

ANALYSIS OF CHROMOSOME V AND THE *ILV1* GENE FROM SACCHAROMYCES CARLSBERGENSIS

by

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Chromosome V of the *Saccharomyces carlsbergensis* lager yeast strain 244, a yeast not amenable to tetrad analysis, was analysed genetically in *S. cerevisiae* genetic standard strains. This was achieved by crossing meiotic progeny of the lager yeast with *S. cerevisiae* strains carrying *kar1* as well as the chromosome V markers *can1*, *ura3*, *his1*, *ilv1*, and *rad3*. From the transitory heterokaryons formed we selected strains retaining the characteristics of the recipient strain but having become prototrophic for uracil, histidine, and isoleucine. The resulting strains were disomic for chromosome V, having acquired a chromosome V from *S. carlsbergensis* in addition to the normal *S. cerevisiae* chromosome complement (chromosome addition strains). They were of two classes: In one class the transferred chromosome hardly recombines with the *S. cerevisiae* chromosome V in the region *CAN1* - *RAD3*, which covers almost the entire known map. In the other class, the transferred chromosome recombined at normal levels. We conclude that *S. carlsbergensis* harbors two structurally different chromosomes V; one being homologous and one homoeologous to the *S. cerevisiae* chromosome. By use of the *CAN1* locus, strains were selected which by mitotic chromosome loss had their normal chromosome V substituted by either the homologous or the homoeologous *S. carlsbergensis* chromosome, showing that these chromosomes are fully functional in *S. cerevisiae*. Tetrad analysis of the chromosome substitution strains confirmed the very different genetic behavior of the two *S. carlsbergensis* chromosomes V. In spite of the almost complete absence of recombination between the homoeologous chromosome and the *S. cerevisiae* chromosome, disjunction at meiosis appears normal, as indicated by high spore viability.

Genomic Southern hybridizations with the probes *CAN1*, *URA3*, *CYC7*, and *ILV1* could not detect any nucleotide sequence differences between these loci on the recombining *S. carlsbergensis* chromosome and the *S. cerevisiae* alleles. Under standard stringency (68 °C, 0.1×SSC), hybridization of the probes to DNA from the strain with the homoeologous chromosome was only observed in the case of *ILV1*, where weak hybridization was found, indicating a considerable difference in nucleotide sequence.

To further study the extent of nucleotide sequence inhomology, the two different *ILV1* genes of *S. carlsbergensis*

were cloned in λ vectors. Mapping of 16 restriction enzyme sites showed identity between the allele located on the recombining chromosome and the *ILV1* gene of *S. cerevisiae*. The nucleotide sequence of the *ILV1* gene of the non-recombining chromosome was by restriction site mapping found to be very different from that of the *S. cerevisiae* allele.

1. INTRODUCTION

Saccharomyces carlsbergensis strain 244 is used for lager beer production. Like many other industrial yeasts it does not express a mating type and sporulates poorly with irregular asci containing mostly unviable ascospores. The germinating spores produce strains with either a, α , or non-mating phenotypes. Crosses of such meiotic segregants with mating type to haploid genetic standard strains give hybrids which produce more regular asci, but still a very low proportion of the ascospores is viable (7). Because of this problem we use the single chromosome transfer technique (21) for the genetic characterization of *S. carlsbergensis* instead of conventional tetrad analysis of hybrids between the brewing strain and laboratory tester strains. This technique makes use of the *kar1* mutation (4) which strongly reduces the frequency of karyogamy after zygote formation. It has been found (5, 20) that one or a few chromosomes may be transferred from one nucleus to another in heterokaryons formed in *KAR1* \times *kar1* crosses. Also in the case of crosses between *S. carlsbergensis* and a *kar1* carrying *S. cerevisiae* strain, heterokaryons are formed and single chromosomes may be transferred. Thus, it is possible to isolate a *S. carlsbergensis* chromosome in a genetically well marked *S. cerevisiae* strain, where the chromosome subsequently can be analysed by tetrad analysis (21). With this method we have demonstrated that (i) *S. carlsbergensis* contains two structurally rather different chromosomes III (9, 21), (ii) at least one of the chromosomes is functionally homologous to the *S. cerevisiae* chromosome III, and (iii) recombination with *S. cerevisiae* chromosome III occurs only in a limited region. By molecular hybridization using probes of cloned DNA from *S. cerevisiae* we showed that the non-recombining part of the chromosome contains genes (*HML*, *HIS4*, *LEU2*, and *MAT*) which differ in base sequence from the equivalent *S. cerevisiae* genes (9).

In the present communication we present genetic as well as molecular evidence for the presence in *S. carlsbergensis* of two different chromosomes V. Both types of chromosome are functional in *S. cerevisiae*, i.e. all essential genes normally harbored on chromosome V are also present on the *carlsbergensis* chromosomes. One of the chromosomes is homologous to the *S. cerevisiae* chromosome as normal recombination frequencies are obtained. We call the other chromosome homoeologous to the *S. cerevisiae* chromosome as virtually no recombination with the latter is found. Parts of these results have been presented previously (11, 13, 22, 29).

2. MATERIALS AND METHODS

2.1. Strains, media, and genetic methods

All genetic methods employed in this study have been described in earlier publications (9, 20, 21). Canavanine medium is a synthetic complete medium lacking arginine and containing 60 mg/l canavanine.

A list of yeast strains used in this study is found in Table I.

2.2. Molecular hybridization

Molecular hybridization (Southern blot analysis (28)) was carried out as described by HOLMBERG (9).

2.3. Molecular cloning of *ILV1* genes from *S. carlsbergensis*

The *ILV1* alleles of *S. carlsbergensis* were cloned in bacteriophage λ by standard methods (16). Phage vectors NM781 (19) and L47 (15), respectively, were used in two different experiments. Recombinant phages with *ILV1* sequences were identified by the plaque hybridization method of BENTON and DAVIS (1). The probe was plasmid pC519 containing a 6.1 kb HindIII – Sall DNA fragment with the entire

Table I. Yeast strains used in chromosome transfer and genetic crosses.

Strain	Genotype	Source
Saccharomyces carlsbergensis strains		
244	<i>Saccharomyces carlsbergensis</i> , brewing strain	(7)
C80-CG110	Spore derived clone from strain 244, <i>MATα</i>	(7)
C80-CG7	Spore derived clone from strain 244, <i>MATα</i>	(7)
kar1 recipients		
C80-II-17	<i>MATα can1 ura3 his1 ilv1 rad3 ade2 cyh2 kar1-1</i>	This study
090780-BIV-37	<i>MATα can1 ura3 his1 ilv1 rad3 ade2 cyh2 leu2 kar1-1</i>	“ “
080182-B51	<i>MATα ura3 his1 ilv1 rad3 ade2 cyh2 kar1-1</i>	“ “
Tester strains		
240380B-55	<i>MATα ura3 his1 ilv1 rad3 trp1 leu2</i>	“ “
040380I-56	<i>MATα ura3 his1 ilv1 rad3 leu2</i>	“ “
230480CI-14	<i>MATα ura3 his1 can1 trp1 cyh2</i>	“ “
040380I-47	<i>MATα ura3 ilv1 rad3 trp1 leu2</i>	“ “
Chromosome V addition strains		
IXX18	<i>MATα +/can1 +/ura3 +/his1 +/ilv1 +/rad3 ade2 cyh2 kar1-1</i>	“ “
IXX11	<i>MATα +/can1 +/ura3 +/his1 +/ilv1 +/rad3 ade2 cyh2 kar1-1</i>	“ “
IXX3	<i>MATα can1/can1 +/ura3 +/his1 +/ilv1 +/rad3 ade2 cyh2 kar1-1</i>	“ “
C80-1607	<i>MATα can1/can1 +/ura3 +/his1 +/ilv1 +/rad3 ade2 cyh2 kar1-1</i>	“ “
Chromosome V substitution strains		
C80-1609	<i>MATα CAN1 URA3 HIS1 ILV1 RAD3 ade2 cyh2 kar1-1</i>	“ “
250382-4A-1	<i>MATα can1 URA3 HIS1 ILV1 RAD3 ade2 cyh2 kar1-1</i>	“ “
Transformable strain		
C81-1431	<i>MATα ilv1-23 trp1 ura4 arg4 ade2</i>	(24)

ILV1 gene from *S. cerevisiae* (24) in pBR322 (see Figure 4). Plasmid pCI-1 is pC508 (pBR325-*TRP1-ARS1*) (24), with a 12 kb *Bam*HI fragment containing a *S. carlsbergensis* *ILV1* allele recovered from the recombinant phage λ L47-R6. pC551 is pUR222 with a *S. cerevisiae* 1.077 kb *Pst*I fragment (14) containing 54% of the *ILV1* coding region. pC552 is pUR222 with a *S. cerevisiae* 1.7 kb *Pst*I fragment (24) 5' to the *ILV1* coding region.

