

Research article

Open Access

# Involvement of the YneS/YgiH and PlsX proteins in phospholipid biosynthesis in both *Bacillus subtilis* and *Escherichia coli*

Mika Yoshimura, Taku Oshima and Naotake Ogasawara\*

Address: Graduate School of Information Science, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan

Email: Mika Yoshimura - y-mika@is.naist.jp; Taku Oshima - taku@bs.naist.jp; Naotake Ogasawara\* - nogasawa@bs.naist.jp

\* Corresponding author

Published: 24 July 2007

Received: 21 January 2007

BMC Microbiology 2007, 7:69 doi:10.1186/1471-2180-7-69

Accepted: 24 July 2007

This article is available from: <http://www.biomedcentral.com/1471-2180/7/69>

© 2007 Yoshimura et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Abstract

**Background:** Phospholipid biosynthesis commences with the acylation of glycerol-3-phosphate (G3P) to form 1-acyl-G3P. This step is catalyzed by the PlsB protein in *Escherichia coli*. The gene encoding this protein has not been identified, however, in the majority of bacterial genome sequences, including that of *Bacillus subtilis*. Recently, a new two-step pathway catalyzed by PlsX and PlsY proteins for the initiation of phospholipid formation in *Streptococcus pneumoniae* has been reported.

**Results:** In *B. subtilis*, 271 genes have been reported to be indispensable, when inactivated singly, for growth in LB medium. Among these, 11 genes encode proteins with unknown functions. As part of a genetic study to identify the functions of these genes, we show here that the *B. subtilis* ortholog of *S. pneumoniae* PlsY, YneS, is required for G3P acyltransferase activity, together with PlsX. The *B. subtilis* genome lacks *plsB*, and we show in vivo that the PlsX/Y pathway is indeed essential for the growth of bacteria lacking *plsB*. Interestingly, in addition to *plsB*, *E. coli* possesses *plsX* and the *plsY* ortholog, *ygiH*. We therefore explored the functional relationship between PlsB, PlsX and YgiH in *E. coli*, and found that *plsB* is essential for *E. coli* growth, indicating that PlsB plays an important role in 1-acyl-G3P synthesis in *E. coli*. We also found, however, that the simultaneous inactivation of *plsX* and *ygiH* was impossible, revealing important roles for PlsX and YgiH in *E. coli* growth.

**Conclusion:** Both *plsX* and *yneS* are essential for 1-acyl-G3P synthesis in *B. subtilis*, in agreement with recent reports on their biochemical functions. In *E. coli*, PlsB plays a principal role in 1-acyl-G3P synthesis and is also essential for bacterial growth. PlsX and YgiH also, however, play important roles in *E. coli* growth, possibly by regulating the intracellular concentration of acyl-ACP. These proteins are therefore important targets for development of new antibacterial agents.

## Background

Phospholipids are major components of the cell membrane. Glycerol-3-phosphate (G3P) forms the backbone of all phospholipid molecules [1]. Phospholipid biosynthesis begins with two steps of G3P acylation, leading to the formation of phosphatidic acid (PA). In bacteria, PA is converted to CDP-diacylglycerol, the precursor of the

three major phospholipids, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CA) [2] (Figure 1). Earlier biochemical and genetic analyses disclosed that G3P and 1-acylglycerol-phosphate (1-acyl-G3P) acyltransferase are encoded by the *plsB* and *plsC* genes, respectively, in *Escherichia coli* [3-5]. Interestingly, while *plsC* is universally conserved in eubacteria, *plsB* has

been identified in only a limited number of species, mainly belonging to the gamma proteobacterial group. Another gene homologous to *E. coli plsC*, possibly encoding G3P acyltransferase, *plsD*, has been cloned from the *Clostridium butyricum* genome, based on its ability to complement *plsB* defects in *E. coli*. However, the role of *plsD* in *C. butyricum* cells is currently unclear [6].

*E. coli* PlsB utilizes acyl-ACP (Acyl Carrier Protein) and acyl-CoA as acyl donors to synthesize 1-acyl-G3P [7]. Recently, Lu et al. [8,9] have reported a new two-step pathway that utilizes a novel fatty acid intermediate for the initiation of phospholipid formation in *Streptococcus pneumoniae*. They demonstrated biochemically that PlsX produced a unique activated fatty acid by catalyzing the synthesis of fatty acyl-phosphate from acyl-ACP, and then showed that PlsY transferred the fatty acid moiety from acyl-phosphate (acyl-PO<sub>4</sub>) to G3P. The *plsX* gene is widely conserved in eubacteria and it had been suggested that this gene was involved in fatty acid and/or phospholipid synthesis in *E. coli*, although the exact role of the gene remained unknown [3]. In *B. subtilis*, PlsX is essential for growth [10] and PlsX expression is controlled by FapR, a protein that regulates fatty acid and phospholipid biosynthesis genes [11]. Although the *plsY* gene, encoding a membrane protein around 200 amino acids long, is also widely conserved in eubacteria [8,9], the *plsY* gene function had been completely unknown.

In the gram-positive spore-forming bacterium, *Bacillus subtilis*, we showed that 271 genes, including *plsC* (*yhdO*) and *plsX*, when inactivated singly, were indispensable for growth in LB medium [10]. Among these genes, 11 encoded proteins with unknown functions, and these proteins are therefore targets for development of new antibacterial agents. As part of a genetic study to identify the functions of these genes, here we show that the *B. subtilis* ortholog of *S. pneumoniae* PlsY, YneS, is required for G3P acyltransferase activity, together with PlsX. The *B. subtilis* genome lacks *plsB*, and our results demonstrate in vivo that the PlsX/Y pathway is essential for the growth of bacteria lacking *plsB*. Interestingly, in addition to *plsB*, *E. coli* possesses *plsX* and the *plsY* ortholog, *ygiH*. Thus, we explored the functional relationship between PlsB, PlsX and YgiH in *E. coli*, and found that, although PlsB plays the principal role in 1-acyl-G3P synthesis, PlsX and YgiH are also important for optimal *E. coli* growth.

## Results

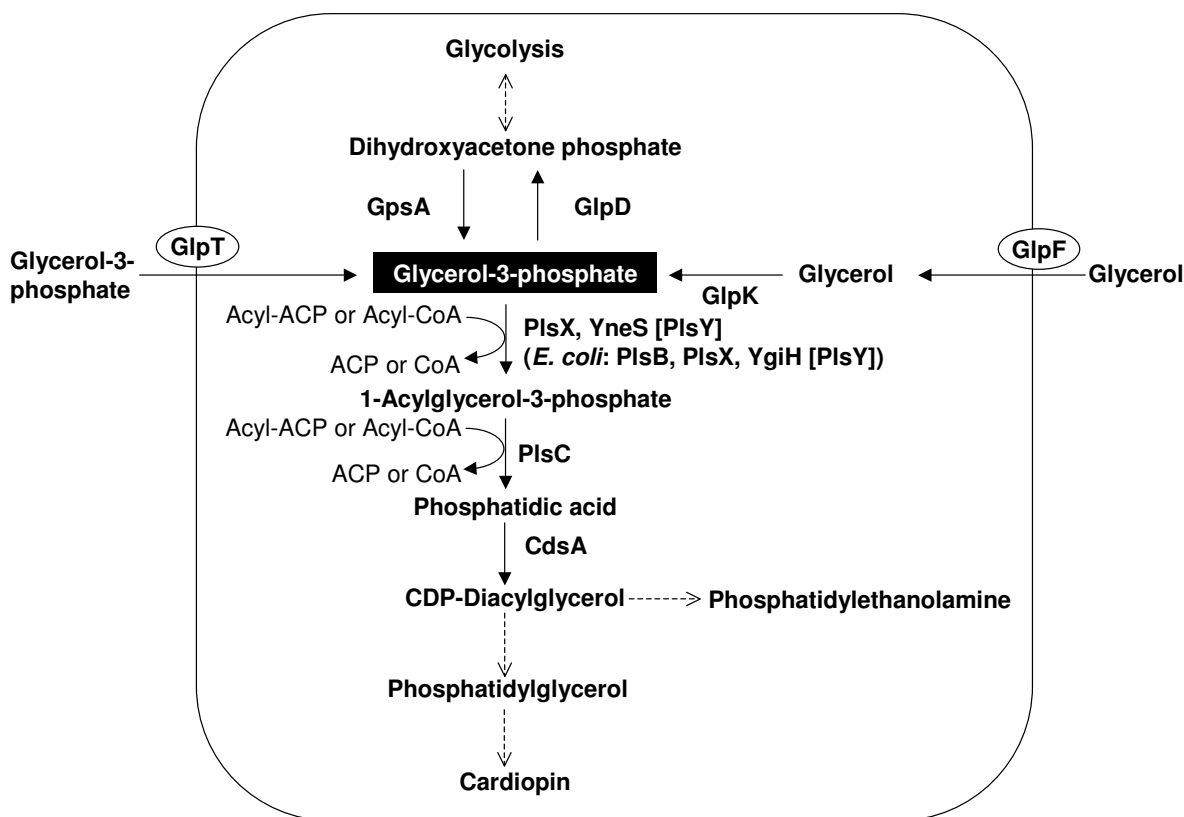
### Isolation of a *B. subtilis yneS-ts* mutant

