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Review Article

Origins of symbiosis: shared mechanisms underlying microbial pathogenesis, commensalism and mutualism of plants and animals

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Abstract

Regardless of the outcome of symbiosis, whether it is pathogenic, mutualistic or commensal, bacteria must first colonize their hosts. Intriguingly, closely related bacteria that colonize diverse hosts with diverse outcomes of symbiosis have conserved host-association and virulence factors. This review describes commonalities in the process of becoming host associated amongst bacteria with diverse lifestyles. Whether a pathogen, commensal or mutualist, bacteria must sense the presence of and migrate towards a host, compete for space and nutrients with other microbes, evade the host immune system, and change their physiology to enable long-term host association. We primarily focus on well-studied taxa, such as *Pseudomonas*, that associate with diverse model plant and animal hosts, with far-ranging symbiotic outcomes. Given the importance of opportunistic pathogens and chronic infections in both human health and agriculture, understanding the mechanisms that facilitate symbiotic relationships between bacteria and their hosts will help inform the development of disease treatments for both humans, and the plants we eat.

Keywords: Symbiosis; host association; *Pseudomonas*; host-microbe interactions; colonization; outer membrane modifications; biofilm; two-component signaling

Introduction: symbiosis is shared trait among pathogens, commensals, and mutualists

Symbiosis means 'living together' and includes pathogens, mutualists and commensals. Regardless of symbiotic lifestyle, bacteria must be adapted to a host environment, and so perhaps unsurprisingly, pathogens and mutualists share a number of host-association factors. Even across very different hosts, there are aspects of a host environment that are shared including the presence of primary and specialized metabolites, and an innate immune system (Haney et al. 2014, Mermigka et al. 2020). Consequentially, there may be common mechanisms, such as evasion or tolerance of immunity, and competition for nutrients, that promote host association across phylogenetically diverse hosts and bacterial lifestyles. While mechanisms of bacterial virulence or mutualism have been extensively reviewed (Huisman and Geurts 2020, Kim et al. 2020, Nyholm and McFall-Ngai 2021), this review explores the shared adaptations among pathogens, commensals and mutualists that allow certain bacterial taxa to be ubiquitously symbiotic. We propose that the process of becoming symbiotic might be the initial evolutionary innovation that predisposes bacteria to becoming mutualists or pathogens.

Here we review common features of how plant- and animal-associated pathogenic and mutualistic bacteria (i) find a host and use host-derived nutrients, (ii) compete within a host niche, (iii) suppress and evade the host immune system, (iv) establish chronic host association, and (v) use signaling cascades to integrate host signals with bacterial physiology (Fig. 1; Table S1). We emphasize examples from *Pseudomonas* and other well-studied bacteria that have diverse lifestyles on phylogenetically distinct hosts. This review will also touch on how we can use our knowledge of host association to either deter pathogen infection, or to promote colonization of beneficial microbes to improve human health and the health of plant resources which humans depend on to survive.

How bacteria find a host and use host-derived nutrients

The selective pressure for bacteria to become host-associated is largely independent of the effect they have on the host (Rohmer et al. 2011). Bacteria free-living in the environment often face a wide range of stressors, including scarce amounts of essential nutrients (Haruta and Kanno 2015). One way in which bacteria can attempt to alleviate or avoid these stresses is to find a host, which through autotrophic or heterotrophic lifestyles can provide a homeostatic, nutrient rich environment. Nutrient acquisition or exchange is a major driver of symbiosis: mutualistic symbioses are largely driven by nutrient sharing (Noë and Kiers 2018, Kim et al. 2020), and even pathogenic bacteria may disseminate into host organs to acquire nutrients (Rohmer et al. 2011). As a result, there are selective pressures for microbes to live symbiotically irrespective of the outcome on host health.

Finding a Host: bacterial Motility and Chemotaxis

Hosts exude a large number of chemoattractants, which include molecules that are rare or unstable in the environment, and so

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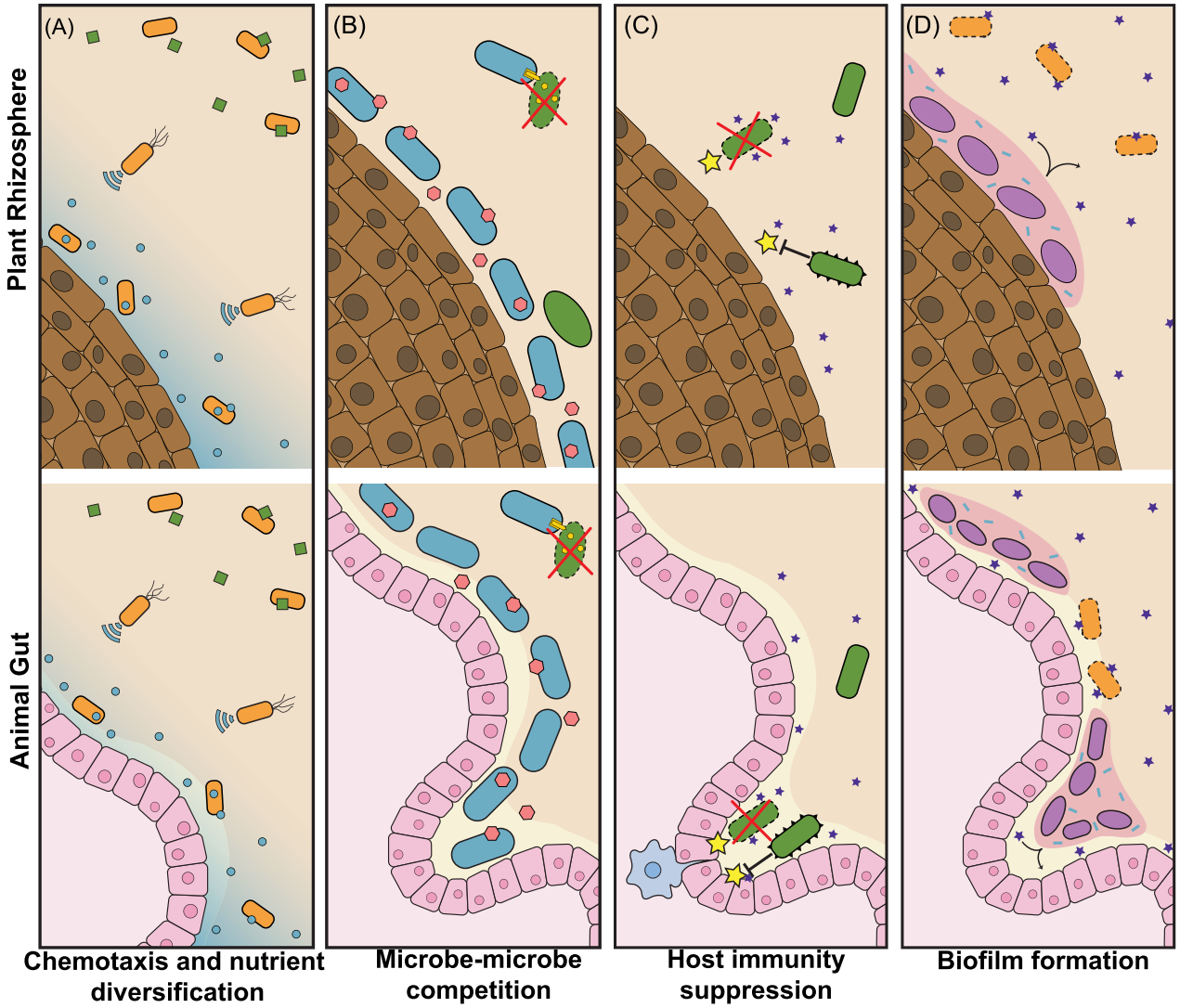

