J Mol Evol (2000) 51:299–302
DOI: 10.1007/s002390010091

JOURNAL OF MOLECULAR EVOLUTION

What Is Characteristic of Fungal Lysine Synthesis Through the α-Aminoadipate Pathway?

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Received: 18 February 2000 / Accepted: 19 June 2000

Abstract. Recent finding that a prokaryote synthesizes lysine through the α-aminoadipate pathway demonstrates that the lysine synthesis through the α-aminoadipate pathway is not typical of fungi. However, the fungal lysine biosynthesis is not completely the same as the prokaryotic one. We point out that α-aminoadipate reductase is a key enzyme to the evolution of fungal lysine synthesis. In addition, fungi have two different saccharopine dehydrogenases, which is also characteristic of fungi.

Key words: Lysine biosynthesis — α-Aminoadipate pathway — α-Aminoadipate reductase — Saccharopine dehydrogenase — Fungal evolution

Introduction

Many mycologists have believed that lysine synthesis from 2-oxoglutarate and acetyl-CoA through the α-aminoadipate pathway is one of specific characters of fungi (e.g., Broquist 1971; Vogel 1964, 1965). Recently, the gene cluster for the lysine synthesis of an extremely thermophilic Gram-negative bacterium, *Thermus thermophilus*, was found (Kosuge and Hoshino 1998; Kobashi et al. 1999), and the bacterium was shown to synthesize lysine through the α-aminoadipate pathway. This was the first discovery of lysine biosynthesis through the α-aminoadipate pathway in bacteria.

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Among microorganisms whose whole genome sequences have been completely determined, hyperthermophilic archaea, *Pyrococcus horikoshii* [www.bio.nite.go.jp/ot3db_index.html] and *P. abyssi* [www.genoscope.cns.fr/Pab/] were found to contain gene cluster closely related to that for the bacterial lysine biosynthesis through the α-aminoadipate pathway. The evolutionary analysis about the gene clusters of *T. thermophilus* and *P. horikoshii* has been already reported in detail (Nishida et al. 1999). Thus, lysine synthesis through the α-aminoadipate pathway is shown to be distributed among all three domains, proposed by Woese and Fox (1977), of organisms.

However, the prokaryotic lysine biosynthetic pathway is not completely identical to that of fungi (Nishida et al. 1999). The synthesis of α-aminoadipate from 2-oxoglutarate proceeds in the same way in both organisms, but the fungal process to synthesize lysine from α-aminoadipate is different from the prokaryotic one (Fig. 1). This suggested that the four steps for synthesis of lysine from α-aminoadipate must contain fungal specific enzymes. The enzymes related to these last four steps are LYS1, LYS2, LYS5, and LYS9 in the ascomycetous yeast *Saccharomyces cerevisiae* (Fig. 1). In this report we describe that α-aminoadipate reductase is a key enzyme to the evolution of fungal lysine synthesis.

Materials and Methods

We performed a homology search using BLAST (Altschul et al. 1990) with the given parameter values on the DNA data bank of Japan (DDBJ). This program compares a given query amino acid sequence

