



MICROBIOLOGICAL IMAGE

Scanning electron microscopic observations of early stages of interaction of *Trichoderma harzianum*, *Gliocladium virens* and *Bacillus subtilis* with *Acaulospora colombiana*



Observaciones por microscopía electrónica de barrido de estadios tempranos de la interacción de *Trichoderma harzianum*, *Gliocladium virens* y *Bacillus subtilis* con *Acaulospora colombiana*

Emilia M. Ortiz^a, Jessica Duchicela^{a,*}, Alexis Debut^b

^a Departamento de Ciencias de la Vida, Universidad de las Fuerzas Armadas – ESPE, P.O. Box 171102, Sangolquí, Ecuador

^b Centro de Nanociencia y Nanotecnología, Universidad de las Fuerzas Armadas – ESPE, P.O. Box 171102, Sangolquí, Ecuador

Received 16 February 2017; accepted 17 June 2017

Available online 10 November 2017

Arbuscular mycorrhizal fungi (AMF) establish symbiosis between most vascular plant roots, and play a key role in facilitating nutrient uptake¹; they have been applied as microbial inoculants in low-input farming sustainable systems. In this context, other microbial inoculants are used as biocontrol agents of plant-pathogenic fungi, the most common in commercial inoculants being *Trichoderma harzianum*, *Gliocladium virens* and *Bacillus subtilis*.^{2,3} Those applications have shown beneficial effects on plant health and productivity. However, the understanding of the interactions between biological control agents and AMF requires special attention because of the possibility that these fungal antagonists could also interfere with AMF activity.

Here we performed scanning electron microscopic (SEM) observations of *Acaulospora colombiana* spores exposed to *T. harzianum*, *G. virens* and *B. subtilis*, after 30 days of interaction under *in vitro* conditions.

The micrographs revealed that the outer hyaline spore layer was strongly eroded and that the spore surface was covered with mucilaginous products. Moreover, the outer hyaline layer showed an early stage of degradation in all the interactions of biocontrol agents and AMF in contrast with the control (Fig. 1). *T. harzianum* and *G. virens* hyphae grew alongside the spore adhered to the laminated layer or embedded in the sloughing hyaline layer (Figs. 2 and 3). *A. colombiana* spore wall was covered with bacterial bacillus-shaped cells of different sizes (Fig. 4A). Moreover, rough and mucilaginous material covered 50% of the spore surface (Fig. 4B). Decaying material complicated the observation of bacterial cells because they appeared to be covered with their own mucilage.

* Corresponding author.

E-mail address: jiduchicela@espe.edu.ec (J. Duchicela).

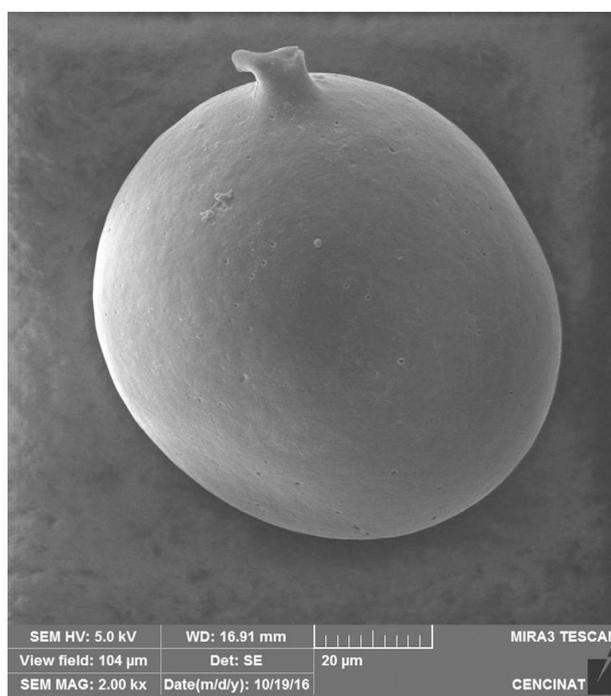


Figure 1 Scanning electron micrograph of *A. colombiana*.

