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Organization of the *SUC* Gene Family in *Saccharomyces*

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The *SUC* gene family of yeast (*Saccharomyces*) includes six structural genes for invertase (*SUC1* through *SUC5* and *SUC7*) found at unlinked chromosomal loci. A given yeast strain does not usually carry SUC^+ alleles at all six loci; the natural negative alleles are called suc^0 alleles. Cloned *SUC2* DNA probes were used to investigate the physical structure of the *SUC* gene family in laboratory strains, commercial wine strains, and different *Saccharomyces* species. The active SUC^+ genes are homologous. The suc^0 allele at the *SUC2* locus ($suc2^0$) in some strains is a silent gene or pseudogene. Other *SUC* loci carrying suc^0 alleles appear to lack *SUC* DNA sequences. These findings imply that *SUC* genes have transposed to different chromosomal locations in closely related *Saccharomyces* strains.

The *SUC* (sucrose fermentation) genes of *Saccharomyces* appear from genetic studies to be a dispersed family of genes. Six *SUC* genes (*SUC1* through *SUC5* and *SUC7*) have been found at unlinked loci on at least four chromosomes (for review see reference 11; 6). The *SUC1* through *SUC6* loci were identified by segregational analysis of different *Saccharomyces* strains (9, 11, 18; D. Hawthorne, Ph.D. thesis, University of Washington, Seattle, 1955); *SUC4* and *SUC6* were later found to be allelic (D. Hawthorne, personal communication). We recently identified the *SUC7* locus (6). The loci are on the following chromosomes: *SUC1*, VII; *SUC2*, IX; *SUC3*, II; *SUC4*, not mapped; *SUC5*, IV; *SUC7*, not mapped (10, 12; G. Kawasaki, Ph.D. thesis, University of Washington, Seattle, 1979).

Each *SUC* gene encodes both a secreted and an intracellular form of the sucrose-hydrolyzing enzyme invertase (3), and thus a single SUC^+ allele confers the ability to ferment sucrose. An unusual feature of this gene family is that closely related *Saccharomyces* strains often differ in *SUC* genotype; for example, Gilliland (9) and Winge and Roberts (18) showed that *Saccharomyces chevalieri* contains three SUC^+ genes and *Saccharomyces italicus* has none. Most yeast strains do not have SUC^+ alleles at all six *SUC* loci, but rather carry negative alleles at some or all *SUC* loci in their genomes. These natural negative alleles are called suc^0 alleles (to distinguish them from negative mutations [suc^-] derived from a SUC^+ gene in the laboratory).

The variability in the number and location of active SUC^+ genes in different yeast genomes could be explained by three possible models for the structure of the *SUC* gene family. One possibility is that all *SUC* loci in the genome of a yeast strain carry *SUC* genetic information, although only one or a few loci carry active SUC^+ genes. According to this idea, the suc^0 alleles are *SUC* genes that are not expressed or that encode a defective product, in other words, silent genes or pseudogenes. Another possibility is that in a given yeast strain only those *SUC* loci carrying SUC^+ alleles contain *SUC* gene information and that the *SUC* loci bearing suc^0 alleles are "empty sites" containing no *SUC* DNA sequences. The third possibility, which we show here to be the correct one, is that both types of suc^0 alleles exist.

We have previously reported genetic evidence that a suc^0 allele at the *SUC2* locus ($suc2^0$) is a silent gene; this allele can mutate to an active state and can recombine with three different *suc2* amber mutations to yield an active $SUC2^+$ gene (6). No other silent suc^0 genes were detected by genetic methods.

In this work we have examined directly the physical structure of the *SUC* gene family in different *Saccharomyces* genomes by using cloned *SUC2* DNA probes to detect homologous sequences. We show that the six active SUC^+ genes are homologous and confirm that the silent $suc2^0$ allele identified genetically does in fact contain *SUC* gene sequences. No *SUC* DNA sequences corresponding to suc^0 alleles at

other loci were detected in a variety of strains. These results suggest that the presence of active *SUC*$^+$ genes at loci other than *SUC2* must result from movement of *SUC*$^+$ information during the evolution of *Saccharomyces* species and strains.

MATERIALS AND METHODS

Strains and genetic methods. Yeast strains and genotypes are listed in Table 1. Strains with the *SUC1*$^+$, *SUC2*$^+$, *SUC3*$^+$, *SUC4*$^+$, *SUC5*$^+$, and *SUC7*$^+$ genes were previously shown each to carry a *SUC*$^+$ gene at a different locus (6). The following wine yeasts were obtained from Robert Mondavi Winery (Oakville, Calif.): Woodbridge, French white, French red, Chateau Epernay, Bresiach 12, Bresiach 15, Geisenheim 49, Geisenheim 74, Moet/Chandon, Steinberg, 51, 505, 522X, 595, and 679.

