1

Agricultural Microbiomes

Functional and Mechanistic Aspects

CONTENTS

Microbiomics and Sustainable Crop Production, First Edition. Mohammad Yaseen Mir and Saima Hamid.

1.1 Introduction

In recent years, the microbial environment has gotten a lot of attention because lower sequencing costs have allowed for more in-depth study of the structure and dynamics of host-associated microbiota. It is widely acknowledged that microbes have immense ability to improve host well-being in both humans and plants. Targeted application of beneficial microbial cocktails can be a sustainable way to mitigate biotic and abiotic stress conditions and maintain yield stability in the potential vision of precision agriculture. Many beneficial microbes, on the other hand, have similar pathogenic relatives, and it is unknown how the plant immune system distinguishes between pathogenic and beneficial microbes in order to combat infection by the former and promote colonization by the latter. It is possible that even the earliest eukaryotes were overwhelmed by a variety of prokaryotes, and that eukaryotic immune systems developed to distinguish between beneficial and pathogenic bacteria. As a result, a deep and complex interaction between microbes and hosts is predicted, affecting every aspect of eukaryote biology. Traditional as well as systems biological 'omics' and computational modelling methods would be needed to understand microbe–host interactions.

1.2 Model Microbiome–Plant Systems

Plant microbiome researchers must design new model systems as well as draw on existing systems to integrate microorganism populations as an emerging group. To create culture collections, large-scale microbial isolation activities and genome sequencing programmes will be required, as will concerted group efforts to build a set of uniform protocols and growth platforms. A small flowering angiosperm in the mustard family, *Arabidopsis thaliana*, is an example of a popular, albeit non-agricultural, model for plant microbiome study. The *Arabidopsis* scheme, however, has shortcomings, including a lack of symbiotic relationships with nodulating nitrogen-fixers and mycorrhizal fungi, as well as genomic and phenotypic variations from essential monocot crops. As a result, there would be a need for multiple model systems. To figure out which processes can transfer to crops at different evolutionary distances, a collection of model plant species is needed [1]. The legumes *Medicago* [2], *Populus* [3], rice [4], *Sorghum* [5], *Miscanthus* [6], maize [7], and tomato [8] have all

made progress towards the development of model host–microbiome systems. Many of these model organisms have completely sequenced genomes and burgeoning scientific populations, allowing them to be used in more microbiome studies. The complete maturation of model systems necessitates coordinated initiatives to create public services, such as repositories and curated databases for sequenced culture collections of related microbiota. A multidisciplinary group of academic and commercial plant microbiome scientists, with funding from funding agencies, is needed to develop successful model systems for elucidating plant–microbiome interactions with full interoperability – a key move towards building a knowledge base for long-term high-yielding agriculture innovation.

1.2.1 Plant Perception of Microbes

Pathogens and endophytes must first resolve systemic obstacles such as cell walls [9], waxy epidermal cuticles [10], and constitutive antimicrobial compounds like phytoanticipins. The evolutionary proximity of beneficials and pathogens can be explained in part by this common criterion. Plant surface receptors called pattern-recognition receptors (PRRs) detect the presence of microbes close to the cell membrane [11]. As per findings of Boller and Felix [12] and Macho and Zipfel [13], in the defence mechanism of pathogen- or microbe-triggered immunity (PTI/MTI), the intracellular signalling helps in culmination of conserved pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs); for example bacterial flagellin or elongation factor thermo unstable (EF-Tu) as MTI mechanism embrace production of reactive oxygen species (ROS), nitrogen oxide species (NO_x), nutrient allocation, metabolite release against microbial protection, and release signalling molecules for defence purposes and other functions, including transcriptional modifications. Beneficials and pathogens, on the other hand, have common, if not identical, molecular patterns, making separation by unique PRRs impossible. FLS2, which recognizes flg22, the most conserved motif in bacterial flagellin, is one of the most important models for studying PRR function [14]. BAK1 is a coreceptor that FLS2 has to activate downstream signalling. BAK1 is also a coreceptor for BRI1, a leucine-rich repeat receptor kinase (LRR-RK) that detects plant brassinosteroids (BR) and serves as an integrator between defence and growth signalling [15]. Certain portions of the protein are recognized by other receptors. Tomato can sense flgll-28 in an FLS2-independent manner by FLS3 [16], and the rice pathogen *Acidovorax avenae* has a separate flagellin motif, CD2-1, whose receptor is still unknown [17]. Interestingly, some *A. avenae* strains are resistant to flagellin glycosylation detection [18]. In comparison to viruses, certain beneficials have epitopes that resist detection by either or both receptors [19]. Apart from MAMP-masking or avoidance processes, certain beneficials are possibly identified by their flagellin and inhibit full-fledged immune responses

by yet unexplained mechanisms. Most genes caused by experience of distilled flg22 in *Arabidopsis* were downregulated in response to colonization by the commensal *Rhizobium* sp. 129E [20]. According to their findings, this commensal is capable of interfering with MAMP-induced transcriptional responses through alternative pathways. Since this *Rhizobium* strain lacks the type III secretion system (T3SS) or Nod factor biosynthesis genes, signalling from other heteromeric PRR complexes is likely to play a role.

1.2.2 Molecular Plant

Interactions between symbionts and plants point to pathways that underpin the distinction between friend and foe. When arbuscular mycorrhizal fungi (AMF) and rhizobia come into contact for the first time, they cause temporary defence-like responses that are easily suppressed [21]. Signalling by the Myc and Nod factors is thought to be essential in this repression [22]. Both symbiotic signals are characterized by their ability to induce nuclear calcium oscillations through a signalling cascade involving many conserved symbiotic proteins [23]. Lysine motif (LysM) receptors, like kinases (RLK), detect Nod factors in hosts, and it is thought that related receptors exist for Myc factors [24]. Any of these receptors tend to be involved in pathogen identification. OsCERK1 is a LysM-RLK that is needed for the establishment of mycorrhizal root symbiosis as well as resistance to rice blast fungus [25], indicating that it functions as a 'molecular transition' between symbiotic and defence responses. According to Gourion et al. [22] the exact functioning is not clear, although LysM-RLK is found to be of some specific mechanism with frequent occurrence. NFP is a Nod factor receptor found in *Medicago truncatula* that regulates vision and defence against the fungus *Colletotrichum trifolii*, as well as the oomycetes *Aphanomyces euteiches* and *Phytophthora palmivora* [26, 27]. Combinatorial physical interactions between receptors and coreceptors are essential for signal specificity and integration, according to detailed studies of exemplary PRRs and LysM-RLK. In nature, plant roots come into contact with a slew of MAMPs as well as a smorgasbord of signalling molecules. As a result, it is conceivable, if not likely, that a personalized response to complex microbial assemblages is installed through a network of interacting receptors' combinatorial and quantitative perceptions of the various signalling molecules. As a result, PRR signalling would include the use of interconnected global systems approaches. Smakowska-Luzan et al. [28] performed a proteome-scale interactome analysis, which is a significant step towards a complete understanding of this important plant perception mechanism. Also, 225 LRR-RKs (CSILRR) formed a physical cell surface interaction network in *A. thaliana*, which they mapped using biochemical pull-down experiments. CSILRR showed that all LRR-RKs are highly interconnected, clustering into many modules of unknown biological significance.

Importantly, the authors demonstrated that not only direct connections but also indirect network effects modulate downstream signalling performance, and that the entire network contributes to the plant immune system's well-balanced responses. Understanding plant immunity would need a better understanding of the LRR-RK network's automated information processing.

1.2.3 Bacterial Signalling: Quorum Sensing and Symbiosis Factors

Plants use metabolites, volatiles, symbiosis cues, and quorum sensing (QS) molecules to detect bacterial contact in addition to sensing conserved microbial patterns [29]. *N*-Acyl homoserine lactones (AHL) are important components of bacterial contact that plants can detect. This was shown with the beneficial *Acidovorax radicis* N35, where the AHL-producing wild form was able to dampen the barley defence response, while flavonoid defence was unregulated after inoculation with the non-AHL-producing mutant [30]. Other examples demonstrate the growth-promoting and priming effects of AHLs on host plants such as *Medicago*, tomato, *Arabidopsis*, and barley [31].

According to Cha et al. [32] and von Bodman et al. [33], since pathogenic bacteria often manufacture AHL, these signalling substances are unlikely to provide enough information for the plant to modulate its defensive responses on their own. It is possible that the QS molecule compositions and concentrations suggest an unbalanced microbial composition. Although the biochemical effects of AHLs have been studied in depth, the processes and mechanisms by which plants perceive these bacterial molecules are yet to be discovered [34]. Even in plants that do not form symbiosis, lipochitooligosaccharides, i.e. Myc and Nod symbiosis factors, may promote root production, seed germination, and plant growth. As a result, the detection and signalling mechanism for symbiosis factors is partly independent of the host's symbiosis competence. More research is required to understand how the plant interprets the wide range of rhizosphere signals emitted by microorganisms, and how different molecules can interact synergistically or antagonistically to affect plant growth and stress resistance [35].

1.2.4 Hormone Signalling in Microbe–Host Interactions

Signalling by phytohormones is crucial to almost all plant processes. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are the primary mediators of defence responses. JA and ET mediate induced systemic resistance (ISR) and protection against necrotrophs and insects, while SA mediates structural activity relationships (SAR) and defence against biotrophic and hemibiotrophic pathogen invasion [36]. Auxin, gibberellins (GA), BR, or cytokinins (CK) are hormones that primarily regulate developmental processes or abiotic stress

responses (abscisic acid [ABA]). Hormone signalling is heavily integrated, and multiple hormones control every mechanism of concern, beyond these seemingly clean classifications [37]. Phytohormones are therefore essential for bidirectional communication between plants and microbes. Strigolactones are exuded from roots in response to phosphate or nitrogen deficiency in order to attract AM fungi, and their biosynthesis is downregulated after the fungus has colonized [38]. GA, SA, and ET, on the other hand, inhibit both AM and root nodule symbiosis, while auxin and ABA promote AM production in a concentration-dependent manner. For nodule forming, CK and localized auxin signalling are necessary. Depending on the circumstances and plant organisms, the position of JA in symbiosis establishment is uncertain and can be positive, negative, or neutral. Beneficial and pathogenic bacteria also influence the hormone signalling pathway. Coronatine (COR) is a toxin developed by the pathogenic *Pseudomonas syringae* pv. tomato DC3000 (Pst) that is similar to plant JA-isoleucine (JA-Ile) but is more active [39]. The required SA-mediated defences against the hemibiotrophic Pst are suppressed as a result of the activation of JA-dependent defence mechanisms [40]. Pathogens control plant signalling in general to inhibit defensive responses and redirect nutrients to infested tissues for long-term pathogenic colonization [41]. According to Singh et al. [42] and Li et al. [43], presence of beneficial strains including *Pseudomonas fluorescens Pf4*, *Pseudomonas aeruginosa Pag*, or *Bacillus velezensis* LJ02 enhances endogenous SA levels while some strains are proved to show opposed results in *A. thaliana* as well as decreasing JA-Ile levels as reported by Srivastava et al. [44]; however, *Paraburkholderia phytofirmans* PsJN decreases expression of JA-biosynthesis and wound-induced JA accumulation [45]. As a result, phytohormones derived from microbes have a wide range of effects, based on the plant–microbe mix. While various studies show contradictory findings, the SA signalling mechanism appears to be important for shaping the root microbiome. SA mutants had mild effects on microbiome structure, according to one report [46]. On the other hand, according to Lebeis et al. [47] the rhizosphere microbiota of *A. thaliana* mutants lacking SA synthesis or vision was altered, whereas the corresponding JA and ET mutants had no such impact. Many plant-growth promoting rhizobacteria (PGPRs) influence plant development, especially root growth, by producing auxins, GA, or CK, in addition to influencing defences, which is common to pathogens and beneficials. To unravel the underlying complexity, systems biology tools such as metabolomics, global network analysis, hormone profiling, and specialized quantitative modelling of molecular processes in plants and soil will be necessary. Auxin signalling in the plant root is being extensively investigated, and sophisticated models are available [48]. Detailed mechanistic information as well as fluorescent auxin reporters that offer time-resolved data on auxin distribution permitted the construction of

such quantitative models [49]. Quantitative time-resolved models are built on the foundation of both. Although preliminary evidence on the effects of auxin concentrations on receptor pairs is available, quantitative data on the chemicals and receptors that convert a given auxin concentration into specific transcriptional responses are often lacking [50]. A model of the SA signalling system will be useful in studying microbe–host interactions. NPR1, NPR3, and NPR4 are newly discovered SA receptors that work together to modulate responses to various SA concentrations [51]. BOP1 and BOP2, on the other hand, appear to have no role in SA signalling but have been linked to developmental programmes in legumes such as blooming and nodule formation [52]. Simultaneously, the biochemical control of NPR1 and presumably its paralogs is complicated, including several cellular compartments, redox potential, phosphorylation, and degradation. Although critical aspects for model creation such as thermogravimetric analysis (TGA) transcription factors and signalling network components are known [53], our knowledge of this important immune signalling system is still limited. For quantitative modelling of SA signalling, the creation of fluorescent SA sensors as well as quantitative protein level and binding data are crucial. All hormone signalling pathways are linked, and only a few biological reactions are mediated by a single hormone. The integrative research of Tsuda et al. [54] constitutes great efforts in unravelling the interaction of SA, JA, and ET during immunity in *Arabidopsis*. They separated the hormone signalling network into four sectors (SA, JA, ET, and PAD4) and tested immunity in all potential mutants from each sector following stimulation with a panel of MAMPs and effectors. Their findings revealed substantial hormone network component interactions, including additive, synergistic, and compensatory interactions. Later research by the same group led to the conclusion that the PTI signalling network is strongly buffered against interference, such as pathogen effectors [55].

1.2.5 Interactome Network Analysis

Molecular interaction network techniques can be useful in the absence of quantitative dynamic models for identifying modules, routes, components, and system-level patterns of molecular host–microbe interactions [56]. A reference protein network is necessary to situate host–microbe interaction data in the context of host biology. With the publishing of the first experimental map of physical protein–protein interactions among several thousand *Arabidopsis* proteins, plant interactome analysis began:

Arabidopsis Interactome-1 (AI-1) [57] offered a first integrated organizational view of plant molecular connectivity. Since then, numerous specialized and complementary maps have been developed to aid in the investigation of certain processes. Using the split-ubiquitin technique, a map of roughly 12 000 protein–protein

interactions was created for membrane proteins [58]. A G-protein interactome showed a novel role for G-proteins in the regulation of cell-wall modification, a critical defensive mechanism [59]. Interolog mapping was used to create a protein–protein interaction network for the fungus *Phomopsis longicolla*, which causes *Phomopsis* seed damage in soybean [60]. To control plant defence and physiology, pathogens and beneficial microorganisms can release hundreds of (virulence) effector proteins into the cytosol and apoplast of the host plant [12]. To fully appreciate host–microbe interactions, their functions must be comprehended in a holistic and time-resolved manner. Small-scale investigations of plant-targeted pathogen effectors revealed that virulence effectors affect host protein activities to interfere with immune responses and cause illness, a phenomenon known as effector-triggered susceptibility (ETS) [61]. Effector-triggered immunity (ETI) is initiated when a pathogen's effectors are recognized by a host resistance protein (R protein) [61]. A large-scale interactome study (PPIN-1) mapped the interactions of virulence effectors from the bacterial pathogen Pst and the oomycete pathogen *Hyaloperonospora arabidopsidis* with proteins in the AI-1 host network to get a systems-level perspective on effector functions [62]; a follow-up study later added interactions of effectors from the biotrophic ascomycete *Golovinomyces orontii* [63]. The findings demonstrated that pathogen effectors partially converge on common host proteins, many of which are highly linked hubs in the host network. The host proteins showed genetic validation rates ranging from 100% for the most strongly targeted proteins to 40% for the less highly targeted proteins, depending on the degree of convergence. In addition to convergence, numerous effectors targeted proteins throughout the host network, most likely as a result of the immunological signalling network's high buffering [55]. Positive and balanced selection was seen in the immediate network neighbourhood of the highly targeted proteins, according to population genetic analysis. As a result, pathogen-induced selection pressure appears to be absorbed by the network that surrounds the effector targets [63]. These data support the idea that host–microbe interactions are mediated by a complex network that can only be partially comprehended by looking at individual routes. Pathogens appear to alter host networks rather than deconstructing network integrity, according to research on the *Yersinia pestis* interactome [64]. Pathogens are not the only ones that produce effector proteins. Plant immune responses and symbiotic relations can be modulated by mycorrhizal fungi, endophytic fungi, and nitrogen-fixing rhizobia effector proteins [65]. Many PGPRs, such as *Pseudomonas simiae* WCS417, and many proteobacterial strains are anticipated to contain functioning T3SS and effectors in complex microbiome data sets [66]. It is recognized that virulence effectors are necessary for productive and beneficial interactions between the beneficial fungus *Serendipita indica* and rhizobial bacteria. *Bradyrhizobium elkanii* T3SS-delivered effectors even allowed soybean nodulation without the need for

Nod factor [67]. Many proteobacteria contain type IV and type VI secretion systems, which may transfer bacterial protein into hosts and other microorganisms in addition to T3SS. *P. simiae* WCS417 possesses two T6SS loci [66] and may send effectors to both its plant host and other competing microorganisms to alter the microbiota. Proteomic techniques can help researchers better understand the variety of bacteria effector repertoire [68]. In a study comparing the genomes of a beneficial soil fungus, *Colletotrichum tofieldiae*, and a closely related pathogenic counterpart, *Colletotrichum incanum*, researchers discovered that while their secretomes were similar, the beneficial fungus had 50% fewer effector genes and lower activation of pathogenicity-related genes in plants [69]. As a result, microbial secretomes, as well as the quantity and kind of secreted effectors, may serve as a key point of distinction between beneficials and pathogens. Non-pathogenic interactions are most likely influenced by the beneficial effectors' complement. Understanding the global dynamics of effectors targeting different areas of the host network, and how this dynamic connects to ETS and ETI, as well as the systems-level and dynamic variations between pathogen and helpful effectors' secretion, will be a major issue for systems biology. RNA, which is transported to the host through extracellular vesicles, has emerged in recent years as a key communication molecule between hosts and microorganisms (EVs). EVs were initially discovered in mammalian cells and are now found in bacteria, archaea, and eukaryotes. In *Arabidopsis*, small RNA from the fungus *Botrytis cinerea* was shown to target host defence genes [70]. Plant EVs and multivesicular bodies accumulate around plasmodesmata during fungal infections to facilitate callose deposition at infection sites via host-induced gene silencing (HIGS) using dsRNA. Another layer of communication is formed by EVs and their RNA payload, whose relevance is just becoming apparent [71].

1.2.6 Transcriptional Regulatory Networks

Transcriptional profiling is frequently utilized, and the findings of significant research are referenced throughout this article. While comparative transcriptomics is prevalent, causal regulatory networks and co-expression correlation networks are less so. Co-expression networks are based on the idea that time series transcript profiles might reveal causal links between transcripts. Weighted Gene Correlation Network Analysis (WGCNA) is a widely used approach for grouping genes into co-expression modules using hierarchical clustering [72]. Signalling network connections, metabolic pathways, and phenotypic features are all used to compare these modules. Saelens et al. [73] examined 42 alternative clustering, decomposition, biclustering, and iterative network inference approaches in addition to WGCNA. In *Arabidopsis*, these methods were used. Kim et al. [74] used *A. thaliana* and other plants including maize and wheat to study their interactions with microorganisms.

The discovered modules give a preliminary look at genes with similar functions and can aid in the understanding of processes related to infection or commensalism.

1.2.7 Metabolic Exchanges and Nutrient Competition in the Soil

Metabolic exchanges are one of the core concepts of microbiome–host interactions. Plant roots deliver up to 40% of the complex carbons generated by photosynthesis to the rhizosphere, nourishing the microbiome [75]. Fungi and bacteria, on the other hand, aid in the solubilization and absorption of critical elements like phosphorus, nitrogen, and iron by the plant [76]. Plant reprogramming by pathogens via effectors and hormone signalling aims to relocalize nutrients as the metabolism of an individual organism has been studied using genome-scale metabolic modelling, and community-level response modelling is in progress but difficult [77]. Metabolic modelling of prokaryotes is increasingly commonplace [78]; metabolic models for *Arabidopsis*, barley, maize, sorghum, sugarcane, and canola have been developed on the plant side. By comparing metabolic capacities of beneficials and pathogens, the metabolic capacities of beneficials and pathogens may be assessed [79]. *P. syringae* has evolved to be metabolically adapted for a plant-pathogenic lifestyle, according to Mithani et al. [80]. The pathogenic *P. syringae* is metabolically extremely similar to its benign cousin *P. fluorescens* Pf-5, according to a comparison of metabolic networks for nine *Pseudomonas* strains, suggesting that metabolism may not be a crucial differentiating trait. A genome-scale metabolic model for the oomycete *Phytophthora infestans* was recently created, which predicts biochemical processes in various cellular compartments and in the context of the pathogen's stage [81]. To provide a more exact picture of the metabolic changes caused in plant and microbe during colonization, these models will need to be constrained by metabolite levels.

1.2.8 Integrated Multi-omics Modelling

Although reciprocal benefit between plants and their microbiome is evident, and a 'cry for assistance' might recruit bacteria to aid the host, it is unknown how the plant combines microbial identification with nutrient-related signals at this time. Phosphorus is frequently abundant in soil, while plant-absorbable orthophosphate is rare [82]. Castrillo et al. [83] explored the relationship between diet and defence in a stunning multi-omics systems biology exercise. They demonstrated that the plant phosphate starvation response (PSR) plays a crucial role in modifying the root microbiome by combining 16S rRNA sequencing, genome-wide expression analysis, analysis and modelling of SynComs, and functional experiments. When phosphate uptake-deficient and phosphate hyperaccumulating *Arabidopsis* mutants were compared to wild type, they found that they constructed distinct root-associated microbiomes. The transcription factors PHR1, and

probably PHL1, are integrators of PSR and immune responses, as phr1 and phr1; phl1 mutant plants were more resistant to the oomycete and bacterial pathogens. The relationship between PSR and plant immunity appears to be influenced not only by the surrounding microbiota but also by pathogens, bringing new issues concerning the distinctions between helpful and pathogenic bacteria [84].

