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GENETIC DIFFERENCES BETWEEN SACCHAROMYCES CARLSBERGENSIS AND S. CEREVISIAE. ANALYSIS OF CHROMOSOME III BY SINGLE CHROMOSOME TRANSFER

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Tetrad analysis of most Saccharomyces strains used in beer production is impossible due to a low yield of viable spores. The present paper describes the use of single chromosome transfer in the genetic analysis of a brewer's yeast.

The technique employs the *kar1* mutation, which reduces karyogamy after conjugation. In rare cases *kar1* × *KAR* crosses yield progeny resulting from the transfer of one chromosome or a limited number of chromosomes from a nucleus of one parent to one of the other. S. cerevisiae strains with an extra S. carlsbergensis chromosome III have thus been isolated from crosses between spore derived clones of the brewing strain and haploid *kar1* S. cerevisiae strains carrying several auxotrophic markers. When the disomics were crossed to other haploid S. cerevisiae strains a normal spore viability was obtained, allowing tetrad analysis.

High functional homology was found between the transferred S. carlsbergensis chromosome and chromosome III of S. cerevisiae. All genes essential for viability on the latter are represented on the former as are also *HIS4*, *LEU2*, *MAT* and *THR4*. Despite the functional homology, the transferred chromosome had a structure that was substantially different from that of standard S. cerevisiae strains. It did not recombine with S. cerevisiae chromosomes III, except in a certain region, where recombination was normal. Furthermore, restriction endonuclease analysis showed that the variant chromosome has a nucleotide sequence in the *HIS4* region different from that of S. cerevisiae. The S. carlsbergensis brewing strain is heterozygous for this sequence variation, containing also a *HIS4* region with a sequence identical or close to that of S. cerevisiae.

Abbreviations: NM = non-mater, NPD = non-parental ditype, PD = parental ditype, TT = tetratype, YPD = yeast extract peptone dextrose.

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1. INTRODUCTION

Most commercial brewer's yeasts (Saccharomyces carlsbergensis and S. cerevisiae) show no mating type and sporulate poorly, giving few viable ascospores (1, 3). Therefore, these strains have been poorly characterized genetically, in contrast to laboratory strains of S. cerevisiae.

In attempts to genetically characterize a S. carlsbergensis brewing strain GJERMANSEN and SIGSGAARD (4) managed to sporulate it and to recover some viable spores. Some of their clones expressed mating type. Crosses of these clones to haploid laboratory strains produced hybrids, many of which gave 4-spored asci. However, the spore viability of these hybrids was still low.

Another approach to the genetic analysis of Saccharomyces strains of low spore viability has been suggested by NILSSON-TILLGREN et al. (13). It is based on the use of the *kar1* mutation, which decreases the frequency of nuclear fusion after mating (2). We have observed that one or a few chromosomes may be transferred from one nucleus to another in transient heterokaryons caused by *kar1* (12, 13). Transient heterokaryons between a mating spore derived clone of a brewing strain and a laboratory strain carrying the *kar1* mutation can be formed. Selection of segregants with the nucleus of the laboratory strain which has received a dominant wild type marker from the brewing strain allows the recovery of a chromosome of the brewing strain in the genetic background of strains that can be readily crossed and subjected to tetrad analysis.

In the present study we have used this approach in the characterization of a S. carlsbergensis chromosome which turned out to be functionally similar to S. cerevisiae chromosome III.

Table I

Yeast strains used in this study

S. carlsbergensis strains:		
244	Brewing strain. Production strain of the Carlsberg Breweries	
C80–CG7	Spore derived clone of 244, mating type **a**	(4)
C80–CG110	Spore derived clone of 244, mating type α	(4)
S. cerevisiae strains:		
K5–5A	*MATα his4–15 ade2–1 can1 kar1–1*	J. Conde (2)
C79–992	*MATα his4–644 leu2 thr4 ura4*	(13)
C79–993	*MATa his4–644 leu2 thr4 ura4*	(13)
C79–994	*MATa leu2 thr4*	(13)
C79–995	*MATα leu2 thr4*	(13)
C80–1396	*MATa his4–15 leu2 thr4 ade2–1 can1 kar?*	T. Nilsson-Tillgren
IV–1	*MATa his4–260 trp1*	(10)
S. cerevisiae strains containing a S. carlsbergensis chromosome:		
C80–1253	K5–5A, chromosome III substituted with a chromosome from C80–CG7	This study
C80–1263	K5–5A with an additional chromosome III from C80-CG7	This study
C80–1391	C80–1396 with an additional chromosome III from C80–CG110	This study
C80–1389	*HIS4 LEU2 MATα THR4/his4 LEU2 MATa thr4 ade2 can1*, disomic spore derived clone from the cross C80–1263 × C79–993	This study
C80–1390	*HIS4 LEU2 MATα THR4/his4 LEU2 MATα THR4 ura4*, disomic spore derived clone from the cross C80–1263 × C79–993	This study

[a] *kar* phenotype weak or absent, but *kar* genotype uncertain, since this strain is derived from a tetrad with only one spore having a clear *kar* phenotype.

