Characterization of a *lysK* gene as an *argE* homolog in *Thermus thermophilus* HB27

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Abstract We conducted a chromosome walk to obtain a DNA fragment downstream of *lysJ* and found an *argE* homolog in a putative operon composed of *lysJ–orfC–orfD–argE* homologs. A knockout mutant of the *argE* homolog showed significantly slow growth on a minimal medium, and the growth was markedly improved by addition of lysine. We therefore termed this gene *lysK*. Purified LysK protein has deacetylating activities for both N^2-acetyllysine and N^2-acetylornithine at almost equal efficiency. These results suggest that *lysK* which may share an ancestor with *argE* functions not only for the lysine biosynthesis, but also for arginine biosynthesis in *Thermus thermophilus*.

Key words: Lysine biosynthesis; *argE*; Arginine biosynthesis; *Thermus thermophilus*

1. Introduction

There are two pathways for lysine biosynthesis. One is the diaminopimelate (DAP) pathway starting from aspartate via DAP, as seen in most bacteria and plants, the other is the α-aminoadipate (AAA) pathway from 2-oxoglutarate via AAA, as seen in yeast [1] and fungi [2,3]. In our recent studies, however, an extremely thermophilic bacterium, *Thermus thermophilus* HB27, was shown to synthesize lysine not via DAP, but through AAA [4]. We had cloned a gene cluster involved in lysine biosynthesis through AAA from *T. thermophilus* HB27, and found that some of the genes in the lysine AAA biosynthetic gene cluster encode proteins which have significant identity in amino acid sequence to those encoded by genes involved in arginine biosynthesis [5]. In addition, there are no homologs of LYS2, LYS5, LYS9 and LYS1, which are involved in the conversion process of AAA to lysine through an intermediate, saccharopine, in *S. cerevisiae*, in the cluster of *T. thermophilus* HB27. Consistent with this, we have never isolated lysine auxotrophic mutants that require saccharopine for growth (unpublished data). Then, we have proposed that in *T. thermophilus* HB27, conversion from AAA to lysine does not include saccharopine as a biosynthetic intermediate as seen in yeast and fungi, but proceed in a manner similar to that from glutamate to ornithine in arginine biosynthesis [6]. We also succeeded in cloning of a homolog of *argD* encoding N^2-acetylornithine aminotransferase (EC 2.6.1.11) [7] and found that the homolog named *lysJ* is essential for lysine biosynthesis in this bacterium. Kinetic analysis revealed that LysJ actually catalyzed the transaminating reaction for N^2-acetyllysine as the substrate. The catalytic efficiency for this substrate was, however, significantly lower than that for N^2-acetylornithine, an intermediate of arginine biosynthesis. Those results suggest that the lysine biosynthetic pathway in this bacterium shares a common ancestor with the arginine biosynthetic pathway, and may function in both pathways.

To elucidate the whole pathway for lysine biosynthesis and its regulation, it is necessary to clone all the genes involved in the lysine biosynthesis in *T. thermophilus* HB27. By our previous cloning experiments [4,5,7], most of the genes involved in lysine biosynthesis of *T. thermophilus* HB27 had been cloned. Although the enzyme activity to deacetylate N^2-acetyllysine, which was putatively the last step of the lysine biosynthesis, was detected in the crude extract of *T. thermophilus* HB27 (data not shown), none of the cloned DNA fragments contains a gene homologous to *argE* that is involved in the last step of the arginine biosynthesis. In the putative lysine biosynthetic gene cluster of *Pyrococcus* species, *argE* homolog (*PH1715*) is present just downstream of the gene corresponding to *lysJ* (*PH1716*) [5,8]. Downstream of the *lysJ* gene in the *Bam*HI fragment, however, the open reading frame (ORF) corresponding to *argE* was absent, but two short ORFs that were probably co-transcribed as a single mRNA with *lysJ* gene are found. This prompted us to clone the region further downstream of the two ORFs. In this report, we describe cloning of an *argE* homolog termed *lysK* and its characterization. We also discuss roles of *lysK* and evolutionary relationship of the newly identified lysine biosynthesis and arginine biosynthesis.

