Interspecific formation of the antimicrobial volatile schleiferon

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Microorganisms release a plethora of volatile secondary metabolites. Up to now, it has been widely accepted that these volatile organic compounds are produced and emitted as a final product by a single organism e.g. a bacterial cell. We questioned this commonly assumed perspective and hypothesized that in diversely colonized microbial communities, bacterial cells can passively interact by emitting precursors which non-enzymatically react to form the active final compound. This hypothesis was inspired by the discovery of the bacterial metabolite schleiferon A. This bactericidal volatile compound is formed by a non-enzymatic reaction between acetoin and 2-phenylethylamine. Both precursors are released by *Staphylococcus schleiferi* cells. In order to provide evidence for our hypothesis that these precursors could also be released by bacterial cells of different species, we simultaneously but separately cultivated *Serratia plymuthica 4Rx13* and *Staphylococcus delphini 20771* which held responsible for only one precursor necessary for schleiferon A formation, respectively. By mixing their headspace, we demonstrated that these two species were able to deliver the active principle schleiferon A. Such a joint formation of a volatile secondary metabolite by different bacterial species has not been described yet. This highlights a new aspect of interpreting multispecies interactions in microbial communities as not only direct interactions between species might determine and influence the dynamics of the community. Events outside the cell could lead to the appearance of new compounds which could possess new community shaping properties.

Bacterial communities represent very diverse and dynamic systems. This applies especially to densely populated soil, marine and plant- or human-associated habitats where bacterial cells experience a highly competitive environment regarding living space, water and nutrients. Survival, growth and flourishing depend on their abilities for quick acclimations to changing living conditions and their strategies to outcompete co-habitants. Consequently, bacteria developed a tight network of interactive patterns consisting of cooperative traits, but also competitive and antagonistic action modes. Antagonism is often realized by the release of secondary metabolites. Research of recent years has shown that many of these secondary metabolites are volatile compounds. Because of their properties (small molecules with a high vapor pressure), volatiles do not only easily diffuse into areas close to the emitting organism, but also travel over longer distances. Therefore, they represent antagonistic and/or signalling compounds with a potential to manipulate physiological processes in other bacteria, as well as in fungi and plants. The recognition of volatiles enables bacteria to adjust to developmental processes that take place in microbial communities, thereby influencing the motility, biofilm formation and sporulation. Furthermore, antibiotic resistances and other stress responses can be enhanced due to contact with bacterial volatiles. On the other side, volatile metabolites can exhibit direct bacteriostatic and bactericidal activities. Volatile compounds with such a potential are schleiferons (3-(phenylamino)butan-2-one and 3-(phenylimino)butan-2-one, schleiferon A and B, respectively). They are produced by *Staphylococcus schleiferi* isolates. If present in the environment, schleiferons dramatically decrease the growth of Gram-positive bacteria and affect quorum-sensing controlled phenotypes of Gram-negative bacteria. Schleiferon A is formed by a spontaneous reaction between acetoin and 2-phenylethylamine (Schulz et al. unpublished), which are both synthesized by the bacterium *S. schleiferi*. Schleiferon B represents the oxidation product of A (Schulz et al. unpublished).

Both acetoin and 2-phenylethylamine are volatile compounds themselves. They are produced by a broad range of bacterial species. A bacterial community in general can consist of several different bacterial species. These species have the potential to synthesize precursors of final volatile products. This fueled the hypothesis that precursors like acetoin or 2-phenylethylamine released by different species into the headspace of a microbial community will spontaneously react to form an active principle like schleiferon A (and B). The goal of our experiments was to introduce a mechanism of how new compounds could arise within a habitat environment.
Results

The in vitro headspace reaction. A preliminary investigation was necessary in order to evaluate whether acetoin and 2-phenylethylamine react in an aerial environment. We separately dropped both compounds into two Petri dishes. These Petri dishes were simultaneously incubated in an analysis chamber for 24 hours. Subsequently, we funneled an air stream through the chamber onto an adsorbent and analyzed the eluted compounds by GC/MS (Fig. S1a). As shown in Fig. S1b, schleiferon A was also formed from acetoin and 2-phenylethylamine when incubated under aerial conditions. Since it lingered in the headspace of the Petri dish, most of it was oxidized to schleiferon B. Consequently, we could search for an acetoin producing bacterial strain that would not be able to emit 2-phenylethylamine and vice versa a bacterial isolate that emits solely 2-phenylethylamine.

