



HAL
open science

Aminolipids in bacterial membranes and the natural environment

Shengwei Liu, Eleonora Silvano, Mingyu Li, Michaela Mausz, Branko Rihtman, Richard Guillonneau, Otto Geiger, David J Scanlan, Yin Chen

► **To cite this version:**

Shengwei Liu, Eleonora Silvano, Mingyu Li, Michaela Mausz, Branko Rihtman, et al.. Aminolipids in bacterial membranes and the natural environment. *The International Society of Microbiological Ecology Journal*, 2024, 18 (1), 10.1093/ismejo/wrae229 . hal-04837000

HAL Id: hal-04837000

<https://hal.science/hal-04837000v1>

Submitted on 13 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Aminolipids in bacterial membranes and the natural environment

Shengwei Liu¹, Eleonora Silvano¹, Mingyu Li¹, Michaela Mausz¹, Branko Rihtman¹, Richard Guillonneau², Otto Geiger³, David J. Scanlan¹, Yin Chen^{4,5,*}

¹School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, United Kingdom

²Faculty of Science and Technology, Nantes Université, CNRS, US2B, UMR 6286, Nantes F-44000, France

³Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Avenida Universidad s/n, Colonia Chamilpa, Cuernavaca, Morelos 62210, México

⁴School of Biosciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

⁵Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, United Kingdom

*Corresponding author: Yin Chen, School of Biosciences, University of Birmingham, Birmingham B15 2TT, United Kingdom. Email: y.chen.22@bham.ac.uk

Abstract

Our comprehension of membrane function has predominantly advanced through research on glycerophospholipids, also known as phosphoglycerides, which are glycerol phosphate-based lipids found across all three domains of life. However, in bacteria, a perplexing group of lipids distinct from glycerol phosphate-based ones also exists. These are amino acid-containing lipids that form an amide bond between an amino acid and a fatty acid. Subsequently, a second fatty acid becomes linked, often via the 3-hydroxy group on the first fatty acid. These amide-linked aminolipids have, as of now, been exclusively identified in bacteria. Several hydrophilic head groups have been discovered in these aminolipids including ornithine, glutamine, glycine, lysine, and more recently, a sulfur-containing non-proteinogenic amino acid cysteinolic acid. Here, we aim to review current advances in the genetics, biochemistry and function of these aminolipids as well as giving an ecological perspective. We provide evidence for their potential significance in the ecophysiology of all major microbiomes, i.e. gut, soil, and aquatic as well as highlighting their important roles in influencing biological interactions.

Keywords: aminolipids, bacterial membrane, environmental lipidomics

Introduction

Lipids comprise the building blocks for all biological membranes, providing a fluid and dynamic environment for hosting integral and peripheral membrane proteins that are critical for nutrient acquisition, defense, and cell signaling [1]. Of all lipids, glycerol phosphate based glycerophospholipids are probably the best studied given they are ubiquitous in all three domains of life. Indeed, it has been proposed that the most ancient life forms likely had a glycerophospholipid-based membrane [2]. Over recent years environmental microbiology has focused on the transformation between glycerophospholipids and non-phosphorus lipids such as betaine lipids and glycolipids [3–5]. However, a unique group of lipids which appears confined to bacteria are the amino acid containing aminolipids, often comprised of an amide linked 3-hydroxy fatty acid (R1) and an ester linked fatty acid (R2) attached to the 3-hydroxy group at R1 (Fig. 1A). These aminolipids can be integrated into both inner and outer membranes of bacterial cells [6, 7]. A noticeable structural difference between aminolipids and glycerophospholipids is that glycerophospholipids are made from a glycerol backbone whereas aminolipids are synthesized from a 3-hydroxy acyl fatty acid backbone. Several proteinogenic amino acids are found as the hydrophilic headgroup in these aminolipids, including glycine, glutamine and lysine.

Non-proteinogenic amino acids have also been found including ornithine and more recently cysteinolic acid [8–10].

It is important to differentiate the aminolipids that are discussed here from aminoacyl phospholipids (Fig. 1B), the latter including lysyl-phosphatidylglycerol (PG), alanyl-PG and arginyl-PG [11]. A common feature of these aminoacyl phospholipids is to neutralize the negative charge of bacterial phospholipids (such as PG) using aminoacylated transfer RNAs (tRNAs), thus allowing pathogenic bacteria to successfully evade host immunity by preventing the action of cationic antimicrobial peptides (CAMPs). However, interested readers should look elsewhere for elegant and comprehensive reviews on amino acid decoration of phospholipids [11, 12].

Two-step biosynthesis of aminolipids

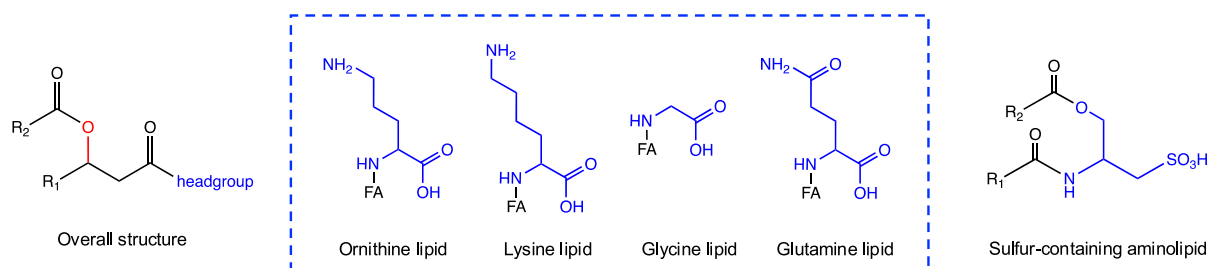
The biosynthesis of aminolipids typically involves two steps to form the mature lipid that is composed of two fatty acid chains, although these lipids can undergo subsequent modifications such as hydroxylation, methylation and transamination. Readers interested in this topic are encouraged to refer to recent reviews for additional information [13, 14]. In the first step, fatty acids are activated before they are conjugated to an amino acid to form an

Received: 30 May 2024. Revised: 1 October 2024. Accepted: 7 November 2024

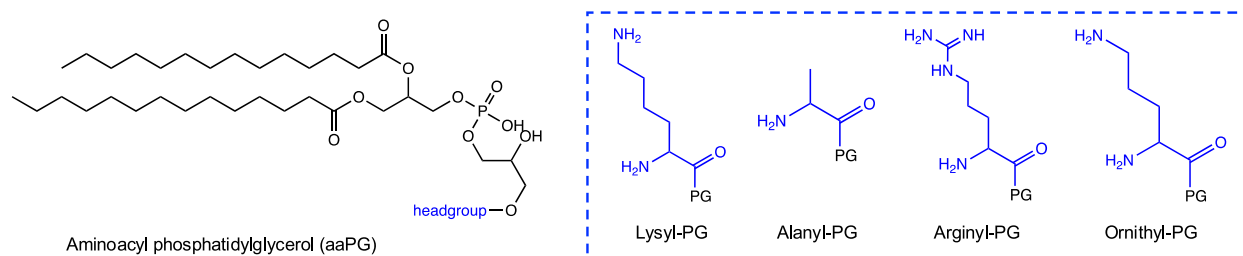
© The Author(s) 2024. Published by Oxford University Press on behalf of the International Society for Microbial Ecology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

