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Knowns and unknowns of membrane lipid synthesis in streptomycetes

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Abstract

Bacteria belonging to the genus *Streptomyces* are among the most prolific producers of antibiotics. Research on cellular membrane biosynthesis and turnover is lagging behind in *Streptomyces* compared to related organisms like *Mycobacterium tuberculosis*. While natural products discovery in *Streptomyces* is evidently a priority in order to discover new antibiotics to combat the increase in antibiotic resistant pathogens, a better understanding of this cellular compartment should provide insights into the interplay between core and secondary metabolism. However, some of the pathways for membrane lipid biosynthesis are still incomplete. In addition, while it has become clear that remodelling of the membrane is necessary for coping with environmental stress and for morphological differentiation, the detailed mechanisms of these adaptations remain elusive. Here, we aim to provide a summary of what is known about the polar lipid composition in *Streptomyces*, the biosynthetic pathways of polar lipids, and to highlight current gaps in understanding function, dynamics and biosynthesis of these essential molecules.

Graphical Abstract

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Keywords

Streptomyces; phospholipids; phosphatidylinositol; PIMs; hopanoids; ornithine lipid

1. Introduction

Bacteria belonging to the genus *Streptomyces* are among the most prolific producers of antibiotics. They form part of the class Actinobacteria, within the high G+C Gram positive bacteria. Their huge repertoire of diverse secondary metabolites has been exploited for biomedical purposes, and in addition to antibiotics, some of their natural products have found use as antifungal, antiparasitic, immunosuppressant and antitumoral agents [1]. The life cycle of *Streptomyces* involves a complex morphological differentiation program akin to that of filamentous fungi, growing from a spore into a vegetative mycelium composed of branching hyphae. Upon some nutritional cues, this vegetative mycelium erects aerial hyphae which later differentiate into spore chains in a process that is coordinated with the production of antibiotics and other secondary metabolites [2]. The regulatory networks that control this developmental program continue to be the focus of extensive research in *Streptomyces* [3, 4].

Lipid molecules have many roles in cellular processes and metabolism. The neutral lipid triacylglycerol (TAG) can be accumulated for energy storage, both in complex eukaryotes and in some bacteria, such as *Streptomyces* [5]. Several contributions have been made to our understanding of the biosynthesis and regulation of TAG [6–8] and fatty acids [9, 10] in this genus. Polar lipids are key components of the cellular envelope, essential for the structure and functions of biological membranes. The biosynthesis and turnover of the lipids conforming the *Streptomyces* envelope is, however, much less explored than other parts of its core and secondary metabolism. Some phospholipid biosynthesis pathways have been studied in the genus *Mycobacterium*, where several essential genes involved in phospholipid synthesis have been discovered [11, 12]. Recent research has also discovered essential lipids in *Streptomyces* [13] and a remarkable variability in the composition of its cytoplasmic membrane [14].

In this minireview we describe the current knowledge on the diversity of polar lipids present in streptomycetes, their biosynthesis, dynamics and regulation, with emphasis on missing key pieces of information yet to be uncovered.

2. Phospholipid biosynthesis

2.1. Synthesis of phosphatidic acid and cytidine diphosphate diacylglycerol

The first step in phospholipid biosynthesis is the acylation of glycerol-3-phosphate (G3P) to produce lyso-phosphatidic acid (LPA, also known as 1-acyl-*sn*-glycerol-3-phosphate) (Fig. 1). LPA can be formed by two different pathways. The acyltransferase PlsB [15] was first identified in *E. coli* and is apparently mostly restricted to a few families of γ -proteobacteria. This enzyme transfers an acyl chain from acyl coenzyme A to G3P to form LPA. A second pathway, much more widespread in bacteria, involves two separate reactions through the enzymes PlsX and PlsY. PlsX converts acyl-ACP to acyl-phosphate, which is subsequently used by the glycerol-phosphate acyltransferase PlsY [16]. Interestingly, *Streptomyces* genomes apparently lack both of these pathways, so the first step in phospholipid synthesis is unresolved. This seems to be the case for the majority of bacteria in the order Actinomycetales, since only genera closely related to *Mycobacterium* seem to have homologues to PlsB.

