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An unprecedented glutamate epimerase for bacterial peptidoglycan biosynthesis

○Ruoyin FENG<sup>1</sup>, Yasuharu Satoh<sup>1</sup>, Yasushi Ogasawara<sup>1</sup>, Tohru Yoshimura<sup>2</sup>, Tohru Dairi<sup>1</sup>  
(<sup>1</sup>Hokkaido Univ., <sup>2</sup>Nagoya Univ.)

## Background and Object of Research

Bacterial cell walls contain D-glutamate (D-Glu) as a component of peptidoglycans. In general, D-Glu is synthesized from L-Glu by Glu racemase (MurI, EC 5.1.1.3). In *Bacillus*, D-amino acid aminotransferase (EC 2.6.1.21) supplies D-Glu by transamination from D-alanine to  $\alpha$ -ketoglutarate. However, by extensive and detailed bioinformatic analysis, we revealed that *Xanthomonas oryzae* has no orthologs of the Glu racemase and the D-Glu aminotransferase in spite of the fact that *X. oryzae* is prototroph for D-Glu. This fact strongly suggested that the bacterium would have an alternative D-Glu biosynthetic pathway. This research aims to examine the D-Glu biosynthetic pathway in *X. oryzae*.

## Methods and results

To investigate the genes responsible for D-Glu biosynthesis in *X. oryzae*, we performed shotgun cloning experiments with a D-Glu auxotrophic *Escherichia coli* mutant as host and *X. oryzae* as DNA donor. As the result, we obtained two complementary genes, XOO\_1319 and XOO\_1320, which are annotated as a hypothetical protein and MurD (UDP-MurNAc-L-Ala-D-Glu synthetase), respectively. By detailed *in vitro* analysis with recombinant enzymes, we revealed that XOO\_1320 is an enzyme to ligate L-Glu to UDP-MurNAc-L-Ala for the first example of MurD utilizing L-Glu. In addition, XOO\_1319 is a novel enzyme catalyzing epimerization of the terminal L-Glu of the product in the presence of ATP and Mg<sup>2+</sup>.

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発表責任者 : 大利徹 (dairi@eng.hokudai.ac.jp)