Functional Annotation of *hisC* (Q88P86, PP_0967) in *Pseudomonas putida* KT2440

Gene: *hisC* (OrderedLocusName PP_0967) **Protein:** Histidinol-phosphate aminotransferase (HisC); AltName: imidazole-acetol-phosphate transaminase **UniProt:** Q88P86 · **EC:** 2.6.1.9 · **KEGG Ortholog:** K00817 **Organism:** *Pseudomonas putida* (strain ATCC 47054 / DSM 6125 / NCIMB 11950 / **KT2440**)

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1. Summary (Answer to the Research Question)

hisC encodes **histidinol-phosphate aminotransferase (HisC, EC 2.6.1.9)**, a soluble, cytoplasmic, pyridoxal-5′-phosphate (PLP)-dependent **class-II aminotransferase** that catalyzes the **seventh step of de novo L-histidine biosynthesis**. Working as a **homodimer**, it performs a reversible transamination that, in the biosynthetic direction, transfers the α-amino group of **L-glutamate onto imidazole-acetol phosphate (3-(imidazol-4-yl)-2-oxopropyl phosphate)** to yield **L-histidinol phosphate + 2-oxoglutarate**. Its substrate specificity is dominated by recognition of the substrate **phosphate group**; members of this subfamily can additionally act as aromatic-amino-acid aminotransferases. In *P. putida* the gene sits in a compact **hisG–hisD–hisC** cluster and is **conditionally essential** — its loss causes histidine auxotrophy on minimal medium.

The identity of the target was rigorously verified: gene symbol, organism, EC number, protein family, and catalytic residues are all mutually consistent across UniProt, KEGG, and the primary structural literature on close orthologs. **No gene-symbol ambiguity was encountered.**

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2. Identity Verification

Attribute	Provided target	Confirmed by this study
Gene symbol	*hisC*	UniProt Q88P86; KEGG ppu:PP_0967 (SYMBOL hisC)
Enzyme	Histidinol-phosphate aminotransferase, EC 2.6.1.9	UniProt catalytic activity; KEGG KO K00817; EC 2.6.1.9
Organism	*P. putida* KT2440	KEGG ORGANISM ppu; UniProt organism
Family	Class-II PLP-dependent aminotransferase	UniProt SIMILARITY; KEGG BRITE "Aminotransferase Class II"; Pfam Aminotran_1_2
Locus/ position	PP_0967	KEGG POSITION 1,106,849–1,107,895

All identifiers converge on a single, well-characterized enzyme family. The verification requirement is satisfied.

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3. Primary Function: Reaction Catalyzed and Substrate Specificity

3.1 The reaction

HisC catalyzes the PLP-dependent, reversible transamination (UniProt Q88P86 catalytic activity):

> **L-histidinol phosphate + 2-oxoglutarate ⇌ 3-(imidazol-4-yl)-2-oxopropyl phosphate (imidazole-acetol phosphate) + L-glutamate**

Physiologically, the enzyme operates in the **biosynthetic (amination) direction**: "histidinol-phosphate aminotransferase catalyzes the transfer of the amino group from glutamate to imidazole acetol-phosphate producing 2-oxoglutarate and histidinol phosphate" (Fernández et

al., 2004,). P 15007066

This is the **seventh step in the synthesis of histidine within eubacteria** (Sivaraman et al., 2001,), P 11518529 corresponding to **step 7 of 9** from 5-phospho-α-D-ribose-1-diphosphate (PRPP) in the UniProt/KEGG pathway map (KEGG module M00026).

3.2 Substrate specificity

- The **natural substrate pair** is L-histidinol phosphate / 2-oxoglutarate (amino donor L-glutamate in the biosynthetic direction).
- The **substrate phosphate group is the principal specificity determinant**. In *Corynebacterium glutamicum* HisC, "the hydrogen bond between the side chain of this residue [Tyr21] and the phosphate group of His-P is important for recognition of the natural substrate and discrimination against other potential amino donors such as phenylalanine and leucine" (Marienhagen et al., 2008,). P 18560156
- **Family-level substrate promiscuity / moonlighting:** In organisms lacking dedicated aromatic aminotransferases, HisC also transaminates aromatic amino acids. The *Thermotoga maritima* enzyme "accepts histidinol phosphate, tyrosine, tryptophan, and phenylalanine, but not histidine, as substrates" (Fernández et al., 2004,). P 15007066
  Consistent with this, **KEGG maps P. putida PP_0967 not only to histidine metabolism (ppu00340) but also to tyrosine (ppu00350), phenylalanine (ppu00360), and aromatic amino acid biosynthesis (ppu00400)** pathways. However, this mapping reflects **family-level catalytic capacity rather than a demonstrated dedicated role**: *P. putida* KT2440 encodes **two dedicated aromatic-amino-acid aminotransferases (PP_1972 and PP_3590; KEGG K00832, EC 2.6.1.57)**, whereas *T. maritima*—where HisC broadens to aromatic substrates—lacks such enzymes. Any aromatic-amino-acid transamination by *P. putida* HisC is therefore likely **biochemically possible but physiologically redundant/minor**, and has not been directly demonstrated.

