Progress in Lipid Research 49 (2010) 46–60

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Amino acid-containing membrane lipids in bacteria

Otto Geiger *, Napoleón González-Silva, Isabel M. López-Lara, Christian Sohlenkamp

Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Apdo. Postal 565-A, Cuernavaca, Morelos CP62210, Mexico

ARTICLE INFO

Keywords:
Bacterial membrane
Amino acid-containing lipid
Phosphatidylserine
Lysyl-phosphatidylglycerol
Betaine lipid
Ornithine-containing lipid
Acyl-oxyacyl lipid
Sulfonolipid

ABSTRACT

In the bacterial model organism *Escherichia coli* only the three major membrane lipids phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin occur, all of which belong to the glycerophospholipids. The amino acid-containing phosphatidylserine is a major lipid in eukaryotic membranes but in most bacteria it occurs only as a minor biosynthetic intermediate. In some bacteria, the anionic glycerophospholipids phosphatidylglycerol and cardiolipin can be decorated with aminoacyl residues. For example, phosphatidylglycerol can be decorated with lysine, alanine, or arginine whereas in the case of cardiolipin, lysine or D-alanine modifications are known. In few bacteria, diacylglycerol-derived lipids can be substituted with lysine or homoserine. Acyl-oxyacyl lipids in which the lipidic part is amide-linked to the α-amino group of an amino acid are widely distributed among bacteria and ornithine-containing lipids are the most common version of this lipid type. Only few bacterial groups form glycine-containing lipids, serineglycine-containing lipids, sphingolipids, or sulfonolipids. Although many of these amino acid-containing bacterial membrane lipids are produced in response to certain stress conditions, little is known about the specific molecular functions of these lipids.

Contents

* Corresponding author. Tel.: +52 7773 290815; fax: +52 7773 175581.
E-mail address: otto@ccg.unam.mx (O. Geiger).

0163-7827/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plipres.2009.08.002

1. Introduction

A primary role of lipids in cellular function is to form the lipid bilayer permeability barrier of cells. Glycerophospholipids are the primary building blocks of membranes but other lipids are important components. In the bacterial model organism *Escherichia coli* only the three major membrane lipids phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin occur [1]. In addition, *E. coli* and almost all other Gram-negative bacteria usually have the lipid A-containing lipopolysaccharide in the outer monolayer of their outer membrane and lipid A modification systems have been reviewed recently [2]. However, in other bacteria, additional and alternative membrane lipids are found and in many cases neither their biosyntheses nor their functionalities are understood. Some Gram-negative bacteria have phosphatidylcholine [3] or sphingolipids [4] in their standard repertoire, whereas many Gram-positives have glycosylated diacylglycerols [5] and lysyl-phosphatidylglycerol [6] in their membranes. Notably, phosphatidylinositol is an essential lipid for *Mycobacterium tuberculosis* [7]. Steroid and hopanoid lipids only occur in some bacteria [8]. Under certain stress conditions specific membrane lipids can be formed and in some cases existing membrane lipids can suffer modifications in order to minimize the stress exerted. For example, under phosphorus-limiting conditions of growth, some bacteria form membrane lipids lacking phosphorus such as glycolipids, sulfolipids, betaine lipids, or ornithine-containing lipids [9]. Challenge of proteobacteria with acid causes modifications of membrane lipids, such as formation of lysyl-phosphatidylglycerol [10] or hydroxylation of ornithine-containing lipids [11].

Numerous examples of bacterial lipids containing amino acids or peptides are known [12] and many of them display interesting properties as antibiotics [13] and biosurfactants [14]. However, in order to form lipidic bilayer membranes, amphiphilic lipids usually need to have at least two long-chain acyl or alkyl residues and the molecules should be roughly of cylindric shape, i.e. in cross-section, the area covered by their hydrophilic head group should be similar to the area covered by the hydrophobic acyl or alkyl chains and therefore upon assembly of monomers, bilayer instead of micelle formation is favoured [15]. In this review we will focus on membrane-forming lipids containing aminoacyl residues.

2. Amino acid-containing glycerophospholipids

2.1. Phosphatidylserine and its derivates

In bacteria, CDP-diacylglycerol is the central activated intermediate for the biosynthesis of glycerophospholipids [3] (Fig. 1). Distinct and specific enzymes belonging to the CDP-alcohol phosphotransferase family condense alcohols such as glycerol-3-phosphate, inositol, choline, or serine to CDP-diacylglycerol forming phosphatidylglycerol phosphate, phosphatidylinositol, phosphatidylcholine, or phosphatidylserine (PS), respectively, (Fig. 1). The only amino acid converted by a CDP-alcohol phosphotransferase into a phospholipid head group is serine, and the condensation of CDP-diacylglycerol with serine to form phosphatidylserine (PS) constitutes the first step for the synthesis for phosphatidylethanolamine (PE) [1,16]. In most bacteria, this condensation is catalyzed by the membrane-bound type II PS synthase (Pss) but in some Gram-negative bacteria, like the *Enterobacteriaceae*, PS is synthesized by a type I Pss, which are soluble enzymes that form part of a distinct superfamily that furthermore includes cardiolipin (CL) synthases, poxvirus envelope proteins, phospholipases D, and nucleases [17]. In a second step, the decarboxylation of PS is catalyzed by PS decarboxylase (Psd) to yield PE. Although PS accounts for 5–15% of the phospholipids in eukaryotic cells [18], in most bacteria, PS is a biosynthetic intermediate and is a very minor membrane lipid. In some bacteria, however, the pool of PS seems to be larger and in *Bacillus megaterium* PS comprises some 5–10% [19] of the total membrane lipids. Also, in *Bdellovibrio bacteriovorus*, which parasitizes larger Gram-negative bacteria, PS is a major membrane phospholipid [20]. Psd-deficient bacterial mutants are unable to form PE, and as PS is not consumed they accumulate significant amounts of PS. In such mutants, PS can comprise up to 34% in *E. coli* [21], or up to 29% in *Bacillus subtilis* [22] of the total membrane lipids. A Psd-deficient mutant of *Sinorhizobium meliloti* lacks PE but forms up to 18% PS [23]. This sinorhizobial Psd-deficient mutant resembles in many vegetative aspects a Pss-deficient mutant of *S. meliloti* [16] which also lacks PE but does not form PS either. Surprisingly, Pss-deficient mutants lacking PE form nitrogen-fixing root nodules on alfalfa host plants nearly as efficiently as the wild type. In contrast, the Psd-deficient sinorhizobial mutant accumulates significant amounts of PS and only few empty nodules that are unable to fix nitrogen are formed by this mutant with much delay on the host plant [23]. In animal systems, PS plays a key role in physiological and pathological events. For example, PS exposed on activated platelets promotes the blood coagulation cascade and the aggregation of platelets, and the externalization of PS to the cell surface is a hallmark of apoptotic cells [24]. Although PS is a major membrane lipid in plants [25] its specific roles or functions are unknown. However, the presence of PS in the Psd-deficient sinorhizobial mutant interferes with the accommodation of this mutant bacterium inside the nodule, possibly due to a plant-mediated mechanism. In *E. coli*, the PS formed is distributed equally between the inner and outer membrane [26], but it is not clear whether bacteria expose their PS on the outer surface of the outermost membrane.

2.2. Aminoacyl modifications of phosphatidylglycerol

Phosphatidylglycerol (PG) and cardiolipin (CL) are the major anionic membrane lipids in most bacteria and their synthesis is well understood [1]. Modified forms of PG and CL have been described in different bacteria. Lysyl-phosphatidylglycerol (lysyl-PG) and other aminoacyl esters of PG, such as alanyl-PG, or ornithyl-PG, are major membrane lipids in several Gram-positive bacteria (firmicutes) [28]. Lysyl-PG constitutes a major membrane lipid in *Staphylococcus aureus* [29], *B. subtilis* [30], *Bacillus anthracis* [31], *Listeria monocytogenes* [32], and *Lactococcus plantarum*. In addition to lysyl-PG, some bacteria form ornithyl-PG (*M. tuberculosis*) or alanyl-PG (*Clostridium perfringens*) [6]. Moreover, aminoacylation of PG with arginine [33] or glycine [34] has been described. A large variety of PG-derived lipids are present in *Enterococcus faecalis* (formerly known as *Streptococcus faecalis*) which probably has alanyl-PG, 2′-lysyl-PG, 3′-lysyl-PG, 2′,3′-dilysyl-PG, arginyl-PG, and a diglucosyl derivative of PG [35]. As with other acylated glycerol derivatives, a 2′-lysyl-PG can undergo acyl migration to yield 3′-lysyl-PG [36]. The protein MprF ("multiple peptide resistance factor"), responsible for lysyl-PG formation, was first described in *S. aureus* during a screen for transposon mutants more susceptible to cationic peptides of the innate immune response than the wild type [37]. MprF from *S. aureus* is able to transfer lysine from charged lysyl-tRNA to PG forming lysyl-PG [38,39] (Fig. 1). Also in *B. subtilis* [40] or in *B. anthracis* [31], MprF is required for the synthesis of lysyl-PG and for resistance to cationic antimicrobial peptides. It has been thought that the presence of lysyl-PG is restricted mainly to Gram-positive bacteria although lysyl-PG is present in a strain of *Pseudomonas aeruginosa* [41] and in *Caulobacter crescentus* [42]. In screens for mutants more susceptible to acidic growth conditions, Reeve et al. [43] and Vinuesa et al. [44] identified genes coding for MprF homologues in the α-proteobacteria *Sinorhizobium medicae* and *Rhizobium tropici* that are called *lpiA* ("low pH-induc-

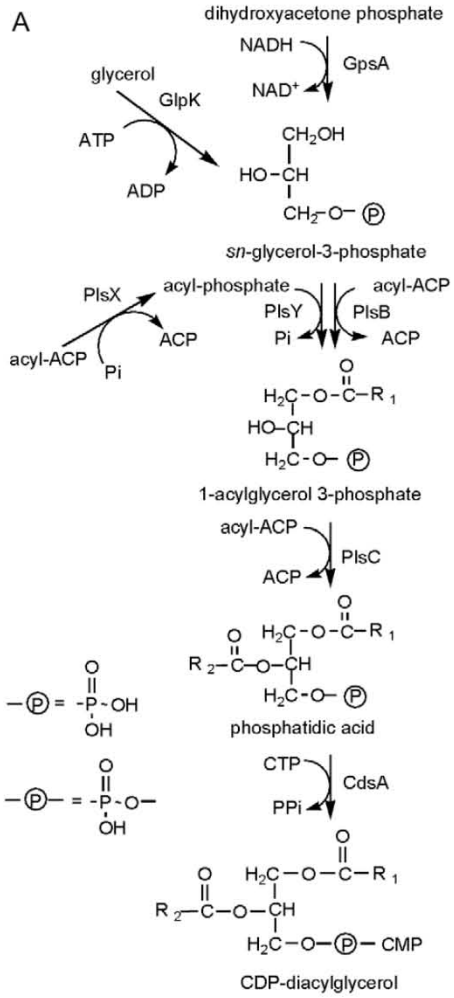

Fig. 1. Biosynthesis of glycerophospholipids in bacteria. (A) Formation of the activated intermediate CDP-diacylglycerol. Glycerol-3-phosphate forms the backbone of all glycerophospholipid molecules and it can be synthesized by two different pathways, either from glycerol directly by glycerol kinase (GlpK) or by reduction of the glycolytic intermediate dihydroxyacetone phosphate with NADH catalyzed by biosynthetic glycerol-3-phosphate dehydrogenase (GpsA) [1]. In *E. coli*, the glycerol-3-phosphate acyltransferase PlsB can use acyl-CoA or acyl-ACP as acyl donors and is the major activity for catalyzing the first acylation at position 1 of glycerol-3-phosphate thereby forming 1-acyl-glycerol-3-phosphate. However, the more widespread pathway to achieve the initial acylation of glycerol-3-phosphate among bacteria seems to involve PlsX and PlsY [27]. In this pathway, PlsX catalyzes the conversion of acyl-ACP and inorganic phosphate to acyl-phosphate and ACP. In a second step, PlsY transfers the acyl group from acyl-phosphate to glycerol-3-phosphate forming inorganic phosphate (Pi) and 1-acyl-glycerol-3-phosphate [27]. The second fatty acyl residue is added by another enzyme, the 1-acyl-glycerol-3-phosphate acyltransferase PlsC, to form phosphatidic acid. The conversion of phosphatidic acid to CDP-diacylglycerol (CDP-diglyceride) is catalyzed by CDP-diglyceride synthase CdsA [1]. (B) Diversification of phospholipid head groups. The first step in the synthesis of phosphatidylethanolamine (PE) is the condensation of CDP-diacylglycerol with serine to form phosphatidylserine (PS) catalyzed by PS synthase (Pss). In a second step, the decarboxylation of PS is catalyzed by PS decarboxylase (Psd) to yield PE. A well-known pathway for PC formation occurs by threefold methylation of PE using *S*-adenosylmethionine (SAM) as methyl donor and catalyzed by phospholipid *N*-methyltransferase (PmtA). Many PC-containing bacteria have a second pathway for PC formation, catalyzed by PC synthase (Pcs), in which choline is condensed directly to CDP-diacylglycerol forming PC and CMP [3]. In the initial step of phosphatidylglycerol (PG) and cardiolipin (CL) biosynthesis, phosphatidylglycerol phosphate synthase (PgsA) transfers glycerol-3-phosphate to CDP-diacylglycerol under the release of CMP thereby producing phosphatidylglycerol phosphate (PGP). There are at least two enzymes with PGP phosphatase activity (PgpA and PgpB) in *E. coli*, releasing inorganic phosphate from PGP to form phosphatidylglycerol (PG) [1]. Lysyl-phosphatidylglycerol (lysyl-PG) is a well-known membrane lipid in many Gram-positive bacteria and MprF can transfer lysine from charged lysyl-tRNA to PG forming lysyl-PG. The pathogen *Clostridium perfringens* has two phylogenetically distinct MprF paralogues, one responsible for the formation of lysyl-PG (MprF1) and the other causing the synthesis of alanyl-phosphatidylglycerol (MprF2) [6]. In *E. coli* and probably most other bacteria, a cardiolipin synthase (Cls) condenses two PG molecules to yield cardiolipin (CL) and free glycerol in a transesterification reaction. Although a MprF homologue is required for the lysinylation of CL, it is not known whether lysyl-CL is formed by lysinylation of CL or by Cls-catalyzed condensation of lysyl-PG with PG. The causative agent of tuberculosis *Mycobacterium tuberculosis* has phosphatidylinositol (PI) and derivatives thereof as major components in its membrane. In *M. tuberculosis*, PI is formed by condensing *myo*-inositol to CDP-diacylglyceride in a reaction catalyzed by PI synthase [7].

ible A"). In both organisms, *lpiA* is transcriptionally induced under acidic growth conditions [43–45]. Later, genes encoding for homologues of LpiA were identified in several other Gram-negative bacteria, like *Agrobacterium tumefaciens*, *Aeromonas hydrophila*, *Xanthomonas campestris*, *Xylella fastidiosa* and several species of the genera *Brucella*, *Burkholderia*, *Pseudomonas*, among others. Interestingly, most of these species interact with eukaryotic hosts as symbionts, pathogens, or commensals. Lysyl-PG formation in *R. tropici* increases the resistance to cationic peptides [10]. Notably, in most Gram-negative bacteria the *lpiA* gene probably forms an operon with the gene *atvA* ("acid tolerance and virulence A"). The biochemical function of AtvA is not known.

