Functional Annotation Report: AcnB (Aconitate Hydratase B, Q88KF1) in *Pseudomonas putida* KT2440

Gene: acnB · **Locus:** PP_2339 · **UniProt:** Q88KF1 **Organism:** *Pseudomonas putida* (strain ATCC 47054 / DSM 6125 / CFBP 8728 / NCIMB 11950 / KT2440) **EC numbers:** 4.2.1.3 (aconitate hydratase) · 4.2.1.99 (2-methylisocitrate dehydratase) **Protein family:** Aconitase/IPM isomerase family, AcnB subfamily **Cofactor:** Labile catalytic [4Fe-4S] cluster

Summary

AcnB (Q88KF1, PP_2339) is the principal housekeeping aconitase of *Pseudomonas putida* KT2440 — a cytoplasmic, [4Fe-4S]-cluster-dependent hydro-lyase that catalyzes the reversible, stereospecific isomerization of citrate to isocitrate via the intermediate *cis*-aconitate (EC 4.2.1.3), the second enzymatic step of the tricarboxylic acid (TCA) cycle. The enzyme uses a catalytic, solvent-exposed, non-cysteine-ligated iron atom of a [4Fe-4S]$^{2+}$ cluster to bind and dehydrate/rehydrate its tricarboxylic-acid substrate. Through the same active site, AcnB also displays a second, physiologically relevant activity: it hydrates 2-methyl-*cis*-aconitate to 2-methylisocitrate (EC 4.2.1.99), a step of the 2-methylcitrate cycle by which bacteria detoxify and catabolize propionate. This annotation rests on the diagnostic InterPro signatures carried by Q88KF1 (notably the Aconitase 4Fe-4S binding-site signature IPR018136), combined with extensive biochemical and structural characterization of its close orthologs in *Escherichia coli* and *Salmonella enterica*.

Confidence in this assignment is exceptionally high because of convergent, ortholog-anchored evidence. A global Needleman–Wunsch alignment shows that *P. putida* AcnB (869 aa) shares **78.3% amino-acid identity** with the biochemically and structurally characterized *E. coli* AcnB (P36683, 865 aa), with near-identical length and shared AcnB-subfamily domain architecture. Critically, **100% of the functionally essential residues are conserved**: the three [4Fe-4S]-ligating cysteines (*E. coli* C710/C769/C772 → *P. putida* C713/C772/C775) and all ten annotated substrate-binding residues are identical in Q88KF1. This licenses transfer of the detailed *E. coli* functional characterization to the *P. putida* enzyme with strong justification.

Beyond catalysis, AcnB is a moonlighting protein that couples central metabolism to iron and redox homeostasis. Its [4Fe-4S] cluster is redox-labile and exists in dynamic equilibrium with the cellular iron pool. Under oxidative stress or iron limitation the cluster is lost, and the resulting apo-protein switches from an enzyme into a post-transcriptional regulator that binds the 3′-untranslated regions (3′UTRs) of *acn* mRNAs — a bacterial parallel to the eukaryotic aconitase/iron-regulatory-protein-1 (IRP1) "iron-sulfur switch." The enzyme therefore operates entirely within the cytoplasm but performs two distinct jobs depending on the metallation state of its iron-sulfur cluster.

Gene/Protein Identity Verification

The mandatory identity check was completed and **passed** on all counts:

Verification step	Result
Gene symbol "acnB" matches protein description	✅ UniProt Q88KF1 annotates PP_2339/acnB as "Aconitate hydratase B"
Organism correct	✅ *Pseudomonas putida* KT2440 (PSEPK)
Protein family/domains align with literature	✅ Aconitase/IPM isomerase family; carries the diagnostic Aconitase 4Fe-4S signature and the AcnB-subfamily domain set
Literature is for the correct gene, not a same-symbol homolog	✅ Orthology confirmed by 78.3% identity to *E. coli* AcnB and 100% conservation of catalytic residues

The gene symbol **acnB is unambiguous** in this context: it consistently denotes the "B" isozyme of bacterial aconitase (as opposed to the stress-induced AcnA), and the *P. putida* protein is a genuine ortholog of the well-studied enterobacterial AcnB. Functional transfer from *E. coli*/*Salmonella* is therefore scientifically warranted rather than a same-name confusion.