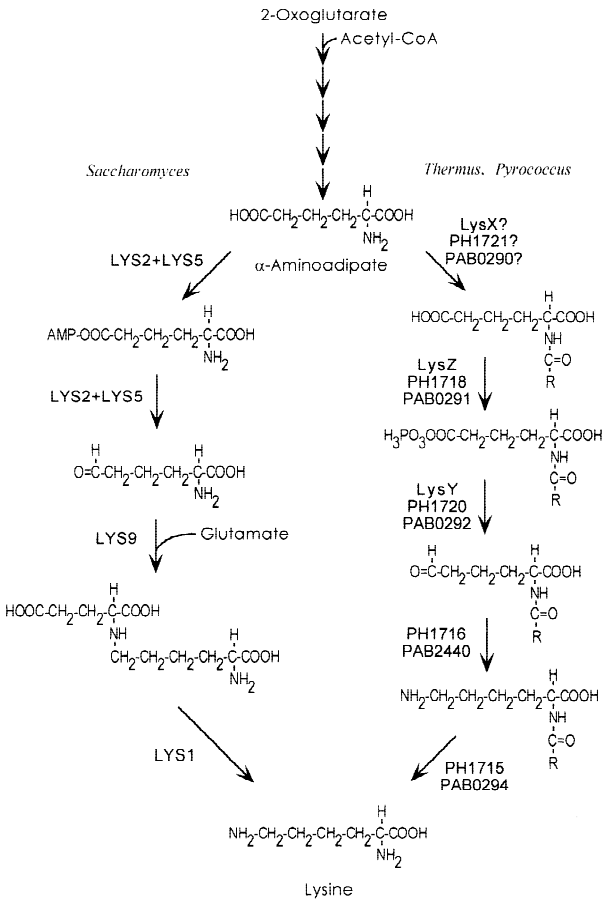

Fig. 1. Comparison of lysine synthetic pathways through the α-aminoadipate.

against all other proteins in the databases to identify related sequences. The query amino acid sequences were those for LYS1, LYS2, LYS5, and LYS9 of *S. cerevisiae*. Then, each of the multiple alignments was created using the CLUSTAL W (Thompson et al. 1994) on DDBJ among a query sequence and high-scoring sequences found in the BLAST result. Finally, the phylogenetic tree from p-distance estimation by the neighbor-joining method (Saitou and Nei 1987) with 1000 bootstrap analyses (Felsenstein 1985) was constructed based on the multiple alignment using MEGA version 1.01 (Kumar et al. 1993).

Results and Discussion

The results of this homology search and the multiple alignments in this study are available at http://iam.u-tokyo.ac.jp/misyst/LYS1259.html and http://iam.u-tokyo.ac.jp/misyst/LYS1259aln.html, respectively.

The last two steps of the lysine biosynthesis in fungi are known to be catalyzed by two different enzymes having the same name, saccharopine dehydrogenase. One saccharopine dehydrogenase (glutamate forming), LYS9, catalyzes the reaction to produce saccharopine using both 2-aminoadipate 6-semialdehyde and glutamate as the substrates (Munich Information Center for protein sequence, www.mips.biochem.mpg.de). The other saccharopine dehydrogenase (lysine forming), LYS1, then converts saccharopine to lysine and 2-oxoglutarate (Garrad et al. 1994).

The BLAST result showed that LYS1 had similarity with the proteins from 3 fungi (*Candida albicans, Yarrowia lipolytica,* and *Schizosaccharomyces pombe*), 4 animals (*Caenorhabditis elegans, Mus musculus, Homo sapiens,* and *Bos taurus*), 1 plant (*Zea mays*),

