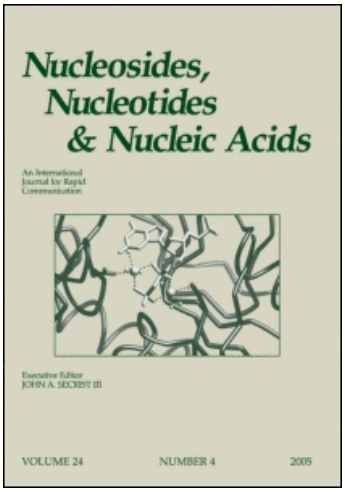

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Thymidine Kinase Diversity in Bacteria

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THYMIDINE KINASE DIVERSITY IN BACTERIA

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□ *Thymidine kinases (TKs) appear to be almost ubiquitous and are found in nearly all prokaryotes, eukaryotes, and several viruses. They are the key enzymes in thymidine salvage and activation of several anti-cancer and antiviral drugs. We show that bacterial TKs can be subdivided into 2 groups. The TKs from Gram-positive bacteria are more closely related to the eukaryotic TK1 enzymes than are TKs from Gram-negative bacteria.*

Keywords Thymidine kinase; Deoxyribonucleoside kinase; Nucleosides; Pyrimidines; Evolution; Nucleic acids precursors

INTRODUCTION

Deoxyribonucleoside kinases (dNKs), the key enzymes of deoxyribonucleoside salvage, phosphorylate deoxyribonucleosides (dNs) to the corresponding monophosphates in the first of the three phosphorylating steps leading to deoxyribonucleotide triphosphates (dNTPs).[1] Furthermore, dNKs have proven their medical importance as activators of a variety of deoxyribonucleoside analogs used in antiviral and anticancer treatment and as suicide genes in suicide gene therapy.[2] Two super families of dNKs exist, the thymidine kinase 1 (TK1)-like and the non-TK1-like family.[3] The

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TK1-like family is comprised only of dNKs with homology to human TK1. These enzymes are specific only for thymidine (dT) and deoxyuridine (dU). The non-TK1-like family comprises of dNKs that share no homology with the human TK1 except for the P-loop region at the N-terminus. The dNKs of the non-TK1-like family are rather unspecific compared to the TK1s, typically phosphorylating at least 2 of the native dNs. Recently, these 2 super families were shown to have a completely different structural scaffold. The TK1 family contains a structural zink and very rigid substrate selectivity,[4] as opposed to the non-TK1-family, which has great plasticity with respect to the substrates they accept or can accept upon mutagenesis.[5,6] Anyhow, the modern dNK functions of the 2 super families have evolved in parallel using different structural modules.

Here, we present the identification and phylogenetic relationship among a variety of TK1-like kinases of the bacterial origin and compare them with eukaryotic TK1s.

MATERIALS AND METHODS

Open reading frames (ORFs) of putative dNKs were identified by searching sequenced bacterial genomes available at NCBI (www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). In some cases the sequence was determined by additional sequencing. Amino acid sequence alignment of the identified TKs and selected known TK1s was performed using Clustal X 1.81[7] and phylogenetic reconstruction based on this alignment was done with Treecon.[8] Graphical visualization of the aligned sequences was done in BioEdit. The PDB file 1XBT, containing the coordinates for the human TK1 structure, was used with Swiss PDB viewer[9] for structural analysis and representation.

RESULTS

Several sequenced bacterial genomes (*Clostridium perfringens, Clostridium acetobutylicum, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Salmonella typhi, Deinococcus radiodurans, Yersinia enterocolitica, Listeria monocytogenes, Xanthomonas campestris, Ureaplasma parvum, Streptomyces coelicolor, Pseudomonas aeruginosa, and Helicobacter pylori*) were searched for genes with homology to the known TKs using the genome BLAST service at the NCBI website. Our search resulted in the identification of 12 putative TKs with 13.6% to 27.7% identity to the human TK1. The TKs originated from bacteria of both Gram-negative and Gram-positive origin meaning that the TK was present in the common ancestor. However, *Pseudomonas aeruginosa* and *Helicobacter pylori* do not contain a TK1-like gene, suggesting that dNKs might have been lost in some bacterial lineages. From the