2. Materials and methods

2.1. Strains, media and chemicals

T. thermophilus HB27 was cultivated as described previously [1,9,10]. *Escherichia coli* DH5α and JM105 [11] were used for DNA manipulation and *E. coli* BL21-CodonPlus (DE3)-RIL [F^-, *ompT*, *hsdS* (r_B^-, m_B^-), *dcm*$^+$, Tet^r, *gal*, λ (DE3), *endA*, Hte, [*argU*, *ileY*, *leuW*, Cam^r]] (Stratagene, La Jolla, CA, USA) was used as the host for gene expression. 2×YT medium [11] was generally used for culti-

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vation of *E. coli* cells. Antibiotics and isopropyl-β-D-thiogalactopyranoside (IPTG) were added to the medium when required. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Kanto Chemicals (Tokyo, Japan). Enzymes for DNA manipulation were purchased from Takara Shuzo (Kyoto, Japan).

2.2. Cloning of the downstream region of lysJ gene

Chromosomal DNA of *T. thermophilus* HB27 was prepared according to the methods in the literature [11]. Southern hybridization was carried out with a Random Primer Fluorescein Labeling kit (New England Nuclear, Boston, MA, USA) against genomic DNA of *lysJ*-deficient mutant of *T. thermophilus* HB27, RV4 [7], using a *Hin*dIII/*Bam*HI fragment of 342 bp from pRDBamL [7] as the probe. A *Kpn*I fragment of approximately 4.5 kb that was positive in hybridization assay was ligated into pUC18 previously digested with *Kpn*I, and introduced into *E. coli* DH5α. A colony that was positive in the colony hybridization assay using the same probe was selected. A plasmid was recovered from the colony and named pREKpnL. The nucleotide sequence was determined by the method of Sanger et al. [12].

2.3. Disruption of three genes in T. thermophilus HB27 and auxotrophic complementation test

To introduce the *Aor*51HI site into *lysK*, two independent PCRs using OrfDN1/MutREN and MutREC/RE-MidNN as primers were performed. PCR conditions used are: (step 1) 95°C for 2 min, (step 2) 95°C for 1 min, (step 3) 65°C for 1 min, (step 4) 72°C for 2 min, (step 5) 72°C for 7 min; step 2 to step 4 were repeated 30 times. A portion of each reaction mixture was mixed and subjected to additional PCR using orfDN1/RE-MidNN as primers under the same PCR conditions. Amplified fragment was digested with *Sph*I and *Eco*RI and ligated with pUC18 previously digested with the same enzymes. Resulting plasmid named pDFRE was digested with *Aor*51HI, treated with calf intestine alkaline phosphatase, and ligated with blunt-ended *Hin*dIII fragment of pUC39-442KmR [13] that contains heat-stabilized kanamycin nucleotidyltransferase gene [14]. The resulting plasmid was named pDFREKmR and used for knockout of the *lysK* gene of *T. thermophilus* HB27 as described previously [7]. In the same way, two other plasmids, pDFOCKmR and pDFODKmR that were used for knockout of *orfC* and *orfD*, respectively, were constructed. For introduction of an *Aor*51HI site into *orfC* and *orfD*, a pair of PCRs using orfDN1/MutOCN and MutOCC/RE-MidNN, and another pair of PCRs using orfDN1/MutODN and MutODC/RE-MidNN were performed. All the primers used for PCR were listed in Table 1. Disruption of the genes was confirmed by Southern hybridization, and auxotrophic complementation tests for the mutants were carried out as described previously [7]. Growth of the cells was analyzed by monitoring OD_{600} of the cell culture taken at an appropriate interval.

2.4. Construction of plasmid for expression of lysK in E. coli

A plasmid for the expression of *lysK* gene was constructed as follows. Two independent PCRs were performed using ARGENN/RE-MidNN and RECC2/RE-MidCC as primers. The former and the latter PCRs were used to amplify the first and second halves of *lysK*-coding region, respectively. Each amplified fragment was blunt-ended with T4 DNA polymerase, treated with T4 polynucleotide kinase, and ligated into pBluescript II SK (+) (Stratagene) previously digested with *Eco*RV. These fragments were digested with *Nde*I/*Eco*RI or *Eco*RI/*Hin*dIII, respectively, and inserted into pET26b (+) (Novagen) previously digested with *Nde*I and *Hin*dIII. The resulting plasmid, pETTRENH, was used for the expression of *lysK* gene.

