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Review Article

Microbiology Section

The Application of Pulsed Field Gel Electrophoresis in Clinical Studies

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ABSTRACT

Pulsed-field gel electrophoresis is a method applied in separating large segments of deoxyribonucleotide using an alternating and cross field. In a uniform magnetic field, components larger than 50kb pass a route through the gel and since the movement of DNA (Deoxyribonucleic acid) molecules are in a Zigzag form, separation of DNAs as bands carried out better via gel. PFGE in microbiology is a standard method which is used for typing of bacteria. It is also a very useful tool in epidemiological studies and gene mapping in microbes and mammalian cell, also motivated development of large-insert cloning system such as bacterial and yeast artifical chromosomes. In this method, close and similar species in terms of genetic patterns show alike profiles regarding DNA separation, and those ones which don't have similarity or are less similar, reveal different separation profiles. So this feature can be used to determine the common species as the prevalence agent of a disease. PFGE can be utilized for monitoring and evaluating different micro-organisms in clinical samples and existing ones in soil and water. This method can also be a reliable and standard method in vaccine preparation. In recent decades, PFGE is highly regarded as a powerful tool in control, prevention and monitoring diseases in different populations.

Keywords: Clinical research tool, DNA size, Typing

INTRODUCTION

To date, many methods have been developed to identify and classify bacteria but all these methods are not applicable. One of the practical methods that enable researchers to detect, identify and classify genome of micro and macro organisms accurately and sensitively is Pulsed-field gel electrophoresis (PFGE) [1-3].

Hospital, prison, private hospitals, child care institutions (kindergartens - schools, etc) homeless shelters all are exposed to a greater danger of diseases outbreak that are caused by bacteria [3,4]. In addition, in some places such as hospitals, there are people who have weakened immune system, in such cases and also in older people; diseases are easily spreadable [4-6].

Hospitals are one of the greatest threats in terms of introduction of environmental bacteria into wounds after surgery, IVF (Invitro Fertilization) and catheterization. In suspicious cases of an outbreak, determining the exact time by which spread occurs is important because the source of the outbreak can be a hospital worker or hospital food [6].

PFGE is a useful way to discover the causing agents concerning unilateral diseases and tracking the effects of these agents through a single source. This technique as first explained by Schwartzand and Cantorand (1983) is different from the common gel electrophoresis and with the alternating change of electricity current causes DNA migration. This technique gives researchers the ability to separate linear DNAs more than 10mb [7] (Mega Base pair) [3-6].

TYPES OF PFGE

The use of electric current with the speed and alternate rotations in the magnetic field leads to the emergence of various types of PFGE. Methods such as FIGE, TAFE, CHEF, OFAG, PHOGE and PACE are separate means for isolating and typing of the DNA molecule with the large pieces. The selection of these tools is dependent on the financial issue and the purpose of study [1,2]. Below are some of PFGE briefly described:

Contour Clamped Homogeneous Electric Field (CHEF): The original system of CHEF was developed by Clark et al., and today these devices are widely used. In this system, distortion is created on the edge of the chamber and passive electrodes by a complex method, the electrodes of gel electrophoresis system can be controlled automatically and 24 electrodes are allowed to find hexagonal arrangement. There is no non-passive electrode in CHEF system and all the electrodes are connected to the power supply through a series of identical ring resistors. These rings are responsible for regulating the voltage on all electrodes arranged hexagonally in a unit electric field. CHEF system voltage is set at 24 points. CHEF uses the positive electrode to the negative electrode from the angle of orientation 120 degrees. Molecules over 7000 kb can be separated in this system. In this system, the size, location, coordination, stability and continuity of the electric field are precisely controlled, and this allows the separation DNA fragments with different size [7].

Field-Inversion Gel Electrophoresis (FIG): This procedure was introduced by Carle, Frank and Olson for the first time in 1986. In this method, there are two fields with separated straight angle that periodically turn upside down. Forward movement of molecules is provided by shorter time pulses or less traction for the reverse movement of molecules. The movement of DNA molecules more than 2mb is not always consistent; for example, molecules in different sizes and labeled sometimes can move and immigrate together in FIGE [6,7]. Work with FIGE is easy and simple. FIGE is a very popular nowadays due to separation of smaller parts. FIGE provides acceptable resolution, over 800 Kb.

