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Chromosome Polymorphisms among Strains of *Hansenula polymorpha* (syn. *Pichia angusta*)

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Contour-clamped homogeneous electrophoresis and an embedded-agarose method of sample preparation were combined to carry out an analysis of the chromosome sets of nine strains of *Hansenula polymorpha* (syn. *Pichia angusta*). Chromosomal DNA molecules could be separated into a series of bands ranging, approximately, from 650 up to 2,200 kb in size. Polymorphism of the electrophoretic pattern was demonstrated among the strains investigated in this study. Cross-hybridization between *H. polymorpha* and *Saccharomyces cerevisiae* ribosomal DNA was also observed.

The advent of pulsed-field gel electrophoresis technology for separating high-molecular-weight DNA molecules has provided a novel means of characterizing the chromosome sets of lower eukaryotes (3, 22). Fundamentally new information about the basic organization of the genomes of many species, including *Schizosaccharomyces pombe*, *Candida albicans*, *Neurospora crassa*, and *Cryptococcus neoformans*, has been published (7, 14, 16–18).

Hansenula polymorpha (syn. *Pichia angusta*) (12) is a methylotrophic yeast assuming a primary role as a host for high-level expression of heterologous genes (9). At present, little information is available regarding the genomic structure and organization of this yeast species (10, 11). In 1986 De Jonge et al. (6), using orthogonal-field-alternation gel electrophoresis, observed three distinct chromosomal mobility groups in one strain of *H. polymorpha*, all of which were larger than 1,000 kb. In this work we show that with pulsed-field gel electrophoresis modified for contour-clamped homogeneous field gel electrophoresis (5), chromosomal DNA from *H. polymorpha* strains can be separated into two to six distinct bands ranging from 650 to 2,200 kb in size.

Chromosome-size DNA molecules were prepared from the following *H. polymorpha* strains: ATCC 14754, ATCC 14755, ATCC 18208, ATCC 26012, ATCC 34438, ATCC 58401, ATCC 64209, ATCC 66057, and LR9 (19). Intact chromosomal DNAs were prepared by growing *Hansenula* strains in yeast extract-peptone-glucose medium (4) for 14 to 18 h at 30°C with shaking. Growth conditions were such to maintain the yeast as a stable haploid (10). Cells (10 ml) were harvested by centrifugation, washed twice with TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), suspended in 4 ml of TE, and transferred to a 50-ml round-bottom flask. Embedding of cells in the agarose matrix and the lysis procedure were as described by Carle and Olson (4). Soon after lysis, agarose microbeads were prepared for digestion as described by Sambrook et al. (21). DNA preparations showed significant degradation of the large DNA molecules within 2 weeks of storage at 4°C even though they were stored in 0.5 M EDTA. Restriction endonucleases were obtained from Boehringer (Mannheim, Germany). Enzymatic reactions were performed according to the supplier's instructions.

Electrophoresis was performed in the 2015 PULSAPHOR apparatus (LKB-Pharmacia Biotechnology, Uppsala, Sweden). Samples were electrophoresed through 1% (wt/vol) agarose gels in 0.5× TAE buffer (21) at 9°C. Electrophoresis of chromosomes was carried out for 15 h with a 60-s pulse time and then for 9 h with a 90-s pulse time at 13 V/cm. Electrophoresis of chromosomal DNA digests was carried out for 20 h with a 30-s pulse time at 10 V/cm. Gels were stained with ethidium bromide (0.5 μg/ml) for 20 min, destained in distilled water, and photographed under UV light. Chromosomes and restriction fragment sizes were determined from their mobilities by using *Saccharomyces cerevisiae* YNN295 chromosomes (Bio-Rad Laboratories, Richmond, Calif.) and phage lambda concatemers (CLONTECH Laboratories, Inc., Palo Alto, Calif.) as reference molecular weight markers. Southern blot hybridizations were carried out by standard methods (21). The 2.4-kb *Eco*RI fragment of 25S ribosomal DNA (rDNA) of *S. cerevisiae* (23) was used as a probe for localization of the rDNA genes in *H. polymorpha* chromosomes. A 5.5-kb *Pvu*II fragment from pYAC3, which includes the *CEN4* sequence of *S. cerevisiae* (2), was also used as a probe. Probes were labeled with ^{32}P by the random-primer method (8) using a commercial kit (Boehringer). [α-^{32}P]dCTP (specific activity, 800 Ci/mmol, 10 μCi/μl) was purchased from Amersham (Amersham, Little Chalfont, United Kingdom).