2.4. Transformation

For transformation of yeast cells the Li-acetate method of ITO et al. (12) was used. *Escherichia coli* strain HB101 was transformed according to MANIATIS et al. (16).

2.5. Electrophoretic karyotyping

The method described by CARLE and OLSON (3) was used for the electrophoretic separation of chromosomal DNA.

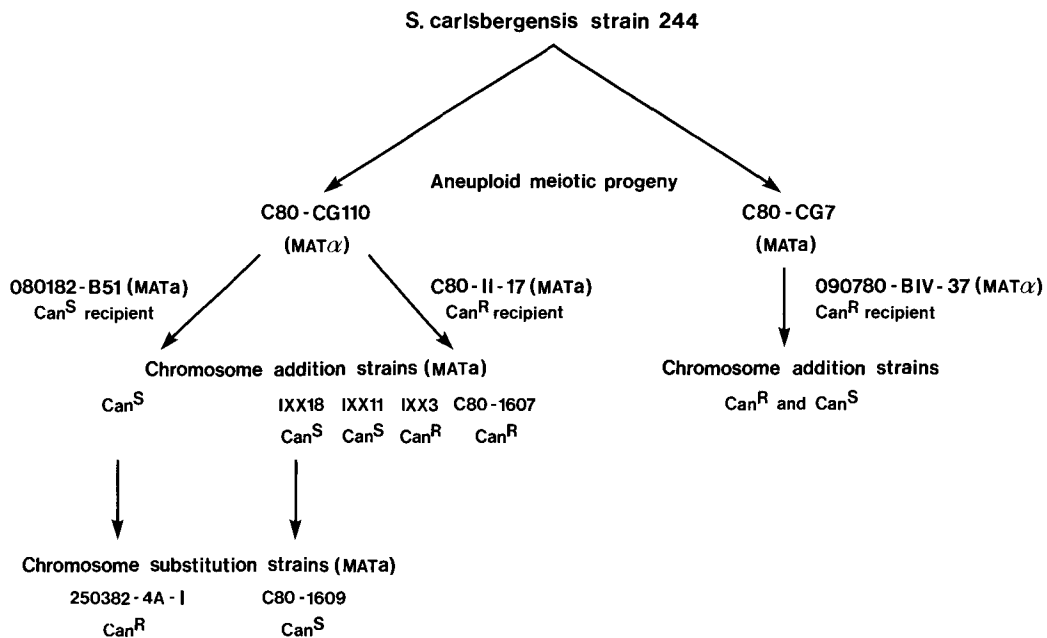


Figure 1. The construction of chromosome V addition and substitution strains from meiotic progeny of the *S. carlsbergensis* lager brewing strain 244.

3. RESULTS

3.1. Transfer of chromosome V

Strain C80-CG110 is a meiotic segregant of the *S. carlsbergensis* brewing strain 244 (see Figure 1). It grows well on both synthetic and complex media and expresses mating type α efficiently. This strain was chosen as donor for the transfer of chromosome V from *S. carlsbergensis* to *S. cerevisiae*. The recipient strain, C80-II-17, is a haploid laboratory strain carrying the *kar1* mutation, several chromosome V markers (*can1*, *ura3*, *ilv1*, *his1*, and *rad3*), a recessive gene for resistance to cycloheximide (*cyh2*) located on chromosome VII and the chromosome XV marker *ade2* which gives a red colony colour. Approximately equal numbers of log phase cells from the two strains were mixed and plated densely on a YPD plate which was left for 6 hours at 30 °C followed by 20 hours at 20 °C. The cells were then washed off the plate, washed twice in water, diluted and plated on YPD as well as on media selective for chromosome V markers: cycloheximide containing synthetic complete medium lacking histidine,

uracil, and isoleucine, singly or in combinations. Products of the transfer of chromosome V should be histidine, uracil and/or isoleucine prototrophic, while retaining cycloheximide resistance and red colony colour. Neither donor nor recipient can grow on these media. The same is true for hybrid progeny resulting from complete karyogamy, since *cyh2* is recessive. Red as well as white colonies appeared after incubation for several days at 30 °C. On the selective plates the red colonies were about 10 times more frequent than the white ones. The latter category was not further analysed; most of them are supposed to be diploids that have become homozygous for *cyh2* by mitotic recombination. True hybrids between the donor and the recipient strains form white colonies on YPD that are bigger than the white colonies of the donor strain. This makes it possible to calculate the frequency of red His^+ , Ura^+ , Ile^+ , Cyh^R colonies relative to true hybrids. This frequency was found to be 2×10^{-3} . In a control experiment involving only *S. cerevisiae* strains this frequency was in the same order, 1.2×10^{-3} .

Four hundred and fifty-eight red colonies were isolated from the cycloheximide containing plates and tested for auxotrophic requirements, canavanine resistance and mating type. They were all His⁺, Ile⁺, and Ura⁺, no matter which of the media they were selected on. Ninety percent of them had retained the mating type of the recipient strain and almost all of the remaining 10% were non-maters and non-sporulators. Unexpectedly, a large fraction (38%) were canavanine resistant, a result analyzed in 3.2. The red cycloheximide resistant colonies have received genetic information from *S. carlsbergensis* making them prototrophic for histidine, uracil, and isoleucine, while they for the *ade2* and *cyh2* genes, and most often also for the *MAT* gene, have retained the phenotype of the recipient strain. Since the mutations complemented in the recipient strain are all located on the *S. cerevisiae* chromosome V, the prototrophic colonies were supposed to be chromosome addition strains, i.e. they are carrying a *S. cerevisiae* complement with an additional chromosome V from *S. carlsbergensis*. This notion was supported by the fact that reversion to auxotrophy for one of the three nutrients was always associated with a reversion for the two others. Such reversion by mitotic chromosome loss was observed with a frequency of approximately 1% after growth in non-selective medium for 8-10 generations.

Very similar results were obtained when strain C80-GC7, another meiotic offspring of strain 244, was used as a donor (Figure 1). In this case, strain 090780-BIV-37 was the recipient and again both Can^S and Can^R colonies were found among the histidine, isoleucine, and uracil prototrophs.

3.2. Tetrad analysis of presumptive chromosome addition strains

To further investigate the nature of the prototrophic colonies described above, we crossed two Can^S (strains IXX18 and IXX11) and two Can^R isolates (strains IXX3 and C80-1607) (see Figure 1) to chromosome V tester strains. The resulting diploids, presumed to be trisomic for chromosome V, were sporulated and subjected to tetrad analysis. The two Can^S clones were

both crossed to strain 240380B-55. The results are shown in Table II. Except for *can1*, which will be dealt with in more detail below, all markers investigated segregated 2:2. The absence of 1:3 segregation for any of the chromosome V genes indicates that the *S. carlsbergensis* chromosome did not participate in any cross-over (cf. ref. 6). That this is indeed the case is shown by the distribution of different tetrad types. Note the almost complete absence of TT and NPD types of asci for the markers *HIS1*, *ILV1*, and *URA3*. The genes appear to be tightly linked and, except for one case, no recombination takes place between the *URA3*, *HIS1*, and *ILV1* genes introduced from *S. carlsbergensis* and the equivalent *S. cerevisiae* genes on chromosome V of the tester or recipient strain.