Asymmetric Field Inversion Gel Electrophoresis (AFIGE): The method is used for the detection of DNA double-strand breaks (DSBs), a reverse- asymmetrical field gel electrophoresis. DNAs extracted from cells are fixed in Agarose Plugs and transferred into AFIGE system and the rate of DNA breakage is measured under an inverted and asymmetric electric field, quantitatively. In this procedure, DNA is longer period of time exposed to restriction endonuclease such as XhoI and the amount of fractures increases, accidentally. It seems that this method is specific for double-stranded DNA detection [7,8].

Orthogonal-Field Alternation Gel Electrophoresis (OFAGE): This system was reported by Clark and Olsen in 1984 that is a vertical alternating field electrophoresis, which uses regular spot electrodes and the final immigration of samples is changed by creating nonlinear and dissimilar electric fields [3]. The major drawback of this device is the use of non-unit electric fields that the angle among the electric fields is different throughout the gel; so the migration of DNA will vary depending on the situation in the gel. This is problematic particularly in mapping the genome of mammalian cells, because a continuous distribution of the different components is produced in it that cannot be distinguished clearly. The angle among the electric fields is variable, less than 180 degrees and more than 90 degrees. DNA molecules between 1000 kb and 2000 kb can be separated by this method [7,8].

Rotating Gel Electrophoresis (RGE): This method was introduced by Southern in 1987. In this way, the gel rotates between two angles while the power supply is turned off. RGE has a monotonous electric field and direct separation is done because only one set of electrodes are used. Because of the tedium of the flow, the intensity of the voltage can be added to be isolated in a shorter time; Change the angle of rotation is also easily possible. Work with this method is easy and suitable for the separation of DNA with 50 - 6000 Kb [9].

Pulsed-Homogeneous Orthogonal Field Gel Electrophoresis (PHOGE): The difference among this method and other methods of PFGE with homogeneous fields is the orientation angle of 90 degrees in field. DNA molecules are moved four times per cycle instead of twice. DNA lines do not follow a straight line because of the use of multiple electric fields. This system separates DNA over 1Mb [3].

Programmable Autonomously-Controlled Electrodes(PACE): PACE electrophoresis system by independent adjustment of voltage, on 24 electrodes arranged in a closed contour controls all the parameters of the electric field. PACE system flexibility is due to its ability to produce an unlimited number of controlled homogeneous electric field, voltage gradient, direction and duration of flow. PACE system would be preferable than the other alternating electrophoresis methods (such as FIGE, OFAG and PHOGE). In this system, DNA fragments from 100 bp to more than 6Mb are separable. The ability to change the angle among the reorientation of alternating currents provides increasing the speed of separation of large DNA Molecules [9].

SUMMARY OF PFGE PROCEDURE

At the beginning of the process through cultivation of the intended bacterial cells within 24 hours, we prepare a pure culture, then, a loop full of the product from culture content was sampled and a suspension was prepared using a suitable buffer. At first, release of DNA from cells is required. To do this process, we mix cell suspension (microbial) with a protease (an enzyme that through attack cell membrane proteins can tear up the membrane), agarose and SDS (a detergent that removes proteins) with each other. The mixture of enzyme - detergent will denature proteins of cell membrane so that a pore is formed across the cell through which the chromosomal DNA will be free [1-3].

Agarose will keep the immersed DNA within the matrix-gel. Then plug (agarose fragmentation after washing) is washed several times and therefore proteases and cell debris are removed [8]. This will cause the large DNA molecules to be scattered more easily in the gel-agarose matrix. Removing of proteases is very important because they are effective in the subsequent stages of the process, in DNA breaking with restriction enzymes [7,8].

One of the critical steps during the preparation process for PFGE is incubation of plugs with restriction enzymes. Restriction enzymes identify the specific sequence of nucleotide and wherever found the desired sequence, cut the two DNA strings from the exact place. Restriction enzymes' analysis during PFGE steps is very important because the length of DNA fragments derived from the analysis, will predict a pattern to distinguish between two bacterial strains. The applied restriction enzymes in this process, identify sites of 8 base pairs or larger so that only a limited number of segments will be produced. If cells have been resulted from the same bacterial isolated samples, their chromosomes will have the same nucleotide sequence. So the restriction enzyme cuts DNA if it has the ability to identify the place of the particular sequence, released chromosome parts of the two bacteria must be equal [1-3]. When the generated DNA fragments by enzymes – that usually are 8 base pairs or a little more - if they are much larger than this size, a special form of electrophoresis will be needed to separate them [1]. Plugs (the sectioned and fragmented agarose) within gel agarose, are located in electrophoretic cavity and flow (voltage) is established. Important support of this approach is ability of DNA to travel throughout the gel and create differences based on travel length. Electric current, forces DNA which is negatively charged, to move towards the positive pole within the gel. Smaller pieces of DNA in comparison with larger pieces have more freely movement. Therefore, in direct electric current will move ahead of larger parts [1-3].

