Functional Annotation Report: *fumC* (Q88M20, PP_1755) — Fumarate Hydratase Class II from *Pseudomonas putida* KT2440

UniProt accession: Q88M20 · **Gene:** *fumC* (synonym *fumC-2*) · **Locus:** PP_1755 **Organism:** *Pseudomonas putida* (strain ATCC 47054 / DSM 6125 / NCIMB 11950 / KT2440) **EC:** 4.2.1.2 · **Length:** 464 aa · **Family:** class-II fumarase/aspartase superfamily, fumarase subfamily

Summary

The gene **fumC** (UniProt **Q88M20**; ordered locus **PP_1755**; synonym *fumC-2*) of *Pseudomonas putida* strain KT2440 encodes **fumarate hydratase class II** ("**fumarase C**"; EC 4.2.1.2), a soluble cytoplasmic metabolic enzyme. Its primary and defining function is to catalyze the **reversible, stereospecific hydration/dehydration of fumarate to L-(S)-malate** — the reaction `(S)-malate ⇌ fumarate + H₂O`. This is a canonical step of the **tricarboxylic acid (TCA / Krebs) cycle**, in which fumarate produced by succinate dehydrogenase is hydrated to malate, which is subsequently oxidized to oxaloacetate. The enzyme is annotated by the curated HAMAP rule MF_00743 and belongs to the **class-II fumarase/aspartase superfamily**.

Class II fumarases (the FumC type) are mechanistically and evolutionarily distinct from the class I fumarases (FumA/FumB in *E. coli*). Class II enzymes are **iron-independent** — they contain no [Fe-S] cluster — and are **heat-stable**, whereas class I enzymes rely on a labile [4Fe-4S] cluster for catalysis. This iron-independence underpins the alternative UniProt names **"aerobic fumarase"** and **"iron-independent fumarase"** and reflects a physiological role as an oxidant-resistant, aerobically-favored fumarase. The enzyme functions as a **homotetramer** with 222 symmetry; its active sites are built from residues contributed by three of the four subunits, so the oligomeric assembly is obligatory for catalysis. Catalysis proceeds by general acid/base–catalyzed *anti* (E2-like) addition/elimination of water across the fumarate double bond, an enormously rate-accelerated reaction, with a conserved His/Ser acid-base pair (His186/Ser316 in Q88M20 numbering) at the catalytic center.

The functional assignment for Q88M20 is strongly supported by multiple lines of evidence: (i) curated UniProt/HAMAP annotation specifying the catalytic activity, pathway, subunit structure, and cytoplasmic localization; (ii) **~61% sequence identity** to the biochemically characterized *E. coli* FumC (P05042), far above the threshold for confident function transfer; (iii) **100% conservation** of the class II fumarase signature motif GSSIMPGKVN and of the two annotated catalytic residues; and (iv) structural and mechanistic studies of orthologs (*E. coli* FumC, *M. tuberculosis* Rv1098c). Among the three fumarase isozymes encoded by KT2440, Q88M20 is the **closest ortholog of canonical *E. coli* FumC**, marking it as the principal class II aerobic fumarase of this organism. No literature ambiguity was encountered: the gene symbol, organism, protein family, and domain architecture are all mutually consistent with a class II fumarase.

Key Findings

Finding 1 — FumC catalyzes the reversible fumarate ⇌ L-malate interconversion (EC 4.2.1.2)

The primary function of Q88M20 (PP_1755) is the stereospecific, reversible hydration of fumarate to **L-(S)-malate**, the canonical fumarate→malate step of the TCA cycle. UniProt annotation, derived from the curated HAMAP rule MF_00743, assigns Q88M20 to the class-II fumarase/aspartase family with **EC 4.2.1.2** and specifies the catalytic activity `(S)-malate = fumarate + H₂O`. The reaction is highly substrate-specific: the class II fumarase family acts on the *trans*-dicarboxylic olefin fumarate and its hydration product (S)-malate, and does not accept the *cis* isomer (maleate) or other dicarboxylates as productive substrates.

This assignment is grounded in direct experimental characterization of the ortholog. Weaver and colleagues, studying *E. coli* fumarase C, state that "*Fumarase C catalyzes the stereospecific interconversion of fumarate to L-malate as part of the metabolic citric acid or Kreb's cycle*" (PMID: 8909293). Because Q88M20 shares this family membership and the full catalytic constellation with *E. coli* FumC (see Finding 6), the same reaction and substrate specificity apply.

