Variation of electrophoretic karyotypes in genetically different strains of *Saccharomycopsis fibuligera* and *Yarrowia lipolytica*

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***Saccharomycopsis fibuligera* is a dimorphic yeast, which is both saccharolytic and fermentative, that is used in the production of rice wine. It has a predominant diploid phase. When grown on solid agar *S. fibuligera* strains develop different morphological forms. In previous studies, intergeneric hybrids between *S. fibuligera* and a related yeast, *Yarrowia lipolytica*, were obtained which were able to utilize starch and tributyrin. A putative haploid mitotic segregant, N14i60 *met*, was obtained from the intergeneric hybrid between *S. fibuligera* 193 *met* and *Y. lipolytica* A *his1*. Auxotrophic mutants were readily isolated following UV mutagenesis of N14i60 *met*. The auxotrophic mutants and N14i60 have a similar morphology. Protoplast fusants were produced between the auxotrophic mutant A6 *met lys1 arg1* and *Y. lipolytica* 21501-4 B *lys5 leu2 ade1 xpr2* and between specific pairs of the auxotrophic mutant strains. Differences and similarities in the DNA banding patterns of these genetically different strains of *S. fibuligera* and *Y. lipolytica* were demonstrated and it was established that four distinct types consistent with their specific positions in the pedigree chart could be clearly distinguished. Furthermore, by a study of the patterns of hybridization signals for specific genes of *S. fibuligera* for an intergeneric hybrid and its mitotic segregants, genetic segregants with different characteristics were obtained. The segregants from the intergeneric hybrid and the protoplast fusants probably arose by a process of chromosomal assortment at mitosis. The intergeneric hybrid, the protoplast fusants and their mitotic segregants showed a similar karyotype. Together these studies provide an explanation for the basis of phenotypic differences of some of the yeast strains studied.**

Keywords: *Saccharomycopsis fibuligera*, *Yarrowia lipolytica*, electrophoretic karyotypes

INTRODUCTION

Saccharomycopsis fibuligera was first isolated from chalky bread by Lindner in 1907 and it was then named *Endomyces fibuliger* Lindner. In 1984, it was re-classified in the genus *Saccharomycopsis* (Lodder, 1970; Kreger-van Rij, 1984). Strains of *S. fibuligera* have been isolated from ragi tape and macaroni (Barnett *et al.*, 1983). It is a homothallic yeast, with a dominant diploid phase and a brief haploid phase. It is dimorphic, and exhibits both a multicellular mycelial form with branched septate hyphae which contain plasmodesmata, and individual budding cells. *S.*

fibuligera produces hat-shaped ascospores that are formed in spheroidal asci borne usually on lateral branches of the main hyphae.

When cultivated on yeast extract/phosphate/soluble starch agar (YPSS), strains of *S. fibuligera* give white sporulation. On prolonged culture the colonies often produce mitotic sectors. *S. fibuligera* is a protein secretor, and produces and secretes glucoamylase and α -amylase. It is a slow sugar-fermenting yeast; sugars such as glucose and maltose, but not galactose and sucrose, are converted to alcohol and carbon dioxide. *S. fibuligera* is also able to hydrolyse cellobiose by its extracellular β -glucosidase (Machida *et al.*, 1988). These characteristics make it extremely suitable for use in brewing industries to

Abbreviation: PFGE, pulsed-field gel electrophoresis.

