Chemical ecology: Bacteria–fungi interaction for plant biocontrol

Kovács, Á.T.

better suited for a seer of Baba Vanga's caliber. But, does this limitation really matter, when, along the way, we learn so much about the interesting biology of species we might otherwise only know from a chance encounter in a sulfidic spring?

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Wikipedia Baba Vanga. https://en.wikipedia.org/wiki/Baba_Vanga.
2. Tobler, M., and Plath, M. (2011). Living in extreme environments. In Ecology and Evolution of Poeciliid Fishes, J.P. Evans, A. Pilastro, and S. Ingo, eds. (Chicago, IL: University of Chicago Press), pp. 120–127.
3. Greenway, R., Barts, N., Henpita, C., Brown, A.P., Rodriguez, L.A., Rodríguez Peña, C.M., Arndt, S., Lau, G.Y., Murphy, M.P., Wu, L., *et al*. (2020). Convergent evolution of conserved mitochondrial pathways underlies repeated adaptation to extreme environments. Proc. Natl. Acad. Sci. USA *117*, 16424–16430.
4. Tobler, M., Palacios, M., Chapman, L.J., Mitrofanov, I., Bierbach, D., Plath, M., Arias-Rodriguez, L., García de León, F.J., and Mateos, M. (2011). Evolution in extreme environments: replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. Evolution *65*, 2213–2228.
5. Greenway, R., De-Kayne, R., Brown, A.P., Camarillo, H., Delich, C., McGowan, K.L., Nelson, J., Arias-Rodriguez, L., Kelley, J.L., and Tobler, M. (2024). Integrative analyses of convergent adaptation in sympatric extremophile fishes. Curr. Biol. *34*, 4968–4982.e7.
6. Láruson, Á.J., Yeaman, S., and Lotterhos, K.E. (2020). The importance of genetic redundancy in evolution. Trends Ecol. Evol. *35*, 809–822.
7. Bohutínská, M., and Peichel, C.L. (2024). Divergence time shapes gene reuse during repeated adaptation. Trends Ecol. Evol. *39*, 396–407.
8. Barghi, N., Hermisson, J., and Schlötterer, C. (2020). Polygenic adaptation: a unifying framework to understand positive selection. Nat. Rev. Genet. *21*, 769–781.
9. Jones, F.C., Grabherr, M.G., Chan, Y.F., Russell, P., Mauceli, E., Johnson, J., Swofford, R., Pirun, M., Zody, M.C., White, S., *et al*. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. Nature *484*, 55–61.
10. James, M.E., Wilkinson, M.J., Bernal, D.M., Liu, H., North, H.L., Engelstadter, J., and Ortiz-Barrientos, D. (2021). Phenotypic and genotypic parallel evolution in parapatric ecotypes of Senecio. Evolution *75*, 3115–3131.
11. Morales, H.E., Faria, R., Johannesson, K., Larsson, T., Panova, M., Westram, A.M., and Butlin, R.K. (2019). Genomic architecture of parallel ecological divergence: Beyond a single environmental contrast. Sci. Adv. *5*, eaav9963.
12. Corbett-Detig, R.B., Russell, S.L., Nielsen, R., and Losos, J. (2020). Phenotypic convergence is not mirrored at the protein level in a lizard adaptive radiation. Mol. Biol. Evol. *37*, 1604–1614.
13. Blount, Z.D., Lenski, R.E., and Losos, J.B. (2018). Contingency and determinism in evolution: Replaying life's tape. Science *362*, eaam5979.
14. Fraser, B.A., and Whiting, J.R. (2020). What can be learned by scanning the genome for molecular convergence in wild populations? Ann. N. Y. Acad. Sci. *1476*, 23–42.
15. Bomblies, K., and Peichel, C.L. (2022). Genetics of adaptation. Proc. Natl. Acad. Sci. USA *119*, e2122152119.
16. Bolnick, D.I., Barrett, R.D.H., Oke, K.B., Rennison, D.J., and Stuart, Y.E. (2018). (Non) parallel evolution. Annu. Rev. Ecol. Evol. Syst. *49*, 303–330.
17. Hämälä, T., Gorton, A.J., Moeller, D.A., and Tiffin, P. (2020). Pleiotropy facilitates local adaptation to distant optima in common ragweed (Ambrosia artemisiifolia). PLoS Genet. *16*, e1008707.
18. Feldman, C.R., Brodie, E.D., Brodie, E.D., and Pfrender, M.E. (2012). Constraint shapes convergence in tetrodotoxinresistant sodium channels of snakes. Proc. Natl. Acad. Sci. USA *109*, 4556–4561.
19. Pearless, S.M., and Freed, N.E. (2024). Unravelling the factors of evolutionary repeatability: insights and perspectives on predictability in evolutionary biology. Front. Ecol. Evol. *12*, 1335452.
20. Roesti, M., Roesti, H., Satokangas, I., Boughman, J., Chaturvedi, S., Wolf, J.B.W., and Langerhans, R.B. (2024). Predictability, an Orrery, and a Speciation Machine: Quest for a Standard Model of Speciation. Cold Spring Harb. Perspect. Biol. *16*, a041456.

