Phylogenetic analysis of *Photobacterium* species based on the nucleotide sequences of *gyrB* gene

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Abstract
Phylogenetic analysis is widely used to determine evolution and function of gene, to identify the genus of bacteria based on gene sequences. The use of protein-coding genes that are known to evolve much faster than rRNAs seems to be more appropriate for the phylogenetic analysis of closely related bacteria. We were thus interested in using *gyrB* (the DNA gyrase B subunit genes) sequences in molecular systematics. In this study, we carried out a phylogenetic analysis of 21 *Photobacterium* species by using their gyrB sequences. *Photobacterium* is gram-negative bioluminescent bacterium.In this research , we used NCBI database and CLUSTAL V and MEGA4 softwares.Evolutionay trees based on Nucleotide sequences and translated amino acid sequences of *gyrB* were constructed. This study has shown that analysis of *gyrB* can be a rapid and effective method for the identification and classification of bacterial species in various situations.

Key words: Phylogenetic Analysis, *gyrB* gene, *Photobacterium*, Evolutionary tree

Introduction

Phylogenetic analysis is widely used to determine evolution and function of gene, to identify the genus of bacteria based on gene sequences(Tian . 2008) . The nucleotide sequences of small-subunit rRNA (16srRNA) have been used most frequently to deduce the phylogenetic relationships between bacteria. However,the results of 16s rRNA sequence analysis have often disagreed with the results using DNA reassociation, which is considered to be an absolute measure of relatedness. The major reason for this discrepancy may be the low rate of base substitution in 16s rRNA genes: the numbers of substituted bases between rRNA genes from closely related bacteria are small, and a comparison of almost identical sequences is accompanied by a large statistical error(Yamamoto, 1996). The use of protein-coding genes that are known to evolve much faster than rRNAs seems to be more appropriate for the phylogenetic analysis of closely related bacteria(LaDuc ,2003). We were thus interested in using *gyrB* (the DNA gyrase B subunit genes) sequences in molecular systematics in view of the following criteria: (1) it is distributed universally among bacteria, (2) it can readily be sequenced without subcloning, (3) it is not transmitted horizontally at a significant frequency, and (4) a single copy is present on each genome. DNA gyrase is the bacterial type II topoisomerase that regulates the level of supercoiling of double-stranded DNA and is indispensable for DNA replication and horizontal transmission of this gene may be as rare as that of rRNA genes (Yamamoto, 2002).
Photobacterium is gram-negative bioluminescent bacterium. In this study, we carried out a phylogenetic analysis of 21 *Photobacterium* species by using their gyrB sequences.

Materials and Methods

The *gyrB* sequences were gotten from NCBI database. The nucleotide sequences of *gyrB* were aligned by using the CLUSTAL V computer program. Evolutionary trees were constructed with the MEGA4 software, using the neighbor-joining method.

Result and Discussion

The pattern of nucleotide substitutions is shown in Table 1. The percentage of nucleotide substitutions in the *gyrB* genes varied from 1.2 to 40.8 %.

Table 1 - Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution [1]

	A	**T**	**C**	**G**
A	-	*1.7*	*1.26*	**6.89**
T	*1.93*	-	**30.26**	*1.41*
C	*1.93*	**40.84**	-	*1.41*
G	**9.42**	*1.7*	*1.26*	-

NOTE: Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Only entries within a row should be compared. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The nucleotide frequencies are 0.306 (A), 0.27 (T/U), 0.2 (C), and 0.224 (G).

The phylogenetic tree based on the *gyrB* nucleotide sequences is shown in Fig. 1. *Ph. aquimaris* strain LC2-038 and *Ph. Aquimaris* strain LC2-068 formed one cluster, while *Ph. Angustum* , *Ph. Leiognathi* strain lunch 13.1 and *Ph. Mandapensis* strain ajapo formed another cluster.
The phylogenetic tree based on the amino acid sequences of GyrB is shown in Fig. 2. The branching order of theGyrB-based tree was very similar to that based on the *gyrB*nucleotide sequences.