Table 1. Descriptions of yeast strains used in this study

Category	Strain	Origin	Description of colonies grown on YPSS
I	<i>S. fibuligera</i> 2610 <i>met</i> 8014 <i>met</i> }	Stock culture	Convex white colonies with erose margin Convex white colonies with erose margin
II	<i>S. fibuligera</i> 193 <i>met</i>	UV-induced mutant of 8014 <i>met</i>	Pulvinate brownish colonies with erose margin
III	<i>S. fibuligera</i> 56 <i>met</i>	Mitotic sector of 193 <i>met</i>	Pulvinate white colonies with erose margin
IV	<i>S. fibuligera</i> N14i60 <i>met</i>	Mitotic sector of the intergeneric hybrid 193 <i>met</i> /1529 <i>A his1</i>	Thin, filmy white colonies with less aerial mycelia than strains of categories I, II and III
	A6 <i>met lys1 arg1</i> } A18 <i>met ade1</i> } I12 <i>met his1</i> }	UV-induced mutants of N14i60 <i>met</i>	
V	<i>S. fibuligera</i> /Y. <i>lipolytica</i> intergeneric hybrid A6/21501-4B } A18/21501-4B }	Protoplast fusion	Colonies with the appearance of 193 <i>met</i>
VI	Fusants between auxotrophic mutants A6/A18 } A18/I12 }	Protoplast fusion	Colonies like those of IV but thicker in appearance and with more aerial mycelia
VII	Mitotic sectors of intergeneric hybrids		In general, thin, filmy white colonies like those of category IV; occasionally, sectors with a distinctly slow growth rate One sector, S6, obtained from A6/21501-4B appeared wrinkled and non-hairy had less aerial hyphae than the other sectors from the same hybrid
VIII	Mitotic sectors of fusants Y. <i>lipolytica</i> 1529 <i>A his1</i> Y. <i>lipolytica</i> 21501-4B <i>lys5 leu2</i> <i>ade1 xpr2</i> Y. <i>lipolytica</i> <i>A ura3 leu2 lys11</i>		In general, thin, filmy white colonies like those of category IV; occasionally, sectors with a distinctly slow growth rate

produce wine from starchy substrates (Merican & Yeoh, 1989).

Very little is known about the genetics of this yeast and, as far as we are aware, only two papers which reported studies of intergeneric hybrids between *Saccharomycopsis* and *Yarrowia* have previously been published (Nga *et al.*, 1992, 1994). We are particularly interested in developing a system for genetic analysis in *S. fibuligera* which requires a haploid strain. For this purpose an intergeneric hybrid between *S. fibuligera* 193 *met* and a strain of the closely related dimorphic yeast, *Y. lipolytica* 1529 *A his1* was produced by mass mating. The intergeneric hybrid was extremely unstable in prolonged vegetative culture on YPSS and produced numerous mitotic segregants. A large number of the mitotic segregants were mitotically unstable. Among the mitotic segregants, however, there was a mitotically stable, putative haploid strain, N14i60 *met* (Nga *et al.*, 1994). By UV mutagenesis of this strain, auxotrophic mutants were readily obtained. Diploid fusants were obtained between pairs of these mutants by protoplast fusion. When single colonies of the fusants

were grown on YPSS agar for 14 d at 28 °C mitotic sectors, some of which were stable and haploid, were obtained. By systematic analysis of the phenotypic classes of the haploid mitotic sectors of the fusants, linkage relationships between the genetic markers of the parent strains were determined (Nga *et al.*, 1994). As we now have a pedigree of *S. fibuligera* strains in our stock, we set out to determine if these strains could be classified by differences in their electrophoretic karyotypes. We also studied the pattern of hybridization signals for specific genes of *S. fibuligera* for the intergeneric hybrid, A6/21501-4B, and its mitotic segregants.

METHODS

Strains of yeast. The origin and characteristics of the strains of *S. fibuligera* (8014 *met*, 193 *met*, 2610, 56 *met*, N14i60 *met*, A6, A18 and I12) and of *Y. lipolytica* (1529 *A his1*, 21501-4B *leu2 lys5 ade1 xpr2* and *A ura3 leu2 lys11*) studied are given in Table 1. The interrelationships of the yeast strains of direct significance to this study are presented in the pedigree chart in Fig. 1.

Fig. 1. Pedigree of yeast strains used in this study.

Saccharomyces cerevisiae SH964 and *Schizosaccharomyces pombe* L975 were from the stock of Y. Oshima, Department of Biotechnology, Faculty of Engineering, Osaka University, Japan. They were used to provide the standard size markers for the pulsed-field gel electrophoresis (PFGE) runs.

Media. The media for the growth of the strains used in these studies were those of Nga *et al.* (1992). The recipes for complex yeast extract/peptone/dextrose medium (YEFD), and Difco Yeast Nitrogen Base (without amino acids) (YNB) minimal medium were described by Nga *et al.* (1992). Supplementation of YNB when required (for classification) was as follows: 1.5% (w/v) starch, 2% (w/v) glucose, 1% (v/v) tributyrin, 20 mg l⁻¹ each of adenine, arginine and methionine, and 30 mg l⁻¹ each of leucine and lysine. The recipes of YPSS and yeast extract agar (YEA) were described by Nga *et al.* (1992).

