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CHARACTERIZATION OF TYPICAL AND ATYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* (EPEC) STRAINS OF THE CLASSICAL O55 SEROGROUP BY RAPD ANALYSIS

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ABSTRACT

The genetic diversity of 41 typical and atypical enteropathogenic *Escherichia coli* (EPEC) strains of the serogroup O55 was analyzed by using the random amplified polymorphic DNA (RAPD) method. All typical EPEC O55 strains were grouped in two clusters (A and C) and belonged to the serotype O55:H6, while cluster B included all atypical strains, which were of the serotype O55:H7. The three groups also included non-motile strains. RAPD may be a useful method for epidemiological studies on *E. coli* O55 infection.

Key words: Enteropathogenic *Escherichia coli*, EPEC, genetic diversity, RAPD analysis

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) is a leading cause of diarrhea in many countries. Typically, EPEC strains possess a pathogenicity island, named LEE (locus of enterocyte effacement) and a 50-70 MDa plasmid, designated EAF (EPEC adherence factor). The LEE pathogenicity island contains the *eae* gene encoding an outer membrane adhesin (intimin) and all the other genes involved in formation of the attaching and effacing (A/E) lesion, characterized by bacterial adherence and destruction of intestinal microvili. The EAF plasmid encodes the BFP fimbriae (bundle-forming pilus) and some regulatory proteins. Routinely, LEE is detected by the *eae* gene probe and the EAF plasmid by the EAF probe (reviewed in 7).

It has been reported that the EAF plasmid can be lost during storage or even during infection (3, 6), but the absence of this plasmid in certain serotypes (i.e, O111:H9 and O26:H11) seems to be a natural occurrence. Natural EAF-negative strains belong to distinct electrophoretic types or clones (2, 9). Kaper, in 1996, proposed the designation of atypical EPEC for these strains (4).

E. coli O55 is one of the most important of the classical EPEC O serogroups not only because of its

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isolation frequency, but also because this serogroup includes strains with variable virulence properties (8, 9). O55 strains may be motile or non-motile and when motile they usually belong to serotypes O55:H6 and O55:H7. Most strains of the serotype O55:H6 possess the EAF plasmid while the plasmid has not yet been found in O55:H7 strains (9, 10). When studied by multilocus enzyme electrophoresis (MLEE), O55:H6 and O55:H7 strains are separated in two clonal groups, both including non-motile strains. The non-motile strains in O55:H7 clonal group may be EAF-negative or EAF-positive or express other virulence characteristics (9). In this study we investigated whether EAF-positive and EAF-negative O55 strains, isolated in different places and periods of time, could be distinguished by RAPD analysis. RAPD is a reproducible and inexpensive method that has been used to characterize both bacterial groups and strains (1).

MATERIALS AND METHODS

Bacterial strains. A total of 41 *E. coli* strains of the serogroup O55 were studied. Of these 26 were *eae*+ EAF+ and 15 were *eae*+ EAF–, as determined by the corresponding gene probes (9). None of the strains produce Shiga toxins as verified by tissue culture assay in Vero cells (5). Six strains were of serotype O55:H7, 5 of the serotype O55:H6 and 27 were non-motile. In 3 strains the H antigen could not be determined. With the exception of some H7 strains that were isolated in other countries, the remaining strains were isolated in São Paulo and in Rio de Janeiro from children with diarrhea between 1965 and 1994 (Fig. 2). From the time of isolation to the beginning of the studies, the strains were kept at room temperature or in glycerinated broth at -70ºC.

RAPD reaction. Genomic DNA was extracted and purified from bacterial cultures in Luria-Bertani broth as described previously (1). PCR for the RAPD reaction was performed in 20 µl reaction volumes containing: 10 ng of DNA, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM of dNTP (dATP, dCTP, dGTP, dTTP, Gibco BRL), 0.3 µM of random primer (OPE-16: 5'-GGTGACTGTG-3'; OPK-01: 5'-CATTCGAGCC-3'; OPK-04: 5'-CCGCCCAAAC-3'; OPP-03: 5'-CTGATACGCC-3'; Operon Technologies), 1.5 unit of Taq DNA polymerase (Gibco BRL) and overlaid with mineral oil. Amplification reactions were performed in a thermalcycler (Perkin-Elmer model 480, Cetus) and included one previous step at 94ºC for 5 min and 40 cycles with the following steps: denaturation at 95ºC for 1 min, annealing at 35ºC for 1 min and extension at 72ºC for 2 min. An additional extension step of 72ºC for 7 min was included at the end of the PCR cycles. Amplified products were electrophoresed in 1.4% agarose gels, stained with ethidium bromide and visualized by using UV light. The 1kb and 123 bp DNA ladders (Gibco BRL) were used as molecular size markers in all gels.

