Trends in Plant Science

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REVIEWS

Opinion

Yeast–Bacterium Interactions: The Next Frontier in Nectar Research

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Beyond its role as a reward for pollinators, floral nectar also provides a 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.

Highlights

Floral nectar is inhabited by specialized and opportunistic yeasts and bacteria that can alter the mutualistic relationships between plants and animals.

Most previous studies in nectar microbiology have focused on either yeasts or bacteria without consideration of yeast–bacterium interactions.

Potential mechanisms of yeast–bacterium interactions in floral nectar include the formation of physical complexes, nutritional interactions, antibiosis, signaling-based interactions, and horizontal gene transfer.

The combined action of these mechanisms might have unexpected consequences for the host plants and their floral visitors.

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** (see Glossary) 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. Through 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.

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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].

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 *Metschnikowia reukaufii*, and *Leuconostoc* sp. with *M. reukaufii* [22]. Co-occurrence might be facilitated by resource partitioning between yeasts and bacteria in nectar. For example, *Metschnikowia* spp. and the nectar acinetobacters *Acinetobacter nectaris* and *Acinetobacter 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). Similar

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 they 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 nonlegitimate 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 the plant is blooming [27,35,91]. Outside of the flowering season, flower-visiting animals 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

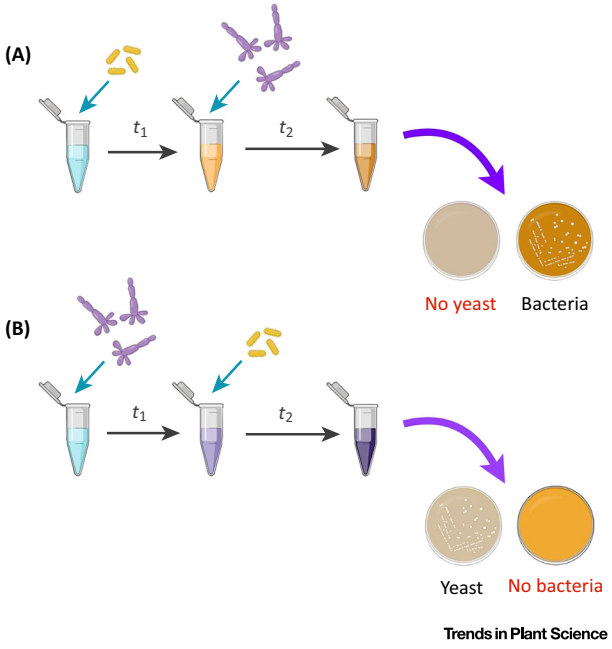

Figure 1. Typical Setting of a Microcosm Experiment to Test for Priority Effects between Nectar Microorganisms [34,94,95]. Sequential microbial dispersal events to flowers is mimicked using plastic microtubes loaded with sterile synthetic nectar (or, alternatively, filtered natural nectar). In the example 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).

priority effects were found between *M. reukaufii* and the acetic acid 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 wherein 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 common) 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 contrasting effects 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.

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].

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

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 that, 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 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).

Bacteria not only attach to fungal cells but also can 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