In the crosses in Table II, the *can1* gene was difficult to score because of the presence of intermediate phenotypes. Nine tetrads of the upper cross were more closely investigated for the segregation of *can1* by following the growth after replica plating to canavanine medium. For each of all 9 tetrads, it was found that one His, Ura, and Ile auxotrophic spore gave rise to a clone which was classified as resistant after 24 hours, while cells from a second spore needed two days to grow up. The two remaining spores were canavanine sensitive. The slowly emerging resistant cells were all coming from spores that were originally scored as His⁺, Ile⁺, and Ura⁺, but testing of the phenotypes of the cells that came up on canavanine showed that they had now become auxotrophic for all three nutrients. These resistant clones were thus all produced from disomic strains by mitotic chromosome loss of the *S. carlsbergensis* *CAN1* gene and subsequent selection on the canavanine plates. In summary, the results show that *CAN1* and *URA3*, *HIS1* and *ILV1* are linked also in *S. carlsbergensis* and that strains IXX18 and IXX11 contain an additional chromosome from this species not recombining with the normal *S. cerevisiae* chromosome V.

Results from tetrad analysis of the two canavanine resistant isolates (IXX3 and C80-1607) are shown in Tables III and IV. In crosses involving the *HIS1* tester strain (Table III), the segregation of *HIS1* shows a significant number of 4:0 asci, while the rest of the chromosome V

Table II. Tetrad analysis of two canavanine sensitive chromosome addition strains (crosses *MATa*⁺/*can1*⁺/*ura3*⁺/*his1*⁺/*ilv1*⁺/*rad3 ade2 cyh2 kar1* × *MATα CAN1 ura3 his1 ilv1 rad3 trp1 leu2*).

IXX18 × 240380B-55					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>			49		
<i>ILV1:ilv1</i>			49		
<i>URA3:ura3</i>			49		
<i>TRP1:trp1</i>			49		
<i>LEU2:leu2</i>			49		
<i>ADE2:ade2</i>			49		
<i>MATa:MATα</i>			49		
		PD	NPD	TT	
<i>ILV1:HIS1</i>		49	0	0	
<i>HIS1:URA3</i>		48	0	1	
<i>URA3:TRP1</i>		23	24	2	
IXX11 × 240380B-55					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>			16		
<i>URA3:ura3</i>			16		
<i>ILV1:ilv1</i>			16		
<i>ADE2:ade2</i>			16		
<i>TRP1:trp1</i>			16		
<i>LEU2:leu2</i>			16		
<i>MATa:MATα</i>			16		
		PD	NPD	TT	
<i>ILV1:HIS1</i>		16	0	0	
<i>HIS1:URA3</i>		16	0	0	
<i>URA3:TRP1</i>		11	5	0	

markers segregate normally. This shows that the diploid contained two copies of *HIS1* (one from 040480I-47 and one from *S. carlsbergensis*) and one *his1* (from the *kar1* recipient C80-II-17). The distribution of tetrad types shown in Tables III and IV makes it clear that the *HIS1*, *URA3*, *ILV1*, and *RAD3* wild-type genes introduced from *S. carlsbergensis* are indeed linked. However, in this case the introduced chromosome is able to recombine with a *S. cerevisiae* chromosome. The genetic distances observed between these genes are similar to what is observed in crosses between laboratory strains (18) and fit also well with the distances found in the crosses performed to construct the recipient and tester

strains used in this study. It should be pointed out that these distances are determined in a trisomic diploid. As was found in the crosses of the two canavanine sensitive, primary isolates, the *can1* gene was difficult to score on the canavanine medium. In this case we suspected the presence of two copies of the *can1* gene in strains IXX3 and C80-1607, as they express the recessive phenotype (*can1*) of the recipient strain. Complete absence of a *CAN1* locus as a result of e.g. a translocation would also result in a canavanine resistant chromosome addition strain under the present conditions. Segregation of two *can1* and one *CAN1* gene in the trisomic diploids would result in a predominantly 2:2

Table III. Tetrad analysis of two canavanine resistant chromosome addition strains (crosses *MAT α can1/can1⁺ura3⁺/his1⁺/ilv1⁺/rad3 ade2 cyh2 kar1* \times *MAT α CAN1 ura3 HIS1 ilv1 rad3 trp1 leu2*)

IXX3 \times 0403801-47					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>	3	3	3		
<i>ILV1:ilv1</i>			9		
<i>URA3:ura3</i>			9		
<i>RAD3:rad3</i>			8	1	
<i>TRP1:trp1</i>			9		
<i>LEU2.leu2</i>			9		
<i>ADE2:ade2</i>			9		
		PD	NPD	TT	
<i>URA3:ILV1</i>		3	1	5	
<i>ILV1:RAD3</i>		2	1	5	
<i>URA3:TRP1</i>		2	4	3	
C80-1607 \times 0403801-47					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>	4	7	4		
<i>ILV1:ilv1</i>			15		
<i>URA3:ura3</i>			15		
<i>RAD3:rad3</i>			13	2	
<i>TRP1:trp1</i>		1	14		
<i>ADE2:ade2</i>			15		
		PD	NPD	TT	
<i>URA3:ILV1</i>		4	2	9	
<i>ILV1:RAD3</i>		3	0	10	
<i>URA3:TRP1</i>		4	7	3	

distribution of sensitive to resistant colonies. This was also observed in most tetrads (Table IV), but this cross also produced many tetrads showing the ratio of one Can^R to three Can^S, as well as asci with four Can^S spores. Such a segregation should not be compatible with the hypothesis of the presence of two *can1* genes. However, the canavanine resistance introduced from *S. carlsbergensis* is not as strong as the one from *S. cerevisiae*. Therefore, some slowly growing *can1* colonies will be scored as Can^S, explaining the presence of the 4:0 and 3:1 asci. The distribution of tetrad types for *CAN1-URA3* in the cross described in Table IV does not seem to indicate linkage of the genes as the number of NPD tetrads (2 Ura⁺Can^S:2 Ura⁻Can^R) is too high. However, in this particular cross we fol-

lowed the segregation of two *S. cerevisiae* chromosomes, marked with *can1 ura3* (recipient) and *CAN1 ura3* (tester), together with a *S. carlsbergensis* chromosome carrying *URA3* and, presumably, *can1*. With this distribution of genes between the different chromosomes, NPD tetrads will appear not only as a consequence of double cross-over, but also when for instance the *S. carlsbergensis* chromosome in the first meiotic division segregates together with a *S. cerevisiae* chromosome carrying *CAN1*. Although the meiotic analysis of IXX3 and C80-1607 did not prove that *can1* and *URA3* of *S. carlsbergensis* origin were linked, we consider this to be the most likely explanation for the high number of Can^R colonies obtained after transfer of chromosome V from *S. carlsbergensis*.

Table IV. Tetrad analysis of the canavanine resistant chromosome addition strain C80-1607 after crossing to 240380B-55 (*MAT α* *CAN1 ura3 his1 ilv1 rad3 trp1 leu2*).

Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>			58	4	
<i>ILV1:ilv1</i>			61	1	
<i>URA3:ura3</i>		1	60	1	
<i>RAD3:rad3</i>		2	57	3	
<i>CAN1:can1</i>	4	9	47	2	
<i>TRP1:trp1</i>			62		
<i>LEU2:leu2</i>			62		
<i>ADE2:ade2</i>			62		
<i>MATα.MATα</i>			62		
		PD	NPD	TT	cM ^a
<i>HIS1:ILV1</i>		37	0	21	18
<i>HIS1:URA3</i>		30	7	22	54
<i>ILV1:RAD3</i>		27	3	26	39
<i>URA3:CAN1</i>		12	9	26	n.l.
<i>URA3:TRP1</i>		26	23	11	9 ^b

n.l. = no linkage detectable

^aGenetic distances were calculated according to the formula of PERKINS (23).

