DNA SEQUENCE POLYMORPHISMS IN THE GENUS SACCHAROMYCES IV. HOMOELOGOUS CHROMOSOMES III OF SACCHAROMYCES BAYANUS, S. CARLSBERGENSIS, AND S. UVARUM

by

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Single chromosome III transfers have been accomplished from the two species *S. bayanus* and *S. uvarum* into genetically marked *S. cerevisiae* strains. Incompatibilities between *S. bayanus* or *S. uvarum*, and *S. cerevisiae* strains may account for the very few chromosome addition lines obtained. The preliminary genetic map for *S. bayanus* chromosome III is similar to the genetic map for a *S. carlsbergensis* chromosome III from Danish lager strain BK2208. Recombination is absent between the transferred chromosomes and the auxotrophically marked chromosome from *S. cerevisiae* in the interval *HIS4* to *LEU2* but recombination does occur in the interval *LEU2* to *THR4*. The mapped *S. bayanus* chromosome III carries *HIS4* pattern III while the mapped chromosome III from the lager strain contains *HIS4* pattern II. Transfer of a chromosome III from *S. uvarum* to *S. cerevisiae* has been recognised by restriction endonuclease fragment patterns, OFAGE chromosome separations and molecular hybridization, but a genetic map could not be constructed for an *S. uvarum* chromosome due to instability of the chromosome addition lines. The hypothesis is put forward that the *S. carlsbergensis* type strain and the lager strains are sibling species and species hybrids both produced by hybridization of an *S. cerevisiae* top fermenting strain and the bottom fermenting strain *S. monacensis*. The lager strains and the authentic type strain are designated *S. carlsbergensis*. From the electrophoretic karyotypes of type strains of *Saccharomyces* and molecular hybridization patterns it is deduced that *S. bayanus*, *S. inusitatus*, *S. pastorianus* and *S. uvarum* are closely related but distinct from the bottom fermenting strains.

1. INTRODUCTION

Base composition, DNA-RNA homology, DNA-DNA reassociation kinetics and restriction endonuclease fragment length polymorphisms have proven useful in the taxonomic

evaluations of genera and species as well as in the diagnosis of closely related yeast strains (3, 4, 15, 21, 23, 24, 25, 26, 27, 28, 30, 40). For taxonomic as well as breeding purposes the establishment of genetic linkage groups and their assignment to

Abbreviations: OFAGE = Orthogonal field alternation gel electrophoresis; TBE = 0.089 M-Tris-borate, 0.089 M-boric acid, 0.002 M-EDTA; SSC = 0.15 M-NaCl, 15 mM-Na citrate.

Figure 1. Genetic map of chromosome III from four *Saccharomyces* strains. The distances in cM are calculated on the basis of the recombination values between the three prototrophic chromosomes and a standard *S. cerevisiae* chromosome III carrying the appropriate mating type and auxotrophic markers. Chromosome B according to (21), and C and D to this study. Strains B and C differ by their *Ty* element patterns. B (Carlsberg lager strain) contains pattern I, while C (Danish lager strain BK2208) contains pattern IIa (27).

cytologically or electrophoretically recognisable chromosomes is desirable. By selectable transfer of a single or a few chromosomes from an

uncharacterised yeast strain or species into a well marked genetic strain knowledge of linkage groups and chromosome homology can be established. This has been demonstrated for the Carlsberg lager strain (20, 21). By genetic and molecular analyses the lager strain was found to be amphiploid for homoeologous chromosomes apparently originating from two different species (21, 22). In the present study the transfer of chromosome III from *Saccharomyces bayanus* BK4505, from the Danish lager strain BK2208

Table I. Characteristics of *S. bayanus*, *S. carlsbergensis*, *S. monacensis*, *S. pastorianus*, and *S. uvarum*. Restriction fragment patterns of the *RDNI*, *HIS4*, *LEU2* and *Ty* genes are according to (24, 27 and this study). -: Indicates very weak to no hybridization to *Ty1* p14.

Strains:	<i>RDNI</i>	<i>HIS4</i>	<i>LEU2</i>	<i>Ty</i>	Karyotypes:
<i>S. bayanus</i> group:					
<i>S. bayanus</i> CBS380	III	II+III	IV	-	Figure 2
<i>S. bayanus</i> NCYC374	III	II+III	IV	-	Figures 2, 6
<i>S. pastorianus</i> ATCC12752	III	II+III	IV	-	Figure 2
<i>S. pastorianus</i> NCYC392	III	II+III	IV	-	Figure 2
Bottom fermenting yeast group:					
<i>S. carlsbergensis</i> C84-AJL248	III	I+IIa	III+IV	III	Figures 2, 3, 4
<i>S. carlsbergensis</i> CBS1513	III	I+IIa	III+IV	III	Figure 2
<i>S. carlsbergensis</i> NCYC396	III	I+IIa	III+IV	III	Figure 2
<i>S. monacensis</i> CBS1503	III	II	IV	IV	Figure 3, 4
Pschorr lager strain BK1101	II	II	III+IV	IIa	Figure 4
Danish lager strain BK2208	II	I+II	III+IV	IIa	Figures 8, 9
Tuborg lager strain BK2224	II	I+II	III+IV	II	Figures 3, 4, 9
Carlsberg lager strain BK2246	II	I+II	III+IV	I	Figures 3, 4, 9
<i>S. uvarum</i> group:					
<i>S. inusitatus</i> CBS1546	III	III	IV	-	Figure 9
<i>S. uvarum</i> C81-1510 (CBS395)	III	IIIa	IV	-	Figure 2
<i>S. uvarum</i> C81-1511	III	III	IV	-	Figures 2, 9

Table II. Characteristics of strains used in the genetic and molecular studies.

