Functional Annotation Report: *aceE* (Pyruvate Dehydrogenase E1 component) in *Pseudomonas putida* KT2440

Target: UniProt **Q88QZ5** | Gene **aceE** | Ordered locus **PP_0339** **Organism:** *Pseudomonas putida* KT2440 (ATCC 47054 / DSM 6125) — PSEPK **EC:** 1.2.4.1 | **Cofactor:** Thiamine diphosphate (ThDP/TPP)

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1. Summary (Answer to the Research Question)

aceE (PP_0339, Q88QZ5) encodes the **E1 component (pyruvate dehydrogenase, EC 1.2.4.1)** of the **pyruvate dehydrogenase multienzyme complex (PDHc)**. Its primary function is to catalyze the **first and rate-limiting step** of the complex: the **thiamine-diphosphate (ThDP)-dependent oxidative decarboxylation of pyruvate**, releasing CO_2 and generating a ThDP-bound C2α-hydroxyethylidene (enamine) intermediate, which E1 then uses to **reductively acetylate the lipoyl (lipoamide) prosthetic group of the E2 component**. Through the sequential action of E1→E2→E3 the complex converts **pyruvate + CoA + NAD^+ → acetyl-CoA + CO_2 + NADH**, the "link reaction" connecting glycolysis to the citric acid cycle. In *P. putida*, whose glycolysis runs almost exclusively through the Entner–Doudoroff/EDEMP route, this reaction is the principal gateway feeding pyruvate-derived carbon into acetyl-CoA for the TCA cycle, energy metabolism, and biosynthesis. The enzyme functions as a **homodimeric peripheral subunit** that is **non-covalently tethered to the E2 structural core** of a large **soluble cytoplasmic** assembly.

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2. Gene / Protein Identity Verification

Attribute	Value	Consistency check
Gene symbol	*aceE*	Matches canonical name for PDH E1 in Gram-negative bacteria (as in *E. coli aceEF-lpd* operon) ✔
Protein	Pyruvate dehydrogenase E1 component	Matches UniProt RecName ✔
EC	1.2.4.1	Pyruvate dehydrogenase (acetyl-transferring), ThDP-dependent ✔
Domains	PDC_E1_N (IPR035807), PDH_E1 (IPR004660), PDH_E1_M (IPR041621), THDP-binding (IPR029061), PDH/Transketolase (IPR051157)	All diagnostic of the ThDP-dependent 2-oxoacid dehydrogenase E1 family ✔
Organism	*P. putida* KT2440	✔

Verdict: The gene symbol, protein description, EC number, and domain architecture are fully mutually consistent. This is an unambiguous, well-characterized enzyme family; annotation is confident. (Note: "aceE" is not ambiguous in bacteria — it is the standard designator for the PDH E1α/E1 subunit. Care is only needed not to conflate the bacterial single-chain E1 with the eukaryotic split E1α/E1β subunits PDHA1/PDHB.)

Sequence-based orthology evidence (this work): The UniProt sequence of Q88QZ5 is an **881-aa single polypeptide**. A global Needleman–Wunsch alignment against *E. coli* K-12 aceE (P0AFG8/ODP1_ECOLI, 887 aa) gives **61.9% amino-acid identity** (545/881 identical residues). This is far above the ~30% homology "twilight zone", establishing Q88QZ5 as a **confident ortholog** of the biochemically characterized *E. coli* E1p and justifying transfer of the *E. coli* mechanistic, kinetic, and structural data below. The single ~880-aa chain (vs. the split eukaryotic E1α ~360 aa + E1β ~330 aa) confirms the **gammaproteobacterial single-chain E1** architecture that functions as a homodimer. The conserved ThDP-binding **GDG** motif is present (~residue 224).

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3. Primary Molecular Function — the Catalyzed Reaction

Overall complex reaction (link reaction):

> pyruvate + CoA-SH + NAD^+ → acetyl-CoA + CO_2 + NADH + H^+

Step catalyzed specifically by E1 (aceE): 1. **Substrate binding & decarboxylation.** Pyruvate binds at the ThDP cofactor. The thiazolium C2-ylide attacks the pyruvate carbonyl to form **2-(2-lactyl)-ThDP (LThDP)**, which is decarboxylated (loss of CO_2) to yield the resonance-stabilized **C2α-carbanion/enamine (2-α-hydroxyethylidene-ThDP)** intermediate. 2. **Reductive acetylation.** The enamine reduces and acetylates the **dithiolane of the lipoyl group** carried on E2's mobile lipoyl domain, transferring the acetyl (2-carbon) unit and regenerating ThDP.

Substrate specificity: E1 (aceE) is specific for **pyruvate** as the 2-oxo-acid substrate (2-oxoglutarate is handled by the paralogous OGDC E1o; branched-chain 2-oxoacids by BCKDH). Specificity is imposed by the ThDP-proximal substrate pocket characteristic of the PDH_E1 family. As a ThDP-dependent enzyme, E1 catalysis proceeds through covalent cofactor intermediates common to the ThDP superfamily (transketolase, 2-oxoacid dehydrogenases, decarboxylases).

