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Review Article

DNA Fingerprinting – Microbiologists Perspective: Review

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Abstract

DNA fingerprinting as emerged to be an excellent valuable tool in the field of forensics, unique identification of person and paternity disputes and issues. Genetics are the base criteria of identification. Microbiology runs hand to hand in evaluating these scenarios by the microflora detected. This review explains about the basics, methods, sequencing and role of microbiologists and their perspective in DNA fingerprinting.

Keywords: Microbial forensics, Fingertip microflora, Microbial DNA analysis, Microbial fingerprint.

INTRODUCTION

Background

DNA fingerprinting also termed as DNA profiling or genetic fingerprinting. This concept was first figured out by Alec Jeffery in 1984. DNA sequencing is the basic unit for same. There are long linear segments of DNA where in which present are genes. 99.9% of base pair sequence of DNA or genes are same in individuals. Separation is done on density gray gradient. Gene makes the protein which further corrects the prototype. Satellite DNA or Repetitive DNA is categorized on basis of A:T/C:G ratio, length of sequence and number of times the sequence repeats i.e. copy number. Satellite DNA is divided into Minisatellites or VNTR and microsatellites. In DNA fingerprinting non-coding regions makes differences in which are short tandem repeats and variable number tandem repeats, previously restriction fragment length polymorphism was also used. Letters of DNA repeats over and over but short tandem repeats will be different in the numbers. The variability between all individuals is detected by the variability in STR. Restriction enzymes are used to differentiate them into pieces and amplify them using polymerase chain reaction. Separation of STR is done by gel electrophoresis. On collecting all the DNA or STR together of separate individual they get cationic and anionic charge. This corresponds to migration of little fragments of DNA. This is called as DNA fingerprinting which will be replaced by DNA sequencing. This is used for Forensics, unique identification of person and paternity disputes and issues. DNA fingerprinting methods are isolation of DNA, Digestion of DNA by endonuclease followed by gel electrophoresis and blotting in a nitrocellulose membrane. Hybridization with radiolabeled VNTR and Autoradiography is the final method.

Molecular genetics

Molecular genetics has dramatically altered the field of human forensics analysis by providing one of the most powerful and definitive tools for the legal system. The process of sequencing DNA and the huge technical advances stimulated by the Human Genome Project and the discovery of PCR forever changed forensics [1, 2]. In only a few decades, DNA analysis has become the gold standard of forensic investigation. Before these advances, the identification and detection of variable human genetic markers required complex and tedious genetic cloning and DNA probing techniques. Nowadays, a human DNA signature from a latent, nearly invisible sample (sometimes as small as a single cell) can be analyzed and compared with great ease with large genetic databases. Human fingertip microflora is transferred to touched objects and may provide forensically relevant information on individual hosts, such as on geographic origins, if endogenous microbial skin species/strains would be retrievable from physical fingerprints and would carry geographically restricted DNA diversity. The dynamic fingerprint

microflora challenges human host inferences for forensic purposes including geographic ones. Human fingerprint microflora is too dynamic to allow for forensic marker developments for retrieving human information. In the coming years we can expect a steep increase in the use of molecular fingerprinting techniques, such as DGGE/TGGE and T-RFLP, and in the use of fluorescent PCR products in these techniques. However, although successful in the study of community dynamics, we have to keep in mind that none of the methods is perfect and that they all have their strong and weak points [3].

