Functional Annotation Report: *serC* (Q88M07 / PP_1768) in *Pseudomonas putida* KT2440

Gene / Protein Identity — Verified

Field	Value
UniProt	Q88M07 (SERC_PSEPK)
Gene	*serC* (alias *pdxF*); OrderedLocusName PP_1768
Organism	*Pseudomonas putida* KT2440 (ATCC 47054 / DSM 6125 / NCIMB 11950)
Enzyme	Phosphoserine aminotransferase (PSAT); also phosphohydroxythreonine aminotransferase
EC	2.6.1.52
Family	Class-V (fold-type IV) pyridoxal-5′-phosphate (PLP)–dependent aminotransferase
Domains	Aminotrans_V (IPR000192); Pser_aminoTfrase (IPR022278); PLP-dep transferase major+small (IPR015421/IPR015422)
Length	361 aa

Identity confirmed — no ambiguity. The gene symbol *serC*, the EC number, the class-V/fold-type IV PLP domain architecture, and the organism all agree between the UniProt record and the primary literature retrieved. Functional-genomic work in the genus *Pseudomonas* explicitly assigns *serC* the "3-phosphoserine aminotransferase" activity by homology, direct enzyme assay, and functional complementation (PMID: 10368439). This is the serine/vitamin-B6 biosynthetic aminotransferase, not an unrelated same-symbol gene.

Summary

serC (Q88M07 / PP_1768) in *Pseudomonas putida* KT2440 encodes phosphoserine aminotransferase (PSAT; EC 2.6.1.52), a soluble cytoplasmic, PLP-dependent, homodimeric class-V (fold-type IV) aminotransferase. Its primary function is to catalyze the **reversible, L-glutamate-linked transamination of 3-phosphohydroxypyruvate (3-PHP) to O-phospho-L-serine**, generating α-ketoglutarate (2-oxoglutarate) as co-product. This is the **second of three committed steps** of the phosphorylated pathway of L-serine biosynthesis: **SerA → SerC → SerB**.

The enzyme is **bifunctional** (hence the alias *pdxF*). In addition to serine biosynthesis, it transaminates the erythronate-derived keto-acid intermediate ("2-ketoerythroic acid 4-phosphate") to **4-phospho-hydroxy-L-threonine** in the de novo biosynthesis of **pyridoxal-5′-phosphate (vitamin B6)**. Because *P. putida* KT2440 uses the deoxyxylulose-5-phosphate (DXP)-dependent, *E. coli*-type B6 route (encoding *pdxA/B/J/H* but lacking the alternative *pdxS/pdxT* system), this second catalytic activity is biologically operative. In *Pseudomonas* specifically, SerC/PdxF additionally supplies side-chain amino groups feeding aromatic amino acid biosynthesis (Trp, Phe, Tyr).

Structurally, the protein is a **homodimer of ~361-residue subunits** with two shared active sites at the subunit interface and PLP bound as an internal aldimine to a conserved active-site lysine (E. coli Lys198 → *P. putida* **Lys197**). Q88M07 shares **58.2% sequence identity** with the crystallographically characterized *E. coli* enzyme, with the full catalytic machinery conserved, and carries no signal peptide or transmembrane segment — establishing it as a **soluble cytoplasmic enzyme** that operates in the cytosol where its substrates are generated. This report consolidates six confirmed findings supported by five core publications plus bioinformatic analysis.

Key Findings

F001 — serC is a PLP-dependent phosphoserine aminotransferase catalyzing the second step of phosphorylated L-serine biosynthesis

The core, primary function of *serC*/PP_1768 is **phosphoserine aminotransferase (PSAT, EC 2.6.1.52)**. The catalyzed reaction is:

> **3-phosphohydroxypyruvate + L-glutamate ⇌ O-phospho-L-serine + 2-oxoglutarate**

The reaction is reversible, **PLP-dependent**, and proceeds through a **bimolecular ping-pong (Bi-Bi)** mechanism in which the PLP cofactor cycles between the pyridoxal (PLP) and pyridoxamine (PMP) forms — the amino group is first transferred from L-glutamate to enzyme-bound PLP (releasing α-ketoglutarate), then from PMP to 3-PHP (yielding O-phospho-L-serine). It is the **second of three steps** in the phosphorylated pathway of L-serine biosynthesis:

