

CORRELATION BETWEEN API 20 STREP AND MULTIPLEX PCR FOR IDENTIFICATION OF *ENTEROCOCCUS* SPP. ISOLATED FROM BRAZILIAN FOODS

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ABSTRACT

We evaluated the suitability of API 20 STREP and multiplex PCR to speciate 52 *Enterococcus* spp. obtained from Brazilian foods. A high percentage of isolates (78.9%) presented discrepant results between evaluated tests. Similar results were obtained for six *E. faecalis* and five *E. faecium*. The PCR multiplex was more effective than API 20 STREP for complete identification of the isolates.

Key words: *Enterococcus* spp., identification, API 20 STREP, PCR multiplex, foods

Enterococci are commonly found in soil, water, plants, vegetables and foods (6,9). This genus includes more than 20 species, being *E. faecium* and *E. faecalis* the most commonly found (7,8). However, failure in the identification of other species is a matter of concern specially due to the emergence of antibiotic resistant enterococcal strains (12).

Identification of enterococci species is generally done by conventional phenotypic and biochemical tests even so molecular methods are becoming the choice tests for bacterial identification (1-3).

There are some kits available for a rapid identification of *Enterococcus* spp. such as ID 32 STREP, API 20 STREP, API 50CH, *Api zym* (BioMérieux, France) and Phene Plate PhP Plate System (PhPlate Microplate Techniques, Sweden). However, according to Facklam *et al.* (7) these tests should be carefully used since most of them only correctly identify *E. faecalis*. The API 20 STREP strip contains 20 microtubes with dehydrated substrates necessary to detect enzymatic activity and fermentation of sugars. The results are obtained within 24 - 48 hours, and the identification is based on numerical analysis after a visual interpretation of color changes (BioMérieux, France).

The purpose of the present work was to compare the biochemical test - API 20 STREP system and a multiplex

Polymerase Chain Reaction (PCR) using specific primers for identification of *Enterococcus* spp. obtained from Brazilian foods.

Fifty two *Enterococcus* spp. isolates were initially identified in our lab at genus level based on Gram stain, catalase reaction, growth in de Man Rogosa Sharpe broth - MRS (Oxoid, UK) at 10°C and 45°C, MRS broth with 6.5% NaCl, MRS broth pH 9.6 and production of PYR (L-pyrrolidonyl-β-naphthylamide). The presumptive enterococcal isolates were speciated by API 20 STREP (BioMérieux, France), used for identification of streptococci and enterococci, according to the protocol provided by the manufacturer.

Multiplex PCR was performed according to protocol described by Dutka-Malen *et al.* (5). Primers were used to amplify the genes *ddl* of *E. faecalis*, *ddl* of *E. faecium*, *vanC-1* of *E. gallinarum*, *vanC-2* and *vanC-3* of *E. casseliflavus*. Overnight cultures of *Enterococcus* spp. were harvested from trypticase soy agar with 0.6% yeast extract (TSAYE) and transferred to tubes containing 100 µl of PCR water (w-3500-Sigma, USA). This suspension was vortexed, centrifuged for ca. 15 seconds and the supernatant was used as template. The reaction was performed on a DNA thermal cycler (Mastercycler - Eppendorf, Germany) in a final volume of 25 µl containing 60

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ng of DNA as template, 25 pmol of each primer, 0.2 mM of each nucleotide, 2 mM of buffer with MgCl₂ and 0.625 U of *Taq* DNA polymerase. Amplification conditions were: initial denaturation at 94°C - 2 min, 30 cycles at 94°C - 1 min, 54°C - 1 min, 72°C - 1 min, and final extension at 72°C - 10 min. PCR products were electrophoresed on 1.0% agarose and stained with ethidium bromide (5). The strains *E. faecalis* NCTC 775, *E. faecium* NCTC 7171, *E. gallinarum* NCTC 12359 and *E. casseliflavus* NCTC 12361 were used as controls.

Table 1 shows the comparison of the results obtained using the two methodologies for identification of 52 *Enterococcus* spp. isolates. Only six *E. faecalis* isolates (11.5%) and five (9.6%) *E. faecium* isolates presented the same result by using API 20 STREP and PCR. The remaining 41 isolates (78.9%) presented discrepant results. Eight out of eleven isolates identified as *E. durans* by API 20 STREP were identified as *E. faecium* in the PCR, and the remaining three isolates could not be identified using the primers used in the study.