TABLE 1. Yeast strains

Strain	Genotype or species	Source or reference
S288C	*MATα SUC2*$^+$	G. Fink
FL100	*MATa SUC7*$^+$	F. Lacroute
DBY615	*MATa SUC7*$^+$ *ura3-34*	F. Lacroute
DBY938	*MATa suc2*^{0} *ade2-101*	(6)
DBY939	*MATα suc2-215 ade2-101*	(3)
DBY940	*MATα suc2-215 lys2-801 his4-539*	This work
DBY962	*MATα SUC2*$^+$ *lys2-801 his4-539*	This work
DBY1046[a]	*MATα suc2*^{0} *his4-619*	This work
MCY135[a]	*MATa suc2*^{0} *ade2-101*	This work
R251-4A	*MATα SUC1*$^+$ *ura1 ade2*	Yeast Genetic Stock Center
1412-4D	*MATa SUC3*$^+$ *MAL3*$^+$ *MEL1*$^+$ *MGL2*$^+$ *MGL3*$^+$ *GAL*$^+$ *ade2*	Yeast Genetic Stock Center
SS-12A	*MATα SUC4*$^+$ *his4*	Yeast Genetic Stock Center
2080-8C	*MATa SUC5*$^+$ *ade6*	Yeast Genetic Stock Center
N422-8C[b]	*MATα SUC5*$^+$ *his4 ade6 lys2 leu2 ura4 trp1 tyr1 arg4 thr4 gal7*	G. Kawasaki
77-104	*S. bayanus*	H. Phaff
C247	*S. oviformis*	H. Phaff
51-242	*S. kluyveri*	H. Phaff
55-99	*S. diastaticus*	H. Phaff
57-47	*S. carlsbergensis*	H. Phaff
C258	*S. cerevisiae*	H. Phaff
61-22	*S. chevalieri*	H. Phaff
C105	*S. italicus*	H. Phaff
Y12633	*S. chevalieri*	C. Kurtzman

[a] These strains are congenic to S288C as a result of 13 crosses to S288C-derived strains.

[b] N422-8C contains a *SUC*$^+$ gene which G. Kawasaki mapped to chromosome IV (Ph.D. thesis) and which we showed to be allelic to *SUC5* (6).

Standard yeast genetic procedures of crossing, sporulation, and tetrad analysis were followed (11, 15). Media and scoring for ability to ferment sugars have been described previously (5).

Preparation of DNAs. Plasmid DNAs were prepared by cesium chloride-ethidium bromide equilibrium centrifugation. Yeast DNAs were isolated by a modification of the method of Cryer et al. (7) or by a procedure adapted from that of Cameron et al. (2).

Restriction enzyme digestion and gel electrophoresis of DNAs. Restriction enzymes were purchased from New England BioLabs. Agarose gel electrophoresis of DNA fragments was carried out in 89 mM Tris-OH–89 mM boric acid–2.5 mM EDTA (pH 8.3) (13).

Gel transfer hybridization. DNA fragments were transferred from agarose gels to nitrocellulose filters by the method of Southern (16). Except where otherwise noted, filters were incubated before hybridization for several hours at 65°C in 0.6 M NaCl–0.075 M sodium citrate–0.1 M sodium phosphate (pH 7) containing 0.5% sodium dodecyl sulfate. Hybridization with ^{32}P-labeled probes prepared by nick translation of plasmid DNAs (14) was carried out overnight under the same conditions, but with the addition of sonicated carrier DNA (100 μg/ml). Nitrocellulose filters were washed at 65°C in 0.75 M NaCl–0.075 M sodium citrate and allowed to expose Kodak XR-5 or XAR-5 film at −70°C with Du Pont Lightning Plus screens.

RESULTS

***suc*^{0} alleles with different structures.** The structures of *SUC*$^+$ and *suc*^{0} alleles present in the genomes of different yeast strains were investigated by the gel transfer hybridization method of Southern (16) by using probes specific for *SUC* DNA sequences. We previously cloned *SUC2* DNA by complementation of a *suc2*$^-$ mutation in yeast; a *suc2* amber allele was cloned, and complementation depended on the presence of an amber suppressor (3). We also mapped the structural gene and subcloned segments of the gene (3). Figure 1 shows maps of the *SUC2* DNA segments subcloned in plasmids pRB59, pRB117, and pRB118, which were used to prepare probes.

We first investigated two common *Saccharomyces cerevisiae* laboratory strains, each carrying one active *SUC*$^+$ allele: S288C (*SUC2*$^+$) and FL100 (*SUC7*$^+$). Each of these strains carries *suc*^{0} alleles at all loci other than the *SUC*$^+$ locus. Previous genetic evidence indicated that the *suc2*^{0} allele of FL100 is a silent gene or pseudogene (6). We expected to detect the *SUC2*$^+$ gene in the S288C genome and to detect both the *suc2*^{0} gene and the active *SUC7*$^+$ gene (presuming homology between *SUC2*$^+$ and *SUC7*$^+$) in the FL100 genome. The other *suc*^{0} alleles in these genomes might or might not contain *SUC* gene information.

