

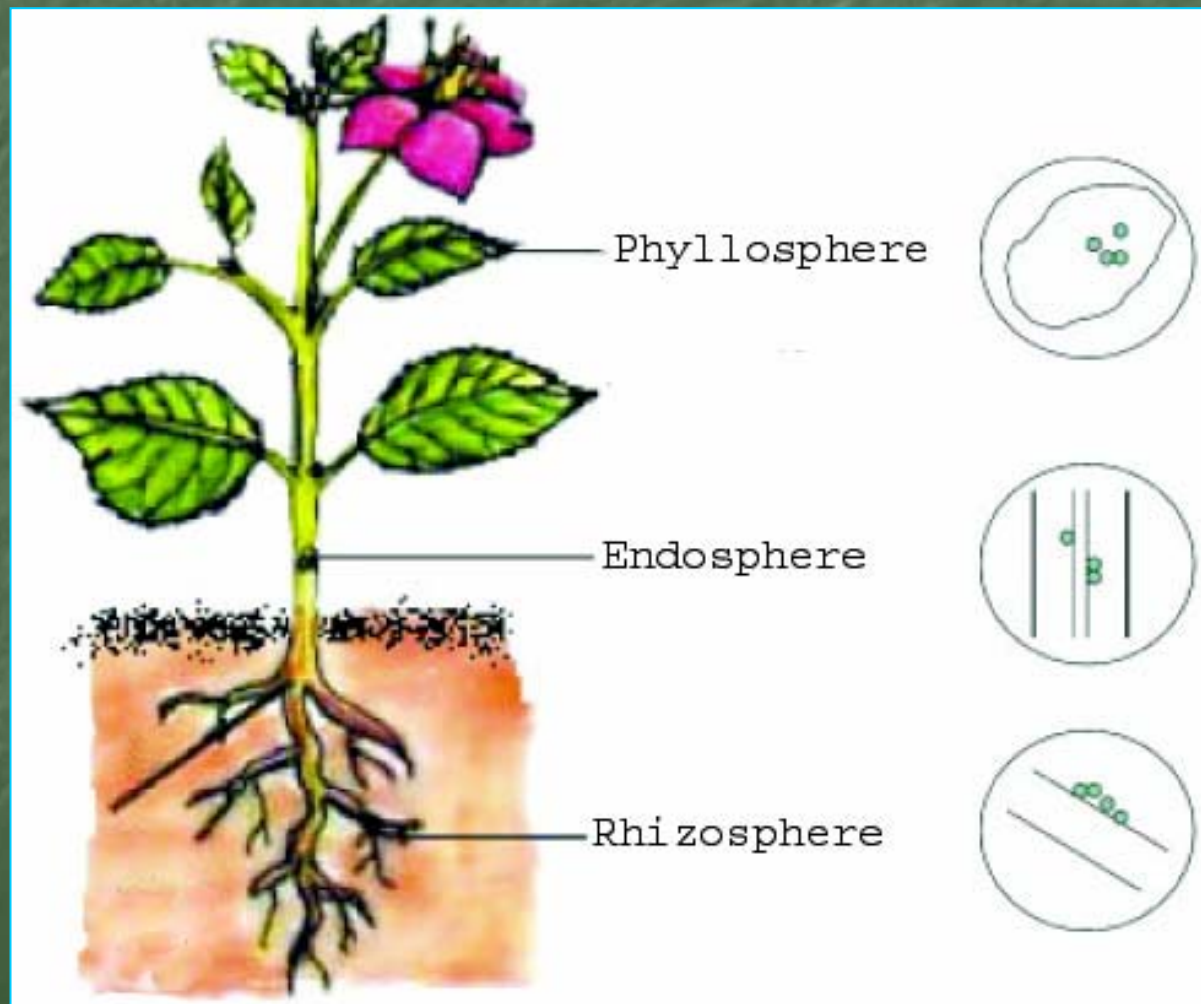
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University of Antwerp

Visualisation and identification of endophytic bacterial communities of *Phyllostachys* and *Fargesia*.