The best resolution of chromosome-size DNA molecules was obtained by applying electrophoresis conditions that adequately separated *S. cerevisiae* chromosomes. As shown in Fig. 1a and 2, the number of separable bands for *H. polymorpha* ranged from two to six. Their sizes, compared with the known sizes of *S. cerevisiae* chromosomes, were estimated to range between 650 and 2,200 kb. Three groups of chromosomal bands could be resolved: bottom bands (650 to 900 kb in size, present only in two strains), middle bands (1,000 to 1,600 kb in size, present in nearly all strains examined), and top bands (>1,600 kb in size, present in all the strains examined) (Fig. 2).

When the chromosomes separated according to the preceding conditions were blotted and probed with the *S. cerevisiae* rDNA probe, hybridization signals corresponding to bands with the following approximate sizes were observed: 1,100 kb for ATCC 26012, ATCC 34438, ATCC 58401, ATCC 64209, and ATCC 66057; 1,300 and 1,600 kb for ATCC 14754; 1,900 kb for ATCC 18208; 1,300 kb for

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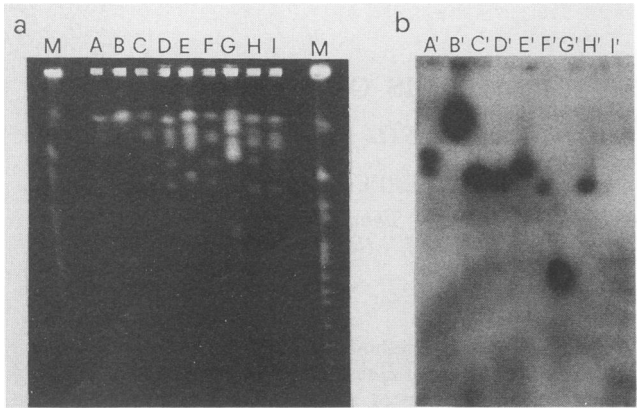

FIG. 1. (a) Ethidium bromide-stained gel of chromosome-size DNA fragments from *H. polymorpha* strains. Lanes: A, ATCC 14754; B, ATCC 18208; C, ATCC 26012; D, ATCC 34438; E, LR9; F, ATCC 58401; G, ATCC 14755; H, ATCC 64209; I, ATCC 66059; M, molecular weight markers (*S. cerevisiae* YNN295 chromosomes). (b) Autoradiograph of a Southern blot of the gel in panel a probed with the 2.4-kb *Eco*RI fragment of *S. cerevisiae* 25S rDNA. The absence of the rDNA signal for strain 66057 (lane I') was due to poor blotting.

strain LR9; and 800 kb for ATCC 14755 (Fig. 1b and 2). The fact that two hybridization signals were observed for the chromosome which carries rDNA genes when separating the haploid genome of ATCC 14754 (Fig. 1b, lane A') suggests the presence of two homologs and subsequently aneuploid conditions. Similarly, the overly strong hybridization signals observed for strains ATCC 18208 and ATCC 64209 (Fig. 1b, lanes B' and G') could be due to the presence of more than one homolog carrying rDNA comigrating together. Comigration of more than one chromosome (either homologous or nonhomologous) could also be the cause of the unusual brightness and thickness of some bands (Fig. 1a, lanes B and G) and explain the abnormally small number of bands present in the electrokaryotype of ATCC 18208 (Fig. 1a, lane B).