DNA was prepared from FL100 and from

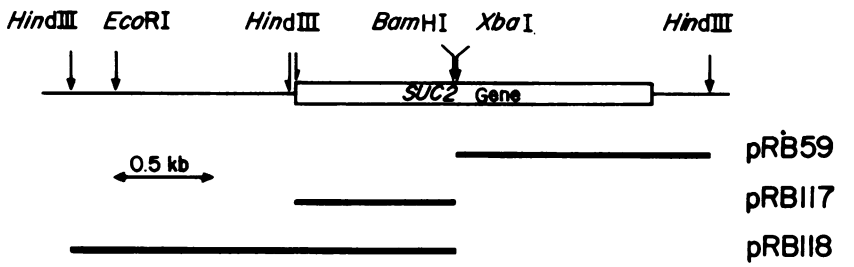

FIG. 1. Maps of cloned *SUC2* DNA. A restriction map of the *SUC2* gene is shown with the 5′ end of the gene at the left (3). Plasmids pRB59, pRB117, and pRB118 contain the indicated *Bam*HI-*Hin*dIII fragments subcloned into pBR322 (1). There are no *Bgl*II, *Pst*I, *Sal*I, *Pvu*I, or *Xho*I sites in this region.

DBY939, a strain derived directly from S288C by mutation. DBY939 has a simple point (amber) mutation at *SUC2* (the *suc2-215* allele [5]). DNA from each strain was digested with endonuclease *Bam*HI, and the resulting fragments were separated by agarose gel electrophoresis. The gel transfer hybridization technique was used to detect sequences homologous to a *SUC2* DNA probe prepared from plasmid pRB59 (Fig. 1). One DNA fragment homologous to the pRB59 probe was detected in DBY939, and two such fragments were detected in FL100 (Fig. 2). A variety of different restriction enzymes were used (*Sal*I, *Pst*I, *Bgl*II, *Pvu*I, *Xba*I, *Xho*I), and in each case one homologous fragment was detected in DBY939 and two fragments were detected in FL100 (data not shown). The simple interpretation of these results is that DBY939 contains the *suc2* gene and no other *SUC* gene information and that FL100 contains the $SUC7^+$ gene and the silent $suc2^0$ gene; however, fragments of the same size could, in principle, be derived from *SUC* DNA sequences at more than one chromosomal locus. To test this possibility, we examined the meiotic segregation of the *SUC* DNA sequences which give rise to these fragments. Strains derived directly from S288C and FL100 were crossed, and the four spores produced by meiosis of a single diploid cell were recovered by dissection. DNA samples prepared from the four spore clones were analyzed by gel transfer hybridization; endonuclease *Bam*HI and probe pRB59 were used for this experiment so that fragments derived from the *suc2-215*, $suc2^0$, and $SUC7^+$ loci would be distinguishable by size. Figure 2 shows that each of the bands is present in two spores of the tetrad. This result means that each band is composed of fragments derived from one chromosomal *SUC* locus and therefore that S288C carries *SUC* gene information at one locus and FL100 at two loci. These results were confirmed by analysis of another tetrad from this cross and a tetrad from the cross of MCY135 by DBY962 (see below and Fig. 2). Although the possibility that two tightly linked loci contribute fragments to the same band cannot be ruled out without analysis of many tetrads, our independent evidence regarding the number of loci based on use of a variety of restriction enzymes makes this possibility an unlikely one. In any case, the six identified *SUC* loci are genetically unlinked. Thus, no sequence corresponding to the $suc1^0$, $suc3^0$, $suc4^0$, $suc5^0$,