Qualified bacterial aspirants. The genus Serratia is known to produce acetoin during fermentation of sugars. We analyzed the volatile compounds released by S. plymuthica 4Rx13 growing in complex liquid medium supplemented with glucose in a closed dynamic airflow system. Already after 24 hours of growth, acetoin (#1) was detected (Fig. S2a). The emission quickly increased during the exponential growth phase and peaked after 48h of incubation, which corresponded with the early stationary phase of bacterial growth (Fig. S2c). Subsequently, the acetoin level declined and reached the lowest value after 120 hours of growth. Most importantly, 2-phenylethylamine (#2) was not detected at all during these sampling periods.

While screening for a 2-phenylethylamine producer, S. delphini DSMZ 20771 attracted our attention. This strain emitted only 2-phenylethylamine during cultivation in tryptic soy broth (TSB) (#2, Fig. S2b). The emission started after 96h and even increased after 120 hours of growth (Fig S2d). S. delphini DSMZ 20771 did not release acetoin (#1) during these sampling intervals.

Interspecific bacterial formation of schleiferons. Based on the results of the mono-cultivation described above, we developed a VOC collection system where both bacterial isolates were concurrently cultivated in separated vessels. A split air stream entered simultaneously one culture flask inoculated with a 24h old culture of S. plymuthica 4Rx13 (flask 1) and one flask with a 120 h old culture of S. delphini DSMZ 20771 (flask 2). The two VOC-enriched air streams were reunited to allow the chemical reaction of acetoin produced by S. plymuthica 4Rx13 and 2-phenylethylamine produced by S. delphini DSMZ 20771 (Fig. 1a). We used three controls. In control 1, both flasks were filled with NBII liquid medium supplemented with glucose and TSB, respectively. Sets with one culture flask inoculated with S. plymuthica 4Rx13 while the other flask contained TSB and one flask inoculated with S. delphini DSMZ 20771 while the other flask contained NBII plus glucose served as control 2 and 3, respectively. The experiments were performed in triplicate. As expected, the precursor molecule acetoin (#1) was emitted by S. plymuthica 4Rx13 (control 2). The precursor 2-phenylethylamine (#2) was present in the VOC profile of S. delphini DSMZ 20771 (control 3). Both controls did not contain schleiferons. Control 1 did not contain precursors nor schleiferons. Small amounts of the precursors were still present in the concurrent culture of both bacterial isolates, however, most interesting was the detection of schleiferon A (#4) and B (#3) in the reunified bacterial headspace of this S. plymuthica/S. delphini concurrent culture after 24h and 48h of cultivation (Fig. 1b). Since S. plymuthica 4Rx13 released other compounds co-eluting with schleiferon B (#3), we verified the TIC result by an EIC m/z 105 representing the base peak of schleiferon A (#4) and B (#3) (Fig. 1c). The mass feature of m/z 105 at the retention time of schleiferon B was unique for the S. plymuthica/S. delphini concurrent culture.

