Molecular signaling of bacteria on root surfaces: Computer aided analysis of the *in situ* "calling distance"

(together with BIOP and with external cooperations)

The communication of bacterial populations is of great importance for the regulation of cellular activities and adaptation to a changing conditions. We used computer-assisted microscopy at single cell resolution to quantify the *in situ* spatial scale of N-acylhomoserine lactone (AHL)-mediated cell-to-cell communication of *Pseudomonas putida* colonizing tomato and wheat root surfaces. The results of this *in situ* quantification study on natural surfaces challenge the conventional view of a quorum group requirement of high cell densities for this type of bacterial communication. *In situ* image analysis indicated that the effective 'calling distance' on root surfaces was most frequent at 4–5 µm, extended to 37 µm in the root tip/elongation zone and further out to 78 µm in the root hair zone. The spatial scale of these calling distances is very long-range in proportion to the size of individual bacteria. Geostatistical modeling analysis implicated the importance of AHL-gradients mediating effective communication between remote cells. We conclude that AHL-mediated cell-to-cell communication occurs not only within dense populations, but also in very small groups and over long ranges between individual bacteria, and therefore this cellular activity is more commonplace and effective than hitherto predicted. We propose that this cell-to-cell communication is governed more by the *in situ* spatial proximity of cells within AHL-gradients than the requirement for a quorum group of high population density.

Further experiments: Currently, calling distances of AHL-signaling on the root surface are analyzed three dimensionally with newly constructed sensors and special attention is paid to the impact of cell clustering and restricted diffusion. These experiments are performed in the frame of the additional funding project "Molecular interaction in the rhizosphere" in cooperation with image analysis and mathematical modelling (IBB) and high resolving chemical analysis (IÖC).

Gantner S., Schmid Michael, Dürr C., Schuhegger R., Steidle A., Hutzler P., Langebartels C., Eberl L., Hartmann A., and Dazzo F. B. (2006). *In situ* spatial scale of calling distances and population density-independent *N*-Acylhomoserine Lactone-mediated communication by rhizobacteria colonized on plant roots. FEMS Microbiol. Ecol., **56(2):** 188-194