2.5. Purification and assay of the recombinant LysK

E. coli BL21-CodonPlus (DE3)-RIL harboring pETTRENH was cultured in 1.2 l of 2×YT medium containing 50 μg/ml kanamycin and 30 μg/ml chloramphenicol. When the *E. coli* cells were grown to an OD_{600} of 0.6, IPTG (final 0.2 mM) was added. The culture was continued for an additional 12 h at 25°C after the induction. *E. coli* cells of 7 g were suspended in 14 ml of buffer II (20 mM potassium phosphate buffer, pH 7.5) and disrupted by sonication. The supernatant prepared by centrifugation at 40 000×*g* for 20 min was heated at 70°C for 20 min, and denatured proteins from *E. coli* cells were removed by the centrifugation as described above. Supernatant fractions were applied onto an anion-exchange column (DE-52; Whatman, Tokyo, Japan) pre-equilibrated with buffer II and passing fractions were pooled. After addition of ammonium sulfate to the pooled fractions at a final concentration of 60%, the resultant precipitate was collected by centrifugation at 40 000×*g* for 30 min. The precipitate was suspended in buffer III (20 mM Tris–HCl, pH 8.5), dialyzed against buffer III, and loaded onto an anion-exchange column, ResourceQ (Pharmacia Biotech, Tokyo, Japan) equilibrated with buffer III. Proteins adsorbed were eluted with a linear gradient of 0–0.3 M of NaCl. Active fractions were pooled, concentrated, and applied onto a Hi-load Superdex 200 prep grade column (Pharmacia Biotech) equilibrated with buffer II containing 0.2 M NaCl.

Purity of the recombinant enzyme was verified by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis (SDS–PAGE). Protein concentration was determined by the method of Bradford [15] using a Bio-Rad protein assay kit (Nippon Bio-Rad, Tokyo, Japan). Sedimentation equilibrium analysis of the purified enzyme was carried out with a Beckman Optima XL-A analytical ultracentrifuge fitted with a Beckman An-60Ti analytical rotor. The oligomeric structure of the enzyme was analyzed according to the procedure of Van Holde and Baldwin [16].

Assay of the purified LysK was performed according to the literature [17] with minor modifications. Enzyme reaction was started by adding 10 μl of the enzyme (0.2 mg/ml) to a reaction mixture (1 ml, 50 mM potassium phosphate buffer, pH 7.0, 0.2 mM $CoSO_4$, 0.2–0.6 mM N^2-acetyllysine or 0.2–0.6 mM N^2-acetylornithine). Decrease in absorption at 214 nm was monitored at 60°C. Kinetic parameters were calculated by using an initial velocity program, HYPER, of Cleland [18].

2.6. Nucleotide sequence accession number

The nucleotide sequence data reported in this paper will appear in the DDBJ sequence database under the accession number AB063579.

3. Results

3.1. Cloning of the downstream region of lysJ gene

When the chromosomal DNA was digested with several restriction enzymes and subjected to Southern hybridization using a DNA fragment containing the downstream region of *lysJ* as the probe, several hybridization-positive DNA frag-