More recently, genes encoding proteins responsible for the formation of alanyl-PG have been identified. The pathogen *C. perfringens* has two phylogenetically distinct MprF paralogues, one responsible for the formation of lysyl-PG and the other for the synthesis of alanyl-PG [6] (Fig. 1). Alanyl-PG also occurs in *P. aeruginosa* and the responsible gene is identified [46]. Formation of

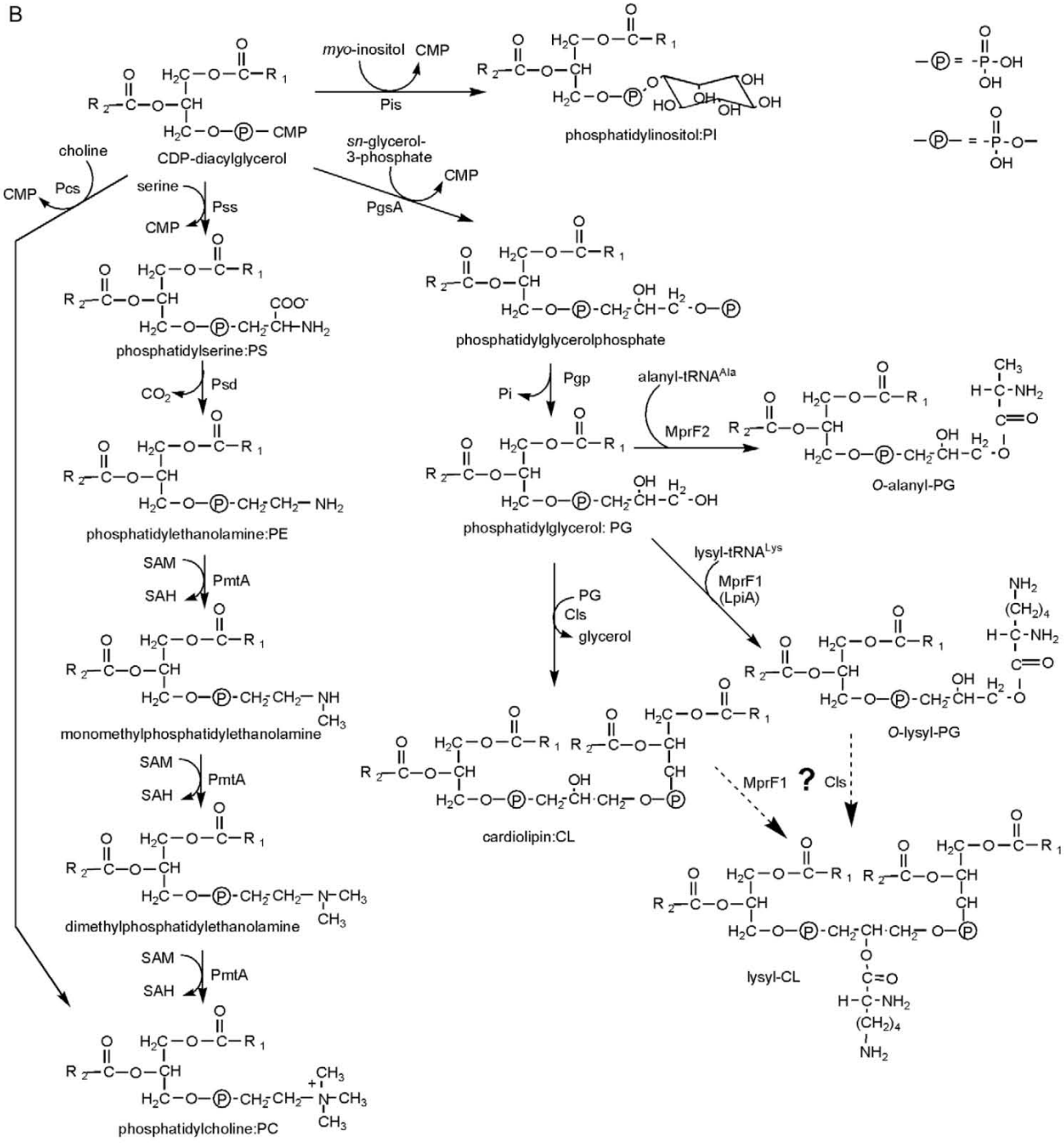

Fig. 1 (*continued*)

2′-alanyl-PG in *P. aeruginosa* is increased under acidic growth conditions. Alanyl-PG is found in the inner and the outer membrane of *P. aeruginosa* and it increases the resistance of the bacterium towards the β-lactam antibiotic cefsulodin, the heavy metal ion Cr^{3+}, the osmolyte sodium lactate, and the cationic antimicrobial peptide protamine sulphate [46].

Two genes coding for LpiA/MprF homologues are also present in *Sphingomonas wittichii* and *Enterococcus faecium* and even three LpiA/MprF homologues are encoded by the *Kineococcus radiodurans* genome, but nothing is known about their functions. Although the presence of aminoacylated PG has been described only in bacteria, a gene coding for an LpiA/MprF homologue is present in the genome of the moss *Physcomitrella patens*.

2.3. Aminoacyl modifications of cardiolipin

Cardiolipin (CL) can also be substituted on the *sn*-2 hydroxyl of the middle glycerol moiety with α-D-glucopyranosyl, D-alanyl, or L-lysyl residues [47,48] in the group N streptococcus *Vagococcus fluvialis* or with L-lysyl in *Listeria* species [32] (Fig. 1). The MprF homologue Lmo1695 of *L. monocytogenes* is required for the lysinylation of both, PG and CL [49]. However, it is not known whether lysyl-CL is formed by lysinylation of CL or by CL synthase-catalyzed condensation of lysyl-PG with PG. Therefore, the precise biosynthetic steps that form these modified versions of CL are not clear. Usually, CL is only partially ionized at physiological conditions due to the hydrogen bonding of the *sn*-2 hydroxyl group of the middle glyc-

erol moiety with the neighboring phosphate groups. Therefore, CL can function as a proton sink or a conduit for protons in transfer processes [15]. Upon derivatization of the *sn*-2 hydroxyl of the middle glycerol with the above-mentioned residues the hydrogen bonding should be impeded and the special property of CL as a proton sink will be lost.

3. Amino acid-containing diacylglycerols

3.1. Homoserine-containing betaine lipids

Although PC is known to be the major membrane lipid in eukaryotes, some lower eukaryotic organisms possess the betaine lipid diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS) instead. DGTS occurs in a wide variety of lower green plants (green algae, bryophytes and pteridophytes), chromophytes, fungi, and amoebae (reviewed in [3]). In some α-proteobacteria, DGTS acts as a phosphorus-free membrane lipid [50] that substitutes for PC under conditions of phosphate limitation [51]. There is an apparent reciprocity between the content of PC and the content of DGTS, i.e. when PC is a major membrane lipid often no DGTS is detected in the same organism, whereas in organisms where DGTS is a major lipid, PC is only found in trace levels. This correlation suggests, that DGTS and PC, both zwitterionic at physiological pH, are interchangeable at least with regard to essential functions for the respective organism. The mutual replacement of PC or DGTS occurs in culture conditions but also in natural environments [51a]. Phytoplankton communities from the phosphorus-poor Sargasso Sea have much higher betaine lipid/PC ratios than communities from the phosphorus-replete South Pacific due to the respective adjustments in eukaryotic phytoplankton [51a].

Two structural genes from *Rhodobacter sphaeroides*, *btaAB*, coding for two enzymes BtaA and BtaB involved in DGTS biosynthesis have been characterized [52,53]. The BtaA *S*-adenosylmethionine/diacylglycerol 3-amino-3-carboxypropyl transferase converts diacylglycerol (DAG) into diacylglyceryl-homoserine (DGHS) and during the formation of the ether bond, *S*-adenosylmethionine functions as donor of the homoseryl group. Subsequently, the *S*-adenosylmethionine: diacylglyceryl-homoserine-*N*-methyltransferase BtaB catalyzes threefold methylation of DGHS in order to yield DGTS (Fig. 2). Orthologues of BtaA and BtaB exist in *S. meliloti*, and a BtaA-deficient mutant of *S. meliloti* is unable to produce DGTS [54]. In the eukaryotic green alga *Chlamydomonas reinhardtii*, the betaine lipid synthase $BTA1_{Cr}$ is a bifunctional protein that can perform the homoseryl modification of diacylglycerol as well as the subsequent methylations of the homoseryl amino group [55]. In other lower eukaryotes where DGTS is present, genes coding for homologues of the bifunctional *C. reinhardtii* enzyme are present. Heterologous expression of $BTA1_{Cr}$ in *E. coli* leads to DGTS accumulation and the two domains of $BTA1_{Cr}$ are functionally equivalent to BtaA and BtaB [55].

The wealth of genome sequencing data indicates that the occurrence of DGTS-like betaine lipids is limited in bacteria. Homologues of rhodobacterial BtaA (above 42% identity and 55% similarity) and BtaB are mainly found in some orders of the α-proteobacteria, such as the Rhodobacterales (*Rhodobacter, Roseobacter, Sagittula, Stappia*), the Sphingomonadales (*Sphingomonas, Erythrobacter*), the Rhizobiales (*Rhizobium, Agrobacterium, Sinorhizobium, Ochrobactrum, Mesorhizobium, Beijerinckia, Rhodopseudomonas*), and in members of the Planctomycetes (*Planctomyces, Blastopirellula, Rhodopirellula*) (Fig. 3). More distantly related genes are present in the δ-proteobacterium *Plesiocystis pacifica* SIR-I and in *Chthoniobacter flavus* (Chlamydia/Verrucomicrobia). Genes coding for homologues of the bifunctional enzymes from *Chlamydomonas reinhardtii* are present in several lower eukaryotes such as *Candida albicans*, *Cryptococcus neoformans*, *Neurospora crassa* and *Physcomitrella patens* (Fig. 3). Planctomycetes represent a distinct bacterial phylum as they show absence of a peptidoglycan cell wall and extensive cell compartmentalization, in some cases even a

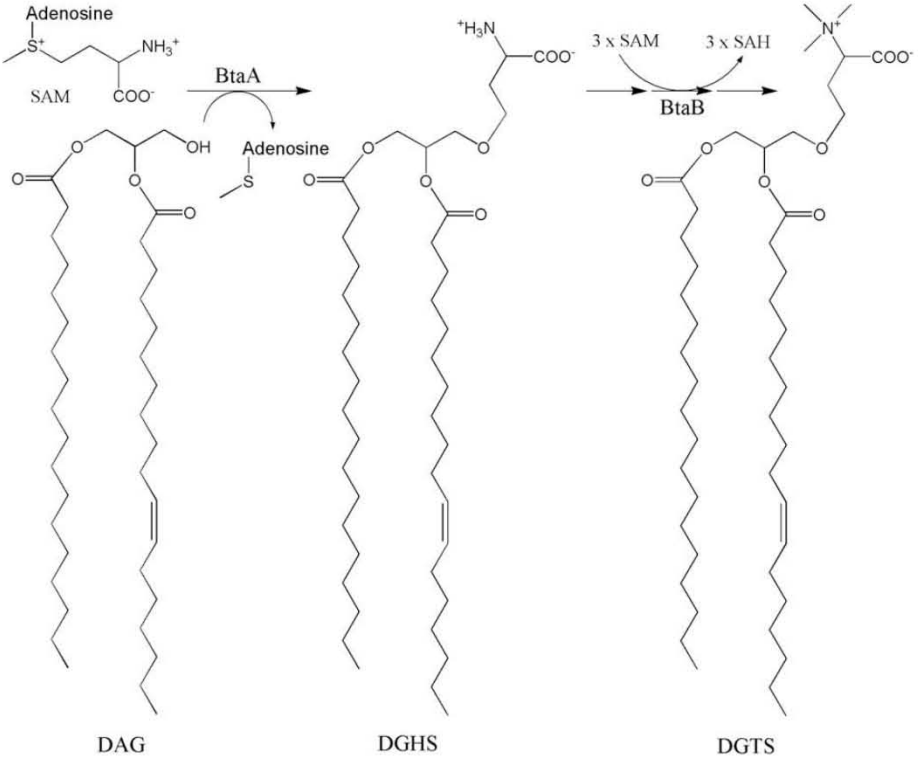

Fig. 2. Biosynthesis of diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS) [52,53]. DAG: diacylglycerol; SAM: *S*-adenosylmethionine; SAH: *S*-adenosylhomocysteine; DGHS: diacylglyceryl-homoserine.

