CHARACTERIZATION METHODS FOR BACTERIA

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ABSTRACT

Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. Bacterial identification is used in a wide variety of applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies. So in this review we have discussed several methods for identification of bacteria.

Keywords: Bacteria, Phenotype , Genotype, Plasmid, Rybotype, DNA, PCR

INTRODUCTION

Traditional methods of bacterial identification rely on phenotypic identification of the causative organism using gram staining, culture and biochemical methods. However, these methods of bacterial identification suffer from two major drawbacks. First, they can be used only for organisms that can be cultivated in vitro. Second, some strains exhibit unique biochemical characteristics that do not fit into patterns that have been used as a characteristic of any known genus and species. In the past decade or so, molecular techniques have proven beneficial in overcoming some limitations of traditional phenotypic procedures for the detection and characterization of bacterial phenotypes (Cullimore, 2000; Barrow and Feltham, 2004).

Through the early part of the twenty-first century, there appeared to be a general feeling that the same observations and tests could be used to characterize and identify any kind of bacterium. But as different, "exotic" types of bacteria were discovered, it was found that they would tend not to grow in the standard test media nor even in the usual conditions of incubation. Obligate parasites and strict anaerobes were among the emerging groups of bacteria needing special methods for growth and characterization (Cullimore, 2000; Barrow and Feltham, 2004). By the 1930s, a standard descriptive chart was developed for uniformity in recording the characteristics of the "aerobic saprophytes" (which are equivalent to what we call the "commonly-found" or "easy-to-grow" chemoheterotrophs in our general courses today).

MORPHOLOGICAL METHODS

In identifying bacteria, the morphological features are of importance in that they constitute the first step in characterization. The form, diameter, elevation and margin of colonies, pigment formation can be observed directly. Cellular morphology, Gram status, sporulation and motility of an isolate can be determined by

microscope after different staining methods. Phase contrast microscopy is also used to determine spore presence and cellular morphology without staining.

Phenotypic Methods

Phenotypic methods include biotyping, antibiogram, phage typing, serotyping, PAGE/immunoblotting and multilocus enzyme electrophoresis (MLEE). In biotyping an organism can be identified and classified at the genus and/or species level using various biochemical reagents and parameters. Antibiogram includes the analysis of the growth of strain in the presence of antibiotic. However it is not very discriminatory and for example antibiogram patterns can change with transformation of plasmids (Busch and Nitschko, 1999). In phage typing, a particular bacterium is infected by a specific phage. Thus, the isolates can be differentiated based on the infection capability. Serotyping involves identification of microorganisms according to their reaction to a given antiserum. Proteins from whole-cell lysate can be separated by SDS-PAGE. Protein patterns are used in classification of strains (Busch and Nitschko, 1999).

Genotypic Methods

Genotypic methods are based on DNA analysis of chromosomal or extrachromosomal (plasmid) genetic material. The main advantages can be summarized as follows: - They are able to distinguish between two closely related strains: High discriminatory power. - All strains are typeable since it is always possible to extract DNA from bacteria - Analytical strategies can be applied to DNA of any source since it is always possible to extract DNA from bacteria. - The composition of DNA is not affected by cultural conditions or preparation methods. - Resulting data can be analyzed statistically (Farber, 1996). Genotypic methods include plasmid typing, pulse-field gel electrophoresis, ribotyping, polymerase chain reaction based methods, nucleotide sequencing, DNA-DNA hybridization (Farber, 1996, Goodfellow, 2000). Some of these methods were summarized below.

Plasmid Typing

Plasmids are self-replicating, extra chromosomal, usually supercoiled genetic elements (Bush and Nitschko, 1999, Farber, 1996). Plasmids generally encodes for products and/or functions which modify the phenotype of the cell. In plasmid typing, plasmids are isolated from bacterial strains, then their number and size are determined by gel electrophoresis. However different plasmids can be of the same size. Using restriction enzymes, this problem can be solved. Different plasmids will give different fragment patterns (Farber, 1996). The main drawback of the method is the transfer of plasmid between strains and species

Chromosomal DNA Restriction Endonuclease Analysis

In this method, DNA is cut with a frequent-cutting restriction enzyme and the fragments are electrophoresed on agarose gel. A difference in fragment patterns between isolates is referred to restriction fragment length polymorphism (RFLP). Different patterns are due to DNA composition variations. This method is rapid, inexpensive, relatively easy to perform and universally applicable. But interpretation of fragment pattern is not easy since numerous fragments are obtained and they are closely spaced on agarose. In order to obtain interpretable results, several restriction enzymes must be used.

