Opinion

Yeast-bacterium interactions: the next frontier in nectar research

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ABSTRACT

Beyond its role as reward for pollinators, floral nectar also provides habitat for specialized and opportunistic yeasts and bacteria. These microbes modify nectar chemistry, often altering mutualistic relationships between plants and pollinators in ways that we are only beginning to understand. Many studies on this multi-partite system have focused on either yeasts or bacteria without consideration of yeast-bacterium interactions, but recent evidence suggests that such interactions drive the assembly of nectar microbial communities and its consequences for pollination. Unexplored potential mechanisms of yeast-bacterium interactions include the formation of physical complexes, nutritional interactions, antibiosis, signaling-based interactions, and horizontal gene transfer. We argue that studying these mechanisms can elucidate how nectar microbial communities are established and affect plant fitness via pollinators.

MAIN TEXT

Microbial ecology of floral nectar

Virtually all ecosystems contain both fungi and bacteria. They interact with each other via diverse mechanisms ranging from trophic interactions to biofilm formation and even the interchange of genetic information, to name just a few [1,2]. These interactions are receiving increasing attention as we understand more about how the roles of fungi and bacteria as decomposers, nitrogen fixers, pathogens, and mutualistic partners of plants and animals are modified by fungus-bacterium interactions [3–8].

In this context, one emerging focus of plant science is the study of floral nectar as a habitat for both fungi (particularly yeasts) and bacteria that can withstand high **osmotic pressure** and secondary compounds (Box 1). Recent studies indicate that these microorganisms reach high densities in nectar (up to $>10^5$ cells/mm^3 for yeasts and $>10^7$ cells/mm^3 for bacteria [9–11]) and modify nectar chemistry in ways that alter pollinator foraging and, consequently, seed set and other fecundity parameters of plants [12–20]. Likewise, it has been shown that microbe-induced changes in nectar chemistry can affect longevity and other life-history characteristics of nectar-feeding insects [21].

Although bacteria and yeasts are both found frequently in floral nectar [22–24] and can have contrasting effects on nectar traits [19,25], most studies so far have focused on either bacteria or yeasts [9–12,26–32], and much remains unknown about interactions between these two microbial groups. In this Opinion article, we briefly review the current knowledge of yeast-bacterium interactions and identify potential mechanisms of the interactions that we believe would be worthwhile to study. With this article, we hope to stimulate more research on yeast-

bacterium interactions, which we believe will be necessary to fully understand the effects of nectar microbes on plants and their pollinators.

Current evidence for yeast-bacterium interactions and consequences for plants

The microbiome of floral nectar is species-poor relative to that of other parts of plants (Box 2). However, an increasing number of recent studies suggest strong associations between yeasts and bacteria in floral nectar. For example, a survey of nectar microorganisms associated with diverse species of Mediterranean plants in southern Spain found that culturable bacteria and yeasts co-occurred more often than would be expected by chance and identified three significant and relatively frequent positive bacterium-yeast associations: *Acinetobacter* spp. with *Metschnikowia gruessii*, *Acinetobacter* spp. with *M. reukaufii*, and *Leuconostoc* sp. with *M. reukaufii* [22]. Co-occurrence might be facilitated by resource partitioning between yeasts and bacteria in nectar. For instance, *Metschnikowia* spp. and the nectar acinetobacters *Acinetobacter nectaris* and *A. boissieri* may have complementary carbon assimilation profiles, with the yeast depleting glucose and enriching floral nectar in fructose and the bacteria preferentially using the latter monosaccharide [33].

Recent laboratory experiments, however, suggested **priority effects** (Figure 1) between *A. nectaris* and *M. reukaufii*, in which *A. nectaris* decreased the abundance of *M. reukaufii* when introduced to nectar earlier than the yeast and, conversely, *M. reukaufii* decreased *A. nectaris* abundance when the order of introduction was reversed (T. Fukami *et al.*, unpublished results). Similar priority effects were found between *M. reukaufii* and the acid acetic bacterium *Neokomagatea* (formerly *Gluconobacter*) sp., both isolated from the floral nectar of *Diplacus (Mimulus) aurantiacus* (Phrymaceae, sticky monkey-flower) [34]. Priority effects have also been found in a field experiment, where inoculation of *D. aurantiacus*

nectar with *Neokomagataea* sp. resulted in this bacterium dominating the nectar communities across multiple floral generations. *Neokomagataea* sp. dominance even led to exclusion of *M. reukaufii* despite *M. reukaufii* being common in nearby plants to which *Neokomagataea* sp. was not introduced [35].

Antagonistic interactions between yeasts and bacteria in nectar were also suggested by Tsuji and Fukami [36]. This study showed that reduced animal visitation caused a decline in yeast (mostly *M. reukaufii*) frequency and abundance in the nectar of male flowers of the dioecious shrub *Eurya emarginata* (Pentaphylacaceae) and an increase in bacterial (mostly *A. nectaris* and *A. boissieri*) abundance. This result was interpreted as possible competitive release of bacteria from yeasts, which, curiously, was not found in female flowers of the same shrub (where yeasts were never frequently found) nor for *Eurya japonica* plants in the region [36].