No hybridization signal was observed when a DNA fragment of pYAC3 containing the *S. cerevisiae CEN4* sequence was used as a probe (data not shown).

Analysis of chromosomes from three *Hansenula* strains (ATCC 26012, ATCC 58401, and LR9) yielded fragments ranging from a few kilobases up to about 500 kb in size after cleavage by the rare cutting enzymes *Not*I and *Sfi*I (13). *Not*I generated at least two fragments greater than 485 kb in the ATCC 26012 and ATCC 58401 and four in the LR9 strain, while *Sfi*I produced only one fragment greater than 485 kb in ATCC 26012 and two in LR9 (data not shown). In *Not*I and *Sfi*I chromosomal digests, only one band for each of the three strains was apparently recognized by the rDNA probe (the band size ranged from 150 to about 500 kb), indicating that neither enzyme cut regularly in the rDNA unit (data not shown).

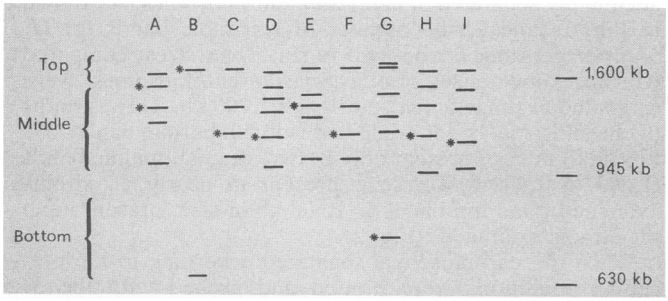

FIG. 2. Schematic representation of the electrokaryotypes of *H. polymorpha* strains. Lanes: A, ATCC 14754; B, ATCC 18208; C, ATCC 26012; D, ATCC 34438; E, LR9; F, ATCC 58401; G, ATCC 14755; H, ATCC 64209; I, ATCC 66059. The asterisks denote the bands which hybridized with the rDNA probe.

Analysis of *H. polymorpha* strains by contour-clamped homogeneous field gel electrophoresis gives better resolution with respect to previous work (6) and shows up to six mobility groups of DNA molecules. By this technique, it was also possible to establish a molecular weight range for the chromosome-size DNA molecules resolved. As compared with *S. cerevisiae* with chromosomes ranging from 260 to 2,200 kb (15, 22), *C. albicans* with chromosomes ranging from 1,000 to 5,000 kb (14), and *Schizosaccharomyces pombe* with chromosomes ranging from 3,000 to 9,000 kb (7), *H. polymorpha* chromosomes apparently range from about 650 to 2,200 kb. The exact number of *H. polymorpha* chromosomes remains to be determined, since it cannot simply be inferred from the number of bands. Apart from comigrating chromosomal DNA molecules, very large or unusually folded molecules that do not enter the gel might escape detection (14). Moreover, because only one linkage group has so far been described for *H. polymorpha* (11), it is not possible yet to establish, by using appropriate probes, a relationship between the electrokaryotype and classical genetic analysis. It should be noted that great electrokaryotype variability was observed among the strains investigated in this study, notwithstanding the fact that they all are ascribed to the same species. Similar observations have also been reported for other yeast species (1, 20). Since *H. polymorpha* is capable of mating (11), it would be interesting to know whether viable meiotic progenies are obtained from crosses of the strains with diverse electrokaryotypes. For this purpose, generation of auxotrophic mutants from some of the investigated strains is currently in progress.

In *H. polymorpha* the rDNA showed different localizations in the nine genomes studied, suggesting that chromosomal changes could involve, as it happens in *C. albicans* (20), the chromosome which carries rDNA genes.