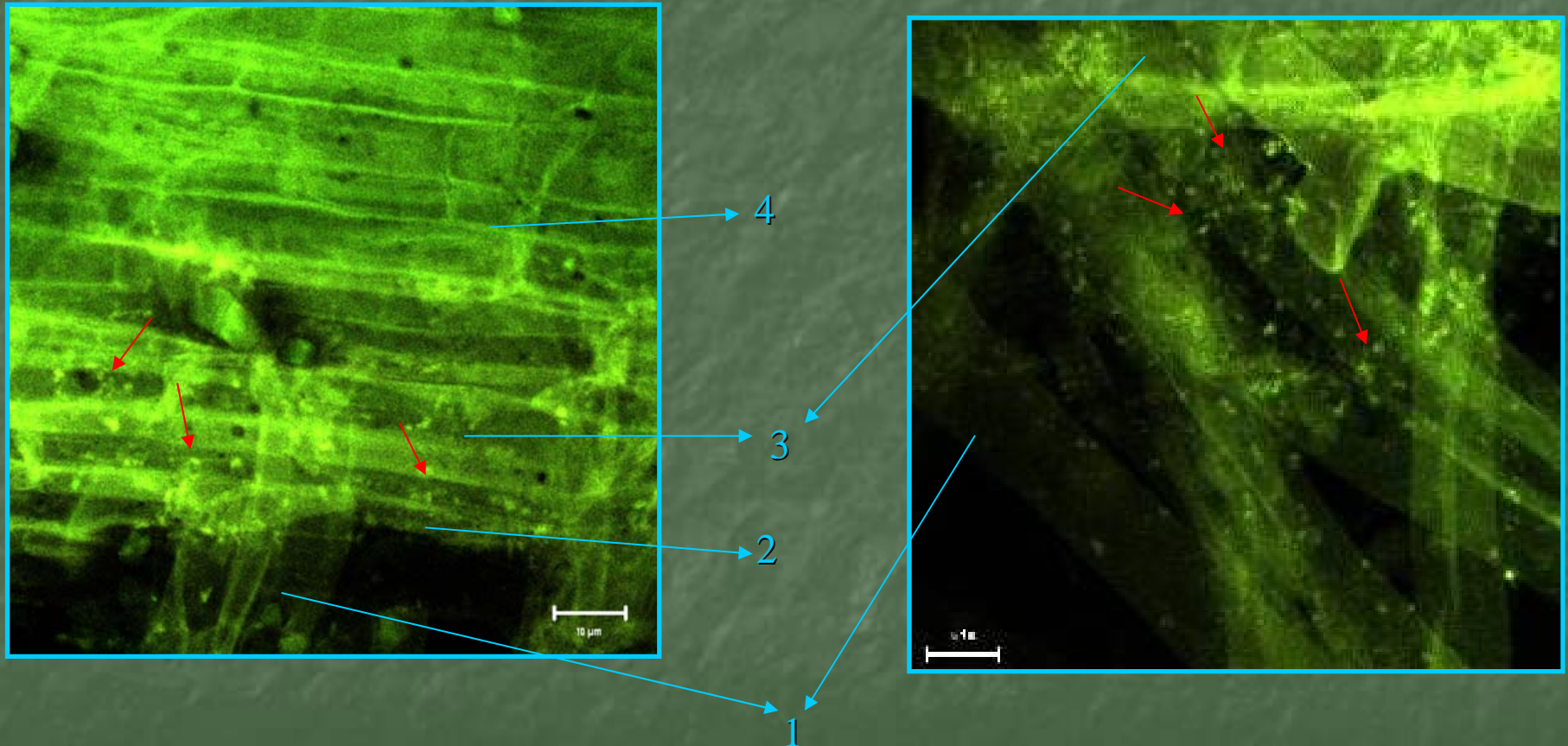
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Phytosphere is a complex multi-component niche



What are endophytes and why do they need?



The endorhizosphere of tomato (*Solanum lycopersicum*) colonised with an artificial biocontrol community. CLSM, SYBR green staining: 1 – root hairs; 2 – rhizodermis; 3 – exodermis; 4 – secondary root.

Endophytes as a feature of biotechnology

- Endophytes are involved in maintaining internal plant homeostasis, keeping the stressors low and the defence against possible pathogens.
- Modification of endophytes can be of use in designing phytotechnical solutions for environmental problems. Eg: soil cleanup, water purification.
- The main goal was to investigate the microbial community of certain bamboo plants.