^bDistance between *URA3* and the centromere.

In summary, the meiotic analysis of colonies carrying *HIS1*, *URA3*, and *ILV1* from the brewing strain showed that they are disomic for chromosome V and that two different types of this chromosome are present in *S. carlsbergensis*.

3.3. Construction and tetrad analysis of chromosome substitution strains

In order to establish if the two different types of chromosome V present in *S. carlsbergensis* C80-CG1 10 are functionally homologous to the *S. cerevisiae* chromosome V and to be able to measure genetic distances in euploid strains, we constructed strains where the normal *S. cerevisiae* chromosome V was substituted by a chromosome from *S. carlsbergensis*. The strategy utilized the location of the *CAN1* gene on chromosome V. The disomic strain IXX18 contains a mutant *can1* gene from the recipient strain in addition to a wild-type *CAN1* gene from *S. carlsbergensis*. As the gene giving resistance to canavanine is recessive, IXX18 is sensitive, but resistant subclones will be produced if the *CAN1*

gene is lost by either mitotic recombination or chromosome loss. Thus, if a colony is replica plated to canavanine medium, no growth is seen after 24 hours of incubation but after a further 24 hours, the few cells that have lost the carlsbergensis chromosome (about 1%) will have gone through a sufficient number of cell divisions to allow the detection of growth. If instead the *can1* gene had been lost in the cell that formed the original colony, resistant growth can only appear in the much rarer cases of a mutation. To screen for the appearance of a chromosome substitution strain by the spontaneous loss of the *can1* carrying *S. cerevisiae* chromosome V, IXX18 was grown in YPD to late logarithmic phase and plated on YPD at a density of approximately 400 colonies per plate. After three days of growth the colonies were replica plated to canavanine medium. Colonies that did not give rise to growth after 4 days on canavanine were selected and retested to confirm the lack of ability to produce Can^R papillae. One such isolate, C80-1609 (see Figure 1), was crossed to tester strains that were wild-type for either *HIS1* or *ILV1* and a tetrad analysis was carried out.

Table V. Tetrad analysis of the canavanine sensitive chromosome V substitution strain C80-1609 (*MAT α CAN1 URA3 HIS1 ILV1 RAD3 ade2 cyh2 kar1*).

C80-1609 \times 040380I-47 (<i>MATα ura3 ilv1 rad3 trp1 leu2</i>).					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>	13				
<i>ILV1:ilv1</i>			13		
<i>URA3:ura3</i>			13		
<i>RAD3:rad3</i>			13		
<i>TRP1:trp1</i>			13		
<i>LEU2:leu2</i>			13		
<i>ADE2:ade2</i>			13		
<i>MATα:MATα</i>			13		
		PD	NPD	TT	
<i>ILV1:RAD3</i>		13	0	0	
<i>ILV1:URA3</i>		13	0	0	
<i>URA3:TRP1</i>		6	6	1	
C80-1609 \times 230480CI-14 (<i>MATα can1 ura3 his1 trp1 cyh2</i>)					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>	2	1	48		
<i>ILV1:ilv1</i>	51				
<i>URA3:ura3</i>	2		49		
<i>CAN1:can1</i>	2		49		
<i>TRP1:trp1</i>			51		
<i>ADE2:ade2</i>			51		
<i>MATα:MATα</i>			51		
		PD	NPD	TT	
<i>URA3:CAN1</i>		48	0	1	
<i>HIS1:URA3</i>		48	0	0	
<i>URA3:TRP1</i>		23	24	3	

Both these genes are located on the right arm of chromosome V while *CAN1* is on the left arm. The complete absence of segregation of *his1* or *ilv1* in these crosses (Table V) demonstrates that chromosome V of the *kar1* recipient strain C80-II-17 has indeed been lost. Thus, strain C80-1609 is a substitution strain with a *S. carlsbergensis* chromosome instead of a *S. cerevisiae* chromosome V. The strain grows normally on complete, as well as minimal medium, showing that all essential genes located on chromosome V are also present on the substituting chromosome. The presence of *RAD3* was not investigated in the disomic strain IXX18, but the data shown in Table V (upper cross) and

Table VI demonstrate that also this chromosome V gene is present and functional on the non-recombining chromosome V of *S. carlsbergensis*.

C80-1609 is mitotically unstable and has a strong tendency to produce clones with two chromosomes V. This is seen in the lower cross presented in Table V, where two out of the 51 analysed asci are found to segregate 4:0 for the chromosome V markers because of the presence of two *S. carlsbergensis* chromosomes in the diploid. Possibly, some function important for growth rate or viability is encoded by a gene located on chromosome V and the *S. carlsbergensis* gene provides limiting function if it is not

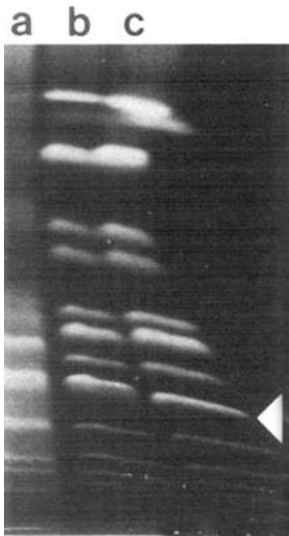


Figure 2. Electrophoretic chromosome separation of DNA from *S. carlsbergensis* and *S. cerevisiae* strains. DNA from *S. carlsbergensis* strain C80-CG7 (lane a), the chromosome substitution strain C80-1609 (lane b) and the *S. cerevisiae* strain C80-II-17 (lane c) was extracted and separated according to the method of CARLE and OLSON (3). An arrow marks the position of chromosome V and VIII in *S. cerevisiae* (3).

present in double dosis.

An electrophoretic chromosome separation according to CARLE and OLSON (3) was made with DNA from strains C80-CG7, C80-1609 and C80-II-17 (Figure 2). Although the DNA of the *S. carlsbergensis* strain has not been especially well resolved in this experiment, it is evident from the figure that several of the chromosomal bands have a different mobility compared to those of the *S. cerevisiae* strain C80-II-17. As expected, the band patterns of the latter strain and the substitution strain (C80-1609) are almost completely identical. Only in the case of the band representing chromosomes V and VIII (3), indicated by an arrow in Figure 2, is it possible to observe a difference. Comparing the band pattern of all three strains, it is evident that chromosome V of *S. carlsbergensis* (present in C80-CG7 and C80-1609) has a slightly lower electrophoretic mobility than chromosome V of *S. cerevisiae*. Furthermore, the intensity of the chromosome V band of the substitution strain is

consistent with the notion that this chromosome is present in two copies. Chromosome VIII of *S. cerevisiae* is seen to migrate slightly faster than the *carlsbergensis* chromosome V in the substitution strain.

The isolate of C80-1609 in our strain collection was later found to have become disomic for chromosome V. We have now constructed a new strain monosomic for chromosome V by crossing C80-1609 to a canavanine resistant strain (strain 230480-CI-14). After sporulation and dissection, tetrads with four living spores were selected and tested for auxotrophic requirements and canavanine resistance. As expected, all four spores were *His*⁺, *Ura*⁺, *Ile*⁺, and canavanine sensitive after 24 hours of incubation. However, further incubation of the canavanine containing plates showed that two of the spores of each tetrad produced resistant papillae. The papillae were *His*⁻, *Ura*⁻, and *Ile*^v and were thus signalling that these two spores, in addition to the *S. carlsbergensis* chromosome V from C80-1609, also contained the chromosome V of strain 230480-CI-14. The other two spores of the tetrads did not, even after prolonged incubation, produce any canavanine resistant growth and were therefore assumed to be monosomic for chromosome V of *S. carlsbergensis*. Subsequent tetrad analysis confirmed this notion and showed that the new strains retained the properties of the original isolate of C80-1609, including the pronounced mitotic instability (data not shown).

