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(Article begins on next page)



# UNIVERSITÀ DEGLI STUDI DI TORINO

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## **The endless tale of endobacteria: a conversation with Paola Bonfante**

Paola Bonfante

January 1974: In the dark of the electron microscope room, Paola Bonfante started up and looked again at the greenish screen; she was indeed looking at a round body very similar to a bacterium. Unexpectedly, this faint body was inside the cytoplasm of a mycorrhizal symbiotic fungus, which was inside the root cells of a plant.

This unexpected observation marked the start of my second, parallel career hunting for the endobacteria that live inside mycorrhizal fungi. At that time, I had already established my career studying mycorrhizae, the plant–fungal symbioses central to the balance of natural and agronomical ecosystems. Emerging research has recognized the primary importance of mycorrhizal fungi for the environment and for sustainable agriculture, since they act as biofertilizer for their host plants, increasing nutrient uptake in exchange for fixed carbon. Mycorrhizae may be the ultimate biological tool in our efforts to help feed the world.

When did you decide on your research topics?

Starting in the 1970s, after a seminal training period in Paris at the École Normale Supérieure (1980), I spent a long time using cellular and molecular approaches to study the intimate interactions between mycorrhizal fungi and plants. Symbiosis has always fascinated me and Lynn Margulis remains one of my favorite scientists. Her concept that symbiosis leads to biological novelties and thus acts as a powerful driver of evolution strongly influenced my scientific path. Applying cell biology to mycorrhizal systems allowed me to describe some of the plant responses in the pre-molecular era; for example, my mentor, Professor Silvano Scannerini, and I were among the first to describe how arbuscular mycorrhizal fungi exist inside the cortical cells of the host, leading to the creation of a new cellular compartment that we called the interface [1]. Later work showed that this compartment requires the deposition of new cell-wall molecules and membranes. Many of the discoveries that Andrea Genre of my group made in later years, using in vivo imaging and in collaboration with David Barker's team in Toulouse, derive from those early electron microscopy observations.

Early in the 1990s, I applied emerging DNA technologies to contribute to our knowledge of mycorrhizal diversity in natural and cultivated fields, developing probes that allowed the identification of mycorrhizal fungi. When genomics approaches became available, enabling researchers to examine how fungal metabolism contributes to the physiology of their hosts and to plant communities, I applied these new technologies to both ecto- and endomycorrhizal fungi, developing various projects, some in collaboration with Francis Martin from the Institut National de la Recherche Agronomique (INRA). Thanks to these collaborative networks, the genomes of fascinating fungi like the black truffle (*Tuber melanosporum*) and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* were sequenced, shedding light on these enigmatic organisms and their evolutionary history 2 and 3.

Forty years working on mycorrhizae! What motivates you long term?

Mycorrhizae are a fascinating world and an ecologically relevant system; nearly all plant species have established interactions with mycorrhizal fungi, beginning at least

450 million years ago. Therefore, research on mycorrhizae is a primary, crucial issue for plant biology. Looking at the biology of the system, to understand how two eukaryotes interact and communicate, remains very challenging: this communication is a sort of dance in the dark of the soil that leads to impressive changes in both partners. Mycorrhizal fungi affect the plant's roots where they live and they also affect the plant's entire physiology. For example, mycorrhizal symbiosis in the roots affects the properties of tomato fruits [4]; this exciting new knowledge represents one of the next frontiers for mycorrhizal research. Lastly, science has to cater for human needs. Wider and more scientifically informed use of mycorrhizae might help us to respond to some of the big questions that society puts to science on subjects such as protection of the environment, minimizing the use of chemicals, plant health, and food quality.

I have never been bored; I have the feeling that studying mycorrhizae is a topic highly relevant to biology and society and it makes sense to invest energy in it. Also, observing and following arbuscule development inside a plant cell remains exciting, just as it was 40 years ago.

Coming back to the endobacteria, why do you define this investigation as a second career?