2. MATERIALS AND METHODS

2.1. Media and strains

All media used in this study have been described previously (10). The strains employed are presented in Table I.

2.2. Chromosome transfer

The mating technique used for chromosome transfer was as follows. Log phase cells in YPD of the two strains to be mated were harvested, washed and resuspended in fresh medium to a density of 2–4 $\times$ 10^8 cells $\cdot$ ml^{-1}. Equal numbers of cells of the two strains (total volume 0.4–0.6 ml) were spread on a YPD plate, which was incubated at 30 °C for 6 hours. The cells were rinsed off, washed twice with distilled water and plated on selective media.

2.3. Crosses and tetrad analyses

Crosses for recovery of rare mating products (forced matings) were performed as described previously (13). Standard techniques were used for sporulation and tetrad analyses (7).

2.4. Molecular hybridization analysis

DNA extraction, treatment with restriction endonucleases and electrophoretic separation were performed as described previously (8, 9, 10). Detection of specific DNA sequences in the electropherograms by molecular hybridization was carried out according to SOUTHERN (14) with modifications (9). The hybridization probe was a S. cerevisiae PstI restriction fragment purified by electrophoresis (16) from the plasmid pC503 (9).

3. RESULTS

3.1. Transfer of chromosome III from a spore derived clone of brewing strain 244 to a S. cerevisiae strain

S. carlsbergensis brewing strain 244 was sporulated, yielding asci with never more than 3 spores. From the low percentage of viable spores some clones were isolated that showed efficient **a** or α mating ability (4). One such clone, C80–CG7 (mating type **a**) was chosen for further analysis.

Strain C80–CG7 was crossed to the haploid S. cerevisiae strain K5–5A (*MATα his4–15 ade2–1 can1 kar1–1*), and the mating mixture was densely plated on minimal medium containing canavanine and adenine in order to select for K5–5A cells that had received *HIS4*. This medium will not allow growth of the parental strains. Neither will it allow growth of normal hybrids, since the gene for canavanine resistance (*can1*) is recessive. Red as well as white colonies appeared with low frequency. The white colonies may represent normal hybrids that have undergone a mitotic recombination in the *can1* locus resulting in homozygosity for *can1*. They were not further studied. The red colonies must have received the *HIS4* gene from C80–CG7, but not the *ADE2* and *CAN1* genes. One hundred red colonies were screened for sporulation. Eighty-nine of them were sporulators; they were not further studied since they could be partial hybrids having received enough chromosomes from C80–CG7 to be able to sporulate. The 11 non-sporulating, histidine prototrophic, red, canavanine resistant colonies were reisolated from single colonies on YPD and further analysed. Nine of them were non-maters, but rare mating was possible to **a** and α strains with varying efficiency. The remaining two were maters. One of these two was **a**, whereas the other was α. The **a** strain, C80–1253, can be interpreted as having lost chromosome III of K5–5A, thereby expressing the mating type of C80–CG7. The α strain must have lost the chromosome of C80–CG7 with *MAT*a. Interestingly this isolate had simultaneously become His^-, suggesting that *his4* and *MAT* are linked in the brewing strain, as is the case for these genes in S. cerevisiae (Figure 1). The chromosome loss must have taken place during the non-selective growth in YPD. One of the 9 non-maters, C80–1263, was chosen for further analysis.

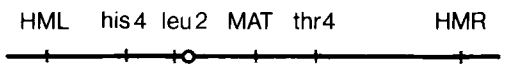

Figure 1. Map of relevant markers of chromosome III of S. cerevisiae (5, 11).

3.2. Mitotic behaviour of C80–1263

After 8 generations in YPD one cell out of 2918 cells had become α His^- and one was **a**

His$^+$. Again the simultaneous loss of *MAT*a and *HIS4* indicates that these two genes are located in the same linkage group in the brewing strain. The spontaneous occurrence of mating colonies explains the rare mating behaviour of C80–1263 with both **a** and α strains. In mating behaviour and mitotic stability C80–1263 is identical to S. cerevisiae strains disomic for chromosome III (13).

