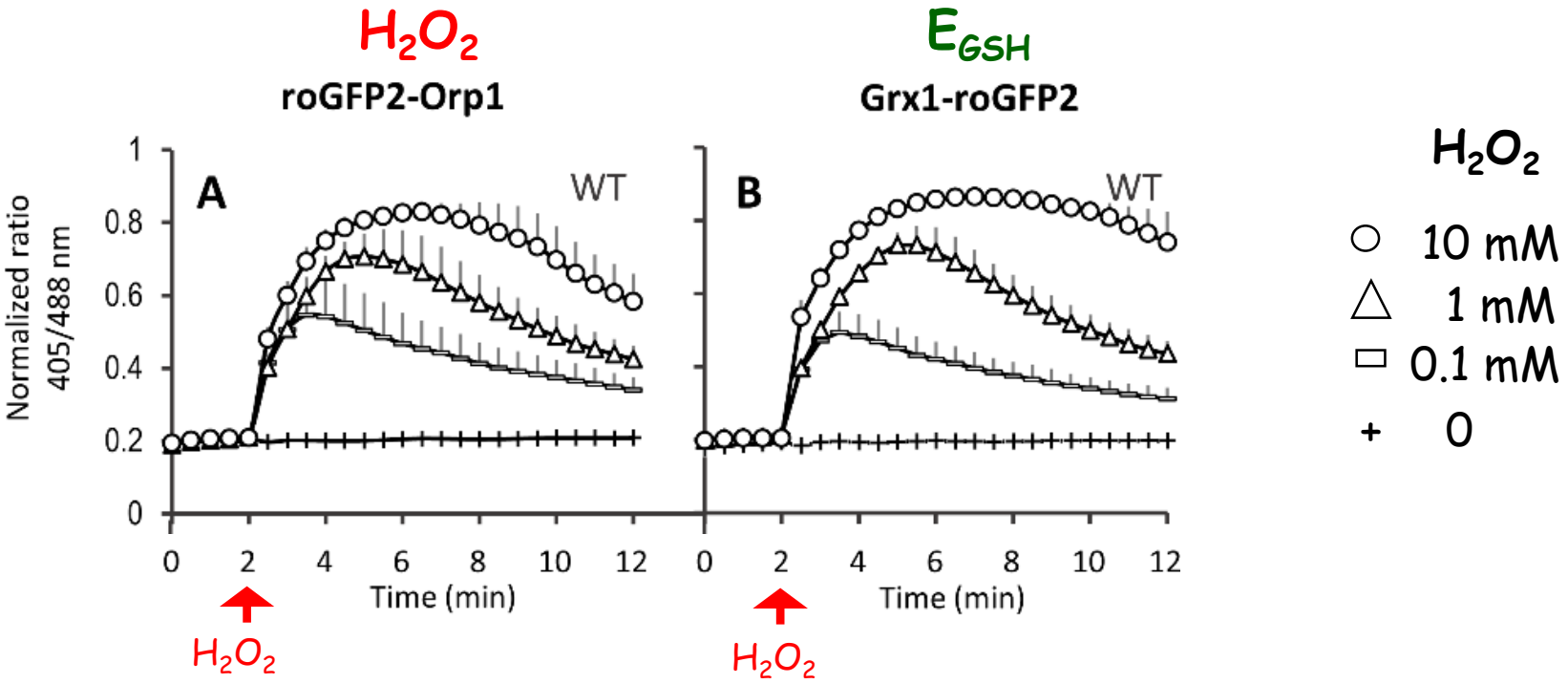
Measure of intracellular H_2O_2

roGFP2-Orp1
(H_2O_2 specificity for oxidation)

The biosensors were introduced into *S. meliloti* under the control of a strong promoter (Ptrp)