Figure 1. Shared features of bacterial symbiosis across pathogens and mutualists that associate with plants and animals. **(A)** In both plant roots and the human gut, the host secretes chemoattractants (blue gradient), which alert the bacteria to their presence. Bacteria use chemoreceptors to sense these molecules and move towards the host. Once the bacteria are in close proximity to the environment, they can diversify their metabolism to use host-derived nutrients (blue circles) instead of environmental nutrients (green squares). **(B)** Once bacteria are in close proximity to a host, they must compete with other bacteria. They can do this by secreting toxins (pink pentagons) into the environment which inhibit competitors, or using T6SS to secrete toxins (yellow pentagons) directly into neighboring bacteria. **(C)** Close proximity to a host also triggers a host innate immune response including the production of a reactive oxygen species (ROS) burst (yellow stars) and the production of antimicrobial peptides (purple stars). Bacteria can alter their outer membrane to help avoid triggering a host immune response, and make their membrane less permeable to inhibitory host-derived compounds **(D)** Biofilm formation on a plant root or in the gut allows for chronic association with a host and provides protection against many stressors including host-secreted antimicrobial peptides (purple stars).

alert microbes to a host's presence (Fig. 1A). These chemoattractants may include both primary and specialized metabolites that may act as nutrients or signaling molecules (Lowe-Power et al. 2018, Shi et al. 2019). Primary metabolites include hormones, proteins, steroids, sugars, and amino acids and frequently serve a dual role as both chemoattractants and nutrients (Hughes and Sperandio 2008). The diffusion of signals leads to the formation of a concentration gradient, which bacteria sense and use to move towards the host (Sourjik and Wingreen 2012). In turn, many bacteria, ranging from mutualistic *Rhizobium leguminosarum* to commensal *Pseudomonas fluorescens* and pathogenic *Campylobacter jejuni* are chemoattracted to nutrients including amino acids, sugars, and organic acids (Singh and Arora 2001, Vegge et al. 2009). Because bacteria may be limited for carbon and nitrogen in the environment, there may be a strong selective pressure for diverse bacteria to couple chemotaxis with adaptations that would allow them to colonize a host.

In order to take advantage of nutrient-rich host environments, it is necessary for bacteria to sense the presence of a host and move towards it. While some symbiotic bacteria are acquired from an organism's mother (vertical transition), many are obtained from the environment (horizontal transmission). In animals, skin and gut bacteria are acquired during birth and shaped by the surrounding environment (Blaser and Dominguez-Bello 2016, Wang et al. 2020). In plants, vertical transmission makes a relatively small contribution to the microbiome, and most microbes are transmitted from the air or the soil (Torres-Cortés et al. 2018, Trivedi et al. 2020). Bacterial sensing and adaptation to a host

environment occurs through two related mechanisms: sensing of compounds produced by the host (chemosensing), and movement towards the host (chemotaxis) (Fig. 1A).

Bacterial chemotaxis and motility is broadly required for symbiosis by both pathogens (Josenhans and Suerbaum 2002) and mutualists (Raina et al. 2019) (Table S1A). Diverse plant-associated nitrogen-fixing mutualistic rhizobia require specialized motility and chemotaxis machinery to sense and initiate symbiosis (reviewed in Aroney et al. 2021). Rhizobia can sense host-produced metabolites including carbohydrates, phenolics, sugars and organic acids often using methyl accepting chemotaxis proteins (Mcp) that can sense chemotactic environmental signals common across both prokaryotes and archaea (Gaworzewska and Carlile 1982, Yost et al. 1998, Salah Ud-Din and Roujeinikova 2017). Genetic screens for plant colonization genes by the commensal *P. fluorescens* also identified genes involved in chemotaxis and motility (de Weger et al. 1987). Similarly, chemotaxis (*cheY*) and motility (*fliGHI*) genes are required for animal pathogens, like *Salmonella,* to cause disease (Stecher et al. 2004). While chemotaxis and motility are also required for non-host associated environments, the ability to sense and move towards a potential host is a necessary step for many environmental organisms to initiate symbiosis.