Protoplast fusion. Preparation of protoplasts of yeast strains and protoplast fusion between pairs of yeast strains were by the techniques of Nga *et al.* (1992). Following the treatment of the protoplasts of the two strains with 4 ml 25% (v/v) PEG in osmotic stabilizing buffer (OSB) and incubation at 30 °C for 30 min, an equal volume of OSB was added to the suspension, followed by centrifugation. The pellet was washed once in 2 ml OSB and resuspended in 2 ml OSB. Aliquots from this preparation, and diluted portions thereof, were dispensed onto 3.5 ml molten YNBG agar containing 1 M sorbitol (YNBGS) and overlaid onto YNBGS agar plates. Depending on the specific crosses, the YNBGS in the overlay was appropriately supplemented with lysine, adenine or methionine.

Mitotic sectoring of fusants. *S. fibuligera* 193 *met*/Y. *lipolytica* 1529 A *his1* intergeneric hybrid, and the protoplast fusants between pairs of auxotrophic mutant strains derived from N14i60 *met*, were inoculated individually onto the centre of YPSS plates and incubated at 28 °C for 14 d. These strains could be distinguished from the haploid strains as the latter were stable and did not give sectors in mitotic sectoring tests.

Mass mating. Crossing between *S. fibuligera* 193 *met* and *Y. lipolytica* 1529 A *his1* was by the method of Gaillardin *et al.* (1973). Intergeneric hybrids were obtained as described by Nga *et al.* (1992).

PFGE. The protocol for preparing DNA of yeast strains to be separated by PFGE was that of De Jonge *et al.* (1986). Resolution of the yeast chromosomal DNA was carried out by using the LKB-Pharmacia Pulsaphor apparatus equipped with a hexagonal electrode array (Koh, 1993; Yip, 1993). The running conditions of the Pulsaphor consisted of three sequential separating steps as follows: 80 V with a 1200 s pulse time for 48 h; 50 V with a 1800 s pulse time for 48 h; and then 50 V with a 2700 s pulse time for 48 h. Electrophoresis was carried out in 1% (w/v) agarose gel with 0.5 × Tris/acetate/EDTA cooling buffer at 14 °C.

Labelling with isotope, hybridization probe and preparation of autoradiograms. Synthesis of uniformly labelled probes was with [α -³²P]dCTP by the random priming method. Southern hybridizations of nylon membrane impregnated with DNA and preparation of autoradiograms with Kodak XAR-5 X-ray film were carried out by standard methods (Sambrook *et al.*, 1989). The α -amylase (*ALP1*) gene probe was prepared from the 1.88 kb *XhoI*-*StuI* fragment of the plasmid pSfALP1 of Yamashita *et al.* (1985a). The glucoamylase (*GLU1*) gene probe was the 2.54 kb *PstI* fragment of the plasmid pSfGLU ES1 of Yamashita *et al.* (1985b). The rDNA gene probe was the 7.7 kb *HindIII* fragment of plasmid pINA 24 obtained from van Heerikhuizen *et al.* (1985).

RESULTS AND DISCUSSION

Characteristics of the intergeneric hybrids and protoplast fusants between auxotrophic mutants

In the case of the intergeneric hybrid, A6/21501-4B, the fusant was isolated on YNB supplemented with glucose and lysine (Chiu, 1992). The hybrid grew only when YNBG was supplemented with lysine. With the intergeneric hybrid A18/21501-4B, as expected, the fusant was able to grow on YNBG supplemented with adenine. This result could be explained on the basis that in both the parent strains, lesions had occurred in the gene for the same enzyme in the biosynthetic pathway of adenine. Protoplast fusants, A6/A18 and A18/I12, were able to grow on YNBG.

A distinctive characteristic of the intergeneric hybrids and the protoplast fusants between auxotrophic mutants was that they were mitotically unstable in vegetative culture on YPSS at 28 °C for 14 d.

