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Short Report

Genetic Diversity of the Internal Transcribed Spacers (ITS) and 5.8S rRNA Genes among the Clinical Isolates of *Candida parapsilosis* in Brazil and Japan

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Abstract

The internal transcribed spacer (ITS) region including 5.8S rDNA sequences of 58 isolates of *Candida parapsilosis* in Brazil and Japan was analyzed. Although most of the *C. parapsilosis* strains tested were confirmed to belong to three already reported genetically distinct groups (I, II and III) based on their ITS region sequences, 5 strains of the Brazilian isolates showed different sequences from those heretofore reported and suggested a presence of new genotype. For these strains of *C. parapsilosis*, we proposed a new genetic group (IV). The sequence similarities of this new group of IV to I, II and III were 87.4%, 94.7% and 87.3% in the ITS1 region, respectively. Genetic diversity in ITS regions of the remaining *C. parapsilosis* strains in Brazil and Japan was also discussed.

Key words: *Candida parapsilosis*, clinical isolates, Brazil, Japan, ITS region, genetic diversity

Introduction

Candida parapsilosis is a frequent cause of opportunistic infection, associated with high morbidity and mortality rates in hospitalized immunocompromised patients[1, 2]. Lin *et al.*[2] reported the presence of three genetically distinct groups (genetic groups I, II and III) for *C. parapsilosis* among clinical isolates in USA on the basis of their internally transcribed spacer (ITS) region sequences. Identification of a common source of infection and determination of genetic relatedness among the strains involved in outbreaks are important for infection control[2-5].

In this paper, we describe ITS region sequences of 58 clinical isolates of *C. parapsilosis* in Brazil and Japan, which demonstrate the presence of a new genetic group as well as genetic diversity based on the ITS region sequence information. The sequence of the D1 and D2 domains of the large-subunit ribosomal DNA region[6] of the representative genetic groups was also analyzed and used to confirm the presence of this new group (genetic group IV, G IV).

C. parapsilosis strains: 24 and 34 isolates from clinical specimens in Brazil and Japan, respectively, were used. These strains were isolated during the years of 1996 and 2001 in Brazil and Japan: isolated hospitals were Campinas University Hospital and Sao Paulo University Hospital in Brazil, and Juntendo University Hospital and Tokyo University Hospital in Japan. All of them were isolated from blood samples. These clinical isolates were identified as *C. parapsilosis* in each regional hospital on the basis of their cultural characteristic of colony on CHROMagar *Candida* (Kanto Kagaku Co., Tokyo, Japan). The fungus was also confirmed to be *C. parapsilosis* by the slide agglutination

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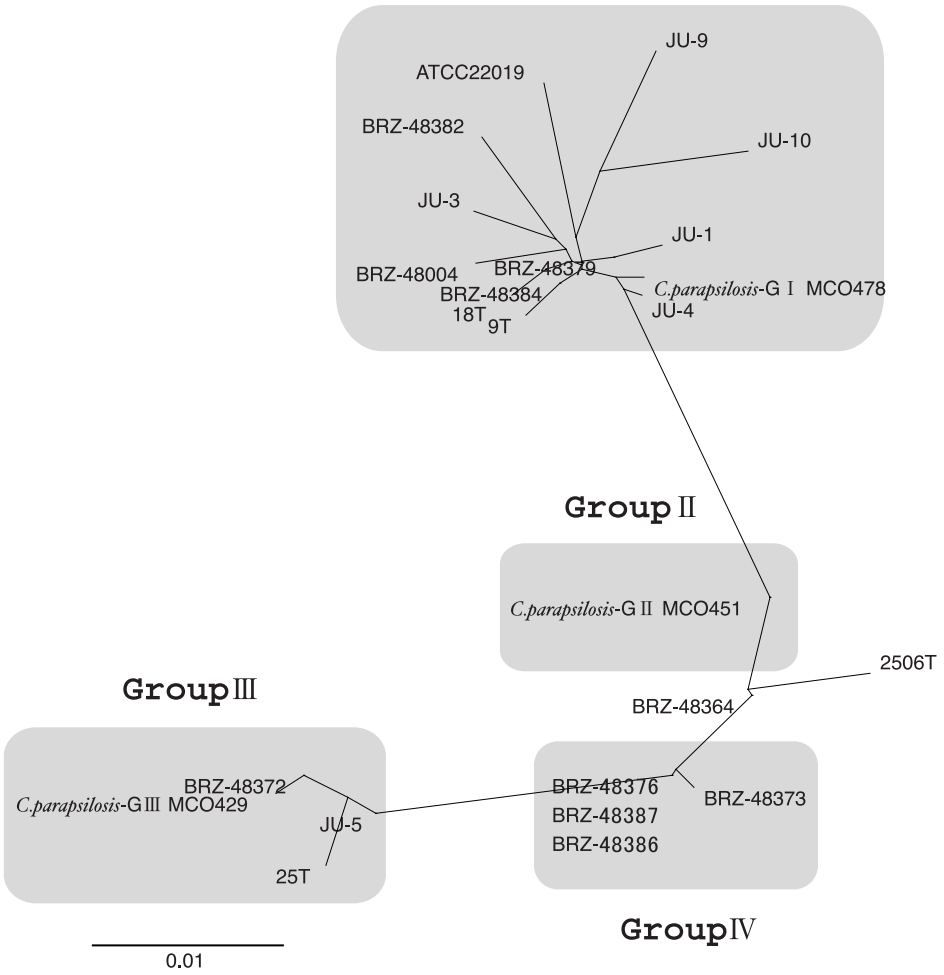