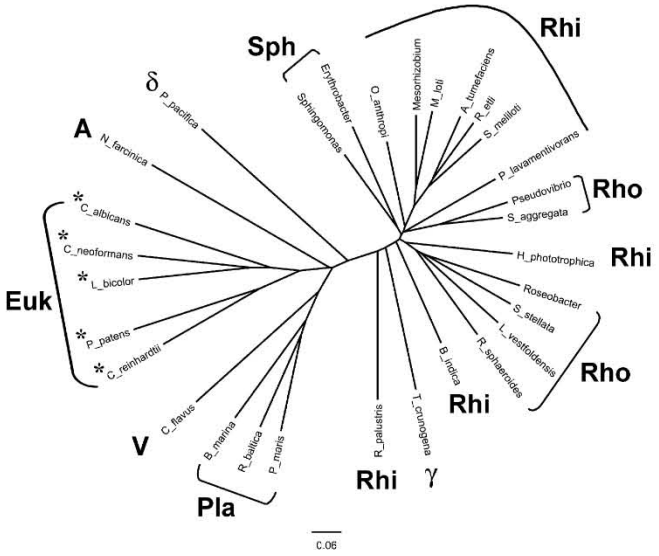

Fig. 3. Unrooted phylogenetic tree of *Rhodobacter sphaeroides* BtaA, *Sinorhizobium meliloti* BtaA, the *N*-terminal domain of *Chlamydomonas reinhardtii* $Bta1_{CR}$ and BtaA-like ORFs from other genomes. The tree was constructed using the program CLUSTALW (http://www.expasy.ch/). Distances between sequences are expressed as 0.06 changes per amino acid residue. The asterisks indicate that only the *N*-terminal domains of the respective sequences corresponding to BtaA were used for the construction of the tree. Accession numbers are as follows: *Rhodobacter sphaeroides* BtaA (ABA80038), *Sinorhizobium meliloti* 1021 BtaA (NP_386300), *Chlamydomonas reinhardtii* $Bta1_{CR}$ (XP_001700879), *Agrobacterium tumefaciens* str. C58 (NP_355081), *Beijerinckia indica* subsp. *indica* ATCC 9039 (YP_001834100), *Blastopirellula marina* DSM 3645 (ZP_01088661), *Candida albicans* SC5314 (XP_713069), *Chthoniobacter flavus* Ellin428 (ZP_03133405), *Erythrobacter* sp. NAP1 (ZP_01039410), *Hoeflea phototrophica* DFL-43 (ZP_02168757), *Laccaria bicolor* S238N-H82 (XP_001877704), *Loktanella vestfoldensis* SKA53 (ZP_01002837), *Mesorhizobium loti* MAFF303099 (NP_103130), *Mesorhizobium* sp. BNC1 (YP_674579), *Nocardia farcinica* IFM 10152 (YP_117653), *Ochrobactrum anthropi* ATCC 49188 (YP_001370273), *Parvibaculum lavamentivorans* DS-1 (ABS62442), *Physcomitrella patens* subsp. *patens* (XP_001757434), *Planctomyces maris* DSM 8797 (ZP_01856778), *Plesiocystis pacifica* SIR-1 (ZP_01909206), *Pseudovibrio* sp. JE062 (EEA94326), *Rhizobium etli* CFN 42 (YP_470361), *Rhodopirellula baltica* SH 1 (NP_863860), *Rhodopseudomonas palustris* BisB18 (YP_531155), *Roseobacter* sp. MED193 (EAQ46984), *Sagittula stellata* E-37 (EBA07014), *Sphingomonas* sp. SKA58 (ZP_01304163), *Stappia aggregata* IAM 12614 (ZP_01546622), and *Thiomicrospira crunogena* XCL-2 (YP_390494). A – Actinomycetales, Euk – Eukaryotes, V – Verrucomicrobia, Pla – Planctomycetales, Rhi – Rhizobiales, Rho – Rhodobacterales, Sph – Sphingomonadales, γ – gamma proteobacteria, δ – delta proteobacteria.

membrane-enclosed nuclear structure [56]. Interestingly, the BtaAB homologues from the order *Planctomycetales* seem to be closer related to the sequences from eukaryotic origin than to the other bacterial sequences (Fig. 3). It has been speculated that the biosynthesis of certain phosphorus-free membrane lipids such as DGTS might improve the survival of bacteria under phosphorus-depleted conditions [54]. The fact that intracellular pathogens of the genus *Brucella* apparently lack the genes needed for DGTS biosynthesis but that the closely related, and normally free-living (opportunistic) pathogen *Ochrobacter anthropi* has the respective genes supports this idea.

3.2. Mycobacterial lysine-containing lipid

A diacylglycerol-based lysine-containing lipid was isolated from *Mycobacterium phlei* strain IST [57]. Lysine is esterified to 1,2-diglyceride via an ester linkage (Fig. 4) and the major fatty acyl substitutions are palmitic and tuberculostearic acid [57]. The mycobacterial lysine-containing lipid in strain IST is not detected it in a reference strain (ATCC 19249), and it has been suggested that this lipid is involved in lysine uptake to the cell [57].

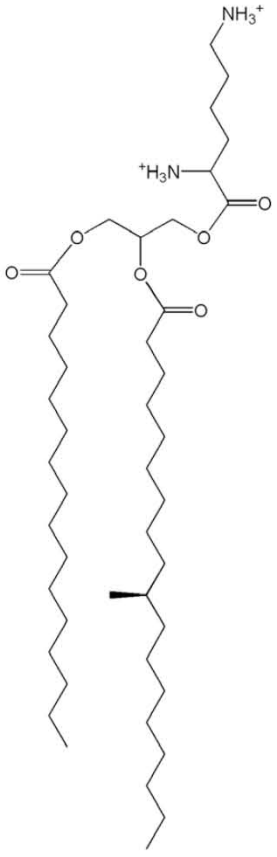

Fig. 4. Structure of mycobacterial lysyl-diacylglycerol.

4. Amino acid-containing acyl-oxyacyl lipids

4.1. Ornithine-containing lipids

4.1.1. Distribution and structure of ornithine-containing lipids

Ornithine-containing lipids (OL) are widespread among Gram-negative bacteria and have been reported in some Gram-positives, like *Mycobacterium* and *Streptomyces* species (reviewed in [9]) but are absent in *Archaea* and *Eukarya*. The α-*N*-(acyloxyacyl)-ornithines have a 3-hydroxyfatty acyl group that is attached in amide linkage to the α-amino group of ornithine [51,58]. A second fatty acyl group is ester-linked to the 3-hydroxy position of the first fatty acid (Fig. 5A). In some bacteria the fatty acyls joined by ester linkage are hydroxylated at the 2 or 3 positions [12]. The configuration of the asymmetric carbon of 3-hydoxyfatty acyls of the OLs is D or (*R*) [59]. Although OLs are found in both membranes of Gram-negative bacteria, they seem to be enriched in the outer membrane [60].

4.1.2. Biosynthesis of ornithine-containing lipids

The biosynthesis of OLs occurs in two-steps. The *N*-acyltransferase OlsB catalyzes the transfer of a 3-hydroxy fatty acyl group from 3-hydroxy fatty acyl–acyl carrier protein to the α-amino group of ornithine forming lyso-ornithine lipid [61]. Next, the *O*-acyltransferase OlsA catalyzes the transfer of an acyl group from fatty acyl–acyl carrier protein to the hydroxy group of lyso-ornithine lipid forming OL [62] (Fig. 5A).

OlsB-deficient mutants of *S. meliloti* [61] or *Rhodobacter capsulatus* [63] are unable to form OL. Expression of *olsB* from *S. meliloti* in *Escherichia coli* causes the formation of lyso-ornithine lipid [61]. The OlsB of *S. meliloti* is predicted to be a water-soluble protein of 296 amino acids that encodes an *N*-acyltransferase converting ornithine

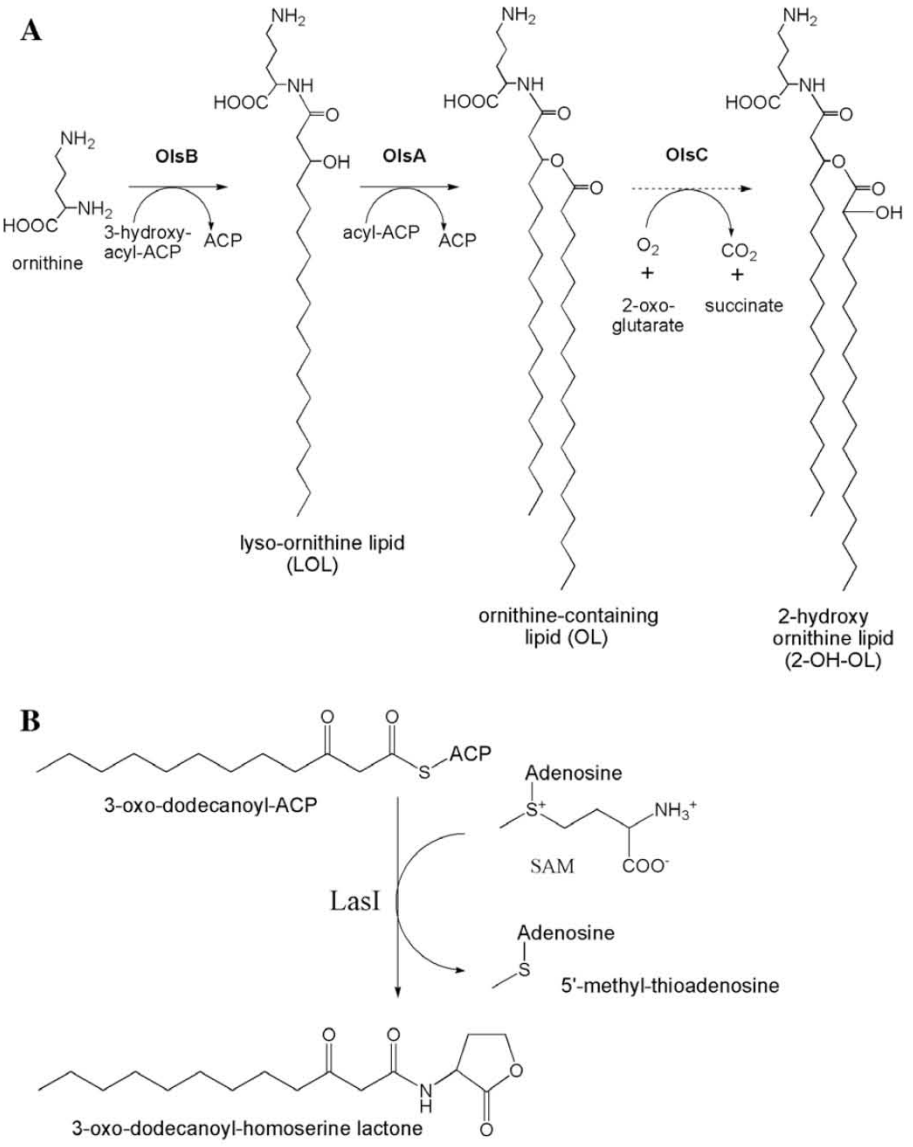

Fig. 5. Biosyntheses of ornithine-containing lipids (A) and of the *Pseudomonas aeruginosa* quorum sensing signal 3-oxo-dodecanoyl-homoserine lactone (B).

to lyso-ornithine lipid thereby catalyzing first step of OL biosynthesis. OlsB defines a concrete function for a whole cluster of orthologous group of proteins (COG3176) previously assigned as hypothetical or as putative hemolysins [61]. OlsB belongs to the acyl-CoA *N*-acyltransferase superfamily [64]. Heath and Rock [65] described a consensus peptide motif (H(X)4D) common to glycerolipid acyltransferases and demonstrated that an exchange of this conserved H for A eliminated the activity of *E. coli* PlsB. In OlsB, H87 and D92 might form such a motif that is conserved within OlsB homologues [61]. A search of PhyloFacts with OlsB identifies acyl-homoserine lactone synthases EsaI [66] and LasI [67] and the tertiary structure of OlsB is expected to be quite similar to the autoinducer synthase. Like OlsB, acyl-homoserine lactone synthases are *N*-acyl transferases that use acyl-ACPs and an amino acid derivative (SAM) as substrates (Fig. 5B). The *N*-acyl amino acid synthase FeeM from an uncultured soil microbe binds the acyl carrier protein FeeL, catalyzes the formation of *N*-acyl tyrosine, and its structure resembles that of acyl-homoserine lactone synthases EsaI and LasI [68].

Genome analyses indicate that like many bacterial groups, the order Rhodobacterales have an *olsBA* operon (containing *olsB1*) plus a gene coding for a second homologue of OlsB (*olsB2*) (Fig. 6). The *olsB2* gene is not located physically close to *olsBA*, but at another site in the genome. The OlsB2 homologues of different Rhodobacterales are more closely related to each other than to the OlsB1 homologues (Fig. 6). Interestingly, *R. sphaeroides* forms lipids that contain glutamine in addition to the well-known OL [69]. The initial step in the biosynthesis of glutamine-containing lipids might be catalyzed by the OlsB2 homologue found exclusively in the Rhodobacterales.