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REFERENCES

1. **Bidenne, C., and F. Vezinhet.** 1990. Reliability of electrophoretic karyotyping to identify industrial yeast strains, abstr. D-136. Abstr. 6th Int. Symp. Genet. Ind. Microorganisms GIM 1990.
2. **Burke, D. T., F. G. Carle, and M. V. Olson.** 1987. Cloning of large segments of exogeneous DNA into yeast by means of artificial chromosome vectors. Science **236:**806–811.
3. **Carle, G. F., and M. V. Olson.** 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA **82:**3756–3760.
4. **Carle, G. F., and M. V. Olson.** 1987. Orthogonal-field-alternation gel electrophoresis. Methods Enzymol. **155:**468–482.
5. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:**1582–1585.
6. **De Jonge, P., F. C. M. De Jongh, R. Meijers, H. Y. Steensma, and W. A. Scheffers.** 1986. Orthogonal-field-alternation-gel electrophoresis banding patterns of DNA from yeasts. Yeast **2:**193–204.
7. **Erard, M., D. Barker, and J. Green.** 1987. Is chromosome

condensation a phylogenetic marker?, p. 267–277. *In* G. S. de Hoog, M. T. Smith, and A. C. M. Wejman (ed.), The expanding realm of yeast like fungi. Elsevier Science Publishing, Inc., Amsterdam.
8. **Feinberg, A. P., and B. Volgelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
9. **Gellissen, G., A. J. Zbigniew, A. Merckelbach, M. Piontek, P. Keup, U. Weydemann, C. P. Hollenberg, and A. W. M. Strasser.** 1991. Heterologous gene expression in *Hansenula polymorpha*: efficient secretion of glucoamylase. Bio/Technology **9:**291–295.
10. **Gleeson, M. A., and P. E. Sudbery.** 1988. The methylotrophic yeasts. Yeast **4:**1–15.
11. **Gleeson, M. A., and P. E. Sudbery.** 1988. Genetic analysis in the methylotrophic yeast *Hansenula polymorpha*. Yeast **4:**293–303.
12. **Kurtzman, C. P.** 1984. Synonomy of the yeast genera *Hansenula* and *Pichia* demonstrated through comparisons of deoxyribonucleic acid relatedness. Antonie van Leeuwenhoek **50:**209–217.
13. **Levine, J. D., and C. L. Cech.** 1989. Low frequency restriction enzymes in pulsed field electrophoresis. Bio/Technology **7:**1033–1036.
14. **Mahrous, M., T. J. Lott, S. A. Meyer, A. D. Sawant, and D. G. Ahearn.** 1990. Electrophoretic karyotyping of typical and atypical *Candida albicans*. J. Clin. Microbiol. **28:**876–881.
15. **Mortimer, R. K., and D. Schild.** 1985. Genetic map of *Saccharomyces cerevisiae*, edition 9. Microbiol. Rev. **49:**181–213.
16. **Orbach, M. J., D. Vollrath, R. W. Davis, and C. Yanofsky.** 1988. An electrophoretic karyotype of *Neurospora crassa*. Mol. Cell. Biol. **8:**1469–1473.
17. **Perfect, J. R., B. B. Magee, and P. T. Magee.** 1989. Separation of chromosomes of *Cryptococcus neoformans* by pulsed field gel electrophoresis. Infect. Immun. **57:**2624–2627.
18. **Polacheck, I., and G. A. Lebens.** 1989. Electrophoretic karyotype of the pathogenic yeast *Cryptococcus neoformans*. J. Gen. Microbiol. **135:**65–71.
19. **Roggenkamp, R., H. Hansen, M. Eckart, Z. Janowicz, and C. P. Hollenberg.** 1986. Transformation of the methylotrophic yeast *Hansenula polymorpha* by autonomous replication and integration vectors. Mol. Gen. Genet. **202:**302–308.
20. **Rustchenko-Bulgac, E. P.** 1991. Variations of *Candida albicans* electrophoretic karyotypes. J. Bacteriol. **173:**6586–6596.
21. **Sambrook, J., E. F. Fritsh, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37:**67–75.
23. **Veldman, G. M., J. Klootwijk, V. C. H. F. de Regt, and R. J. Planta.** 1981. The primary and secondary structure of yeast 26S rRNA. Nucleic Acids Res. **9:**6935–6952.