1.2.9 From Systems Biology to Crop Protection

Understanding crop–microbe connections is becoming easier because of conceptual and molecular advancements in microbe–host biology. McGrann et al. [85] utilized a draught genome assembly to estimate a secretome of roughly 1000 proteins in the developing foliar fungal barley pathogen *Ramularia collocygni*, which causes *Ramularia* leaf spot. They postulated that *R. collocygni* first acts as an endophyte without generating disease symptoms before transitioning to a necrotrophic phase, based on the reduced amount of plant cell-wall-degrading enzymes and the presence of genes associated with chitin recognition avoidance. Systems biological analysis will rely heavily on understanding such dynamics as well as the underlying molecular processes and signals. The host specialization of four *Rhynchosporium* species on grasses was studied in another research [85]. *Rhynchosporia* are hemibiotrophic fungi that invade the intercellular matrix of host plants gradually and without causing symptoms. Six unique effector proteins from *Rhynchosporium commune* were revealed to be important for maintaining the biotrophic growth stage in favour of the necrotrophic destructive stage, giving therapy leads. Beneficial microorganisms' impacts on enhanced biomass and greater tolerance to biotic and abiotic stressors in monocot crops were explored in a groundbreaking study that included multi-omics techniques. Using phenotyping, transcriptomic, molecular, and metabolomic techniques, Fiorilli et al. [86] investigated the three-way interactions between *Xanthomonas translucens*, the protective symbiotic AM fungus, and the host. They proposed a two-step process for conferring *Xanthomonas* resistance to AM-treated wheat: first, the activation of a broad-spectrum defence (BSD) response in the roots and leaves of AM-treated plants, and, second, a switch to pathogen-specific defence (PSD) upon bacterial infection, which ultimately leads to pathogen resistance.

1.3 Stability, Resilience, and Assembly of Agricultural Microbiomes

Knowing the identification and functional features of microorganisms present in crop plant 'core' microbiomes is important, but understanding the processes by which microorganisms assemble into such communities is crucial for any attempt

to control or regulate the agricultural microbiome. The tremendous diversity of plant microbial communities creates a formidable barrier to properly comprehending the ecology of plant-associated microbiomes, and as a result, the relationships between microbiome assembly and plant performance remain little known [87]. As a result, we urge that researchers focus their efforts on developing synthetic microbial communities that can colonize plant organs and remain long enough in natural conditions to provide advantages to the host. What characteristics of a synthetic microbial population make it more likely to colonize plant organs is a fundamental topic. Microbial genes that are consistently represented in core metagenomes – and that are enriched in plant microbiomes when compared to soil – are promising candidates for important activities that boost colonization capacity. Community characteristics such as phylogenetic diversity and species richness may also influence colonization success, and these might be investigated in the lab utilizing gnotobiotic model systems and culture collections [88]. What qualities enable a synthetic community to survive invasion and displacement by plentiful microorganisms in the surrounding environment once it has colonized a plant and what characteristics provide resistance to abiotic stresses, or at the very least the capacity to recover from them, both are questionable. These traits might be the same as or different from the ones that allowed the colonization to begin in the first place. All of these issues might be investigated in the lab using gnotobiotic systems before being tested in greenhouse and outdoor studies. Years of agronomic work refining single-strain distribution via seed coatings, clay particles, and peat have shown that, in addition to genetic features of the microbiome, the technique of microbiome inoculation may play a large role in its effectiveness. Seeding numerous described microbial species into resilient particles for distribution by air, water, soil, or new delivery mechanisms such as insect vectors might be used to develop successful microbiome inoculation tactics for agriculture. However, attempts to increase colonization ability should also guarantee that synthetic communities do not overrun local ecosystems and have a detrimental impact on soil health, nearby plants, or future crops [89]. Finally, what external variables influence a positive synthetic microbiome's success? Synthetic communities may need to be adaptable to changes in host phenotypic features that affect assembly, or they may need to be customized to colonize certain crop species. Similarly, the environment will influence a community's ability to colonize or its resilience. Any attempt to regulate agricultural microbiomes would require an understanding of both abiotic (e.g. temperature, light, acidity, nutrient, and water availability) and biotic (e.g. competition, predation, parasitism, and mutualism) factors within the microbiome elements impacting its assembly [89]. It will be especially challenging to test the impact of biotic factors on microorganisms that cannot be cultivated independently. Strong interactions, for example, can 'link' organisms to other microorganisms (e.g. mycoparasitism).

Network analysis is particularly beneficial for discovering related microorganisms and 'hub' microorganisms, similar to keystone species that interact with a large number of other bacteria and hence have a significant influence on the community's structure and function [90]. Testing the effects of biotic variables on microorganisms that cannot be grown on their own will be particularly difficult. For example, strong interactions can 'link' organisms to other microbes (e.g. mycoparasitism). Network analysis is particularly useful for identifying related microorganisms and 'hub' microorganisms, which are comparable to keystone species in that they interact with a large number of other bacteria and so have a considerable impact on the structure and function of the community [90].

1.4 Core Plant Microbiome and Metagenome

Targeted investigations of agricultural microbiomes in the field, in addition to model systems and culture collections, provide critical fundamental information that can lead to innovation in a variety of ways. First, identifying the 'core' microbiome – the set of microbial species identified in the majority of samples of a given set of plants [91–95] – will aid in identifying plant-associated microorganisms that should be prioritized for future research, inclusion in culture collections, and manipulation studies. Although the plant microbiota is varied, not all of these microorganisms play functionally essential roles in the biology of their hosts. Researchers can filter out transitory connections and focus on stable taxa that have a better chance of impacting host phenotype by defining the core microbiome. In contrast to very deep sequencing of a few plant microbiomes, culture-independent surveys (such as sequencing internal transcribed spacer (ITS) and 16S rRNA amplicons) of large numbers of microbiomes of the same plant species from a variety of environments would improve progress towards this goal and could be followed-up by selective culturing of candidate core microbiota. Second, finding a functional core microbiome in addition to a taxonomically defined core microbiome based on phylogenetic differences is critical since component bacteria are likely to be adaptable. Using metagenomic and metatranscriptomic techniques to uncover shared projected functions that are likely relevant for the collection of plants investigated, the functional core may be found [96]. Extrapolating functional information from phylogenetic marker genes like ITS offers only a limited amount of information while metatranscriptomics, metaproteomics, and metabolomics, on the other hand, show the functional community phenotype, whereas focused metagenomics of functional genes and shotgun metagenomics provide a deeper understanding of community functional potential [97]. We can detect fundamental community functionality and the level of taxonomic functional redundancy using a combination of multi-omic techniques across large sample sizes of plants. Third,

comparing core microbiomes or metagenomes between important plant groups and genotypes of the same plant species may reveal host-driven variations in microbiome assembly. The soil is the most important source of microorganisms that make up the plant microbiome [93], while there have been rare reports of seed-borne vertical transmission [98], and the atmosphere also contributes to above-ground plant microbiota [99]. Plants of various species or genotypes generate a mainly shared core microbiota, derived from the same environmental 'inoculum' at taxonomic levels of family and above. To what extent does the host impact microbiome assembly, and is within-species host genetic variation sufficient for breeding superior microbiome associations? Broad-sense heritability is often found to be between 5 and 7% [7, 100], implying that the potential of microbiome-related variables to react to conventional artificial selection may be restricted. Microbiomes might, however, be altered to boost heredity [101], and focused breeding efforts might be successful if we understand the host molecular basis of microbiome construction. Fourth, comparing the core microbiota of genetically diverse plant groupings may uncover plant genes and functional features that drive microbiome formation. Although plant functional features such as cuticle composition [102], root length and exudates [103, 104], and plant defences (immunity) [105] have been implicated, the methods by which hosts winnow the ambient community to establish their microbiota are not entirely known. Individual microorganisms (e.g. rhizobia, mycorrhizae) may have indirect impacts on later-arriving microorganisms, whereas cuticle and root characteristics should directly impact colonization by a wide variety of microbial species [106]. Furthermore, crop species or cultivars that consistently assemble various microbiomes may be relying on their microbiota to meet a variety of demands, especially if the plants are suited to distinct environmental difficulties [107].

1.5 Interactions Among the Microbes, Environment, and Management

All organisms are inhabited by microorganisms including archaea, bacteria, fungi, and viruses; this microbiota plays a key role in host health and development [104, 108]. The microbiome associated with plants is considered its second genome. It is determinant for plant health, growth, fitness, and consequently productivity [109]. Each environment associated with the plant – rhizosphere, endosphere, and phyllosphere – presents a specific microbial community with specific functions [4].

These culture-independent methods show that plant microbiome can reach densities greater than the number of plant cells and also greater expressed genes than the host cells. Metagenomics analysis using next-generation sequencing

technologies shows that only 5% of bacteria have been cultured by current methods, revealing how many microorganisms and their functions remain unknown [110]. The first step in plant–microbe interaction is microbial recognition of plant exudates in the soil. There is a hypothesis that plants are able to recruit microorganism by plant exudates, which are composed of amino acids, carbohydrates, and organic acids that can vary according to the plant and its biotic or abiotic conditions [111]. Different plants select specific microbial communities as reported by Berg et al. [104] when comparing rhizosphere colonization of two medicinal plants: chamomile (*Matricaria chamomilla*) and nightshade (*Solanum distichum*); despite being cultivated under similar conditions, they presented different structural (analysing 16S rRNA genes) and functional (analysing nitrogen-fixing nifH genes) microbial communities. Moreover, plant exudate of the same plant varies according to plant developmental stages selecting specific microbial communities [112]. Researchers already identified some plant exudate compounds responsible for specific interactions such as flavonoids in Legume-Rhizobia [48] and Strigolactone as a signal molecule for AMF [113].

Reinhold-Hurek et al. [114] proposed a model for microorganism colonization. In bulk soil, the microbial community presents a great diversity and is influenced only by soil type and environmental factors. Getting closer to plant roots (rhizosphere), where there are root exudates, there are fewer species and a more specialized community. And only a few species are able to enter plant root and establish in the plant. Furthermore, after entering the plant, microbial community varies among the different organs: top leaves, fruits, bottom leaves, flowers, stems, and roots [115]. Mutualistic microorganisms can protect plants from pathogen either by inducing plant resistance or by antibiosis. The ISR in plants leads to high tolerance to pathogens. There are soils that even if there is the pathogen the disease does not occur; the mechanisms of these disease suppressives are still being investigated. In this way, Mendes et al. [116] analysed the microbiome of a soil suppressive to the fungal pathogen *Rhizoctonia solani* that causes damping off in several agricultural crops. Using a 16S rDNA oligonucleotide microarray (PhyloChip), they were able to identify more than 33 000 bacterial and archaeal taxa in the sugar beet seedlings rhizosphere grown in suppressive soil and in conductive soil. These analyses revealed the bacterial groups present only in the suppressive soil. The authors reported that γ-proteobacteria, especially Pseudomonadaceae, were all more abundant in suppressive soil than in conductive soil, focusing thereby on this bacterial group. Using random transposon mutagenesis technic in *Pseudomonas* sp. they were able to identify genes responsible for the biosynthesis of an antifungal: nine amino acid chlorinated lipopeptide produced by *Pseudomonas* sp. and that controls the pathogen. From the same PhyloChip diversity analysis, Cordovez et al. [117] identified other antifungal, this time produced by rhizosphere-associated streptomycetes. These *Streptomyces* isolates were able

to produce chemically diverse volatile organic compounds (VOCs) with an antifungal activity as well as plant growth-promoting properties. Showing that different bacteria groups can have similar roles in the same environment, another example was reported by Ardanov et al. [118] who showed that the inoculation of *Methylobacterium* strains also protected plants against pathogen attack and affected endophyte communities. Therefore, using this concept, researchers started inoculating plants with a pool of microorganism with complementary traits, for example with different mechanisms of control; however, it is a challenge to find the right players to be inoculated [119].

In order to define which microorganisms should be inoculated, several approaches were used. The first approach seeks to define a core microbiome of a healthy host, or understand the function of microbiomes by sequencing approach, that can be followed by experiments on gnotobiotic host manipulating the microbiome with a selection factor (for example, antibiotics, salinity, and UV light) or transferring microbiomes between hosts [101]. In this way, researchers are starting to study 'microbiome engineering', modulating microbial community. This modulation can occur either by performing plant breeding programmes selecting a beneficial interaction between plant lines and rhizosphere microbiome or by redirecting rhizosphere microbiome by stimulating or introducing beneficial microorganisms [101, 119]. The microbiome engineering can occur by altering ecological processes such as modulation in community diversity and structure changing microbe–interaction networks and by altering the evolutionary processes that include extinction of microbial species in the microbiome, horizontal gene transfer, and mutations that can restructure microbial genomes [120].

Summarizing, plant phenotype is the sum of plant response to the environment and to the present microbiome (including endophytes and pathogens); this microbiome also responds to the environment and interacts with each other [121]. Mendes and Raaijmakers [108] suggest a similarity between gut and plant rhizosphere microbiomes. They are both open systems, with a gradient of oxygen, water, and pH resulting in a large number and diversity of microorganism due to the different existing conditions. There are differences between gut and plant rhizosphere microbiome composition, therefore there are some similarities related to nutrient acquisition, immune system modulation, and protection against infections. Berg et al. [122] point seven similarities between host-associated microbiome ecology, among them: different abiotic conditions shape the structure of microbial communities; host and its microbiome co-evolute; core microbiome can be transmitted vertically; during life cycle, the microbiome structure varies; host-associated microbiomes are composed of bacteria, archaea, and eukaryotic microorganisms; functional diversity is key in a microbiome; and microbial diversity is lost by human interventions.

1.5.1 Secondary Metabolism

Microorganisms produce a large variety of compounds known as secondary metabolites that do not play an essential role in growth, development, and reproduction of the producing organism. Nevertheless, these metabolites are often bioactive compounds and can perform important functions in defence, competition, signalling, and ecological interactions [123, 124]. To establish a microbial interaction network, microorganisms usually respond by metabolic exchange, which leads to complex regulatory responses involving the biosynthesis of secondary metabolites. These interactions can be parasitic, antagonistic, or competitive and the metabolites involved and their functions have been specially studied recently as a result of the advent of tools such as metabolomics and imaging mass spectrometry (IMS) technology [125, 126]. Siderophores are related to competitive and cooperative microbial interactions and can also play other roles, such as signalling and antibiotic activity [127, 128]. Hopanoids play an important role in bacterial interaction, conferring tolerance and improving the adaptation of bacteria in different environments [129, 130]. In fungi, the compounds differentially regulated in an interaction are often bioactive secondary metabolites, such as diketopiperazines, trichothecenes, atranones, and polyketides [131, 132]. Nevertheless, there is still a lot to understand about the mechanisms involved and the role of many secondary metabolites and genes differentially expressed during the interaction. In this section, we present examples of studies on secondary metabolites involved in different types of microbial interactions.

1.5.2 Endophyte–Phytopathogen–Plant Interaction

The metabolites and mechanisms involved in the interactions between endophyte, phytopathogen, and host plant are still very unclear and are predicted to involve many secondary metabolites. Endophytic fungi are known to produce a large variety of bioactive secondary metabolites [133, 134] that are probably related to the endophyte complex interactions with the host and the phytopathogens and can perform important ecological functions, for example, in the plant development (as growth promoters) and in defence, acting against phytopathogens [135, 136]. This interaction has been studied in co-cultures of the phytopathogen *Moniliophthora roreri* and the endophyte *Trichoderma harzianum* that cohabit in cacao plants [127]. *T. harzianum* is extensively used as a biocontrol agent and has known ability to antagonize *M. roreri*. Tata et al. [127] identified four secondary metabolites (T39 butenolide, harzianolide, sorbicillinol, and an unknown substance) whose production was dependent on the phytopathogen presence and was spatially localized in the interaction zone [127]. T39 butenolide and harzianolide have been reported to have antifungal activity. Sorbicillinol is

an intermediate in the biosynthesis of bisorbicillinoids, a family of secondary metabolites that present diverse activities [137].

Trichoderma atroviride, commonly used as a biocontrol agent, produces acetic acid-related indole compounds that may stimulate plant growth. Colonization of *Arabidopsis* roots by *T. atroviride* promotes growth and enhances systemic disease resistance conferring resistance against hemibiotrophic and necrotrophic phytopathogens [138]. Other co-cultured studies were performed with bacteria. Araújo et al. [139] isolated a great number of *Methylobacterium* strains from asymptomatic citrus plants (with *Xylella fastidiosa* but without disease); then Lacava et al. [140] showed that *Methylobacterium mesophilicum* SR1.6/6 and *Curtobacterium* sp. ER1.6/6 isolated from health and asymptomatic plants inhibited the growth of the phytopathogen *X. fastidiosa*, the causal agent of citrus variegated chlorosis. Moreover, transcriptional profile of *X. fastidiosa* was evaluated during in vitro co-cultivation with a citrus endophytic strain of *M. mesophilicum*. It was shown that genes related to growth, such as genes involved in DNA replication and protein synthesis, were downregulated, while genes related to energy production, stress, transport, and motility, such as fumarate hydratase, dihydrolipoamide dehydrogenase (Krebs cycle), pilY transporter, clpP peptidase, acriflavin resistance, and toluene tolerance genes, were upregulated [141].

Another approach to study endophyte–phytopathogen plant interaction is based on the genome sequencing and transposon mutagenesis of an endophyte strain of *Burkholderia seminalis*, which suppresses orchid leaf necrosis by *Burkholderia gladioli*, which revealed eight loci related to biological control. A web cluster related to the synthesis of extracellular polysaccharides of the bacterial capsule was identified [142]. Extracellular polysaccharides are known to be key factors in bacterial–host interactions [143, 144]. In addition, gene clusters putatively related to indole-acetic acid and ET biosynthesis were identified in the sequenced genome of the endophyte strain, suggesting that this strain might interact with the plant by altering hormone metabolism [142].

1.5.3 Hopanoid

Hopanoids compose the cell membrane of some bacteria, [145] presenting the same function of eukaryotes cholesterol. They are responsible for stabilization of the membrane and regulate its fluidity and permeability [146]. Experiments that knockout biosynthesis genes such as hnpF (squalene hopene cyclase: shc) gene show that the absence of hopanoids does not influence bacterial growth [146, 147] but affects tolerance to several stress conditions, such as extremely acidic environments [148] or toxic compounds such as dichloromethane (DCM) [149]; it also affects the resistance to antibiotics [64] and antimicrobial lipopeptide [63], playing a role in multidrug transport [83] and bacterial motility [84]. Hopanoids act in increasing bacteria tolerance to

adverse environments, conferring resistance to stress conditions including extreme pH and temperature and exposure to detergents and antibiotics [129, 130]. In this way, hopanoids may be involved in bacteria–plant interaction, being responsible for adaptation of bacteria in aerobic microenvironment and low pH culture medium [131] as well as involved in nitrogen metabolism in *Frankia* sp. [150]. For example, a type of hopanoids produced by the nitrogen-fixing bacteria *Bradyrhizobium diazoefficiens* is essential for its symbiosis with the host *Aeschynomene afraspera*, a tropical legume. In this case, the synthesis of C35 hopanoids is related to evasion of plant defence, utilization of host photosynthates, and nitrogen fixation [151].

1.5.4 Parasitic Interaction

The study of the mycoparasitic interaction between *Stachybotrys elegans* and *R. solani* revealed many secondary metabolites differentially expressed in the interaction [132]. During the interaction, *S. elegans* produces cell-wall-degrading enzymes and expresses genes associated with parasitism [152, 153] while *R. solani* responds with an elevated level of the pyridoxal reductase-encoding gene [152]. A metabolomic study showed the profile of the induced secondary metabolites during the interaction. It showed a significant effect of the mycoparasite on *R. solani* metabolism: the biosynthesis of many antimicrobial compounds were downregulated, possibly as a result of the interaction, and only a few diketopiperazines were induced [132]. Diketopiperazines are known to have antimicrobial properties, among others biological activities [154]. The mycoparasite *S. elegans* produced several mycotoxins, mainly trichothecenes and atranones. It was hypothesized that the trichothecenes were triggered by *R. solani* and were responsible for the alteration in its metabolism, growth, and development [132]. Trichothecenes are a major class of mycotoxins and have been reported to inhibit eukaryotic protein biosynthesis and generate oxidative stress.

1.5.5 Microbial Community's Interaction

Actinomycetes are noteworthy as producers of many natural products (NPs) with a wide range of bioactivities [126]. A study on *Streptomyces coelicolor* interacting with other actinomycetes showed that most of the compounds produced in each interaction was unique, revealing a differential response in each case. Many unknown molecules and an extended family of acyl-desferrioxamine siderophores never described before in *S. coelicolor* were identified. They identified 227 compounds differentially produced in interactions; half of these were known metabolites: prodiginines, actinorhodins, coelichelins, and acyl-desferrioxamines. Thus, actinomycetes interspecies interaction seems to be very specific and complex [155]. It has been shown that fungal–bacterial interactions can lead to the production of

specific fungal secondary metabolites and not only diffusible compounds act in this communication but also there is a contribution from physical interaction [156]. Schroeckh et al. [157] demonstrated that an intimate physical interaction between *Aspergillus nidulans* and the actinomycete *Streptomyces rapamycinicus* leads to the activation of fungal secondary metabolite genes related to the production of aromatic polyketides, which were otherwise silent. A PKS gene required for the biosynthesis of the archetypal polyketide orsellinic acid, lecanoric acid (typical lichen metabolite), and the compounds F-9775A and F-9775B (cathepsin K inhibitors) was identified [156]. It was later reported that alterations in fungal histone acetylation via the Saga/Ada complex are triggered by the actinomycete leading to the induction of the otherwise silent PKS cluster. This result shows that bacteria can trigger alterations of histone acetylation in fungi [156].