Chemical ecology: Bacteria–fungi interaction for plant biocontrol

Ákos T. Kovács
Institute of Biology, Leiden University, 2333 BE Leiden, The Netherlands
Correspondence: a.t.kovacs@biology.leidenuniv.nl
https://doi.org/10.1016/j.cub.2024.09.071

Secondary metabolites mediate a broad variety of interactions between bacteria and fungi. Testing two plant growth-promoting microorganisms, a rhizobacterium and an arbuscular mycorrhizal fungus, a new study reveals their chemical interaction and an enhanced systemic induction of plant immunity.

Plant growth-promoting microorganisms are utilized to mitigate the use of synthetic chemical fertilizers and their detrimental impact on our environment. The mode of action of these microbes is most often mediated by secondary or specialized metabolites that either nourish the growth and development of plants or restrict pathogenic (micro) organisms. For example, these microbes produce a plethora of bioactive secondary metabolites that inhibit plant-pathogenic microorganisms. In the loose symbiotic relationship between a plant and its beneficial microbiome, plants excrete a mixture of metabolites that are utilized by the rhizoplane (directly attached biomass on the root)

and rhizosphere (soil region around the root) microbiome[1]. However, in addition to the broadly explored anti-fungal effect of rhizobacteria, certain species of bacteria and fungi might synergistically work together to benefit plants. Indeed, bacteria and fungi are also jointly utilized in certain plant promoting biofertilizer products. Detailed understanding of the underlying chemical interactions among bacteria, fungi, and their plant host will aid improvement of existing formulations and development of new combinations of plant growth-promoting microorganisms[2].

In this issue of *Current Biology*, Anckaert, Declerck and colleagues dissect the chemical ecology between the rhizobacterium *Bacillus velezensis* and the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis*[3]. They demonstrate that, in analogy to plant root exudate-mediated recruitment of specific microbial taxa, *R. irregularis* attracts *B. velezensis* to the hyphosphere, the soil region that is influenced by fungal hyphae (Figure 1). At the same time, production of the antifungal secondary metabolite fengycin is attenuated in the bacterium. Downregulation of antifungal lipopeptides has also been observed in a close relative of *B. velezensis*, *Bacillus subtilis*, during attachment on *Aspergillus niger* mycelia[4] and during two weeks of incubation with *Setophoma terrestris*[5], suggesting an overarching response and precise modulation of secondary metabolite production by *B. subtilis* group species in the presence of specific fungal taxa. However, production of the bacterium-produced amphiphilic lipopeptide surfactin is maintained by *B. velezensis* in the hyphosphere[3]. It remains to be discovered how rhizobacteria, like those of the *B. subtilis* group species, differentiate between different fungi — which one is friend and which one is foe? Identification of fungal-derived cues that modulate the production of secondary metabolites in *Bacilli* will aid predictability of *B. subtilis* group species compatibility with fungi. Moreover, it is also important to dissect how different fungi cope with the different lipopeptides produced by *Bacilli*, as certain fungal taxa might be sensitive to surfactin, and others to fengycins[6]. While fengycin production is reduced in *B. velezensis* upon AMF colonization, another secondary metabolite, di-peptide bacilysin, protects *R. irregularis* against its harmful competitor fungus, *Trichoderma harzianum*[3]. Similarly, the growth of another harmful bacterium, *Collimonas fungivorans*, is suppressed by *B. velezensis*, although independently of the *Bacillus* lipopeptides and bacilysin. These observations emphasize the complexity of the antimicrobial activity range of secondary metabolites.

