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Short communication

Electrophoretic karyotype of environmental isolates of *Paracoccidioides brasiliensis*

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Five of the 12 environmental isolates of the dimorphic fungus *Paracoccidioides brasiliensis* known to date, were analysed by contour-clamped homogeneous electric field gel electrophoresis (CHEF). The electrophoretic pattern was shown to consist of five bands, with molecular size ranging from 3·2 to 10 Mb, a model quite similar to the one found in the clinical isolates previously tested and used here as controls. However, one of the bands in the environmental isolates had a lesser weight (7·2 Mb), than the one corresponding to the clinical counterparts (8·8 Mb). This resulted in a smaller genome, approximately 29·7 Mb. The small differences that were found indicate the presence of chromosome polymorphism in this fungus.

Keywords *Paracoccidioides brasiliensis*, karyotype, environmental isolates

Introduction

Paracoccidioides brasiliensis is a thermally dimorphic fungus and the pathogen responsible for paracoccidioidomycosis, an important systemic mycosis in Latin America [1].

Until recently, it was thought that man was the only host susceptible to *P. brasiliensis*; however, several studies have shown that the nine band armadillo (*Dasypus novemcinctus*), also becomes infected with the fungus, as demonstrated by organ histology and isolation of the fungus in culture [2,3]. Additionally, *P. brasiliensis* has been isolated from the faeces of an Antarctic penguin [4] and the chow consumed by a dog with gastrointestinal problems [5]. Other non-human cultures are represented by isolates from soils collected in coffee farms in Brazil and Venezuela [6,7]. The isolates corresponding to the first infected armadillo [8] and the penguin faeces [9,10], have been analysed by various mycological and immnulogical methods and found to correspond to human isolates of *P. brasiliensis*.

Pulse-field gel electrophoresis (PFGE) as well as some of its modifications [11], have allowed characterization of large DNA molecules from fungi and other microorganisms previously non-amenable to cytogenetic methods [12].

Few studies have been conducted to determine the karyotype of *P. brasiliensis*. In a previous work [13], chromosomal DNA molecules from eight clinical isolates of this fungus were analysed. Under the conditions specified, five bands were visualized in seven of the patients' isolates tested; one of the isolates had four bands. Chromosomal bands were in the range of 3·2 to 10 Mb and the genoma size was approximately 30 Mb. There were, however, two different patterns in the five-band group. Recently two clinical isolates of *P. brasiliensis*, B-339 and 113, were analysed by PFGE. The electrophoretic conditions employed in this study allowed separation of four chromosomal bands in the range of 2 to 10 Mb and the haploid genome size was estimated to be in the range of 23–27·6 Mb [14].

The above studies indicate that there are variations in *P. brasiliensis* electrophoretic patterns [13,14]. Compari-

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sons among such patterns, could furnish important information concerning genetic relatedness and epidemiological trends of the fungus. This would help to define *P. brasiliensis* behavior in its various hosts and habitats.

In the present work we have analysed five of the 12 known non-human *P. brasiliensis* isolates with the aim of comparing their electrophoretic patterns.

Materials and methods

The five non-humans isolates of *P. brasiliensis* were identified on the basis of their macro- and microscopical aspects, as well as on their thermal dimorphism; they were maintained by periodic subculturing in slanted tubes with the modified synthetic medium of McVeigh–Morton (MMcM) as described previously by our group [15].

Intact DNA was prepared from the five environmental isolates (Table 1) and also, from four clinical isolates that served as controls; these controls were from the collection of the Corporación para Investigaciones Biológicas (Medellín, Colombia), and are designated 11762, 29068, 24243, and 24527. The intact DNA molecules were obtained as described earlier [13].

DNA extraction was performed during the early stages of the yeast to mycelial transformation process when germ tubes were being produced as enzyme treatment was indicated to be more effective at this particular growth stage (Dr Garry T. Cole, Medical College Ohio, USA; personal communication, 1993). The optimal electrophoretic conditions employed in this study were similar to the ones used by Montoya *et al.* [13], as follows: 1·4 V cm, switching at 60 min for 68 h with circulating buffer and temperature maintained at 10–12 °C.

Determination of the band's molecular size was based on the genomic DNA migration of the control employed (*Schizosaccharomyces pombe*), which was run in the same gel. This control DNA was obtained in our laboratory and checked against a commercial control (Megabase III DNA standard, cat. no. 5629SA GIBCO BRL, Grand Island, NY, USA). Bands of molecular size larger than those of the control were determined by linear regression analysis and the total genome size was calculated by adding the molecular weights of each band.