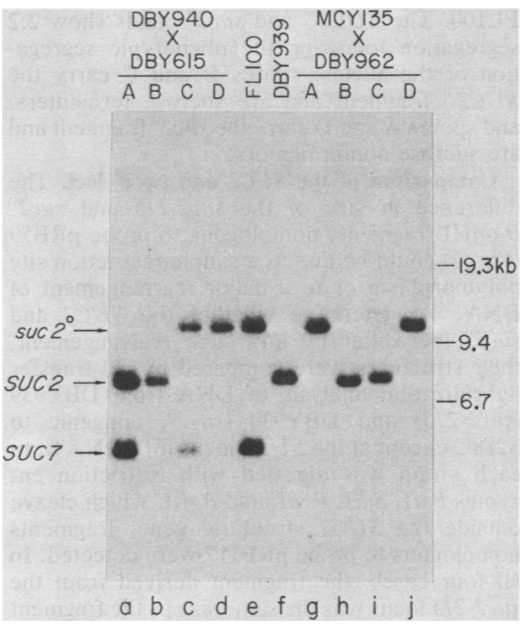

FIG. 2. Gel transfer hybridization analysis of *SUC* genes in strains derived from S288C and FL100. Total DNAs from the following strains were digested with *Bam*HI and electrophoresed in a 0.5% agarose gel: (a through d) the four spore clones (A, B, C, and D) of a tetrad from the cross of DBY940 (*suc2-215*; derivative of S288C) with DBY615 ($SUC7^+$ $suc2^0$; derivative of FL100); (e) FL100 ($SUC7^+$ $suc2^0$); (f) DBY939 (*suc2-215*); (g through j) the four spore clones (A, B, C, and D) of a tetrad from the cross of MCY135 ($suc2^0$) with DBY962 ($SUC2^+$). The DNA fragments were transferred to nitrocellulose and hybridized to ^{32}P-labeled pRB59. An autoradiograph is shown.

or *suc7*^{0} allele was detected with cloned *SUC2* DNA probes.

The assignment of the *SUC7* and *suc2*^{0} bands in the FL100 genome is based on the following evidence. First, the bands labeled *SUC2* and *suc2*^{0} segregate from one another in meiosis; two spores (A and B) carry the *SUC2* (*suc2-215*) band, and the remaining two spores (C and D) carry the *suc2*^{0} band, as expected for Mendelian segregation of alleles. Second, the spore (D) which carries the *suc2*^{0} band and no other *SUC* information is unable to ferment sucrose, and the two spores (A and C) that carry the *SUC7*$^+$ band do ferment sucrose. The identity of the *suc2*^{0} band was confirmed by using a strain (MCY135) constructed to be congenic to S288C at all loci except *SUC2*, where it carries the *suc2*^{0} allele rather than the *SUC2*$^+$ allele (6). MCY135 was crossed to DBY962 (*SUC2*$^+$) and DNAs from four spore clones of a tetrad were digested with *Bam*HI and analyzed by gel transfer hybridization (Fig. 2). This experiment shows that the MCY135 genome contributes one fragment homologous to the *SUC2* DNA probe which comigrates with the *suc2*^{0} fragment from FL100. The *SUC2*$^+$ and *suc2*^{0} bands show 2:2 segregation following the phenotypic segregation of the alleles; spores B and C carry the *SUC2*$^+$ fragment and are sucrose fermenters, and spores A and D carry the *suc2*^{0} fragment and are sucrose nonfermenters.

Comparison of the *SUC2* and *suc2*^{0} loci. The difference in size of the *suc2-215* and *suc2*^{0} *Bam*HI fragments homologous to probe pRB59 (Fig. 2) could be due to a simple restriction site polymorphism or to a major rearrangement of DNA. To determine whether the *SUC2* and *suc2*^{0} loci differ by any such rearrangement, their structures were compared by gel transfer hybridization analysis of DNA from DBY939 (*suc2-215*) and DBY938 (*suc2*^{0}; congenic to S288C except at the *SUC2* locus [6]). DNA from each strain was digested with restriction enzymes *Pst*I, *Sal*I, *Pvu*I, and *Bgl*II, which cleave outside the *SUC2* structural gene; fragments homologous to probe pRB117 were detected. In all four cases, the fragment derived from the *suc2-215* locus was the same size as the fragment from the *suc2*^{0} locus (data not shown). Thus, no large deletions, insertions, or rearrangements were detected as differences between the two loci. The size difference between the *suc2-215* and *suc2*^{0} *Bam*HI fragments homologous to pRB59 is therefore due to a restriction site polymorphism.

We showed that this polymorphism occurs at a *Bam*HI site outside the *SUC2* gene by the following experiment. The *SUC2* gene contains a single *Bam*HI site; the fragment subcloned in pRB59 lies to the right of this *Bam*HI site, and the fragment subcloned in pRB117 lies to the left (Fig. 1). *Bam*HI-digested DNA from the *suc2-215* and *suc2*^{0} strains was hybridized with probe pRB117. The fragment detected in the *suc2*^{0} DNA was the same size as the fragment in the *suc2-215* DNA (data not shown). This result implies that the *Bam*HI site in the *SUC2* gene and the site to the left of the gene are both conserved in the *suc2*^{0} allele. The polymorphism detected with pRB59 must occur at a *Bam*HI site located outside the gene to the right.

As a measure of the sequence divergence between the *SUC2*$^+$ and *suc2*^{0} alleles, restriction site polymorphisms within or close to the structural gene were investigated by using endonucleases which cleave within the gene. DNAs from DBY939 (*suc2-215*) and the congenic strain DBY1046 (*suc2*^{0}) were digested with *Hin*dII, *Ava*II, *Ava*I, *Msp*I, *Hae*III, and *Sau*3AI; fragments homologous to *SUC2* DNA probes were analyzed by gel transfer hybridization. Although most of the restriction sites are conserved between the two alleles, several polymorphisms were detected with probe pRB59 (Fig. 3); similar results were obtained with probe pRB118 (data not shown). The fact that polymorphisms are so readily detected suggests considerable sequence divergence between *SUC2* and *suc2*^{0}. DNA from FL100 (*suc2*^{0} *SUC7*$^+$) was also included in this analysis, and several fragments unique to the *SUC7*$^+$ locus were detected.