Ribotyping

Ribotyping is based on the use of nucleic acid probes to recognize ribosomal genes. In a prokaryotic ribosome there are three types of RNA (23S, 16S and 5S rRNA). The genes coding for rRNA sequences are highly conserved and multiple copies of the rRNA

operon exist in most bacteria. Thus, chromosomal fragments containing a ribosomal gene are revealed after hybridization with probes. Resulting hybridization bands (approximately 1 to 15) are compared between isolates. Ribotyping refers to the grouping of bacteria based on this method.

PCR-Based Methods

PCR is based on the amplification of DNA by a heat stable DNA polymerase enzyme. Depending on the special primer used, the region of interest is amplified. Reaction includes repeated cycles of high temperature for denaturation of the DNA, oligonucleotide (primer) annealing and an extension step mediated by thermostable DNA polymerase. Amplification cycle is repeated 25-35 times to produce a >106 fold amplification of the target DNA. The amount of the target DNA is exponentially increased.

PCR-Ribotyping

In a prokaryotic ribosome genes coding for rRNA are separated by spacer regions which are variable in length or sequence at both the genus and species level (Farber, 21 1996). Thus, multiple bands are obtained after amplification of spacer regions in different rRNA coding operons for a particular strain. Besides, spacer region between 16S-23S rRNA or 23S-5S can be amplified and amplification products can be compared on agarose gel. In this method, availability of universal primers is the major advantage. Sequence variation between ribosomal operons, described especially in the ISR between the 16S and 23S rDNA genes in individual strains (Gürtler and Stanisich, 1996), has been used for bacterial identification (Jensen *et al.*, 1993, Tilsala-Timisjarvi and Alatossava, 1997). Ribosomal internal spacer regions have been found to be more variable than 16S and 23S rDNA between bacterial species (Barry *et al*., 1997). For example, closely related species *B. subtilis* and *B. atrophaeus* have been differentiated by comparing ISRs (Nagpal *et al*., 1998). Flint *et al*. (2001) have shown that ISR sequences have varied in length among the different lactic acid bacterial species and have varied also within some strains of the same species.

LH-PCR (Length Heterogeneity Analysis of Polymerase Chain Reaction Amplified DNA)

This method is based on the natural length variation within 16S rDNA genes. The variable region is amplified by PCR with fluorescently labeled universal primers that recognize the region in all eubacteria (Tiirola *et al*., 2003).

PCR-RFLP

In this method PCR amplicons are digested with suitable restriction enzymes. Digested amplicon is run onto an agarose gel and DNA fingerprint results are obtained. 16S, 23S and 16S-23S rRNA spacer regions have been used for locus specific RFLP (Olive and Bean, 1999, Caccamo, 2001).

Genomic DNA Based RFLP

PFGE (Pulse Field Gel Electrophoresis) is a very discriminating and reproducible typing method. In this method, intact cells are embedded in agarose plugs to 22 prevent the shearing of DNA during DNA extraction. Then, these plugs are treated with detergent and enzymes to isolate the DNA. In the following step, the isolated DNA is cut with an infrequently cutting restriction endonuclease which recognizes specific 8-base cutter or 6-base cutter sequences. These enzymes are chosen depending on the G+C content of the bacterial genome. After digestion, very large DNA fragments (10-800 kb) are obtained. Bacterial plugs are inserted into agarose gel and they are subjected to electrophoresis. In PFGE system the electrical field is alternated at predetermined intervals. At these intervals, called switch time or pulse time, the direction

of electrical field is changed. Consequently, the separation of high molecular weight DNA fragments is performed. Agarose concentration, buffer concentration, pulse times, voltage and electrophoresis run time are important parameters which affect the separation of fragments (Olive and Bean, 1999, Busch and Nitschko, 1999, Farber, 1996).

DNA Sequencing

In this method, the nucleotide composition of a DNA molecule is determined. Generally the 16S rRNA gene or the 16S rRNA is sequenced because they contain variable and conserved regions within bacterial species. Besides, sequencing of whole genome is not practical. Evolutionary trees are constructed based on 16S rRNA.

DNA-DNA Hybridization

Denatured complementary strands of DNA can reassociate to form native duplexes under suitable experimental conditions. Nucleic acid fragments are paired according to the similar linear arrangements of the bases along the DNA. Nucleotide sequence similarity between bacterial strains can be detected by measuring the amount of molecular hybrid and its thermal stability (Goodfellow, 2000). Microbial species are extensively delineated using DNA-DNA relatedness data (Stackebrandt and Goebel, 1994).

CONCLUSION

In this review we report a comparative study between conventional and unconventional identification methods for bacterial identification in the Clinical Microbiology Laboratory. Bacterial clinical isolates identification obtained by DNA sequencing shows excellent correlation with identification obtained by conventional microbiological methods. Moreover, DNA sequencing allows the identification of bacteria from colonies grown on agar culture plates in just a few minutes, with a very simple methodology and hardly any consumable costs, although the financial costs of this experiment can be high.

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