The linkage data shown in Tables V and VI agree completely with what was found for the disomic parental strain IXX18, in that all chromosome V markers appear tightly linked. Among the 87 tetrads of the three crosses, only one ascus is found with recombination between the lager yeast and the laboratory yeast chromosomes. This low frequency of recombination is not due to the presence of inversions in the *S. carlsbergensis* chromosome as the spore viability in each of the crosses was between 80 and 90%.

The *CAN1* gene was also used for the construction of a substitution with the recombining chromosome. The strains IXX3 and C80-1607, which harbor this chromosome, show a canavanine resistant phenotype, as neither the recipient strain nor the recombining *S. carlsbergensis*

Table VI. Tetrad analysis of the canavanine sensitive chromosome V substitution strain C80-1609 after crossing to 040380I-56 (*MAT α* *CAN1* *ura3* *his1* *ilv1* *rad3* *leu2*).

Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>			23		
<i>ILV1:ilv1</i>			23		
<i>URA3:ura3</i>			23		
<i>RAD3:rad3</i>			23		
<i>LEU2:leu2</i>			21	2	
<i>ADE2:ade2</i>			23		
<i>MATα:MATα</i>			23		
		PD	NPD	TT	
<i>HIS1:ILV1</i>		23			
<i>ILV1:RAD3</i>		23			
<i>HIS1:URA3</i>		23			
<i>URA3:LEU2</i>		9	8	4	

chromosome carry information for a functional arginine permease. To obtain a disomic strain heterozygous for *can1*, we performed a new chromosome transfer experiment with a recipient strain (080182B-51) which was similar to the strain used in the first experiment, but this time canavanine sensitive. Again, red, cycloheximide resistant, His⁺, Ura⁺, and Ile⁺ colonies were selected. Approximately 50% of these colonies gave spontaneously rise to canavanine resistant papillae after growth on canavanine medium, indicating that they contained cells with a *can1* gene from *S. carlsbergensis*. Ten such colonies were picked and grown to early stationary phase and plated on YPD. After 3 days of growth at 30 °C they were replica plated to canavanine medium and after 24 hours of incubation the plates were inspected for the appearance of colonies with vigorous growth. All ten selected clones produced at least one such colony which was resistant to canavanine and one colony from each clone was purified and crossed to strain 040380I-47. Tetrad analysis showed that four of the isolates were chromosome substitution strains that had become canavanine resistant by loss of the *S. cerevisiae* chromosome with *CAN1*. Two of the remaining six strains could not be analysed because of poor spore viability. The rest were chromosome addition strains which probably had become resistant by mitotic

recombination leading to homozygosity for *can1*. An extended tetrad analysis was carried out on one of the chromosome substitution strains (strain 250382-4AI) (see Figure 1). As can be seen in Table VII (upper cross) no segregation of *his1* is seen when 250383-4AI is crossed to a tester strain that is wild-type for this gene. As *HIS1* and *CAN1* map on each side of the centromere, this provides strong evidence for the loss of chromosome V of the recipient strain. The results from a cross to a tester strain, recessive for all five chromosome V markers used in this study, are given in the lower half of Table VII. From the data in the table it can be seen that the distances measured between chromosome V loci in strain 250382-4A-I resemble the distances obtained in pure *S. cerevisiae* crosses. Comparing the results obtained from the disomic strain C80-1607 and the substitution strain, a considerable difference in the region *ILV1-RAD3* (Tables VII and IV) is seen. While no linkage can be detected between these markers in the substitution strain (distance > 50 cM), the low number of NPD tetrads in the case of the chromosome addition strain clearly indicates that the genes are on the same chromosome. The other gene distances are not influenced to any considerable extent by the presence of a third chromosome.

Table VII. Tetrad analysis of the canavanine resistant chromosome V substitution strain 250382-4A-I (*MAT α can1 URA3 HIS1 ILV1 RAD3 ade2 cyh2 kar1*).

250382-4A-I \times 040380I-47 (<i>MATα ura3 ilv1 rad3 trp1 leu2</i>)					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>	50				
<i>ILV1:ilv1</i>		2	48		
<i>URA3:ura3</i>			50		
<i>CAN1:can1</i>		1	46	3	
<i>RAD3:rad3</i>			50		
<i>LEU2:leu2</i>			50		
<i>ADE2.ade2</i>			50		
		PD	NPD	TT	cM ^a
<i>URA3:CAN1</i>		20	1	25	34
<i>URA3:ILV1</i>		12	4	32	58
<i>ILV3:RAD3</i>		7	8	32	n.l.
<i>URA3:TRP1</i>		23	21	6	6 ^b
250382-4A-I \times 240380B-55 (<i>MATα ura3 his1 ilv1 rad3 trp1 leu2</i>)					
Marker	Segregation				
	4:0	3:1	2:2	1:3	0:4
<i>HIS1:his1</i>		2	125	1	
<i>ILV1:ilv1</i>		2	126		
<i>URA3:ura3</i>		1	127		
<i>CAN1:can1</i>		3	124	1	
<i>RAD3:rad3</i>		2	125	1	
<i>TRP1:trp1</i>		3	124	1	
<i>LEU2:leu2</i>		7	120	1	
<i>ADE2.ade2</i>			128		
<i>MATα:MATα</i>			128		
		PD	NPD	TT	cM ^a
<i>HIS1:ILV1</i>		73	1	49	22
<i>ILV1:RAD3</i>		22	23	78	n.l.
<i>HIS1:URA3</i>		43	6	76	45
<i>URA3:CAN1</i>		64	3	56	30
<i>URA3:TRP1</i>		58	44	21	9 ^b

n.l. = no linkage detectable

^aGenetic distances were calculated according to the formula of PERKINS (23).

^bDistance between *URA3* and the centromere.

3.4. Molecular analysis of four loci on chromosome V

In two earlier publications (9, 21) we have shown that the absence of recombination between a region of chromosome III from *S. carlsbergensis* strain 244 and standard laboratory strains is correlated with nucleotide sequence inhomology. In contrast, genes mapping in the region where recombination takes place are

indistinguishable from the genes of the laboratory strains. To investigate whether there is an analogous difference between the non-recombining and recombining chromosomes V of *S. carlsbergensis* we analysed four different loci, *ilv1*, *ura3*, *cyc7*, and *can1*, by molecular hybridization according to the technique of SOUTHERN (28). DNA from strains C80-II-17 (recipient), 244 (*S. carlsbergensis*), C80-1609