***SUC* DNA sequences in other yeast strains of defined *SUC* genotype.** We next examined the *SUC* DNA sequences present in four other laboratory strains, each carrying one active *SUC*$^+$ allele at the *SUC1*, *SUC3*, *SUC4*, or *SUC5* locus (and *suc*^{0} alleles at all other loci). These experiments were undertaken to characterize the relationships among different *SUC*$^+$ genes and to investigate the *suc*^{0} alleles in these strains. Figure 4 shows a blot hybridization analysis of DNA prepared from these strains and digested with endonucleases *Bgl*II, *Xba*I, *Pst*I, and *Sal*I; similar results were obtained previously with *Bam*HI (4). In all cases, two fragments homologous to the *SUC2* DNA probe were detected. One of the fragments generated from each strain comigrates with the fragment derived from the *suc2*^{0} allele of FL100 (not all data shown), which suggests that each of these strains contains a silent *suc2*^{0} allele in addition to its active *SUC*$^+$ gene. We have previously reported genetic evidence that strain R251-4A (*SUC1*$^+$) contains a silent *suc2*^{0} gene which can mutate to confer the ability to ferment sucrose (6). The other fragment produced in each digest presumably corresponds to the active *SUC*$^+$ allele in the genome. In many cases this second fragment comigrates with the fragment carrying the *SUC7*$^+$ gene of FL100. The sizes of the

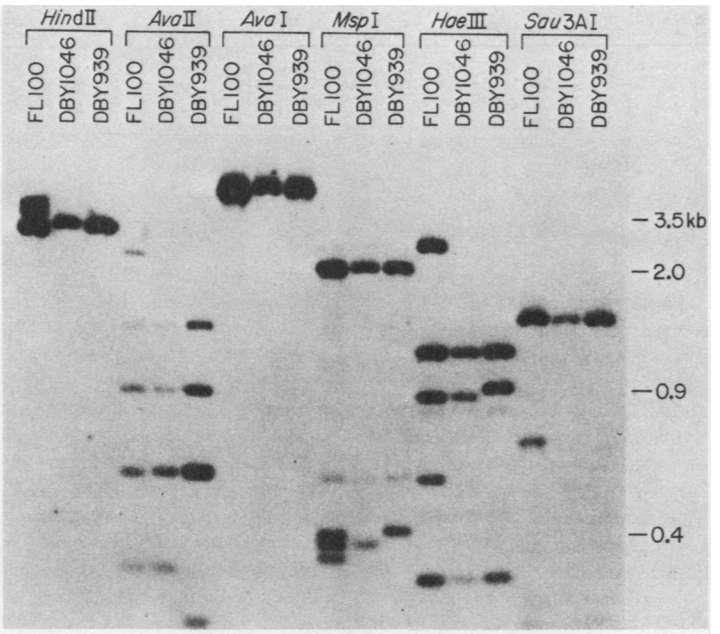

FIG. 3. Comparison of *SUC2*$^+$, *suc2*^{0}, and *SUC7*$^+$ alleles. Gel transfer hybridization of DNAs from DBY939 (*suc2-215* mutation of the *SUC2*$^+$ gene), DBY1046 (*suc2*^{0}), and FL100 (*SUC7*$^+$ *suc2*^{0}) digested with the indicated restriction enzymes and electrophoresed in a 1.7% agarose gel. The probe was ^{32}P-labeled pRB59, and hybridization was carried out as described by Wahl et al. (17) with dextran sulfate. An autoradiograph is shown. The fragments from FL100 DNA are derived from both the *SUC7*$^+$ and *suc2*^{0} alleles.

fragments indicate that restriction sites spaced 5 to 10 kilobase pairs apart are conserved at several different *SUC*$^+$ loci. The failure to detect hybridization of the *SUC2* DNA probe to any additional sequences suggests that these strains each contain one active *SUC*$^+$ gene and one *suc*^{0} pseudogene and that their other *suc*^{0} alleles lack homology to *SUC2* DNA. The use of five different restriction enzymes minimizes the possibility that an additional pseudogene escaped detection because of fortuitous comigration of restriction fragments.

***SUC* genes in different *Saccharomyces* species.** The yeast strains examined thus far are all haploid laboratory stocks of defined *SUC* genotype. It was important to ascertain whether these strains are representative of the majority of *Saccharomyces* species and strains with respect to the structure of the *SUC* gene family. We therefore obtained from H. Phaff (University of California, Davis) isolates of different closely related *Saccharomyces* species: *S. bayanus*, *S. oviformis*, *S. kluyveri*, *S. diastaticus*, *S. carlsbergensis*, *S. cerevisiae*, *S. chevalieri*, and *S. italicus*. All strains except *S. italicus* ferment both sucrose and raffinose, which is another substrate of some invertases (6, 11).

