Functional Annotation Report: *argD* / PP_4481 (UniProt P59319) in *Pseudomonas putida* KT2440

Summary

The gene annotated **argD** (locus **PP_4481**, UniProt **P59319**) in *Pseudomonas putida* KT2440 encodes a **cytoplasmic, homodimeric, pyridoxal-5′-phosphate (PLP)-dependent class-III aminotransferase** of the acyl-ornithine aminotransferase family. Its molecular function is that of an **acyl-ornithine:2-oxoglutarate aminotransferase** — it removes the amino group from the side-chain (δ) carbon of an N-acylated ornithine and transfers it to 2-oxoglutarate, generating an N-acylglutamate-5-semialdehyde and L-glutamate. This is a reversible, PLP-Schiff-base–dependent transamination characteristic of the class-III PLP-dependent aminotransferase fold.

There is an important **annotation conflict** that this investigation resolved. UniProt, via the automated HAMAP rule MF_01107, names P59319 the **biosynthetic acetylornithine aminotransferase (ArgD/ACOAT, EC 2.6.1.11)**, the enzyme that catalyzes the fourth (transamination) step of L-arginine biosynthesis. However, three independent lines of evidence — (i) genomic synteny placing PP_4481 inside the **arginine succinyltransferase (AST) catabolic operon** (PP_4478–PP_4481), (ii) KEGG orthology assignment (K00840, EC 2.6.1.81, *astC*), and (iii) **~80 % pairwise sequence identity to the experimentally characterized *P. aeruginosa* AruC** — converge on the conclusion that the **primary physiological role of PP_4481 is catabolic**: it is the **N^2-succinyl-L-ornithine 5-aminotransferase (AstC/AruC, EC 2.6.1.81)**, catalyzing the third step of the aerobic pathway that degrades arginine and ornithine to glutamate for use as carbon and nitrogen sources. The dedicated **biosynthetic** ACOAT step in *P. putida* is instead attributable to a distinct paralog, **PP_0372** (UniProt Q88QW2), and the pathway operates through the *argJ* (PP_1346) acetyl-recycling route.

Because catabolic AstC-type and anabolic ArgD-type enzymes are only ~58–60 % identical yet have been shown to be **functionally interchangeable** and mutually promiscuous toward both N-acetyl- and N-succinyl-ornithine, PP_4481 most likely retains measurable activity on acetylornithine as well. Nonetheless, the weight of genomic, phylogenetic, and structural

evidence assigns its **primary cellular function to arginine/ornithine catabolism via the AST pathway**, not to arginine biosynthesis. The enzyme acts in the **cytoplasm** and prefers **N-acylated ornithine over free ornithine**, a specificity that arises from steric and desolvation effects at the active site.

Key Findings

Finding 1 — Molecular identity: a class-III PLP-dependent acyl-ornithine aminotransferase

UniProt P59319 describes a 406-residue class-III PLP-dependent aminotransferase of the ArgD/acetylornithine-aminotransferase subfamily. The canonical annotated reaction (EC 2.6.1.11) is the reversible transamination:

> N^2-acetyl-L-ornithine + 2-oxoglutarate ⇌ N-acetyl-L-glutamate 5-semialdehyde + L-glutamate

with **pyridoxal 5′-phosphate (PLP)** as the essential cofactor, covalently bound as an internal aldimine (Schiff base) to the active-site lysine (mapped to **Lys255** in P59319). Substrate- and cofactor-binding residues are annotated at positions 108, 141, 144, 226, 283 and 284, all consistent with the conserved class-III active site. The core aminotransferase chemistry — abstraction of the substrate α/δ-amino group via a PLP ketimine/quinonoid intermediate and its transfer to 2-oxoglutarate — is deeply conserved across bacteria including *E. coli*, *M. tuberculosis*, *Erwinia*, *Corynebacterium* and cyanobacteria.

The biosynthetic ArgD reaction was confirmed verbatim in the literature: ArgD "*catalyzes the reversible conversion of N-acetylornithine and 2 oxoglutarate into glutamate-5-semialdehyde and L-glutamate*" (PMID: 34922100), and N-acetylornithine aminotransferase "*(EC 2.6.1.11, ACOAT) catalyzes the conversion of N-acetylglutamic semialdehyde to N-acetylornithine, the forth step involved in the L-arginine biosynthetic pathways*" (PMID: 22016985). These establish the **enzyme class and chemistry**; the subsequent findings establish which *physiological* reaction PP_4481 performs.

