IDENTIFICATION OF ENTEROBACTERIA COLLECTION BY PHENOTYPICAL CRITERIA AND ANALYSIS OF THE 16S rDNA GENE SEQUENCES

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Introduction. In recent years microbial identification had been made with different aproximations, which include phenotypical traits but also the analysis of their 16S rDNA sequences (1). Phylogenetic relationships among prokaryotes can be inferred from comparisons of their 16S rRNA (or 16S rDNA) sequences. This has had an enormous repercussion on bacterial taxonomy, leading to the currently applied system of classification, and allowing a rapid and precise identification of bacteria. (2, 3).

The objective of this work was to ensure the identity of fifteen Enterobacteria strains collection by means of traditional phenotypical tests and with the 16S rDNA sequencing using universal primers.

Methods. A collection of fifteen Enterobacteria (Table 1), were used in this work. Strains grown in agar Luria (37°C/24h) were used for phenotypic identification with the system API 20E™. Results were analyzed in the the APIweb database (2.0.1 v.), and were complemented with conventional tests. Genotypic identification was performed extracting the bacterial chromosomal DNA. The 16S rDNA sequence from each strain was PCR-amplified using the fd1 and rd1 universal primers (3). A 50µL PCR mixture reaction and conditions were performed as previously reported (4). 16S rDNA PCR-products were sequenced, and the identity was obtained with representative 16S rRNA sequences from GenBank with de BLAST tool of the NCBI site. A multiple alignment of them was performed. This alignment was use for the construction of a 16S rDNA phylogenetic tree by the Neighbor-Joining Method (data not shown).

Results. The identity for strains *C. freundii*, *E.coli, E. aerogenes, K. oxytoca, K.pneumoniae P. mirabilis, P. vulgaris, S.enteritidis, S.* Typhi, *S. Typhimurium, S. marcescens,* and *Y. enterocolitica* based on phenotypical and 16S sequencing shown good to excellent quality, and it was posible to achieve this at species level. In contrast, with both methods it was only possible to achieve at genus level the identitiy of the strain *S.nitra*. Finally for the strain *E.cloacae* both methods indicated the same genus but each one determinated a different species taxon (Table 1)

Table 1. Identity of the Enterobacteria collection by API20E and 16S rDNA sequencing methods

		Identificación fenotípica (API 20E)			Identificación molecular (Secuencias del gen ADNr 16S)	
No.	Microorganismo analizado	Calidad de identificación	% ID	Microorganismo (Reportado por API web)	Microorganismo (Reportado en GenBank)	% ID
1	*Citrobacter freundii*	Excelente identificación	99.9	*Citrobacter freundii*	*Citrobacter freundii*	90
2	*Escherichia coli* ATCC *11229*	Buena identificación	99.1	*Escherichia coli* 1	*Escherichia coli*	97
3	*Escherichia coli* ATCC *25922*	Buena identificación	84.9	*Escherichia coli* 2	*Escherichia coli*	97-98
4	*Enterobacter aerogenes*	Buena identificación	96	*Enterobacter aerogenes*	*Enterobacter aerogenes*	100
5	*Enterobacter cloacae*	Buena identificación	84.9	*Enterobacter gergoviae*	*Enterobacter hormaechei*	90
6	*Klebsiella oxytoca* ATCC *49131*	Excelente identificación	97.4	*Klebsiella oxytoca*	*Klebsiella oxytoca*	99
7	*Klebsiella pneumoniae*	Buena identificación	97.8	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	98
8	*Proteus mirabilis*	Excelente identificación	99.9	*Proteus mirabilis*	*Proteus mirabilis*	100
9	*Proteus vulgaris*	Buena identificación	97.8	*Proteus vulgaris*	*Proteus vulgaris*	100
10	*Salmonella enteritidis*	Buena identificación	93.4	*Salmonella choleraesuis*	*Salmonella enteritidis*	98
11	*Salmonella nitra*	Buena identificación	94	*Salmonella* spp	*Salmonella* spp	99
12	*Salmonella Typhi* ATCC *9992*	Excelente identificación	99.9	*Salmonella* spp	*Salmonella* Typhi	100
13	*Salmonella Typhimurium* ATCC *14028*	Excelente identificación	99.9	*Salmonella Typhimurium*	*Salmonella Typhimurium*	93
14	*Serratia marcescenes*	Buena identificación	97.5	*Serratia liquefaciens*	*Serratia marcescenes*	97
15	*Yersinia enterocolitica*	Excelente identificación	99.9	*Yersinia enterocolitica*	*Yersinia enterocolitica*	97-98

Conclusions. Both methods aimed to ensure the identity of the Enterobacteria collection. In general, it was concluded that both of them are complementary to each other.

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