1.5.6 Siderophore

The production and acquisition of siderophores by microorganisms is a crucial mechanism to obtain iron. Many microorganisms secrete siderophores in the environment that when loaded are recognized by cell surface receptors and then transported into the microbial cell [158]. Thus, they are related to competitive and cooperative microbial interactions. In addition, many siderophores can also present other functions, for example, they can function as sequesters of a variety of metals and even heavy metal toxins, as signalling molecules, as agents in regulating oxidative stress, and as antibiotics, which were reviewed by Johnstone and Nolan. In some *Pseudomonas* species, a group of siderophores called pyoverdines is essential for infection and biofilm formation, probably helping to regulate bacterial growth [159]. Pyoverdines have been reported to act as signalling molecules triggering a cascade that results in the production of several virulence factors, such as exotoxin A, PrpL endoprotease, and pyoverdine itself [160].

In the marine environment, exogenous siderophores affect the synthesis of induced siderophores and other iron acquisition mechanisms by other microbial species, working as signalling compounds that influence the growth of marine bacteria under iron-limited conditions. Many strains of marine bacteria were reported to produce siderophores and iron-regulated outer membrane proteins only in the presence of exogenous siderophores produced by other species, such as *N*,*N*-bis(2,3-dihydroxybenzoyl)-*O*-serylserine from a *Vibrio* sp., even under very low iron concentrations [161].

1.5.7 Symbiotic Interaction

A remarkably complex inter-kingdom interaction is the symbiotic relationship between *Burkholderia*, a genus of bacteria, and *Rhizopus*, a genus of phytopathogen

fungi that causes rice seedling blight. The endosymbiotic bacteria *Burkholderia* spp. is responsible for the production of the phytotoxin rhizoxin, the causal agent of rice seedling blight [162]. It was reported that in the absence of the endosymbiont, *Rhizopus* is not capable of producing spores, indicating that the fungus is dependent on factors produced by the symbiont to complete its life cycle [163]. This complex symbiont–pathogen–plant interaction is still poorly understood regarding the metabolites and mechanisms involved in the communication and interaction. A study on exopolysaccharide (EPS), which usually plays key roles in interactions, produced by *Burkholderia rhizoxinica* described a previously unknown structure of EPS. However, the loss of EPS production did not affect the endosymbiotic interaction with *Rhizopus microsporus*, as shown by a targeted knockout mutant experiment [164]. *B. gladioli* produces enacyloxins (polyketides with potent antibiotic activity) in co-culture with *R. microsporus*. The fungus induces the growth of *B. gladioli* resulting in an increased production of bongkrekic acid, which inhibited the growth of the fungus [164].

1.6 Microbiome Innovation in Agriculture: Insect Pest Management

Insects are associated with diverse microbial communities and in many cases, these associations are crucial for insect survival and development. Symbiotic microbes in the gut, hemolymph, as well as in specialized cells carry an arsenal of enzymes that provide specialized services to the insect hosts [165]. Supplies of essential nutrients (particularly amino acids and B vitamins) by endosymbionts have been well documented in a number of crop pests, particularly plant sap-sucking Hemipteran insects such as aphids, whiteflies, and psyllids [166–169], and in human disease vectors and urban pests such as tsetse flies in the genus *Glossina* and the common bed bug (*Cimex lectularius*). Some symbionts can degrade complex polysaccharides or recycle nitrogen for insects, such as the termites [170, 171] and cockroaches [172, 173]. The production of antimicrobials by symbionts aids the immune system to fight against invading pathogens, as was shown in the beewolf digger wasps [174] and cotton leafworm [175]. Besides nutritional and immune services, symbionts can shape the ecological interactions between insects and their natural enemies. For instance, the secondary symbiont of aphids *Hamiltonella defensa* increased the chance of host survival from parasitoid wasp attacks by disrupting wasp embryogenesis, mediated by its bacteriophage-encoded toxins [176–180]. *H. defensa* was also shown to attenuate volatile release in aphid-infested plants, thus reducing parasitic wasp recruitment [181]. Similarly, symbiont manipulation of plant physiology that facilitates insect colonization was observed in whiteflies and the Colorado potato beetle [182, 183]. Modification of body colour by facultative symbionts may determine aphid susceptibility to

predation or parasitism [184]. In particular, *Rickettsiella* infection in the pea aphid *Acyrthosiphon pisum* increased the synthesis of blue-green polycyclic quinone pigments, turning the host from red to green. This symbiotic-dependent colour variation is believed to affect the aphid's relative risks between predation and parasitism, as their predators such as the ladybird beetles preferentially prey on the red morphs, while parasitoids preferentially attack the green morphs [185–187]. Termites [171, 172] and cockroaches have symbionts that can digest complex polysaccharides or recycle nitrogen for them [173]. As proven in the beewolf digger wasps [174] and cotton leafworms [174], symbionts produce antimicrobials that help the immune system combat invading infections [175]. In different insect pests, symbionts were also shown to impact pesticide resistance. Resistance to organophosphorous pesticides is related to the direct detoxification by their symbionts in the beans bug (*Riptortus pedestric*) and the eastern fruit fly (*Bactrocera dorsalis*) [176, 177]. *Bacillus thuringiensis* (Bt) insecticide action was demonstrated to depend on the presence of symbiotic mid-gout bacteria in gypsy moth larvae *Lymantria dispar* [188]. Field microbiome studies showed that Bt resistance in bollworm cotton (*Helicoverpa armigera*) was related with different microbiome compositions [178–180]. The diamond back moth (*Plutella xylostella*) was diagnosed with varying levels of sensitivity to Chlorpyrifos treated with antibiotics and then decolonized with various gut-associated bacteria [189, 190]. In mosquitoes that demonstrated decreased lethality of pesticides, various intestinal commensal bacteria were found to have been contributing to pesticide resistance [191]. Collective data suggest that bacteria have a greater role as previously assumed in the formation of insect behaviour [192]. Long-term scatter, egg position, mattress, hosted search, and kin recognition are insect behaviours that are found to be altered by microorganisms [193]. Studies further imply that microbiomes might alter host behaviour by the formation of host neuro-endocrine circuitacted metabolites [194–196], a phenomenon termed the 'gut-brain axis'. There has been a lot of study on the gut-brain axis, with the majority of it being on mammalian systems. This field, however, is still in its infancy. A recent study found that the microbiome of the *Drosophila melanogaster* alters the host's olfactory-guided foraging preferences towards meals with various microbial content [197]. Farine et al. [198] and Qiao et al. [199] showed similar microbiome-priming effects on fly behaviour in following investigations. Scientists have achieved two breakthroughs in insect microbiome research thanks to advances in high-throughput sequencing and functional genomics: (i) investigate previously unidentified microbiomes in a wider range of insects, resulting in a better knowledge of the host and environmental variables that influence insect microbiome diversity and composition – some examples include microbial communities associated with Drosophilid and Tephritid fruit flies [200–202], ants [203, 204], bees [205], mosquitoes [206, 207], ticks [208], beetles [209], and midges [210], among others;

and (ii) assign particular microbial taxa or consortia with microbiome roles. While insect microbiomes differ in terms of diversity and stability, there is widespread agreement that microbial effect on insect invasive characteristics exists. For example, the invasiveness of the sweet potato whitefly (*Bemisia tabaci*) was aided by the introduction of a *Rickettsia* sp. into the pest population (from 1% infected in 2000 to 97% in 2006), which resulted in faster development, a higher survival rate to adulthood, and increased host fecundity [197, 211–214]; it was found that the microbiome of the strong lab model *D. melanogaster* accelerates larval development, impacts host foraging choice, and reproduction [197, 213]. *Drosophila suzukii*, often known as spotted wing drosophila (SWD), is a tiny fruit pest that relies on the microbiome to flourish [215]. Microbial symbiosis was initially discovered in the olive fruit fly *Bactrocera oleae*, a very damaging agricultural pest belonging to the Tephritidae family [216]. Unlike other fruit-feeding *Bactrocera* species, *B. oleae* has an obligatory bacterial symbiont (Candidatus *Erwinia dacicola*) that is maintained in the midgut caeca of the larvae. The symbiont is yet to be grown, but studies have demonstrated that it aids fly growth and reproduction by delivering necessary amino acids and metabolizing urea from a variety of sources, including bird droppings, making nitrogen accessible to adult flies [217, 218]. It also aids the development of larvae in unripe olives by inhibiting the production of oleuropein, a plant defence molecule [217, 219]. The symbiont was shown to be missing in domesticated *B. oleae* grown on antibiotic-laced artificial medium, illustrating the influence of upbringing on symbiont selection [220]. *Pantoea* sp. and *Burkholderia* sp. are two more bacterial species found in the intestines of *B. oleae*, although their nutritional significance is unknown [202]. The medfly (*Ceratitis capitata*) and apple maggot fly both have microbiome-dependent larval development (*Rhagoletis pomonella*). Diazotrophs that express the nitrogen reductase gene (nifH) in the stomach of medflies are involved in microbial food supply [221]. *Citrobacter*, *Klebsiella*, *Pectobacteria*, *Enterobacter*, and *Pantoea* are among the bacteria found in medflies [200]. When provided as probiotics, the community has been found to boost fly growth, reproduction, and lifespan, as well as boost male copulatory success [200, 218]. A modest but persistent population associated with the medfly stomach contains *Pseudomonas* spp., in addition to the dominating Enterobacteriaceae. Malacrin et al. [222] used a metabarcoding strategy to find changes in the microbial community at different instar stages of the medfly [222]. *Burkholderia* was discovered to be prevalent in early instars and adults, and it has been postulated that it may play a role in nitrogen fixation in *Tetraponera* ants [223]. Similarly, bacteria from the genera *Sphingomonas* and *Pseudomonas*, as well as an unidentified bacterium from the family Methylobacteraceae, were found to be more abundant in late instars of the medfly, whereas bacteria from the families *Leuconostoc*, *Weissella*, *Acetobacter*, *Gluconobacter*, and an unidentified bacterium from the family Xanthomonadaceae,

were more abundant at pupal stage. In addition, medflies fed on different host plants had a diverse microbial community. Malacrin et al. [222] observed that medfly larvae fed *Ficus carica* fruits had *Acinetobacter* and *Gluconobacter*, whereas *Acetobacter* and *Leuconostoc* were more prevalent when given *Prunus persica* (peaches). It has been proposed that *Acinetobacter* and *Gluconostoc* are involved in phenolic glycoside detoxification [224]. Similarly, enterobacteria such as *Pantoea*, *Klebsiella*, and *Enterobacter* are found in the guts of apple maggot flies [225]. During oviposition, microorganisms are deposited into the fruit, supplying necessary nutrients and proteins for larval growth [225, 226]. Symbiotic bacteria enhance larval development in several Tephritids of the subfamilies Dacinae and Trypetinae by metabolizing carbohydrates, raising organic nitrogen levels, and producing vitamins [217, 225]. However, their functions in adult flies are unclear [218].

1.6.1 Manipulation of Insect-Associated Microbiomes for Pest Management

The contribution of microbiomes to a wide range of insect invasiveness-related features implies a wealth of resources that might be targeted for pest control. The use of biochemicals to eradicate or disrupt insect symbiosis is a straightforward method [227]. Antibiotics such as tetracycline and penicillin, for example, have been demonstrated to render tsetse flies infertile by inhibiting the obligatory mutualist *Wigglesworthia*, obstruct the development of young ticks, and reduce adult tick reproduction by lowering their symbiont load [228]. Antimicrobial peptides (AMPs) have also been investigated for manipulating insect symbionts, albeit they are more typically utilized to combat human or plant infections transmitted by insects [229]. Insect innate immunity relies heavily on AMPs (which include a variety of amphiphilic and cationic oligopeptides). They provide immunity against bacteria, fungi, and viruses, among other microorganisms. Cecropin was the first AMP recovered from *Hylaophora cecropia* pupae [230, 231], and since then over 150 AMPs have been purified from insects [232]; for example, the α-helical peptides (e.g. moricin and cecropin), cysteine-rich peptides (e.g. defensin and drosomycin), proline-rich peptides (e.g. apidaecin, drosocin, and lebocin), and glycine-rich proteins (e.g. apidaecin, drosocin [e.g. attacin and gloverin). Some AMPs (such as defensins, cecropins, attacins, and proline-rich peptides) are found in all insect orders, whereas others (such as moricin and gloverin) are exclusively found in particular insect orders (e.g. Lepidoptera). AMPs' antibacterial activity is due to their positively charged surface, which allows them to bind negatively charged microbial surfaces via charge–charge interactions, disrupting bacterial cell wall integrity [233]. Resistance to bacterial, fungal, and certain eukaryotic parasites has been conferred by AMPs in plants and insects. Insect

defensins (gallerimycin from *Galleria mellonella*) and cecropin (sarcotoxin-IA from *Sarcophaga peregrine*), for example, have been found to provide harmful fungal resistance in transgenic tobacco [234]. In rice and tomato plants, transgenic production of cecropins has also been demonstrated to give resistance to fungal and bacterial diseases [235, 236]. Cecropins have also been shown to be effective against protozoan parasites including *Plasmodium* and *Trypanosoma* [237, 238]. The use of transgenic cecropin expression in *Anopheles gambiae*, a human parasite vector, has been found to lower the quantity of *Plasmodium berghei* oocysts by 60% [239]. Furthermore, transgenic co-expression of defensin-A and cecropin-A in *Aedes aegypti* has been found to prevent *Plasmodium* parasite transmission cooperatively [240]. Non-target effects, which can lead to disturbance of the natural microbiome of non-target insects, are a significant drawback of utilizing antibiotics or AMPs. Low bioavailability, instability, and antimicrobial resistance are some of the other drawbacks of employing AMPs [241]. Nonetheless, approaches such as fusion with antigen-specific antibody fragments [242], protein engineering, and synthetic biology approaches can be used to create AMPs with improved specificities (e.g. substitution of amino acids, chemical modifications) [243]. Nanotechnology-assisted distribution of AMPs to many biological systems is also being investigated [244]. Insect trait manipulation can also be accomplished by introducing a foreign microbe or replacing a symbiont with another bacterium. In stink bugs and aphids, experimental substitution of certain cultivated and uncultured insect symbionts has been demonstrated. According to Hosokawa et al. [245], exchanging the Ishikawaella symbionts between the stink insect *Megacopta punctatissima*, a common pest of soybean and other legumes, and a closely related non-pest species, *Megacopta cribraria*, resulted in poor *M. punctatissima* egg hatching on the plants. The major symbiont Buchnera was replaced with a new genotype by microinjection in the pea aphid in one experiment, and the pest's heat tolerance was altered [246].

1.6.2 Incompatible Insect Technique (IIT)

Several techniques have been developed to produce gnotobiotic insects in the lab, including *Drosophila* [213], mosquitoes [247], and honey bees [248]. Cleaning insect eggs to remove maternally deposited bacteria on the surface or treating larval or adult insects with antibiotics, followed by feeding on food seeded with cultured microbes or microbe-laden materials were all common parts of the technique (e.g. faecal transplantation). While the gnotobiotic approach has aided in the discovery of microbiome impact on insect traits such as development, physiology, behaviour, and insecticide resistance [190, 213, 247–249], its application in pest management is still largely theoretical. The use of *Wolbachia* to control mosquitoes and mosquito-borne disease pathogens is one notable exception. *Wolbachia* is

a widespread, vertically transmitted endosymbiont found in arthropods, infecting nearly 60% of all insects [250]. The bacteria are most recognized for inducing cytoplasmic incompatibility (CI), feminization, parthenogenesis, and male death in its hosts [251]. *Wolbachia*-infected females have a reproductive advantage over uninfected females because their sex ratios are skewed towards females, making it easier for them to spread across a community. *Wolbachia*-induced CI is used in the incompatible insect technique (IIT) to reduce mosquitoes and other insect pests [259[251, 252]. CI causes embryonic death [253] and can be caused unidirectionally in crossings between *Wolbachia*-infected males and uninfected females, or bidirectionally in crosses between infected animals carrying different *Wolbachia* strains [254]. As demonstrated in *A. aegypti*, *Wolbachia*-induced sterility has little effect on male mating competitiveness or survival [255]. *Wolbachia*-infected males are returned into the wild on a regular basis at IIT [256, 257]. Several insect pests, including *Rhagoletis cerasi*, *C. capitata*, the tsetse fly, and disease vectors, such as *Culex pipiens*, *Aedes albopictus*, and *Culex quinquefasciatus*, have been studied extensively using IIT [256, 257]. *Wolbachia* can be transinfected into a new host that is not natively infected with CI-inducing *Wolbachia* strains [258–260]. *Wolbachia* strain wSuz, as naturally, infects *D. suzukii* but does not cause CI [261–263]. *D. suzukii* has been successfully introduced to two CI-inducing *Wolbachia* strains (wHa and wTei) from different *Drosophila* species, paving the way for IIT for this pest [261, 264]. By distorting sex ratios toward females, Wolbachia-infected females have a reproductive advantage over uninfected females, facilitating their propagation in a population. The incompatible insect technique (IIT) employs Wolbachia-induced CI as a strategy to control mosquitoes and other insect pests [251, 252]. In addition, because host genotype influences *Wolbachia* density and phenotypic manifestation of infection in hosts, including CI, the genotype of IIT insects should be examined [265, 266]. Recent research has made substantial progress in identifying the molecular mechanisms that cause *Wolbachia*-mediated CI. When expressed dually in uninfected males, the wMel genes cifA and cifB (encoded by WO prophage) functionally repeated CI, according to LePage et al. [267]. Both genes are unable to cause CI on their own. Further research revealed that transgenic production of the cifA gene in *Drosophila* recovers CI and eliminates the embryonic lethality induced by wMel *Wolbachia* [268]. Beckmann et al. [269] found that the *Wolbachia* deubiquitylating enzymes (DUB) cidA and cidB combine to cause CI in transgenic *Drosophila*. Aside with CI, *Wolbachia*'s pathogen-blocking capabilities are another key feature for mosquito control. When transinfected with *Wolbachia* generated from *Drosophila* or other mosquitoes including *A. albopictus* and *C. quinquefasciatus*, *A. aegypti*, the vector for several clinically important arboviruses, showed dramatically decreased competence for dengue, chikungunya, yellow fever, and Zika viruses [270–273], as well as *Plasmodium* and filarial nematodes [274, 275].

The specific mechanism of *Wolbachia*-mediated pathogen inhibition is still being researched. Priming of the immune system, alterations in cholesterol and lipid droplet formation and trafficking [276], and (viral) RNA degradation are among the possibilities that have been offered [277]. In the year 2011, in the wild in Cairns, Australia, *A. aegypti* harbouring the wMel strain were released, marking the first experiment of microbiome modification of a wild insect population with the objective of lowering vector competence [278]. The *Wolbachia* infection has firmly established in the mosquito population, according to a follow-up experiment two years later [278]. More crucially, the discharge effectively halted dengue transmission in Cairns and its environs in northern Queensland, Australia. Meanwhile, *Wolbachia* frequencies in the original Cairns populations are at 95%, with a 96% decline in dengue incidence as of late 2019. *Wolbachia* has now been found in northern Queensland, as well as in Yogyakarta, Indonesia, and Kuala Lumpur, Malaysia, as a result of further releases [279–281].