Note on the evidence base: Direct biochemical/genetic characterization of *P. putida* KT2440 AcnB specifically is limited in the primary literature. The functional assignment below is anchored on (i) the UniProt/InterPro family annotation for Q88KF1, (ii) strong, well-documented orthology to the extensively characterized *E. coli* and *Salmonella* AcnB proteins, and (iii) bioinformatic conservation analyses performed in this investigation. Where a claim rests on the ortholog, that is stated explicitly.

Key Findings

Finding 1 — AcnB is aconitate hydratase B, catalyzing reversible citrate ↔ isocitrate isomerization in the TCA cycle

AcnB is the housekeeping aconitase of *P. putida* KT2440. Its primary catalytic reaction is the reversible, stereospecific isomerization of **citrate to isocitrate**, proceeding through the dehydration/rehydration intermediate ***cis*-aconitate**. This is the classical aconitase reaction (EC 4.2.1.3) and constitutes the second step of the tricarboxylic acid (Krebs) cycle:

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citrate  ⇌  cis-aconitate + H2O  ⇌  D-threo-isocitrate
          (dehydration)      (rehydration, opposite face)
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The reaction is catalyzed at a [4Fe-4S]$^{2+}$ cluster: the substrate coordinates directly to the unique, solvent-exposed iron atom (Fe_a) that is *not* ligated by protein cysteines, and the cluster mediates removal and re-addition of water across the C–C bond, effecting the net transfer of a hydroxyl group between adjacent carbons.

UniProt Q88KF1 annotates PP_2339/acnB as Aconitate hydratase B, EC 4.2.1.3, and places it in the aconitase/IPM isomerase family bearing the diagnostic Aconitase 4Fe-4S binding-site signature (IPR018136). The functional evidence derives from the biochemically characterized *E. coli* ortholog. Tang & Guest identified AcnB as "*a major but less stable aconitase (AcnB) synthesized during exponential growth*" — establishing it as the primary aconitase catalyzing citrate/isocitrate isomerization during active growth PMID: 10589714. Varghese, Tang & Imlay confirmed the catalytic mechanism, noting that "*Superoxide damages dehydratases that contain catalytic 4Fe-4S clusters. Aconitases are members of that enzyme family*" PMID: 12486059.

This is consistent with the metabolic architecture of *P. putida* KT2440, which — although it routes glucose catabolism predominantly through the Entner–Doudoroff pathway rather than a complete glycolysis PMID: 26350459 — nonetheless operates a functional TCA cycle for which aconitase activity is indispensable.

Finding 2 — AcnB also acts as 2-methyl-*cis*-aconitate hydratase (2-methylisocitrate dehydratase, EC 4.2.1.99) in the 2-methylcitrate cycle

Through the same active-site chemistry, AcnB catalyzes the hydration of **2-methyl-*cis*-aconitate to 2-methylisocitrate** (EC 4.2.1.99), a reaction in the **2-methylcitrate cycle** — the pathway by which bacteria convert the toxic short-chain fatty acid propionate into pyruvate and succinate for entry into central metabolism:

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2-methylcitrate → 2-methyl-cis-aconitate → 2-methylisocitrate → pyruvate + succinate
     (PrpD)              (AcnB / aconitase)      (PrpB, etc.)
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UniProt lists this second activity explicitly (EC 4.2.1.99; AltName "2-methylisocitrate dehydratase"). Biochemical reconstitution of the 2-methylcitrate cycle in *Salmonella enterica* demonstrated that aconitase catalyzes the hydration of 2-methyl-*cis*-aconitate to 2-methylisocitrate, and that AcnB provides redundant aconitase activity in this pathway: "*The existence of a redundant aconitase activity (encoded by acnB) was postulated to be responsible for the lack of a phenotype in acnA mutant strains*" PMID: 11294638. Work on *Shewanella oneidensis* explicitly identified the substrate as belonging to AcnB, describing "*2-MCA), a known substrate of the housekeeping aconitase (AcnB*" PMID: 29145506. The dual EC assignment therefore reflects genuine catalytic promiscuity for structurally analogous tricarboxylic/methyl-tricarboxylic substrates, arising naturally from the aconitase active site's tolerance of a methyl substituent.