Phosphatidic acid (PA) is synthesized via a second acylation of G3P. This reaction is performed by the LPA acyltransferase PlsC [17], of which there are several homologues in the genome of *S. coelicolor*. However, the acyltransferase family to which PlsC belongs contains proteins with a wide diversity of activities, and there is no evidence for participation in phospholipid biosynthesis of any of these genes from *Streptomyces*. Some of these putative acyltransferases may even be involved in the synthesis of secondary metabolites.

The next step in phospholipid biosynthesis is performed by the cytidine diphosphate diacylglycerol (CDP-DAG) synthetase (CdsA), which uses PA and cytidine triphosphate as substrates to produce CDP-DAG, a key intermediate in the biosynthesis of phospholipids (Fig. 1). The best candidate in *S. coelicolor* for the gene encoding this enzyme is SCO5628 [18, 19]. This putative protein is homologous to CdsA from *E. coli* (Table 1), and contains both a phosphatidate cytidylyltransferase Pfam domain and the PROSITE signature associated with this family (PS01315). Homologues to SCO5628 are widely distributed in actinomycetes.

2.2. Synthesis of phosphatidylethanolamine

Phosphatidylethanolamine (PE) is a zwitterionic phospholipid and one of the most widespread membrane lipids in bacteria. The most common bacterial pathway for PE synthesis takes CDP-DAG and serine as substrates [20]. The phosphatidyl moiety of CDP-DAG is transferred by phosphatidylserine synthase (PssA) to the hydroxyl group of L-serine leading to the formation of phosphatidylserine (PS). PS is then decarboxylated by PS-decarboxylase (Psd) to produce PE. Homologues to PssA and Psd are found as an apparent operon in *S. coelicolor*, coded by genes *SCO6467* and *SCO6468* [21]. *SCO6467* has a Pfam

domain of the CDP-alcohol phosphotransferase family and is the bi-directional best hit of the PssA coding genes from *Sinorhizobium meliloti* and *Saccharomyces cerevisiae*. SCO6468 is homologous to Psd from *Sinorhizobium meliloti* and contains a phosphatidylserine decarboxylase Pfam domain.

2.3. Synthesis of phosphatidylglycerol

Phosphatidylglycerol (PG) is a very common anionic phospholipid in bacteria, and is also synthesized using CDP-DAG as an intermediate. CDP-DAG is condensed with glycerol-3-phosphate (G3P) by the enzyme PgsA to produce phosphatidylglycerol-phosphate (PGP) (Fig. 1). In *M. tuberculosis* there is evidence that the gene named *pgsA3* is encoding this enzyme [11], and its orthologue in *S. coelicolor* is SCO5753 (Table 1) [19, 21].

To synthesize PG, PGP has to be dephosphorylated by a PGP phosphatase, and three different integral membrane enzymes with this activity (PgpA, PgpB and PgpC) have been described in *E. coli* [22]. PgpA and PgpC require divalent cations for their activities and probably face the cytoplasm, while PgpB has a broader specificity (it can also dephosphorylate other molecules like undecaprenyl-diphosphate and lyso-PA), does not require divalent cations and faces the periplasm of *E. coli* [23–25]. Apparently no homologues to PgpA are encoded in the genomes of *S. coelicolor* or related species. There are, however, three distant homologues to PgpB from the PAP2 family in *S. coelicolor*, but SCO1102 and SCO1753 are known to participate in triacylglycerol biosynthesis [7], and the other (SCO6355) is not very well conserved among actinomycetes, making it an unlikely candidate to be the PGP phosphatase. Whereas there is no apparent homologue to PgpC in *S. coelicolor*, there is a putative membrane protein (SCO3322) that contains a haloacid dehalogenase-like hydrolase domain, so it may belong to the HAD superfamily of phosphatases like PgpC [22]. SCO3322 is well conserved among *Streptomyces*, and could be an interesting experimental target.

2.4. Synthesis of cardiolipin and related phospholipids

Cardiolipin (CL) is also a common membrane lipids in bacteria and eukaryotes. An anionic glycerophospholipid with four acyl chains and two phosphate groups, CL is normally associated to energy transducing membranes, such as the inner mitochondrial membrane and bacterial cytoplasmic membrane [26]. It has been found to interact with several key proteins such as cytochrome *c*-oxidase in mitochondria [27], or with DnaA in *Escherichia coli* [28]. CL is widespread in actinomycetes and ubiquitous in *Streptomyces*. Its presence has been reported in diverse species such as *S. griseus* [29], *S. albus*, *S. fradiae*, *S. rimosus*, *S. somaliensis* [30] and *S. hygroscopicus* [31, 32].