Phenotypes of the intergeneric hybrid, A6/21501-4B, the protoplast fusants A6/A18 and A18/I12, and some of their mitotic sectors

S. fibuligera produces glucoamylase and α -amylase. Glu⁺ colonies produced an opaque halo on starch agar due to formation of turbid precipitates, whereas Alp⁺ colonies produced a clear halo. Glucoamylase catalyses the release of glucose molecules from the non-reducing ends of starchy materials and other α -glucopyranosides. α -Amylase randomly hydrolyses α -1,4-glucoside linkages of amylose and amylopectin, but not α -1,6-glucoside link-

Fig. 2. Hydrolysis of starch of yeast strains on YPSS medium observed as haloes around the colonies. A, *Y. lipolytica* A *his1*; B, *Y. lipolytica* 21501-4B *leu2 lys5 ade1 xpr2*; C, *S. fibuligera* A6; D, *S. fibuligera* A18; E, *S. fibuligera*/Y. *lipolytica* intergeneric hybrid A6/21501-4B; F, *S. fibuligera* S5; G, *S. fibuligera* S6; H, *S. fibuligera* S14; I, *S. fibuligera* S17; J, *S. fibuligera* S19.

ages of amylopectin. For the colonies of A6 and A18, clear haloes were observed after growth on YPSS for 2 d. Colonies of the intergeneric hybrid between A6 and *Y. lipolytica* 21501-4B also showed a clear halo around the colony on YPSS. On this medium, colonies of *Y. lipolytica* 21501-4B did not show a halo; this result was expected as *Y. lipolytica* is not amylolytic. Strains A6, A18 and A6/21501-4B showed a similar growth rate on YPSS. When cells of the hybrid, A6/21501-4B, were inoculated onto YPSS as single colonies and incubated for 14 d at 28 °C, mitotic sectors were observed. Some of the sectors were unstable and gave sectors when their cells were inoculated onto YPSS and tested for mitotic sectoring; other sectors from the hybrid were stable when tested for mitotic sectoring. Six stable mitotic segregants obtained from the intergeneric hybrid, A6/21501-4B, showed a slower growth rate than the parent hybrid. Colonies of one of the segregants, S6, showed a distinctly slower growth rate with less aerial hyphae when compared with the other five segregants, S5, S14, S17, S18 and S19 (Table 1; Yip, 1993). Furthermore, the surface of the colonies of S6 appeared wrinkled and non-hairy, i.e. similar to *Y. lipolytica*. However, colonies of S6 on YPSS could be easily distinguished from those of *Y. lipolytica* 21501-4B on the same medium. Colonies of the other five segregants had aerial hyphae, a non-wrinkled appearance and amylolytic activity, and thus resembled *S. fibuligera* A6.

All six mitotic segregants were considered to be putative haploids as they were mitotically stable and did not give sectors when inoculated onto YPSS. When five of the segregants were grown on YPSS as single colonies for 2 d at 28 °C, haloes were observed, but with sizes smaller than those of A6, A18 and A6/21501-4B (Fig. 2; Yip, 1993)

with strain S6 giving a distinctly smaller halo than the others. Strains S5, S14 and S17 gave haloes which were smaller than S19 but larger than S6. A distinctive feature was that the four strains, S5, S6, S14 and S17, gave haloes which had a cloudy appearance in contrast to the bright or clear haloes of A6, A18, A6/21501-4B and S19. To explain this phenomenon more precisely, studies were made of the electrophoretic karyotypes of these strains and of Southern hybridization patterns with the probes for the *ALP1* and *GLU1* genes (see below). These results are in agreement with the view that the haploid mitotic segregants arose from the diploid parent hybrid by a process of haploidization during which chromosomal assortment had occurred.

As for the protoplast fusants, A6/A18 and A18/I12, all the stable mitotic segregants obtained from them from mitotic sectoring had, in general, a thin, filmy white phenotype (Table 1). Colonies of the segregants on YPSS had aerial hyphae, were non-wrinkled and amylolytic and resembled *S. fibuligera* strains A6 and A18. Mitotic segregants with parental and non-parental phenotypes were obtained from these fusants (Chiu, 1992).