Fig. 1. The phylogenetic relationship among 45 strains of *C. parapsilosis* based on the aligned sites of ITS1-5.8S-ITS2 region sequence. Sequences in the box show the one conserved in each genetic group. Representative strains of each group are included. The new group IV is composed of four strains of Brazilian isolates of *C. parapsilosis* (BRZ-48376, BRZ-48373, BRZ-48387 and BRZ-48386). The tree was constructed using the neighbor-joining method.

test (IATRON Candida Check, Iatron Co., Tokyo). DNA extraction was carried out by the procedure described by Tamura *et al.*[7] using GPT reagent (6 M guanidine thiocyanate in 50 mM Tris [pH 8.3]) and Tris (pH 8.0)-buffered phenol solution. The DNA concentration was adjusted to 5 μg/m*l*.

DNA sequencing of ITS1-5.8S-ITS2 region and D1/D2 domains of 26S rDNA were the same as those described by Tamura *et al.*[7], and Kurtzman and Robnett[6], respectively. The PCR products were sequenced directly with a Big Dye terminator reagent kit with *Taq* polymerase and by the protocol recommended by the manufacturer of the model 310 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Neighbor-joining (NJ) trees were generated using Kimura's two parameter methods[8, 9]. The confidence values of branches were determined by a bootstrap analysis[10].

Results and Discussion

The ITS region sequences including 5.8S rRNA gene of 58 isolates from Brazil (24 strains) and Japan (34 strains) were determined, and their information was deposited in the DNA data bank of Japan (DDBJ) as accession numbers from AB109223 to AB109236, and from AB109248 to AB109292. All strains (58 isolates) showed identical sequences in their 5.8S rRNA gene. The ITS1 region in the majority strains was from 1 to 157 bp long, while the ITS 2 was from 316 to 470 bp long, and 5.8S rDNA was from 158 to 315 bp long in most of the strains tested, respectively. However, a diversity of ITS region sequences was observed in the remaining strains, and the ITS region length in the longest group was 481 bp and the shortest was 458.

Fig. 1 shows the molecular phylogenetic relationship among the 58 strains of *C.*

parapsilosis based on ITS region sequence. The trees were constructed using the neighbor-joining (NJ) and maximum parsimony (MP) methods. The scale indicates one base change per 200 nucleotide positions. Three reference strains were confirmed to be classified into group I (G I, *C. parapsilosis* MCO478), II (G II, *C. parapsilosis* MC0451) and III (G III, *C. parapsilosis* MCO429) as reported by Lin *et al.*[2].