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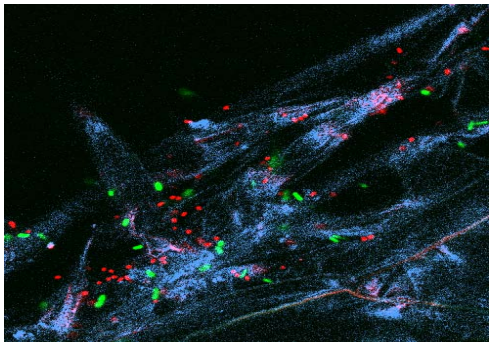
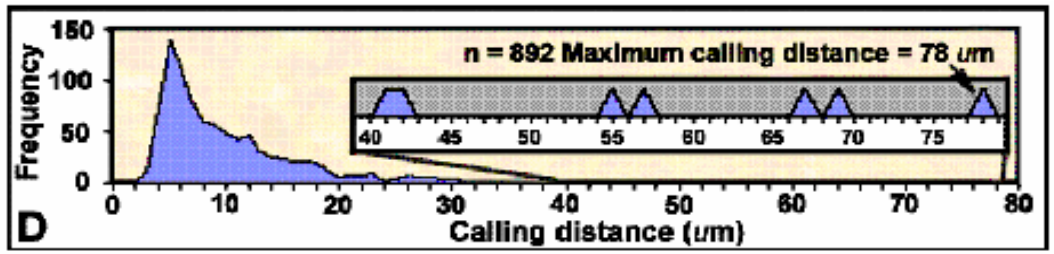
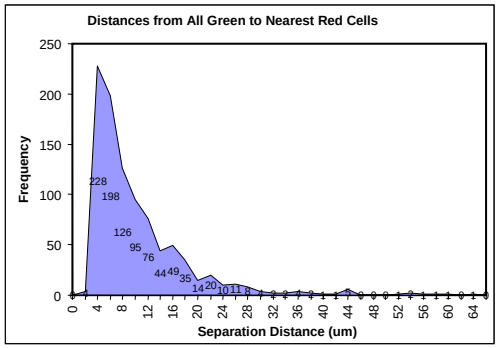
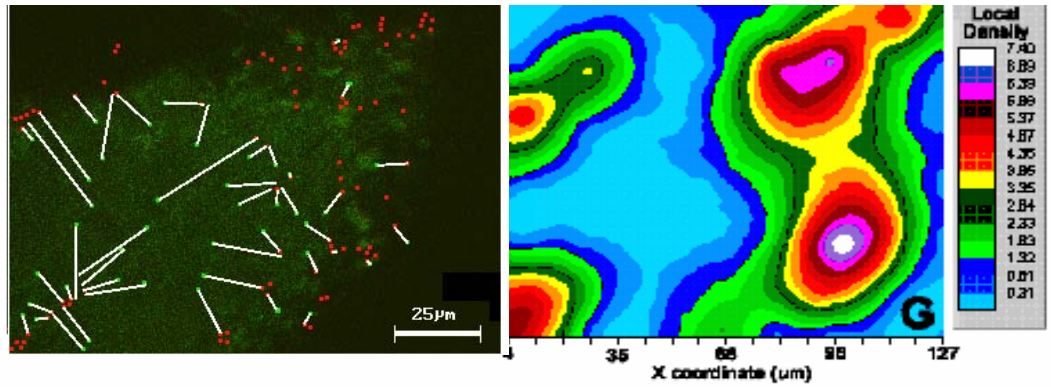
Center for Microbial Ecology Image Analysis System

***In situ* signaling with AHL-molecules in the rhizoplane: Quantitative estimation of the „calling distance“**









Gantner *et al.* (2006). FEMS Microbiol. Ecol., **56(2):** 188-194

Molecular signaling of bacteria on root surfaces: Effects on plants

Interaction of AHL-producing rhizobacteria with the plant

- Tomato roots inoculated with AHL-producing *S. liquefaciens* MG1 or its AHL-negative mutant MG44
- Infection of leaves with the fungal pathogen *Alternaria alternata*

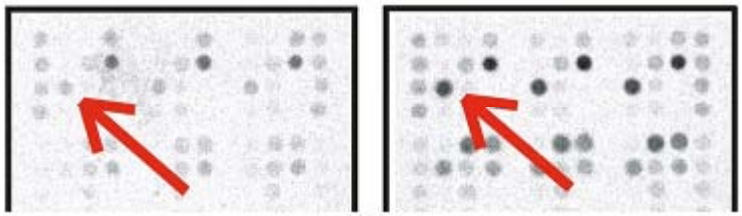
not inoculated | with MG1 (WT) | with MG44 (mutant)

Transcriptional response to AHL by tomato plants

Microarray analysis

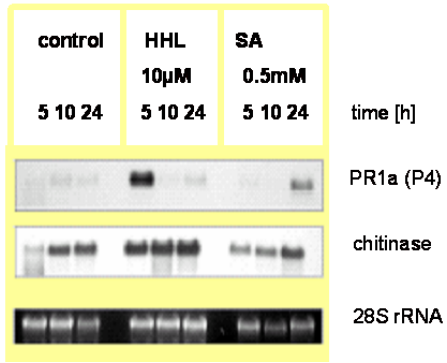
Using the BIOP-Pathochip (96 genes) involved in

- Ethylene biosynthesis and signaling
- Pathogenesis-related proteins
- Phenylpropanoid metabolism
- Antioxidant enzymes
- Wound-induced proteins
- Senescence-associated genes
- Fruit development proteins