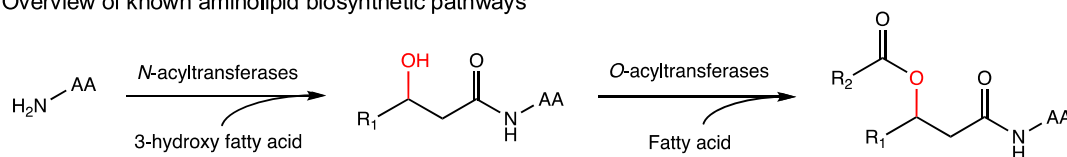
A Structure of known aminolipids



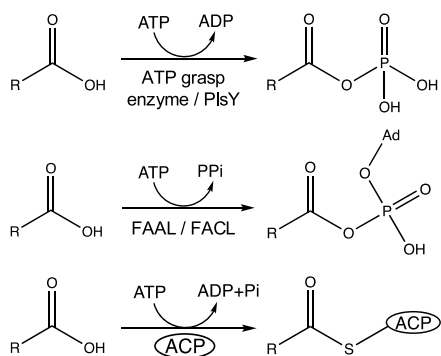
B Examples of aminoacyl phospholipids



C Overview of known aminolipid biosynthetic pathways



D Known activation mechanisms



E Examples of N-acylated amino acids

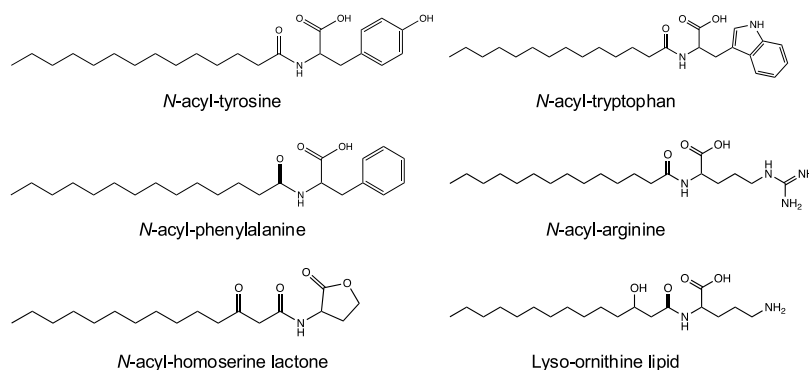


Figure 1. Representative structures of aminolipids and related compounds, including common fatty acid activation pathways. (A) Selected structures of several known aminolipids. (B) Examples of aminoacyl phospholipids. (C) Overview of currently known 2-step aminolipid biosynthetic pathways. (D) Currently known pathways for fatty acid activation in biological systems. (E) Selected examples of *N*-acylated amino acids, including *N*-acylhomoserine lactones and lyso-aminolipids. The 3-hydroxy group in the aminolipid is highlighted in red. FA, fatty acid chains; PG, phosphatidylglycerol; ad, adenosine; FAAL, fatty acyl-AMP ligase; FACL, fatty acyl-CoA ligase; ACP, acyl carrier protein; AA, amino acid.

amide bond (Fig. 1C). Subsequently in the second step, another fatty acid is conjugated to create the mature aminolipid (Fig. 1C). Fatty acids are generally inert chemicals and require specific biological mechanisms for activation to make them biologically available for condensation with an amino acid. Several mechanisms exist for the formation of an amide bond between fatty acids and amino acids, including acyl-phosphate, acyl-adenylate and acyl-ACP (acyl carrier protein) (Fig. 1D).

The direct condensation of ATP with a carboxylate group in fatty acids can be catalyzed by ATP grasp enzymes (Pfam 13535) involving acyl-phosphate as the key intermediate, as seen in the formation of acyl-histidine in *Legionella pneumophila*, the causative

agent of Legionnaires' disease [15, 16]. However, it is worth noting that whether this type of system can effectively use 3-hydroxy fatty acids as a substrate has not been tested. Perhaps a more relevant example of acyl-phosphate serving as the fatty acid donor is observed in glycerophospholipid synthesis, where the PlsY enzyme transfers the acyl group from acyl-phosphate to glycerol-3-phosphate to form lyso-PG [17]. Fatty acids can also be activated to form an acyl-adenylate intermediate, e.g. by a fatty acid AMP ligase (FAAL) or a fatty acid CoA ligase (FACL) [18, 19]. However, whether FAAL/FACL enzymes can be utilized in aminolipid synthesis has yet to be established. Curiously, it appears that acyl-ACP is the donor of choice for aminolipid biosynthesis, which

has been experimentally confirmed in the synthesis of ornithine-containing aminolipids [14, 20].

It is important to highlight that 3-hydroxy fatty acids are often required in the final step of ester bond formation for attaching the second fatty acid in several aminolipids (Fig. 1A). It thus appears that the aminolipid biosynthesis pathway might have “hijacked” 3-hydroxy fatty acyl-ACP from the type II fatty acid synthesis pathway, an essential intermediate in the elongation step mediated by the FabG reductase, to supply the intermediate required for aminolipid biosynthesis [21]. Similarly, several acyl-transferases involved in lipid A biosynthesis, such as LpxA and LpxM, all require 3-hydroxy fatty acyl-ACP as the preferred substrate [22, 23]. Subsequent formation of lyso-aminolipids between 3-hydroxy fatty acyl-ACP and an amino acid is often carried out by an *N*-acyl transferase (Pfam 13 444), the archetype of which in ornithine lipid biosynthesis is OlsB [20].

The first step in aminolipid biosynthesis, i.e. the formation of an amide bond between the carboxylate functional group of an activated fatty acid and the amine group of an amino acid, closely resembles the biosynthesis of fatty acyl amides that are widely recognized for their role in cell signaling [24]. A large group of fatty acyl amides known as *N*-acylated amino acids, where an amino acid provides the functional amine group (Fig. 1E), are produced by a variety of organisms, including humans. Perhaps the best known of these is anandamide which is capable of binding to human cannabinoid receptors (reviewed in Ezzili et al., 2010 [25]). It is worth noting that many bacteria are also able to produce *N*-acylated amino acids although their *in vivo* function is poorly understood [26–28]. In these microbes, *N*-acylated amino acids can be synthesized through a group of GNAT acyltransferases that show some similarity to enzymes involved in aminolipid biosynthesis (see below). However, as it stands, it has not been reported whether these *N*-acylated amino acids can be further acylated to produce an aminolipid-like molecule that can be incorporated into microbial membranes. These *N*-acylated amino acids identified from soil microorganisms often do not have a 3-hydroxygroup which can be readily acylated further to form a mature membrane lipid.

Phylogenetically, *N*-acyltransferases involved in aminolipid biosynthesis form a separate branch distinct from other acyl-transferases, most noticeably *N*-acylated amino acid synthase and acyl-homoserine lactone synthase (Fig. 2). Domain analysis suggests that all enzymes involved in aminolipid biosynthesis (see below), along with some involved in *N*-acyl amino amide synthesis, share the common PF13444 domain. Both *N*-acylated amino acid synthase and acyl-homoserine lactone synthase use acyl-ACP for the initial step of amide bond formation. Curiously, enzymes catalyzing the amide bond formation between aromatic and non-aromatic amino acids are clearly separated phylogenetically. However, it remains to be investigated whether aromatic amino acids can indeed lead to the formation of mature aminolipids.

The second step in aminolipid biosynthesis typically involves an *O*-acyltransferase (Fig. 3), which shares some sequence similarity to the PlsC enzyme responsible for glycerophospholipid phosphatidic acid biosynthesis [39]. This family of *O*-acyltransferases also contains several enzymes involved in lipopolysaccharide (LPS) synthesis and modification, as well as the PatA acyltransferase involved in modifying membrane lipids in mycobacteria. Together, these proteins form a large group known as the lysophospholipid acyltransferase (LPLAT) family. Thus, it is likely that different functional domains are capable of *O*-acylation in the second step of aminolipid synthesis in different

organisms and there may be new sequence motifs yet to be discovered. This consideration is important when interpreting omics-data in the context of aminolipid biosynthesis (see below).