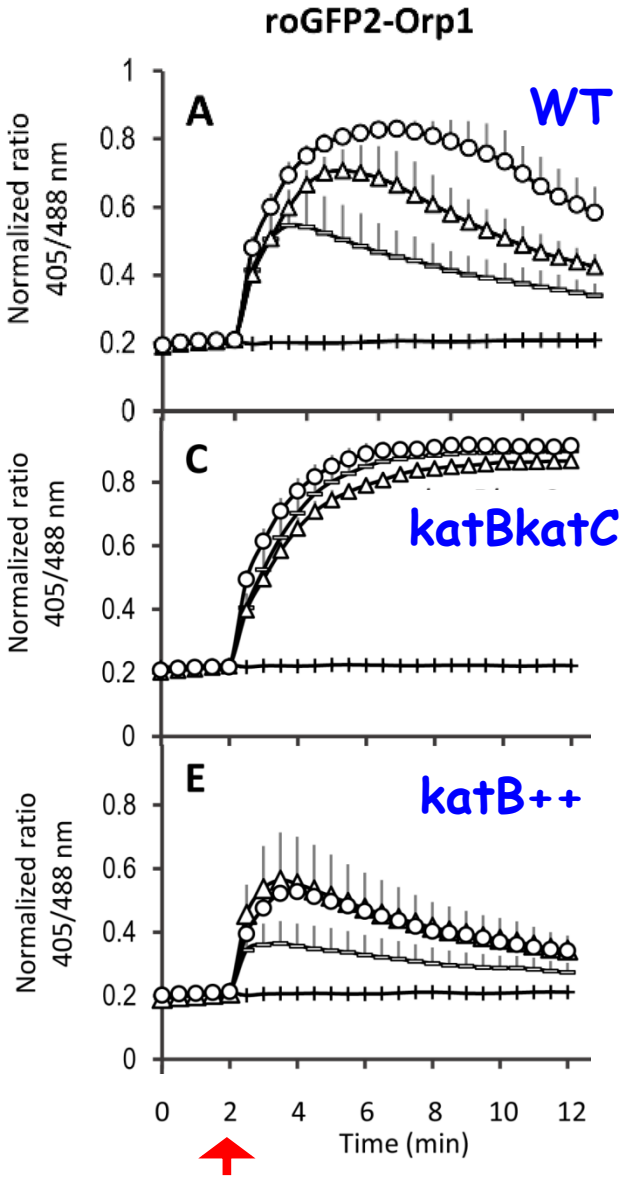
Effect of H_2O_2 treatment in the free-living bacteria

Emission was measured by fluorimetry



real-time measurements of *S. meliloti* GSH redox potential/H_2O_2 intracellular levels

