Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 2 |APR-JUN |2013|493-495

Review Article
Biological Sciences

FUNGAL KARYOTYPING: A MINIREVIEW

Thulasi G. Pillai[1,*], Asha Chandran[2] & Anaz Muhammad K[3]

[1*, 2, 3]*Division of forest Ecology and Biodiversity Conservation, Kerala Forest Research Institute, Peechi 680 653, Kerala, India.*

*Corresponding AuthorEmail:*thulasigpilla@yahoo.co.in*

ABSTRACT

The Kingdom fungi include an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies. Little is known of their true biodiversity which has been estimated at 1.5 million species, with about 5% of these having been formally classified according to their morphology (e.g., characteristics such as spore color or microscopic features) or physiology. Advances in molecular genetics have opened the way for DNA analysis to be incorporated into taxonomy. Recent studies have shown that fungi are source of metabolites which have therapeutic potentials. Molecular karyotyping will help to better understand about the fungi. The review is on electrophoretic karyotyping of fungi (Pulse field gel electrophoresis (PFGE)).

KEY WORDS

Kingdom fungi, Molecular Genetics, DNA analysis, Pulse Field Gel Electrophoresis.

Mini Review

Though 1.5 [1] million fungi are known to exist on earth only 70,000 has been discovered so far. The fungal wealth is immense and its potentials are enormous. Among studies on diversity of fungi, much work has to be done, to explore them fully. Fungi are excellent tools in innovative biotechnology. Molecular techniques in identifying fungi, is a promising tool. The present review focus on karyotyping of fungi. Filamentous fungal genomes are relatively small and contain a remarkably consistent number of genes. Their genomes range in size from 30 to 100 Mbp and contain 10,000 to 13,000 predicted genes [1]. Their reduced complexity and small size relative to most eukaryotes makes them amenable to assessing the suitability of new sequencing technologies

Fungal nuclei are too small and only few attempts to visualise fungal chromosomes have been successful. The number of chromosomes per nucleus, estimated by conventional light visualisation and stained with standard dyes like Giemsa or aceto-orceine, usually does not exceed ten. A method developed in the late 1980s called 'germ tube burst' [2] enables the discharge of condensed chromosomes from the hyphal cell and their spread on the surface of a slide. This method, usually gives resolution of chromosomes. It was used with conventional light microscopy dyes as well as in fluorescent microscopy or for in situ hybridisation.

The idea of using the electrophoretic fractionation of intact chromosomal DNA molecules as an alternative to classical karyotyping has been explored for many years. However, efforts to apply electrophoresis to the separation of very large DNA molecules achieved only moderate success until 1982, when it was reported by Schwartz et al. [3] that molecules of several hundred kilo base pairs had strongly size-dependent mobilities when they migrated through agarose gels in the presence of two alternately applied, approximately orthogonal electrical fields. The technique is named as Pulse field gel electrophoresis (PFGE). Schwartz and Cantor have introduced a technique for releasing DNA from yeast spheroplasts that had been embedded in agarose, thereby preserving the intactness of the yeast chromosomal DNA molecules, and they also showed

that several single-copy DNA-DNA hybridization probes hybridized to specific bands that could be separated by the orthogonal-field-alternation technique [4].

Filamentous fungal genomes are relatively small and contain a remarkably consistent number of genes. Their genomes range in size from 30 to 100 Mbp and contain 10,000 to 13,000 predicted genes [5]. Their reduced complexity and small size relative to most eukaryotes makes them amenable to assessing the suitability of new sequencing technologies. This technique may be expected to provide fundamentally new information about the basic organization of the genomes of many species, since numerous members of such taxonomic groups as the fungi have proven intractable both to genetic and cytogenetic analysis. Fungal cytogenetics has been hampered by the small size of the chromosomes, the lack of known sexual stages in many medically and industrially important species, and the occurrence of endomitosis [6]. The chromosomes of most fungi, however, are small enough to be separated by using pulsed field gel electrophoresis (PFGE) [7].

Electrophoretic karyotyping of different fungi has shown that chromosome length polymorphisms (CLPs) are a common and prominent feature of these organisms. Two mechanisms have been proposed for the generation of these polymorphisms: increasing the copy numbers of particular sequences, such as the ribosomal DNA (rDNA) [8-9] and subtelomeric repeats [10,11] and mitotic and meiotic recombination processes [12-13]. In many cases, however, these CLPs seem to have minor genetic consequences, since many different karyotypes are found in a given species. For some species, it has been reported that CLPs within a given strain will eventually disappear by chromosome recombination [13]. Separation of the chromosomes on the gel enables the determination of their number, estimation of genome size, chromosome length polymorphism and the presence of supernumerary chromosomes, which are usually too small to visualise in nuclei.

