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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 identifyieng most unknown bacteria to the genus level because the highly conserved 16Ss 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 glomerulonephrities, rhematic fever, urinary tract infections and endocardities but *lactobacillus* is not pathogenic. They are used in the production of fermented vegetable foods, sour dough bread (Guarner *et al*., 2005) and sausase 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,

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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 (Higuera *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.

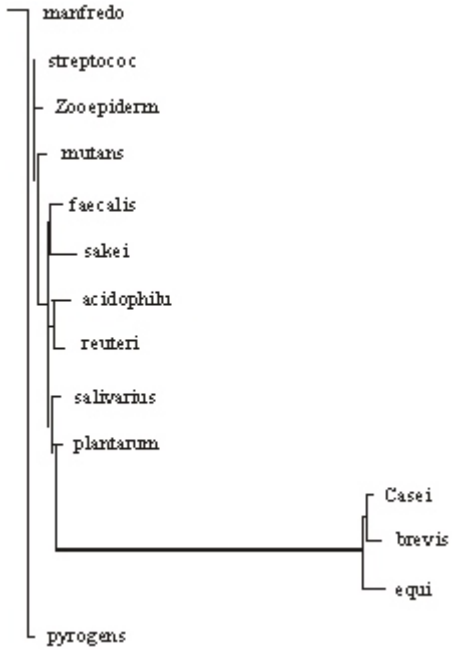

Fig. 1: Phylogenetic tree based on the nucleotide sequences of 16s rRNA genes. The tree was constructed by the neighbor-joining method, using the computer program PHYLIP. The sequence data for phylogenetic analysis were taken from the Genbank nucleotide sequence database for the following strains: *Enterococcus 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*

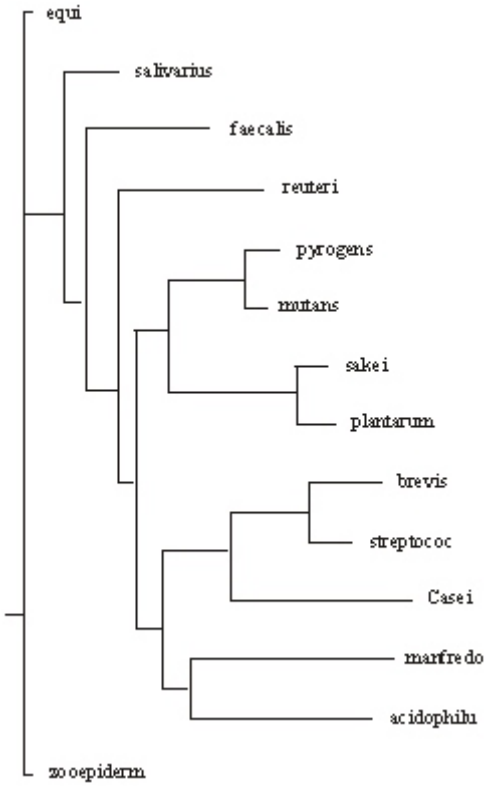

Fig 2: Phylogenetic tree based on the nucleotide sequences of *abc* (ATP binding protein) genes. The tree was constructed by the neighbor-joining method, using the computer program PHYLIP. The sequence data for phylogenetic analysis were taken from the Genbank nucleotide sequence database for the following 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,

Fig 3: CLUSTALX result of 16s rRNA gene sequence

Fig. 4: CLUSTALX result of *abc* (ATP binding protein) gene sequence

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.

Comparision 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

Bentley, R.W., J.A. Leigh and M.D. Collins, 1991. Intrageneric structure of *Streptococcus* based on comparative analysis of small subunit rRNA sequences. Int. J. Syst. Bacteriol., 41: 487-494.

Blaser, M.J. and J.M. Musser, 2001. Bacterial polymorphisms and disease in humans. J. Clin. Invest., 107: 391-392.

Brenner, D.J., 1984. Order Lactobacillales, Bergey's Manual of Systematic Bacteriology, Vol. 1, Williams 7 Wilkins, Baltimore, Md.

Cilia, V., B. Lafay and R. Christen, 1996. Sequence heterogeneities among 16S ribosomal RNA sequences and their effect on phylogenetic analyses at the species level. Mol. Biol. Evol., 13: 451-461.

Guarner, F., G. Perdigon, G. Corthier, S. Salminen, B. Koletzko and L. Morelli, 2005. Should yogurt cultures be considered probiotic? Br. J. Nutr., 93: 783-786.

Higuera, A., J. Liebana, J. Gutierrez, A. Garcia-Mendoza and A. Castillo, 1995. Evaluation of the Automicrobic system for the identification of *Streptococcus* mutans. Eur. J. Clin. Microbiol. Infect. Dis., 14: 1102-1105.

Kumar, S., K. Tamura and M. Nei, 1993. MEGA: Molecular evolutionary genetic analysis, version 1.01. The Pennsylvania State University, University Park.

Ljungh, A. and T. Wadstrom, 2009. Lactobacillus Molecular Biology: From Genomics to Probiotics. Caister Academic Press. ISBN 978-1-904455-41-7

Maidak, B.L., N. Larsen, M.J. McCaughey, R. Overbeek, G.J. Olsen, K. Fogel, Y.J. Bland and C.R. Woese, 1994. The ribosomal database project. Nucl. Acids Res., 22: 3485-3487.

Musser, J.M., 1996. Molecular population genetic analysis of emerged bacterial pathogens: selected insights. Emerg. Infect. Dis., 2: 1-17.

Saarela, M., S. Alaluusua, T. Takei and S. Asikainen, 1993. Genetic diversity within isolation of mutans streptococci recognized by an rRNA gene probe. J. Clin. Microbiol., 31: 584-587.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic tree. Mol. Biol. Evol., 4: 406-425.

Stevens, D.L., 1992. Invasive group a streptococcus infections. Clin. Infect. Dis., 14: 2-13.

Teng, L.J., P.R. Hsueh, Y.H. Wang, H.M. Lin, K.T. Luh and S.W. Ho, 2001. Determination of Enterococcus faecalis groESL full-length sequences and application for species identification. J. Clin. Microbiol., 39: 3326-3331.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins, 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res., 25(24): 4876-4882.

Wood, B., 1998. Microbiology of Fermented Foods. Blackie, London.