Anckaert, Declerck and colleagues[3] developed a unique experimental system to decipher bacterial colonization, fungal cell biology, and interaction of AMF both with plant roots and with distant bacteria (Figure 1). Time-lapse imaging demonstrated that, through colonization of the AMF hyphae, *B. velezensis* forms a biofilm and expands along the extraradical mycelium of *R. irregularis* and reaches the plant root more promptly than by spreading through the soil. Formation of a sessile biofilm by *B. velezensis* on the AMF mycelia depends on the matrix components[3], as observed for *B. subtilis* group species[7,8]. Importantly, surfactin contributes to AMF colonization, and at the same time boosts cytoplasmic translocation within the hyphae, while surfactin is transported by the fungus to distal regions of the mycelia. Surfactin displays a plethora of ecological functions; in addition to its antimicrobial properties, it facilitates spreading on semi-solid surfaces and colonization of the rhizosphere, functions as a paracrine signaling molecule, induces systemic resistance in plants against pathogens, and influences establishment in microbial communities[9,10]. Due to its chemical properties, surfactin can penetrate lipid membranes and change fluidity. While surfactin disrupts intercellular vesicles of *A. niger*, including secretory vesicles that are transported towards the tip of the fungal mycelium[6], Anckaert, Declerck *et al.* did not observe any alteration of fungal membrane integrity by surfactin within the concentration range they tested.

Facilitation of bacterial spreading along the surface of fungal mycelia is a potentially conserved phenomenon that promotes the maintenance of mutualism between the interacting bacteria and fungi, including nutrient sharing and cross-feeding[11,12].

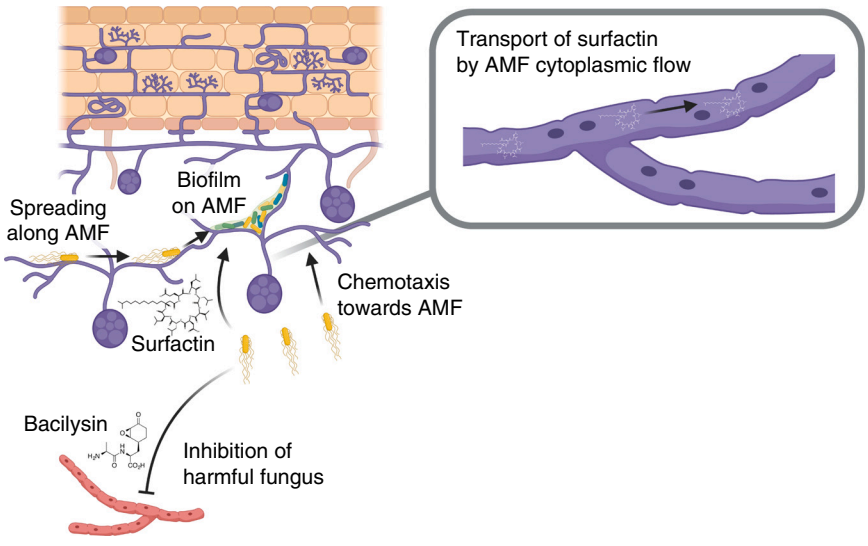

Figure 1. Bacteria–fungi interaction in the rhizosphere.
The various interaction mechanisms between the rhizobacterium *B. velezensis* and the AMF *R. irregularis* are depicted as described by Anckaert, Declerck *et al.*[3]. The example includes *B. velezensis* chemotaxis towards AMF, enhanced bacterial spreading along the hyphae and biofilm formation, protection of AMF against harmful fungi, and transport of the lipopeptide surfactin by the cytoplasmic translocation within the AMF mycelia. (This figure was created with BioRender.com.)