The only other examples where multiple *olsB* homologues are present within one genome are the α-proteobacteria *Magnetospirillum magneticum* and *M. magnetotacticum* (Fig. 6). In addition to OL, another unknown amino lipid has been described in the magnetosome membrane of *Magnetospirillum* which might be synthesized by the second homologue [70]. Remarkably, heterologous expression of microbial DNA extracted from environmental samples led to the identification of long-chain *N*-acyl derivatives of tyrosine, phenylalanine, tryptophan, and arginine, all of which have antibiotic activity [13,71,72]. It is possible that some of the long-chain *N*-acyl transferases involved in the formation of these compounds catalyze initial steps in the biosyntheses of other still unknown acyl–oxyacyl membrane lipids.

Genome sequencing data indicate, that in several organisms from the order *Alteromonadales* and in the ε-proteobacterium

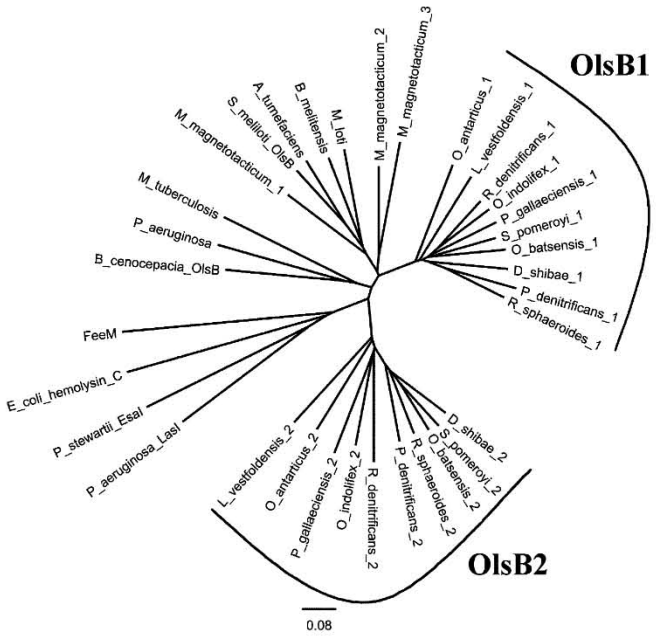

Fig. 6. Unrooted phylogenetic tree of *Sinorhizobium meliloti* OlsB, *Burkholderia cenocepacia* OlsB and OlsB-like ORFs. The tree was constructed using the program CLUSTALW (http://www.expasy.ch/). Distances between sequences are expressed as 0.08 changes per amino acid residue. Accession numbers are as follows: *Sinorhizobium meliloti* 1021 OlsB (NP_384499), *Burkholderia cenocepacia* J2315 OlsB (YP_002230419), *Escherichia coli* CFT073 HlyC (NP_755444), *Pantoea stewartii* subsp. *stewartii* EsaI (AAA82096), *Pseudomonas aeruginosa* PAO1 LasI (NP_250123), *N*-acyl transferase FeeM from an uncultured bacterium (AAM97306), *Agrobacterium tumefaciens* str. C58 (NP_353376), *Brucella melitensis* 16M (NP_540717), *Dinoroseobacter shibae* DFL 12 (YP_001532815 and YP_001533050), *Loktanella vestfoldensis* SKA53 (ZP_01003503 and ZP_01003690), *Magnetospirillum magnetotacticum* MS-1 (ZP_00050271, ZP_00055184, and ZP_00053729), *Mesorhizobium loti* MAFF303099 (NP_104372), *Mycobacterium tuberculosis* H37Rv (NP_217543), *Oceanicola batsensis* HTCC2597 (ZP_00999281 and ZP_00999194), *Oceanobulbus indolifex* HEL-45 (ZP_02153706 and ZP_02153839), *Octadecabacter antarticus* 307 (EDY79302 and EDY80135), *Paracoccus denitrificans* PD1222 (ZP_00631241 and ZP_00629627), *Phaeobacter gallaeciensis* 2.10 (ZP_02148428 and ZP_02148788), *Pseudomonas aeruginosa* PAO1 (NP_253040), *Rhodobacter sphaeroides* 2.4.1 (YP_354511 and YP_352676), *Roseobacter denitrificans* OCh 114 (YP_682889 and YP_683344), and *Silicibacter pomeroyi* DSS-3 (YP_167215 and YP_167705). OlsB1 and OlsB2 assign the two different subgroups of OlsB homologues present in bacteria from the order Rhodobacterales.

Arcobacter butzleri, the OlsB protein is *N*-terminally fused to a hypothetical protein domain of unknown function, suggesting that OL biosynthesis might turn out to be more complicated. The presence of OL has not been described in any of these species.

Also mutants of *S. meliloti* [62] or *R. capsulatus* [63] deficient in OlsA are unable to form OL. Overexpression of *olsB* in an *olsA*-deficient mutant of *S. meliloti* leads to the accumulation of lyso-ornithine lipid [61]. The OlsA of *S. meliloti* is a protein of 292 amino acids with a probable transmembrane helix close to the *N*-terminus. An alignment of OlsA with some prokaryotic enzymes displaying lysophosphatidic acid acyltransferase activities [62] demonstrates that there are two conserved regions (amino acids 67–83 and 139–154) where OlsA has the highest similarity to other members of this group. In lysophosphatidic acid acyltransferases, two motifs, NHQS and PEGTR, are conserved [73], and they are found in modified forms (NHVS, amino acids 72–75; PEGTT, amino acids 143–147) in the OlsA sequence [62]. Based on the consensus peptide motif (H(X)4D) [65] common to glycerolipid acyltransferases, in OlsA, H73 and D78 might form such a motif. It is striking, however, that sequences coding for enzymes with lysophosphatidic acid acyltransferase activities are overall quite dissimilar. For example, in the bacterium *Neisseria meningitidis* there are three enzymes (NlaA, NlaB, and a third activity detectable in *nlaA*-, *nlaB*-deficient double mutants) with lysophosphatidic acid acyltransferase activity in vitro [74]. Though NlaA and NlaB are from the same organism they are quite dissimilar and it is interesting to note that *nlaA*-deficient mutants and *nlaB*-deficient mutants show different phenotypes suggesting that NlaA and NlaB perform different biochemical functions in *N. meningitidis* in vivo. From its sequence, OlsA clearly groups within the present lysophosphatidic acid acyltransferases. Because *olsA*-deficient mutants are unable to form OL, but show no accumulation of lysophosphatidic acid and no impairment of glycerophospholipid biosynthesis, there must be a PlsC activity in *S. meliloti* responsible for these latter functions, presumably SMc00714 (http://sequence.toulouse.inra.fr/meliloti.html). OlsA is required for the enzymatic activity of a lyso-ornithine lipid- and acyl-AcpP-dependent *O*-acyltransferase that converts lyso-ornithine lipid into OL [62]. In addition to OlsA, *Pseudomonas fluorescens* possesses two more lysophosphatidic acid acyltransferase homologues, HdtS and PatB [75]. Either HdtS or PatB complement an *E. coli* PlsC-deficient mutant for growth, while the OlsA from *P. fluorescens* does not. Although HdtS or PatB can provide the PlsC function in vivo, they are not functionally identical. Mutants lacking PatB show reduced growth at elevated temperatures while HdtS-deficient mutants are affected in growth, motility and have reduced amounts of *cis*-vaccenic acid [75]. Also, *Rhodobacter capsulatus* possesses three lysophosphatidic acid acyltransferase homologues, OlsA, PlsC316, and PlsC3498 [76]. Either OlsA or PlsC316 from *R. capsulatus* complement an *E. coli* PlsC-deficient mutant for growth, while PlsC3498 does not. A PlsC316-deficient mutant has reduced amounts of C16 fatty acids [76]. Therefore, OlsA from *R. capsulatus* is able to acylate 1-acyl-*sn*-glycerol-3-phosphate in addition to lyso-ornithine lipid and therefore exhibits relaxed substrate specificity towards the acyl acceptor substrate [76]. It is expected that future studies of the present group of "lysophosphatidic acid acyltransferases" will reveal numerous subgroups with slightly different biochemical activities.

Little is known about the functions of OLs. *S. meliloti* mutants deficient in OL biosynthesis do not show any alteration of their macroscopic phenotype. The unability to form OL must be combined with deficient DGTS biosynthesis to obtain reduced cell yields when *S. meliloti* is grown under phosphorus-limiting conditions [54]. In *R. capsulatus*, OLs are required for optimal steady-state amounts of *c*-type cytochromes [63].

4.1.3. Hydroxylated ornithine-containing lipids

In bacteria like *Burkholderia cepacia*, *Flavobacterium* [12,77], *Thiobacillus* [58], *Gluconobacter* [78], *Streptomyces* [12], some *Ralstonia* spec. [79], and *R. tropici* [11] OL also have ester-linked fatty acyl groups with a hydroxyl group at the 2-position. The 2-hydroxyfatty acyl residues are not formed during standard fatty acid biosynthesis and specific enzymatic activities are required to introduce a hydroxyl group onto the 2-position of a fatty acyl residue. Similar *S*-2-hydoxyfatty acyl moieties are integral parts of *Salmonella typhimurium* lipid A and are thought to be of importance for pathogenesis of this organism. The *S*-2-hydroxylation is introduced after the fatty acyl group had been attached to the lipid A molecule and is catalyzed by the $Fe^{2+}/O_2/\alpha$-ketoglutarate-dependent LpxO-encoded dioxygenase [80,81]. It has been speculated that the hydroxyl groups might increase hydrogen bonding between adjacent lipid A molecules decreasing the outer membrane's permeability to lipophilic compounds under some growth conditions [81]. A similar dioxygenase (OlsC) might be responsible for the introduction of 2-hydroxy substitutions on the ester-linked fatty acyl group of OL [11] (Fig. 5). 2-Hydroxy substitutions on ester-linked fatty acyl groups occur also in bacterial sphingolipids and PE, other major components of the outer membrane in Gram-negative bacteria. PE is 2-hydroxylated on its *sn*-2-fatty acyl residue in *Burkholderia* [82,83]. Homologues of *S. typhimurium*

LpxO are found in the genome of *Burkholderia cenocepacia* J2315 (BCAM1214 and BCAM2401) and they might be candidates for introducing hydroxyl groups into the esterified fatty acyl residue of OL.

Rhizobium tropici CIAT899, an efficient symbiont of bean plants, is highly tolerant to acid, and produces four different classes of OL (termed S1, S2, P1, and P2) [11]. A mutant deficient in *olsC* is symbiotically defective and does not form P1 or P2. Overexpression of the *olsC* gene in the *olsC*-deficient mutant yielded P1 and P2 as major OLs, coupled with a near-complete lack of S1 and S2 and an acid-sensitive phenotype [11]. These results suggest that some classes of OL are important for acid tolerance (S1 and S2) and others for symbiotic effectiveness (P1 and P2), but in order to optimize both traits, an adequate balance of the four distinct classes of OLs is required [11]. The product encoded by *olsC* is a putative LpxO-like dioxygenase that might convert the two less polar forms of OLs (S1 and S2) to the two more polar forms (P1 and P2) [11] by hydroxylation at an unknown position. The OlsC of *R. tropici* is predicted to be a water-soluble protein of 281 amino acids [11].

4.1.4. Tauro-ornithine- and lysine-containing lipids

In *Gluconobacter cerinus*, ornithine-containing lipids hydroxylated in the 2-position of the ester-linked fatty acyl residue (2-OH-OL) are partially modified with a taurine residue that is amide-linked to the α-carboxy group of ornithine [78] (Fig. 7). The particulate fraction from *G. cerinus* requires ATP and Mn^{2+} to condense taurine to 2-OH-OL leading to the formation of tauro-ornithine lipid [84]. This tauro-ornithine lipid is also called cerilipin after the species of the bacterium from which it was isolated [78]. The gene encoding the taurine-condensing activity is unknown.

A lysine-containing lipid (LL) from an *Agrobacterium tumefaciens* strain [85] has the α-amino group of lysine *N*-acylated with a 3-hydroxypalmitoyl residue that is esterified with a fatty acid (Fig. 7). This LL is analogous to the OL with lysine instead of ornithine as a building block.

4.2. Glycine-containing lipids

The glycine-containing lipids (GLs) were identified in the gliding bacterium *Cytophaga johnsonae* C21 and the Gram-negative sea-water bacterium *Cyclobacterium marinus* WH [86,87]. GLs consist of the amino acid glycine and two fatty acyl residues, using the acyl-oxyacyl or piggyback structure. The structure of GL from *C. marinus* WH is principally a *N*-[3-D-(13-methyltetradecanoyloxy)-15-methylhexadecanoyl]glycine [87]. In this structure (Fig. 8), an iso-3-hydroxyfatty acyl group is amide-linked to glycine and its 3-hydroxy group is esterified to another iso-fatty acid. The absolute configuration of the hydroxy ester is 3-D [87]. This type of GL is called cytolipin because it was initially identified in the genus *Cytophaga* [86]. It constitutes about 6% and 5% of the total lipids in *C. johnsonae* C21 [86] and *C. marinus* WH [87], respectively. Based on chromatographic methods and specific stains it was assumed that lipoamino acids structurally similar to GL are presents in several gliding bacteria of the genus *Cytophaga* [88], and in some strains of Gram-negative fresh-water bacteria belonging to the genera *Arcocella* [89] and *Flectobacillus* [90] related to the genus *Cyclobacterium*. The three latter genera are systematically distant from the family *Cytophagaceae* and therefore it was suggested that GL might be widely distributed among Gram-negative aquatic bacteria [87]. However, an alternative explanation might be that the structural genes for GL formation were transferred horizontally.

