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Hopanoid lipids: from membranes to plant–bacteria interactions

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Abstract

Lipid research represents a frontier for microbiology, as showcased by hopanoid lipids. Hopanoids, which resemble sterols and are found in the membranes of diverse bacteria, have left an extensive molecular fossil record. They were first discovered by petroleum geologists. Today, hopanoid-producing bacteria remain abundant in various ecosystems, such as the rhizosphere. Recently, great progress has been made in our understanding of hopanoid biosynthesis, facilitated in part by technical advances in lipid identification and quantification. A variety of genetically tractable, hopanoid-producing bacteria have been cultured, and tools to manipulate hopanoid biosynthesis and detect hopanoids are improving. However, we still have much to learn regarding how hopanoid production is regulated, how hopanoids act biophysically and biochemically, and how their production affects bacterial interactions with other organisms, such as plants. The study of hopanoids thus offers rich opportunities for discovery.

Lipids are the forgotten molecules of the microbial sciences. Despite their central role in defining cellular membranes, lipids have been routinely understudied, largely because they cannot be measured, synthesized or modified as easily as nucleic acids and amino acids. Although recent technical advances are beginning to simplify these tasks (for example,

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LIPID MAPS[1]), much of the functional importance of the rich chemical diversity of bacterial lipids remains a mystery.

Here, we introduce a particular class of membrane lipids — the hopanoids — as a model for modern lipid research. Hopanoids are found in diverse bacteria and in a handful of lichens and plants. Similar to eukaryotic sterols, hopanoids are planar, polycyclic hydrocarbons containing five rings compared with the four rings in sterols, and they have a variety of polar and nonpolar side chains[2]. This structural analogy underlies their functional similarities. Both lipid classes modulate the fluidity and permeability of membranes, and they have other, subtler biological functions that are beginning to be elucidated.

Unusual for biological molecules, hopanoids were first discovered in ancient rocks rather than in living cells. Hopane skeletons, which are pentacyclic carbon backbones lacking complex side chains, are produced by hopanoid degradation during rock formation, and they were recognized as components of oil shales many years before their biological source was found[3]. These ‘fossil’ hopanoids are now known to be staggeringly ubiquitous in rocks. They are common elements of sedimentary rocks formed as early as 1.64 billion years ago[4] and are estimated to have a greater mass than all other natural products from living organisms combined[5].

The landmark discovery of hopanoids in the bacteria *Methylococcus capsulatus* and *Alicyclobacillus* (formerly *Bacillus*) *acidocaldarius*[6,7] sparked a new field of hopanoid research. Since then, much work in this field has been motivated by a geobiological question: how can understanding the distribution and the biology of hopanoids in living microorganisms inform our interpretations of ancient bacteria on the basis of hopane biomarkers in the rock record? So far, a simple answer has proved elusive. As the number of sequenced bacteria and the availability of genetic approaches have expanded, it has become clear that remarkably diverse organisms produce hopanoids. It is therefore difficult to attribute hopanes in the rock record to the advent of a specific phylogenetic group, metabolic process or environmental context. Reviews of the efforts to interpret hopanoids in the rock record are available in the literature[8,9].

Here, we discuss the state of hopanoid research from a biological and biochemical perspective. First, we summarize what is known about the structural diversity and biosynthesis of hopanoids. Work on these topics has led to the characterization of the genes that are required to make hopanoids. Identifying these genes is a necessary first step for understanding the distribution of hopanoid production among bacteria and has provided a handle for genetic manipulation of hopanoid biosynthesis. In the second section, we discuss the functional consequences of disrupting hopanoid biosynthesis from the membrane scale to the population level and for bacteria in host-associated environments. In the latter context, we focus on how hopanoids may facilitate beneficial plant–bacteria interactions, an agronomically and ecologically important issue.

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Hopanoid structure and biosynthesis

The terpenoids, also known as isoprenoids, are formed by condensation of two or more isoprene units. They are one of the world's largest groups of natural products[10] and occur in all domains of life. In some organisms, terpenoids regulate essential metabolic processes, including growth, photosynthesis and respiration. More commonly, terpenoids form secondary metabolites that mediate the interactions between organisms and their environments.

Hopanoids belong to the cyclic triterpenoid subclass, a structurally diverse group of molecules synthesized by the cyclization of the branched terpenoid hydrocarbon squalene and its oxygenated derivative 2,3-oxidosqualene[11] (FIG. 1). These chemically complex reactions are catalysed by the triterpenoid cyclase superfamily of enzymes. Over a dozen triterpenoid cyclase families exist, which produce various classes of monocyclic and polycyclic triterpenoids[11,12].

The largest group of triterpenoid cyclases contains the epoxysqualene or oxidosqualene cyclases (OSCs). This family of triterpenoid cyclases is highly specific for the oxygenated squalene intermediate 2,3-oxidosqualene[13], and as a result, its products bear signature oxygen groups on ring A (FIG. 1). Collectively, the OSCs can produce a broad spectrum of chemically distinct cyclic triterpenoids, and they are usually divided into subfamilies based on their primary end products. Virtually all OSC subfamilies are unique to eukaryotes, with the exception of the sterol synthases, which have been identified and characterized in a handful of aerobic methanotrophs and myxobacteria. Recent analyses of environmental samples suggest that sterol production in bacteria is more widespread than previously thought[14].

Another major family of triterpenoid cyclases contains the squalene-hopene cyclases (SHCs). These enzymes can synthesize cyclic triterpenoids directly from squalene in an oxygen-independent process; they can also cyclize 2,3-oxidosqualene in vitro[15], although the organisms containing SHCs generally do not produce this substrate. Similarities in the sequences and structures of the active sites of OSCs and SHCs have suggested that these enzyme families are evolutionarily related[16–18]. Although the higher substrate promiscuity of SHCs than of OSCs has been used to suggest that OSCs evolved from SHCs[19], some evolutionary models of SHC and OSC relatedness have favoured the idea that SHCs and OSCs diverged from a common ancestor[20].

Whereas SHCs can accommodate more diverse substrates than OSCs, their known products are more limited. SHCs primarily synthesize the hopanoids diploptene and diplopterol, which are also known as C_{30} hopanoids (FIG. 1). In a few bacterial genera, SHC enzymes can also produce tetrahymanol through an additional tetrahymanol synthase enzyme. This pathway of tetrahymanol synthesis is unique to bacteria and predominantly occurs in the Bradyrhizobiaceae family of nitrogen-fixing legume symbionts[21].

Roughly 10% of bacteria can produce hopanoids (that is, they encode *shc*)[22–24]. The group with the highest conservation of *shc* genes is the Proteobacteria, particularly the alphaproteobacterial Bradyrhizobiaceae family and the betaproteobacteria genus

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Burkholderia, which includes both plant-associated members and human pathogens; *shc* genes are also well conserved among the Firmicutes, especially the plant-associated and human-associated Bacilli as well as the Frankiaceae family of plant-symbiont Actinobacteria. Other groups in which *shc* genes are common include the Cyanobacteria, Acidobacteria and Planctomycetes.

Although hopanoids are often assumed to be bacteria-specific, many molecules sharing the pentacyclic ring structure and core methylation sites of hopanoids have been isolated from various tropical trees, grasses, lichens and ferns[25,26]. Some of these structures are identical to bacterial hopanoids. For example, several members of the Polypodiales (polypod) order of ferns encode an SHC enzyme[24] that seems to exclusively produce the C_{30} hopanoid diplopterol in vitro[27]. It is likely that these ferns acquired *shc* by horizontal transfer from bacteria[28]. Other hopanoid-like molecules in plants contain additional accessory groups not observed in bacterial hopanoids (FIG. 1). The most common of these accessory groups is a C3 oxygen group, indicating that these molecules are 2,3-oxidosqualene derivatives[26,29]. Given the paucity of *shc* genes in plants, we speculate that plants encode an as-yet-uncharacterized OSC subfamily member that produces hopanoid-like lipids as a primary product.

Side chain formation.

In most organisms, the *shc* gene is part of a conserved *hpn* (hopanoid biosynthesis) operon that encodes enzymes for upstream terpenoid biosynthesis and downstream hopanoid structural modifications[30]. The most common hopanoid modification is the addition of hydrocarbon side chains by the enzyme hopanoid biosynthesis-associated radical SAM protein HpnH, which is found in almost all hopanoid-producing bacteria[31]. Hopanoids made by HpnH are known as the extended or C_{35} hopanoids, and they are formed by the addition of a C_5 ribose moiety to C_{30} hopanoids[32,33] (FIG. 1). These groups can be processed to produce diverse side groups by other *hpn* gene products, although the fitness advantages conferred by specific side chains are generally unknown[31,34–36] (FIG. 1).

Core modification.

Both C_{30} and extended hopanoids can be methylated at either the C2 position by the enzyme hopanoid 2-methyltransferase HpnP[37], forming the 2-methyl-hopanoids (2Me-hopanoids), or at the C3 position by the enzyme hopanoid C3 methylase (HpnR)[38], forming the 3-methyl-hopanoids (3Me-hopanoids) (FIG. 1). These methylation sites are more geostable than hopanoid side chains, and they have therefore occupied a central position in hopanoid geobiology. Genes encoding HpnP are largely restricted to the Cyanobacteria, Proteobacteria and Acidobacteria[39], whereas HpnR is even less common and is present in only a handful of species among the Proteobacteria, Nitrospirae and Actinobacteria[38]. Given that 2Me-hopanoids and 3Me-hopanoids have non-overlapping distributions, they may be functionally redundant.

In a few organisms, desaturation of one or more bonds in the pentacyclic hopanoid core also occurs, although the enzymes responsible are not known[40–44]. In general, little attention has been paid to hopanoid catabolism

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Biological functions of hopanoids

Unlike proteins and nucleic acids that can be easily probed with fluorescent dyes, antibodies or hybridization, detection of lipids in live cells can be technically complex. Most lipids can be extracted from cells using organic solvents and partially purified with chromatography[45], and protocols for hopanoid purification are well established. Great progress has also been made in identifying specific hopanoid structures by high-temperature gas chromatography[46] or reverse-phase liquid chromatography[47] coupled to mass spectrometry. Although it remains challenging to precisely quantify the abundance of hopanoids, synthetic isotope-labelled standards for some hopanoids have been developed[48]. Based on the results of these approaches, hopanoids seem to constitute less than 1% up to 90% of bacterial membranes, depending on the species and growth condition, and they are present in peripheral, intracellular and extracellular membranes, such as the outer membranes of cyanobacterial akinetes, cyanobacterial thylakoid membranes and the nitrogen-fixing vesicles of *Frankia* spp.[49–53]. In Gram-negative bacteria, hopanoids are enriched in the outer membrane by the hopanoid biosynthesis-associated RND transporter-like protein HpnN[54–56] (FIG. 2), although other factors regulating hopanoid localization are unknown so far.

Membrane fluidity.

