

Table S1. Gradients used in amino acid enantiomer HPLC analysis

Amino acid (D and L)	Gradient
Thr, Pro, Lys, Phe, Cys, Leu, Tyr, Ile, Ala, Trp	Start at 80%:20% TEAP buffer (pH3.0):acetonitrile; ramp to 70%:30% buffer:acetonitrile over 5 minutes; ramp to 50%:50% buffer:acetonitrile over 15 minutes; ramp to 80%:20% buffer:acetonitrile over 10 minutes; (injection volume: 10µl; flow rate: 0.5ml; detection wavelength: 340nm)
Ser, Asp, Glu, Val, HyPro	Start at 80%:20% TEAP buffer (pH3.0):acetonitrile; ramp to 70%:30% buffer:acetonitrile over 15 minutes; ramp to 50%:50% buffer:acetonitrile over 5 minutes; ramp to 80%:20% buffer:acetonitrile over 10 minutes; (injection volume: 10µl; flow rate: 0.5ml; detection wavelength: 340nm)
Arg	Start at 90%:10% TEAP buffer (pH3.0):acetonitrile; ramp to 80%:20% buffer:acetonitrile over 15 minutes; ramp to 50%:50% buffer:acetonitrile over 5 minutes; ramp to 90%:10% buffer:acetonitrile over 10 minutes; (injection volume: 10µl; flow rate: 0.5ml; detection wavelength: 340nm)
Met	Start at 80%:20% TEAP buffer (pH3.0):acetonitrile; ramp to 70%:30% buffer:acetonitrile over 5 minutes; ramp to 60%:40% buffer:acetonitrile over 15 minutes; ramp to 80%:20% buffer:acetonitrile over 10 minutes; (injection volume: 10µl; flow rate: 0.5ml; detection wavelength: 340nm)
Asn, Gln, His	Start at 85%:15% TEAP buffer (pH3.0):acetonitrile; ramp to 75%:25% buffer:acetonitrile over 15 minutes; ramp to 50%:50% buffer:acetonitrile over 5 minutes; ramp to 85%:15% buffer:acetonitrile over 10 minutes; (injection volume: 10µl; flow rate: 0.5ml; detection wavelength: 340nm)

TABLE S2. Screening results from *P. putida* KT2440 genomic libraries^a

Supplemented amino acid	Genome region	Gene range	Annotated function of genes of interest
D-Pro	1	PP1251-PP1256	PP1255: FAD-dependent oxidoreductase
	2	PP1257-PP1259	PP1258: proline racemase^b
	3	PP3588-PP3597	PP3590: aromatic amino acid aminotransferase PP3591: Δ^1 -piperideine-2-carboxylate reductase PP3596: FAD-dependent oxidoreductase
D-Lys	1	PP1162-PP1165	PP1163: FAD-binding oxidoreductase
	2	PP3719-PP3727	PP3721: aspartate aminotransferase PP3722: alanine racemase/Air
D-Leu	1	PP3589-PP3593	PP3590: aromatic amino acid aminotransferase PP3591: Δ^1 -piperideine-2-carboxylate reductase
	2	PP3593-PP3598	PP3596: FAD-dependent oxidoreductase
	3	PP3717-PP3724	PP3721: aspartate aminotransferase PP3722: alanine racemase/Air
	4	PP5264-PP5271	PP5269: alanine racemase/DadX PP5270: D-amino acid dehydrogenase/DadA
D-Phe	1	PP2244-PP2253	PP2246: FAD-dependent oxidoreductase

^aThe genes from each region are designated according to their genomic locus tag, and the range of genes indicates the minimal unit identified for each region. Genes of interest from these regions include those whose predicted products belong to enzyme families known to catalyze reactions characteristic of amino acid metabolism. The minimal genomic region does not imply that more than one gene from this region is required for recovery of the phenotype. The coverage for the entire screening with each amino acid exceeded 500× genome coverage.

^bORFs in **bold** are the focus of the current study.

Figure S1. Genomic synteny. *dadAX* region (A) and *proR* region (B) in *P. aeruginosa* PAO1, *P. protegens* Pf-5, *P. putida* KT2440, and *P. putida* GB-1. Gene numbers and annotations were obtained from Pseudomonas Genome Database.