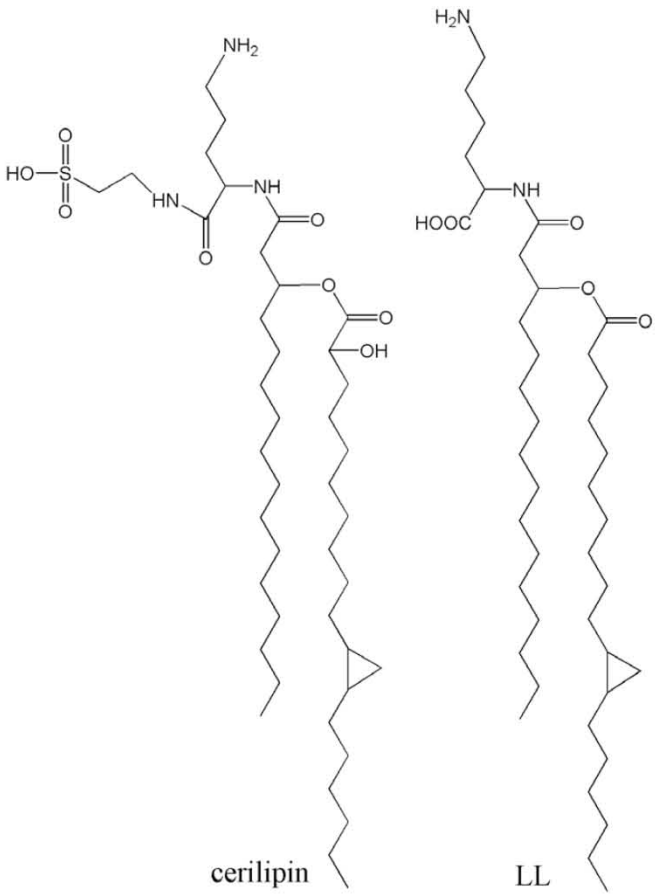

Fig. 7. Structures of tauro-ornithine-containing lipid (cerilipin) and of agrobacterial lysine-containing lipid (LL).

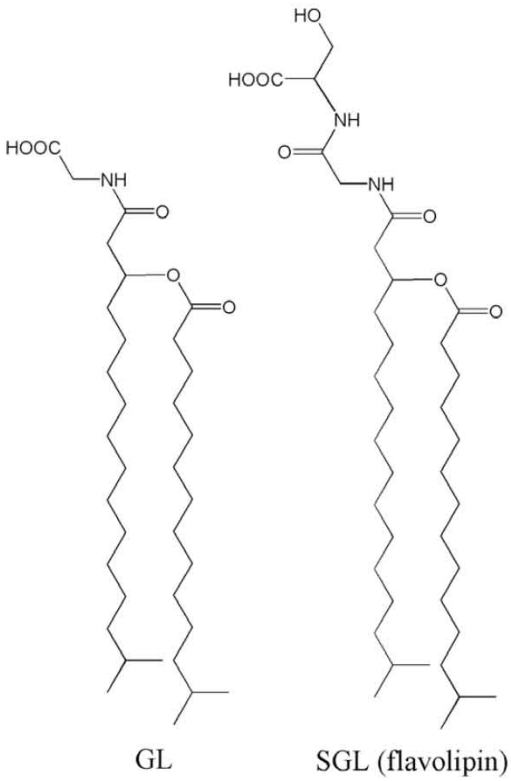

Fig. 8. Structures of glycine-containing lipid (GL) and of serineglycine-containing lipid (SGL; flavolipin).

The genes involved in the GL biosynthesis are not known although one might expect that they are similar to the genes involved in OL biosynthesis, due to the structural similarity between both molecules. Homologues to the above-described *olsB* fusions detected in species of the *Alteromonadales* are also found in genomes of several members of the genus *Cytophaga*. So far nothing is known about functions associated with GL.

4.3. Serineglycine-containing lipids

A serineglycine-containing lipid (SGL) was isolated from the opportunistic pathogen *Flavobacterium meningosepticum* [77]. This SGL was called "flavolipin" based on the genus name of the bacterium from which it was first isolated [77]. The initially proposed flavolipin structure of an *N*-(3-acyloxyacyl)serine, was incorrect [77,91] because it lacked a glycine residue. The synthesis of flavolipin to study its biological activities led to the correct structural assignment as a serineglycine-containing lipid (SGL) (Fig. 8) [92]. Flavolipin is not unique to *Flavobacterium* species, is also found in *C. marinus* WH [93], and therefore might be present in other sea-water bacteria as well. Flavolipin constitutes about 21% and 11% of the total lipids in *F. meningosepticum* [77] and *C. marinus* WH [93], respectively. Flavolipin shares the GL basic structure but has an additional serine residue (Fig. 8) which suggests that GL is a direct biosynthetic precursor for flavolipin formation in *C. marinus* WH [87]. In contrast to *C. marinus*, the seven species analyzed from the *Cytophaga* genus produce no flavolipin [88]. The genes involved in flavolipin biosynthesis are not known.

4.4. The innate immune response to amino acid-containing acyl-oxyacyl lipids

Bacterial lipids with an acyl-oxyacyl structure are recognized by toll-like receptors (TLRs) as pathogen-associated molecular patterns and trigger the innate immune response of mammals. The 3-acyl-oxyacylamide structure with (*R*)-configuration is present in OL, SGL, and lipid A. The best studied example is the bacterial endotoxin lipid A. Lipid A is the reactive part of LPS that stimulates Toll-like receptor 4 (TLR4) and the nuclear factor κB (NF-κB) to produce inflammatory cytokines. MD-2, a molecule that physically associates with TLR4 on the cell surface, confers the LPS responsiveness on the TLR4 receptor [94]. OL and SGL also induce inflammatory immune responses, measured by the formation of PGE_2, IL-1β, and tumor necrosis factor α by macrophages [95]. A recent study suggests that even the physical state of lipid A or OL affect their biological activities [96]. OL and SGL can be used as adjuvants [59,97–101], and when injected into mice before exposure to the endotoxin lipid A they prevent the lethal effects of the latter [102]. Because of the structural similarities between the two molecules, OL might function as an antagonistic blocker of lipid A-provoked events [102]. Like LPS, the inflammatory immune response-causing SGL signal is transduced via the TLR4–MD-2 complex [103].

5. Bacterial sphingolipids

Although sphingolipids are not amino acid-containing lipids in a strict sense, they are formed by condensing an amino acid (serine) to the fatty acyl-CoA forming the sphingolipid precursor 3-oxo-sphinganine, CoA and CO_2 (Fig. 9A). In eukaryotes, sphingolipids are ubiquitous and essential components of the plasma membrane and are crucial for signaling and organization of lipid rafts. In contrast, sphingolipids occur only in few bacteria, particularly some anaerobes, where they functionally replace other bacterial membrane lipids. Sphingolipids are found in the genera *Pedobacter* [104], *Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Sphingomonas*, *Sphingobacterium*, *Bdellovibrio*, *Cystobacter*, *Mycoplasma*, *Flectobacillus*, and possibly *Acetobacter* [4]. Their

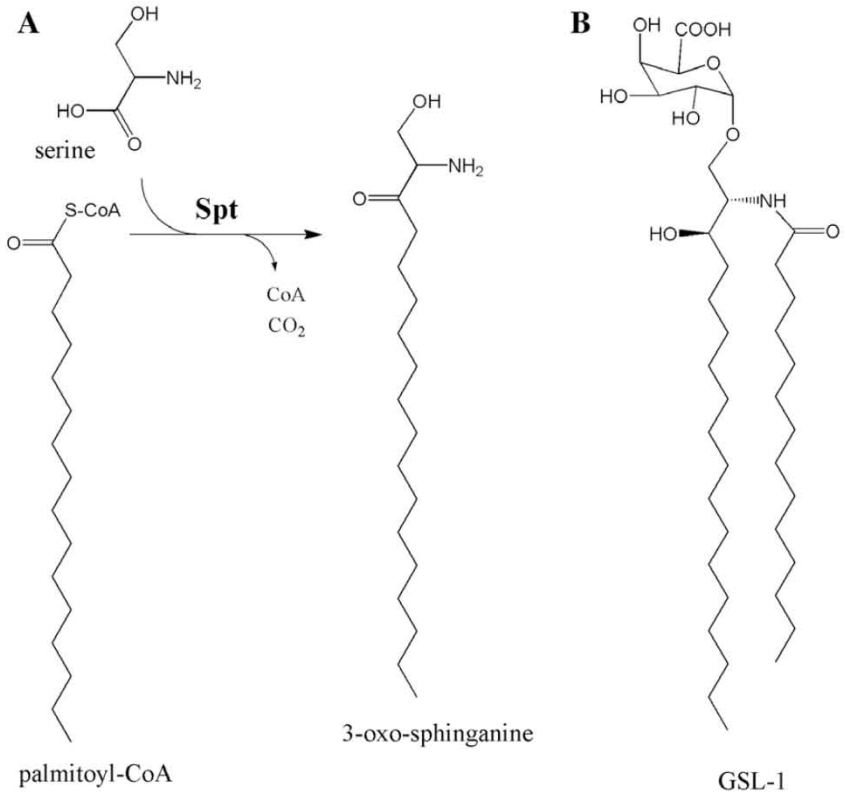

Fig. 9. Bacterial sphingolipids. The serine palmitoyl transferase (Spt)-catalyzed initial step of sphingolipid biosynthesis in bacteria (A) and the structure of glycosphingolipid GSL-1 from *Sphingomonas* (B).

occurrence in bacteria is thought so unusual that the genus name of the respective bacterium harbours the prefix "Sphingo", i.e. in *Sphingomonas* and *Sphingobacterium*. Some Gram-negative bacteria, such as *Sphingomonas capsulata*, lack lipopolysaccharide in their outer membrane and instead have glycosphingolipids as functional replacements. In *Sphingomonas paucomobilis*, two glycosphingolipids differing in their ceramide structures are substituted with the tetrasaccharide Man-Gal-GlcNAc-GlcA [105]. The chirality at carbon atoms C-2 and C-3 of the sphingoid base is D-erythro [4,106]. The variability of glycosphingolipids in the outer bacterial membrane of the *Sphingomonadaceae* is considerable [107], and only some of these glycosphingolipids, such as GSL-1 (Fig. 9B), are recognized by natural killer T cells which provide an innate-type immune response towards glycosphingolipid-containing bacteria [107,108]. Major molecular species of ceramides in sphingobacteria have been identified as 2-*N*-2′-hydroxy-13′-methyltetradecanoyl-15-methylhexadecasphinganine, 2-*N*-13′-methyltetradecanoyl-15-methylhexadecasphinganine, and 2-*N*-13′-methyltetradecanoyl-hexadecasphinganine [109–111]. Many *Bacteroides* species have two types of phosphosphingolipids, ceramide phosphorylethanolamine and ceramide phosphorylglycerol [112].

In eukaryotes, the biosynthesis of sphingolipids takes place in five stages. It begins with the condensation of serine and a fatty acyl-CoA to form 3-oxo-sphinganine (Stage 1), followed by its reduction to sphinganine (Stage 2), acylation to *N*-acylsphinganine (dihydroceramide) (Stage 3), and desaturation to ceramide (Stage 4) [106,113]. In Stage 5, ceramide is modified with different polar groups to form the great diversity of sphingolipids. Although the eukaryotic genes involved in the sphingolipid biosynthetis are known [114,115], little is known in bacteria. An exception is sphingolipid biosynthesis Step 1 catalyzed by serine palmitoyltransferase (EC 2.3.1.50) (Fig. 9A). Like other oxoamine synthases, the bacterial soluble serine palmitoyltransferase is pyridoxal 5′-phosphate-dependent and performs a Claisen condensation between serine and the acyl-CoA thioester with concomitant decarboxylation [116]. Although the serine palmitoyltransferase from *Sphingomonas* seems to be cytosolic, the serine palmitoyltransferases from *Sphingobacterium multivorum* and from *Bdellovibrio stolpii* are peripherically associated with the cytoplasmic side of the inner membrane [117,118]. The *S. paucimobilis* serine palmitoyltransferase crystal structure [119] at 1.3 Å resolution shows that the enzyme is a symmetrical homodimer with two active sites composed of monomers consisting of three domains. The pyridoxal 5′-phosphate cofactor is bound covalently to lysine 265 as an internal aldimine/Schiff base, and the active site is composed of residues from both subunits, located at the bottom of a deep cleft. Other bacterial α-oxoamine synthases are 8-amino-7-oxononanoate synthase (BioF; EC 2.3.1.47) which catalyzes the formation of 8-amino-7-oxononanoate from 6-carboxyhexanoyl-CoA and L-alanine during biotin biosynthesis, 5-aminolevulinate synthase (HemA; EC 2.3.1.37), which catalyzes the formation of 5-aminolevulinate from succinyl-CoA and glycine during tetrapyrrole and heme biosynthesis in α-proteobacteria [120], and 2-amino-3-oxobutyrate coenzyme A ligase (Kbl; EC 2.3.1.29), which cleaves 2-amino-3-oxobutyrate into acetyl-CoA and glycine during threonine degradation [116]. Phylogenetic analysis of bacterial α-oxoamine synthases (Fig. 10) suggests that distinct subgroups of serine palmitoyltransferases exist and that the encoding genes frequently form an operon with a putative acyl carrier protein gene. This finding suggests specialized acyl carrier proteins, instead of CoA, are used in some cases during the initial step of sphingolipid biosynthesis in bacteria. Based on our analysis (Fig. 10), the ability to form sphingolipids is more widespread in α-proteobacteria (*Gluconobacter, Granulibacter, Caulobacter*) than previously thought and might occur even in the β-proteobacterium *Nitrosomonas* and