3.3. Tetrad analysis of strain C80–1263 after forced mating to tester strains shows that it contains an extra chromosome III

The occurrence of histidine prototrophic mitotic segregants of mating type **a** raises the possibility that all essential functions of the S. cerevisiae chromosome III may be provided by a single chromosome of S. carlsbergensis. However, the possibility that several S. carlsbergensis chromosomes are present in C80–1263 can not be excluded.

In order to find out whether this strain carries a S. carlsbergensis chromosome homologous to chromosome III of S. cerevisiae, rare mating products were recovered from crosses with haploid tester strains of both mating types. In analogy to the S. cerevisiae crosses described (13), both *his4* and *HIS4* strains were used. In addition the strains carried the chromosome III markers *leu2* and *thr4* (cf. Figure 1). The matings resulted in rare prototrophic colonies, which were isolated and sporulated. The spore viability was normal (ranging from 78 to 92%) and the results of the tetrad analyses are shown in Tables II–V.

Table II

Tetrad analysis of the cross C80–1263 × C79–995 *(MATα leu2 thr4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4	14				
*MAT*a/*MAT*α			14		
LEU2/leu2			14		
THR4/thr4		1[a]	12	1[a]	
ADE2/ade2			14		
	PD		NPD		TT
MAT – leu2	14				
leu2 – thr4	5				7
MAT – thr4	5				7
leu2 – ade2	1		3		10
ade2 – thr4	3		1		8

[a] Interpreted to represent a meiotic gene conversion.

Table III

Tetrad analysis of the cross C80–1263 × C79–992 *(MATα his4 leu2 thr4 ura4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4			16		
LEU2/leu2			16		
*MAT*a/*MAT*α			16		
THR4/thr4		3[a]	13		
can1/CAN1			15	1[a]	
ADE2/ade2			16		
URA4/ura4	1[b]		15		
	PD		NPD		TT
his4 – leu2	16				
his4 – MAT	16				
his4 – thr4	8				5
his4 – ura4	6				9
his4 – can1	1		1		13
thr4 – can1	3				9
thr4 – ade2	1		2		10
thr4 – ura4	3				9

[a] Interpreted to represent meiotic gene conversions
[b] Interpreted to represent a mitotic gene conversion

From each of the crosses to C79–995 *(MATα HIS4)* and C79–992 *(MATα his4)* a single isolate was analysed (Tables II and III). In both crosses a 2:2 segregation was observed for all chromosome III markers, except *HIS4* in the cross to C79–995 (Table II). Therefore the rare mating must have been preceded, and made possible, by a mitotic loss of the S. cerevisiae chromosome III (with *his4–15*) in C80–1263.

In the linkage data of these crosses (lower parts of Tables II and III) it can be seen that *HIS4*, *LEU2*, *MAT*a, and *THR4* from the brewing strain are linked. This observation together with the normal spore viability confirms that all essential functions of S. cerevisiae chromosome III are provided by the transferred chromosome. An absolute linkage was observed between *his4*, *leu2* and *MAT* in these crosses (Tables II and III), whereas recombination between the S. cerevisiae and S. carlsbergensis

Table IV

Tetrad analysis of the cross C80–1263 × C79–993 (*MAT*a *his4 leu2 thr4 ura4*)

Prototroph 1	4:0	3:1	2:2	1:3	0:4
HIS4/his4			7		
LEU2/leu2		1	6		
THR4/thr4	2	5			
can1/CAN1			7		
ADE2/ade2	1[b]		6		
URA4/ura4			7		
			2α:1**a**:1NM		
MAT			7		

The phenotypes NM His^-, **a** His^+ and Leu^-His^+ did not occur

Prototroph 2	4:0	3:1	2:2	1:3	0:4
HIS4/his4			6	3	1
LEU2/leu2	2	4	4		
THR4/thr4			10		
can1/CAN1			9	1[a]	
ADE2/ade2			10		
URA4/ura4	1[b]	1[a]	7		1[b]
	2α:2NM	2α:1**a**:1NM		1α:1**a**:2NM	2α:2**a**
MAT	1	5		1	3

The phenotypes NM His^-, **a** His^+ and Leu^-His^+ did not occur; His and Thr segregated independently

[a] Interpreted to represent a meiotic gene conversion
[b] Interpreted to represent a mitotic gene conversion

chromosomes was observed between *MAT* and *thr4*.

In Table IV are presented tetrad analyses of two prototrophic products from the cross C80–1263 × C79–993. Since the tester strain in this cross is *MAT*a *his4*, a prototroph can only be formed if the mating cell in the C80–1263 culture has the phenotype α His^+. Such a phenotype can not arise by a simple chromosome loss, but requires a shift or loss of *MAT*a on the S. carlsbergensis chromosome. The two isolates represent two different ways that such a shift can take place.