Known aminolipids: their synthesis and function

Several pathways for aminolipid biosynthesis in bacteria have been studied in detail in recent years. Arguably the best example is ornithine lipid [14, 40] whose synthesis is often carried out by two genes *olsB/olsA* usually found adjacent to each other on the chromosome. OlsB is an *N*-acyltransferase that forms the amide bond and OlsA an *O*-acyltransferase that forms the ester bond. In some bacteria, particularly Bacteroidetes and Flavobacteria, one single protein encoded by *olsF* is responsible for ornithine biosynthesis. While the C-terminus of OlsF shows a high degree of similarity to OlsB, the N-terminus differs from OlsA. Ornithine lipid appears widely distributed in bacteria and previous genome analyses have found more than half of bacterial genomes appear to have pathways involved in its biosynthesis.

There has been long-standing speculation that OlsB homologues are likely responsible for the initial step in the synthesis of various other aminolipids [40]. This was indeed the case for at least two other aminolipids, the glutamine lipids found in marine roseobacter clade bacteria that are important players in oceanic biogeochemical cycling [29], as well as glycine lipids found in an important group of gut microbes [30]. It is unfortunate that these two independent studies, published in the same year, both designated the *N*-acyltransferase involved in glutamine and glycine aminolipid biosynthesis as GlsB. Given this may lead to confusion in the literature we thus propose to rename the *N*-acyltransferase involved in glutamine synthesis as GluB to avoid confusion in the future. Intriguingly, these lipids appear to have a relatively restricted distribution, as determined through genome analysis. GluB is predominantly found in marine roseobacters (Alphaproteobacteria) [29] whilst GlsB is prevalent in gut associated Bacteroides [30]. The second step of the synthesis of glutamine and glycine lipids involves an *O*-acyltransferase similar to that known for the synthesis of ornithine lipids. Glycine aminolipid and its lyso form have been identified in previous omics studies of the gut microbiome [41, 42]. Indeed, the discovery of GlsB was instrumental in unveiling the synthesis of these microbiota-derived lipids for the first time. Phylogenetic analysis indicates that *gluB* genes may have arisen from a gene duplication event involving *olsB*, whereas *glsB* genes could potentially have emerged from the duplication of the C-terminus of OlsF (Fig. 2).

More recently, a new sulfur-containing aminolipid (SAL) was discovered in marine bacteria named SAL [8]. This lipid comprises a non-proteinogenic amino acid containing sulfur, cysteinolic acid. The first synthesis step, however, does not appear to be carried out by an OlsB *N*-acyltransferase homolog and the gene responsible for this step is still awaiting identification. However, the final step of *O*-acylation is performed by the *salA* gene which is homologous to *olsA*. Both OlsA and SalA share common sequence motifs with PlsC [8, 28]. However, it is worth noting that the N-terminus of OlsF involved in ornithine lipid biosynthesis in some flavobacteria and *Serratia* spp. lacks sequence similarity with PlsC [31]. Indeed, the OlsA/PlsC proteins feature a characteristic Pfam 01553 domain, whereas the N-terminus of OlsF and recently discovered *O*-acyltransferase GlyA have a different Pfam 19 576 domain (Fig. 3). Previous genome analysis has shown that *salA* appears to be confined to the marine roseobacter clade for

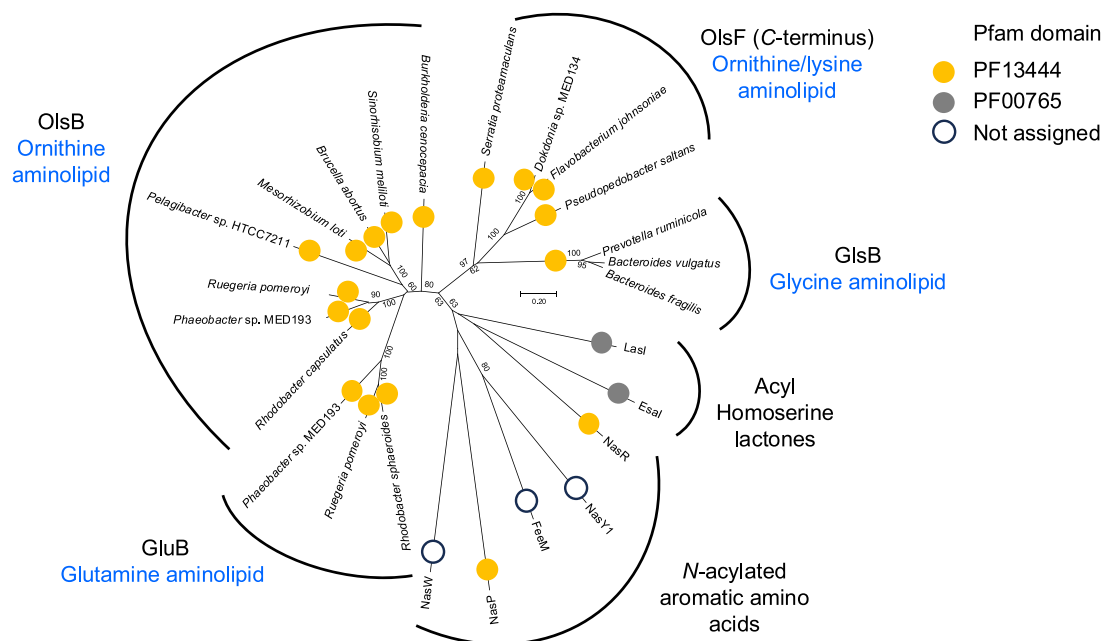


Figure 2. The phylogeny of N-acyltransferases involved in aminolipid biosynthesis together with those involved in homoserine lactone synthesis and N-acylated aromatic amino acid synthesis. The evolutionary history was inferred using a neighbor joining algorithm from a multiple sequence alignment obtained with ClustalX. Bootstrap values greater than 50% are shown. A total of 304 positions were used in the final dataset. Evolutionary analysis was conducted in MEGA7. Protein domain analysis was obtained from the InterProScan at EBI (<https://www.ebi.ac.uk/interpro/search/sequence/>). Sequences included in this analysis include OlsB (BAB1_0147 *Brucella abortus*; SMC01127 *Sinorhizobium meliloti*; SPO1980 *Ruegeria pomeroyi*; MED193_03912 *Phaeobacter* sp. MED193; BCAL1281 *Burkholderia cenocepacia*; mlr3216 *Mesorhizobium loti*; HTCC7211_00011000 *Pelagibacter* sp. HTCC7211; MED193_02489 *R. pomeroyi*; MED193_22491 *Phaeobacter* sp. MED193; LAZ29_20365 *Rhodobacter sphaeroides* [29]), GlsB (locus tags – BVU_RS07720 *Bacteroides vulgatus*; BM023_RS02715 *Prevotella ruminicola*; HMPREF1203_RS01905 *Bacteroides fragilis* [30]), OlsF (Spro_2569 *Serratia proteamaculans*; MED134_08291 *Dokdonia* sp. MED134; PEDSA_2277 *Pseudopedobacter saltans*; Fjoh_0833 *Flavobacterium johnsoniae* [31]), LasI (PDB 1R05) and EsaI (PDB 1KZF) involved in SAM-dependent homoserine lactone synthesis [32, 33], and FeeM (PDB 2G0B), NasW (AAO64420), NasP (ABB76600), NasY1 (AAG53691), and NasR (AAO64421) involved in N-acylated amino acid synthesis [28, 34].

reasons that are unclear [8]. Perhaps this restriction is related to the unique ability of these bacteria to produce and metabolize a range of C₃ sulfonates [43, 44], including cysteinolic acid which forms the hydrophilic head group of this unusual lipid [9].

Another lesser-known aminolipid features lysine as the head-group. This lipid, along with its hydroxylated forms, have been found in the soil bacterium *Pseudopedobacter saltans* as well as *Agrobacterium* spp. and *Pedobacter* spp. [45, 46]. However, the exact synthesis mechanism remains unclear although it is speculated that an OlsF homolog is likely involved [31, 46]. Similar to the hydroxylation of ornithine lipid by OlsE, it is hypothesized that an OlsE homolog is responsible for lysine lipid hydroxylation [46]. An OlsE homolog is also found in some marine roseobacters, such as SPO0328 in *Ruegeria pomeroyi* DSS-3 (i.d. 35%, e-value e⁻⁵⁴), but it remains to be seen whether this gene is responsible for hydroxylation of the ornithine lipid or the SAL lipid found in this bacterium [8].