As a professor of plant biology, I consider my study of endobacteria to be another adventure in a different domain of life. I tried to develop this work as a parallel project, but this was very hard to achieve with the limited resources available. Irrespective of resources, I believe that endobacteria, which live inside a fungus that lives inside a plant (Figure 1A), offer a nice story that has given us a lot of new ideas on interdomain communication. Endobacteria are widespread among animals; in particular, the endobacteria living in insect cells have been investigated in depth. The endobacteria living in fungal cells have been investigated much less, although the endobacteria of Glomeromycota are currently one of the most thoroughly investigated types. On the basis of morphological observations, we identified two types of endosymbiont in Glomeromycota (Figure 1B): a rod-shaped, Gram-negative bacterium and a coccoid bacterium with a Gram-positive-like wall [5]. However, since they are uncultivable microbes, I had to wait until Kary Mullis invented PCR and won the Nobel Prize; only with this new tool could we name the rod bacterium, which we found to be a beta-proteobacterium [*Candidatus Glomeribacter gigasporarum* (CaGg)]. We also found that the coccoid bacterium is a currently undescribed taxon of the Mollicutes-related endobacteria (Mre). It also became clear that the endobacteria are widely present: the Mre have a broad distribution across the Glomeromycota and CaGg commonly occurs in several species of Gigasporales. Recently, we also spotted some African isolates that harbor both microbial communities, suggesting that mycorrhizal fungi host a complex intracellular microbiota [6].

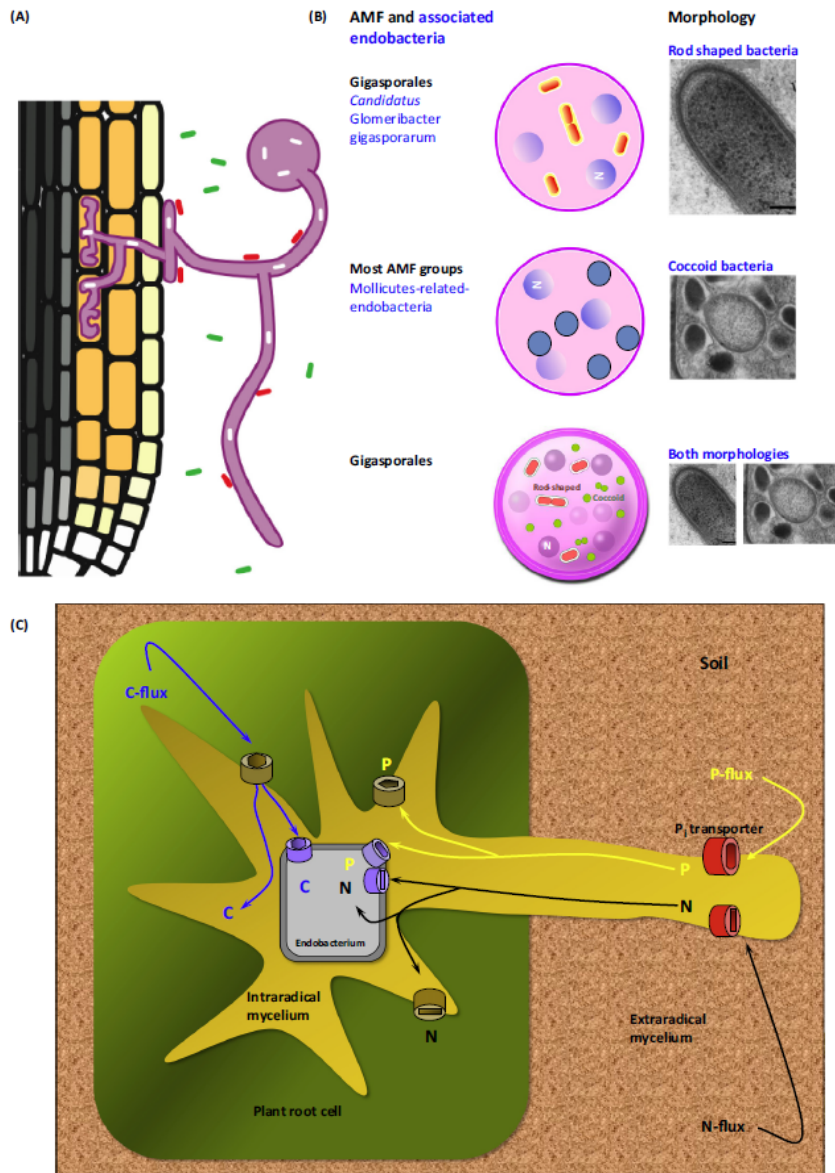


Figure 1.