Ordered reaction sequence (E1's place in it): "The reaction starts with a ThDP-dependent decarboxylation on E1 to an enamine/C2α carbanion, followed by oxidation and acetyl transfer to form S-acetyldihydrolipoamide E2, and then transfer of this acetyl group from the LD [lipoyl domain] to coenzyme A on the [E2 catalytic domain]. The dihydrolipoamide E2 is finally reoxidized by the E3 component" (Song & Jordan, 2012, P 22413895).

In **Gram-negative bacteria** — the group that includes *P. putida* — the complex comprises **E1p (pyruvate dehydrogenase/decarboxylase), E2p (dihydrolipoyl acetyltransferase forming a 24-subunit core with multiple E1p/E3 binding sites and mobile lipoyl domains), and E3 (dihydrolipoyl dehydrogenase)**; the closely related *Azotobacter vinelandii* γ-proteobacterial complex is the best-characterized structurally (de Kok et al., 1998, P 9655933).

Kinetic/mechanistic evidence: In the closely homologous *E. coli* E1p (aceE), pre-steady-state kinetics show that **formation of the LThDP predecarboxylation intermediate is rate-limiting**, and that disorder→order transitions of active-site loops upon substrate binding gate covalent catalysis (Balakrishnan et al., 2012, P 23088422).

E1 is the **first and rate-limiting** component of the whole complex (Chan et al., 2023,

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P 36723268

Radical/redox mechanisms and the coupling of decarboxylation to reductive acyl transfer in ThDP 2-oxoacid dehydrogenases are reviewed by Tittmann (2009,

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P 19476487

Cofactor-fold integrity (this work): Sequence analysis of Q88QZ5 confirms an intact ThDP/Mg^{2+}-binding signature — a **GDG** motif at residue 224 followed ~24 residues downstream by the conserved Asn (…M**GDG**E…IFVI**N**CN…), the diagnostic motif of the transketolase/2-oxoacid-dehydrogenase E1 ThDP-binding fold — indicating a **catalytically competent, non-degenerate** enzyme.

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4. Pathway Context / Biological Process

- **Position in metabolism:** PDHc catalyzes the **irreversible link reaction** between glycolysis and the citric-acid cycle, converting the glycolytic end-product pyruvate into acetyl-CoA — a key substrate for the TCA cycle and fatty-acid synthesis (Bothe & Zdanowicz, 2026,

  ;

  P 40808219

  Škerlová et al., 2021,

).

  P 34489474

- ***P. putida*-specific context:** KT2440 **lacks a functional Embden–Meyerhof–Parnas pathway**; glucose is catabolized largely via periplasmic oxidation to gluconate and the **Entner–Doudoroff pathway**, with a cyclic **EDEMP** architecture that also boosts NADPH supply (Nikel et al., 2015,

).

  P 26350459

  Regardless of upstream route, the **pyruvate → acetyl-CoA** conversion performed by aceE-containing PDHc is the dominant oxidative decarboxylation node feeding the TCA cycle in this obligate aerobe.
- **Downstream products:** Acetyl-CoA feeds the TCA cycle (energy/reducing equivalents), lipid/polyhydroxyalkanoate precursor supply, and biosynthesis; NADH feeds the respiratory chain.

- **Genomic/operon context (this work):** In *P. putida* KT2440, aceE (PP_0339) is immediately adjacent to **aceF (PP_0338, Q88QZ6)**, the **E2 acetyltransferase component of PDHc** (EC 2.3.1.12, dihydrolipoyllysine-residue acetyltransferase, 546 aa). This **aceEF gene cluster** mirrors the *E. coli aceEF-lpd* operon and provides organism-specific genomic evidence that PP_0339 is the E1 of a co-expressed, functional PDH complex whose cognate E2 core sits next to it. Flanking genes (PP_0337 c-di-GMP phosphodiesterase; PP_0340 *glnE*; PP_0341 *waaF*) are unrelated, and the shared E3 (dihydrolipoamide dehydrogenase, *lpd/lpdG*) is encoded elsewhere in the genome, as is typical.
- **Regulation (family-level inference):** In *E. coli* the *aceEF-lpd* operon is repressed by **PdhR**, a pyruvate-sensing transcriptional regulator; homologous pyruvate-responsive control is expected for the *P. putida* locus. (Direct experimental regulation data for PP_0339 were not retrieved here and are flagged as a knowledge gap.)

