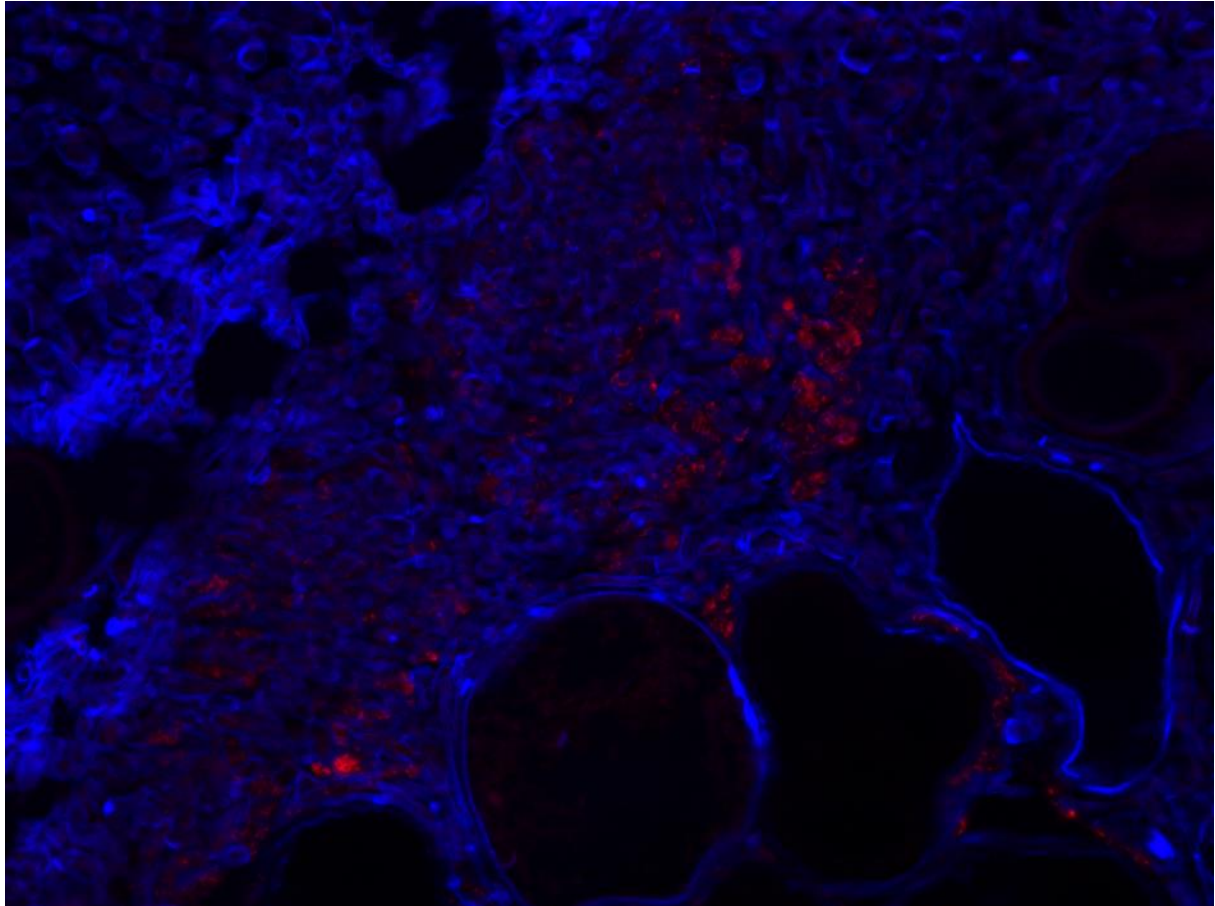


BACTOTRUF



Functional characterization of bacterial communities of the black truffle *Tuber melanosporum* along its life cycle

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Partners: EEF, BEF, LAE

Collaboration: R. Splivallo (Frankfort University)

Context — As a belowground organism, black truffle is in constant interaction with a large diversity of bacteria, some of which can colonize the outer and inner tissues of truffle sporocarps. Up to a 100 million of bacteria can be found per gram of truffle. Bacterial groups colonizing truffle ascocarps change along ascocarp development and maturation, suggesting an intimate interaction between the microorganisms. How can we explain this selection of specific strains inside truffles ? Are these bacteria opportunists which solely use fungal nutrients for their growth or do they participate also to the development of the fungus by providing nutrients for example ?