Analysis of data. The statistical analysis of the data was performed by using the NTSYS-pc program (Numerical Taxonomy and Multivariate Analysis System) 1.7 version (Exeter Software, Setaked, N.Y.).

RESULTS AND DISCUSSION

Each primer yielded distinct polymorphism for the 41 strains and discriminated relatively well between EAF-positive and EAF-negative strains. This is exemplified in Fig. 1, which shows the

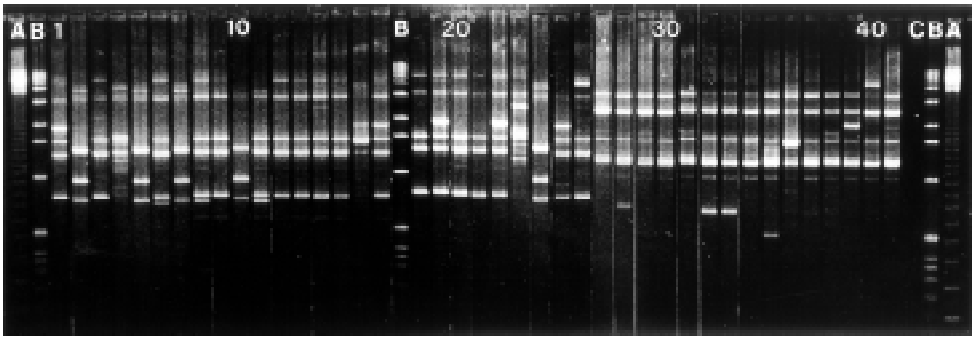

Fig. 1. RAPD fingerprints patterns of 41 *E. coli* O55 strains with primer OPE16. Lanes 1-26: EAF+ strains and lanes 27-41: EAF- strains. Molecular weight markers: 123bp (lane A) and 1kb (lane B) DNA ladders. In lane C no template DNA was added.

polymorphism and discrimination obtained with primer OPE16. Preliminary analysis of the data showed that better discrimination could be obtained by using the polymorphism's yielded by the four primers. Analyzing all 61 polymorphism, using the UPGMA method, we constructed a matrix and dendogram (Fig. 2), which showed that the 41 strains could be divided into three clusters designated A, B, and C. Clusters A and C included all EAF-positive strains and cluster B, all the EAF-negative ones. The two São Paulo O55:H6 strains in cluster A were isolated in 1966 and 1968 and the three ones in cluster C were isolated in 1981-1985.

The results of this study show that the O55 EAF-positive and the O55 EAF-negative strains are genetically distinct since they belonged to different RAPD clusters (Fig. 2). These results confirm previous findings obtained by MLEE and are consistent with the concept that typical and atypical EPEC are distinct bacterial lineages. The occurrence of the São Paulo O55:H6 strains in clusters A and C may represent the existence of two clones in this serotype predominating in different periods of time. The results of this study provide further evidence that O55:H6 and O55:H7 strains correspond to typical and atypical EPEC, respectively and show that RAPD provides an informative and simple tool that can be applied for epidemiological studies.

It has been shown that the occurrence of EAF-negative strains is rather frequent in several EPEC O serogroups and that these strains may be more important than the EAF-positive ones in some countries (i.e. England) (10). Further characterization of these strains is required to gain better

