APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2003, p. 2161–2165 Vol. 69, No. 4
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.4.2161–2165.2003

Karyotype Rearrangements in a Wine Yeast Strain by *rad52*-Dependent and *rad52*-Independent Mechanisms

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Received 2 August 2002/Accepted 18 January 2003

Yeast strains isolated from the wild may undergo karyotype changes during vegetative growth, a characteristic that compromises their utility in genetic improvement projects for industrial purposes. Karyotype instability is a dominant trait, segregating among meiotic derivatives as if it depended upon only a few genetic elements. We show that disrupting the *RAD52* gene in a hypervariable strain partially stabilizes its karyotype. Specifically, *RAD52* disruption eliminated recombination at telomeric and subtelomeric sequences, had no influence on ribosomal DNA rearrangement rates, and reduced to 30% the rate of changes in chromosomal size. Thus, there are at least three mechanisms related to karyotype instability in wild yeast strains, two of them not requiring *RAD52*-mediated homologous recombination. When utilized for a standard sparkling-wine second fermentation, Δ*rad52* strains retained the enological properties of the parental strain, specifically its vigorous fermentation capability. These data increase our understanding of the mechanisms of karyotype instability in yeast strains isolated from the wild and illustrate the feasibility and limitations of genetic remediation to increase the suitability of natural strains for industrial processes.

Karyotype instability during vegetative growth is common in many naturally occurring yeast strains (4, 5, 10, 13, 18, 21). This phenotype can be monitored only by examining karyotypes of large numbers of clones isolated after several generations of vegetative growth. There is no good model for this phenotype in standard laboratory strains, for which many genetic tools are applicable.

We previously analyzed karyotype instability in strain DC5 (4). This karyotypically unstable strain produced karyotypically stable meiotic products with high frequency. From these results we inferred that karyotype instability might be governed by relatively few genetic elements and that it might be possible to stabilize the karyotype of unstable strains by disrupting one, or more, of the genes involved.

Mitotic and meiotic karyotype variations in natural and industrial yeast strains have been related to chromosomal translocations due to ectopic recombination between homologous sequences interspersed in the yeast genome, such as Ty elements, delta elements, or Y′ elements (5, 18, 21). A direct prediction of this model is that chromosomal rearrangements require a functional *RAD52* gene for homologous recombination (16). Our objectives in the present study were (i) to obtain and characterize a Δ*rad52* derivative of an unstable yeast strain to determine the role of homologous recombination in karyotype variability during vegetative growth and (ii) to determine whether the fermentation abilities of the disrupted strain have been altered.

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MATERIALS AND METHODS

Culture medium and conditions. All strains were grown in YEPS medium (5 g of yeast extract/liter, 20 g of sucrose/liter, 10 g of peptone/liter; Pronadisa, Madrid, Spain) and were incubated at 30°C with continuous shaking (250 rpm). YPD plates contained 5 g of yeast extract/liter, 20 g of glucose/liter, 10 g of peptone/liter, and 20 g of Bacto agar (Pronadisa)/liter.

Serial cultures. Strain DC5 was isolated and characterized among a collection of wine yeast strains from El Penedès, located 50 km southwest of Barcelona, Spain (4, 14, 13). Serial cultures were grown in 2 ml of YEPS at 30°C in 15-ml culture tubes. After 24 h of culture in a roller, cultures reached near saturation (optical density at 600 nm [OD_{600}] of >10) and were used to inoculate fresh tubes to an OD_{600} of 0.05. The growth and subculturing process was repeated until these serial cultures completed 100 doublings (ca. 10 to 15 transfers). A sample from the last culture was spread on a YPD plate and incubated at 30°C for 2 days. At least nine clones were picked from each plate, grown in YPD, and stored at −80°C after the addition of 50% glycerol. The frozen stocks were used for all further analyses.

