

**LysY: the best marker of prokaryotic lysine biosynthesis through the amino adipate pathway**

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## **Abstract**

Phylogenetic study is a powerful approach for interpreting genomic sequences as well as showing evolutionary relationships. The extremely thermophilic bacterium *Thermus thermophilus* performs lysine biosynthesis in a unique way. However, the genes involved in this lysine biosynthetic pathway are distributed among many organisms. Which gene is characteristic of this pathway? In this paper, the phylogenetic study reveals that LysY is a key enzyme to the evolution of prokaryotic lysine biosynthesis through the aminoadipate pathway, and is the most useful marker of this pathway.

## Introduction

The extremely thermophilic bacterium *Thermus thermophilus* synthesizes lysine through the aminoadipate pathway, although all bacteria and plants were believed to synthesize lysine through the diaminopimelate. The first half of the biosynthesis (from 2-oxogultarate to 2-aminoadipate) proceeds in the same manner as that of fungi (Nishida et al. 1999; Nishida and Nishiyama 2000). The reactions were also suggested to be similar to those of leucine biosynthesis and a part of the tricarboxylic acid cycle (Irvin and Bhattacharjee 1998; Nishida et al. 1999; Velasco et al. 2002). The second half of the biosynthesis from 2-aminoadipate to lysine is similar to the conversion of glutamate to ornithine in arginine biosynthesis (Miyazaki et al. 2002). This second half pathway is typical of the lysine biosynthetic pathway of *T. thermophilus*. The enzymes related to this pathway are LysX, LysY, LysZ, LysJ, and LysK (Nishida et al. 1999; Miyazaki et al. 2001; Miyazaki et al. 2002).

## Materials and Methods

We performed a homology search for each of the five proteins using the BLAST program (Altschul et al. 1990) with the given parameter values on the Kyoto Encyclopedia Genes and Genomes ([www.genome.ad.jp/kegg](http://www.genome.ad.jp/kegg)) or the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). The multiple-alignment of LysY and the 24 extensive homologous sequences was created using the CLUSTAL W (Thompson et al. 1994) on the DNA Data Bank of Japan. The phylogenetic tree by the neighbor-joining method with 1,000 bootstrap analyses was constructed based on the alignment using MEGA version 2.1 (Kumar et al. 2001).

## Results and Discussion

The result of the homology search showed that some prokaryotes had more than two extensive homologous sequences (a value of  $E < 10^{-10}$ ) to each of LysX, LysZ, LysJ, or LysK. On the other hand, all organisms that we used in this study, except for *Deinococcus radiodurans* and *T. thermophilus*, had a single or no extensive homologous sequence to LysY. The extremely radioresistant bacterium *D. radiodurans* has two extensive homologous sequences to LysY. Therefore, LysY and the extensive

homologous sequences are suggested to be orthologous.

We assume that LysY catalyzes the conversion of *N*-acetylaminoadipate 5-phosphate to *N*-acetylaminoadipate 5-semialdehyde in lysine biosynthesis of *T. thermophilus* (Nishida et al. 1999). On the other hand, ArgC catalyzes the conversion of *N*-acetylglutamate 5-phosphate to *N*-acetylglutamate 5-semialdehyde in arginine biosynthesis. These enzymes share structural and functional similarities and are therefore thought to have diverged from a common ancestor (Nishida et al. 1999). In this study, no organism was found, which had both ArgC and LysY separately, except for *D. radiodurans* and *T. thermophilus*.

The phylogenetic tree (Fig. 1) showed that LysY clustered together with the gene products from *Aeropyrum pernix*, *D. radiodurans*, *Ferroplasma acidarmanus*, *Pyrococcus abyssi*, *P. furiosus*, *P. horikoshii*, *Sulfolobus solfataricus*, and *S. tokodaii*. The bootstrap analysis indicated 98% support values for monophyletic lineage of this cluster. *Deinococcus radiodurans* has homologues of the genes involved in the lysine biosynthetic pathway of *T. thermophilus*, but these genes are not clustered (Nishida 2001; Nishida and Narumi 2002). However, *Aeropyrum*, *Ferroplasma*, *Pyrococcus*, and *Sulfolobus* have a similar gene cluster to that of *T. thermophilus* (Brinkman et al. 2002). This suggests that the proteins from these archaea are involved in both arginine and lysine biosyntheses. On the other hand, the other proteins are suggested to be ArgCs in arginine biosynthesis. In any case, we conclude that LysY is a key enzyme to the evolution of prokaryotic lysine biosynthesis through the aminoadipate pathway. Therefore, LysY is the best marker of prokaryotic lysine biosynthesis through the aminoadipate pathway.

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## Legend of figure

Fig. 1. Phylogenetic relationships of LysY and the 24 extensive homologous amino acid sequences. A total of 230 amino acid sites were considered without gap regions in the alignment. The bar indicates 20% difference of amino acid sequence.