Anckaert, Declerck and colleagues suggest that facilitated spreading ensures homogeneous distribution of *B. velezensis* along the AMF mycelium[3], unlike the preferential zones along the plant roots where bacterial macrocolonies are formed[13]. However, spatial distribution of bacterial cells on mycelia might be dependent on the species or condition used, as distal localization of bacterial biofilm on a fungal colony has been reported for the ectomycorrhizal fungus *Laccaria bicolor*[14]. Imaging fungal mycelia dynamics during interaction with bacterial partners is a powerful tool applied in the last decade to understand bacterial–fungal interactions at the single-cell level while mimicking the structural properties of soil[15]. The work of Anckaert, Declerck *et al.* expands on this approach to discover the mechanistic details of tripartite interactions among bacteria, fungi, and the plant host[3].

While colonization and spreading along the extraradical mycelium of *R. irregularis* allows *B. velezensis* to arrive at a plant root more promptly, the chemical interaction within this cross-kingdom system has additional implications. Surfactin produced by *B. velezensis* induces systemic resistance against microbial infections in various plants[16]. Co-inoculation of *B. velezensis* and *R. irregularis* on tomato plants increased disease resistance against the foliar pathogen *Botrytis cinerea* compared with the lower protection observed when the plants were treated with only the bacterium or the fungus[3]. Importantly, *B. velezensis* was not present in the phyllosphere, suggesting that the reduced disease severity was surfactin-induced immune activation in the tomato plants, expedited by AMF. This prime example of bacterium–AMF-mediated plant protection against pathogens highlights the importance of a holistic view, considering supplementation of bacteria together with the application of AMF for promoting crop yield[17], where understanding the molecular details of this bacterium–fungus interaction could facilitate specific engineering of plant microbiomes.

In summary, the study by Anckaert, Declerck *et al.* uncovers a complex mutualistic interaction between a rhizobacterium and an AMF towards promotion of plant growth and health. To fully understand the interactions in the hyphosphere, cell biology and natural chemistry need to be combined, as presented in this work, in order to reveal the intricate functionalities of secondary metabolites in microbial communities. Future technical developments could potentially increase the spatial and temporal resolution of secondary metabolite detection, including their transfer and biotransformation in microbial communities.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Compant, S., Cassan, F., Kostić, T., Johnson, L., Brader, G., Trognitz, F., and Sessitsch, A. (2024). Harnessing the plant microbiome for sustainable crop production. Nat. Rev. Microbiol., in press. https://doi.org/10.1038/s41579-024-01079-1.

2. Zhou, Y., Wang, H., Xu, S., Liu, K., Qi, H., Wang, M., Chen, X., Berg, G., Ma, Z., Cernava, T., *et al*. (2022). Bacterial-fungal interactions under agricultural settings: from physical to chemical interactions. Stress Biol. *2*, 22.

3. Anckaert, A., Declerck, S., Poussart, L.-A., Lambert, S., Helmus, C., Boubsi, F., Steels, S., Argüelles-Arias, A., Calonne-Salmon, M., and Ongena, M. (2024). Deciphering the biology and chemistry of a mutualism between a soil bacterium and a mycorrhizal fungus. Curr. Biol. *34*, 4934–4950.e8.

4. Benoit, I., van den Esker, M.H., Patyshakuliyeva, A., Mattern, D., Blei, F., Zhou, M., Dijksterhuis, J., Brakhage, A.A., Kuipers, O.P., de Vries, R.P., *et al*. (2015). *Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism. Environ. Microbiol. *17*, 2099–2113.

5. Albarracín Orio, A.G., Petras, D., Tobares, R.A., Aksenov, A.A., Wang, M., Juncosa, F., Sayago, P., Moyano, A.J., Dorrestein, P.C., and Smania, A.M. (2020). Fungal–bacterial interaction selects for quorum sensing mutants with increased production of natural antifungal compounds. Commun. Biol. *3*, 670.