Karyotype analysis. Yeast cells from late-exponential-phase cultures were embedded in low-melting-point agarose (Pronadisa). The resulting plugs were incubated first with Lyticase (Sigma, St. Louis, Mo.) and then with proteinase K (Sigma) to digest both yeast wall and yeast proteins, as previously described (7). Yeast chromosomes were separated by pulsed-field gel electrophoresis in a Hula-Gel apparatus (Hoefer Instruments, San Francisco, Calif.) at 200 V by using a pulse ramp from 60 to 150 s for 50 h in ×0.5 TBE buffer (100 mM Tris-hydroxymethylaminomethane borate, 5 mM EDTA; pH 8.4) at 12°C.

Calculation of rearrangement rates. The rate of chromosomal rearrangements per generation R was calculated from the fraction of clones showing a karyotype pattern identical to the input strain after 100 doublings (P_i) according to the following formula (4): $R = 1 - P_i^{0.01}$.

Statistical analyses. Significance tests between assays were performed as 2×2 contingency tables. Significance values were calculated by the χ^2 function with 1 degree of freedom.

PCR protocols. DNA sequences for the *kan*^{r} gene and *nat1* (nourseothricin *N*-acetyltransferase) genes, conferring resistance to Geneticin and nourseothricin, respectively, were amplified by PCR from plasmids pFA6-kanMX4 (23) and pAG25 (natMX4) (9), respectively, by using the following primers (*RAD52* sequences are capitalized): RAD52Δ-up (5′-GAAGTTGCAGCCTTAGCTGTAACAAAGGTgcataggccactagtggatctg-3′) and RAD52Δ-lo (5′-TAGGACCTGAGTATATCTCCAAGAGAGTTGGGTTTGGAcagctgaagcttcgtacgc-3′).

A *nat1*-disrupted endogenous *rad52* locus from a transformed yeast strain was reamplified with the following primers: rad52b-up (5′-TTACGCGACCGGTATCGA-3′) and rad52b-lo (5′-TATTTGTTTCGGCCAGGAAG-3′).

PCR conditions. PCR was performed with 1 U of DyNazyme Ext DNA polymerase (Finnzymes, Espoo, Finland), either 0.1 ng of DNA (plasmids) or 10 ng of genomic DNA, and 10 pmol of each primer. After an initial denaturation step at 5 min for 94°C, primers were annealed for 1 min at 48°C and extension was allowed to proceed for either 1.5 min (disruption cassette) or 3 min (disrupted genomic fragment from the heterozygote) at 72°C. After redenaturation for 1 min at 94°C, the cycle was repeated 30 times.

Yeast transformation. Strain DC5 was transformed with the different PCR products by the lithium acetate method (8, 19), with minor modifications. Yeast transformants were selected in YPD plates containing 200 mg of Geneticin (Sigma) or 100 mg of clonNat (Hans-Knöll Institute für Naturstoff Forschung, Jena, Germany)/liter. Double transformants were isolated on plates containing both antibiotics.

DNA isolation. DNA was extracted as previously described (20) with some modifications. A dense culture was washed in 50 mM EDTA (pH 7.5) and treated with Lyticase (1 mg/ml; Sigma) and RNase A (20 mg/ml; Sigma) for 1 h at 37°C. After centrifugation (15,000 × *g*, 1 min), the cell pellet was resuspended in 800 μl of lysis buffer (50 mM Tris HCl, 10 mM EDTA, 2% sodium dodecyl sulfate; pH 8.0). Upon addition of 150 μl of 5 M potassium acetate at pH 4.8, the cells were placed on ice for 1 h and pelleted by centrifugation at 15,000 × *g* for 15 min. The supernatant was extracted with phenol three times, once with phenol-chloroform-isoamyl alcohol (25:24:1), precipitated with two volumes of ethanol at −20°C for 30 min, and air dried.