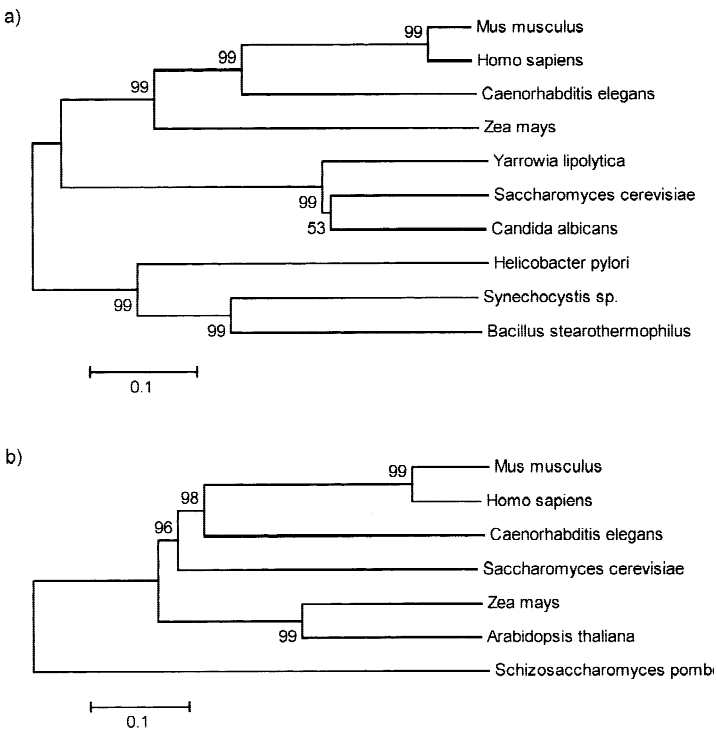

Fig. 2. **a** Phylogenetic relationships among the amino acid sequence of the LYS1 from *Saccharomyces cerevisiae* and high-scoring amino acid sequences from the result of homology search using BLAST. A total of 336 amino acid sites were considered without gap regions in alignment. The bar indicates 10% difference of amino acid sequence. The DAD (DDBJ amino acid sequence databases) accession nos. used in this phylogenetic analysis are U13233-1, M34929-2, AF038615-5, AJ224761-1, AC006020-2, AE000639-10, D90900-95, AF003551-1, and M33299-1. The LYS1 homologue of *Schizosaccharomyces pombe* (Z50142-1) is too short (only 49 aa) to include in this analysis. The prokaryotic data (*Helicobacter pylori, Synechocystis* sp., and *Bacillus stearothermophilus*) are alanine dehydrogenases. The others are saccharopine dehydrogenases. **b** Phylogenetic relationships among the amino acid sequence of the LYS9 from *S. cerevisiae* and the related sequences. A total of 430 amino acid sites were considered. The DAD accession nos. used in this phylogenetic analysis are AL022244-3, AC006020-2, AF038615-5, AJ224761-1, AL035525-8, and AF003551-1.

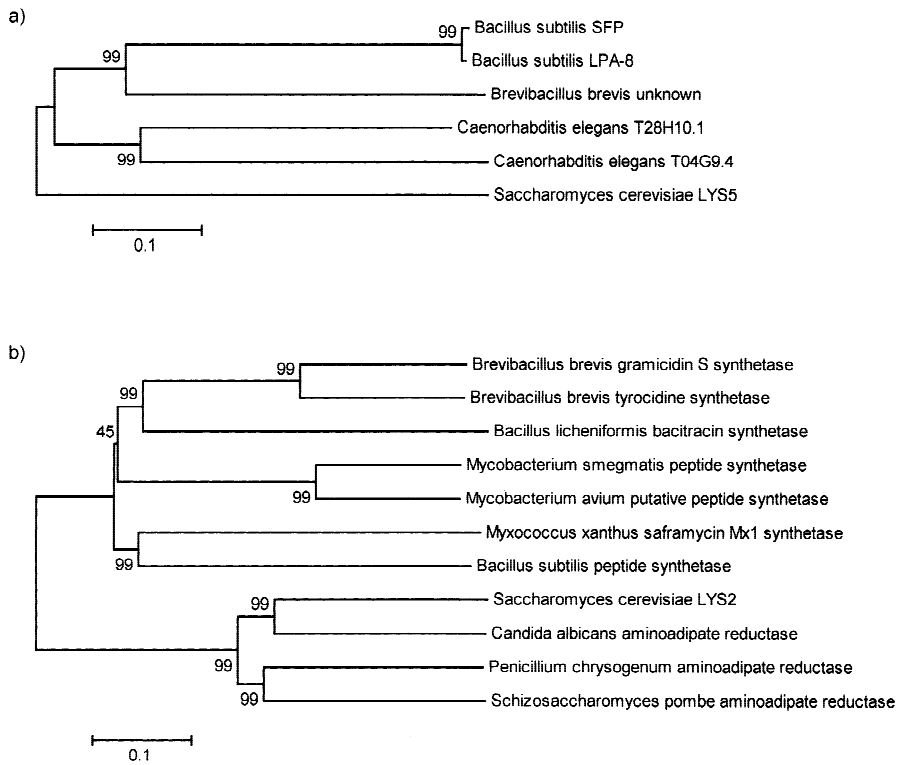

Fig. 3. **a** Phylogenetic relationships among the amino acid sequence of the LYS5 from *S. cerevisiae* and the related sequences. A total of 207 amino acid sites were considered. The DAD accession nos. used in this phylogenetic analysis are Z75551-1, U41274-4, X63158-1, D50562-1, and X15577-1. **b** Phylogenetic relationships among the amino acid sequence of the LYS2 from *S. cerevisiae* and the related sequences. A total of 893 amino acid sites were considered without gap regions in alignment. The DAD accession nos. used in this phylogenetic analysis are U58133-1, Y13967-1, U15923-1, U24657-2, AF007865-2, D29676-1, AF004835-3, AJ238027-1, AF143772-36, and AF184977-1.

and 9 prokaryotes. Interestingly, the phylogenetic tree (Fig. 2a) showed that saccharopine dehydrogenase (lysine forming) had a common ancestor with alanine dehydrogenases of prokaryotes. At present we could find no sequence for the alanine dehydrogenase from eukaryotes in the international DNA/Protein databases, so we suggested that eukaryotic saccharopine dehydrogenase (lysine forming) had been evolved from a common ancester with bacterial alanine dehydrogenase.