Electrophoretic karyotypes of yeast strains

The chromosomal DNA banding patterns of the yeast strains used in this study are represented by electrophoretic karyotypes in Fig. 3. In these studies, standard size markers for the chromosomes were provided by the DNA of *Schiz. pombe* and *Sacc. cerevisiae*. The results showed that four chromosomal banding patterns for the *S. fibuligera* strains could be distinguished as follows: I, 2610 and 8014 *met*, with seven bands; II, 193 *met*, with eight bands; III, 56 *met*, with six bands; and IV, N14i60 *met* and the auxotrophic mutants derived from it, the intergeneric hybrids A6/21501-4B and A18/21501-4B, the protoplast fusants between pairs of the auxotrophic mutants, and the mitotic segregants of the hybrids and fusants, with seven bands. The mobilities of the seven bands of these strains correspond with one another. *Y. lipolytica* 1529 A *his1* had six bands and 21501-4B six bands. Chromosomal DNA bands 5 and 6 of *Y. lipolytica* 21501-4B are of sizes which corresponded with bands 6 and 7 of the type IV strains, respectively. As can be observed in Fig. 3, strain 8014 *met* differs from 193 *met* in that it lacks a band corresponding to band 6 of the latter. Bands 1–4 of strains 193 *met* and 8014 *met* correspond with one another. Band 2 of both these strains is larger than band 2 of the type IV strains. Band 6 of the type IV strains corresponds with band 7 of 193 *met* and band 6 of 8014 *met*, and the band 5 of the type IV strains corresponds with band 5 of 193 *met*, also being smaller than band 5 of 8014 *met* (Koh, 1993; Yip, 1993).

Probing by Southern hybridization with α -amylase (*ALP1*) and glucoamylase (*GLU1*) genes

A comparison of the electrophoretic karyotypes of the mitotic segregants with their phenotypes revealed that although these strains were clearly distinguishable phenotypically, they could not be separated by their DNA

Fig. 3. PFGE karyotyping of yeast strains. (a) Lane 1, *Sacc. cerevisiae*; lane 2, *Schiz. pombe*; lane 3, *S. fibuligera* 8014 met; lane 4, *S. fibuligera* 193 met; lane 5, *S. fibuligera* 56 met; lane 6, *S. fibuligera* N14i60 met; lane 7, *Y. lipolytica* A his1. (b) Lane 1, *Sacc. cerevisiae*; lane 2, *Schiz. pombe*; lane 3, *S. fibuligera* A6; lane 4, *S. fibuligera* fusant A6/A18; lanes 5 and 6, mitotic sectors 116 and 117 of A6/A18; lane 7, *S. fibuligera* fusant A18/112; lane 8, *S. fibuligera* 112; lane 9, mitotic sector C15 of A18/112. (c) Lane 1, *Sacc. cerevisiae*; lane 2, *Schiz. pombe*; lane 3, *S. fibuligera* 193 met; lane 4, *S. fibuligera* N14i60 met; lane 5, *S. fibuligera* A18; lane 6, *Y. lipolytica* 21501-4B *leu2 lys5 ade1 xpr2*; lane 7, *S. fibuligera*/*Y. lipolytica* intergeneric hybrid A18/21501-4B; lanes 8, 9 and 10, mitotic sectors M23, M30 and M39 of A18/21501-4B.

banding patterns in PFGE studies. This agrees with the results of a recent study by Rustchenko-Bulgac & Howard (1993) in which the phenotypic variability of a number of characters in strains of *Candida albicans* did not correspond with the specific patterns of chromosomal alterations in PFGE studies.

In the study on Southern hybridization, the DNA bands of the intergeneric hybrid A6/21501-4B and the four mitotic segregants, S5, S6, S14 and S17, were probed with *ALP1* and *GLU1* (Yip, 1993). In the case of *ALP1*, hybridization signals were observed for DNA band 1 for strains A6, A6/21501-4B, and the four segregants, but not for *Y. lipolytica* 21501-4B (Fig. 4). The reduced amyolytic activity of strains S5, S6, S14 and S17 as compared with A6 was therefore not due to the absence of the *ALP1* gene in chromosome 1. With the *GLU1* probe hybridization signals were observed for bands 6 and 7 for A6 and A6/21501-4B, indicating that their chromosomal bands 6 and 7 were similar to those of strain A6, whereas in the cases of the segregants S6 and S14 signals were only observed for band 7 (Fig. 5). In the cases of S5 and S17, hybridization signals were observed only for band 6. No hybridization signals were observed for the DNA bands of *Y. lipolytica* 21501-4B. These results suggest that chromosomal assortment occurred at mitosis, giving rise to haploid strains with different haploid complements of chromosomes.