There are few methodological approaches were used in order to catch endophytes

- Microbiological: intact plants were used as a basis for the classic isolation strategy of the bacteria inside;
- Molecular: whole DNA was extracted (of plant and bacteria alike) to go for a direct identification.
- Plastic film sampling method after Cholodny: plastic film strips made with polyethylene terephthalate were used as a matrix for the attachment and direct growth of the bacterial communities, maintaining the original (*in situ*) spatial shape and structure of these communities.

- Endophytes from the overground tissues are:
 - Bacillus (B. amyloliquefaciens, B. subtilis, B. mojavensis);
 - Mycobacterium (M. palustre, M. lentiflavum, M. avium complex, M. arosiense);
 - *uncultured bacterium clones*

- Endophytes from the underground tissues are:
 - Agrobacterium/Rhizobium;
 - *Burkholderia* (*spp.*, *B. cepacia complex*);
 - Pseudomonas (P. fluorescens, P. fuscovaginae);
 - Paenibacillus (*spp.*, P. chondroitinus);
 - Microbacterium (*spp.*, M. laevaniformans);
 - *Leifsonia*

- Endophytes from *in vitro* tissues are:
 - Achromobacter;
 - Acinetobacter

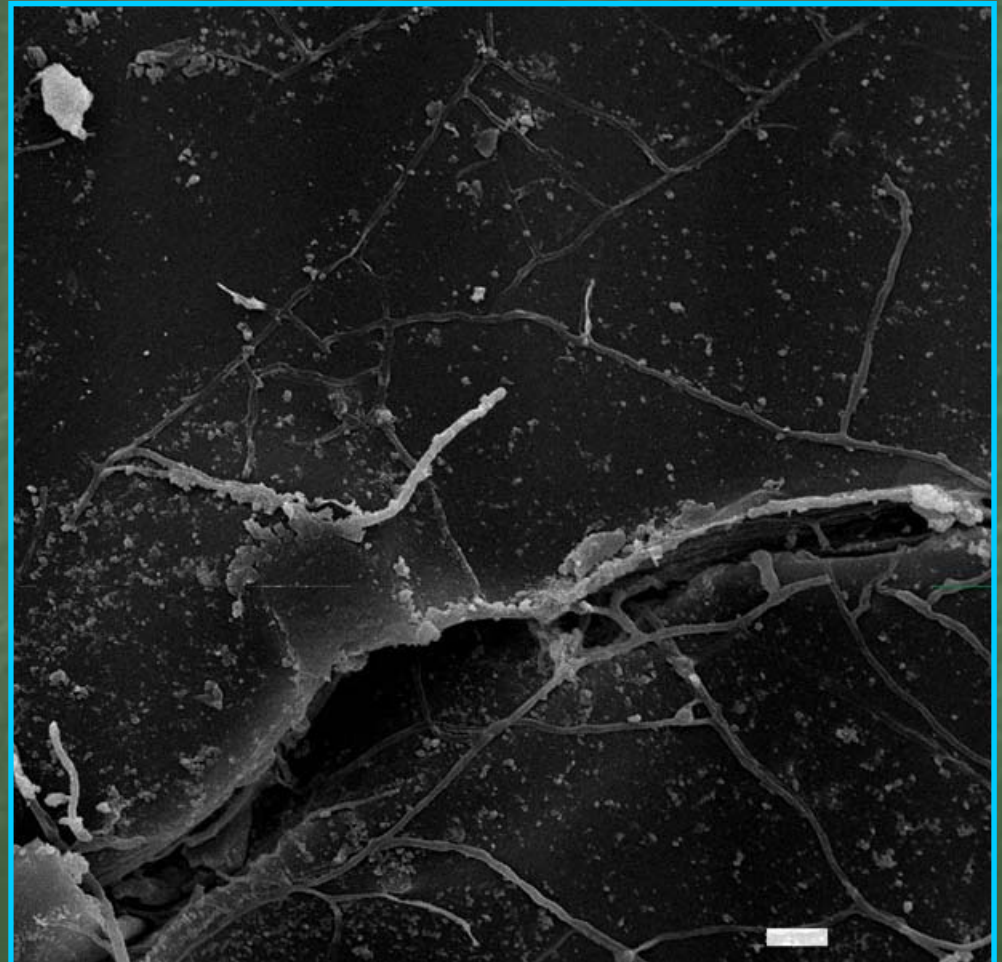
A plastic film made with PET (polyethylene terephthalate) as a carrier for real microbial community formation *in situ*.