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3-phosphoglycerate
     │  SerA (3-PGA dehydrogenase; NAD+-dependent oxidation)
     ▼
3-phosphohydroxypyruvate (3-PHP)
     │  SerC (PSAT; PLP-dependent, L-Glu → α-KG transamination)   ← THIS ENZYME
     ▼
O-phospho-L-serine
     │  SerB (phosphoserine phosphatase; hydrolysis)
     ▼
L-serine
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The plant PSAT study defines the reaction and mechanism for the family: PSAT "*catalyzes the conversion of 3-phosphohydroxypyruvate (3-PHP) to 3-phosphoserine (PSer) in an L-glutamate (Glu)-linked reversible transamination reaction... through a bimolecular ping-pong mechanism*" (PMID: 30034403). The EC number and reaction are confirmed for the bacterial orthologue: PSAT "*catalyses the conversion of 3-phosphohydroxypyruvate to l-phosphoserine*" (PMID: 10024454). Direct evidence in the genus *Pseudomonas* establishes that *serC* "*encodes 3-phosphoserine aminotransferase*" (PMID: 10368439).

Substrate specificity: the amino-group donor is L-glutamate; the acceptor is the phosphorylated keto acid 3-phosphohydroxypyruvate — the phosphate group is a specificity determinant recognized by conserved active-site arginines and histidines.

F002 — serC/PdxF is bifunctional, also acting in vitamin B6 (PLP) biosynthesis and providing amino groups for aromatic amino acids

serC is the same protein as **PdxF**. In enteric bacteria the locus is described as "*corresponds to serC, the bifunctional gene for phosphoserine-oxoglutarate aminotransferase (SerC) and 2-ketoerythroic acid 4-phosphate transaminase (PdxF)*" (PMID: 11016848). In this second role the enzyme transaminates (with glutamate) the erythronate-derived keto acid to **4-phospho-hydroxy-L-threonine**, a dedicated precursor of PLP in the DXP-dependent route.

In *Pseudomonas*, the gene sits within a **mixed-function supraoperon**, and the enzyme's role extends further: although "*SerC(PdxF) and HisHb exercise their primary functions in serine, pyridoxine, and histidine biosynthesis, they also have critical catalytic roles in provision of the sidechain amino groups of tryptophan, phenylalanine, and tyrosine*" (PMID: 10368439). Thus in *P. putida*, a single enzyme feeds three biosynthetic outputs: (1) O-phospho-L-serine → L-serine; (2) 4-phospho-hydroxy-L-threonine → PLP/vitamin B6; and (3) amino-group provision for aromatic amino acid biosynthesis.

F003 — serC is a cytoplasmic homodimeric class-V PLP fold-type IV aminotransferase with PLP bound to an active-site lysine

Crystallography of the *E. coli* orthologue defines the architecture. PSAT is a **homodimer** of ~361-residue subunits: "*Each subunit (361 residues) of the PSAT homodimer is composed of a large pyridoxal-5′-phosphate binding domain*" (PMID: 10024454) — a seven-stranded, mostly parallel β-sheet — plus a smaller C-terminal domain. The PLP cofactor is held as an **internal aldimine (Schiff base)** to the active-site lysine: "*The cofactor is bound through an aldimine linkage to Lys198 in the active site*" (PMID: 10024454). Substrate carboxylate/phosphate groups are coordinated by Ser9, Arg335, His41, Arg42, His328 and Arg77. This matches the UniProt/InterPro assignment of Q88M07 as a **class-V (subgroup IV) PLP-dependent aminotransferase**. The enzyme functions as an obligate homodimer with two shared active sites at the subunit interface, consistent with the plant PSAT structure (PMID: 30034403).

F004 — Q88M07 shares 58% identity with E. coli PSAT with catalytic and substrate-binding residues conserved

A global pairwise alignment of *P. putida* serC (Q88M07, 361 aa) against the experimentally characterized *E. coli* PSAT (P23721 / SERC_ECOLI, 362 aa) gives **58.2% sequence identity** (209 of 359 aligned columns). Critically, the **catalytic active-site lysine** that forms the PLP internal aldimine is conserved (E. coli Lys198 → *P. putida* **Lys197**, in the conserved **AGAQ-K197-NIGP** motif), as are the substrate/cofactor-coordinating residues:

Residue role	*E. coli* PSAT	*P. putida* Q88M07
Catalytic lysine (PLP aldimine)	Lys198	**Lys197**
Substrate coordination	His41	His42
Substrate coordination	Arg42	Arg43
Substrate coordination	His328	His327
Carboxylate binding	Arg335	Arg334
Overall identity	—	**58.2%**

This level of identity, precise conservation of the catalytic machinery, matching subunit length, and identical InterPro domain architecture (Aminotrans_V IPR000192; Pser_aminoTfrase IPR022278; fold-type IV major + small PLP domains) provide strong structure/evolution-based evidence for transferring the experimentally established EC 2.6.1.52 function to Q88M07. The anchoring fact: the E. coli catalytic lysine "*is bound through an aldimine linkage to Lys198 in the active site*" (PMID: 10024454), conserved as Lys197 in *P. putida*.