Table 1
Oligonucleotides used in this study

Oligonucleotide	Sequence	Restriction site
ARGENN	5′-AAAAAACATATGAGCAAAAGCGCCTTG-3′	*Nde*I
RECC2	5′-TTTTAAGCTTACCCCTCCTTCTCCCC-3′	*Hin*dIII
RE-MidNN	5′-CTCGCGGCCGAAGAATTCCAGCTCAATCGT-3′	*Eco*RI
RE-MidCC	5′-ACGATTGAGCTGGAATTCTTCGGCCGCGAG-3′	*Eco*RI
orfDN1	5′-GGCGCGGAGCATGCCCAAGGGGGGGCACGG-3′	*Sph*I
MutOCN	5′-GAGGAGTTCCTCAGCGCTTTGGGCCTGGGT-3′	*Aor*51HI
MutOCC	5′-ACCCAGGCCCAAAGCGCTGAGGAACTCCTC-3′	*Aor*51HI
MutODN	5′-CCGAAGGCGCACAGCGCTTCCCCGGACCCA-3′	*Aor*51HI
MutODC	5′-TGGGTCCGGGGAAGCGCTGTGCGCCTTCGG-3′	*Aor*51HI
MutREN	5′-CCTCCCAGCCCGAGCGCTCCCCGATCACCG-3′	*Aor*51HI
MutREC	5′-CGGTGATCGGGGAGCGCTCGGGCTGGGAGG-3′	*Aor*51HI

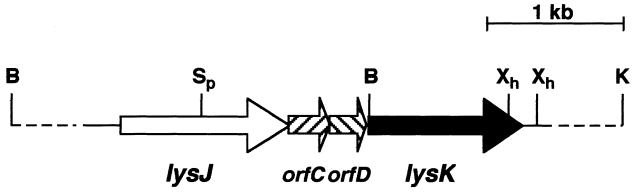

Fig. 1. Newly cloned lysine biosynthetic gene cluster composed of *lysJ–orfC–orfD–lysK*. Abbreviations: K, *Kpn*I; X_h, *Xho*I; B, *Bam*HI; S_p, *Sph*I.

ments were detected. We first tried to clone some of them showing positive hybridization. Several attempts to clone the DNA fragments, however, have so far failed for unknown reasons. On the other hand, we previously generated a knockout mutant (RV4) for the *lysJ* gene of *T. thermophilus* HB27 [7]. In the *lysJ* region of the genome of the mutant, a single additional *Kpn*I site was introduced as the result of the disruption. Southern hybridization against the chromosomal DNA of the mutant gave a positive signal of 4.5 kb when it was digested with *Kpn*I (data not shown). The DNA fragment was recovered and inserted into pUC18. A clone positive in colony hybridization assay was picked and the plasmid contained in the transformant was named pREKpnL. Sequencing analysis revealed that the insert contains a 3′-terminal portion of *lysJ* gene, *orfC* and *orfD* as expected from our previous result [7]. In addition to the three ORFs, another ORF overlapping with the 3′-terminus of 29 bp of *orfD* was found (Fig. 1). The ORF was a homolog of the *argE* gene encoding N^2-acetylornithine deacetylase (EC 3.5.1.16) [19]. Although the DNA fragment was cloned from the *lysJ* knockout mutant, we later confirmed that the cloned DNA fragment possesses the original nucleotide sequence of the wild-type *T. thermophilus* by sequencing of PCR products using chromosomal DNA of the wild-type as the template (data not shown). Putative catalytic and metal binding residues proposed for ArgE [20] are completely conserved (Fig. 2). Among ArgE and their homologs, the ArgE homolog from *T. thermophilus* HB27 shows significant identity in amino acid sequence to DR1413 (50%) from *Deinococcus radiodurans* [21], PH1715 (39%) from *Pyrococcus horikoshii*, and PAB0294 (39%) from *Pyrococcus abyssi*. The two *Pyrococcus* homologs are both found in the putative lysine biosynthetic gene cluster of these archaea [5,8]. *D. radiodurans* was recently suggested to possess the lysine biosynthesis similar to that of *T. thermophilus* [22]. These results suggest that the homolog that we cloned is involved in the last reaction of the lysine biosynthesis in *T. thermophilus* HB27 (Fig. 3).