Finding 2 — Subcellular localization and quaternary structure

P59319 is annotated (HAMAP MF_01107) as a **cytoplasmic, homodimeric** enzyme with PLP covalently bound via a Schiff base to Lys255. This is fully consistent with the class-III PLP aminotransferase fold, which functions as an obligate dimer with the active site formed at the subunit interface. The physiological role is therefore carried out **inside the cell**, in the cytoplasm, where the arginine/ornithine catabolic and biosynthetic intermediates reside.

The essentiality of *argD*-type activity for a functional arginine pathway is well demonstrated in related bacteria: in *Erwinia amylovora*, an *argD* transposon-insertion mutant "*was an arginine auxotroph that did not cause fire blight in apple and had reduced virulence in immature pear fruits*," and the *argD* gene "*encodes a predicted N-acetylornithine aminotransferase enzyme, which is involved in the production of the amino acid arginine*" (PMID: 25172854). Note that this evidence pertains to the *biosynthetic* role of ArgD in *Erwinia*; for *P. putida* PP_4481 specifically, the localization (cytoplasm) and fold (homodimeric PLP enzyme) hold, but the physiological pathway is catabolic (see Findings 4–8).

Finding 3 — Dual biosynthetic capability of the ArgD/ACOAT family (substrate promiscuity)

A key feature of this enzyme family, directly relevant to interpreting PP_4481's substrate specificity, is **catalytic promiscuity**. Purified *E. coli* ArgD exhibits both N-acetylornithine aminotransferase and **N-succinyl-L,L-diaminopimelate aminotransferase (DapC/SDAP-AT)** activities with *similar specificity constants*: the enzyme "*exhibits both NAcOATase and DapATase activity, with similar specificity constants for N-acetylornithine and N-succinyl-L,L-DAP, suggesting that it can function in both lysine and arginine biosynthesis*" (PMID: 10074354). Genetic dissection in *E. coli* further showed extensive aminotransferase redundancy: "*The enzymes with N-acetylornithine aminotransferase (ACOAT) activity in arginine synthesis were ArgD, AstC, GabT and PuuE; the major anaerobic ACOAT was ArgD*" (PMID: 25243376). This demonstrates that members of this family — including the catabolic AstC — routinely accept multiple acylated amino-acid substrates. This promiscuity is the root cause of the cross-annotation between *argD* and *astC*.

Finding 4 — Genomic synteny places PP_4481 in the AST catabolic operon

The decisive contextual evidence is genomic. PP_4481 sits within a tight, co-oriented **arginine succinyltransferase (AST) catabolic operon** on the *P. putida* KT2440 chromosome:

Locus	Gene	Enzyme	Role in AST pathway
PP_4478	*astD*	N-succinylglutamate-5-semialdehyde dehydrogenase	Step 4
PP_4479	*astA-I* (aruAI)	arginine/ornithine N-succinyltransferase subunit	Step 1
PP_4480	*astA-II* (aruAII)	arginine/ornithine N-succinyltransferase subunit	Step 1
PP_4481	**astC (this gene)**	**succinylornithine aminotransferase**	**Step 3**

KEGG annotates PP_4481 as *astC*, **K00840, EC 2.6.1.81**, within module **M00879 "Arginine succinyltransferase pathway, arginine ⇒ glutamate."** This arrangement mirrors the *P. aeruginosa* PAO1 *aru* cluster, in which "*The arginine succinyltransferase (AST) pathway is the major arginine and ornithine utilization (aru) pathway under aerobic conditions in Pseudomonas aeruginosa*" and the cluster encodes "*N2-succinylornithine 5-aminotransferase, N-succinylglutamate 5-semialdehyde dehydrogenase, N2-succinylarginine dihydrolase, and N-succinylglutamate desuccinylase*" (PMID: 9393691). The neighboring genes of PP_4481 correspond precisely to these AST enzymes, strongly indicating that PP_4481 is the operon's **succinylornithine aminotransferase (AruC/AstC)**, a catabolic enzyme.