The segregation of *thr4* and *MAT* in the tetrads of prototroph 1 shows that this isolate was trisomic for chromosome III (one S. carlsbergensis and two S. cerevisiae chromosomes). Two of the *MAT* genes are *MAT*α and one is *MAT*a. This demonstrates that the *MAT*a gene on the S. carlsbergensis chromosome has shifted to *MAT*α, possibly by mitotic recombination. The 2:2 segregation of *his4* is consistent with the presence of only one *HIS4* gene. The absence of NM His^-, **a** His^+ and Leu^-His^+ spores means that the S. carlsbergensis chromosome III never went alone to one pole in the first meiotic division, i.e., the normal meiotic disjunction always took place between the two S. cerevisiae chromosomes III. The absence of these phenotypes is also consistent with the absence of crossing over between the S. carlsbergensis chromosome and S. cerevisiae chromosomes in the *his4* – *MAT* region, as observed in the first two crosses (Tables II and III).

The predominant 2:2 segregation of *leu2* in asci from prototroph 1 is unexpected, since the

trisomic should contain two *LEU2* genes. However, we regard this to be fortuitous since histidine prototrophic spores of this cross were confirmed to contain two segretating *LEU2* genes in subsequent crosses described in section 3.4.

The His^+ spores *(HIS4/his4)* from prototroph 1 resemble the parent C80–1263 in their high stability for the His^+ phenotype.

The shift in mating type which led to the isolation of prototroph 2 (Table IV) was of a different nature. As in prototroph 1, *MAT, his4* and *leu2* showed aberrant segregation, whereas *thr4* segregated 2:2. This is not consistent with simple trisomy for chromosome III. Mating type shift in the C80–1263 culture may have occurred by a gross rearrangement of the S. carlsbergensis chromosome III. Such rearrangements resulting in mating type shift have been described in S. cerevisiae by HAWTHORNE (6) and STRATHERN et al. (15). Thus, we take the segregation data as indication for the presence in prototroph 2 of two complete chromosomes III from S. cerevisiae and a fragment of the S. carlsbergensis chromosome lacking a region including *THR4*. The presence of a fragment with *HIS4, LEU2* and *MATα* was confirmed by a mitotic analysis of the His^+ spore clones. Twenty-four such clones (including those from asci with less than four viable spores) turned out to segregate His^- cells at a high frequency: After about 8 generations of non-selective growth the frequency of His^- cells was between 1.0 and 5.1% (total number of colonies 10247, median frequency of His^- 2.2%). Nine His^+ spore clones were chosen at random and analysed for mitotic loss of other markers together with *HIS4*. In several cases it could be seen that *HIS4, LEU2* and *MATα* were lost together. In the remaining cases the retainment of the Leu^+ or α mating type could, with one exception, be ascribed to the presence of *LEU2* and *MATα* from K5–5A, as revealed by the phenotypes of the His^- spores in the investigated tetrads. The exceptional spore may be explained by a gene conversion. High frequency of mitotic loss of a circular derivative of chromosome III from S. cerevisiae has been reported (15).

The cross of C80–1263 to the *HIS4 MATa* strain (Table V) was more trivial. The deviations from a 2:2 segregation were so few that they are ascribed to meiotic gene conversion. A cell of the C80–1263 culture had in this case lost the S. carlsbergensis chromosome III with the result that it could mate as an α cell to C79–994. Recombination between markers of the two S. cerevisiae chromosomes III is observed at normal levels.

Table V

Tetrad analysis of the cross C80–1263 × C79–994 *(MATa leu2 thr4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4		1[a]	11		
LEU2/leu2		1[a]	10	1[a]	
MATa/MATα			12		
THR4/thr4			12		
can1/CAN1			11	1[a]	
ADE2/ade2			12		
	PD		NPD		TT
his4 – leu2	7				2
leu2 – MAT					10
MAT – thr4	7				5
his4 – can1	2		1		7
his4 – ade2	3		1		7

[a] Interpreted to represent a meiotic gene conversion.

3.4. Non-random disjunction of S. carlsbergensis and S. cerevisiae chromosomes in trisomic strains confirmed by further crosses

In the tetrads of the trisomic prototroph 1 presented in Table IV it was observed that disjunction took place in a non-random fashion: The S. carlsbergensis chromosome III never went alone to one pole in the first meiotic division. In that cross only 7 tetrads were analysed and we wished to extend the material.