Owing to their planar, hydrophobic structures, eukaryotic sterols and hopanoids can intercalate into lipid bilayers and interact with other lipids to alter the biophysical properties of membranes (FIG. 2). Early experiments in synthetic membranes and liposomes demonstrated that both extended hopanoids and cholesterol can condense, thicken and decrease the permeability of membranes, although with different efficiencies[57–64]. These data have shown that, although the membrane-modifying behaviours of sterols and hopanoids are broadly similar, they are not functionally interchangeable. Indeed, although certain hopanoids can compensate partially for the absence of cholesterol in *Mycoplasma mycoides*, the growth rate of these cells is markedly impaired, indicating that the functions of hopanoids and cholesterol are not identical[65].

Just as hopanoids differ from cholesterol, extended hopanoids with distinct side chains can also differ in their membrane-modifying capacities[66,67]. These structural differences between hopanoids may modulate their ability to interact with other lipids in the bilayer and, perhaps, their distribution in bacterial membranes. Molecular dynamics simulations have been performed to model the orientation of the C_{30} hopanoid diploptene and the extended hopanoid bacteriohopanetetrol (BHT) in membranes in silico[67]. In these simulations, BHT adopts an upright orientation inside the lipid bilayer, where it can interact with other membrane lipids to condense the membrane (FIG. 2). By contrast, diploptene localizes between the two leaflets, partitioning to the midplane. This latter positioning is expected to decrease membrane permeability by providing an added layer of protection to the cell. In vivo analyses of how different hopanoids affect membrane fluidity partially support these molecular dynamics results. In synthetic vesicles, diplopterol, which differs from diploptene by the addition of a single hydroxyl group, had no effect on membrane fluidity, whereas BHT decreased fluidity to a similar degree as cholesterol[53].

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The effects of hopanoid methylation on membrane fluidity are less clear and less widely studied than the effects of extended hopanoids. In synthetic membranes, 2-methyl-diplopterol outperformed cholesterol in decreasing membrane fluidity, suggesting that methylation is the most relevant modification for condensing the bilayer[53]. Yet this finding was not supported by measurements in live cells. In the purple non-sulfur bacterium *Rhodopseudomonas palustris* TIE-1, membranes from the Δ*hpnP* mutant, which lacks all 2Me-hopanoids, are equally as fluid as wild-type membranes[53]. It has been shown that hopanoid mutants overproduce other membrane-condensing lipids[68–70], which may explain this discrepancy. Regardless, factors affecting membrane fluidity in vivo are clearly more complex than what can be captured in vitro.

Lipid rafts.

Another well-noted feature of sterols is their ability to interact with sphingolipids to promote the formation of a liquid-ordered (L_o) phase in eukaryotic membranes[71–73] in which lipids are packed into a highly ordered organization but can still move freely throughout the bilayer. This property is important because it creates a platform for the formation of lipid rafts, which are specialized membrane microdomains that compartmentalize cellular processes by recruiting specific lipids and membrane-associated proteins. Although the existence of lipid rafts was once hotly debated, it is now accepted that lipid rafts in eukaryotes have important roles in signal transduction, endocytosis, ion homeo stasis and cell growth as well as in lipid–protein and protein–protein interactions at the membrane[73,74] (FIG. 2).

Fewer studies have been performed on the formation of lipids rafts in bacteria, and the topic remains controversial[75–77]; however, there is evidence that hopanoids could support this process[78–80]. In hopanoid-producing Gram-negative bacteria, hopanoids typically are abundant in the outer membrane, of which lipid A is the major component (FIG. 2). Inspired by the structural similarities between lipid A and the eukaryotic sphingolipid sphingomyelin (that is, amide-linked acyl chains and hydroxyl groups), Sáenz et al. showed that diplopterol can interact with the saturated chains of lipid A to induce the formation of a L_o phase in synthetic membranes[78,79], which promotes a fluid but mechanically robust membrane (FIG. 2). The ability of the hopanoids to modulate outer membrane properties through their interactions with lipid A suggests that they facilitate membrane compartmentalization into functional domains. Additionally, hopanoids appear to localize to membrane microdomains in vivo. In the marine cyanobacterium *Crocosphaera watsonii*, hopanoids are present in insoluble, detergent-resistant membrane fractions, as is common for raft-forming lipids in eukaryotes[80]. Microanalysis of isotope-labelled hopanoids in the cyanobacterium *Nostoc punctiforme* and in *R. palustris* TIE-1 also showed that hopanoids preferentially localize to regions of high membrane curvature, where high degrees of membrane tension may require thicker, more condensed membranes[81]. Although these collective data are promising, further biophysical analyses of live membranes will be needed to determine whether hopanoids indeed form lipid rafts in bacterial cells.

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Stress tolerance.

Many studies in diverse bacteria show that hopanoids facilitate bacterial survival under stress (for example, at high temperatures, low pH and high osmotic pressures and during antibiotic or detergent treatments), although the specific subset of conditions for which hopanoids are beneficial varies between bacterial strains[82–87]. Under these conditions, hopanoid content is typically increased, and it has long been proposed that hopanoids are regulated by canonical stress response pathways[88]. The alphaprotobacterial general stress response pathway regulates the *hpnP* gene in *R. palustris* TIE-1, although *shc* appears to be unregulated in this organism[89]. Regulation of hopanoids in other bacteria has not been studied.

The relative contributions of hopanoid structural modifications to survival under stress vary. 2Me-hopanoids seem to be generally associated with pH stress in diverse bacteria but have negligible or very subtle effects in the presence of other stressors, depending upon the bacterial strain[37,86,89–91]. Extended hopanoids are more important in this context, as they improve growth during exposure to bile salts, high temperatures, low pH, detergents, osmotic stress and antimicrobial peptides[34,86]. In *Bradyrhizobium diazoefficiens*, extended hopanoids are also essential for hypoxic growth in rich media[86].

The role of hopanoids in stress tolerance is particularly apparent in bacteria that form specialized, stress-associated cell types. In *N. punctiforme*, hopanoids do not affect growth of vegetative cells under stress but primarily localize to stress-associated akinete cells[52,90]. Similarly, in *Streptomyces coelicolor*, hopanoids are not detectable in vegetative cells but are massively upregulated during sporulation[92]. Tetracyclic isoprenoids, which are called sporulenes, are synthesized during spore formation in *Bacillus subtilis* and provide resistance to oxidative stress[93]; it is possible that hopanoids may have a similar role.

It is unknown whether the role of hopanoids in stress tolerance is simply a consequence of their impact on membrane fluidity and permeability or whether more specific stress response mechanisms are at play. In the case of hopanoid-dependent pH tolerance, it was suggested that lowered membrane permeability due to hopanoids prevents leakage of cations and protons across the membrane[87]. Similarly, the high content of hopanoids in membranes of thermotolerant and thermophilic bacteria[88] likely reflects their ability to reinforce the membrane bilayer against thermal disorder or thermolysis while maintaining its fluidity. Molecular dynamics simulations show that BHT has a strongly temperature-dependent effect on lipid tails, whereas its effect on the polar heads is negligible[94].

Other evidence suggests that hopanoids can promote the stress response beyond membrane stabilization. In *Methylobacterium extorquens*, a hopanoid-deficient mutant is impaired in multidrug efflux, hinting that hopanoids may modulate the action of membrane-associated protein complexes, possibly in subcellular membrane compartments[79,95]. Hopanoids also facilitate the formation of intracellular nutrient storage granules in *N. punctiforme*, a process that does not have an obvious, direct requirement for a more stable membrane but may result from an indirect metabolic effect[90].

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Nitrogen fixation.

Many hopanoid-producing bacteria are capable of nitrogen fixation, including *Anabaena* spp., *Azotobacter* spp., *Beijerinckia* spp., *Bradyrhizobium* spp., *Burkholderia* spp., *Frankia* spp. and *Nostoc* spp.[96–99]. It has long been suggested that hopanoids facilitate this metabolism[96], though few direct tests of this hypothesis have been performed. Almost all nitrogen-fixing hopanoid producers associate with plants; some, such as the endosymbiotic non-photosynthetic *Bradyrhizobium* spp., are wholly reliant on their hosts for cofactors that are essential for nitrogen fixation[100]. Others, such as *Nostoc* spp., *Anabaena* spp. and *Frankia* spp., are plant symbionts but can also fix nitrogen in the free-living state. A few of these nitrogen fixers are exclusively free living, most notably *Azotobacter vinelandii*.

Nitrogen fixation typically occurs under a narrow range of environmental conditions. Oxygen irreversibly inhibits the nitrogenase enzyme, and as a result, nitrogen fixation typically occurs in a low oxygen atmosphere. Nitrogen fixation is less often observed at higher oxygen concentrations, as this requires sophisticated mechanisms for nitrogenase protection. During bacterial nitrogen-fixing symbioses with plants, the hosts can provide this protection. For example, in the root nodule symbioses that occur between rhizobia and legumes (BOX 1), plants express leghaemoglobin molecules that maintain low-nanomolar levels of free oxygen[101].

In other cases of nitrogen fixation under high oxygen concentration, the mechanisms of nitrogenase protection are less clear. The free-living obligate aerobe *A. vinelandii* is presumed to both consume oxygen rapidly and create an extracellular alginate barrier to prevent oxygen diffusion into the cell[102]. Uniquely in *Frankia* spp., nitrogenase is sequestered into vesicles with a multi-layered lipid envelope that is presumed to form an oxygen diffusion barrier, as the thickness of the vesicle envelope is directly correlated to the oxygen concentration[103,104] (FIG. 3). Up to 90% of these vesicle envelopes consist of BHT and a phenylacetyl monoester of BHT (phenylacetic acid (PAA)-BHT), at an estimated PAA-BHT:BHT ratio between 30:70 and 50:50 (REFS 51,105). Purified PAA-BHT and BHT seem to optimally stabilize membranes at those relative abundances in vitro, and so this mixture of PAA-BHT and BHT may promote vesicle stability[66]. Intriguingly, PAA-BHT in *Frankia alni* can also be cleaved to release PAA, which is a plant auxin involved in development[106,107]. It is thus possible that during the interaction of *Frankia* spp. with plants, hopanoids also have indirect roles by releasing symbiotic signals.

Plant–bacteria association.

Beyond *Frankia* spp., the correlation between hopanoid biosynthesis and plant-associated nitrogen fixation may reflect a more general role for hopanoids in promoting bacterial survival in the rhizosphere, regardless of whether cells are free-living in the soil or associated with plants. Bacteria encounter diverse stressors in these habitats, and teasing out the specific selective pressures that underpin this correlation is a priority for future research. Intriguingly, an analysis of *hpnP* and *shc* distributions in metagenomes found that hopanoid production is enriched in plant-associated environments and is correlated with markers for nitrogen fixation as well as methanol utilization, another metabolic activity common in plant-associated bacteria[108]. Hopanoid production is also common among beneficial

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members of the Bacilli class[24], which do not fix nitrogen but are nonetheless known for promoting plant growth[109].