Metabolic Innovations for Symbiosis

Once a bacterium successfully finds a host, and a suitable niche within that host, it must be able to use the available nutrients. While autotrophic or phototrophic eukaryotic hosts provide an opportunity for microbes to access nutrients that would otherwise be limiting, the ability to access and compete for these nutrients might require unique evolutionary innovations. For instance, host-associated carbon and nitrogen is more likely to be in the form of peptides or complex carbohydrates than inorganic nitrogen or simple sugars (Rowland et al. 2018, Vives-Peris et al. 2020). As a result, diversification of catabolic enzymes involved in acquiring specialized and complex carbon and nitrogen sources may be a key component of establishing symbiosis.

In addition to carbon and nitrogen, iron acquisition and tolerance plays a pivotal role in symbiosis; iron is required for both host immune function and for bacterial growth (Fig. 1B). Iron is an essential nutrient, is a cofactor for an estimated 2% of proteins, and is essential in the biosynthesis of heme, the production of iron sulfur clusters (Andreini et al. 2018, Liu et al. 2020), and the synthesis of chlorophyll (Betoudji et al. 2020). However iron also reacts with hydrogen peroxide to generate reactive oxygen through Fenton chemistry, which plants and animals both harness to combat pathogens (De Gara et al. 2003, Verbon et al. 2017). As a result, iron tension is central to the outcome of host-microbe interactions across diverse hosts.

Microbial siderophore production is essential for virulence, mutualism and commensalism (Mirleau et al. 2000, Bairwa et al. 2017). In *P. aeruginosa*, the siderophore pyoverdine is essential for virulence and both sequesters iron in a host environment (Meyer et al. 1996, Minandri et al. 2016) and also acts as a signaling molecule to trigger the bacteria producing it to upregulate the production of virulence factors (Wilderman et al. 2001, Lamont et al. 2002). Siderophore production is also essential to microbial virulence in other pathogens ranging from the human fungal pathogen *Cryptococcus*, to the plant pathogen *Erwinia* (Bairwa et al. 2017, Verbon et al. 2017). These findings indicate that a robust ability to compete for iron is essential to establishing host association across host environments.

As eukaryotic-associated iron is often sequestered in enzyme active sites, heme, or organelles, symbiosis may require specialized adaptations to acquire specialized iron. Indeed, human fungal pathogens, such as *Cryptococcus* spp., can uptake heme using the CIG1 transporter for use (Won et al. 2008). The pathogen *P. aeruginosa* is similarly able to use heme as an iron source, as well as *Vibrio* species that range in their interactions from mutualistic symbioses with marine organisms, to pathogens of humans and fish (Cornelis and Dingemans 2013, Payne et al. 2016). These findings suggest that the ability to use heme and other host-specific sources of iron may predispose groups of bacteria to diverse host-associated lifestyles.

For bacteria encountering a new host, evidence suggests that they can rapidly adapt to make use of novel metabolites available in host environments. This occurs through mutations that can lead to diversification of bacterial metabolism, allowing for survival in previously uninhabitable niches and continued proliferation and success as a species (Boles et al. 2004, Li et al. 2021). Directed evolution experiments with plants or insects have shown that beneficial mutations, including changes in nutrient utilization potential, can occur in just a few iterations of host selection (Rafaluk-Mohr et al. 2018, Li et al. 2021, Koga et al. 2022). This increased metabolic versatility can be considered a virulence factor if this allows for increased virulence of the bacterium in new niches (Rohmer et al. 2011). This suggests that host-associated bacteria can rapidly fine tune specific traits that allow them to better adapt to new environments.

A number of bacterial clades that are largely host associated can rapidly adapt to new environments by optimizing nutrient usage. For example, *Vibrio cholerae* can vary in their ability to use sialic acid, a nutrient which is available in a host gut (Almagro-Moreno and Boyd 2009). The genes required for sialic acid utilization are encoded on an excisable region of the genome making this trait easily shared between different strains of *Vibrio,* allowing rapid acquisition of these genes and adaptation to a new environment (Murphy and Boyd 2008). Similarly, the commensal bacterium *Lactobacillus plantarum* has the ability to rapidly adapt to nutrient utilization in the *Drosophila melanogaster* gut with just a single mutation to the acetate kinase gene *ackA* (Martino et al. 2018). This single mutation allows the bacterium to utilize acetate present in the fly gut to produce greater amounts of N-acetyl-glutamine, promoting bacterial growth in the *Drosophila* gut (Martino et al. 2018). Collectively these findings show that sensing and using host-derived nutrients may predispose certain bacterial taxa to symbiosis. The ability of bacteria to rapidly fine tune metabolic diversity may further contribute to host- or niche-specific adaptation or transitions between bacterial lifestyles.

How bacteria compete within a host niche

In addition to the ability to use specific host-derived nutrients, bacterial ability to physically deter their competition is essential to compete in a crowded host environment. Microbial warfare can occur through the production of antimicrobials or toxins that physically harm potential competitors. These toxins can either be secreted into the environment causing damage to the bacterial outer membrane, or internal structures if they are taken up by the bacteria and allowed into the cell (Granato et al. 2019). Growing evidence suggests that both fungal and bacterial pathogens possess virulence factors that specifically target microbiota to promote their own virulence (Shyntum et al. 2019; Snelders et al. 2021), and so the ability to effectively suppress

competition may be an essential component of virulence across diverse hosts.

Some widely conserved microbial warfare tactics allow diverse bacteria to survive in diverse host-associated environments. Type six secretion systems (T6SS) allow bacteria to deliver effectors and toxins directly into the cytoplasm of potential competitors. For instance, *Pseudomonas protegens,* which is an effective gut pathogen of diverse insects, uses its T6SS to kill members of the microbiome and colonize the insect gut (Vacheron et al. 2019). Similarly, the bacterial potato pathogen *Pectobacterium carotovorum* uses T6SS to outcompete commensal microbes on potatoes (Shyntum et al. 2019). Diverse gut commensal bacteria also require T6SS to persist in a host in the absence of disease (reviewed in Coyne and Comstock 2019). The extracellular Contractile Injection System (eCIS) is a toxin delivery system that shares structural similarity with T6SS (Sarris et al. 2014). It is necessary for bacterial-insect interactions and is enriched in the genomes of bacteria isolated from diverse hosts (Geller et al. 2021). Collectively these findings indicate that acquisition of T6SS and similar toxin delivery systems may predispose bacteria be effective competitors in crowded host-associated environments (Table S1B).