Two disomic spores from prototroph 1, C80–1389 *(HIS4 LEU2 MATα THR4/his4 LEU2 MATa thr4 ade2 can1)* and C80–1390 *(HIS4 LEU2 MATα THR4/his4 LEU2 MATα THR4 ura4)* were crossed to tester strains (Tables VI–VIII). Table VI shows the result of a rare mating caused by loss of the S. cerevisiae chromosome III of C80–1389 in analogy to the events presented in Table III. The absence of recombination between the S. carlsbergensis and S.

Table VI

Tetrad analysis of the cross C80–1389 *(HIS4 LEU2 MATα THR4/his4 LEU2 MATa thr4 ade2 can1)* **× C79–993** *(MATa his4 leu2 thr4 ura4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4			6		
LEU2/leu2			6		
MATa/MATα			6		
THR4/thr4		1[a]	5		
URA4/ura4			6		
can1/CAN1			6		
	PD		NPD		TT
his4 – leu2	6				
his4 – MAT	6				
his4 – thr4	5				
his4 – ura4	1				5
his4 – can1					6
thr4 – can1					5
thr4 – ura4	1				4

[a] Interpreted to represent a meiotic gene conversion

cerevisiae chromosomes III in the *his4 – MAT* region is confirmed. Table VII shows the result of another rare mating of C80–1389, this time to a *MATα his4* tester strain. The segregation in the 14 tetrads confirms the conclusions drawn from the analysis of prototroph 1 of the cross C80–1263 × C79–993 (Table IV). The segregation of all four chromosome III markers is consistent with the trisomic nature of the sporulated prototroph. The absence of α His^+, NM His^- and Leu^-His^+ spores confirms the non-random disjunction as well as the absence of recombination described above. The other disomic His^+ spore that was analysed (C80–1390, Table VIII) was a mater. Evidence for disomy of C80–1390, non-random disjunction and the absence of recombination in the *his4 – MAT* region is seen in the same way as in the cross shown in Table VII.

The segregation of *LEU2* in Tables VII and VIII shows that the *LEU2* gene from S. carlsbergensis segregates equally well with either of the two S. cerevisiae alleles.

3.5. Isolation and analysis of another product of chromosome transfer, C80–1391

During the course of the experiments described above, several new, well growing spore derived clones of brewing strain 244 were isolated. One of these, C80–CG110, had a mating type opposite to that of C80–CG7 and was chosen as another donor of chromosome III in an experiment analogous to that described in section 3.1. Thus it was crossed to the S. cerevisiae strain C80–1396 *(MATa his4–15 leu2 thr4 ade2–1 can1 kar?)* and the mixture was plated on complete medium with canavanine, lacking histidine and arginine. Eleven red colonies were picked and tested for nutritional requirements as well as mating and sporulation phenotypes. All of them were His^+, Leu^+, Thr^+

Table VII

Tetrad analysis of the cross C80–1389 *(HIS4 LEU2 MATα THR4/his4 LEU2 MATa thr4 ade2 can1)* **× C79–992** *(MATα his4 leu2 thr4 ura4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4			14		
LEU2/leu2	7	2	5		
THR4/thr4			11	3	
URA4/ura4			14		
can1/CAN1		1[a]	13		
		2a:2α	2a:1α:1NM		2a:2NM
MAT		3	8		3

The phenotypes α His^+, NM His^- and Leu^-His^+ did not occur

[a] Interpreted to represent a meiotic gene conversion

Table VIII

Tetrad analysis of the cross C80–1390 *(HIS4 LEU2 MATα THR4/his4 LEU2 MATα THR4 ura4)* **× C80–1396** *(MAT***a** *his4 leu2 thr4 ade2 can1)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4			8		
LEU2/leu2	4		4		
THR4/thr4	2	5	1		
can1/CAN1			8		
ADE2/ade2			8		
URA4/ura4			8		
	3α:1NM		**2a**:2α	2α:2NM	2α:1**a**:1NM
MAT	1[a]		2	1	4

The phenotypes **a** His^+ and NM His^- did not occur

[a] The appearance of three α clones in this tetrad is interpreted by mitotic loss of a S. cerevisiae chromosome in the clone of a NM His^+ spore.