The *SUC* genes carried by these strains were characterized by gel transfer hybridization analysis of genomic DNA digested with *Bgl*II, *Pst*I, or *Bam*HI. Figure 5 shows the *Bgl*II and *Pst*I fragments containing *SUC* DNA sequences; essentially similar results were obtained when probes prepared from either pRB59 or pRB118 were used to detect homologous *Bam*HI fragments (data not shown). Overall, the hybridization patterns of all strains except *S. kluyveri* resemble those observed for the genetically defined laboratory strains: only one or a few fragments are homologous to the *SUC2* DNA probe. One fragment from each digest comigrates with the *suc2*^{0} fragment of FL100 (and also the *SUC2*$^+$ fragment of S288C; data not shown), suggesting the presence of a *suc2*^{0} or *SUC2*$^+$ allele. A fragment from *S. carlsbergensis* DNA is the same size as the *SUC7*$^+$ fragment (and the *SUC3*$^+$ and *SUC5*$^+$ fragments [Fig. 4]).

The cases of *S. italicus* and *S. kluyveri* deserve special mention. The fragment detected from *S. italicus* must correspond to a *suc*^{0} pseudogene because this strain does not ferment sucrose. DNA from *S. kluyveri*, which ferments both sucrose and raffinose, failed to hybridize to the *SUC2* DNA probe. We assayed this strain for invertase by electrophoresing a crude extract of glucose-derepressed cells on a polyacrylamide gel and staining the gel for invertase activity (5, 8). Two sucrose-cleaving activities

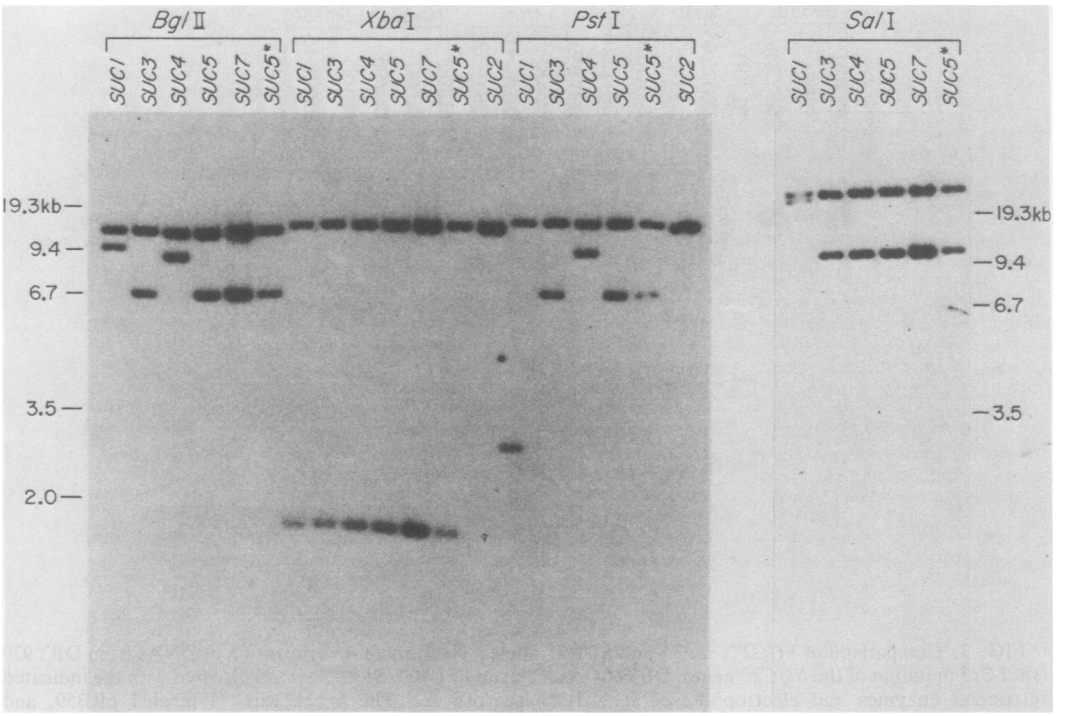

FIG. 4. Gel transfer hybridization analysis of strains carrying different *SUC*$^+$ genes. DNAs from the following strains carrying one active *SUC*$^+$ allele were digested with the indicated enzyme, electrophoresed in a 0.5% agarose gel, and analyzed by gel transfer hybridization with ^{32}P-labeled probe prepared from pRB59: R251-4A (*SUC1*$^+$), DBY939 (*suc2-215*), 1412-4D (*SUC3*$^+$), SS-12A (*SUC4*$^+$), 2080-8C (*SUC5*$^+$), N422-8C (*SUC5*$^+$), and FL100 (*SUC7*$^+$). An autoradiograph is shown, and the lanes are labeled with the *SUC* genotypes of the strains. Lanes with N422-8C DNA are marked with an asterisk. The fragments corresponding to the *suc2*^{0} allele of FL100 were identified in other experiments as the upper band in each lane (data not shown).

