Plant-exuded Choline Is Used for Rhizobial Membrane Lipid Biosynthesis by Phosphatidylcholine Synthase*

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Phosphatidylcholine is a major lipid of eukaryotic membranes, but found in only few prokaryotes. Enzymatic methylation of phosphatidylethanolamine by phospholipid N-methyltransferase was thought to be the only biosynthetic pathway to yield phosphatidylcholine in bacteria. However, mutants of the microsymbiotic soil bacterium Sinorhizobium (Rhizobium) meliloti, defective in phospholipid N-methyltransferase, form phosphatidylcholine in wild type amounts when choline is provided in the growth medium. Here we describe a second bacterial pathway for phosphatidylcholine biosynthesis involving the novel enzymatic activity, phosphatidylcholine synthase, that forms phosphatidylcholine directly from choline and CDP-diacylglycerol in cell-free extracts of S. meliloti. We further demonstrate that roots of host plants of S. meliloti exude choline and that the amounts of exuded choline are sufficient to allow for maximal phosphatidylcholine biosynthesis in S. meliloti via the novel pathway.

Eukaryotes can synthesize the membrane lipid phosphatidylcholine (PC) by two alternative biosynthetic pathways (1). In the CDP-choline pathway, choline is activated to choline phosphate and subsequently to CDP-choline, that condenses with diacylglycerol (DAG) to obtain PC. In the methylation pathway, however, phosphatidylethanolamine (PE) is provided in the growth medium. Here we describe a second bacterial pathway for phosphatidylcholine biosynthesis involving the novel enzymatic activity, phosphatidylcholine synthase, that forms phosphatidylcholine directly from choline and CDP-diacylglycerol in cell-free extracts of S. meliloti. We further demonstrate that roots of host plants of S. meliloti exude choline and that the amounts of exuded choline are sufficient to allow for maximal phosphatidylcholine biosynthesis in S. meliloti via the novel pathway.

Phosphatidylcholine synthase provides a second pathway for phosphatidylcholine biosynthesis and with the help of a novel enzyme activity, phosphatidylcholine synthase, S. meliloti can condense choline directly with CDP-diacylglycerol to form PC in one step. We also demonstrate that abundant choline is provided by host plants and can be used for PC biosynthesis by the guest bacterium S. meliloti.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—S. (Rhizobium) meliloti 1021 (wild type) and derived mutants KDR309 (pmt-deficient), KDR500 (betCBA-deficient), and KDR508 (pmt- and betCBA-deficient) were grown at 29 °C in tryptone/yeast extract (TY) medium (7) or in MOPS minimal medium containing 40 mM MOPS, 20 mM KOH, 20 mM NH4Cl, 100 mM NaCl, 2 mM MgSO4, 1.2 mM CaCl2, 0.3 mg biotin/l, 15 mM succinate, 10 mM potassium phosphate buffer, pH 7. Antibiotics were added when required to obtain the following final concentrations in mg/liter medium: 400 spectinomycin, 20 piperaclillin, 40 gentamycin, and 2 tetracycline for S. meliloti, and 200 spectinomycin, 10 gentamycin, and 20 tetracycline for Escherichia coli.

Construction of betCBA-deficient Strains—A plasmid (pHY109), which harbors an insertion of the spectinomycin resistance–confering ϕ interposon in the EcoRI restriction sites of the tetracycline-resistant IncP plasmid pCHO341, thereby replacing the betCBA genes (8), was conjugated in S. meliloti 1021 wild type and the pmt-deficient mutant KDR309 by triparental mating, respectively. The insertion was recombined into the wild type and mutant genomes by the plasmid-incompatibility technique as described (8). The betCBA-deficient phenotype of tetracycline-sensitive double recombinants was confirmed by their ability to use glycine betaine but not choline as sole carbon source. Recombinant betCBA-deficient strains derived from S. meliloti 1021 wild type and pmt-deficient mutant KDR309 were named KDR500 and KDR508, respectively.