3.2. Disruption of orfC, orfD and argE homolog

We previously showed that a knockout mutant of *lysJ* could not grow on a minimal medium and exogenous addition of lysine restored the growth on the medium [7]. We then analyzed the roles of three other genes, presumably forming an operon with *lysJ* in the lysine AAA biosynthesis, by disruption of the chromosomal copy of *T. thermophilus* HB27. Knockout mutants of *orfC* and *orfD* could grow normally in the minimal medium, indicating that these genes are not essential for the lysine biosynthesis in *T. thermophilus* HB27 (data not shown). However, a knockout mutant of the *argE* homolog showed significantly slow growth in the minimal medium (Fig. 4). The slow growth was at least partially restored by exogenous addition of 0.1 mM lysine. The result indicated that this *argE* homolog is involved in the lysine biosynthesis of *T. thermophilus* HB27. We, therefore, named this gene *lysK*. Interestingly, the addition of both lysine and ornithine to the minimal medium gave obviously better growth of the *Thermus* mutant, when compared to the case for the addition of only lysine.

3.3. Expression and purification of LysK

To characterize *lysK* gene product, we expressed the gene in *E. coli* and obtained a purified preparation of LysK by using a T7 promoter expression system. LysK was accumulated as insoluble inclusion bodies when the *E. coli* BL21-CodonPlus (DE3)-RIL cells carrying pETTRENH were cultured in the presence of 1 mM IPTG at 37°C. By lowering the temperature for the expression to 25°C, a portion of LysK proteins was produced in soluble fraction. Through four steps of purification procedure, LysK of 13 mg was prepared from 1.2 l culture with more than 95% purity on SDS–PAGE (data not shown). To estimate the quaternary structure of LysK, we carried out sedimentation equilibrium analysis. By using a partial specific volume of 0.7404 that was calculated from the amino acid composition, we estimated that LysK was present as a mixture of homotetramer (75%) and homooctamer (25%) when the enzyme concentration was in the range 0.125–0.5 mg/ml (data not shown).

3.4. Kinetic properties of LysK activity

To elucidate the catalytic properties of LysK, kinetic analysis was carried out using N^2-acetyllysine, a putative intermediate of lysine AAA biosynthesis, and N^2-acetylornithine, an intermediate of arginine biosynthesis, as the substrates. The result shows that LysK recognizes both the substrates at almost equal efficiency (Table 2).

	67	125	149	327
T. thermophilus LysK	LLGHTDT	ATEEEAP	VIGEPSG	-LDHTPY
E. coli ArgE	LAGHTDT	TADEETS	IIGEPTS	NQAHQPD
E. coli SDAP	FAGHTDV	TSDEEAS	LVGEPSS	ATIHKIN
Pseudomonas sp. Cpg2	LMSHMDT	NTDEEKG	LSFEPTS	FGYHSDK
Porcine ACY1	LNSHTDV	VPDEEVG	LD-EGLA	VLLHDHD
Human ACY1	LNSHTDV	VPDEEVG	LD-EGIA	VLLHDHD
		*		

Fig. 2. Alignment of the amino acid sequence of LysK from *T. thermophilus* HB27 with those of *E. coli* ArgE [8], *E. coli* succinyl-L,L-diaminopimelate desuccinylase (DapE) (P24176), *Pseudomonas* sp. Cpg2 (P06621), porcine AcyI (X68564), and human AcyI (Q03154). The residues involved in the metal binding sites determined for Cpg2 [20] are boxed. The putative active site residue that acts as a general acid is marked with an asterisk. Numbers of amino acid sequence for LysK are given.