- Genetic corroboration of the pathway step: *hisC* mutants (in *Micrococcus luteus*) "accumulated imidazoleacetol" (Kane-Falce & Kloos, 1975, P 1126626), confirming HisC acts at the imidazole-acetol(-phosphate) node.

4. Mechanism, Cofactor, and Quaternary Structure

- **Cofactor:** pyridoxal 5′-phosphate (PLP), covalently bound as an **internal aldimine (Schiff base) at Lys210** of *P. putida* HisC (UniProt "N6-(pyridoxal phosphate)lysine" at residue 210).
- **Catalytic cycle:** classic aminotransferase **ping-pong (two half-reaction)** mechanism cycling between the PLP and pyridoxamine-5′-phosphate (PMP) forms. In the *E. coli* ortholog, a covalent tetrahedral complex "consisting of PLP and l-histidinol phosphate attached to Lys214" was captured crystallographically, resembling the transient gem-diamine intermediate (Sivaraman et al., 2001, P 11518529). PMP-enzyme complexes have been trapped in both *E. coli* (P 11518529) and *C. glutamicum* (P 18560156).
- **Quaternary structure: homodimer**. "HisC is a dimeric enzyme with a mass of approximately 80 kDa" (Sivaraman et al., 2001, P 11518529); UniProt lists Q88P86 as a homodimer. Each monomer comprises a large α/β/α PLP-binding domain plus a small domain, with an N-terminal arm contributing to dimerization; the active site lies at the dimer interface.
- **Conserved catalytic apparatus in Q88P86 (direct sequence evidence):** A global alignment of Q88P86 against the crystallographically characterized *E. coli* HisC (P06986) shows the active-site residues are conserved — **Tyr55→Tyr57, Asp184→Asp181, Tyr187→Tyr184, Ser213→Ser209, Lys214→Lys210, Arg222→Arg218**. The reference set is described as: "Residues that interact with the PLP cofactor, including Tyr55, Asn157, Asp184,

Tyr187, Ser213, Lys214 and Arg222, are conserved in the family of aspartate, tyrosine and histidinol phosphate aminotransferases" (Sivaraman et al., 2001,

P 11518529

).

The mapped catalytic lysine (Lys214→**Lys210**) matches UniProt's independent PLP-attachment annotation exactly, validating the alignment. Asp181 stabilizes the protonated PLP pyridinium nitrogen (a fold-type-I/class-II hallmark), Arg218 binds the substrate α-carboxylate/2-oxoglutarate, and Tyr57 contributes to substrate/phosphate binding. This upgrades the annotation from database transfer to **residue-level evidence that Q88P86 is a catalytically competent HisC**.

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5. Subcellular Localization

HisC acts in the **cytoplasm** as a soluble enzyme. UniProt Q88P86 shows **no signal peptide, transmembrane segment, or lipidation/anchor**; all characterized bacterial orthologs (*E. coli*, *Salmonella typhimurium*, *C. glutamicum*, *T. maritima*) are soluble proteins purified from soluble extracts and crystallized as such (e.g., "Crystalline L-histidinol phosphate aminotransferase from *Salmonella typhimurium*", Henderson & Snell, 1973,

P 4632247

).

Its substrates are cytosolic phosphorylated intermediates and glutamate/2-oxoglutarate. The entire de novo histidine biosynthetic pathway is cytoplasmic, so HisC exerts its function there.

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6. Pathway Context and Biological Role

- **Pathway:** de novo **L-histidine biosynthesis** (PRPP → histidine), an unbranched, ancient pathway. HisC provides the **transamination that installs the α-amino group of the histidine backbone**, converting imidazole-acetol phosphate to L-histidinol phosphate. The immediately downstream enzyme HisD (histidinol dehydrogenase) then oxidizes L-histidinol phosphate/L-histidinol to L-histidine. (Pathway architecture reviewed in Alifano et al., 1996, *Microbiol. Rev.*,

P 8852895

.)

- **Amino-donor coupling:** By consuming L-glutamate and releasing 2-oxoglutarate, HisC links histidine biosynthesis to the cell's central **glutamate/2-oxoglutarate nitrogen pool**.
- **Genomic organization / co-regulation:** In *P. putida* KT2440, *hisC* (PP_0967) lies immediately downstream of **hisG** (PP_0965, ATP phosphoribosyltransferase — the first, feedback-regulated step) and **hisD** (PP_0966, histidinol dehydrogenase — the terminal steps), all on the same strand. *hisD* and *hisC* are separated by only **2 bp**, indicating translational coupling and operon-like co-transcription of a **hisG–hisD–hisC** cluster. This physically and transcriptionally embeds HisC within the histidine biosynthetic program.
- **Physiological requirement (organism-specific):** A genome-wide mini-Tn5 transposon screen of *P. putida* KT2440 identified de novo amino-acid biosynthesis genes as **conditionally essential** on glucose minimal medium — "Auxotrophs for all amino acids predicted by the in silico models were found" (Molina-Henares et al., 2010,

  P 20158506

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  Because HisC catalyzes an obligatory, non-bypassable step of the single linear histidine pathway (no isozyme), *hisC* loss yields **histidine auxotrophy**: required for prototrophic growth but dispensable when histidine is supplied.

