MICROBIAL DNA CHALLENGE STUDIES OF PCR-BASED SYSTEMS USED IN FORENSIC GENETICS

Fernández-Rodríguez A., Alonso A., Albarrán C., Martín P., Iturralde M.J., Montesino M., Sancho M.

Sección de Biología, Instituto Nacional de Toxicología, Ministerio de Justicia e Interior, C) Luis Cabrera nº9, 28002 Madrid, España.

INTRODUCTION

The analysis of polymorphic DNA loci by PCR-based methods has extended DNA typing to a significant proportion of biological evidences that contain scarce and even highly degraded DNA. However, in many cases, biological samples presented for DNA analysis have also been subjected to contamination by microorganisms and, as a result of that, DNA isolated from such sources can potentially be a mixture of human and microbial DNA. To our knowledge only a few studies have been performed to address the specificity of the PCR-based systems against microbial DNA. (Budowle et al. 1995, Cosso et al. 1995).

The purpose of this study is to evaluate the influence of this source of foreign DNA on human PCR-based DNA typing by testing the specificity of 10 PCR-based systems widely used in forensic genetics (HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, D1S80, HUMTH01, HUMTPOX and HUMCSF1PO) against microbial DNA templates obtained from 30 different microorganisms isolated from forensic casework or reference strains.

MATERIAL AND METHODS

A total of 30 microorganisms (10 Gram-negative bacterial strains, 16 Gram-positive bacterial strains and 4 yeasts) that can be usually found in forensic samples, as both environmental contaminants of biological samples and indigenous flora of skin and vagina was used in this study. The standard strains were: *Bacillus cereus* ATCC, *Escherichia coli* ATCC, *Klebsiella pneumoniae* ATCC, *Citrobacter freundii* ATCC, *Enterobacter aerogenes* ATCC, *Pseudomonas aeruginosa* ATCC, *Pseudomonas stutzeri* ATCC, *Salmonella enteritidis* ATCC, *Vibrio alginolyticus* ATCC, *Micrococcus luteus* ATCC, *Staphylococcus aureus* ATCC, *Staphylococcus epidermidis* ATCC, *Staphylococcus saprophyticus* ATCC, *Enterococcus faecalis* ATCC, *Streptococcus pyogenes* ATCC, *Streptococcus sanguis* ATCC, *Clostridium perfringens* ATCC, *Corynebacterium sp.* ATCC, *Saccharomyces cerevisiae* ATCC, *Candida albicans* ATCC, *Candida parasilopsis* ATCC, *Candida tropicalis* ATCC (kindly provided by the Spanish Collection of Culture Type) and *Listeria monocytogenes* L028 serotype 1,2 kindly provided by Dr. Pérez-Díaz (Ramón y Cajal Hospital, Madrid, Spain). The strains isolated from evidentiary items were: *P. stutzeri, V. alginolyticus, S. epidermidis, S. saprophyticus,* two strains of *Bacillus sp.* and one strain of *Staphylococcus sp.*

All the microorganisms were grown overnight in liquid Luria-Bertani (LB) culture medium at optima temperatures. High molecular weight DNA was isolated by the standard phenol/chloroform procedure after treatment with proteinase K and also by adding lysozime (10 mg/ml) (SIGMA) in the case of Gram-positive and yeasts strains. Genomic DNA was quantitated by fluorimetry. The absence of human DNA on the microbial DNA extracts was verified by slot-blot hybridization with the human-specific

D17Z1 probe (Walsh et al. 1992) using the QuantiBlot™ Kit (Perkin Elmer Corporation, Norwalk, CT).

The amplification and typing of the HLA-DQA1 and PM systems were performed according to the manufacturer's recommendations using the Amplitype PM and HLA-DQA1 forensic DNA amplification and typing kits (Perkin Elmer Corporation, Norwalk, CT). The amplification of HUMTH01, HUMTPOX and HUMCSF1PO was performed by a multiplex PCR reaction according to the manufacturer's recommendations using the GenePrint STR System (Promega Corporation, Madison, WI, USA). The amplification of D1S80 locus was performed using the AmpliFLP D1S80 PCR Amplification Kit (Perkin Elmer, Corporation, Norwalk, CT). In all the cases, PCR products were first analyzed by agarose (2-3% Nusieve, FMC) gel electrophoresis in the presence of ethidium-bromide. D1S80 PCR products were also analyzed by vertical polyacrylamide gel electrophoresis and silver stain (Budowle et al. 1991). All the extraction, amplification and detection procedures were performed twice by two different analyzers.

RESULTS AND DISCUSSION

Some nonspecific amplification products were observed in the post-amplification yield gel when some bacterial DNA templates were used for the amplification of the HLA-DQA1 and PM systems (Fig. 1). However, in no case false-positive results were found by the reverse dot-blot hybridization system used for the typing (data not shown). No PCR products were observed with the HUMTH01/ HUMTPOX/ HUMCSF1PO multiplex STR system for none of the microbial DNA templates tested (data not shown).