Quantification of Lipids Synthesized in S. meliloti Strains—The lipid compositions of S. meliloti strains were determined following labeling with [1-14C]choline for 16 h as described previously (6). The strains were labeled in MOPS minimal medium cultures (1 ml) inoculated from fresh overnight cultures. TY components (2.5% tryptone, 1.5% yeast extract, w/v), choline (1 mM), or root exudate of Medicago sativa after 7 days incubation (75 μl of root exudate containing 14.7 μM choline to 1 ml of minimal medium) were added as sterile filtrates to the final concentrations indicated. The in vitro incorporation of choline into PC of S. meliloti was determined following labeling with betCBA, betCBA-deficient (C16) (55 μCi/mmole; Amersham Pharmacia Biotech). At a cell density of 2 × 108/ml in MOPS minimal medium, betCBA-deficient (C16) was added to a final concentration of 100 μM. Lipids were analyzed by one-dimensional thin layer chromatography (TLC) as described previously (6).

Determination of Specific Phosphatidylcholine Synthase Activity—The in vitro incorporation of radiolabeled choline into PC was determined in cell-free extracts of the betCBA- and pmt-deficient double mutant S. meliloti KDR508. The strain was inoculated in 400 ml of MOPS minimal medium from a fresh overnight culture and grown. At a cell density of 4 × 108/ml, cells were harvested by centrifugation, and cell-free extracts were prepared as described previously (6) in 100 mM Tris/HC1, pH 8. The initial reaction mixture to detect phosphatidylethanolamine synthase activity contained, in a total volume of 50 μl in Eppendorf tubes, 125 μg of protein, 50 mM Tris/HC1, pH 8, and 100 μM [methyl-14C](55 μCi/mmole). Final concentrations of additional compounds when applied were 10 mM MgCl2, 10 mM MnCl2, 345 μM CDP-

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1 The abbreviations used are: PC, phosphatidylcholine; DAG, diacylglycerol; Pmt, phospholipid N-methyltransferase; TLC, thin layer chromatography; PE, phosphatidylethanolamine; MMPE, monomethylphosphatidylethanolamine; DMPE, dimethylphosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; Pcs, phosphatidylcholinesynthase; Ps, phosphatidylserinesynthase; MOPS, 4-morpholinoacetate; Pe, phosphatidylethanolamine; MMPE, monomethylphosphatidylethanolamine; Bis, bisphenol A; TriTris, 2[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

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FIG. 1. Choline-dependent biosynthesis of phosphatidylcholine in S. meliloti. The strains S. meliloti 1021 (wild type) (lanes 1 and 3), KDR209 (pmt-deficient) (lanes 2, 4, 5, and 7), and KDR508 (pmt- and betCBA-deficient) (lanes 6 and 8) were cultivated in TY medium or in MOPS minimal medium with the additions indicated in the presence of [14C]acetate. Lipids were isolated, separated by TLC, and are indicated: PE, PG, CL, MMPE, DMPE, and PC.

FIG. 2. Time course of choline incorporation into phosphatidylcholine by S. meliloti strains KDR500 (betCBA-deficient) (○) and KDR508 (betCBA- and pmt-deficient) (●) during growth in MOPS minimal medium. Radiolabeled choline was added, and lipids were analyzed after different times.

DAG, 0.1% (w/v) Triton X-100, 340 μM PC, 340 μM DAG, 20 mM EDTA, 10 mM ATP, 5 mM CTP. Cell-free extracts of E. coli overexpressing the yeast enzymes choline kinase (9) and choline-phosphate cytidylyltransferase (10) were prepared in a similar way as the Sinorhizobium extracts and 45 μg protein of the choline kinase-containing extract or 63 μg of protein of the choline-phosphate cytidylyltransferase-containing extract were added in the cases indicated. The mixtures were incubated for 2 h in a 30 °C water bath. Samples of 10 μl were taken at different time points to confirm the linear character of choline incorporation. Lipids were analyzed by one-dimensional TLC as described previously (6). Incorporation of radiolabel into PC was determined by scraping the TLC material and by quantifying it with lipophilic scintillation fluid.

After performing kinetic studies with the sinorhizobial phosphatidylcholine synthase, it was realized that in the initial reaction mixture substrate inhibition by choline as well as by CDP-DAG occurred. Substrate inhibition can be observed at concentrations higher than 100 μM choline or higher than 75 μM CDP-DAG (data not shown). Therefore conditions for an optimized standard assay were established where no substrate inhibitions occur in order to allow quantification of phosphatidylcholine synthase activity. The optimized standard assay contained in a total volume of 50 μl in Eppendorf tubes was 50 μg of protein, 50 mM Tris/Cl, pH 8.0, 10 mM MnCl2, 20 μM CDP-DAG, 0.2% (w/v) Triton X-100, and usually 50 μM [methyl-14C]choline (55 mCi/mmol). The mixtures were incubated for 15 min in a 30 °C water bath and stopped by mixing with 188 μl of methanol/chloroform (2:1; v/v). Addition of 63 μl chloroform and 63 μl water led to phase separation, and after washing the chloroform phase once with another 100 μl of water, it was dried and quantified in a scintillation counter. Under such conditions the only radioactive compound detectable in the chloroform phase was PC. The linearity of the enzyme reaction during the incubation period was ensured.