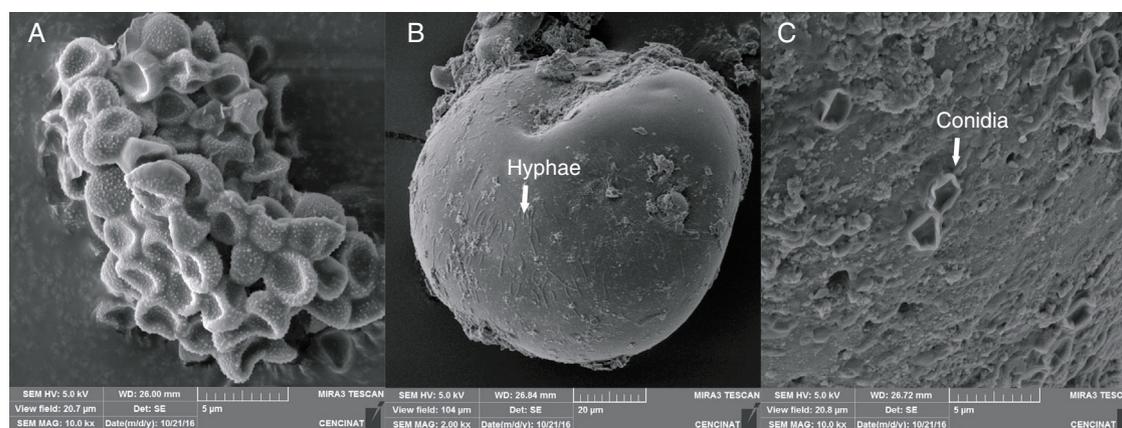


Figure 2 Scanning electron micrographs of *T. harzianum* and surface of *A. colombiana* spores. (A) Phialospores which are verrucose at high magnification. (B) *T. harzianum* hyphae and (C) conidia adhered to *A. colombiana* spore after 10 days of inoculation.

In our study, evidence of fungal and bacterial AMF spore saprophytic activity was suggested by SEM observations. An understanding of these effects is essential for obtaining the maximum benefit for plant growth and health in the context of agroecosystem sustainability.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Acknowledgement

We are grateful to Ing. Andrea Vaca (Nanomaterials Characterization Laboratory, Center of Nanoscience and Nanotechnology – CENCINAT) for providing technical support to take the SEM micrographs.



Figure 3 Scanning electron micrographs of *G. virens* adhered to *A. colombiana* spore after 30 days of inoculation.

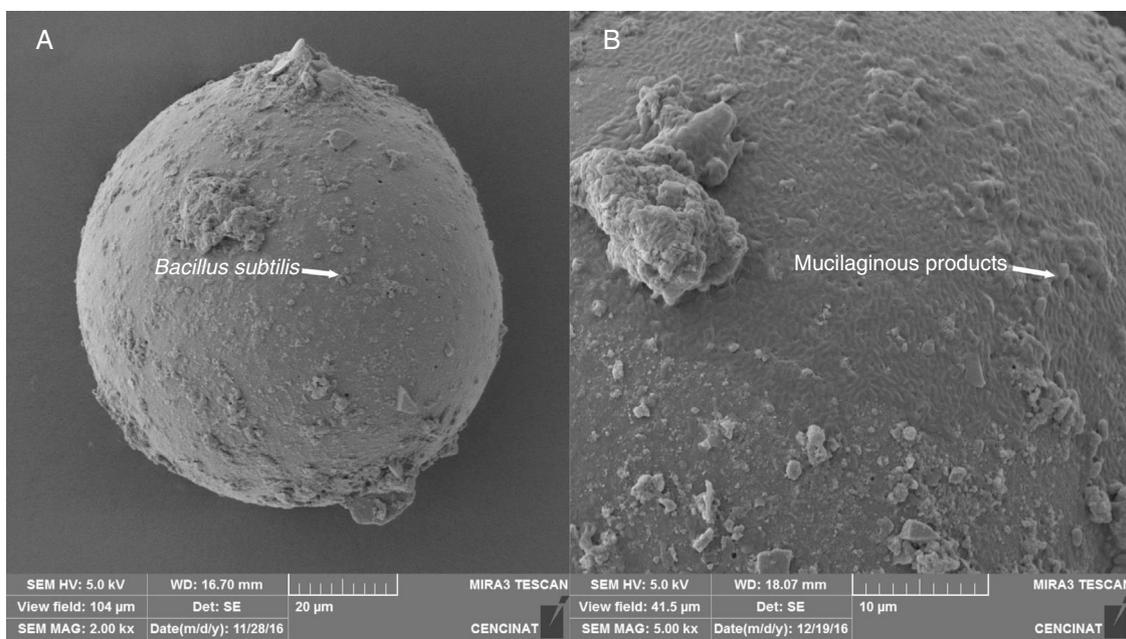


Figure 4 Scanning electron micrographs of surface of *A. colombiana* spores. (A) *B. subtilis* adhering to the surface of the laminated layer of the spore surface after 15 days of inoculation. (B) Mucilaginous outer hyaline layer starting to “peel off” and being replaced by mucilaginous products (arrow).

References

1. Berruti A, Balestrini R, Bianciotto V. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Front Microbiol.* 2015;6:1–13.
2. Ongena M, Jacques P. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol.* 2008;16:115–25.
3. Papavizas GC. *Trichoderma* and *gliocladium*: biology, ecology, and potential for biocontrol. *Annu Rev Phytopathol.* 1985;23: 23–54.