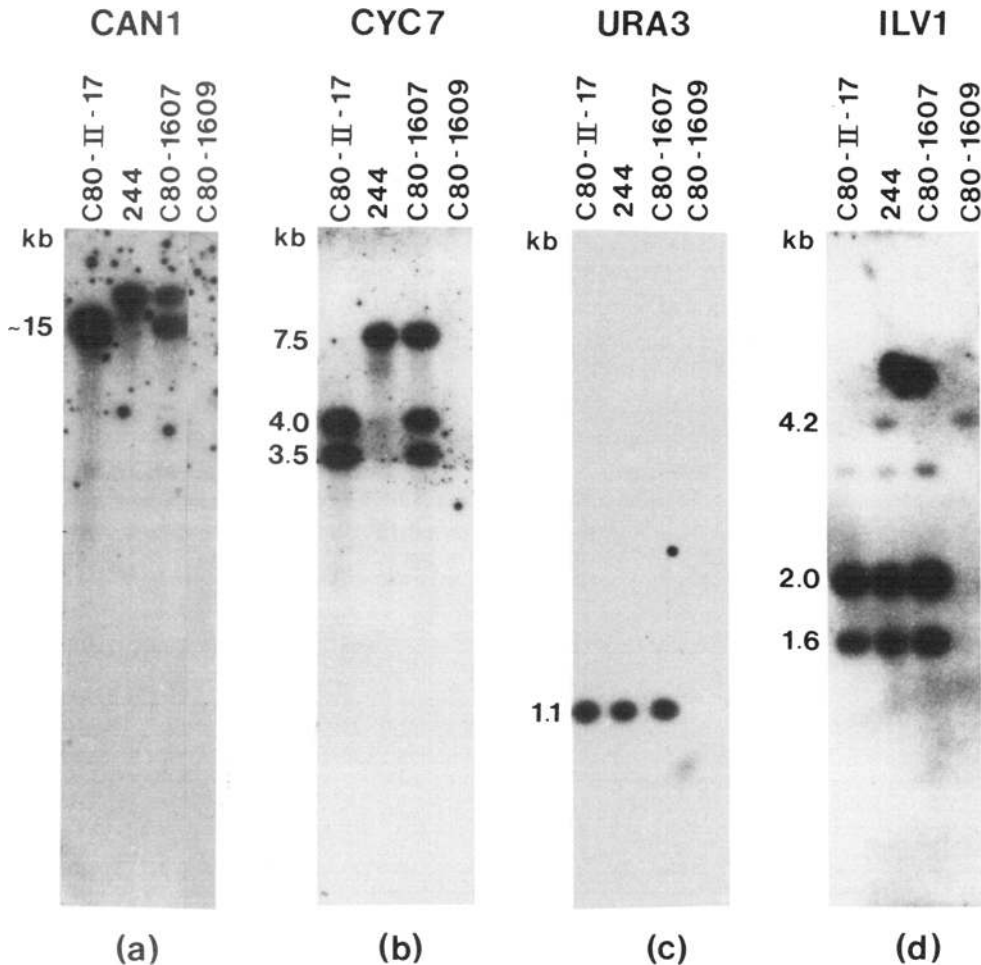


Figure 3. Molecular hybridization analysis of four chromosome V genes. DNA from strains 244 (*S. carlsbergensis*), C80-II-17 (*kar1* recipient *S. cerevisiae*), C80-1607 (chromosome addition strain with recombining chromosome) and C80-1609 (chromosome substitution strain with non-recombining chromosome) was digested with restriction enzymes EcoRI (a and d) or HindIII (b and c). The resulting fragments were separated by electrophoresis, transferred to nitrocellulose membranes, and hybridized to the following ³²P-labelled probes: (a) *CAN1*, a 0.7 kb BamHI-EcoRI fragment subcloned from plasmid TLC-1 (2). (b) *URA3*, a 1.1 kb HindIII fragment from plasmid MB1068. (c) *CYC7*, plasmid pAB25 (17). (d) *ILV1*, a 6.1 kb HindIII-SalI fragment from plasmid pC519.

Hybridization was in 3×SSC at 68 °C followed by washing in 0.1×SSC at the same temperature.

(substitution strain with non-recombining chromosome), and C80-1607 (addition strain with recombining chromosome) was cut with restriction endonucleases EcoRI, HindIII, BamHI, and PstI. After agarose gel electrophoresis the fragments were transferred to nitrocellulose filters and analysed by molecular hybridization to *in vitro* ³²P-labelled probes. For the *ilv1* locus the

two adjacent fragments (3.2 kb HindIII-HindIII and 2.9 HindIII-SalI) prepared from plasmid pC519 (see Figure 4) were used as probe. The probe for the *ura3* locus was a 1.1 kb HindIII fragment prepared from plasmid MB1068 obtained from T. Petes (University of Chicago, Illinois, USA). The *cyc7* locus was analysed with plasmid pAB25 (kindly supplied by F. SHER-

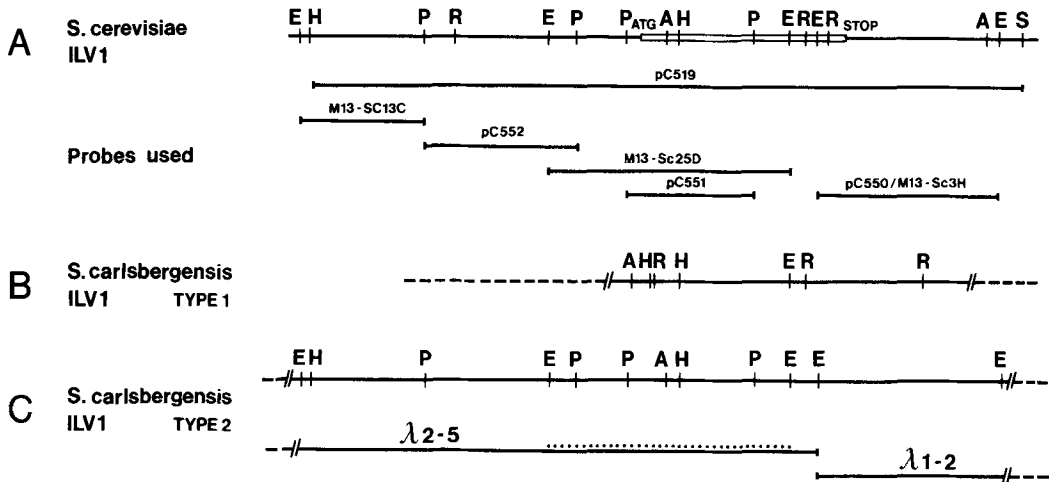


Figure 4. Restriction endonuclease map of the *ILV1* genes of *S. carlsbergensis* and *S. cerevisiae*.

(A): The *ILV1* gene of *S. cerevisiae* (14). The box shows the coding region of the gene. Below the gene are shown the size and location of the probes used for identifying recombinant λ phages and for the localization of the coding region in plasmid pCI-1 as well as in the recombinant phages λ 1-2 and λ 2-5.

(B): The *ILV1* allele (type 1) found on the homeologous chromosome V of *S. carlsbergensis* and cloned in plasmid pCI-1 and phage λ L47-R6. No PstI sites are present in the depicted region.

(C): The *ILV1* allele (type 2) found on the homologous chromosome V of *S. carlsbergensis* and cloned in the phages λ 1-2 and λ 2-5. The extent of these two phages covering the *ILV1* region is depicted below the restriction site map. The region of λ 2-5 which was mapped with enzymes AccI, AhaIII, AvaI, FokI, PvuII and XhoI is marked by a stippled line. The two phages, λ 1-2 and λ 2-5, were not mapped with the enzyme EcoRV.

The following signatures are used: A = AccI; E = EcoRI; R = EcoRV; H = HindIII; P = PstI; S = SalI. No difference in restriction enzyme sites were detected between the cloned fragments of *S. carlsbergensis* *ILV1* type 2 and the *S. cerevisiae* gene.

MAN, University of Rochester, New York, USA). This plasmid contains the *CYC7* gene on a 7.4 kb fragment in pBR322 (17). Finally, a sub-cloned 0.7 kb BamHI-EcoRI fragment from plasmid TLC-1 (2) (kindly supplied by J.R. BROACH, Princeton University, New Jersey, USA) was used for the *can1* region. The results in Figure 3 show that all the probes hybridize very poorly with DNA from strain C80-1609 at the chosen stringency (hybridization at 68 °C in 3×SSC, washings in 0.1×SSC at the same temperature). Only in the case of the *ilv1* probe (Figure 3d), a faint band of 4.2 kb can be seen. The same results are obtained with the three other restriction enzymes used in the experiment (data not shown). In contrast, strong hybridization is obtained with DNA from the three other strains. The patterns obtained for strain 244 probed with *ilv1* DNA contain bands

identical to those of the recipient strain in addition to the faint bands specific for the non-recombining *S. carlsbergensis* chromosome. In the chromosome addition strain C80-1607, only the *S. cerevisiae* like pattern is observed. As mentioned above, the *ura3* probe does not hybridize to strain C80-1609 and the pattern observed with strains 244 and C80-1607 is identical to the pattern of *S. cerevisiae* for all four enzymes tested. In the case of the *cyc7* and *can1* loci, the autoradiograms disclose a slightly altered nucleotide sequence in the *S. cerevisiae* like chromosome of strain 244 (Figures 3a and b). Thus, a HindIII and a PstI site were found to be absent in the region covered by the *cyc7* probe, resulting in the formation of a larger fragment. Polymorphism for the HindIII site has been reported in genetic standard strains (17). As expected, the chromosome addition strain

C80-1607 shows the pattern of the recipient strain as well as that of *S. carlsbergensis*. In the case of *can1*, a difference in restriction enzyme fragment length was observed between the recipient and strain 244 for the enzymes EcoRI (Figure 3a) and HindIII (data not shown). To summarize, the results from the molecular hybridization experiments confirm the genetic data, showing the presence in *S. carlsbergensis* of two structurally different chromosomes V.