Discussion

Our experiments strongly indicate that in a microbial community volatile products can arise from microbial precursor molecules which originate from cells of different species. We used the bacterial isolates S. plymuthica 4Rx13 and S. delphini DSMZ 20771 as model organisms in order to illustrate the principle. Although these particular isolates did not originate from the same habitat, it can be assumed that acetoin producing bacterial species and 2-phenylethylamine producers can inhabit the same ecological niches. The 2-phenylethylamine producer Enterococcus faecalis and the acetoin producer Serratia sp. both found to be ubiquitous constituents of the gastrointestinal microbiome, represent only one example. It should be noted that our in vitro experiment required certain nutrient conditions for each strain, in order to provoke the synthesis of the precursor molecules. This situation might ostensibly not be expected when bacteria share the same ecological niche in a natural environment. However, particularly in densely populated microhabitats like soil pores of the rhizosphere or the zone along root hairs, nutrient conditions can be different. Plants secrete metabolites depending on species, age, developmental stage and root zone. Adjacent living cells of different bacterial species might therefore experience different nutrient conditions and accordingly produce a certain spectrum of volatile metabolites, which could undergo a chemical reaction as we could show for acetoin and 2-phenylethylamine. Furthermore, in a populated hotspot where cells of different species mingle even under comparable nutrient supplies, cells of certain species could cause a modification of the ambient milieu in terms of secreting metabolites that serve as nutrient for other species or change conditions (i.e. pH-value) in the microhabitat. Those changing conditions can also provoke a changing spectrum of volatile metabolites of certain species. New volatile metabolites emitted into the headspace again could have the potential to initialize chemical reactions. Similar scenarios can be assumed for intestines of animals. A changing pH-value in gut systems and a different status of nutrient digestion might challenge symbiotic gut bacteria in a similar way. As those specific alterations in the microbial habitat might facilitate beneficial chemical reactions, natural environmental conditions, such as water and oxygen saturation, temperature, salinity, chemical composition of the habitat and the pH-value could in general affect headspace reactions. This influence should be questioned in further investigations.
In conclusion, our results show a new aspect of volatile production in microbial communities. We suggest that not only a single organism i.e. a bacterial cell of a certain species, participates in the synthesis of a final volatile metabolite which is released into the environment. Volatile metabolites could also be formed in the headspace of a microbial community by a non-enzymatic reaction employing precursors that could originate from cells of different species. Consequently, the microbial community itself has to be considered for VOC production. The synthesis of volatile precursors and perhaps even the non-enzymatic reaction might be a result of interaction between species. It is already known that microbial interactions can strongly influence the spectrum of volatiles metabolites emitted by a microbial community. However, even if the reaction would be a random result of emitted precursors of accidentally coexisting organisms it could be of the benefit of one or even both producer species. In addition, interspecifically formed volatile secondary metabolites could impair third party organisms i.e. cells of other bacterial or fungal species. Therefore, they could contribute to survival, growth and flourishing of precursor producers in a community (Fig. 2). Producers of matching precursors might seek neighbourly contact for the benefit of both. Since fungi can also form 2-phenylethylamine, inter-kingdom communicative traits can be expected. Hence, the headspace of habitats should not be considered as a static environment but rather as a dynamic system. Although, these conclusions have to be confirmed, they represent an important and non-negligible aspect to describe, evaluate and analyze interactions in a multispecies community and there is plenty of room for further research.

Figure 1. Schleiferon formation in concurrent cultures of physically separated Serratia plymuthica and Staphylococcus delphini. (a) VOC-collection system: Charcoal purified and sterilized air was split into two air streams of which each entered one culture flask containing either S. plymuthica 4Rx13 or S. delphini 20771. After VOC-enrichment, the two air streams were reunited and funneled over a VOC adsorbent (Porapak) (b) TIC-GC/MS chromatograms of media (blue line), S. delphini mono-culture (pink line), S. plymuthica mono-culture (black line) and S. plymuthica/S. delphini concurrent cultured for 24 hours (red line) (c) EIC-GC/MS chromatograms of m/z 105 representing the base peak of schleiferon A and B (color code for experimental setup see (b)). #1: acetoin, #2: 2-phenylethylamine, #3: schleiferon B, #4: schleiferon A; IS = internal standard (N-nonyl acetate, 5 ng); n = 3.
Methods