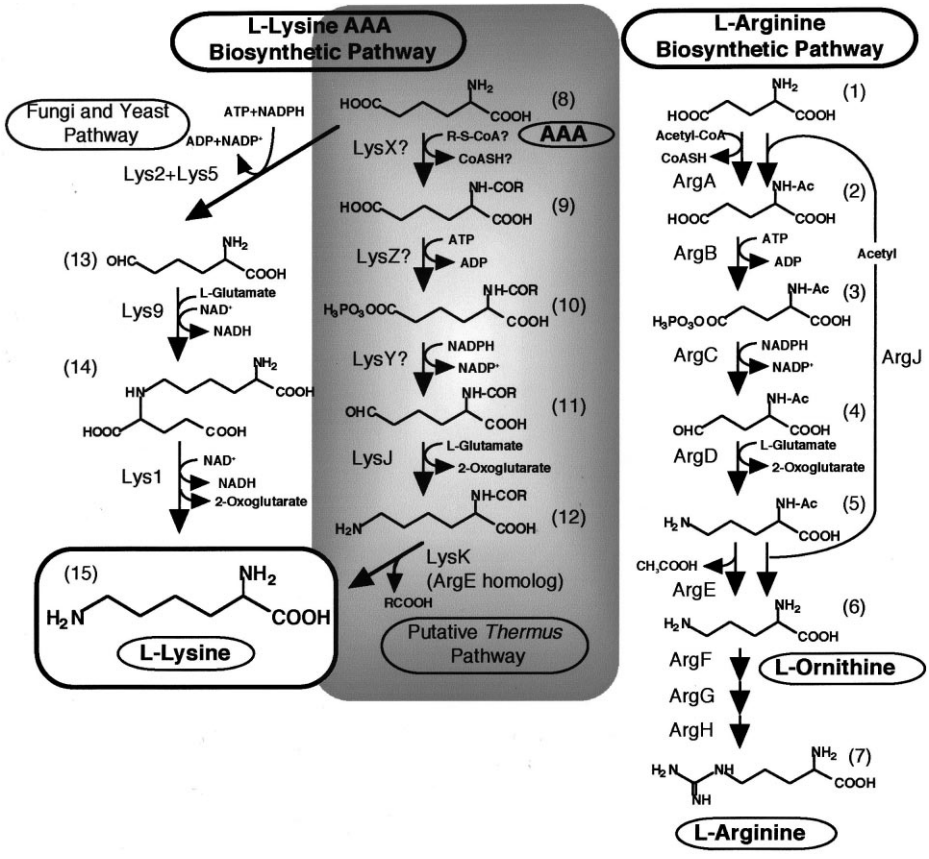

Fig. 3. Lysine AAA biosynthetic pathway after AAA and arginine biosynthetic pathway. The shadow indicates the biosynthetic pathway in *T. thermophilus* HB27. (1) L-glutamate; (2) N^2-acetyl-L-glutamate; (3) N^2-acetyl-L-glutamyl-γ-phosphate; (4) N^2-acetyl-L-glutamate semialdehyde; (5) N^2-acetyl-L-ornithine; (6) L-ornithine; (7) L-arginine; (8) AAA; (9) N^2-acetyl-α-aminoadipate (probably); (10) N^2-acetyl-α-aminoadipyl-δ-phosphate (probably); (11) N^2-acetyl-α-aminoadipate semialdehyde (probably); (12) N^2-acetyl-L-lysine (probably); (13) AAA semialdehyde; (14) L-saccharopine; (15) L-lysine.

4. Discussion

In this study, we cloned the downstream region of *lysJ* gene and found an *argE* homolog, *lysK*. The phylogenetic tree among the ArgE family shows that LysK from *T. thermophilus* HB27 forms a group with DR1413 from *D. radiodurans* [21], PH1715 from *P. horikoshii* [8], and PAB0294 from *P. abyssi* (G75160) (Fig. 5). The fact that the group containing LysK is separated from a lineage containing ArgEs from organisms such as *E. coli*, which was shown to be involved in arginine biosynthesis, suggests that all the members of the LysK-containing group have a function in lysine biosynthesis through AAA, and therefore these microorganisms produce lysine through the newly identified AAA pathway.

Phenotypic analysis of the knockout mutant of the homolog suggests that the homolog is indeed involved in lysine biosynthesis of *T. thermophilus* HB27. Among four genes, *lysJ*, *orfC*, *orfD*, and *lysK*, only *lysJ* and *lysK* were shown to be involved in the lysine biosynthesis. Gene arrangement suggests that *orfC* and *orfD* are also co-transcribed with *lysJ* and *lysK*. Therefore, the fact that the knockout of these two ORFs showed no effect on the cell growth may look curious. However, *PH1716* and *PH1715* genes which are putative counterparts of *lysJ* and *lysK*, respectively, in *P. horikoshii*, are present in a tandem manner in the distal end of the putative lysine biosynthetic gene cluster, and there are no ORFs between these two genes and no ORFs homologous to ORFs C and D in the cluster [5,8]. This may suggest that these two ORFs have no critical role in lysine biosynthesis. Since *orfC* and *orfD* have no homologous genes whose functions are identified in databases, we at present do not know whether or not these two ORFs actually have some functions in the lysine biosynthesis.