Microbiology in DNA fingerprinting

Microbes can negatively interfere with the postmortem assessment of alcohol abuse and in this way pose problems for forensic investigators [4]. However, microbial forensics is often chiefly associated with the detection of highly pathogenic microbes to which humans are deliberately exposed in cases of biological terrorism [5, 6]. However, human fingertip microflora left behind on touched objects at crime scenes may potentially contain forensically relevant information that may be useful for human host inferences accessible via microbial DNA fingerprinting of physical fingerprints. For example, if endogenous microbial skin species/strains with a geographically restricted distribution could be retrieved from touched objects via microbial DNA analysis, the geographic origin of the human host individual could be determined indirectly. Information about the geographic region of origin can be relevant in suspect-less forensic cases where the evidence DNA sample does not match either a suspect's DNA profile or any in a criminal DNA database. In such cases, geographic information derived from crime scene samples is expected to reduce the potential pool of suspects by allowing police investigations to concentrate on specific groups of people, i.e., those from a restricted geographic region. Numerous human genetic markers have been suggested for inferring human genetic ancestry mostly to the continental level 7-10] and a recent study indicated that inferring the subregion of origin of an unknown European may be feasible from autosomal genetic data [11]. However, direct ancestry inference based on human genetic markers is currently far from perfect, initiating the question whether microbial DNA may be used to supplement human DNA markers in reliable ancestry reconstruction of unknown persons. Recently, it has been shown that the gastric pathogenic bacteria Helicobacter pylori have intimately coevolved with its human host [12, 13]. Although this example may be of limited direct relevance for forensics, because samples containing H. pylori are usually not found at crime scenes (with the exception of bodies in cases of missing persons), it shows that in principle human geographic signatures are inferable from microbial genomes. The human skin is a complex microbial ecosystem consisting of multiple niches, which can differ drastically from each other [14]. Interactions between skin microbes and the human host, as well as between the microbial occupants, are still poorly understood. The current knowledge on skin microbiota primarily derives from cultivation-based studies [15, 16], although molecular fingerprinting techniques have been employed more recently [17, 18]. If a comparable relationship exists between humans and their skin microbiota, as has been observed for H. pylori, new methods for human geographic origin determination could be developed based on DNA analysis of fingertip microflora, with interesting new applications to molecular analyses of physical fingerprints left at crime scenes. One study tested the suitability of physical fingerprints for revealing human host information, with geographic inference as example, via microbial DNA fingerprinting. They showed that the transient exogenous fingertip microflora is frequently different from the resident endogenous bacteria of the same individuals. In only 54% of the experiments, the DNA analysis of the transient fingertip microflora allowed the detection of defined, but often not the major, elements of the resident microflora. Although they found microbial persistency in certain individuals, time-wise variation of transient and resident microflora within individuals was also observed when resampling fingerprints after 3 weeks. While microbial species differed considerably in their frequency spectrum between fingerprint samples from volunteers in Europe and southern Asia, there was no clear geographic distinction between Staphylococcus strains in a cluster analysis, although bacterial genotypes did not overlap between both continental regions [19].

Data generation

Data generated from microbial fingerprinting methods are used to understand which microorganisms are present and how they are intrinsically coupled to their environmental conditions [20]. For example, geochemical conditions (such as the availability of electron acceptors) influence which microorganisms are present and active at a site, while the microbial activities (such as electron acceptor consumption) can strongly impact the site geochemistry. A microbial fingerprinting method therefore can provide valuable information as to whether subsurface conditions are conducive to bioremediation and in evaluating the effectiveness of monitored natural attenuation (MNA) [21]. Most engineered bioremediation strategies involve the addition of an amendment to stimulate the growth and activity of specific groups of microorganisms capable of performing desired processes. Microbial fingerprinting methods can also be used to track the overall changes in the microbial community over time or in response to remediation activities. Data gathered from the microbial fingerprinting methods then can be used to evaluate the performance of the bioremediation strategy [22].

Advantages

The microbial fingerprinting methods are cultivation independent, meaning that they do not require growth of the microorganisms in the laboratory. Laboratory cultivation is difficult, time-consuming, and not always possible for several important microorganisms. In general, microbial fingerprinting methods require little prior knowledge about which microorganisms are of interest. So, these methods may be useful for emerging contaminants (i.e., contaminants for which little information is currently available) [23]. Microbial fingerprinting methods can capture the presence and activity of uncultured and previously unidentified microorganisms. PLFA analysis provides a direct measure of viable biomass in addition to a biochemical profile of the microbial community. PLFA analysis can be used in conjunction with SIP to document that biodegradation is occurring (see the SIP Fact Sheet for more information) [24]. Fingerprinting techniques based on DNA can also be used with SIP but often require greater quantities of the labeled compound. The genetic fingerprinting methods allow identification of some members of the microbial community to the family or genus level [25].

Limitations

PLFA analysis cannot be used to identify specific microorganisms. Genetic fingerprinting methods (e.g., DGGE, T-RFLP) can be used to identify specific microorganisms. However, the number of microorganisms that can be identified depends on the complexity of the microbial community. The genetic fingerprinting methods are not quantitative [26]. See the Quantitative Polymerase Chain Reaction (qPCR) Fact Sheet for quantification of a specific functional gene or group of microorganisms. Important microbial processes may be performed by a numerically small portion of the total community (<1%) that is not detected in a DGGE profile. Interpretation of microbial community fingerprints is somewhat subjective and less straightforward than for other EMDs [27].

CONCLUSION

Microbial forensics is a naïve branch that involves multi-disciplinary approach for the detection, tracing and evidencing, with a predominant microbiological approach. This field is emerging as a necessity for civil security rather than luxury. In 21st century, pathogens are readily accessible, and technology is making their use as a weapon more feasible. As a preventive measure, it is important to strengthen microbial forensic capabilities. An effective program will require development and validation of all aspects of the forensic investigative process, from sampling to interpretation of results. There is a need to rely on other existing and emerging capabilities beyond the traditional forensic laboratory and its practitioners. The sharing of data collected and validated will greatly improve the practice of microbial forensics. Forensic scientists worldwide should contribute to the field of microbial forensics and enhance its capabilities to aid in bringing perpetrators of these heinous attacks to justice. International and National collaborative approaches can be done by setting up a national and international reference laboratory, transparency of analysis.

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