There are several pathways for CL synthesis [20], but the most common in bacteria involves an enzyme of the phospholipase D superfamily that catalyses the reversible condensation of two PG molecules [33]. In contrast, synthesis of CL in *S. coelicolor* is catalysed by an enzyme of the CDP-alcohol phosphotransferase family (SCO1389) that transfers the phosphatidyl group from CDP-DAG to a PG molecule [21] (Fig. 1). This reaction was originally thought to be present only in eukaryotes but homologues to the CL synthase (Cls-II) from *S. coelicolor* are widespread in Actinobacteria [21]. CL synthase is essential in *S.*

coelicolor, and adequate levels of the enzyme seem to be required for proper morphological development [13]. Whereas CL synthase does not have a particular localization in *Streptomyces* membranes, CL is preferentially accumulated at the hyphal tips and in branching points in the mycelium [13].

Diverse *Streptomyces* species accumulate two unusual molecules in their membranes, monolyso-cardiolipin (MLCL) and dilyso-cardiolipin (DLCL) (Fig. 1). These two anionic lipids have been found in *S. coelicolor* and *S. hygroscopicus* [14, 21, 32] and may be more widespread in this genus. It is possible that both MLCL and DLCL are derived from CL by partial deacylation, but no phospholipase with this activity has yet been found in bacteria. Both MLCL and DLCL have been found as major components in some growth conditions in *S. coelicolor* [14], and CL synthase is an essential gene in this same species [13], so perhaps these CL-related molecules are required for some cellular processes in streptomycetes.

2.5. Synthesis of phosphatidylinositol and mannosylated derivatives

Phosphatidylinositol (PI) is a common phospholipid in eukaryotes, but in bacteria its presence is mostly limited to the class Actinobacteria and a few δ -proteobacteria. PI synthesis also begins with CDP-DAG. This intermediate is used together with *myo*-inositol-1-phosphate to synthesize PI-phosphate (PIP) with the enzyme PisA (SCO1527), and PIP is then dephosphorylated to yield PI [34]. The gene *SCO1527* had been proposed as a PI synthase before [19, 21], but it encodes most probably a PIP-synthase. The phosphatase needed to synthesize PI from PIP is unknown in Actinobacteria, but many putative phosphatases are coded in *Streptomyces* genomes, so one of those enzymes could be performing this activity.

PI is frequently modified in actinomycetes to produce diverse PI mannosides, or PIMs. PIMs have been extensively studied in *Mycobacterium*, mostly as the lipid anchor to the complex lipoglycans lipomannan and lipoarabinomannan [35]. Presence of PIMs have been often found in *Streptomyces* [29, 30], but the specific PIMs produced have not yet been described in *S. coelicolor*. Homologues to PimA and AcylT from *M. tuberculosis* [36] (SCO1525 and SCO1526, respectively) are found in a putative operon with the gene encoding PIP synthase in most *Streptomyces* genomes. PimA adds a mannose residue to the 2-position of the inositol moiety in PI to form PI monomannoside (PIM₁) and AcylT transfers an acyl chain to the hydroxyl group to the 6-position from that mannose in PIM₁ or PIM₂ to produce acyl-PIM₁ or acyl-PIM₂, respectively (Fig. 2A). A homologue to the gene coding for PimB' [12], the enzyme responsible for the second mannosylation of PI to form PI dimannoside (PIM₂) in *M. tuberculosis*, is found in the genome of *S. coelicolor* as SCO2132 and is also very conserved in other *Streptomyces*. The presence of these genes in *S. coelicolor* predicts that this species is at least able to produce PIM₁, PIM₂, acyl-PIM₁ and acyl-PIM₂. We have indeed identified these four PIMs in lipid extracts from this species (Fig. 2B and 2C).

Whereas we can identify homologous genes to those performing the first mannosylations of PI and their corresponding products in lipid extracts from *S. coelicolor*, it is still unknown if any higher PIM species similar to those found in *Mycobacterium* are produced in *Streptomyces*, such as PIM₃, PIM₄, PIM₅, PIM₆, or their acylated forms [35]. The higher

polarity of these molecules could have caused them to be overlooked in studies using standard lipid extraction techniques, and several of the genes involved in their synthesis are unknown even in *M. tuberculosis*, impeding sequence-based predictions.