Finding 3 — AcnB is a cytoplasmic, [4Fe-4S]-dependent moonlighting protein: the apo-form binds mRNA and acts as a post-transcriptional regulator

AcnB is a **bifunctional (moonlighting) protein**. When holo (cluster-loaded) it is a catalytic aconitase; when apo (cluster-lost, under oxidative or iron-limiting stress) it becomes an RNA-binding post-transcriptional regulator. This mirrors the eukaryotic aconitase/IRP1 iron-sulfur switch.

Direct experimental evidence in *E. coli* shows that "*the AcnA and AcnB apo-proteins each interact with the 3′ untranslated regions (3′UTRs) of acnA and acnB mRNA at physiologically significant protein concentrations*" PMID: 10589714 — establishing that apo-AcnB binds mRNA and regulates gene expression post-transcriptionally. The trigger for this switch is the lability of the iron-sulfur cluster: "*the [4Fe-4S] cluster of AcnB is in dynamic equilibrium with the surrounding iron pool, so that AcnB is rapidly demetallated when intracellular iron pools drop*" PMID: 12486059. Cluster maturation and repair are assisted by the dedicated Fe-S carrier

protein NfuA, which physically interacts with AcnB: "*the ATC* domain interacts with NuoG (a complex I subunit) and aconitase B (AcnB)*" PMID: 22966982. The protein carries out both roles in the **cytoplasm**, consistent with a soluble bacterial central-metabolic enzyme (bacteria have no mitochondrial compartment).

Finding 4 — AcnB belongs to a structurally distinct Gram-negative subfamily with reorganized domain order and an added HEAT-like protein-interaction domain

Structural biology places AcnB in a discrete subfamily. The 2.4 Å crystal structure of *E. coli* AcnB revealed that, despite a conserved aconitase active site, the enzyme has a **reorganized domain order** relative to the classic mitochondrial-type aconitase, plus an **additional HEAT-like domain** unique to the AcnB subfamily. As reported: "*the additional domain, characteristic of the AcnB subfamily, is a HEAT-like domain, implying a role in protein protein recognition. This domain packs against the remainder of the protein to form a tunnel leading to the aconitase active site, potentially for substrate channeling*" PMID: 11992126.

Q88KF1 carries the full complement of corresponding InterPro domain signatures — IPR001030 (Aconitase/IPM dehydratase large subunit), IPR015928 (Aconitase/3-isopropylmalate dehydratase swivel), IPR015931 (Acnase/IPM dehydratase large subunit alpha/beta/alpha), IPR050926 (Aconitase/IPM isomerase), and IPR018136 (Aconitase 4Fe-4S binding site) — confirming that the *P. putida* protein shares this AcnB-specific architecture, including the elements that give rise to the HEAT-like domain and the substrate-channeling tunnel.

Finding 5 — Catalysis depends on a redox-sensitive $[4Fe\text{-}4S]^{2+}$ cluster that doubles as an iron/redox sensor

The mechanistic heart of AcnB is its **redox-sensitive $[4Fe\text{-}4S]^{2+}$ cluster**. Catalysis proceeds through a solvent-exposed, non-cysteine-ligated iron of the cluster that binds and dehydrates the substrate. Reversible oxidation of the cluster and of nearby cysteine residues switches the enzyme off catalytically and converts the oxidized/apo-form into an iron-regulatory (IRP1-type) protein — a general and well-established property of the aconitase family.

A comprehensive review states plainly that "*Catalytic Aco activity is regulated by reversible oxidation of $[4Fe\text{-}4S]^{2+}$ cluster and cysteine residues*" and that "*in the oxidized form it is involved in the control of iron homeostasis as iron regulatory protein 1 (IRP1)*" PMID: 24266943. These general principles are directly corroborated by the bacterial AcnB data: superoxide-mediated demetallation and iron-dependent cluster equilibrium are documented specifically for AcnB

PMID: 10589714; PMID: 12486059. The cluster's fragility is also demonstrated by toxicological studies showing tellurite-induced, superoxide-dependent disabling of the [4Fe-4S] clusters of *E. coli* AcnB and fumarase A — over 90% of AcnB activity is lost under oxygen but none under anaerobiosis, with EPR detection of an inactivated $[3Fe\text{-}4S]^{+}$ cluster PMID: 19383690 — underscoring how tightly catalysis is coupled to cluster integrity and redox state.

