Primers	Sequences ^a
Cloning of C14ofr149 gene into pETDuet-1	
P1 (BamHI)	5'-catggatccgGAGAGCGCGCTGGCGGTGCCCCGGC-3'
P2 (PstI)	5'-tggctgcagTCACTTGAGAAGAAATCCATCCCTC-3'
Cloning of PaLhpD genes into pETDuet-1	
P3 (BamHI)	5'-cat <u>ggatcc</u> gATCCGAATGACGCTGGACGAGGTCCGCG-3'
P4 (HindIII)	5'-att <u>aagctT</u> CACTCCAGCAACGCCAGCAGCTCCCGG-3'
Cloning of AbLhpH gene into pETDuet-1	
P5 (BamHI)	5'-cat <u>ggatcc</u> gAAAATTACCCGATCCCTTTCCACCGTCG-3'
P6 (HindIII)	5'-aatt <u>aagctT</u> CAGGCGATGTTTTGCTCGGCCGACCAGCTGGC-3'
Cloning of CpLhpH gene into pETDuet-1	
P7 (BamHI)	5'-cat <u>ggatcc</u> gACAAAAAATATAGCGCAAGCAGCTG-3'
P8 (PstI)	5'-tgg <u>ctgcag</u> TTATCTTAATAAAAACCATATTTC-3'
Cloning of AbLhpI into pETDuet-1	
P9 (BamHI)	5'-cat <u>ggatcc</u> gACCGCTCTTTCCCCGATTCCCGTTTTCG-3'
P10 (HindIII)	5'-att <u>aagctT</u> CAGCCGGCCCGGCGTGCGGCCAGCG-3'
Cloning of CpLhpI into pETDuet-1	
P11 (BamHI)	5'-cat <u>ggatcc</u> gAAAATTATTAGCGCCGAACAAGTCCACC-3'
P12 (HindIII)	5'-tgg <u>ctgcag</u> TTAATCTGCTAACTTTTCTACTACGCTATG-3'
Disruptant construction of AbLhpI	
P13 (SacII)	5'-cat <u>ccgcgg</u> GTCACGACGTTGTAAAACGACGGCC-3'
P14 (SacII)	5 ′ -tgg <u>ccgcgg</u> TTTCACACAGGAAACAGCTATGACC-3 ′
P15 (MfeI)	5'-cat <u>caattg</u> ACCGCTCTTTCCCCGATTCCCGTTTTCG-3'
P16 (MfeI)	5'-att <u>caattg</u> CAGCCGGCCCGGCGTGCGGCCAGCG-3'

Supplementary Table S1. Primers used in this study.

^a Small letters indicate additional bases for introducing digestion sites of restriction enzymes in parentheses.



FIGURE S1. Growth curves of *P. putida* (A) and *P. aeruginosa* (B) on glucose (*black*), L-proline (*green*), D-proline (*light-green*), T4LHyp (*red*), C4DHyp (*blue*), T3LHyp (*yellow*) and D-lysine (*aqua*) as a sole carbon source (30 mM).



FIGURE S2. Elution profile of purified recombinant proteins during gel filtration. AbLhpH, *red;* CpLhpH, *blue;* C14orf149, *orange;* PaLhpD, *green;* AbLhpI, *light-green;* CpLhpI, *aqua.*



FIGURE S3. Phylogenetic analysis of T3LHyp dehydratase. (A) Phylogenetic tree of proline racemase superfamily. The *number* on each *branch* indicates the bootstrap value. T3LHyp dehydratases and T4LHyp epimerases from the same organisms are in the colored box. Proteins with *black, white* and *gray-colored circles* in T3LHyp dehydratase subfamily are from mammalian, fungi and bacteria (discovered in this study), respectively. (B) Partial multiple sequence alignment of deduced amino acid sequences. Catalytic cysteine and/or threonine residues are shown as *white letters in black boxes*. *Gray-shaded letters* indicate highly conserved amino acid residues.



FIGURE S4. Metabolic pathways around Pyr2C/Pip2C reductase. Homologous genes are indicated in the same color, and correspond to Fig. 1ABD. *Gray-colored routes* indicate L- to D-epimerization (racemization) of amino acid consisting of two distinct FAD- and NAD(P)⁺-dependent dehydrogenase (oxidase).