Unlike glycerophospholipids, the physiological functions of these aminolipids are generally not well understood and no clear pattern has yet emerged for these lipids. Indeed, it appears that in different bacteria their roles are noticeably dissimilar. For example, in the soil bacterium *Sinorhizobium meliloti*, the production of ornithine lipids is believed to be at least in part a response to phosphorus (P) deficiency. However, this transition from phospholipids to ornithine lipids does not appear to be relevant for root nodulation on legume host plants [39]. In *Pseudomonas aeruginosa*, ornithine lipid production is also enhanced under P limitation, and this lipid seems to play a role in the resistance of *Pseudomonas aeruginosa* to antimicrobial

peptides [47]. However, ornithine lipids are not tightly regulated by P availability in other bacteria, e.g. *Burkholderia* sp. [48] and *Ruegeria* sp. [29]. Thus, the specific role of these lipids may differ significantly depending on the organism under investigation.

The function of glycine-containing aminolipids has been primarily studied in the context of infection and immunity, particularly in the gut microbiome (recently reviewed by Ryan et al., [49]) although many environmental bacteria are also known to produce them [10]. Both glycine and ornithine aminolipids are known to induce an immune response, but through different pathways. For example, synthetic ornithine lipids composed of saturated C14:0 fatty acids are able to bind to Toll like receptor 4 (TLR4), which is the best-known receptor for lipopolysaccharides (LPS) of Gram-negative bacteria, thus reducing the LPS-induced immune response [50, 51]. In contrast, glycine lipid such as flavolipin interacts with TLR2, a receptor known to interact with lipopeptides [51, 52]. The lyso form of flavolipin (mono-acylated) activates G-protein coupled receptors (GPCR) through internal signaling cascades [42]. Lyso glycine aminolipids display hemolytic activity and can lyse membranes [53]. This membrane-disrupting activity is similar to that of N-acylated amino acids that are able to disrupt bacterial membranes and exhibit antimicrobial activity [27].

In the marine environment, two unusual aminolipids, SAL and the glutamine-containing aminolipid, are largely confined to the marine roseobacter group [8, 29]. These bacteria are well known for their metabolic versatility and their ability to form various interactions with algae, marine animals and other organisms [54]. As such, we have hypothesized that these aminolipids in marine roseobacter may have a role in cell-cell interactions. This has

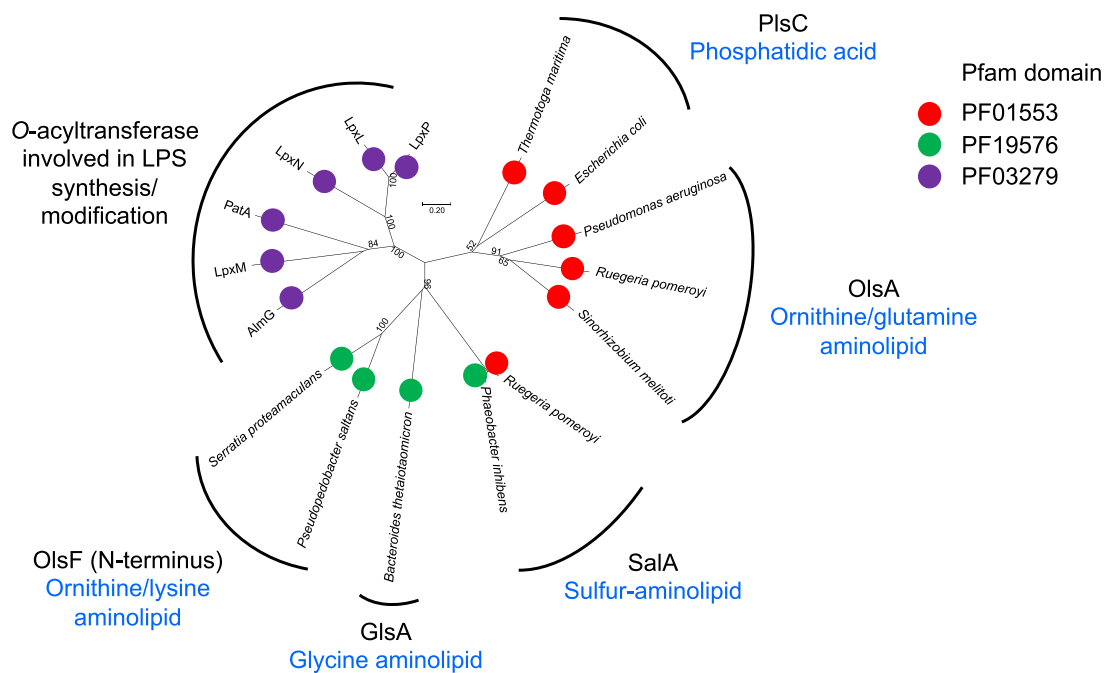


Figure 3. The phylogeny of O-acyltransferases of the lysophospholipid acyltransferase (LPLAT) family involved in the synthesis of phosphatidic acid, aminolipids as well as those involved in lipopolysaccharide (LPS) biosynthesis and modification. The evolutionary history was inferred using a neighbor joining algorithm from a multiple sequence alignment obtained with ClustalX. Bootstrap values greater than 50% are shown. A total of 557 positions were used in the final dataset. Evolutionary analysis was conducted in MEGA7. Protein domain analysis was obtained from the InterProScan at EBI (<https://www.ebi.ac.uk/interpro/search/sequence/>). Sequences included in this analysis include LpxP (NP_416879), LpxL (NP_415572), LpxN (NP_229869), PatA (PDB 5F2Z, [35]), LpxM (PDB 5KN7, [23]), AlmG (NP_231217, [36]), PlsC (PDB 5KYM, [37]), PlsC (NC_011748), OlsA from *Pseudomonas aeruginosa* (PA4351, [38]), *Ruegeria pomeroyi* (SPO1979, [29]), *Sinorhizobium meliloti* (SMc01116, [39]), SalA from *R. pomeroyi* (SPO0716, [8]) and *Phaeobacter inhibens* (PGA1_c01210), GlsA (BVU_RSO7715, [30]), and OlsF from *Serratia proteamaculans* (Spro_2569), *Pseudopedobacter saltens* (PEDSA_2277).

been partially validated. For example, changes in membrane lipid composition in roseobacters seems to influence the dynamics of bacteria-protist interactions [55]. However, unlike the formation of betaine-containing lipids through membrane lipid remodeling [55], the absence of SAL lipids does not appear to affect grazing of *R. pomeroyi* DSS-3 by *Uronema* sp. (unpublished data). The loss of two aminolipids, ornithine and glutamine lipids in *R. pomeroyi* DSS-3, however, significantly alters membrane protein composition and completely changes the dynamics of bacteriophage attachment [6]. These studies suggest that the physiology of lipids is likely influenced by the interplay between lipids and integral or peripheral membrane proteins, and possibly other macromolecules that are attached to the lipid membrane, e.g. LPS. Perhaps, it is this uncertainty regarding how alterations in lipid composition can impact local protein assembly and function that makes predicting the function of aminolipids in various organisms somewhat challenging.