The role of hopanoids in beneficial plant–bacteria interactions has been studied best in legume–rhizobia root nodule symbioses. Hopanoid production occurs predominantly in rhizobia that associate with tropical legumes[110–112], and in the association of *Bradyrhizobium* spp. with tropical *Aeschynomene* legumes, they seem to support rhizobial survival within hosts (BOX 1). In the photosynthetic *Bradyrhizobium* sp. BTAi1, a Δ*shc* mutant that lacked hopanoids showed severe defects in outer membrane integrity and was both slower growing under optimal growth conditions and less resistant to stresses mimicking the root nodule microenvironment (such as low pH, hypoxia, high osmolarity and antimicrobial peptides) than wild-type bacteria[113]. In association with *Aeschynomene evenia*, this Δ*shc* mutant induced nodules showing normal development and wild-type nitrogen fixation activity but a shorter lifespan, prematurely triggering a plant senescence programme in which the symbiotic tissue is digested by the host[113]. This may reflect an inability to maintain a persistent host infection, perhaps owing to a long-term survival defect.

Information on the roles of different hopanoid modifications during root nodule symbiosis has been obtained in *B. diazoefficiens* USDA 110 (formerly named *Bradyrhizobium japonicum*[114]) in association with *Aeschynomene afraspera*[86]. In this symbiosis, loss of 2Me-hopanoids (Δ*hpnP*) has no discernible impact, while loss of extended hopanoids (Δ*hpnH*) induces nodules that fix nitrogen poorly and display morphological disorders such as disorganization of the nodule tissue and triggering of plant defence responses. Given that extended hopanoids contribute strongly to survival under stress for free-living *B. diazoefficiens*, we suggest that the Δ*hpnH* mutant is similarly challenged inside hosts.

Interestingly, hopanoids seem to be irrelevant for the symbiosis between the nitrogen-fixing cyanobacterium *N. punctiforme* and the (non-hopanoid-producing) fern hosts *Azolla* spp.[90]. In this symbiosis, bacteria associate with the plant surface only and do not experience such harsh changes in their environment; it is likely that the promotion of stress survival by hopanoids is not relevant in this context. However, it is also possible that the relevance of hopanoids in *Bradyrhizobium* spp. symbioses can be attributed to the presence of an unusual lipid A structure: hopanoid-lipid A (HoLA), in which lipid A is covalently attached to an extended hopanoid polyol[113,115,116] (FIG. 4). Although the physiological benefit of the attachment of hopanoids to lipid A remains to be determined, the impact of HoLA on the physiochemical properties of model membranes is known. The very long chain fatty acids (VLCFAs) of HoLA span the entire bilayer, helping tightly link the two membrane leaflets and inducing compact packing of the lipid tails of the bilayer, which increases the mechanical strength of the membrane[113]. The covalently linked hopanoic acid is positioned within the inner leaflet, further enhancing the mechanical robustness of the outer membrane[113]. Yeasts and archaea likely adopt similar strategies to regulate their membrane properties, as they also have very long lipid chains[117,118]. Archaeal membrane lipids consist of saturated isoprenoid chains between 20 and 40 carbons long, which span the bilayer and contribute to the thermal stability of the membrane[118]. In yeast, sphingolipids containing hydroxylated VLCFAs are interdigitated into the opposing leaflet of the bilayer and interact

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with ergosterol[117]. Despite these intriguing in vitro findings, identifying the enzyme that joins hopanoids to lipid A will be essential for assessing HoLA functions and distribution within bacteria and for assessing whether this molecule is sufficient to explain the phenotype of hopanoid iosynthesis mutants during symbiosis with legumes.

Outlook

Hopanoid biology represents a frontier for the microbial sciences. Although hopanoid biosynthetic pathways are known, much remains to be discovered about their biochemistry, biophysics and how they impact bacterial fitness in the context of plant and animal hosts. We have paid particular attention to the relationship between hopanoids and plants in this Review, not only because hopanoid-producing bacteria are abundant in the rhizosphere but also because hopanoids are critical for the development of certain plant–microorganism symbioses. Given the need to better understand what makes these symbioses robust in light of a warming climate, it is important to identify the mechanisms whereby hopanoids mediate these relationships.

The chemical similarity between hopanoids made by bacteria and pentacyclic triterpenoids made by lichens and plants is striking (FIG. 1). It is worth exploring whether this similarity is coincidental or, perhaps more plausibly, reflects an underlying functional connection. Our expanding ability to analyse the chemistry of hopanoids has revealed a plethora of structures and triggered numerous questions. What is the functional importance of this chemical diversity? Do certain hopanoids interact with membrane proteins, enabling their proper localization and/or function? How do they enable bacterial survival within the nodule microenvironment? Can hopanoids mediate the exchange of signals or nutrients between bacteria and plants? Could it be that bacterial hopanoids and plant cyclic triterpenoids engage in crosstalk, during which one can substitute the other? Might the diversity of side-chain modifications regulate the degree to which functional exchange could occur? The fact that hopanoids themselves might serve as carriers for auxin-like compounds that affect plant development is fascinating and merits further study.

In closing, we remind the reader of the words of John F. Kennedy when explaining what was needed to send astronauts to the Moon. “We choose to go to the Moon! … We choose to go to the Moon in this decade and do the other things, not because they are easy, but because they are hard; because that goal will serve to organize and measure the best of our energies and skills…”. Replacing the words ‘go to the Moon’ with ‘study lipids’ provides fitting inspiration for tackling one of the next great challenges in microbiology. It is time for lipids to get the attention they deserve, and the study of hopanoids is an excellent place to begin.

Note added in proof

While the proofs of this Review were being finalized, a new study[140] was published that identified the dominant bacterial taxa in global soils, including organisms from the *Bradyrhizobium* genus, further motivating efforts to understand whether hopanoid production by this genus contributes to its ecological success.

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References

1. Fahy E , Sud M , Cotter D & Subramaniam S LIPID MAPS online tools for lipid research. Nucleic Acids Res 35, W606–W612 (2007).17584797
2. Rohmer M , Bouvier P & Ourisson G Molecular evolution of biomembranes: structural equivalents and phylogenetic precursors of sterols. Proc. Natl Acad. Sci. USA 76, 847–851 (1979).284408
3. Burlingame AL , Haug P & Belsky T Calvin M Occurrence of biogenic steranes and pentacyclic triterpanes in an Eocene shale (52 millions years) and in an early Pre-Cambrian shale (2.7 billion years): a preliminary report. Proc. Natl Acad. Sci. USA 54, 1406–1412 (1965).16591314
4. Brocks JJ & Banfield J Biomarker evidence for green and purple sulphur bacteria in a stratified Paleoproterozoic sea. Nature 437, 866–870 (2005).16208367
5. Ourisson G & Albrecht P Geohopanoids: the most abundant natural products on Earth? Acc. Chem. Res 25, 398–402 (1992).
6. Bird CW , Lynch JM , Pirt SJ & Reid WW The identification of hop-22(29)-ene in prokaryotic organisms. Tetrahedron Lett 12, 3189–3190 (1971).
7. De Rosa M , Gambacorta A , Minale L & Bu'Lock JD Bacterial triterpenes. J. Chem. Soc. D Chem. Commun, 619–620 (1971).References 6 and 7 were the first papers to discover hopanoids in bacteria.
8. Newman DK , Neubauer C , Ricci JN , Wu C-H & Pearson A Cellular and molecular biological approaches to interpreting ancient biomarkers. Annu. Rev. Earth Planet. Sci 44, 493–522 (2016).
9. Pearson A in Treatise on Geochemistry 2nd edn (eds Falkowski PG & Freeman KH) 291–336. (Elsevier, London, 2014).
10. Vranová E , Coman D & Gruissem W Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu. Rev. Plant Biol 64, 665–700 (2013).23451776
11. Abe I Enzymatic synthesis of cyclic triterpenes. Nat. Prod. Rep 24, 1311–1321 (2007).18033581
12. Christianson DW Structural biology and chemistry of the terpenoid cyclases. Chem. Rev 106, 3412–3442 (2006).16895335
13. Barton DH et al. Biosynthesis of 3beta-hydroxytriterpenoids and -steroids from (3S)-2,3-epoxy-2,3-dihydrosqualene. J. Chem. Soc. Perkin Trans 1 12, 1134–1138 (1975).
14. Wei JH , Yin X & Welander PV Sterol synthesis in diverse Bacteria. Front. Microbiol 7, 259–219 (2016).26973633
15. Rohmer M , Anding C & Ourisson G Non-specific biosynthesis of hopane triterpenes by a cell-free system from *Acetobacter pasteurianum*. Eur. J. Biochem 112, 541–547 (1980).7460938
16. Poralla K The possible role of a repetitive amino acid motif in evolution of triterpenoid cyclases. Bioorg. Med. Chem. Lett 4, 285–290 (1994).
17. Ourisson G & Nakatani Y The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. Chem. Biol 1, 11–23 (1994).9383366
18. Syrén P-O , Henche S , Eichler A , Nestl BM & Hauer B Squalene-hopene cyclases — evolution, dynamics and catalytic scope. Curr. Opin. Struct. Biol 41, 73–82 (2016).27336183
19. Ourisson G , Albrecht P & Rohmer M Predictive microbial biochemistry — from molecular fossils to procaryotic membranes. Trends Biochem. Sci 7, 236–239 (1982).
20. Fischer WW & Pearson A Hypotheses for the origin and early evolution of triterpenoid cyclases. Geobiology 5, 19–34 (2007).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Banta AB , Wei JH & Welander PV A distinct pathway for tetrahymanol synthesis in bacteria. Proc. Natl Acad. Sci. USA 112, 13478–13483 (2015).26483502
22. Fischer WW , Summons RE & Pearson A Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. Geobiology 3, 33–40 (2005).
23. Ourisson G , Albrecht P & Rohmer M The hopanoids: palaeochemistry and biochemistry of a group of natural products. Pure Appl. Chem 51, 709–729 (1979).This paper is a concise compendium of key early references that describe the discovery of fossil hopanes in sedimentary rocks and their origin as bacterial hopanoids.
24. Racolta S , Juhl PB , Sirim D & Pleiss J The triterpene cyclase protein family: a systematic analysis. Proteins 80, 2009–2019 (2012).22488823
25. Devon TK & Scott AI in Handbook of Naturally Occurring Compounds Vol. 2 281–389 (Academic Press, New York, 1972).
26. Inayama S , Hori H & Pang GM Isolation of a hopane-type triterpenoid, zeorin, from a higher plant, *Tripterygium regelii*. Chem. Pharm. Bull 37, 2836–2837 (1989).This work provides a short review of hopanoid-like triterpenoids found in plants and lichens.
27. Shinozaki J , Shibuya M , Masuda K & Ebizuka Y Squalene cyclase and oxidosqualene cyclase from a fern. FEBS Lett 582, 310–318 (2008).18154734
28. Frickey T & Kannenberg E Phylogenetic analysis of the triterpene cyclase protein family in prokaryotes and eukaryotes suggests bidirectional lateral gene transfer. Environ. Microbiol 11, 1224–1241 (2009).19207562
29. Lopes D , Villela CT , Mac Kaplan & Carauta J Moretenolactone, a β-lactone hopanoid from Ficus insipida. Phytochem 34, 279–280 (1993).
30. Perzl M et al. Cloning of conserved genes from *Zymomonas mobilis* and *Bradyrhizobium japonicum* that function in the biosynthesis of hopanoid lipids. Biochim. Biophys. Acta 1393, 108–118 (1998).9714766
31. Bradley AS , Pearson A , Sáenz JP & Marx CJ Adenosylhopane: The first intermediate in hopanoid side chain biosynthesis. Org. Geochem 41, 1075–1081 (2010).
32. Flesch G & Rohmer M Prokaryotic hopanoids: the biosynthesis of the bacteriohopane skeleton. Formation of isoprenic units from two distinct acetate pools and a novel type of carbon/carbon linkage between a triterpene and D-ribose. Eur. J. Biochem 175, 405–411 (1988).3136017
33. Rohmer M , Sutter B & Sahm H Bacterial sterol surrogates. Biosynthesis of the side-chain of bacteriohopanetetrol and of a carbocyclic pseudopentose from 13C-labelled glucose in *Zymomonas mobilis*. J. Chem. Soc. Chem. Commun 19, 1471–1472 (1989).
34. Welander PV et al. Identification and characterization of Rhodopseudomonas palustris TIE-1 hopanoid biosynthesis mutants. Geobiology 10, 163–177 (2012).22221333This work shows that hpnH is essential for formation of all extended hopanoids.
35. Liu W et al. Ribosylhopane, a novel bacterial hopanoid, as precursor of C35 bacteriohopanepolyols in Streptomyces coelicolor A3(2). ChemBioChem 15, 2156–2161 (2014).25155017
36. Schmerk CL et al. Elucidation of the *Burkholderia cenocepacia* hopanoid biosynthesis pathway uncovers functions for conserved proteins in hopanoid-producing bacteria. Environ. Microbiol 17, 735–750 (2014).24888970This work identifies hopanoid biosynthesis-associated glycosyltransferase protein HpnI, hopanoid biosynthesis-associated radical SAM protein HpnJ and hopanoid biosynthesis-associated protein HpnK as enzymes that modify extended hopanoid tails.
37. Welander PV , Coleman ML , Sessions AL , Summons RE & Newman DK Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. Proc. Natl Acad. Sci. USA 19, 8537–8542 (2010).
38. Welander PV & Summons RE Discovery, taxonomic distribution, and phenotypic characterization of a gene required for 3-methylhopanoid production. Proc. Natl Acad. Sci. USA 109, 12905–12910 (2012).22826256
39. Ricci JN , Michel AJ & Newman DK Phylogenetic analysis of HpnP reveals the origin of 2-methylhopanoid production in Alphaproteobacteria. Geobiology 13, 267–277 (2015).25630231