1.6.3 Paratransgenesis

Paratransgenesis, a similar method that has gained popularity in recent years, involves genetically modifying microorganisms to express desired effects in insects [282–284]. Paratransgenesis avoids the problems of fitness cost associated with introducing a transgene into insects and transgenic instability in insect genomes by not changing the insects (i.e. transgenesis). This method is best for bacteria that can be cultivated, altered, and reintroduced into insect hosts with ease. Although paratransgenesis was first postulated in the early 1990s, the majority of study has focused on human disease vectors and a few Hemipteran crop pests. Beard et al. [285, 286] demonstrated that the triatomine bug's gut symbiont *Rhodococcus rhodnii* may be genetically engineered to express effector molecules (cecropin A and similar pore-forming molecules) against the protozoan *Trypanosoma cruzi*, which causes Chagas disease. Inoculating eggshells or food with excrement seeded with the designed symbiont allows the symbiont to be introduced to insect offspring. Durvasula et al. [287] used an anti-trypanosome single-chain antibody to alter the symbiont and found a considerable decrease in parasite burden. Following the positive findings of laboratory investigations, field trials were conducted to assess the transmission efficiency of modified *R. rhodnii* to the triatomine bug using CRUZIGARD, a simulated triatomine-faecal substance made of an inert guar gum matrix painted with India ink [288]. To manage *Rhodnius prolixus*, a study recently combined paratransgenesis with RNA interference (RNAi) technology. In *R. prolixus*, oral administration of an *Escherichia coli* strain HT115 or *R. rhodnii* engineered to express dsRNA targeting the antioxidant genes-heme-binding protein (RHBP) and catalase (CAT) genes caused systemic RNAi to silence these genes, resulting in poor nymph development and

reduced female fecundity [289]. Using the engineered symbiont *Sodalis glossinidius*, which produced antigen-binding molecules targeting *Trypanosoma brucei*, the causative agent of sleeping sickness, similar paratransgenic techniques have been explored on tsetse flies. *Sodalis* may be transferred vertically by the milk glands and is present in the hemolymph, midgut, and milk gland [290, 291]. Using bacteria and fungi obtained from mosquito midguts and ovaries, different paratransgenic techniques have been investigated in mosquitoes to limit the transfer of malaria-causing *Plasmodium* parasites. The Gram-negative *Asaia bogorensis* was chosen for paratransgenesis against *P. berghei* because it has been demonstrated to survive in mosquito midguts and spread swiftly both vertically and horizontally within a population [292, 293]. The siderophore receptor gene was fused with anti-plasmodial effector genes to create genetically engineered *Asaia* strains. The scorpine AMP and a synthetic antiPbs21 scFv-Shiva1 immunotoxin made up of a single-chain antibody (scFv) against the *P. berghei* ookinete surface protein 21-Shiva1 fusion protein were among the genes identified. *Anopheles stephensi* mosquitoes fed with the altered *Asaia* and challenged with *P. berghei*-infected blood showed a substantial reduction in parasite growth [294]. *Pantoea agglomerans*, common mosquito symbiotic bacteria, was previously modified to produce anti-*Plasmodium* effector proteins utilizing an *E. coli*-derived Type I hemolysin secretion system. In the midgut of *Anopheles* mosquitoes, these modified *P. agglomerans* strains were shown to suppresses the growth of *P. falciparum* and *P. berghei* [295]. *Metarhizium anisopliae*, an entomopathogenic fungus, has also been engineered to release the antibiotic scorpine and anti-plasmodial peptide SM1, which inhibits *Plasmodium* parasite growth [196]. Novel *Serratia* sp. AS1 colonizes the ovaries and gut of *A. stephensi* as AS1 strain was both sexually and vertically transmitted, persisting for at least three generations. Mosquitoes infected with an engineered *Serratia* AS1 containing five different anti-plasmodium effector molecules (Shiva1, a cecropin-like synthetic AMP; MP2, midgut peptide 2; EPIP, enolase–plasminogen interaction peptide (lysine-rich enolase peptide); scorpine, scorpion *Pandinus imperator* venom AMP; and mPLA2, inactive bee venom phospholipase A2) displayed a reduction in the oocyte load by 93% [296]. The Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, demonstrates the potential of paratransgenesis in crop protection against insect pests or insect-vectored illnesses. GWSS is a vector for *X. fastidiosa*, a bacterial pathogen that causes Pierce's disease in grapes by manufacturing EPSs, which assist the infection to colonize the xylem of its host plant and restrict the flow of the xylem [297–299]. *Alcaligenes xylosoxidans* var. denitrificans (Axd) was identified as a candidate for genetic modification among the several bacterial species identified from GWSS. It has always been detected in the xylem of host plants, in the same spot where the pathogen lives. GWSS was successfully given with genetically engineered Axd harbouring a DsRed fluorescent protein gene from

injected stems. It was discovered to invade the foregut of insects, implying that a paratransgenic method to eradicate *X. fastidiosa* from GWSS is possible [298]. However, as this genus of bacteria has been recognized as a nosocomial human pathogen linked in causing lung infection in cystic fibrosis patients, the usage of altered Axd in plants has significant downsides [300]. To alleviate the safety concern, an endophytic bacteria from grapes, *P. agglomerans* E325 (an EPA-approved agent for managing fire blight in pears and apples), was genetically engineered to express anti-*Xylella* effector proteins melittin and a scorpine-like AMP, and colonized the foregut of GWSS using an artificial feeding system (AFS) [301]. In addition, in simulated field settings, targeted administration of recombinant *P. agglomerans* E325 to the gut of GWSS utilizing a microencapsulation approach was established to treat Pierce's illness. The microencapsulation approach might be effective in the field since it might restrict the transmission of alien genetic material [301]. Leonard et al. [302] devised a paratransgenic technique in honeybees and showed in the lab that it increased bee survival against viral infection and *Varroa* mites. The researchers created symbiotic gut bacteria called *Snodgrassella alvi* that produces dsRNA that targets bee, virus, and mite genes. The selection of microorganisms, the genetic design, and the implementation of the treated insects all have a role in the success of gnotobiotic or paratransgenic techniques for insect pest management. Another barrier is the association's stability, which should be particular to target insects or innocuous to non-target hosts. Although persistent association guarantees that microbial-mediated effects on host insects are long-lasting, certain microorganisms may be 'lost' from the insects owing to environmental selection pressure or antagonistic interactions with other germs. Despite these cautions, it is expected that gnotobiotic and paratransgenic insect research and development will continue to expand.

1.6.4 Exploiting the Chemical Inventories of Microbiomes to Develop New Biopesticides

The evolution of pesticide resistance is a major barrier for crop security, since many insect pests have no other treatment options. The difficulties of turning a lead compound into a product that can pass tight environmental and safety laws have made the development of new synthetic pesticides more expensive and complicated. According to a recent research, developing a synthetic pesticide in the United States currently costs over $300 million and takes about 12 years [200]. The demand for novel insecticidal chemicals is necessitated by the need to address resistance issues and improve sustainable agriculture. The effort to produce innovative insecticides with little environmental effect has sparked renewed interest in biopesticides in recent years (i.e. pesticides based on living organisms or their NPs, including their genes and metabolites). The global market for biopesticides is now estimated at

$3 billion and is predicted to expand by 15% over the next four years, exceeding the growth of synthetics by a factor of 10 [303]. Biopesticides are divided into three types: biochemical pesticides, which are naturally occurring chemicals that can change pest behaviour or physiology; microbial pesticides, which use pathogenic or toxic microbes as the active ingredient; and plant-incorporated protectants (PIPs), which are transgenic plants that produce pesticidal compounds [304].

1.6.5 Microbial Insecticides and Plant-Incorporated Protectants

Historically, microbial insecticides have taken the form of living microbes or spores applied directly to the field. Among the most common microbial insecticides are the entomopathogenic fungi, or EPFs, which are used in half of all classical biological control programmes [305]. Several attributes of EPFs make them ideal candidates for biological control agents: many can be mass cultured in vitro, fungal spores have a long shelf life compared to other biological control agents, and they are often capable of persisting in the host population without repeated introductions [306]. *Metarhizium anisopliae*, *Beauveria bassiana*, and *Entomophaga maimaiga* are among the most commercially important soil-dwelling Hypocreales. Entomopathogenic nematodes (EPNs) from the Steinernematidae and Heterorhabditidae families, as well as their bacterial symbionts *Xenorhabdus* and *Photorhabdus*, are essential biological control agents for insects. EPNs infect a wide spectrum of hosts, kill quickly, and actively seek for new victims to infect. They do, however, have a limited shelf life and are susceptible to environmental factors such as low moisture levels and ultraviolet (UV) radiation [303]. Entomopathogens have several advantages over conventional insecticides as biological control agents: they are often host-specific, reducing non-target species impact, and are usually harmless to humans; they can be used in organic farming and Integrated Pest Management Programmes; and they are less susceptible to pest resistance issues than conventional products. They are, however, difficult and costly to mass produce, as many of them require a host to complete their life cycle; they are particularly sensitive to environmental variables such as sunlight and humidity; and they have a short shelf life. *B. thuringiensis* (Bt), a Gram-positive, spore-forming species often found in soils, is the source of the majority of bacterial insecticidal compounds (Table 1.1). In 1901, Bt was identified as the cause of sotto sickness in silkworms (*Bombyx mori*) [306]. However, until the 1950s, when it was found that the Cry proteins of certain strains are poisonous to insects, the mechanism of Bt-induced insect death was unknown [313]. Until 1995, when transgenic crops expressing Cry proteins were commercially accessible, Bt remained a niche product utilized exclusively in forestry and organic farming. Bt application was fundamentally revolutionized with the development of Bt transgenic crops: the toxin became constitutively produced, covered all tissues, and

Table 1.1 List of some of entomopathogens.

Pathogen	Target pests	Mechanism	Virulence factors	Commercial application	References
Bacillus thuringiensis various strains	Lepidoptera, Coleoptera, and Diptera	Colonization and perforation of midgut epithelium	Cry proteins; Vegetative Insecticidal Proteins (VIP)	Vegetative Insecticidal Proteins (VIP); Spore suspension (liquid and granular); Cry protein expression in GM crops	[307]
Serratia entomophila	*Costelytra giveni*	Colonization of foregut and cessation of feeding	sepA, sepB, and sepC on pADAP	Soil-applied granules	[308]
Bacillus popilliae; *Bacillus lentimorbus*	*Popillia japonica*	Spores germinate in the larval gut; vegetative cells penetrate into the haemocoel leading to death			[309]
Beauveria bassiana	Aphids, fungus gnats, mealy bugs, mites, thrips, and whiteflies	Conidia land on host and germinate into cuticle; mortality caused by fungal toxicity and colonization of vital tissues		Spore suspension; promotion of existing natural populations	[307]
Verticillium lecanii	*Trialeurodes vaporariorum*, Hemiptera: Aphididae	Conidia land on host and germinate into cuticle; mortality caused by fungal toxicity and colonization of vital tissues		Spore suspension	[310]

(*Continued*)

Table 1.1 (Continued)

Pathogen	Target pests	Mechanism	Virulence factors	Commercial application	References
Nosema locustae	European corn borer caterpillars, grasshoppers, and mormon crickets	Infects fat body tissues, disrupting host metabolism and energy storage		Spores applied to bait	[311]
Ns NPV (*Neodiprion sertifer* nuclear polyhedrosis virus)	Pine sawfly	Capsid dissolves in the alkaline midgut to release the virus particle causing cell lysis	Polyhedral capsid	Suspension concentration of virus	[312]

non-target effects were eliminated because only insects that devour the plants were harmed [314]. Bt maize, cotton, soy, and eggplant types are extensively farmed now, and new Bt varieties are being created all the time [315]. Bt maize and cotton types are grown on more than 80% of maize and cotton land in the United States [316]. Moreover, approximately 300 different variants of the cry gene have been found so far, with specificity for lepidopteran, dipteran, coleopteran, or nematode hosts [306]. However, the evolution of Bt resistance has posed a danger to the efficacy of Bt crops in recent years, highlighting the relevance of insect resistance management (IRM) and the need to find novel microbial insecticides. Spinosad is a commercial pesticide developed from microbial NPs that has proven to be effective. It is the outcome of Dow Agrosciences' natural product discovery initiative, which began in the mid-1980s. The larval mosquitoes and Lepidoptera were exposed to fermentation broths made from soil samples as part of the programme. Insecticidal activity was found in a soil sample taken from a Virgin Islands rum distillery, which was shown to be caused by secondary metabolites generated by an unknown actinomycete eventually identified as *Saccharopolyspora spinosa* [317]. The spinosyns, which are metabolites of nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptors, have been discovered to be broad-spectrum insecticides with minimal vertebrate toxicity and unique method of action. Spinosad, a formulation that combines spinosyn A and spinosyn D in a 5 : 1 ratio, was authorized for over 150 conventional and organic crops in the United States by 2004 [318]. This small number of microbial insecticides is unlikely to reflect the full variety of microbial insecticidal compounds. Insecticidal screenings of microbial NPs are uncommon in the literature; the bulk

of NP discovery efforts are focused on potential medicines. Furthermore, because of the rapid pace of rediscovery of known compounds over the last 30 years, detecting new NPs via traditional, culture-dependent screens has become more difficult [319]. Given that the fraction of successfully cultivated microbial species is typically considered to be less than 1%, many bioactive microbial NPs are likely to remain undiscovered [307]. In the realm of NP discovery, the emergence of culture-independent, high-throughput metagenomics approaches has ushered in a revival. Extracting DNA from environmental samples, cloning onto plasmids, and expressing in recombinant expression systems might be used to 'mine' microbial toxins. This strategy, however, implies that the expression system has the molecular engineering required to heterologously express and ply the desired genetic products, including regulatory elements (for example, promotors and ribosome sites), chemicals, and biosynthetic precursors. For example, a Gabor et al. [320] investigation recovered only <40% of enzyme activities in the *E. coli* vector by shotgun cloning of environmentally microbial DNA. This problem can be avoided by doing functional tests in several expression systems, often by using a shuttle vector that is particularly developed [319].

1.6.6 Microbial Semiochemicals

To adapt their comportments in reaction to the environment, insects rely on chemical communication. These include foraging, matching, risk prevention, identification of children, and social interactions. Semiochemicals are defined as substances that transmit signal between organisms that cause changes in the behaviour of recipients. The use of these semiochemicals to modify insect behaviour is a sustainable technique for plague management. Pheromones of the insects represent a big market share of attractants for plant and urban pests, and are classified as semiochemical products that promote inter-specific contact. Both the monitoring and management of a range of agricultural and forestry and urban pests, such as the *Anthonomus grandis* cotton boll, the *L. dispar* gypsum moth, and Musca home-fly, are utilized for pheromone boiled traps [320–323]. Field applications of synthetic pheromones also showed that multiple lepidopteran pesticides, including *Cydia pomonella* codling moth, *Pectinophora gossypiella* rose bollworm, and diamond bolt moth, can be effectively controlled through an impediment to their mates, a process called a disruption of the mating [324]. Insect pheromones are not many commercially utilized semi-organic products, but organic plant or microbial volatile compounds (mVOCs) [325]. VOCs are a vast family of low-molecular weight chemicals that are characterized by low vapour and high volatility. VOCs may be made by various metabolisms, such as fermentation, catabolism, and reduction of sulphur and terpenoid biosynthesis [325, 326]. A lot of VOCs are efficient semiochemicals that only require little

concentrations to cause insect reactions, which have very sensitive (particularly olfactory) chemosensory systems. VOCs enable broad communication through air transport and act as significant methods for the sense of the environment. The discovery and deployment of mVOCs has recently become more and more relevant as a tool for pest management. This tendency has been supported by several causes. First, an online library of over 2000 microbial volatiles from about 1000 microbial species is reported as mVOC 2.0 [326, 327]; <10% of known mVOCs are studied or assigned with functions. Since an estimated 1018 microbial species are expected to exist on earth, many VOCs still remain to be identified. Second, the demand for NPs is growing in the field of agriculture pesticide management, because they are generally better perceived by the public in terms of safety and environmental sustainability than synthetic chemicals.

Over three billion years, microbes have existed and their metabolism has been very varied and dynamic through evolutionary processes. The metabolism of microbiomes often goes much beyond their eukaryotic hosts' capacity. Genetic engineering techniques such as recombinant and synthetic approaches can also be used to improve microbial metabolism. This means that microbial production of desirable metabolites (including mVOCs) may easily be scaled up to suit commercial requirements. In a variety of ways, advances in 'omics' tools and computational biology for studying microbial metabolism can aid in the discovery of mVOCs. High-throughput sequencing, in particular, is shedding light on the metabolic potential of microbiomes found in plants, soils, and other natural resources that are not farmed. Assembling complicated metagenomes and metatranscriptomes as well as predicting biosynthetic gene clusters from large data sets of DNA/RNA sequences are all becoming more computationally feasible [328, 329].

Biologically active NPs can be obtained from uncultured microbes by cloning environmental DNA into plasmid vectors and expressing in recombinant systems such as *E. coli*, yeast, or baculovirus [329]. In recent years, this technique has been used to screen for new antibiotics and medicinal medications, and it may theoretically be extended to the discovery of microbial-based agricultural compounds, such as novel semiochemicals and biopesticides [326]. Microbes can impact insect behaviour through modifying plant VOC emission patterns, in addition to directly affecting insects. In response to stimulation by JA, a key plant hormone involved in anti-pest defence, growth, and development, lima bean plants colonized with Rhizobia produced a distinct VOC profile from non-colonized plants [330]. Increased indole production was related to the unique VOC profile of colonized plants, which corresponded to considerably stronger *Epilachna varivestis* (Mexican bean beetle) repellency [330]. Several symbiotic fungi, such as endophytic fungi and arbuscular mycorrhizas, have also been demonstrated to influence plant volatile emissions and, as a result, plant susceptibility to certain insect pests [331, 332]. These findings suggest that symbiotic microorganisms may have indirect positive impacts on plant

defence that may be turned into plant probiotics. Some pathogenic microorganisms, on the other hand, are known to alter plant volatile emissions in order to attract pests and vectors. Huanglongbing (HLB), commonly known as citrus greening disease, is caused by the bacteria *Candidatus Liberibacter asiaticus*, which infects citrus trees. Citrus trees infected with *Candidatus L. asiaticus* produced more methyl salicylate, which was thought to attract the Asian citrus psyllid (*Diaphorina citri*), its carrier insect [333]. A variety of plant viruses, including the Cucumber mosaic virus and the Barley yellow dwarf luteovirus, have been discovered to alter plant volatile emissions in order to attract their vector insects (aphids) and therefore promote transmission [334]. Laboratory research and field experiments have supported the use of microbial-based attractants and repellents in pest management. The invasive *D. suzukii*, for example, is a major pest of tiny fruits, and it differs from other non-pest *Drosophila* species in that it affects intact, mature, and maturing fruits [335]. *Drosophila* species are attracted to fermentation products [335], and adding fermenting sugar–yeast combination to a popular non-microbial trap bait (apple cider vinegar + 10% ethanol) boosted *D. suzukii* capture rate by up to 15-fold in both laboratory and field circumstances [336]. In addition, it has been observed that yeast wine–vinegar–sugar mixes are more effective attractants than wine–vinegar alone. Ishii et al. [337] genetically engineered the acetic acid bacteria (AAB) *Komagataeibacter europaeus* to produce more acetoin (a major element in vinegar) and found that the mutant strain captured substantially more *Drosophila* than other strains put on sticky traps. Tephritid fruit flies are attracted to microbial semiochemicals in the same way [338–340]. For example, alcohols, ketones, pyrazines, phenols, and acids were found in *Klebsiella pneumoniae* fermented broth that attracted the Mexican fruit fly (*Anastrepha ludens*) [333]. Female Caribbean fruit flies (*Anastrepha suspensa*) were shown to be attracted to the VOCs 3-methyl-1-butanol and ammonia released by *Enterobacter agglomerans*, a bacterium isolated from larval-infected fruits and fly adults [340]. Insect repellents might be made using some mVOCs, particularly those generated by fungus. Mould emits geosmin, which *D. melanogaster* detects and avoids [338]. It was eventually discovered that it causes unpleasant behaviours in SWD, and that it may be used to prevent oviposition on crops [341]. The development of particular fungus species (*Phoma* spp., *Fusarium* spp., or *Rhizopus* spp.) on chicken faeces inhibited oviposition by the housefly, *Musca domestica*, and the impact was linked to dimethyl trisulphide and 2-phenylethanol emission [342]. VOCs might come from plant-associated symbionts as well. *Muscodor vitigenus*, for example, generates naphthalene and repels insects as an endophyte of the tropical liana *Paullinia paullinoides* [343]. Microbial catalysis of repellent chemicals has been established in fungi such as *Penicillium*, *Aspergillus*, and *Fusarium*, which can convert dihydrojasmone and *cis*-jasmone to bioactive molecules like [+]-[*R*]-4-hydroxydihydrojasmone and [–]-4-hydroxyjasmone [344]. In a food-choice behavioural experiment, these chemicals were found to repel aphids. [±]-β-aryl-γ-et

hylidene-γ-lactones are the compounds that have previously been determined to have phagodeterrent, antifungal, antibacterial, and anticancer activities, which were enantioselectively hydrolysed by a strain of *Aspergillus ochraceus* to form [−]-[*S*]-γ-ethylidene-γ-lactones and [+]-[*R*]-γ-ketoacids [345]. The smaller mealworm *Alphitobius diaperinus* was used to test the phagodeterrent/repellent capabilities of these derived compounds. Many entomopathogenic bacteria, including *B. thuringiensis* (Bt), *Pseudomonas entomophila*, and nematode-associated *Xenorhabdus* and *Photorhabdus* species, have been proven to repel insects [346]. A recent study found that a cocktail of bioactive secondary metabolites (mainly fabclavines) derived from *Xenorhabdus budapestensis* was more effective than DEET (chemical name, N,N-diethyl-meta-toluamide) in repelling *A. aegypti* and required a lower dose to produce the same repellent effect [347]. Leroy et al. [347] have documented several examples of microbial semiochemicals and their interactions with insects. Scientists will be able to make reliable inferences about microbial regulatory networks in mVOC generation by identifying distinct pathways from large-scale omics data. Dissecting synergistic multi-species interactions (i.e. consortia of microorganisms) in metabolite synthesis is another developing subject [348]. These findings suggest that microbial-based insect attractants or repellents might lead to more diversified, effective, and versatile agricultural products. Incorporating microbial-based semiochemicals into push–pull tactics is one of the applications. A target pest is repelled or discouraged away ('pull') from a protected source (a valuable crop or farm animal) using unpleasant stimuli in a standard push–pull system. In the meantime, the pest is drawn away from the protected supply by appealing stimuli ('pull'). Different crop plants (intercrops or trap crops), visual cues, pheromones, synthetic volatiles, phagostimulants/repellents, and anti-feedants are all typical stimuli [349]. To increase the efficacy of future push–pull techniques, microbial products can be utilized instead of or in addition to these stimuli.

1.6.7 Combining Microbial-Based Biopesticides with Nanotechnologies

Nanotechnology advancements are supporting the successful and long-term usage of biopesticides in field applications. When entomopathogenic bacteria, such as Bt and *Photorhabdus luminescens*, were administered to insect pests in the form of nanoparticles, their efficacy rose dramatically [350]. It has been found that larvae exposed to Bt nanoparticles died quicker and more often than larvae exposed to un-homogenized Bt powder, owing to greater Cry toxin solubility in the alkaline midguts. Furthermore, the development of nanoformulation delivery technologies such as nanoemulsion, nanocapsule, and nanosuspension has improved the longevity and stability of biopesticides in a variety

of environments, including UV radiation and humidity [351]. Because they are frequently constructed of biodegradable natural polymers, these delivery techniques are environmentally beneficial. Smart nanopesticides are an emerging subject stemming from nanoformulation research, in which active chemicals are contained in stimulus-responsive carriers and their release is regulated by stimuli like light, temperature, humidity, or pH [352, 353]. The stimuli-responsive administration strategy allows for more accurate spatial administration while reducing pesticide dosage and frequency, resulting in less environmental effect. For VOCs, nanoformulations offer the potential to fine-tune the compounds' thermal stability, resulting in optimal release and semiochemical lifetime. There have been several comprehensive reviews written on various types of nanopesticides and stimuli-responsive delivery systems [339]. The application of nanotechnology in agrochemicals is still in its early stages. Commercial items in this category, on the other hand, are starting to appear on the market. Seltima R, a fungicide introduced by BASF in 2016 to protect rice crops, is one example. Seltima encapsulates pyraclostrobin, a fungicide agent that is very poisonous to aquatic organisms, using humidity-responsive polymers. This encapsulation is water resistant, enabling for regulated delivery of the fungicide to the rice leaf surface while reducing pollution of the aquatic habitats nearby. There will be unanticipated barriers in the processing, storage, and distribution of these products for agricultural purposes, based on the anticipation that new microorganisms and microbiome-derived compounds will be found at an exponential rate. The combination of microbial products with nanotechnology offers a number of options for improving efficacy and stability while also regulating environmental dispersal. Microbiome mining and nanotechnology, when coupled, have a lot of promise for developing breakthrough bioinsecticide applications in the near future.