Southern blot. Purified DNA was resuspended in TE and digested with appropriate enzymes. DNA fragments were separated in 0.8% agarose-TBE-gel electrophoresis, denatured, and blotted onto Hybond-N+ filters (Amersham Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. The *RAD52* probe was obtained by amplification of DNA of the laboratory strain W303a (from the Yeast Stock Center, American Type Culture Collection, Manassas, Va.) with the primers rad52b-up and rad52b-lo. The Y′ probe was obtained from plasmid pEL42H10+7 4.8 (11). Both probes were labeled with fluorescein-12-UTP (Roche, Mannheim, Germany) by the random primer protocol (Ready-to-Go; Amersham Pharmacia). Prehybridization was performed in 50% formamide–0.25 M sodium phosphate buffer (pH 7.2)–7% sodium dodecyl sulfate–1 mM EDTA–50 μg of salmon sperm DNA (Sigma)/ml at 42°C for 4 h. Hybridization was performed at 42°C overnight in the prehybridization solution plus the labeled DNA probe. The fluorescein-labeled probe was detected by an alkaline phosphatase-linked antibody (Fluorx-AP; Tropix, Bedford, Mass.), according to the manufacturer's instructions, by using CDP-Star (Boehringer, Mannheim, Germany) in 0.1 M diethanolamine (pH 10)–1 mM $MgCl_2$ as a chemiluminiscent substrate. Chemiluminiscence was recorded by exposing Kodak X-Omat AR (Kodak, Ltd., London, United Kingdom) films for 2 to 15 min, at room temperature.

Experimental fermentations. Yeast strains were propagated in heat-treated grape juice (15 min at 110°C) and then adapted and grown in the base wine, according to standard procedures (pied de cup [1]). Yeast growth was followed by turbidimetry (Hach ratio and xr Turbidimeter; Hach Company, Loveland, Colo.). All trials were performed in heat-treated, 2000 vintage base wine. This base wine was a blend of young wines from Chardonnay, Macabeu, and Perellada grape cultivars; its composition was determined by standard enological determinations (6). Sparkling wine second fermentations were performed with this base wine in autoclaved standard 750-ml bottles modified to withstand up to 10 bar. Bottles were filled with a mixture of base wine, sucrose, and pied de cup containing 10% ethanol, 6 g of titrable acidity, 24 g of sucrose/liter, and 10^6 viable yeast cells per ml. Fermentation progress was monitored with pressure gauges.

RESULTS

Generation of a Δ*rad52* DC5 derivative. We attempted to disrupt both copies of *RAD52* of DC5 by using two noncomplementary, non-mutually interfering antibiotic resistance markers, *kan*^{r} and *nat1* (9) (Fig. 1A). We amplified cassettes encompassing the resistance markers with chimeric primers encompassing the relevant sequences of the corresponding plasmids, as well as base positions 185 to 213 (upper primers) and 1044 to 1070 (lower primers) from the *RAD52* open reading frame (positions relative to the first ATG), whereas we obtained single disruptions at relatively high frequency with both selection strategies. Simultaneous or sequential disruption of the two *RAD52* alleles by transforming with these PCR fragments failed. The second copy of the *RAD52* gene in a *RAD52*/Δ*rad52::kan*^{r} strain was disrupted by replacement by a DNA fragment encompassing the natMX4 cassette (9) flanked by 195 bp upstream and 504 bp downstream sequences from the *RAD52* gene (Fig. 1B). All double disruptants were sensitive to 0.015% methyl methanesulfonate, a typical phenotype for Δ*rad52* mutants (25). The data demonstrated the existence of two, and only two, copies of *RAD52* in the parental strain, a finding which is consistent with the DNA content of DC5 being close to 2C (4).