When the phylogenetic position of the present 58 strains was studied, 49 strains including 18 Brazilian and 31 Japanese isolates of *C. parapsilosis* were classified in group I. The type strain of *C. parapsilosis* ATCC 22019 belonged to this group. In addition to these 49 strains, 4 strains of 1 Brazilian (BRZ-48384) and 3 Japanese isolates (JU-9, JU-1 and JU-10) were considered to belong to this group although the sequences of these 4 strains were slightly different from the reference group I. These studies suggested that the majority (about 85%) of *C. parapsilosis* strains isolated from Brazil and Japan belong to this group (G I).

No *C. parapsilosis* strains which could be classified into group II, were not observed among the present 58 isolates. One Brazilian strain (BRZ-48372) and two Japanese strains (25T and JU-5) were considered to belong to group III despite showing genetic diversity in their ITS region sequences because such diversities were viewed as a range-difference within a genetic group.

Four Brazilian isolates (BRZ-48376, BRZ-48387, BRZ-48386 and BRZ-48373) were found to belong to a different phylogenetic group, and tentatively designated as group IV (G IV, Fig. 1). These strains were differentiated from other groups by their sequence similarity. The sequence similarity of this new group of IV to I, II and III was 87.4, 94.7 and 87.3% in ITS1 region, and 98.1, 98.7 and 97.5% in total ITS2 region, respectively. Based on this information, we proposed a new group IV for these strains of *C. parapsilosis* (Fig. 1). The tree created using maximum likelihood analysis supported the tree derived from ITS region sequences prepared by the neighbor-joining method[11].

Two isolates, one each from Brazil (BRZ-48364) and Japan (2506T) could not be clearly classified into any group. However, since the sequences of both isolates showed similarities to both group II and IV, and the number of these strains were small, they were tentatively classified into an intermediate group. Coupled with the increase of the *C. parapsilosis* numbers used, these strains may coalesce to group II or IV.

Thus, although the 58 isolates from Brazil and Japan were classified into 4 genetic groups, the 2 above strains (BRZ-48364 and 2506T) were tentatively grouped into an intermediate one. In addition, there were some strains which were unreasonably classified into the 4 groups and showed genetic diversity within each group. The present studies confirmed the extensive diversity of ITS region sequences in *C. parapsilosis* strains.

In these sequences, each group of strains showed a characteristic sequence, and it was easily separable from other groups using the sequence information shown in Fig. 2. The new group IV strains of *C. parapsilosis* (strain numbers BRZ-48376, BRZ-48373, BRZ-48387 and BRZ-48386) were easily separable using the combined information of sequences in the boxes suggested by the arrow in Fig. 2. Genetic heterogeneity in the ITS region of *C. parapsilosis* has been reported in the strains isolated in USA[2]. Such heterogeneity was also confirmed by Roy and Meyer[12] using the DNA relatedness and restriction fragment length polymorphisms patterns of whole-cell DNA, and they demonstrated three genetic groups. Genetic diversity in ITS regions was also confirmed in the isolates from Japan as well as Brazil by the present experiment.

Molecular phylogenetic analysis data based on D1/D2 region analysis of the genetic group IV (BRZ-48386 [AB199908], BRZ-48373 [AB199909], BRZ-48387 [AB199907] and BRZ-48376 [AB-199906]) also supported the separation of genetic group IV from other genetic groups due to ITS region information (data not shown).

Da Silva *et al.*[13] reported that *C. parapsilosis* is an increasingly important bloodstream pathogen in neonatal intensive care units (NICU), and they concluded that nosocomial transmission occurred and that neonates under intensive care may represent a risk group for this pathogen. The grouping on the ITS sequences in *C. parapsilosis* reported in this experiment could be used to trace the locus of an infection due to this fungus.

During the revision of our present paper, we knew that Tavanti *et al.*[14] had proposed two new species, *Candida orthopsilosis* and *Candida metapsilosis*, which replace the exiting designation of *C. parapsilosis* groups II and III, respectively. Therefore, further detailed molecular and taxonomic studies on *C. parapsilosis* group IV designated in this manuscript are expected to lead to a proposal of a third new