Analysis of H_2O_2 treatments in catalase mutant strains



H_2O_2

○ 10 mM

△ 1 mM

▭ 0.1 mM

\+ 0

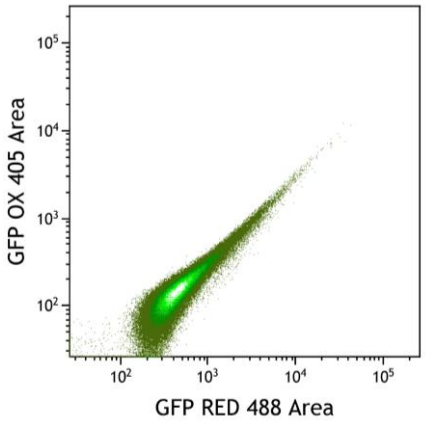
Lower H_2O_2 detoxification

Higher H_2O_2 detoxification

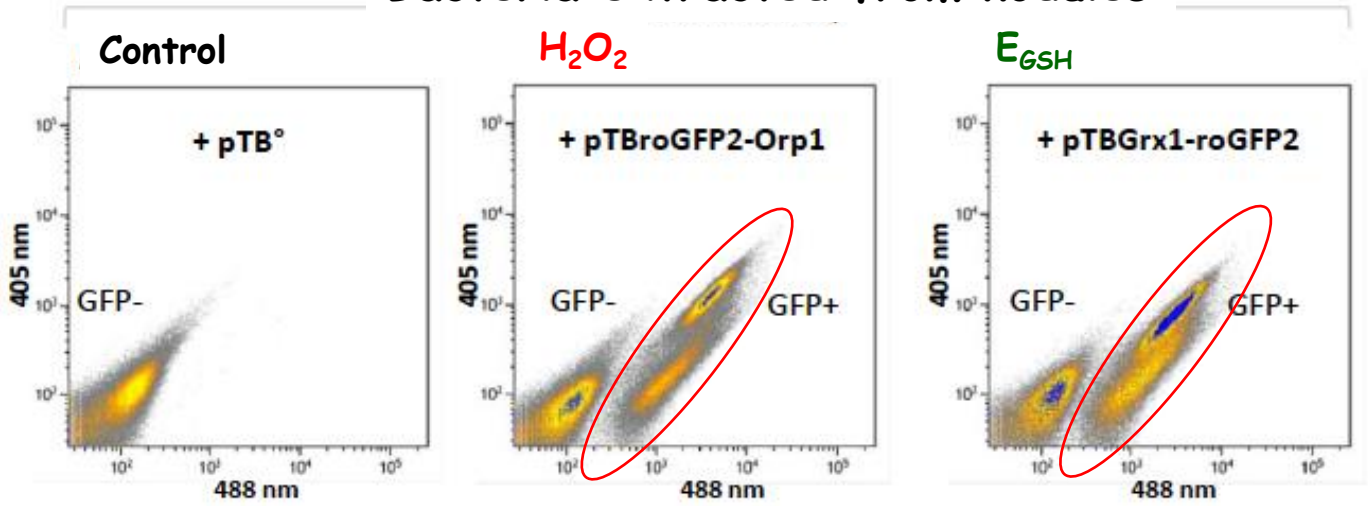
Validation of the probes in *S. meliloti*