Examples of PFGE in which DNA fragments are very large, (if the genomes of bacteria to be cut by one or two enzymes), too much time is required for DNA to move throughout the gel. With the help of large pieces of DNA movement, the flow is reversed periodically at the poles so that make DNA fragments move in different directions. This back and forth movement allows large pieces to cover their path like a snake [7,8].

So, PFGE can also determine the DNA fragments length related to other used samples. PFGE can also estimate fragments' length by comparing their location within gel [1-3].

APPLICATION OF PFGE

PFGE is used for analysing bacterial genomes when classical genetic techniques are not responsive to our questions [9]. Genome structural maps of many bacterial kinds are plotted using PFGE, in which, large parts are produced to the limited numbers through digestion action by restriction enzymes and thus segregation and separation are done [10].

Information that is obtained from plotting a genome structural map provides information about size and shape (circular or linear) of bacteria genomes [9-11]. Structural maps which are created by PFGE can be used for genetic mapping; this means that via hybridization of cloned genes from the produced parts in structural maps, genetic maps are obtained [12].

By comparing the structural and genetic maps, information about re-arrangement, decrease or increase in chromosomes of associated species and strain can be achieved [10]. In addition to the genotyping and fingerprinting, other applications of PFGE include the use of it in aetiologic studies of bacteria, identification of bacteria isolated from environmental or clinical samples, identification of antibiotics resistant strains (e.g. MRSA strains) as well as the classification (taxonomy) of bacteria. Identification of infectious agents and their origin using this method as a standard method is superior in epidemiological studies. The use of this method in the detection of DNA viruses that have large genomes (e.g. HSV) is allowed. Viral genomes that are healthy are separated on the basis of size. In the study conducted by Zhung and colleagues (in 1994) the FIGE method was used to identify viral DNA replication intermediates. Viral DNA fingerprinting often has been used by means of PFGE on viruses isolated from the environment. PFGE also is a useful tool in order to determine the relationship among the different strains of a single species, an efficient method to estimate the size and

chromosomal mechanism, to describe and explain the genome of eukaryotes and prokaryotes, to help the physical mapping of genes, to allow analysis of large DNA fragments and detection of primary origin of genes in fungi, protozoa, bacteria and even mammals. As well as this method is used in the preparation of Yeast artificial chromosome libraries (YAC). Another advantage of this method is the transgenic mice [9].

PFGE AND CLINICAL TRIALS

Pulsed-field gel electrophoresis is used more in laboratorial researches which require special analysis of samples. This method is a valuable tool to determine the cases and source of incidence. Examples of two different researches confirm the trueness of the above-mentioned subject.

Using PFGE can determine epidemiological or genetic relationships. Separated isolates from patients, environment or animal that are epidemiologically associated, may be resulted from a common source [10]. Epidemiological studies should be considered in genetic relationship between environmental and clinical strains in each geographical area where PFGE enable ribotype all the samples. There are some methods such as Electerophoresis by which the clonal relationship of bacteria between and within the host species and also the source and transmission route of bovine *S. aureus* [10]. Gradually became widespread use of PFGE for epidemiological studies, especially to determine the prevalence of bacteria causing hospital infections. Alfizah, using PFGE, indicated that the outbreak in ICU hospital clonal expansion of a single strain of *S. marcescens* reason [11]. This can help to decide on the prevention and proper treatment. One of the important rules in research studies is to evaluate the accuracy of PFGE analysis in comparison with other methods in depicting bacterial strains. An example of these studies was about a research that analysed the presence of *Neisseria meningitidis* Serogroup C in isolated samples from four occurred outbreaks during 1993 and 1995 years in the United States of America. The obtained results by PFGE were compared with the other formerly used typing methods such as Stereotyping (using antibodies to distinguish strains) or other DNA-based methods [12]. All these methods are not cost effective for comparing prevalent strains in an outbreak. One aim of this study is to clarify the fact that whether the PFGE method is better than other methods or not. PFGE can also detect genetically related bacteria on nursing unit and hospital staff so that the appropriate precaution can be made by the study [13].