The amount, composition, and timing of nectar production can influence the array of animals that the flower attracts and their foraging behavior, but all these parameters can be affected by factors that are not entirely under the control of the producing plant, which include the activity of bacteria and yeasts in nectar [37–39]. Vannette and Fukami [25] have recently demonstrated that *M. reukaufii* and *Neokomagatea* sp. can have a significant and somewhat contrasting impact on the floral nectar traits of *D. aurantiacus*. Specifically, *M. reukaufii* reduced the concentration and altered the composition of amino acids in nectar, but had no significant effect on the total nectar volume produced by the plant or its sugar composition, whereas bacteria increased the amino acid concentration, enhanced the proportion of monosaccharides, and reduced the total volume of nectar [25]. However, combined inoculation of yeasts and bacteria was not carried out in this or previous similar studies [13,19], overlooking potential effects of yeast-bacterium interactions on nectar traits.

Yeasts and bacteria may also differentially alter secondary metabolites in nectar, including volatile compounds [15,40]. Nectar microorganisms can produce blends of volatile compounds that attract or deter pollinators [15,40,41]. In turn, this effect on pollinators might have consequences on microbial and plant fitness and the dispersal of microorganisms from flower to flower [15]. Furthermore, other nectar-consuming animals can also be affected by the volatile-producing activity of nectar microbes, as recently demonstrated for the generalist aphid parasitoid *Aphidius ervi* (Hymenoptera) [42]. However, this line of research has also been focused on the separate effects of bacteria and yeasts, rather than the potential combined effects.

All in all, studies so far suggest that yeast-bacterium interactions in floral nectar can be strong enough to affect plant-pollinator mutualism, but that the direction and strength of yeast-bacterium interactions might depend on many factors, including the microbes involved, the plant hosts, their intra-species variability in floral traits, environmental conditions [34], and the order of arrival of microbes to floral nectar, which, in turn, depends on the dispersal activity of pollinators and other floral visitors [43]. To explain the conditions under which yeasts and bacteria interact and affect plants and pollinators, what is needed now is a better understanding of the mechanisms that underlie yeast-bacterium interactions in floral nectar.

Unexplored potential mechanisms of yeast-bacterium interactions in floral nectar

Potential mechanisms of yeast-bacterium interactions include the formation of physical complexes, nutritional interactions, **antibiosis**, signaling-based interactions and **horizontal gene transfer** between yeast and bacterial cells [1,2]. Although the importance of these

mechanisms in nectar is currently unknown, they may operate simultaneously, and potentially result in unexpected consequences even for host plants and floral visitors (Figure 2).

Formation of physical complexes

Fungi and bacteria often form assemblies in which participating cells display physical and physiological properties distinct from free-living cells [44]. These associations are found in a variety of microbial habitats in and on plants and vary in their degree of complexity and intimacy, ranging from loose and disordered cell aggregates to multi-species biofilms held together by an extracellular matrix and highly specific **endosymbiotic** associations [2,8,44]. Inspection of a nectar drop under the microscope makes clear that simple forms of physical association (e.g. polymicrobial groups of cells) are common in nectar microbial communities. Similarly, although polymicrobial biofilms in floral nectar have not been documented, they are widespread in the **rhizosphere** and the **phylloplane** [45]. There is no reason to discard their possible occurrence on nectary surfaces. If they do occur, the extracellular matrix surrounding the microbes might protect them against osmotic pressure, toxins, and other stressors that limit microbial growth [46]. Formation of microbial biofilms on the surface of pollinator's mouthparts may also be possible, given the anchor-like morphology of the aggregates of *M. gruessii* cells [28] and the stickiness of the colonies of bacteria such as *A. nectaris* and *Rosenbergiella* spp. (S. Álvarez-Pérez *et al.*, unpublished results).

Bacteria do not only attach to fungal cells, but can also colonize them intracellularly, as seen in diverse species of soil, rhizophere, and phylloplane fungi [1,2,8]. Examples of endosymbiotic bacteria hosted within yeast partners are scarce in the literature, but Siavoshi *et al.* [47] reported that diverse osmotolerant yeasts isolated from whole flowers, fruits, and honeybees contained in their vacuoles bacterial cells identified as *Helicobacter pylori* and

hypothesized that this intracellular establishment could be an adaptation to the stressful conditions of sugar-rich environments. If such intracellular bacteria were found in nectar yeasts, the study of the consequences for both microbial partners (e.g. genome signatures, transmission during yeast mitosis and/or meiosis, yeast-bacteria co-evolution) and the plant-animal system would open exciting new avenues in nectar research.

Nutritional interactions

Competition for nutrients may drive yeast-bacterium interactions in nectar [34]. In particular, *M. reukaufii* seems to have undergone extensive gene duplications, especially in high-capacity amino acid transporter genes, allowing the yeast to exert strong priority effects against other microbes in nitrogen-poor habitats such as nectar [48,49]. An opposite trend in genome evolution might have taken place for *A. nectaris* and *A. boissieri*, whose genome sizes are well below the average value for the genus *Acinetobacter* (2.7 *vs.* 3.9 Mb) [50]. Such a difference in genome size between the *A. nectaris*/*boissieri* clade and most other acinetobacters could reflect adaptation to the carbohydrate-rich condition of floral nectar and the digestive tract of pollinators. A similar scenario has been hypothesized for some insect-associated bacteria such as *Lactobacillus kunkeei*, whose genome is remarkably smaller than those of other species of *Lactobacillus* and seems to have lost a substantial part of the genetic repertoire encoding for amino acid metabolism and carbohydrate metabolism and transport [51].