Analysis of roGFP2 nodule bacteria by flow cytometry

Free bacteria



Bacteria extracted from nodules



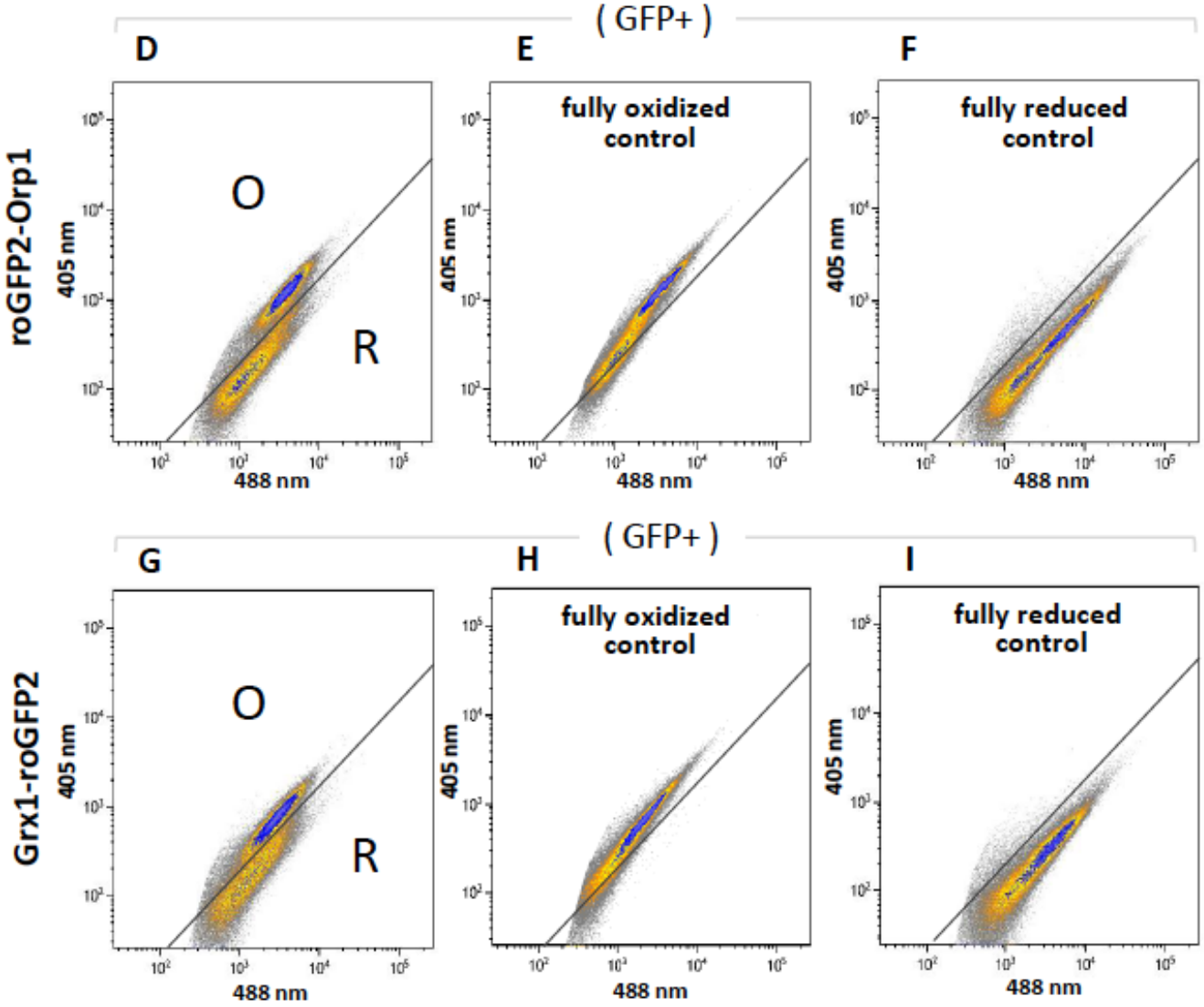
Use of N-ethylmaleimide (NEM) to maintain the cellular redox state

Selection and analysis of GFP+ bacteria

Analysis of roGFP2 nodule bacteria by flow cytometry

H_2O_2

E_{GSH}

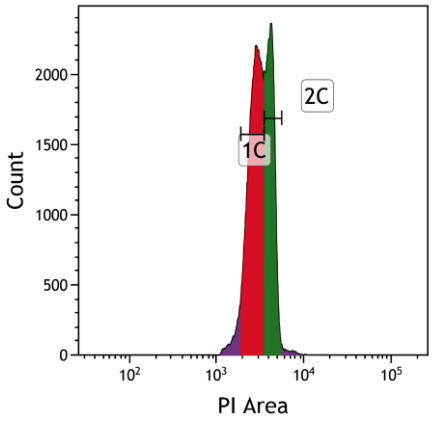


2 populations with distinct redox states in nodule bacteria

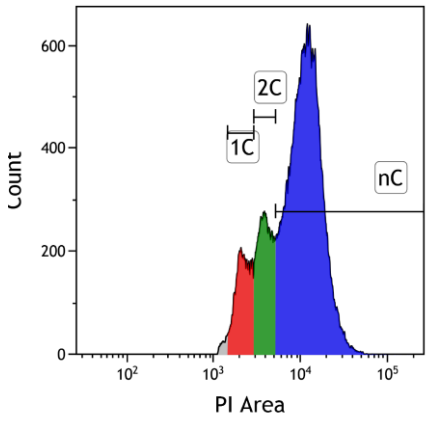
Analysis of roGFP2 nodule bacteria by flow cytometry