The BLAST result also showed that LYS9 had similarity with the proteins from 1 fungus (*S. pombe*), 3 animals (*H. sapiens, C. elegans,* and *M. musculus*), 3 plants (*Arabidopsis thaliana, Z. mays,* and *Brassica napus*), and 18 prokaryotes. Interestingly, animals and plants have a single saccharopine dehydrogenase that is a bifunctional enzyme, in which the first half of the protein had a similarity with LYS1 and the second half had a similarity with LYS9. In the course of eukaryotic evolution, genes encoding saccharopine dehydrogenase (lysine forming) and saccharopine dehydrogenase (glutamate forming) (Fig. 2b) were fused to code for a single-chain polypeptide in animals and plants, whereas such an event has not occurred in fungi. Considering that animals and plants could not synthesize lysine but could catabolize it, it is possible to assume that the single-chain saccharopine dehydrogenase (bifunctional) is adapted for the degradation of lysine, and the separation of the saccharopine dehydrogenases may be fit for the synthesis of lysine. At present we do not know the reason for the separation and/or combination of the two saccharopine dehydrogenases. Recently, the presence in mice of monofunctional lysine-degrading enzyme was reported (Papes et al. 1999). Cumulative information on the amino acid sequences of saccharopine dehydrogenases from various sources along with their functions could be required for elucidation of the regulation of the lysine biosynthetic pathway.

In the lysine biosynthetic pathway of *S. cerevisiae*, complex of LYS2 and LYS5 serves as a multifunctional enzyme, aminoadipate reductase, which converts α-aminoadipate to 2-aminoadipate 6-semialdehyde via an adenosylated derivative (Miller and Bhattacharjee 1996; Morris and Jinks-Robertson 1991). This process has been found only in fungi. The BLAST results showed that LYS2 had similarity with proteins from 7 fungi (*C. albicans, Penicillium chrysogenum, S. pombe, Emericella nidulans, Tolypocladium inflatum, Metarhizium anisopliae,* and *Alternaria alternata*) and 21 prokaryotes; LYS5 had similarity with the proteins from 9 animals (*C. elegans, H. sapiens, M. musculus, Rattus norvegicus, Gallus gallus, Danio rerio, Xenopus laevis, Manduca sexta,* and *Oryctolagus cuniculus*), 1 plant (*A. thaliana*), and 7 prokaryotes.

Although LYS2 had no homologue in other eukaryotes, LYS5 had some homologues in *C. elegans.* The phylogenetic tree (Fig. 3a) showed that the two eukaryotic LYS5 homologues (T28H10.1 and T04G9.4 from *C. elegans*), which showed the highest score among eukaryotes in the BLAST result, were far from LYS5 of *S. cerevisiae.* This suggests that the functions of the homologues are probably different from that of LYS5. In addition, some antibiotic synthetases and peptide syntheta-

ses of bacteria had similarity with LYS2 as already been reported by Suvarna et al. (1998), suggesting that aminoadipate reductase in fungal lysine synthesis is evolutionarily related to some bacterial antibiotic/peptide synthetases. However, in addition to the difference in the molecular size of both enzymes (bacterial antibiotic/peptide synthetases are much bigger than α-aminoadipate reductase), our phylogenetic tree (Fig. 3b) indicates that each cluster is completely monophyletic with deep diverging point between the clusters. At the moment, it is unlikely that direct horizontal gene transfer happened between fungi and bacteria. In this paper, we cannot elucidate whether the eukaryotic origin had aminoadipate reductase or not. Nevertheless, we conclude that α-aminoadipate reductase is a key enzyme to the evolution of fungal lysine synthesis, which is unique character of *S. cerevisiae* and probably other fungi.

Acknowledgments. We thank Wellyzar Sjamsuridzal for reading through the manuscript and correcting the English.

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