Strains:			source:
S. cerevisiae genetic standard strains:			
S288C	α	<i>SUC2 CUP1 gal2</i>	
K5-5A	α	<i>his4-15 ade2-1 can1 kar1-1</i>	CONDE & FINK (8)
C79-992	α	<i>his4-644 leu2 thr4 ura4</i>	NILSSON-TILLGREN (20)
C79-993	α	<i>his4-644 leu2 thr4 ura4</i>	NILSSON-TILLGREN (20)
C81-1611 (090780B-II-A)	α	<i>ura3 his1 ilv1-92 can1 rad3 ade2 cyh2 kar1-1</i>	NILSSON-TILLGREN
S. bayanus NCYC374 spore clones:			
TSP-374-4A, -4B, -5B, -6A, -6B, -7A and -7B			This study
S. bayanus BK4505 (Subculture of NCYC374)			
S. bayanus chromosome III addition line (receptor K5-5A):			
CYT-TSP-4505-3A-5	a/α	<i>HIS4 LEU2 THR4 ade2-1 can1</i>	This study
Lager strain BK2208 chromosome III substitution line (receptor K5-5A):			
CYT-MSP-2208-2	α	<i>HIS4 LEU2 THR4 ade2-1 can1</i>	This study
Lager strain BK2208 chromosome III addition line (receptor K5-5A):			
CYT-MSP-2208-7	a/α	<i>HIS4 LEU2 THR4 ade2-1 can1</i>	"
S. uvarum C81-1511	nm	HO prototroph	
S. uvarum C81-1511 spore clones:			
TSP-1511-4A, -4B, -4C and 4D		HO prototroph	"
S. uvarum C81-1511 chromosome III addition line (receptor K5-5A):			
CYT-TSP1511-4C-3	a/α	<i>HIS4 LEU2 THR4 ade2-1 can1</i>	This study
Hybrids between S. uvarum C81-1511 chromosome III addition line and S. cerevisiae genetic standard strains:			
CYT-TSP-1511-4C-3 $\times$ C79-992 : -1, -2, -3, -4, -5, -6, -7, -8			"
CYT-TSP-1511-4C-3 $\times$ C79-993 : -1, -2, -3, -4, -5, -6, -7, -8, -9, -10			"

(S. carlsbergensis) and from S. uvarum (C81-1511) into a Saccharomyces cerevisiae strain with appropriate recessive markers was attempted. Using in the recipient the *kar1-1* gene to prevent karyogamy, appropriate chromosome addition and/or substitution lines are obtained. These are characterised by genetic analysis and for restriction endonuclease fragment patterns of the *HIS4* and *LEU2* regions. Separation of the chromosomes by orthogonal field alternation gel electrophoresis in conjunction with molecular hybridization of Southern transfers to the chromosome III *HIS4* pC503 probe (14) established basic features of the karyotypes of the three species studied. A map of chromosome III with the relevant markers is given in Figure 1.

2. MATERIALS AND METHODS

2.1. Strains and media

The Saccharomyces species used in the present study are listed in Table I together with

their known DNA characteristics. Genetic standard strains and chromosome addition or substitution lines are tabulated in Table II. In Tables III and IV are shown the ascus analyses of the transferred chromosomes III from S. bayanus BK4505 and Danish lager strain BK2208. Agar plates with complete medium (YPD), sporulation media plates (SPOR) and defined minimal plates for genetic studies were made up according to SHERMAN, FINK, and HICKS (32). Plates used for selection of chromosome III transfers were prepared according to (18, 20).

2.2. Preparation of DNA

Total DNA from yeast cells was isolated according to PEDERSEN (26) and plasmid DNA was prepared and isolated in preparative scale according to HOLMES and QUIGLEY (16). DNA preparations for OFAGE separations were made according to SCHWARTZ and CANTOR (31), with the modifications reported by PEDERSEN (27).

Figure 2. Panel A: OFAGE separations of chromosomes from nine *Saccharomyces* species, which all contain *RDNI* form III (cf ref. (24, 27) and Table I). Lane a: *S. uvarum* CBS395, lane b: *S. uvarum* C81-1511, lane c: *S. carlsbergensis* NCYC396, lane d: *S. carlsbergensis* C84-AJL248 (mother strain for NCYC396 and CBS1513), lane e: *S. carlsbergensis* CBS1513, lane f: *S. pastorianus* NCYC392, lane g: *S. pastorianus* ATCC12752, lane h: *S. bayanus* NCYC374, and lane i: *S. bayanus* CBS380. The karyotypes of *S. bayanus*, *S. pastorianus*, and *S. uvarum* seem to be closely related, since the distribution of chromosome bands is almost identical. The three clones of the *S. carlsbergensis* bottom fermenting strain are identical, and the chromosomal band patterns are significantly different from the other three species. Arrowheads point to the chromosomes which hybridize with the *HIS4* gene probe. Panel B: Corresponding Southern filter of the gel in panel A hybridized with the pC503 *HIS4* probe used to monitor the chromosome III's in the different species. Even though the electrophoretic karyotypes of the three *S. carlsbergensis* clones are identical one difference between NCYC396 and the other clones C84-AJL248 and CBS1513 is seen. The uppermost of the three to *HIS4* hybridizing bands in *S. carlsbergensis* C84-AJL248 and CBS1513 are missing in NCYC396.

The apparatus was constructed after the descriptions of CARLE and OLSON (5, 6).

2.3. Molecular hybridization analysis

Restriction endonucleases used in this study were obtained from Boehringer Mannheim and used according to MANIATIS, FRITSCH, and SAMBROOK (19). Restricted DNA molecules were separated by gel electrophoresis in 0.7% agarose, $1 \times$ TBE buffer, while chromosome length DNA was separated by OFAGE in 1% agarose, $0.5 \times$ TBE buffer. The gels were stained with ethidium bromide in a concentration of $1 \mu\text{g} \cdot \text{ml}^{-1}$ in the appropriate electrophoresis buffer. Conditions for OFAGE separations are as in (27). DNA fragments were transferred to nitrocellulose (Millipore HAWP 00010 or to Schleicher and Schuell BA85) by Southern transfer in $20 \times$ SSC (33). The probes used in this study are pC503 (*HIS4*) (11, 13) and the 0.9 kb *EcoRI*-*Sall* fragment (39) of pC512 (*LEU2*) (1, 2, 7, 10, 15, 34). Both genes originate from chromosome III in *S. cerevisiae*. Labelling of the probes was done by nick translation according to RIGBY et al. (29). Molecular hybridization of labelled probes to filterbound DNA was done in $3 \times$ SSC + 0.2% Denhardt solution (9) + pre-boiled salmon sperm DNA ($50 \mu\text{g} \cdot \text{ml}^{-1}$) (17) at 60°C for 18-24 hours (27). Exposure of autoradiographs was done at -80°C with KODAK X-Omatic screens on KODAK XRP1 or XAR5 films.