Analysis of bacteroid differentiation

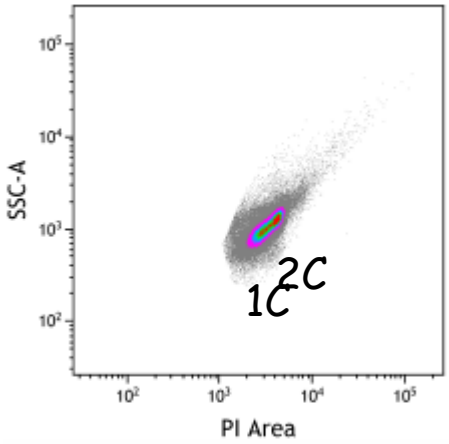
Free bacteria

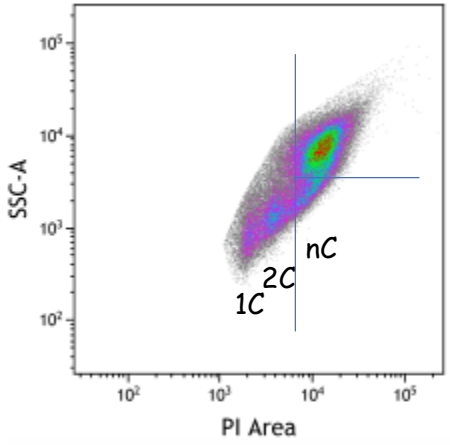


Nodule bacteria



Endoreduplication – DNA quantity (coloration with propidium iodide PI)





Granularity-complexity side scatter (SSC)

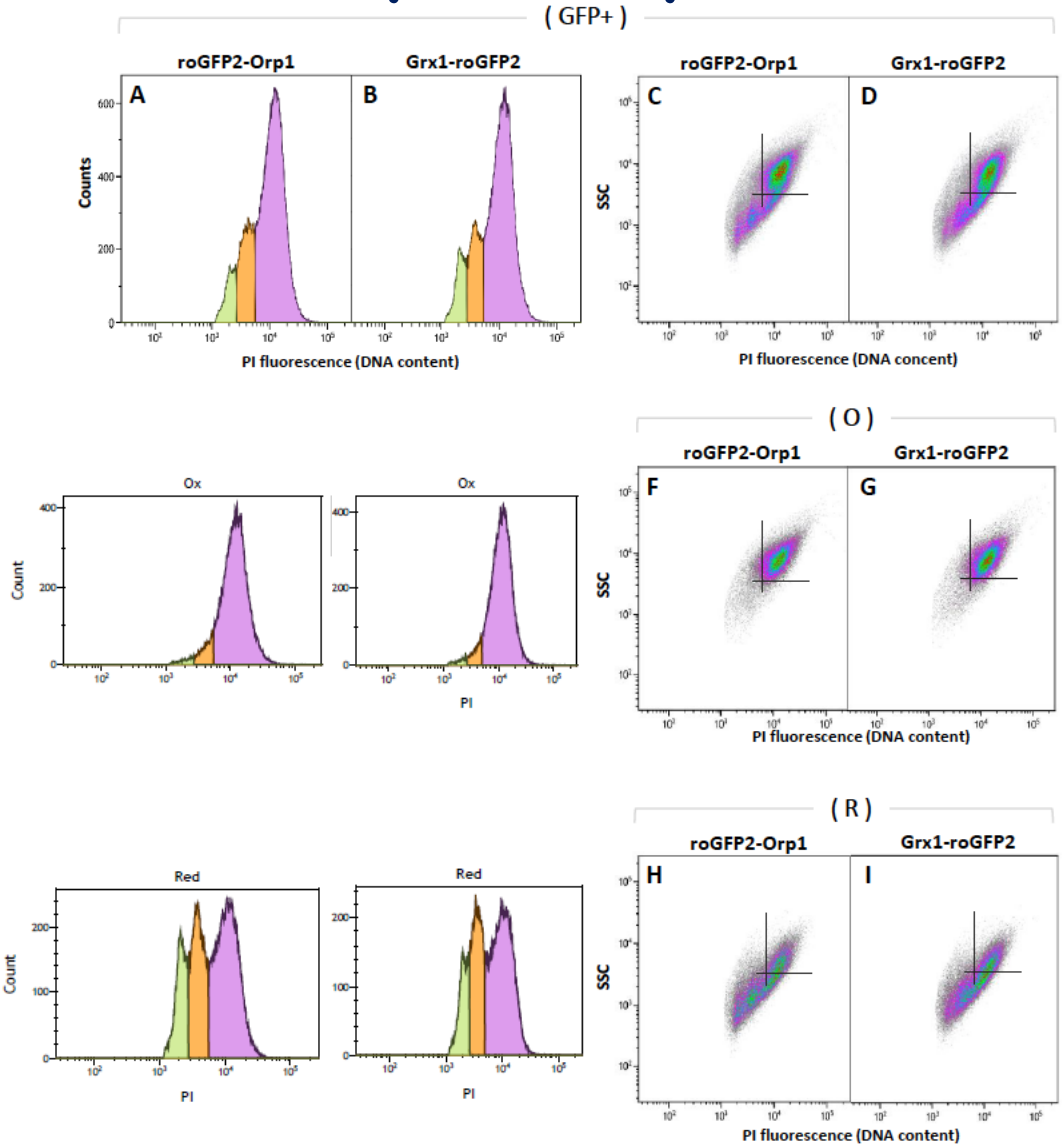
PI treatment and SSC allow to separate bacteroids from bacteria