phylogenetic point of view the bacterial TKs comprise 2 groups, one from Gram-positive bacteria, which seems to have high identity with the eukaryotic TK1 and another group that is mainly comprised of TKs from Gram-negative bacteria (Figure 1). However, in the group of Gram-negative TKs we also find TKs similar to that from Gram-positive bacteria, *L. monocytogenes, C. perfringens*, and *S. coelicolor*. It is surprising that TKs from C. acetobutylicum and C. perfringens are located in the 2 separate groups. We would expect that the Clostridiae, which are highly identical to each other,[10] also have very similar TKs. Several of the motifs that are conserved in the eukaryotic and the Gram-positive TKs are degenerated in the Gram-negative group (Figure 2). The P-loop motif G—GK[ST] is highly conserved in the Gram-positive group, as are Ser30, Glu35, Arg38, Arg39, Arg42 (human TK1 numbering). In human TK1, which is similar to the *Ureaplasma* TK,[4] the aforementioned amino acids are involved in the formation of hydrogen bonds between the A and B, and C and D chains in the tetrameric enzyme. Ser30 of chain B hydrogen binds to Arg38 of chain A. Arg38 in turn hydrogen binds to Glu35 and Glu35 to Arg39. Arg42 of the A chain hydrogen binds to the main chain hydroxyl group of Thr150 in Chain B (Figure 3). The Gram-negative TKs presented here do not have any of these residues conserved (Figure 2). The importance of these differences remains unclear, however it has not escaped our notion that the immediate

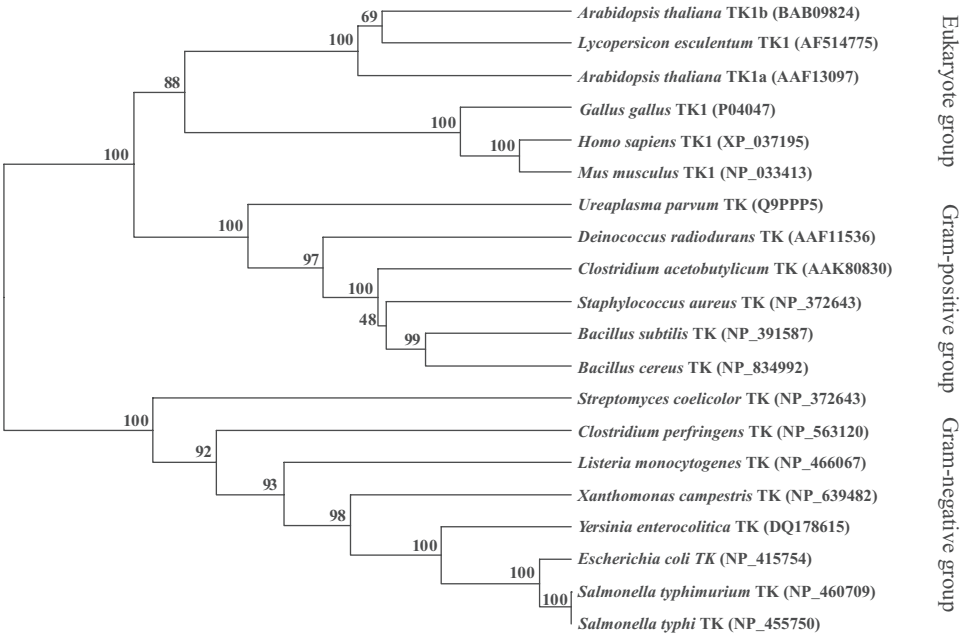

FIGURE 1 Phylogenetic tree of the TK1-like superfamily. Three major groups can be found, eukaryote TK1s, Gram-positive TKs, and Gram-negative TKs. *L. monocytogenes, C. perfringens*, and *S. coelicolor* have adopted the Gram-negative TK. Accession numbers are in brackets.