PFGE has many applications that include karyotyping, strain identification of similar species, characterization of transformed strains, building of linkage maps, and preparation of DNA for genomic analysis. Electrophoretic separation of chromosomes is an empiric process in which the initial concentration of intact chromosome-sized DNA and the optimization of electrophoretic parameters are the most important experimental variables. Fungi display a surprising amount of intraspecific variation in both chromosome number and size, making it difficult to establish a standard "reference" karyotype for many species. Although PFGE is not a panacea for bringing genetics to species that lack classical genetic systems, it often does provide a way for developing a molecular linkage map in the absence of a formal genetic system. It is far faster than parasexual analysis in the discovery of linkage relationships. For genomics projects, DNA can be recovered from pulsed field gels and used to prepare chromosome-specific libraries.

Pulsed field gel electrophoresis (PFGE), or electrophoretic karyotyping, separates chromosomal-sized pieces of DNA in agarose gels where the orientation of the electric field is periodically altered. This technique has revealed that many fungi have a high degree of chromosomal length polymorphisms. Often the only isolates with identical karyotypes are derived from a single clone, thus PFGE provides a 'genetic fingerprint' for them. The size range and number of chromosomes within isolates of a particular species are usually constant, hence PFGE can distinguish between morphologically similar fungi. This technique can also be used to follow inheritance of chromosomal length polymorphisms and shows that in some fungi novel-sized chromosomes are produced during meiosis.

PFGE has advantages in accurate measurement of chromosome size, detection of minute chromosomes and analysis of karyotype polymorphisms. PFGE enables separation of fungal chromosomes upto several megabases and is a worthful tool for fungal karyotyping. This technique is based on direct current electric field that periodically alters direction and or intensity. Due to hexagonal geometry of electrode array (CHEF – contour clamped homogenous electric field) the PFGE apparatus generates a 120^0 reorientation field as well as periodical changes in the strength and the direction of electric field, allowing

large DNA molecules to migrate through the agarose matrix by a zig-zag movement. Migration of small DNA molecules in the gel matrix require relatively short pulse time in the direction of electric field, whereas relatively long pulse time enable migration of large DNA molecules. PFGE is used efficiently to determine chromosome length polymorphism and chromosome nuclear polymorphism.

As somatic chromosomes of interphase nuclei are analysed by PFGE, this method is applicable to a wide range of fungi. Owing to this new technology, karyotypes of imperfect fungi that have not been amenable to conventional karyotypic analysis are now analysable. PFGE is fairly accurate in the measurement of chromosome size and detection of small size chromosomes. Karyotyping of several fungal species have enhanced the knowledge on structural genomics in fungal biology including identification of horizontal chromosomal transfer among related fungal species.

REFERENCES

1. Hawksworth DL. (2006). "The fungal dimension of biodiversity: magnitude, significance, and conservation". Mycological Research 95 (6): 641–655.
2. Shirane N, Masuko M and Hayashi Y. (1988). Nuclear behavior and division in germinating conidia of Botrytis cinerea, Phytopathology 78, 1627-1630.
3. Schwartz, D. C., Saffran, W., Welsh, J., Haas, R.,Goldenberg, M. & Cantor, C. R. (1982) Cold Spring Harbor Symp. Quant. Biol. 47, 189-195.
4. Schwartz, D. C. & Cantor, C. R. (1984) Cell 37, 67-75.
5. Properties of Eukaryotic Genome Sequencing Projects. [http://www.ncbi.nlm.nih.gov/genomes/leuks.cgi].
6. Carle, G. F. & Olson, M. V. (1984) Nucleic Acids Res. 12, 5647-5664.
7. Mills, D., and K. McCluskey. 1990. Electrophoretic karyotypes of fungi: the new cytology. Mol. Plant-Microbe Interact. 3:351–357.
8. Pukkila, P. J., and C. Skrzynia. 1993. Frequent changes in the number of reiterated ribosomal RNA genes throughout the life cycle of the basidiomycete Coprinus cinereus. Genetics 133:203–211.
9. Sonnenberg, A. S. M., P. W. J. de Groot, P. J. Schaap, J. J. P. Baars, J. Visser, and L. J. L. D. van Griensven. 1996. Isolation of expressed sequence tags of Agaricus bisporus and their assignment to chromosomes. Appl. Environ. Microbiol. 62:4542–4547.
10. Farman, M. L., and S. A. Leong. 1995. Genetic and physical mapping of telomeres in the rice blast fungus Magnaporthe grisea. Genetics 140:479–492.
11. Louis, E. J., E. S. Naumova, A. Lee, G. Naumov, and J. E. Haber. 1994. The chromosome end in yeast: its mosaic nature and influence in recombinational dynamics. Genetics **136:**789–802.
12. Zolan, M. E. 1995. Chromosome-length polymorphism in fungi. Microbiol. Rev. 59:686–698.
13. Zolan, M. E., N. K. Heyler, and N. Y. Stassen. 1994. Inheritance of chromosome-length polymorphisms in Coprinus cinereus. Genetics 137:87–94

***Corresponding Author:**
Thulasi G. Pillai*
Division of Forest Ecology and Biodiversity Conservation, Kerala Forest Research Institute, Peechi, Thrissur-690653, Kerala, India.
E-Mail:thulasigpilla@yahoo.co.in
Tel : 91-487-2690100, Fax : 91-487-2690100