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7. Evidence Summary

Claim	Evidence type	Source
EC 2.6.1.9; His-P aminotransferase; step 7 of His biosynthesis	Database annotation + primary structural lit.	UniProt Q88P86; KEGG K00817; P 11518529 P 15007066
PLP cofactor at Lys210; ping-pong (PLP↔PMP) mechanism	UniProt residue annotation + ortholog crystal structures	UniProt Q88P86; P 11518529 P 18560156
Homodimer, ~80 kDa	Ortholog biochemistry/ crystallography	P 11518529; UniProt
Catalytic residues conserved in Q88P86	Bioinformatic alignment (this study)	vs P06986; P 11518529
Phosphate-group specificity; aromatic-AA moonlighting	Site-directed mutagenesis + substrate assays	P 18560156 P 15007066; KEGG pathway mapping
Cytoplasmic, soluble	Sequence features + ortholog purification	UniProt Q88P86; P 4632247
hisGDC cluster / co-regulation	Genome coordinates	KEGG ppu genome
Conditionally essential (His auxotrophy)	Genome-wide transposon screen	P 20158506

8. Supported and Refuted Hypotheses

Supported: - H1 — *hisC* encodes a functional PLP-dependent histidinol-phosphate aminotransferase (EC 2.6.1.9). **Strongly supported** (database + conserved catalytic residues incl. Lys210-PLP). - H2 — HisC operates in the cytoplasm as a soluble homodimer. **Supported.** - H3 — HisC is embedded in a co-regulated histidine operon (hisG–hisD–hisC) and is required for de novo His synthesis. **Supported** (genomic + auxotrophy evidence).

Partially supported / open: - H4 — *P. putida* HisC physiologically moonlights as an aromatic-amino-acid aminotransferase. **Plausible but unproven, and likely minor**: supported by family behavior (*T. maritima*) and KEGG pathway mapping, but no direct *P. putida* enzymology exists. *P. putida* encodes **two dedicated aromatic-amino-acid aminotransferases (PP_1972, PP_3590; K00832)**, making any HisC moonlighting role physiologically redundant/minor in this organism.

Refuted / ruled out: - The protein is **not** a membrane transporter, structural protein, or signaling molecule; it is a soluble metabolic enzyme (no TM/signal features). - HisC does **not** transaminate free histidine (orthologs do not accept histidine as substrate).

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9. Limitations and Future Directions

- **No direct enzymology exists for the *P. putida* KT2440 protein itself.** All mechanistic and kinetic detail is transferred from orthologs (*E. coli*, *C. glutamicum*, *T. maritima*, *Salmonella*) plus bioinformatic residue mapping specific to Q88P86. This is standard and well-justified for a HAMAP-ruled housekeeping enzyme, but *P. putida*-specific kinetics (Km for His-P, imidazole-acetol phosphate, glutamate) and any aromatic-AA side activity remain to be measured.
- The **conditional-essentiality inference** for *hisC* rests on pathway logic plus a genome-wide screen that reports amino-acid auxotrophs collectively; a targeted *hisC* knockout with His-supplementation rescue would confirm it directly.
- **Future work:** solve/model the *P. putida* HisC structure (AlphaFold model is available), test aromatic-amino-acid transaminase activity, and define operon boundaries and HisG feedback regulation experimentally.

References (PMIDs)

- Sivaraman J. et al. (2001) Crystal structure of histidinol phosphate aminotransferase (HisC) from *E. coli*.

  P 11518529

- Fernández F.J. et al. (2004) Structural studies of the catalytic reaction pathway of a hyperthermophilic HisC (*T. maritima*).

  P 15007066

- Marienhagen J. et al. (2008) Substrate recognition by HisC from *C. glutamicum*.

  P 18560156

- Kane-Falce & Kloos (1975) Histidine biosynthesis in *Micrococcus luteus*.

  P 1126626

- Henderson & Snell (1973) Crystalline His-P aminotransferase from *S. typhimurium*.

  P 4632247

- Alifano P. et al. (1996) Histidine biosynthetic pathway and genes.

  P 8852895

- Molina-Henares M.A. et al. (2010) Conditionally essential genes for growth of *P. putida* KT2440.

  P 20158506

- Rutkiewicz M. et al. (2023) Plant HISN6 substrate specificity/structure.

  P 36842242

Database sources: UniProt Q88P86; KEGG ppu:PP_0967 (K00817); Pfam Aminotran_1_2; InterPro IPR005861 (HisP_aminotrans), IPR050106.