Uncovering aminolipid biosynthesis in the environment through lipidomics and metagenomics

Environmental lipidomics (a.k.a. metalipidomics) has emerged as a potent tool for revealing the vast array of membrane lipids within various environmental settings, and elucidating how the environment can influence microbiome dynamics across diverse climate scenarios [56]. Nonetheless, it is puzzling that only a few studies have documented the detection of aminolipids in environmental samples through mass spectrometry-based lipidomics investigations, despite genomic analyses indicating the

abundance of genes involved in aminolipid biosynthesis across many bacterial genomes. Only a handful of studies have reported the presence of ornithine aminolipids or its methylated derivatives [57–62]. This scarcity of aminolipid reports in an environmental context may be partially attributed to the insufficient utilization of extensive mass spectrometry fragmentation analyses, essential for assigning environmental lipidomics data (comprising intact lipid m/z and MS^n fragmentation patterns) to distinct aminolipid species. It is perhaps unsurprising that ornithine lipids are comparatively better documented, as a diagnostic m/z 115 fragment originating from dehydrated cyclized ornithine amino acid is observed in positive-mode electrospray ionization mass spectrometry [63], facilitating the identification of this aminolipid in environmental metalipidomics studies. The identification of aminolipids heavily relies on the availability of reference ion component spectra, underscoring the urgent need for further research to discover and catalogue novel aminolipid species. Current lipid databases, e.g. the Global Natural Product Social Molecular Networking (GNPS) library [64] and the LIPID MAPS Structure Database [65], are deficient in comprehensive aminolipid references, limiting their use in structural annotation of aminolipids. Employing advanced techniques such as fragment network analysis through platforms such as the GNPS could help characterization of aminolipids in future environmental lipidomics studies [59].

It is noteworthy that the precursor for aminolipid synthesis, 3-hydroxy fatty acids, is also commonly employed as a biomarker indicative of the presence of bacterial LPS [66], which has been extensively used in paleoclimate research. Comprehensive soil, sediment, and aquatic environment samplings have revealed the

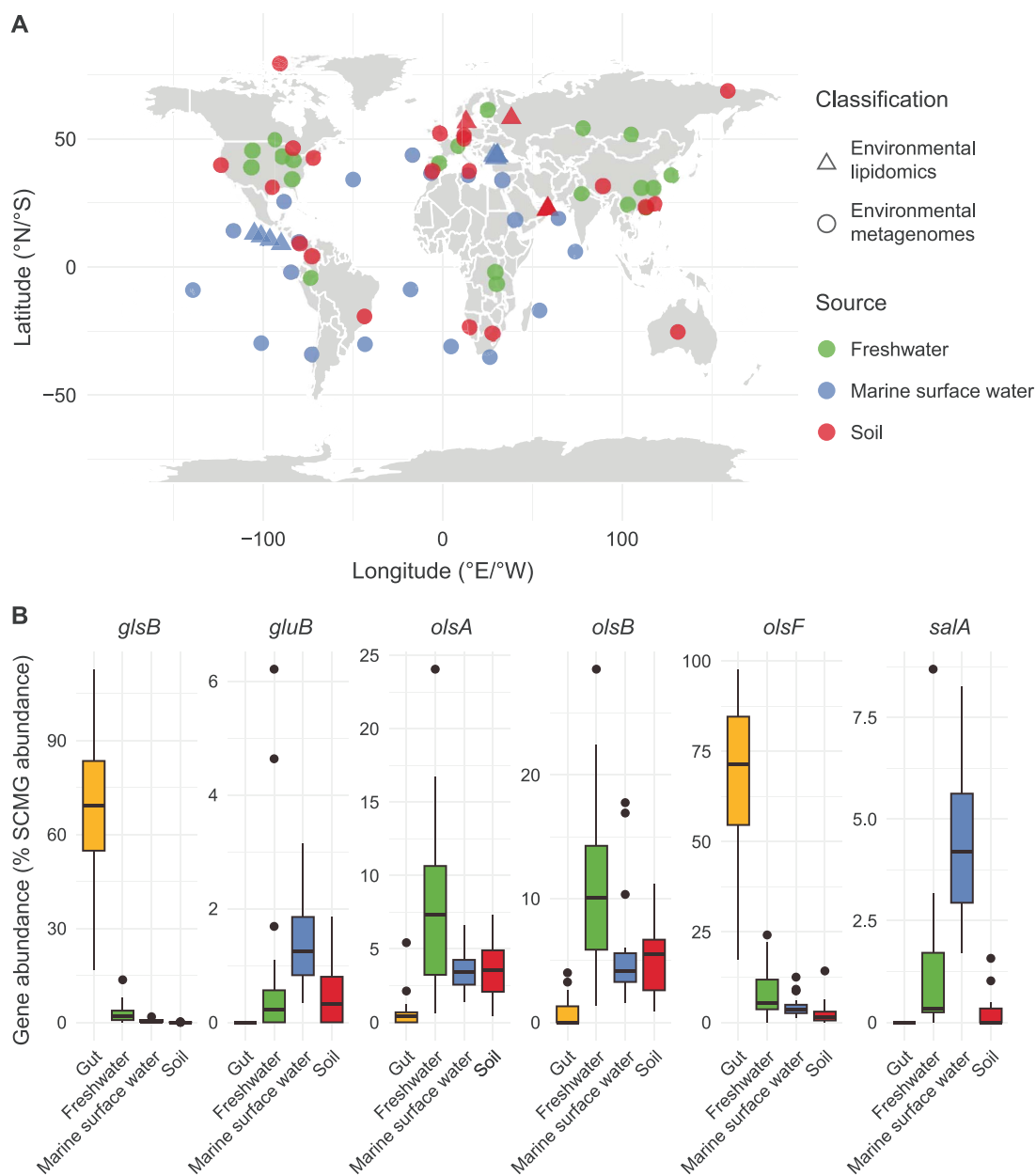


Figure 4. The global distribution of microbial aminolipid biosynthesis potential. (A) Detection of aminolipids through environmental lipidomics surveys, represented by triangles, and the global distribution of key genes involved in aminolipid biosynthesis in representative environmental microbiomes, including freshwater ($n = 20$), marine surface water ($n = 20$) and soil ($n = 20$) represented by green, blue and red circles, respectively. The environmental lipidomics dataset included in this figure is shown in [Supplementary Table S1](#). Globally distributed metagenome datasets were retrieved from the NCBI, and basic sample and quality information is presented in [Supplementary Table S2](#). (B) The relative abundance of aminolipid biosynthesis genes across different ecosystems, expressed as a percentage of whole-community single-copy marker gene (SCMG) abundances. Gut microbiome metagenome datasets ($n = 20$), with human DNA sequences removed using BMTagger, were obtained from the human microbiome project [67] accessible at <https://www.hmpdacc.org/HMASM/>. Briefly, raw data was trimmed to clean the data using Trimmomatic v0.39 [68], which was then used to assemble contigs using MEGAHIT v1.2.9 [69] with a k -mer range from 21 to 119. Gene predictions for contigs were made by prodigal v2.6.3 [70]. The aminolipid biosynthesis genes and ten bacterial single-copy housekeeping genes were searched by HMMER v3.4 [71], applying an optimized cut-off (e -value $< 1e-40$). Relative gene abundances were calculated as reads per kilobase per million mapped reads (RPKM) using CoverM v0.7.0 [72]. Then, the relative abundance of aminolipid biosynthesis genes was normalized by dividing by the median abundance of ten bacterial SCMGs [73]. The taxonomy of these aminolipid biosynthesis genes was affiliated against the IMG's unrestricted isolate genomes (IUIG) dataset (dated 11/20/2023) using DIAMOND blastp v2.1.9.163 [74] with an e -value $< 1e-5$. These data are summarized in [Supplementary Table S3](#).

widespread occurrence of 3-hydroxy fatty acids across global ecosystems. However, whether or not these molecules are truly derived from bacterial LPS is questionable, given that aminolipids are also likely prevalent in the environment. Despite the limited number of studies measuring aminolipids in the environment, recent advances in uncovering genes involved in aminolipid biosynthesis have facilitated analysis of various environmental

metagenomics datasets for the presence of these genes. This analysis included representative microbiomes from marine surface water, freshwater, and soil ecosystems, as well as the human gut. The data presented in [Fig. 4](#) reveal intriguing patterns. Whilst genes responsible for ornithine lipid biosynthesis appear ubiquitous across all ecosystems studied, the distribution of other aminolipids seems confined to specific ecosystems. For

instance, the *salA* gene responsible for the synthesis of the SAL lipid is particularly abundant in marine surface water microbiomes, whereas the glycine lipid biosynthesis gene *glsB* is most prevalent in the human gut. Moreover, genes like *gluB* (required for glutamine lipid biosynthesis) and *salA* which are relatively abundant in marine bacteria, are completely absent in the human gut.

