Phylogenetic Analysis of Enterococcus, Lactobacillus and Streptococcus Strains on the Basis of abc (Atp Binding Protein) Gene Sequences

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Abstract: Phylogenetic analysis of about 14 strains of Enterococcus, Lactobacillus and Streptococcus was carried out using the nucleotide sequence of the gene for abc (ATP binding protein). The result establish a new phylogenetic tree for the classification of Enterococcus, Lactobacillus and Streptococcus. In comparison with 16s rRNA analysis, the abc sequence indicated a greater evolutionary divergence for the bacteria. Thus, in screening for the presence of bacteria, the abc gene might be a useful tool for differentiating between closely related species of bacteria such as Lactobacillus species and Streptococcus. At present, 16s rRNA sequence analysis is an accurate and rapid method for identifying most unknown bacteria to the genus level because the highly conserved 16S rRNA region is easy to amplify; however, analysis of the more variable abc sequence region can identify unknown bacteria to the species level. In summary, we have shown that abc sequence analysis is a useful alternative to 16s rRNA analysis for constructing the phylogenetic relationships of bacteria, in particular for the classification of closely related bacterial species.

Key words: Phylogeny, Enterococcus, binding protein, Lactobacillus and Streptococcus

INTRODUCTION

Streptococcus and Enterococcus are pathogens that cause sore throat, acute glomerulonephritities, rheumatic fever, urinary tract infections and endocarditis but lactobacillus is not pathogenic. They are used in the production of fermented vegetable foods, sour dough bread (Guarnier et al., 2005) and sausage etc., they are called lactic acid bacteria because lactate is as their major or sole fermentation product (Brenner, 1984; Wood, 1998) in general, the chromosomes of Enterococcus, Lactobacillus and Streptococcus genome sizes range from 1.1 to 2.6 Mb and they are belonging to the order lactobacillales. There are Lactobacillus species (Ljungh and Wadstrom, 2009) which have to analyze, Lactobacillus sakei subsp. sakei 23K, Lactobacillus casei ATCC 334, Lactobacillus salivarius UCC118, Lactobacillus acidophilus NCFM, Lactobacillus plantarum WCFS1, Lactobacillus reuteri JCM 1112, Lactobacillus brevis ATCC 367.

For Streptococcus, there are six species Streptococcus pyogenes M1 GAS, Streptococcus equi subsp. Zooepidemicus, Streptococcus pyogenes MGAS315, Streptococcus mutans UA159 and Streptococcus equi subsp. equi 4047, Streptococcus pyogenes str. Manfredo. Streptococcus pyogenes is a member of the group A streptococci (GAS) and is known to be responsible for wide variety of human diseases. Recently, there has been a remarkable increase in the rate of severe invasive infections caused by the GAS and more than 70% of the GAS strains associated with these infections were of the M1 and M3 (Stevens, 1992; Musser, 1996; Blaser, 2001). Finally, for Enterococcus there is only one species Enterococcus faecalis V583. Enterococcus, Lactobacillus and Streptococcus strains are often extremely difficult to separate biochemically because they are facultative anaerobes, nonmotile, lactic acid producing bacteria. It would be useful to classify these types of bacteria to aid the treatment of bacterial infections or uses of bacteria in food and dairy industries.

MATERIALS AND METHODS

Phylogenetic analysis: The phylogenetic data described below were obtained by alignment and phylogenetic analysis of the bacterial sequences. The nucleotide sequences of 16s rRNA and abc were aligned by using the CLUSTAL X (Thompson et al., 1997) computer program Fig 3 and Fig 4. A neighbor joining (Saitou and Nei, 1987) analysis was used to reconstruct phylogenetic trees with the PHYLIP computer program.

RESULTS

Phylogenetic analysis and genetic distance of 16s rRNA: Data for the phylogenetic analysis were obtained from sequences contained in the GenBank nucleotide sequences database (Maidak et al., 1994). The following strains were examined Enterococcus faecalis V583 (Teng et al., 2001), Lactobacillus sakei subsp. sakei 23K, Lactobacillus casei ATCC 334, Lactobacillus salivarius UCC118, Lactobacillus acidophilus NCFM.
Lactobacillus plantarum WCFS1, Lactobacillus reuteri JCM 1112, Lactobacillus brevis ATCC 367, Streptococcus pyogenes M1 GAS, Streptococcus equi subsp. Zooepidemicus, Streptococcus pyogenes MGAS315, Streptococcus mutans UA159 (Higuerà et al., 1995, Saarela et al., 1993) Streptococcus equi subsp. equi 4047, Streptococcus pyogenes str. Manfredo (Bentley et al., 1991, Kumar et al., 1993). Alignment of the 16s rRNA nucleotide sequences was performed by the computer program PHYLIP (PHYLIP package documentation mirror site).