Results

All environmental isolates presented five bands. In comparison with the clinical isolates there were four common bands (3·2, 4·1, 5·2 and 10 Mb) but the 8·8 Mb in the clinical isolates was replaced by a 7·2 Mb in the environmental cultures. Fig. 1 illustrates the band pattern in four of the isolates tested.

The whole genome for the environmental isolates was estimated to be 29·7 in comparison with 31·3 Mb in the clinical isolates.

Discussion

As previously reported [13], the electrophoretic pattern of the clinical isolates, revealed the presence of five identical chromosomal DNA molecules, with individual chromosomes showing a molecular size ranging form 3·2 to 10 Mb. These data imply that, in general, at least 30 Mb of the *P. brasiliensis* clinical isolates genome are organized as chromosomal macromolecules.

The five environmental isolates studied closely resembled the clinical isolates. There were five large-sized chromosomal bands of similar molecular constitution. However, there was a slight difference between the 5·2 (clinical) and 7·2 Mb (environmental) band. This difference altered the genome's molecular mass, from 31·3 to 29·7 Mb, respectively. It is unlikely that this small difference could change the species position of the non-human isolates some of which have already been defined by other criteria (mycological, immunological) as identical to *P. brasiliensis* patient isolates [8–10].

In a previous report, Nogueira *et al.* [14] presented differences in the electrophoretic mobilities of bands in their isolates of *P. brasiliensis*, as compared with our previous publication [13], although this variation can also be explained by the difference in the electrophoresis techniques used, PFGE vs. CHEF, both reports indicate that chromosome polymorphism does exist in *P. brasiliensis*.

Table 1 Environmental isolates analysed

Isolate No.	Country of Origin	Source	Year reported	Author
1	Venezuela	Soil (Paracotos)	1971	Albornoz *et al.*
2	Brazil	Armadillo	1986	Naiff *et al.**
3	Uruguay	Penguin faeces	1989	Gezuele
4	Brazil	Dogfood	1990	Ferreira *et al.*
5	Brazil	Soil (IBIA)	1998	Silva *et al.*

* Kindly donated by Dr R. P.Mendes, Botucatu, Brazil.

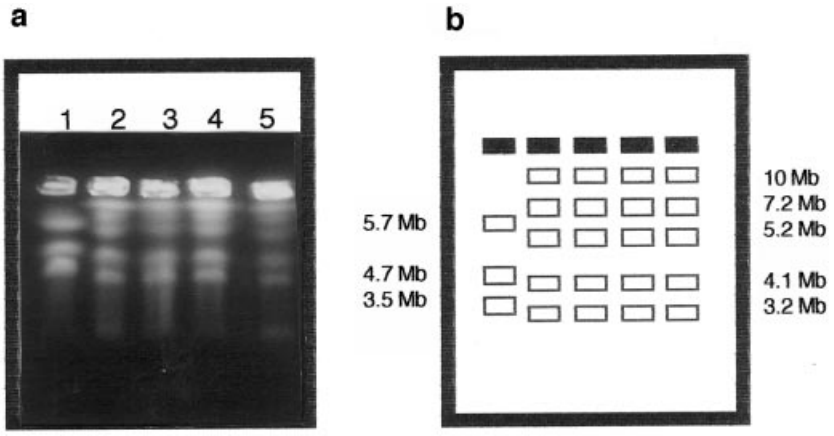

Fig. 1 (a) Electrophoretic karyotype. Lane 1 *S. pombe*, lane 2 soil isolate [7], lane 3 Armadillo [2], lane 4 dogfood [5], lane 5 penguin faeces [4]. (b) Computer diagram representing the electrophoretic pattern.

For some fungi it as been demonstrated that isolates of the same species have a similar although not identical, chromosomal band pattern [16]. This study confirms that the difference observed between individual bands corresponds to a polymorphism along the length of the chromosomes; such polymorphism could be due to genetic translocations, deletions or rearrangements during growth, as indicated for *Candida albicans* and other fungi [16].

Chromosomal aberrations and rearrangements may play an important role in the genetic variability and evolution of the different isolates. Similar structural aberrations may have caused the small difference observed in the patterns of the clinical and non-clinical isolates of *P. brasiliensis* [13,14].

The karyotype of *P. brasiliensis* is the starting point in the study of genomic organization of this pathogen and, together with other molecular procedures, such as blotting for the assignment of specific genes, restriction analysis of individual chromosomes and production of chromosomes-specific libraries, [17] may contribute to further define the genetic, taxonomic and epidemiological characteristics of *P. brasiliensis*.

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