The *lysK*-deficient mutant exhibited significantly slow growth on the minimal medium but did not show the complete lysine auxotrophic phenotype. This suggests that other *argE* homologs or genes with similar function that partially compensate for the lack of *lysK* are present. Until now 'real

Table 2
Kinetic parameters of LysK

Substrate	K_m (mM)	k_{cat}^a (s^{-1})	k_{cat}/K_m^a (s^{-1} M^{-1})
N^2-Acetyl-L-lysine	1.3 ± 0.5	51.8 ± 13.7	4.1×10^4
N^2-Acetyl-L-ornithine	0.5 ± 0.1	28.1 ± 3.4	5.3×10^4

[a]Calculated on the assumption that LysK forms a homotetramer.

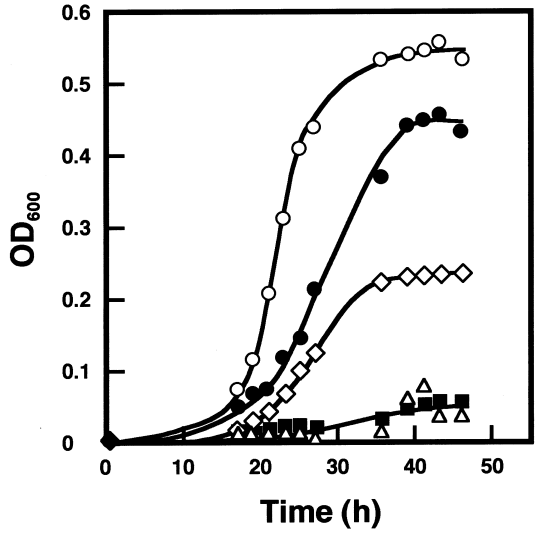

Fig. 4. Growth curve of wild-type and *lysK*-deficient mutant of *T. thermophilus* HB27. Growth of wild-type of *T. thermophilus* HB27 and *lysK*-deficient mutant in a minimal medium is shown by open circles and squares, respectively. Growth of *lysK*-deficient mutant in a minimal medium supplemented with 0.1 mM ornithine, 0.1 mM lysine, and both the amino acids (0.1 mM each) is shown by triangles, diamonds, and filled circles, respectively.

argE' that is critical for the synthesis of ornithine has not yet been cloned. Recently, *argJ* gene encoding ornithine acetyltransferase (EC 2.3.1.35) was isolated from *T. thermophilus* HB27 [23]. This enzyme catalyzes the transfer of an acetyl group from N^2-acetylornithine to glutamate, and produces ornithine and N^2-acetylglutamate that is subsequently used as the second intermediate in the arginine biosynthesis [6] (Fig. 3). This enzyme is a possible candidate that may catalyze the final reaction step for lysine biosynthesis, although this hypothesis remains to be elucidated for the future study.

The results shown in Fig. 4 also suggest another role for the *lysK* gene. Addition of lysine to the culture actually improved the growth of the *lysK*-deficient mutant on the minimal medium. On the contrary, exogenous addition of ornithine had only a negligible effect on the growth of the *lysK*-deficient mutant. Therefore, it is evident that *lysK* is mainly involved in the lysine biosynthesis. However, the growth of the mutant was much improved by simultaneous addition of lysine and ornithine. This suggests that *lysK* is involved in not only the lysine biosynthesis but also the arginine biosynthesis in *T. thermophilus* HB27. Consistent with this, LysK catalyzes deacetylation of N^2-acetylornithine as well as N^2-acetyllysine at similar efficiency (Table 2). Ornithine is an intermediate of arginine biosynthesis. Furthermore, the compound is also used as a precursor of polyamines that were involved in cellular processes and an indispensable component of the cell wall in *T. thermophilus* [24,25]. Requirement of ornithine for several cellular functions may have developed several supporting systems to produce ornithine. In our previous studies, we showed that LysJ that is responsible for transamination of N^2-acetyllysine also recognizes the corresponding compound in arginine biosynthesis [7]. The previous result may also coincide well with the above hypothesis. Thus, it is interesting to examine the substrate specificity of other enzymes involved in the lysine biosynthesis to understand the relationship between substrate specificity, evolution, and cellular functions of the enzymes.