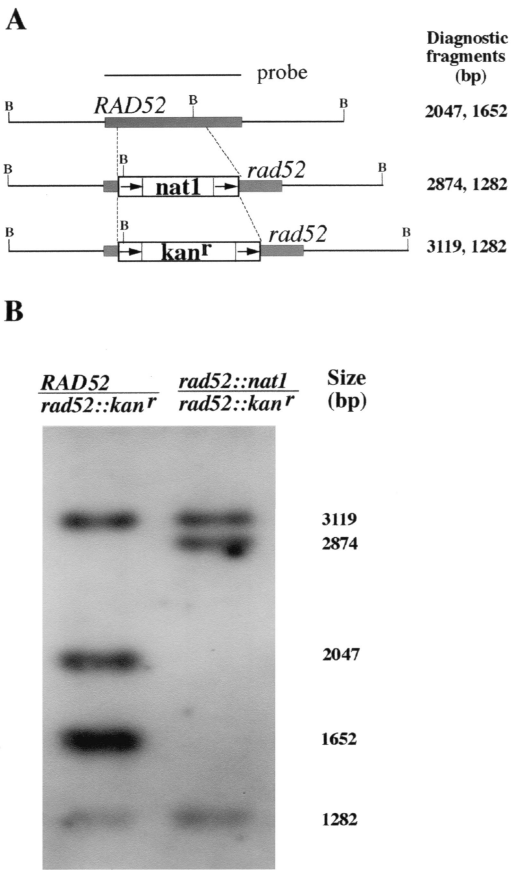

FIG. 1. Disruption of both *RAD52* alleles of DC5. (A) Diagram of the disruption. The resident *RAD52* gene is indicated as a black box at the top. *Bam*HI sites as deduced from the sequence at the *Saccharomyces* Genome Database (http://genome-www4.stanford.edu/cgi-bin/SGD) are indicated by the letter B. Disruption cassettes conferring resistance to clonNat (middle) or to Geneticin (bottom) are represented as white boxes; arrows indicate the sequences corresponding to promoter (left) and terminator (right) sequences. Predicted sizes of a *Bam*HI digestion of the strain harboring the disrupted Δ*rad52* allele are indicated. (B) Southern blot of genomic DNA from a geneticine-resistant heterozygote (left) and a double disruptant (right), digested with *Bam*HI and probed with a *RAD52* probe (Fig. 2A, top). The original DC5 strain gave only two bands of 2,047 and 1,562 bp (not shown).

Karyotype stability of Δ*rad52* strains. We compared karyotypes of clones from DC5 and from two (A1 and A4) Δ*rad52* clones isolated after 100 doublings in rich medium (Fig. 2). As previously described (4), a subset of highly variable chromosomal bands appeared in the upper part of the gel. These bands were identified as variants of chromosome XII by hy-

