

Karyotype Analysis of *Cryptococcus Neoformans* with Pulse Electrophoresis

Xiaodong She, Weida Liu*, Shaoxi Wu, Ningru Guo

Abstract: Pulse electrophoresis was used to isolate chromosomes DNA in 20 strains of *Cryptococcus neoformans* including 3 varieties and 5 serotypes. The results showed that genomes of *Cryptococcus neoformans* were composed of 10~20 chromosomes with sizes from 0.4~2.1 Mb and full length of 15 Mb. Association of electrophoresis karyotype and serotype was found with some distinctive bands in some strains. It indicated that karyotype analysis can be used as a new genotype method to distinguish different varieties and species.

Key words: *Cryptococcus neoformans*; electrophoresis; pulsed field gel

INTRODUCTION

Cryptococcus neoformans is a yeast-like fungus with capsule. It can cause serious infections, especially in immunocompromised patients^[1,2]. In human immunodeficiency virus-seropositive individuals, an infection with *C. neoformans* indicates progression to AIDS and the incidence rate can reach 15%. So it is a major pathogen of fatal fungal infection in these patients^[3]. The traditional identification of *C. neoformans*, lacking of stability and reproducibility, was mainly based on the phenotype characters which is easily influenced by the circumstances and the antropic factors. Moreover, phenotypic method is imprecise that affected the researches on epidemiology and virulence of this fungus. Pulsed field gel electrophoresis technique developed in the late 1980s' could separate macromolecule DNA, and made a big progress in genome mapping and analyzing^[4]. We first used PFGE to separate DNA of *C. neoformans* in China and gained the fundamental data of genome structure and electrophoretic karyotype of *C. neoformans*. Finally, we investigated the possibility of using PFGE in its identification and categorization.

INFORMATION AND METHODS

The strains (Table 1) were obtained from Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (ID). The strains were maintained in the slant of SDA medium after routine phenotype identification at 40 °C.

Preparations of the whole chromosome gene were performed as described by Polacheck^[5] and Smith^[6] and adjusted reasonably.

1. After activation, colonies were picked out and inoculated in 10ml YPD (2% glucose, 2% peptone, 1% yeast extract), then cultured with vibration for 18h at 30 °C. Spores were washed in 50 mmol/L EDTA (pH7.5) and SCE (1 mol/L sorbitol, 0.1 mol/L sodium citrate, pH 5.8, 10 mmol/L EDTA) once respectively. Yeast cell

* Corresponding to Weida Liu (1957-), male, doctor tutor, professor of Institute of Dermatology, Chinese Academy of Medical Sciences; Main research field: medical fungus; Address: Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, Postcode: 210042.

concentration was adjusted to about 5×10^8 /ml.

Table 1 Strains employed in this study

Experiment serial	Serotype	Source serial
Saccharomyces cerevisia	AB972	Genetics institute of FuDan university
C. neoformans var. gattii		
6	C	NIH18
12	B	T13
C. neoformans var. neoformans		
10	A	Nanjing urease negative
11	A	ATCC 64532 urease negative
27	A	NCN-1
29	A	NCN-3
30	A	NCN-4
32	A	NCN-II
33	A	NCN-III
34	A	NCN-I
7	D	NIH B3501
8	D	NIH 52
9	AD	IFM-5889
15	AD	II-4b
16	AD	I-3c
17	AD	II-4c
25	AD	CH 92
26	AD	CH 6
C. neoformans var. Shanghai		
CH	B	CH var.
SH	B	SH 8012

2. To obtain protoplasts, cells were treated with 6~8 mg/ml lysing enzymes Novozym234 (Novo, Biolabs) at 37 °C for 1 h, 1 ml protoplasm suspension was extracted and mixed with 1 ml 1.5% low melting point agarose (BRL) (first dissolved in 0.125 mol/ml EDTA, pH 7.5), then the mixture was added into holes of pre-cooled tooting and coagulated for 30 min.

3. Solidified gels were incubated for 2 h at 60 °C in 3 ml 0.25 mol/L EDTA (pH, 7.5) containing 1% SDS, then the gels were placed into 3 ml ESP (0.5 mmol/L, pH 9.0, 1% (Fluka), 0.5 mg/ml protease K (GIBCO)) for 24 h at 50 °C. After being washed, the gels were conserving in 0.5 mol/L EDTA pH 9.0 for later use.