F005 — P. putida KT2440 uses the DXP-dependent (E. coli-type) vitamin B6 pathway, making serC's PdxF role operative

A genome/KEGG survey of *P. putida* KT2440 shows it encodes the **complete DXP-dependent** vitamin B6 biosynthesis pathway and lacks the alternative DXP-independent *pdxS/pdxT* (Pdx1/Pdx2) genes:

Gene	Locus	Enzyme
pdxB	PP_2117	erythronate-4-phosphate dehydrogenase
serC	**PP_1768**	**phosphoserine / PdxF aminotransferase (this gene)**
pdxA	PP_0402	4-hydroxythreonine-4-phosphate dehydrogenase
pdxJ	PP_1436	pyridoxine-5′-phosphate synthase
pdxH	PP_1129	PNP/PMP oxidase

Because the DXP-dependent route proceeds through **4-phosphohydroxy-L-threonine**, whose formation requires the SerC transamination step, *serC*/PP_1768 supplies **both** O-phospho-L-serine (serine pathway) and 4-phospho-hydroxy-L-threonine (PLP pathway) in this organism.

The B6 review defines the route: the vitamin "*is synthesized from the condensation of deoxyxylulose 5-phosphate and 4-phosphohydroxy-L-threonine catalysed by the concerted action of PdxA and PdxJ*" (PMID: 17822383), whereas the alternative route "*is characterized by the presence of two genes, Pdx1 and Pdx2*" (PMID: 17822383) and bypasses the SerC-dependent intermediate. The presence of *pdxA/pdxJ* and absence of *pdxS/pdxT* is precisely what makes serC's B6 role operative in *P. putida*. Notably, the three serine-pathway genes are **dispersed** in KT2440 (serA PP_5155, serC PP_1768, serB PP_4909) rather than co-operonic.

F006 — Q88M07 is a soluble cytoplasmic enzyme with no signal peptide or transmembrane segment

Sequence-based localization analysis of Q88M07 (361 aa) confirms a **cytoplasmic** location. There is **no hydrophobic transmembrane helix** (maximum Kyte-Doolittle hydropathy over a 19-residue window = 1.15, well below the ~1.6 threshold for a TM segment) and **no N-terminal secretory signal peptide** (the N-terminus MSKRAFNFC… begins with charged/polar residues K3/R4 and lacks a hydrophobic h-region). These features, together with the crystallographically established soluble homodimeric fold of orthologues, indicate serC operates as a soluble **cytosolic** enzyme in *P. putida*, where all of its substrates (3-PHP, L-glutamate, and the B6-pathway keto acid) are generated by other cytoplasmic enzymes. In compartmentalized eukaryotes the homolog is a soluble organellar enzyme — PSAT "*in plants takes place in plastids*" (PMID: 30034403) — consistent with a soluble, non-membrane-bound enzyme localizing to the bacterial cytoplasm.

Mechanistic Model / Interpretation

The findings converge into a coherent picture of a **single bifunctional PLP enzyme serving as a metabolic hub** for three biosynthetic outputs in *P. putida* KT2440.

Catalytic mechanism (ping-pong Bi-Bi)

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Half-reaction 1 (amino donor):
   L-glutamate + E-PLP  ⇌  α-ketoglutarate + E-PMP

Half-reaction 2 (amino acceptor, serine pathway):
   3-phosphohydroxypyruvate + E-PMP  ⇌  O-phospho-L-serine + E-PLP