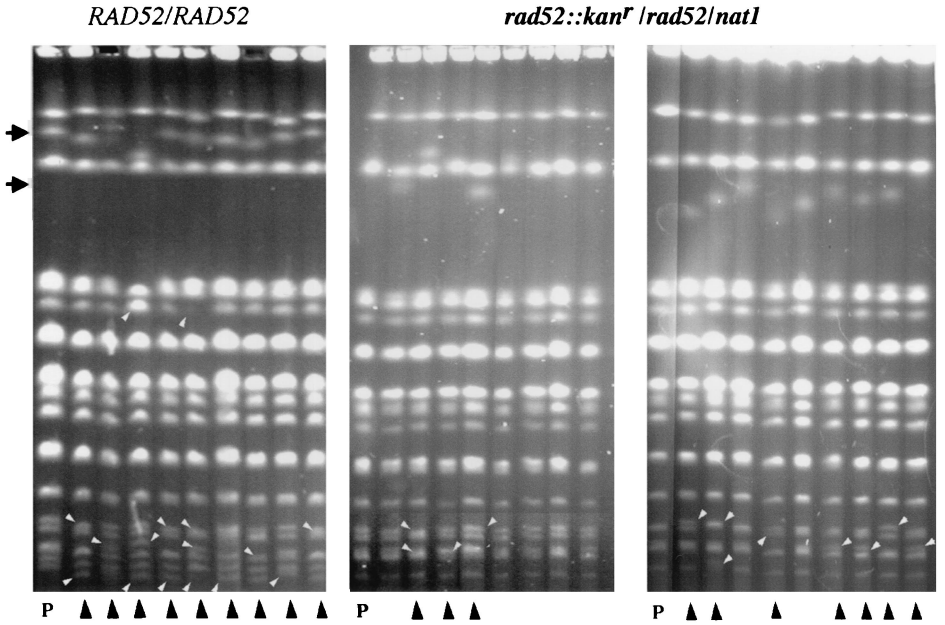

FIG. 2. Analysis of karyotype instability of DC5 and of its Δ*rad52* derivatives. Cultures of DC5 strain (left panel) and of two independent DC5Δ*rad52* derivatives (middle and right panels) were maintained during 100 doublings in reach medium. The resulting culture was spread on a plate, and nine clones were randomly picked for karyotype analysis by pulsed-field gel electrophoresis. The figure also includes the karyotypes of the parental input strains for each culture (indicated by the letter P on the bottom). Arrows at the side of the gels indicate the region where chromosome XII hypervariable bands run. White arrowheads indicate other karyotype variations relative to the parental input strains. Black triangles at the bottom indicate clones whose karyotypes differed from that of the corresponding input strain. The lower part of the gels was digitally enhanced to better reproduce small chromosomal bands.

bridization with ribosomal DNA (rDNA) probes (4, 13). Size variants of this chromosome reflect changes in the number of rDNA repeats present in this chromosome, a phenomenon genetically unlinked to size variations in the rest of chromosomes (4, 17, 22). This particular kind of karyotype variability will not be considered for the rest of considerations that follow.

Table 1 shows rearrangement rates for several independently analyzed Δ*rad52* derivatives (4) (chromosome XII excluded). Their combined rearrangement rates, 6.4×10^{-3} changes per clone per generation, is significantly lower than that of the parental DC5 strain (2.1×10^{-2}, $P = 2.2 \times 10^{-3}$) or of the combined variable monosporidic derivatives of DC5 (1.3×10^{-2}, $P = 6.4 \times 10^{-5}$) but higher than that for constant meiotic derivatives from DC5 (8.4×10^{-4}, $P = 9.1 \times 10^{-17}$ [Table 1] [4]). Independent Δ*rad52* derivatives showed similar rearrangement rates, ranging from 3.9×10^{-3} to 8.3×10^{-3} changes per clone per generation. The Δ*rad52* deletion did not suppress the chromosome XII hypervariability, a result consistent with the previous observation that chromosome XII rearrangements are genetically unrelated to size variations in the rest of the chromosomes (4).

TABLE 1. Statistics of rearranged clones in Δ*rad52* strains

Group	No. of clones		Rearrangement rate (changes · clone^{-1} · generation^{-1})
	Total	Rearranged	
DC5	17	15	2.1×10^{-2}
Δ*rad52* clones			
A1	25	8	3.9×10^{-3}
A4	39	22	8.3×10^{-3}
B1	9	4	5.9×10^{-3}
B2	9	5	8.1×10^{-3}
Total	82	39	6.4×10^{-3}
Monosporidic derivatives[a]			
Constant derivatives	273	22	8.4×10^{-4}
Variable derivatives	181	132	1.3×10^{-2}

[a] Carro and Piña (4).

Analysis of subtelomeric recombination in Δ*rad52* strains. Chromosome rearrangements that occur during vegetative growth are especially evident in the subtelomeric regions, where most changes accumulate (3, 12). We evaluated variations in the subtelomeric ends in Δ*rad52* strains through restriction fragment length polymorphism analysis (RFLP) of Y′ sequences (2). DC5 clones isolated after 100 doublings have considerable polymorphism in their Y′ sequences (Fig. 3, top), comparable to that of the chromosomal bands for the same clones (not shown, see Fig. 2 for comparison). In contrast, a similar experiment with Δ*rad52* strains showed an uniform Y′ RFLP pattern (Fig. 3, bottom), even in clones that were rearranged on the basis of their karyotype (not shown). Analysis of a total of 20 independent Δ*rad52* clones isolated after 100 doublings showed no polymorphism in their Y′ RFLP pattern, which sets an upper limit value for variability of the Y′ pattern of 5×10^{-4} changes per clone per generation. We conclude that variation in the subtelomeric regions depends on *RAD52* (presumably, through ectopic recombination) but that at least