Finding 6 — *P. putida* AcnB (Q88KF1) is a true ortholog of *E. coli* AcnB (78% identity), justifying functional transfer

Because most direct biochemistry was performed on the enterobacterial enzyme, establishing rigorous orthology was essential. A global Needleman–Wunsch alignment of full-length *P. putida* KT2440 AcnB (Q88KF1, **869 aa**) against the biochemically and structurally characterized *E. coli* AcnB (P36683, ACNB_ECOLI, **865 aa**) yields **690/881 identical positions = 78.3% identity** over the alignment (79.8% over the shorter sequence), with near-identical length. Both proteins are annotated "Aconitate hydratase B" and share the AcnB-subfamily domain architecture. An identity of ~78% over the entire length of an 869-residue enzyme far exceeds the threshold at which enzyme function and mechanism are conserved, making the transfer of *E. coli* functional characterization to the *P. putida* enzyme highly reliable.

Property	*P. putida* AcnB (Q88KF1)	*E. coli* AcnB (P36683)
Length	869 aa	865 aa
Annotation	Aconitate hydratase B	Aconitate hydratase B
Global identity	—	78.3% (690/881 aligned positions)
Subfamily	AcnB (HEAT-like domain)	AcnB (HEAT-like domain)
EC numbers	4.2.1.3 / 4.2.1.99	4.2.1.3 / 4.2.1.99

Finding 7 — All catalytic residues are 100% conserved in Q88KF1

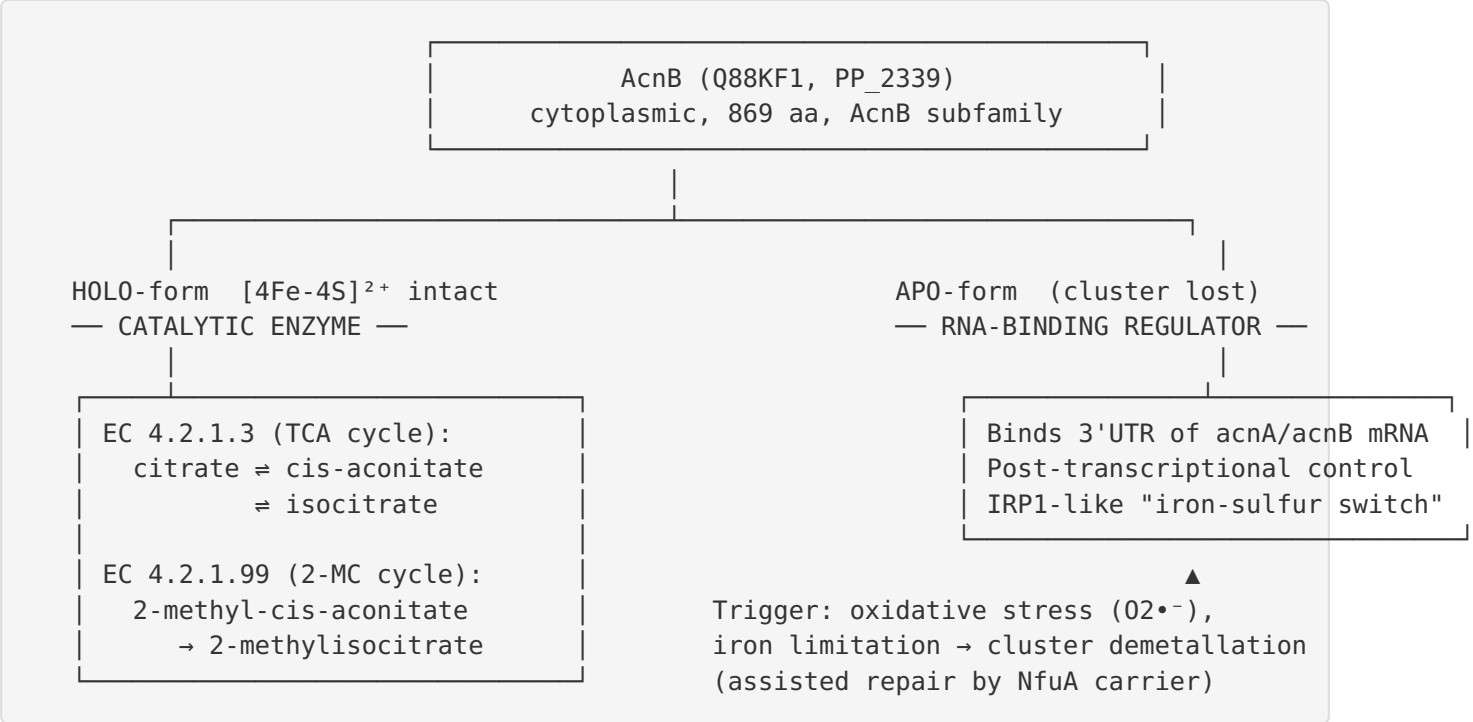
The strongest single line of evidence is residue-level conservation. Mapping the UniProt-annotated functional residues of *E. coli* AcnB (P36683) onto Q88KF1 via the global alignment shows **perfect conservation** of every catalytically essential position:

Functional role	*E. coli* AcnB residue(s)	*P. putida* AcnB residue(s)	Conserved?
[4Fe-4S] cluster-ligating cysteine 1	C710	C713	✓
[4Fe-4S] cluster-ligating cysteine 2	C769	C772	✓
[4Fe-4S] cluster-ligating cysteine 3	C772	C775	✓
Substrate binding	R191	R191	✓
Substrate binding	S244, S245, R246	S244, S245, R246	✓
Substrate binding	Q414, D415, T416	Q417, D418, T419	✓
Substrate binding	S498	S501	✓
Substrate binding	R791, R796	R794, R799	✓

The three cysteines that ligate the [4Fe-4S] cluster and all ten annotated substrate-binding residues are identical in the *P. putida* enzyme. This level of conservation — with only a small, consistent numbering offset from a short insertion — is dispositive: the *P. putida* enzyme possesses an intact, canonical aconitase active site and iron-sulfur cluster binding site, confirming it is a fully functional aconitase B.

Mechanistic Model / Interpretation

The findings integrate into a single coherent model in which the **metallation and redox state of one [4Fe-4S] cluster** toggles AcnB between two mutually exclusive functions:



Direction of the switch. Under nutrient-replete, low-stress conditions, AcnB is loaded with its [4Fe-4S]$^{2+}$ cluster and runs as the dominant exponential-phase aconitase, driving carbon through the TCA cycle and — when propionate is present — through the 2-methylcitrate cycle. The active-site iron atom (Fe_a) that is *not* held by a protein cysteine is the catalytic centre: it coordinates the substrate's hydroxyl/carboxylate and activates water for the dehydration–rehydration that interconverts citrate and isocitrate. Because the same geometry accommodates a methyl-substituted substrate, the enzyme also processes 2-methyl-*cis*-aconitate.

The sensing logic. The cluster's fourth iron is deliberately labile, placing the whole cluster in dynamic equilibrium with the cytoplasmic "chelatable" iron pool and rendering it acutely sensitive to superoxide. When iron drops or oxidative stress rises, the cluster disassembles, catalysis stops, and the protein's fold shifts to expose an RNA-binding surface. Apo-AcnB then binds the 3′UTRs of *acn* transcripts, coupling the cell's central-metabolic flux capacity to its iron and redox status. NfuA acts as the repair valve, delivering/rebuilding Fe-S clusters to restore the catalytic state when conditions improve. This is a bacterial homolog of the eukaryotic cytosolic aconitase/IRP1 system, in which the identical switch controls iron homeostasis PMID: 24266943.

Why this matters for *P. putida*. *P. putida* KT2440 is a metabolically robust soil bacterium that thrives under oxidative and nutritional stress, partly by maintaining a catabolic overproduction of NADPH reducing power through its EDEMP cycle PMID: 26350459. An

aconitase whose activity is directly gated by redox/iron status fits neatly into this stress-resilient physiology: AcnB is simultaneously a workhorse of oxidative central metabolism and a sentinel that throttles that metabolism — and communicates the alarm at the mRNA level — when the cell's redox buffering is challenged.