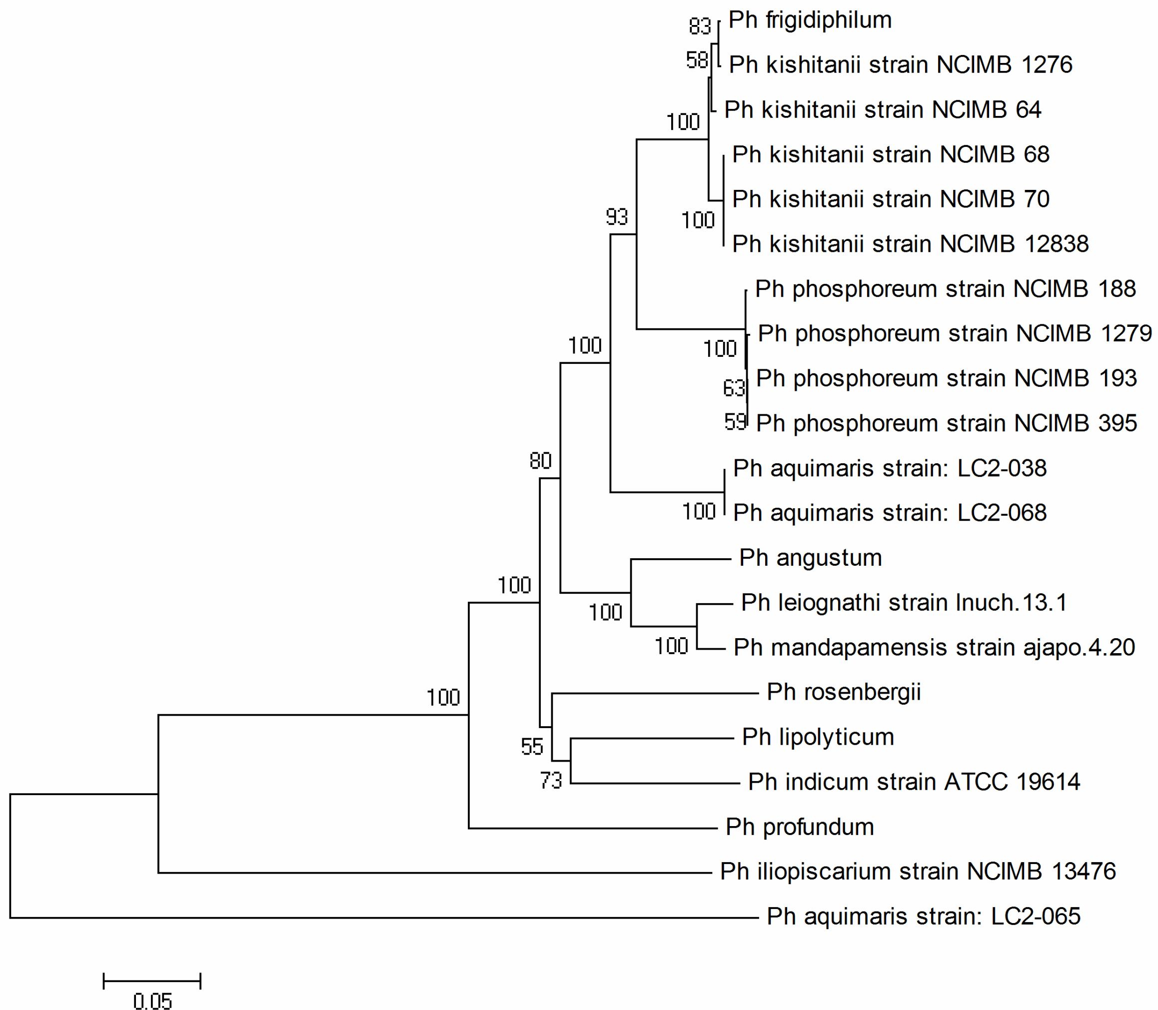

FIG. 1. Phylogenetic tree for the species of *Photobacterium* based on the *gyrB* nucleotide sequences by the neighbor-joining method. The numbers shown next to the nodes indicate percent bootstrap values of the 500 replicates. Genetic distances were computed by using p-distance model .