Competition among nectar microbes for iron and other micronutrients is also possible. Yeasts such as *Metschnikowia pulcherrima* [52] and species of bacterial genera such as *Acinetobacter* and *Pseudomonas* [53,54] can produce **chelators** that allow them to efficiently acquire iron from the environment and make it unavailable for other microbes. Moreover,

bacterial **mycophagy** [55] and **bacterial farming** by fungi [56] have not yet been reported to occur in the nectar microbiota, but given the high cell densities that yeasts and bacteria can reach in floral nectar [9−11], these types of nutritional interactions might be likely. Similarly, the possibility that nectar microbes engage in **cross-feeding** and **syntrophic interactions** [57] cannot be discarded.

Antibiosis and signaling-based interactions

Some species of *Metschnikowia* and other yeasts prevalent in nectar exhibit antimicrobial activity against plant pathogens [58,59], suggesting that antibiosis might shape nectar microbial communities. Likewise, diverse bacterial genera found in nectar (e.g. *Pseudomonas* and *Pantoea*) produce antifungal substances and **bacteriocins** [60,61]. Tucker and Fukami [34] demonstrated that environmental variability could counteract the inhibitory effects of some substances generated by nectar microbes (e.g. H^+ ions, which reduce nectar pH and hinder yeast growth), thus promoting coexistence of yeasts and bacteria in floral nectar. As floral nectar is a dynamic system where biotic and abiotic conditions are highly variable during a flower's lifespan [39,62], the role of inhibitory substances on yeast-bacterium interactions might be difficult to predict. A better knowledge (e.g. through metabolomic and transcriptomic analyses) of the metabolites produced by microbes when colonizing nectar alone or in interactions, supplemented with mathematical modelling of microbial community assembly [34], would be of great help in this regard.

Apart from their role in affecting the foraging behavior of floral visitors, some metabolites of microbial origin can act as signaling molecules in interactions among microbes and between these microbes and their host plants [63]. These **semiochemicals** can affect the behavior, population dynamics, and gene expression of other microorganisms [2,63]. In addition, some

semiochemicals of fungal origin can alter bacterial **quorum sensing**, affecting population density-dependent activities of the target species, including effects on morphogenesis, biofilm formation, antibiotic production, and interactions with animal and plant hosts [2,64,65]. Although quorum sensing was originally considered in bacteria, similar signaling mechanisms can occur in fungi, and even several cases of inter-kingdom quorum sensing have been reported [64,65]. Farnesol, a major quorum sensing molecule in diverse fungal species [41,64,65], is also a component of insect pheromones that mediate foraging, sexual attraction, and other behavioral responses, and has been found in the flowers of some plants [66–68]. Even though the study of semiochemical production by nectar microbes is still in its infancy [15,40,42] and, to our knowledge, farnesol release by nectar yeasts remains to be demonstrated, it seems possible that microbe-microbe communication changes floral visitors' behavior as a side effect.

Horizontal gene transfer

Horizontal gene transfer is prevalent in plant-associated bacteria [69,70]. Numerous cases of horizontal gene transfer from bacteria to fungi have also been described, although it seems less frequent than horizontal gene transfer among bacteria [70,71]. Although horizontal gene transfer has not been reported for nectar microbes, the genome of *A. nectaris* contains sequences encoding **transposases** and **prophage** sequences [50]. Additionally, it has been demonstrated that *Acinetobacter baylyi*, which is also found in floral nectar ([10]; S. Álvarez-Pérez *et al.*, unpublished results), can speed up horizontal gene transfer by actively killing other bacteria to extract and take up parts of their DNA, and that this phenomenon is more effective when *A. baylyi* outnumbers its "victim" and also when both co-exist for a short time [72]. Furthermore, other nectar bacteria such as *Pseudomonas* spp. and acetic acid bacteria have a complex history of genome evolution, which might include horizontal gene transfer

events with yeasts [70,73–75]. Future research should therefore focus on finding possible hallmarks of passive and active (e.g. killing-enhanced, as in *A. baylyi*) horizontal gene transfer in the genome of nectar microbes.

Concluding Remarks and Future Perspectives

The conventional view that floral nectar is merely a reward that angiosperms offer pollinators has been challenged in recent years. Floral nectar is now routinely seen also as the habitat of specialized yeasts and bacteria capable of overcoming high sugar concentrations and other hurdles inflicted by plants, and opportunistic microbes profiting from the activity of the former. We have argued here that elucidating the mechanisms of yeast-bacterium interactions will be essential to advancing the understanding of the effects that these microorganisms have on the behavior of pollinators and other floral visitors and, eventually, plant fitness. Many questions remain to be addressed (see some examples in Outstanding Questions) regarding the ecology and evolution of the nectar inhabitants and their interactions with animals and plants. Because pollination is a critical component of many agricultural crops, better knowledge on yeast-bacterium interactions that will be gained by answering outstanding questions has the potential to facilitate improved plant breeding and crop production.