1.6.8 Microbial Interventions to Improve Fitness of Mass-Reared Insects for Autocidal Programmes

Engel and Moran [193] have described probiotics ways for promoting beneficial insect populations (e.g. pollinators, natural enemies of pests). The IIT (discussed in Section 1.6.1) and the sterile insect technique (SIT) are two microbiological techniques for promoting insect development for autocidal programmes. SIT is an environmentally acceptable insect pest management technology that involves mass-rearing the target pests and generating sterile male insects using non-lethal ionizing radiation exposure [354]. The pest population is then suppressed or eradicated by releasing large numbers of totally infertile males to mate with wild females [354]. Inherited sterility (IS), a derivation of the SIT, was created for insect pests, mostly lepidopterans, which require a high-radiation dosage to establish total

sterility. In IS, partly sterile males are created by exposing them to sub-sterilizing amounts of radiation and then mating them with wild females, resulting in low egg viability and mostly male progeny [355]. This method eliminates the negative effects of high-dose radiation on the insects. For some lepidopteran (moth) species, studies have demonstrated that males with hereditary sterility reduce wild populations more successfully than entirely sterile males. The primary screwworm (*Cochliomyia hominivorax*), the medfly (*C. capitata*), the Mexican fruit fly (*A. ludens*), several Lepidoptera (moths), and tsetse flies have all been successfully controlled by SIT over the years [356]. However, because huge numbers of treated insects must be released to compete with wild males, and often repeated releases are required, the expense of pest management through autocidal programmes is a substantial drawback. SIT necessitates a ratio of up to 100 sterile insects per wild insect. Although there are fewer data on IIT, a study on *Wolbachia*-infected mosquitoes found that releasing sterile to wild males at a 10 : 1 ratio was enough to suppress the local mosquito population [357]. Irradiation and handling in mass raising facilities further jeopardize insect survival and performance, adding to the high expense of autocidal operations. The alteration of mass-reared and irradiated males' gut microbiomes has been associated with lower performance, such as competence in attracting and mating with wild females. Laboratory-reared agricultural pests and disease vectors have fewer gut microbial taxa than their wild counterparts [358]. Insects are thought to obtain their gut bacteria from their natural habitats and food sources; however, these bacteria are usually absent from the artificial environment and diets, resulting in a loss in species richness or variety [359]. Furthermore, the insect gut microbiome composition is subjected to a different selection pressure due to artificial rearing. Antimicrobials are routinely added as preservatives to insect diets in the laboratory, for example. As demonstrated by the *B. oleae* example, these antimicrobials are capable of wiping out the majority of bacteria normally associated with insects. If the microbiome is seen as an ecosystem, decreases in species richness or variety may result in poor host outcomes, particularly if the bacteria offer specialized services to the host. While there is a substantial amount of literature on the effects of artificial rearing on the microbiomes of various insects, nothing is known about how irradiation affects the microbiome of insects. Because numerous prominent pests belonging to this category have been the focus of SIT applications, the majority of the study has been undertaken on them. In medflies, recently eclosed irradiated males showed decreased levels of some prominent gut bacteria (particularly *Klebsiella* sp.) but a greater level of *Pseudomonas* sp., which was not found in wild flies [360]. Following a few days after irradiation, however, some of these bacteria appeared to rebound in numbers. Irradiation therapy increased the number of *Lactococcus* and *Orbus* but decreased *Lactobacillus*, members of the Orbacecae family, and *Morganella* in the oriental fruit fly [361]. More study is needed to figure out how these temporary alterations in the gut microbiota affect fly recovery after

irradiation, as well as their long-term implications on fly fitness. Scientists have been looking at the impact of nutritional supplementation with bacteria on the performance of irradiated tephritid fruit flies, based on the notion that reversing the microbiome alterations associated with artificial rearing or irradiation therapy might increase the insects' fitness. The variations in these trials show that the effects of probiosis therapy for insects such as host age, genotype, microbial strain, nutrition, and raising conditions may be affected by diverse aspects. It is also worth mentioning that previous research has only looked at single bacteria or bacterial mix supplements. Other methods for manipulating the microbiomes of artificially grown or irradiated insects are still being investigated. Microbiome transplanting, either by growing the treated insects on a diet seeded with excrement from newly caught wild insects or by co-housing treated insects with wild insects, might be one way; however, it would be difficult to scale up for mass-rearing. Another alternative is to change the diet formula to encourage the growth of beneficial microorganisms in the insect gut ('prebiotics'), which would include adjusting the nutritional content and maybe integrating natural food ingredients into their meals. It is obvious that further study still has to be done to determine the fitness of sterile, artificially grown probiotics and to find criteria for treatment that impact the results of insects in response to the various ways indicated. Insects are good models for dissecting the intricacies of microbial interactions and processes from a fundamental research standpoint, because their microbiomes are often less diversified than in mammalian systems, where much probiotics research has been undertaken.

References

1 Chang, C., Bowman, J.L., and Meyerowitz, E.M. (2016). Field guide to plant model systems. *Cell* 167 (2): 325–339. https://doi.org/10.1016/j.cell.2016.08.031 PMID: 27716506.

2 Stanton-Geddes, J., Paape, T., Epstein, B. et al. (2013). Candidate genes and genetic architecture of symbiotic and agronomic traits revealed by whole-genome, sequence based association genetics in Medicago truncatula. *PLoS One* 8 (5): e65688. https://doi.org/10.1371/journal.pone.0065688 PMID: 23741505.

3 Hacquard, S. and Schadt, C.W. (2015). Towards a holistic understanding of the beneficial interactions across the populus microbiome. *New Phytol.* 205 (4): 1424–1430. https://doi.org/10.1111/nph.13133 PMID: 25422041.

4 Spence, C., Alff, E., Johnson, C. et al. (2014). Natural rice rhizospheric microbes suppress rice blast infections. *BMC Plant Biol.* 14: 130. https://doi.org/10.1186/1471-2229-14-130 PMID: 24884531.

5 Ramond, J.-B., Tshabuse, F., Bopda, C.W. et al. (2013). Evidence of variability in the structure and recruitment of rhizospheric and endophytic bacterial

communities associated with arable sweet sorghum (Sorghum bicolor (L) Moench). *Plant Soil* 372 (1–2): 265–278.
6 Li, D., Voigt, T.B., and Kent, A.D. (2016). Plant and soil effects on bacterial communities associated with Miscanthus X giganteus Rhizosphere and Rhizomes. *GCB Bioenergy* 8 (1): 183–193.
7 Peiffer, J.A., Spor, A., Koren, O. et al. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci.* 110 (16): 6548–6553.
8 Tian, B.-Y., Cao, Y., and Zhang, K.-Q. (2015). Metagenomic insights into communities, functions of endophytes, and their associates with infection by root-knot nematode, meloidogyne incognita, in tomato roots. *Sci. Rep.* 5: 17087.
9 Miedes, E., Vanholme, R., Boerjan, W., and Molina, A. (2014). The role of the secondary cell wall in plant resistance to pathogens. *Front. Plant Sci.* 5: 358.
10 Yeats, T.H. and Rose, J.K.C. (2013). The formation and function of plant cuticles. *Plant Physiol.* 163: 5–20.
11 VanEtten, H.D., Mansfield, J.W., Bailey, J.A., and Farmer, E.E. (1994). Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". *Plant Cell* 6: 1191.
12 Boller, T. and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60: 379–406.
13 Macho, A.P. and Zipfel, C. (2014). Plant PRRs and the activation of innate immune signaling. *Mol. Cell* 54: 263–272.
14 Chinchilla, D., Bauer, Z., Regenass, M. et al. (2006). The Arabidopsis receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. *Plant Cell* 18: 465–476.
15 Nam, K.H. and Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* 110: 203–212.
16 Fliegmann, J. and Felix, G. (2016). Immunity: flagellin seen from all sides. *Nat. Plants* 2: 16136.
17 Katsuragi, Y., Takai, R., Furukawa, T. et al. (2015). CD2-1, the C-terminal region of flagellin, modulates the induction of immune responses in rice. *Mol. Plant-Microbe Interact.* 28: 648–658.
18 Hirai, H., Takai, R., Iwano, M. et al. (2011). Glycosylation regulates specific induction of rice immune responses by Acidovorax avenae flagellin. *J. Biol. Chem.* 286: 25519–25530.
19 Gomez-Gomez, L., Felix, G., and Boller, T. (1999). A single locus determines sensitivity to bacterial flagellin in Arabidopsis thaliana. *Plant J.* 18: 277–284.
20 Garrido-Oter, R., Nakano, R.T., Dombrowski, N. et al. (2018). Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host Microbe* 24: 155–167.e5.

21 Libault, M., Farmer, A., Brechenmacher, L. et al. (2010). Complete transcriptome of the soybean root hair cell, a single-cell model, and its alteration in response to Bradyrhizobium japonicum infection. *Plant Physiol.* 152: 541–552.

22 Gourion, B., Berrabah, F., Ratet, P., and Stacey, G. (2015). Rhizobiumlegume symbioses: the crucial role of plant immunity. *Trends Plant Sci.* 20: 186–194.

23 Singh, S. and Parniske, M. (2012). Activation of calcium- and calmodulin-dependent protein kinase (CCaMK), the central regulator of plant root endosymbiosis. *Curr. Opin. Plant Biol.* 15: 444–453.

24 Buendia, L., Wang, T., Girardin, A., and Lefebvre, B. (2016). The LysM receptor-like kinase SlLYK10 regulates the arbuscular mycorrhizal symbiosis in tomato. *New Phytol.* 210: 184–195.

25 Zhang, X., Dong, W., Sun, J. et al. (2015). The receptor kinase CERK1 has dual functions in symbiosis and immunity signalling. *Plant J.* 81: 258–267.

26 Rey, T., Chatterjee, A., Buttay, M. et al. (2015). Medicago truncatula symbiosis mutants affected in the interaction with a biotrophic root pathogen. *New Phytol.* 206: 497–500.

27 Rey, T., Nars, A., Bonhomme, M. et al. (2013). NFP, a LysM protein controlling Nod factor perception, also intervenes in Medicago truncatula resistance to pathogens. *New Phytol.* 198: 875–886.

28 Smakowska-Luzan, E., Mott, G.A., Parys, K. et al. (2018). An extracellular network of Arabidopsis leucine-rich repeat receptor kinases. *Nature* 553: 342–346.

29 Jourdan, E., Henry, G., Duby, F. et al. (2009). Insights into the defenserelated events occurring in plant cells following perception of surfactin-type lipopeptide from Bacillus subtilis. *Mol. Plant-Microbe Interact.* 22: 456–468.

30 Han, S., Li, D., Trost, E. et al. (2016). Systemic responses of barley to the 3-hydroxy-decanoyl-homoserine lactone producing plant beneficial endophyte Acidovorax radicis N35. *Front. Plant Sci.* 7: 1868.

31 Mathesius, U., Mulders, S., Gao, M. et al. (2003). Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc. Natl. Acad. Sci. U. S. A.* 100: 1444–1449.

32 Cha, C., Gao, P., Chen, Y.C. et al. (1998). Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria. *Mol. Plant-Microbe Interact.* 11: 1119–1129.

33 von Bodman, S.B., Bauer, W.D., and Coplin, D.L. (2003). Quorum sensing in plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* 41: 455–482.

34 Schikora, A., Schenk, S.T., and Hartmann, A. (2016). Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the N-acyl homoserine lactone group. *Plant Mol. Biol.* 90: 605–612.

35 Tanaka, K., Cho, S.H., Lee, H. et al. (2015). Effect of lipochitooligosaccharide on early growth of C4 grass seedlings. *J. Exp. Bot.* 66: 5727–5738.

36 Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43: 205–227.

37 Nguyen, D., Rieu, I., Mariani, C., and van Dam, N.M. (2016). How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Mol. Biol.* 91: 727–740.

38 Yoneyama, K., Xie, X., Kim, H.I. et al. (2012). How do nitrogen and phosphorus deficiencies affect strigolactone production and exudation? *Planta* 235: 1197–1207.

39 Katsir, L., Schilmiller, A.L., Staswick, P.E. et al. (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc. Natl. Acad. Sci. U. S. A.* 105: 7100–7105.

40 Wasternack, C. and Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Ann. Bot.* 111: 1021–1058.

41 Ma, K.-W. and Ma, W. (2016). Phytohormone pathways as targets of pathogens to facilitate infection. *Plant Mol. Biol.* 91: 713–725.

42 Singh, U.P., Sarma, B.K., and Singh, D.P. (2003). Effect of plant growthpromoting Rhizobacteria and culture filtrate of Sclerotium rolfsii on phenolic and salicylic acid contents in chickpea (Cicer arietinum). *Curr. Microbiol.* 46: 131–140.

43 Li, Y., Gu, Y., Li, J. et al. (2015). Biocontrol agent Bacillus amyloliquefaciens LJ02 induces systemic resistance against cucurbits powdery mildew. *Front. Microbiol.* 6: 883.

44 Srivastava, S., Chaudhry, V., Mishra, A. et al. (2012). Gene expression profiling through microarray analysis in Arabidopsis thaliana colonized by Pseudomonas putida MTCC5279, a plant growth promoting rhizobacterium. *Plant Signal. Behav.* 7: 235–245.

45 Pinedo, I., Ledger, T., Greve, M., and Poupin, M.J. (2015). Burkholderia phytofirmans PsJN induces long-term metabolic and transcriptional changes involved in Arabidopsis thaliana salt tolerance. *Front. Plant Sci.* 6: 466.

46 Bodenhausen, N., Bortfeld-Miller, M., Ackermann, M., and Vorholt, J.A. (2014). A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genet.* 10: e1004283.

47 Lebeis, S.L., Paredes, S.H., Lundberg, D.S. et al. (2015). PLANT MICROBIOME. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349: 860–864.

48 Clark, N.M., de Luis Balaguer, M.A., and Sozzani, R. (2014). Experimental data and computational modeling link auxin gradient and development in the Arabidopsis root. *Front. Plant Sci.* 5: 328.

49 Liao, C.Y., Smet, W., Brunoud, G. et al. (2015). Reporters for sensitive and quantitative measurement of auxin response. *Nat. Methods* 12: 207–210.

50 Fendrych, M., Leung, J., and Friml, J. (2016). TIR1/AFB-Aux/IAA auxin perception mediates rapid cell wall acidification and growth of Arabidopsis hypocotyls. *elife* 5: e19048.

51 Castello, M.J., Medina-Puche, L., Lamilla, J., and Tornero, P. (2018). NPR1 paralogs of Arabidopsis and their role in salicylic acid perception. *PLoS One* 13: e0209835.

52 Magne, K., George, J., Berbel Tornero, A. et al. (2018). Lotus japonicus NOOT-BOPCOCH- LIKE1 is essential for nodule, nectary, leaf and flower development. *Plant J.* 94: 880–894.

53 Seyfferth, C. and Tsuda, K. (2014). Salicylic acid signal transduction: the initiation of biosynthesis, perception and transcriptional reprogramming. *Front. Plant Sci.* 5: 697.

54 Tsuda, K., Sato, M., Stoddard, T. et al. (2009). Network properties of robust immunity in plants. *PLoS Genet.* 5: e1000772.

55 Hillmer, R.A., Tsuda, K., Rallapalli, G. et al. (2017). The highly buffered Arabidopsis immune signaling network conceals the functions of its components. *PLoS Genet.* 13: e1006639.

56 Marin-de la Rosa, N. and Falter-Braun, P. (2015). Primer on protein–protein interaction maps. *eLS.* https://doi.org/10.1002/9780470015902.a0006205.pub2.

57 Consortium, Arabidopsis (2011). Evidence for network evolution in an "Arabidopsis" interactome map. *Science* 333: 601–607.

58 Jones, A.M., Xuan, Y., Xu, M. et al. (2014). Border control–a membrane-linked interactome of Arabidopsis. *Science* 344: 711–716.

59 Klopffleisch, K., Phan, N., Augustin, K. et al. (2011). Arabidopsis G-protein interactome reveals connections to cell wall carbohydrates and morphogenesis. *Mol. Syst. Biol.* 7: 532.

60 Li, S., Musungu, B., Lightfoot, D., and Ji, P. (2018). The interactomic analysis reveals pathogenic protein networks in Phomopsis longicolla underlying seed decay of soybean. *Front. Genet.* 9: 104.

61 Dou, D. and Zhou, J.M. (2012). Phytopathogen effectors subverting host immunity: different foes, similar battleground. *Cell Host Microbe* 12: 484–495.

62 Mukhtar, M.S., Carvunis, A.R., Dreze, M. et al. (2011). Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science* 333: 596–601.

63 Wessling, R., Epple, P., Altmann, S. et al. (2014). Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. *Cell Host Microbe* 16: 364–375.

64 Crua Asensio, N., Munoz Giner, E., de Groot, N.S., and Torrent Burgas, M. (2017). Centrality in the host-pathogen interactome is associated with pathogen fitness during infection. *Nat. Commun.* 8: 14092.

65 Miwa, H. and Okazaki, S. (2017). How effectors promote beneficial interactions. *Curr. Opin. Plant Biol.* 38: 148–154.

66 Berendsen, R.L., Vismans, G., Yu, K. et al. (2018). Disease-induced assemblage of a plant beneficial bacterial consortium. *ISME J.* 12: 1496–1507.

67 Okazaki, S., Kaneko, T., Sato, S., and Saeki, K. (2013). Hijacking of leguminous nodulation signaling by the rhizobial type III secretion system. *Proc. Natl. Acad. Sci. U. S. A.* 110: 17131–17136.

68 Schumacher, J., Waite, C.J., Bennett, M.H. et al. (2014). Differential secretome analysis of Pseudomonas syringae pv tomato using gel-free MS proteomics. *Front. Plant Sci.* 5: 242.

69 Hacquard, S., Spaepen, S., Garrido-Oter, R., and Schulze-Lefert, P. (2017). Interplay between innate immunity and the plant microbiota. *Annu. Rev. Phytopathol.* 55: 565–589.

70 Weiberg, A., Wang, M., Lin, F.M. et al. (2013). Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342: 118–123.

71 An, Q., Ehlers, K., Kogel, K.H. et al. (2006). Multivesicular compartments proliferate in susceptible and resistant MLA12-barley leaves in response to infection by the biotrophic powdery mildew fungus. *New Phytol.* 172: 563–576.

72 Langfelder, P. and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinf.* 9: 559.

73 Saelens, W., Cannoodt, R., and Saeys, Y. (2018). A comprehensive evaluation of module detection methods for gene expression data. *Nat. Commun.* 9: 1090.

74 Kim, M.S., Zhang, H., Yan, H. et al. (2018). Characterizing co-expression networks underpinning maize stalk rot virulence in Fusarium verticillioides through computational subnetwork module analyses. *Sci. Rep.* 8: 8310.

75 Whipps, J.M. (1990). Carbon economy. In: *The Rhizosphere* (ed. J.M. Lynch), 59–97. Chichester, UK: Wiley.

76 Jacoby, R., Peukert, M., Succurro, A. et al. (2017). The role of soil microorganisms in plant mineral nutrition current knowledge and future directions. *Front. Plant Sci.* 8: 1617.

77 Topfer, N., Kleessen, S., and Nikoloski, Z. (2015). Integration of metabolomics data into metabolic networks. *Front. Plant Sci.* 6: 49.

78 Heavner, B.D. and Price, N.D. (2015). Comparative analysis of yeast metabolic network models highlights progress, opportunities for metabolic reconstruction. *PLoS Comput. Biol.* 11: e1004530.

79 Botero, D., Alvarado, C., Bernal, A. et al. (2018). Network analyses in plant pathogens. *Front. Microbiol.* 9 (35).

80 Mithani, A., Hein, J., and Preston, G.M. (2011). Comparative analysis of metabolic networks provides insight into the evolution of plant pathogenic and nonpathogenic lifestyles in *Pseudomonas*. *Mol. Biol. Evol.* 28: 483–499.

81 Rodenburg, S.Y.A., Seidl, M.F., de Ridder, D., and Govers, F. (2018). Genome-wide characterization of *Phytophthora infestans* metabolism: a systems biology approach. *Mol. Plant Pathol.* 19: 1403–1413.

82 Raghothama, K.G. (1999). Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 665–693.

83 Castrillo, G., Teixeira, P.J., Paredes, S.H. et al. (2017). Root microbiota drive direct integration of phosphate stress and immunity. *Nature* 543: 513–518.

84 Lu, Y.T., Li, M.Y., Cheng, K.T. et al. (2014). Transgenic plants that express the phytoplasma effector SAP11 show altered phosphate starvation and defense responses. *Plant Physiol.* 164: 1456–1469.

85 McGrann, G.R., Andongabo, A., Sjokvist, E. et al. (2016). The genome of the emerging barley pathogen Ramularia collo-cygni. *BMC Genomics* 17: 584.

86 Fiorilli, V., Vannini, C., Ortolani, F. et al. (2018). Omics approaches revealed how arbuscular mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in wheat. *Sci. Rep.* 8: 9625.

87 van der Heijden, M.G.A. and Hartmann, M. (2016). Networking in the plant microbiome. *PLoS Biol.* 14 (2): e1002378. https://doi.org/10.1371/journal.pbio.1002378 PMID: 26871440.

