Functional Annotation Report: *tal* (PP_2168, UniProt Q88KX1) — Transaldolase of *Pseudomonas putida* KT2440

0. Gene/Protein Identity Verification

The target is **unambiguous**. The UniProt record (Q88KX1) describes: - **Protein:** Transaldolase (RecName), **EC 2.2.1.2** - **Gene:** *tal*; ordered locus **PP_2168** - **Organism:** *Pseudomonas putida* strain KT2440 (ATCC 47054 / DSM 6125) - **Family/domains:** Transaldolase family, **Type 1 subfamily**; Aldolase_TIM (IPR013785), TAL/FSA (IPR001585), Transaldolase_1 (IPR004730), Transaldolase active-site signature (IPR018225), Pfam **PF00923** (TAL_FSA).

Every one of these annotations is internally consistent: the gene symbol *tal*, the EC number 2.2.1.2, the Pfam/InterPro transaldolase signatures, and the "Type 1 subfamily" classification all point to the same well-characterized enzyme, transaldolase. There is **no ambiguity** and no conflicting gene using the same symbol. Research therefore proceeds directly on transaldolase.

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1. Summary (Answer to the Research Question)

tal / PP_2168 encodes transaldolase (EC 2.2.1.2), a soluble cytoplasmic enzyme of the non-oxidative branch of the pentose phosphate pathway. Its primary function is to catalyze the reversible transfer of a three-carbon **dihydroxyacetone (C3) unit** from a ketose-phosphate donor (sedoheptulose-7-phosphate or fructose-6-phosphate) to an aldose-phosphate acceptor (glyceraldehyde-3-phosphate or erythrose-4-phosphate). The signature reaction is **sedoheptulose-7-P + glyceraldehyde-3-P ⇌ erythrose-4-P + fructose-6-P**. It is a cofactor-less class I aldolase that acts through a covalent Schiff-base intermediate on a conserved active-site lysine. In *P. putida* KT2440 — a bacterium that runs glycolysis almost entirely through the

Entner–Doudoroff route — transaldolase supplies the sugar-phosphate interconversion capacity that allows operation of the cyclic **EDEMP** metabolic architecture, balancing carbon between catabolism and biosynthesis and contributing to NADPH supply.

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2. Primary Function: Reaction Catalyzed and Substrate Specificity

Transaldolase (TAL) is a near-ubiquitous enzyme of central carbon metabolism that **"transfers a dihydroxyacetone group from donor compounds (fructose 6-phosphate or sedoheptulose 7-phosphate) to aldehyde acceptor compounds"** (Samland & Sprenger, 2009,).

P 19401148

- **Group transferred:** a C3 dihydroxyacetone moiety (from the ketose donor's C1–C3).
- **Donors (ketoses):** sedoheptulose-7-phosphate (S7P), fructose-6-phosphate (F6P).
- **Acceptors (aldoses):** glyceraldehyde-3-phosphate (G3P), erythrose-4-phosphate (E4P).
- **Canonical net reaction (EC 2.2.1.2; Rhea RHEA:17053):** `D-sedoheptulose-7-P + D-glyceraldehyde-3-P ⇌ D-erythrose-4-P + β-D-fructose-6-P`
- **Reversibility:** the reaction is freely reversible; direction is set by mass action / cellular metabolic demand.

The UniProt/HAMAP curated function statement for Q88KX1 (Rule MF_00492) matches exactly: *"Transaldolase involved in the non-oxidative phase in the pentose phosphate pathway. Catalyzes the reversible conversion of sedoheptulose-7-phosphate and D-glyceraldehyde 3-phosphate into erythrose-4-phosphate and beta-D-fructose 6-phosphate. Transaldolase is important for the balance of metabolites in the pentose-phosphate pathway."* It is assigned to the non-oxidative PPP as **step 2 of 3** (generating G3P + F6P from ribose-5-P and xylulose-5-P).

Together with transketolase (which transfers C2 glycolaldehyde units), transaldolase performs the reversible carbon-shuffling reactions that interconvert 3-, 4-, 5-, 6-, and 7-carbon sugar phosphates in the **non-oxidative pentose phosphate pathway (PPP)** (Samland & Sprenger, 2009). This lets the cell (i) route pentose phosphates back into glycolytic hexose/triose phosphates and (ii) generate erythrose-4-phosphate (aromatic amino acid / vitamin precursor) and ribose-5-phosphate (nucleotide precursor) from glycolytic intermediates.

The Q88KX1 assignment to the **Type 1 transaldolase subfamily** is meaningful: within the transaldolase superfamily, five subfamilies are distinguished — three with proven TAL activity, one of unclear function, and a fifth comprising the related **fructose-6-phosphate aldolases** (Samland & Sprenger, 2009). Type 1 corresponds to the classical, catalytically confirmed transaldolases (the *E. coli* TalB type), so the substrate specificity above applies directly.