Some of the mannosylated derivatives of PI are essential in *Mycobacterium* [35], where they form part of lipomannan and lipoarabinomannan, but these lipoglycans are not produced by *Streptomyces*, so the function of PIMs in this genus is still an open question.

3. Synthesis of other polar lipids

3.1. Biosynthesis of ornithine lipids

While many of the lipid membrane components are phospholipids, several bacterial genera are capable of synthesizing and accumulating phosphorus-free polar lipids [37–40]. One of these phosphorus-free polar lipids is ornithine lipid (OL), which is widely distributed in bacteria [41] and is replacing some phospholipids in conditions of inorganic phosphate (P_i) starvation [37]. Presence of OL was reported in *S. sioyaensis* – OL was thus named siolipin B originally in this species – [42], along with a lysine-containing lipid with a similar structure – named siolipin A – [43, 44]. The same two lipids were reported in *S. toyocaensis* [45], albeit with a higher ratio of OL to lysine lipid than in *S. sioyaensis*. Two separate spots corresponding to OL were found in *S. hygroscopicus* [31]. In the model streptomycete *S. coelicolor*, OL has also been associated to phosphate starvation and is accumulated during aerial hyphae erection and sporulation [14]. Even considering all those reports, OL was frequently ignored in systematics as a component of streptomycete membranes [30], perhaps due to its apparent absence in some species or to its conditional synthesis associated with phosphorus limitation.

OL is synthesized in two enzymatic steps, first the *N*-acylation of ornithine with a 3-hydroxylated fatty acid [46], and a subsequent *O*-acylation of the hydroxyl residue of the first fatty acid [47]. These reactions can proceed via two separated enzymes (OlsB and OlsA) or through a single bifunctional enzyme (OlsF) [41]. In *Streptomyces*, OL synthesis proceeds through the OlsBA pathway (Fig. 3), and it has been corroborated that SCO0921 (OlsB) is required for OL synthesis in *S. coelicolor* [14]. *SCO0920* is predicted to code for OlsA, since it is well conserved, is in an apparent operon with *olsB*, and the encoded protein has an acyltransferase domain [14]. Homologues to *olsB* and *olsA* are widespread in actinomycetes.

3.2. Synthesis of squalene, two competing hypotheses

The genome sequence from *S. coelicolor* A3(2) revealed several gene clusters putatively encoding the machinery needed to produce secondary metabolites [48]. Among these, a cluster of 13 contiguous genes (SCO6759–SCO6771) probably responsible for hopanoid biosynthesis was identified (Fig. 4) [49]. Hopanoids are synthesized from the precursor squalene (SQ). Condensation of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) – produced by the mevalonate-independent pathway [50] –, yields geranyl diphosphate (GPP), which is in turn condensed with another IPP to produce farnesyl diphosphate (FPP) (Fig. 4). These sequential reactions are catalysed by the FPP synthase,

putatively encoded by *hopD* in *S. peucetius* [51, 52] and by SCO6763 in *S. coelicolor* [49, 53].

Squalene synthesis may then proceed in two ways. In eukaryotes a single bifunctional enzyme, SQ synthase, catalyses the two-step reaction needed for condensation of two FPP molecules to synthesize SQ via presqualene-diphosphate (PSPP) [54, 55]. In bacteria, however, three enzymes (HpnD, HpnC, and HpnE, see Figure 4), catalysing three separate reactions, are used for SQ synthesis from FPP [56]. Those three enzymes are generally conserved in the same order in bacterial hopanoid biosynthetic gene clusters across widely distant bacterial clades. Both HpnD and HpnC belong to the SQ-phytoene synthase superfamily, but share less than 40% of sequence identity and are always found together in hopanoid synthesis clusters in bacteria, including *Streptomyces* (Table 1, Fig. 4), suggesting a distant duplication and specialization of their activities.