6. Richter, A., Blei, F., Hu, G., Schwitalla, J.W., Lozano-Andrade, C.N., Xie, J., Jarmusch, S.A., Wibowo, M., Kjeldgaard, B., Surabhi, S., *et al*. (2024). Enhanced surface colonisation and competition during bacterial adaptation to a fungus. Nat. Commun. *15*, 4486.

7. Kjeldgaard, B., Listian, S.A., Ramaswamhi, V., Richter, A., Kiesewalter, H.T., and Kovács, Á.T. (2019). Fungal hyphae colonization by *Bacillus subtilis* relies on biofilm matrix components. Biofilm *1*, 100007.

8. Xie, J., Sun, X., Xia, Y., Tao, L., Tan, T., Zhang, N., Xun, W., Zhang, R., Kovács, Á.T., Xu, Z., *et al*. (2024). Bridging the Gap: biofilm-mediated establishment of *Bacillus velezensis* on *Trichoderma guizhouense* mycelia. Preprint at bioRxiv, https://doi.org/10.1101/2024.06.06.597722.

9. Balleux, G., Höfte, M., Arguelles-Arias, A., Deleu, M., and Ongena, M. (2024). *Bacillus* lipopeptides as key players in rhizosphere chemical ecology. Trends Microbiol., in press. https://doi.org/10.1016/j.tim.2024.08.001.

10. Lozano-Andrade, C.N., Dinesen, C., Wibowo, M., Arenos Bach, N., Hesselberg-Thomsen, V., Jarmusch, S.A., Strube, M.L., and Kovács, Á.T. (2024). Surfactin facilitates establishment of *Bacillus subtilis* in synthetic communities. Preprint at bioRxiv, https://doi.org/10.1101/2024.08.14.607878.

11. Abeysinghe, G., Kuchira, M., Kudo, G., Masuo, S., Ninomiya, A., Takahashi, K., Utada, A.S., Hagiwara, D., Nomura, N., Takaya, N., *et al*. (2020). Fungal mycelia and bacterial thiamine establish a mutualistic growth mechanism. Life Sci. Alliance *3*, e202000878.

12. Worrich, A., Stryhanyuk, H., Musat, N., König, S., Banitz, T., Centler, F., Frank, K., Thullner, M., Harms, H., Richnow, H.H., *et al*. (2017). Mycelium-mediated transfer of water and nutrients stimulates bacterial activity in dry and oligotrophic environments. Nat. Commun. *8*, 15472.

13. Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O.H., and Aharoni, A. (2017). Live imaging of root–bacteria interactions in a microfluidics setup. Proc. Natl. Acad. Sci. USA *114*, 4549–4554.

14. Miquel Guennoc, C., Rose, C., Labbé, J., and Deveau, A. (2018). Bacterial biofilm formation on the hyphae of ectomycorrhizal fungi: a widespread ability under controls? FEMS Microbiol. Ecol. *94*, fiy093.

15. Aleklett, K., Ohlsson, P., Bengtsson, M., and Hammer, E.C. (2021). Fungal foraging behaviour and hyphal space exploration in micro-structured Soil Chips. ISME J. *15*, 1782–1793.

16. Pršić, J., Gilliard, G., Ibrahim, H., Argüelles-Arias, A., Rondelli, V., Crowet, J.M., Genva, M., Luzuriaga-Loaiza, W.P., Deboever, E., Nasir, M.N., *et al*. (2023). Mechanosensing and sphingolipid-docking mediate lipopeptide-induced immunity in Arabidopsis. Preprint at bioRxiv, https://doi.org/10.1101/2023.07.04.547613.

17. Lutz, S., Bodenhausen, N., Hess, J., Valzano-Held, A., Waelchli, J., Deslandes-Hérold, G., Schlaeppi, K., and van der Heijden, M.G.A. (2023). Soil microbiome indicators can predict crop growth response to large-scale inoculation with arbuscular mycorrhizal fungi. Nat. Microbiol. *8*, 2277–2289.