3. RESULTS

3.1. Electrophoretic karyotypes of *S. bayanus*, *S. carlsbergensis*, *S. inusitatus*, *S. pastorianus*, and *S. uvarum*

The electrophoretic karyotypes of the five species are shown in Figures 2 and 9. *S. pastorianus* and *S. bayanus* are considered according to VAN DER WALT (35) to be strains of the same species and restriction endonuclease fragment length polymorphisms at *RDNI*, *HIS4*, and *LEU2* support this view (24, 27). The four strains included in the *S. bayanus* group (Table I) are: *S. pastorianus* NCYC392, *S. pastorianus* ATCC12752, *S. bayanus* NCYC374, and *S. bayanus* CBS380. The karyotypes of the two *S. pastorianus* strains are identical (Figure 2, lanes f and g), in agreement with the statement in culture collection catalogues (Centraalbureau voor Schimmelcultures, Baarn 1983, List of Cultures 30th edition, 1983, CBS; American Type Culture Collection, Catalogue of strains I, 15th ed. 1982, ATCC; National Collection of Yeast Cultures, June 1981, NCYC) that both *S. pastorianus* strains are subclones of the authentic *S. pastorianus* (HANSEN). The major difference between *S. bayanus* (Figure 2, lanes h and i) and *S. pastorianus* (Figure 2, lanes f and g) is found in the number and intensity of the fastest moving chromosome bands. While there are three fast moving bands in *S. bayanus* there are only two in *S. pastorianus*. After hybridizing a blot of the gel in panel A to the pC503 *HIS4* probe the autoradiogram of the filter (Figure 2B) identifies chromosomes III in identical positions

in the two species, i.e. in the second respectively third fastest moving chromosome band (arrow-heads in lanes f to i of Figure 2A). The karyotype of the two *S. uvarum* accessions CBS395 (C81-1510 and C81-1511) is identical with regards to the seven fastest moving bands (lanes a, b in Figure 2A) and the autoradiogram of the filter probed with *HIS4* identifies chromosome III in the same position as found for *S. bayanus* and *S. pastorianus*. The *S. uvarum* strain C81-1511 (Figure 2, lane b) lacks a chromosome band between the very long and short chromosomes. *S. inusitatus* CBS1546 also has a single chromosome III band with a migration property comparable to *S. uvarum* (Figure 9, lane k versus lane b).

In contrast to the species just discussed the three *S. carlsbergensis* strains have more than one chromosome band containing a *HIS4* gene (Figure 2, lanes c, d, and e). The authentic *S. carlsbergensis* bottom fermenting strains No. I in lane d (C84-AJL248, mother strain for NCYC396 and CBS1513) and lane e (CBS1513) has a karyotype with three chromosome bands hybridizing to the pC503 *HIS4* probe. In this line there are apparently three homoeologous chromosomes III and its electrophoretic karyotype as a whole is very different from the karyotypes of the species in the *S. bayanus* or *S. uvarum* groups. Also derived from the authentic *S. carlsbergensis* bottom fermenting strain No. I is the culture NCYC396. This strain has only two chromosome bands, which hybridize with *HIS4* (Figure 2, lane c). It lacks the longest band of the three which hybridize in the two other *S. carlsbergensis* accessions. Instead, there is a more intense hybridization and mass in the longer of the two chromosome bands containing a *HIS4* gene. This may indicate the occurrence in this culture of a deletion in the longest of the three homoeologous chromosomes III.

In Figure 3 are compared the electrophoretic karyotypes of *S. monacensis* (CBS1503), which is the Carlsberg bottom fermenting yeast No. II (lane a), with those of the bottom fermenting yeast No. I (lane b), the Tuborg lager strain BK2224 (lane c), and the Carlsberg lager strain BK2246 (lane d). Interestingly, the Carlsberg and Tuborg lager strains have a single *HIS4* probe hybridizing chromosome III band which

Figure 3. Comparison of chromosomes hybridizing to the *HIS4* gene probe in the bottom fermenting strains: Lane a: *S. monacensis* CBS1503, lane b: *S. carlsbergensis* C84-AJL248, lane c: Tuborg lager strain BK2224, and lane d: Carlsberg lager strain BK2246. *S. monacensis* shows genomic relationship to both *S. carlsbergensis* C84-AJL248 and the lager strains, since the *S. monacensis* strain contains two chromosome bands hybridizing to *HIS4* of which the larger one has the same size as that hybridizing in the two lager strains and the shorter one is of a size corresponding to a *HIS4* hybridizing chromosome in *S. carlsbergensis* C84-AJL248. Panel A: Total electrophoretic karyotypes, panel B: Southern filter hybridized with pC503.

has the same mobility as the longer of the two chromosome III bands in *S. monacensis*. The second shorter and more weakly hybridizing band in *S. monacensis* has a mobility comparable to a chromosome III band present in the *S. carlsbergensis* bottom fermenting yeast No. I.