Some opportunistic pathogens secrete diverse toxins that have antimicrobial activity against many other species of bacteria or fungi, making them strong competitors in host-associated environments (Tarkka et al. 2009; Hamad et al. 2020). *Pseudomonas aeruginosa* strains have the ability to outcompete other microorganisms, and can therefore readily colonize the plant rhizosphere or cause disease even in the face of potential competitors (Anjaiah et al. 2003; Spago et al. 2014; Yasmin et al. 2017). For example, *P. aeruginosa* PNA1 can produce two different phenazine antibiotics, phenazine-1-carboxylic acid and oxychloroaphin, which are required for PNA1-mediated protection of pigeonpea and chickpea plants against fusarium wilt (Anjaiah et al. 2003). Similarly, phenazine production is essential for microbe-microbe competition in acute and chronic *P. aeruginosa* infections in animals (Trejo-Hernández et al. 2014; Bisht et al. 2020). Thus, some bacteria such as *Pseudomonas* spp. may be broadly antagonistic against many bacteria predisposing them to survival in diverse host environments.

In some cases, production of toxins, and resistance to those toxins can result in a complex molecular arms race in host-associated environments. *Pseudomonas protegens* species CHA0 can kill the closely-related strain *P. protegens* Pf-5 through production of a phage-like tail particles called tailocins (Heiman et al. 2022). Heiman et al. (2022) found that the CHA0-produced tailocin kills Pf-5 in an O-antigen-dependent manner (a modification to the lipopolysaccharide found on the outer leaflet of the outer membrane). The study further found that the presence of the O-antigen makes *P. protegens* strains more susceptible to distinct tailocins encoded by closely-related strains, but helps the bacteria evade animal immunity and colonize the *Galleria mellonella* gut. In contrast, the absence of the O-antigen makes *P. protegens* strains more susceptible to more distantly-related *P. chloraphis* tailocins. This indicates that presence of microbial toxins may drive gain and loss of O-antigen production, which in turn may change interactions with a host. In addition the presence of the O-antigen has been shown to be required for evasion of immunity in both plants and animals; for instance, rhizobia strains lacking O-antigen cannot form successful mutualistic symbiosis, and *Brucella* lacking O-antigen are poorer colonizers of mammalian cells (discussed below). Consequently, toxins produced by one microbe may drive evolution of others in the environment, which in turn may have major consequences for host association.

How bacteria suppress and evade the host immune system

Plants and animals alike must be able to defend against both well-adapted and opportunistic pathogens, and avoid overgrowth of microbiota. Not surprisingly, presented with a common challenge, and a similar molecular toolbox, plants and animals have evolved a number of parallels in innate function (Haney et al. 2014; Mermigka et al. 2020). Plants and animals can recognize conserved microbe-associated molecular patterns (MAMPs) that include flagellin, specific peptides, peptidoglycan, and lipopolysaccharides (LPS) (Boller and Felix 2009). Despite the evolutionary distance between plants and animals, they sense and respond to MAMPs through functionally similar mechanisms (Haney et al. 2014; Mermigka et al. 2020). In plants and animals, bacterial MAMPS are sensed by leucine rich repeat (LRR) domains that are components of membrane-associated receptor kinases, or cytoplasmic Nod-like receptors. Perception of MAMPs results in a mitogen activated protein kinase (MAPK) cascade and transcriptional changes. While many of these responses are distinct between plants and animals, immune responses in both plants and animals similarly rely on production of reactive oxygen species and antimicrobial peptides (Haney et al. 2014; Roudaire et al. 2021). Because of the functional similarities between innate immunity in plants and animals, similar adaptations allow bacteria to evade and tolerate the immune responses of diverse eukaryotic hosts.

As plants and animals both produce a suite of antimicrobial chemicals and peptides following MAMP recognition, bacteria must suppress or tolerate these defenses to persist in association with a host. There are diverse mechanisms by which bacteria do so including cloaking MAMPs to evade of elicitation of immunity in the first place (Reddick and Alto 2014). Specific secreted polysaccharides may also help suppress host immunity, or evade detection; for instance cyclic beta glucans produced by pathogenic *Brucella* and mutualistic *S. meliloti* can dampen immune responses in macrophages and plants respectively (Guidolin et al. 2018). While bacterial mechanisms to evade and suppress host immunity have been extensively reviewed (Hooper 2009, Reddick and Alto 2014, Thakur et al. 2019, Wang et al. 2022), here we focus on outer membrane modifications in Proteobacteria that may predispose groups of bacteria for host association, and that may be shared across plant- and animal- associated taxa (Table S1C).

Proteobacteria have modified the outer leaflet of their outer membrane with lipopolysaccharide (LPS), which may help protect them from environmental stress and host immune responses (Fig. 2). The presence of the O-antigen, a modification to LPS with repeating sugar moieties (Fig. 2B) is most commonly demonstrated to help bacteria, ranging from pathogens to mutualists, to evade host immunity and establish commensalism or infection (Priebe et al. 2004, Wang et al. 2010, Kim et al. 2016, Heiman et al. 2022). The O-antigen may contribute to immune evasion in part through cloaking MAMPs; for instance, loss of the O-antigen results in induction of innate immune responses including TLR4 activation in mice (Duerr et al. 2009) and enhanced ROS burst in plants (Rapicavoli et al. 2018) indicating that the O-antigen helps protect bacteria from detection by the host immune system.

