Chapter 14
Anthosphere Microbiome and Their Associated Interactions at the Aromatic Interface

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Abstract The American quote "snug as a bug in a rug" (which means very comfortable and everyone has their own tastes) fits perfectly for the relation between plant and microbes with their associated interactions. Microbes interact at anthosphere, caulosphere, carposphere, phyllophane, rhizosphere, and spermosphere regions of the plants, and the plant-microbe interface acts as a medium of communication between these two diversified living systems. The interface is influenced by an extensive variety of biotic and abiotic determinants responsible for shaping plant-associated habitats, considerably modifying the active composition of the microbial communities, which alter themselves according to the environment for beneficial interactions. The microbiome of root and leaf interactions is most studied as evident from the availability of humongous literature; however, even a small microhabitat such as the anthosphere has its own group of associated microbes obtained from autochthonous or allochthonous. In addition, these microhabitats are contiguous with mutualistic pollinators, florivores, and nectar robbers, which alter the dynamic microbial inhabitants of these aromatic interfaces. To attain sustainability in plant conservation, food, and agriculture, an in-depth understanding of the entire plant-microbe environment is crucial. This chapter was written to provide an overview of the different interfaces, in particular, the anthosphere region of the phyllosphere.

14.1 Introduction to the Plant-Microbe Interface

The emergence, structural formation, and development of biological systems depend on complex signal exchanges between the systems over space and time (Adam et al. 2018). Plant-microbe interface (PMI) is a point where the two diversified living systems, plants and microbes, meet or more specifically the dynamic environment in

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which plants and microbes interact. A detailed signal exchange exists between the microbe and plant even before they engage in any physical contact, and PMI acts as a medium of long-distance chemical communication. The knowledge on inherent molecular, chemical, and physical processes occurring at the interface has facilitated the understanding of diversified microbe-plant interactions. The interface is considerable because microorganisms have evolved over time for possible microbial interactions based on the environment (Felestrino et al. 2017). Colonizing microorganisms have obtained most of their housekeeping genes from plants through horizontal gene transfer, and the most promising explanation for this evolution is to form a successful plant-microbe interaction (Kaneko et al. 2010) and hence gaining the importance toward PMI studies. The interface mainly occurs in the rhizosphere (a narrow zone influenced by plant roots), spermosphere (a zone or habitat surrounding the seeds where the soil, germinating seeds, and the microbial communities interact), phyllosphere (the total aerial aboveground plant surfaces, particularly the leaves), and anthosphere (an adjacent zone around the flowers, a subdivision of phyllosphere) (Fürnkranz et al. 2012; Remus-Emsermann and Schlechter 2018).

The interface is influenced by an extensive variety of biotic (Table 14.1) and abiotic (Fig. 14.1) factors responsible for shaping plant-associated habitats, considerably modifying the active composition of the microbial communities, which alter themselves according to the environment for beneficial interactions (Aleklett et al. 2014; Ushio et al. 2015; Schiltz et al. 2015; Santoyo et al. 2017; Bumroongsook 2018). The interfaces may remain disconnected or sometimes interconnected to each other and can also considerably influence the interactions of microbial communities. The below- and aboveground parts of plants create specific habitats for various microbial communities without interference, which was confirmed using a metaproteogenomic approach on the phyllosphere and rhizosphere microbiota of domestic cultivars (Knief et al. 2012). Substantial research has proved that variance in environmental factors across any plant surfaces can affect the distribution of microbial communities around the other plant parts (Aleklett et al. 2014), thereby influencing the plant ecosystem functions under different environmental conditions (Kembel et al. 2014). On the basis of the pertaining studies on these interaction habitats, this chapter aimed to provide an overview of the different interfaces, in particular, the anthosphere region of the phyllosphere.