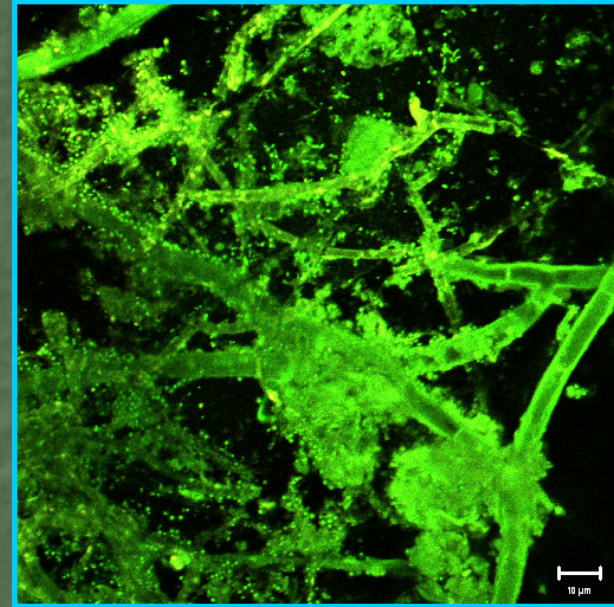
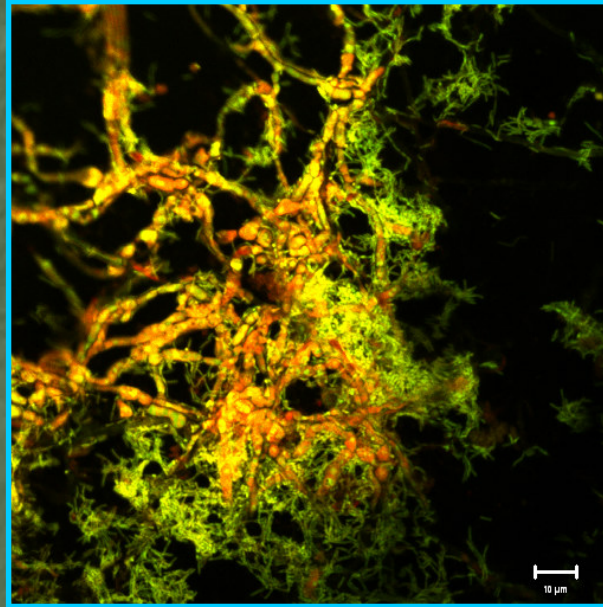
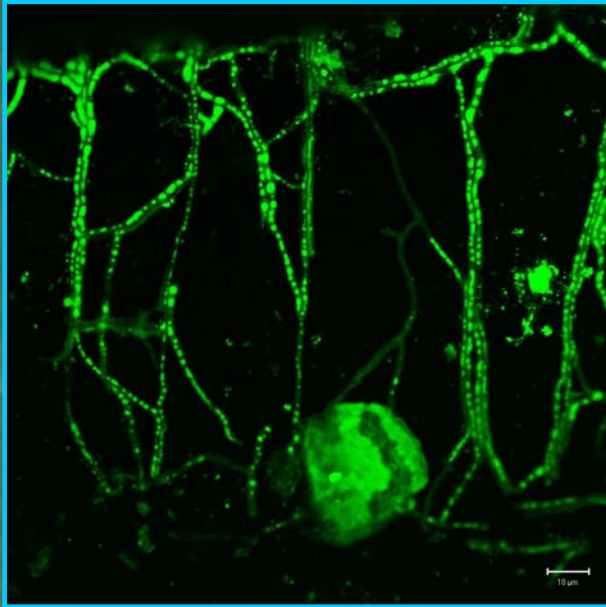
- chemically inert
- biologically neutral
- nontoxic
- transparent
- flexible
- more hydrophilic than other plastics
- permeable for oxygen and carbon dioxide



PET films were used to study the endophytic microbial communities of bamboo (*Phyllostachys atrovaginata*)