In Figure 4 are shown electrophoretic karyotypes of *S. carlsbergensis* No. I (248), *S. monacensis* (1503), the Pschorr lager strain (1101), the Tuborg lager strain (2224), and the Carlsberg lager strain (2246). The roman numerals on the left correspond to the positions of the chromosomes in the genetic standard strain K5-5A. The Tuborg, the Carlsberg, and the Pschorr strain have identical chromosome band patterns except for the band identified with double-headed arrow, which in the Pschorr strain moves slower than in the other two strains. The *S. monacensis*

Figure 4. Electrophoretic karyotypes of five bottom fermenting strains: Lane 248: *S. carlsbergensis* C84-AJL248, lane 1503: *S. monacensis* CBS1503, lane 1101: Pschorr lager strain BK1101, lane 2224: Tuborg lager strain BK2224 and lane 2246: Carlsberg lager strain BK2246. The white arrows point at chromosomal bands in 248 and 1503 which are absent in the lager strains. The double headed arrow at lanes 1101 and 2224 points to a chromosomal band which in BK1101 and BK2224 differs in mobility. The roman numbers refer to band positions of the chromosomes in *S. cerevisiae* K5-5A.

and *S. carlsbergensis* No. I karyotypes differ by a number of bands (marked with arrows in Figure 4). Both of these strains have karyotypes distinguishable from the three subsequently employed lager strains.

3.2. Segregation of chromosomes III from *S. bayanus* NCYC374

S. bayanus NCYC374 could be sporulated on YPD medium as well as on sporulation medium at 20 °C. Tetrads were dissected and individual spores grown. No more than two spores per

ascus germinated. Homothallic spore clones were analysed for their chromosome III constitution in Southern blots with the *HIS4* probe (Figure 5). *S. bayanus* (lane 374) is characterized by six *SalI HIS4* fragments, two of which are known as pattern II (6.7 kb and 2.3 kb), while the other four fragments (6.4 kb, 5.5 kb, 2.1 kb, and 1.6 kb) originate from a chromosome designated pattern III. The five spore clones analyzed in Figure 5 reveal the segregation of these two types of chromosomes: Clones -4A, -4B, and -5A contain only chromosomes with pattern III and clone -7B contains exclusively pattern II. The

Figure 5. *HIS4* Sall restriction endonuclease fragment patterns of *S. bayanus* NCYC374, of *S. cerevisiae* S288C = Reference A, of *S. cerevisiae* K5-5A *his4-15* = Reference B and of clones from ascospores TSP-374-4A, -4B, -5A, -7A and -7B. NCYC374 contains *HIS4* patterns II and III, while spore clones -4A, -4B and -5A contain *HIS4* pattern III and -7B contains pattern II. Spore clone TSP-374-7A carries both *HIS4* patterns II and III. 4505-3A-5 is a chromosome addition line containing patterns I *his4-15* and III together. Fragment sizes are in thousand of basepairs (kb).

clone -7A has both types of chromosomes i.e. pattern II and III. To confirm that the spore clones are actual meiotic products from *S. bayanus* electrophoretic karyotypes were established for the *S. bayanus* parental strain and its progeny (Figure 6A). Segregation of several chromosomes are seen in the spore clones (Figure 6A, white arrow heads). The *HIS4* probe pC503 has been hybridized to the corresponding Southern filter of the gel (Figure 6B). NCYC374 contains a chromosome III doublet (Figure 6A, double white arrow heads) which has segregated in its progeny. Thus the spore clones -4A, -4B, and -5A with the *HIS4* pattern III have only the upper chromosome of the doublet, while the spore clone -7B containing only pattern II carries the lower chromosome of the doublet. It is also obvious that the spore clone -7A with both patterns II+III contains the doublet. That the latter is a meiotic segregant is shown by the absence of at least one chromosome (corre-

sponding in size to chromosome VI of the reference strain).

3.3. Genetic mapping of a *S. bayanus* chromosome III

S. bayanus strain BK4505 is a subculture of NCYC374 and has been kept for several years at the Department of Brewing Chemistry. It sporulates on YPD medium like NCYC374. It was sporulated and yielded one ascus with four spores. The four spore clones of this ascus were homothallic and contained only the *HIS4* pattern III. One of the clones was sporulated and mixed with a large surplus of cells from the chromosome III receptor strain K5-5A (α , *his4-15*, *ade2-1*, *can1*, *kar1-1*) and the mixture was plated on a YPD plate and incubated at 27 °C for 18 hours. After the incubation the cells were washed off and plated on a defined complete medium plate minus histidine plus canavanine

Figure 6. Electrophoretic karyotypes of ten *Saccharomyces* strains. Panel A: Ref. lane: *S. cerevisiae* genetic standard strain C81-1611, lane 374: *S. bayanus* 374, lanes 4A to 7B: Surviving tetraspores from dissected asci of *S. bayanus* NCYC374. Roman numerals correspond to chromosomes in *S. cerevisiae* C81-1611. White arrow heads point at chromosomal bands in NCYC374 which have segregated in the ascospore clones. Panel B: Corresponding Southern filter hybridized with *HIS4* probe pC503, thus revealing the position of chromosome III in *S. bayanus* NCYC374.

Table III. Ascus analysis of cross between *S. bayanus* chromosome III addition line CYT-TSP-4505-3A-5 and *S. cerevisiae* chromosome tester strain C79-992:

	4:0	3:1	2:1	2:2	1:2	1:3	0:3	0:4
<i>HIS4/his4</i>			6	10	2	1		
<i>LEU2/leu2</i>			6	10	2	1		
<i>THR4/thr4</i>			7	11	1			
<i>ADE2/ade2</i>			3	10	5	1		
<i>URA4/ura4</i>			1	2	5	5	2	4
			PD	NPD	TT			
<i>HIS4-LEU2</i>			19					
<i>LEU2-THR4</i>			6		13			

and adenine. Red actively growing colonies were selected after a week. Their growth was dependent on the transfer of an *S. bayanus* chromosome III with a functional complementing *HIS4* gene. The colony designated CYT-TSP-4505-3A-5 (Figure 5) reveals in the Southern blot the *HIS4* pattern I-15 characteristic for the *his4-15* allele with its 500 base pair deletion (14) including a *Sall* site and pattern III from the *S. bayanus* chromosome transferred. In Figure 5 is also given the comparison of a wild type *HIS4* pattern I and the pattern I-15 of the *his4-15* recipient strain. Due to the elimination of a *Sall* site the latter has a restriction fragment of 10.4 kb instead of the two fragments with the size of 9.3 and 1.5 derived from the *HIS4* wild type gene.