To explore the functions of essential genes of unknown function, we adopted a strategy to isolate temperature-sensitive (*ts*) mutants of these genes and then to seek extragenic suppressors of the *ts* genes. To generate a *ts* mutation in *yneS*, we initially introduced a nonfunctional

chloramphenicol-resistant gene (*cat*) downstream of *yneS*, and transformed cells with the *yneS*-functional *cat* fragment mutagenized by PCR in vitro. Chloramphenicol-resistant transformants were selected at 30°C, and colonies that were unable to grow above 42°C were analyzed. In this manner, we isolated a *ts* mutant of *yneS* (strain MY103) that had a one-base substitution altering Val190 to Glu in the C-terminal cytoplasmic region of the protein.

### Mapping of the suppressor mutation site in the *yneS-ts sup-1* strain

Next, we isolated a spontaneous extragenic suppressor mutant of the *ts* phenotype (strain MY105, *yneS-ts sup-1*, Figure 2) by cultivation at the restrictive temperature. Transformation of wild-type strain 168 cells with chromosomal DNA of MY105 led to the *ts* growth of more than 90% of chloramphenicol-resistant transformants, indicating that the *sup-1* mutation was not linked to the *yneS* gene. MY105 cells lost sporulation ability, and the Spo phenotype was maintained in MY107 cells (Pspac::*yneS sup-1*) in which the *yneS-ts* gene was replaced with wild-type *yneS* under control of the IPTG-dependent Pspac promoter. A DNA fragment (ca. 8 kb) that complemented the Spo phenotype of MY107 in trans was isolated from a *B. subtilis* genomic DNA library in which an *MboI* partial digest was ligated with the phage vector,  $\phi$ CM, as described previously [12]. The 8 kb fragment was cut into 2.5 kb and 5 kb fragments. The 5 kb fragment with the *cat* gene of the phage vector was cloned into pBR322, while the 2.5 kb fragment was ligated into pCA191 containing the *cat* gene [13]. MY107 cells were transformed with these plasmids for integration into the chromosome via single crossovers. The results indicated that the 5 kb fragment allowed recovery of the ability to sporulate, while the 2.5 kb fragment did not. Sequencing of the 5 kb fragment revealed three genes. These were the 3'-terminal region of *glpF*, encoding the glycerol uptake facilitator, and full-length *glpK* and *glpD*, encoding glycerol kinase and glycerol-3-phosphate dehydrogenase, respectively. Because *glpF* and *glpK* constitute an operon, and because the cloned 8 kb fragment did not contain the promoter sequence [14], *glpK* was possibly not expressed from the fragment cloned in  $\phi$ CM. The *glpD* promoter between *glpK* and *glpD* has, however, been identified [15]. These results suggested that the mutation(s) responsible for the Spo phenotype mapped in the *glpD* gene. To confirm this theory, full-length wild type *glpD* (in pCA191*glpD*-FL), the 5'-terminal half of the gene (in pCA191*glpD*-N), and the 3'-terminal half (in pCA191*glpD*-C), were separately cloned into pCA191. The resulting constructs were integrated into the MY107 chromosome by single crossovers and the sporulation abilities of transformants were examined. The 5'-terminal region of *glpD*, and the full-length gene, conveyed the ability to sporulate, but the 3'-terminal *glpD*

**Figure 1**

**Phospholipid synthesis pathway in *B. subtilis*.** Abbreviations: GpsA, NAD(P)H-dependent glycerol-3-phosphate dehydrogenase; GlpD, glycerol-3-phosphate dehydrogenase; GlpT, glycerol-3-phosphate permease; GlpF, glycerol uptake facilitator protein; GlpK, glycerol kinase; PlsC, 1-acylglycerol-3-phosphate O-acyltransferase; CdsA, phosphatidate cytidylyltransferase.

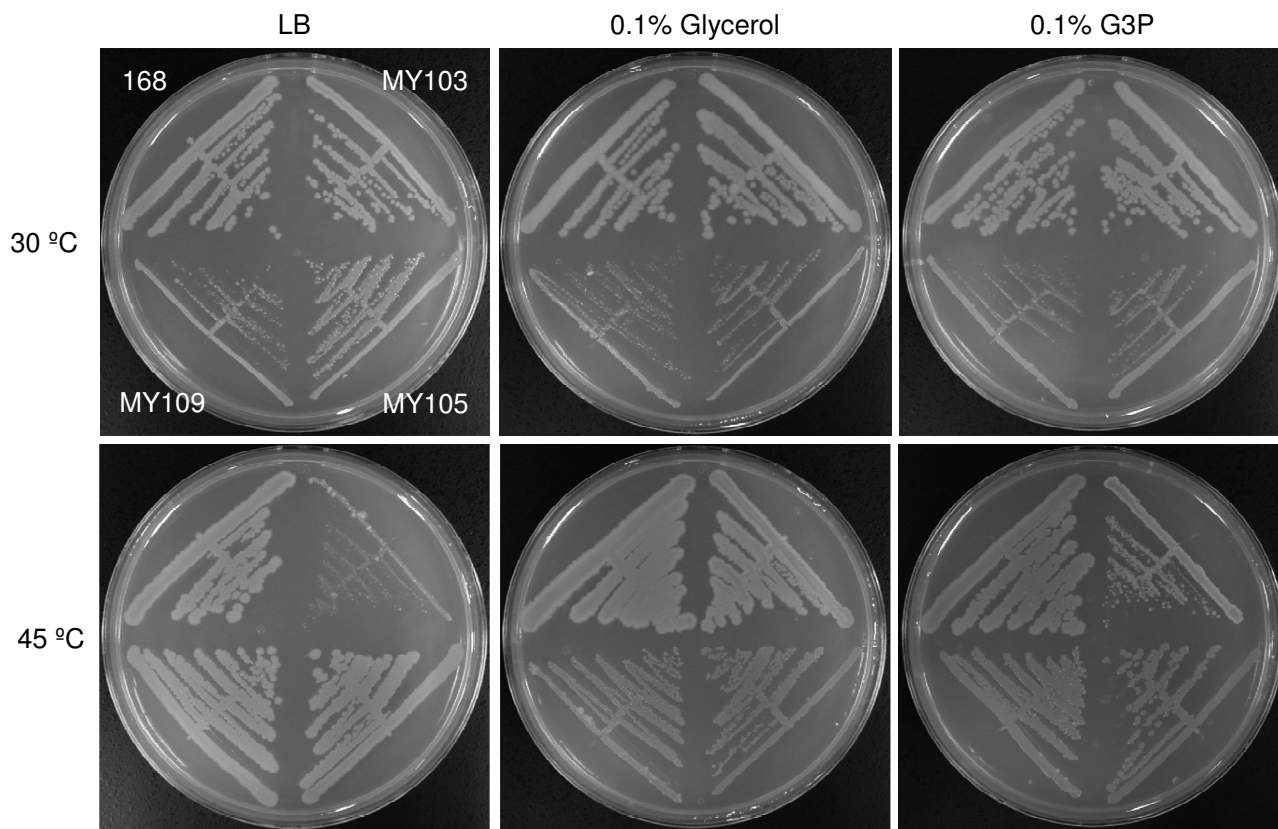
region did not. Sequencing of the 1.2 kb 5'-terminal half of *glpD* in MY103 (*yneS-ts*) revealed a single base (G) insertion between nucleotides C 492 and G 493, which resulted in inactivation of the gene. This result was consistent with the previous finding that *glpD* inactivation impairs sporulation ability [16].

We further confirmed that *glpD* inactivation suppressed the *yneS-ts* phenotype by insertion of a plasmid, pMutinNC [17], carrying an internal segment of the *glpD* gene, into the *glpD* gene of *yneS-ts* cells (strain MY109, *yneS-ts*,  $\Delta glpD::pMutin NC$ , Figure 2).