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

40. Rohmer M , Bouvier P & Ourisson G Non-specific lanosterol and hopanoid biosynthesis by a cell-free system from the bacterium *Methylococcus capsulatus*. Eur. J. Biochem 112, 557–560 (1980). 6780348

41. Herrmann D A non-extractable triterpenoid of the hopane series in *Acetobacter xylinum*. FEMS Microbiol. Lett 135, 323–326 (1996).

42. Cvejic J et al. Bacterial triterpenoids of the hopane series as biomarkers for the chemotaxonomy of *Burkholderia*. *Pseudomonas* and *Ralstonia* spp. FEMS Microbiol. Lett 183, 295–299 (2000). 10675600

43. Cvejic J et al. Bacterial triterpenoids of the hopane series from the methanotrophic bacteria *Methylocaldum* spp.: phylogenetic implications and first evidence for an unsaturated aminobacteriohopanepolyol. FEMS Microbiol. Lett 182, 361–365 (2000).10620693

44. Rohmer M & Ourisson G Unsaturated bacteriohopanepolyols from *Acetobacter aceti ssp. xylinum*. J. Chem. Res (S), 356–357; (M), 3037–3059 (1976).

45. Bligh E & Dyer W A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol 37, 911–917 (1959).13671378

46. Sessions AL et al. Identification and quantification of polyfunctionalized hopanoids by high temperature gas chromatography-mass spectrometry. Org. Geochem 56, 120–130 (2013). 24496464This work establishes an optimized protocol for identification of structurally diverse hopanoids by gas chromatography.

47. Talbot H et al. Analysis of intact bacteriohopanepolyols from methanotrophic bacteria by reversed-phase high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. A 921, 175–185 (2001).11471801This work establishes an optimized protocol for identification of various extended hopanoids by high-performance liquid chromatography.

48. Wu C-H et al. Quantitative hopanoid analysis enables robust pattern detection and comparison between laboratories. Geobiology 13, 391–407 (2015).25865768

49. Tahara Y , Yamashita T , Kondo M & Yamada Y Isolation of Zymomonas mobilis mutant deficient in tetrahydroxybacteriohopane biosynthesis. Agr. Biol. Chem 52, 3189–3190 (1988).

50. Jürgens UJ , Simonin P & Rohmer M Localization and distribution of hopanoids in membrane systems of the cyanobacterium *Synechocystis* PCC 6714. FEMS Microbiol. Lett 1097, 90723–90722 (1992).

51. Kleemann G , Alskog G , Berry AM & Huss-Danell K Lipid composition and nitrogenase activity of symbiotic Frankia (Alnus incana) in response to different oxygen concentrations. Protoplasma 183, 107–115 (1994).

52. Doughty DM , Hunter RC , Summons RE & Newman DK 2-Methylhopanoids are maximally produced in akinetes of *Nostoc punctiforme*: geobiological implications. Geobiology 7, 524–532 (2009).19811542

53. Wu C-H , Bialecka-Fornal M & Newman DK Methylation at the C-2 position of hopanoids increases rigidity in native bacterial membranes. eLife 4, e05663 (2015).This work describes the specific effects of 2Me-hopanoids on the fluidity of membranes and compares the contribution of hopanoids to fluidity among inner, outer and native membranes.

54. Doughty DM et al. The RND-family transporter, HpnN, is required for hopanoid localization to the outer membrane of *Rhodopseudomonas palustris* TIE-1. Proc. Natl Acad. Sci. USA 108, E1045–1051 (2011).21873238

55. Kumar N et al. Crystal structures of the *Burkholderia multivorans* hopanoid transporter HpnN. Proc. Natl Acad. Sci. USA 114, 6557–6562 (2017).28584102

56. Sandhu P & Akhter Y Evolution of structural fitness and multifunctional aspects of mycobacterial RND family transporters. Arch. Microbiol 10.1007/s00203-017-1434-6 (2017).

57. Kannenberg E , Poralla K & Blume A A hopanoid from the thermo-acidophilic *Bacillus acidocaldarius* condenses membranes. Naturwissenschaften 67, 458 (1980).

58. Poralla K , Kannenberg E & Blume A A glycolipid containing hopane isolated from the acidophilic, thermophilic *Bacillus acidocaldarius*, has a cholesterol-like function in membranes. FEBS Lett 113, 107–110 (1980).7380001This work provides the first evidence that hopanoids can condense and increase the stability of lipid monolayers similarly to cholesterol.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

59. Kannenberg E , Blume A McElhaney RN & Poralla K Monolayer and calorimetric studies of phosphatidylcholines containing branched-chain fatty acids and of their interactions with cholesterol and with a bacterial hopanoid in model membranes. Biochim. Biophys. Acta Biomembr 733, 111–116 (1983).
60. Leonard A & Milon A Modulation of membrane hydrophobic thickness by cholesterol, cycloartenol and hopanoid. A solid state 2H-NMR Study. Bull. Magn. Reson 15, 124–127 (1993).
61. Nagimo A , Sato Y & Suzuki Y Electron spin resonance studies of phosphatidylcholine interacted with cholesterol and with a hopanoid in liposomal membrane. Chem. Pharm. Bull 39, 3071–3074 (1991).1666027
62. Chen Z , Tanno N & Takenaka S Effects of bacteriohopane-32-ol on the stability of various kinds of liposomal membranes. Biol. Pharm. Bull 18, 600–604 (1995).7655435
63. Sato Y , Chen Z & Suzuki Y Thermodynamic effects of hopanoids on synthetic and bacterial phospholipid membranes. Chem. Pharm. Bull 43, 1241–1244 (1995).
64. Chen Z , Sato Y , Nakazawa I & Suzuki Y Interactions between bacteriohopane-32, 33, 34, 35-tetrol and liposomal membranes composed of dipalmitoylphosphatidylcholine. Biol. Pharm. Bull 18, 477–480 (1995).7550109
65. Kannenberg E & Poralla K The influence of hopanoids on growth of *Mycoplasma mycoides*. Arch. Microbiol 133, 100 (1983).
66. Stroeve P , Pettit CD , Vasquez V , Kim I & Berry AM Surface active behavior of hopanoid lipids: bacteriohopanetetrol and phenylacetate monoester bacteriohopanetetrol. Langmuir 14, 4261–4265 (1998).
67. Poger D & Mark AE The relative effect of sterols and hopanoids on lipid bilayers: when comparable is not identical. J. Phys. Chem. B 117, 16129–16140 (2013).24299489
68. Neubauer C et al. Lipid remodeling in Rhodopseudomonas palustris TIE-1 upon loss of hopanoids and hopanoid methylation. Geobiology 13, 443–453 (2015).25923996
69. Bradley AS et al. Hopanoid-free Methylobacterium extorquens DM4 overproduces carotenoids and has widespread growth impairment. PLoS ONE 12, e0173323 (2017).28319163
70. Gruszecki WI & Strzalka K Carotenoids as modulators of lipid membrane physical properties. Biochim. Biophys. Acta 1740, 108–115 (2005).15949676
71. Pike LJ The challenge of lipid rafts. J. Lipid Res 50 (Suppl.), S323–S328 (2009).18955730
72. Gumí-Audenis B et al. Structure and nanomechanics of model membranes by atomic force microscopy and spectroscopy: insights into the role of cholesterol and sphingolipids. Membranes 6, 58 (2016).
73. Brown DA & London E Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J. Biol. Chem 275, 17221–17224 (2000).10770957
74. Sezgin E , Levental I , Mayor S & Eggeling C The mystery of membrane organization: composition, regulation, and roles of lipid rafts. Nat. Rev. Mol. Cell Biol 18, 361–374 (2017). 28356571
75. Farnoud AM , Toledo AM , Konopka JB , Del Poeta M & London E Raft-like membrane domains in pathogenic microorganisms. Curr. Top. Membr 75, 233–268 (2015).26015285
76. Bramkamp M & Lopez D Exploring the existence of lipid rafts in bacteria. Microbiol. Mol. Biol. Rev 9, 81–100 (2015).
77. Strahl H & Errington J Bacterial membranes: structure, domains and function. Annu. Rev. Microbiol 71, 519 (2017).28697671
78. Sáenz JP , Sezgin E , Schwille P & Simons K Functional convergence of hopanoids and sterols in membrane ordering. Proc. Natl Acad. Sci. USA 109, 14236–14240 (2012).22893685This work demonstrates that hopanoids can confer membrane order via formation of liquid-ordered states in synthetic liposomes.
79. Sáenz JP et al. Hopanoids as functional analogues of cholesterol in bacterial membranes. Proc. Natl Acad. Sci. USA, 112, 11971–11976 (2015).26351677This work demonstrates that hopanoids facilitate ordering of bacterial outer membranes via interaction with saturated lipids such as lipid A as well as via enhancing multidrug efflux.
80. Sáenz JP Hopanoid enrichment in a detergent resistant membrane fraction of *Crocosphaera watsonii*: implications for bacterial lipid raft formation. Org. Geochem 41, 853–856 (2010).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

81. Doughty DM , Dieterle M , Sessions AL , Fischer WW & Newman DK Probing the subcellular localization of hopanoid lipids in bacteria using nanoSIMS. PLoS ONE 9, e84455 (2014). 24409299

82. Flesch G & Rohmer M Growth inhibition of hopanoid synthesizing bacteria by squalene cyclase inhibitors. Arch. Microbiol 147, 100–104 (1987).