The disomic chromosome III addition line was crossed by rare mating with C79-992 (α , *his4-644*, *leu2*, *thr4*, *ura4*) and plated on minimal medium which will select for a strain containing the α , *his4-644* carrying chromosome, together with chromosome III from *S. bayanus*. A prototrophic colony was picked, grown, and sporulated. The ascus analysis is presented in Table III. In 19 asci analysed no recombinants were found in the interval *HIS4-LEU2*, while 13 tetratypes and 6 parental di-types were counted when the *LEU2-THR4* region was analysed. This corresponds to 34 cM, which indicates that the recombination frequency in this interval is similar to that observed in the *S. cerevisiae* chromosome III and the *S. carlsbergensis* chromosome III with the *HIS4* pattern II. The latter chromosome is also charac-

terised by the absence of crossing over in the *HIS4-LEU2* interval. Chromosome transfer from tetraspores TSP-374-4B (*HIS4* pattern III) and TSP-374-7B (*HIS4* pattern II) has been attempted, but only one very unstable reddish colored histidine prototroph cytoductant was found in the cross between TSP-374-4B and K5-5A.

3.4. Chromosome III transfer from the Danish lager strain BK2208

Lager strain BK2208 has formerly been used as a production strain at the Tuborg brewery. As seen from Table I the molecular markers *RDN1*, *HIS4*, and *LEU2* of this strain are those characteristic for the bottom fermenting yeasts. The strain has the *Ty* pattern IIa. A culture of this strain was sporulated and harvested spores were mixed with a surplus of cells from the chromosome III receptor strain K5-5A. A chromosome addition line was obtained in the same way as described above in the *S. bayanus* cross and a substitution line identified by the expression of the α mating type. The DNA of these two strains was digested with *Sall* and the separated restriction fragments probed for the *HIS4* and *LEU2* patterns (Figure 7). The chromosome substitution line CYT-MSP-2208-2 contains only *HIS4* pattern II (from strain BK2208) while the addition line CYT-MSP-2208-7 contains both patterns II and I-15 from the receptor strain. In agreement with these results the substitution line CYT-MSP-2208-2 carries *LEU2* fragment IV, while the addition line also has fragment I from

Figure 7. Restriction endonuclease fragment patterns of *HIS4* and *LEU2* of chromosome substitution line CYT-MSP-2208-2 and chromosome addition line CYT-MSP-2208-7 together with *S. cerevisiae* reference strains S288C and K5-5A. The restriction pattern analyses show that *LEU2* fragment IV is linked to *HIS4* pattern II in BK2208.

the receptor strain. This shows that fragments III, IV and I segregate independently.

The ascus analysis of the cross of the substitution line with the *S. cerevisiae* tester strain C79-992 (Table IV) gave the following results: Only four of the 27 tetrads contained four viable spores, the rest had three viable spores. Since the classes 2:1 and 1:2 are of the same size, 2:2 segregation of the chromosomes has been assumed. No recombination has occurred between *HIS4* and *LEU2* or between *LEU2* and *MAT*. For the recombination between *MAT* and *THR4* a genetic distance of 22 cM is calculated on the basis of 26 asci.

The result with the chromosome III transfer from strain BK2208 are in agreement with the result in (21) for the Carlsberg lager strain BK2246. Electrophoretic karyotypes reveal the following about the size of the genetically mapped chromosome III in the BK2208 lager strain. Chromosome III in the receptor strain K5-5A is marked in Figure 8 and also present in the CYT-MSP-2208-7 addition line. This chromosome is absent in the substitution line and the donor strain, both of which have a shorter chromosome III. The autoradiogram in Figure 9B of the Southern blot identifies the two chromosomes in the addition line (lane g), while K5-5A in lane e has the long chromosome III and the donor BK2208 in lane h the short chromosome III, which is also present in lane f containing the chromosomes of the substitution line.

Table IV. Ascus analysis of cross between *S. carlsbergensis* chromosome III substitution line CYT-MSP-2208-2 and *S. cerevisiae* tester strain C79-992:

	4:0	3:1	3:0	2:1	2:2	1:2	0:3	1:3	0:4
<i>HIS4/his4</i>				11	4	12			
<i>LEU2/leu2</i>				11	4	12			
<i>MATa/MAT α</i>				11	4	12			
<i>THR4/thr4</i>	1	1		13	2	10			
<i>ADE2/ade2</i>				12	4	11			
<i>URA4/ura4</i>				15	4	8			
			PD	NPD	TT				
<i>HIS4-LEU2</i>			27						
<i>LEU2-MAT</i>			27						
<i>MAT-THR4</i>			14		12

Figure 8. OFAGE chromosomal separations of chromosomal addition and substitution lines together with parental strains. From left to right: *S. cerevisiae* K5-5A receptor strain for chromosome III, Danish lager strain BK2208, chromosome III substitution line CYT-MSP-2208-2, chromosome III addition line CYT-MSP-2208-7, *S. cerevisiae* chromosome size reference strain C81-1611, addition line CYT-TSP-1511-4C-3 for chromosome III from *S. uvarum* C81-1511. Preferential separation of short chromosomes, 1.5% agarose and 35 seconds pulsetime.

3.5. Transfer of chromosome III from *S. uvarum* C81-1511 to *S. cerevisiae*

Chromosome III from *S. uvarum* has been transferred to *S. cerevisiae* in cytoductants with the aid of the *kar1* gene. The mother strain was sporulated and asci with four surviving spores obtained. The receptor strain K5-5A was mated with the homothallic spore isolates TSP-1511-4A, -4B, -4C, and -4D. Only one chromosome addition line in the form of a red histidine prototroph was obtained, namely CYT-TSP-1511-4C-3 (Figure 8). The karyotype reveals it to be disomic for chromosome III. Figure 9 demonstrates in lane b that chromosome III of *S.*

uvarum is shorter than chromosome III of the receptor strain K5-5A (lane e). The addition line karyotype in Figure 8 contains both these chromosomes and in addition at least two other chromosomes, one with a faster migration than chromosome III in the receptor strain and another one with a size intermediate between chromosome IX and VIII.