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3. Catalytic Mechanism and Structural Basis

Transaldolase is a **class I aldolase**: it is **cofactor-less** (no metal ion, no thiamine/PLP) and **"proceeds with a Schiff base intermediate (bound dihydroxyacetone)"** (Samland & Sprenger, 2009,).

P 19401148

Direct structural evidence comes from the *E. coli* transaldolase B (TalB) reduced-intermediate crystal structure (Jia et al., 1997,):

P 9007983

- The **dihydroxyacetone moiety is covalently linked to the ε-amino group of Lys132** at the active site — the trapped Schiff-base intermediate. - Surrounding residues position the substrate: the C1 hydroxyl H-bonds to Asn154 and Ser176; the C3 hydroxyl interacts with Asp17 and Asn35. - A reaction mechanism for the whole class I aldolase family was deduced from this complex.

Mechanistically: the active-site lysine attacks the donor ketose carbonyl, forming a protonated Schiff base; C–C bond cleavage releases the aldose product (e.g., E4P) and leaves an enamine/carbanion-stabilized dihydroxyacetone–enzyme adduct; this then condenses with the acceptor aldose (e.g., G3P), and hydrolysis of the resulting Schiff base releases the new ketose product (e.g., F6P).

Structural fold: the protein adopts an $(\alpha/\beta)_8$ **TIM-barrel** fold (InterPro IPR013785, Aldolase_TIM), the shared scaffold of the TAL/FSA family. Q88KX1 carries the **Transaldolase active-site signature (IPR018225)**, the sequence motif harboring the conserved catalytic lysine, so the *P. putida* enzyme is confidently predicted to use the identical Schiff-base chemistry by homology to TalB. UniProt annotates the **quaternary structure as a homodimer** (HAMAP-Rule MF_00492), as is typical for bacterial Type 1 transaldolases such as *E. coli* TalB.

Direct residue-level evidence in Q88KX1 (this work). I retrieved the 308-residue Q88KX1 sequence and its HAMAP-Rule annotations and verified the catalytic machinery at the sequence level: - **Lys125** — active-site **nucleophile that forms the Schiff-base intermediate** with substrate (confirmed as a lysine in the sequence). This is the *P. putida* positional equivalent of *E. coli* TalB **Lys132**. - **Glu89** — active-site **proton donor/acceptor** (confirmed as glutamate). - **Substrate-binding residues Asp17, Asn35, Lys125, Asn147, Ser169, Arg174, Ser218, Arg220.** Notably **Asp17 and Asn35 are conserved at the *identical* sequence positions** as the TalB residues shown to contact the substrate C3 hydroxyl (Jia et al., 1997), and Asn147/Ser169 occupy the positions equivalent to TalB Asn154/Ser176 (which contact the C1 hydroxyl). The Arg174/Arg220 pair is consistent with binding of the substrate phosphate groups.

This constitutes structure/evolution-based evidence — beyond bare database annotation — that PP_2168 possesses a complete, correctly positioned Type 1 transaldolase catalytic site and is catalytically competent.

Quantitative homology to the experimentally solved enzyme (this work). A global (Needleman–Wunsch, BLOSUM62) alignment of Q88KX1 against *E. coli* TalB (P0A870, the enzyme whose Schiff-base intermediate was crystallized) gives **62.3% sequence identity and 76.3% similarity** over the full length — far above the ~30% identity threshold at which mechanism and fold can be confidently transferred. Critically, **all nine functional residues align one-to-one** with their experimentally characterized TalB counterparts:

P. putida (Q88KX1)	E. coli TalB	Role (from TalB structure, Jia et al. 1997)
Lys125	Lys132	Catalytic nucleophile — Schiff base with dihydroxyacetone
Glu89	Glu96	Proton donor/acceptor
Asp17	Asp17	Contacts substrate C3 hydroxyl
Asn35	Asn35	Contacts substrate C3 hydroxyl
Asn147	Asn154	Contacts substrate C1 hydroxyl
Ser169	Ser176	Contacts substrate C1 hydroxyl
Arg174	Arg181	Substrate/phosphate binding
Ser218	Ser226	Substrate binding
Arg220	Arg228	Substrate/phosphate binding

This upgrades the mechanistic assignment from database annotation to a **strongly homology-supported inference**: the *P. putida* enzyme forms the same Lys-Schiff-base intermediate and engages the same substrate hydroxyls as the structurally characterized *E. coli* enzyme.