14.2 Rhizosphere Interface

The immediate thin layer of soil surrounding plant roots is the rhizosphere and rhizoplane (the zone on the root surface) (Shrivastava et al. 2014; Prasad et al. 2015). Microorganisms attach to these rhizo-zones for possible interactions using the special appendages (fimbriae and flagella) and secretions (surface polysaccharides) (Mwajita et al. 2013). This continuum (layers separated by an extremely slim boundary) of rhizosphere and rhizoplane layers is extensively studied because of

Table 14.1 Biotic factors governing the plant-microbe interactions at the plant-microbe interface

S. no.	Interface	Role player	Factors involved	References
1.	Rhizosphere	Plant	Root length/density/depth, root exudates, foliar leaching, MAMP (microbe-associated molecular pattern), chemoattractants, carbon sources, defense metabolites, and enzyme secretion	Mommer et al. (2016), Garcia and Kao-Kniffin (2018)
		Microbes	Quorum-sensing-involved biofilm formation, phytohormone biosynthesis, virulence of pathogenic bacteria, production of antimicrobial compounds, microbial effectors, soil, and the rhizosphere microbiome	Gianfreda (2015), Santoyo et al. (2017)
2.	Phyllosphere	Plant	Phenological stage of the plant, plant phenotype and genotype, biochemical secretions, leaf characteristics, leaf food resources, phytohormones, green leaf volatiles, and plant traits	Whipps et al. (2008), Kembel et al. (2014), Hacquard et al. (2017)
		Microbes	PPFM (pink pigmented facultative methylotrops) characteristics, microbial fitness (surfactants and extracellular polysaccharides), metabolic response, phyllosphere, and allochthonous microbiome through insect-, atmosphere-, seed-, or even animal-borne sources	El-Gawad et al. (2015), Remus-Emsermann and Schlechter (2018)
3.	Spermosphere	Plant	Seed exudations (nature and composition), seed genotype, seed carbon deposition, germination pattern, seed-borne pathogens, host-dependent microbiome	Schiltz et al. (2015), Chohan et al. (2017)
		Microbes	Chemotaxis, tropic and signal-mediated interactions, evolutionary traits for colonization, and spermosphere-dependent microbiome	Tian et al. (2015), Lemanceau et al. (2017)

its importance in root activity and metabolism. A diversified group of microorganisms co-occurs and multiplies in the rhizosphere where bacteria are abundant (Saharan and Nehra 2011). The rhizosphere interface is pooled with biochemical secretions backed by molecular pattern mechanisms for possible interaction with the soil microorganisms. Root-soil interface is constantly exposed to a vast array of stresses, and the interface responds to these abiotic and biotic stresses by secreting an admixture of root exudates to enhance positive interactions and protect the interface

