Chromosome-Sized DNA of Malassezia pachydermatis by Pulsed-Field Gel Electrophoresis
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ABSTRACT. The genome of Malassezia pachydermatis isolates from dogs was resolved into six chromosomes by using pulsed-field gel electrophoresis and their molecular sizes were calculated as 820, 1,100, 1,400, 1,470, 1,660 and 1,820 Kb, respectively. Comparison of electrophoretic patterns suggested that the chromosomes of M. pachydermatis were homozygous. Key words: chromosome-sized DNA, M. pachydermatis.


The genus Malassezia has been classified into the Cryptococceae and includes two species, Malassezia furfur (M. furfur) and Malassezia pachydermatis (M. pachydermatis) [1]. M. pachydermatis has been isolated from men, dogs, cats, pigs, camels, horses, cattle, elephants and black bears [2, 3, 5, 6, 9, 10].

Our knowledge on the genome of the genus Malassezia is poor, and few results about the G+C content and DNA-DNA reassociation experiments on Malassezia were reported [4]. Using pulsed-field gel electrophoresis we examined the numbers and sizes of chromosomal DNA in the genome of M. pachydermatis isolated from dogs.

Various strains of M. pachydermatis were grown to stationary phase (48–72 hr) with shaking in 100 ml of YPD (YPD: 1% yeast extract, 2% dextrose and 2% bacteropeptone) at 37°C. Chromosomal DNA was prepared by lysing cells suspended in solid agarose inserts. Cells were harvested by centrifugation and washed twice with TE buffer (10 mM Tris-Cl, pH 7.6, and 50 mM EDTA). 0.5 ml of 1% low melting temperature agarose (InCert™, FMC. Inc.) in 50 mM EDTA (pH 7.5) was added to 0.5 ml (0.5 × 10⁹ cells), followed by the addition of 7.5 µl of Zymolyase 100T (Kiriin Breweries, 2 mg per ml of 10 mM sodium phosphate containing 50% glycerol). The mixture was held at 42°C and poured into the insert mold. After the inserts have solidified, those inserts were added to LET buffer (500 mM EDTA, 10 mM Tris-Cl, pH 7.6), plus 1% 2-mercaptoethanol. Inserts were incubated overnight at 37°C and then incubated in ESP solution (0.5 mM EDTA, pH 9, 1% sodium lauroyl sarcosinate, and 1 mg

![Fig. 1. Separation of chromosomal DNAs of M. pachydermatis. The Saccharomyces cerevisiae YNN295 was used as a reference (lane 1). The samples were prepared from M. pachydermatis strains AV1 (lane 2), AV2 (lane 3), AV3 (lane 4), AV7 (lane 5), AV8 (lane 6), AV9 (lane 7), AV10 (lane 8), AV11 (lane 9), AV12 (lane 10), AV14 (lane 11), AV15/16 (lane 12), AV48 (lane 13), 84264 (lane 14), IFO-0995 (lane 15), CBS-1879 (lane 16).](image-url)

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per ml of Proteinase K) at 50°C for 48 hr. The blocks were washed twice with TE buffer containing 1 mM phenylmethylsulfonylfluoride (PMSF) at 50°C for 60 min and were washed two more times under the same conditions without PMSF.

Agarose blocks were cut in quarters and placed in the slots (1.5 mm thick and 5.0 mm width) of 1.3% agarose gel (SeaKem GTG agarose, FMC, Inc.).

Electrophoresis of the intact DNA was performed on the CFGE (Closed-Field Gradient Gel Electrophoresis) apparatus (ATTO, Japan) that is the modification of the RGE (Rotary Gel Electrophoresis) with the following parameters: 100 V, 5-min pulse time, 8°C buffer, 60-hr run time. The buffer was 0.05 M TBE (1 M TBE is 1 M Tris-base, 1 M boric acid and 20 mM EDTA). DNA was visualized after soaking gels in 0.5 µg of ethidium bromide per ml of distilled water. These conditions have given good electrophoretic resolution throughout the gel for *M. pachydermatis*.

The genome of *M. pachydermatis* was resolved into six chromosomes and their molecular sizes were calculated as 820, 1,100, 1,400, 1,470, 1,660 and 1,820 Kb, respectively (Fig. 1).

It was assumed that *Candida albicans* (*C. albicans*) has 8 chromosomes with haploid form [7, 8]. But when the chromosomal DNA of the diploid form of *C. albicans* was electrophoresed by pulsed-field gel electrophoresis, from 8 to 16 chromosomal bands appeared depending on whether it was a heterozygote or not. Asakura *et al.* [1] reported that the chromosomal DNA of a clinically isolated *C. albicans* was resolved into 7 to 12 bands and their molecular sizes were from 0.42 Mb to 3.0 Mb.

Since there were no variations in numbers and sizes of chromosomal DNA bands of *M. pachydermatis*, we assumed that their chromosomes were homozygous. The numbers and sizes of chromosomal DNA bands of *M. furfur* by pulsed-field gel electrophoresis were different from those of *M. pachydermatis* (data not shown). Moreover, *M. pachydermatis* is known to be different by 11% in the G+C content, suggesting considerable divergence from *M. furfur* [4].

We should make further studies of the genus *Malassezia* from the aspect of molecular biology.

REFERENCES