were detected, which most likely correspond to the two forms of invertase commonly synthesized from a *SUC* gene (data not shown). *S. kluyveri* is more distantly related to *S. cerevisiae* than the other species analyzed, and it appears that its *SUC* gene or genes have diverged sufficiently that no homology can be detected under the hybridization conditions used.

The hybridization pattern of *S. chevalieri* was of particular interest because the *SUC1*, *SUC2*, and *SUC3* genes were first isolated genetically by Winge and Roberts (18) from a strain of *S. chevalieri*. The *S. chevalieri* strain used in this analysis does not appear to contain the *SUC1*$^+$ and *SUC3*$^+$ genes, as judged by the absence of labeled bands of the expected mobility. Although it is possible that the fragments derived from the *SUC1*$^+$ and *SUC3*$^+$ loci of this strain fortuitously comigrate with the *suc2*^{0} (and *SUC2*) fragment, it seemed more likely that our strain differs in *SUC* genotype from that of Winge and Roberts. The idea that the two strains are different is supported by the observation that strain 61-22 ferments maltose, whereas their *S. chevalieri* strain did not. We obtained another isolate of *S. chevalieri*, Y12633, which ferments sucrose but not maltose, from C. Kurtzman (Northern Regional Research Laboratory, Peoria, Ill.). Gel transfer hybridization analysis showed that this isolate contains at least two (probably three) *SUC* genes; two fragments were detected in *Sal*I or *Bgl*II digests and three fragments were detected in *Bam*HI digests (data not shown). In each digest one of the fragments comigrated with the *SUC1* fragment of strain R251-4A, suggesting that strain Y12633 carries a *SUC1* gene.

We also investigated the *SUC* genes present in the genomes of 15 commercial wine yeasts obtained from the Robert Mondavi Winery (see above for a list of the strains). All strains ferment sucrose and raffinose. Gel transfer hybridization of *Bam*HI-digested DNA revealed patterns very similar to those observed in previous experiments; one or a few fragments homologous to pRB59 were detected in all strains except French red, in which no homologous fragment was detected (data not shown).

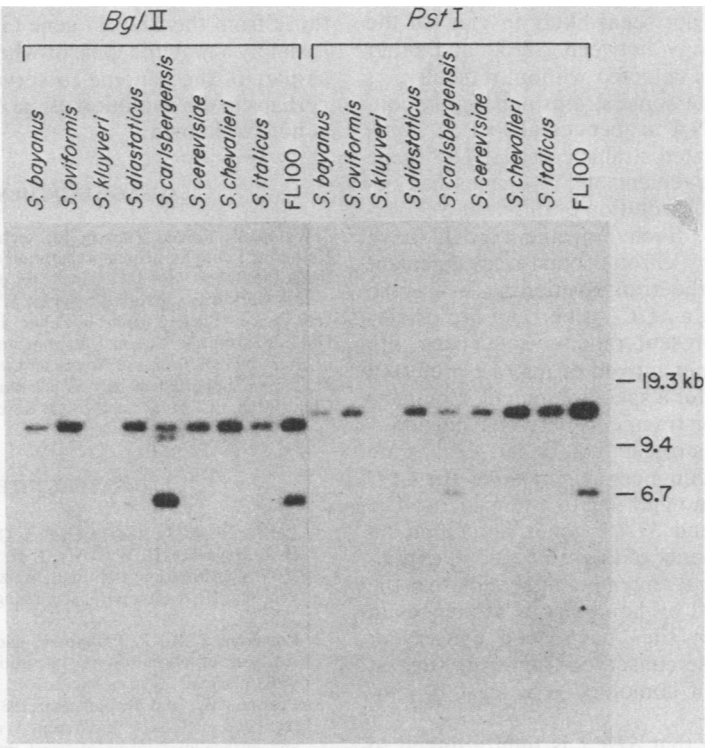

FIG. 5. *SUC* genes in different *Saccharomyces* species. Fragments generated by *Bgl*II and *Pst*I digestion from the DNAs of the indicated *Saccharomyces* species were electrophoresed in a 0.5% agarose gel. Fragments homologous to ^{32}P-labeled pRB59 DNA were detected by gel transfer hybridization and autoradiography.