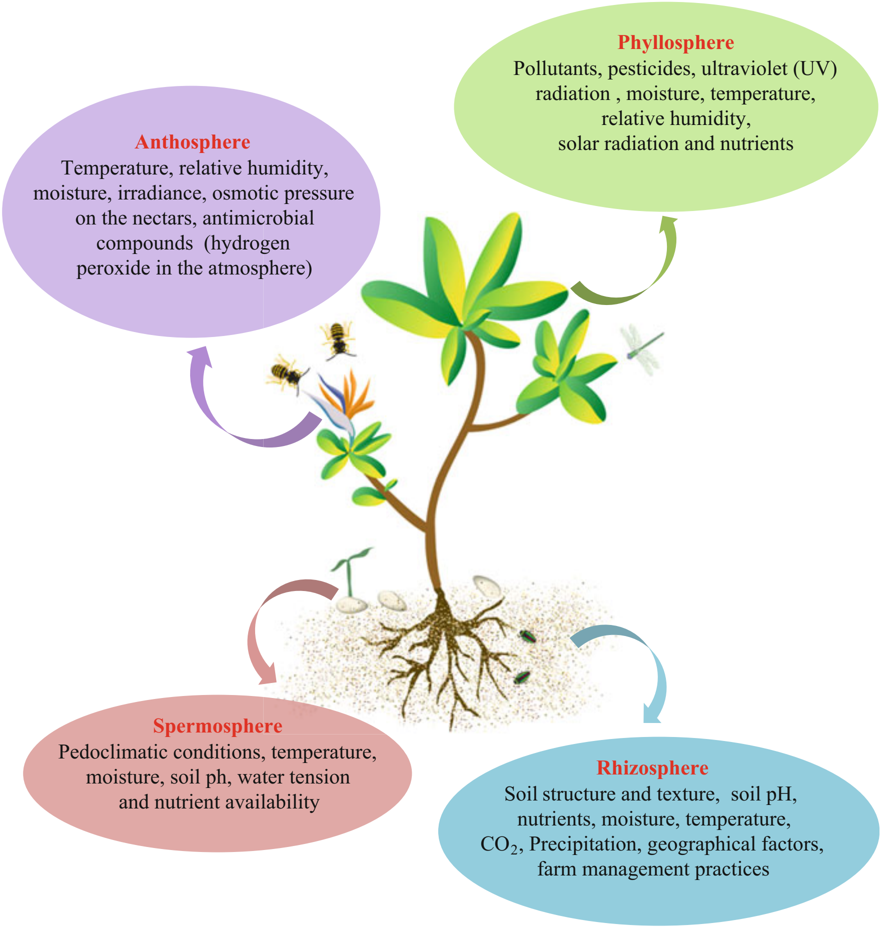

Fig. 14.1 Major abiotic factors affecting plant-microbe interactions at the plant-microbe interface

against harmful negative influences (Badri and Vivanco 2009). In addition, abiotic factors such as light, soil structure or texture, temperature, soil moisture, and soil pH highly modulate root exudation (a biotic factor), which in turn modulates the microbial interactions. For example, neutral soils tend to show a greater microbial diversity by providing an environment for controlled root exudation, whereas acidic soils show lower diversity indices (Rousk et al. 2010). Likewise, the genetic diversity of nitrogen-fixing rhizobacterium is influenced by soil type and other geographical factors as reported by Santoyo et al. (2017). The bioavailability of soil nutrients at the rhizosphere interface has both direct (toxic effects) and indirect (plant exudates) effects on the abundance and diversity of the rhizosphere microbiome by reducing the possibility of interactions (Berendsen et al. 2012).

The biotic factors include the secretion of root exudates, enzymes for metabolism, antimicrobial compounds, phytotoxic chemicals, and processes including molecular plant-pathogen detection at the interface (Field et al. 2006). At the plant-pathogen interface, either at rhizo- or phyllosphere, the first line of defense is the secretion of biochemical compounds (antimicrobial or phytotoxic compounds), failure of which leads to the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). Plants can identify microbe-associated molecular patterns (MAMPs) or PAMPs through the effectors, which are pathogen-specific signatures. Plants maintain a complex genetic system for recognizing effectors (flagella proteins, elongation factor Tu, peptidoglycan, and lipopolysaccharides) as signals of invasion leading to PTI either at the interface or initial interaction (Newman et al. 2013). However, in case of positive beneficial interactions, microbial effectors form a biotrophic interface complex, which acts as suppressors of plant defense machinery (Pellegrin et al. 2015). In addition, a combined effect of living and nonliving factors is reported; for example, the biomolecules and clay minerals form natural nanoprecipitates at the soil-root interface, acting as an active microsite for favorable root-microbe interactions (Violante and Caporale 2015).

14.3 Spermosphere Interface

The spermosphere represents the soil adjacent to a germinating seed: a habitat with a short span but a microbiologically dynamic and rapidly changing zone. When the germination starts, various carbon compounds are released as seed exudations (fatty acids, carbohydrates, amino acids, and organic acids) into the soil. These exudations alter and control the microbial activities that occur in the spermosphere interface, and also these microbial interactions can continue for a short time or the whole life cycle of the plant (Nelson 2004). This interconnection may occur during the seed development in the fruit, dormancy, or germination, and these interrelations are rather planted species specific and microorganism specific. These interrelations may be casual and nonspecific, but in most cases, they are beneficial (root nodulation) or pathogenic (seed-borne fungal diseases) (Chohan et al. 2017). The dynamic associations between plant-microbe interactions around a germinating seed are governed by certain extrinsic (temperature, moisture, and biotic habitat of the soil) and intrinsic factors (plant genotype and phenotype) prevailing at the interface (Simon et al. 2001). Schiltz et al. (2015) reported that the nature and quantity of the seed exudates are dependent on the plant species and abiotic factors such as soil pH, temperature, and type, thereby indirectly influencing the microbial community of the spermosphere.