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REFERENCES

1. Deveau, A. *et al.* (2018) Bacterial–fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiol. Rev.* 42, 335–352
2. Frey-Klett, P. *et al.* (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol. Mol. Biol. Rev.* 75, 583–609
3. de Boer, W. (2017) Upscaling of fungal–bacterial interactions: from the lab to the field. *Curr. Opin. Microbiol.* 37, 35–41
4. Duran, P. *et al.* (2018) Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell.* 175, 973-983.e14
5. Kim, D. *et al.* (2017) *Candida albicans* stimulates *Streptococcus mutans* microcolony development via cross-kingdom biofilm-derived metabolites. *Sci. Rep.* 7, 41332
6. van der Heijden, M.G. *et al.* (2016) A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J.* 10, 389–399
7. Worrich, A. *et al.* (2017) Mycelium-mediated transfer of water and nutrients stimulates bacterial activity in dry and oligotrophic environments. *Nat. Comm.* 8, 15472
8. Jambon, I. *et al.* (2018) Harnessing plant-bacteria-fungi interactions to improve plant growth and degradation of organic pollutants. *J. Plant Interact.* 13, 119–130
9. de Vega, C. *et al.* (2009) Yeasts in floral nectar of some South African plants: quantification and associations with pollinator type. *S. Afr. J. Bot.* 75, 798–806

10. Fridman, S. *et al.* (2012) Bacterial communities in floral nectar. *Environ. Microbiol. Rep.* 4, 97–104
11. Herrera, C.M. *et al.* (2009) Yeasts in floral nectar: a quantitative survey. *Ann. Bot.* 103, 1415–1423
12. Canto, A. *et al.* (2017) Nectar-living yeasts of a tropical host plant community: diversity and effects on community-wide floral nectar traits. *PeerJ* 5, e3517
13. Good, A.P. *et al.* (2014) Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut. *PLoS One* 9, e86494
14. Herrera, C.M. *et al.* (2013) Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. *Ecology* 94, 273–279
15. Rering, C.C. *et al.* (2018) Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytol.* 220, 750–759
16. Schaeffer, R.N. and Irwin, R.E. (2014) Yeasts in nectar enhance male fitness in a montane perennial herb. *Ecology* 95, 1792–1798
17. Schaeffer, R.N. *et al.* (2014) Nectar yeasts in the tall larkspur *Delphinium barbeyi* (Ranunculaceae) and effects on components of pollinator foraging behavior. *PLoS One* 9, e108214
18. Schaeffer, R.N. *et al.* (2017) Consequences of a nectar yeast for pollinator preference and performance. *Funct. Ecol.* 31, 613–621
19. Vannette, R.L. *et al.* (2013) Nectar bacteria, but not yeast, weaken a plant-pollinator mutualism. *Proc. Biol. Sci.* 280, 20122601
20. Yang, M. *et al.* (2019) Nectar yeasts enhance the interaction between *Clematis akebioides* and its bumblebee pollinator. *Plant Biol.* DOI: 10.1111/plb.12957 (https://onlinelibrary.wiley.com/journal/14388677)

21. Lenaerts, M. *et al.* (2017) Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry. *Funct. Ecol.* 31, 2061–2069
22. Álvarez-Pérez, S. and Herrera, C.M. (2013) Composition, richness and non-random assembly of culturable bacterial–microfungal communities in floral nectar of Mediterranean plants. *FEMS Microbiol. Ecol.* 83, 685–699
23. Jacquemyn, H. *et al.* (2013) Among-population variation in microbial community structure in the floral nectar of the bee-pollinated forest herb *Pulmonaria officinalis* L. *PLoS One* 8, e56917
24. Jacquemyn, H. *et al.* (2013) Microbial diversity in the floral nectar of seven *Epipactis* (Orchidaceae) species. *Microbiologyopen* 2, 644–658
25. Vannette, R.L. and Fukami, T. (2018) Contrasting effects of yeasts and bacteria on floral nectar traits. *Ann. Bot.* 121, 1343–1349
26. Álvarez-Pérez, S. *et al.* (2012) Zooming-in on floral nectar: a first exploration of nectar-associated bacteria in wild plant communities. *FEMS Microbiol. Ecol.* 80, 591–602
27. Belisle, M. *et al.* (2012) Flowers as islands: spatial distribution of nectar-inhabiting microfungi among plants of *Mimulus aurantiacus*, a hummingbird-pollinated shrub. *Microb. Ecol.* 63, 711–718
28. Brysch-Herzberg, M. (2004) Ecology of yeasts in plant–bumblebee mutualism in Central Europe. *FEMS Microbiol. Ecol.* 50, 87–100
29. Chappell, C.R. and Fukami, T. (2018) Nectar yeasts: a natural microcosm for ecology. *Yeast* 35, 417–423
30. Mittelbach, M. *et al.* (2015) Nectar sugars and bird visitation define a floral niche for basidiomycetous yeast on the Canary Islands. *BMC Ecol.* 15, 2
31. Pozo, M.I. *et al.* (2011) Species richness of yeast communities in floral nectar of southern Spanish plants. *Microb. Ecol.* 61, 82–91

32. Samuni-Blank, M. *et al.* (2014) The role of abiotic environmental conditions and herbivory in shaping bacterial community composition in floral nectar. *PLoS One* 9, e99107
33. Álvarez-Pérez, S. *et al.* (2013) *Acinetobacter nectaris* sp. nov. and *Acinetobacter boissieri* sp. nov., isolated from floral nectar of wild Mediterranean insect-pollinated plants. *Int. J. Syst. Evol. Microbiol.* 63, 1532–1539
34. Tucker, C.M. and Fukami, T. (2014) Environmental variability counteracts priority effects to facilitate species coexistence: evidence from nectar microbes. *Proc. Biol. Sci.* 281, 20132637
35. Toju, H. *et al.* (2018) Priority effects can persist across floral generations in nectar microbial metacommunities. *Oikos* 127, 345–352
36. Tsuji, K. and Fukami, T. (2018) Community-wide consequences of sexual dimorphism: evidence from nectar microbes in dioecious plants. *Ecology* 99, 2476–2484
37. Aleklett, K. *et al.* (2014) The microbial ecology of flowers: an emerging frontier in phyllosphere research. *Botany* 92, 253–266
38. Junker, R.R. *et al.* (2011) Composition of epiphytic bacterial communities differs on petals and leaves. *Plant Biol.* 13, 918–924
39. Parachnowitsch, A.L. *et al.* (2018) Evolutionary ecology of nectar. *Ann. Bot.* DOI: 10.1093/aob/mcy132 (https://academic.oup.com/aob)
40. Rering, C.C. *et al.* (2018) Quantitative assessment of nectar microbe-produced volatiles. *ACS Symp. Ser.* 1294, 127–142
41. Dzialo, M.C. *et al.* (2017) Physiology, ecology and industrial applications of aroma formation in yeast. *FEMS Microbiol. Rev.* 41, S95–S128
42. Sobhy, I.S. *et al.* (2018) Sweet scents: nectar specialist yeasts enhance nectar attraction of a generalist aphid parasitoid without affecting survival. *Front. Plant. Sci.* 9, 1009