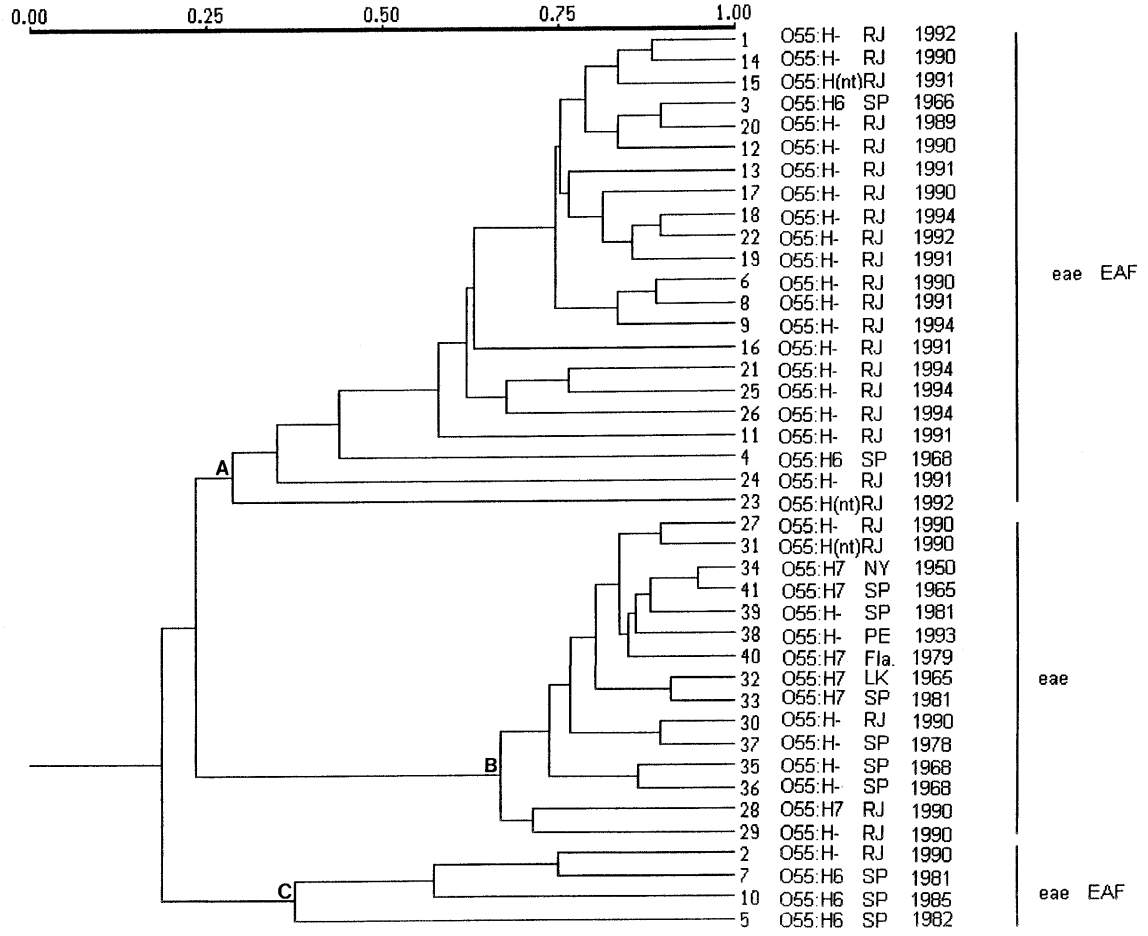

Figure 2. Dendogram based on UPGMA cluster analysis of Jaccard coefficients. Column 1 shows the numbers of the strains, column 2 shows their serotypes, columns 3 and 4 indicate the location and the year of isolation, respectively. Symbols: SP, São Paulo (Brazil); RJ, Rio de Janeiro (Brazil); Fla, Flórida (USA); NY, New York (USA); PE, Pernambuco (Brazil); LK, Srilanka (India).

understanding of their evolution and pathogenic properties, since there are other evidences suggesting that atypical EPEC strains may be ancestral to the typical EPEC and EHEC bacteria (11).

RESUMO

Caracterização de amostras de *Escherichia coli* enteropatogênica (EPEC) típicas e atípicas do sorogrupo O55 através da análise por RAPD

Quarenta e uma amostras de *Escherichia coli* enteropatogênica (EPEC) típicas e atípicas do sorogrupo O55 foram analisadas com relação à diversidade genética através da técnica de RAPD (random amplified polymorphic DNA). Todas amostras de EPEC O55 típicas ficaram localizadas em 2 grupos (A e C) e pertenciam ao sorotipo O55:H6, enquanto que o grupo B compreendia todas as amostras atípicas, as quais eram do sorotipo O55:H7. Os três grupos apresentavam também amostras imóveis. A técnica de RAPD pode ser uma ferramenta de grande utilidade em estudos epidemiológicos da infecção pela *E. coli* O55.

Palavras-chave: *Escherichia coli* enteropatogênica, EPEC, diversidade genética, análise por RAPD

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