88 Bai, Y., Muller, D.B., Srinivas, G. et al. (2015). Functional overlap of the arabidopsis leaf and root microbiota. *Nature* 528: 364–369.

89 Schlaeppi, K. and Bulgarelli, D. (2014). The plant microbiome at work. *Mol. Plant-Microbe Interact.* 28 (3): 212–217.

90 Nemergut, D.R., Schmidt, S.K., Fukami, T. et al. (2013). Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* 77 (3): 342–356. https://doi.org/10.1128/MMBR.00051-12 PMID: 24006468.

91 Agler, M.T., Ruhe, J., Kroll, S. et al. (2016). Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* 14 (1).

92 Bulgarelli, D., Rott, M., Schlaeppi, K. et al. (2012). Revealing structure and assembly cues for arabidopsis root-inhabiting bacterial microbiota. *Nature* 488 (7409): 91–95. https://doi.org/10.1038/nature11336. PMID: 22859207.

93 Lundberg, D.S., Lebeis, S.L., Paredes, S.H. et al. (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature* 488 (7409): 86–90. https://doi.org/10.1038/nature11237. PMID: 22859206.

94 Bulgarelli, D., Schlaeppi, K., Spaepen, S. et al. (2013). Structure and functions of the bacterial microbiota of plants. In: *Annual Review of Plant Biology*, vol. 64 (ed. S.S. Merchant). Palo Alto: Annual Reviews, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106. PMID: 23373698.

95 Hacquard, S., Garrido-Oter, R., Gonzalez, A. et al. (2015). Microbiota and host nutrition across plant and animal kingdoms. *Cell Host Microbe* 17 (5): 603–616.

96 Vandenkoornhuyse, P., Quaiser, A., Duhamel, M. et al. (2015). The importance of the microbiome of the plant holobiont. *New Phytol.* 206 (4): 1196–1206. https://doi.org/10.1111/nph. 13312. PMID: 25655016.

97 Louca, S., Jacques, S.M.S., Pires, A.P.F. et al. (2016). High taxonomic variability despite stable functional structure across microbial communities. *Nat. Ecol. Evol.* 1: 15.

98 Truyens, S., Weyens, N., Cuypers, A., and Vangronsveld, J. (2015). Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environ. Microbiol. Rep.* 7 (1): 40–50.

99 Ottesen, A.R., Gorham, S., Reed, E. et al. (2016). Using a control to better understand phyllosphere microbiota. *PLoS One* 11 (9): e0163482. https://doi.org/10.1371/journal.pone.0163482. PMID: 27669159.

100 Wagner, M.R., Lundberg, D.S., Coleman-Derr, D. et al. (2014). Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild arabidopsis relative. *Ecol. Lett.* 17 (6): 717–726. https://doi.org/10.1111/ele.12276. PMID: 24698177.

101 Mueller, U.G. and Sachs, J.L. (2015). Engineering microbiomes to improve plant and animal health. *Trends Microbiol.* 23 (10): 606–617. https://doi.org/10.1016/j.tim.2015.07.009. PMID: 26422463.

102 Ritpitakphong, U., Falquet, L., Vimoltust, A. et al. (2016). The microbiome of the leaf surface of arabidopsis protects against a fungal pathogen. *New Phytol.* 210 (3): 1033–1043.

103 Lakshmanan, V. (2015). Root microbiome assemblage is modulated by plant host factors. In: *Plant Microbe Interactions*, Advances in Botanical Research, vol. 75 (ed. H. Bais and J. Sherrier), 57–79.

104 Berg, G., Rybakova, D., Grube, M., and Koberl, M. (2016). The plant microbiome explored: implications for experimental botany. *J. Exp. Bot.* 67 (4): 995–1002. https://doi.org/10.1093/jxb/erv466. PMID: 26547794.

105 Lebeis, S.L., Paredes, S.H., Lundberg, D.S. et al. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349 (6250): 860–864. https://doi.org/10.1126/science.aaa8764. PMID: 26184915.

106 Werner, G.D. and Kiers, E.T. (2015). Order of arrival structures arbuscular mycorrhizal colonization of plants. *New Phytol.* 205 (4): 1515–1524. https://doi.org/10.1111/nph.13092. PMID: 25298030.

107 Yuan, Z., Druzhinina, I.S., Labbe, J. et al. (2016). Specialized microbiome of a halophyte and its role in helping non-host plants to withstand salinity. *Sci. Rep.* 6: 32467.

108 Mendes, R. and Raaijmakers, J.M. (2015). Cross-kingdom similarities in microbiome functions. *ISME J.* 9: 1905–1907.

109 Lakshmanan, V., Selvaraj, G., and Bais, H.P. (2014). Functional soil microbiome: belowground solutions to an above ground problem. *Plant Physiol.* 166: 689–700.

110 Stuart, L.M., Paquette, N., and Boyer, L. (2013). Effector-triggered versus pattern-triggered immunity: how animals sense pathogens. *Nat. Rev. Immunol.* 13: 199–206.

111 Haldar, S. and Sengupta, S. (2015). Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. *Open Microbiol. J.* 9: 1–7.

112 Chaparro, J.M., Badri, D.V., Bakker, M.G. et al. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One* 8: e55731.

113 Akiyama, K., Matsuzaki, K., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824–827.

114 Reinhold-Hurek, B., Bunger, W., Burbano, C.S. et al. (2015). Roots shaping their microbiome: global hotspots for microbial activity. *Annu. Rev. Phytopathol.* 53: 403–424.

115 Ottesen, A.R., Pena, A.G., and White, J.R. (2013). Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum (tomato). *BMC Microbiol.* 13: 114.

116 Mendes, R., Kruijt, M., and de Bruijn, I. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332: 1097–1100.

117 Cordovez, V., Carrion, V.J., and Etalo, D.W. (2015). Diversity and functions of volatile organic compounds produced by Streptomyces from a disease-suppressive soil. *Front. Microbiol.* 6: 1081.

118 Ardanov, P., Sessitsch, A., Haggman, H. et al. (2012). Methylobacterium-induced endophyte community changes correspond with protection of plants against pathogen attack. *PLoS One* 7: e46802.

119 Mendes, R., Garbeva, P., and Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 37: 634–663.

120 Jimenez, P.N., Koch, G., Thompson, J.A. et al. (2012). The multiple signaling systems regulating virulence in Pseudomonas aeruginosa. *Microbiol. Mol. Biol. Rev.* 76: 46–65.

121 Hardoim, P.R., van Overbeek, L.S., and Berg, G. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.* 79: 293–320.

122 Berg, G., Krause, R., and Mendes, R. (2015). Cross-kingdom similarities in microbiome ecology and biocontrol of pathogens. *Front. Microbiol.* 6: 1311.

123 Keller, N.P., Turner, G., and Bennett, J.W. (2005). Fungal secondary metabolism – from biochemistry to genomics. *Nat. Rev. Microbiol.* 3: 937–947.

124 Yin, W. and Keller, N.P. (2011). Transcriptional regulatory elements in fungal secondary metabolism. *J. Microbiol.* 49: 329–339.

125 Demain, A.L. and Fang, A. (2000). The natural functions of secondary metabolites. *Adv. Biochem. Eng. Biotechnol.* 69: 1–39.

126 Bilyk, O. and Luzhetskyy, A. (2016). Metabolic engineering of natural product biosynthesis in actinobacteria. *Curr. Opin. Biotechnol.* 42: 98–107.

127 Tata, A., Perez, C., Campos, M.L. et al. (2015). Imprint desorption electrospray ionization mass spectrometry imaging for monitoring secondary metabolites production during antagonistic interaction of fungi. *Anal. Chem.* 87: 12298–12304.

128 Johnstone, T.C. and Nolan, E.M. (2015). Beyond iron: non-classical biological functions of bacterial siderophores. *Dalton Trans.* 44: 6320–6339.

129 Schmerk, C.L., Welander, P.V., and Hamad, M.A. (2015). Elucidation of the Burkholderia cenocepacia hopanoid biosynthesis pathway uncovers functions for conserved proteins in hopanoid-producing bacteria. *Environ. Microbiol.* 17: 735–750.

130 Malott, R.J., Steen-Kinnaird, B.R., Lee, T.D., and Speert, D.P. (2012). Identification of hopanoid biosynthesis genes involved in polymyxin resistance in Burkholderia multivorans. *Antimicrob. Agents Chemother.* 56: 464–471.

131 Lopez-Lara, I.M., Sohlenkamp, C., and Geiger, O. (2003). Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. *Mol. Plant-Microbe Interact.* 16: 567–579.

132 Chamoun, R., Aliferis, K.A., and Jabaji, S. (2015). Identification of signatory secondary metabolites during mycoparasitism of Rhizoctonia solani by Stachybotrys elegans. *Front. Microbiol.* 6: 353.

133 Prakash, V. (2015). Endophytic fungi as resource of bioactive compounds. *Int J Pharm. Bio. Sci* 6: 887–898.

134 Suryanarayanan, T.S. and Shaanker, R.U. (2015). Fungal endophytes – biology and bioprospecting preface. *Curr. Sci.* 109: 37–38.

135 Schulz, B. and Boyle, C. (2005). The endophytic continuum. *Mycol. Res.* 109: 661–686.

136 Strobel, G.A. (2003). Endophytes as sources of bioactive products. *Microbes Infect.* 5: 535–544.

137 Abe, N., Sugimoto, O., Arakawa, T. et al. (2001). Sorbicillinol, a key intermediate of bisorbicillinoid biosynthesis in Trichoderma sp USF-2690. *Biosci. Biotechnol. Biochem.* 65: 2271–2279.

138 Salas-Marina, M.A., Silva-Flores, M.A., Uresti-Rivera, E.E. et al. (2011). Colonization of Arabidopsis roots by Trichoderma atroviride promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur. J. Plant Pathol.* 131: 15–26.

139 Araujo, W.L., Marcon, J., Maccheroni, W. Jr. et al. (2002). Diversity of endophytic bacterial populations and their interaction with Xylella fastidiosa in citrus plants. *Appl. Environ. Microbiol.* 68: 4906–4914.

140 Lacava, P.T., Araujo, W.L., Marcon, J. et al. (2004). Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria Xylella fastidiosa, causal agent of citrus-variegated chlorosis. *Lett. Appl. Microbiol.* 39: 55–59.

141 Dourado, M.N., Santos, D.S., Nunes, L.R. et al. (2015). Differential gene expression in Xylella fastidiosa 9a5c during co-cultivation with the endophytic bacterium Methylobacterium mesophilicum SR1.6/6. *J. Basic Microbiol.* 55: 1357–1366.

142 Araujo, W.L., Creason, A.L., and Mano, E.T. (2016). Genome sequencing and transposon mutagenesis of Burkholderia seminalis TC3.4.2R3 identify genes contributing to suppression of orchid necrosis caused by B. gladioli. *Mol. Plant-Microbe Interact.* 29: 435–446.

143 Kim, H.S., Schell, M.A., and Yu, Y. (2005). Bacterial genome adaptation to niches: divergence of the potential virulence genes in three Burkholderia species of different survival strategies. *BMC Genomics* 6: 174.

144 Sim, B.M., Chantratita, N., and Ooi, W.F. (2010). Genomic acquisition of a capsular polysaccharide virulence cluster by non-pathogenic Burkholderia isolates. *Genome Biol.* 11: R89.

145 Bradley, A.S., Pearson, A., Sáenz, J.P., and Marx, C.J. (2010). Adenosylhopane: the first intermediate in hopanoid side chain biosynthesis. *Org. Geochem.* 41: 1075–1081.

146 Welander, P.V., Hunter, R.C., Zhang, L. et al. (2009). Hopanoids play a role in membrane integrity and pH homeostasis in Rhodopseudomonas palustris TIE-1. *J. Bacteriol.* 191: 6145–6156.

147 Seipke, R.F. and Loria, R. (2009). Hopanoids are not essential for growth of Streptomyces scabies 87-22. *J. Bacteriol.* 191: 5216–5223.

148 Jones, D.S., Albrecht, H.L., and Dawson, K.S. (2012). Community genomic analysis of an extremely acidophilic sulfur-oxidizing biofilm. *ISME J.* 6: 158–170.

149 Muller, E.E., Hourcade, E., Louhichi-Jelail, Y. et al. (2011). Functional genomics of dichloromethane utilization in Methylobacterium extorquens DM4. *Environ. Microbiol.* 13: 2518–2535.

150 Nalin, R., Putra, S.R., Domenach, A.M. et al. (2000). High hopanoid/total lipids ratio in Frankia mycelia is not related to the nitrogen status. *Microbiology* 146: 3013–3019.

151 Kulkarni, G., Busset, N., and Molinaro, A. (2015). Specific hopanoid classes differentially affect free-living and symbiotic states of Bradyrhizobium diazoefficiens. *MBio* 6: e01251–e01315.

152 Morissette, D.C., Driscoll, B.T., and Jabaji-Hare, S. (2003). Molecular cloning, characterization, and expression of a cDNA encoding an endochitinase gene from the mycoparasite Stachybotrys elegans. *Fungal Genet. Biol.* 39: 276–285.

153 Morissette, D.C., Dauch, A., Beech, R. et al. (2008). Isolation of mycoparasitic-related transcripts by SSH during interaction of the mycoparasite Stachybotrys elegans with its host Rhizoctonia solani. *Curr. Genet.* 53: 67–80.

154 Martins, M.B. and Carvalho, I. (2007). Diketopiperazines: biological activity and synthesis. *Tetrahedron* 63: 9923–9932.

155 Traxler, M.F., Watrous, J.D., Alexandrov, T. et al. (2013). Interspecies interactions stimulate diversification of the Streptomyces coelicolor secreted metabolome. *MBio* 4.

156 Nutzmann, H.W., Reyes-Dominguez, Y., and Scherlach, K. (2011). Bacteria-induced natural product formation in the fungus Aspergillus nidulans requires Saga/Ada-mediated histone acetylation. *Proc. Natl. Acad. Sci. U. S. A.* 108: 14282–14287.

157 Schroeckh, V., Scherlach, K., and Nutzmann, H.W. (2009). Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in Aspergillus nidulans. *Proc. Natl. Acad. Sci. U. S. A.* 106: 14558–14563.

158 Faraldo-Gomez, J.D. and Sansom, M.S. (2003). Acquisition of siderophores in gram-negative bacteria. *Nat. Rev. Mol. Cell Biol.* 4: 105–116.

159 Visca, P., Imperi, F., and Lamont, I.L. (2007). Pyoverdine siderophores: from biogenesis to biosignificance. *Trends Microbiol.* 15: 22–30.

160 Lamont, I.L., Beare, P.A., Ochsner, U. et al. (2002). Siderophore-mediated signaling regulates virulence factor production in Pseudomonas aeruginosa. *Proc. Natl. Acad. Sci. U. S. A.* 99: 7072–7077.

161 Guan, L.L., Kanoh, K., and Kamino, K. (2001). Effect of exogenous siderophores on iron uptake activity of marine bacteria under iron-limited conditions. *Appl. Environ. Microbiol.* 67: 1710–1717.

162 Partida-Martinez, L.P. and Hertweck, C. (2005). Pathogenic fungus harbours endosymbiotic bacteria for toxin production. *Nature* 437: 884–888.

163 Partida-Martinez, L.P., Monajembashi, S., Greulich, K.O., and Hertweck, C. (2007). Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. *Curr. Biol.* 17: 773–777.

164 Uzum, Z., Silipo, A., Lackner, G. et al. (2015). Structure, genetics and function of an exopolysaccharide produced by a bacterium living within fungal hyphae. *Chembiochem* 16: 387–392.

165 Ross, C., Opel, V., Scherlach, K., and Hertweck, C. (2014). Biosynthesis of antifungal and antibacterial polyketides by Burkholderia gladioli in coculture with Rhizopus microsporus. *Mycoses* 57: 48–55.

166 Blow, F. and Douglas, A.E. (2019). The hemolymph microbiome of insects. *J. Insect Physiol.* 115: 33–39. https://doi.org/10.1016/j.jinsphys.2019.04.002.

167 Douglas, A.E. (1998). Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria buchnera. *Annu. Rev. Entomol.* 43: 17–37. https://doi.org/10.1146/annurev.ento.43.1.17.

168 Thao, M.L. and Baumann, P. (2004). Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. *Appl. Environ. Microbiol.* 70: 3401–3406. https://doi.org/10.1128/AEM.70.6.3401-3406.2004.

169 Thao, M.L., Moran, N.A., Abbot, P. et al. (2000). Cospeciation of psyllids and their primary prokaryotic endosymbionts. *Appl. Environ. Microbiol.* 66: 2898–2905. https://doi.org/10.1128/AEM.66.7.2898-2905.2000.

170 Luan, J.B., Chen, W., Hasegawa, D.K. et al. (2015). Metabolic coevolution in the bacterial symbiosis of whiteflies and related plant sap-feeding insects. *Genome Biol. Evol.* 7: 2635–2647. https://doi.org/10.1093/gbe/evv170.

171 Raychoudhury, R., Sen, R., Cai, Y. et al. (2013). Comparative metatranscriptomic signatures of wood and paper feeding in the gut of the termite reticulitermes flavipes (*Isoptera: Rhinotermitidae*). *Insect Mol. Biol.* 22: 155–171. https://doi.org/10.1111/imb.12011.

172 Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nat. Rev. Microbiol.* 12: 168–180. https://doi.org/10.1038/nrmicro3182.

173 Berlanga, M., Llorens, C., Comas, J., and Guerrero, R. (2016). Gut bacterial community of the *Xylophagous cockroaches Cryptocercus punctulatus* and *Parasphaeria boleiriana*. *PLoS One* 11: e0152400. https://doi.org/10.1371/journal.pone.0152400.

174 Kroiss, J., Kaltenpoth, M., Schneider, B. et al. (2010). Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. *Nat. Chem. Biol.* 6: 261–263. https://doi.org/10.1038/nchembio.331.

175 Shao, Y., Chen, B., Sun, C. et al. (2017). Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. *Cell Chem. Biol.* 24: 66–75. https://doi.org/10.1016/j.chembiol.2016.11.015.

176 Oliver, K.M., Moran, N.A., and Hunter, M.S. (2005). Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proc. Natl. Acad. Sci. U. S. A.* 102: 12795–12800. https://doi.org/10.1073/pnas.0506131102.

177 Oliver, K.M., Russell, J.A., Moran, N.A., and Hunter, M.S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc. Natl. Acad. Sci. U. S. A.* 100: 1803–1807. https://doi.org/10.1073/pnas.0335320100.

178 Vorburger, C., Sandrock, C., Gouskov, A. et al. (2009). Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host–parasitoid interaction. *Evol. Int. J. Organ. Evol.* 63: 1439–1450. https://doi.org/10.1111/j.1558-5646.2009.00660.x.

179 Schmid, M., Sieber, R., Zimmermann, Y.-S., and Vorburger, C. (2012). Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. *Funct. Ecol.* 26: 207–215. https://doi.org/10.1111/j.1365-2435.2011.01904.x.

180 Brandt, J.W., Chevignon, G., Oliver, K.M., and Strand, M.R. (2017). Culture of an aphid heritable symbiont demonstrates its direct role in defence against parasitoids. *Proc. R. Soc. B Biol. Sci.* 284: 20171925. https://doi.org/10.1098/rspb.2017.1925.

181 Frago, E., Dicke, M., and Godfray, H.C.J. (2012). Insect symbionts as hidden players in insect–plant interactions. *Trends Ecol. Evol.* 27: 705–711. https://doi.org/10.1016/j.tree.2012.08.013.

182 Chung, S.H., Rosa, C., Scully, E.D. et al. (2013). Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proc. Natl. Acad. Sci. U. S. A.* 110: 15728–15733. https://doi.org/10.1073/pnas.1308867110.

183 Su, Q., Oliver, K.M., Xie, W. et al. (2015). The whitefly-associated facultative symbiont *Hamiltonella defensa* suppresses induced plant defences in tomato. *Funct. Ecol.* 29: 1007–1018. https://doi.org/10.1111/1365-2435.12405.

184 Xu, H., Zheng, X., Liu, S. et al. (2009). The role of endosymbionts in insect host resistance against adverse factors. *Chin. Bull. Entomol.* 46: 350–354.

185 Libbrecht, R., Gwynn, D.M., and Fellowes, M.D.E. (2007). *Aphidius ervi* preferentially attacks the green morph of the pea aphid, acyrthosiphon pisum. *J. Insect Behav.* 20: 25–32. https://doi.org/10.1007/s10905-006-9055-y.

186 Tsuchida, T., Koga, R., Fujiwara, A., and Fukatsu, T. (2014). Phenotypic effect of "Candidatus Rickettsiella viridis," a facultative symbiont of the pea aphid (*Acyrthosiphon pisum*), and its interaction with a coexisting symbiont. *Appl. Environ. Microbiol.* 80: 525–533. https://doi.org/10.1128/AEM.03049-13.

187 Tsuchida, T., Koga, R., Horikawa, M. et al. (2010). Symbiotic bacterium modifies aphid body color. *Science* 330: 1102–1104. https://doi.org/10.1126/science.1195463.

188 Moran, N.A., Russell, J.A., Koga, R., and Fukatsu, T. (2005). Evolutionary relationships of three new species of enterobacteriaceae living as symbionts of aphids and other insects. *Appl. Environ. Microbiol.* 71: 3302–3310. https://doi.org/10.1128/AEM.71.6.3302-3310.2005.

189 Xia, X., Sun, B., Gurr, G.M. et al. (2018). Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). *Front. Microbiol.* 9 (25). https://doi.org/10.3389/fmicb.2018.00025.

190 Xia, X., Zheng, D., Zhong, H. et al. (2013). DNA sequencing reveals the midgut microbiota of diamondback moth, *Plutella xylostella* (L.) and a possible relationship with insecticide resistance. *PLoS One* 8: e68852. https://doi.org/10.1371/journal.pone.0068852.

