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Electrophoretic Karotypes of Wine Strains of Saccharomyces cerevisiae

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Abstract

Chromosomal profiles of wine strains of *Saccharomyces cerevisiae* were compared to those of a standard laboratory karyotype. Qualitative analysis supported previous genetic findings that two of the wine strains are aneuploid and two diploid. Obstacles to direct and meaningful quantitation of the electrophoretic profiles are discussed.

Methods

Yeast Strains

The homothallic wine yeasts UCD Enology 505 (California Champagne), UCD Enology 595 (Pasteur Champagne), UCD Enology 522 (Montrachet), and 522X[14] have been described.[1] 2407-1a (a haploid isogenic to X2180-1A), 2407 (diploid), and 2794 (triploid) are wildtype heterothallic laboratory strains.

Pulsed-Field Gel Electrophoresis

The electrophoretic apparatus (CHEF-DR® II cell, Bio-Rad Laboratories) has been described.[6] Standard conditions were a 30-hour run (23 hours with a 60 second switching interval followed by 7 hours with 100 second intervals) at 200 volts, 1.8 liters of 0.5X TBE buffer, 65 ml of 1% agarose (Ultra Pure DNA Grade, Bio-Rad Laboratories) cast in a 5" x 5.5" mold, and a buffer temperature maintained at 12° C by constant recycling through a cooling bath. Samples of cell-containing agarose beads, 35 µl, were loaded into wells which were subsequently sealed by addition of a few drops of molten 1% Low Melt Preparative Grade Agarose (Bio-Rad Laboratories). Gels were stained in used buffer containing 0.5 µg/ml ethidium bromide for 30 minutes and destained overnight.

Cell Preparation

Cells were prepared by the agarose bead encapsulation method[11] with minor modifications. A 200 ml YEPD culture was grown at 30° C to an $O.D._{600}$ of 1.0 and centrifuged at 400–500 x g for 5 minutes at 4° C. Following removal of the supernatant, cells were resuspended in 10 ml of SE (75 mM NaCl, 25 mM Na_2 EDTA, pH 8.0), recentrifuged, washed twice, and resuspended in 4 ml SE. In a 45° C water bath, cells were mixed with 5 ml 1% low melt agarose (Bio-Rad Laboratories) in SE, prior to addition of 20 ml temperature-equilibrated mineral oil (Squibb). The resultant emulsion was mixed vigorously for 30 seconds before it was quickly poured into a beaker—kept in an ice bath—containing 100 ml of ice-cold SE and a stir bar stirring at medium speed. The mixture was stirred for several minutes before being transferred to several graduated polypropylene tubes which were centrifuged as before at room temperature. The mineral oil was removed and any beads not pelleted were dispersed by repeated pipeting with a large-bore pipette.

After recentrifugation and removal of excess SE and beads that did not pellet, the remaining pellets were combined into a single tube and centrifuged. The supernatant was removed, the inside of the tube wiped with a tissue to remove excess mineral oil, the volume brought to 20 ml with 25 mM Na_2EDTA and the final SDS concentration to 1%. After repeated pipeting with a wide-bore pipette to break up clumps, the tube was taped to a platform shaker and shaken for 10 minutes at room temperature. Following recentrifugation and removal of the supernatant, 0.5 ml of 2-mercaptoethanol and 10 mg of zymolyase-100T (Seikagaku Kogyo Co.) were added, the volume brought to 10 ml in SE, and the beads incubated at 37 °C for 2 to 24 hours. Following incubation, the beads were pelleted as before, resuspended in 20 ml of 25 mM Na_2EDTA pH 8.0, containing 1% sarkosyl (Na salt of N-lauroylsarcosine, Sigma) and 50 µg/ml proteinase K (Boehringer Mannheim), and incubated overnight at 50 °C. The next day they were centrifuged and washed several times in 20 ml aliquots of 50 to 150 mM EDTA, pH 7.5 to 8.0, before being stored as a slurry (approximately 10 ml) in the EDTA solution at 4 °C for as long as 2 years.

The following substitutions[9] have since produced identical results: SDS instead of sarkosyl; 1 mg/ml pronase E (Sigma P 6911) at 37 °C for 2 to 24 hours instead of 50 µg/ml proteinase K overnight at 50 °C; stationary instead of log phase cultures; use of cells from individual colonies from a YEPD plate (0.25 ml agarose per colony); and a scaled-down but analogous cell work-up with microfuge tubes and 5-second microcentrifuge spins instead of 50 ml tubes and 5-minute low-speed spins.