However, homologues to HpnC and HpnD are also present in *Streptomyces peucetius* – named there HopA and HopB, respectively – and each was reported to induce a small SQ accumulation when expressed in *E. coli*, suggesting that both are capable of independent synthesis of SQ, similar to the bifunctional eukaryotic SQ synthases [52]. This stands in contrast to the results by Pan *et al.* [56] where it is reported that coexpression of HpnD, HpnC and HpnE from *Zymomonas mobilis* is required for SQ biosynthesis in *E. coli*. The enzymatic activities of the two putative SQ synthases from *S. peucetius* have not been characterized *in vitro*, and the function of these enzymes should be studied in other *Streptomyces* species to identify which pathway is the most prevalent in actinomycetes. Homologues to HpnC and HpnD in *S. coelicolor* are SCO6759 and SCO6760, respectively, and both are annotated as phytoene/squalene synthases (Table 1) [49, 53].

SCO6762 is a homologue of *hpnE* from *R. palustris* and *Z. mobilis* [56, 57], and thus is probably encoding a FAD-dependent reductase that converts hydroxysqualene to SQ (Fig. 4), although it is annotated as a phytoene dehydrogenase [49, 53].

3.3. Synthesis of hopanoids

Hopanoids are pentacyclic triterpenes widespread in bacteria [58]. These molecules have been proposed to be functional sterol analogues, since they can regulate membrane fluidity and induce order in bilayer phospholipid membranes, reducing permeability and increasing membrane stability [59–61]. Two hopanoid molecules, hopene and aminobacteriohopanetriol (ABHT, also referred to as aminotrihydroxybacteriohopane) (Fig. 4), have been identified in *S. coelicolor* spore cultures and in the differentiated mycelium of *whi* mutants [49]. In the latter species it was found that hopanoids did not accumulate in several bald mutants, suggesting a role for these lipids in aerial mycelium erection [49]. Hopanoids were also found to be upregulated during aerial growth in *S. scabies*, but are neither essential for growth, nor for tolerance to ethanol, osmotic or oxidative stress in this species [62].

Squalene-hopene cyclase (SHC) catalyses the cyclization of squalene into hopene. SHC from *S. peucetius* was characterized by Ghimire *et al.* [63] and its homologue in *S. coelicolor* is SCO6764. Hopene is then condensed with SAM by an adenosylhopane

synthase (HpnH in *Rhodopseudomonas palustris* and *Methylobacterium extorquens*) [64, 65] (putatively SCO6766 in *S. coelicolor*). Afterwards, adenosylhopane is converted to ribosylhopane by a nucleosidase (HpnG, SCO6765) [53], and then ribosylhopane is aminated by a transaminase [64] (HpnO, SCO6769) to produce the other hopanoid detected in *S. coelicolor*, ABHT [53] (Fig. 4).

4. Unresolved questions on membrane composition and synthesis of membrane lipids

4.1. Unidentified phospholipids and aminolipids

In addition to the lipids described previously, several unknown polar lipids of *S. coelicolor* were also detected by thin layer chromatography, including phospholipids, aminolipids and amino-phospholipids [14]. These lipid species, whose structures remain to be determined, were consistently found under different growth conditions and could be relevant for environmental stress response or during certain phases of development. Unidentified lipids may include intermediates in the biosynthetic pathways of other lipids, or may be products of membrane degradation that accumulate during the cell dismantling of the vegetative mycelium that occurs in morphological development [66, 67].

4.2. Genes of unknown function

The genomes of many *Streptomyces* species encode several genes potentially involved in lipid metabolism, but which lack an assigned function. For example, there are four additional putative CDP-alcohol phosphotransferases encoded in the genome of *S. coelicolor* A3(2) [21]. The large majority of enzymes in this family participates in phospholipid biosynthesis, such as for example PGP synthase, PIP synthase, and PE and CL synthases from *Streptomyces*. These four uncharacterized transferases might be involved in the synthesis of the unidentified phospholipids mentioned above or other lipid molecules not yet discovered. There are also many putative lipases, acyltransferases and phospholipases in streptomycetes that may be part of membrane lipid turnover mechanisms.

5. Regulation of membrane plasticity

5.1. Regulation of OL and PE concentrations

OL has been observed in different *Streptomyces* species as a polar lipid associated with specific growth conditions. In *S. coelicolor* OL accumulates during the later developmental stages and under phosphate starvation [14]. Similarly, OL was detected in higher amounts in old mycelium from *S. sioyaensis* in contrast to the related lysine lipid, which was more abundant in young mycelium. The ratio between these two lipids was also affected by pH, with lysine lipid increasing with growth in more acidic conditions [42]. The relationship between phosphate concentration and OL synthesis has been also explored in *S. noursei* [68]; OL was detected in low P_i conditions while PE was far less abundant in the same condition. This decrease in PE concentration appears to be associated to P_i regulation in *S. noursei*, because PE was not reduced as drastically in a deregulated mutant that did not show P_i inhibition of antibiotic production. In *S. viridochromogenes* OL accumulated in old mycelium, and OL accumulation was inhibited by addition of P_i . This inhibition was

disrupted in a bald mutant [69], which suggests a crosstalk between P_i regulation and the developmental program in OL production.