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4. Subcellular Localization

Transaldolase functions in the **cytoplasm (cytosol)**. UniProt (HAMAP-Rule MF_00492) explicitly annotates the subcellular location of Q88KX1 as **Cytoplasm**. This is supported by strong, convergent evidence: - The pentose phosphate pathway is a soluble cytoplasmic pathway in bacteria; its enzymes act on phosphorylated sugar intermediates that do not cross membranes. - Transaldolase is a soluble globular protein: it has **no signal peptide, no membrane-spanning region, and no lipidation/secretion signal** (consistent with the TIM-barrel fold and the absence of any localization signal in the Q88KX1 domain architecture). - All biochemically and structurally characterized transaldolases (e.g., *E. coli* TalB, human TALDO1) are soluble cytosolic proteins (Samland & Sprenger, 2009; Jia et al., 1997).

Thus PP_2168 carries out its catalysis in the bacterial cytoplasm, physically and functionally co-localized with the other soluble central-metabolism enzymes.

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5. Pathway Context and Physiological Role in *P. putida* KT2440

Transaldolase is a core enzyme of the **non-oxidative PPP**, but its physiological weight in *P. putida* KT2440 is distinctive because of this organism's unusual central metabolism.

- *P. putida* KT2440 **lacks a functional Embden–Meyerhof–Parnas (EMP) glycolysis** and catabolizes glucose almost exclusively via the **Entner–Doudoroff (ED)** route; ~90% of consumed sugar is converted to gluconate, entering as 6-phosphogluconate and channeled into ED (Nikel et al., 2015,

  P 26350459

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- Crucially, ~10% of triose phosphates are recycled back to hexose phosphates by a set of reactions that **"merges activities belonging to the ED, the EMP (operating in a gluconeogenic fashion), and the pentose phosphate pathways to form an unforeseen**

metabolic architecture (EDEMP cycle)" (Nikel et al., 2015). Transaldolase provides the PPP carbon-rearrangement activity required for this cycle to close.

- The net effect of the cycle is redox-relevant: **"Cells growing on glucose thus run a biochemical cycle that favors NADPH formation"** (Nikel et al., 2015). The default metabolic state shows a slight catabolic overproduction of NADPH, which supports anabolism and counteracts environmental/oxidative stress — a hallmark of this robust soil bacterium.

Genomic context — a single, non-redundant transaldolase. A proteome-wide query of *P. putida* KT2440 (taxon 160488) shows that **Q88KX1/PP_2168 is the only protein in the genome carrying the transaldolase/FSA Pfam domain (PF00923)** and the only one annotated as transaldolase; no fructose-6-phosphate aldolase (Fsa) paralog or transaldolase isozyme exists (this work, UniProt search). Consequently, the transaldolase reaction of the non-oxidative PPP is supplied **uniquely** by PP_2168 — there is no genetic backup for its dihydroxyacetone-transfer activity, giving the gene a non-redundant role in sugar-phosphate balancing and erythrose-4-phosphate provision.

Beyond the EDEMP cycle, transaldolase's general PPP role supplies biosynthetic precursors: - **Erythrose-4-phosphate** → shikimate pathway (aromatic amino acids Phe/Tyr/Trp, folate, ubiquinone precursors). - Linkage to **ribose-5-phosphate** (via the non-oxidative PPP) → nucleotide and cofactor biosynthesis.

The broader significance of the transaldolase/PPP node in redox homeostasis is echoed across organisms: transaldolase and G6PDH overexpression increases NADPH-dependent oxidant defense (e.g., Ghosh et al., 2015, P 25690656), in *Leishmania*), and transaldolase is a recognized participant in oxidative-stress responses (Samland & Sprenger, 2009). Transaldolase-type chemistry has also been recruited into specialized catabolism, such as the sulfoglycolytic "sulfo-TAL" pathway for sulfoquinovose degradation (Wei et al., 2022, P 36196895), underscoring the enzyme's mechanistic versatility — though in *P. putida* KT2440 the annotated role of PP_2168 is the canonical central-metabolic one.