Large epidemic in which enterobacteriaceae family is the causative agent of the disease has been repeated throughout history. In a study in India, *V. cholerae* isolated from the sporadic and outbreak cases were typing by PFGE, Rep-PCR and ribotyping. PFGE revealed better than similarity and genetic relationship bitween some clinical strains isolated and environmental samples [14]. Advantage of PFGE compared to other typing methods mentioned in numerous studies [15]. Evaluation of antibiotic resistance of pathogenic strains is critical for follow the healing process and even specifies the dominant strains that are prevalent in society. Several studies performed each year on antibiotics resistance in different diseases. In one of these, *S.aureus* strains that were resistant or sensitive for methicillin gene, by PFGE methods were typing [16]. PFGE-based typing has been proved to be reliable methods for dissemination of MRSA and MSSA either in endemic or epidemic situation [17].

Using PFGE to identify genes resistant to antibiotics has increased in recent decades. Such as Carbapenemase Gene of *Acinetobacter baumannii* that cause of the spread of hospital infections, Panton-Valentine Leukocidine gene in gram positive bacteria especially in *Staphylococcus aureus* in medical centers, has provided treatment problems with the help of PGGE, can be examined the pattern of antibiotic-resistant genes [18-21].

In several studies using PFGE, this has been become possible that using different samples obtained from food, clinical samples or even isolated samples from environment (water, soil, sewage, and etc.) and well able to distinguish between different strains [21]. In 2006, in a study that Sandt was done on prevalence of transmitted *Salmonella* from foods in Pennsylvania which implies to key role of PFGE in determining bacteria multi serotype. In this study, five serotypes of *Salmonella enterica* were detected that was the agent of most transmitted infections through food in this country [22]. Separation of human pathogens of animal is another electrophoresis application [23-25]. Find different strains of pathogenic species is very important in terms of making the right decision for treatment and prevention also that is useful in clinical and epidemiology studies [26,27].

One of the recent studies in the field of study is Khosravi. That investigated the genetic diversity of separated *Mycobacterium tuberculosis* strains from patients with tuberculosis and its relationship with prevalence of the disease, using PFGE method, they found that only 15% of isolated cases genetic diversity was linked to epidemic of the disease [28]. In another study that investigated the genetic diversity pattern of *Bacillus cereus* and *Bacillus weihenstephan* in different samples [29].

Studies shows that PFGE is powerful with very high sensitivity in differentiation between involved strains in one outbreak and to investigate the possibility of bacterial outbreak [27]. Also a report based on other highlighted advantages of PFGE is as following: reproduction of products which is very important. It is good to know that factors and agent such as different sources of reactants, level of individual experience and any small deviation from policy of this method can have significant effect on laboratorial results [28]. However, following established protocols, standardized PFGE is a reliable method with the ability of repetition and secure in terms of making data relevant with each other [27]. This is a very important step that will allow different laboratories to compare their results and then it is benefited in order to identify and depicting the prevalence of various diseases. The point is that all researchers suggest PFGE as the best method and performing this method for the first time in a laboratory is difficult because it is difficult to release DNAs within the agarose plugs. But PFGE is a very accepted method that uses the characteristics of bacterial strain and diverse of giant viruses for identification and isolation [27,28,30,31].

CONCLUSION

After more than 30 years of development and introduction of PFGE, these methods are still popular in many countries and studies for molecular typing and identification of pathogens in the prevalence of certain diseases and are considered the golden standard method to identification of some bacteria. In fact PFGE methods are able to detect 90 percent of the bacterial genome and are powerful tools for monitoring genetic changes in bacteria at a global level. For example, any changes in antibiotic-resistant genes in important clinical and hospital infections bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Mycobacterium tuberculosis*, etc.) in regional and global level can cause in order to provide appropriate solutions to deal with the phenomenon of antibiotic resistance. Regardless of the high cost of materials and equipments and the time-consuming nature of this method, PFGE is still presented as a practical and applicable typing method.

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