83. Horbach S , Neuss B & Sahm H Effect of azasqualene on hopanoid biosynthesis and ethanol tolerance of *Zymomonas mobilis*. FEMS Microbiol. Lett 79, 347–350 (1991).

84. Schmerk CL , Bernards MA & Valvano MA Hopanoid production is required for low-pH tolerance, antimicrobial resistance, and motility in *Burkholderia cenocepacia*. J. Bacteriol 193, 6712–6723 (2011).21965564

85. Seipke RF & Loria R Hopanoids are not essential for growth of *Streptomyces scabies* 87–22. J. Bacteriol 191, 5216–5223 (2009).19502399

86. Kulkarni G & Busset N et al. Specific hopanoid classes differentially affect free-living and symbiotic states of Bradyrhizobium diazoefficiens. mBio 6, e01251–15 (2015).26489859This work identifies extended hopanoids as the most important hopanoid structural class for stress resistance and symbiosis of Bradyrhizobium diazoefficiens.

87. Welander PV et al. Hopanoids play a role in membrane integrity and pH homeostasis in *Rhodopseudomonas palustris* TIE-1. J. Bacteriol 191, 6145–6156 (2009).19592593

88. Poralla K , Härtner T & Kannenberg E Effect of temperature and pH on the hopanoid content of *Bacillus acidocaldarius*. FEMS Microbiol. Lett 23, 253–256 (1984).

89. Kulkarni G , Wu C-H & Newman DK The general stress response factor EcfG regulates expression of the C-2 hopanoid methylase HpnP in *Rhodopseudomonas palustris* TIE-1. J. Bacteriol 195, 2490–2498 (2013).23524612

90. Ricci JN , Morton R , Kulkarni G , Summers ML & Newman DK Hopanoids play a role in stress tolerance and nutrient storage in the cyanobacterium Nostoc punctiforme. Geobiology 15, 173–183 (2016).27527874

91. Garby TJ et al. Lack of methylated hopanoids renders the cyanobacterium *Nostoc punctiforme* sensitive to osmotic and pH Stress. Appl. Environ. Microbiol 83, e00777–17 (2017).28455341

92. Poralla K , Muth G & Hartner T Hopanoids are formed during transition from substrate to aerial hyphae in *Streptomyces coelicolor* A3(2). FEMS Microbiol. Lett 189, 93–95 (2000).10913872

93. Bosak T , Losick RM & Pearson A A polycyclic terpenoid that alleviates oxidative stress. Proc. Natl Acad. Sci. USA 105, 6725–6729 (2008).18436644

94. Caron B , Mark AE & Poger D Some like it hot: the effect of sterols and hopanoids on lipid ordering at high temperature. J. Phys. Chem. Lett 5, 3953–3957 (2014).26276476

95. Lopez D & Koch G Exploring functional membrane microdomains in bacteria: an overview. Curr. Opin. Microbiol 36, 76–84 (2017).28237903

96. Vilcheze C , Llopiz P & Neunlist S Prokaryotic triterpenoids: new hopanoids from the nitrogen-fixing bacteria *Azotobacter vinelandii*. *Beijerinckia indica* and *Beijerinckia mobilis*. Microbiology 140, 2749–2753 (1994).

97. Rohmer M , Bouvier-Nave P & Ourisson G Distribution of hopanoid triterpenes in prokaryotes. J. Gen. Microbiol 130, 1137 (1984).

98. Kannenberg E , Perzl E & Härtner T The occurrence of hopanoid lipids in Bradyrhizobium bacteria. FEMS Microbiol. Lett 127, 255–261 (1995).

99. Rosa-Putra S , Nalin R , Domenach A-M & Rohmer M Novel hopanoids from Frankia spp. and related soil bacteria: squalene cyclization and significance of geological biomarkers revisited. Eur. J. Biochem 268, 4300 (2001).11488925

100. Hakoyama T et al. Host plant genome overcomes the lack of a bacterial gene for symbiotic nitrogen fixation. Nature 462, 514–517 (2009).19940927

101. Appleby CA Leghemoglobin and rhizobium respiration. Annu. Rev. Plant Physiol 35, 443–478 (1984).

102. Sabra W , Zeng AP & Lünsdorf H Effect of oxygen on formation and structure of *Azotobacter vinelandii* alginate and its role in protecting nitrogenase. Appl. Environ. Microbiol 66, 4037–4044 (2000).10966426

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

103. Parsons R , Silvester W , Harris S , Gruijters W & Bullivant S Frankia vesicles provide inducible and absolute oxygen protection for nitrogenase. Plant Physiol 83, 728–731 (1987).16665327
104. Abeysekera RM , Newcomb W , Silvester WB & Torrey JG A freeze-fracture electron microscopy study of *Frankia* in root nodules of *Alnus incana* grown at three oxygen tensions. Can. J. Microbiol 36, 97–108 (1990).
105. Berry AM , Moreau RA & Jones AD Bacteriohopanetetrol: abundant lipid in *Frankia* cells and in nitrogen-fixing nodule tissue. Plant Physiol 95, 111–115 (1991).16667936
106. Fries L Growth regulating effects of phenylacetic acid and phydroxy-phenylacetic acid on Fucus spiralis L. (Phaecophyceae. Fucales) in axenic culture. Phycology 16, 451–455 (1977).
107. Hammad Y et al. A possible role for phenyl acetic acid (PAA) on Alnus glutinosa nodulation by Frankia. Plant Soil 254, 193 (2003).This work finds that the auxinomimetic plant hormone PAA can be released from PAA-conjugated hopanoid lipids to regulate nodulation in Frankia symbioses.
108. Ricci JN et al. Diverse capacity for 2-methylhopanoid production correlates with a specific ecological niche. ISME J 8, 675–684 (2013).24152713This work identifies a important association between 2Me-hopanoids and plant-associated environments.
109. Kloepper JW , Ryu CM & Zhang S Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94, 1259–1266 (2004).18944464
110. Lemaire B et al. Biogeographical patterns of legume-nodulating *Burkholderia* spp.: from African Fynbos to continental scales. Appl. Environ. Microbiol 82, 5099–5115 (2016).27316955
111. Moulin L , James EK , Klonowska A , Daria SM & Simon MF in Biological Nitrogen Fixation (ed. de Bruijn FJ) 177–190 (John Wiley & Sons, Hoboken, NJ, USA, 2015).
112. Sy A et al. Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. J. Bacteriol 183, 214–220 (2001).11114919
113. Silipo A et al. Covalently linked hopanoid-lipid A improves outer-membrane resistance of a Bradyrhizobium symbiont of legumes. Nat. Commun 5, 5106 (2014).25355435This work describes a novel HoLA structure and provides the first evidence that hopanoids affect legume–rhizobia symbioses.
114. Delamuta JRM et al. Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* sp. nov. Int. J. Syst. Evol. Microbiol 63, 3342–3351 (2013).23504968
115. Komaniecka I et al. Occurrence of an unusual hopanoid-containing lipid A among lipopolysaccharides from *Bradyrhizobium* species. J. Biol. Chem 289, 35644–35655 (2014). 25371196
116. Busset N et al. The very long chain fatty acid (C26:25OH) linked to the lipid A Is important for the fitness of the photosynthetic Bradyrhizobium strain ORS278 and the establishment of a successful symbiosis with Aeschynomene legumes. Front. Microbiol 8, 1821 (2017).28983292
117. Shevchenko A , Schwille P & Simons K Yeast lipids can phase-separate into micrometer-scale membrane domains. J. Biol. Chem 285, 30224–30232 (2010).20647309
118. Boyd ES , Hamilton TL , Wang J , He L & Zhang CL The role of tetraether lipid composition in the adaptation of thermophilic archaea to acidity. Front. Microbiol 4, 62 (2013).23565112
119. Cooper JE Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. J. Appl. Microbiol 103, 1355–1365 (2007).17953546
120. D'Haeze W et al. Reactive oxygen species and ethylene play a positive role in lateral root base nodulation of a semiaquatic legume. Proc. Natl Acad. Sci. USA 100, 11789–11794 (2003). 12975522
121. Pauly N et al. Reactive oxygen and nitrogen species and glutathione: key players in the legume-Rhizobium symbiosis. J. Exp. Bot 57, 1769–1776 (2006).16698817
122. Pierre O et al. Peribacteroid space acidification: a marker of mature bacteroid functioning in *Medicago truncatula* nodules. Plant Cell Environ 36, 2059–2070 (2013).23586685
123. Miller KJ & Wood JM Osmoadaptation by rhizosphere bacteria. Annu. Rev. Microbiol 50, 01–36 (1996).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