LPS is also critical for resistance to antimicrobial peptides, and this is largely determined by the structure of the lipid A head (Fig. 2); as diverse plants and animals produce antimicrobial peptides, these modifications may have evolved to tolerate host defenses. Modifications to the charge and size of the lipid A head can make the outer membrane more or less permeable to

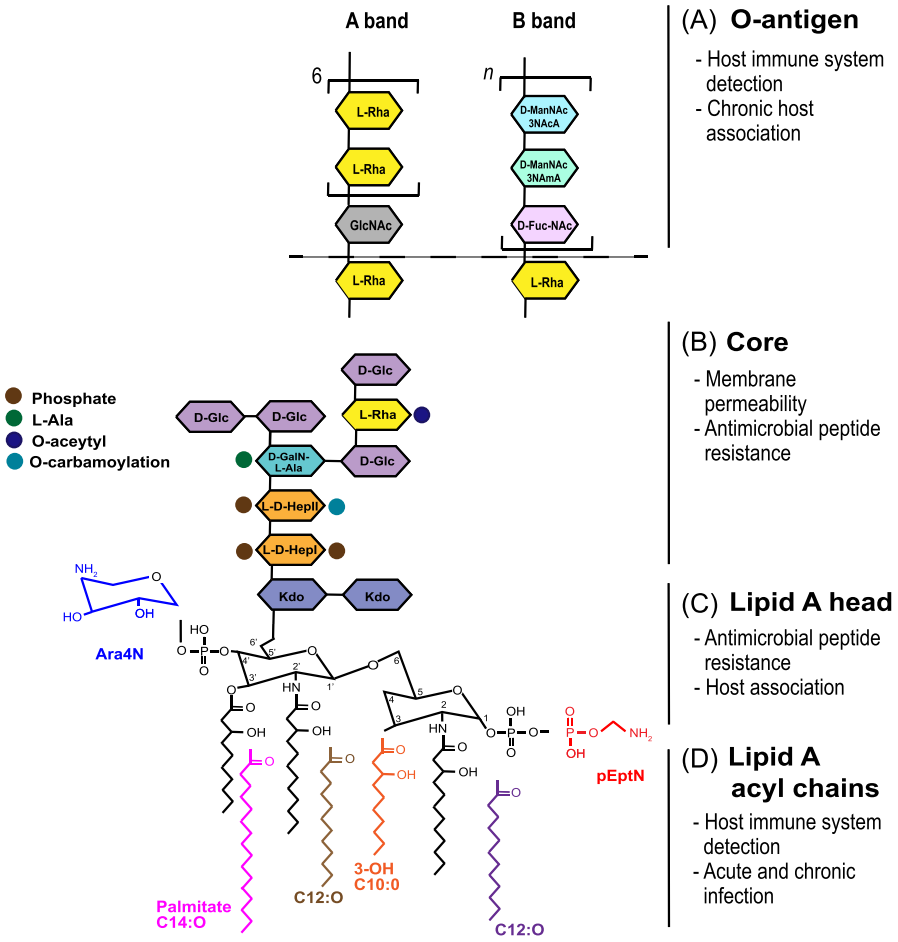

Figure 2. Structure of LPS and roles of LPS modifications in host association. **(A)** The presence of the O-antigen, consisting of either A or B bands, is known to contribute to bacterial evasion of the host innate immune response. The A band is often composed of a single GlcNAc and 12 L-Rha sugars and the B band often consists of one to tens of sugar repeats. **(B)** Modifications to the core sugars are known to affect both bacterial membrane permeability, as well as host association. Common core sugars as well as known possible sugar modifications are shown. **(C)** The charge of the LPS lipid A head affects resistance to cationic peptides and is composed of a β(1,6)-linked glucosamine disaccharide attached to phosphate groups on either side which can be reversibly modified at the 1' and 4' positions with Ara4N (aminoarabinose) and pEptN (phosphoethanolamine). **(D)** Variability in the number and composition of the acyl chains affects LPS detection by the host immune system and different modifications contribute to acute and chronic host association. The acyl chains shown in black are irreversibly attached to the head, while the coloured acyl chains are reversibly attached at the positions indicated. L-Rha: L-rhamnose; GlcNAc: glycose-acetamido; D-ManNAc3NAcA: di-N-acetylated mannuronic acid; D-ManNAc3NAmA: di-N-acylated aetylmuramic acid; D-Fuc-NAc: d-2-amino-2,6-dideoxy-galactose-acetamido; D-Glc: D-glucose; D-GalN-L-Ala: d-galactosamine-l-alanyl; L-D-HepI/II: L-glycero-d-manno-heptose; Kdo: 3-deoxy-d-manno-oct-2-ulosonic acid.

harmful agents such as antimicrobial compounds (Fig. 2C; Simpson and Trent 2019). For example, modification of the *Salmonella* or *P. aeruginosa* lipid A with aminoarabinose (arn) increases bacterial resistance to the antimicrobial cationic peptide polymyxin B, as well as other classes of host-derived cationic peptides, and metals including aluminum and iron (Moskowitz et al. 2004; Nishino et al. 2006). At the same site, the lipid A head can be alternatively modified with phosphoethanolamine, a modification which contributes to polymyxin B resistance in *Salmonella* but not *P. aeruginosa*, and is required for host association in *P. fluorescens* (Lee et al. 2004, Wiesmann et al. 2022). Collectively these findings indicate that the ability to alter membrane permeability through modification of lipid A allows bacteria to tolerate host immune responses.

Plants and animals can both detect the acyl chains attached to lipid A (Fig. 2C) which directly interact with the mammalian MAMP receptor TLR4 and the plant receptor LORE, resulting in bacterial detection and innate immune response activation (Maeshima and Fernandez 2013, Kutschera et al. 2019). As a consequence, the length, number, and composition of these acyl chains affect detection by the host immune system (Needham and Trent 2013) where hypo- or hyper-acylated forms are less potent, or can even antagonize, TLR4 activation (Teghanemt et al. 2005, Li et al. 2013). Collectively, these results demonstrate how modification of LPS can allow bacteria to associate with diverse hosts.