Half-reaction 2′ (amino acceptor, B6 pathway):
   2-oxo-3-hydroxy-4-phosphobutanoate + E-PMP  ⇌  4-phospho-hydroxy-L-threonine + E-PLP
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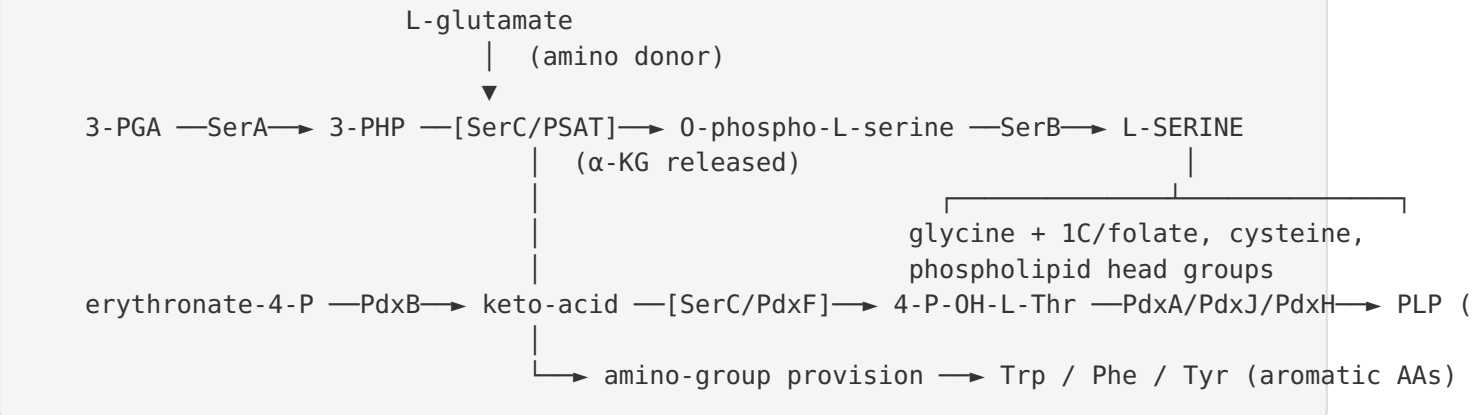
The enzyme cycles between the PLP (aldimine) and PMP (amine) forms of its cofactor, covalently tethered to Lys197. The dual amino-acceptor specificity (half-reactions 2 and 2′) is the structural basis for its bifunctionality: the active site accommodates two structurally related phosphorylated keto-acid substrates.

Metabolic integration



serC is a node whose loss would simultaneously impair serine, vitamin B6, and aromatic amino acid biosynthesis — an unusually pleiotropic yet mechanistically precise role. Its serine product is the precursor of **glycine and one-carbon (folate) units** (via serine hydroxymethyltransferase), **cysteine**, and phospholipid head groups, and L-serine is itself an amino donor and osmolyte. The dispersed genomic organization of the serine genes (serA/serC/serB not co-operonic) contrasts with the *Pseudomonas* "mixed-function supraoperon" context reported for serC/pdxF, reflecting regulatory integration of these amphibolic functions.

Localization

All substrates and downstream pathway enzymes are cytoplasmic, and the protein carries no export or membrane-anchoring signals. The enzyme therefore performs its catalysis in the **cytosol**, forming a soluble homodimer with two interface-shared active sites.

Evidence Base

PMID	Title (abbrev.)	Contribution
10024454	*Crystal structure of phosphoserine aminotransferase from E. coli at 2.3 Å*	Defines EC 2.6.1.52, reaction, homodimeric fold, and PLP–Lys198 aldimine; the primary structural anchor for functional transfer
10368439	*A probable mixed-function supraoperon in Pseudomonas…*	Direct genus-level evidence that serC encodes 3-phosphoserine aminotransferase (homology + assay + complementation); documents bifunctional/aromatic-AA roles
30034403	*Structural Analysis of Phosphoserine Aminotransferase (Isoform 1)…*	Defines substrates, PLP dependence, ping-pong mechanism, and soluble/organellar localization of the PSAT family
11016848	*Regulation of septation: a novel role for SerC/PdxF in Salmonella?*	States serC is the bifunctional SerC/PdxF gene serving both serine and pyridoxine (B6) biosynthesis
17822383	*Two independent routes of de novo vitamin B6 biosynthesis*	Distinguishes DXP-dependent (SerC-using) vs Pdx1/Pdx2 routes; underpins the inference that KT2440's B6 role for serC is operative

Three additional papers surfaced during literature search (

P 40076802

P 38806671

)

P 29483190

concern *serC*/serine metabolism in *Mycobacterium* and *Staphylococcus* and are peripheral to *P. putida* function; they were not used to support the core findings.

