Communication/Comunicação

PCR-RFLP of 16S ribosomal DNA to confirm the identification of *Enterococcus gallinarum* and *Enterococcus casseliflavus* isolated from clinical and food samples

PCR-RFLP do 16S DNA ribossomal para confirmar a identificação de *Enterococcus gallinarum* e *Enterococcus casseliflavus* isolados de amostras clínicas e alimentares

Aline Weber Medeiros[1], Pedro d'Azevedo[2], Rebeca Inhoque Pereira[3], Ana Paula Cassenego[1], Sueli Van Der Sand[3], Jeverson Frazzon[4] and Ana Paula Guedes Frazzon[3]

ABSTRACT

Introduction: This study aimed to confirm the identification of *Enterococcus gallinarum* and *Enterococcus casseliflavus* isolated from clinical and food samples by PCR-RFLP. **Methods:** Fifty-two strains identified by conventional biochemical exams were submitted to PCR amplification and digested with *HinfI.* Only 20 (38.5%) of the 52 strains showed a DNA pattern expected for *E. gallinarum a*nd *E. casseliflavus.* **Results:** Analysis of the results of this study showed that *E. gallinarum* and *E. casseliflavus* are occasionally erroneously identified and confirmed the potential application of 16S rDNA analysis for accurate identification of these species. **Conclusions**: A correct identification is important to distinguish between intrinsic and acquired vancomycin resistance.

Key-words: PCR-RFLP of 16S rDNA. *Enterococcus gallinarum. Enterococcus casseliflavus*.

RESUMO

Introdução: O objetivo deste estudo foi confirmar a identificação de amostras clínicas e alimentos de *Enterococcus gallinarum* e *Enterococcus casseliflavus* por PCR-RFLP. **Métodos**: Cinquenta e duas cepas identificadas por exames bioquímicos convencionais foram submetidos a amplificação por PCR e digestão com *HinfI*. Apenas 20 (38,5%) das 52 amostras apresentaram um padrão de DNA esperado *E. gallinarum* e *E. casseliflavus*. **Resultados**: Analise dos resultados deste estudo demonstraram que, algumas vezes *E. gallinarum* e *E. casseliflavus* são erroneamente identificados e confirmaram a potencial aplicação da análise do 16S rDNA para identificação exata destas espécies. **Conclusões**: A correta identificação é importante a fim de distinguir entre resistência intrínseca e adquirida à vancomicina.

Palavras-chaves: PCR-RFLP de 16S rDNA. *Enterococcus gallinarum. Enterococcus casseliflavus.*

1. Graduate Program of Agricultural and Environmental Microbiology, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. 2. Department of Basic Health Sciences, Microbiology Department, Federal Health Sciences University of Porto Alegre, Porto Alegre, RS, Brazil. 3. Microbiology Department, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. 4. Science and Food Technology Institute, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Address to: Dr. Ana Paula Guedes Frazzon. ICBS/UFRS. Campus do Centro. Av. Sarmento Leite 500/ sala 158, 90050-170 Porto Alegre, RS.
Tel: 55 51 3308-4111; Fax: 55 51 3308-4111
e-mail: ana.frazzon@ufrgs.br

Received in 16/11/2009
Accepted in 13/01/2010

INTRODUCTION

Enterococci are opportunistic pathogens and well known as the principal microorganisms associated with the development of infections, especially in immunosuppressed patients. Furthermore, strains have been recognized as emerging human pathogens mostly associated with nosocomial infections[1]. The emergence of enterococci in nosocomial infections has grown in parallel with the rise in strains resistant to a large number of antimicrobial drugs used in the treatment of human infections. *Enterococcus gallinarum* and *Enterococcus casseliflavus* exhibit low-level intrinsic resistance to vancomycin, conferred by the *van*C-1 gene[2]. Commercial kits for species identification of *Enterococcus* are unable to distinguish *E. gallinarum* and *E. casseliflavus* from other enterococci[3]. Rapid and reliable differentiation of these species in patients infected with vancomycin resistant enterococci (VRE) is essential for an infection control program. The aim of this work was to confirm the identification of *E. gallinarum* and *E. casseliflavus* using the PCR-restriction fragment length polymorphism (PCR-RFLP) technique.

METHODS

In the current study, *E. gallinarum* (n=32) and *E. casseliflavus* (n=20) isolated from clinical samples and food identified by conventional biochemical were analyzed. Two references strains *E. gallinarum* (PAD 262) and *E. casseliflavus* (PAD 71) were obtained from the culture collection at the laboratory of microbiology of the Federal University of Health Sciences (*Universidade Federal de Ciências da Saúde*) of Porto Alegre and used as controls (**Table 1**). Extraction of total DNA from cells followed the method described by Riboldi et al[4] The amplifications were performed with a thermal

Table 1. *Enterococcus gallinarum* and *Enterococcus casseliflavus* isolated from clinical and food samples in South Brazil.