#### **Increases in intracellular G3P concentration suppress the *yneS-ts* phenotype**

The intracellular G3P concentration is known to be elevated in *glpD* mutants [16]. We speculated that increases in the G3P level might suppress the *yneS-ts* phenotype. Indeed, we found that supplementation of the growth

medium (LB) with 0.1% (w/v) G3P, or glycerol, complemented the *yneS-ts* phenotype (Figure 2). Growth of MY105 (*yneS-ts sup-1*) and MY109 (*yneS-ts*,  $\Delta glpD::pMutinNC$ ) cells was impaired compared to *glpD*<sup>+</sup> cells (wild type and *yneS-ts* cells), when glycerol or G3P was supplemented in the growth medium. This phenotype was also observed in MY108 ( $\Delta glpD::pMutinNC$ ) cells (data not shown). Given that accumulation of G3P is known to result in abnormal septation and inhibition of sporulation [18,19], an increase in the intracellular concentration of G3P would explain the growth impairment of cells without *glpD*. It should be noted that G3P and glycerol did not complement the growth defect resulting from YneS depletion in the IPTG-dependent *yneS* mutant cells, strain YNESp [10] (data not shown). The YneS-ts mutant protein will retain a reduced activity at high temperature, although the activity is insufficient to support cell growth. Residual activity would be enhanced by increases in the intracellular concentrations of G3P or glycerol.

**Figure 2**

**Growth properties of *B. subtilis* *yneS-ts* and suppressor mutants.** *B. subtilis* wild-type (168), *yneS-ts* (strain MY103), *yneS-ts sup-1* (strain MY105), and *yneS-ts ΔglpD::pMutinNC* (strain MY109) cells were grown in LB, LB with 0.1% (v/v) glycerol, and LB with 0.1% (w/v) glycerol-3-phosphate, at 30°C and 45°C.

#### **Phospholipid synthesis in *B. subtilis* *plsC-ts*, *yneS-ts*, and IPTG-dependent *plsX* mutants, under restrictive conditions**

G3P is a substrate for G3P acyltransferase, and inhibition of G3P acyltransferase activity as a result of simultaneous mutations in *plsB* and *plsX* in *E. coli* led to G3P auxotrophy [3]. Accordingly, we examined the involvement of YneS in 1-acyl-G3P synthesis by analyzing phospholipid synthesis in *yneS-ts* cells at the restrictive temperature. We additionally constructed mutations in genes possibly involved in phospholipid synthesis. These included a *ts* mutation in *plsC*, in which 7 C-terminal residues were deleted (strain MY112), and an IPTG-dependent *plsX* mutation in strain MY111.

To determine phospholipid biosynthesis rates, *B. subtilis* cells were pulse-labeled with  $^{32}$  [Pi] for 5 min. Phospholi-

pids were extracted using the method of Bligh and Dyer [20], and separated by two-dimensional thin layer chromatography [5]. We detected PA, PG, PE, and CL in wild-type cells growing at either 30°C or 42°C (Figure 3A). When *plsC-ts* cells were labeled at 30°C, a pattern similar to that of wild-type cells was obtained. At the restrictive temperature, however, 1-acyl-G3P accumulation was observed, and phospholipid synthesis did not take place (Figure 3B). This is consistent with the known 1-acyl-G3P acyltransferase activity of PlsC. In contrast, no phospholipids were detectable in *yneS-ts* cells following a shift to the restrictive temperature (Figure 3C). Our data indicate that YneS inactivation results in blockage of the first step in phospholipid synthesis in *B. subtilis* cells. Furthermore, PlsX depletion in IPTG-dependent mutant cells induces a similar change in phospholipid composition (Figure 3D). The results are consistent with the two-step mechanism of

1-acyl-G3P synthesis reported by Lu *et al.* [8], and show that the PlsX/Y pathway is essential for growth of *B. subtilis* lacking *plsB*.

#### **Functional relationship between *plsB*, *plsX* and *yneS*/*ygiH* in *E. coli***

It is generally believed that *E. coli plsB* is essential for bacterial growth, although experimental evidence supporting this hypothesis has yet to be obtained, and transposon mutagenesis experiments suggested that *plsX* is dispensable in *E. coli* [21]. The ortholog of *S. pneumoniae plsY* and *B. subtilis yneS* in *E. coli*, *ygiH*, is yet another dispensable gene [21]. Accordingly, we determined the functional relationship between *plsB*, *plsX*, and *ygiH*, in *E. coli*.

We initially attempted to systematically inactivate *E. coli* genes either singly or in combination. For this purpose, we replaced the *plsX* and *ygiH* genes with a kanamycin-resistance gene (*kan*) using the method of Datsenko and Wanner [22]. Inactivation of *plsB* was performed in the presence of plasmid-borne *plsB*. Phage P1 transduction frequencies of each allele into cells of various genetic backgrounds were measured to avoid possible effects of secondary mutations arising during gene inactivation (the *kan* cassette was removed, using FLP recombinase, in recipient cells for P1 transduction). The results, summarized in Table 1, show that *plsB* is essential for *E. coli* growth, while single deletions of either *plsX* or *ygiH* do not affect cell growth. These results suggested that PlsB plays a principal role in G3P acyltransferase activity in *E. coli*. Additionally, to examine whether *plsB* function might be complemented by overexpression of other genes, we introduced derivatives of plasmid pSTV28 or pSTV29 (12–15 copies per cell) harboring *E. coli plsX* and *ygiH*, and *B. subtilis plsX* and *yneS* into recipient strains for P1 transduction, without restoration of *plsB* function. Unexpectedly, although single deletions of *plsX* or *ygiH* did not affect cell growth, we found that the simultaneous inactivation of *plsX* and *ygiH* was impossible (Table 2), indicating that *plsX* and *ygiH* also have important roles in *E. coli* growth. Next, we expressed *B. subtilis plsX* and *yneS* genes in *E. coli*, and also the corresponding *E. coli* genes (as controls). The lethal phenotype of the *plsX* and *ygiH* double deletion was suppressed by *B. subtilis plsX* or *yneS*, indicating that these genes were able to complement the *plsX* and *ygiH* *E. coli* gene activities. In addition, a *plsX* and *ygiH* double deletion was possible when *plsB* was overexpressed from pSTV29*plsB*. To obtain further insights into the functional relationships between *plsB*, *plsX* and *ygiH*, we next introduced a plasmid harboring a mutated *plsB* gene (*plsB26*) into *E. coli*, and then attempted to inactivate *plsB*, *plsX* or *ygiH* (Table 3). The *plsB26* gene has a point mutation that changes Gly1045 to Ala, reducing the affinity of PlsB26 for G3P [23]. In the presence of plasmid-borne *plsB26*, genomic *plsB* could be inactivated, indicating that PlsB26

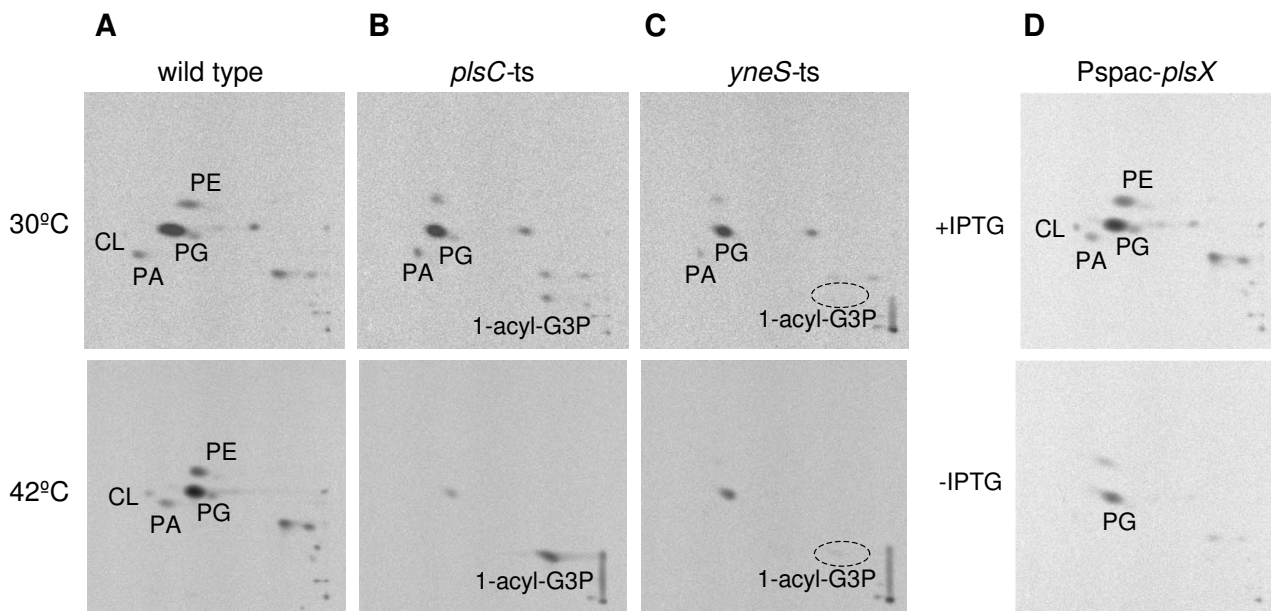
protein expressed from the plasmid-borne gene could support *E. coli* growth. The plasmid-borne gene could not, however, complement the lethality of the *plsX* and *ygiH* double deletion. Furthermore, while native *plsB* could be inactivated in the presence of pSVT29*plsB26* in cells lacking functional *ygiH*, *plsB* inactivation became impossible in cells with inactivated *plsX*, indicating that PlsX activity becomes indispensable for the growth of cells when the *plsB* activity is supplied by a plasmid copy of *plsB26*.