Analysis of roGFP2 nodule bacteria by flow cytometry

total cells

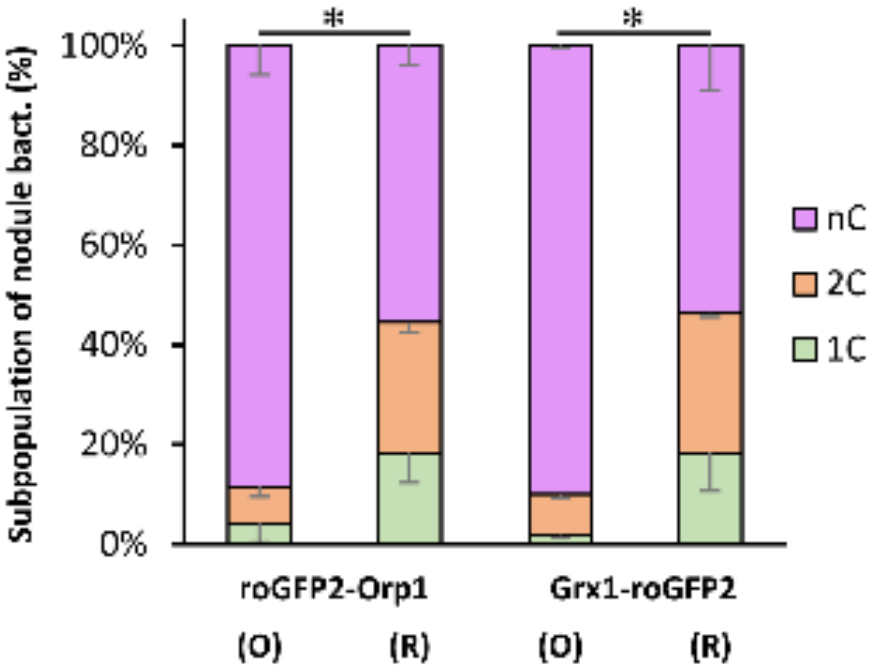
O cells (-245 mV)

R cells (-295 mV)