On the other hand, D1S80 amplifications from six of the bacterial DNAs analyzed showed some nonspecific amplification products of different sizes that were located within the range of length variability of the human D1S80 alleles, as analyzed by native polyacrylamide gel electrophoresis and silver stain (Fig. 2a). The amplification of bacterial DNA at different quantities (40 ng-100 pg) demonstrated that these nonspecific D1S80 PCR products were obtained even from trace amounts (100 pg) of bacterial DNA (Fig. 2b).

In conclusion, our results validate the specificity of the majority of PCR-based systems analyzed against microbial DNA templates, but also suggest that microbial DNA could be a potential source of extra bands when D1S80 typing is carried out from forensic biological samples subjected to contamination by microorganisms.

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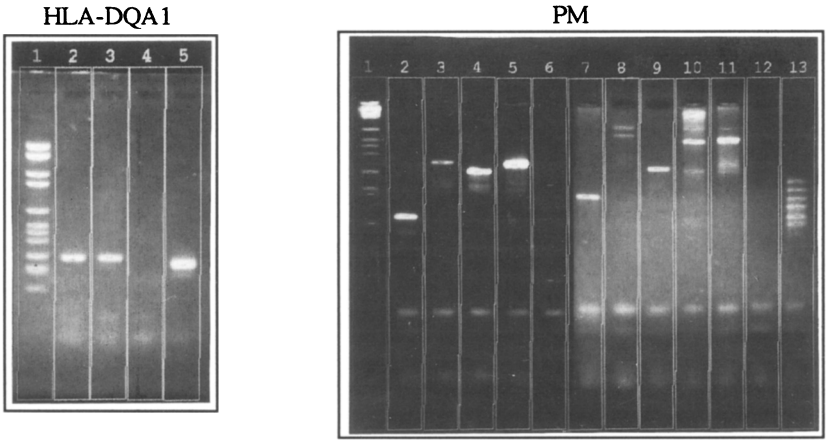

Figure 1: Nonspecific PCR products obtained with microbial DNA templates for HLA-DQA1 and PM loci. **HLA-DQA1:** Samples: (1) Molecular weight marker. (2) *V. alginolyticus* from an evidentiary item. (3) *V. alginolyticus* ATCC. (4) Negative control. (5) Human positive control. **PM:** Samples: (1) Molecular weight marker. (2) *C. perfringens* ATCC. (3) *K. pneumoniae* ATCC. (4) *S. saprophyticus* from an evidentiary item. (5) *P. stutzeri* from an evidentiary item. (6) *S. cerevisiae* ATCC. (7) *S. enteritidis* ATCC. (8) *C. parapsilosis* ATCC. (9) *C. tropicalis* ATCC. (10) *Corynebacterium sp.* ATCC. (11) *S. pyogenes* ATCC. (12) Negative control. (13) Human positive control.

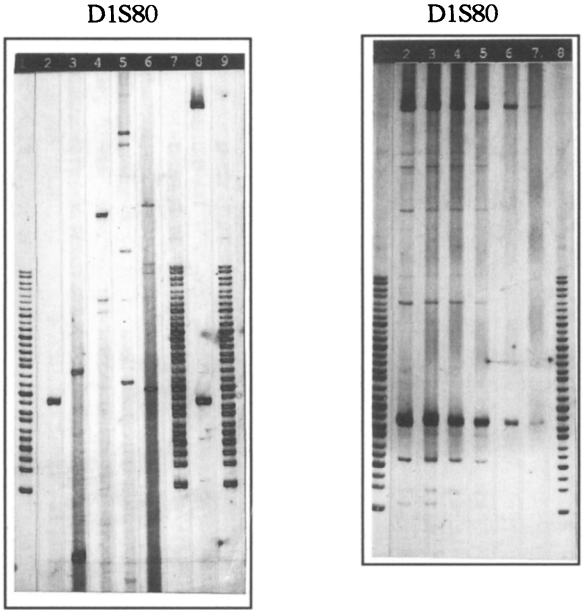

Figure 2a: Nonspecific PCR products obtained with bacterial DNA templates for D1S80 system as analyzed by polyacrylamide gel electrophoresis and silver stain. Samples: (1) Allelic ladder. (2) *Corynebacterium sp.* ATCC. (3) *S. sanguis* ATCC. (4) *M. luteus* ATCC. (5) *K. pneumoniae* ATCC. (6) *P. stutzeri* ATCC. (7) Allelic ladder. (8) *P. stutzeri* from an evidentiary item. (9) Allelic ladder.
2b: Nonspecific D1S80 PCR products obtained from different quantities of bacterial DNA template extracted from *P. stutzeri* . Samples: (1) Allelic ladder. (2) 40 ng of bacterial DNA template. (3) 20 ng. (4) 10 ng. (5) 5 ng. (6) 1 ng. (7) 0,1 ng. (8) Allelic ladder.