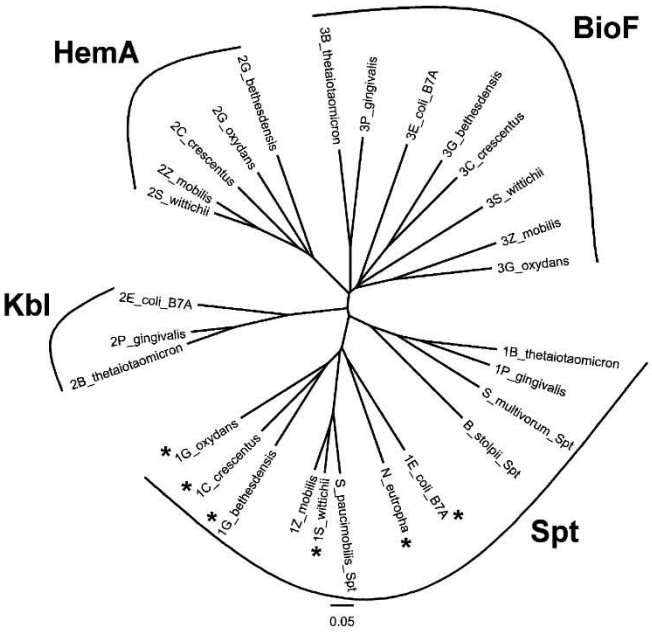

Fig. 10. Unrooted phylogenetic tree of selected bacterial serine palmitoyltransferases and other α-oxoamine synthases from bacteria. The tree was constructed using the program CLUSTALW (http://www.expasy.ch/). Distances between sequences are expressed as 0.05 changes per amino acid residue. The asterisks label species in which the serine palmitoyltransferase gene forms an operon with a putative acyl carrier protein. Accession numbers are as follows: *Bacteroides thetaiotaomicron* VPI-5482 (NP_809783, NP_810284, NP_810356), *Bacteriovorax stolpii* Spt (BAF73753), *Caulobacter crescentus* CB15 (NP_419978, NP_420168, NP_420387), *Escherichia coli* B7A (EDV60350, ZP_03029941, ZP_03030227), *Gluconobacter oxydans* 621H (AAW61792, YP_192033, YP_191153), *Granulibacter bethesdensis* CGDNIH1 (YP_744060, YP_744129, YP_744319), *Nitrosomonas eutropha* C91 (YP_746703), *Porphyromonas gingivalis* ATCC 33277 (YP_001929837, YP_001929605, YP_001929054), *Sphingobacterium multivorum* Spt (BAF73751), *Sphingomonas paucimobilis* Spt (BAB56013), *Sphingomonas wittichii* RW1 (YP_001264383, YP_001264306, YP_001261757), and *Zymomonas mobilis* subsp. *mobilis* ZM4 (YP_163005, YP_162933, YP_163652). Annotations of genes are: 8-amino-7-oxononanoate synthase (BioF), 2-amino-3-ketobutyrate coenzyme A ligase (Kbl), 5-aminolevulinate synthase (HemA), and serine palmitoyltransferase (Spt).

in several pathogenic *Escherichia coli* strains, i.e. in the enterotoxigenic *E. coli* (ETEC) B7A.

6. Sulfonolipids in the *Cytophaga* group

Gram-negative bacteria of the *Cytophaga* group move by gliding. Major lipids in the membranes of *Cytophaga johnsonae* are sulfonolipids, OL, and PE. Sulfonolipids and OL are predominantly localized to the outer membrane whereas PE is the predominant lipid of the inner membrane [121]. Sulfonolipids contain capnine that is formed by the condensation of cysteate with fatty acyl-CoA under the release of CO_2 [122,123] (Fig. 11A), in a reaction analogous to the one catalyzed by serine palmitoyltransferase [119]. Capnine is then converted to *N*-acyl-capnine, the membrane-forming sulfonolipid. The *N*-acylated residues are C14, C15, and C16 3-hydroxylated iso-fatty acids [124]. Mutants of *C. johnsonae*, deficient in gliding and sulfonolipid biosynthesis, were isolated and restoration of the sulfonolipid content by providing cysteate resulted in recovery of the ability to glide [123]. Therefore, sulfonolipids might be required for gliding motility. A structural variant of capnine exists in another member of the *Cytophaga* group, *Salinibacter ruber* [125]. The *Salinibacter* sulfonolipid contains an extra carboxylate at carbon 2 and an *O*-acyl group at carbon 3 (Fig. 11B) that is diagnostic for this extremely halophilic bacterial genus [125].

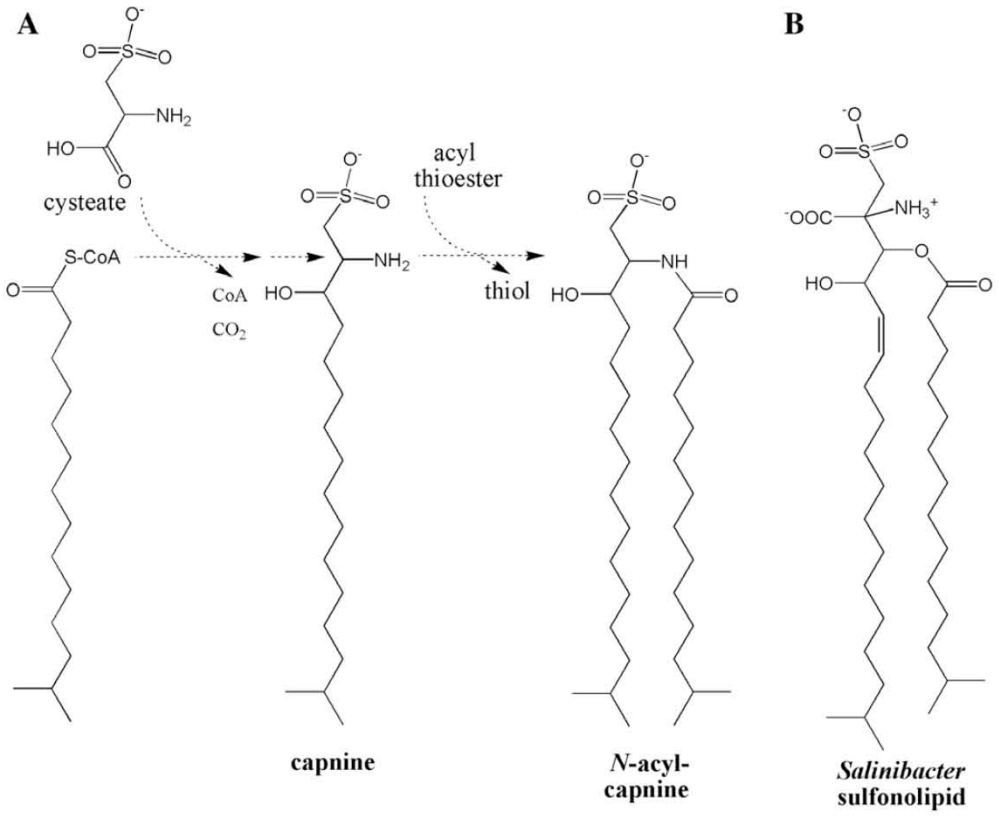

Fig. 11. Proposed pathway for sulfonolipid biosynthesis and an unusual sulfonolipid from *Salinibacter*.

7. Stress causes changes in bacterial membranes

Membrane lipid compositions of bacteria have usually been determined after the organisms had been grown on complex or defined culture media. Such determinations are reproducible and have led to the conviction that membrane lipid compositions are characteristic invariable traits of organisms. Unlike animal cells, however, plant and bacterial cells are not embedded in a controlled environment but are subject to many environmental changes and stresses. The bacterial membrane adapts to changing environments by altering the membrane lipid components by which it is formed.

It has long been known that reduced temperatures [1] or increased hydrostatic pressure [126] cause a reduction of membrane fluidity. In an attempt to maintain the fluidity of their membranes, bacteria include more unsaturated or branched fatty acyl chains into their membrane lipids thereby increasing packing disorder and fluidity of their membranes [127]. Acid stress in proteobacteria causes modifications of membrane lipids, such as formation of lysyl-PG [10], alanyl-PG [46] or the hydroxylation of OL [11]. Under phosphorus-limiting conditions, membrane phospholipids of some bacteria are partially replaced by lipids without phosphorus as demonstrated in *Bacillus subtilis* [128], *Pseudomonas diminuta* [129], *P. fluorescens* [130], and *Rhodobacter sphaeroides* [50]. In *S. meliloti*, these phosphorus-free lipids are sulfoquinovosyl diacylglycerol, OL, and DGTS [51]. In other bacteria, these phosphorus-free lipids include glycolipids as well. The ability to form OL or DGTS contributes to increased cell yields when *S. meliloti* is grown under the phosphorus-limiting condition [54]. In *S. meliloti*, the amounts of OL formed are strongly dependent on growth conditions [61]. However, in clinical isolates of *Flavobacterium* [77], *Burkholderia* isolates [82] and in pathogenic *Brucella* and *Bordetella* species [131], OL are normally major membrane lipids.

Lipid A-containing lipopolysaccharides (LPS) usually cover the outer surface of the outer membrane in Gram-negative bacteria and pose a major permeability barrier for hydrophilic and hydrophobic compounds. It is assumed that the hydrocarbon regions of the outer membrane are in a gel-like state of very low fluidity under physiological conditions [132]. Strong interactions between the lipid molecules forming the outer membrane are probably key to this gel-like behaviour and to its functions as a permeability barrier. Different environments/stresses require adjustments in the outer membrane that are accomplished by certain chemical modifications of LPS. The absence of divalent cations (Mg^{2+} and Ca^{2+}) will destabilize the outer membrane, and low Mg^{2+} concentrations activate the PhoPQ system to trigger a number of modifications of the LPS of *S. typhimurium* to stabilize the outer membrane [133]. Among these membrane-stabilizing modifications is the LpxO-catalyzed 2-hydroxylation of an esterified acyl residue of the lipid A of LPS [80,81]. Introduction of an additional hydroxyl group into the fatty acyl chain of a membrane lipid increases hydrogen bonding with neighboring molecules leading to membrane stabilization. In some bacterial groups, other lipids occur in the outer membrane either in addition to or in place of LPS. These include sphingolipids, sulfonolipids [132], and OLs [60]. Each of these outer membrane lipids have fatty acids with hydroxyl groups at the 2- and/or 3-position to stabilize the membrane. In addition, PE, which is enriched in the outer membrane, is 2-hydroxylated on its *sn*-2 fatty acid in *Burkholderia* [83].

8. Conclusions and perspectives

Membrane lipids act to form the lipid bilayer surrounding every cell and interact with other biomolecules based on their distinct chemical nature. Although phosphatidylserine has been

extensively studied in eukaryotes, other, less universal amino acid-containing membrane lipids are less well-known. Ornithine-containing lipids are formed in a direct two-step pathway whereas more steps are needed to form any of the glycerophospholipids. Therefore, making ornithine-containing lipids and other amino acid-containing acyl-oxyacyl lipids might be an easy way to build membranes in primitive biological systems. However, OL are certainly less resistant to extreme environmental conditions than archaeal ether lipids and probably would not have been of use when life originated on earth. Also, no OL-containing bacterium is known that is totally devoid of glycerophospholipids, leaving the question open whether a functional membrane can be formed by phosphorus-free membrane lipids only. Resolving the genetics and biochemistry of lysyl-phosphatidylglycerol, diacylglyceryl trimethylhomoserine, and ornithine-containing lipids in recent years has revealed the importance of these lipids in adapting to stress conditions and for the survival of bacteria. Nevertheless, we are only beginning to understand the functions of some of the amino acid-containing bacterial membrane lipids. The addition of amino acids into the structure of membrane lipids increases structural and chemical diversity, modifies net charge and polarity, and permits interaction with elements in the environment. For many minor amino acid-containing membrane lipids not much more than their structure and the producing bacterium are known. However, the immense information on bacterial genomes and improved bioinformatic tools will accelerate the detection of structural genes for many amino acid-containing bacterial membrane lipids. In addition to the more traditional approaches, another avenue for discovering new amino acid-containing bacterial membrane lipids will be the expression of metagenomic libraries and a subsequent screening for lipids. Finally, more biochemical studies are needed on the biosynthesis pathways as well as structural studies on the enzymes involved to provide feedback to impove bioinformatic predictions for ORFs involved in the biosynthesis of amino acid-containing bacterial membrane lipids.

Acknowledgments

This review is dedicated to Eugene P. Kennedy on the occasion of his 90th birthday. Work in our laboratory was supported by grants from DGAPA/UNAM (IN200806, IN217907, and IN218009) and the Consejo Nacional de Ciencia y Tecnología de México (CONACyT 46020-N, 49738-Q, and 82614).