A perspective on future research needs

Aminolipids represent an intriguing group of bacterial membrane lipids but we are only just starting to understand their physiological and metabolic roles. Activation of (3-hydroxy) fatty acids through the common bacterial type II fatty acid synthesis pathway enables subsequent adding of the 2nd fatty acid through an ester bond in a piggy-back manner. There it appears that bacteria hijacked fatty acyl-ACP from the type II fatty acid synthesis pathway to make aminolipids. Given the type II fatty acid synthesis pathway is largely found in bacteria, this could help explain the lack of these lipids in archaea and eukaryotes. However, given that the synthesis of the sulfur-containing aminolipids SAL in roseobacters does not require a 3-hydroxy fatty acid, it thus remains to be seen whether similar aminolipids may exist beyond bacteria.

To date, only a handful of amino acids are found in these lipids and questions remain whether other aminolipids exist in bacterial membranes. Noticeably, to date no aromatic amino acids are found in aminolipids (e.g. tyrosine, phenylamine), nor are amino acids with heterocyclic rings found (e.g. histidine, proline) yet their *N*-acyl amino acid counterparts appear common in the biosphere. To address these disparities, a thorough, systematic and meticulous investigation of membrane lipids in a wide range of bacterial isolates and model organisms using high-resolution mass spectrometry is clearly required in future lipidomics studies. For some aminolipids that are known to be present in bacterial membranes, their synthesis pathways also remain to be fully established, e.g. lysine aminolipids and SALs. Uncovering the genes underpinning their biosynthesis would greatly advance our understanding of the biosynthetic potential of particular microbiomes, helping guide the design of metalipidomics surveys to confirm their presence and quantify their abundance.

Functional studies of these lipids in microbial membranes are still in their infancy. Both glycine lipids and ornithine lipids, which are abundant in the human gut microbiome (Fig. 4), have been identified as potential immunomodulators. Ornithine lipids produced by the gut bacterium *Akkermansia muciniphila* likely influence the host's production of pro-inflammatory cytokines and antimicrobial peptides through interaction with the host transcription factor ATF3 [75]. Ornithine itself can also influence gene expression by interacting with ribosomes [76]. Other aminolipids such as SAL and glutamine lipids are not as widely distributed as ornithine lipids, and their functions appear to be group or strain-specific, depending on the model organisms investigated. For example, SAL lipids in *Phaeobacter* sp. play a role in biofilm formation, yet in a closely related marine roseobacter strain *R. pomeroyi* DSS-3, deletion of the *salA* gene had little impact on biofilm formation. Thus, it is entirely possible that the interplay between lipids-lipids and lipids-proteins varies between bacterial strains, making precise prediction of aminolipid function in bacterial physiology not at all straightforward. Moreover, from a fundamental perspective, it is puzzling why bacteria encode the capacity to produce these lipids in the first place. For example, the well-studied model bacterium *Escherichia coli* does not appear

to produce any aminolipids. After all, evolution has selected glycerophospholipids from the beginning of life's existence [2]. Thus, future studies should employ a wide variety of aminolipid-producing models to investigate their roles across a range of bacteria-bacteria and bacteria-host interactions. Only through such extensive work can a pattern for the role of these lipids begin to emerge.

Acknowledgements

The authors acknowledge funding from the Natural Environment Research Council, the Royal Society (UK) and the European Research Council. We are also grateful to the editors of The ISME Journal for their invitation, encouragement and inspiration to write this review.

Supplementary material

Supplementary material is available at *The ISME Journal* online.

Conflicts of interest

The authors have no conflict of interest to report.

Funding

None declared.

Data availability

There are no primary data associated with this review article.

References

- Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. *Nat Rev Mol Cell Biol* 2018;**19**:281–96. <https://doi.org/10.1038/nrm.2017.138>
- Lombard J, López-García P, Moreira D. The early evolution of lipid membranes and the three domains of life. *Nat Rev Microbiol* 2012;**10**:507–15. <https://doi.org/10.1038/nrmicro2815>
- Sebastián M, Smith AF, González JM et al. Lipid remodelling is a widespread strategy in marine heterotrophic bacteria upon phosphorus deficiency. *ISME J* 2016;**10**:968–78. <https://doi.org/10.1038/ismej.2015.172>
- Carini P, Van Mooy BAS, Thrash JC et al. SAR11 lipid renovation in response to phosphate starvation. *Proc Natl Acad Sci USA* 2015;**112**:7767–72. <https://doi.org/10.1073/pnas.1505034112>
- Silvano E, Yang M, Wolterink M et al. Lipidomic analysis of roseobacters of the pelagic RCA cluster and their response to phosphorus limitation. *Front Microbiol* 2020;**11**:552135. <https://doi.org/10.3389/fmicb.2020.552135>
- Stirrup R, Mausz MA, Silvano E et al. Aminolipids elicit functional trade-offs between competitiveness and bacteriophage attachment in *Ruegeria pomeroyi*. *ISME J* 2023;**17**:315–25. <https://doi.org/10.1038/s41396-022-01346-0>
- Wilkinson BJ, Sment KA, Mayberry WR. Occurrence, localization, and possible significance of an ornithine-containing lipid in *Paracoccus denitrificans*. *Arch Microbiol* 1982;**131**:338–43. <https://doi.org/10.1007/BF00411182>
- Smith AF, Silvano E, Päuker O et al. A novel class of sulfur-containing aminolipids widespread in marine roseobacters. *ISME J* 2021;**15**:2440–53. <https://doi.org/10.1038/s41396-021-00933-x>