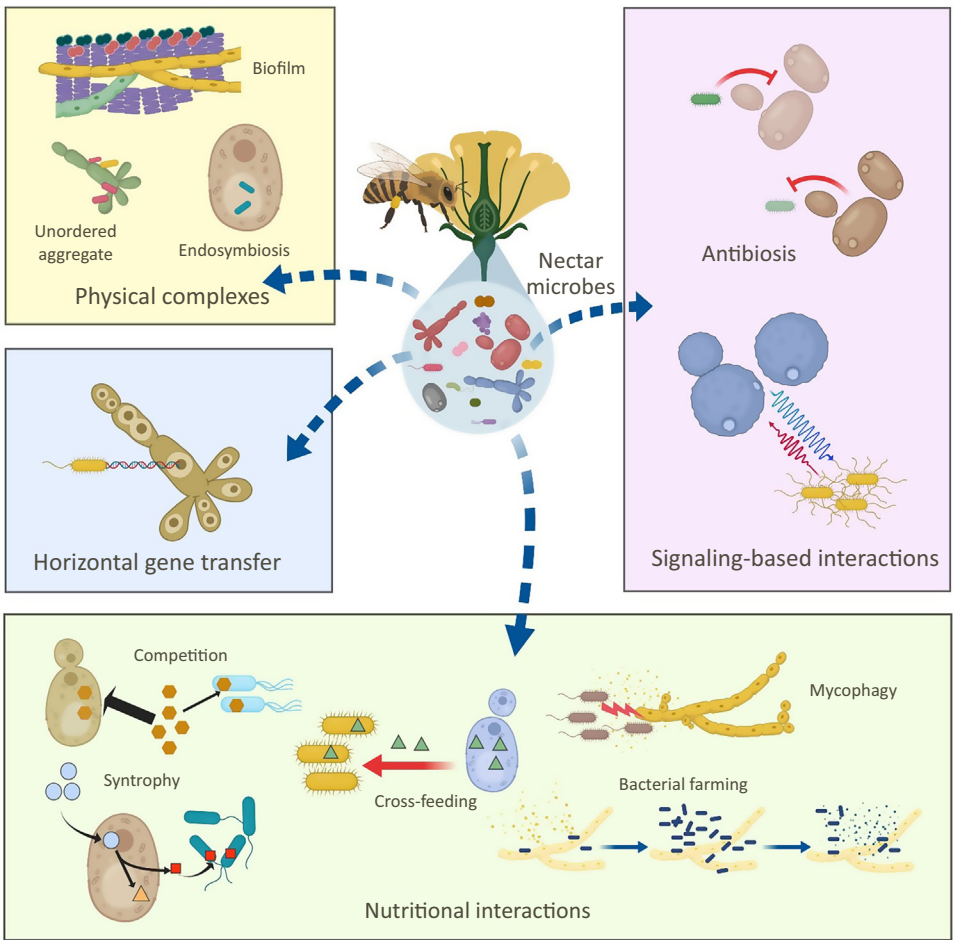

Figure 2. Overview of Potential Mechanisms of Yeast–Bacterium Interactions. This opinion article considers the following potential mechanisms of yeast–bacterium interactions: (i) formation of physical complexes, (ii) antibiosis and signaling-based interactions, (iii) nutritional interactions, and (iv) horizontal gene transfer. Figure created with BioRender (https://biorender.io).

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 modeling 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 it 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]. In addition, it has been demonstrated that *Acinetobacter baylyi*, which is also found in floral nectar ([10]; S. Álvarez-Pérez *et al.*, unpublished), 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 coexist for a short time [72]. Furthermore, other nectar bacteria such as *Pseudomonas* spp. and acetic acid bacteria have a complex history of genome evolution that 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 specialists. 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 Outstanding Questions for some examples) 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.

Outstanding Questions

Is the result of yeast–bacterium interactions between nectar microbes predictable? Which biotic and abiotic factors can alter the outcome of yeast–bacterium interactions?

Do nectar microbes have mechanisms by which they are effectively vectored by insects from flower to flower? Are there any differences between yeasts and bacteria in this regard?

What role have yeast–bacterium interactions played in the evolution of nectar microbes and plant–animal interactions, particularly pollinators?

Do some plants respond adaptively to their nectar microbiota? If so, which mechanisms do they use to select for specific microbial species? Do these mechanisms involve modification of yeast–bacterium interactions?

How can a better understanding of yeast–bacterium interactions in nectar be used to improve pollination and pest control of economically important plants?

Acknowledgments

S.A-P. acknowledges funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant 742964. This work was also supported by the US National Science Foundation (1737758). We thank the editor and reviewers for the constructive comments that contributed to improve our initial manuscript. S.A-P. thanks the members of Fukami lab, Kaoru Tsuji, Marion Donald, and Rachel Vannette for their hospitality and Clara de Vega and Pilar Pérez for their kindness and continuous encouragement.

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