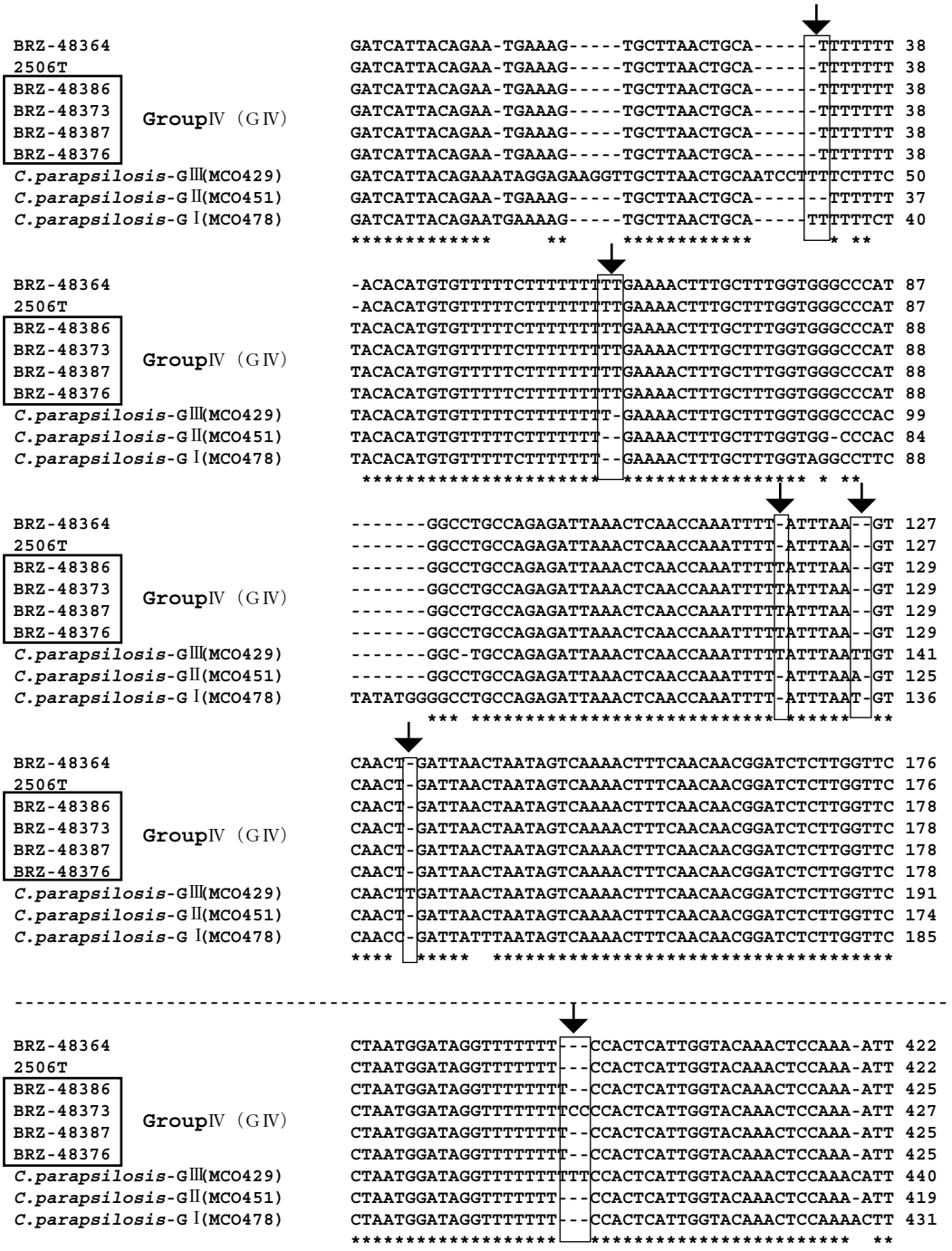

Fig. 2. ITS1 and ITS2 region aligned sequences of representative group strains (group I: *C. parapsilosis* MCO478, group II: *C. parapsilosis* MCO451, and group III: *C. parapsilosis* MCO429), 4 strains of each group IV (BRZ-48376, BRZ-48373, BRZ-48387 and BRZ-48386), and 2 strains of an intermediate group (BRZ-48364, and 2506T).
Asterisks are used when the nucleotide at a particular position is identical in all strains. Dashes represent deletions necessary for alignment.

species to replace this *C. parapsilosis* genetic group (G IV).

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