Table IX

Tetrad analysis of the cross C80–1391 × C79–993 *(MAT***a** *his4 leu2 thr4 ura4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4			17		
LEU2/leu2			17		
*MAT***a**/*MAT*α			16	1[a]	
THR4/thr4			17		
ADE2/ade2			16	1[a]	
URA4/ura4			17		
	PD		NPD		TT
his4 – leu2	17				
his4 – MAT	16				
his4 – thr4	10				7
his4 – ade2	3		2		11
his4 – ura4	4				13

[a] Interpreted to represent a meiotic gene conversion

and non-mating; only one was sporulating. One of the non-sporulating colonies, C80–1391 was chosen for rare mating and tetrad analysis (Tables IX and X). The event leading to the mating presented in Table IX was a loss of the S. cerevisiae chromosome III in C80–1391, whereas that presented in Table X was a mitotic recombination leading to a *MAT***a**/*MAT***a** derivative of C80–1391. The chromosome that was transferred was also in this case functionally a chromosome III and it behaved in the same way as that carrying *MAT***a** (transferred from C80–CG7). Again, no recombination was observed between the S. carlsbergensis and S. cerevisiae chromosomes in the *his4 – MAT* region, but was seen between *MAT* and *thr4*. The absence of α Leu^+ and NM Leu^- spores (Table X) shows that also here the S. carlsbergensis chromosome never went alone to one pole in the first meiotic division.

Table X

Tetrad analysis of the cross C80–1391 × C79–995 *(MATα leu2 thr4)*

	4:0	3:1	2:2	1:3	0:4
HIS4/his4	2	5	2		
LEU2/leu2			9		
THR4/thr4			8	1	
ADE2/ade2			9		
		2**a**:1α:1NM		2**a**:2α	
MAT		7		2	

The phenotypes α Leu^+ and NM Leu^- did not occur

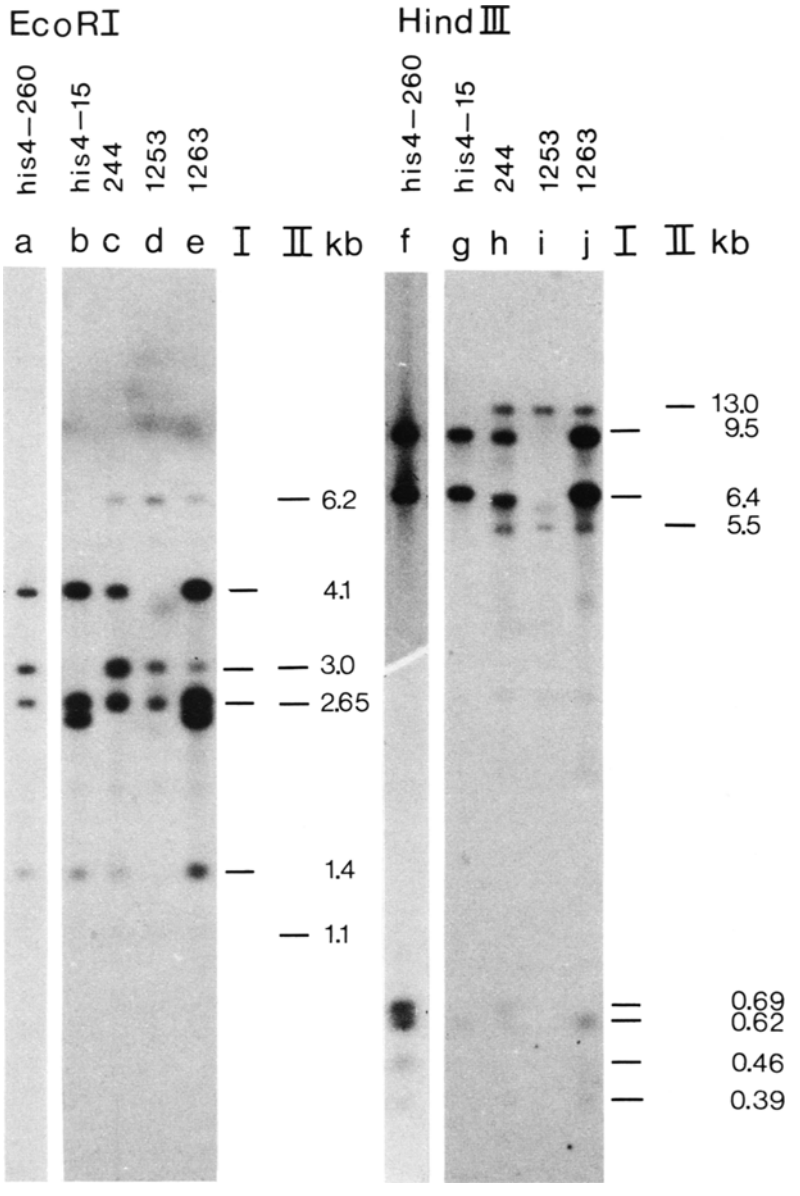

Figure 2. Restriction endonuclease analysis of sequences in the *HIS4* region of S. carlsbergensis brewing strain 244.