Bacterial strains and maintenance. The rhizobacterium *Serratia plymuthica* 4Rx13 was isolated from *Brassica napus* [45]. *Staphylococcus delphini* DSMZ 20771 originated from purulent material from a dolphin [46]. Short-term maintenance of *S. plymuthica* 4Rx13 was performed on nutrient agar plates supplemented with glucose (NAIIG; peptone from casein 3.5 g l\(^{-1}\), peptone from meat 2.5 g l\(^{-1}\), peptone from gelatine 2.5 g l\(^{-1}\), yeast extract 1.5 g l\(^{-1}\), NaCl 5 g l\(^{-1}\), glucose 100 mM, agar-agar 15 g l\(^{-1}\)). *S. delphini* was cultivated on tryptic soy agar plates (TSA; tryptone 17 g l\(^{-1}\), soy meal 3 g l\(^{-1}\), K\(_2\)HPO\(_4\) 2.5 g l\(^{-1}\), glucose 2.5 g l\(^{-1}\), NaCl 5 g l\(^{-1}\), agar-agar 15 g l\(^{-1}\)). Cultures were incubated for 12–24 hours at 30 °C under dim light (1.5 \(\mu\)E m\(^{-2}\)s\(^{-1}\)) and finally stored at 4 °C. For conservation, overnight liquid cultures were supplemented with glycerol (25%) and subsequently stored at –70 °C.

Reaction of acetoin and 2-phenylethylamine in an aerial environment. Acetoin (200 \(\mu\)g dissolved in 1 ml dichloromethane) and 2-phenylethylamine (400 \(\mu\)g dissolved in 1 ml dichloromethane) were separately dropped in a glass Petri dish (Ø30 \(\times\)10 mm). These Petri dishes were placed without a lid in another glass Petri dish (Ø130 \(\times\)30 mm), which had an in- and outlet (analysis chamber). After 24 h of incubation (30 °C and 1.5 \(\mu\)E m\(^{-2}\)s\(^{-1}\)), charcoal-purified, sterile air was sucked through the inlet into the analysis chamber with a constant flow of 0.6 l min\(^{-1}\) provided by a membrane pump (1410 Büh 12 VDC “D”, Gardner Denver Thomas GmbH, Memmingen, Germany). The volatile-enriched air was directed to a trap containing 30 mg of an adsorbent matrix (Porapak; Sigma–Aldrich, Munich, Germany). After defined incubation intervals (24, 48, 72, 96 and 120 h), the volatiles were consecutively eluted with 200 and 100 \(\mu\)l dichloromethane. N-nonyl acetate solution was added as an internal standard (final concentration of 5 ng l\(^{-1}\)).

VOC collection of mono-cultivated bacterial strains. A single bacterial colony of *S. plymuthica* 4Rx13 and *S. delphini* DSMZ 20771 was inoculated into 6 ml nutrient broth II supplemented with glucose (NBIG) and tryptic soy broth (TSB), respectively. Cultures were incubated overnight under agitation (160 rpm; Bühler, Tübingen, Germany) at 30 °C until reaching an OD\(_{600}\) of 1–2. They were diluted into 100 ml medium (NBIG or TSB) to obtain an initial OD\(_{600}\) of 0.05 and further incubated for 120 h using specific Erlenmeyer flasks supplied with an in- and outlet nozzle (30 °C, 1.5 \(\mu\)E m\(^{-2}\)s\(^{-1}\), agitation using a magnetic stirrer). Pure medium served as control. VOC collection was performed as described in Kai et al. 2010 and Lemfack et al. 2016 [42,43]. Charcoal-purified, sterile and humidified air was sucked through the inlet into the flask with a constant flow of 0.6 l min\(^{-1}\) provided by a membrane pump (1410 Büh 12 VDC “D”, Gardner Denver Thomas GmbH, Memmingen, Germany). The volatile-enriched air was directed into a trap containing 30 mg of an adsorbent matrix (Porapak; Sigma–Aldrich, Munich, Germany). After defined incubation intervals (24, 48, 72, 96 and 120 h), the volatiles were consecutively eluted with 200 and 100 \(\mu\)l dichloromethane. N-nonyl acetate solution was added as an internal standard (final concentration of 5 ng l\(^{-1}\)).
eluted with 200 and 100µl dichloromethane. N-nonyl acetate was added as an internal standard (final concentration of 5 ng/l−1). Simultaneously, the living cell number was determined at every 24h by serial dilution in NaCl-solution (0.8%). The dilutions were plated and CFUs were counted. The experiments were conducted in triplicate.