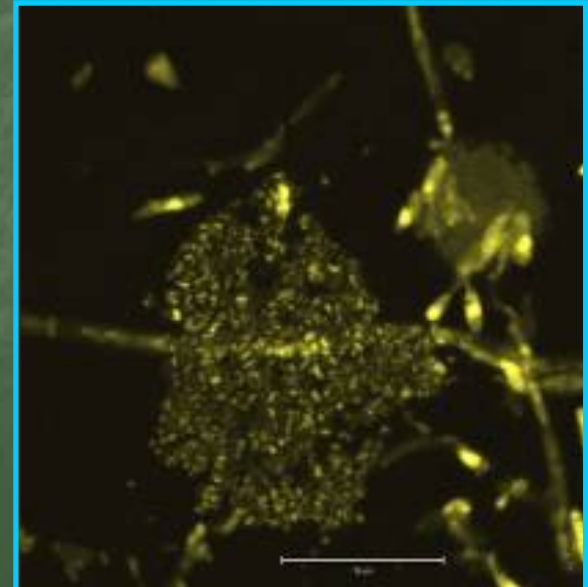
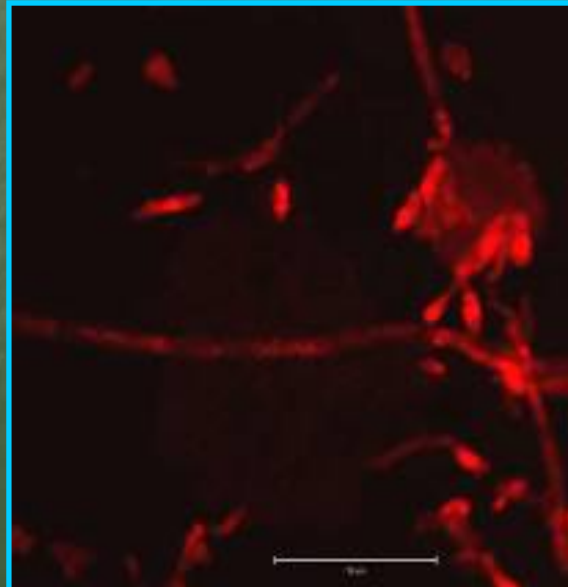
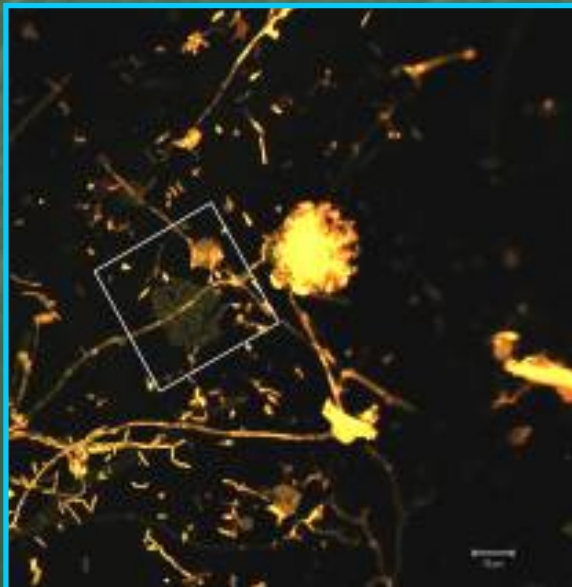