43. Vannette, R.L. and Fukami, T. (2017) Dispersal enhances beta diversity in nectar microbes. *Ecol. Lett.* 20, 901–910
44. Berlanga, M. and Guerrero, R. (2016) Living together in biofilms: the microbial cell factory and its biotechnological implications. *Microb. Cell Fact.* 15, 165
45. Morris, C.E. *et al.* (1997) Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. *Appl. Environ. Microbiol.* 63, 1570–1576
46. Lievens, B. *et al.* (2015) Microbiology of sugar-rich environments: diversity, ecology and system constraints. *Environ. Microbiol.* 17, 278–298
47. Siavoshi, F. *et al.* (2018) Natural fruits, flowers, honey, and honeybees harbor *Helicobacter pylori*-positive yeasts. *Helicobacter* 23, e12471
48. Dhami, M.K. *et al.* (2016) Genetic basis of priority effects: insights from nectar yeast. *Proc. Biol. Sci.* 283, 20161455
49. Herrera, C.M. (2017) Scavengers that fit beneath a microscope lens. *Ecology* 98, 2725–2726
50. Touchon, M. *et al.* (2014) The genomic diversification of the whole *Acinetobacter* genus: origins, mechanisms, and consequences. *Genome Biol. Evol.* 6, 2866–2882
51. Tamarit, D. *et al.* (2015) Functionally structured genomes in *Lactobacillus kunkeei* colonizing the honey crop and food products of honeybees and stingless bees. *Genome Biol. Evol.* 7, 1455–1473
52. Sipiczki, M. (2006) *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. *Appl. Environ. Microbiol.* 72, 6716–6724
53. Butaité, E. *et al.* (2017) Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nat. Commun.* 8, 414

54. Doughari, H.J. *et al.* (2011) The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. *Microbes Environ.* 26, 101–112
55. Leveau, J.H. and Preston, G.M. (2008) Bacterial mycophagy: definition and diagnosis of a unique bacterial-fungal interaction. *New Phytol.* 177, 859–876
56. Pion M., *et al.* (2013) Bacterial farming by the fungus *Morchella crassipes*. *Proc. Biol. Sci.* 280, 20132242
57. Seth, E.C. and Taga, M.E. (2014) Nutrient cross-feeding in the microbial world. *Front. Microbiol.* 5, 350
58. Duffy, B. *et al.* (2006) Biocontrol of *Erwinia amylovora* using a commercial yeast strain mixture. *Acta Hortic.* 704, 363–366
59. Pusey, P.L. *et al.* (2009) Epiphytic bacteria and yeasts on apple blossoms and their potential as antagonists of *Erwinia amylovora*. *Phytopathology* 99, 571–581
60. Parret, A.H. and De Mot, R. (2002) Bacteria killing their own kind: novel bacteriocins of *Pseudomonas* and other γ-proteobacteria. *Trends Microbiol.* 10, 107–112
61. Walterson, A.M. and Stavrinides, J. (2015) *Pantoea*: insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiol. Rev.* 39, 968–984
62. Nicolson, S.W. and Thornburg, R.W. (2007) Nectar chemistry. In *Nectaries and Nectar* (Nicolson, S.W. *et al.*, eds), pp. 215–264, Springer-Verlag
63. Schmidt, R. *et al.* (2015) Volatile affairs in microbial interactions. *ISME J.* 9, 2329–2335
64. Dixon, E.F. and Hall, R.A. (2015) Noisy neighbourhoods: quorum sensing in fungal-polymicrobial infections. *Cell. Microbiol.* 17, 1431–1441
65. Kalia, V.C., ed (2015) *Quorum Sensing vs Quorum Quenching: A Battle with No End in Sight*, Springer.
66. Granero, A.M. *et al.* (2005) Chemical compounds of the foraging recruitment pheromone in bumblebees. *Naturwissenschaften* 92, 371–374