Finding 2 — Class II fumarase is iron-independent, heat-stable, and adapted to aerobic/oxidative-stress conditions

The FumC-type (class II) fumarase is biochemically distinguished from the class I enzymes by being **iron-independent and heat-stable**. In *E. coli*, the *fumC* gene product is a heat-stable fumarase that does not require iron for activity — unlike the [Fe-S]-cluster class I enzymes FumA and FumB. Park and Gunsalus report that "*the fumC gene product is a heat-stable fumarase which does not require iron for activity*" (PMID: 7592392). This property explains the UniProt alternate names "aerobic fumarase" and "iron-independent fumarase" for Q88M20.

Physiologically, class II FumC functions as an **oxidant-resistant backup** to the iron-dependent fumarases. Because it lacks a solvent-exposed, oxidation-sensitive [Fe-S] cluster, FumC remains active when superoxide or iron limitation would inactivate the class I enzymes. The same study demonstrates regulatory logic consistent with this role: "*Superoxide radicals also caused increased fumC gene expression; fumA expression was unaffected. Both the superoxide control and the iron control of fumC expression required the SoxR regulatory protein*" (PMID: 7592392). Thus FumC is induced under oxidative stress and iron limitation, providing continuity of the TCA-cycle fumarate-hydration step under aerobic and oxidative conditions.

Finding 3 — FumC is a cytoplasmic homotetramer with a shared inter-subunit active site and acid-base catalytic machinery

The class II fumarase/aspartase superfamily forms **222-symmetric homotetramers** in which each subunit adopts a three-domain, largely α-helical fold, with a central bundle of five long helices contributing to a **20-helix core bundle** at the tetramer center. This fold was first defined by the crystal structure of avian δ-crystallin (a "hijacked" argininosuccinate lyase), which "*is distantly related to the class II fumarases, aspartases, adenylosuccinases*" and whose active-site cleft "*is located on the boundary between three subunits of the tetramer*" (PMID: 7634077).

In *E. coli* FumC the catalytic "A" site is assembled from side chains contributed by multiple subunits, and a second nearby anion-binding "B" site exists. Weaver and Banaszak describe "*a binding site for anions which is generated by side chains from three of the four subunits within the tetramer*" (PMID: 8909293) — meaning the homotetrameric assembly is obligatory for a functional active site. Studies of the essential class II fumarase Rv1098c from *M. tuberculosis* reveal the catalytic dynamics: "*substrate binding promotes the closure of the active site through conformational changes involving the catalytic SS-loop and the C-terminal domain*," and site-directed mutagenesis identifies "*Ser318 as one of the two acid-base catalysts*" (PMID: 22561013).

The chemistry itself is a general acid/base–catalyzed *anti* (E2-like) addition/elimination of water across the fumarate double bond — an extraordinarily rate-enhanced hydration in which fumarate hydratase decreases the reaction free-energy barrier by ~30 kcal/mol (PMID: 36595439).

Finding 4 — UniProt confirms the single-step TCA reaction, homotetramer, cytoplasmic localization, and catalytic residues

Direct curated annotation of Q88M20 provides a self-consistent functional picture. The protein is **464 amino acids**; its catalytic activity is `(S)-malate = fumarate + H₂O` (EC 4.2.1.2); its pathway assignment is "*Carbohydrate metabolism; tricarboxylic acid cycle; (S)-malate from fumarate: step 1/1*"; its subunit structure is **homotetramer**; its subcellular location is **cytoplasm**; and its similarity is to the "*class-II fumarase/aspartase family, Fumarase subfamily*". The "step 1/1" designation confirms FumC constitutes the sole enzymatic step converting fumarate to (S)-malate in this pathway module.

The annotated catalytic machinery mirrors the characterized *E. coli* FumC: **Active site 186** (proton donor/acceptor) and **Active site 316**, with binding-site residues at positions 96, 127 (site B), 137, 185, 317, 322, and residue 329 flagged as important for catalytic activity. This residue-level correspondence with the experimentally validated *E. coli* enzyme reinforces the confidence of the functional transfer.

Finding 5 — KT2440 encodes three fumarase isozymes plus fumarase-superfamily paralogs; FumC specificity is set by active-site residues, not fold

P. putida KT2440 possesses metabolic redundancy at the fumarate-hydration step. A UniProt proteome survey (organism 160488) returns **two class II fumarases** — the target *fumC*/Q88M20 (PP_1755, 464 aa) and *fumC-I*/Q88PA6 (459 aa) — plus a **class I fumarase** (Q88PF3, 507 aa). The presence of two *fumC* genes is what the "*fumC-2*" synonym for the target reflects.