Three isolates classified as *Aerococcus viridans* by API 20 STREP, when tested by PCR, were identified as *E. gallinarum* (n=2) and *E. casseliflavus* (n=1). Five isolates identified as *Leuconostoc* sp. by API 20 STREP were also classified as *E. faecium* when PCR was used. All five isolates identified as *E. avium* by API 20 STREP, were classified as *E. faecalis* (n=3), *E. faecium* (n=1) and *E. casseliflavus* (n=1).

The multiplex PCR used in the study was more effective than API 20 STREP for identification of the species of several isolates: seven isolates identified only at genus level by API 20 STREP could be identified as *E. faecium* (n=6) and *E. faecalis* (n=1) using PCR. Ten isolates were not identified by API 20 STREP, however the PCR assay identified these strains as: *E. faecalis* (n=3), *E. faecium* (n=5) and *E. casseliflavus* (n=1). One isolate could not be identified regardless the method of identification used.

Our results are in accordance with those obtained by other authors who observed that commercial systems commonly fail to differentiate *Enterococcus* spp. correctly (3,7,12,14).

Robredo *et al.* (11) used the API20 STREP to identify enterococci obtained from foods of animal origin and compared the results with those obtained using colony hybridization. They did not observe discrepant results for *E. faecalis* but observed that eight isolates identified as *E. durans* and *E. casseliflavus* by the API20 STREP were actually *E. faecium* according to the molecular method.

Devriese *et al.* (3) also noted that the API 20 STREP was able to correctly identify *E. faecalis* but did not present all the necessary tests for a correct identification of other species. Morrison *et al.* (10) affirmed that the recognition of new and atypical species of *Enterococcus* spp. creates difficulties for an appropriate identification of this genus and indicates the need of including further tests in the commercially available systems (4). Moreover, according to Willey *et al.* (13), the emergence of antibiotic resistant enterococci demands for more accurate identification of species less commonly associated with clinical infections.

In the present work, the multiplex PCR used to identify *Enterococcus* spp. was based in the amplification of specific D-alanyl-D-alanine ligases of *E. faecalis* and *E. faecium* and related enzymes associated with glycopeptide resistance of *E. gallinarum* and *E. casseliflavus* (5). On the other hand, biochemical tests were based on visual interpretation of results and these readings are subjective and more prone to misinterpretation.

Finally, our study provided strong evidence that a number of *Enterococcus* spp. strains may be misidentified when only biochemical tests are used. Besides this, considering the costs and time of analysis, PCR is a cheaper, faster and more specific method to perform identification of *Enterococcus* spp.

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Table 1. Identification of *Enterococcus* spp. isolates by API 20 STREP (Biomérieux, France) and multiplex PCR

PCR ^a	n	API20 STREP							
		<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. durans</i>	<i>E. avium</i>	<i>Enterococcus</i> spp	<i>Aerococcus viridans</i>	<i>Leuconostoc</i> sp	Unidentified
<i>E. faecalis</i>	13	6			3	1			3
<i>E. faecium</i>	30		5	8	1	6		5	5
<i>E. gallinarum</i>	2						2		
<i>E. casseliflavus</i>	3				1		1		1
Unidentified	4			3					1
Total	52	6	5	11	5	7	3	5	10

^aGold Standard test.

RESUMO

Correlação entre API 20 STREP e PCR multiplex para identificação de *Enterococcus* spp. isolados de amostras de alimentos no Brasil

A identificação das espécies de 52 *Enterococcus* spp. isolados de amostras de alimentos foi realizada empregando-se duas metodologias: sistema API 20 STREP e PCR multiplex. Os resultados obtidos revelaram que 78,9% dos isolados apresentaram resultados diferentes nos dois testes utilizados. Apenas seis *E. faecalis* e cinco *E. faecium* apresentaram resultados concordantes pelos dois métodos. PCR multiplex permitiu a identificação completa de um número significativamente maior de enterococos do que o sistema API 20STREP.

Palavras-chave: *Enterococcus* spp., identificação, API 20 STREP, PCR multiplex, alimentos

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