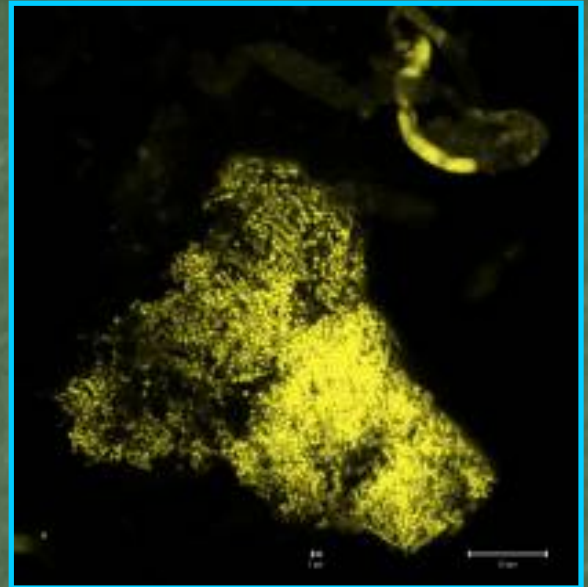
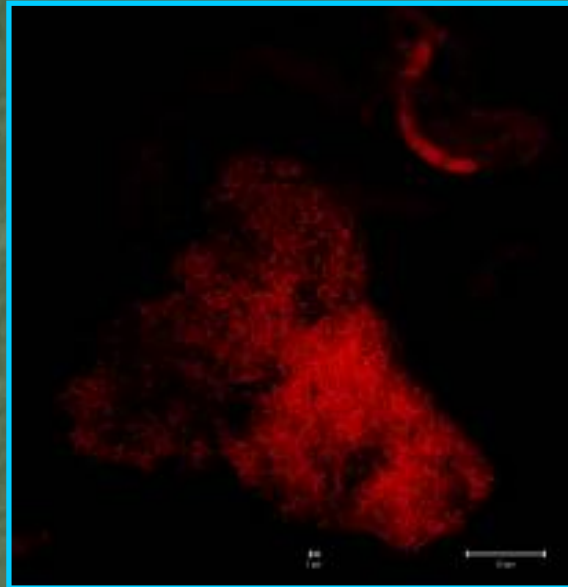
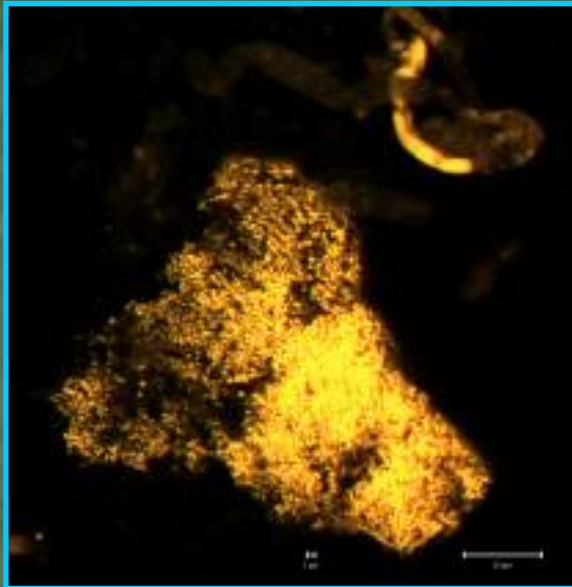