67. Schiestl, F.P. *et al.* (2000) Sex pheromone mimicry in the early spider orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception. *J. Comp. Physiol. A* 186, 567–574
68. Schiestl, F.P. (2010) The evolution of floral scent and insect chemical communication. *Ecol. Lett.* 13, 643–656
69. Kroll, S. *et al.* (2017) Genomic dissection of host–microbe and microbe–microbe interactions for advanced plant breeding. *Curr. Opin. Plant Biol.* 36, 71–78
70. Rolland, T. *et al.* (2009) Insertion of horizontally transferred genes within conserved syntenic regions of yeast genomes. *PLoS One* 4, e6515
71. Lacroix, B. and Citovsky, V. (2016) Transfer of DNA from bacteria to eukaryotes. *MBio* 7, e00863-16
72. Cooper, R.M. *et al.* (2017) Inter-species population dynamics enhance microbial horizontal gene transfer and spread of antibiotic resistance. *Elife* 6, e25950
73. Chouaia, B. *et al.* (2014) Acetic acid bacteria genomes reveal functional traits for adaptation to life in insect guts. *Genome Biol. Evol.* 6, 912–920
74. Hall, C. *et al.* (2005) Contribution of horizontal gene transfer to the evolution of *Saccharomyces cerevisiae*. *Eukaryot. Cell.* 4, 1102–1115
75. Silby, M.W. *et al.* (2011) *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiol. Rev.* 35, 652–680
76. Herrera, C.M. *et al.* (2010) Inhospitable sweetness: nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. *Proc. Biol. Sci.* 277, 747–754
77. Bubán, T. *et al.* (2003) The nectary as the primary site of infection by *Erwinia amylovora* (Burr.) Winslow et al.: a mini review. *Plant Syst. Evol.* 238, 183–194

78. Nepi, M. (2017) New perspectives in nectar evolution and ecology: simple alimentary reward or a complex multiorganism interaction? *Acta Agrobot.* 70, 1704
79. Adler, L.S. (2000) The ecological significance of toxic nectar. *Oikos* 91, 409–420
80. Hillwig, M.S. *et al.* (2010) Petunia nectar proteins have ribonuclease activity. *J. Exp. Bot.* 61, 2951–2965
81. Irwin, R.E. *et al.* (2014) Secondary compounds in floral rewards of toxic rangeland plants: impacts on pollinators. *J. Agric. Food Chem.* 62, 7335–7344
82. Ma, X.L. *et al.* (2017) Floral nectar of the obligate outcrossing *Canavalia gladiata* (Jacq.) DC. (Fabaceae) contains only one predominant protein, a class III acidic chitinase. *Plant Biol.* 19, 749–759
83. Pozo, M.I. *et al.* (2012) Nectar yeasts of two southern Spanish plants: the roles of immigration and physiological traits in community assembly. *FEMS Microbiol. Ecol.* 80, 281–293
84. Vannette, R.L. and Fukami, T. (2016) Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. *Ecology* 97, 1410–1419
85. González-Teuber, M. and Heil, M. (2009) Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal Behav.* 4, 809–813
86. Richardson, L.L. *et al.* (2016) Nectar chemistry mediates the behavior of parasitized bees: consequences for plant fitness. *Ecology* 97, 325–337
87. Wright, G.A. *et al.* (2014) Caffeine in floral nectar enhances a pollinator's memory of reward. *Science* 339, 1202–1204
88. Herrera, C.M. *et al.* (2008) Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology* 89, 2369–2376
89. Aizenberg-Gershtein Y *et al.* (2013) Do honeybees shape the bacterial community composition in floral nectar? *PLoS One* 8, e67556

90. Pozo, M. *et al.* (2015) Impact of microorganisms on nectar chemistry, pollinator attraction and plant fitness. In *Nectar: Production, Chemical Composition and Benefits to Animals and Plants* (Peck, R.L., ed.), pp. 1–40, Nova Science Publishers, Inc.
91. Hausmann, S.L. *et al.* (2017) Solving the puzzle of yeast survival in ephemeral nectar systems: exponential growth is not enough. *FEMS Microbiol. Ecol.* 93(12), doi: 10.1093/femsec/fix150
92. Zemenick, A.T. *et al.* (2018) Legitimate visitors and nectar robbers of *Aquilegia formosa* have different effects on nectar bacterial communities. *Ecosphere* 9, e02459.
93. Pozo, M.I. *et al.* (2018) Surviving in the absence of flowers: do nectar yeasts rely on overwintering bumblebee queens to complete their annual life cycle? *FEMS Microbiol. Ecol.* 94(12), doi: 10.1093/femsec/fiy196.
94. Peay, K. *et al.* (2012) Phylogenetic relatedness predicts priority effects in nectar yeast communities. *Proc. Biol. Sci.* 279, 749–758
95. Vannette, R.L. and Fukami, T. (2014) Historical contingency in species interactions: towards niche-based predictions. *Ecol. Lett.* 17, 115–124
96. Morris, B.E. *et al.* (2013) Microbial syntrophy: interaction for the common good. *FEMS Microbiol. Rev.* 37, 384–406

FIGURE LEGENDS

Figure 1. Typical setting of a microcosm experiment to test for priority effects between nectar microorganisms [34,94,95]. Sequential microbial dispersal events to flowers is mimicked using plastic microtubes loaded with sterile synthetic nectar (or, alternatively, filtered natural nectar). In the example shown, the experiment includes two treatments: (A) "bacteria-first," in which the bacterial species is first introduced and sometime later (t_1) the yeast species is inoculated; and (B) "yeast-first," in which the introduction order is the opposite. In both cases, after a second incubation time (t_2), the content of the microtubes is plated on selective media and colony forming units of yeasts and bacteria counted separately to estimate the final cell density. Control treatments (e.g. only yeasts, only bacteria, and no microbes) are run in parallel. The results of the experiment displayed in the figure depict strong priority effects, as in Tucker and Fukami [34]. Figure created with BioRender (https://biorender.io).