9. Roman D, Meisinger P, Guillonneau R et al. Structure revision of a widespread marine sulfonolipid class based on isolation and total synthesis. *Angew Chem Int Ed Engl* 2024;**63**:e202401195. <https://doi.org/10.1002/anie.202401195>
10. Sohlenkamp C, Geiger O. Bacterial membrane lipids: diversity in structures and pathways. *FEMS Microbiol Rev* 2016;**40**:133–59. <https://doi.org/10.1093/femsre/fuv008>
11. Slavetinsky C, Kuhn S, Peschel A. Bacterial aminoacyl phospholipids – biosynthesis and role in basic cellular processes and pathogenicity. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017;**1862**: 1310–8. <https://doi.org/10.1016/j.bbalip.2016.11.013>
12. Fields RN, Roy H. Deciphering the tRNA-dependent lipid aminoacylation systems in bacteria: novel components and structural advances. *RNA Biol* 2018;**15**:480–91. <https://doi.org/10.1080/15476286.2017.1356980>
13. Sohlenkamp C. Ornithine lipids and other amino acid-containing acyloxyacyl lipids. In: Geiger O. (ed.), *Biogenesis of Fatty Acids, Lipids and Membranes*. Cham: Springer International Publishing, 2019, 109–22.
14. Vences-Guzmán MÁ, Geiger O, Sohlenkamp C. Ornithine lipids and their structural modifications: from A to E and beyond. *FEMS Microbiol Lett* 2012;**335**:1–10. <https://doi.org/10.1111/j.1574-6968.2012.02623.x>
15. Tørring T, Shames SR, Cho W et al. Acyl histidines: new N-acyl amides from *Legionella pneumophila*. *Chembiochem* 2017;**18**: 638–46. <https://doi.org/10.1002/cbic.201600618>
16. Fawaz MV, Topper ME, Firestine SM. The ATP-grasp enzymes. *Bioorg Chem* 2011;**39**:185–91. <https://doi.org/10.1016/j.bioorg.2011.08.004>
17. Li Z, Tang Y, Wu Y et al. Structural insights into the committed step of bacterial phospholipid biosynthesis. *Nat Commun* 2017;**8**:1691. <https://doi.org/10.1038/s41467-017-01821-9>
18. Arora P, Goyal A, Natarajan VT et al. Mechanistic and functional insights into fatty acid activation in *Mycobacterium tuberculosis*. *Nat Chem Biol* 2009;**5**:166–73. <https://doi.org/10.1038/nchembio.143>
19. Schmelz S, Naismith JH. Adenylate-forming enzymes. *Curr Opin Struct Biol* 2009;**19**:666–71. <https://doi.org/10.1016/j.sbi.2009.09.004>
20. Gao J-L, Weissenmayer B, Taylor AM et al. Identification of a gene required for the formation of lyso-ornithine lipid, an intermediate in the biosynthesis of ornithine-containing lipids. *Mol Microbiol* 2004;**53**:1757–70. <https://doi.org/10.1111/j.1365-2958.2004.04240.x>
21. White SW, Zheng J, Zhang Y-M et al. The structural biology of type II fatty acid biosynthesis. *Annu Rev Biochem* 2005;**74**: 791–831. <https://doi.org/10.1146/annurev.biochem.74.082803.133524>
22. Anderson MS, Bull HG, Galloway SM et al. UDP-N-acetylglucosamine acyltransferase of *Escherichia coli*, the first step of endotoxin biosynthesis is thermodynamically unfavorable. *J Biol Chem* 1993;**268**:19858–65. [https://doi.org/10.1016/S0021-9258\(19\)36592-5](https://doi.org/10.1016/S0021-9258(19)36592-5)
23. Dovala D, Rath CM, Hu Q et al. Structure-guided enzymology of the lipid acyltransferase LpxM reveals a dual activity mechanism. *Proc Natl Acad Sci USA* 2016;**113**:E6064–71. <https://doi.org/10.1073/pnas.1610746113>
24. Bhandari S, Bisht KS, Merkler DJ. The biosynthesis and metabolism of the N-acylated aromatic amino acids: N-acylphenylalanine, N-acyltyrosine, N-acyltryptophan, and N-acylhistidine. *Front Mol Biosci* 2022;**8**:801749. <https://doi.org/10.3389/fmolb.2021.801749>
25. Ezzili C, Otrubova K, Boger DL. Fatty acid amide signaling molecules. *Bioorg Med Chem Lett* 2010;**20**:5959–68. <https://doi.org/10.1016/j.bmcl.2010.08.048>
26. Brady SF, Clardy J. N-acyl derivatives of arginine and tryptophan isolated from environmental DNA expressed in *Escherichia coli*. *Org Lett* 2005;**7**:3613–6. <https://doi.org/10.1021/ol0509585>
27. Brady SF, Chao CJ, Clardy J. Long-chain N-acyltyrosine synthases from environmental DNA. *Appl Environ Microbiol* 2004;**70**: 6865–70. <https://doi.org/10.1128/AEM.70.11.6865-6870.2004>
28. Wagoner RMV, Clardy J. FeeM, an N-acyl amino acid synthase from an uncultured soil microbe: structure, mechanism, and acyl carrier protein binding. *Structure* 2006;**14**:1425–35. <https://doi.org/10.1016/j.str.2006.07.005>
29. Smith AF, Rihtman B, Stirrup R et al. Elucidation of glutamine lipid biosynthesis in marine bacteria reveals its importance under phosphorus deplete growth in *Rhodobacteraceae*. *ISME J* 2019;**13**:39–49. <https://doi.org/10.1038/s41396-018-0249-z>
30. Lynch A, Tammireddy SR, Doherty MK et al. The glycine lipids of *Bacteroides thetaiotaomicron* are important for fitness during growth *in vivo* and *in vitro*. *Appl Environ Microbiol* 2019;**85**:e02157–18. <https://doi.org/10.1128/AEM.02157-18>
31. Vences-Guzmán MÁ, Guan Z, Escobedo-Hinojosa WI et al. Discovery of a bifunctional acyltransferase responsible for ornithine lipid synthesis in *Serratia proteamaculans*. *Environ Microbiol* 2015;**17**:1487–96. <https://doi.org/10.1111/1462-2920.12562>
32. Gould TA, Schweizer HP, Churchill MEA. Structure of the *Pseudomonas aeruginosa* acyl-homoserinylactone synthase LasI. *Mol Microbiol* 2004;**53**:1135–46. <https://doi.org/10.1111/j.1365-2958.2004.04211.x>
33. Watson WT, Minogue TD, Val DL et al. Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol Cell* 2002;**9**:685–94. [https://doi.org/10.1016/S1097-2765\(02\)00480-X](https://doi.org/10.1016/S1097-2765(02)00480-X)
34. Craig JW, Cherry MA, Brady SF. Long-chain N-acyl amino acid synthases are linked to the putative PEP-CTERM/exosortase protein-sorting system in Gram-negative bacteria. *J Bacteriol* 2011;**193**:5707–15. <https://doi.org/10.1128/JB.05426-11>
35. Albesa-Jové D, Svetlíková Z, Tera M et al. Structural basis for selective recognition of acyl chains by the membrane-associated acyltransferase PatA. *Nat Commun* 2016;**7**:10906. <https://doi.org/10.1038/ncomms10906>
36. Henderson JC, Herrera CM, Trent MS. AlmG, responsible for polymyxin resistance in pandemic *Vibrio cholerae*, is a glycytransferase distantly related to lipid acyltransferases. *J Biol Chem* 2017;**292**:21205–15. <https://doi.org/10.1074/jbc.RA117.000131>
37. Robertson RM, Yao J, Gajewski S et al. A two-helix motif positions the lysophosphatidic acid acyltransferase active site for catalysis within the membrane bilayer. *Nat Struct Mol Biol* 2017;**24**: 666–71. <https://doi.org/10.1038/nsmb.3436>
38. Lewenza S, Falsafi R, Bains M et al. The *olsA* gene mediates the synthesis of an ornithine lipid in *Pseudomonas aeruginosa* during growth under phosphate-limiting conditions, but is not involved in antimicrobial peptide susceptibility. *FEMS Microbiol Lett* 2011;**320**:95–102. <https://doi.org/10.1111/j.1574-6968.2011.02295.x>
39. Weissenmayer B, Gao J-L, López-Lara IM et al. Identification of a gene required for the biosynthesis of ornithine-derived lipids. *Mol Microbiol* 2002;**45**:721–33. <https://doi.org/10.1046/j.1365-2958.2002.03043.x>
40. Geiger O, González-Silva N, López-Lara IM et al. Amino acid-containing membrane lipids in bacteria. *Prog Lipid Res* 2010;**49**: 46–60. <https://doi.org/10.1016/j.plipres.2009.08.002>