Species (number of isolates)	Genotype by PCR-RFLP	
	Positive (%)	Negative (%)
Enterococcus gallinarum PAD 1262* (1)	1 (100%)	0
Enterococcus casseliflavus PAD 71* (1)	1 (100%)	0
Enterococcus gallinarum (32)	15 (47%)	17 (53%)
Enterococcus casseliflavus (20)	5 (25%)	15 (75%)
Total	**22**	**32**

* PAD culture collection at the Laboratory of Microbiology of Federal University of Health Sciences of Porto Alegre.

cycler (Eppendorf Mastercycler Personal). The primers 16Sent-F (5'-CTGACGCTGAGGCTCGAAAGCG-3') and 16Sent-R (5'-TGTGACGGGCGGTGTGTACAAGGGGG-3') corresponded to nucleotide sequences of 16SrDNA of the genus *Enterococcus*. The PCR product of 661 bp amplified was submitted to digestion with the restriction enzyme *Hinf*I (Jena Bioscience GmbH, Germany), according to the manufacturer's instructions. The DNA fragments obtained were resolved by electrophoresis on 2% agarose gel stained by ethidium bromide.

RESULTS AND DISCUSSION

The PCR-RFLP results from reference strains of *E. gallinarum* and *E. casseliflavus* showed two distinguishable DNA fragments of 589bp and 72bp (**Figure 1**). PCR-RFLP from the 52 strains tested demonstrated that 47% (15/32) of *E. gallinarum* and 25% (5/20) showed the expected PCR-RFLP patterns (**Figure 1**). Two PCR-RFLP positive *E. gallinarum* and one *E. casseliflavus* were analyzed by the SDS-PAGE method and confirmed the results obtained. On the other hand, 53% (17/32) of *E. gallinarum* and 75% (15/20) of *E. casseliflavus* strains showed three DNA fragments of 504, 85 and 72bp (**Figure 1**). These strains were resubmitted to a new set of biochemical tests and reclassified as: *E. faecium, E. faecalis* and *Enterococcus* sp. The 16S rDNA gene has been useful for the identification of *Enterococcus* genus and species[5,6]. All 16S rDNA sequences deposited in GenBank of the NCBI of *E. gallinanum* and *E.casseliflavus* have a conserved thymidine (T) at position 1248, while other species of enterococci predominantly present a cytosine (C) or T at the equivalent position. A single conserved base substitution in this position in *E. gallinarum* and *E. casseliflavus* eliminates the restriction endonuclease site for *Hinf*I. The present results demonstrate that *E. gallinarum* and *E. casseliflavus* are occasionally erroneously identified and confirmed the potential application of 16S rDNA analysis to accurately identify these species. Correct identification is very important to discriminate between natural and VRE strains.

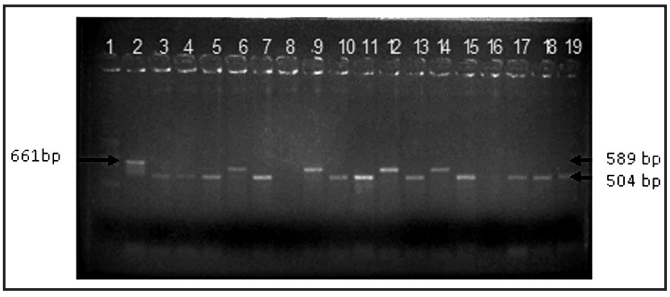

FIGURE 1 - Restriction DNA fragments obtained with the digestion of PCR amplification products with the enzyme *Hinf*I. (1) Ladder 100 bp; (2) PCR product from *E. gallinarum* non digested; (3) *E. galllinarum* PAD 1262; (4) *E. casseliflavus* PAD 71; (5) *E. galllinarum* clinical isolate; (6) misidentified *E. galllinarum* clinical isolate; (7) *E. casseliflavus* clinical isolate; (8) Negative control; (9) misidentified *E casseliflavus* clinical isolate; (10-11) *E. casseliflavus* isolated from food; (12) misidentified *E. casseliflavus* isolated from food; (14) *E. galllinarum* isolated from food; (15-19) misidentified *E. galllinarum* isolated from food.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

The authors grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico, the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior and the Fundação de Amparo a Pesquisa do Rio Grande do Sul for their financial support.

REFERENCES

1. d'Azevedo PA, Dias CA, Teixeira LM. Genetic diversity and antimicrobial resistance of enterococcal isolates from Southern region of Brazil. Rev Inst Med Trop São Paulo 2006; 48,11-16.
2. Clark NC, Teixeira LM, Facklam RR, Tenover FC. Detection and differentiation of *van C-1, van C-2*and *van C-3*, glycopeptide resistance genes in enterococci. J Clin Microbiol 1998; 36: 2294-2297.
3. Facklam RR, Carvalho MGS, Teixeira LM. History, taxonomy, biochemical characteristics, and antibiotic susceptibility testing of enterococci. In *The enterococci: pathogenesis, molecular biology, and antibiotic resistance*. Eds, Washington: ASM Press; 2002. p 1-54.
4. Riboldi GP, Frazzon J, d'Azevedo PA, Frazzon APG. Antimicrobial resistance profile of Enterococcus spp isolated from food in Southern Brazil. Braz J Microbiol 2009; 40:125-128.
5. Fortina MG, Ricci G, Borgo F, Manachini PL. Rapid identification of *Enterococcus italicus* by PCR with primers targeted to 16S rRNA gene. Lett Appl Microbiol 2007; 44:443-446
6. Patel R, Piper KE, Rouse MS, Steckelberg JM, Uhl JR, Kohner P, et al. Determination of 16S rRNA sequences of enterococci and application to species identification of nonmotile *Enterococcus gallinarum* isolates. J Clin Microbiol 1998; 36: 3399-3407