Pulse electrophoresis: CHEF system (CHEF-DR II, Bio-Rad) was adopted. Electrophoresis buffer was $0.5 \times$ TBE, with which 1% electrophoresis gels were prepared. Temperature was about 10 °C. Application of sample was 1/2 gel block (containing about 4 µg DNA). Conditions of electrophoresis were composed by different parameters in 4 grades: (1) voltage: 110V, pulse time: 5 min, electrophoresis time: 20 h; (2) 90V, 10 min, 20 h; (3) 70V, 3 min, 10 h. Gels were stained with EB for 15min after electrophoresis; then, were rinsed with double distilled water for 1 h, finally observed and photographed under the ultraviolet lamp.

RESULTS

Fig.1 showed electrophoresis results. From the figures we could see the biggest chromosome of *C. neoformans* was similar to that of *saccharomyces cerevisia* AB972 strain (2.2 Mb). Total 10~12 chromosome bands were separated; chromosome sizes varied from 2.1 to 0.41 Mb. The whole length of genome chromosome was about 12~15.7 Mb.

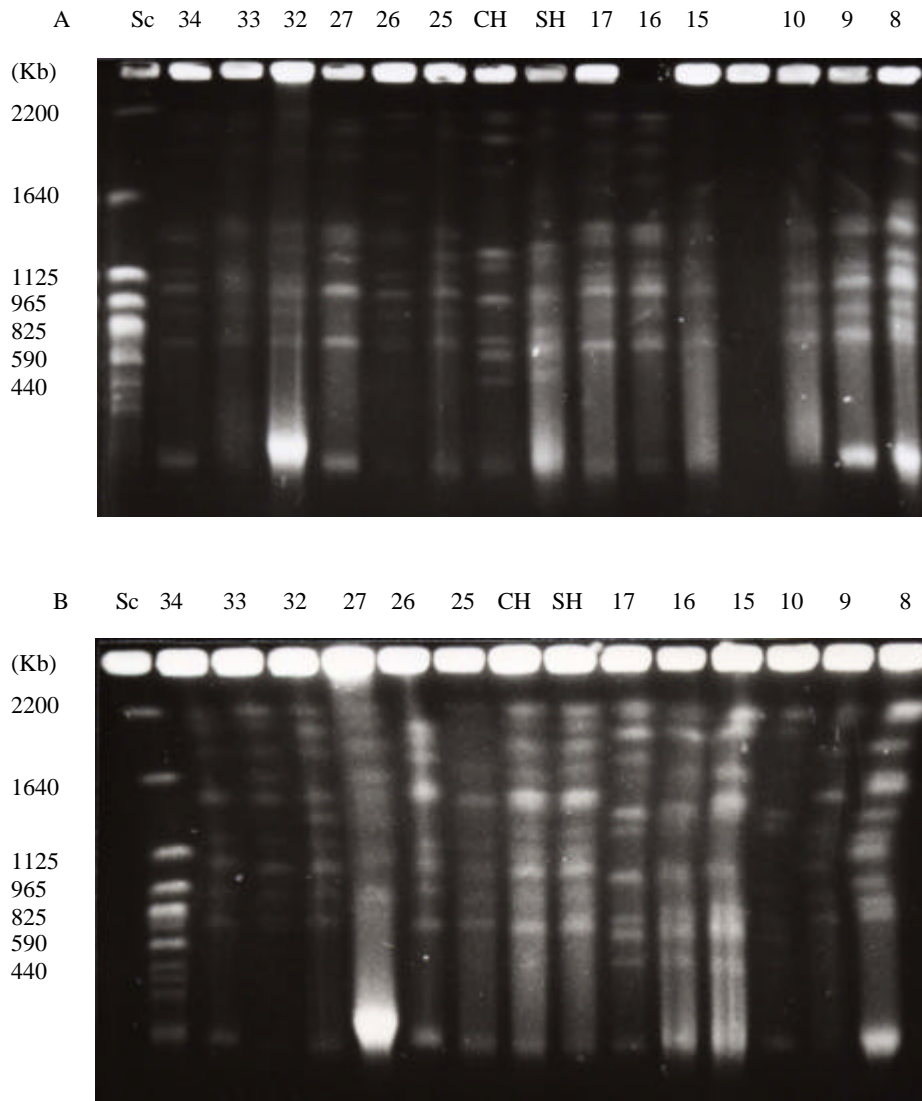


Fig. 1 PFGE of *C. neoformans* var. *gattii* and *C. neoformans* var. *neoformans* and *C. neoformans* var. *Shanghai* strains (A and B). The experiment serials are indicated above the lanes. Sc, *Saccharomyces cerevisia* AB972 strain; kb, kilobase pair.

Previous studies of the electrophoretic karyotype of *C. neoformans* have shown extensive genetic variation in chromosome number and size [7-9] which representing the different varieties of *C. neoformans* [10, 11]. Similar to their findings, we could find that all strains had the different band forms from our results; namely, they were generally polymorphism. But there were still some significant characteristic distributions. In the variety level, an obvious distinction was showed between the strains of *C. neoformans* var. *neoformans* and var. *gattii* strains. An additional chromosome band less than 500 kb was seen in the all strains of *C. neoformans* var. *gattii* and var. *Shanghai*. As for different serotypes, type A, B and D all had 10 bands, type AD had 12 bands, otherwise type C and *C. neoformans* var. *Shanghai* strains had 11 bands. Especially, electrophoresis band forms of different strains belonging to one serotype were very similar which indicated that EK correlates with serotype.

In addition, from the figures we found, every chromosome was roughly separated in electrophoresis conditions of 4 grades, and band forms were clear. Although there was slight DNA degradation, it didn't affect the

analysis.

DISCUSSION