3.5. Molecular cloning of the *ILV1* gene of *S. carlsbergensis*

To be able to get a more precise knowledge of the structure of the different wild-type alleles of a gene located on chromosome V, we cloned the two *ILV1* alleles of strain 244. In a first attempt, DNA from this strain was partially digested with EcoRI and ligated into the λ -EcoRI vector NM781 (19). Two recombinant phages, λ 1-2 and λ 2-5, respectively, were recovered with sequences homologous to the *S. cerevisiae* *ILV1* probe pC519 (see Figure 4). One of the recombinant phages, λ 1-2 (Figure 4C) was found to have, in its insert, a 1.5 kb EcoRI fragment equivalent in size and homologous to the EcoRI fragment containing the 3' end of the *ILV1* gene of *S. cerevisiae* (24). Like in *S. cerevisiae*, this fragment did not contain any restriction endonuclease sites for the enzymes PstI and HindIII. The other phage, λ 2-5 (Figure 4C), contained an insert that covered the rest of the gene and several kb upstream of the translation startpoint. This clone contains three EcoRI-HindIII fragments which hybridize to pC519. They are identical, both in size and in the positions of four PstI sites, to three contiguous EcoRI - HindIII fragments of *ILV1* of *S. cerevisiae* (14). Two of the three EcoRI - HindIII fragments cover almost the entire coding region of the *S. cerevisiae* gene. They were isolated by gel electrophoresis and subjected to restriction enzyme mapping with AccI, AhaIII, AvaI, FokI, PvuII, and XhoI. In this region a total of 16 sites for these enzymes are present in the *S. cerevisiae* gene as determined by sequencing (14). All 16 sites were found to be present in the two fragments. Thus, strain 244 contains one allele of the *ILV1* gene which is identical or very closely

related to the *S. cerevisiae* allele. The approximate position of the coding region at the *S. carlsbergensis* *ILV1* gene in Figure 4C was localized by a Southern analysis (28): λ 1-2 and λ 2-5 were treated with the restriction enzymes EcoRI, HindIII, and PstI in single or double digestion and probed with three different ³²P-labelled M13-*ILV1* DNAs. The location of the three probes, M13-Sc13C, M13-Sc25D, and M13-Sc3H are depicted in Figure 4 (14). As expected, M13-Sc25D, which contains most of the *S. cerevisiae* *ILV1* coding region, hybridized to all restriction enzyme fragments with endpoints within the 2.1 kb EcoRI fragment of λ 2-5.

The other *ILV1* allele of strain 244 was cloned from the substitution strain C80-1609. Partially MboI digested DNA was ligated to purified BamHI digested λ L47 arms (15, 16). One recombinant phage, λ L47-R6 was isolated with an insert of approximately 18 kb containing the *ILV1* gene as judged by hybridization to pC519. Further analysis of the insert in this phage showed that the gene was located on an approximately 12 kb BamHI fragment. This fragment was subcloned in plasmid pC508 (pBR325-*TRP1-ARS1*), and the resulting plasmid, pCI-1, was able to transform a *S. cerevisiae* *ilv1* strain (C81-1431) to isoleucine prototrophy with high frequency. Thus, the entire gene is contained on the BamHI fragment. A map of the recognition sites for the enzymes AccI, EcoRI, EcoRV, HindIII, and PstI in the gene is shown in Figure 4B. Colinearity of the type I *ILV1* allele as drawn in Figure 4B was established by probing BamHI, HindIII, EcoRI, and PstI restriction enzyme digests of pCI-1 with labelled pC552, pC551, and pC550 (10) (see Figure 4). The map has been finally positioned relative to the *S. cerevisiae* gene from the conserved locations of an EcoRI, a HindIII, and an EcoRV site in the nucleotide sequence (C. GJERMANSSEN, in preparation).

4. DISCUSSION

The genetic and molecular analysis of the strains obtained from the single chromosome transfer experiments clearly demonstrates the presence of two different chromosomes V in *S. carlsbergensis*. One of these chromosomes appears to be homologous to chromosome V of

laboratory strains in that genetic recombination between them is unimpaired. The data from the molecular hybridization experiments confirm the great similarity between these two chromosomes. It is unexpected that there are similar distances between the markers observed with the recombining chromosome V whether it is analysed in a disomic or trisomic situation, i.e. together with one or two homologous *S. cerevisiae* chromosomes: If it is assumed that in a trisomic strain the total probability of physical exchange between two genes is the same as in a normal diploid, one would expect to register a reduced frequency of recombination in our experimental set up, as recombination between the two *S. cerevisiae* chromosomes can not be seen (both are marked with recessive alleles). As the recombination frequency in the *can1-ilv1* region is normal, it is as if genetic exchange is stimulated in our trisomic strains, with the homologous chromosome V from *S. carlsbergensis*. Such stimulation has previously been found in trisomic *S. cerevisiae* strains by SHAFER et al. (27). Only in the case of the region *ilv1-rad3* do we observe a strong map contraction when measured in a trisomic strain. Possibly this reflects a different structure in the region leading to a preferential pairing between the two *S. cerevisiae* homologues and thereby a reduced possibility for the *S. carlsbergensis* chromosome to recombine with one of the *S. cerevisiae* chromosomes.

The other chromosome V of *S. carlsbergensis* is also functional in the recipient strains, but quite different in physical structure as well as in genetic properties. This homoeologous chromosome does not recombine with the laboratory strain chromosome V and the molecular analysis shows that its nucleotide sequence is different. Since it is generally believed that chromosome pairing and crossing-over are needed for proper disjunction at meiosis, it is noteworthy that a high spore viability is obtained in crosses involving a strain carrying the homoeologous chromosome and genetic standard strains. Possibly, pairing and chiasma formation take place outside the most distal markers (*rad3* and *can1*) used in this investigation. In the case of chromosome III from *S. carlsbergensis*, genetic recombination with *S. cerevisiae* chromosome III only

takes place in a distal region of the right arm (21) where the nucleotide sequence is homologous to the *S. cerevisiae* chromosome (9). In spite of the reduced recombination between these chromosomes disjunction seems to be unimpaired as judged by the high frequency of asci with 3 or 4 viable spores (21). That only a small region of homology is needed for proper disjunction of chromosomes has been demonstrated for the mammalian sex chromosomes (26). Moreover, recombination takes place in this homologous region with very high frequency (26). Another possibility is that, if just a single pair of chromosomes does not recombine, they can disjoin properly. D. DAWSON and J. SZOSTAK (personal communication) have found that in vitro constructed minichromosomes with little homology can disjoin efficiently. Such a phenomenon may be related to distributive pairing in *Drosophila* (8). As was the case with chromosome III absence of recombination in *S. carlsbergensis* chromosome V is correlated with a difference in nucleotide sequence between the involved chromosomes. The type I *ILV1* gene in plasmid pCI-1 has been sequenced (C. GJERMANSEN, in preparation). In the coding region the homology with the *S. cerevisiae* gene is approximately 80%, with stretches of up to 29 nucleotides of perfect homology. The results of the molecular hybridization with *CAN1*, *CYC7*, and *URA3* suggest that a similar degree of homology is found also in the rest of the chromosome. Apparently, this is not enough to promote meiotic recombination to any reasonable level. It is unknown whether the absence of recombination is caused by reduced or absent synapsis of the chromosomes, or by a hindrance in formation of sufficiently long stretches of heteroduplex DNA.