191 Barnard, K., Jeanrenaud, A.C.S.N., Brooke, B.D., and Oliver, S. (2019). The contribution of gut bacteria to insecticide resistance and the life histories of the major malaria vector *Anopheles arabiensis* (Diptera: Culicidae). *Sci. Rep.* 9: 9117. https://doi.org/10.1038/s41598-019-45499-z.

192 McFall-Ngai, M., Hadfield, M.G., Bosch, T.C. et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. U. S. A.* 110: 3229–3236. https://doi.org/10.1073/pnas.1218525110.

193 Engel, P. and Moran, N.A. (2013). The gut microbiota of insects – diversity in structure and function. *FEMS Microbiol. Rev.* 37: 699–735. https://doi.org/10.1111/1574-6976.12025.

194 Adamo, S.A. (2013). Parasites: evolution's neurobiologists. *J. Exp. Biol.* 216: 3–10. https://doi.org/10.1242/jeb.073601.

195 Hemarajata, P. and Versalovic, J. (2013). Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Ther. Adv. Gastroenterol.* 6: 39–51. https://doi.org/10.1177/1756283X12459294.

196 Wang, Y. and Kasper, L.H. (2014). The role of microbiome in central nervous system disorders. *Brain Behav. Immun.* 38: 1–12. https://doi.org/10.1016/j.bbi.2013.12.015.

197 Wong, A.C.-N., Wang, Q.-P., Morimoto, J. et al. (2017). Gut microbiota modifies olfactory-guided microbial preferences and foraging decisions in *Drosophila*. *Curr. Biol.* 27: 2397–2404.e4. https://doi.org/10.1016/j.cub.2017.07.022.

198 Farine, J.-P., Habbachi, W., Cortot, J. et al. (2017). Maternally-transmitted microbiota affects odor emission and preference in drosophila larva. *Sci. Rep.* 7: 6062. https://doi.org/10.1038/s41598-017-04922-z.

199 Qiao, H., Keesey, I.W., Hansson, B.S., and Knaden, M. (2019). Gut microbiota affects development and olfactory behavior in drosophila melanogaster. *J. Exp. Biol.* 222: jeb192500. https://doi.org/10.1242/jeb.192500.

200 Behar, A., Yuval, B., and Jurkevitch, E. (2008). Gut bacterial communities in the mediterranean fruit fly (*Ceratitis capitata*) and their impact on host longevity. *J. Insect Physiol.* 54: 1377–1383. https://doi.org/10.1016/j.jinsphys.2008.07.011.

201 Wong, C.N.A., Ng, P., and Douglas, A.E. (2011). Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* 13: 1889–1900. https://doi.org/10.1111/j.1462-2920.2011.02511.x.

202 Ras, E., Beukeboom, L.W., Cáceres, C., and Bourtzis, K. (2017). Review of the role of gut microbiota in mass rearing of the olive fruit fly, bactrocera oleae, and its parasitoids. *Entomol. Exp. Appl.* 164: 237–256. https://doi.org/10.1111/eea.12609.

203 Ramalho, M., Bueno, O., and Saux Moreau, C. (2017). Species-specific signatures of the microbiome from camponotus and colobopsis ants across developmental stages. *PLoS One* 12: e0187461. https://doi.org/10.1371/journal.pone.0187461.

204 Shin, B., Park, S.H., Kim, B.-Y. et al. (2017). Deinococcucins A–D, aminoglycolipids from *Deinococcus* sp., a gut bacterium of the carpenter ant camponotus japonicus. *J. Nat. Prod.* 80: 2910–2916. https://doi.org/10.1021/acs.jnatprod.7b00426.

205 Engel, P., Kwong, W.K., McFrederick, Q. et al. (2016). The bee microbiome: impact on bee health and model for evolution and ecology of host-microbe interactions. *MBio* 7: e02164–15. https://doi.org/10.1128/mBio.02164-15.

206 Minard, G., Mavingui, P., and Moro, C.V. (2013). Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasit. Vectors* 6: 146. https://doi.org/10.1186/1756-3305-6-146.

207 Novakova, E., Woodhams, D.C., Rodríguez-Ruano, S.M. et al. (2017). Mosquito microbiome dynamics, a background for prevalence and seasonality of west nile virus. *Front. Microbiol.* 8: 526. https://doi.org/10.3389/fmicb.2017.00526.

208 Narasimhan, S. and Fikrig, E. (2015). Tick microbiome: the force within. *Trends Parasitol.* 31: 315–323. https://doi.org/10.1016/j.pt.2015.03.010.

209 Hulcr, J., Rountree, N.R., Diamond, S.E. et al. (2012). Mycangia of ambrosia beetles host communities of bacteria. *Microb. Ecol.* 64: 784–793. https://doi.org/10.1007/s00248-012-0055-5.

210 Halpern, M. and Senderovich, Y. (2015). Chironomid microbiome. *Microb. Ecol.* 70: 1–8. https://doi.org/10.1007/s00248-014-0536-9.

211 Himler, A.G., Adachi-Hagimori, T., Bergen, J.E. et al. (2011). Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* 332: 254–256. https://doi.org/10.1126/science.1199410.

212 Ridley, E.V., Wong, A.C.-N., Westmiller, S., and Douglas, A.E. (2012). Impact of the resident microbiota on the nutritional phenotype of *Drosophila melanogaster*. *PLoS One* 7: e36765. https://doi.org/10.1371/journal.pone.0036765.

213 Ridley, E.V., Wong, A.C.N., and Douglas, A.E. (2013). Microbe-dependent and nonspecific effects of procedures to eliminate the resident microbiota from *Drosophila melanogaster*. *Appl. Environ. Microbiol.* 79: 3209–3214. https://doi.org/10.1128/AEM.00206-13.

214 Morimoto, J., Simpson, S.J., and Ponton, F. (2017). Direct and trans-generational effects of male and female gut microbiota in drosophila melanogaster. *Biol. Lett.* 13: 20160966. https://doi.org/10.1098/rsbl.2016.0966.

215 Bing, X., Gerlach, J., Loeb, G., and Buchon, N. (2018). Nutrient-dependent impact of microbes on drosophila suzukii development. *MBio* 9: e02199-17. https://doi.org/10.1128/mBio.02199-17.

216 Petri, L. (1910). Untersuchung uber die darmbakterien der olivenfliege. *Zentralblatt Bakteriol. Parasitenkd. Infekt. Hyg.* 26: 357–367.

217 Capuzzo, C., Firrao, G., Mazzon, L. et al. (2005). 'Candidatus erwinia dacicola', a coevolved symbiotic bacterium of the olive fly bactrocera oleae (Gmelin). *Int. J. Syst. Evol. Microbiol.* 55: 1641–1647. https://doi.org/10.1099/ijs.0.63653-0.

218 Ben-Yosef, M., Pasternak, Z., Jurkevitch, E., and Yuval, B. (2014). Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. *J. Evol. Biol.* 27: 2695–2705. https://doi.org/10.1111/jeb.12527.

219 Estes, A.M., Hearn, D.J., Bronstein, J.L., and Pierson, E.A. (2009). The olive fly endosymbiont, "Candidatus erwinia dacicola," switches from an intracellular existence to an extracellular existence during host insect development. *Appl. Environ. Microbiol.* 75: 7097–7106. https://doi.org/10.1128/AEM.00778-09.

220 Estes, A.M., Hearn, D.J., Burrack, H.J. et al. (2012). Prevalence of *Candidatus erwinia dacicola* in wild and laboratory olive fruit fly populations and across developmental stages. *Environ. Entomol.* 41: 265–274. https://doi.org/10.1603/EN11245.

221 Behar, A., Yuval, B., and Jurkevitch, E. (2005). Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly ceratitis capitata. *Mol. Ecol.* 14: 2637–2643. https://doi.org/10.1111/j.1365-294X.2005.02615.x.

222 Malacrinò, A., Campolo, O., Medina, R.F., and Palmeri, V. (2018). Instar- and host-associated differentiation of bacterial communities in the mediterranean fruit fly *Ceratitis capitata*. *PLoS One* 13: e0194131. https://doi.org/10.1371/journal.pone.0194131.

223 Borm, S., van Buschinger, A., Boomsma, J.J., and Billen, J. (2002). Tetraponera ants have gut symbionts related to nitrogen–fixing root–nodule bacteria. *Proc. R. Soc. Lond. B Biol. Sci.* 269: 2023–2027. https://doi.org/10.1098/rspb.2002.2101.

224 Mason, C.J., Couture, J.J., and Raffa, K.F. (2014). Plant-associated bacteria degrade defense chemicals and reduce their adverse effects on an insect defoliator. *Oecologia* 175: 901–910. https://doi.org/10.1007/s00442-014-2950-6.

225 Behar, A., Ben-Yosef, M., Lauzon, C.R. et al. (2009). Structure and function of the bacterial community associated with the mediterranean fruit fly. *Insect Symbiosis* 3: 251–271. https://doi.org/10.1201/9781420064117.ch11.

226 Lauzon, C. (2003). Symbiotic relationships of tephritids. In: *Insect Symbiosis*, vol. 2 (ed. K. Bourtzis and T. Miller), 115–129. Boca Raton: CRC. https://doi.org/10.1201/9780203009918.ch8.

227 Baumann, P. (2005). Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* 59: 155–189. https://doi.org/10.1146/annurev.micro.59.030804.121041.

228 Zhong, J., Jasinskas, A., and Barbour, A.G. (2007). Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. *PLoS One* 2: e405. https://doi.org/10.1371/journal.pone.0000405.

229 Carter, V., Underhill, A., Baber, I. et al. (2013). Killer bee molecules: antimicrobial peptides as effector molecules to target sporogonic stages of plasmodium. *PLoS Pathog.* 9: e1003790. https://doi.org/10.1371/journal.ppat.1003790.

230 Hultmark, D., Steiner, H., Rasmuson, T., and Boman, H.G. (1980). Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of hyalophora cecropia. *Eur. J. Biochem.* 106: 7–16. https://doi.org/10.1111/j.1432-1033.1980.tb05991.x.

231 Steiner, H., Hultmark, D., Engström, A. et al. (1981). Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* 292: 246–248. https://doi.org/10.1038/292246a0.

232 Yi, H.-Y., Chowdhury, M., Huang, Y.-D., and Yu, X.-Q. (2014). Insect antimicrobial peptides and their applications. *Appl. Microbiol. Biotechnol.* 98: 5807–5822. https://doi.org/10.1007/s00253-014-5792-6.

233 Wu, Q., Patočka, J., and Kuča, K. (2018). Insect antimicrobial peptides, a mini review. *Toxins* 10: 461. https://doi.org/10.3390/toxins10110461.

234 Mitsuhara, I., Matsufuru, H., Ohshima, M. et al. (2000). Induced expression of sarcotoxin IA enhanced host resistance against both bacterial and fungal pathogens in transgenic tobacco. *MPMI* 13: 860–868. https://doi.org/10.1094/MPMI.2000.13.8.860.

235 Sharma, A., Sharma, R., Imamura, M. et al. (2000). Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori*, confers enhanced resistance to bacterial leaf blight in rice. *FEBS Lett.* 484: 7–11. https://doi.org/10.1016/S0014-5793(00)02106-2.

236 Jan, P.-S., Huang, H.-Y., and Chen, H.-M. (2010). Expression of a Synthesized gene encoding cationic peptide cecropin B in transgenic tomato plants protects against bacterial diseases. *Appl. Environ. Microbiol.* 76: 769–775. https://doi.org/10.1128/AEM.00698-09.

237 Rodriguez, M.D., Zamudio, F., Torres, J.A. et al. (1995). Effect of a cecropin-like synthetic peptide (shiva-3) on the sporogonic development of *Plasmodium berghei*. *Exp. Parasitol.* 80: 596–604. https://doi.org/10.1006/expr.1995.1075.

238 Fieck, A., Hurwitz, I., Kang, A.S., and Durvasula, R. (2010). *Trypanosoma cruzi*: synergistic cytotoxicity of multiple amphipathic anti-microbial peptides to *T. cruzi* and potential bacterial hosts. *Exp. Parasitol.* 125: 342–347. https://doi.org/10.1016/j.exppara.2010.02.016.

239 Kim, W., Koo, H., Richman, A.M. et al. (2004). Ectopic expression of a cecropin transgene in the human malaria vector mosquito anopheles gambiae (*Diptera: Culicidae*): effects on susceptibility to plasmodium. *J. Med. Entomol.* 41: 447–455. https://doi.org/10.1603/0022-2585-41.3.447.

240 Kokoza, V., Ahmed, A., Woon Shin, S. et al. (2010). Blocking of *Plasmodium* transmission by cooperative action of cecropin A and defensin A in transgenic *Aedes aegypti* mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 107: 8111–8116. https://doi.org/10.1073/pnas.1003056107.

241 Shen, W., He, P., Xiao, C., and Chen, X. (2018). From antimicrobial peptides to antimicrobial poly(α-amino acid)s. *Adv. Healthc. Mater.* 7: e1800354. https://doi.org/10.1002/adhm.201800354.

242 Peschen, D., Li, H.-P., Fischer, R. et al. (2004). Fusion proteins comprising a fusarium -specific antibody linked to antifungal peptides protect plants against a fungal pathogen. *Nat. Biotechnol.* 22: 732–738. https://doi.org/10.1038/nbt970.

243 Cao, J., de la Fuente-Nunez, C., Ou, R.W. et al. (2018). Yeast-based synthetic biology platform for antimicrobial peptide production. *ACS Synth. Biol.* 7: 896–902. https://doi.org/10.1021/acssynbio.7b00396.

244 Biswaro, L.S., da Costa Sousa, M.G., Rezende, T.M.B. et al. (2018). Antimicrobial peptides and nanotechnology, recent advances and challenges. *Front. Microbiol.* 9: 855. https://doi.org/10.3389/fmicb.2018.00855.

245 Hosokawa, T., Kikuchi, Y., Shimada, M., and Fukatsu, T. (2007). Obligate symbiont involved in pest status of host insect. *Proc. Biol. Sci.* 274: 1979–1984. https://doi.org/10.1098/rspb.2007.0620.

246 Moran, N.A. and Yun, Y. (2015). Experimental replacement of an obligate insect symbiont. *Proc. Natl. Acad. Sci. U. S. A.* 112: 2093–2096. https://doi.org/10.1073/pnas.1420037112.

247 Coon, K.L., Vogel, K.J., Brown, M.R., and Strand, M. (2014). Mosquitoes rely on their gut microbiota for development. *Mol. Ecol.* 23: 2727–2739. https://doi.org/10.1111/mec.12771.

248 Kešnerová, L., Mars, R.A.T., Ellegaard, K.M. et al. (2017). Disentangling metabolic functions of bacteria in the honey bee gut. *PLoS Biol.* 15: e2003467. https://doi.org/10.1371/journal.pbio.2003467.

249 Wong, A.C.-N., Chaston, J.M., and Douglas, A.E. (2013). The inconstant gut microbiota of drosophila species revealed by 16S rRNA gene analysis. *ISME J.* 7: 1922–1932. https://doi.org/10.1038/ismej.2013.86.

250 Hilgenboecker, K., Hammerstein, P., Schlattmann, P. et al. (2008). How many species are infected with wolbachia? – a statistical analysis of current data. *FEMS Microbiol. Lett.* 281: 215–220. https://doi.org/10.1111/j.1574-6968.2008.01110.x.

251 Stouthamer, R., Breeuwer, J.A., and Hurst, G.D. (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53: 71–102. https://doi.org/10.1146/annurev.micro.53.1.71.

252 Werren, J.H. (1997). Biology of wolbachia. *Annu. Rev. Entomol.* 42: 587–609. https://doi.org/10.1146/annurev.ento.42.1.587.

253 Bourtzis, K., Dobson, S.L., Braig, H.R., and O'Neill, S.L. (1998). Rescuing wolbachia have been overlooked. *Nature* 391: 852–853. https://doi.org/10.1038/36017.

254 Saridaki, A. and Bourtzis, K. (2010). Wolbachia: more than just a bug in insects genitals. *Curr. Opin. Microbiol.* 13: 67–72. https://doi.org/10.1016/j.mib.2009.11.005.

255 Segoli, M., Hoffmann, A.A., Lloyd, J. et al. (2014). The effect of virus-blocking wolbachia on male competitiveness of the dengue vector mosquito, *Aedes aegypti*. *PLoS Negl. Trop. Dis.* 8: e3294. https://doi.org/10.1371/journal.pntd.0003294.

256 Alam, U., Medlock, J., Brelsfoard, C. et al. (2011). Wolbachia symbiont infections induce strong cytoplasmic incompatibility in the tsetse fly glossina morsitans. *PLoS Pathog.* 7: e1002415. https://doi.org/10.1371/journal.ppat.1002415.

257 Berasategui, A., Shukla, S., Salem, H., and Kaltenpoth, M. (2016). Potential applications of insect symbionts in biotechnology. *Appl. Microbiol. Biotechnol.* 100: 1567–1577. https://doi.org/10.1007/s00253-015-7186-9.

258 Zabalou, S., Apostolaki, A., Pattas, S. et al. (2008). Multiple rescue factors within a wolbachia strain. *Genetics* 178: 2145–2160. https://doi.org/10.1534/genetics.107.086488.

259 Zhang, L., Zhang, X., Zhang, Y. et al. (2016). A new formulation of *Bacillus thuringiensis*: UV protection and sustained release mosquito larvae studies. *Sci. Rep.* 6: 39425. https://doi.org/10.1038/srep39425.

260 O'Connor, L., Plichart, C., Sang, A.C. et al. (2012). Open release of male mosquitoes infected with a wolbachia biopesticide: field performance and

infection containment. *PLoS Negl. Trop. Dis.* 6: e1797. https://doi.org/10.1371/journal.pntd.0001797.

261 Cattel, J., Kaur, R., Gibert, P. et al. (2016). Wolbachia in European populations of the invasive pest drosophila suzukii: regional variation in infection frequencies. *PLoS One* 11: e0147766. https://doi.org/10.1371/journal.pone.0147766.

262 Ometto, L., Cestaro, A., Ramasamy, S. et al. (2013). Linking genomics and ecology to investigate the complex evolution of an invasive drosophila pest. *Genome Biol. Evol.* 5: 745–757. https://doi.org/10.1093/gbe/evt034.

263 Siozios, S., Cestaro, A., Kaur, R. et al. (2013). Draft genome sequence of the wolbachia endosymbiont of *Drosophila suzukii*. *Genome Announc.* 1: e00032-13. https://doi.org/10.1128/genomeA.00032-13.

264 Cattel, J., Nikolouli, K., Andrieux, T. et al. (2018). Back and forth wolbachia transfers reveal efficient strains to control spotted wing drosophila populations. *J. Appl. Ecol.* 55: 2408–2418. https://doi.org/10.1111/1365-2664.13101.

265 Hamm, C.A., Begun, D.J., Vo, A. et al. (2014). Wolbachia do not live by reproductive manipulation alone: infection polymorphism in *Drosophila suzukii* and *D. subpulchrella*. *Mol. Ecol.* 23: 4871–4885. https://doi.org/10.1111/mec.12901.

266 Mouton, L., Henri, H., Charif, D. et al. (2007). Interaction between host genotype and environmental conditions affects bacterial density in wolbachia symbiosis. *Biol. Lett.* 3: 210–213. https://doi.org/10.1098/rsbl.2006.0590.

267 LePage, D.P., Metcalf, J.A., Bordenstein, S.R. et al. (2017). Prophage WO genes recapitulate and enhance wolbachia-induced cytoplasmic incompatibility. *Nature* 543: 243–247. https://doi.org/10.1038/nature21391.

268 Shropshire, J.D., On, J., Layton, E.M. et al. (2018). One prophage WO gene rescues cytoplasmic incompatibility in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* 115: 4987–4991. https://doi.org/10.1073/pnas.1800650115.

269 Beckmann, J.F., Ronau, J.A., and Hochstrasser, M. (2017). A wolbachia deubiquitylating enzyme induces cytoplasmic incompatibility. *Nat. Microbiol.* 2: 17007. https://doi.org/10.1038/nmicrobiol.2017.7.

270 Bian, G., Xu, Y., Lu, P. et al. (2010). The endosymbiotic bacterium wolbachia induces resistance to dengue virus in aedes aegypti. *PLoS Pathog.* 6: e1000833. https://doi.org/10.1371/journal.ppat.1000833.

271 Van den Hurk, A.F., Hall-Mendelin, S., Pyke, A. et al. (2012). Impact of wolbachia on infection with chikungunya and yellow fever viruses in the mosquito vector aedes aegypti. *PLoS Negl. Trop. Dis.* 6: e1892. https://doi.org/10.1371/journal.pntd.0001892.

272 Aliota, M.T., Peinado, S.A., Velez, I.D., and Osorio, J. (2016). The *w*Mel strain of *Wolbachia* reduces transmission of zika virus by *Aedes aegypti*. *Sci. Rep.* 6: 28792. https://doi.org/10.1038/srep28792.

273 Carrington, L.B., Tran, B.C.N., Le, N.T.H. et al. (2018). Field- and clinically derived estimates of wolbachia-mediated blocking of dengue virus transmission potential in *Aedes aegypti* mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 115: 361–366. https://doi.org/10.1073/pnas.1715788115.

274 Kambris, Z., Cook, P.E., Phuc, H.K., and Sinkins, S.P. (2009). Immune activation by life-shortening wolbachia and reduced filarial competence in mosquitoes. *Science* 326: 134–136. https://doi.org/10.1126/science.1177531.

275 Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A. et al. (2009). A wolbachia symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and plasmodium. *Cell* 139: 1268–1278. https://doi.org/10.1016/j.cell.2009.11.042.

276 Geoghegan, V., Stainton, K., Rainey, S.M. et al. (2017). Perturbed cholesterol and vesicular trafficking associated with dengue blocking in wolbachia-infected aedes aegypti cells. *Nat. Commun.* 8: 526. https://doi.org/10.1038/s41467-017-00610-8.