Since CYT-TSP-1511-4C-3 is disomic for chromosome III low mating efficiency is seen. The line was mated with both C79-992 (α) and C79-993 (α). In Figure 10 is shown a Southern blot of the *SalI* digested DNA from all hybrids obtained on minimal media plates (lanes a to h,

Figure 9. OFAGE chromosome separations of parental strains and chromosome III addition and substitution lines. Lane a: *S. carlsbergensis* Tuborg lager strain BK2224, lane b: *S. uvarum* C81-1511, lane c: hybrid strain CYT-TSP-1511-4C-3 $\times$ C79-992-3A, lane d: hybrid strain CYT-TSP-1511-4C-3 $\times$ C79-992-3B, lane e: *S. cerevisiae* chromosome III receptor strain K5-5A, lane f: substitution line CYT-MSP-2208-2 (partially degraded), lane g: addition line CYT-MSP-2208-7, lane h: *S. carlsbergensis* BK2208, lane i: Carlsberg lager strain BK2246, lane j: *S. cerevisiae* genetic standard strain S288C, and lane k: *S. inusitatus* CBS1546. Panel A: Total electrophoretic karyotypes and panel B: Corresponding Southern filter hybridized with *HIS4* probe pC503.

CYT-TSP-1511-4C-3 $\times$ C79-992 and lanes i to r, CYT-TSP-1511-4C-3 $\times$ C79-993. The blot was probed with the nick translated *HIS4* gene and the patterns of the two parents are given at right. The trisomic sum of the two patterns and

the wild type *HIS4* pattern from C79-992 or C79-993 is seen in e.g. lane d and lanes i to r. When mated to C79-992 the *his4-15* allele is absent in some progenies (lanes a, b, and c) leaving the *S. uvarum* chromosome III together

Figure 10. *SalI* restriction endonuclease treated DNAs hybridized with *HIS4* probe pC503. Lanes a to h: offspring from the cross between CYT-TSP-1511-4C-3 $\times$ C79-992. Seven of the strains contain *HIS4* patterns I and III with traces of pattern I-15. One strain (-5) has lost pattern III. Lanes i to r: The progeny from the cross CYT-TSP-1511-4C-3 $\times$ C79-993 all seem to contain *HIS4* patterns I, I-15 and III and are thus trisomic for chromosome III.

with the *S. cerevisiae* chromosome III. In lane e a hybrid is depicted, which has lost the *S. uvarum* chromosome. The eight hybrid offsprings from the mating between the cytoductant and C79-992 were obviously unstable, since they show a variety of colony colours and shapes. The hybrid strain CYT-TSP-1511-4C-3 $\times$ C79-992-3 was chosen for further analyses since it seemed to contain only the *S. uvarum* chromosome III together with the chromosome III from C79-992. It was reisolated several times and divided into two lines, a "brown" -3A and a "white" -3B line. In Figure 9, lanes c and d are shown the OFAGE separations of the two lines and as indicated by the arrow heads each one has retained only two chromosomes III. Line -3B has lost chromosome III from *S. uvarum* and has retained chromosome III from K5-5A, while the -3A line shows the reverse situation, it has kept the *S. uvarum* chromosome III and has lost the K5-5A chromosome. The other chromosomal bands seem to be unaltered. The CYT-TSP-1511-4C-3 $\times$ C79-992-3A hybrid was picked and analysed. Very few, three and four spored asci

formed upon sporulation, so a genetic map could not be derived for the transferred *S. uvarum* chromosome III.

In Figure 11 are shown the *Saccharomyces* lines which have been used for characterization of the *S. uvarum* C81-1511 chromosome III. The Southern blot of the restricted DNAs have been hybridized to the nick translated *HIS4* probe. Each strain has been digested separately by *EcoRI*, *SalI*, and *HindIII*. In lanes a and b are shown the -3A and -3B hybrid lines with and without the *S. uvarum* chromosome III, in lanes c and d are shown C81-1511 (*S. uvarum*) and TSP-1511-4C (*S. uvarum* spore clone), while in lanes e, f, and g are presented DNAs from CYT-TSP-1511-4C-3 (*S. uvarum* chromosome III addition line), K5-5A and C79-992. In lane a is seen *HIS4* pattern I and III together, in b *HIS4* pattern I-15 and I. *HIS4* pattern III alone is seen in *S. uvarum* DNA in lane c and in its spore clone in d, while pattern I-15 and III are together in the DNA of the addition line in lane e. The two *S. cerevisiae* strains in lanes f and g contain pattern I-15 and I respectively.

Figure 11. Restriction endonuclease fragment patterns of DNA from seven *Saccharomyces* strains, digested with *EcoRI*, *Sall* or *HindIII*, respectively, and probed with *HIS4* pC503. Strain a: hybrid strain CYT-TSP-1511-4C-3 $\times$ C79-992-3A ("brown" line), strain b: hybrid strain CYT-TSP-1511-4C-3 $\times$ C79-992-3B ("white" line), strain c: *S. uvarum* C81-1511, strain d: *S. uvarum* tetraspore TSP-1511-4C, strain e: chromosome addition line CYT-TSP-1511-4C-3, strain f: *S. cerevisiae* chromosome III receptor strain K5-5A, and strain g: *S. cerevisiae* tester strain C79-992. The hybrid strain in lane a contains both *HIS4* from *S. cerevisiae* C79-992 and from *S. uvarum* C81-1511, while the hybrid in lane b contains the two *S. cerevisiae* *HIS4* alleles, *HIS4* pattern I-15 from K5-5A, and *HIS4* pattern I from C79-992. Lanes c and d contain *HIS4* pattern III, while lane e contains *S. uvarum* *HIS4* pattern III together with *S. cerevisiae* *HIS4* pattern I-15.