(Wood, 1998). Fig. 1 shows the phylogenetic tree of 16s rRNA for Enterococcus, Lactobacillus and Streptococcus strains. In this tree, Lactobacillus casei ATCC 334 and Lactobacillus brevis ATCC 367 have maximum similarity to each other; and it’s similarity to Streptococcus equi subsp. equi 4047 have more than other strains. These results indicate that Lactobacillus casei ATCC 334 and Lactobacillus brevis ATCC 367 are more closely related to Streptococcus equi subsp. equi 4047 than others.

**Fig 2:** Phylogenetic tree based on the nucleotide sequences of abc (ATP binding protein) genes. The tree was constructed by the neighbor-joining method, using the computer program PHYLIP. The sequence data for phylogenetic analysis were taken from the Genbank nucleotide sequence database for the following strains: Enterococcus faecalis V583, Lactobacillus sakei subsp. sakei 23K, Lactobacillus casei ATCC 334, Lactobacillus salivarius UCC118, Lactobacillus acidophilus NCFM, Lactobacillus plantarum WCFS1, Lactobacillus reuteri JCM 1112, Lactobacillus brevis ATCC 367, Streptococcus pyogenes M1 GAS, Streptococcus equi subsp. Zooepidemicus, Streptococcus pyogenes MGAS315 (Streptococcus), Streptococcus mutans UA159, Streptococcus equi subsp. equi 4047 (equi), Streptococcus pyogenes str. Manfredo.

**Phylogenetic analysis and genetic distance of abc genes:** The following strains were examined: Enterococcus faecalis V583, Lactobacillus sakei subsp. sakei 23K, Lactobacillus casei ATCC 334, Lactobacillus salivarius UCC118, Lactobacillus acidophilus NCFM, Lactobacillus plantarum WCFS1, Lactobacillus reuteri JCM 1112, Lactobacillus brevis ATCC 367, Streptococcus pyogenes M1 GAS, Streptococcus equi subsp. Zooepidemicus, Streptococcus pyogenes MGAS315, Streptococcus mutans UA159, Streptococcus equi subsp. equi 4047, Streptococcus pyogenes str. Manfredo. Alignment of the abc nucleotide sequences was performed by the computer program PHYLIP. Fig. 2 shows the phylogenetic tree for these species based on the abc gene sequence. As indicated in Fig. 2,
Lactobacillus brevis ATCC 367 and Streptococcus pyogenes MGAS315 have maximum similarity to each other; and its similarity to Lactobacillus casei ATCC 334 have more than other species.

Comparison of the genetic distance and the phylogenetic tree determined by 16s rRNA and abc:
A direct comparison of the genetic distance and the phylogenetic tree determined by the 16s rRNA sequence with those determined by the abc sequence was not possible because the bacterial strains analyzed were not the same. However, the following observations could be made, first the rate of genetic divergence of the abc sequence differed greatly that of the 16s rRNA sequence. Second, phylogenetic analysis using the abc nucleotide sequence determined a classification of the bacteria different from that determined by 16s rRNA analysis.

DISCUSSION

Phylogenetic-tree analysis is often used as a method to classify organisms. Various genes have been examined for the analysis of phylogenetic relationships of Enterococcus, Lactobacillus and Streptococcus. In general, 16s rRNA is most frequently used for such analyses; however, phylogenetic relationships between closely related species of Lactobacillus and Streptococcus are weakly defined by this approach, as the 16s rRNA sequences of bacteria contain highly conserved regions. In terms of cell biology, Lactobacillus spp. is similar to
Streptococcus. In our phylogenetic - tree analysis of 16s rRNA. Lactobacillus casei ATCC 334 and Lactobacillus brevis ATCC 367 have maximum similarity and similarities respectively; to Streptococcus equi subsp. equi 4047 have more than other. These data are in accordance with the previous study using the 16s rRNA gene sequence, indicating close relationships among these bacteria.

A comparision of Fig. 2 with Fig. 1 highlights the different patterns of bacterial divergence determined through analysis of abc genes and 16s rRNA. In addition, the rate of base substitution was greater for the abc sequence than for the 16s rRNA sequence. Thus, certain bacterial strains were classified differently under the two phylogenetic analyses. It seems likely that phylogenetic analysis using the abc gene sequence will be able to classify some bacteria that can not be classified by their 16s rRNA sequences. 16s rRNA sequences cannot be used to derive phylogenetic-tree analyses among closely related bacteria (Cilia et al., 1996). However, our results indicate that such closely related bacteria might be classified by abc analysis.

The rate of evolution of the abc genetic region is higher than that of the 16s rRNA region and the abc genetic region are found in all bacterial species. We believe that the abc region will have high reliability for identifying pathogenic bacteria. Although the 16s rRNA sequence method is a highly accurate and rapid method for identifying most bacteria to the genus level, the abc sequence method might be more useful for identifying bacteria to the species level.

CONCLUSION

In summary, we have shown that abc sequence analysis is a fruitful approach to determine the phylogenetic relationships of bacteria and may be an alternative to 16s rRNA analysis. In particular, abc analysis of bacteria is an effective means to classify closely related species. Further research on abc sequence analysis will clarify in more detail the classification of bacterial species.

REFERENCES


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