Other Phylogenetic analysis have been done by using this gene. The phylogenetic relationships of 49 *Acinetobacter* strains, 46 of which have previously been classified into 18 genomic species by DNA-DNA hybridization studies were investigated using the nucleotide sequences of *gyrB*(Yamamoto, 1996). Phylogenetic analysis of 20 *Aeromonas* strains was also conducted using the nucleotide sequence of *gyrB*(Soler, 2003).

In conclusion, this study has shown that analysis of *gyrB* can be a rapid and effective method for the identification and classification of bacterial species in various situations. A database of *gyrB* sequences have been created to facilitate easier identification and classification of bacteria (ICB database: http://www.mbio.co.jp/icb/). This database also provides information about the analysis method for *gyrB* genes from various bacterial taxa.

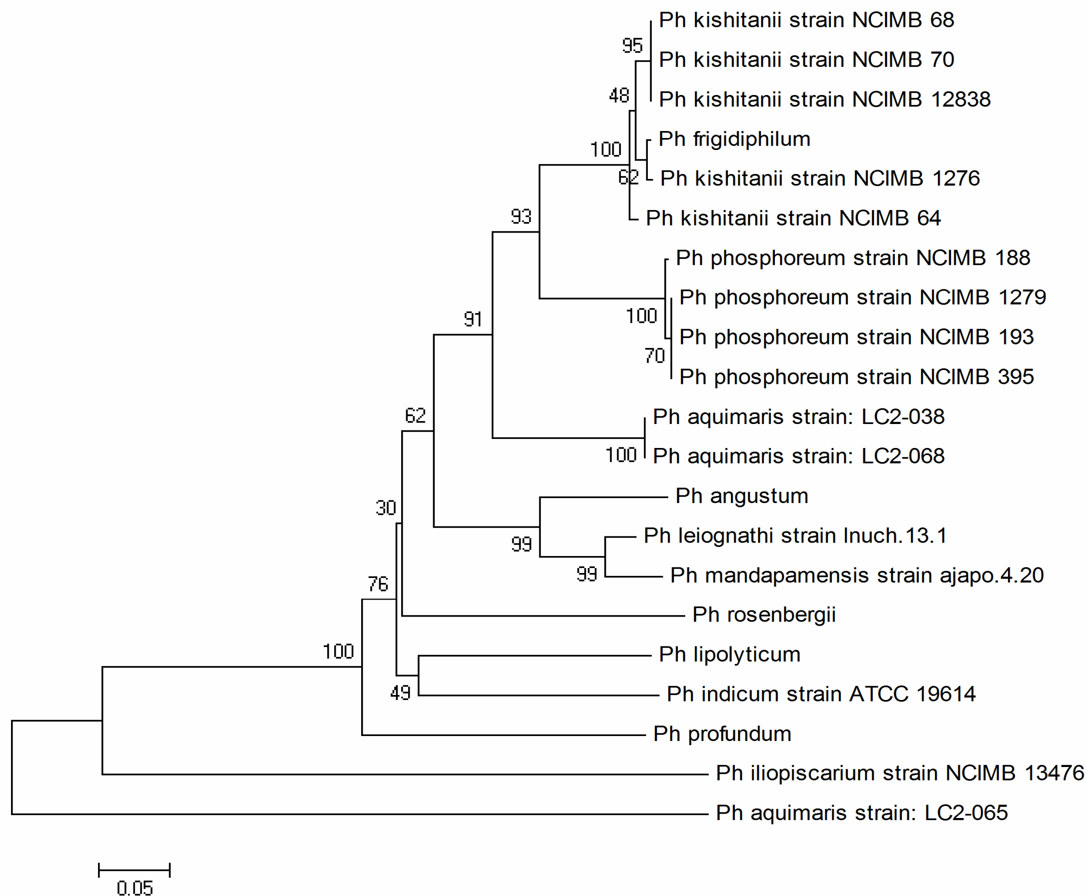

FIG. 2 Phylogenetic tree for the species of *Photobacterium* based on the amino acid sequences of *gyrB* products by the neighbor-joining method. The numbers shown next to the nodes indicate percent bootstrap values of the 500 replicates. Distances were computed by using p-distance model .

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