Preparation of Radiolabeled CDP-diacylglycerol Substrates—[32P]CDP-1,2-diacyl-sn-glycerol was prepared using a two-step enzymatic conversion. First, diacylglycerol kinase was used to prepare [32P]CDP-diacylglycerol preparations were purified by TLC with chloroform/methanol/acetic acid/water (32:45:5:1; v/v) as a running solvent, and the radioactive compound with a RF value between 0.05 and 0.16 was extracted from the silica gel and had a specific radioactivity of 6.4 × 10^6 cpm/nmol.

Similarly, CDP-diacylglycerol that was labeled in its cytidine residue was prepared using [5-3H]CTP and the CDP-diglyceride synthase reaction (12). Before incorporation studies, [3P]CDP-diacylglycerol preparations were purified by TLC with chloroform/methanol/acetic acid/water (32:45:5:1; v/v) as a running solvent, and the radioactive compound with a RF value between 0.44 and 0.47 was extracted from the silica gel and had a specific radioactivity of 8.9 × 10^6 cpm/nmol.

Determination of CMP Formed—For the determination of CMP formed in sinorhizobial extracts, the watery phase of Bligh/Dyer extraction was separated in the same TLC system as [5-3H]CDP-diacylglycerol. Whereas CMP essentially did not migrate under such conditions (RF = 0.01–0.06), CMP chromatographed with an RF value between 0.12 and 0.17.

Determination of Choline in Plant Root Exudates—Seeds of Medicago officinalis, M. sativa, and Melilotus albus were surface-sterilized and germinated as described previously (13). For the production of root

TABLE I

Phosphatidylcholine synthase activity in S. meliloti extracts

| Added compound     | Choline incorporation (pmol/min mg protein) |
|--------------------|--------------------------------------------|
| Mg2+               | <0.01                                      |
| ATP, Mg2+, CKI     | <0.01                                      |
| CTP, Mg2+, CCT     | <0.01                                      |
| ATP, CTP, Mg2+, CKI, CCT | <0.01                               |
| DAG, Mg2+          | <0.01                                      |
| PC, Mg2+           | <0.01                                      |
| CDP-DAG, Mg2+, Triton X-100 | 4.76                           |
| CDP-DAG, Mg2+, Triton X-100, EDTA | <0.01                          |
| CDP-DAG, Mn2+, Triton X-100 | 294.00                         |

TABLE II

Effect of added substances on incorporation of radiolabeled choline into phosphatidylcholine by cell-free extracts of S. meliloti KDR508.

| Substrates | Incorporation into PE | Incorporation into PC | Ratio |
|-----------|-----------------------|-----------------------|-------|
| Choline   | pmol                  | pmol                  |       |
| 0         | 20                    | 181                   | 0     |
| 20        | 20                    | 126                   | 0     |
| 50        | 20                    | 97                    | 188   | 1.01 |
| 100       | 20                    | 65                    | 234   | 1.00 |
| 50        | 10                    | 46                    | 78    | 0.88 |
| 50        | 50                    | 124                   | 250   | 248  | 1.01 |

Effect of added substances on incorporation of radiolabeled choline into phosphatidylcholine by cell-free extracts of S. meliloti KDR508. The initial reaction mixture contained protein, choline, Tris/HCl buffer, pH 8, and various additional compounds in amounts indicated under “Experimental Procedures.” Abbreviations: CKI, choline kinase; CCT, cholinephosphate cytidylyltransferase.
exudates, 20 seedlings of the respective plant were grown in 10 ml of Jensen medium (14) as described previously (13). Small aliquots of exudates were taken at different time points and choline was determined quantitatively after conversion to choline phosphate using $[\gamma-32P]ATP$ and choline kinase (15).