***N*-Hexanoyl-homoserine lactone (HHL) affects gene expression in leaves**

Northern plot analysis



Schuhegger. *et al*. (2006). Plant Cell Environ., **29:** 909-918

Colonization behavior of food borne human pathogens on crop plants

Experimental setup

Model system: Axenic seedlings of barley (*Hordeum vulgare* var. Barke)

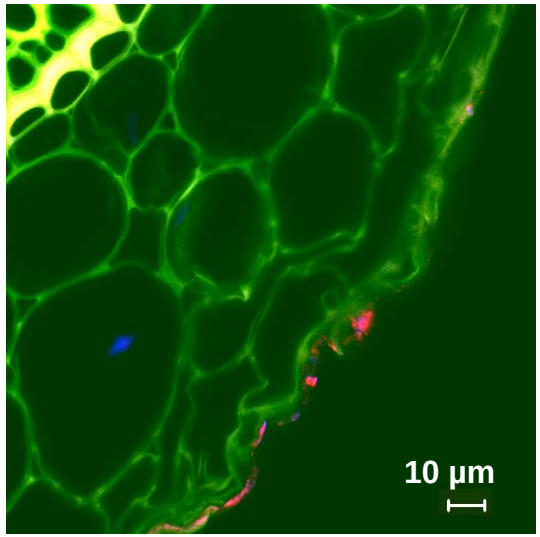
Inoculation with different bacterial strains (inoculation dose 10^8 cells / plant, incubation period about 2 – 4 weeks)

Detection and localization of bacteria in the rhizosphere via FISH/CLSM combination

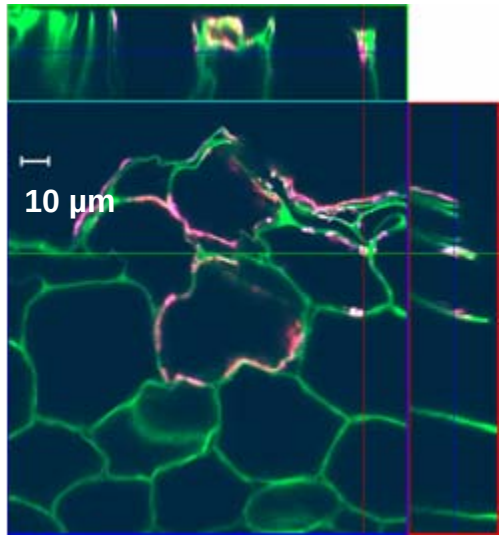
***Bacteria* specific probe EUB-338-I, II, III-Cy5**

***Salmonella* sp. specific probe Salm-63-Cy3**

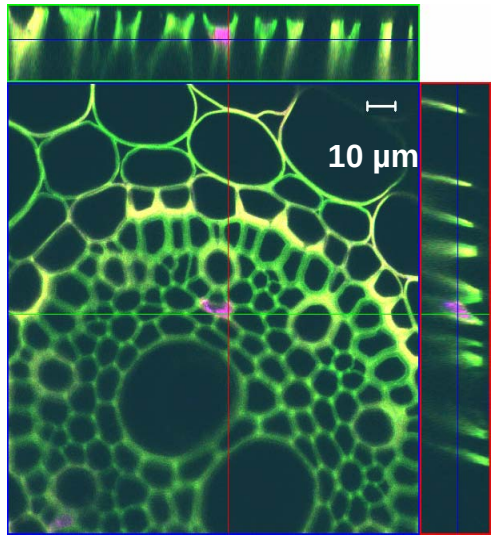
1 week incubation



2 weeks incubation

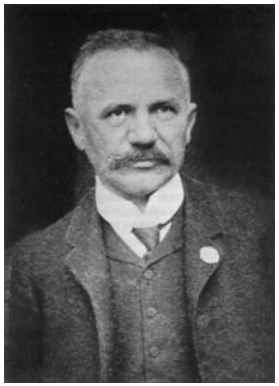


3 weeks incubation



Kutter *et al.*, (2006). FEMS Microbiol. Ecol., **56(2):** 262-271

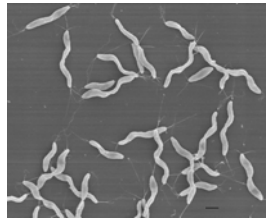
Characterization of a new bacterial species of the genus *Herbaspirillum*