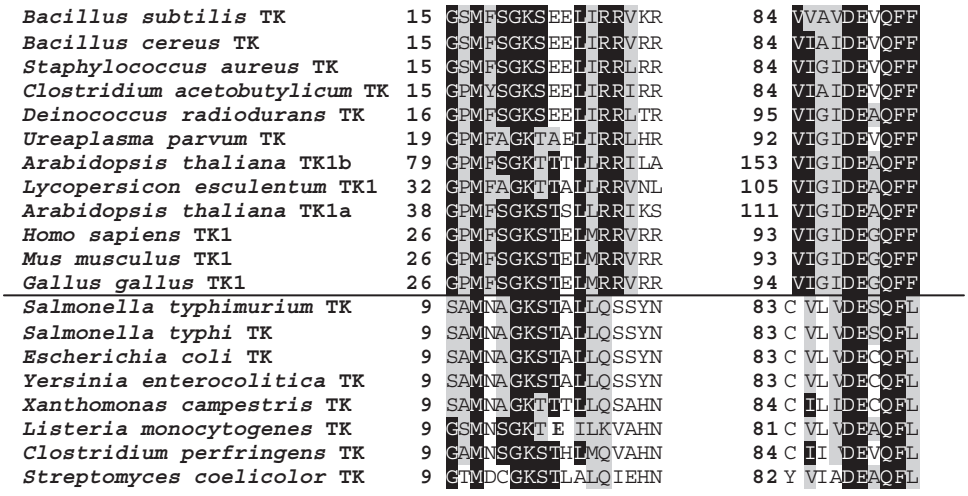

FIGURE 2 Partial alignment of thymidine kinases. Two selected sections of the alignment used for the phylogenetic reconstruction are shown. The horizontal line separates the Gram-negative group (lower) from the Gram-positive and eukaryote group (upper). First section shows the region around the P-loop (G—GK[ST]). This region is degenerated in the gram-negative group. Second section shows the alignment of the VIGIDE region. This region also is degenerated in the gram-negative TKs. Numbers to the left of amino acid sequences indicate residues from the N-terminus.

closeness of these residues to the P-loop and involvement in subunit-subunit interactions suggest a possible way for the ATP-activation/tetramerization of these TK1s, as described for human TK1.[11] Likewise, the eukaryote conserved domain VIGIDE of the central β4 strand is conserved in the Gram-positive TKs, but degenerated in the Gram-negative TKs, where only the DE is conserved. From the structure it appears that the VIGI is responsible for the hydrophobic interactions of the β4 strand with the α3 helix. The 2 hydrophilic residues (DE) at the tip of the β4 strand are directed toward the active center. Most Gram-negative TKs have kept the hydrophobic residues but instead of VIGIDE they have a CVL[VL]DE signature (Figure 2).

DISCUSSION

Two super families, the TK1-like and the non-TK1-like, can be found among dNKs. Here we have identified and compared prokaryotic TK1-like kinases with the eukaryotic and found that from the phylogenetic point of view, the prokaryotic TKs comprise 2 separate groups of which one, containing only TKs from Gram-positive bacteria, appears to be related closely to the eukaryotic TK1s. The other group is predominantly comprised of TKs from Gram-negative bacteria. However, 3 TKs from the Gram-positive *C. perfringens, L. monocytogenes,* and *S. coelicolor* bacteria group together

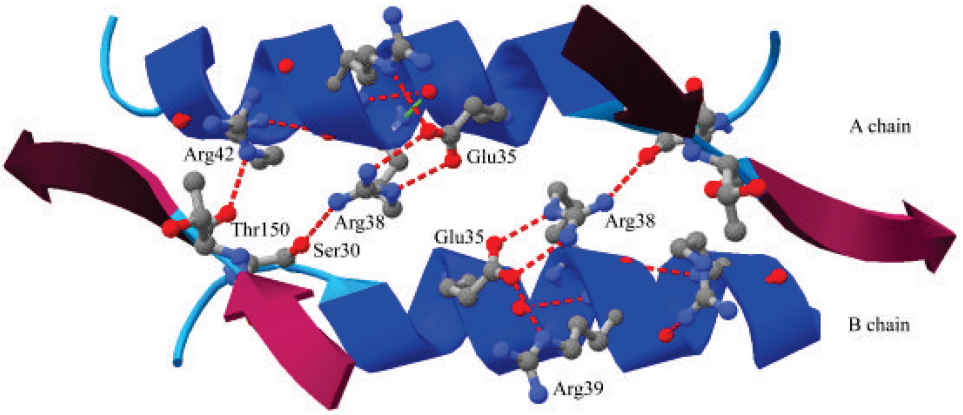

FIGURE 3 Closeup of the interactions between the A and B (or C and D) subunits in the tetrameric form of the human TK1. Dotted lines represent hydrogen bonds. Note that several of the shown residues are different among gram-negative TKs.

with the Gram-negative TKs. The distribution of Gram-positive and Gram-negative TKs could be completely random since our data only operate with a relatively limited number of TKs. It, however, is intriguing that no TKs of Gram-negative bacterial origin are found in the group of Gram-positive TKs. This could indicate that the *C. perfringens, L. monocytogenes,* and *S. coelicolor* TKs are a result of horizontal gene transfer (HGT) from Gram-negative bacteria. In particular, the *C. perfringens* TK supports this, since the *C. acetobutylicum* TK is a true Gram-positive TK. The occurrence of Gram-negative TKs in some Gram-positive bacteria can be explained by the less complex membrane structure of Gram-positive bacteria and the natural competence associated herewith. However, it remains unclear what the advantage, if any, could be to exchange the TK genes/enzymes. As a curiosum, we might add that the P-loop of the TKs belonging to the Gram-negative group, presented here, all begin 9 amino acids from the N-terminal methionine, whereas those of the Gram-positive group are variable. Structurally, the TKs of the Gram-negative group are expected to have the same overall fold as the TKs of human and Gram-positive origin, but several differences are likely to be found in the subunit-subunit interactions and kinetic parameters.

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