Molecular Evolution of Threonine Dehydratase in Bacteria

Xuefei Yu1,2, Ye Li2, Xiaoyuan Wang1,3*
1 State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China, 2 Key Laboratory of Industrial Biotechnology of Ministry of Education, Jiangnan University, Wuxi, China, 3 Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi, China

Abstract
Threonine dehydratase converts L-threonine to 2-ketobutyrate. Several threonine dehydratases exist in bacteria, but their origins and evolutionary pathway are unknown. Here we analyzed all the available threonine dehydratases in bacteria and proposed an evolutionary pathway leading to the genes encoding three different threonine dehydratases CTD, BTD1 and BTD2. The ancestral threonine dehydratase might contain only a catalytic domain, but one or two ACT-like subdomains were fused during the evolution, resulting BTD1 and BTD2, respectively. Horizontal gene transfer, gene fusion, gene duplication, and gene deletion may occur during the evolution of this enzyme. The results are important for understanding the functions of various threonine dehydratases found in bacteria.

Introduction
There are usually two types of threonine dehydratase (TD) in bacteria: the biosynthetic threonine dehydratase (BTD) and the catabolic threonine dehydratase (CTD). They both could convert L-threonine to 2-ketobutyrate, BTD functions in the biosynthetic pathway of L-isoleucine when bacteria grow under the aerobic condition, while CTD plays a role in the degradation of L-threonine to propionate when bacteria grow under the anaerobic condition [1]. BTD usually contains an N-terminal catalytic domain and a C-terminal regulatory domain, while CTD usually contains only the catalytic domain. Sequence and structure analyses have revealed that the C-terminal regulatory domain of BTD is composed of one or two ACT-like subdomains (Fig. 1). BTD containing two ACT-like subdomains (BTD2) encoded by the gene ilvA in *Escherichia coli* is the key enzyme for L-isoleucine biosynthesis, and its activity is inhibited by the end product L-isoleucine but could be countered by L-valine, the product of a biosynthetic pathway [2]. BTD containing one ACT-like subdomain (BTD1) encoded by *ilvA* in *Bacillus subtilis* could be inhibited by L-isoleucine or by high concentrations of L-valine [3]. CTD encoded by the gene ilvB in *Salmonella typhimurium* is insensitive to L-isoleucine or L-valine, but its activity could be activated by AMP and CMP [4]. These examples indicate that the function of TD is closely related to the number of ACT-like subdomains it contains.

The sequence and/or structure of several TDs in bacteria have been characterized [2,5,6], but the differences on the sequence and structure of CTD, BTD1 and BTD2 are not fully understood. In this study, we analyzed the amino acid sequences of all the available TDs in bacteria, and proposed an evolutionary pathway leading to the genes encoding CTD, BTD1 and BTD2 in the present bacteria.

Materials and Methods
Sequential and structural alignment of CTD and BTD
There are 15120 TD sequences in the protein database of NCBI. The number of amino acids in these TDs is mainly around 350, 400 or 510. Because CTD usually contains less amino acids than BTD, we assume that the TDs containing about 350 amino acids are CTD. Thus, all TDs were divided into two groups: BTDs which contain more than 360 amino acids, and CTDs which contain less than 360 amino acids. One BTD and/or CTD sequence was chosen from each genus, and as a result, 546 BTDs and 328 CTDs were chosen. These TDs were further confirmed by using Conserved Domain Architecture Retrieval Tool (CDART) in NCBI [7] to check if they contain the ACT-like subdomain. The sequence alignments of these BTDs and CTDs were further pairwise aligned by using FATCAT model by PyMol. The crystal structure of BTD2 (1TDJ) from *E. coli* and CTD (2GN2) from *S. typhimurium* were obtained from PDB database [10]. The structure of BTD1 coded by gene *ilvA* from *B. subtilis* was modeled by using SWISS-MODEL Web server [11] with default parameters. These structures were used to build the comparison model by PyMol. The crystal structures of *E. coli* BTD2 and *S. typhimurium* CTD were further pairwise aligned by using FATCAT web service [12] with flexible model, and the structural alignment of the PLP binding sites and the substrate binding sites were performed by using PyMol.

Distribution of species containing TD and construction of phylogenetic trees
The distribution of species containing TDs in nature were obtained from the UniProtKB database (http://www.uniprot.org/...
domain and one ACT-like subdomain; BTD2 (shown in blue) contains the catalytic domain and two ACT-like subdomains. CTD (shown in green) contains only the catalytic domain; BTD1 (shown in red) contains the catalytic domain and one ACT-like subdomain; BTD2 (shown in blue) contains the catalytic domain and two ACT-like subdomains.