**VOC collection of physically separated concurrently cultivated bacterial strains.** The pre-cultivation of the bacterial strains and inoculation into the Erlenmeyer flasks was performed as described above. The VOC-collection system was modified as followed. Charcoal-purified and sterile air was sucked with a constant flow of 0.61min−1 (1410 Büh 12 VDC “D”, Gardner Denver Thomas GmbH, Memmingen, Germany). After passing the cotton filter, the air stream was split, humidified and directed through two Erlenmeyer flasks (Fig. 1a). While one Erlenmeyer flask was filled with a liquid culture of *S. plymuthica* 4Rx13 (24 h post inoculation), the second flask contained a *S. delphini* 20771 culture (120 h post inoculation). Three additional set-ups served as control. Flask 1 and 2 contained NBII + glucose and TSB, respectively (control 1). Furthermore, a mono-culture of *S. plymuthica* 4Rx13 in flask 1 was combined with TSB in flask 2 (control 2) and the mono-culture of *S. delphini* 20771 in flask 2 was combined with NBII + glucose in flask 1 (control 3). Cultures were magnetically stirred. Volatile-enriched air streams were reunited and sucked into a trap containing 30 mg of an adsorbent matrix (Porapak; Sigma-Aldrich, Munich, Germany). VOCs were consecutively eluted after 24h and 48 h of cultivation with 200 and 100µl dichloromethane. N-nonyl acetate was added as an internal standard (final concentration of 5 ng/l−1). The experiments were conducted in triplicate.

**Gas-chromatographic separation and mass spectrometric detection of acetoin, 2-phenylethylamine and schleiferons.** Samples were analyzed using a Shimadzu GC/MS-QP5000 system (Kyoto, Japan). Separation was performed on a DB5-MS column (60 m × 0.25 mm × 0.25 µm; J&W Scientific, Folsom, California, USA) connected to a CTC autosampler (CTC Analytics, Zwingen, Switzerland). The sample (1 µl) was splitless injected at 200 °C with a solvent cut of 2 minutes. The initial temperature of the GC column was set at 35 °C. Compounds were separated using a continuous ramp of 10 °C min−1 up to 280 °C with a final hold of 5 minutes. Helium was used as carrier gas (flow rate of 1.1 ml min−1, linear velocity of 28 cm/s).

Electron ionization mass spectra (EIMS) were recorded at an ionization energy of 70 eV with a mass range of m/z 40−280. Schleiferon A and B were identified by comparison of mass spectra and retention times with those of synthesized standards.

### References

1. Gray, J. P. & Herwig, R. P. Phylogenetic analysis of the bacterial communities in marine sediments. *Appl. Environ. Microbiol.* 62, 4049–4059 (1996).

2. Ding, T. & Schloss, P. D. Dynamics and association of microbial community types across the human body. *Nature* 509, 357–360 (2014).

3. Bai, Y. et al. Functional overlap of the *Arabidopsis* leaf and root microbiota. *Nature* 528, 364–369 (2015).

4. Bulgarelli, D. et al. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 17, 392–403 (2015).

5. Hibbing, M. E., Fuqua, C., Parsek, M. R. & Petersen, S. B. Bacterial competition: surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* 8, 15–25 (2010).

6. Haas, D. & Defago, G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* 3, 307–319 (2006).

7. Schulz, S. & Dickshat, J. S. Bacterial volatiles: the smell of small organisms. *Nat. Prod. Rep.* 24, 814–842 (2007).

8. Dai, M. et al. Bacterial volatiles and their action potential. *Appl. Microbiol. Biotechnol.* 81, 1001–1012 (2009).

9. Bos, L. D. J., Sterk, P. J. & Schultz, M. J. Volatile metabolites of pathogens: A Systematic Review. *PloS Pathog.* 9, e1003311 (2013).