124. Czernic P et al. Convergent evolution of endosymbiont differentiation in Dalbergoid and IRLC legumes mediated by nodule-specific cysteine-rich peptides. Plant Physiol 169, 1254–1265 (2015).26286718
125. Haag AF et al. Molecular insights into bacteroid development during Rhizobium-legume symbiosis. FEMS Microbiol. Rev 37, 364–383 (2013).22998605
126. Kondorosi E , Mergaert P & Kereszt A A paradigm for endosymbiotic life: cell differentiation of Rhizobium bacteria provoked by host plant factors. Annu. Rev. Microbiol 67, 611–628 (2013). 24024639
127. Sprent J , Ardley J & James E Biogeography of nodulated legumes and their nitrogen fixing symbionts. New Phytol 215, 40–56 (2017).28211601
128. Parker MA The spread of Bradyrhizobium lineages across host legume clades: from Abarema to Zygia. Microb. Ecol 69, 630–640 (2015).25301497
129. Fowler D et al. Effects of global change during the 21st century on the nitrogen cycle. Atmos. Chem. Phys 15, 13849–13893 (2015).
130. Volkman JK Sterols and other triterpenoids: source specificity and evolution of biosynthetic pathways. Org. Geochem 36, 139 (2005).
131. Killops SD & Killops V in Introduction to Organic Geochemistry 2nd edn Ch. 2 (Blackwell Publishing, Oxford, 2005).
132. Sohlenkamp C & Geiger O Bacterial membrane lipids: Diversity in structures and pathways. FEMS Microbiol. Rev 40, 133–159 (2015).25862689
133. Raetz CR & Whitfield C Lipopolysaccharide endotoxins. Annu. Rev. Biochem 71, 635–700 (2002).12045108
134. Silipo A , De Castro C , Lanzetta R , Parrilli M & Molinaro A in Prokaryotic Cell Wall Compounds: Structure and Biochemistry (eds König H , Claus H & Varma A) 133–153 (Springer, Berlin and Heidelberg, Germany, 2010).
135. Molinaro A et al. Chemistry of lipid A: at the heart of innate immunity. Chemistry 21, 500–519 (2015).25353096
136. Róg T , Pasenkiewicz-Gierula M , Vattulainen I & Karttunen M Ordering effects of cholesterol and its analogues. Biochim. Biophys. Acta 1788, 97–121 (2009).18823938
137. Van Meer G , Voelker DR & Feigenson GW Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol 9, 112–124 (2008).18216768
138. Harriott OT , Khairallah L & Benson DR Isolation and structure of the lipid envelopes from the nitrogen-fixing vesicles of Frankia sp. Strain CpI1. J. Bacteriol 173, 2061–2067 (1991).2002007
139. Berry AM et al. Hopanoid lipids compose the *Frankia* vesicle envelope, presumptive barrier of oxygen diffusion to nitrogenase. Proc. Natl Acad. Sci. USA 90, 6091–6094 (1993).11607408This work identifies hopanoids as the primary component of vesicles in Frankia spp., suggesting that hopanoids limit oxygen diffusion during nitrogen fixation.
140. Delgado-Baquerizo M et al. A global atlas of the dominant bacteria found in soil. Science 359, 320–325 (2018).29348236

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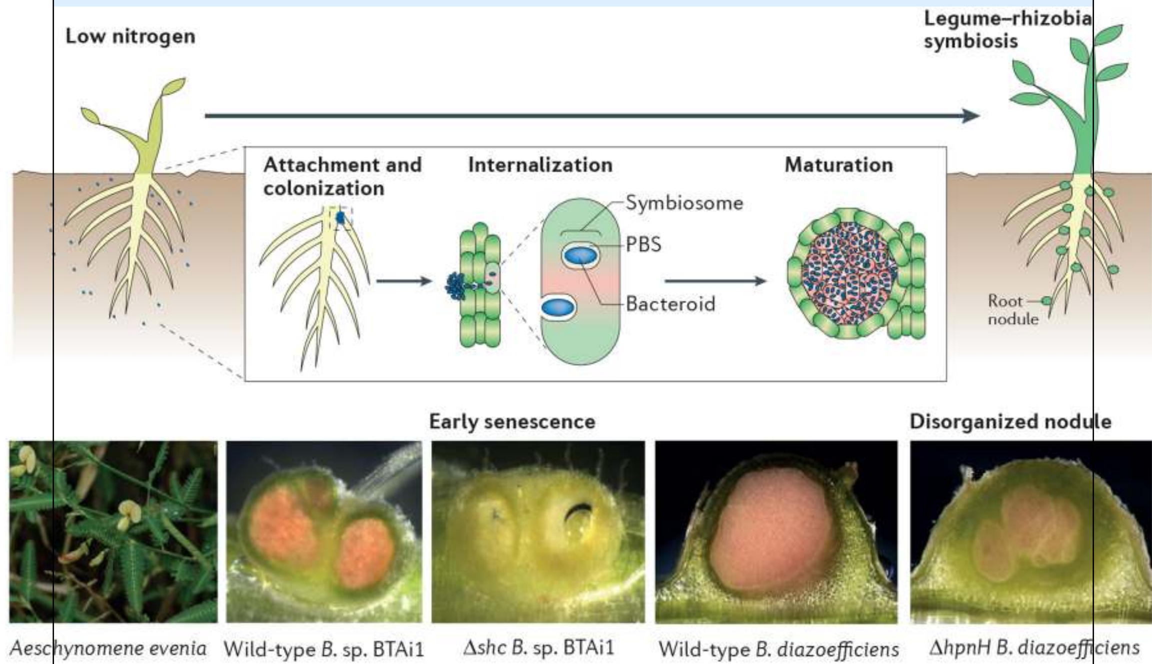
Box 1 |

The role of hopanoids in the development of legume–rhizobia root nodule symbioses

Nitrogen-fixing root nodule symbioses (see the figure), which catalyse the conversion of dinitrogen gas to ammonium, occur between rhizobia and most legumes as well as in a small subset of trees, shrubs and herbaceous plants. These symbioses are initiated under conditions where soil nitrogen levels are too low to support robust plant growth, resulting in the release of pro-symbiotic signals from both plants and rhizobia[119]. These signals suppress the plant immune response to allow rhizobial colonization of the root surface and, subsequently, invasion of the root[120]. The simplest pathways for rhizobial invasion are through root cracks. This process is associated with reactive oxygen species that challenge rhizobial growth but are necessary to sustain the development of symbiosis[121]. Once the bacterial infection progresses to the root interior, host cells internalize the rhizobia through an endocytosis-like process. This creates a new organelle known as a symbiosome containing a rhizobial cell, now known as a bacteroid, surrounded by a plant-derived membrane. The region between the bacteroid and symbiosome membranes is known as the peribacteroid space (PBS). The environment of the PBS is harsh and drastically different from that of free-living rhizobia. Some of this harshness is necessary for efficient nitrogen fixation, including the creation of a low pH environment to provide protons for nitrogenase[122] and concentrated carbon osmolytes for increased bacteroid metabolism[123]. Other stresses result from host control mechanisms, such as antimicrobial nodule-specific cysteine-rich peptides that change the morphology, proliferation and gene expression of bacteroids to maximize their productivity[124–126]. Bacteroid-containing host cells undergo many rounds of proliferation after the initial internalization event to form the mature root nodule. Root nodule formation occurs simultaneously across the entire root system until the supply of nitrogen to the host is sufficient to support plant growth. In some plant hosts, only a single rhizobial cell is internalized per nodule, resulting in a clonal population of bacteroids, whereas in other hosts, multiple strains of bacteria may coexist in the same nodule. It is unknown whether hopanoids can facilitate competitiveness of rhizobia under mixed-species infections; answering this question is an important next step in determining the relevance of hopanoids for real-world agricultural conditions. Hopanoid production is most conserved in root nodule symbionts from the *Bradyrhizobium* and *Burkholderia* genera, which are known to inhabit high-temperature, low-pH soils[127,128], suggesting that hopanoids facilitate adaptation to these conditions. In the context of global warming[129], in which temperature and soil acidity are predicted to increase globally, hopanoid-producing rhizobia may become more dominant. In the symbioses between *Bradyrhizobium* spp. and legumes, hopanoids seem to be important for maintaining a chronic infection within this stressful environment. In the symbiosis between the tropical legume *Aeschynomene evenia* and its native symbiont *Bradyrhizobium* sp. BTAi1, infection of hosts with a hopanoid-free Δ*shc* mutant elicits nodules that initially develop normally but trigger an early onset of nodule senescence (see the figure), a process in which nodule tissue is degraded and recycled by the host. This senescence presents as air-filled cavities in the central nodule tissue and a decrease in nitrogen fixation, which is reflected by a lighter pink colour that is indicative of low

leghaemoglobin levels[113]. In association with *Aeschynomene afraspera*, loss of extended hopanoids (Δ*hpnH*) results in a slight reduction in nitrogen fixation levels and a disorganization of the central nodule tissue[86] (see the figure).



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Hopanoids

Pentacyclic lipids with C_6 A-D rings and a C_5 E ring, among which six core methyl groups are distributed. Hopanoids are also characterized by the formation of accessory groups at their C2, C3 and C30 positions.

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Sterols

Tetracyclic lipids with C_6 A-C rings and a C_5 D ring. The parent compounds for all sterols contain oxygen groups at the C3 position.

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Terpenoids

Molecules assembled from two or more C_5 isoprene units that share the core formula $(C_5H_8)_n$. They are also known as isoprenoids.

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Triterpenoid

Molecules derived from assemblies of six isoprene units with the core formula $(C_5H_8)_6$ or $C_{30}H_{48}$. They may be acyclic or cyclic.

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Squalene

An acyclic triterpene with an irregular (tail-to-tail) linkage between two 3-isoprene units; a parent molecule of cyclic triterpenoids.

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Triterpenoid cyclase

A superfamily of enzymes that convert acyclic triterpenoids, including squalene and 2,3-oxidosqualene, into various cyclic products.

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Oxidosqualene cyclases

(OSCs). A family of predominantly eukaryotic 3-β-hydroxytriterpene cyclases that transform 2,3-oxidosqualene into sterols and diverse other cyclic triterpenoids.

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Squalene-hopene cyclases

A family of predominantly bacterial 3-deoxytriterpene cyclases that cyclize squalene to primarily form hopanoids.

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C_{30} hopanoids

Short hopanoids containing no additional carbon atoms that are not derived from squalene.

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Tetrahymanol

A hopanoid-like compound with a C_6 E-ring. In bacteria, it is made from E-ring expansion of the C_{30} hopanoid diploptene.

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Legume

Legumes are flowering plants of the Fabaceae (previously known as Leguminosae) family, which includes economically important crops such as soybeans, common beans and peanuts.

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C_{35} hopanoids

Extended hopanoids that contain ribose-derived hydrocarbon side chains at their C30 position.

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2-Methyl-hopanoids

(2Me-hopanoids). Hopanoids containing an accessory methyl group at the C2 position.

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3-Methyl-hopanoids

(3Me-hopanoids). Hopanoids containing an accessory methyl group at the C3 position.

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Liposomes

Spherical vesicles surrounded by one or more lipid bilayers that can be produced from cellular membranes in vivo or synthetically by sonication or extrusion of lipids into aqueous solution.

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Bacteriohopanetetrol

(BHT). A common C_{35} hopanoid containing a tetra-hydroxylated C_5 side chain.

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Membrane fluidity

The rotational and diffusional freedom of movement of molecules within a membrane.

Lipid rafts

Membrane microdomains with high stability that are thought to recruit specific membrane- associated proteins to spatially regulate their functions.

