TECHNICAL NOTE

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Microbial DNA Challenge Studies of Variable Number Tandem Repeat (VNTR) Probes Used for DNA Profiling Analysis

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ABSTRACT: DNA probes commonly used for forensic DNA profiling analysis were hybridized to *Hinfl* digested DNA isolated from various common microbial species. Extended exposures to light sensitive film failed to detect any DNA fragments of a microbial origin following hybridizations to radio-labeled DNA insert single-locus probes (SLPs) and nonisotopically labeled oligonucleotide SLPs.

KEYWORDS: pathology and biology, DNA, DNA profiling, VNTR probes

DNA analysis of evidentiary materials found at a scene of crime can help establish identity. Methods and materials are now available to forensic scientists, enabling the widespread application of DNA profiling to casework [*1–3*]. Although the technology is developing rapidly, and alternative methods of assaying DNA polymorphisms incorporating the polymerase chain reaction (PCR) are available [*4–7*], single-locus probes (SLPs) still offer reliability and robustness.

Numerous polymorphic markers cloned from variable number tandem repeat (VNTR) sequences in the human genome have been described and used for individual identification purposes [*8–10*]. These clones usually consist of several kilobases of VNTR sequence suitable for isotopic labeling. More recently however synthetic oligonucleotides complementary to VNTRs have been used as DNA probes [*11*]. Reporter molecules such as biotin, digoxigenin, and alkaline phosphatase can be covalently linked to the oligonucleotide thus facilitating the use of nonisotopic detection methods [*12,13*].

Both types of locus-specific probes are widely used in forensic science laboratories around the world for characterization of various body fluids and tissues left at a scene of crime. However evidentiary materials presented for DNA analysis have often been subjected to various environmental insults and contaminated by microbial growths. DNA isolated from such sources can potentially be a mixture of human and nonhuman DNA.

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Previous studies on the effects of environmental insults and contamination, on the results of DNA analysis using cloned DNA SLPs have been reported [*14*].

This study specifically addresses the issue of microbial contaminants and the specificity of both double-stranded DNA cloned and synthetic single-stranded oligonucleotide SLPs for human DNA. Probes were challenged with DNA isolated from bacterial, yeast and fungal sources common to evidentiary samples submitted for DNA analysis. The bacteria selected were *Streptococcus faecalis*-NCIB (National Collection of Industrial Bacteria) 11508 and *Escherichia coli*-NCIB 9517 (fecal), *Staphyloccus aureus*-BCC (Bioproducts Culture Collection) 662 (wounds and lesions), and *Bacillus subtilis*-NCIB 3610 (environmental). *Candida albicans*-CBS (Centraalbureau voor Schimmelcultures) 2723 represented a yeast contaminant of vaginal origin. The environmentally ubiquitous fungi species *Aspergillus* and *Mucor* completed the microbial challenge set. The potential for false-positive results being obtained was tested.

Materials and Methods

Samples

Streptococcus faecalis, Bacillus subtilis, Escherichia coli, Staphylococcus aureus and *Candida albicans* were obtained from the Bioproducts Culture Collection, ICI Biological Products Billingham England. *Aspergillus oryzae* and *Mucor fuscus* DNA was purchased from the International Mycological Institute, Kew, London. The SLPs are commercially available from Cellmark Diagnostics, Abingdon Business Park, Abingdon, Oxfordshire, England, apart from YNH24, which was purchased from the Promega Corporation, Madison, Wis.

Methods

High molecular weight DNA was isolated by phenol/chloroform lysis from bacterial cultures, and high molecular weight DNA from human volunteer blood samples was isolated as previously described [*15*]. Genomic DNA from each source (bacteria, yeast, fungi, and human) was quantitated by spectrofluorimetry, digested with *Hinfl* restriction endonuclease and electrophoresed following standard procedures as described [*15*]. Electrophoresis was terminated when a 2.3 kilobase restriction fragment of Lambda DNA had migrated 15 cm from the origin.