The essential nature of *plsB*, and the lethality of the *plsX* and *ygiH* double deletion, were further confirmed using inducible forms of these genes. To this end, we placed the coding sequences of *plsB*, *plsX* and *ygiH* under the control of an IAA (3 $\beta$ -indoleacrylic acid)-inducible *trp* promoter (Pw), integrated the genes into the chromosome as described in Methods, and then deleted the native copies of either *plsB* alone, or both *plsX* and *ygiH*. As expected, strains MEC201 (Pw-*plsB*,  $\Delta$ *plsB*), MEC306 (Pw-*plsX*,  $\Delta$ *plsX*,  $\Delta$ *ygiH*) and MEC307 (Pw-*ygiH*,  $\Delta$ *plsX*,  $\Delta$ *ygiH*) cells displayed IAA-dependent growth (Figure 4A and 4B).

The PlsB and PlsX enzymes utilize acyl-ACP as acyl-donor, and abnormal accumulation of acyl-ACP has been shown to result in growth impairment [24]. To examine acyl-ACP accumulation in cells with inactivated *plsX* and *ygiH* genes, we introduced a derivative of pUC18, harboring the *E. coli tesA* gene encoding thioesterase I [25], into cells of strains MEC306 and MEC307. Interestingly, the growth defects of these strains in the absence of IAA were suppressed by the additional supply of thioesterase I (TesA hydrolyses acyl-ACP) (Figure 4C). We have not yet succeeded in demonstrating the expected *tesA*-dependent suppression of the inability to achieve *plsX* inactivation when *plsB* activity is supplied by a plasmid encoding *plsB26*. This is because it is difficult to construct an appropriate strain in which to examine the hypothesis.

#### **Complementation of the PlsB activity by a combination of PlsX and YneS/YgiH**

As we found that the combination of PlsX and YneS conferred the ability to synthesize 1-acyl-G3P upon *B. subtilis*, we expected that simultaneous overexpression of these two proteins would complement a *plsB* mutation in *E. coli*. To test this hypothesis, we introduced various combinations of plasmids harboring *plsX* and *yneS*/*ygiH* into *E. coli* cells. In these experiments, we employed derivatives of plasmid pMW118 (ca. 5 copies per cell), whose replication is compatible with that of pSTV28/29 derivatives, when more than one cloned gene was to be expressed. We successfully complemented the *E. coli plsB* mutation by expression of *B. subtilis plsX* and *yneS* (Table 4). The complementation of the *E. coli* PlsB activity by the *B. subtilis* PlsX and YneS combination was also demonstrated using an IAA-inducible *plsB* gene (Figure 4A). Other gene com-



**Figure 3**  
**Phospholipid synthesis in *B. subtilis* *yneS-ts*, *plsC-ts*, and IPTG-dependent *plsX* mutants, under restrictive conditions.** Labeling with  $^{32}$  [Pi] for 5 min, lipid isolation, lipid separation using two-dimensional TLC, and phospholipid detection, are described in Methods. (A) Wild-type cells labeled at 30°C and 42°C. (B) *plsC-ts* cells (strain MY112) labeled at 30°C and 42°C. (C) *yneS-ts* cells (strain MY103) labeled at 30°C and 42°C. (D) *Pspac-plsX* cells (strain MY111) labeled in the presence and absence of IPTG. Abbreviations: 1-acyl-G3P, 1-acylglycerol-phosphate; PA, phosphatidic acid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CA, cardiolipin.

binations, including those with *E. coli* genes, did not complement *plsB*. Plasmid pMW118*ygiH* complemented the growth defect of MEC306 and MEC307 cells in the absence of IAA (data not shown), indicating that functional YgiH is expressed from pMW118*ygiH*. Although we could not determine the expression levels of the various proteins due to the lack of appropriate antibodies, the specific activities of *E. coli* PlsX and YgiH may be lower than those of their *B. subtilis* counterparts.

## Discussion

Our genetic studies have shown that PlsX and YneS (now renamed PlsY) are essential for G3P acyltransferase activity in *B. subtilis* cells, and that the combination of the two *B. subtilis* proteins complements PlsB activity in *E. coli* cells. Our findings are consistent with the two-step mech-

anism of 1-acyl-G3P synthesis reported by Lu *et al.* [8], and we have shown that the PlsX/Y pathway is essential for the growth of *B. subtilis* lacking *plsB*. The combination of *plsX* and *plsY* is widely conserved in eubacteria, but most eubacteria lack *plsB* [8], strongly suggesting that *plsX/Y* will be essential genes in these bacteria.

A number of bacteria, belonging mainly to the gamma group of proteobacteria, possess both *plsB* and *plsX/plsY*, however. Another important finding reported here is that, in such cases, the relationship between the two 1-acyl-G3P synthesis pathways may be variable and complex. Although *plsB* has been reported to be dispensable in *Pseudomonas aeruginosa* [26], we found that the gene is indispensable in *E. coli*, indicating that PlsB is principally responsible for 1-acyl-G3P synthesis in *E. coli*. On the

**Table 1: Frequency of PI transduction of *plsB::kan*, *plsX::kan*, and *ygiH::kan* mutations into W3110 cells harboring various plasmids**

Recipient strain									Relative transduction frequency*		
Strain	Chromosomal gene		Harboring plasmids						PI donor strain		
	<i>plsX</i>	<i>ygiH</i>	pSTV29	pSTV29 <i>plsB</i>	pSTV28 <i>EcplsX</i>	pSTV29 <i>ygiH</i>	pSTV28 <i>BsplsX</i>	pSTV29 <i>yneS</i>	$\Delta$ <i>plsB::kan</i>	$\Delta$ <i>plsX::kan</i>	$\Delta$ <i>ygiH::kan</i>
W3110	+	+	-	-	-	-	-	-	0	100	100
MEC104	+	+	+	-	-	-	-	-	0	50	64.3
MEC105	+	+	-	+	-	-	-	-	100	65.5	58.9
MEC106	+	+	-	-	+	-	-	-	0	80	50.9
MEC107	+	+	-	-	-	+	-	-	0	100	78
MEC108	+	+	-	-	-	-	+	-	0	63.6	100
MEC109	+	+	-	-	-	-	-	+	0	81.8	70.6

\*Transduction frequencies are expressed as percentages of the highest number of Km<sup>r</sup> colonies recorded in each experiment, and are depicted as blocks.

other hand, we found that simultaneous inactivation of both PlsX and PlsY is impossible, and that this lethality was complemented by overexpression of PlsB. As single deletions in either *plsX* or *ygiH* did not affect cell growth, the lethality of the *plsX* and *ygiH* double deletion cannot be because of inactivation of a minor pathway (using acyl-PO<sub>4</sub>) for 1-acyl-G3P synthesis.