277 Thomas, S., Verma, J., Woolfit, M., and O'Neill, S.L. (2018). Wolbachia-mediated virus blocking in mosquito cells is dependent on XRN1-mediated viral RNA degradation and influenced by viral replication rate. *PLoS Pathog.* 14: e1006879. https://doi.org/10.1371/journal.ppat.1006879.

278 Hoffmann, A.A., Montgomery, B.L., Popovici, J. et al. (2011). Successful establishment of wolbachia in aedes populations to suppress dengue transmission. *Nature* 476: 454–457. https://doi.org/10.1038/nature10356.

279 Nazni, W.A., Hoffmann, A.A., NoorAfizah, A. et al. (2019). Establishment of wolbachia strain wAlbB in malaysian populations of *Aedes aegypti* for dengue control. *Curr. Biol.* 29: 4241–4248.e5. https://doi.org/10.1016/j.cub.2019.11.007.

280 Ryan, P.A., Turley, A.P., Wilson, G. et al. (2020). Establishment of wMel wolbachia in aedes aegypti mosquitoes and reduction of local dengue transmission in cairns and surrounding locations in northern Queensland, Australia. *Gates Open Res.* 3: 1547. https://doi.org/10.12688/gatesopenres.13061.2.

281 Tantowijoyo, W., Andari, B., Arguni, E. et al. (2020). Stable establishment of wMel wolbachia in *Aedes aegypti* populations in yogyakarta, Indonesia. *PLoS Negl. Trop. Dis.* 14: e0008157. https://doi.org/10.1371/journal.pntd.0008157.

282 Aksoy, S., Weiss, B., and Attardo, G. (2008). Paratransgenesis applied for control of tsetse transmitted sleeping sickness. In: *Transgenesis and the Management of Vector-Borne Disease. Advances in Experimental Medicine and Biology*, vol. 627 (ed. S. Aksoy), 35–48. New York: Springer. https://doi.org/10.1007/978-0-387-78225-6_3.

283 Coutinho-Abreu, I.V., Zhu, K.Y., and Ramalho-Ortigao, M. (2010). Transgenesis and paratransgenesis to control insect-borne diseases: current status and future challenges. *Parasitol. Int.* 59: 1–8. https://doi.org/10.1016/j.parint.2009.10.002.

284 Caragata, E.P. and Walker, T. (2012). Using bacteria to treat diseases. *Expert. Opin. Biol. Ther.* 12: 701–712. https://doi.org/10.1517/14712598.2012.677429.

285 Beard, C.B., Durvasula, R.V., and Richards, F.F. (2000). Bacterial symbiont transformation in Chagas disease vectors. In: *Insect Transgenesis: Methods and Applications* (ed. A.M. Handler and A.A. James), 289–303. Boca Raton: CRC Press. https://doi.org/10.1201/9781420039399.ch16.

286 Beard, C., O'Neill, S.L., Tesh, R. et al. (1993). Modification of arthropod vector competence via symbiotic bacteria. *Parasitol. Today* 9: 179–183. https://doi.org/10.1016/0169-4758(93)90142-3.

287 Durvasula, R.V., Kroger, A., Goodwin, M. et al. (1999). Strategy for introduction of foreign genes into field populations of chagas disease vectors. *Ann. Entomol. Soc. Am.* 92: 937–943. https://doi.org/10.1093/aesa/92.6.937.

288 Hurwitz, I., Hillesland, H., Fieck, A. et al. (2011). The paratransgenic sand fly: a platform for control of Leishmania transmission. *Parasit. Vectors* 4: 82. https://doi.org/10.1186/1756-3305-4-82.

289 Taracena, M.L., Oliveira, P.L., Almendares, O. et al. (2015). Genetically modifying the insect gut microbiota to control chagas disease vectors through systemic RNAi. *PLoS Negl. Trop. Dis.* 9: e0003358. https://doi.org/10.1371/journal.pntd.0003358.

290 De Vooght, L., Caljon, G., De Ridder, K., and Van Den Abbeele, J. (2014). Delivery of a functional anti-trypanosome nanobody in different tsetse fly tissues via a bacterial symbiont, *Sodalis glossinidius*. *Microb. Cell Factories* 13: 156. https://doi.org/10.1186/s12934-014-0156-6.

291 De Vooght, L., Caljon, G., Stijlemans, B. et al. (2012). Expression and extracellular release of a functional anti-trypanosome nanobody® in *Sodalis glossinidius*, a bacterial symbiont of the tsetse fly. *Microb. Cell Factories* 11: 23. https://doi.org/10.1186/1475-2859-11-23.

292 Bisi, D.C. and Lampe, D.J. (2011). Secretion of anti-plasmodium effector proteins from a natural pantoea agglomerans isolate by using PelB and HlyA secretion signals. *Appl. Environ. Microbiol.* 77: 4669–4675. https://doi.org/10.1128/AEM.00514-11.

293 Dinparast Djadid, N., Jazayeri, H., Raz, A. et al. (2011). Identification of the midgut microbiota of an. stephensi and an. maculipennis for their application as a paratransgenic tool against malaria. *PLoS One* 6: e0028484. https://doi.org/10.1371/journal.pone.0028484.

294 Bongio, N.J. and Lampe, D.J. (2015). Inhibition of plasmodium berghei development in mosquitoes by effector proteins secreted from *Asaia* sp. Bacteria using a novel native secretion signal. *PLoS One* 10: e0143541. https://doi.org/10.1371/journal.pone.0143541.

295 Fang, W., Vega-Rodríguez, J., Ghosh, A.K. et al. (2011). Development of transgenic fungi that kill human malaria parasites in mosquitoes. *Science* 331: 1074–1077. https://doi.org/10.1126/science.1199115.

296 Hopkins, D. and Purcell, A. (2002). *Xylella fastidiosa*: cause of pierce's disease of grapevine and other emergent diseases. *Plant Dis.* 86: 1056–1066. https://doi.org/10.1094/PDIS.2002.86.10.1056.

297 Hackett, K.J. (2003). Investigating invasives: it takes a team. *Agric. Res.* 51: 2.

298 Bextine, B.R., Harshman, D., Johnson, M.C., and Miller, T.A. (2004). Impact of pymetrozine on glassy-winged sharpshooter feeding behavior and rate of *Xylella fastidiosa* transmission. *J. Insect Sci.* 4: 34. https://doi.org/10.1673/031.004.3401.

299 Killiny, N., Martinez, R.H., Dumenyo, C.K. et al. (2013). The exopolysaccharide of *Xylella fastidiosa* is essential for biofilm formation, plant virulence, and vector transmission. *Mol. Plant-Microbe Interact.* 26: 10444–10453. https://doi.org/10.1094/MPMI-09-12-0211-R.

300 Saiman, L., Chen, Y., Tabibi, S. et al. (2001). Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. *J. Clin. Microbiol.* 39: 3942–3945. https://doi.org/10.1128/JCM.39.11.3942-3945.2001.

301 Arora, A.K., Forshaw, A., Miller, T.A., and Durvasula, R. (2015). A delivery system for field application of paratransgenic control. *BMC Biotechnol.* 15: 59. https://doi.org/10.1186/s12896-015-0175-3.

302 Leonard, S.P., Powell, J.E., Perutka, J. et al. (2020). Engineered symbionts activate honey bee immunity and limit pathogens. *Science* 367: 573–576. https://doi.org/10.1126/science.aax9039.

303 USEPAO (2015). *What are Biopesticides? US EPA*. https://www.epa.gov/pesticides/biopesticides#what (accessed February 2020).

304 Hajek, A.E. and Delalibera, I. (2010). Fungal pathogens as classical biological control agents against arthropods. *BioControl* 55: 147–158. https://doi.org/10.1007/s10526-009-9253-6.

305 Lacey, L.A. and Georgis, R. (2012). Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *J. Nematol.* 44: 218–225.

306 Ibrahim, M.A., Griko, N., Junker, M., and Bulla, L.A. (2010). *Bacillus thuringiensis*. *Bioeng. Bugs* 1: 31–50. https://doi.org/10.4161/bbug.1.1.10519.

307 Lacey, L.A., Frutos, R., Kaya, H.K., and Vail, P. (2015). Insect pathogens as biological control agents: back to the future. *J. Invertebr. Pathol.* 132: 1–41. https://doi.org/10.1006/bcon.2001.0938.

308 Hurst, M.R., Glare, T.R., Jackson, T.A., and Ronson, C.W. (2000). Plasmid-located pathogenicity determinants of Serratia entomophila, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of Photorhabdus luminescens. *J. Bacteriol.* 182: 5127–5138. https://doi.org/10.1128/jb.182.18.5127-5138.2000.

309 Rippere, K.E., Tran, M.T., Yousten, A.A. et al. (1998). Bacillus popilliae and Bacillus lentimorbus, bacteria causing milky disease in Japanese beetles and

related scarab larvae. *Int. J. Syst. Bacteriol.* 48: 395–402. https://doi.org/10.1099/00207713-48-2-395.

310 Sinha, K.K., Choudhary, A.K., and Kumari, P. (2016). Entomopathogenic fungi. In: *Ecofriendly Pest Management for Food Security*, 475–505. Amsterdam: Elsevier Inc.

311 Solter, L.F., Becnel, J.J., and Oi, D.H. (2012). Chapter 7: microsporidian entomopathogens. In: *Insect Pathology*, 2nde (ed. F.E. Vega and H.K. Kaya), 1–45. San Diego, CA: Elsevier.

312 Chiu, E., Coulibaly, F., and Metcalf, P. (2012). Insect virus polyhedra, infectious protein crystals that contain virus particles. *Curr. Opin. Struct. Biol.* 22: 234–240. https://doi.org/10.1016/j.sbi.2012.02.003.

313 Bravo, A., Gill, S.S., and Soberón, M. (2007). Mode of action of bacillus thuringiensis cry and cyt toxins and their potential for insect control. *Toxicon* 49: 423–435. https://doi.org/10.1016/j.toxicon.2006.11.022.

314 Schnepf, E., Crickmore, N., Van Rie, J. et al. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62: 775–806. https://doi.org/10.1128/MMBR.62.3.775-806.1998.

315 Sanchis, V. (2011). From microbial sprays to insect-resistant transgenic plants: history of the biospesticide *Bacillus thuringiensis:* a review. *Agron. Sustain. Dev.* 31: 217–231. https://doi.org/10.1051/agro/2010027.

316 Mertz, F.P. and Yao, R.C. (1990). *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar mill rum still. *Int. J. Syst. Evol. Microbiol.* 40: 34–39. https://doi.org/10.1099/00207713-40-1-34.

317 Wechsler, S. (2018). *USDA ERS - Recent Trends in GE Adoption*. *USDA*. https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx (accessed July 2020).

318 Racke, K.D. (2006). A reduced risk insecticide for organic agriculture: spinosad case study. In: *Crop Protection Products for Organic Agriculture: Environmental, Health and Efficacy Assessment, ACS Symposium Series*, vol. 947 (ed. A.S. Felsot and K.D. Racke), 92–108. Washington, DC: American Chemical Society. https://doi.org/10.1021/bk-2007-0947.ch007.

319 Katz, M., Hover, B.M., and Brady, S.F. (2016). Culture-independent discovery of natural products from soil metagenomes. *J. Ind. Microbiol. Biotechnol.* 43: 129–141. https://doi.org/10.1007/s10295-015-1706-6.

320 Gabor, E.M., Alkema, W.B.L., and Janssen, D.B. (2004). Quantifying the accessibility of the metagenome by random expression cloning techniques. *Environ. Microbiol.* 6: 879–886. https://doi.org/10.1111/j.1462-2920.2004.00640.x.

321 Hofer, U. (2018). The majority is uncultured. *Nat. Rev. Microbiol.* 16: 716–717. https://doi.org/10.1038/s41579-018-0097-x.

322 Witzgall, P., Kirsch, P., and Cork, A. (2010). Sex pheromones and their impact on pest management. *J. Chem. Ecol.* 36: 80–100. https://doi.org/10.1007/s10886-009-9737-y.

323 Cardé, R.T. and Minks, A.K. (1995). Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol.* 40: 559–585. https://doi.org/10.1146/annurev.en.40.010195.003015.

324 Davis, T.S., Crippen, T.L., Hofstetter, R.W., and Tomberlin, J. (2013). Microbial volatile emissions as insect semiochemicals. *J. Chem. Ecol.* 39: 840–859. https://doi.org/10.1007/s10886-013-0306-z.

325 Lemfack, M.C., Gohlke, B.-O., Toguem, S.M.T. et al. (2017). mVOC 2.0: a database of microbial volatiles. *Nucleic Acids Res.* 46: D1261–D1265. https://doi.org/10.1093/nar/gkx1016.

326 Choudoir, M., Rossabi, S., Gebert, M. et al. (2019). A phylogenetic and functional perspective on volatile organic compound production by actinobacteria. *MSystems* 4: e00295-18. https://doi.org/10.1128/mSystems.00295-18.

327 Medema, M.H., Blin, K., Cimermancic, P. et al. (2011). antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* 39: W339–W346. https://doi.org/10.1093/nar/gkr466.

328 Weber, T., Blin, K., Duddela, S. et al. (2015). antiSMASH 3.0 – a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res.* 43: W237–W243. https://doi.org/10.1093/nar/gkv437.

329 Rappé, M.S. and Giovannoni, S.J. (2003). The uncultured microbial majority. *Annu. Rev. Microbiol.* 57: 369–394. https://doi.org/10.1146/annurev.micro.57.030502.090759.

330 Ballhorn, D.J., Kautz, S., and Schädler, M. (2013). Induced plant defense via volatile production is dependent on rhizobial symbiosis. *Oecologia* 172: 833–846. https://doi.org/10.1007/s00442-012-2539-x.

331 Clardy, J., Fischbach, M.A., and Walsh, C.T. (2006). New antibiotics from bacterial natural products. *Nat. Biotechnol.* 24: 1541–1550. https://doi.org/10.1038/nbt1266.

332 Fontana, A., Reichelt, M., Hempel, S. et al. (2009). The effects of arbuscular mycorrhizal fungi on direct and indirect defense metabolites of plantago lanceolata L. *J. Chem. Ecol.* 35: 833–843. https://doi.org/10.1007/s10886-009-9654-0.

333 Li, L., Chen, J., Yang, Z. et al. (2015). The effect of nano-Mg $(OH)_2$ on insecticidal activity and UV resistance of bacillus thuringiensis protein. *J. Agric. Biotechnol.* 23: 1452–1457.

334 Sharifi, R., Lee, S.-M., and Ryu, C.-M. (2018). Microbe-induced plant volatiles. *New Phytol.* 220: 684–691. https://doi.org/10.1111/nph.14955.

335 Keesey, I.W., Knaden, M., and Hansson, B.S. (2015). Olfactory specialization in *Drosophila suzukii* supports an ecological shift in host preference from rotten to fresh fruit. *J. Chem. Ecol.* 41: 121–128. https://doi.org/10.1007/s10886-015-0544-3.

336 Lasa, R., Tadeo, E., Toledo-Hérnandez, R.A. et al. (2017). Improved capture of drosophila suzukii by a trap baited with two attractants in the same device. *PLoS One* 12: e0188350. https://doi.org/10.1371/journal.pone.0188350.

337 Ishii, Y., Akasaka, N., Goda, I. et al. (2015). Effective trapping of fruit flies with cultures of metabolically modified acetic acid bacteria. *Appl. Environ. Microbiol.* 81: 2265–2273. https://doi.org/10.1128/AEM.03678-14.

338 Stensmyr, M.C., Dweck, H.K.M., Farhan, A. et al. (2012). A conserved dedicated olfactory circuit for detecting harmful microbes in *Drosophila*. *Cell* 151: 1345–1357. https://doi.org/10.1016/j.cell.2012.09.046.

339 Huang, B., Chen, F., Shen, Y. et al. (2018). Advances in targeted pesticides with environmentally responsive controlled release by nanotechnology. *Nanomaterials* 8: 102. https://doi.org/10.3390/nano8020102.

340 Epsky, N.D., Heath, R.R., Dueben, B.D. et al. (1998). Attraction of 3-methyl-1-butanol and ammonia identified from *Enterobacter agglomerans* to *Anastrepha suspensa*. *J. Chem. Ecol.* 24: 1867–1880. https://doi.org/10.1023/A:1022363718193.

341 Wallingford, A.K., Hesler, S.P., Cha, D.H., and Loeb, G. (2016). Behavioral response of spotted-wing drosophila, *Drosophila suzukii* matsumura, to aversive odors and a potential oviposition deterrent in the field. *Pest Manag. Sci.* 72: 701–706. https://doi.org/10.1002/ps.4040.

342 Lam, K., Tsang, M., Labrie, A. et al. (2010). Semiochemical-mediated oviposition avoidance by female house flies, musca domestica, on animal feces colonized with harmful fungi. *J. Chem. Ecol.* 36: 141–147. https://doi.org/10.1007/s10886-010-9741-2.

343 Daisy, B.H., Strobel, G.A., Castillo, U. et al. (2002). Naphthalene, an insect repellent, is produced by muscodor vitigenus, a novel endophytic fungus. *Microbiology* 148: 3737–3741. https://doi.org/10.1099/00221287-148-11-3737.

344 Gliszczyńska, A., Gladkowski, W., Dancewicz, K., and Gabryś, B. (2015). Enantioselective microbial hydroxylation as a useful tool in the production of jasmonate derivatives with aphid deterrent activity. *Curr. Microbiol.* 71: 83–94. https://doi.org/10.1007/s00284-015-0831-9.

345 Skrobiszewski, A., Gladkowski, W., Mazur, M. et al. (2018). Microbial hydrolysis of racemic β-Aryl-γ-ethylidene-γ-lactones and antifeedant activity of the products against alphitobius diaperinus panzer. *Molecules* 23: 1516. https://doi.org/10.3390/molecules23071516.

346 Kajla, M.K., Barrett-Wilt, G.A., and Paskewitz, S.M. (2019). Bacteria: a novel source for potent mosquito feeding-deterrents. *Sci. Adv.* 5: eaau6141. https://doi.org/10.1126/sciadv.aau6141.

347 Leroy, P.D., Sabri, A., Verheggen, F.J. et al. (2011). The semiochemically mediated interactions between bacteria and Insects. *Chemoecology* 21: 113–122. https://doi.org/10.1007/s00049-011-0074-6.

348 Schulz-Bohm, K., Zweers, H., de Boer, W., and Garbeva, P. (2015). A fragrant neighborhood: volatile mediated bacterial interactions in soil. *Front. Microbiol.* 6: 1212. https://doi.org/10.3389/fmicb.2015.01212.

349 Cook, S.M., Khan, Z.R., and Pickett, J.A. (2006). The use of push-pull strategies in integrated pest management. *Annu. Rev. Entomol.* 52: 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407.

350 Kim, J.S. and Je, Y.H. (2012). Milling effect on the control efficacy of spray-dried *Bacillus thuringiensis* technical powder against diamondback moths. *Pest Manag. Sci.* 68: 321–323. https://doi.org/10.1002/ps.2330.

351 Vassilev, N., Vassileva, M., Martos, V. et al. (2020). Formulation of microbial inoculants by encapsulation in natural polysaccharides: focus on beneficial properties of carrier additives and derivatives. *Front. Plant Sci.* 11: 270. https://doi.org/10.3389/fpls.2020.00270.

352 Khot, L.R., Sankaran, S., Maja, J.M. et al. (2012). Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Prot.* 35: 64–70. https://doi.org/10.1016/j.cropro.2012.01.007.

353 Kumar, S., Nehra, M., Dilbaghi, N. et al. (2019). Nano-based smart pesticide formulations: emerging opportunities for agriculture. *J. Control. Release* 294: 131–153. https://doi.org/10.1016/j.jconrel.2018.12.012.

354 Dyck, V.A., Hendrichs, J., and Robinson, A.S. (ed.) (2006). *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, 39–68. Dordrecht: Springer https://doi.org/10.1007/1-4020-4051-2.

355 Vreysen, M.J., Saleh, K., Mramba, F. et al. (2014). Sterile insects to enhance agricultural development: the case of sustainable tsetse eradication on Unguja Island, Zanzibar, using an area-wide integrated pest management approach. *PLoS Negl. Trop. Dis.* 8: e2857. https://doi.org/10.1371/journal.pntd.0002857.

356 Munhenga, G., Brooke, B.D., Gilles, J.R. et al. (2016). Mating competitiveness of sterile genetic sexing strain males (GAMA) under laboratory and semi-field conditions: steps towards the use of the sterile insect technique to control the major malaria vector anopheles arabiensis in South Africa. *Parasit. Vectors* 9: 122. https://doi.org/10.1186/s13071-016-1385-9.

357 Harris, A.F., McKemey, A.R., Nimmo, D. et al. (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat. Biotechnol.* 30: 828–830. https://doi.org/10.1038/nbt.2350.

358 Raza, M.F., Yao, Z., Bai, S. et al. (2020). Tephritidae fruit fly gut microbiome diversity, function and potential for applications. *Bull. Entomol. Res.* 110: 423–437. https://doi.org/10.1017/S0007485319000853.

359 Drew, R.A. and Yuval, B. (2000). The evolution of fruit fly feeding behavior. In: *Fruit Flies (Tephritidae) Phylogeny and Evolution of Behavior* (ed. M. Aluja and A.L. Norrbom), 749–768. Boca Raton: CRC Press. https://doi.org/10.1201/9781420074468.ch27.

360 Ami, E.B., Yuval, B., and Jurkevitch, E. (2010). Manipulation of the microbiota of mass-reared mediterranean fruit flies ceratitis capitata (diptera: tephritidae) improves sterile male sexual performance. *ISME J.* 4: 28–37. https://doi.org/10.1038/ismej.2009.82.

361 Stathopoulou, P., Asimakis, E.D., Khan, M. et al. (2019). Irradiation effect on the structure of bacterial communities associated with the oriental fruit fly, bactrocera dorsalis. *Entomol. Exp. Appl.* 167: 209–219. https://doi.org/10.1111/eea.12770.