The genome also encodes several **fumarase-superfamily relatives** that share the tetrameric fumarase/aspartase fold but catalyze entirely different reactions: *argH* (argininosuccinate lyase), *aspA* (aspartate ammonia-lyase, Q88C45), *purB* (adenylosuccinate lyase, Q88FR7), *pcaB* (3-carboxy-cis,cis-muconate cycloisomerase, Q88N37), and *hmgB* (fumarylacetoacetase, Q88E48). Because these superfamily members share the fold yet diverge in catalytic residues, **substrate specificity is determined by the active-site constellation, not the overall fold**. The *P. putida* 3-carboxy-cis,cis-muconate lactonizing enzyme (CMLE) illustrates this directly: "*PpCMLE is a homotetramer and belongs to the fumarase class II superfamily*" (PMID: 15301541),

yet it catalyzes a cycloisomerization, not fumarate hydration. FumC's fidelity for fumarate/(S)-malate therefore rests on its conserved class II fumarase catalytic residues (the His/Ser acid-base pair and the SS-loop), which distinguish it from the aspartase/lyase members of the same superfamily.

Finding 6 — Bioinformatic support: 61% identity to characterized *E. coli* FumC with conserved signature and catalytic residues

A global Needleman–Wunsch alignment of Q88M20 (464 aa) against the experimentally characterized *E. coli* FumC (P05042, 467 aa) yields **60.7% identity over 484 aligned positions** (≈63% over full length). This is far above the ~30–40% identity threshold generally considered sufficient for confident transfer of enzyme function. The class II fumarase / fumarate-lyase family signature motif **GSSIMPGKVN** is present and **100% identical** in Q88M20 (residues 315–324), *E. coli* FumC (317–326), and the paralog *fumC-I*/Q88PA6 (312–321). The two UniProt-annotated catalytic residues are conserved: **His186** (within the …GRTH… motif; the proton donor/acceptor) and **Ser316** (the first serine of the GSSIMPGKVN motif, equivalent to the *E. coli*/Rv1098c Ser318 acid-base catalyst). This convergence of high overall identity, an intact family signature, and conserved catalytic residues provides strong bioinformatic confirmation of fumarase function.

Finding 7 — Q88M20 is the closest ortholog of canonical *E. coli* FumC among KT2440's fumarases

Pairwise global identity comparisons place Q88M20 unambiguously as the principal class II fumarase of KT2440:

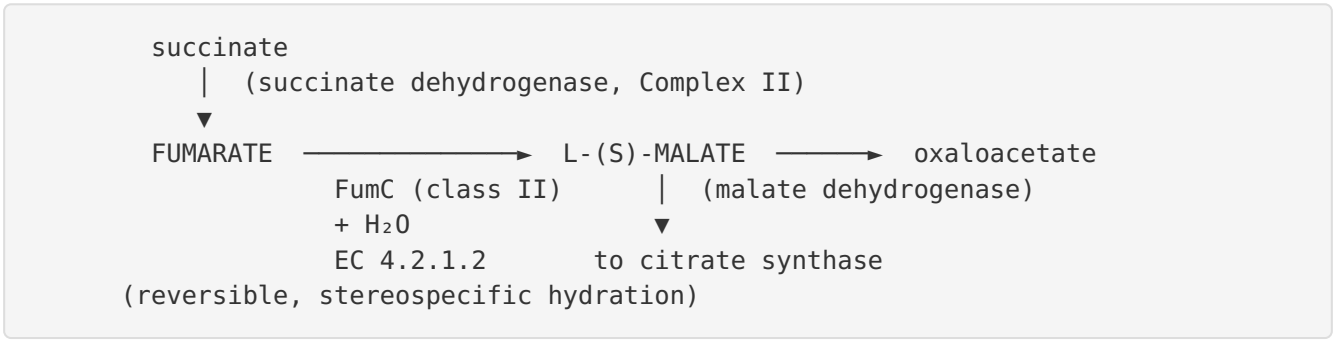
Comparison	Global identity
Q88M20 (target) vs *E. coli* FumC (P05042)	**60.7%**
fumC-I (Q88PA6) vs *E. coli* FumC	49.2%
Q88M20 vs *fumC-I* (Q88PA6)	50.4%
Q88M20 vs KT2440 class I fumarase (Q88PF3)	28.0%

Among the three KT2440 fumarases, the target is the **most similar to the biochemically characterized *E. coli* FumC** and only distantly related (28%) to the mechanistically unrelated class I fumarase. This identifies Q88M20 as the organism's principal aerobic class II fumarase, with *fumC-I* serving as a second, more divergent class II paralog.