The presence of a pair of homoeologous chromosomes V in *S. carlsbergensis* resembles the situation described for chromosome III (9, 21). In the present study, genetic and molecular data have shown the presence of two different chromosomes, one which is *S. cerevisiae* like, and one which is so structurally different that no recombination takes place over the entire known genetic map. Such homoeologous pairs of chromosomes have also been found for chromosomes XII and XIII (25) as well as for

chromosome VII (unpublished data) and X (G.P. CASEY, Carlsberg Res. Commun., this issue). All these observations agree with our earlier proposition that the Carlsberg lager yeast is composed of two different *Saccharomyces* genomes (22, 25), and that the low degree of recombination between its set of homoeologous chromosomes could be a cause of improper disjunction at meiosis and the production of aneuploid spores with low viability.

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REFERENCES

1. BENTON, W D & R. W. DAVIS: Screening λ gt recombinant clones by hybridization to single plaques in situ. *Science* 196, 180-182 (1977)
2. BROACH, J. R., J. N. STRATHERN & J. B. HICKS: Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. *Gene* 8, 121-133 (1979)
3. CARLE, G F. & M V. OLSON: An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci. USA* 82, 3756-3760 (1985)
4. CONDE, J. & G. R. FINK: A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc. Natl. Acad. Sci. USA* 73, 3651-3655 (1976)
5. DUTCHER, S. K.: Internuclear transfer of genetic information in *kar1-1/KAR1* heterokaryons in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 1, 245-253 (1981)
6. FINCHAM, J. R. S., P. R. DAY & A. RADFORD: *Fungal Genetics*. Fourth edition. Blackwell Scientific Pub-

- lications. Oxford, London, Edinburgh and Melbourne (1979)
7. GJERMENSEN, C & P. SIGSGAARD: Construction of a hybrid brewing strain of *Saccharomyces carlsbergensis* by mating of meiotic segregants. *Carlsberg Res. Commun.* 46, 1-11 (1981)
8. GRELL, R. F.: A new hypothesis on the nature and sequence of meiotic events in the female of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 48, 165-172 (1962)
9. HOLMBERG, S.: Genetic differences between *Saccharomyces carlsbergensis* and *S. cerevisiae* II. Restriction endonuclease analysis of genes in chromosome III. *Carlsberg Res. Commun.* 47, 233-244 (1982)
10. HOLMBERG, S., M. C. KIELLAND-BRANDT, T. NILSSON-TILGREN & J. G. L. PETERSEN: The *ILV1* gene in *Saccharomyces cerevisiae*: 5' and 3' end mapping of transcripts and their regulation. *Carlsberg Res. Commun.* 50, 163-178 (1985)
11. HOLMBERG, S., J. G. L. PETERSEN, T. NILSSON-TILGREN, C. GJERMENSEN & M. C. KIELLAND-BRANDT: Biosynthesis of isoleucine and valine in *Saccharomyces*. Gene structure and regulation. In: *Biological Research on Industrial Yeasts*, vol. 1, G. G. Stewart, I. Russel, R. D. Klein & R. R. Hiesch, eds. Uniscience Series, CRC press, Boca Raton (in press)
12. ITO, H., Y. FUKADA, K. MURATA & A. KIMURA: Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153, 163-168 (1983)
13. KIELLAND-BRANDT, M. C., C. GJERMENSEN, T. NILSSON-TILGREN, J. G. L. PETERSEN, S. HOLMBERG & P. SIGSGAARD: Genetics of a lager production yeast. *Tech. Q. MBAA* 18, 185-187 (1981)
14. KIELLAND-BRANDT, M. C., S. HOLMBERG, J. G. L. PETERSEN & T. NILSSON-TILGREN: Nucleotide sequence of the gene for threonine deaminase (*ILV1*) of *Saccharomyces cerevisiae*. *Carlsberg Res. Commun.* 49, 567-575 (1984)
15. LOENEN, W A. M. & W J. BRAMMAR: A bacteriophage lambda vector for cloning large DNA fragments made with several restriction enzymes. *Gene* 10, 249-259 (1980)
16. MANIATIS, T., E F. FRITSCH & J. SAMBROOK: *Molecular cloning. A laboratory manual*. Cold Spring Harbor Laboratory, New York (1982)
17. MCKNIGHT, G L, T S. CARDILLO & F. SHERMAN: An extensive deletion causing overproduction of yeast iso-2-cytochrome c. *Cell* 25, 409-419 (1981)
18. MORTIMER, R K & D. SCHILD: Genetic map of *Saccharomyces cerevisiae*. *Microbiol. Rev.* 44, 519-571 (1980)
19. MURRAY, N E., W J. BRAMMAR & K. MURRAY: Lambdoid phages that simplify the recovery of in

- vitro recombinants. *Mol. Gen. Genet.* 150, 53-61 (1977)
20. NILSSON-TILLGREN, T., J. G. L. PETERSEN, S. HOLMBERG & M. C. KIELLAND-BRANDT: Transfer of chromosome III during *kar* mediated cytoduction in yeast. *Carlsberg Res. Commun* 45, 113-117 (1980)
 21. NILSSON-TILLGREN, T., C. GJERMANSSEN, M. C. KIELLAND-BRANDT, J. G. L. PETERSEN & S. HOLMBERG: Genetic differences between *Saccharomyces carlsbergensis* and *S. cerevisiae*. Analysis of chromosome III by single chromosome transfer. *Carlsberg Res. Commun.* 46, 65-76 (1981)
 22. NILSSON-TILLGREN, T., M. C. KIELLAND-BRANDT, S. HOLMBERG, J. G. L. PETERSEN & C. GJERMANSSEN: Is lager yeast a species hybrid? Utilization of intrinsic genetic variation in breeding. In: *Genetics of Industrial Microorganisms*. Y. Ikeda & T. Beppu, eds., pp. 143-147, Kodansha Ltd., Tokyo (1983)
 23. PERKINS, D. D.: Biochemical mutants in the smut fungus *Ustilago maydis*. *Genetics* 34, 607-626 (1949)
 24. PETERSEN, J. G. L., S. HOLMBERG, T. NILSSON-TILLGREN & M. C. KIELLAND-BRANDT: Molecular cloning and characterization of the threonine deaminase (*ILV1*) gene of *Saccharomyces cerevisiae*. *Carlsberg Res. Commun.* 48, 149-159 (1983)
 25. PETERSEN, J. G. L., M. C. KIELLAND-BRANDT, S. HOLMBERG, T. NILSSON-TILLGREN & C. GJERMANSSEN: Homeologous *ILV* genes in *Saccharomyces*. In: *Gene Expression in Yeast*. M. Korhola & E. Vaisainen, eds., Proc. of the Alko Yeast Symp., Helsinki 1983. Foundation for Biotechnical and Industrial Fermentation Research 1, 201-207 (1983)
 26. ROUYER, F., M. -C. SIMMLER, C. JOHNSSON, G. VERGNAUD, H. J. COOKE & J. WEISSENBACH: A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. *Nature* 319, 291-295 (1986)
 27. SHAFFER, B., I. BREARLEY, R. LITTLEWOOD & G. R. FINK: A stable aneuploid of *Saccharomyces cerevisiae*. *Genetics* 67, 483-495 (1971)
 28. SOUTHERN, E. M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98, 503-517 (1975)
 29. WETTSTEIN, D. VON, C. GJERMANSSEN, S. HOLMBERG, M. C. KIELLAND-BRANDT, T. NILSSON-TILLGREN, M. B. PEDERSEN, J. G. L. PETERSEN & P. SIGSGAARD: Genetic engineering in the improvement of brewers yeast. *Tech. Q. MBAA* 21, 45-61 (1984)

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