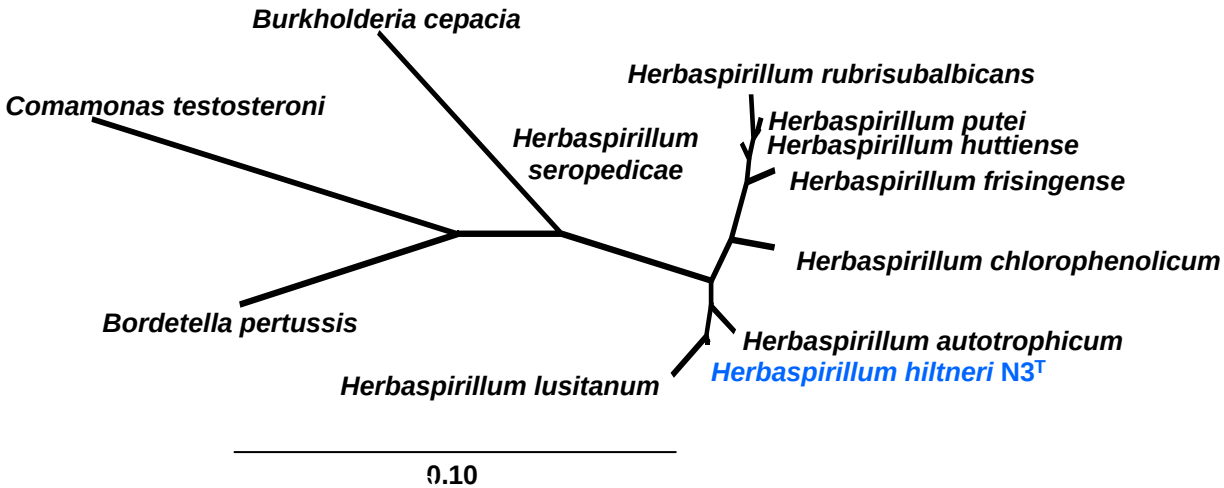


Prof. Dr. Lorenz Hiltner (1862-1923)

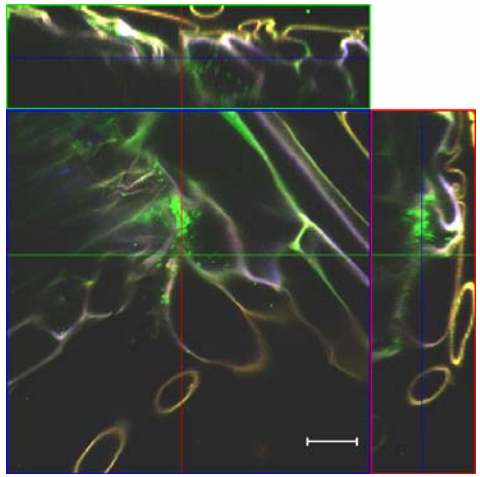
Pioneer of rhizosphere microbiology
Founding director of the royal bavarian agriculture-botanical Institute in Munich (1902-1923)

Scanning electron micrographs of *Herbaspirillum hiltneri* isolate N3^T. Black bars indicate 1 µm.





Phylogenetic tree of 16S rDNA of ß-proteobacteria with special focus on the genus *Herbaspirillum* and *H. hiltneri*.



In situ localization of *Herbaspirillum hiltneri* N3 (gfpmut3-labelled) at wheat roots

 Rothballer *et al.* (2006). Int. J. Syst. Evol. Microbiol., **56:** 1341 - 1348