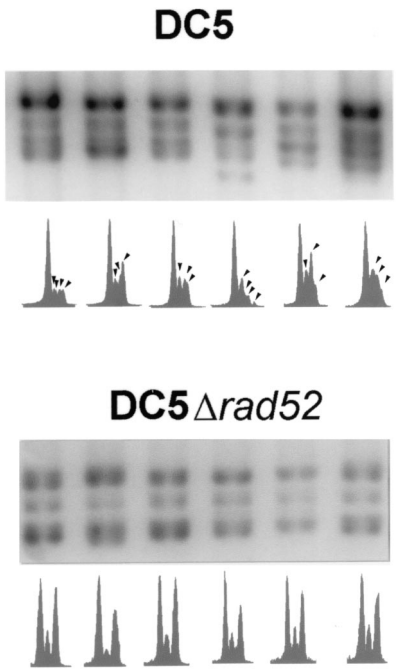

FIG. 3. Analysis of Y′ sequence polymorphism in DC5 and DC5Δ*rad52*. Genomic DNA from six clones (originated as in Fig. 2) from DC5 (top) and from a DC5Δ*rad52* strain (bottom) were digested with *Xho*I, run in a TBE agarose gel, blotted, and hybridized with a Y′ probe. At the bottom of each tract there is the corresponding densitometric profile, running from left to right; small arrowheads indicate bands whose mobility changes among the different clones (only observed in the DC5 strain).

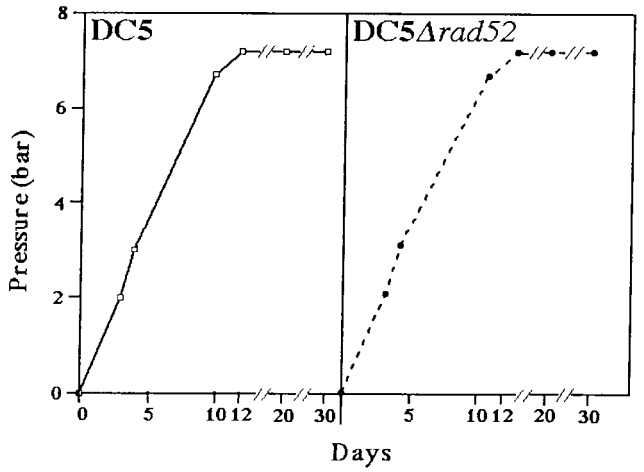

FIG. 4. Small-scale fermentation trials for DC5 and DC5Δ*rad52*. The graphic shows the increase in pressure in the bottle in a typical sparkling wine second fermentation. Note the completion of the fermentation (at ~7.5 bar) after only 10 days of fermentation for both strains. Data are averages of two independent clones for DC5 (left) and six independent DC5Δ*rad52* clones. Standard deviations were under 5% of the mean values in both cases.

some of the chromosomal rearrangements observed originate from an alternative mechanism.

Fermentation capacity of the Δ*rad52* strains. Strain DC5 was isolated following selection for yeast strains for sparkling wine production, which requires an extremely high fermentation capability (13, 14). Both the original DC5 and the *rad52* double disruptant performed the typical sparkling wine second fermentation with very similar, if not identical, kinetics (Fig. 4). Preliminary organoleptic analyses revealed no major differences between the two fermentation products (data not shown). Viable cells were recovered after the completion of the fermentation, i.e., when the pressure in the bottles reached at least 7 bar. Phenotypic analysis of 25 surviving clones from the bottles inoculated with the Δ*rad52* strain indicated that all 25 of them maintained both resistance markers (*nat1* and *kan*^{r}), indicating that Δ*rad52* cells were responsible for the observed fermentation in the bottles.