Microbial attachment to seeds is mainly through biofilm formation or by bacterial adhesins and is regulated by quorum sensing (Tian et al. 2015). Biofilm formations by the colonizers provide resistance to various antimicrobial compounds produced by the seed during germination (Nelson 2004). In the spermosphere, the hydration state of the seed during germination acts as a leading factor behind plant-microbe interactions. In addition, seed carbon deposition and germination of dicot (epigeal germination) and monocot seeds (i.e., hypogeal germination) influence the microbial behavior in the seed habitat (Lemanceau et al. 2017). Only microorganisms with specific traits (trophic and signal-mediated interactions) can succeed in colonizing germinating seeds because of the high competition prevailing within the spermosphere for resources and space. These competitive trophic interactions include the judicious consumption of available resources, chemotaxis motility toward seed exudates (amino acids and organics acids), and versatile metabolic potential to colonize and sustain spermosphere competence (Barret et al. 2015); spermosphere bacterial taxa including *Pseudomonas*, *Bacillus*, and *Rhizobium* with specific evolutionary traits for colonization were found to be abundant in the spermosphere during germination (Lemanceau et al. 2017). Hence, the biochemical secretions of the seeds triggered by their internal genomic and external environmental factors coupled with the microbial evolutionary characters at the interface confirm the nature and survival of the interactions (Truyens et al. 2015).

14.4 Phyllosphere Interface

The total aerial aboveground plant surfaces or phyllosphere is an ubiquitous environment for harboring diversified microscopic living organisms. In agricultural and native plants, phyllosphere is dominated by the bacterial members of the phyla *Firmicutes*, *Proteobacteria*, and *Actinobacteria* (Williams and Marco 2014). The quantitative microbial proposition of each taxon may vary depending on the geographical location, plant phenotype and genotype, human intervention, and seasonal variation (Maignien et al. 2014), which make the phyllosphere interface crucial for qualitative and quantitative plant-microbe interactions. Lettuce plants grown indoors confirmed that the leaf microbiota of laboratory-grown plants is distinct and low compared with that of field-grown plants (Williams and Marco 2014). In addition, similar studies on indoor-grown plants have reported low cell numbers with little diversity under laboratory conditions, confirming the intervention of natural factors before and after interaction (Reisberg et al. 2012). Recent genomics and metabolomics studies have characterized the interaction and survival of phyllosphere microbial communities with regard to the ecological, utilitarian, and structural properties of host plants and environment properties, such as changing climate dynamics and composition of trace gases in the surrounding atmosphere (Bringel and Couée 2015). Functional plant traits such as plant stature, leaf dry matter content, leaf mass per area, height and diameter, wood density, relative growth, and mortality rate have a functional role in plant-microbe interactions (Kembel et al. 2014).

Green leaf volatiles, that is, small organic compounds such as methane to higher complex compounds (fatty acid derivative and sesquiterpenes), are formed at the interface for the recruitment of microbes (Matsui 2006). Pink-pigmented facultative methylotrophic bacteria are capable of growing on small carbon compounds such as formate, formaldehyde, and methanol, which are constantly available on the leaf surface, which in turn renders systemic resistance against diseases, produce plant growth regulators, and offer drought resistance to young leaves (El-Gawad et al. 2015). Field and greenhouse experiments performed by plant pathologists confirmed that fungal species found on a leaf, such as *Cladosporium*, are potential antagonist against *Septoria nodorum*, *Alternaria zinniae*, *Cochliobolus sativus*, and *Botrytis cinerea* (Rodríguez et al. 2001). Certain green leaf volatile organic compounds (VOCs) have growth-inhibiting effects on some microbial strains; however, leaf-colonizing bacteria have developed adaptation mechanisms for their survival. Likewise, some coniferous species prevent the establishment of airborne bacteria on needles through volatile compounds, and the denseness of microbes in the surrounding atmosphere of the conifer stands was considerably reduced by VOCs in addition to some epiphytic communities (Gao et al. 2005). Similarly, in order to colonize the plant internal tissues, plant growth-promoting bacterial endophytes have developed a complex genome compared with other microbes; however, the molecular basis of endophytic microbes to overcome the plant defense is still not well understood, but it is well confirmed that the sensing of MAMPs by pattern recognition receptors in plants control the endophytic load on the leaves (Hacquard et al. 2017).