(A) The figure illustrates how arbuscular mycorrhizal fungi (AMF) colonize their host root, producing extra- and intraroot structures. Hyphae thriving in the soil are in contact with many bacterial communities (green rods) but may also host endobacteria in their cytoplasm (white rods). Reproduced, with permission, from [5]. (B) Endobacterial typologies so far identified and their distribution in AMF taxa. (C) Model of plant–fungus–endobacterium interaction (Courtesy of M. Novero). Genome-sequencing results for *Candidatus Glomeribacter gigasporarum* indicate that the bacterium fully depends on the fungal metabolism [i.e., for carbon (C), phosphate (P), and nitrogen (N) metabolism]. By contrast, the fungus depends on its green host for carbon uptake only. Thus, a network of nutritional and energetic interactions among species from three different kingdoms is emerging, although the potential benefits to the fungal host remain obscure.

However, these biodiversity studies did not provide biological insights: why have these endobacteria been conserved during evolution? To answer this question, we started a project that aimed to sequence the CaGg genome [7]. We learned many things: CaGg has a reduced genome, confirming its uncultivable status; also, it lacks metabolic pathways leading to important amino acids, but has many amino acid permeases for uptake of nutrients from the host ( Figure 1C).

The genomic data show that the fungus feeds the endobacteria, while the fungus also depends (at least for carbon) on its plant host. Thus, the fungus pays a cost for maintaining the endobacterium inside its cytoplasm, but how does the bacterium affect the fungus? The bacterium has the correct information for producing vitamin B12, but is this useful for its partners? The biological-market theory claims that a stable symbiotic relationship requires reciprocal benefits for host and symbiont [8]. We are currently using RNA sequencing (RNA-seq) to investigate these issues. Thanks to the development of an endosymbiont-free cured line, which allows us to directly check the impact of the endobacterium, we already know that the cured line can colonize its plant host but is impaired in mycelial growth and spore production.

Therefore, this is my next goal: the story remains ongoing!  
What advice would you give to young scientists, on the basis of your long experience?

After 40 years, the mystery of fungal endobacteria has not been completely deciphered, so this does not sound like a very successful story. However, I have learned that, even when asking the right questions, the time may not be right to provide the appropriate experimental design or technologies to answer the questions. As an example, the existence of unculturable microbes is a relatively recent concept, unknown in the 1990s. The sequencing of such microbes has become a relatively easy task, but in 2006, when I started the sequencing work, 454 sequencing was just in its infancy. Certainly, science requires creativity, but the capacity to evaluate whether the experiment is feasible is also needed. The study of fungal–bacterial interactions is now an emerging field. We are becoming aware that the presence of endobacteria inside fungi is probably much more widespread than expected. For example, Greg Bonito from Duke University has found a CaGg-related endobacterium living inside *Mortierella*, which is a fungus associated with poplar. Also, Alessandro Desirò, a previous PhD student of mine, has detected *Mre* in a fungus, *Endogone*, that is currently considered to be older than the Glomeromycota. These novel results suggest that plant-associated fungi may possess or have possessed phylogenetically diverse bacteria as a sort of evolutionary marker. It seems that the endless tale of endobacteria has gone trans-generational.

June 2014: In the dark of the night in her small office at home, Paola Bonfante is puzzling over some spreadsheet files showing RNA-seq comparisons between *Gigaspora* hosting CaGg and the line cured of its mycorrhizal symbiont. Hmm, what could these data mean?

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