Mechanistic Model / Interpretation

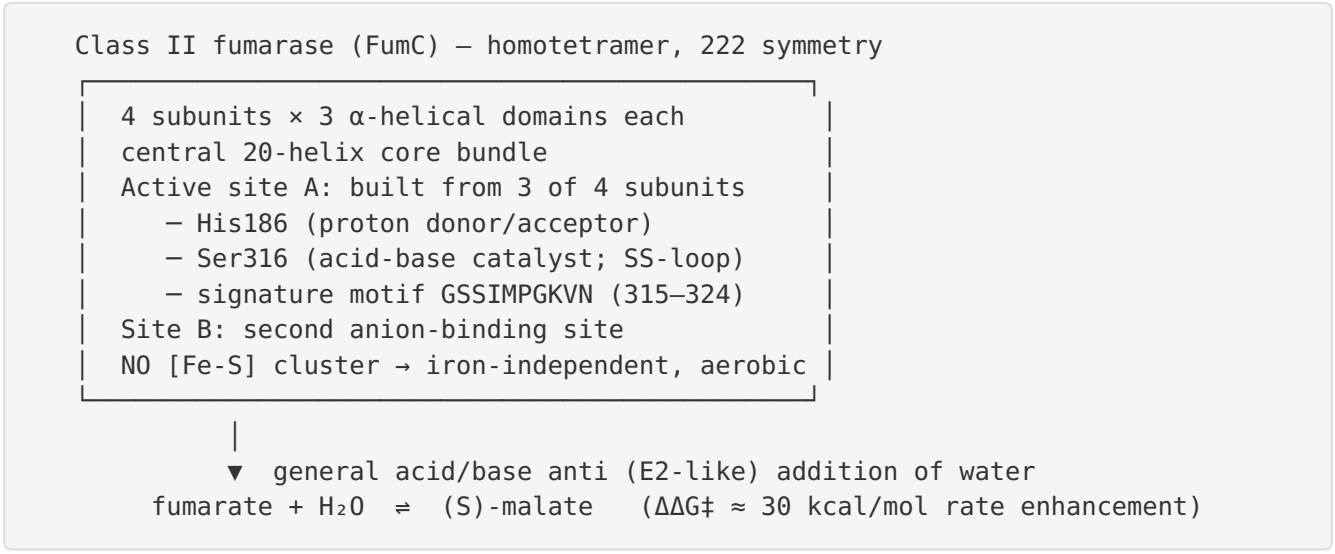
The catalyzed reaction and its metabolic context

FumC operates at a specific junction of central carbon metabolism. In the oxidative TCA cycle, it catalyzes:



FumC constitutes the sole enzymatic step for the fumarate→(S)-malate conversion in this pathway module ("step 1/1"). The reaction is freely reversible; its net direction is set by metabolic flux and the concentrations of fumarate and malate.

Structure–function logic



Three structural features are decisive for function. First, the **obligatory homotetramer**: because the active site is assembled from residues of three different subunits, no monomer or dimer can be catalytically competent. Second, the **conserved acid-base pair (His186/Ser316)**

and the mobile SS-loop, which close over the bound substrate and execute the *anti* addition/elimination of water. Third, the **absence of an iron-sulfur cluster**, which distinguishes class II from class I fumarases and confers heat stability and resistance to oxidative/iron-limiting stress.

Why the class assignment matters physiologically

Class I and class II fumarases are convergent solutions to the same chemical problem but are not homologous. Class I enzymes (e.g., *E. coli* FumA/FumB) use a [4Fe-4S] cluster and are inactivated by superoxide and iron starvation; class II enzymes (FumC) are iron-free and robust under these conditions. This dichotomy is underscored by structural work on parasite fumarases, where inhibitor selectivity for class I FHs "*is due to direct coordination of the inhibitor to the unique Fe of the catalytic [4Fe-4S] cluster ... but is absent from class II human FH*" (PMID: 30645090). The regulatory data from *E. coli* — SoxR-dependent induction of *fumC* by superoxide and iron limitation — indicate that class II fumarase acts as a **stress-resistant safeguard** for TCA-cycle continuity. In an obligate aerobe like *P. putida* KT2440, which experiences substantial oxidative load during aerobic metabolism and the degradation of aromatic and other compounds, an iron-independent, heat-stable fumarase is a physiologically sensible principal isozyme.