Interestingly, PlsX activity becomes indispensable for growth of *E. coli* cells in which PlsB activity is supplied from a plasmid copy of *plsB26*. Growth, in minimal medium, of *E. coli* SJ22 cells harboring the *plsB* mutation on the chromosome is dependent on a supply of exoge-

nous G3P. Depletion of G3P in the growth medium has been found to result in accumulation of acyl-ACP due to the blockage of 1-acyl-G3P synthesis, leading to a severe reduction in fatty acid biosynthesis because accumulated acyl-ACP causes feedback inhibition of enzymes in the biosynthetic pathway [27,28]. G3P-acyltransferase activity expressed from a plasmid-borne copy of *plsB26* may be less than that in wild type cells, and acyl-ACP may therefore accumulate in such cells. PlsX may be able to suppress the deleterious accumulation of acyl-ACP by converting acyl-ACP to acyl-PO<sub>4</sub>. PlsY may also be involved in control of the intracellular acyl-ACP concentration. *E. coli* PlsB has been shown to utilize both acyl-ACP and acyl-

**Table 2: Frequency of PI transduction of *plsB::kan*, *plsX::kan*, and *ygiH::kan* mutations into strains MEC102 and MEC103 harboring various plasmids**

Recipient strain									Relative transduction frequency*		
Strain	Chromosomal gene		Harboring plasmids						PI donor strain		
	<i>plsX</i>	<i>ygiH</i>	pSTV29	pSTV29 <i>plsB</i>	pSTV28 <i>EcplsX</i>	pSTV29 <i>ygiH</i>	pSTV28 <i>BsplsX</i>	pSTV29 <i>yneS</i>	$\Delta$ <i>plsB::kan</i>	$\Delta$ <i>plsX::kan</i>	$\Delta$ <i>ygiH::kan</i>
MEC102	-	+	-	-	-	-	-	-	0	100	0
MEC103	+	-	-	-	-	-	-	-	0	0	100
MEC110	-	+	+	-	-	-	-	-	0	55.9	0
MEC111	-	+	-	+	-	-	-	-	100	53.5	59.8
MEC112	-	+	-	-	+	-	-	-	0	56.3	75.3
MEC113	-	+	-	-	-	+	-	-	0	83.1	100
MEC114	-	+	-	-	-	-	+	-	0	45.1	84.2
MEC115	-	+	-	-	-	-	-	+	0	100	92.7
MEC116	+	-	+	-	-	-	-	-	0	0	69.8
MEC117	+	-	-	+	-	-	-	-	100	63.7	85.9
MEC118	+	-	-	-	+	-	-	-	0	99.5	100
MEC119	+	-	-	-	-	+	-	-	0	85.1	73.8
MEC120	+	-	-	-	-	-	+	-	0	36.3	60.5
MEC121	+	-	-	-	-	-	-	+	0	100	96

\*Transduction frequencies are expressed as percentages of the highest number of Km<sup>r</sup> colonies recorded in each experiment, and are depicted as blocks.

**Table 3: Frequency of PI transduction of *plsB::kan*, *plsX::kan*, and *ygiH::kan* mutations into W3110, strain MEC102, and strain MEC103, harboring pSTV29 -*plsB* and *plsB26***

Strain	Recipient strain					Relative transduction frequency*		
	Chromosomal gene		Harboring plasmids			PI donor strain		
	<i>plsX</i>	<i>ygiH</i>	pSTV29	pSTV29 <i>plsB</i>	pSTV29 <i>plsB26</i>	$\Delta plsB::kan$	$\Delta plsX::kan$	$\Delta ygiH::kan$
MEC104	+	+	+	-	-	0	66.4	76.9
MEC105	+	+	-	+	-	100	100	100
MEC288	+	+	-	-	+	61.9	67.7	55.1
MEC110	-	+	+	-	-	0	98.2	0
MEC111	-	+	-	+	-	100	75	100
MEC289	-	+	-	-	+	0	100	0
MEC116	+	-	+	-	-	0	0	100
MEC117	+	-	+	-	-	100	100	89
MEC290	+	-	-	-	+	59.3	0	61.7

\*Transduction frequencies are expressed as percentages of the highest number of Km<sup>r</sup> colonies recorded in each experiment, and are depicted as blocks.

CoA as acyl donors in the synthesis of 1-acyl-G3P [7], but does not use these donors to make acyl-PO<sub>4</sub> [8]. On the other hand, *S. pneumoniae* PlsY has been shown to synthesize 1-acyl-G3P using both acyl-ACP and acyl-PO<sub>4</sub>. These results suggest that *E. coli* PlsY, in the absence of PlsX, may synthesize 1-acyl-G3P using acyl-ACP, and this activity would then contribute to consumption of acyl-ACP. It is therefore possible that both PlsX and PlsY contribute independently to the maintenance of an appropriate intracellular acyl-ACP concentration, and simultaneous inactivation of both proteins therefore results in deleterious accumulation of acyl-ACP. Overexpression of PlsB increases 1-acyl-G3P synthesis activity and may suppress the accumulation of acyl-ACP resulting from the *plsX* and *ygiH* double deletion. Suppression of growth defects due to the *plsX* and *ygiH* double deletion by overexpression of thioesterase I strongly supports our hypothesis.

Acyl-ACP has been shown to be not only a central cofactor for fatty acid and phospholipid synthesis, but also a regulatory molecule, coordinating the synthesis of these lipids with cell growth. Our results strongly suggest that PlsX, PlsY and PlsB form a complex network functioning to supply appropriate levels of lipid biosynthetic precursors, especially acyl-ACP. In turn, this controls the synthesis of 1-acyl-G3P in *E. coli* cells, resulting in appropriate levels of 1-acyl-G3P under various growth conditions. Further studies are needed to reveal the precise roles of PlsX, PlsY and PlsB.

**Conclusion**

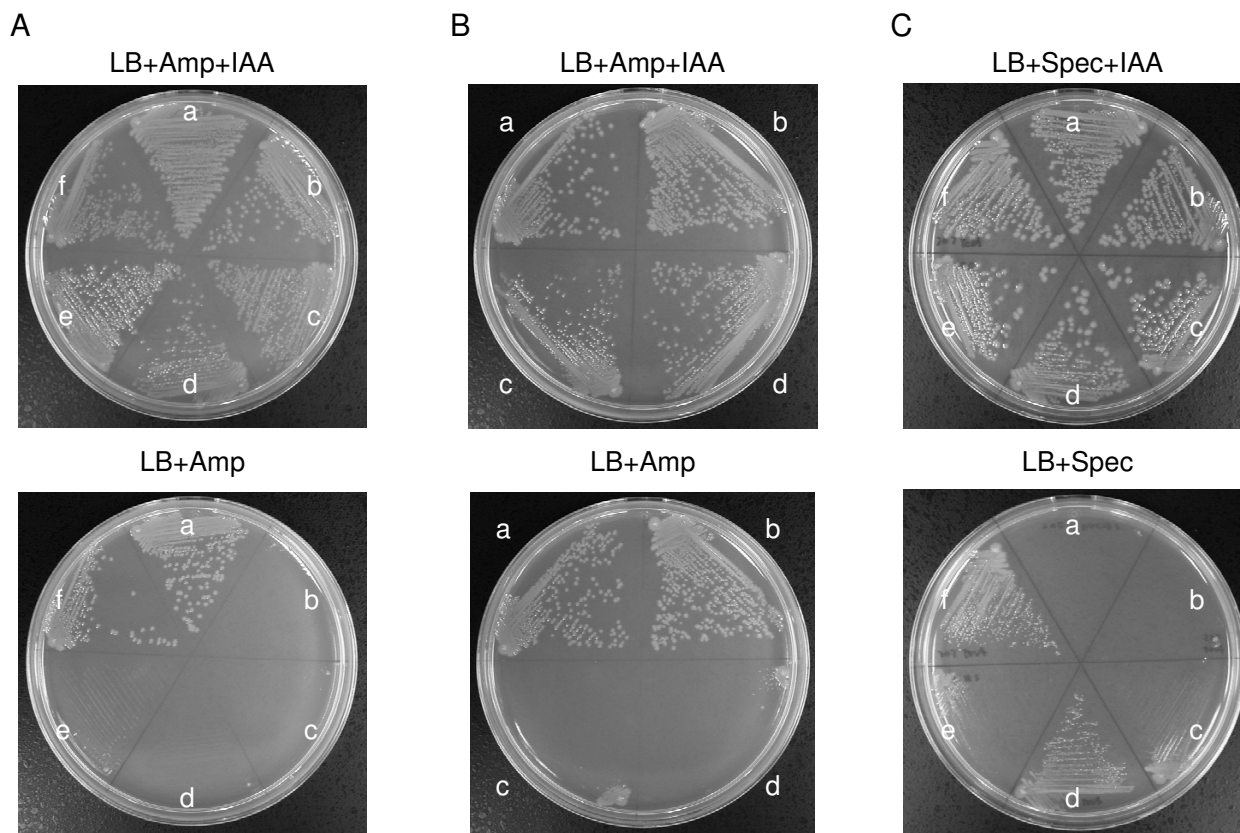
Both *plsX* and *yneS* are essential for 1-acyl-G3P synthesis in *B. subtilis* cells lacking *plsB*, in agreement with recent reports on the biochemical functions of these genes. Both genes will be essential for bacterial growth lacking *plsB*. In