RESULTS AND DISCUSSION

Choline-dependent PC Biosynthesis in S. meliloti—For the determination of lipid compositions, S. meliloti strains were grown on complex medium or on a MOPS minimal medium in the presence of radiolabeled acetate, and lipid extracts were subsequently separated by TLC. When a S. meliloti mutant (KDR309) with a defective methylation pathway of PC biosynthesis (6) is grown on complex medium containing tryptone and yeast extract, the mutant still forms PC in wild type amounts (Fig. 1, lane 2). The methylated intermediates of the methylation pathway, monomethylphosphatidylethanolamine (MMPE) and dimethylphosphatidylethanolamine (DMPE), are not formed in the mutant, and these results suggest the existence of a second, methylation-independent pathway for PC biosynthesis in S. meliloti. Cultivation of S. meliloti in defined minimal medium demonstrates that a similar lipid spectrum is formed by the wild type strain (Fig. 1, lane 3) as found after growth on complex medium (Fig. 1, lane 1). The nonresolved lipids phosphatidylglycerol (PG), cardiolipin (CL), and phosphatidylethanolamine (PE), the methylated lipids MMPE, DMPE, and PC are formed. The pmt-deficient mutant KDR309, however, forms neither MMPE, nor DMPE, nor PC under such growth conditions (Fig. 1, lane 4). Therefore one or several components of the complex medium must be required for the second pathway of PC biosynthesis in S. meliloti. The mutant strain was cultivated in defined minimal medium with different additional components in order to identify the substance(s) required for PC biosynthesis (data not shown). Only when choline was added to the minimal medium the Pmt-deficient mutant formed PC (Fig. 1, lane 5), eliminating the possibility that choline degradation was required for choline-dependent PC formation.

![Figure 3](http://www.jbc.org/) Effects of pH, manganese or magnesium, and Triton X-100 on Pcs activity. A, phosphatidycholine synthase was measured at the indicated pH values with 50 mM Tris/HCl (○) or BisTris/HCl (●). B, phosphatidycholine synthase was assayed with the indicated concentrations of MnCl$_2$ (●) or MgCl$_2$ (○). C, phosphatidycholine synthase was assayed with 20 μM CDP-DAG and the indicated concentrations of Triton X-100. Otherwise, conditions of the standard assay were used, and activity was measured by following the incorporation of $[^{14}C]$choline into chloroform-soluble product as described in the text.

### TABLE III

Choline-dependent release of CMP from CDP-DAG in sinorhizobial cell-free extracts

| Substrates | Extract from S. meliloti | CMP release from CDP-DAG |
|------------|-------------------------|-------------------------|
| Choline    | μM | CDP-DAG |
| 0          | +  | 359      |
| 20         | +  | 396      |
| 50         | +  | 412      |
| 100        | +  | 424      |
| 50         | 10 | +        | 282 |
| 50         | 50 | +        | 623 |
| 50         | 20 | –        | 72  |

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Choline is taken up efficiently by S. meliloti (16) and can be utilized as sole carbon and nitrogen source (17). The proposed pathway of choline degradation (17) is initiated by two subsequent oxidation steps catalyzed by choline dehydrogenase (BetB) and glycine betaine aldehyde dehydrogenase (BetA) leading to the formation of glycine betaine, which can be metabolized further (16). During growth at high osmolarity glycine betaine accumulates in S. meliloti and functions, like in other organisms, as an osmoprotectant or compatible solute. In order to eliminate the possibility that choline degradation was required for choline incorporation in PC, we constructed strains that were unable to degrade choline. The S. meliloti double mutant KDR508, deficient in Pmt and choline oxidation (data not shown), was still able to synthesize PC when choline was added to the minimal growth medium (Fig. 1, lane 6), eliminating the possibility that choline degradation was required for choline-dependent PC formation.
Choline Is a Biosynthetic Precursor for Methylation-independent PC Biosynthesis in S. meliloti—In order to search for intermediates of the choline-dependent pathway of PC biosynthesis, we labeled S. meliloti strains with radioactive choline and analyzed aliquots of the cell suspensions after various time points. Specifically, we searched for intermediates of the CDP-choline pathway (choline phosphate and CDP-choline), but we were unable to detect either compound in aqueous phases of Bligh/Dyer (18) extracts (data not shown). Rather we observed a rapid incorporation of radiolabeled choline into the lipid-containing chloroform phases of Bligh/Dyer extracts and nearly exclusively in PC, as previously found by Sherr and Law (19) for the close relative Agrobacterium tumefaciens. In a S. meliloti strain unable to oxidize choline (KDR500), the time course of choline incorporation into PC (Fig. 2) is rapid (within minutes), nearly linear, and no delay in incorporation is observed, suggesting that the incorporation might not proceed via any biosynthetic intermediate but might occur in one step. Incorporation of choline into PC of the double mutant KDR508, which cannot oxidize choline and which is unable to form PC via the methylation pathway, occurs twice as fast as in KDR500 (Fig. 2), suggesting that the choline-dependent pathway of PC biosynthesis is working more actively in KDR508 and can compensate for the lack of PC biosynthesis by the methylation pathway.

