Note

Separation of Chromosomal DNA Molecules of *Candida maltosa* on Agarose Gels Using the OFAGE Technique

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Candida maltosa can assimilate *n*-alkanes, and one of the aims of our experiments using this yeast has been to elucidate the molecular mechanisms underlying regulation of the expression of the genes involved in *n*-alkane assimilation. We have established host-vector systems in this yeast for the purpose of isolating these genes.^{1,2)} If we could clone these genes, one of the next steps would be to determine the locations of these genes on the chromosomes of *C. maltosa*. Recently, a technique called orthogonal field alternation gel electrophoresis (OFAGE) has been developed to separate large pieces of DNA molecules.^{3,4)} The present paper describes the results of ex-

periments performed to determine the conditions for the application of the OFAGE technique to the separation of chromosomal DNA molecules of *C. maltosa*, and to examine the possibility of assigning a gene of interest to a specific chromosomal band on a gel through molecular hybridization.

The strains used were *C. maltosa* IAM12247⁵⁾ and *S. cerevisiae* AH22.⁶⁾ Chromosomal DNA was prepared by the procedure of Schwartz and Cantor,⁷⁾ modified by Carle and Olson.⁴⁾ The OFAGE apparatus we constructed has a similar structure to that described by Carle and Olson.³⁾ During a run for 20~26 hr, the current was maintained at 200 mA, and the temperature in the electrophoresis tank was kept between 16~24°C. For a DNA-DNA hybridization experiment, the gel was soaked in 0.25 M HCl for 15 min before blotting to increase the DNA transfer efficiency. The following DNA probes derived from the *C. maltosa* genome were labeled with ³²P by the nick translation method⁸⁾: an ARS sequence which replicates autonomously in both *C. maltosa* and *S. cerevisiae* (a 3.8 kb *Bam*HI fragment from pBR-TRA¹⁾), the *C-LEU2* gene (a 3.0 kb *Bam*HI-*Xho*I fragment of pCMK303^{9,10)}), which complements the *leu2* mutation of *S. cerevisiae*, and the *C-HIS5* gene (a 2.3 kb *Sal*I fragment of pCMH10, unpublished data), which complements the *his5* mutation of *S. cerevisiae*.

Figure 1 shows the electrophoretic patterns of DNA from *C. maltosa* IAM12247, and from *S. cerevisiae* AH22 (haploid) as a reference. Under the conditions where good separation of the chromosomes of *S. cerevisiae* is obtained (Fig. 1A), six distinct bands (designated as bands-1 to -6, in order of increasing size) were observed in the case of *C. maltosa*. Furthermore, by de-

FIG. 1. Electrophoretic Patterns of Chromosomal DNA Molecules from *C. maltosa* and *S. cerevisiae*.

Chromosomal DNA molecules of *C. maltosa* (Cm) and *S. cerevisiae* (Sc) were separated under three different sets of conditions. The agarose concentration in the gel and the switching interval of the current in (A), (B) and (C) were 1.5% and 90 sec, 0.8% and 60 sec, and 0.3% and 45 sec, respectively. The designated band-numbers are shown on the left of the photographs.

FIG. 2. Estimation of the Molecular Weights of Chromosomal DNA Molecules from *C. maltosa* with a Calibration Curve Constructed Using Those for *S. cerevisiae* as a Standard.

A calibration curve was constructed to estimate the molecular weights of chromosomal DNA molecules from the migration distance in a gel using chromosomal DNA molecules of *S. cerevisiae*³⁾ as a standard (O). Then, the molecular weights of chromosomal DNA molecules of *C. maltosa* were estimated ($\leftarrow$).

creasing both the gel concentration and the switching interval for electrophoresis, two new bands, called bands-7 and -8, were detected in addition to the above six bands, although the bands of lower molecular weights were broad, as can be seen in Fig. 1B and 1C. The brightness of the original well for *C. maltosa* in Fig. 1C was about the same as that for *S. cerevisiae*, strongly suggesting that the number of chromosomes in *C. maltosa* is eight. However, as this brightness might also be affected by the efficiency of protoplast formation, we have concluded that the number of chromosomes in this yeast strain is eight plus a few. That the average sizes of chromosomes are higher while the chromosome numbers are lower in *Candida* yeasts than in *S. cerevisiae* has been reported by de Jonge *et al.*,¹¹⁾ and Magee *et al.*,¹²⁾ in agreement with the results in Fig. 1.

We tentatively calculated the sizes of eight chromosomal DNA molecules of *C. maltosa* in the eight bands to be as shown in Fig. 2. The total genome size was calculated to be larger than 14,000 kb (sum of the molecular weights of the eight bands, assuming that those of bands-6, -7 and -8 are 2,000 kb), and probably smaller than 17,000 kb (sum of the molecular weights of the eight bands, assuming that those of bands-6, -7 and -8 are 3,000 kb), which is approximately the same as the genome size of *S. cerevisiae* (haploid type). We have previously suggested¹³⁾

FIG. 3. DNA-DNA Hybridization with the ARS Sequence as a Probe.

The conditions for electrophoresis were the same as in Fig. 1(A). After electrophoresis (A), DNA-DNA hybridization was carried out with the ³²P-labeled ARS sequence as a probe (B).

that *C. maltosa* may have the same DNA content per cell as that of a diploid strain of *S. cerevisiae*. Although the exact genome size in *C. maltosa* cannot be determined from the data in Fig. 2, it is possible to speculate that *C. maltosa* may have a diploidic or a aneuploidic karyotype, and that a pair of chromosomes may be contained in some of the bands on the gel.

A DNA-DNA hybridization experiment was performed to assign each of the three isolated genes to a specific chromosomal band on the gel. The results using the ARS sequence as a probe are shown in Fig. 3. The strongest signal was observed for band-2, and there is another signal for band-5. In our previous study,¹⁾ we showed, using the ARS sequence as a probe, that there are at least two sequences in the *C. maltosa* genome hybridizable to this sequence, the ARS sequence itself and a highly homologous sequence. So, it is suggested that the ARS sequence is located in band-2, and the highly homologous sequence is located in band-5. The same kind of experiment indicated that the *C-LEU2* gene is located in band-4, and the *C-HIS5* gene in band-5 (data not shown). It is concluded that we will be able to assign any gene of interest to a specific chromosomal band of *C. maltosa* under the experimental conditions described in this paper, if we can clone such a gene.

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