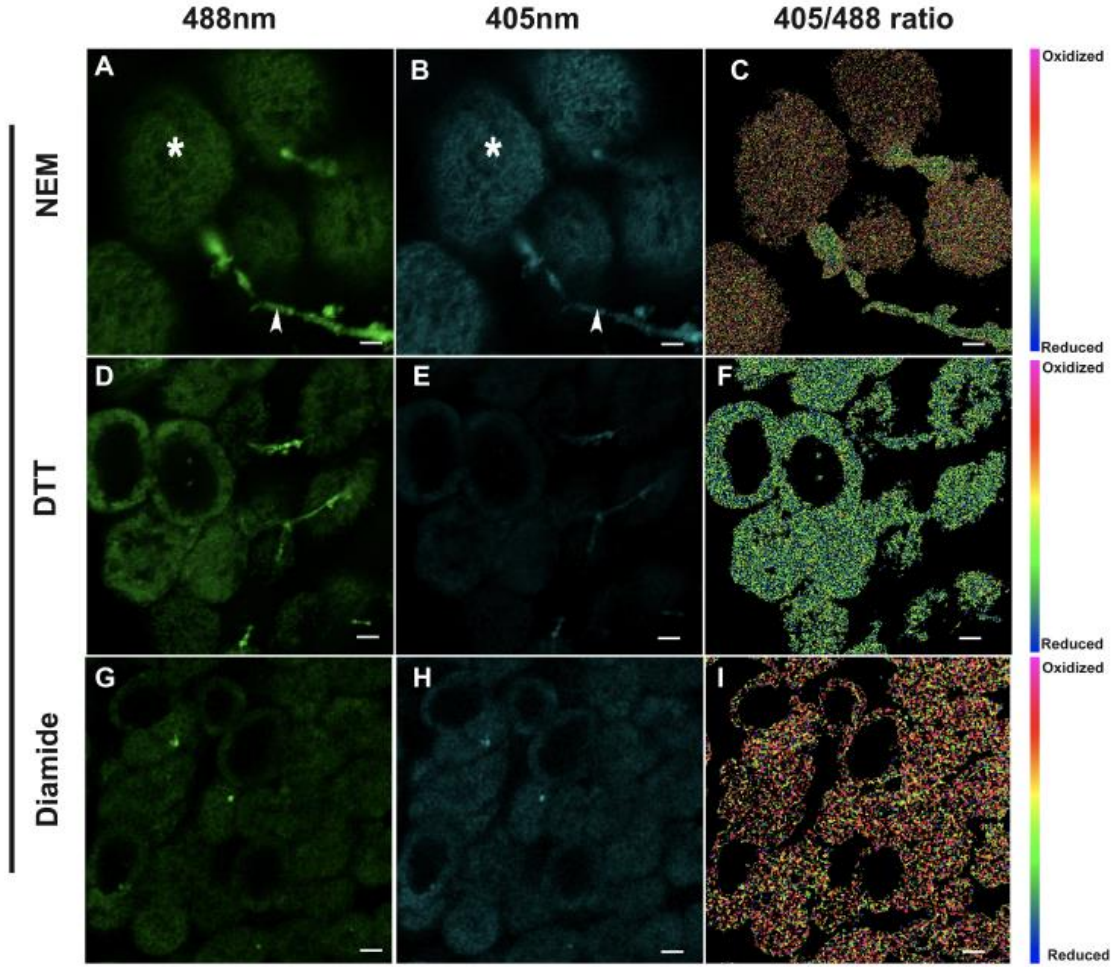
Bacteroids presented a more oxidized state than bacteria

Analysis of roGFP2 bacteroids by flow cytometry



Analysis of nodule roGFP2 bacteria suggest that the oxidized population contains 90% bacteroids

Analysis of nodules sections by confocal microscopy



E_{GSH}

Control for reduction

Control for oxidation

The microscopic analysis confirms the oxidized state of the bacteroids compared to bacteria present in the infection threads