DISCUSSION

We have investigated the physical structure of the *SUC* gene family by using cloned *SUC2* DNA probes to detect homologous sequences in a variety of yeast strains including different *Saccharomyces* species, commercial wine strains, and laboratory stocks of defined *SUC* genotype. Similar patterns of restriction fragments containing *SUC* DNA sequences were generated from the genomes of all these strains, indicating that the laboratory stocks are representative of "wild" *Saccharomyces* strains with respect to *SUC* gene family organization.

Gel transfer hybridization analysis of strains with defined *SUC* genotypes showed that all the active SUC^+ genes ($SUC1^+$ through $SUC5^+$ and $SUC7^+$) are homologous in sequence. In addition, a suc^0 allele containing *SUC* genetic information was detected in each strain except the $SUC2^+$ strain. Previous genetic evidence indicated that the $suc2^0$ alleles in FL100 and R251-4A are silent genes or pseudogenes (6). The studies reported here provide physical evidence for such silent genes and confirm that the suc^0 DNA sequence detected in FL100 is the $suc2^0$ allele by demonstrating linkage to $SUC2^+$. Analysis of restriction fragments suggests that the suc^0 pseudogenes in the other strains are also $suc2^0$. The sucrose-nonfermenting strain *S. italicus* contains a suc^0 pseudogene which on the basis of restriction fragment sizes appears to be $suc2^0$. The presence of a pseudogene in *S. italicus*, a natural isolate, argues that pseudogenes occur in "wild" yeast strains and are not peculiar to laboratory stocks. The other *Saccharomyces* species and the commercial wine strains may in fact also carry pseudogenes; even strains in which only one labeled band is detected by gel transfer hybridization may carry active *SUC* genes and pseudogenes which give rise to fragments of identical size.

In contrast to the *SUC2* locus, other *SUC* loci carrying suc^0 alleles appear to contain no *SUC* genetic information. In the six strains of defined *SUC* genotype which were analyzed, no *SUC* DNA sequences corresponding to other suc^0 alleles were detected. In other words, the suc^0 alleles (besides $suc2^0$) appear to be "empty sites." We cannot exclude the possibility that these suc^0 alleles contain *SUC* DNA sequences which have diverged so greatly that we cannot detect homology to cloned *SUC2* DNA under the hybridization conditions used; however, this

possibility does not seem likely in view of the fact that homology between *SUC2* and other *SUC*$^+$ genes was detected without difficulty.

The finding that some strains apparently contain no *SUC* DNA sequences at loci at which other closely related strains carry a *SUC*$^+$ gene suggests that movement of *SUC* genes has occurred during the evolution of different *Saccharomyces* strains. Such movement could have been mediated by chromosomal rearrangement or perhaps by the transposition of a specific element carrying a *SUC*$^+$ gene. The *suc*^{0} alleles may simply represent random sequences into which *SUC*$^+$ genes moved or may be preferred integration sites for a specific element. Comparisons of restriction fragments derived from different *SUC*$^+$ loci suggest that, in at least some cases, flanking sequences accompanied the *SUC* structural gene in migrating to a new locus. The *SUC3*, *SUC5*, and *SUC7* genes are found on restriction fragments of the same size in experiments with several enzymes, indicating that the genes are flanked by homologous sequences in which restriction sites have been conserved. The sizes of these conserved fragments suggest that the region of homology is at least 10 kilobase pairs.

It is intriguing that *SUC1* is tightly linked to *MAL1* and that *SUC3* is tightly linked to *MAL3* and *MGL2* (10). The *MAL* (maltose fermentation) and *MGL* (α-methylglucoside fermentation) genes are gene families concerned with sugar utilization and are at least superficially analogous to the *SUC* gene family in organization (11). The close linkage of these genes, and perhaps of other *SUC*, *MAL*, and *MGL* genes as yet unmapped, suggests the possibility that they have been dispersed through the genome as a unit or by related mechanisms resulting in movement to the same chromosomal loci.

The *suc2*^{0} pseudogene found in the FL100 genome has now been partially characterized both physically and genetically. Genetic studies showed that the *suc2*^{0} allele can mutate to confer the ability to produce invertase and ferment sucrose and also that *suc2*^{0} can provide functional information to rescue amber mutations of *SUC2* by recombination (6). The physical studies reported here indicate that the *suc2*^{0} and *SUC2*$^+$ genes do not differ by a major rearrangement, deletion, or insertion. Taken together, the physical and genetic evidence suggests that the *suc2*^{0} allele may be derived from a *SUC2*$^+$ gene by simple point mutations. It is likely that *suc2*^{0} encodes a defective invertase because the lesion maps in the middle of the structural gene by recombination analysis (6; M. Carlson and B. Osmond, unpublished data), and that the gene is transcribed under normal glucose regulation to produce two RNAs of the same sizes as those from the *SUC2*$^+$ gene (3). Whether *suc2*^{0} is simply a mutated gene or whether it is retained as part of the genome to serve some function, perhaps in the evolution of the *SUC* gene family, is not yet known.

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