DISCUSSION

Genetic remediation of natural and industrial yeast strains is complicated by their lack of selectable genetic markers and by their aneuploid nature. The use of dominant markers, e.g., antibiotic resistance, can overcome the first of these problems. We found that the nourseothricin resistance gene *nat1* is compatible with the commonly used *kan*^{r} gene, making this pair of markers suitable for complete disruption of both alleles at a gene in diploid strains. Targeting the second resistance marker to a nondisrupted allele required extended sequence homology to both flanks of the nondisrupted allele. An easy way to obtain this extended homology is to amplify a previously disrupted allele from a heterozygotic strain by PCR. This strategy should be applicable for many protocols in which disruption of both alleles in a diploid strain is needed.

The current hypothesis to explain chromosomal rearrangements in natural and industrial strains of *Saccharomyces* relies on recombination between nonallelic homologous sequences dispersed across the yeast genome, including Y′, delta, and Ty sequences, to generate the observed results (5, 13, 18, 21). This model predicts that chromosomal rearrangements require a functional *RAD52* gene (15, 16). We found that karyotype instability during vegetative growth is only partially dependent on *RAD52*, since chromosomal rearrangement rates in Δ*rad52* strains were significantly lower than that of its parental strain DC5 but still at least five times higher than the rates associated with stable strains. In contrast, recombination at subtelomeric regions was dependent on *RAD52*, indicating that they probably occur through homologous recombination between nonhomologous loci. Recombination at subtelomeric sequences may play a role in the generation of chromosomal polymorphisms, both in mitosis and in meiosis (2, 5, 12), but our results suggest that this mechanism is not responsible for much of the karyotype variation observed. We hypothesize that at least two additional chromosomal rearrangement mechanisms can result in nonhomologous, *RAD52*-independent recombination processes. One of these mechanisms would account for at least a third of the observed changes in chromosome sizes during vegetative growth. The second one is involved in rDNA rearrangements, which occur genetically independent from rearrangements of the rest of the genome (4, 24).

The long-term objective of our research is to demonstrate the feasibility of genetic remediation for reducing chromosomal instability of natural strains without compromising their

industrial performance. For historical reasons, we were particularly interested in yeast strains that can perform the so-called second fermentation of sparkling wine, which involves a refermentation of a base wine in the typical sparkling wine bottles (13, 14). This process requires a very efficient fermentation by the yeast due to the stringent conditions under which it proceeds, including a low pH (2.9 to 3.1), an ethanol concentration of >10%, low levels of nutrients, the presence of SO_2, a moderate temperature (15 to 20°C), and an increase in CO_2 pressure up to 7.5 bar (1). That the Δ*rad52* strains perform similarly to the parental strain in fermentation trials suggests that this approach is feasible for this type of yeast strain and that similar strategies of genetic remediation in other industrial yeast-based fermentations need to be considered.

ACKNOWLEDGMENTS

This work was supported by grants from the Spanish Ministry of Education and Science (PB98-0469, BMC2001-0246, GEN2001-4707-C8-08, and AGL2000-0133-P4-03) with additional support from the Generalitat de Catalunya (SGQ97-062 and SGR99-189) and from the Alexander von Humboldt Stiftung (Germany) to B.P. D.C. is a recipient of a predoctoral fellowship from the Generalitat de Catalunya. This work was carried out within the framework of the "Centre de Referència en Biotecnologia" of the Generalitat de Catalunya.