References

[1] Rock CO. Fatty acid and phospholipid metabolism in prokaryotes. In: Vance DE, Vance JE, editors. Biochemistry of lipids lipoproteins and membranes. Amsterdam: Elsevier; 2008. p. 59–96.
[2] Raetz CR, Reynolds CM, Trent MS, Bishop RE. Lipid A modification systems in Gram-negative bacteria. Annu Rev Biochem 2007;76:295–329.
[3] Sohlenkamp C, López-Lara IM, Geiger O. Biosynthesis of phosphatidylcholine in bacteria. Prog Lipid Res 2003;42:115–62.
[4] Olsen I, Jantzen E. Sphingolipids in bacteria and fungi. Anaerobe 2001;7:103–12.
[5] Jorasch P, Wolter FP, Zähringer U, Heinz E. A UDP glucosyltransferase from *Bacillus subtilis* successively transfers up to four glucose residues to 1,2-diacylglycerol: expression of ypfP in *Escherichia coli* and structural analysis of its reaction products. Mol Microbiol 1998;29:419–30.
[6] Roy H, Ibba M. RNA-dependent lipid remodeling by bacterial multiple peptide resistance factors. Proc Natl Acad Sci USA 2008;105:4667–72.
[7] Jackson M, Crick DC, Brennan PJ. Phosphatidylinositol is an essential phospholipid in mycobacteria. J Biol Chem 2000;275:30092–9.
[8] Summons RE, Bradley AS, Jahnke LL, Waldbauer JR. Steroids, triterpenoids and molecular oxygen. Philos Trans Roy Soc B 2006;361:951–68.
[9] López-Lara IM, Sohlenkamp C, Geiger O. Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. Mol Plant Microbe Interact 2003;16:567–79.
[10] Sohlenkamp C, Galindo-Lagunas KA, Guan Z, Vinuesa P, Robinson S, Thomas Oates J, et al. The lipid lysyl-phosphatidylglycerol is present in membranes of *Rhizobium tropici* CIAT899 and confers increased resistance to polymyxin B under acidic growth conditions. Mol Plant Microbe Interact 2007;20:1421–30.
[11] Rojas-Jiménez K, Sohlenkamp C, Geiger O, Martínez-Romero E, Werner D, Vinuesa P. A ClC chloride channel homolog and ornithine-containing membrane lipids of *Rhizobium tropici* CIAT899 are involved in symbiotic efficiency and acid tolerance. Mol Plant Microbe Interact 2005;18:1175–85.
[12] Asselineau J. Bacterial lipids containing amino acids or peptides linked by amide bonds. Fortschr Chem Org Naturst 1991;56:1–85.
[13] Brady SF, Chao CJ, Clardy J. New natural product families from an environmental DNA (eDNA) gene cluster. J Am Chem Soc 2002;124:9968–9.
[14] Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 1997;61:47–64.
[15] Dowhan W, Bogdanov M, Mileykovskaya E. Functional roles of lipids in membranes. In: Vance DE, Vance JE, editors. Biochemistry of lipids, lipoproteins and membranes. Amsterdam, The Netherlands: Elsevier; 2008. p. 1–37.
[16] Sohlenkamp C, de Rudder KEE, Geiger O. Phosphatidylethanolamine is not essential for growth of *Sinorhizobium meliloti* on complex culture media. J Bacteriol 2004;186:1667–77.
[17] Koonin EV. A duplicated catalytic motif in a new superfamily of phosphohydrolases and phospholipid synthases that includes poxvirus envelope proteins. Trends Biochem Sci 1996;21:242–3.
[18] Vance DE, Vance JE. Phospholipid biosynthesis in eukaryotes. In: Vance DE, Vance JE, editors. Biochemistry of lipids, lipoproteins and membranes. Amsterdam, The Netherlands: Elsevier; 2008. p. 213–44.
[19] Langley KE, Yaffe MP, Kennedy EP. Biosynthesis of phospholipids in *Bacillus megaterium*. J Bacteriol 1979;140:996–1007.
[20] Nguyen NA, Sallans L, Kaneshiro ES. The major glycerophospholipids of the predatory and parasitic bacterium *Bdellovibrio bacteriovorus* HID5. Lipids 2008;43:1053–63.
[21] Hawrot E, Kennedy EP. Phospholipid composition and membrane function in phosphatidylserine decarboxylase mutants of *Escherichia coli*. J Biol Chem 1978;253:8213–20.
[22] Matsumoto K, Okada M, Horikoshi Y, Matsuzaki H, Kishi T, Itaya M, et al. Cloning, sequencing, and disruption of the *Bacillus subtilis psd* gene coding for phosphatidylserine synthase. J Bacteriol 1998;180:100–6.
[23] Vences-Guzmán MA, Geiger O, Sohlenkamp C. *Sinorhizobium meliloti* mutants deficient in phosphatidylserine decarboxylase accumulate phosphatidylserine and are strongly affected during symbiosis with alfalfa. J Bacteriol 2008;190:6846–56.
[24] Vance JE, Steenbergen R. Metabolism and functions of phosphatidylserine. Prog Lipid Res 2005;44:207–34.
[25] Rontein D, Wu WI, Voelker DR, Hanson AD. Mitochondrial phosphatidylserine decarboxylase from higher plants. Functional complementation in yeast, localization in plants, and overexpression in *Arabidopsis*. Plant Physiol 2003;132:1678–87.
[26] Langley KE, Hawrot E, Kennedy EP. Membrane assembly: movement of phosphatidylserine between the cytoplasmic and outer membranes of *Escherichia coli*. J Bacteriol 1982;152:1033–41.
[27] Lu Y-J, Zhang Y-M, Grimes KD, Qi J, Lee RE, Rock CO. Acyl-phosphates initiate membrane phospholipid synthesis in Gram-positive pathogens. Mol Cell 2006;23:765–72.
[28] O'Leary WM, Wilkinson SG. Gram-positive bacteria. In: Ratledge S, Wilkinson SG, editors. Microbial lipids. London: Academic Press; 1988. p. 117–201.
[29] Nahaie MR, Goodfellow M, Minnikin DE, Hajek V. Polar lipid and isoprenoid quinine composition in the classification of *Staphylococcus*. J Gen Microbiol 1984;130:2427–37.
[30] Op den Kamp JA, Redai I, van Deenen LL. Phospholipid composition of *Bacillus subtilis*. J Bacteriol 1969;99:298–303.
[31] Samant S, Hsu FF, Neyfakh AA, Lee H. The *Bacillus anthracis* protein MprF is required for synthesis of lysylphosphatidylglycerols and for resistance to cationic antimicrobial peptides. J Bacteriol 2009;191:1311–9.
[32] Fischer W, Leopold K. Polar lipids of four Listeria species containing L-lysylcardiolipin, a novel lipid structure, and other unique phospholipids. Int J Syst Bacteriol 1999;49:653–62.
[33] Houtsmuller UM, van Deenen L. Identification of a bacterial phospholipid as an O-ornithine ester of phosphatidyl glycerol. Biochim Biophys Acta 1963;70:211–3.
[34] Gould RM, Lennarz WJ. Biosynthesis of aminoacyl derivatives of phosphatidylglycerol. Biochem Biophys Res Commun 1967;26:512–4.
[35] Dos Santos Mota JM, Op den Kamp JAF, Verheij HM, van Deenen LLM. Phospholipids of *Streptococcus faecalis*. J Bacteriol 1970;104:611–9.
[36] Tocanne JF, Verheij HM, den Kamp JA, van Deenen LL. Chemical and physicochemical studies of lysylphosphatidylglycerol derivatives. Occurrence of a 2′ yields 3′ lysyl migration. Chem Phys Lipids 1974;13:389–403.
[37] Peschel A, Jack RW, Otto M, Collins VL, Staubitz P, Nicholson G, et al. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. J Exp Med 2001;193:1067–76.
[38] Oku Y, Kurokawa K, Ichihashi N, Sekimuzu K. Characterization of the *Staphylococcus aureus mprF* gene, involved in lysinylation of phosphatidylglycerol. Microbiology 2004;150:45–51.
[39] Staubitz P, Neumann H, Schneider T, Wiedemann L, Peschel A. MprF-mediated biosynthesis of lysylphosphatidylglycerol, an important

determinant in staphylococcal defensing resistance. FEMS Microbiol Lett 2004;231:67–71.
[40] Salzberg LI, Helmann JD. Phenotypic and transcriptomic characterization of *Bacillus subtilis* mutants with grossly altered membrane composition. J Bacteriol 2008;190:7797–807.
[41] Kenward MA, Brown MRW, Fryer JJ. The influence of calcium or manganese on the resistance to EDTA, polymyxin B or cold shock, and the composition of *Pseudomonas aeruginosa* grown in glucose- or magnesium-depleted batch cultures. J Appl Bacteriol 1979;47:489–503.
[42] Jones DE, Smith JD. Phospholipids of the differentiating bacterium *Caulobacter crescentus*. Can J Biochem 1979;57:424–8.
[43] Reeve WG, Tiwari RP, Worsley PS, Dilworth MJ, Glenn AR, Howieson JG. Constructs for insertional mutagenesis, transcriptional signal localisation and gene regulation studies in root nodule and other bacteria. Microbiology 1999;145:1307–16.
[44] Vinuesa P, Neumann-Silkow F, Pacios-Bras C, Spaink HP, Martinez-Romero E, Werner D. Genetic analysis of a pH-regulated operon from *Rhizobium tropici* CIAT899 involved in acid tolerant and nodulation competitiveness. Mol Plant Microbe Interact 2003;16:159–68.
[45] Reeve WG, Tiwari RP, Bräu l, Castelli J, Garau G, Dilworth MJ, et al. The *Sinorhizobium medicae* WSM419 *lpiA* gene is transcriptionally activated by low pH and regulated by FsrR. Microbiology 2006;152:3049–59.
[46] Klein S, Lorenzo C, Hoffmann S, Walther JM, Storbeck S, Piekarski T, et al. Adaptation of *Pseudomonas aeruginosa* to various conditions includes tRNA-dependent formation of alanyl-phosphatidylglycerol. Mol Microbiol 2009;71:551–65.
[47] Fischer W, Arneth-Seifert D. D-alanylcardiolipin, a major component of the unique lipid pattern of *Vagococcus fluvialis*. J Bacteriol 1998;180:2950–7.
[48] Peter-Katalinic J, Fischer W. A-D-Glucopyranosyl-, D-alanyl- and L-lysylcardiolipin from Gram-positive bacteria: analysis by fast atom bombardment mass spectrometry. J Lipid Res 1998;39:2286–92.
[49] Thedieck K, Hain T, Mohamed W, Tindall BJ, Nimtz M, Chakraborty T, et al. The MprF protein is required for lysinylation of phospholipids in listerial membranes and confers resistance to cationic antimicrobial peptides (CAMPs) on *Listeria monocytogenes*. Mol Microbiol 2006;62: 1325–39.
[50] Benning C, Huang ZH, Gage DA. Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. Arch Biochem Biophys 1995;317:103–11.
[51] Geiger O, Röhrs V, Weissenmayer B, Finan TM, Thomas-Oates JE. The regulator gene phoB mediates phosphate stress-controlled synthesis of the membrane lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine in *Rhizobium (Sinorhizobium) meliloti*. Mol Microbiol 1999;32:63–73.
[51a] Van Mooy BA, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblízek M, et al. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 2009;458:69–72.
[52] Klug RM, Benning C. Two enzymes of diacylglyceryl-O-4′-(*N*,*N*,*N*,-trimethyl) homoserine biosynthesis are encoded by btaA and btaB in the purple bacterium *Rhodobacter sphaeroides*. Proc Natl Acad Sci USA 2001;98: 5910–5915.
[53] Riekhof WR, Andre C, Benning C. Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria. Arch Biochem Biophys 2005;441:96–105.
[54] López-Lara IM, Gao JL, Soto MJ, Solares-Pérez A, Weissenmayer B, Sohlenkamp C, et al. Phosphorus-free membrane lipids of *Sinorhizobium meliloti* are not required for the symbiosis with alfalfa but contribute to increased cell yields under phosphorus-limiting conditions of growth. Mol Plant Microbe Interact 2005;18:973–82.
[55] Riekhof WR, Sears BB, Benning C. Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: discovery of the betaine lipid synthase $BTA1_{Cr}$. Eukaryot Cell 2005;4:242–52.
[56] Madigan MT, Martinko JM, Dunlap PV, Clark DP. Brock biology of microorganisms. 12th ed. San Francisco, CA: Pearson Benjamin Cummings; 2008.
[57] Lerouge P, Lebas MH, Agapakis-Caussé C, Promé JC. Isolation and structural characterization of a new non-phosphorylated lipoamino acid from *Mycobacterium phlei*. Chem Phys Lipids 1988;49:161–6.
[58] Knoche HW, Shively JM. The structure of an ornithine-containing lipid from *Thiobacillus thiooxidans*. J Biol Chem 1972;247:170–8.
[59] Kawai Y, Nakagawa Y, Matuyama T, Akagawa K, Itagawa K, Fukase K, et al. A typical bacterial ornithine-containing lipid Nα-(D)-[3-(hexadecanoyloxy)hexadecanoyl]-ornithine is a strong stimulant for macrophages and a useful adjuvant. FEMS Immunol Med Microbiol 1999;23:67–73.
[60] Dees C, Shively JM. Localization and quantitation of the ornithine lipid of *Thiobacillus thiooxidans*. J Bacteriol 1982;149:798–9.
[61] Gao JL, Weissenmayer B, Taylor AM, Thomas-Oates J, López-Lara IM, Geiger O. 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.
[62] Weissenmayer B, Gao JL, López-Lara IM, Geiger O. Identification of a gene required for the biosynthesis of ornithine-derived lipids. Mol Microbiol 2002;45:721–33.
[63] Aygun-Sunar S, Mandaci S, Koch HG, Murria IVJ, Goldfine H, Daldal F. Ornithine lipid is required for optimal steady-state amounts of *c*-type cytochromes in *Rhodobacter capsulatus*. Mol Microbiol 2006;61:418–35.
[64] Gough J, Karplus K, Hughey R, Chothia C. Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure. J Mol Biol 2001;313:903–19.
[65] Heath RJ, Rock CO. A conserved histidine is essential for glycerolipid acyltransferase catalysis. J Bacteriol 1998;180:1425–30.
[66] Watson WT, Minogue TD, Val DL, Beck von Bodman S, Churchill MEA. Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. Mol Cell 2002;9:685–94.
[67] Gould TA, Schweizer HP, Churchill MEA. Structure of the *Pseudomonas aeruginosa* acylhomoserine lactone synthase LasI. Mol Microbiol 2004;53:1135–46.
[68] Van Wagoner RM, 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.
[69] 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.
[70] Schüler D. Molecular analysis of a subcellular compartment: the magnetosome membrane in *Magnetospirillum gryphiswaldense*. Arch Microbiol 2004;181:1–7.
[71] 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.
[72] Clardy J, Brady SF. Cyclic AMP directly activates NasP, an *N*-acyl amino acid antibiotic biosynthetic enzyme cloned from an uncultured β-proteobacterium. J Bacteriol 2007;189:6487–9.
[73] West J, Tompkins CK, Balantac N, Nudelman E, Meengs B, White T, et al. Cloning and expression of two human lysophosphatidic acid acyltransferase cDNAs that enhance cytokine-induced signaling responses in cells. DNA Cell Biol 1997;16:691–701.
[74] Shih GC, Kahler CM, Swartley JS, Rahman MM, Coleman J, Carlson RW, et al. Multiple lysophosphatidic acid acyltransferases in *Neisseria meningitidis*. Mol Microbiol 1999;32:942–52.
[75] Cullinane M, Baysse C, Morrissey JP, O'Gara F. Identification of two lysophosphatidic acid acyl transferase genes with overlapping function in *Pseudomonas fluorescens*. Microbiology 2005;151:3071–80.
[76] Aygun-Sunar S, Bilaloglu R, Goldfine H, Daldal F. *Rhodobacter capsulatus* OlsA is a bifunctional enyzme active in both ornithine lipid and phosphatidic acid biosynthesis. J Bacteriol 2007;189:8564–74.
[77] Kawai Y, Yano I, Kaneda K. Various kinds of lipoamino acids including a novel serine-containing lipid in an opportunistic pathogen *Flavobacterium*. Their structures and biological activities on erythrocytes. Eur J Biochem 1988;171:73–80.
[78] Tahara Y, Kameda M, Yamada Y, Kondo K. A new lipid; the ornithine and taurine-containing "cerilipin". Agric Biol Chem 1976;40:243–4.
[79] Galbraith L, Jonsson MH, Rudhe LC, Wilkinson SG. Lipids and fatty acids of *Burkholderia* and *Ralstonia* species. FEMS Microbiol Lett 1999;173:359–64.
[80] Gibbons HS, Lin S, Cotter RJ, Raetz CR. Oxygen requirement for the biosynthesis of the S-2-hydroxymyristate moiety in *Salmonella typhimurium* lipid A. Function of LpxO, a new Fe^{2+}/alpha-ketoglutarate-dependent dioxygenase homologue. J Biol Chem 2000;275:32940–9.
[81] Gibbons HS, Reynolds CM, Guan Z, Raetz CRH. An inner membrane dioxygenase that generates the 2-hydroxymyristate moiety of *Salmonella* lipid A. Biochemistry 2008;47:2814–25.
[82] Phung LV, Tran TB, Hotta H, Yabuuchi E, Yano I. Cellular lipid and fatty acid compositions of *Burkholderia pseudomallei* strains isolated from human and environment in Viet Nam. Microbiol Immunol 1995;39:105–16.
[83] Taylor CJ, Anderson AJ, Wilkinson SG. Phenotypic variation of lipid composition in *Burkholderia cepacia*: a response to increased growth temperature is a greater content of 2-hydroxy acids in phosphatidylethanolamine and ornithine amide lipid. Microbiology 1998;144:1737–45.
[84] Tahara Y, Shinmoto K, Yamada Y, Kondo K. Enzymatic synthesis of tauro-ornithine lipid in *Gluconobacter cerinus*. Agric Biol Chem 1978;42:205–6.
[85] Tahara Y, Yamada Y, Kondo K. A new lysine-containing lipid isolated from *Agrobacterium tumefaciens*. Agric Biol Chem 1976;40:1449–50.
[86] Kawazoe R, Okuyama H, Reichardt W, Sasaki S. Phospholipids and a novel glycine-containing lipoamino acid in *Cytophaga johnsonae* Stanier strain C21. J Bacteriol 1991;173:5470–5.
[87] Batrakov SG, Nikitin DI, Mosezhnyi AE, Ruzhitsky AO. A glycine-containing phosphorus-free lipoaminoacid from the Gram-negative marine bacterium *Cyclobacterium marinus* WH. Chem Phys Lipids 1999;99:139–43.
[88] Kawazoe R, Monde K, Reichardt W, Okuyama H. Lipoamino acids and sulfonolipids in *Cytophaga johnsonae* Stanier strain C21 and six related species of *Cytophaga*. Arch Microbiol 1992;158:171–5.
[89] Nikitin DI, Oranskaya MS, Pitryuk IA, Chernykh NA, Lysenko AM. A new ring-forming bacterium *Arcocella aquatica* gen. et sp. nov. Microbiology (Moscow) 1994;63:87–90 [Engl. Transl.].
[90] Raj HD, Maloy SR. Proposal of *Cyclobacterium marinus* gen. nov., comb. nov. for a marine bacterium previously assigned to the genus *Flectobacillus*. Int J Syst Bacteriol 1990;40:337–47.
[91] Shiozaki M, Deguchi N, Ishikawa T, Haruyama H, Kawai Y, Nishijima M. Revised structure of flavolipin and synthesis of its isomers. Tetrahedron Lett 1998;39:4497–500.
[92] Shiozaki M, Deguchi N, Mochizuki T, Wakabayashi T, Ishikawa T, Haruyama H, et al. Revised structure and synthesis of flavolipin. Tetrahedron 1998;54:11861–76.