Objectives — This study will have two aims: first, to understand mechanisms by which specific groups of bacteria are selected inside truffle fruiting bodies; second to understand the potential role of bacteria in the development and maturation of black truffle.

Approach — A library of bacterial strains isolated from truffle ascocarps, soil and ectomycorrhizae – the symbiotic organ connecting the plant roots and the fungus – will be characterized at the taxonomic (species identification) and functional levels. Using functional tests, we will more specifically focus

on the impact of those bacteria on truffle development, truffle nutrition, aroma production and spore dissemination.

Key results —

- 330 strains were characterized at the taxonomic level. They belong to 31 genera distributed into 7 classes
- Several factors of structuration of the truffle bacterial communities were identified: metabolic specificities of bacteria colonizing truffles, an increased chitinolytic ability that correlates with spore release, a high resistance to Cu and Al that correlates with an important accumulation of these metals inside ascocarps
- None of the bacteria isolated have the ability to fix atmospheric nitrogen
- Bacteria are most likely important contributors to truffle aroma
- A compound that allow to cure *T. melanosporum* mycelium from symbiotic bacteria have been identified. Preliminary data suggest that this compound stimulates the growth of the fungus.

Main conclusions including key points of discussion — Our data indicate that bacteria colonizing *T. melanosporum* ascocarps have specific metabolic activities and an increased resistance to heavy metals that are accumulated by truffles. We thus hypothesize that these two factors could be involved in the selection of specific bacterial communities inside truffles. Whether these bacteria participate to the development of truffles is still an open question. However our results support the hypothesis that some of these bacteria first participate to the growth of the fungus thanks to the production of growing factor that we identified. Second, we suspect that some bacteria would also contribute to the release of the spores thanks to their chitinolytic activities. Last but not least, they would contribute to the truffle aroma.

Future perspectives — The modelling of the networks of interactions between microorganisms in the Black truffle is still on going and will be pursued in the frame of a collaboration with statisticians (IECL, CRAN) and computer scientist (INRIA) funded by PEPS Mirabelle (Trufinet Project). In addition, further research will be performed to confirm if the compound suspected to be produced by *T. melanosporum* associated bacteria is responsible for the mutualistic interaction between the bacteria and the fungus and if it also responsible for the mutualistic interaction between bacteria and *T. magnatum*.

Valorisation —

Publications

Vahdatzadeh M., Deveau A., Splivallo R. (2015) The role of the microbiome of truffles in aroma formation: a meta-analysis approach. *Applied Environmental Microbiology*. 81(20) :6946-52.

Splivallo, R., Deveau, A., Valdez, N., Kirchhoff, N., Frey-Klett, P., Karlovsky, P. (2015). Bacteria associated with truffle-fruited bodies contribute to truffle aroma. *Environmental Microbiology*, 17 (8), 2647-2660.

Deveau A., Antony-Babu S., Le Tacon F., Robin C., Frey-Klett P., Uroz S. (2016) Temporal changes of bacterial communities in the *Tuber melanosporum* ectomycorrhizosphere during ascocarp development. *Mycorrhiza Journal*. 26 (5), 389-399.

4. Uroz, S., Buée, M., Deveau, A., Mieszkin, S., Martin, F. (2016). Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. *Soil Biology and Biochemistry*, 103, 471-488.

Scientific conference

Deveau A., Splivallo, R., Kirchoff N., Nicolitch O., Bontemps C., Antony-Babu S., Le Tacon F., Frey-Klett P., Kohler A., Uroz S. The Black Truffle : more than a niche for bacteria ? International

Mycology Conference, Bangkok, Session « Fungal-Bacterial Interaction », 3- 8 aout 2014. (Invited talk)

Deveau A., Taxonomic and functional characterization of the truffle microbiome MEM INRA meeting, 18.01.2017 (Invited talk)

Deveau A., Splivallo R., Palin B., Niccolitch O., Kohler A., Uroz S. Black truffles as a model in microbial ecology to analyse fungal-bacterial interactions. ISME Conference. 20-27.08.16. Montreal Canada. (Selected talk)

Public conference

Journées techniques des trufficulteurs de la région Est, 3-4 avril 2014 : « Les bactéries, des partenaires méconnus de la truffe »

Fête de la Truffe Jarnac, 21-22 janvier 2017 : « Les bactéries, des partenaires méconnus de la truffe »