Bacteria (II) Plants: discovery of a novel class of Fe(II) oxidases that are involved in lysine catabolism via high-throughput fitness screening

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Lysine is an essential amino acid produced on a scale of one million tons per year to meet agricultural and nutritional demands: however, many pathways of lysine metabolism remain unresolved. In *Pseudomonas putida* lysine is degraded by two pathways specific for either the L- or D- isomer. While the initial steps of the D-lysine catabolic pathway that produce 2-aminoadipate (2AA) are known, the basis of further catabolism have not been characterized. Using Random Barcode Transposon Sequencing, a three enzyme pathway that converts L-2AA to 2-oxoglutarate (2OG) was identified. One of these enzymes, PP_5260, is a DUF1338 family protein that catalyzes a novel 2-oxoadipate (2OA) to 2-hydroxyglutarate (2HG) reaction. Initial characterization of PP_5260 revealed it to be an Fe(II)-dependent decarboxylase widely distributed across bacteria, fungi, and plants.

To identify the mechanism of action, the structure of PP_5260 was solved to 1.1 Å. Structural similarity to hydroxymandelate synthase suggested PP_5260 might function as a dioxygenase that catalyzes a decarboxylation followed by an intramolecular hydroxylation. This model was supported by results showing 1:1 stoichiometric consumption of O_2 and 2OA, and O^{18} labelling incorporation. Further biochemical study revealed that PP_5260 is highly specific towards 2OA as a substrate. Co-crystallization of PP_5260 and 2OA revealed this specificity is largely mediated by a single amino acid residue, R74. Bioinformatic analysis of all DUF1338 proteins showed that >99% of homologs that contained the conserved metal binding triad also retained the R74 residue, suggesting that the biochemical function in this family is both highly specific and highly conserved.

In plants lysine catabolism also goes through 2OA, though further catabolism remained undefined. Analysis of transcripts across multiple plants showed that DUF1338 homologs were significantly co-expressed with known lysine catabolic genes. Purification of the rice DUF1338 homolog FLO7 revealed identical activity to that of PP_5260.