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Lipopolysaccharides

(LPSs). Complex, heat-stable amphiphilic lipids that are the main component of the external leaflet of the outer membrane of Gram-negative bacteria.

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Lipid A

The lipophilic moiety of lipopolysaccharides.

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Hypoxic

An environment with a low concentration of oxygen (usually less than 30% saturation).

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Nitrogen fixation

The conversion of dinitrogen gas into fixed or bioavailable nitrogen sources such as ammonia.

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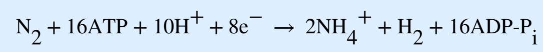
Nitrogenase

A bacterial enzyme complex that performs the following reaction:



$$N_2 + 16ATP + 10H^+ + 8e^- \rightarrow 2NH_4^+ + H_2 + 16ADP\text{-}P_i$$

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Root nodule

A specialized root organ that is generated by most legume plants to house nitrogen-fixing symbionts and create a specialized microenvironment to support bacterial nitrogen fixation.

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Rhizobia

A paraphyletic group of nitrogen-fixing soil bacteria that can engage in symbioses with legumes.

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Leghaemoglobin

An oxygen-carrying haem protein expressed in the root nodules of rhizobial host plants.

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Aeschynomene

A genus of tropical legumes that is broadly distributed globally and is used for livestock grazing.

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Hopanoid-lipid A

(HoLa). An extended hopanoid that is covalently attached to lipid A and appears to be unique to the Bradyrhizobiaceae.

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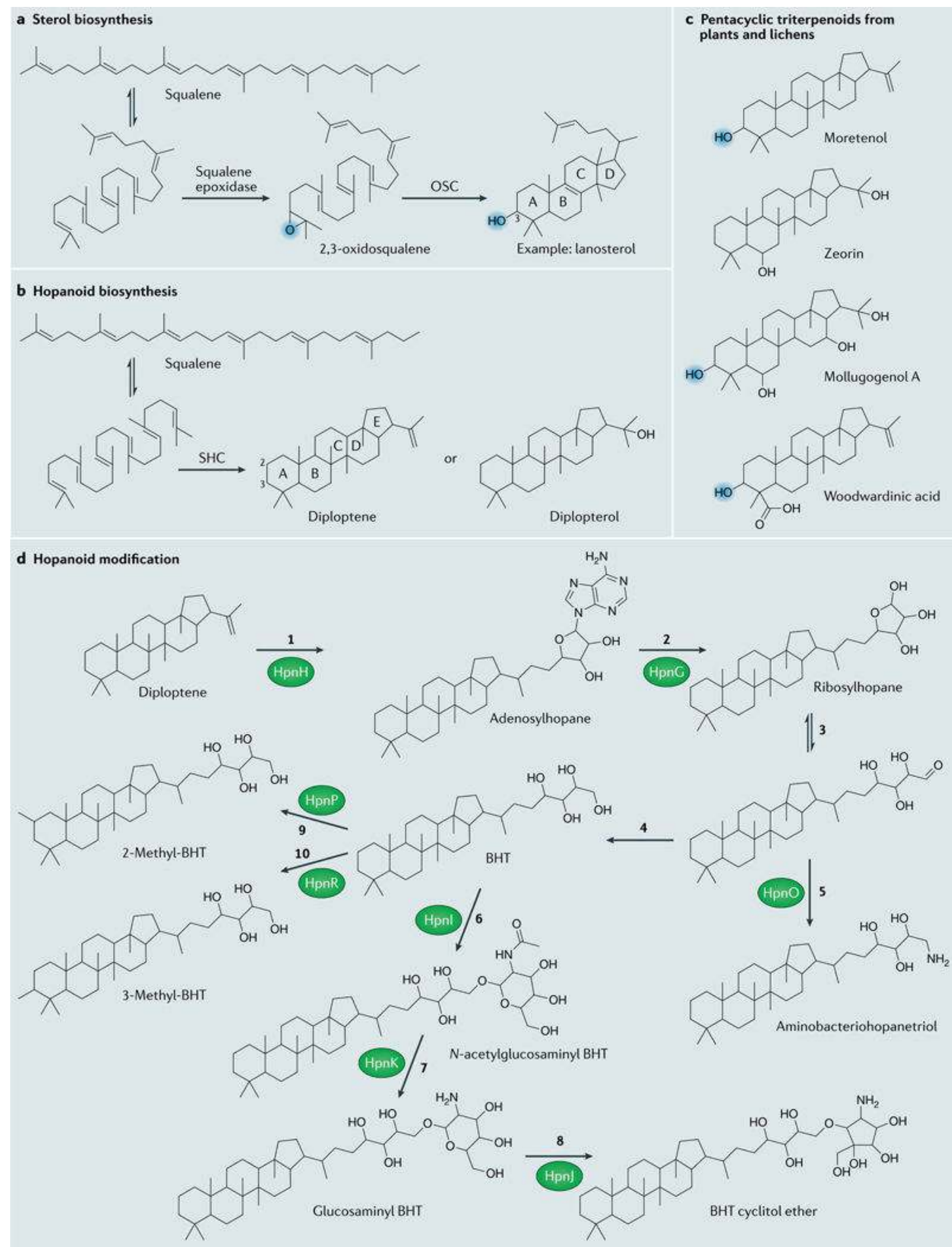

Figure 1 |. Pathways of sterol and hopanoid biosynthesis.

Sterols and hopanoids are synthesized from squalene through oxygen-dependent and oxygen-independent mechanisms, respectively[130]. **a |** To produce the cholesterol precursor lanosterol, squalene is oxygenated by the squalene epoxidase enzyme to form 2,3-oxidosqualene, which is then cyclized by the oxidosqualene cyclase (OSC) family member lanosterol synthase. **b |** During hopanoid biosynthesis, squalene-hopene cyclase (SHC) enzymes directly cyclize squalene to form one of two C_{30} hopanoids — diploptene or diplopterol. **c |** Interestingly, many hopanoid-like compounds made by plants seem to

originate from a hybrid cyclization, in which 2,3-oxidosqualene cyclization results in a molecule that contains a hopanoid core with a C3 oxygen signature. **d | ** Bacterial C_{30} hopanoids, which are produced directly by SHC (as in part b), also can be modified to form C_{35} hopanoids. The first steps of C_{35} hopanoid formation are the addition of adenosine by hopanoid biosynthesis-associated radical SAM protein HpnH (step 1) and the removal of adenine by putative hopanoid-associated phosphorylase HpnG (step 2), which generates a ribosylhopanoid with a ribose side chain. This ribose moiety can be readily interconverted between the cyclic and acyclic forms (step 3), from which the signature-extended hopanoid bacteriohopanetetrol (BHT) can be produced by an unknown enzyme (step 4). The side chains of the extended hopanoids can be further altered by the *hpn* gene products hopanoid biosynthesis-associated glycosyltransferase protein HpnI, hopanoid biosynthesis-associated protein HpnK, hopanoid biosynthesis-associated radical SAM protein HpnJ and aminotransferase HpnO to generate *N*-acetylglucosaminyl BHT, glucosaminyl BHT, BHT cyclitol ether and aminobacteriohopanetriol (steps 5,6,7 and 8). Hopanoids can also be methylated at the C2 or C3 positions by hopanoid 2-methyltransferase HpnP and hopanoid C3 methylase HpnR, respectively (steps 9 and 10); although only BHT methylation is shown, it can occur for any C_{35} or C_{30} hopanoid. For details of triterpenoid stereochemistry, we refer the reader to REF. 131. Part d is adapted from REF. 132, CC BY.

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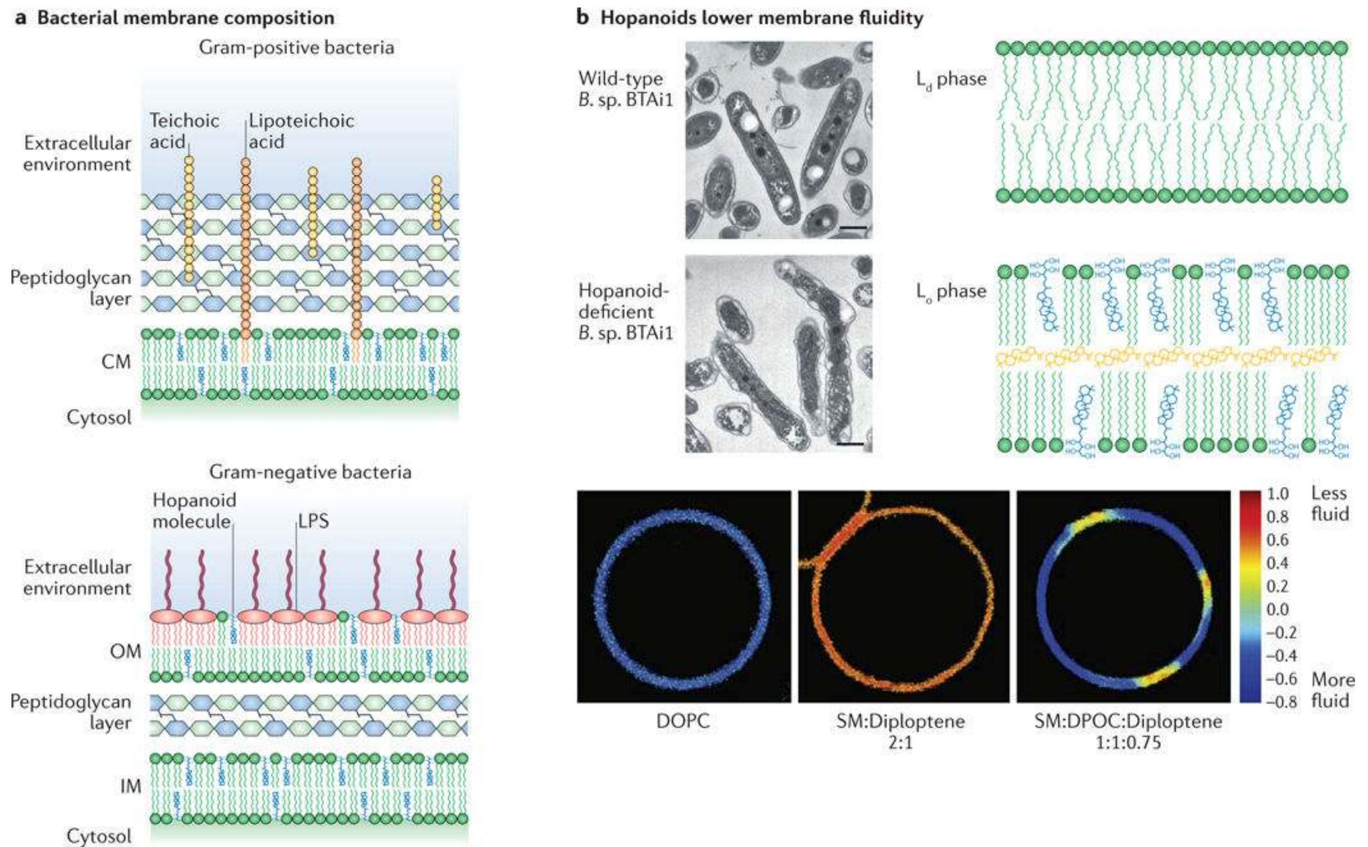