**Table 4: Frequency of PI transduction of *plsB::kan*, *plsX::kan*, and *ygiH::kan* mutations into W3110 cells harboring various pairs of plasmids.**

Strain	Recipient strain								Relative transduction frequency*		
	Chromosomal gene		Harboring plasmids						PI donor strain		
	<i>plsX</i>	<i>ygiH</i>	pSTV29	pSTV28 <i>EcplsX</i>	pSTV28 <i>BsplSx</i>	pMW118	pMW118 <i>ygiH</i>	pMW118 <i>yneS</i>	$\Delta plsB::kan$	$\Delta plsX::kan$	$\Delta ygiH::kan$
MEC130	+	+	+	-	-	+	-	-	0	56.1	39.4
MEC134	+	+	+	-	-	-	+	-	0	53.7	41.3
MEC136	+	+	+	-	-	-	-	+	0	100	81.7
MEC137	+	+	-	+	-	+	-	-	0	87.8	69.2
MEC139	+	+	-	+	-	-	+	-	0	73.2	100
MEC140	+	+	-	-	+	+	-	-	0	90.2	46.2
MEC142	+	+	-	-	+	-	-	+	100	97.6	90.4

\*Transduction frequencies are expressed as percentages of the highest number of Km<sup>r</sup> colonies recorded in each experiment, and are depicted as blocks.



**Figure 4**

**Growth properties of *E. coli* strains harboring IAA-inducible genes.** *E. coli* strains harboring IAA-inducible genes were grown in LB medium containing 50 µg/ml ampicillin, with or without IAA (100 µg/ml), at 37°C. (A) a; MEC199 (Pw-*plsB*), b; MEC201 (Pw-*plsB* Δ*plsB*), c; MEC212 (Pw-*plsB* Δ*plsB* pSTV29 pMW118), d; MEC214 (Pw-*plsB* Δ*plsB* pSTV29 pMW118yneS), e; MEC218 (Pw-*plsB* Δ*plsB* pSTV29Bs*plsX* pMW118), and f; MEC220 (Pw-*plsB* Δ*plsB* pSTV29Bs*plsX* pMW118yneS). (B) a; MEC308 (Pw-*EcplsX*), b; MEC309 (Pw-*ygiH*), c; MEC306 (Pw-*EcplsX* Δ*plsX* Δ*ygiH*), and d; MEC307 (Pw-*ygiH* Δ*plsX* Δ*ygiH*). (C) *E. coli* strains harboring pUC18S or pUC18S *tesA* were grown in LB medium containing 50 µg/ml spectinomycin, with or without IAA (50 µg/ml), at 30°C. a; MEC323 (Pw-*plsB* Δ*plsB* Δ*ygiH* pUC18S), b; MEC324 (Pw-*plsB* Δ*plsB* Δ*ygiH* pUC18S *tesA*), c; MEC325 (Pw-*EcplsX* Δ*plsX* Δ*ygiH* pUC18S), d; MEC326 (Pw-*EcplsX* Δ*plsX* Δ*ygiH* pUC18S *tesA*), e; MEC327 (Pw-*ygiH* Δ*plsX* Δ*ygiH* pUC18S), e; MEC328 (Pw-*ygiH* Δ*plsX* Δ*ygiH* pUC18S *tesA*).

*E. coli*, PlsB plays a principal role in 1-acyl-G3P synthesis and is also essential for bacterial growth. PlsX and YgiH also, however, play important roles in *E. coli* growth, probably by regulating the intracellular concentrations of acyl-ACP at appropriate levels. These proteins are therefore important targets for the development of new anti-bacterial reagents.

## Methods

### Materials and strains

The *B. subtilis* and *E. coli* strains used in this study are listed in Tables S1 [see Additional file 1] and S2 [see Additional file 2], respectively. Primers and plasmids are specified in

Tables S3 [see Additional file 3] and S4 [see Additional file 4], respectively. *B. subtilis* cells were transformed as described previously [29]. *E. coli* strains DH5α (Takara) and JM105 (Takara), were used throughout as cloning hosts.

Full-length sequences of *E. coli* *plsB*, *plsX* (*EcplsX*), and *ygiH*, and *B. subtilis* *plsX* (*BsplsX*) and *yneS*, were amplified and cloned into plasmids pSTV28 or pSTV29 (Takara, 12–15 copies per cell) as summarized in Table S3 [see Additional file 3]. A DNA fragment containing a mutated *plsB* sequence, *plsB26*, was PCR-amplified from genomic DNA of *E. coli* strain TL84 [3], and cloned into pSTV29.

Inserts of pSTV29ygiH and pSTV29yneS were transferred into pMW118 (Nippon Gene, ca. 5 copies per cell), because the replication of pMW118 is compatible with that of pSTV28 and pSTV29.

The IAA (3 $\beta$ -indoleacrylic acid, Sigma)-inducible *trp* promoter fragment was PCR amplified from W3110 chromosomal DNA. The PCR product was digested with the restriction enzymes *MunI* and *EcoRI*, and cloned into the *EcoRI* site of pMC1403 [30], generating pMC1403Pw. Full-length sequences of *E. coli* *plsB*, *plsX*, and *ygiH* were amplified and cloned into the *EcoRI/BamHI*, *BamHI*, or *EcoRI/BamHI* sites of pMC1403-Pw, respectively, to obtain pMC1403Pw-*plsB*, pMC1403Pw-*plsX* and pMC1403Pw-*ygiH*.

The full-length sequence of *E. coli* *tesA* was amplified and cloned into plasmid pUC18S. Plasmid pUC18S was created by replacing the *AatII/Eam1105I* fragment (containing the ampicillin-resistance gene) of pUC18 (Takara) with an *AatII/Eam1105I* fragment (containing the spectinomycin-resistance gene) amplified, by PCR, from pAPNC213 [17].

*B. subtilis* strains were grown in Luria-Bertani (LB) medium, or DSM, supplemented with chloramphenicol (5  $\mu$ g/ml), kanamycin (5  $\mu$ g/ml), or erythromycin (0.5  $\mu$ g/ml), as appropriate. *E. coli* strains were grown in LB or M9 minimal media, supplemented with ampicillin (50  $\mu$ g/ml), chloramphenicol (5  $\mu$ g/ml), spectinomycin (50  $\mu$ g/ml), or kanamycin (25  $\mu$ g/ml), as appropriate.

#### Isolation of the *B. subtilis* yneS-ts mutant

The *yneS-ts* mutant was isolated according to the procedure of F. Kawamura (personal communication). The *B. subtilis* strain MY101, in which the sequence 45–103 bp downstream of the termination codon of *yneS* (in the coding sequence of *yneR*, which is downstream of *yneS*) was replaced with a *cat* gene, was generated as follows. The regions upstream and downstream of the insertion site were amplified by PCR using a *yneS-F1* and *yneS-R1* primer pair, and a *yneS-F2* and *yneS-R2* primer pair, respectively. The *cat* gene of pCBB31 [31] was amplified using primers *cat-F* and *cat-R*. The primer pairs *yneS-R1* and *cat-R*, and *cat-F* and *yneS-F2*, contain overlapping sequences, and the three fragments obtained were ligated by recombinant PCR using *yneS-F1* and *yneS-R2* primers. *B. subtilis* strain 168 was transformed with the resulting fragment, and chloramphenicol-resistant transformants were selected (MY101,  $\Delta yneR::cat^+$ ). Next, the pCH11 plasmid containing an inactive *cat* gene (a nonsense mutation is located in the gene), a kanamycin-resistance gene, and a *ts* origin of replication, was introduced into MY101. We selected for kanamycin resistance and chloramphenicol sensitivity in order to isolate cells with the inactive *cat*

gene on the chromosome, which occurred when gene conversion from the plasmid copy was successful [32]. The introduced plasmid was removed by cultivation at the temperature restrictive for pCH11 replication, to generate strain MY102 ( $\Delta yneR::cat$ ). PCR mutagenesis of the *yneS-cat* fragment was performed using *rTaq* polymerase (Takara) with MY101 chromosomal DNA as template. The mutated *yneS-cat* fragment was used to transform strain MY102. Chloramphenicol-resistant transformants were selected at 30°C, and colonies that were unable to grow above 42°C were screened to isolate strain MY103 (*yneS-ts*).