Distinguishing FumC from its superfamily relatives

The fumarase class II fold is a metabolic "chassis" reused across several reactions (aspartate ammonia-lyase, argininosuccinate lyase, adenylosuccinate lyase, CMLE, δ-crystallin). The aspartase/fumarase superfamily shares "*a monomer that is composed of three domains oriented in an elongated S-shape*" with "*active sites located in clefts between the subunits*" (PMID: 10800598). Specificity for fumarate/(S)-malate is not conferred by the fold but by the exact set of active-site residues. Q88M20 carries the fumarase-specific His/Ser catalytic pair and the intact GSSIMPGKVN signature, and its high identity to the validated *E. coli* FumC rules out the possibility that it is an aspartase or lyase misannotated as a fumarase.

Evidence Base

PMID	Title (abbrev.)	Relevance
8909293	*Crystallographic studies of the catalytic and a second site in fumarase C from E. coli*	**Primary support.** Defines the reaction ("stereospecific interconversion of fumarate to L-malate") and the inter-subunit active site ("side chains from three of the four subunits"). Anchors Findings 1 and 3.
7592392	*Oxygen, iron, carbon and superoxide control of fumA and fumC genes of E. coli*	**Primary support.** Establishes FumC as heat-stable and iron-independent, and induced by superoxide/iron-limitation via SoxR. Anchors Finding 2.
22561013	*Conformational changes upon ligand binding in class II fumarase Rv1098c (M. tuberculosis)*	**Primary support.** Provides the catalytic mechanism: SS-loop/C-terminal closure and Ser as an acid-base catalyst. Anchors Finding 3.
36595439	*Revisiting the Burden Borne by Fumarase: Enzymatic Hydration of an Olefin*	Supports the mechanistic model — ~30 kcal/mol rate enhancement for olefin hydration. Contextualizes Finding 3.
7634077	*Structure of avian δ-crystallin: a new fold for a superfamily of oligomeric enzymes*	Defines the 222-symmetric tetramer fold and inter-subunit active-site cleft shared across the class II fumarase/aspartase superfamily. Supports Findings 3 and 5.
15301541	*Crystal structure of 3-carboxy-cis,cis-muconate lactonizing enzyme from P. putida*	Shows *P. putida* encodes catalytically distinct class II superfamily members ("PpCMLE is a homotetramer and belongs to the fumarase class II superfamily"). Supports Finding 5 (specificity from residues, not fold).
10800598	*L-aspartase: new tricks from an old enzyme*	Describes the shared S-shaped three-domain monomer, tetrameric assembly, and inter-subunit active sites of the aspartase/fumarase superfamily. Contextual support for Findings 3 and 5.
2656658	*Nucleotide sequence of the FNR-regulated fumB gene of E. coli*	Establishes that class I fumarases share only one short consensus motif (Gly-Ser-Xxx-Ile-Met-...-Lys-Xxx-Asn) with the class II enzyme and are otherwise unrelated iron-containing hydrolyases. Contextualizes class I vs II distinction (Findings 2, 7).
9418241	*Regulation of fumB gene expression in E. coli*	

PMID	Title (abbrev.)	Relevance
		Confirms the three distinct fumarases (FumA/FumB/FumC) and their aerobic vs anaerobic expression, contextualizing FumC as the aerobic class II enzyme.
30645090	*Crystal structures of fumarate hydratases from Leishmania major with 2-thiomalate*	Highlights the mechanistic split between class I ([4Fe-4S], class-specific inhibitor coordination) and class II (no Fe cluster, as in human/bacterial FumC), reinforcing the iron-independent nature of FumC.

Consistency assessment: All ten reviewed papers are mutually consistent with the assignment of Q88M20 as a class II fumarase. No literature was found describing a different gene product under the *fumC* symbol that would create ambiguity. The gene symbol (*fumC*), organism (*P. putida* KT2440), protein family (class-II fumarase/aspartase), and InterPro domains (Fum_hydII IPR005677; Fumarase_C_C IPR018951; Fumarate_lyase_CS IPR020557) all align.