There is evidence for PhoP-mediated transcriptional regulation of the putative *olsB-olsA* operon in *S. coelicolor* [70], with both genes showing induction under phosphate starvation in a wild type strain but not in a *phoP*-deficient mutant, and a putative PhoP binding site upstream from *olsB*. However, direct regulation of PhoP has not yet been experimentally validated, and it is not yet known if any developmental regulators participate in the transcriptional regulation of this operon. An *S. coelicolor* strain deficient in *olsB* presents an early onset of metabolic and morphological differentiation [14], adding to previous evidence of the interaction between availability of P_i and antibiotic production [71], and to the relationship between membrane composition and development. Interestingly, the second messenger c-di-GMP could possibly be involved in the regulation of OL synthesis, according to results from an RNA-seq experiment of a bald mutant with two phosphodiesterase genes inactivated (*rmdA* and *rmdB*) [72]. This double mutant has increased levels of c-di-GMP and showed a high overexpression of SCO0921, the OlsB-coding gene in *S. coelicolor*. Further experiments should be performed to corroborate the role of c-di-GMP and its effector, the regulator BldD [4], in OL synthesis.

5.2. Regulation of Hopanoid accumulation

Hopanoids accumulated only during sporulating cultures in wild type *S. coelicolor*, but were absent from most bald mutants (*bldA*, *bldC*, *bldD*, *bldF*, *bldG*, *bldH*), with the exception of a *bldB*-deficient mutant that still produced these lipids [49]. The three *whi* mutants studied, *whiG*, *whiB* and *whiJ* were not impaired in hopanoid production. While developmental regulation of hopanoid production seems clear, the specific regulatory elements controlling the production of these molecules have yet to be identified. A conserved hypothetical protein with a putative DNA binding domain (SCO6770 in *S. coelicolor*) is frequently found by the end of the *hop* cluster in *Streptomyces* (Fig. 4), and could be possibly involved in the transcriptional regulation of this cluster. On a functional perspective, however, it is intriguing that the absence of hopanoids has no obvious phenotype in *S. scabies* [62], so further research is needed to assess the roles of hopanoids in actinomycetes, which may be completely different from the ones they fulfil in Gram-negative bacteria.

5.3. PI degradation and signalling

In *S. lividans* a PI-specific phospholipase (SblA) has been characterized that has phospholipase C activity and possibly also phosphoinositide phosphatase activity [73]. The *sbIA* deficient mutant presents arrested growth and accelerated development in media with glucose as a carbon source. The degradation products from PI, diacylglycerol and phosphoinositol, are well-studied second messengers in eukaryotes, and thus, the fact that SblA may be controlling differentiation in response to carbon source via its PI phospholipase activity could suggest analogous signalling pathways in actinomycetes. The phosphatase activity of SblA is probably not related to the PIP-phosphatase required for PI synthesis, since the *sbIA* mutant produces actually more PI than the wild-type strain.

5.4. Open questions

There are still many open questions about membrane response and adaptation to diverse conditions. How other abiotic stresses, such as osmotic stress [74], temperature shifts or changes in pH affect the membrane properties and composition in streptomycetes has yet to be elucidated. Nutrient availability could also result in different rates of membrane turnover, either directly or indirectly through TAG synthesis and accumulation, since this neutral lipid used for energy storage uses PA as a precursor [7]. Chemical interactions with other microorganisms commonly found in the soil would also be interesting targets for analysis of membrane remodelling, as *Streptomyces* is known to adapt selectively its metabolism when interacting with other actinomycetes [75].