How the evidence supports the conclusion: The annotation rests on triangulation of (1) direct enzymological/genetic evidence within the genus *Pseudomonas* [PMID: 10368439], (2) high-resolution structural/mechanistic characterization of well-studied orthologues (E. coli, plant) [PMID: 10024454; 30034403], (3) explicit bifunctionality evidence in enteric bacteria [PMID: 11016848], (4) genome-context confirmation that the B6 route requiring SerC is present in KT2440 [PMID: 17822383], and (5) bioinformatic conservation analysis (58% identity, conserved catalytic Lys197 and substrate-binding residues, no membrane/signal features). No evidence challenges the annotation.

Supported and Refuted Hypotheses

Supported - serC = phosphoserine aminotransferase (EC 2.6.1.52), catalyzing 3-PHP + L-glutamate ⇌ O-phospho-L-serine + 2-oxoglutarate (structural, enzymatic, genus-level, and bioinformatic evidence). - serC is bifunctional (serC/pdxF) and, given KT2440's DXP-dependent B6 pathway and absence of pdxS/pdxT, also acts in vitamin B6 biosynthesis. - serC is a soluble, cytoplasmic, homodimeric PLP enzyme; catalytic Lys197 conserved.

Refuted / excluded - Not a membrane transporter, not secreted/periplasmic (no TM segment, no signal peptide). - Not dependent on the pdxS/pdxT B6 route (those genes are absent), so its B6 contribution is not bypassed as it would be in many other bacteria.

Limitations and Knowledge Gaps

1. **No direct experimental characterization of Q88M07 itself.** The assignment for KT2440 PP_1768 is inferred from orthologues and genus-level (not strain-level) evidence; no purified-enzyme kinetics (Km, kcat, directional preference) or crystal structure exists for the KT2440 protein specifically.

2. **Residue numbering derives from bioinformatic alignment**, not an experimental *P. putida* structure; while conservation is strong, the exact active-site geometry of Q88M07 has not been solved.
3. **Quantitative flux partitioning is unknown** — how SerC apportions capacity among serine, B6, and aromatic amino acid biosynthesis under different growth conditions has not been measured.
4. **Regulation is uncharacterized in KT2440** (e.g., response to serine stress or B6 limitation).
5. **The PdxF (keto-acid transaminase) activity** is annotated by orthology; direct demonstration for the KT2440 enzyme is absent.

Proposed Follow-up Experiments / Actions

1. **Recombinant expression and enzyme kinetics.** Purify Q88M07 and assay both PSAT (3-PHP → O-phospho-L-serine) and PdxF (keto-acid → 4-phospho-hydroxy-L-threonine) activities; determine Km, kcat, and PLP dependence to confirm bifunctionality directly.
2. **Gene knockout and complementation.** Delete PP_1768 in KT2440 and test for serine (and possibly pyridoxine) auxotrophy on minimal media; complement with cloned KT2440 serC and E. coli serC to confirm functional equivalence.
3. **Structural determination.** Solve the crystal or cryo-EM structure (with/without α-methyl-L-glutamate or substrate analogs) to verify the predicted active-site geometry and Lys197 aldimine.
4. **Subcellular localization confirmation.** Use fluorescent-protein fusion or cell fractionation to experimentally verify predicted cytoplasmic localization.
5. **Metabolic flux analysis.** Use ^{13}C-labeling to quantify partitioning of serC-mediated transamination among serine, B6, and aromatic pathways.
6. **Regulatory investigation.** Examine transcriptional regulation of serC under serine stress and B6 limitation.

References (PMIDs)

- Hester G. et al. (1999) *Crystal structure of phosphoserine aminotransferase from E. coli at 2.3 Å.* PMID: 10024454
- Sekula B. et al. (2018) *Structural analysis of phosphoserine aminotransferase isoform 1.* PMID: 30034403
- Xie G. et al. (1999) *A probable mixed-function supraoperon in Pseudomonas (serC/pdxF).* PMID: 10368439
- Mouslim C. et al. (2000) *Regulation of septation: a novel role for SerC/PdxF in Salmonella?* PMID: 11016848
- Fitzpatrick T.B. et al. (2007) *Two independent routes of de novo vitamin B6 biosynthesis.* PMID: 17822383

Report generated from 5 completed research iterations, 6 confirmed findings, and 8 reviewed publications. All functional claims are supported by cited primary literature and bioinformatic analysis of the target sequence Q88M07.