#### Construction of the *B. subtilis* plsC-ts mutant

As the growth of cells in which *gfp* (encoding green fluorescent protein) was translationally fused to the C-terminus of PlsC became temperature-sensitive (data not shown), we predicted that the required *plsC-ts* mutant could be obtained by modification of the C-terminal region of the *plsC* gene. We therefore created a series of mutants with deletions of different numbers of amino acids from the C-terminus of PlsC and found that deletion of the 7 end residues resulted in a *ts* phenotype (strain MY112). To generate strain MY112, the 3' end of the *plsC* coding region (with the 7 codons deleted) was amplified, and a termination codon was added by PCR using the primers *plsC-F1* and *plsC-CA7-R1*. The region downstream of *plsC* was amplified with the oligonucleotides *plsC-F2* and *plsC-R2*. The two fragments were ligated to the 5' and 3' ends of the *cat* gene (amplified from pCBB31 using *plsC-cat-F* and *plsC-cat-R* primers) by recombinant PCR with overlapping sequences contained within the *plsC-CA7-R1* and *plsC-cat-F* primer pair, and the *plsC-F2* and *plsC-cat-R* primer pair. The resulting fragment was used to transform *B. subtilis* strain 168 with selection for chloramphenicol resistance.

#### Construction of an IPTG-dependent mutant of *B. subtilis* plsX

The *plsX* gene is located in the fatty acid biosynthesis operon composed of *fapR*, *plsX*, *fabD*, *fabG*, and *acpA*. To generate cells in which *plsX* expression was under the control of the IPTG-inducible Pspac promoter, we initially integrated Pspac-*plsX* into the *aprE* locus of the bacterial chromosome. The *plsX* gene was amplified by PCR using the pAP-*plsX-F* and pAP-*plsX-R* primers, and inserted between the Pspac promoter and the kanamycin-resistance gene (*kan*) on pAPNCK, that contains sequences upstream and downstream of *aprE*, flanking Pspac and *kan*. Plasmid pAPNCK was created by replacing the *HindIII* fragment (containing the spectinomycin-resistance gene) of pAPNC213 [17] with the *HindIII* fragment (containing the *kan* gene) of pDG780 [33]. The resulting plasmid was transformed into *B. subtilis* cells and selection of a double crossover using kanamycin resistance yielded

strain MY110 ( $\Delta aprE::Pspac-plsX-kan$ ). Next, the native *plsX* gene was replaced with the *cat* gene, without the promoter and terminator sequences, to ensure the correct expression of downstream essential genes. To achieve this, upstream and downstream regions of *plsX* were amplified using the *plsX*-F1 and *plsX*-R1 primer pair, and the *plsX*-F2 and *plsX*-R2 primer pair, respectively. Fragments were ligated to the 5' and 3' ends of the *cat* gene (amplified from pCBB31 using primers *cat*-F-p and *cat*-R-t) by recombinant PCR using overlapping sequences in the *plsX*-R1 and *cat*-F-p primer pair, and the *cat*-R-t and *plsX*-F2 primer pair. The resulting fragment was used to transform strain MY110, and chloramphenicol-resistant transformants were selected to generate strain MY111 ( $\Delta aprE::Pspac-plsX-kan \Delta plsX::cat-p-t$ ).

#### Analysis of phospholipid composition

Cells of *B. subtilis* strains 168, *yneS-ts* (MY103), and *plsC-ts* (MY112) were cultured in LB medium at 30°C until an OD<sub>600</sub> of 0.4 was attained, and the temperature was then shifted to 42°C. Cells were pulse-labeled with <sup>32</sup>[Pi] (50 μCi/ml, Amersham Biosciences) for 5 min after 1 h of cultivation at 42°C. Incorporation of the label was terminated by the addition of 0.8 ml of culture to 3.0 ml of chloroform:methanol (1:2, v/v). Lipids were extracted using the method of Bligh and Dyer [20]. The phospholipids produced were examined by two-dimensional thin-layer chromatography on Silica Gel 60 plates (Merck) developed with chloroform:methanol:water (65:25:4) in the first dimension and chloroform:methanol:acetic acid (65:25:10) in the second dimension [5]. The incorporation of label into lysophosphatidic acid and other lipids was determined and quantitated using the BAS2500 image analyzer (Fuji).

*Pspac-plsX* (MY111) cells were cultured in LB medium containing 50 μM IPTG at 37°C until an OD<sub>600</sub> value of 0.4 was attained. Cells were harvested, washed twice with LB medium, and suspended in 5 mL LB medium with or without 1 mM IPTG at final densities (OD<sub>600</sub> values) of 0.06 or 0.1, respectively. Next, cells were pulse-labeled with <sup>32</sup>[Pi] (50 μCi/ml) for 5 min after 1 h of cultivation at 37°C. Lipid extraction and analysis were performed as described above.

#### Gene disruption in E. coli

Deletions of the *plsB*, *plsX*, and *γgiH* genes were achieved using a one-step chromosomal gene inactivation method involving the phage λ Red recombination system, as developed by Datsenko and Wanner [22,34]. The *E. coli* strain, BW25113, was used. The primers used for target gene disruptions are listed in Table S4 [see Additional file 4]. The 5' end of each primer contained 60–70 bp of sequences upstream or downstream of the target gene, while the 3' end contained specific 20-nucleotide

sequences from the template, pKD13. The primer pairs *plsB*delup and *plsB*deldown, *plsX*delup and *plsX*deldown, and *γgiH*delup and *γgiH*deldown, were used to generate  $\Delta plsB::kan$  (in strain MEC001),  $\Delta plsX::kan$  (in strain MEC002), and  $\Delta \gamma giH::kan$  (in strain MEC003) mutations, respectively. Because *E. coli plsB* was essential for growth, inactivation of *plsB* was performed in the presence of a plasmid harboring the gene (pSTV28*plsB-p*). Deletion mutations were confirmed using colony PCR with a *kan*-specific primer (k1 or k2) [22] and the locus-specific primers listed in Table S4 [see Additional file 4]. The deletion mutations,  $\Delta plsX::kan$  and  $\Delta \gamma giH::kan$ , were transferred into prototrophic strain W3110 by P1*vir* transduction to obtain strains MEC005 and MEC006, respectively. The *kan* cassettes in strains MEC005 and MEC006 were removed with FLP recombinase, expressed by plasmid pCP20, as described previously [22], to obtain strains MEC102 and MEC103, respectively.

#### Construction of E. coli strains harboring IAA-inducible genes

To introduce *plsB*, *plsX* and *γgiH* genes, under the control of IAA-inducible *trp* promoters, into the *E. coli* chromosome, cloned gene fragments in pMC1403-based plasmids were transferred into the λRZ5 phage by *recA*-mediated homologous recombination as previously described [35]. The technique involves double crossovers between common upstream ampicillin-resistance genes and downstream *lac* operon sequences. The resulting λPw-*plsB*, λPw-*plsX*, and λPw-*γgiH* phages were lysogenized into the W3110 chromosome, to obtain strains MEC199, MEC308, and MEC309, respectively. The  $\Delta plsB::kan$  mutation was transferred into strain MEC199 by P1*vir* transduction, and the *kan* cassette was then removed with FLP recombinase, expressed from plasmid pCP20, to obtain strain MEC201. The λPw-*plsX* and λPw-*γgiH* phages were also lysogenized into the strain MEC102 chromosome, to generate strains MEC303 and MEC304, respectively. The  $\Delta \gamma giH::kan$  mutation was then transferred into strains MEC303 and MEC304 by P1*vir* transduction.

#### Abbreviations

G3P, Glycerol-3-phosphate; 1-acyl-G3P, 1-acyl-glycerol-phosphate; PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; CL, cardiolipin; IAA, 3β-indoleacrylic acid.

#### Authors' contributions

All experimental work was carried out by MY under the supervision of TO and NO. TO provided the *E. coli* transduction system. MY and TO wrote the draft manuscript. NO finalized the analysis and completed the manuscript. All authors read and approved the final manuscript.

## Additional material

### Additional file 1

*B. subtilis* strains used in this study, and their genotypes. List of *B. subtilis* strains used in this study.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2180-7-69-S1.pdf>]

### Additional file 2

*E. coli* strains used in this study, and their genotypes. List of *E. coli* strains used in this study.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2180-7-69-S2.pdf>]

### Additional file 3

Plasmids used in this study. List of plasmids used in this study.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2180-7-69-S3.pdf>]

### Additional file 4

Oligonucleotides used in this study. List of oligonucleotides used in this study.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2180-7-69-S4.pdf>]

## Acknowledgements

We are grateful to Fujio Kawamura (Rikkyo University) for providing plasmid pCHI1. TL84 (ME8400) was supplied by the National BioResource Project for *E. coli* (National Institute of Genetics). We thank Toshifumi Inada (Nagoya University) for helpful discussions. This work was supported by KAKENHI (Grant-in-Aid for Scientific Research) on Priority Areas "Systems Genomics" from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid for JSPS Research Fellowships for Young Scientists to MY.