Three micrograms of DNA from the S. cerevisiae *his4–15* deletion strain (K5–5A, lanes b and g), the brewing strain (244, lanes c and h), and the two products of chromosome transfer, C80–1253 (lanes d and i) and C80–1263 (lanes e and j), were digested with restriction endonuclease EcoRI or HindIII and subjected to agarose gel electrophoresis (8, 9). Fragments which carry sequences of the *HIS4* region were detected by molecular hybridization (14) with 10^8 cpm of a ^{32}P-labelled isolated cloned PstI fragment carrying the *HIS4* gene (9, 10). Lanes a and f show the wild type patterns (I) of S. cerevisiae obtained with strain IV–1 carrying the point mutation *his4–260* (8). Note the presence of both pattern I and pattern II in the brewing strain.

3.6. Brewing strain 244 is heterozygous for nucleotide sequences in the *HIS4* region

The absence of recombination in the *his4 – MAT* region between the transferred S. carlsbergensis chromosome and chromosome III of standard laboratory strains may be due to nucleotide sequence inhomologies.

The sequence arrangement around the *HIS4* gene in brewing strain 244 was studied by molecular hybridization according to SOUTHERN (14). DNA was extracted from the brewing strain (244), a laboratory strain carrying the point mutation *his4–260* (IV–1), a *his4–15* deletion strain (K5–5A) as well as the mating type **a** isolate (C80–1253) and the analysed disomic isolate (C80–1263), both from the cross C80–CG7 × K5–5A. The DNA samples were treated with restriction endonucleases EcoRI or HindIII and subjected to agarose gel electrophoresis. An isolated 9.4 kb PstI restriction fragment carrying the *HIS4* gene previously cloned from S. cerevisiae on a bacterial plasmid (9, 10) was used as radioactive hybridization probe. The autoradiogram is shown in Figure 2. The point mutation *his4–260* shows the S. cerevisiae wild type EcoRI pattern (I); this pattern as well as the pattern of the deletion *his4–15* have been described previously (8). In pattern I the 3.0 and 2.65 kb EcoRI fragments are contiguous and carry the *HIS4* gene, whereas the 4.1 and 1.4 kb fragments contain flanking sequences and extend outside the region represented by the hybridization probe (8). Brewing strain 244 shows in addition to pattern I two bands of 6.2 and 1.1 kb. The two novel bands also appear in DNA from C80–1253 and C80–1263 which have received a *HIS4* gene from the brewing strain. The genetic data (Tables II–V) show that C80–1263 is disomic carrying a chromosome III from the brewing strain as well as a S. cerevisiae chromosome III with *his4–15*. Consistently with this, DNA from C80–1263 is seen to have retained the pattern of *his4–15*. C80–1253, on the other hand, was inferred to have lost the S. cerevisiae chromosome, since it had got the mating type **a**, and indeed, its DNA is seen to have lost the *his4–15* pattern. In this DNA the pattern (II) of the transferred S. carlsbergensis *HIS4* region can therefore be seen alone. It consists of the two new bands plus the two bands (3.0 and 2.65 kb) containing the *HIS4* gene, whereas the 4.1 and 1.4 kb bands are missing. Brewing strain 244 shows a combination of pattern I and pattern II.

The 3.0 and 2.65 kb fragments of the transferred *HIS4* region hybridize with lower efficiency with the radioactive probe than fragments with the corresponding S. cerevisiae sequences. This is best seen in DNA from the disomic strain C80–1263. The 3.0 kb band originating from S. carlsbergensis has a much lower intensity than the 2.5 kb S. cerevisiae band carrying the *his4–15* deletion. The high mitotic stability of this strain ensures that these fragments were present in equimolar amounts. The low intensities of the 3.0 and 2.65 kb bands of the transferred *HIS4* region suggest that the sequence difference is appreciable even within the *HIS4* structural gene.

Also in the HindIII digests two patterns were observed, one characteristic of the transferred S. carlsbergensis *HIS4* region (II) and one characteristic of S. cerevisiae (I). Again, pattern II has a lower intensity and both patterns are present in the brewing strain.

We conclude that the S. carlsbergensis brewing strain is heterozygous for the *HIS4* region, containing one allele similar to the S. cerevisiae *HIS4* region and another which is significantly different in nucleotide sequence.

4. DISCUSSION

In a previous paper (13) we pointed out that *kar* mediated chromosome transfer might become a valuable tool in the improvement of industrial Saccharomyces strains as well as in the analysis of their genetic constitution. The substitution of a whole chromosome, as examplified by C80–1253, a S. cerevisiae strain in which a S. carlsbergensis chromosome III has replaced the original chromosome, demonstrates the potential of this technique in breeding programs. However, the main subject of the present study was the use of chromosome transfer for analytical purposes.