Figure 2 |. Regulation of lipid raft formation by hopanoids.

a | The main constituents of bacterial membranes are amphiphilic lipids, which contain polar headgroups and hydrophobic tails that preferentially self-associate to form lipid bilayers. All bacteria have a cytoplasmic membrane (CM) that delimits the cytoplasm and is enclosed by a protective and rigid layer of peptidoglycan. In Gram-positive bacteria, a thick layer of peptidoglycan encloses the CM and forms the basis of the cell surface. In Gram-negative bacteria, the CM is also referred to as the inner membrane (IM) and the peptidoglycan is surrounded further by an additional asymmetric bilayer, the outer membrane (OM). The inner leaflet of the OM comprises glycerophospholipids, and the external leaflet mainly comprises lipopolysaccharides (LPSs), which can cover up to 75% of the cell exterior[133,134]. LPS structures are highly strain-specific, but they can be roughly divided into three domains: a core oligosaccharide and a O-specific polysaccharide (or O-chain) that together form a hydrophilic heteropolysaccharide, and a lipophilic, membrane-anchoring domain, termed lipid A[135]. The hopanoids shown in the figure are present in some, but not all, Gram-negative and Gram-positive bacteria. **b |** The distributions of specific lipid classes between, and within, the leaflets of the IM and OM determine their physical and biochemical properties. In addition to containing distinct functional groups, lipids differ in the lengths and degrees of saturation of their hydrophobic tails. These properties tune the effective molecular geometries of lipids and in turn determine their packing within the bilayer. A high proportion of unsaturated and variable-length lipids, which pack poorly in the membrane, is associated with a more fluid, liquid-disordered (L_d) phase. When more planar saturated lipids interact with cholesterol (or some hopanoids), they can self-associate within

membranes to form a less fluid, more tightly and regularly packed liquid-ordered (L_o) phase. The interactions leading to the formation of an L_o phase in model membranes are thought to provide the basis for the assembly of raft domains in eukaryotic membranes. The L_o phase is also associated with longer lipid tails, as the increased surface of hydrophobic interactions increases their stability. In Gram-negative bacteria, hopanoids are enriched in the OM where they preferentially interact with lipid A[79,113], and in some organisms, such as *Bradyrhizobium* sp. BTAi1, they greatly enhance the mechanical integrity of the membrane, as evidenced in transmission electron micrographs. Because hopanoids have a rigid, planar ring structure, it is presumed that they generally are found in L_o microdomains, perhaps regulating the relative position of LPSs, thereby facilitating LPS recognition during host–bacteria interactions[136,137]. These membrane microdomains could also affect the localization and dynamics of proteins, restricting their roles in membrane trafficking, signalling and metabolism to functional sub-compartments. The ability of hopanoids to promote lipid rafts has been demonstrated in model membrane vesicles containing eukaryotic phosphatidylcholine (DOPC) and sphingomyelin (SM), in which diploptene addition results in the formation of low-fluidity microdomains. Part b is reproduced from REF. 113, Macmillan Publishers Limited, and with permission from REF. 78, Proceedings of the National Academy of Sciences.

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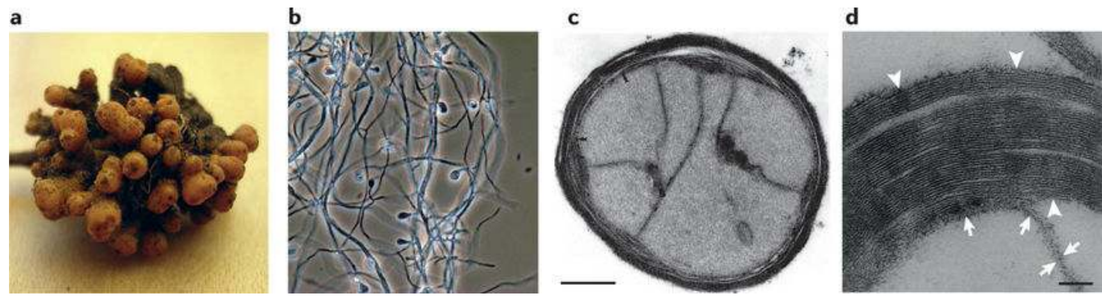

Figure 3 |. Hopanoid-rich vesicles in *Frankia* spp.

a | Root nodules induced by *Frankia* spp. on *Alnus incana* subsp. *rugosa*. Sample is ~1.5 cm in diameter. **b |** Filamentous structure of *Frankia* symbionts containing spherical nitrogen-fixing vesicles. **c |** Detail of cultured *Frankia* vesicles containing nitrogenase (scale bar = 500 nm). **d |** Multilamellate, hopanoid-rich lipid envelope (arrowheads) of the *Frankia* nitrogenase vesicles (scale bar = 50 nm) (arrows show the bacterial cell wall). Parts a and b are courtesy of David R. Benson, University of Connecticut, USA, and part c is amended from *Journal of Bacteriology*, 173, 2061–2067 doi: 10.1128/jb.173.6.2061–2067.1991 (1991) with permission from American Society for Microbiology (REF. 138). Part d is reproduced with permission from REF. 139, Proceedings of the National Academy of Sciences.

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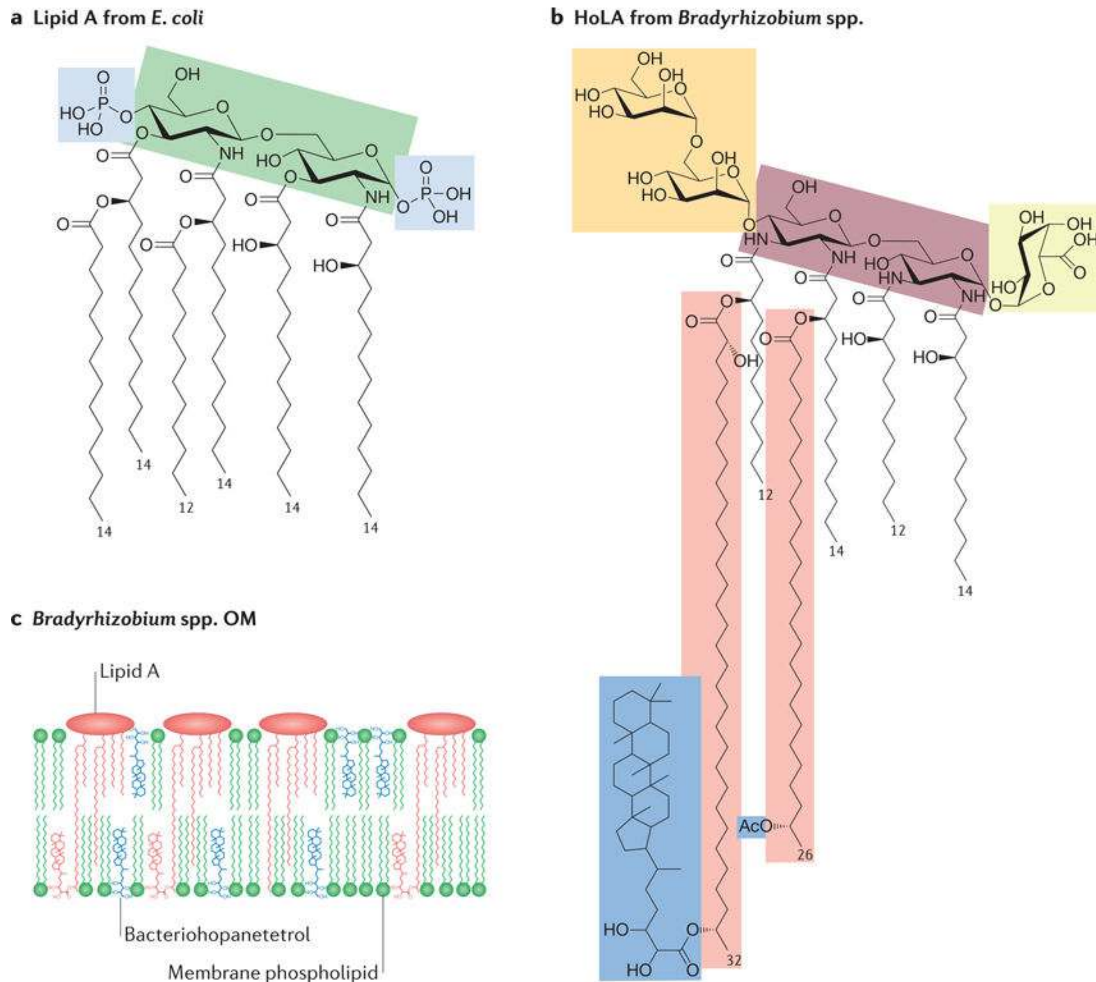

Figure 4 |. Chemical structure of hopanoid-lipid A from *Bradyrhizobium* spp.

a | Hexa-acylated lipid A from *Escherichia coli* contains a bis-phosphorylated (light blue) glucosamine disaccharide backbone (green), which is asymmetrically substituted by six acyl chains. **b |** *Bradyrhizobium* spp. hopanoid-lipid A (HoLA) molecules are a mixture of penta-to-hepta-acylated species, which differ in the number, length and nature of acyl substitutes on the saccharide backbone and are devoid of phosphate groups. *Bradyrhizobium* spp. lipid A has a 2,3-diaminoglucose (DAG) disaccharide backbone (violet), a galacturonic acid (GalA) residue (yellow) on the vicinal DAG and an α-(1, 6)-mannose disaccharide (orange) on the distal DAG. The acyl chains are asymmetrically distributed on the sugar skeleton; the two secondary very long chain fatty acids (VLCFAs; red), one of which is not stoichiometrically hydroxylated at C2, are subsititued at the ω−1 position by a hopanepolyol acid and by an acetyl (Ac) group (blue), respectively. The primary acyl chains on the *E. coli* and *Bradyrhizobium* spp. lipid A backbone have the R configuration at the chiral centre, whereas the VLCFAs have the S configuration at the ω−1 position. The non-stoichiometric 2-hydroxylation of the fatty acids occurs with the S configuration. **c |** A conceptual scheme illustrating the outer membrane (OM) of *Bradyrhizobium* spp. is shown. Lipid A (red) insertion decreases the fluidity and increases the mechanical strength of the membrane; VLCFAs entirely span the lipid bilayer so that the covalently linked hopanoid (red) is

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positioned in the inner leaflet; collectively, this would be expected to order both leaflets, with VLCFAs contributing to tightly link the two. Regular phospholipids are shown in green and free hopanoid molecules are shown in blue.