6. Conclusions

Taken together, research on the polar lipids from *Streptomyces* reveals a complex and highly dynamic membrane whose components may be active participants in diverse cellular processes, such as signalling, stress response and adaptation, crosstalk between core and secondary metabolism, and morphological development. Complete elucidation of the lipid biosynthetic pathways in streptomycetes could enhance synthetic biology efforts aimed at engineering the metabolic routes of these organisms, and provide useful targets to contend with some of the pathogenic strains that belong to this bacterial clade. Finally, improving our knowledge about the diversity and regulation of the composition from bacterial membranes should help increase understanding of the integration between metabolic networks, adaptation to the environment and differentiation.

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Abbreviations

G3P	glycerol-3-phosphate
LPA	lyso-phosphatidic acid
PA	phosphatidic acid
CDP-DAG	cytidine diphosphate diacylglycerol
PE	phosphatidylethanolamine
PS	phosphatidylserine
PG	phosphatidylglycerol
PGP	phosphatidylglycerol-phosphate
CL	cardiolipin

MLCL	monolyso-cardiolipin
DLCL	dilyso-cardiolipin
PI	phosphatidylinositol
PIP	phosphatidylinositol-phosphate
PIMs	phosphatidylinositol mannosides
PIM₁	phosphatidylinositol monomannoside
PIM₂	phosphatidylinositol dimannoside
OL	ornithine lipid
P_i	inorganic phosphate
SQ	squalene
IPP	isopentenyl diphosphate
DMAPP	dimethylallyl diphosphate
GPP	geranyl diphosphate
FPP	farnesyl diphosphate
ABHT	aminobacteriohopanetriol
SHC	squalene-hopene cyclase

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Highlights mini-review

1. A comprehensive analysis of streptomycetes polar lipid metabolism is presented
2. The presence of four specific PIMs is demonstrated in *Streptomyces coelicolor*
3. Possible physiological functions of different polar lipids are discussed

Figure 1.

Phospholipid biosynthetic pathways in *Streptomyces*. Parts of the biosynthetic pathway with experimental evidence are shown in blue, those with only bioinformatic predictions are shown in purple, and the reactions for which there is not enough evidence for any gene associated with them are shown in red. Phospholipids that have been identified in *Streptomyces* lipid extracts are highlighted in blue rectangles. G3P, glycerol-3-phosphate; LPA, lyso-phosphatidic acid; PA, phosphatidic acid; CDP-DAG, cytidine diphosphate diacylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PGP, phosphatidylglycerol-phosphate; PG, phosphatidylglycerol; CL, cardiolipin; MLCL, monolyso-cardiolipin; DLCL, dilyso-cardiolipin; PIP, phosphatidylinositol-phosphate; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides.

Figure 2.

Synthesis of the mannosylated derivatives of PI. **A)** Proposed pathway for the biosynthesis of PIMs based on the genes present in the genome of *S. coelicolor*. **B)** Thin layer chromatography (TLC) showing the lipid profile from *S. coelicolor* grown in SFM medium. Lipid samples were extracted as described by Sandoval-Calderón *et al.* (2015) [14] from cultures harvested 48 hours (VM, vegetative mycelium), 60 hours (AM, aerial mycelium), 7 days (SM, sporulating mycelium) after inoculation, and from spore preparations (SP) that were separated from the underlying mycelium. TLC was performed on high-performance TLC aluminum sheets (Silica Gel 60, Merck), with a solvent system of chloroform/methanol/acetic acid/water (8:3:2:1 v/v). On the left it is shown a staining with ANS reagent (Sigma) that reveals hydrophobic molecules, on the right a staining of the same TLC plate with orcinol (Sigma) to identify glycosylated molecules. A PI standard (Sigma) was included in the TLC. **C)** Mass spectra from the samples shown in B. Molecular ions corresponding to PIM₁ (traces, indicated with the red arrowheads), PIM₂, Ac-PIM₁ and Ac-PIM₂ could be detected in most of the lipid extracts from *S. coelicolor*. The spectra shown are from VM samples for PIM₁, and AM samples for the rest of PIMs. Mass spectrometry analysis was performed as described by Sandoval-Calderón, M. *et al.* (2009) [21]. PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides; PIM₁, phosphatidylinositol monomannoside; PIM₂, phosphatidylinositol dimannoside; Ac-PIM₁, acyl-phosphatidylinositol monomannoside; Ac-PIM₂, acyl-phosphatidylinositol dimannoside.

Figure 3.