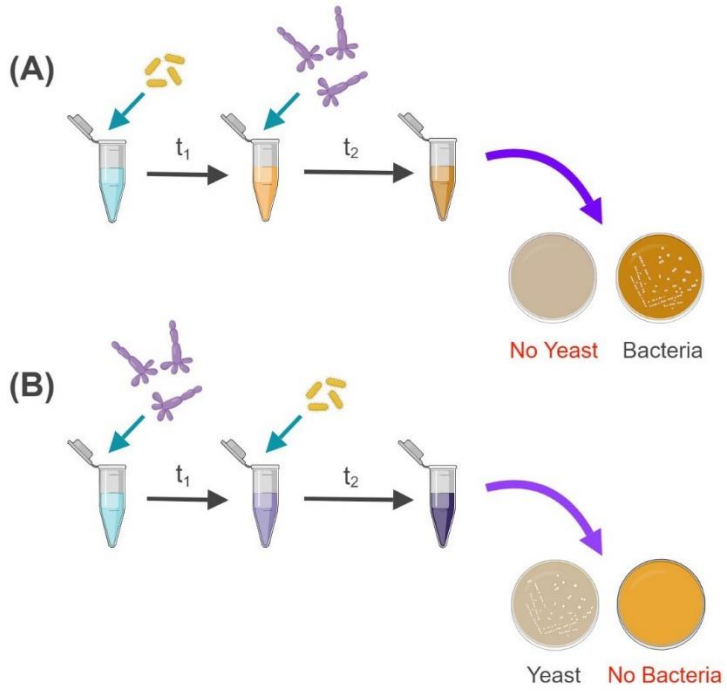
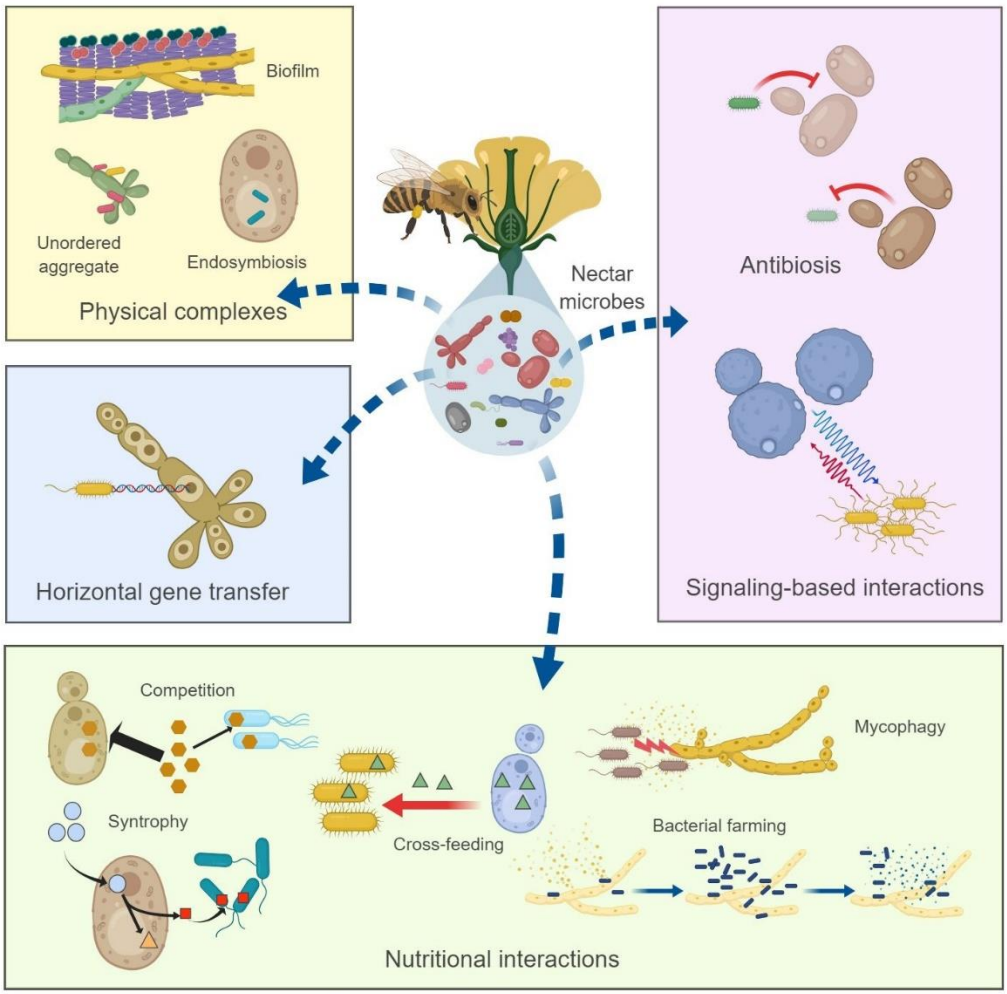


Figure 2. Overview of the potential mechanisms of yeast-bacterium interactions considered in this article: i) formation of physical complexes; ii) antibiosis and signaling-based interactions; iii) nutritional interactions; and iv) horizontal gene transfer. Figure created with BioRender (https://biorender.io).