Finding 5 — Resolution of the UniProt-vs-KEGG annotation conflict via a distinct biosynthetic paralog

P. putida KT2440 encodes at least **two paralogous class-III acyl-ornithine aminotransferases**, and the KEGG orthology cleanly separates their roles:

- **K00821** (biosynthetic acetylornithine aminotransferase, *argD*, EC 2.6.1.11) → **PP_0372** (*aruC*/"Acetylornithine aminotransferase 2", UniProt Q88QW2)
- **K00840** (catabolic succinylornithine aminotransferase, *astC*, EC 2.6.1.81) → **PP_4481** (this gene, UniProt P59319)

UniProt's HAMAP rule MF_01107 applied the biosynthetic ArgD/ACOAT name to P59319 by sequence signature, but the genomic synteny and KEGG orthology assign it the catabolic AstC role. Because AstC also possesses ACOAT activity ("*The enzymes with N-acetylornithine aminotransferase (ACOAT) activity in arginine synthesis were ArgD, AstC, GabT and PuuE*",

PMID: 25243376), the two *P. putida* paralogs likely overlap partially in substrate specificity (acetyl- vs succinyl-ornithine), explaining why an automated rule could not distinguish them. The physiologically dedicated **biosynthetic** step is carried by PP_0372.

Finding 6 — Phylogeny: PP_4481 is the ortholog of *P. aeruginosa* AruC

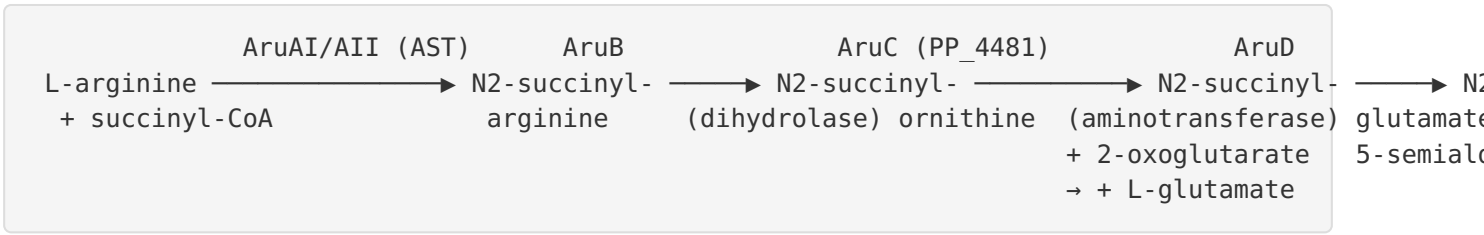
A pairwise sequence analysis (Needleman-Wunsch global alignment of UniProt sequences) quantitatively confirms the orthology:

Comparison	% Identity	Interpretation
PP_4481 vs *P. aeruginosa* AruC (O30508, catabolic N^2-succinylornithine 5-aminotransferase)	**79.8 %**	Highest — direct ortholog
PP_4481 vs *E. coli* ArgD (P18335, anabolic ACOAT)	65.6 %	More distant
PP_4481 vs *E. coli* AstC (P77581, catabolic)	62.6 %	More distant
PP_4481 vs *E. coli* GabT (P22256, GABA-AT outgroup)	35.8 %	Outgroup
E. coli ArgD vs *E. coli* AstC	59.2 %	Close paralogs, interchangeable
PP_0372 (Q88QW2, biosynthetic-argD paralog) vs all four references	~38–40 %	Divergent outlier

The **79.8 % identity to *P. aeruginosa* AruC** — an experimentally characterized AST-pathway succinylornithine aminotransferase — is the single strongest quantitative argument that PP_4481 performs the same catabolic function. AruC is annotated "Succinylornithine transaminase/acetylornithine aminotransferase" and was characterized by Itoh (1997) as the AST-pathway succinylornithine aminotransferase (PMID: 9393691). Notably, PP_0372 (the KEGG-designated biosynthetic paralog) is a divergent outlier at only ~38–40 % identity to all references, confirming it is a *separate* functional class.