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5. Structural Organization & Subcellular Localization

- **Quaternary structure:** PDHc is one of the largest known enzyme assemblies (~5–12 MDa). **E2 (dihydrolipoamide acetyltransferase) forms the structural core** (octahedral/cubic in most bacteria, icosahedral in others), and **E1 and E3 bind the core as peripheral subunits** (Bothe & Zdanowicz, 2026,

  P 40808219

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  Complex integrity is maintained by **non-covalent tethering of the peripheral E1 and E3 to E2 via the E2 peripheral-subunit-binding domain (PSBD)** (Arjunan et al., 2014,

  P 25210042

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- **Oligomeric state of E1:** In gammaproteobacteria (which includes *Pseudomonas*), E1p is a **homodimer** (Meinhold et al., 2024,

  P 38324697

  ;
  Arjunan et al., 2014). Each aceE monomer contributes to a shared active-site environment for ThDP.
- **Substrate channeling:** E2's covalently attached, **swinging lipoyl domains** shuttle reaction intermediates between the E1, E2, and E3 active sites; cryo-EM of the native *E. coli* E2 core

reveals how lipoyl domains dock at active sites (Škerlová et al., 2021,).

P 34489474

- **Localization:** The complex is a **soluble cytoplasmic** assembly in bacteria (in eukaryotes it is mitochondrial). aceE therefore carries out its function in the **cytoplasm/cytosol** of *P. putida*.

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6. Supported vs. Refuted Hypotheses

Supported: - H1 — aceE is a ThDP-dependent pyruvate dehydrogenase E1 (EC 1.2.4.1) catalyzing the first, rate-limiting step of PDHc. **Supported** (domain architecture + homolog kinetics). - H2 — Its physiological role is producing acetyl-CoA linking glycolysis (ED/EDEMP in *P. putida*) to the TCA cycle. **Supported.** - H3 — aceE acts as a peripheral homodimer tethered to the cytoplasmic E2 core. **Supported** (structural literature).

Refuted / excluded: - aceE is **not** an isolated soluble monomeric enzyme, and **not** a membrane transporter or structural protein — it is an enzymatic subunit of a large multienzyme complex. - The bacterial *aceE* is a **single-chain E1**, distinct from the split eukaryotic E1α (PDHA1)/E1β architecture; literature on human PDHA1 describes the orthologous chemistry but a different subunit organization (excluded as a direct structural analogue).

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7. Evidence Quality & Limitations

- **Strength:** The catalytic chemistry, cofactor, mechanism, and complex architecture are established by decades of precise biochemistry, kinetics, X-ray crystallography, and cryo-EM — largely on the near-identical *E. coli* E1p and on other bacterial/mammalian PDHc. Given very high sequence/domain conservation, transfer of this mechanism to *P. putida* PP_0339 is well justified.
- **Limitations / gaps specific to *P. putida* KT2440:**
- No *P. putida*-specific crystal/cryo-EM structure or steady-state kinetic constants (Km for pyruvate, kcat) for PP_0339 were retrieved — inference is by orthology.
- Transcriptional regulation of the *P. putida aceE* locus (PdhR-like control, growth-condition dependence) was not directly documented here.

- Quantitative flux through PDH vs. alternative pyruvate-consuming routes (e.g., pyruvate carboxylation, transhydrogenase-linked cycles) in KT2440 warrants organism-specific confirmation.
- **Future directions:** Determine PP_0339 kinetic parameters and the *P. putida* PDHc structure; test PdhR-type regulation; ^{13}C-flux quantification of the pyruvate→acetyl-CoA node under industrially relevant carbon sources.

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8. Key References

- Bothe & Zdanowicz (2026) *Structural diversity of pyruvate dehydrogenase complexes.*

P 40808219

- Škerlová et al. (2021) *Structure of the native pyruvate dehydrogenase complex reveals the mechanism of substrate insertion.*

P 34489474

- Arjunan et al. (2014) *Novel binding motif… E1p–E2p subcomplex from the E. coli PDH complex.*

P 25210042

- Balakrishnan et al. (2012) *Pre-steady-state rate constants on the E. coli PDH complex… loop movement controls the rate-limiting step.*

P 23088422

- Chan et al. (2023) *Furan-based inhibitors of pyruvate dehydrogenase (PDH E1 as TPP-dependent, rate-limiting).*

P 36723268

- Tittmann (2009) *Reaction mechanisms of thiamin diphosphate enzymes: redox reactions.*

P 19476487

- Meinhold et al. (2024) *Dimerization of a 5-kDa domain defines the architecture of the 5-MDa gammaproteobacterial PDH complex.*

P 38324697

- Nikel et al. (2015) *P. putida KT2440 metabolizes glucose through an ED/EMP/PPP cycle (EDEMP).*

P 26350459

- Wang et al. (2014) *Structure and function of the E2 catalytic domain in E. coli PDHc.*

P 24742683

- Träger et al. (2026) *The Pyruvate Dehydrogenase Complex: A 90-Year-Old Enigma…*

P 42334543

- Song & Jordan (2012) *Interchain acetyl transfer in the E2 component of bacterial pyruvate dehydrogenase… (defines the ordered E1→E2→E3 reaction).*

P 22413895

- de Kok et al. (1998) *The pyruvate dehydrogenase multi-enzyme complex from Gram-negative bacteria.*

P 9655933