Acknowledgments

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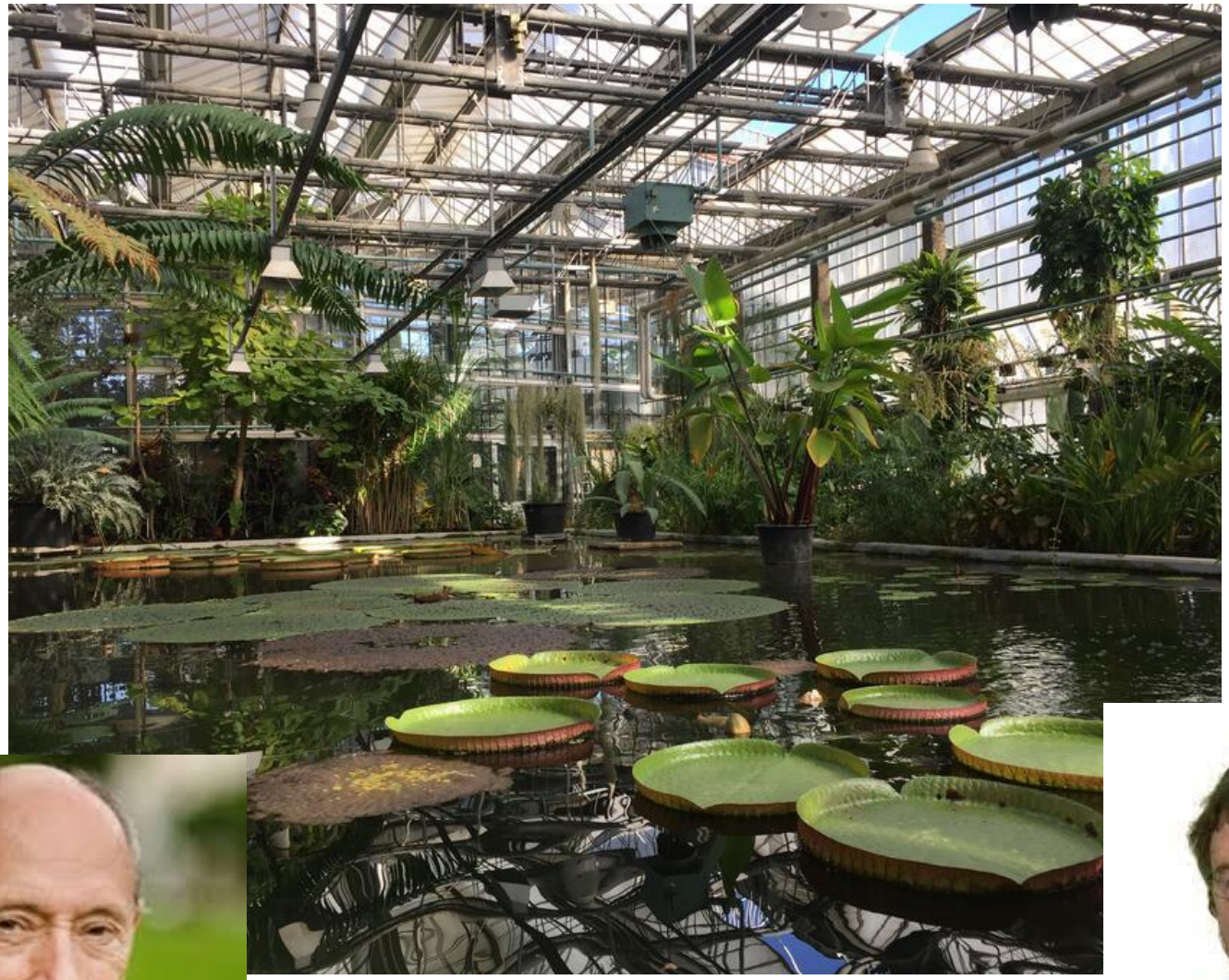
Pacoud et al. (2022)
Free Radic Biol Med. 184:185-195.

1993-1995 : Laboratorium voor Genetica Ledeganckstraat 35, Ghent



Thank you Marc and Dirk