domain composed of ACT-like subdomains. CTD (shown in green) contains only the catalytic domain; BTD1 (shown in red) contains the catalytic domain and one ACT-like subdomain; BTD2 (shown in blue) contains the catalytic domain and two ACT-like subdomains. CTD (shown in green) contains only the catalytic domain; BTD1 (shown in red) contains the catalytic domain and one ACT-like subdomain; BTD2 (shown in blue) contains the catalytic domain and two ACT-like subdomains.
Figure 2. Sequence alignment of CTDs and BTDs and structure alignment of BTD2 (1TDJ) and CTD (2GN2). A. The sequence alignment of CTDs from 328 species of bacteria. B. The sequence alignment of BTDs from 546 species of bacteria. The PLP binding sites and the substrate binding sites are labelled by purple and blue dots, respectively. The other highly conserved residues are labelled by black dots. C. The aligned structure of PLP binding sites of BTD2 and CTD. D. The aligned structure of substrate binding sites of BTD2 and CTD. The amino acid residues directly involved in PLP binding sites and the substrate binding sites are shown in sticks. Residues from CTD are shown in blue and residues from BTD are shown in red. The residues are labeled according to the sequence of CTD coded by tdcs in S. typhimurium [4].

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were selected from a wide range of species and the length of BTDs and CTDs are quite different. Therefore, some bootstrap values on the tree are lower than 50. BTD2 was found mainly in species of \( \alpha \)-and \( \beta \)-Proteobacteria, and a few species of \( \alpha \)-Proteobacteria (Fig. 3B); BTD1-A was found mainly in species of Firmicutes, Actinobacteria and a few species of \( \alpha \)-Proteobacteria (Fig. 3B); BTD1-B and CTD were found in species of all the three phyla: Proteobacteria, Firmicutes and Actinobacteria (Fig. 3C and D). The finding of two distinct BTD1 clusters, BTD1-A and BTD1-B, is interesting. There were 8 species of Firmicutes and Actinobacteria (shown in bold in Fig. 3) containing both BTD1-A and BTD1-B, suggesting that gene duplication of BTD1 might occur in the bacteria. According to the tree, BTD1-A cluster is much closer to BTD2 cluster than to BTD1-B, while BTD1-B cluster is much closer to CTD cluster. Based on these data, CTD might be the common ancestor for all the TDs, and BTD1 and BTD2 might be the gene fusion product of ancestral CTD and ACT-like subdomains because the combination of different domains is an important mechanism for the evolution of multidomain proteins [19]; BTD2 might be derived from ancestral BTD1-A during evolution because it is much closer to BTD1-A cluster than to BTD1-B cluster in the phylogenetic tree. Phylogeny trees were constructed using sequences of ACT-like subdomain of BTD1 and each of the two ACT-like subdomains of BTD2, and the results showed that the first ACT-like subdomain of BTD2 is closer to the ACT-like subdomain of BTD1 than the second ACT-like subdomain of BTD2. This does not mean that the second ACT-like subdomain of BTD2 was generated from a new ACT subdomain, because it could also be duplicated from the ACT-like subdomain of BTD1, considering the duplicated sequences of a protein are usually highly divergent to avoid the misfolding. Moreover, though the regulatory domains of TDs have close structural and functional relationships with ACT family domains [20–21], they have little sequence similarity with ACT family domains, and could not be assigned by PSI-BLAST as ACT family. Thus, the regulatory domains of TDs are named as ACT-like subdomains. Therefore, the second ACT-like subdomain of BTD2 is more likely the result of a duplication of the ACT-like subdomain of BTD1 rather than a fusion of a new ACT subdomain. Since BTDs also exist in Eukaryotes and Archaea, the fusion of CTD and ACT-like domain could be happened before the divergence of three kingdoms.

Figure 3. Phylogenetic tree based on the amino acid sequences of TDs from 82 representative species. Genes encoding the enzymes are represented by arrows. The overall structure of the phylogenetic tree is shown in A. Because it is too big to show in a single page, the detail structure of the phylogenetic tree is divided into three panels (B, C and D). The connecting point of the tree segments in the three panels is marked with a broken line. The strains shown in bold contain both genes encoding for BTD1-A and BTD1-B. \( \alpha \), \( \beta \), \( \delta \), \( \varepsilon \), \( \gamma \), F and A indicate \( \alpha \)-proteobacteria, \( \beta \)-proteobacteria, \( \delta \)-proteobacteria, \( \varepsilon \)-proteobacteria, \( \gamma \)-proteobacteria, Actinobacteria, and Firmicutes, respectively. The tree was constructed with the MEGA 5 software using the neighbor-joining method and 1000 bootstrap replicates. doi:10.1371/journal.pone.0080750.g003
in the bacterium and the numbers next to the arrow show the number of genes that might encode the TD.