Four microgram aliquots of bacterial, yeast or fungal DNA, 1 μg of human DNA (positive control) and a mixture of 1 μg of microbial DNA plus 1 μg of human DNA were electrophoresed through 0.7% agarose (Sigma, U.K.) and Southern blotted onto Hybond-N membranes (Amersham International, Aylesbury, U.K.).

The resulting Southern blots were hybridized to both cloned probe insert labeled with alpha ^{32}P-deoxyguanosine triphosphate and alkaline phosphatase labeled oligonucleotide nonisotopic chemiluminescent enhanced (NICE™), SLPs.

Probe labeling and hybridization procedures were as described by the supplier. Probes MS43A (D12S11), MS1 (D1S7), MS31 (D7S21), MS8 (D5S43), and g3 (D7S22) were labeled both isotopically and nonisotopically, while MS205 (D16S309) and YNH24 (D2S44) were available only as cloned inserts for isotopic labeling. Autoradiographs and lumigraphs were obtained from various exposures, of up to two weeks (Hyperfilm-Amersham International Aylesbury, U.K.).

Results

No DNA sequences of nonhuman origin were visualized on autoradiographs or lumigraphs after extended times of exposure following hybridization to radioactively la-

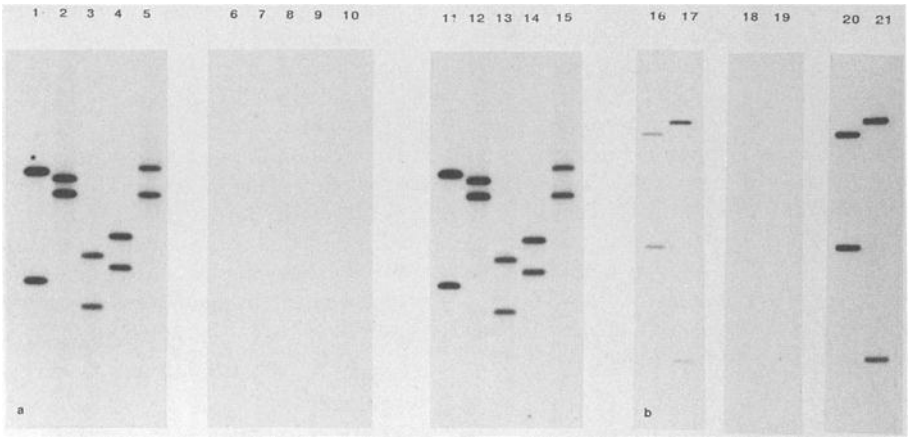

FIG. 1—(a) *Autoradiograph of five 1 μg samples of human DNA (1–5), 4 μg DNA extracted from* Streptococcus faecalis *(6),* Candida albicans *(7),* Staphylococcus aureus *(8),* Bacillus subtilis *(9),* Escherichia coli *(10) and 1:1 mixtures of human/microbial DNA (11–15) probed with* ^{32}*PdGTP labelled MS31 (D7S21).* (b) *Lumigraph of two 1 μg samples of human DNA (16,17), 4 μg DNA extracted from* Aspergillus oryzae *(18),* Mucor fuscus *(19) and 1:1 mixtures of human/fungal DNA (20,21) probed with NICE® MS1 (D1S7).*

belled and nonisotopically labeled SLPs (Fig. 1). A total of 1 μg of human DNA, used as a positive control for hybridization was detected by each probe. Extended exposures, sufficient to detect 10 to 20 ng of human sequence, of up to 2 weeks for radioactive probes and three days for chemiluminescent probes still failed to identify any microbial DNA sequences.

Conditions described here mimic gross contamination of evidentiary samples and demonstrate the specificity of both cloned insert and synthesized oligonucleotide SLPs, commonly used in forensic investigations, if suppliers recommended procedures are followed. From the common micro-organisms selected, no DNA profiles were produced that could have lead to false-positive or false-negative results.

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