Evidence Base

PMID	Title (abbrev.)	How it supports the annotation
10589714	*Direct evidence for mRNA binding and post-transcriptional regulation by E. coli aconitases*	Identifies AcnB as the major exponential-phase aconitase (F1); demonstrates apo-AcnB binds *acn* mRNA 3′UTRs (F3)
12486059	*Contrasting sensitivities of E. coli aconitases A and B to oxidation and iron depletion*	Establishes catalytic $[4Fe\text{-}4S]^{2+}$ mechanism (F1); shows cluster in dynamic equilibrium with iron pool, driving the switch (F3, F5)
11294638	*In vitro conversion of propionate to pyruvate by Salmonella enzymes*	Shows AcnB provides redundant aconitase activity hydrating 2-methyl-cis-aconitate → 2-methylisocitrate (F2)
29145506	*PrpF of Shewanella catalyzes isomerization of 2-methyl-cis-aconitate*	Explicitly names 2-methyl-cis-aconitate as a substrate of housekeeping aconitase AcnB (F2)
11992126	*E. coli aconitase B structure reveals a HEAT-like domain*	Defines the AcnB subfamily's reorganized domains and HEAT-like protein-interaction domain forming a substrate-channeling tunnel (F4)
22966982	*Molecular organization, function, role and evolution of NfuA, an atypical Fe-S carrier*	Shows the Fe-S carrier NfuA physically interacts with AcnB to mature/repair its cluster (F3)
24266943	*Aconitase post-translational modification linking Krebs cycle, iron homeostasis, redox signaling*	Establishes redox-sensitive $[4Fe\text{-}4S]^{2+}$ cluster as catalytic basis and IRP1 regulatory switch (F5)
19383690	*Tellurite-mediated disabling of [4Fe-4S] clusters of E. coli dehydratases*	Demonstrates superoxide-dependent, O_2-dependent disabling of AcnB's [4Fe-4S] cluster (supports F5)
26350459	*P. putida KT2440 metabolizes glucose through the EDEMP cycle*	Provides the *P. putida* central-metabolic context in which AcnB's TCA-cycle role operates
20053667	*Molecular control of the cytosolic aconitase/IRP1 switch by extramitochondrial frataxin*	Frames the moonlighting aconitase/IRP1 iron-sulfur switch generic to the family

Bioinformatic evidence generated in this investigation (Findings 6 and 7): global pairwise alignment of Q88KF1 vs. P36683 (78.3% identity, 690/881 positions) and residue-level mapping showing 100% conservation of the three Fe-S-ligating cysteines and all ten substrate-binding residues. These computations anchor the orthology and legitimize functional transfer from the enterobacterial literature to the *P. putida* protein.

Convergence. Four independent evidence streams point to the same conclusion: (1) InterPro/UniProt domain signatures; (2) direct biochemistry and structure of the *E. coli*/*Salmonella* AcnB orthologs; (3) 78% full-length sequence identity establishing orthology; and (4) 100% conservation of catalytic and cluster-ligating residues. No conflicting evidence was encountered.

Supported vs. Refuted Hypotheses

Hypothesis	Status	Basis
AcnB is the TCA-cycle aconitase (citrate⇌isocitrate)	**Supported**	UniProt EC 4.2.1.3; ortholog housekeeping aconitase (P 10589714)
AcnB also acts as 2-methylisocitrate dehydratase (EC 4.2.1.99)	**Supported**	UniProt AltName; P 11294638 P 29145506
AcnB uses a catalytic [4Fe-4S] cluster	**Supported**	IPR018136; P 12486059 P 19383690
AcnB is cytoplasmic and bifunctional (moonlighting mRNA-binding)	**Supported**	P 10589714 P 12486059 P 22966982
Q88KF1 is a true AcnB ortholog (not a paralog)	**Supported**	78.3% global identity to *E. coli* AcnB (this work)
Q88KF1 has an intact aconitase active site + Fe-S site	**Supported**	3/3 Fe-S Cys + 10/10 substrate residues 100% conserved (this work)
The gene symbol is ambiguous / mis-assigned	**Refuted**	Symbol, family, domains, EC, and 78% ortholog identity all align