## References

- Cronan JE, Rock CO: **Biosynthesis of membrane lipids.** In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* Edited by: Neidhardt FC. American Society for Microbiology; 1987:474-497.
- de Chateau M, Bjorck L: **Protein PAB, a mosaic albumin-binding bacterial protein representing the first contemporary example of module shuffling.** *J Biol Chem* 1994, **269**(16):12147-12151.
- Larson TJ, Ludtke DN, Bell RM: **sn-Glycerol-3-phosphate auxotrophy of *plsB* strains of *Escherichia coli*: evidence that a second mutation, *plsX*, is required.** *J Bacteriol* 1984, **160**(2):711-717.
- Heath RJ, Rock CO: **A conserved histidine is essential for glycerolipid acyltransferase catalysis.** *J Bacteriol* 1998, **180**(6):1425-1430.
- Coleman J: **Characterization of *Escherichia coli* cells deficient in 1-acyl-sn-glycerol-3-phosphate acyltransferase activity.** *J Biol Chem* 1990, **265**(28):17215-17221.
- Heath RJ, Goldfine H, Rock CO: **A gene (*plsD*) from *Clostridium butyricum* that functionally substitutes for the sn-glycerol-3-phosphate acyltransferase gene (*plsB*) of *Escherichia coli*.** *J Bacteriol* 1997, **179**(23):7257-7263.
- Ray TK, Cronan JE Jr: **Acylation of sn-glycerol 3-phosphate in *Escherichia coli*. Study of reaction with native palmitoyl-acyl carrier protein.** *J Biol Chem* 1975, **250**(21):8422-8427.
- Lu YJ, Zhang YM, Grimes KD, Qi J, Lee RE, Rock CO: **Acyl-phosphates initiate membrane phospholipid synthesis in Gram-positive pathogens.** *Mol Cell* 2006, **23**(5):765-772.
- Lu YJ, Zhang F, Grimes KD, Lee RE, Rock CO: **Topology and Active Site of PlsY: THE BACTERIAL ACYLPHOSPHATE:GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE.** *J Biol Chem* 2007, **282**(15):11339-11346.
- Kobayashi K, Ehrlich SD, Albertini A, Amati G, Andersen KK, Arnaud M, Asai K, Ashikaga S, Aymerich S, Bessieres P, et al.: **Essential *Bacillus subtilis* genes.** *Proc Natl Acad Sci USA* 2003, **100**(8):4678-4683.
- Schujman GE, Paoletti L, Grossman AD, de Mendoza D: **FapR, a bacterial transcription factor involved in global regulation of membrane lipid biosynthesis.** *Dev Cell* 2003, **4**(5):663-672.
- Seki T, Miyachi H, Yoshikawa H, Kawamura F, Saito H: **An improved method of prophage transformation in *Bacillus subtilis*.** *J Gen Appl Microbiol* 1986, **32**:73-79.
- Murakami T, Haga K, Takeuchi M, Sato T: **Analysis of the *Bacillus subtilis* *spolIj* gene and its Parologue gene, *yqjG*.** *J Bacteriol* 2002, **184**(7):1998-2004.
- Beijer L, Nilsson RP, Holmberg C, Rutberg L: **The *glpP* and *glpF* genes of the glycerol regulon in *Bacillus subtilis*.** *J Gen Microbiol* 1993, **139**(2):349-359.
- Glatz E, Persson M, Rutberg B: **Antiterminator protein GlpP of *Bacillus subtilis* binds to *glpD* leader mRNA.** *Microbiology* 1998, **144**(Pt 2):449-456.
- Oh YK, Freese EB, Freese E: **Abnormal septation and inhibition of sporulation by accumulation of L-glycerophosphate in *Bacillus subtilis* mutants.** *J Bacteriol* 1973, **113**(2):1034-1045.
- Morimoto T, Loh PC, Hirai T, Asai K, Kobayashi K, Moriya S, Ogasawara N: **Six GTP-binding proteins of the Era/Obg family are essential for cell growth in *Bacillus subtilis*.** *Microbiology* 2002, **148**(Pt 11):3539-3552.
- Freese EB, Oh YK: **Adenosine 5'-triphosphate release and membrane collapse in glycerol-requiring mutants of *Bacillus subtilis*.** *J Bacteriol* 1974, **120**(1):507-515.
- Oh YK, Freese EB, Freese E: **Abnormal septation and inhibition of sporulation by accumulation of L-glycerophosphate in *Bacillus subtilis* mutants.** *J Bacteriol* 1973, **113**(2):1034-1045.
- Bligh EG, Dyer WJ: **A rapid method of total lipid extraction and purification.** *Can J Biochem Physiol* 1959, **37**(8):911-917.
- Gerdes SY, Scholle MD, Campbell JW, Balazsi G, Ravasz E, Daugherty MD, Somera AL, Kyrpides NC, Anderson I, Gelfand MS, et al.: **Experimental determination and system level analysis of essential genes in *Escherichia coli* MGI655.** *J Bacteriol* 2003, **185**(19):5673-5684.
- Datsenko KA, Wanner BL: **One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products.** *Proc Natl Acad Sci U S A* 2000, **97**(12):6640-6645.
- Heath RJ, Rock CO: **A missense mutation accounts for the defect in the glycerol-3-phosphate acyltransferase expressed in the *plsB26* mutant.** *J Bacteriol* 1999, **181**(6):1944-1946.
- Jiang P, Cronan JE: **Inhibition of fatty acid synthesis in *Escherichia coli* in the absence of phospholipid synthesis and release of inhibition by thioesterase action.** *J Bacteriol* 1994, **176**(10):2814-2821.
- Cho H, Cronan JE: ***Escherichia coli* thioesterase I, molecular cloning and sequencing of the structural gene and identification as a periplasmic enzyme.** *J Biol Chem* 1993, **268**(13):9238-9245.
- Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R, et al.: **Comprehensive transposon mutant library of *Pseudomonas aeruginosa*.** *Proc Natl Acad Sci U S A* 2003, **100**(24):14339-14344.
- Rock CO, Jackowski S: **Regulation of phospholipid synthesis in *Escherichia coli*. Composition of the acyl-acyl carrier protein pool in vivo.** *J Biol Chem* 1982, **257**(18):10759-10765.
- Heath RJ, Rock CO: **Regulation of malonyl-CoA metabolism by acyl-acyl carrier protein and beta-ketoacyl-acyl carrier protein synthases in *Escherichia coli*.** *J Biol Chem* 1995, **270**(26):15531-15538.
- Dubnau D, Davidoff-Abelson R: **Fate of transforming DNA following uptake by competent *Bacillus subtilis*. I. Formation and properties of the donor-recipient complex.** *J Mol Biol* 1971, **56**(2):209-221.
- Casadaban MJ, Chou J, Cohen SN: **In vitro gene fusions that join an enzymatically active  $\beta$ -galactosidase segment to amino-**

**terminal fragments of exogenous proteins: *Escherichia coli* plasmid vectors for the detection and cloning of translational initiation signals.** *J Bacteriol* 1980, **143(2)**:971-980.

31. Yamada K: **Characterization of *spoII* genes of *Bacillus subtilis*.** PhD Thesis Hiroshima University; 1989.
32. Kawamura F, Wang LF, Doi RH: **Catabolite-resistant sporulation (*crsA*) mutations in the *Bacillus subtilis* RNA polymerase sigma 43 gene (*rpoD*) can suppress and be suppressed by mutations in *spo0* genes.** *Proc Natl Acad Sci U S A* 1985, **82(23)**:8124-8128.
33. Guerout-Fleury AM, Shazand K, Frandsen N, Stragier P: **Antibiotic-resistance cassettes for *Bacillus subtilis*.** *Gene* 1995, **167(1-2)**:335-336.
34. Oshima T, Aiba H, Masuda Y, Kanaya S, Sugiura M, Wanner BL, Mori H, Mizuno T: **Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12.** *Mol Microbiol* 2002, **46(1)**:281-291.
35. Ito K, Kawakami K, Nakamura Y: **Multiple control of *Escherichia coli* lysyl-tRNA synthetase expression involves a transcriptional repressor and a translational enhancer element.** *Proc Natl Acad Sci U S A* 1993, **90(1)**:302-306.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
[http://www.biomedcentral.com/info/publishing\\_adv.asp](http://www.biomedcentral.com/info/publishing_adv.asp)