Biofilm formation

Once bacteria have successfully begun to colonize a host niche, some bacterial species form a biofilm (Fig. 1D). Through aggregation and production of an extracellular matrix, biofilms help bacteria create a homeostatic environment to protect them from stressors such as host-secreted antimicrobial peptides, osmotic stress, and temperature stress (Anderson and O'Toole 2008, Flemming et al. 2016). While non-host associated bacteria may also form biofilms to protect them from harsh environments, the ability to form biofilm is necessary for chronic host association by many bacteria (Ramey et al. 2004, Chen and Wen 2011).

Microbial cells assembled in a biofilm are physiologically distinct from planktonic cells (Heacock-Kang et al. 2017). These physiological differences result in biofilm-associated microbial cells having enhanced resistance to external physical and chemical stresses (Flemming et al. 2016). For example, *P. aeruginosa* biofilm confers adaptive resistance to antibiotics. In mammalian hosts, *P. aeruginosa* biofilm protects bacteria from phagocytic cells and immune effector cells such as neutrophils (Jesaitis et al. 2003). These characteristics, combined with impaired mucociliary clearance of pathogens in cystic fibrosis (CF) patients, allow *P. aeruginosa* to cause chronic, biofilm-associated infections in CF lungs (Malhotra et al. 2019). Additionally, in phylogenetically diverse bacterial species, biofilm formation by pathogenic and mutualistic bacteria is required for long-term colonization in non-mammalian hosts. For instance, *Salmonella enterica* sv. Typhimurium forms biofilm and downregulates virulence factors to persistently colonize the *C. elegans* gut, and *Vibrio fischeri* forms biofilm in the light organs of the Hawaiian bobtail squid *Euprymna scolopes* (Chavez-Dozal et al. 2012, Desai et al. 2019). Finally, while *Bacillus subtilis* relies on chemotaxis and flagella-mediated motility to initialize early stage root surface (rhizoplane) colonization, long-term colonization of the *Arabidopsis thaliana* rhizoplane requires biofilm formation (Allard-Massicotte et al. 2016). Collectively, these observations indicate that biofilm regulation is crucial for host colonization.

Interestingly, while closely-related strains may share the same structural basis of biofilm, their extracellular matrices can be composed of different biopolymers. For *Pseudomonas* spp., the biofilm extracellular matrix contains adhesins such as flagella, type IV pili (T4P), and specialized adhesion proteins that allow microbial cells to attach to biotic or abiotic surfaces (Tran et al. 2011, Bucior et al. 2012), extracellular DNA as a structural scaffold, and extracellular polysaccharide (EPS) that allow the microbial cells to aggregate (Colvin et al. 2011, Flemming et al. 2016). However, the matrix composition may differ between strains; for example, *P. aeruginosa* strains encode CdrA, a secreted adhesin protein, whereas *P. fluorescens* strains produce a functionally similar, yet non-homologous large adhesin protein, LapA (Hinsa et al. 2003, Borlee et al. 2010). Additionally, *Pseudomonas* spp. produce different forms of polysaccharides. For example, *P. aeruginosa* produces Psl and Pel (Colvin et al. 2011, Jennings et al. 2015) while most *P. fluorescens* strains, however, synthesize Poly-N-acetylglucosamine (PNAG) through the *pgaABCD* gene cluster (Nandi et al. 2016). These examples indicate that even within a genus, individual components of biofilm may be rapidly evolving.

While individual components of biofilm may vary between strains, the signals and regulators that sense a host environment and trigger biofilm formation are often conserved. For instance, sensing of putrescine triggers biofilm formation in both *P. fluorescens* and *P. aeruginosa* (Liu et al. 2018, 2022). Similarly, in *Sinorhizobium* and *Brucella*, orthologous two-component signaling pathways (ChvI/ExoS and BvrR/S respectively) regulate biofilm formation and host association (described below). This suggests that sensing a host and responding with biofilm formation might predispose certain clades to host association, but the specific components of biofilm might be dependent on the specific host or symbiotic lifestyle.

Interestingly, evidence suggests that biofilm must be regulated and reversable for stable host association. Bacterial mutants that form constitutive biofilms can trigger inappropriate immune responses, for instance hyper biofilm formation due to mutations in *morA* or *spuC* in *P. fluorescens* can result in immune activation and reduced fitness (Liu et al. 2018). *Sinorhizobium meliloti* mutants that form too little or too much biofilm are similarly non-symbiotic (Fujishige et al. 2006). Mutations in the biofilm regulator *bifA* in the plant pathogens *Pseudomonas syringae* and *Pseudomonas savastanoi*, result in reduced virulence on tomato and olive respectively (Aragón et al. 2015). These findings indicate that biofilm formation is not only required, but must be tightly integrated with host-derived cues to optimize microbial host association.

Integrating responses to a host environment

To colonize a host, bacteria must be able to sense and adapt to a new environment, and as discussed above, this is a dynamic process that requires continually sensing and adapting to the host. To integrate environmental cues with changes in physiology, bacteria use signalling cascades, which are able to detect a change in the environment and regulate an appropriate downstream response to the perceived change (Fig. 3). It is therefore not surprising that signalling cascades are required for all aspects of symbiosis (Panthel et al. 2003, De Weert et al. 2006, Francis et al. 2017, Fujimoto et al. 2018, Wiesmann et al. 2022) and frequently studied when trying to decipher mechanisms of bacterial adaptation to a host and virulence. While many different types of signalling cascades exist, they are almost all composed of a protein with an extracellular, periplasmic, or cytosolic sensor domain allowing bacteria to sense the environment. They also contain one or more transcription factors that regulate gene expression and allow cells to adapt to the signal perceived, in many cases regulating the transition to a symbiotic lifestyle (Zschiedrich et al. 2016).