41. Cohen LJ, Kang H-S, Chu J et al. Functional metagenomic discovery of bacterial effectors in the human microbiome and isolation of commendamide, a GPCR G2A/132 agonist. *Proc Natl Acad Sci USA* 2015;**112**:E4825–34. <https://doi.org/10.1073/pnas.1508737112>
42. Cohen LJ, Esterhazy D, Kim S-H et al. Commensal bacteria make GPCR ligands that mimic human signalling molecules. *Nature* 2017;**549**:48–53. <https://doi.org/10.1038/nature23874>
43. Moran MA, Durham BP. Sulfur metabolites in the pelagic ocean. *Nat Rev Microbiol* 2019;**17**:665–78. <https://doi.org/10.1038/s41579-019-0250-1>
44. Burchill L, Williams SJ. Chemistry and biology of the aminosulfonate cysteinolic acid: discovery, distribution, synthesis and metabolism. *Org Biomol Chem* 2022;**20**:3043–55. <https://doi.org/10.1039/D2OB00362G>
45. Moore EK, Hopmans EC, Rijkstra WIC et al. Lysine and novel hydroxylysine lipids in soil bacteria: amino acid membrane lipid response to temperature and pH in *Pseudopedobacter saltans*. *Front Microbiol* 2015;**6**:00637. <https://doi.org/10.3389/fmicb.2015.00637>
46. Hölzl G, Sohlenkamp C, Vences-Guzmán MA et al. Head-group hydroxylation by OlsE occurs at the C4 position of ornithine lipid and is widespread in proteobacteria and bacteroidetes. *Chem Phys Lipids* 2018;**213**:32–8. <https://doi.org/10.1016/j.chemphyslip.2018.03.002>
47. Kim S-K, Park S-J, Li X-H et al. Bacterial ornithine lipid, a surrogate membrane lipid under phosphate-limiting conditions, plays important roles in bacterial persistence and interaction with host. *Environ Microbiol* 2018;**20**:3992–4008. <https://doi.org/10.1111/1462-2920.14430>
48. Córdoba-Castro LA, Salgado-Morales R, Torres M et al. Ornithine lipids in *Burkholderia* spp. pathogenicity. *Front Mol Biosci* 2021;**7**:610932. <https://doi.org/10.3389/fmolb.2020.610932>
49. Pizzuto M, Hurtado-Navarro L, Molina-Lopez C et al. Ornithine lipid is a partial TLR4 agonist and NLRP3 activator. *Cell Rep* 2024;**43**:114788. <https://doi.org/10.1016/j.celrep.2024.114788>
50. Pizzuto M, Hurtado-Navarro L, Molina-Lopez C et al. Ornithine lipid activates both TLR4 and the non-canonical NLRP3 inflammasome. *bioRxiv*. 2022; 2022.01.28.477396.
51. Kawai T, Akira S. TLR signaling. *Cell Death Differ* 2006;**13**:816–25. <https://doi.org/10.1038/sj.cdd.4401850>
52. Clark RB, Cervantes JL, Maciejewski MW et al. Serine lipids of *Porphyromonas gingivalis* are human and mouse toll-like receptor 2 ligands. *Infect Immun* 2013;**81**:3479–89. <https://doi.org/10.1128/IAI.00803-13>
53. Lynch A, Crowley E, Casey E et al. The Bacteroidales produce an *N*-acylated derivative of glycine with both cholesterol-solubilising and hemolytic activity. *Sci Rep* 2017;**7**:13270. <https://doi.org/10.1038/s41598-017-13774-6>
54. Buchan A, González JM, Moran MA. Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* 2005;**71**:5665–77. <https://doi.org/10.1128/AEM.71.10.5665-5677.2005>
55. Guillonneau R, Murphy ARJ, Teng Z-J et al. Trade-offs of lipid remodeling in a marine predator–prey interaction in response to phosphorus limitation. *Proc Natl Acad Sci USA* 2022;**119**:e2203057119. <https://doi.org/10.1073/pnas.2203057119>
56. Edwards BR. Lipid biogeochemistry and modern lipidomic techniques. *Annu Rev Mar Sci* 2023;**15**:485–508. <https://doi.org/10.1146/annurev-marine-040422-094104>
57. Moore EK, Villanueva L, Hopmans EC et al. Abundant trimethylornithine lipids and specific gene sequences are indicative of planctomycete importance at the oxic/anoxic interface in *Sphagnum*-dominated northern wetlands. *Appl Environ Microbiol* 2015;**81**:6333–44. <https://doi.org/10.1128/AEM.00324-15>
58. Schubotz F, Xie S, Lipp JS et al. Intact polar lipids in the water column of the eastern tropical North Pacific: abundance and structural variety of non-phosphorus lipids. *Biogeosciences* 2018;**15**:6481–501. <https://doi.org/10.5194/bg-15-6481-2018>
59. Ding S, Bale NJ, Hopmans EC et al. Lipidomics of environmental microbial communities. II: characterization using molecular networking and information theory. *Front Microbiol* 2021;**12**:659315. <https://doi.org/10.3389/fmicb.2021.659315>
60. Rempfert KR, Kraus EA, Nothaft DB et al. Intact polar lipidome and membrane adaptations of microbial communities inhabiting serpentinite-hosted fluids. *Front Microbiol* 2023;**14**:1198786. <https://doi.org/10.3389/fmicb.2023.1198786>
61. Zhang X, Ferguson-Miller SM, Reid GE. Characterization of ornithine and glutamine lipids extracted from cell membranes of *Rhodobacter sphaeroides*. *J Am Soc Mass Spectrom* 2009;**20**:198–212. <https://doi.org/10.1016/j.jasms.2008.08.017>
62. Ding S, von Meijenfildt FAB, Bale NJ et al. Production of structurally diverse sphingolipids by anaerobic marine bacteria in the euxinic Black Sea water column. *ISMEJ* 2024;**18**:wrae153. <https://doi.org/10.1093/ismejo/wrae153>
63. Moore EK, Hopmans EC, Rijkstra WIC et al. Elucidation and identification of amino acid containing membrane lipids using liquid chromatography/high-resolution mass spectrometry. *Rapid Commun Mass Spectrom* 2016;**30**:739–50. <https://doi.org/10.1002/rcm.7503>
64. Wang M, Carver JJ, Phelan VV et al. Sharing and community curation of mass spectrometry data with global natural products social molecular networking. *Nat Biotechnol* 2016;**34**:828–37. <https://doi.org/10.1038/nbt.3597>
65. Conroy MJ, Andrews RM, Andrews S et al. LIPID MAPS: update to databases and tools for the lipidomics community. *Nucleic Acids Res* 2024;**52**:D1677–82. <https://doi.org/10.1093/nar/gkad896>
66. Yang Y, Wang C, Bendle JA et al. Appraisal of paleoclimate indices based on bacterial 3-hydroxy fatty acids in 20 Chinese alkaline lakes. *Org Geochem* 2021;**160**:104277. <https://doi.org/10.1016/j.orggeochem.2021.104277>
67. Human Microbiome Project Consortium. A framework for human microbiome research. *Nature* 2012;**486**:215–21. <https://doi.org/10.1038/nature11209>
68. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;**30**:2114–20. <https://doi.org/10.1093/bioinformatics/btu170>
69. Li D, Liu C-M, Luo R et al. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 2015;**31**:1674–6. <https://doi.org/10.1093/bioinformatics/btv033>
70. Hyatt D, Chen G-L, LoCascio PF et al. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010;**11**:119. <https://doi.org/10.1186/1471-2105-11-119>
71. Eddy SR. Accelerated profile HMM searches. *PLoS Comput Biol* 2011;**7**:e1002195. <https://doi.org/10.1371/journal.pcbi.1002195>
72. Aroney STN, Newell RJP, Nissen J et al. CoverM: read coverage calculator for metagenomics. *Zenodo* 2024;10531254.
73. Milanese A, Mende DR, Paoli L et al. Microbial abundance, activity and population genomic profiling with mOTUs2. *Nat Commun* 2019;**10**:1014. <https://doi.org/10.1038/s41467-019-08844-4>
74. Buchfink B, Reuter K, Drost H-G. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat Methods* 2021;**18**:366–8. <https://doi.org/10.1038/s41592-021-01101-x>

75. Zhang Q, Linke V, Overmyer KA et al. Genetic mapping of microbial and host traits reveals production of immunomodulatory lipids by *Akkermansia muciniphila* in the murine gut. *Nat Microbiol* 2023;**8**:424–40. <https://doi.org/10.1038/s41564-023-01326-w>
76. Herrero del Valle A, Seip B, Cervera-Marzal I et al. Ornithine capture by a translating ribosome controls bacterial polyamine synthesis. *Nat Microbiol* 2020;**5**:554–61. <https://doi.org/10.1038/s41564-020-0669-1>