REFERENCES

1. **Bartra, E.** 1995. Microbiological aspects of sparkling wine processing. Microbiol. SEM **11:**43–50.
2. **Bidenne, C., B. Blondin, S. Dequin, and F. Vezinhet.** 1992. Analysis of the chromosomal DNA polymorphism of wine strains of *Saccharomyces cerevisiae*. Curr. Genet. **22:**1–7.
3. **Carro, D., J. García-Martínez, J. Pérez-Ortín, and B. Piña.** 2003. Structural characterization of chromosome I size variants from a natural yeast strains. Yeast **20:**171–183.
4. **Carro, D., and B. Piña.** 2001. Genetic analysis of the karyotype instability in natural wine yeast strains. Yeast **18:**1457–1470.
5. **Codon, A. C., T. Benítez, and M. Korhola.** 1997. Chromosomal reorganization during meiosis of *Saccharomyces cerevisiae* baker's yeasts. Curr. Genet. **32:**247–259.
6. **European Community Commission.** 1990. Official European Community wine analysis procedure. Reg. CEE no. 2676/90. European Community Commission, Brussels, Belgium.
7. **Gerring, S., C. Connelly, and P. Hieter.** 1991. Positional mapping of genes by chromosome blotting and chromosome fragmentation. Methods Enzymol. **194:**55–57.
8. **Gietz, D., A. St. Jean, R. Woods, and R. Schiestl.** 1992. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **25:**1425.
9. **Goldstein, A., and J. McCusker.** 1999. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:**1541–1553.
10. **Longo, E., and F. Vezinhet.** 1993. Chromosomal rearrangements during vegetative growth of a wild strain of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. **59:**322–326.
11. **Louis, E., and R. Borts.** 1995. A complete set of marked telomeres in *Saccharomyces cerevisiae* for physical mapping and cloning. Genetics **139:**125–136.
12. **Louis, E., E. Naumova, A. Lee, G. Naumov, and J. Haber.** 1994. The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. Genetics **136:**789–802.
13. **Nadal, D., D. Carro, J. B. Fernández-Larrea, and B. Piña.** 1999. Analysis and dynamics of the chromosomal complements of wild sparkling-wine yeast strains. Appl. Environ. Microbiol. **65:**1688–1695.
14. **Nadal, D., B. Colomer, and B. Piña.** 1996. Molecular polymorphism distribution in phenotypically distinct populations of wine yeast strains. Appl. Environ. Microbiol. **62:**1944–1950.
15. **Paques, F., and J. E. Haber.** 1999. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae.* Microbiol. Mol. Biol. Rev. **63:**349–404.
16. **Petes, T., R. Malone, and L. Symington.** 1991. Recombination in yeast, p. 407–521. *In* J. Broach, J. Pringle, and E. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
17. **Petes, T. D.** 1980. Unequal meiotic recombination within tandem arrays of yeast ribosomal DNA genes. Cell **19:**765–774.
18. **Puig, S., A. Querol, E. Barrio, and J. E. Pérez-Ortín.** 2000. Mitotic recombination and genetic changes in *Saccharomyces cerevisiae* during wine fermentation. Appl. Environ. Microbiol. **66:**2057–2061.
19. **Puig, S., D. Ramón, and J. Pérez-Ortín.** 1998. Optimized method to obtain stable food-safe recombinant yeast strains. J. Agric. Food Chem. **46:**1689–1693.
20. **Querol, A., E. Barrio, T. Huerta, and D. Ramón.** 1992. Molecular monitoring of wine fermentations conducted by active dry yeast strains. Appl. Environ. Microbiol. **58:**2948–2953.
21. **Rachidi, N., P. Barre, and B. Blondin.** 1999. Multiple Ty-mediated chromosomal translocation lead to karyotype changes in a wine strain of *Saccharomyces cerevisiae.* Mol. Gen. Genet. **261:**841–850.
22. **Rustchenko-Bulgac, E. P., and F. Sherman.** 1994. Physical constitution of ribosomal genes in common strains of *Saccharomyces cerevisiae*. Yeast **10:**1157–1171.
23. **Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen.** 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:**1793–1808.
24. **Warner, J. R.** 1989. Synthesis of ribosomes in *Saccharomyces cerevisiae*. Microbiol. Rev. **53:**256–271.
25. **Xiao, W., B. Chow, and L. Rathgeber.** 1996. The repair of DNA methylation damage in *Saccharomyces cerevisiae*. Curr. Genet. **30:**461–468.