10. Ruy, C. M. et al. Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 100, 4927–4932 (2003).

11. Effnert, U., Kalderás, J., Warnke, R. & Piechulla, B. Volatile mediated interactions between bacteria and fungi in the soil. *J. Chem. Ecol.* 38, 665–703 (2012).

12. Garbeva, P., Hordijk, C., Gerards, S. & de Boer, W. Volatile-mediated interactions between phylogenetically different soil bacteria. *Front Microbiol.* 5, 289 (2014).

13. Hai, M., Effnert, U. & Piechulla, B. Bacterial-plant-interactions: approaches to unravel the biological function of bacterial volatiles in the rhizosphere. *Front. Microbiol.* 7, 108b (2016).

14. Piechulla, B., Lemfack, M. C. & Hai, M. Effects of discrete bioactive microbial volatiles on plants and fungi. *Plant Cell Environ.* 40, 2042–2067 (2017).

15. Audrain, B., Farag, M., Ryu, C.-M. & Ghigo, J.-M. Role of bacterial volatile compounds in bacterial biology. *FEBS Microbiol. Rev.* 39, 222–233 (2015).

16. Schöller, C. E., Gurtler, H., Pedersen, R., Molin, S. & Wilkins, K. Volatiles metabolites from Actinomycetes. *FEMS Microbiol. Rev.* 39, 222–233 (2015).

17. Nijland, R. & Burgess, J. G. Bacterial ofaction. *Biotechnol. J.* 5, 974–977 (2010).

18. Kim, K. S., Lee, S. & Ryu, C. M. Interspecific bacterial sensing through airborne signals modulates locomotion and drug resistance. *Nat. Commun.* 4, 1809 (2013).

19. Letolle, S., Audrain, B., Bernier, S. P., Delepiere, M. & Ghigo, J.-M. Aerial exposure to the bacterial volatile compound trimethylamine modifies antibiotic resistance of phylogenetically separated bacteria by raising culture medium pH. *mBio* 5, e00944–13 (2014).

20. Bernier, S. C., Letolle, S., Delepiere, M. & Ghigo, J.-M. Biogenic ammonia modifies antibiotic resistance at a distance in physically separated bacteria. *Mol. Microbiol.* 81, 705–711 (2011).

21. Heal, R. D. & Parsons, A. T. Novel intercellular communication system in *Escherichia coli* that confers antibiotic resistance between physically separated populations. *J. Appl. Microbiol.* 92, 1116–1122 (2002).

22. Gurtler, H. et al. Albaflavenone, a sesquiterpene ketone with a zizaene skeleton produced by a streptomycete with a new rope morphology. *J. Antibiot. (Tokyo)* 47, 434–439 (1994).

23. Dandurishvili, N. et al. Broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* suppress *Agrobacterium* crown gall tumours on tomato plants. *J. Appl. Microbiol.* 110, 341–352 (2011).