Signalling cascades regulate all aspects of host adaptation described above, and so different sensor molecules have evolved to sense different signals (Fig. 3). For example, the periplasmic or cytoplasmic sensory domain of chemotaxis response proteins perceive molecules in the environment that alert them to the presence of a host, including host-derived nutrients such as inorganic phosphate, nitrate, amino acids, and host-derived polyamines, triggering a signalling cascade that results in bacterial movement towards a host (Matilla et al. 2021). Physical contact with a host then further triggers chemosensory-like signalling pathways that detect cues such as misfolded outer membrane proteins and damage to LPS (Mitchell and Silhavy 2019). This contact results in the activation of signalling cascades that increase the amount of c-di-GMP in cells, triggering a lifestyle switch from free-living to planktonic and the beginning of biofilm formation and chronic host establishment (Valentini and Filloux 2016).

One bacterial two-component system, ColR/S, is highly conserved across *Pseudomonas* species and is required for host association across different *Pseudomonas* species and in different hosts (De Weert et al. 2006, Wiesmann et al. 2022). This two-component system is required not only for rhizosphere colonization of the beneficial bacterium *P. fluorescens*, and the opportunistic pathogen *P. aeruginosa*, but is also required for mouse abscess formation in *P. aeruginosa* (Wiesmann et al. 2022), and *P. aeruginosa* virulence in *C. elegans* (Garvis et al. 2009). ColS is known to be activated by the presence of high levels of zinc, iron, manganese, and cadmium (Ainsaar et al. 2014, Nowicki et al. 2015), and is required for both zinc and iron tolerance (Ainsaar et al. 2014, Wiesmann et al. 2022), suggesting that this two-component system may be triggered by an increase in the presence of iron. This activation subsequently leads to modification of the outer-membrane in such a way that the bacterium is able to defend against host-derived inhibitory molecules, and/or host conditions, such as low pH (Fig. 3B). This

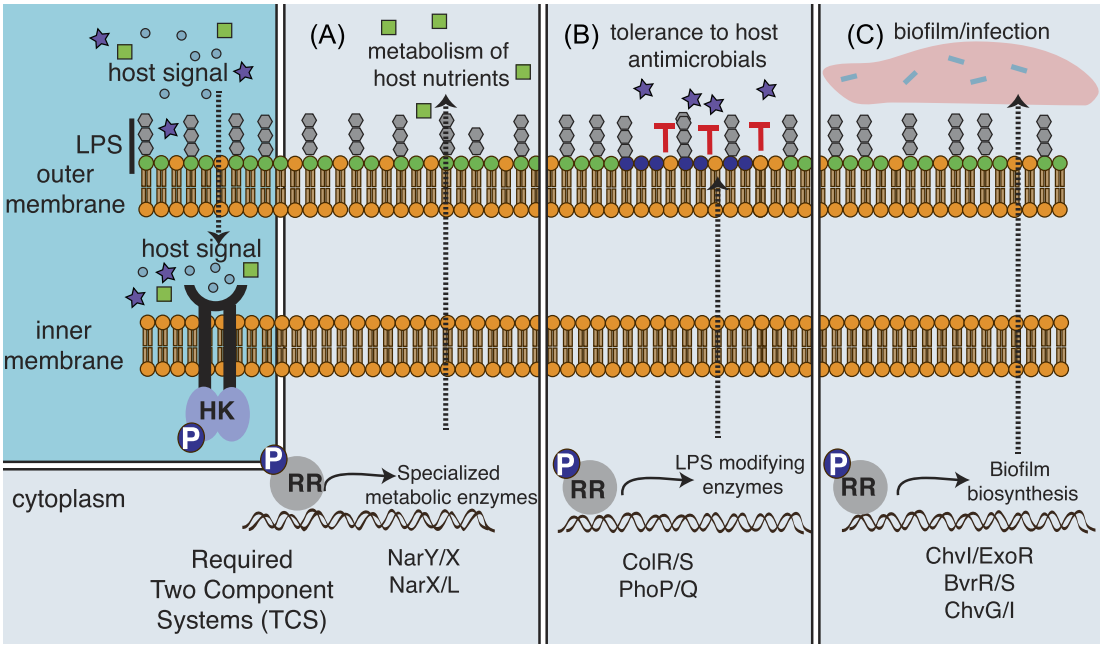

Figure 3. Two-component signaling integrates host sensing with host adaptations. Orthologous two-component systems (TCSs) are required for host association across phylogenetically diverse bacteria. Sensing a host environment results in upregulation of genes required for **(A)** specialized metabolism allowing bacteria to use host-derived molecules, **(B)** outer membrane modification allowing resistance to host-derived antimicrobials and **(C)** biofilm formation helping establish chronic infection. Although the specific genes required for metabolism, outer membrane modification and biofilm formation may vary depending on the bacterial strain and host, orthologous TCSs are necessary to sense a host and respond.