14.5 Anthosphere Interface

The aboveground portions of phyllosphere include the carposphere (fruits), anthosphere (flowers), phyllophane (surface of leaves), and caulosphere (stems), which withhold several peculiar microbial life interactions. In particular, the anthosphere region around flowers is colonized by a vast diversity of microorganisms, which are flower specific; however, some members of the genera *Pseudomonas* and *Acinetobacter* (*Proteobacteria*), *Metschnikowia* (*Ascomycota*), and *Cryptococcus* (*Basidiomycota*) are consistent members of the floral microbiome across many agricultural and ornamental plants. Another most notable feature of this interface is the permanence; this habitat has a shorter life span when compared with other spheres. The studies on apple floral microbiome confirmed the presence of fast-growing bacterial communities (Shade et al. 2013). Novel studies on flower-associated microbial communities have highlighted that the fungal population is the highest in the anthosphere, followed by bacteria (Ushio et al. 2015). The floral components, namely, pollen, nectar, sepals, petals, stamens, style, ovary, and stigmas, act as short-span microsites for the colonization of microorganisms (Fridman et al. 2012). As flowers are dependent on other biotic and abiotic factors to assist in pollination (for fertilization), seed dispersal, and germination, they have every opportunity for inhabitation by harmful, beneficial, and commensal microorganisms. The horizontal transmission of the floral microbiome occurs through the wind in case of wind-pollinated species and mostly through the frequent pollinators in non-wind-pollinated plants (Frank et al. 2017). In addition, anthosphere plays an important role in the biographic interactions among other interfaces (Huisman et al. 2015); hence, the knowledge on possible environmental factors, biochemical compounds, and molecular patterns formed at this interface is of a great importance.

14.5.1 Anthosphere Microbiome and Their Composition

Advanced sequencing techniques offer in-depth knowledge on the composition and diversity of microbial communities in the anthosphere, and these techniques circumvent both laboratory-grown and non-laboratory-grown bacterial species and provide accurate identification up to the genera level (Samuni-Blank et al. 2014). Flowers are always considered whole structures; however, the interactions at this interface are also more organ-specific because the microbiome diversity varies with the floral components. For example, studies on culturable yeast species across different floral parts (nectar, pollen, and inner and outer corollae) found that the floral surface organs were abundant with basidiomycetous yeasts, whereas nectar and pollen were filled with ascomycetous yeast species and nectar was found to host most fungi compared with other floral parts (Pozo et al. 2012), indicating a far down intense separation in the microbial world within the flower.

In pollen, the cultivable bacterial count is abundant, ranging between 10^6 and 10^9, and the diversity and composition vary from species to species because of the difference in nutrient composition, pollen viability, pollen structure, pollen coat antimicrobial peptides, moisture, and the special attachment of the bacterial cells (Frank et al. 2017). The epiphytic bacteria in the anthosphere exist either single or in clusters with the special formation of thin biofilms in certain habitats. The anthosphere microbiome of various plants at different floral components is listed in Table 14.2. Anthosphere interactions are, however, restricted to epiphytic microbes, but novel and biologically active endophytic microbes with potential sources of useful metabolites were also documented among the floral microbiome. Therapeutic metabolites producing endophytic fungi identified as *Pestalotiopsis disseminate*, *Phomopsis* sp., and *Coelomycete* sp. were reported from *Tripterygium wilfordii* flowers, a traditional Chinese medicinal plant that proves the role of endophytes in this unique interface (Kumar and Hyde 2004).

14.5.2 Abiotic and Biotic Determinants of Microbial Colonization

In any living system, both biotic and abiotic factors work in combination to alter species distributions and abundance (Bumroongsook 2018). Compared with other phyllosphere components, floral surfaces provide some unique conditions, such as elevated levels of humidity and moisture, increased irradiance, low pH and high alcohol concentrations (fermentation of nectar sugars by microbes), osmotic pressure, antimicrobial compounds, and some protection against extreme weather conditions similar to some leaf structures (Aleklett et al. 2014). Flowers are exposed to various abiotic stresses such as seasonal variations, geographical factors, rainfall, temperature, and humidity, which alter the microclimate around the flowers (Vega and Marques 2015), directly affecting the flower longevity, pollen viability, and