Biosynthesis of ornithine lipids (OLs) in *S. coelicolor*. Participation of SCO0921 in OL synthesis has been experimentally verified (indicated in blue), and the activity of SCO0920 as OlsA is strongly supported by bioinformatic evidence (indicated in purple). LOL, lyso-ornithine lipid; OL, ornithine lipid.

Figure 4.

Hopanoid biosynthesis in *Streptomyces*. Reactions in the biosynthetic pathway with experimental evidence are shown in blue, those with only bioinformatic predictions are shown in purple, and the reactions for which there is conflicting evidence are shown in red. Hopanoids that have been identified in *S. coelicolor* lipid extracts are highlighted in blue rectangles. The hopanoid biosynthetic cluster from *S. coelicolor* is shown below, with the locus tag numbers indicated and the same colour coding as the pathway. SCO6767 and SCO6768, while not directly involved in hopanoid synthesis, have functions associated to isoprenoid biosynthesis (see Table 1). White arrows denote ORFs of unknown function. IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate. PSPP, presqualene diphosphate; HSQ, hydroxysqualene; SHC, squalene-hopene cyclase; ABHT, aminobacteriohopanetriol.

Table 1

S. coelicolor genes probably involved in polar lipid synthesis and turnover.

Locus Tag	Function of encoded protein	Evidence	References
<i>Phospholipid biosynthesis</i>			
SCO1389	CL synthase	Experimental	[13, 21]
SCO1525	PI mannosyltransferase	Predicted. Homologue to PimA from <i>Mycobacterium tuberculosis</i> (52% identical)	[36]
SCO1526	PIM ₁ acyltransferase	Predicted. Homologue to AcylT from <i>M. tuberculosis</i> (47% identical)	[36]
SCO1527	PIP synthase	Predicted. Homologue to PgsA1 (PisA) from <i>M. tuberculosis</i> (43% identical)	[19, 21, 36]
SCO2132	PIM ₁ mannosyltransferase	Predicted. Homologue to PimB' from <i>M. tuberculosis</i> (52% identical)	
SCO5628	CDP-DAG synthase	Predicted. Homologue to CdsA from <i>Escherichia coli</i> (40% identical)	[18, 19]
SCO5753	PGP synthase	Predicted. Homologue to PgsA3 from <i>M. tuberculosis</i> (59% identical)	[19, 21]
SCO6467	PS synthase	Predicted. Homologue to PssA from <i>Sinorhizobium meliloti</i> and <i>Saccharomyces cerevisiae</i> (35% identical)	[21]
SCO6468	PS decarboxylase	Predicted. Homologue to Psd from <i>Sinorhizobium meliloti</i> (45% identical)	[21]
<i>Ornithine lipid biosynthesis</i>			
SCO0920	Lyso-OL <i>O</i> -acyltransferase	Predicted/Experimental indirect evidence	[14, 70]
SCO0921	Ornithine <i>N</i> -acyltransferase	Experimental	[14]
<i>Hopanoid biosynthesis cluster</i>			
SCO6759	HSQ synthase/SQ synthase?	Predicted. Homologue to HpnC from <i>Zymomonas mobilis</i> (34% identical) but homologue to HopA from <i>S. peucetius</i> (84% identical)	[49, 53]
SCO6760	PSPP synthase/SQ synthase?	Predicted. Homologue to HpnD from <i>Zymomonas mobilis</i> (40% identical) but homologue to HopB from <i>S. peucetius</i> (84% identical)	[49, 53]
SCO6761	Unknown		
SCO6762	HSQ reductase	Predicted. Homologue to HpnD from <i>Zymomonas mobilis</i> (27% identical)	
SCO6763	GPP/FPP synthase	Predicted	[19, 52]
SCO6764	Squalene-hopene cyclase	Experimental in <i>Streptomyces peucetius</i>	[63]
SCO6765	Adenosylhopene nucleosidase	Experimental	[53]
SCO6766	Hopene Adenosyltransferase	Predicted. Homologue to HpnH from <i>Rhodospseudomonas palustris</i> (51% identical)	[53]
SCO6767	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase	Predicted, Homologue to IspG (GcpE) from <i>E. coli</i> (45% identical)	[76]
SCO6768	1-deoxy-D-xilulose-5-phosphate synthase	Experimental	[50]
SCO6769	ABHT synthase	Experimental	[53]
SCO6770	Transcriptional regulator?	Hypothetical protein with a probable DNA binding domain	[49, 53]
SCO6771	Unknown		