The major difference between pulse electrophoresis and common agarose gel electrophoresis is that the former had two electric fields in different directions. During the presetting pulse time, two electric fields change directions alternately which makes big DNA molecules change their shapes and migratory routes. So the big DNA molecules were separated by getting out of the way of small gel pores. Many factors could influence this course, voltage and pulse time were the most important factors. Generally, separating bigger DNA fragments need lower voltage and longer pulse time. In this experiment we designed a set of electrophoresis conditions composed by different parameters in 4 groups to aim directly at different chromosomes in genome of *C. neoformans*. In result, it made each chromosome separated satisfactorily. Polacheck et al. changed gel electrophoresis using right angle field to separate *C. neoformans* chromosome, but they couldn't observe a DNA band less than 580 kb of *C. neoformans* var. *gattii* and a DNA band less than 700 kb of *C. neoformans* var. *neoformans* ^[5]. With our set of electrophoresis conditions, we could separate these bands well. Chromosome number and genome size of Perfect's research results by using CHEF system agreed with that of ours, but they couldn't find serotype C also had a 500 kb small chromosome ^[12]. Further more, comparing with photographs in those papers, our results had more merits such as DNA linear migration, clear band forms and little degradation.

We found there was a good relationship between EK and serotype. Strains belonging to one serotype had similar band forms, but the differences in geographic distribution and host distribution of *C. neoformans* were in rule ^[13]. In addition, EK distributions of 3 varieties had some rules, *C. neoformans* var. *gattii* and Shanghai strains each had a 450kb small chromosome, but *C. neoformans* var. *neoformans* didn't. However, *C. neoformans* var. Shanghai and var. *gattii* also had some difference in band form. *C. neoformans* var. Shanghai had an extra 1700kb DNA band than type B of *C. neoformans* var. *gattii*, and its total band number equal with that of type C but band form had difference. Either these differences attributed to polymorphism in genus or reflected no relations in germ line origin; we need combine phenotype character with gene feature to determine it. In addition, there was no obvious difference in band forms of 2 urease negative strains (type A) and other type A strains which indicated that variations of urease negative didn't reflect in karyotype variations. At last, we noticed that there was chromosome length polymorphism in each strain, but couldn't find identical electrophoresis band form. These differences in gene form had well repeatability and stability; which indicated that EK could be used as an epidemiology mark in finely describing strains ^[14].

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