Table 14.2 List of anthosphere microorganisms of various domestic and wild plants at different floral components

S. no.	Plant host	Flower part	Microbial group	Most abundant microbes	References
1.	*Epilobium canum*	Nectar	Bacteria	*Neokomagataea* sp.	Rering et al. (2017)
2.	*Mimulus aurantiacus*	Nectar	Bacteria	*Asaia astilbes*	Rering et al. (2017)
3.	*Linaria vulgaris*	Nectar	Yeast	*Metschnikowia reukaufii*	Bartlewicz et al. (2016)
4.	*Linaria vulgaris*	Nectar	Bacteria	*Acinetobacter nectaris*	Bartlewicz et al. (2016)
5.	*Malus domestica*	Flower	Archaea	*Deinococcus* sp.	Shade et al. (2013)
6.	*Atropa baetica*	Nectar	Fungi	*Coniochaeta* sp.	Pozo et al. (2012)
7.	*Digitalis obscura*	Pollen	Yeast	*Metschnikowia* sp.	Pozo et al. (2012)
8.	*Amygdalus communis*	Nectar	Bacteria	*Phaseolibacter* sp.	Aizenberg-Gershtein et al. (2013)
9.	*Phleum* sp.	Pollen	Fungi	*Botrytis* sp.	Heydenreich et al. (2012)
10.	*Pulmonaria officinalis*	Nectar	Bacteria	*Rhodococcus* sp.	Jacquemyn et al. (2013)
11.	*Eugeissona tristis*	Nectar	Fungi	*Trichomonascus* sp.	Wiens et al. (2008)
12.	*Atropa baetica*	Nectar	Fungi	*Coniochaeta* sp.	Pozo et al. (2012)
13.	*Helianthus annuus*	Flower	Fungi	*Sclerotinia sclerotiorum*	Rodríguez et al. (2001)
14.	*Epipactis palustris*	Nectar	Bacteria	*Rosenbergiella nectarea*	Lenaerts et al. (2014)
15.	*Protea subvestita*	Nectar	Bacteria	*Tatumella citrea*	Lenaerts et al. (2014)
16.	*Delphinium nuttallianum*	Nectar	Yeast	*Metschnikowia reukaufii*	Schaeffer and Irwin (2014)
17.	*Silene latifolia*	Nectar	Yeast	*Microbotryum violaceum*	Golonka and Vilgalys (2013)
18.	*Mimulus aurantiacus*	Nectar	Bacteria	*Gluconobacter* sp.	Vannette et al. (2013)
19.	*Nicotiana glauca*	Nectar	Bacteria	*Erwinia amylovora*	Fridman et al. (2012)
20.	*Citrus paradisi*	Nectar	Bacteria	*Acinetobacter gerneri*	Fridman et al. (2012)
21.	*Echium leucophaeum*	Nectar	Yeast	*Cryptococcus carnescens*	Mittelbach et al. (2015)
22.	*Helleborus foetidus*	Nectar	Yeast	*Metschnikowia reukaufii*	Pozo et al. (2014)
23.	*Epilobium canum*	Nectar	Endo yeast	*Aureobasidium pullulans*	Rering et al. (2017)
24.	*Iris xiphium*	Nectar	Bacteria	*Rosenbergiella australoborealis*	Álvarez-Pérez and Herrera (2013)

nectar viscosity in turn indirectly modifying the flower-inhabiting microbes. The studies on urban and rural habitat-grown *Linaria vulgaris* (yellow toadflax-late-flowering herb) suggested that environmental changes related to urbanization (landscapes, pollution, and special isolation) may impact inhabiting yeasts in the floral nectar of plants (Bartlewicz et al. 2016). Likewise, the role of rainfall in indirectly shaping the microbial community is evident. The studies on Hindu lotus and East Indian lotus flowers revealed that the thrips population was more abundant in summer than in the rainy season as insect vectors play a major role in the microbiome alteration of the flowers (Bumroongsook 2018). The specific interaction studies on the flowers of the *Rosaceae* family with the blight pathogen, *Erwinia amylovora*, confirmed that a specific combination of relative humidity and temperature on flower surfaces can exert extremely strong specific pressure on the flower microbiome (Aleklett et al. 2014).