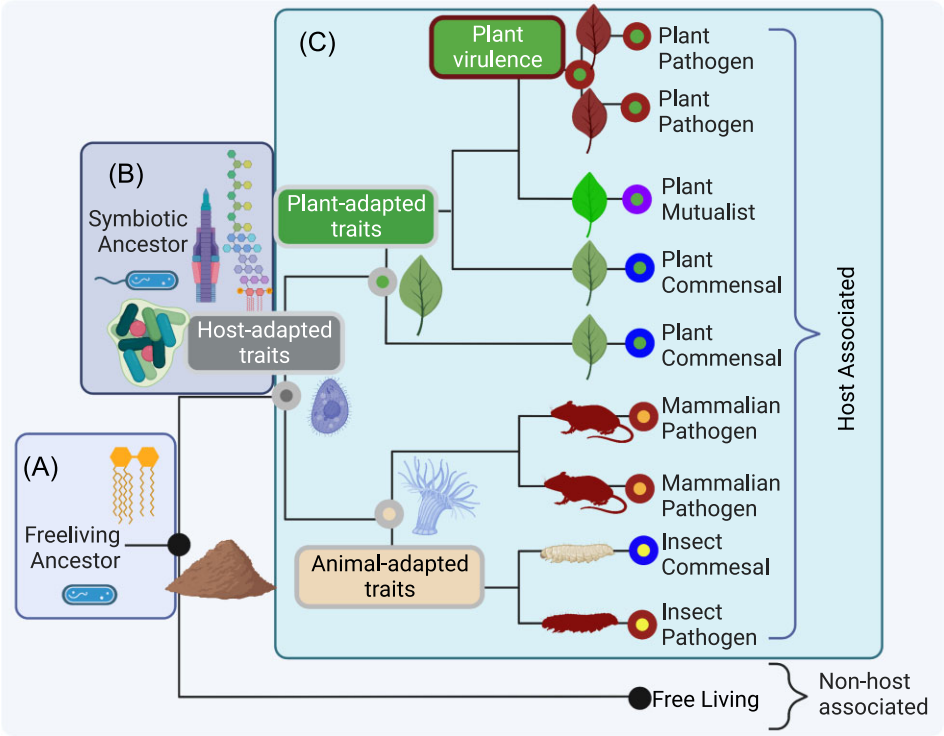

Figure 4. Symbiosis may be an ancestral trait and predate host- and lifestyle-specific adaptations. While it is possible that host association arose multiple times among clades that include symbiotic bacteria, it is more parsimonious if a free living, non-symbiotic ancestor (**A**) acquired adaptations (i.e. motility, T6SS, O-antigen, biofilm formation) that predispose a clade to being host-associated (**B**). This would be followed by more derived traits allowing bacteria to become pathogenic or mutualistic and adapting to specific hosts (**C**).

two-component system is therefore an example of a conserved regulatory cascade required for adaptation to diverse hosts which likely helps bacteria to evade conserved aspects of host defenses.

The orthologous BvrR/S, ExoS/ChvI, and ChvG/ChvI two-component systems are required for virulence in the animal pathogen *Brucella abortus*, the plant mutualist *Sinorhizobium meliloti*, and the plant pathogen *Agrobacterium tumefaciens*, respectively (Guzmán-Verri et al. 2002, Chen et al. 2009). Loss of *bvrR* in *Brucella suis* causes its inability to proliferate in dendritic cells (DCs) (Billard et al. 2005, Billard et al. 2007), as well as macrophages (Sola-Landa et al. 1998, Köhler et al. 2002), two components of the animal innate immune system. BvrR/S are highly similar to ExoS/ChvI in *Sinorhizobium meliloti* and ChvG/ChvI in *A. tumefaciens* (Sola-Landa et al. 1998). Loss of *chvI* in *S. meliloti* leads to many phenotypes including altered biofilm formation and impaired symbiosis with its plant hosts (Wang et al. 2010, Fig. 3C). Disruption of *chvG* and *chvI* in *A. tumefaciens* leads to the inability to form plant tumors (Charles and Nester 1993), and *chvG/chvI* is inducible at low pH (Yuan et al. 2008). Although the ligands are not known in most cases for these two-component regulators, their conserved requirement for host association across hosts suggests they may sense a conserved host-associated molecule to trigger bacterial physiological changes to allow them to adapt to a host environment.

Other bacterial two-component systems have evolved to detect host antimicrobial defences; for example, the *S. typhimurium* PhoP/Q two-component system can detect a number of host-derived compounds including acidic pH, cationic peptides, and divalent metal ions, triggering gene expression changes leading to outer-membrane modifications which decrease permeability to these harmful chemicals (Guo et al. 1997, Hicks et al. 2015). Intracellular mammalian pathogens, such as *Brucella*, can use the NtrY/X two-component system to detect host conditions such as oxygen depletion, leading to upregulation of nitrogen respiration genes (Carrica et al. 2012; Fig. 3A). The NarX/L two-component system plays a similar role in *P. aeruginosa*, regulating adaptation to the nitrogen and salt rich, low oxygen environment often found in the lungs of CF patients (Schobert and Jahn 2010). This sensing and integration of many environmental signals by different signaling cascades over time is essential for allowing bacteria to successfully transition from free-living to host-associated lifestyles.

Concluding remarks

In this review, we touch on the most universal aspects of bacterial symbiosis that are shared across diverse pathogens, commensals and mutualists and diverse hosts. While transitions between symbiotic lifestyles occur over relatively short evolutionary timescales (Drew et al. 2021), specific adaptations may predispose clades of bacteria to symbiosis. This is observed within taxa like *Pseudomonas*, where the majority of strains are host associated, but the specific lifestyles vary between species and even closely-related strains. For instance, *Pseudomonas syringae* are pathogenic on plants, while *P. aeruginosa* are opportunistic pathogens on diverse hosts, and *P. fluorescens* are host-associated with plants and animals and include pathogens and mutualists. Similarly, within the Alphaproteobacteria, *Brucella abortus* (animal pathogen), *Sinorhizobium meliloti* (plant mutualist), and *Agrobacterium tumefaciens* (plant pathogen) are closely-related and share a common ancestor (Sällström and Andersson 2005). While it is possible that these taxa each independently evolved the ability to be host associated, we propose that shared ancestral traits, such as O-antigen acquisition and specific two-component signaling pathways, may have evolved once allowing symbiosis among many members of host-associated bacterial clades. This model, where a bacterial ancestor evolves for symbiosis, followed by host-specific and lifestyle-specific innovations, is summarized in Fig. 4.

Understanding why certain organisms are so effective at symbiosis may provide new insights into treatment of disease. For instance, in individuals with Cystic Fibrosis, or specific immunodeficiencies, certain commensal bacteria can become opportunistic pathogens. While many studies have focused on identifying mechanisms of pathogenesis, relatively few studies have investigated how commensals and opportunistic pathogens are able to form stable host associations in the first place. Understanding what predisposes certain bacteria to stable host association could facilitate targeted treatment, or favor association of non-pathogenic microbiota over those with tendencies towards opportunistic pathogenesis. With the rise of antibiotic resistant chronic infections, as well as emergent agricultural pathogens, understanding how chronic and opportunistic pathogens are able to get a foothold is critical to the development of new treatments and therapies.

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Figure 4 was created with BioRender.

Supplementary data

Supplementary data are available at *FEMSRE* online.

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