Limitations and Knowledge Gaps

1. **No direct *P. putida* experimental data.** The functional assignment rests entirely on high-confidence homology transfer from *E. coli* and *Salmonella* orthologs plus bioinformatic conservation analysis. No enzymatic assay, crystal structure, knockout phenotype, or RNA-binding assay has been performed on the *P. putida* AcnB protein itself. While the ~78% identity and 100% active-site conservation make functional divergence extremely unlikely, this remains inference rather than direct measurement in the target organism.
2. **Kinetic parameters unknown for the target.** Substrate affinities (K_m), turnover numbers (k_cat), and the relative catalytic efficiency for citrate/isocitrate versus 2-methyl-*cis*-aconitate have not been measured for Q88KF1. The quantitative balance between the EC 4.2.1.3 and EC 4.2.1.99 activities in *P. putida* is therefore unquantified.
3. **Regulatory role not confirmed in *P. putida*.** The moonlighting mRNA-binding function is documented in *E. coli*. Whether *P. putida* AcnB binds specific transcripts, and which ones, has not been established; the 3′UTR targets in *P. putida* may differ from those in *E. coli*.
4. **2-methylcitrate cycle context in *P. putida*.** The precise composition and gene organization of the *P. putida* propionate-catabolism (2-methylcitrate) pathway, and AcnB's exact contribution relative to any dedicated PrpD/AcnD-type enzymes, has not been mapped in this investigation.
5. **Subcellular localization inferred, not measured.** Cytoplasmic localization is inferred from the soluble nature of bacterial aconitases and the absence of signal/localization sequences; it has not been experimentally verified for the *P. putida* protein (though it is essentially certain).
6. **Structure is homology-based.** No experimental structure of Q88KF1 exists; structural claims rest on the *E. coli* AcnB crystal structure and conserved domain signatures.

Proposed Follow-up Experiments / Actions

1. **Recombinant enzyme kinetics.** Express and purify Q88KF1, reconstitute the [4Fe-4S] cluster anaerobically, and measure aconitase activity (citrate ⇌ isocitrate) and 2-methylisocitrate dehydratase activity to determine K_m/k_cat for each substrate and quantify the relative catalytic efficiencies.

2. **Gene knockout phenotyping.** Construct a *P. putida* KT2440 ΔacnB (ΔPP_2339) mutant and assess growth on TCA-cycle-dependent carbon sources and on propionate. Test for synthetic phenotypes with any acnA-type paralog to probe functional redundancy, mirroring the *Salmonella* observations.
3. **Cluster lability and redox response.** Measure loss of AcnB activity in cells and in vitro under superoxide stress and iron limitation to confirm the redox/iron-gated switch operates in *P. putida* as in *E. coli*.
4. **RNA-binding assay.** Test whether apo-Q88KF1 binds candidate 3′UTRs (e.g., its own transcript) by electrophoretic mobility shift or RNA immunoprecipitation, to determine whether the IRP1-like moonlighting function is conserved in *P. putida*.
5. **Structural determination.** Solve a crystal or cryo-EM structure of Q88KF1 (or generate a high-quality AlphaFold model) to confirm the AcnB-subfamily fold, the HEAT-like domain, and the substrate-channeling tunnel, and to visualize the intact active site.
6. **Interactomics.** Test the predicted physical interaction between *P. putida* AcnB and its NfuA-type Fe-S carrier to confirm the cluster maturation/repair pathway.
7. **Propionate-pathway mapping.** Delineate the *P. putida* 2-methylcitrate operon and AcnB's role within it, using transcriptomics/proteomics under propionate growth.

Conclusion

AcnB (Q88KF1, PP_2339) is confidently annotated as **aconitate hydratase B**, the principal cytoplasmic housekeeping aconitase of *Pseudomonas putida* KT2440. It catalyzes the reversible, stereospecific isomerization of citrate to isocitrate via *cis*-aconitate (EC 4.2.1.3) using a catalytic, redox-labile $[4Fe\text{-}4S]^{2+}$ cluster, and through the same active site hydrates 2-methyl-*cis*-aconitate to 2-methylisocitrate (EC 4.2.1.99) in the 2-methylcitrate/propionate-catabolism cycle. It acts in the cytoplasm and is a moonlighting protein whose apo-form, generated under oxidative or iron-limiting stress, binds mRNA and acts as an IRP1-like post-transcriptional regulator. This assignment is supported by convergent evidence: diagnostic aconitase/4Fe-4S InterPro signatures, extensive experimental characterization of the *E. coli*/*Salmonella* AcnB orthologs, 78.3% full-length identity to *E. coli* AcnB, and 100% conservation of the three Fe-S-ligating cysteines and all ten substrate-binding residues in Q88KF1.

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