4. DISCUSSION

In Figure 1 a partial genetic map is drawn for the chromosome III of *S. bayanus* which could be transferred into a marked *cerevisiae* line and analysed. It reveals two important features: The *S. bayanus* genes which can functionally complement the *HIS4*, *LEU2*, and *THR4* genes of *cerevisiae* are found on the same chromosome. As is the case for chromosomes III from the Carlsberg lager strain BK2246 (21) and the Danish lager strain BK2208, cross over occurs between the bayanus and *cerevisiae* chromosomes III in the *LEU2* *THR4* interval but not in

the *HIS4*-*LEU2* interval. Although the genetic map of chromosome III of *S. carlsbergensis* and *S. bayanus* are similar, distinct differences between the karyotypes of these two species have been observed. The similarity of the karyotypes of *S. bayanus*, *S. inusitatus*, and *S. uvarum* is attested by the restriction endonuclease fragment patterns of *RDNI*, *HIS4*, *LEU2*, and DNA-DNA reassociation kinetics (4, 23, 24, 25, 27, 30). Since *S. bayanus* NCYC374 shows composite restriction endonuclease fragment patterns at the *HIS4* locus (24, 25, 27), and independent segregation of these patterns in

spore clones more than one chromosome III are present in the genome of *S. bayanus*. A heterologous origin of these chromosomes III is therefore indicated like in the Carlsberg lager strain BK2246 (21, 22, 36, 37). The reason for obtaining only a few chromosome addition lines, when *S. bayanus* or *S. uvarum* is used as donor chromosome to *S. cerevisiae* lines is unknown. WINGE and LAUSTSEN (38) found only 3 germinating spores out of 182 (2% germination) from a sporulated hybrid between *S. cerevisiae* and *S. validus*, while hybrids between Danish bakers yeast and other *S. cerevisiae* strains had a germination frequency of 59% to 94% (38). In *S. bayanus* extensive chromosome length polymorphisms are uncovered when the chromosomes are segregated into spores (cf Figure 6). Further analyses will show to what extent the chromosomal bands in NCYC374 are homoeologous.

There seem to be three major types of bottom fermenting strains, namely: *S. carlsbergensis* C84-AJL248, modern lager strains and *S. monacensis*. Whereas modern lager strains have homoeologous chromosomes III which in electrophoretic separations display uniform length, the type strains of *S. carlsbergensis* C84-AJL248 and *S. monacensis* CBS1503 contain homoeologous chromosomes III of 3 or 2 significantly different sizes (Figures 3 and 4). The chromosome III length of the modern lager strains is comparable to that of one of the chromosomes III of *S. monacensis*, but not found in *carlsbergensis* C84-AJL248. At the level of small segments of the homoeologous chromosomes, the *HIS4*, *LEU2*, and *TyI* restriction endonuclease fragment patterns of *S. carlsbergensis* C84-AJL248 resemble the lager strains closely (27). In short, *S. monacensis* traits are found both in the modern lager strains and *S. carlsbergensis* C84-AJL248. Since the lager strains and *S. carlsbergensis* C84-AJL248 contain *S. cerevisiae* related alleles of at least *HIS4* and *LEU2*, it is suggested that *S. monacensis* is one of the parental strains for the two, and that the other parental strain is a *S. cerevisiae* top fermenting yeast. When E. CHR. HANSEN described the two species *S. carlsbergensis* and *S. monacensis* (12), he had established that the yeast used in 1882-1883 at the Carlsberg brewery was a mixture of

several lines of which *S. carlsbergensis* No. I was one of them and *S. monacensis* another one (line No. II). He suggested that the latter line originated from breweries in Munich (12). Further studies of single chromosome transfers with OFAGE separations of chromosomes accompanied by molecular hybridizations and tetrad analysis will elucidate the genotypic organization and evolution of *Saccharomyces bayanus*, *S. carlsbergensis*, *S. monacensis*, and *S. uvarum*.

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REFERENCES

1. ANDREADIS, A., Y.-P. HSU, G. B. KOHLHAW & P. SCHIMMEL: Nucleotide sequence of yeast *LEU2* shows 5'-noncoding region has sequences cognate to leucine. *Cell* 31, 319-325 (1982)
2. BAICHWAL, V. R., T. S. CUNNINGHAM, P. R. GATZKE & G. B. KOHLHAW: Leucine biosynthesis in yeast. *Curr. Genet.* 7, 369-377 (1983)
3. BARNETT, J. A., R. W. PAYNE & D. YARROW: *Yeasts: Characteristics and identification*. Cambridge University Press (1983)
4. BICKNELL, J. N. & H. C. DOUGLAS: Nucleic acid homologies among species of *Saccharomyces*. *J. Bacteriol.* 101, 505-512 (1970)
5. CARLE, G. F. & M. V. OLSON: Separation of chromosomal DNA molecules from yeast by orthogonal-field alternation gel electrophoresis. *Nucl. Acid Res.* 12, 5647-5664 (1984)
6. CARLE, G. F. & M. V. OLSON: An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci. USA* 82, 3756-3760 (1985)
7. CHINAULT, A. C. & J. CARBON: Overlap hybridization screening: Isolation and characterization of overlapping DNA fragments surrounding the *LEU2* gene on yeast chromosome III. *Gene* 5, 111-126 (1979)
8. CONDE, J. & G. R. FINK: A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc. Nat. Acad. Sci. USA* 73, 3651-3655 (1976)
9. DENHARDT, D. T.: A membrane - filter technique