The microbial model system studies on bacterial communities confirmed that the floral microbes are shaped not only by the abiotic factors in the flower niche but also by the plant genotype and phenotype, nectar allure, pollen surface structure, volatile and nonvolatile organic compounds, microbe-microbe interactions, temporal dynamics of flower communities, visiting pollinators, and insect vectors (Samuni-Blank et al. 2014). The high levels of hydrogen peroxide generated by the nectar proteins (nectarins) and the saponins, alkaloids, terpenoids, and phenolics of the flower keep the colonizing microbes under control (Kessler and Baldwin 2007). In addition, it has been suggested that pollen odors provide defense against pathogens (Basim et al. 2006). The greenhouse and field experiments performed on the fungal pathogens of *Helianthus annuus* (sunflower) anthosphere have confirmed that pollens reduce injury by pathogens, increase the colonization of beneficial microbes, and provide additional protection from pathogens because of competition toward limited anthosphere resource (Rodríguez et al. 2001). In addition, biochemical studies showed a marked difference between the secondary metabolite profiles of the anthosphere and phyllosphere through diverse biochemical pathways such as aliphatics, aromatics, and terpenoids; the flowers maintain distinct organic metabolites in their environment (Knudsen et al. 2006). Nonvolatile metabolites such as proteins, alkaloids and phenolics, and volatile organic compounds such as phenylacetonitrile, 2-phenylethyl alcohol, and sesquiterpene synthesized by the flowers vary among species and even among the flowers of the same plant. Most of these volatile and nonvolatile compounds have specific antimicrobial properties through which the recruitment of microorganisms is thoroughly filtered, and they also offer protection to the nectar-inhabiting indigenous microbes (Junker and Tholl 2013).

14.5.3 *Plant-Microbe-Pollinator Triangle*

Nonpollinating floral herbivores (florivores) and pollinators frequently visit the flowers (de Vega and Herrera 2013), and these visitors carry their own internal

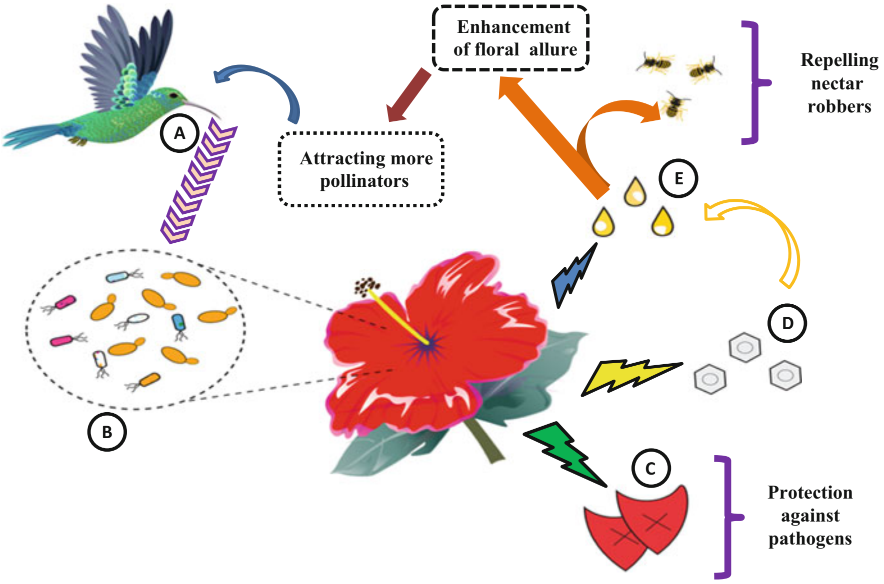

Fig. 14.2 Role of pollinator-transferred microbes in altering the anthosphere; (**a**) pollinators carrying microbes to the flower, (**b**) altering the anthosphere microbiome, (**c**) secretion of antimicrobial compounds, (**d**) altering the secondary metabolites, and (**e**) changing the nectar composition and aroma