Finding 7 — Position and function within the AST catabolic pathway

PP_4481/AruC catalyzes the **third (transamination) step** of the arginine succinyltransferase pathway, which degrades L-arginine to glutamate through N-succinylated intermediates:



This sequence — "*L-arginine→N2-succinylarginine→N2-succinylornithine→N2-succinylglutamate semialdehyde→N2-succinylglutamate→glutamate + succinate*" — was established in *Pseudomonas cepacia* (PMID: 2865249). The pathway allows arginine to serve as **sole carbon and nitrogen source**, and its enzymes are regulated: "*The formation of the enzymes responsible for arginine degradation is regulated not only by induction but also by both carbon and nitrogen catabolite repression*" (PMID: 2865249). In *P. aeruginosa*, "*The aruCFGDB genes appear to form an operon transcribed from a promoter upstream of aruC*" (PMID: 9393691), placing the *aruC* ortholog at the head of an ArgR-controlled catabolic operon. PP_4481 therefore performs the transamination that transfers the amino group from **N^2-succinyl-L-ornithine** to **2-oxoglutarate**, yielding **N^2-succinylglutamate-5-semialdehyde** + **L-glutamate**.

Finding 8 — Structural basis of substrate specificity: preference for acylated ornithine

The catabolic AstC-type enzyme's substrate preference has been defined crystallographically. X-ray structures of *E. coli* AstC were solved in apo, holo-PLP, and **N-succinylornithine-bound** forms (PMID: 23484010). That work established the anabolic/catabolic dichotomy directly: "*Escherichia coli possesses two acyl ornithine aminotransferases, one catabolic (AstC) and the other anabolic (ArgD), that participate in L-arginine metabolism. Although only 58% identical, the enzymes have been shown to be functionally interchangeable*" (PMID: 23484010). Docking of ornithine, succinylornithine and acetylornithine showed that "*AstC has a strong preference for acylated ornithine species over ornithine itself, and suggest that the increase in specificity associated with acylation is caused by steric and desolvation effects*" (PMID: 23484010) — i.e., the enzyme discriminates against free ornithine not through specific acyl-group contacts but through general steric/desolvation penalties.

For PP_4481, UniProt maps the PLP Schiff-base to **Lys255** and cofactor/substrate-binding residues to positions 108, 141, 144, 226, 283 and 284, consistent with this conserved class-III active site. The fold matches the *Salmonella typhimurium* ArgD structure. Thus PP_4481 is expected to bind and turn over **N-acyl-ornithine (succinyl- and, more weakly, acetyl-)** far more efficiently than free ornithine.

Finding 9 — Separation of biosynthetic machinery confirms PP_4481's catabolic role

The biosynthetic arginine genes of *P. putida* KT2440 map to distinct loci, none coinciding with the AST operon:

Step	Gene	Locus
N-acetylglutamate kinase	*argB*	PP_5289
N-acetyl-γ-glutamyl-phosphate reductase	*argC*	PP_0432 (and PP_3633)
Ornithine acetyltransferase / NAGS	*argJ*	PP_1346
Biosynthetic acetylornithine aminotransferase (K00821)	*argD*	**PP_0372**
Acetylornithine deacetylase candidates	*argE*	PP_3571 / PP_5186

The presence of *argJ* (PP_1346) indicates KT2440 uses the **cyclic/acetyl-recycling ornithine biosynthesis route**, in which the biosynthetic acetylornithine aminotransferase (assigned to PP_0372) supplies the transamination step. None of these biosynthetic loci coincide with PP_4481, which is embedded in the catabolic AST operon (PP_4478–4481). This spatial and functional separation reinforces that **biosynthesis and catabolism use different enzymes**, with PP_4481 dedicated to catabolism.