Phosphatidylcholine Synthase, a Novel Phosphatidyltransferase in Cell-free Extracts of S. meliloti—Enzyme activities of the CDP-choline pathway of PC biosynthesis (choline kinase, choline-phosphate cytidylyltransferase) could not be detected in cell-free extracts of S. meliloti KDR508 (data not shown). Even when we tried to stimulate such a potential pathway by adding cell-free extracts from E. coli that contained overexpressed choline kinase (9) or choline-phosphate cytidylyltransferase (10) from yeast, the incorporation of radioactive choline into PC was not stimulated (Table I), although radiolabeled choline phosphate and CDP-choline were produced in large amounts in the reaction mixture (data not shown). We therefore concluded that the CDP-choline pathway was presumably not the second, choline-dependent pathway for PC biosynthesis in S. meliloti.

Other pathways for PC biosynthesis involving acylation of lyso-PC or base exchange are known for plants or animals (20, 21) but not in bacteria. Several potential substrates, involved in the reactions of these pathways, were also tested for in vitro activation of choline incorporation into PC by cell-free extracts of S. meliloti. Neither DAG nor PC had any stimulating effect (Table I). However, addition of CDP-DAG showed a strong stimulating effect, especially when the detergent Triton X-100 was present in concentrations (0.1%; w/v) where micelles are formed. The incorporation of radioactive choline into PC was inhibited by addition of the metal ion chelator EDTA, indicating a requirement for bivalent cations (Table I). Of the different bivalent cations tested (data not shown), Mn$^{2+}$ showed the strongest stimulation of radioactive choline incorporation into phosphatidylcholine by cell-free extracts of S. meliloti (Table I).

Stoichiometry of the Phosphatidylcholine Synthase Reaction—In order to demonstrate that CDP-DAG is the second substrate of this novel enzymatic reaction, the incorporation of the phosphatidyl moiety of $^{32}$P-labeled CDP-DAG into PC was studied (Table II). Without addition of choline no incorporation of phosphatidate into PC is observed. Phosphatidate incorporation into PC is strictly dependent on choline, and during PC formation equimolar amounts of choline and phosphatidate are incorporated into PC. A relatively high incorporation of phosphatidate into PE (181 pmol) can be observed without any amino alcohol added. Surprisingly, addition of choline reduced the amount of phosphatidate incorporation into PE. The reduced incorporation (124 pmol) at high CDP-DAG concentrations (50 $\mu$M) demonstrates that this effect is not due to a lack of substrates but must result from an inhibition of PE formation by the choline added. The mechanism of this down-regulation of PE formation by choline and therefore of the precursor of the methylation pathway of PC biosynthesis is presently under investigation.

In order to show that CMP is the second product of this novel enzymatic reaction, the release of CMP from CDP-DAG that was labeled in its cytidine residue was studied (Table III). Sinorhizobial extracts caused a surprisingly large release (359 pmol) of CMP from CDP-DAG without any amino alcohol added. This release must have been due to phosphatidylserine synthase (Pss) during PE formation and other, uncharacterized CMP-releasing activities. Addition of choline or CDP-DAG increased the amount of CMP released by sinorhizobial extracts. However, the increased amounts of CMP released are significantly less than what is expected if CMP would be released at an equimolar rate as choline or CDP-DAG is incorporated. As we demonstrated above (Table II), however, other CMP-releasing activities, i.e. the Pss-dependent CMP release during the formation of PE, are much reduced when choline is added to the conversion assay, and therefore an increased release of CMP by
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Fig. 5. PC biosynthesis in *S. meliloti*. Abbreviations: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Psd, phosphatidylserine decarboxylase.

The Pcs reaction is partially down-compensated by a reduced release of CMP by the Pss reaction. Therefore a stoichiometric release of CMP during the Pcs reaction cannot be shown in cell-free extracts of *S. meliloti*.