Mating between a spore derived clone of an industrial S. carlsbergensis strain and a laboratory strain carrying multiple markers as well as the *kar1* gene yielded a strain which in addition to its normal complement of S. cerevisiae chromosomes carried an extra chromosome

from S. carlsbergensis. Mitotic as well as meiotic analysis showed that the S. carlsbergensis chromosome could provide all essential functions that are provided by the S. cerevisiae chromosome III. Recombination between the chromosomes of different origin was strongly reduced; it did not occur between *his4* and *MAT*, whereas it was seen at a normal level between *MAT* and *thr4*. The high spore viability would tend to argue against the possibility that the reduced recombination is due to an inversion. General sequence inhomology in the *his4 – MAT* region could also be an explanation. Considerable sequence inhomology was indeed found in the *his4* region. Curiously, the inhomology seemed to be significant also within the *HIS4* structural gene. We are presently investigating other parts of the *his4 – MAT* region for nucleotide sequence differences.

Meiotic disjunction in trisomic strains with one copy of the transferred chromosome occurred always between the two S. cerevisiae chromosomes. This is in accordance with more recombination taking place between these two chromosomes than between the S. carlsbergensis chromosome and either of them. In a trisomic S. cerevisiae strain with two intact chromosomes III and one chromosome carrying a deletion including *MAT* and *thr4* normal spore viability has been observed, showing that the intact chromosomes disjoined preferentially (13). Thus it seems that meiotic disjunction in trisomic strains of S. cerevisiae preferentially takes place between the two chromosomes with greatest homology.

Several mechanisms were observed leading to the appearance of mating cells in the disomic *MAT*a/*MAT*α strains. If not prevented by the requirement for prototrophy of the mating product, the shift always occurred by chromosome loss. In other cases it took place either by gene conversion or transposition of silent mating type information, events that could not be distinguished in the present experiments. In one case the shift took place by a chromosomal rearrangement of the S. carlsbergensis chromosome concomitantly leading to the loss of *THR4*. This rearrangement is different from the *MAT*a – *THR4* deletion we have observed previously in a disomic strain of S. cerevisiae (13), since in the present case the rearranged chromosome contains a functional *MAT* allele. STRATHERN et al. (15) have observed that mating type shift in heterothallic S. cerevisiae strains can be caused by recombinational formation of a circular derivative of chromosome III lacking *THR4* and showing mitotic instability. The mitotic instability of strains carrying the rearranged S. carlsbergensis chromosome points to the possibility that it is analogous to the circular chromosome described by STRATHERN et al. If this is true, there is a locus for silent mating type information distal to *LEU2* and *HIS4* relative to *MAT* on the transferred chromosome, i.e., a locus corresponding to *HML* of S. cerevisiae (Figure 1). In summary, the mechanisms for mating type shift in the S. carlsbergensis chromosome III seem to be similar to those in S. cerevisiae.

Molecular hybridization showed that brewing strain 244 is heterozygous for nucleotide sequences in the *HIS4* region, with one allele significantly deviating from the standard S. cerevisiae sequence. A chromosome with the deviating sequence was transferred to S. cerevisiae giving rise to the disomic C80–1263 (Figure 2). Molecular hybridization analysis of several spore derived clones of strain 244 (to be published) showed that the deviating *HIS4* sequence could occur with both mating types. Strain C80–CG110, which is the donor of the *MAT*α carrying chromosome of C80–1391, has the deviating *HIS4* sequence. Thus it is presently unknown whether the standard *HIS4* gene in strain 244 is located on a chromosome III which will show normal recombination and disjunction behaviour in S. cerevisiae.

In conclusion, single chromosome transfer has allowed the identification of a S. carlsbergensis chromosome III which has a close functional homology to the standard S. cerevisiae chromosome III, but shows significant genetic and molecular deviations.

We wish to point out some features of the technique that contribute to its usefulness. The high spore viability makes a tetrad analysis possible, giving direct information on linkage and meiotic behaviour and avoiding the difficulties in the interpretation of random spore analyses. The analysis of the chromosome III fragment examplifies that close functional homology is not necessary for the use of the technique.

The technique is not dependent on the isolation of auxotrophic mutants in the industrial strain to be investigated, since it makes use of markers present in the recipient and tester strains. Finally, functional as well as structural characteristics of a given chromosome can be studied in a standard genetic background.

We are aware that high spore viability might be obtained by repeated back-crosses to a standard strain, keeping selection for the genes to be studied. However, even if recombination is reduced, such selection will yield chromosomes whose relation to those of the industrial strain is uncertain.

The technique is not limited to chromosome III. Current experiments indicate the presence in brewing strain 244 of a chromosome homologous to chromosome V of S. cerevisiae.

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