Mechanistic Model / Interpretation

The evidence integrates into a coherent model in which *P. putida* KT2440 maintains **two parallel acyl-ornithine aminotransferase systems** built on the same class-III PLP fold but committed to opposite metabolic directions:

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                  ARGININE BIOSYNTHESIS (anabolic)
L-glutamate → N-acetylglutamate → ... → N-acetylglutamate-5-semialdehyde
                                                    │
                                    PP_0372 (ArgD/ACOAT, K00821, EC 2.6.1.11)
                                                    │  ← 2-oxoglutarate / L-glutamate
                                                    ▼
                                         N-acetyl-L-ornithine → (argJ recycle) → L-ornithine → L


                  ARGININE CATABOLISM (catabolic AST pathway)
L-arginine → N2-succinylarginine → N2-succinylornithine
                                                    │
                                PP_4481 (AstC/AruC, K00840, EC 2.6.1.81)   ← THIS GENE
                                                    │  ← 2-oxoglutarate / → L-glutamate
                                                    ▼
                                      N2-succinylglutamate-5-semialdehyde → ... → L-glutamate + succi
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Both enzymes catalyze the same *type* of reaction — transamination between the δ-carbon of an N-acyl-ornithine and 2-oxoglutarate — but on **different acyl groups** (acetyl for biosynthesis, succinyl for catabolism) and in **different physiological directions**. Because the class-III active site tolerates both acyl groups, the enzymes are biochemically promiscuous and, in *E. coli*, functionally interchangeable. The specialization is therefore primarily one of **regulation and metabolic context** (which operon, which inducers, which flux) rather than absolute substrate exclusivity.

For PP_4481 specifically, the primary annotation should be understood as follows:

- **Molecular function:** N^2-succinyl-L-ornithine:2-oxoglutarate 5-aminotransferase (EC 2.6.1.81), PLP-dependent, class-III fold; likely retains secondary acetylornithine aminotransferase activity (EC 2.6.1.11).
- **Biological process:** aerobic **arginine and ornithine catabolism** via the arginine succinyltransferase (AST) pathway, yielding glutamate + succinate as carbon/nitrogen sources.
- **Localization:** cytoplasm; homodimeric holoenzyme with PLP bound at Lys255.
- **Pathway position:** step 3 of 5 in the AST pathway; product feeds AstD (PP_4478).
- **Regulation:** inducible by arginine, subject to carbon and nitrogen catabolite repression, and (by orthology to *P. aeruginosa aruCFGDB*) controlled by the ArgR regulator.

The **UniProt/HAMAP "argD/ACOAT biosynthetic" name is a mis-specialization** driven by sequence-signature promiscuity; the primary physiological role indicated by genomic context, orthology, and pathway membership is **catabolic AstC/AruC**.

Evidence Base

PMID	Title (abbreviated)	How it supports the findings
9393691	*Cloning of the aru genes of the catabolic AST pathway in P. aeruginosa*	Defines *aruC* (79.8 %-identity ortholog of PP_4481) as the AST-pathway succinylornithine aminotransferase; establishes AST as the major aerobic arginine/ornithine utilization route; *aruCFGDB* operon organization. **Central supporting paper.**
2865249	*Succinyl derivatives in arginine catabolism in P. cepacia*	Defines the full AST reaction sequence and the exact substrate/product of the AruC step; documents inducible, catabolite-repressed regulation.
23484010	*Structure of catabolic N-succinylornithine transaminase (AstC) from E. coli*	Crystallographic basis for catabolic-vs-anabolic distinction; AstC/ArgD only 58 % identical yet interchangeable; AstC strongly prefers acylated ornithine (steric/desolvation).
10074354	*Dual biosynthetic capability of N-acetylornithine aminotransferase*	Direct enzymology: ArgD has both ACOAT and succinyl-DAP-AT activity with similar specificity constants — establishes family promiscuity.
25243376	*Redundant aminotransferases in lysine/arginine synthesis in E. coli*	Documents that AstC also has ACOAT activity and quantifies aminotransferase redundancy — explains argD/astC cross-annotation.
34922100	*ArgD of M. tuberculosis is a functional ACOAT*	States the exact reversible ArgD transamination reaction (family chemistry).
22016985	*N-acetylornithine aminotransferase from C. crenatum*	Confirms EC 2.6.1.11 and the fourth-step biosynthetic role of ACOAT.
25172854	*argD mutation causes arginine auxotrophy in E. amylovora*	Genetic evidence that ArgD-type activity is required for arginine biosynthesis (biosynthetic-role context).