Limitations and Knowledge Gaps

1. **No direct biochemical characterization of Q88M20 itself.** The functional assignment rests on curated annotation, sequence homology, and structural/mechanistic studies of orthologs (*E. coli* FumC, *M. tuberculosis* Rv1098c). No published kinetic parameters (kcat, Km for fumarate/L-malate), no purified-protein assay, and no crystal structure specific to the *P. putida* KT2440 enzyme were identified. Function is inferred by homology, albeit at high confidence (~61% identity, intact catalytic residues).
2. **Localization is inferred, not experimentally demonstrated.** Cytoplasmic localization is the UniProt/HAMAP annotation and is consistent with the soluble, non-membrane nature of the class II fumarase fold and its TCA-cycle role, but no proteomic or fluorescence-based localization study specific to PP_1755 was found.
3. **Physiological division of labor between the isozymes is uncharacterized in *P. putida*.** KT2440 encodes two class II fumarases (Q88M20 and *fumC-I*/Q88PA6) plus a class I fumarase (Q88PF3). The specific growth conditions under which each is expressed, their relative flux contributions, and any regulatory hierarchy (analogous to the SoxR/ArcA/FNR control in *E. coli*) have not been directly established for *P. putida*.

4. **Regulatory inference is cross-species.** The oxidative-stress/iron-limitation induction and SoxR dependence are documented in *E. coli*. Whether the same regulatory logic governs *P. putida* PP_1755 remains to be verified experimentally.
5. **Substrate-specificity boundaries not directly tested for the *P. putida* enzyme.** Family-level specificity (fumarate/(S)-malate; exclusion of maleate and non-cognate dicarboxylates) is inferred from the conserved active site and from characterized orthologs, not measured for Q88M20.

Proposed Follow-up Experiments / Actions

1. **Recombinant expression and steady-state kinetics.** Clone PP_1755, express and purify the His-tagged protein, and measure kcat and Km for fumarate hydration and L-malate dehydration. Confirm stereospecificity ((S)- vs (R)-malate) and test candidate non-substrates (maleate, D-malate, aspartate) to delimit specificity.
2. **Iron-independence and thermostability assays.** Verify absence of [Fe-S] cluster (UV-vis, iron content, EPR) and measure thermal stability, directly confirming the class II designation for the *P. putida* enzyme.
3. **Structural determination.** Solve the crystal or cryo-EM structure of Q88M20 (apo and with malate/inhibitor) to confirm the homotetramer, the inter-subunit active site, and the His186/Ser316 catalytic geometry. Alternatively, validate an AlphaFold model against the *E. coli* FumC template using structural-biology tooling.
4. **Site-directed mutagenesis of predicted catalytic residues.** Mutate His186 and Ser316 (and site-B residues) and measure the loss of activity to experimentally confirm their catalytic roles in the *P. putida* enzyme.
5. **Genetic dissection of isozyme roles.** Construct single and combinatorial deletions of PP_1755, *fumC-I* (Q88PA6), and the class I fumarase (Q88PF3), and assess growth on TCA-cycle-dependent carbon sources under aerobic, oxidative-stress (paraquat), and iron-limited conditions to define each isozyme's physiological niche.
6. **Expression profiling under stress.** Use RT-qPCR or RNA-seq to test whether PP_1755 is induced by superoxide generators and iron chelation, probing whether the *E. coli*-like SoxR/oxidative-stress regulation is conserved in *P. putida*.

Conclusion

The gene *fumC* (Q88M20, PP_1755) of *Pseudomonas putida* KT2440 encodes **fumarate hydratase class II (fumarase C, EC 4.2.1.2)**, a **cytoplasmic, iron-independent, heat-stable homotetramer** that catalyzes the **reversible, stereospecific hydration of fumarate to L-(S)-malate** as the fumarate→malate step of the **TCA cycle**. Its active sites are shared across subunits and employ a conserved His186/Ser316 acid-base pair and a mobile SS-loop to perform general acid/base *anti* addition of water. As a class II enzyme it functions as an aerobic, oxidant-resistant fumarase. The assignment is supported by curated annotation, ~61% identity to the biochemically characterized *E. coli* FumC with 100% conservation of the class II catalytic motif and residues, and by structural/mechanistic studies of orthologs — while acknowledging that no direct biochemical characterization of the *P. putida* enzyme itself has yet been published.