[93] Batrakov SG, Nikitin DI, Sheichenko VI, Ruzhitsky AO. A novel sulfonic-acid analogue of ceramide is the major extractable lipid of the Gram-negative marine bacterium *Cyclobacterium marinus* WH. Biochim Biophys Acta 1998;1391:79–91.
[94] Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med 1999;189:1777–82.
[95] Kawai Y, Akagawa K. Macrophage activation by an ornithine-containing lipid or a serine-containing lipid. Infect Immun 1989;57:2086–91.
[96] Okemoto K, Hanada K, Nishijima M, Kawasaki K. The preparation of a lipidic endotoxin affects its biological activities. Biol Pharm Bull 2008;31:1952–4.
[97] Kato H, Goto N. Adjuvanticity of an ornithine-containing lipid of *Flavobacterium meningosepticum* as a candidate vaccine adjuvant. Microbiol Immunol 1997;41:101–6.
[98] Kawai Y, Kamoshita K, Akagawa K. B-lymphocyte mitogenicity and adjuvanticity of an ornithine-containing lipid or a serine-containing lipid. FEMS Microbiol Lett 1991;67:127–9.
[99] Kawai Y, Takasuka N, Inoue K, Akagawa K, Nishijima M. Ornithine-containing lipids stimulate CD14-dependent TNF-alpha production from murine macrophage-like J774.1 and RAW 264.7 cells. FEMS Immunol Med Microbiol 2000;28:197–203.
[100] Kawai Y, Okawarab AI, Okuyama H, Kura F, Suzuki K. Modulation of chemotaxis, O(2)(−) production and myeloperoxidase release from human polymorphonuclear leukocytes by the ornithine-containing lipid and the serineglycine-containing lipid of *Flavobacterium*. FEMS Immunol Med Microbiol 2000;28:205–9.
[101] Kawai Y, Watanabe M, Matsuura M, Nishijima M, Kawahara K. The partially degraded lipopolysaccharide of *Burkholderia cepacia* and ornithine-containing lipids derived from some Gram-negative bacteria are useful complex lipid adjuvants. FEMS Immunol Med Microbiol 2002;34:173–9.
[102] Kawai Y, Kaneda K, Morisawa Y, Akagawa K. Protection of mice from lethal endotoxemia by use of an ornithine-containing lipid or a serine-containing lipid. Infect Immun 1991;59:2560–6 [Erratum: Infect Immun 1992;60:320].
[103] Gomi K, Kawasaki K, Kawai Y, Shiozaki M, Nishijima M. Toll-like receptor 4-MD-2 complex mediates the signal transduction induced by flavolipin, an amino acid-containing lipid unique to *Flavobacterium meningosepticum*. J Immunol 2002;168:2939–43.
[104] Steyn PL, Segers P, Vancanneyt M, Sandra P, Kersters K, Joubert JJ. Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. proposal of the family *Sphingobacteriaceae* fam. nov.. Int J Syst Bacteriol 1998;48:165–77.
[105] Kawahara K, Seydel U, Matsuura M, Danbara H, Rietschel ET, Zähringer U. Chemical structure of glycosphingolipids isolated from *Sphingomonas paucimobilis*. FEBS Lett 1991;292:107–10.
[106] Hanada K. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. Biochim Biophys Acta 2003;1632:16–30.
[107] Kinjo Y, Pei B, Bufali S, Raju R, Richardson SK, Imamura M, et al. Natural *Sphingomonas* glycolipids vary greatly in their ability to activate natural killer T cells. Chem Biol 2008;15:654–64.
[108] Wu D, Zajonc DM, Fujio M, Sullivan BA, Kinjo Y, Kronenberg M, et al. Design of natural killer T cell activators: Structure and function of a microbial glycosphingolipid bound to mouse CD1d. Proc Natl Acad Sci USA 2006;103:3972–7.
[109] Yano I, Tomiyasu I, Yabuuchi E. Long chain base composition of strains of three species of *Sphingobacterium* gen. nov.. FEMS Microbiol Lett 1982;15:303–7.
[110] Yano I, Imaizumi S, Tomiyasu I, Yabuuchi E. Separation and analysis of free ceramides containing 2-hydroxy fatty acids in *Sphingobacterium* species. FEMS Microbiol Lett 1983;20:449–53.
[111] Naka T, Fujiwara N, Yano I, Maeda S, Doe M, Minamino M, et al. Structural analysis of sphingophospholipids derived from *Sphingobacterium spiritivorum*, the type species of genus *Sphingobacterium*. Biochim Biophys Acta 2003;1635:83–92.
[112] Kato M, Muto Y, Tanaka-Bandoh K, Watanabe K, Ueno K. Sphingolipid composition in *Bacteroides* species. Anaerobe 1995;1:135–9.
[113] Nieto FL, Pescio LG, Favale NO, Adamo AM, Sterin-Speziale NB. Sphingolipid metabolism is a crucial determinant of cellular fate in non-stimulated proliferating Madin–Darby canine kidney (MDCK) cells. J Biol Chem 2008;283:25682–91.
[114] Kihara A, Mitsutake S, Mizutani Y, Igarashi Y. Metabolism and biological functions of two phosphorylated sphingolipids, sphingosine 1-phosphate and ceramide 1-phosphate. Prog Lipid Res 2007;46:126–44.
[115] Hirabayashi Y, Furuya S. Roles of L-serine and sphingolipid synthesis in brain development and neuronal survival. Prog Lipid Res 2008;47:188–203.
[116] Kerbarh O, Campopiano DJ, Baxter RL. Mechanism of α-oxoamine synthases: identification of the intermediate Claisen product in the 8-amino-7-oxononanoate synthase reaction. Chem Commun 2006:60–2.
[117] Ikushiro H, Hayashi H, Kagamiyama H. A water-soluble homodimeric serine palmitoyltransferase from *Sphingomonas paucimobilis* EY2395T strain. Purification, characterization, cloning, and overproduction. J Biol Chem 2001;276:18249–56.
[118] Ikushiro H, Islam MM, Tojo H, Hayashi H. Molecular characterization of membrane-associated soluble serine palmitoyltransferases from *Sphingobacterium multivorum* and *Bdellovibrio stolpii*. J Bacteriol 2007;198:5749–61.
[119] Yard BA, Carter LG, Johnson KA, Overton IM, Dorward M, Liu H, et al. The structure of serine palmitoyltransferase; gateway to sphingolipid biosynthesis. J Mol Biol 2007;370:870–86.
[120] Astner I, Schulze JO, van den Heuvel J, Jahn D, Schubert WD, Heinz DW. Crystal structure of 5-aminolevulinate synthase, the first enzyme of heme biosynthesis, and its link to XLSA in humans. EMBO J 2005;24:3166–77.
[121] Pitta TP, Leadbetter ER, Godchaux III W. Increase of ornithine amino lipid content in a sulfonolipid-deficient mutant of *Cytophaga johnsonae*. J Bacteriol 1989;171:952–7.
[122] White RH. Biosynthesis of the sulfonolipid 2-amino-3-hydroxy-15-methylhexadecane-1-sulfonic acid in the gliding bacterium *Cytophaga johnsonae*. J Bacteriol 1984;159:42–6.
[123] Abbanat DR, Leadbetter ER, Godchaux III W, Escher A. Sulphonolipids are molecular determinants of gliding motility. Nature 1986;324:367–9.
[124] Godchaux III W, Leadbetter ER. Sulfonolipids of gliding bacteria: structure of the *N*-acylaminosulfonates. J Biol Chem 1984;259:2982–90.
[125] Corcelli A, Lattanzio VMT, Mascolo G, Babudri F, Oren A, Kates M. Novel sulfonolipid in the extremely halophilic bacterium *Salinibacter ruber*. Appl Environ Microbiol 2004;70:6678–85.
[126] DeLong EF, Yayanos AA. Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure. Science 1985;228:1101–3.
[127] Zhang YM, Rock CO. Membrane lipid homeostasis in bacteria. Nat Rev Microbiol 2008;6:222–33.
[128] Minnikin DE, Abdolrahimzadeh H, Baddiley J. Variation of polar lipid composition of *Bacillus subtilis* Marburg with different growth conditions. FEBS Lett 1972;27:16–8.
[129] Minnikin DE, Abdolrahimzadeh H, Baddiley J. Replacement of acidic phospholipids by acidic glycolipids in *Pseudomonas diminuta*. Nature (London) 1974;249:268–9.
[130] Minnikin DE, Abdolrahimzadeh H. The replacement of phosphatidylethanolamine and acidic phospholipids by an ornithine-amide lipid and a minor phosphorus-free lipid in *Pseudomonas fluorescens* NCMB 129. FEBS Lett 1974;43:257–60.
[131] Thiehle OW, Schwinn G. The free lipids of *Brucella melitensis* and *Bordetella pertussis*. Eur J Biochem 1973;34:333–44.
[132] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593–656.
[133] Murata T, Tseng W, Guina T, Millar SI, Nikaido H. PhoPQ-mediated regulation produces a more robust permeability barrier in the outer membrana of *Salmonella enterica* serovar *typhimurium*. J Bacteriol 2007;189:7213–22.