Supporting context papers on *P. putida* arginine physiology — including the role of arginine as a metabolic signal (PMID: 38429473), arginine biosynthesis modulating pyoverdine and oxidative-stress adaptation (PMID: 31451546), and c-di-GMP/biofilm links (PMID: 27489550) — confirm that arginine metabolism is physiologically prominent in KT2440 but do not directly assay PP_4481.

Limitations and Knowledge Gaps

1. **No direct enzymatic characterization of PP_4481 itself.** The functional assignment rests on orthology (79.8 % identity to *P. aeruginosa* AruC), genomic synteny, and KEGG orthology — not on a purified-enzyme kinetic study of the *P. putida* KT2440 protein. Kinetic constants (kcat, Km for succinyl- vs acetyl-ornithine) have not been measured for this specific protein.
2. **Annotation conflict remains formally unresolved at the database level.** UniProt (HAMAP MF_01107) still names P59319 the biosynthetic ArgD/ACOAT. This report argues, on strong indirect evidence, that the primary role is catabolic AstC — but the two functions are biochemically overlapping, so the enzyme may contribute to both pathways to differing degrees.
3. **The biosynthetic-paralog assignment (PP_0372) is likewise inferential**, based on KEGG KO mapping and sequence divergence, not on a KT2440 knockout/complementation study.
4. **Regulatory details are extrapolated from *P. aeruginosa*.** ArgR control and catabolite repression of the *P. putida* PP_4478–4481 operon are inferred from orthology and from *P. cepacia*/*P. aeruginosa* studies, not directly demonstrated in KT2440.
5. **No structure of the KT2440 protein exists;** active-site residues (Lys255 etc.) are from UniProt feature mapping and homology to *S. typhimurium* ArgD / *E. coli* AstC.

Proposed Follow-up Experiments / Actions

1. **Direct enzyme kinetics.** Heterologously express and purify PP_4481 (P59319) and measure steady-state kinetics with **N^2-succinyl-L-ornithine** vs **N^2-acetyl-L-ornithine** (and free ornithine) using 2-oxoglutarate as the amino acceptor. Compare kcat/Km to test the predicted succinyl-preference and quantify promiscuity.

2. **Genetic dissection.** Construct single (ΔPP_4481, ΔPP_0372) and double deletion mutants in KT2440. Test (a) growth on **L-arginine or L-ornithine as sole carbon/nitrogen source** (predicting ΔPP_4481 is impaired) and (b) **arginine auxotrophy** (predicting ΔPP_0372, not ΔPP_4481, causes auxotrophy). Cross-complementation would test functional interchangeability.
3. **Operon/regulation mapping.** Verify the PP_4478–4481 transcript by RT-PCR/RNA-seq, map the promoter, and test **ArgR dependence** and **carbon/nitrogen catabolite repression** by growth-condition-dependent expression assays.
4. **Structural confirmation.** Solve the crystal structure of PP_4481, ideally with N-succinylornithine bound, to confirm the active-site geometry and the steric/desolvation basis of acyl-ornithine preference.
5. **Database curation.** Submit a curation note to UniProt/KEGG reconciling the *argD* (biosynthetic) vs *astC* (catabolic) assignment for PP_4481, flagging PP_0372 as the biosynthetic ACOAT paralog.

Conclusion

PP_4481 (UniProt P59319) in *Pseudomonas putida* KT2440 is a cytoplasmic, homodimeric, PLP-dependent class-III acyl-ornithine aminotransferase whose **primary physiological function is catabolic**: it is the **N^2-succinyl-L-ornithine 5-aminotransferase (AstC/AruC, EC 2.6.1.81)** that catalyzes the third step of the aerobic arginine succinyltransferase (AST) pathway, degrading arginine/ornithine to glutamate + succinate for use as carbon and nitrogen sources. Despite the UniProt/HAMAP "biosynthetic argD/ACOAT" name, genomic synteny within the AST operon, KEGG orthology, and ~80 % identity to characterized *P. aeruginosa* AruC establish the catabolic assignment, with the dedicated biosynthetic ACOAT role attributable to the distinct paralog PP_0372. The enzyme prefers N-acylated ornithine over free ornithine and is expected to retain secondary acetylornithine aminotransferase activity, reflecting the well-documented promiscuity of this enzyme family.