**Enzymological Properties of Phosphatidylcholine Synthase**—Pcs activity was measured at pH 6.1–7.2 using BisTris/HCl buffer and at pH 7.3–8.8 using Tris/HCl buffer (Fig. 3A). The optimal pH for Pcs in sinorhizobial cell-free extracts was 8.0, and activity decreased as pH was raised or lowered. As shown above the Pcs activity is strictly dependent on the presence of bivalent cations (Table I). The activity increased with higher manganese concentrations. Stimulation of the Pcs activity by manganese, (Fig. 3B). The activity increased with higher magnesium concentrations. The stimulation of the Pcs activity by manganese, however, was much more efficient (more than 20-fold higher) than by magnesium, and 10 mM Mn²⁺ was found to be optimal for Pcs activity. The mild, nonionic detergent Triton X-100 stimulated Pcs activity in the initial assay (Table I). The effect of the Triton X-100 concentration on the standard Pcs assay was studied (Fig. 3C), demonstrating that under these conditions there was a strong Triton X-100 dependence of the Pcs activity with a maximal activity at 3.2 mM (0.2% w/v) Triton X-100. At higher concentrations, Triton X-100 quickly became inhibitory, indicating that substrate dilution kinetics was followed (22). For analysis of Pcs kinetics with respect to CDP-DAG, a mixed micelle system with Triton X-100 was used (Fig. 4). Pcs activity was measured as a function of the surface concentration of CDP-DAG in the micelle. The bulk CDP-DAG concentration was held constant at 20 µM. The molar ratio of CDP-DAG in the micelle was varied by changing the concentration of Triton X-100. At higher concentrations of Triton X-100 (>100 µM) the kinetic curves were sigmoidal, which could be indicative of a positive cooperative effect of CDP-DAG or more likely of substrate inhibition by high concentrations of choline.

The phosphatidyltransferases phosphatidylserine synthase (EC 2.7.8.8) (23) and phosphatidylglycerolphosphate synthase (EC 2.7.8.5) (24) are involved in the biosynthesis of bacterial membrane phospholipids (25, 26). In these reactions the cytidine 5′-monophosphate part of CDP-DAG is replaced by an alcohol like serine or glycerol phosphate. Surprisingly, a phosphatidyltransferase introducing choline as the alcohol was unknown to date, and this “missing link” of phospholipid biochemistry is described here for the first time. In analogy to the known bacterial phosphatidyltransferases, we named this novel enzymatic activity phosphatidylcholine synthase (Pcs). Fig. 5 shows the postulated biosynthetic pathways for PC in *S. meliloti*.

Legume Host Plants Exude Choline That Can Be Used for Rhizobial PC Biosynthesis—The novel Pcs activity enables *S. meliloti* to produce PC from exogenously provided choline, thereby avoiding the energy-demanding, S-adenosyl-l-methionine-consuming methylation steps of PE during the methylation pathway of PC biosynthesis. However, the potential source of such exogenous choline was unclear. Although free choline and choline derivatives are present in plant saps (27) and cells due to the action of specific phospholipases on plant lipids (28), there were no indications to which extent such plant-derived choline might be available for *S. meliloti*. Therefore we studied whether roots of host plants of *S. meliloti* were able to exude choline into their rhizosphere. All three host plants tested (*M. officinalis, M. sativa*, and *M. alba*) exuded 0.5–1 nmol of choline/day/ plant, leading to choline concentrations in the test medium of about 6 µM after 4 days and reaching maximal concentrations (15–30 µM) after 7–18 days. When root exudates were added to Pmt-deficient *S. meliloti* mutants grown in minimal medium without choline, they enabled the mutants to produce PC in wild-type amounts (Fig. 1, lanes 7 and 8). These results suggest that legume host plants exude choline to their rhizosphere where it can be used by the guest bacterium *S. meliloti* as a building block by Pcs to produce PC for the rhizobial membrane.

A surprisingly large number of bacteria, involved in symbiotic or pathogenic interactions with plant or animal hosts, contain PC in their membranes (i.e. *Brucella* (29), *Borrelia* (30), *Treponema* (30)). The presence of abundant choline supplies by the plant or animal (31) hosts makes the biosynthesis of PC by the novel phosphatidylcholine synthase pathway an energetically more favorable alternative for such bacteria.

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