- for the detection of complementary DNA. Biochem. Biophys. Res. Commun. 23, 641-646 (1966)
10. DOBSON, M. J., S. M. KINGSMAN & A. J. KINGSMAN: Sequence variation in the LEU2 region of the *Saccharomyces cerevisiae* genome. *Gene* 16, 133-139 (1981)
 11. FINK, G. R.: A cluster of genes controlling three enzymes in histidine biosynthesis in *Saccharomyces cerevisiae*. *Genetics* 53, 445-459 (1966)
 12. HANSEN, E. CHR.: Recherches sur la physiologie et la morphologie des ferments alcooliques. *Compt. Rend Lab. Carlsberg* 7, 179-217 (1908)
 13. HOLMBERG, S., J. G. L. PETERSEN, T. NILSSON-TILLGREN & M. C. KIELLAND-BRANDT: Molecular characterization of a *Saccharomyces* plasmid containing the *HIS4* gene. *Carlsberg Res. Commun.* 44, 269-282 (1979)
 14. HOLMBERG, S., M. C. KIELLAND-BRANDT, T. NILSSON-TILLGREN & J. G. L. PETERSEN: Molecular characterization of three *his4* deletion mutants in *Saccharomyces cerevisiae*. *Carlsberg Res. Commun.* 44, 283-288 (1979)
 15. 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)
 16. HOLMES, D. S. & M. QUIGLEY: A rapid boiling method for the preparation of bacterial plasmids. *Anal. Biochem.* 114, 193-197 (1981)
 17. JEFFREYS, A. J. & R. A. FLAVELL: A physical map of the DNA regions flanking the rabbit β -globin gene. *Cell* 12, 429-439 (1977)
 18. KIELLAND-BRANDT, M. C., T. NILSSON-TILLGREN, S. HOLMBERG, J. G. L. PETERSEN, & B. A. SVENNINGSSEN: Transformation of yeast without use of foreign DNA. *Carlsberg Res. Commun.* 44, 77-87 (1979)
 19. MANIATIS, T., E. F. FRITSCH & J. SAMBROOK: Molecular cloning. A laboratory Manual. Cold Spring Harbor Laboratory, New York (1982)
 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. *Proc. IV Symp. Genet. Industrial Organisms*, 143-147 (1982)
 23. PEDERSEN, M. B.: Fingerprinting of DNA in the identification of yeast. *Proc. 19th Europ. Brewery Conv. Congr. London*, pp. 457-463 (1983)
 24. PEDERSEN, M. B.: DNA sequence polymorphisms in the genus *Saccharomyces* I. Comparison of the *HIS4* and ribosomal RNA genes in lager strains, ale strains and various species. *Carlsberg Res. Commun.* 48, 485-503 (1983)
 25. PEDERSEN, M. B.: Brewing yeast identification by DNA fingerprinting. *Proc. 20th Europ. Brewery Conv. Congr. Helsinki* (1985)
 26. PEDERSEN, M. B.: DNA sequence polymorphisms in the genus *Saccharomyces* II. Analysis of the genes *RDN1*, *HIS4*, *LEU2* and *Ty* transposable elements in Carlsberg, Tuborg and 22 Bavarian brewing strains. *Carlsberg Res. Commun.* 50, 263-272 (1985)
 27. PEDERSEN, M. B.: DNA sequence polymorphisms in the genus *Saccharomyces* III. Restriction endonuclease fragment patterns of chromosomal regions in brewing and other yeast strains. *Carlsberg Res. Commun.* 51, 163-183 (1986)
 28. PRICE, C. W., G. B. FUSON & H. J. PHAFF: Genome comparison in yeast systematics: Delimitation of species within the genera: *Schwanniomyces*, *Saccharomyces*, *Debariomyces* and *Pichia*. *Microbiol. Rev.* 42, 161-193 (1978)
 29. RIGBY, P. W. J., M. DIECKMANN, C. RHODES & P. BERG: Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* 113, 237-251 (1977)
 30. ROSINI, G., F. FEDERICI, A. E. VAUGHAN & A. MARTINI: Systematics of the species of the genus *Saccharomyces* associated with the fermentation industry. *European J. Appl. Microbiol. Biotechnol.* 15, 188-193 (1982)
 31. SCHWARTZ, D. C. & C. R. CANTOR: Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* 37, 67-75 (1984)
 32. SHERMAN, F., G. R. FINK & J. R. HICKS: Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 11724, pp. 61-64 (1979)
 33. SOUTHERN, E. M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98, 503-517 (1975)
 34. STORMS, R. K., J. B. MCNEIL, P. S. KHANDEKAR, G. AN, J. PARKER & J. D. FRIESEN: Chimeric plasmids for cloning of deoxyribonucleic acid sequences in *Saccharomyces cerevisiae*. *J. Bacteriol.* 140, 73-82 (1979)
 35. WALT, J. P. VAN DER: Genus *Saccharomyces*, In:

- The Yeasts, a Taxonomic Study. J. Lodder, ed. North-Holland Publishing Company. Amsterdam London pp. 555-718 (1970)
36. WETTSTEIN, D. VON: Emil Christian Hansen Centennial Lecture: from pure yeast culture to genetic engineering of brewers yeast. Proc. 19th Europ. Brewery Conv. Congr. London, pp. 97-120 (1983)
37. WETTSTEIN, D. VON, C. GJERMANSEN, S. HOLMBERG, M. C. KIELLAND-BRANDT, T. NILSSON-TILLGREN, M. B. PEDERSEN, J. G. L. PETERSEN & P. SIGTGAARD: Genetic engineering in the improvement of brewers yeast. MBAA Tech. Quart. 21, 45-61 (1984)
38. WINGE, Ø. & O. LAUSTSEN: On 14 new yeast types, produced by hybridization. Compt. Rend. Lab. Carlsberg, Ser. Physiol. 22, 337-352 (1939)
39. YANG, C. A., J. LIS & R. WU: Elution of DNA from agarose gels after electrophoresis. Meth. in Enzymol. 68, 176-182 (1979)
40. YARROW, D. & T. NAKASE: DNA base composition of species of the genus *Saccharomyces*. Antonie van Leeuwenhoek 41, 81-88 (1975)

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