Boyen et al. analyzed the quaternary structure of *E. coli* ArgE by gel filtration [20]. They observed that the ArgE with 43 kDa as a monomer was eluted at various elution times depending on the conditions, and obtained a value of 100 kDa by averaging the apparent molecular mass. From the results, they concluded that ArgE was a homodimer. When we analyzed the quaternary structure of LysK by gel filtration, we also obtained a similar value of 110 kDa for the molecular mass (data not shown). Sedimentation equilibrium indicated that the enzyme was present in highly oligomeric forms, tetramer and octamer, under our experimental conditions. Although we do not know whether or not similar estimation of the quaternary structure is possible for ArgE, similar sedimentation equilibrium would be helpful to elucidate the quaternary structure of ArgE as well.

In this study, we showed that *lysK*, an *argE* homolog, is involved in lysine biosynthesis in *T. thermophilus* HB27. Thus,

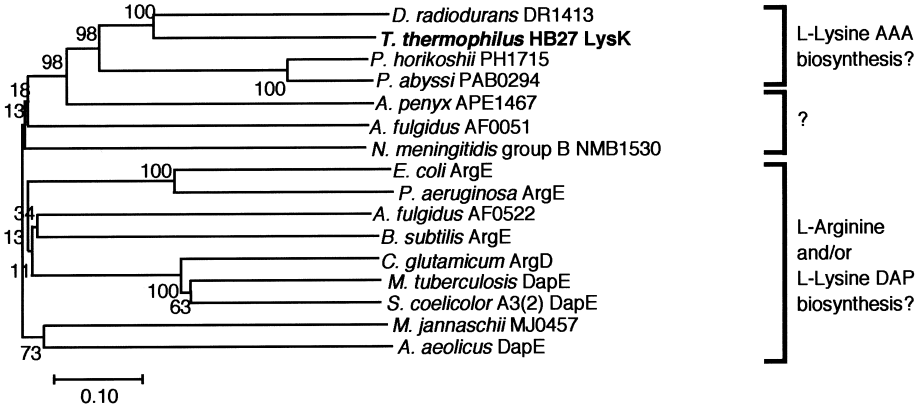

Fig. 5. Phylogenetic tree among various N^2-acetylornithine deacetylases, LysK and related proteins. A phylogenetic tree was constructed by using maximum parsimony and neighbor joining methods. Sequence data for the analysis were obtained from GenBank and PIR databases. The amino acid sequence was aligned by CLUSTAL W [26] on DDBJ. Using these aligned data, a phylogenetic tree was constructed by the computer program Mega [27]. Numbers on selected nodes indicate bootstrap values. This figure includes LysK and related proteins from *D. radiodurans* [21], *P. abyssi* (G75160), *P. horikoshii* [8], *Aeropyrum pernix* (C72626), *Archaeoglobus fulgidus* (C69256, B69315), *Neisseria meningitidis* group B (F81073), *E. coli* [19], *Pseudomonas aeruginosa* (A82996), *Bacillus subtilis* (H69588), *Corynebacterium glutamicum* (Q59284), *Mycobacterium tuberculosis* (H70608), *Streptomyces coelicolor* A3(2) (X81379), *Methanococcus jannaschii* (Q57899), *Aquifex aeolicus* (C70349).

this study has become the second instance where a homolog of enzyme for arginine biosynthesis is directly shown to be involved in lysine biosynthesis. Considering that the corresponding enzymes in both pathways are evolved from a single common ancestral enzyme, these two pathways are diverged from a common ancestral pathway. Through further detailed studies on the lysine biosynthetic pathway in *T. thermophilus* HB27, for example, structural and biochemical analysis, it would be expected to reveal principles for evolution of enzyme along with amino acid biosynthesis.

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