24. Lemfack, M. C. et al. Novel volatiles of skin-borne bacteria inhibit the growth of Gram-positive bacteria and affect quorum-sensing controlled phenotypes of Gram-negative bacteria. *Syst. and Appl. Microbiol.* 39, 503–515 (2016).
25. Xiao, Z. & Xu, P. Acetoin metabolism in bacteria. Crit. Rev. Microbiol. 33, 127–140 (2007).
26. Isfeld, M., Spadafore, M. & Pruß, B. M. 3-phenylethylamine, a small molecule with a large impact. Webmedcentral 4, 4409 (2013).
27. Moons, P., van Houdt, R., Vrijis, B., Michiels, C. & Aertsens, A. Integrated regulation of acetoin fermentation by quorum sensing and pH in Serratia plymuthica RV1H. Appl. Environ. Microbiol. 77, 3422–3427 (2011).
28. Kai, M. et al. Serratia odorifer: analysis of volatile emission and biological impact of volatile compounds on Arabidopsis thaliana. Appl. Microbiol. Biotechnol. 88, 965–976 (2010).
29. Beutling, D. M. & Walter, D. 2-Phenylethylamine formation by enterococci in vitro. Eur. Food Res. Technol. 215, 240–242 (2002).
30. Xiao, Z. & Lu, J. R. Strategies for enhancing fermentative production of acetoin: A review. Biotech. Advances 32, 492–503 (2014).
31. Lebretón F., Willems R. J. L. & Gilmore M. S. Enterococcus diversity, origins in nature, and gut colonization. In: Gilmore MS, Clewell DB, Ike Y, Shankar N (eds). Enterococci: From commensals to leading causes of drug resistant infection [Internet]. Mass Eye Ear Infirmary: Boston, available from: https://www.ncbi.nlm.nih.gov/books/NBK190427/ (2014).
32. Daniel, D. S. Isolation and identification of gastrointestinal microbiota from the short-nosed fruit bat Cynopterus brachyotis. Microbiol. Res. 168, 485–496 (2013).
33. Bulgarelli, D., Schlappi, K., Spaepen, S., van Themaet E., V. L. & Schulze-Lefert, P. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64, 807–838 (2013).
34. Phelan, V. C., Liu, W. T., Pogliano, K. & Dorrestein, P. C. Microbial metabolic exchange—the chemotype-to-phenotype link. Nature Chem. Biol. 8, 26–35 (2011).
35. Jones, S. E. et al. Streptomycines exploration is triggered by fungal interactions and volatile signals. eLife. Science 6, e21738 (2017).
36. Herschend, J. et al. In vitro community synergy between bacterial soil isolates can be facilitated by pH stabilization of the environment. Appl. Environ. Microbiol., https://doi.org/10.1128/AEM.01450-18 Accepted Manuscript (2018).
37. Fallingsborg, J. Intraluminal pH of the human gastrointestinal tract. Dan. Med. Bull. 46, 183–96 (1999).
38. Ma, N. et al. Nutrients Mediate Intestinal Bacteria—Mucosal Immune Crosstalk. Front Immunol. 9, 5 (2018).
39. Schulz-Bohm, K., Zweers, H., de Boer, W. & Garbeva, P. A fragrant neighborhood: volatile mediated bacterial interactions in soil. Front. Microbiol. 6, 1212 (2015).
40. Hol, W. H. et al. Non-random species loss in bacterial communities reduces antifungal volatile production. Ecology 96, 2042–2048 (2015).
41. Tyc, O., Zweers, H., de Boer, W. & Garbeva, P. Volatiles in inter-specific bacterial interactions. Front. Microbiol. 6, 1412 (2015).
42. Schmidt, R. et al. Fungal volatile compounds induce production of the secondary metabolite Sodorifen in Serratia plymuthica PRI-2C. Scientific Reports 7, 862 (2017).
43. Kai, M. & Pichchilla, B. Interspecies interaction of Serratia plymuthica 4Rx13 and Bacillus subtilis B29 alters the emission of sodorifen. FEMS Microbiol. Lett. 365, fny253 (2018).
44. Stüttgen, G., Sya, R. & Dittmar, W. Determination of biogenic amines in fungus-cultures (44. Stüttgen, G., Sya, R. & Dittmar, W. Determination of biogenic amines in fungus-cultures (47. https://www.vecteezy.com/vector-art/146836-silhouette-tree-with-roots-vector. 46. Varaldo, P. E., Kilpper-Bälz, R., Biavasco, F., Satta, G. & Schleifer, K. H. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Appl. Environ. Microbiol. 68, 3328–3338 (2002).
45. Berg, G. et al. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Annu. Rev. Plant. Biol. 59, 331–335 (1978).
46. Varaldo, P. E., Kilpper-Bälz, R., Biavasco, F., Satta, G. & Schleifer, K. H. 2-Phenylethylamine formation by enterococci in vitro. Eur. Food Res. Technol. 215, 240–242 (2002).
47. https://www.vecteezy.com/vector-art/146836-silhouette-tree-with-roots-vector.

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Author Contributions
M.K. conceived and designed the project, performed the experiments and analyzed the data. M.K. produced institutional affiliations.

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