Results

Electrophoretic Karyotype of the Strains

The karyotype (Figure 1) of the laboratory strains (lanes A, B, G, and H) is virtually identical to that previously described[3], and hence the identities of the bands are also presumed to be the same. In the profile of the UCD 522A (lane C), band 1 migrates faster than the laboratory counterpart and although band 4 corresponds well to band 3 (chromosome III) in the laboratory strains, an additional faster migrating band is also present. The doublet corresponding to band 5 in the laboratory strains migrates faster, as does the UCD 522A version of laboratory band 7. Laboratory band 11, which is a doublet, is partially resolved in UCD 522A. The laboratory form of band 12 (chromosome IV) is not present. Instead, a faster-

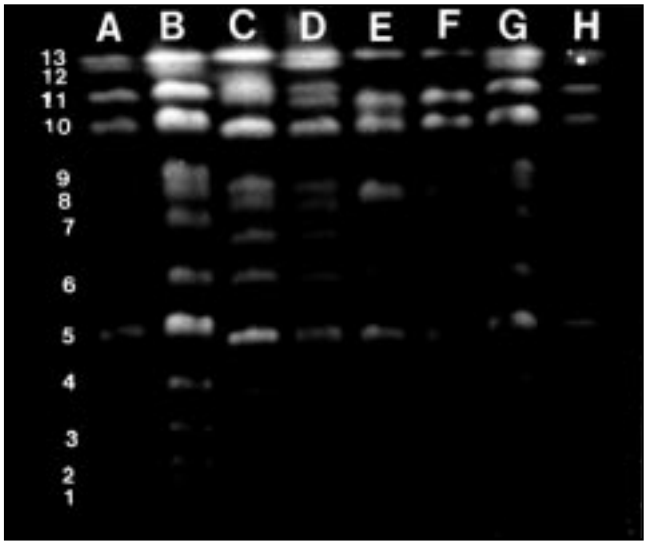

Fig.1. Electrophoretic profiles of laboratory and wine strains. Lanes A and H, 2407-la; B, 2407; C, UCD 522A; D, 522XA; E, UCD 505K; F, UCD 595A; G, 2794.

migrating band is seen which just merges with the partially resolved doublet corresponding to laboratory band 11. The karyotype of the genetically purified and presumably homozygous clone 522XA (lane D) has a different profile than its progenitor, UCD 522A. The band migrating just ahead of laboratory band 3 is absent while laboratory doublet band 11, partially resolved in UCD 522A, is completely resolved.

The profile of UCD 505K (lane E) indicated the presence of laboratory band 3, the faster migrating band seen in the UCD 522A profile, and a unique band migrating between laboratory bands 4 and 5. The faster migrating form of laboratory band 7 seen in UCD 522A and 522XA has a diffuse counterpart in UCD 505K profile. Laboratory bands 10, 11, and 13 have approximate counterparts in UCD 505K, but band 12 is absent.

The profile of UCD 595A (lane F) indicated a very faint counterpart to laboratory band 1, perhaps three merged bands migrating between laboratory bands 2 and 3, and a partially resolved doublet corresponding to laboratory band 5. Laboratory bands 6 through 9 have diffuse and approximate counterparts in this wine strain which may indicate the presence of additional and unresolved chromosomes. As in UCD 505K, laboratory bands 10, 11, and 13 have counterparts in this strain, while band 12 is absent. Instead, a faint band migrates slightly more slowly than the UCD 595A form of laboratory band 11.

While the electrophoretic profiles of UCD 522A, UCD 505K, and UCD 595A provide qualitative evidence for aneuploidy, a more useful quantitative analysis leading to a direct determination of chromosome copy number based on densitometric scanning of an ethidium bromide-stained gel or an X-ray film of a probed blot is complicated by at least four factors: 1) the difficulty of loading DNA samples corresponding to equivalent numbers of cells per strain; 2) variability in chromosome length observed for otherwise apparently homologous chromosomes; 3) the possibility of translocations or deletions which interfere with band identification if probes containing the rearranged sequences are used; and 4) the possibility of sequence heterology of functionally homologous genes resulting in differential hybridization. This has been observed for probes derived from laboratory strains of *S. cerevisiae* used in hybridizations with a distilling strain,[7] lager strains, [4, 8, 12, 10, 13] and a lager strain in which both *S. cerevisiae*- and *S. carlsbergensis*- derived probes were used.[5]

Conclusions

While a simple and direct electrophoretic analysis of genetically complex industrial strains of *S. cerevisiae* provides useful qualitative data concerning their chromosomal constitutions, a more valuable quantitative analysis that might indicate directly the number of copies of each chromosome requires further development. Minimal requirements include inclusion of an internal standard such as an artifical chromosome[2] carrying a marker conferring a known additive phenotype, identification of bands having no laboratory strain counterparts, and a means of interpreting putative doublet bands.

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