Microcolonies of endophytes formed on PET films under *in situ* conditions
(in the plant tissues of *Phyllostachys atrovaginata*)
A lower part of a column.

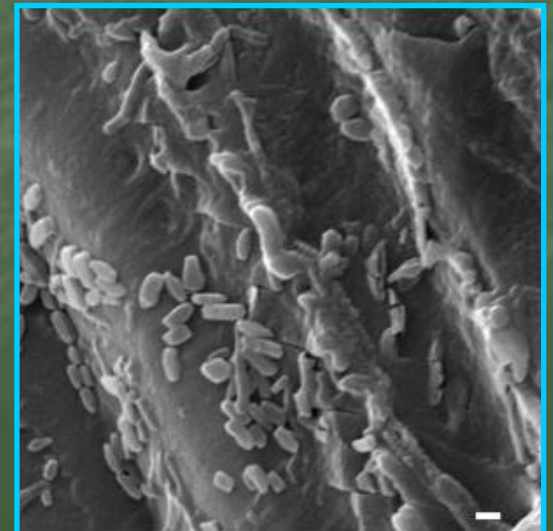
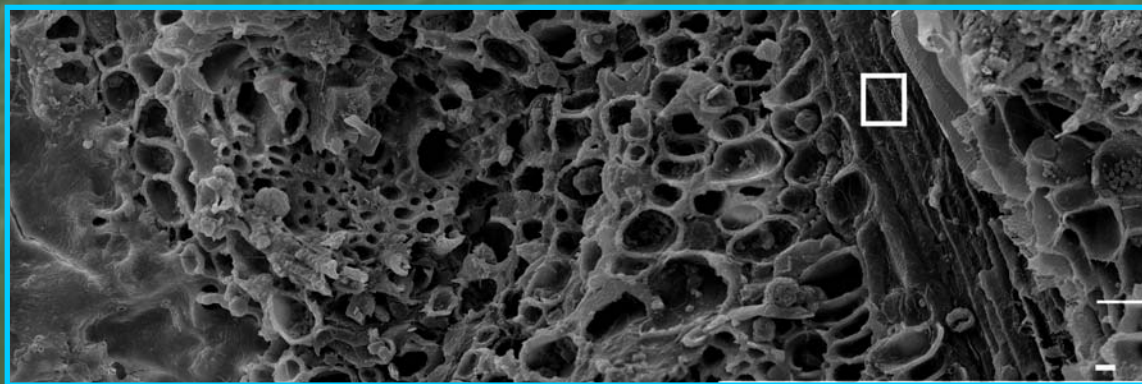
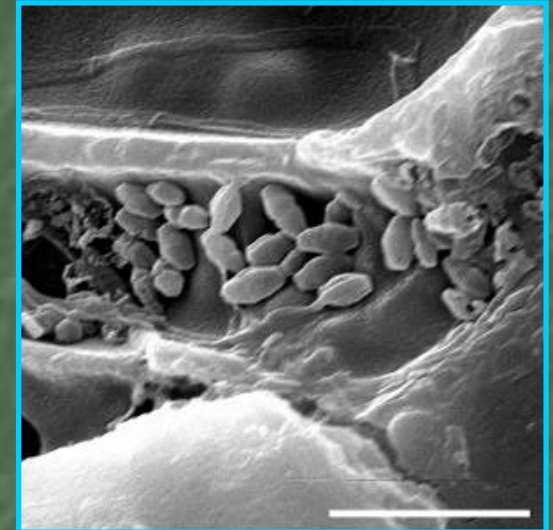
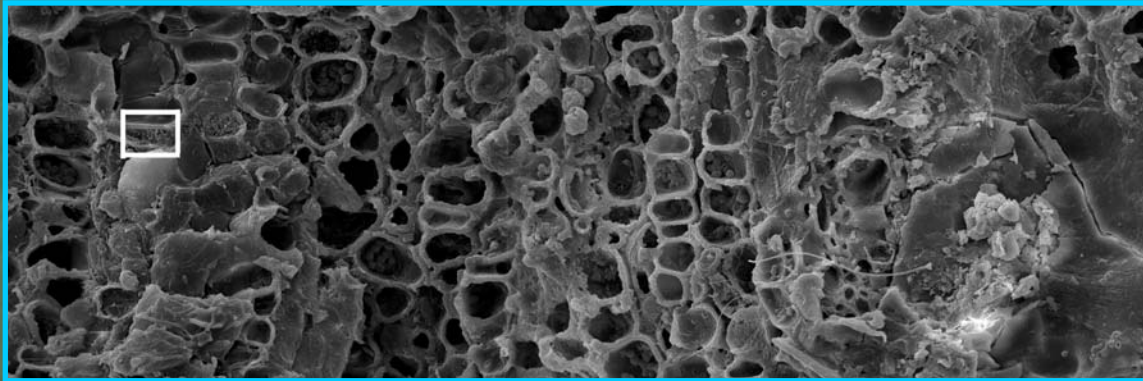


CLSM, scale bar is 10 μm.

Microcolonies of endophytes formed on PET films under *in situ* conditions (in the plant tissues of *Phyllostachys atrovaginata*). A top part of a column.



Endophytic microcolonies formed on PET films and in the plant tissues directly (*Phyllostachys atrovaginata*)



SEM, scale bar is 10 μm .

Underground endophytes were more diverse than the aboveground endophytes.

- Low nucleic acid (LNA)-content bacteria were detected in the upper part of bamboo columns.
- Endophytic populations of plants grown in an artificial soil substrate were more diverse than those from *in vitro* agar-grown plants.
- Gram-positive bacteria of the *Bacillaceae* and *Mycobacteriaceae* families, as well as a variety of unculturable bacteria, were detected in the aboveground samples, while both gram-positive and gram-negative bacteria were found in the underground samples.
- *Achromobacter* sp. and *Acinetobacter* sp. were also isolated from *in vitro* grown plants.
- In total, 18 endophytic bacterial species identified from bamboos.
- For some bacteria, including *Achromobacter* sp., *Acinetobacter calcoaceticus*, *Agrobacterium/Rhizobium* sp., *Bacillus amyloliquefaciens*, *B. mojavensis*, *B. subtilis*, *Microbacterium laevaniformans*, *Mycobacterium arosiense*, *M. avium* complex, *M. lentiflavum*, *M. palustre*, *Paenibacillus chondroitinus*, *Pseudomonas fluorescens*, *P. fuscovaginae*, this is the first report of their isolation as bamboo endophytes.
- Finally, fungal hyphae were found in the endosphere of *Phyllostachys atrovaginata* L. and were frequently associated with bacteria cells.