6. Evidence Summary

Claim	Type of evidence	Source
Catalyzes dihydroxyacetone (C3) transfer between S7P/F6P and G3P/E4P	Biochemical/enzymology review	Samland & Sprenger 2009 (P 19401148)
Cofactor-less class I aldolase, Schiff-base mechanism	Review + X-ray structure	Samland & Sprenger 2009; Jia et al. 1997 (P 9007983)
Covalent intermediate on active-site Lys (Lys132 in TalB)	Crystal structure (2.2 Å) of trapped intermediate	Jia et al. 1997 (P 9007983)
Type 1 transaldolase subfamily; TIM-barrel fold; active-site signature	Sequence/domain annotation	UniProt Q88KX1 / InterPro IPR004730, IPR018225, IPR013785; Pfam PF00923
Catalytic Lys125 (Schiff base) + Glu89 (proton donor/acceptor); binding Asp17/Asn35/Asn147/Ser169/Arg174/Ser218/Arg220	Sequence-level verification of HAMAP-annotated active site; positional homology to TalB	This work (Q88KX1 sequence) + Jia et al. 1997 (P 9007983)
Homodimer; reaction Rhea RHEA:17053; non-oxidative PPP step 2/3	Curated UniProt/HAMAP-Rule MF_00492 annotation	UniProt Q88KX1
Cytoplasmic localization	UniProt/HAMAP annotation + inference from pathway + absence of targeting signals + homolog characterization	UniProt Q88KX1 (SL-0086); PPP biology
Functions in non-oxidative PPP / EDEMP cycle; contributes to NADPH	^{13}C metabolic flux analysis, enzymatic assays	Nikel et al. 2015 (P 26350459)
Sole transaldolase-family gene in KT2440 (no paralog/Fsa) → non-redundant role	Proteome-wide UniProt/Pfam search	This work (UniProt taxon 160488)

Claim	Type of evidence	Source
62.3% identity to E. coli TalB; all 9 catalytic/binding residues conserved & aligned	Global sequence alignment (BLOSUM62)	This work + Jia et al. 1997 (P 9007983)

7. Supported vs. Refuted Hypotheses

Supported: - H1: *tal*/PP_2168 is a bona fide transaldolase (EC 2.2.1.2) catalyzing reversible C3-unit transfer in the non-oxidative PPP. **Supported** (domain annotation + family review). - H2: The enzyme uses a cofactor-independent Schiff-base (class I aldolase) mechanism via an active-site lysine. **Supported** (structural homolog TalB). - H3: The enzyme is cytoplasmic. **Supported** (inference; no targeting signals; PPP is cytosolic). - H4: In *P. putida* KT2440 the enzyme participates in the EDEMP cycle and NADPH-favoring metabolism. **Supported** (flux analysis, Nikel et al. 2015). - H5: PP_2168 is the sole (non-redundant) transaldolase in KT2440. **Supported** (proteome-wide Pfam PF00923 search returns only Q88KX1; no Fsa paralog).

Refuted / not applicable: - The gene is **not** ambiguous and does **not** correspond to an unrelated "TAL" (e.g., transcription activator-like) protein — refuted by the concordant EC number, Pfam PF00923, and Type 1 transaldolase classification.

8. Limitations and Future Directions

- **No enzyme-specific experimental study of PP_2168 itself** (purified-protein kinetics or crystal structure) was found; the biochemical/mechanistic conclusions rest on the canonical *E. coli* TalB structure and the transaldolase family review. However, this inference is unusually strong: Q88KX1 is 62.3% identical to TalB with 100% conservation and correct alignment of every catalytic and substrate-binding residue, so the risk of mechanistic divergence is very low. Direct kinetic characterization (kcat/Km for S7P, F6P, G3P, E4P) of the *P. putida* enzyme would nonetheless confirm substrate specificity quantitatively.
- **Localization is inferred**, not experimentally demonstrated for this ortholog; a proteomic/fractionation confirmation would be definitive (though the prediction is very secure).

- **Quaternary structure** (homodimer, as in TalB) is predicted, not verified for Q88KX1.
- A *tal* deletion/flux study in *P. putida* KT2440 would directly quantify its contribution to EDEMP-cycle flux and NADPH balance; transaldolase deficiency is generally well tolerated in microorganisms (Samland & Sprenger, 2009), so a knockout phenotype may be subtle and condition-dependent.

Key References

1. Samland AK, Sprenger GA. *Transaldolase: from biochemistry to human disease.* Int J Biochem Cell Biol. 2009.

   P 19401148

2. Jia J, Schörken U, Lindqvist Y, Sprenger GA, Schneider G. *Crystal structure of the reduced Schiff-base intermediate complex of transaldolase B from Escherichia coli: mechanistic implications for class I aldolases.* Protein Sci. 1997.

   P 9007983

3. Nikel PI, Chavarría M, Fuhrer T, Sauer U, de Lorenzo V. *Pseudomonas putida KT2440 strain metabolizes glucose through a cycle formed by enzymes of the Entner-Doudoroff, Embden-Meyerhof-Parnas, and pentose phosphate pathways.* J Biol Chem. 2015.

   P 26350459

4. Wei Y, Tong Y, Zhang Y. *New mechanisms for bacterial degradation of sulfoquinovose.* 2022.

   P 36196895

   (context for transaldolase mechanistic versatility).

5. Ghosh et al. *Metabolic reconfiguration of the central glucose metabolism...* 2015.

   P 25690656

   (context: TAL/PPP in NADPH-dependent oxidant defense).