and external surface microbiota that could be shared with the anthosphere (Jacquemyn et al. 2013). The microbial load varies with the pollinators and the flowers they visit. The studies through direct visualization and microbial fingerprint approach on different insect pollinators, such as *Bombus ardens ardens* (bumblebees), *Xylocopa appendiculata circumvolans* (carpenter bees), and *Apis cerana japonica* (honeybees), under field conditions suggested that an average of 12.2×10^5 microbial cells were harbored by individual insects on its surface (Ushio et al. 2015). Reports are available showing that, between flowers, the transport of yeast is facilitated by the pollinators (Belisle et al. 2012) and the diversified yeast speciation and their composition in the nectar are because of the outcome of these diversified vectors. Herrera et al. (2009) highlighted the association between *Zygosaccharomyces* and *Debaryomyces* yeasts in plants and bumblebee mutualism (Brysch-Herzberg 2004). In addition, after colonization, microbial communities play their role in affecting the quality and composition of the nectar, which alters nectar attractiveness to active pollinators (Fig. 14.2); hence, the reproductive success of the flower is directly affected by the floral microbiota (de Vega and Herrera 2013).

In several plant species, flower-insect-yeast interactions have been observed, and insect-mediated microbial dispersal and alteration in the microbiome composition of the anthosphere were inseparable. Nectar-inhabiting microorganisms such as the yeast *Metschnikowia reukaufii* sourced by pollinators produce volatile compounds, which differentially affect the preference of honey bee as reported by Rering et al. (2017). A study involving the shrub *Mimulus aurantiacus* and the nectar yeast, *Metschnikowia reukaufii*, confirmed that the nectar attractiveness (for the pollinator humming bird) was enhanced because of the mutualism, whereas the same plant with bacterial genus *Gluconobacter* sp. decreased the nectar attractiveness by altering the sugar composition (Aleklett et al. 2014). The various volatile and nonvolatile metabolites of the flowers either attract or repel the pollinators, thus preventing the entry of pathogens from infected vectors (Junker and Tholl 2013). At present, researchers have started using the help of pollinators to dispense microbial biocontrol agents for sustainable agriculture production. Recently, entomovectoring (the practice of using bees to spread microbial biocontrol agents) is gaining momentum to reduce the use of harmful fungicides. These new techniques can be successful only by maintaining an attractive interface for the pollinators and their associated microbes (Menzler-Hokkanen and Hokkanen 2017). Likewise, insect species-specific microbes can be transferred from the insect body surface to a floral surface, and these insect-specific microbes can act as a fingerprint of the specified insect, particularly for large-bodied insects (Ushio et al. 2015).

14.6 Interactions Among the Interfaces

The interfaces are connected internally through a series of pathways in the plant life cycle, for example, through foliar leaching, residue decomposition, volatilization, and debris incorporation, the phyllosphere alter the rhizosphere habitat leading to changes in the microbial diversity. Through the floral pathway, the microorganisms are transmitted to the seed compartments and seed coats, which in turn enter the spermosphere region and then to the surrounding soil environment (Singh and Mathur 2004). Evidence suggests that the plant-soil interface is often the preferable site for horizontal gene transfer processes from plant to soil microbes as a result of plant biomass decomposition (Heuer and Smalla 2012). Likewise, the rhizosphere microbes also find their way through dust, water droplets, and agricultural equipment into the other interfaces for possible colonization. For example, *Verticillium dahliae*, a broad host-fungal pathogen, infects the flowers of agricultural crops but always maintains its local reservoir inoculums in spinach seed stock and soil (Maruthachalam et al. 2013). The volatiles phenyl acetonitrile and 2-phenylethyl alcohol emitted by floral structures have strong growth-inhibiting effects on phyllosphere bacterial strains (Junker et al. 2011), proving the interconnection between PMI.

14.7 Inference on the Interface

Our knowledge on the interaction of these microbial communities with the plant kingdom is still insufficient; the hidden process and pathways are still a challenge with an exciting new frontier. The knowledge of interpreting these unanswered questions will help to attain sustainability in various fields such as agriculture, genetic conservation, food safety and security, and the development of genetically modified organisms. The PMI is a region of discrete transience with a mixture of various microbial consortia; in addition, the investigation of these models will provide insights into the general ecology and evolution of various species. However, the archaeal and viral communities of the interface should be classified for their possible role in the interactions; likewise, the role of florivores (flower-feeding nonpollinating herbivores) in the flower microbiota is still unclear and needs elaborate research.

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