TEXT BOX 1. Antimicrobial defenses of floral nectar

The high sugar concentration of floral nectar exerts osmotic pressure on microbes and represents a filter for microbial life [46,76]. However, high sugar concentration can encourage growth of a wide range of osmotolerant microorganisms including plant pathogens [77,78]. Consequently, it has been hypothesized that some plants may resist microbial colonization of nectar by producing high levels of hydrogen peroxide and other reactive oxygen species, toxic secondary metabolites from diverse chemical families (e.g. alkaloids, phenolics and terpenoids), or different lytic enzymes (e.g. chitinases, lipases and RNases) [62,78–82]. These chemicals are geographically and phylogenetically widespread across the plant kingdom, although species may vary in defense mechanisms [62,79]. In turn, many nectar-inhabiting microbes appear to possess catalase activity that might protect them from the toxic action of hydrogen peroxide [23,24,26,83]. Tolerance of nectar yeasts and bacteria to diverse secondary compounds of plant origin has also been reported [83,84]. Antimicrobial chemicals in nectar has also been hypothesized to encourage specialist pollinators, deter nectar robbers, and alter pollinator behavior [79,85–87].

TEXT BOX 2. The nectar microbiome

Evidence indicates that floral nectar is initially sterile, but rapidly colonized by microorganisms after anthesis [28,88] from various sources, including the air, rain drops, dew, pollen, corolla, and especially the body (generally mouthparts) of flower-visiting animals [28,83,89]. Nectar microbial communities are species-poor relative to, for example, the rhizosphere or the phylloplane, and are often dominated by yeasts of the genus *Metschnikowia* and bacteria of the genus *Acinetobacter* [10,22–24,26,27,31,43,90]. Other microbes that are found in nectar include yeast species of the genera *Candida*, *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces*, and bacteria such as *Asaia*, *Erwinia*, *Neokomagataea*, *Pantoea*, *Pseudomonas*, and *Rosenbergiella* (for a detailed list, see [90]). Some of these other species may be opportunistic (i.e. not adapted to the nectar environment) and generally occur in lower frequency than *Metschnikowia* and *Acinetobacter* [76,83,90].

In addition to the filtering effect of the physical and chemical characteristics of nectar (which may be variable even within the same plant [39]) on each microbial species, dispersal limitation [27,43] and microbe-microbe interactions can also determine the species composition of the nectar microbiome. Microbial dispersal and interactions are affected by a variety of factors, including the plant's phenology, the density, longevity, sex, and spatial distribution of flowers, and the activity of legitimate and non-legitimate floral visitors [27,36,91,92]. Nectar secretion patterns may also affect the assembly of the nectar microbiome by providing new nutrients to the microorganisms. All these factors depend to some degree on the abiotic conditions (temperature, water availability, photoperiod, etc., even at microscales). Although individual flowers are ephemeral, the collection of flowers on a plant functions as a microbial metacommunity that lasts longer than individual flowers while

525 the plant is blooming [27,35,91]. Outside of the flowering season, flower-visiting animals
526 may act as reservoirs of nectar microbes [93].

GLOSSARY

Antibiosis: interaction between organisms in which at least one of them is adversely affected by the release of metabolites or cell components from the other.

Bacterial farming: mutualistic association established between bacteria and other organisms (e.g. fungi or social amoeba) in which the bacteria benefit through dispersal and rearing, while the other partner benefits from the harvesting of an additional carbon source and, in some cases, increased stress resistance (e.g. in some filamentous fungi [56]).

Bacteriocin: antibacterial peptide or protein produced by some bacteria that either kills or inhibits the growth of other bacteria.

Chelator: small molecule that binds tightly to metal ions.

Cross-feeding: interactions involving the exchange of metabolites or cofactors between organisms. These interactions can vary in the degree of reciprocity (from completely unidirectional to bidirectional) and cost-benefit balance for the interacting partners.

Endosymbiotic: living within the body or cells of another organism in a mutualistic relationship.

Horizontal gene transfer: sharing of genetic material between organisms that are not in a parent–offspring relationship and may even be members of different species.

Mycophagy: literally "feeding on fungus" and synonymous with "fungivory." Bacterial mycophagy refers to the ability of bacteria to grow at the expense of living fungal cells and/or hyphae.

Osmotic pressure: pressure difference needed to stop the flow of solvents across a semipermeable membrane. It can also be defined as the tendency of solvent molecules to move in the direction of lower solvent activity.

Phylloplane: surface of a leaf considered as a habitat, generally for microorganisms.

Priority effects: effects that the arrival order and initial abundance of species have on the development of assembling communities at a local site (e.g. a flower). These effects of community assembly history occur when species influence one another differently (through resource competition, cross-feeding, and other types of local interactions), depending on arrival order and initial abundance.

Prophage: bacteriophage genome integrated into the genome of a host cell.

Quorum sensing: process of cell-to-cell communication that allows microorganisms (typically bacteria) to share information about cell density and adjust gene expression accordingly. This sharing of information is achieved through the production and release of chemical signal molecules called autoinducers that increase in concentration as a function of cell density.

Rhizosphere: thin soil layer around roots that is directly influenced by root secretions and associated soil microorganisms.

Semiochemical: chemical substance that conveys a signal from one organism to another, of the same or a different species, and frequently modifies the behavior of the recipient organism.

Syntrophy: relationship between the individuals of different species in which one or both benefit nutritionally from the presence of the other. The classical concept of syntrophy refers to the close associations established between microorganisms under anoxic conditions and energy constraints to degrade complex organic compounds, where one of the partners keeps intermediate products (e.g. hydrogen) at low concentrations by active consumption, facilitating further degradation by the other partner. However, other "non-classical" types of syntrophy have also been described [96].

574 **Transposase:** enzyme that binds to the end of a transposon (i.e. DNA sequence that can
575 change its position within a genome) and catalyzes its movement to another part of the
576 genome.