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\( \gamma \)-proteobacteria, but only in 3 species of \( \varepsilon \)-proteobacteria. This suggests that BTD2 might generate within the ancestor of \( \beta \) - and \( \gamma \)-proteobacteria after its divergence from \( \varepsilon \)-proteobacteria, and BTD2 existing in the 3 species of \( \varepsilon \)-proteobacteria could be generated by horizontal gene transfer from species of \( \beta \) - or \( \gamma \)-proteobacteria (Fig. 4A). Although most of the 82 strains exist more than two TDs, BTD2 and BTD1-A were never found in the same strain, suggesting that BTD2 should be derived from the ancestral BTD1-A by fusing with another duplicated ACT-like subdomain. BTD1-B and BTD2 were found in some species of \( \beta \)-proteobacteria, but only BTD2 encoding genes were found in \( \gamma \)-proteobacteria, suggesting that BTD1-B might be deleted in some species after BTD2 was evolved. CTD, BTD1-B and BTD2 were all found in 8 bacterial strains of Proteobacteria but only one or two of them found in other strains, strongly suggesting that the deletion events might happen for TDs in bacteria during the evolution.

Discussion

Based on the homology and phylogenetic analysis, an evolutionary model for TDs was proposed (Fig. 5). The ancestor possessed only a single copy of gene encoding CTD containing only the catalytic domain. Later the gene was duplicated, and the redundant copy was fused with a DNA fragment encoding for ACT-like subdomain, producing the gene encoding for BTD1-B. Then this gene was duplicated, generating a copy encoding for BTD1-A. With the divergence of new species, one or two of the genes encoding for CTD, BTD1-A and BTD1-B were deleted from the genome. The similar duplication and deletion events were also found for the \( lpxH \) gene in Kdo 2 lipid A biosynthesis pathway [22]. The gene \( lpxH \) was duplicated within Proteobacteria, and one of them was lost along with new species generation. Within the ancestor of some species of Proteobacteria, the ACT-like subdomain of BTD1-A might be duplicated, generating BTD2. With the divergence of new species, the gene encoding for CTD, or BTD1-B were deleted from the genome. Two copies of BTD2 were observed in one species of Proteobacteria, suggesting that the duplication of BTD2 could also occur.

Our proposed evolutionary model of TD is consistent with the published theories, which suggest that organisms prefer to generate new genes encoding multiple domain proteins from the pre-existing genes [19,23,24], and new enzymes are usually evolved from enzymes with similar biochemical function rather than in the same biosynthetic pathway [25–28]. CTD exists not only in bacteria, but also in plants and yeast [29–33], suggesting that the pathway of L-threonine degradation may exist in the ancestral cell before the divergence of the three kingdoms. In the primordial soup where organic compounds were rich, the ancestral cell might have more catabolic pathways than biosynthetic pathways, therefore, it might only need CTD for gaining energy under the anaerobic condition [23]. With the increase of the number of primordial cells, the prebiotic supply of amino acids might be exhausted, and 2-ketobutyrate produced by CTD might also be used for L-isoleucine biosynthesis. For better adapting the environment, BTD were created in modern bacterial species by combining CTD and ACT-like subdomain to satisfy the necessary regulation of L-isoleucine and/or L-valine [34]. ACT family domain is widely conserved in bacteria and evolutionarily mobile.

Figure 4. Phylogenetic tree based on 16S rDNA sequences showing the phylogenetic distribution of the TD enzyme. The phylogenetic tree was constructed with MEGA 5 software using sequences from RDP database. Because it is too big to show in a single page, the structure of the phylogenetic tree is divided into two panels (A and B). The connecting point of the tree segments in the two panels is marked with a broken line. The scale bar indicates 0.02 change per nucleotide. The arrows at the right represent the TDs that could
It is always combined with other domains to provide easily regulated enzymes \[21,35\].

The interaction between different domains may lead the enzyme easier to fold correctly \[36\]. Thus BTDD1 or BTDD2 which contains both the catalytic domain and the ACT-like subdomain might be more stable than CTD which contains only the catalytic domain. The activity of BTDD2 might be regulated more easily than that of BTDD1 because BTDD2 contains one more ACT-like subdomain than BTDD1 \[3\]. Flexibility is one important reason for protein evolution, and the mechanical flexibility of proteins are critical for their functions \[37\]. More flexible the structure of an enzyme is more easily its activity could be regulated \[38,39\]. This suggests that the structure of BTDD2 may be more flexible than BTDD1, and BTDD2 might be evolved to benefit bacteria to adapt the more complex environment \[38,40\]. As the activity of BTDD is inhibited by the end product L-isoleucine, constructing feedback resistant BTDD has been used to increase the L-isoleucine production in industrial fermentation \[41–43\]. CTD encoded by tdcB from \textit{E. coli} has been overexpressed in \textit{C. glutamicum} to improve the production of L-isoleucine \[44,45\]. Our results suggest that directly removing the regulatory domain of an enzyme might be an effect way to obtain a feedback-resistant enzyme for the metabolic engineering in bacteria.

**Supporting Information**

**Table S1** The representative bacterial species used in the phylogenetic analysis of TDs.

(\text{DOCX})

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**Author Contributions**

Conceived and designed the experiments: XY XW. Performed the experiments: XY. Analyzed the data: XY YL XW. Wrote the paper: XY XW.

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