Taking all the results into account, three classes of mitotic segregants of the intergeneric hybrid, A6/21501-4B, can be distinguished. As all of the four strains, S5, S6, S14 and S17, have seven chromosomal DNA bands with corresponding mobilities, the differences between these strains cannot be due to aneuploidy. With the *GLU1*

probe, strains S5 and S17 gave hybridization signals with chromosomal band 6, whereas strains S6 and S14 hybridized with chromosomal band 7. In the cases of S5 and S17, chromosomal band 7 of A6 could have been substituted by chromosome 6 of *Y. lipolytica* 21501-4B, whereas the S6 chromosome 6 of A6 could have been replaced by chromosome 5 of *Y. lipolytica* 21501-4B. This result suggests that the *GLU1* gene on chromosomes 6 or 7 may be absent in these segregants with no distinct undesirable or lethal consequence to the strain. Strain S14 is however different from S6 in that S14 has hairy aerial hyphae. Of particular significance was the finding that a few spontaneous segregants were obtained from S14 in mitosis which were strongly amyolytic. No such segregants were obtained from S6. It seems probable from these results that S14 might contain a specific chromosomal rearrangement in its chromosome 6 so that no clear hybridization signals were observed for the *GLU1* probe. Furthermore, strain S6, which has a low amyolytic activity, is characterized by its wrinkled and non-hairy appearance. A plausible explanation for this is that its genome contains a chromosome of *Y. lipolytica* 21501-4B which carries a gene required for the expression of this phenotype. By the same reasoning it can be concluded that this *Y. lipolytica* gene or chromosome is not present in S5, S14 or S17. The substitution of chromosomal band 7 of A6 by band 6 of *Y. lipolytica* 21501-4 in S5 and S17 did not bring about such a clear change in morphological characteristics.

When the rDNA probe of *Y. lipolytica* was used to detect differences among strains A6, S5 and S6, it was found that while A6 gave signals of hybridization for chromosomal bands 6 and 7, in the case of S5 and S6 signals were observed for chromosomal bands 6 (not shown) and 7,

Fig. 4. Hybridization of the *S. fibuligera* ALP1 gene probe (b, d) to a blot of the PFGE (a, c) panel of yeast strains. (a, b) Lane 1, *Schiz. pombe*; lane 2, *S. fibuligera*/Y. *lipolytica* intergeneric hybrid A6/21501-4B; lane 3, *S. fibuligera* A6; lane 4, *S. fibuligera* S5; lane 5, *S. fibuligera* S6; lane 6, *S. fibuligera* S14. (c, d) Lane 1, *Sacc. cerevisiae*; lane 2, *Schiz. pombe*; lane 3, *S. fibuligera* N14i60; lane 4, *S. fibuligera* A2.1; lane 5, *S. fibuligera* A18; lane 6, *S. fibuligera* fusant A18/A2.1; lanes 7, 8 and 9, mitotic sectors 5, 16 and 25 of A18/A2.1; lane 10, *Y. lipolytica* A ura3 leu2 lys11; lane 11, *Y. lipolytica* 1529 A his1.

Fig. 5. Hybridization of the *S. fibuligera* GLU1 gene probe (b) to a blot of the PFGE (a) panel of yeast strains. Lane 1, *S. fibuligera* S5; lane 2, *S. fibuligera* S6; lane 3, *S. fibuligera*/Y. *lipolytica* intergeneric hybrid A6/21501-4B.

Fig. 6. Hybridization of the *Y. lipolytica* rRNA genes (b) to a blot of the PFGE (a) panel of yeast strains. Lane 1, *Schiz. pombe*; lane 2, *S. fibuligera* S6; lane 3, *S. fibuligera* A6.

respectively (Fig. 6). Previous studies (Yip, 1993) showed that, using the same rDNA probe, *Y. lipolytica* 21501-4B gave signals of hybridization for chromosomal DNA bands 1 and 4. These results suggest that the rDNA genes of *S. fibuligera* strains are located on chromosomes 6 and 7. Similar results concerning the chromosomal location of the *GLU1* gene were obtained with the *GLU1* probe. We take this correlation in the patterns of hybridization for the probes *GLU1* and rDNA for strains S5 and S6 to be more definitive evidence in support of our finding that chromosome 7 of S5 and S17 was indeed chromosome 6 of *Y. lipolytica* 21501-4B, and that chromosome 6 of S6 was chromosome 5 of 21501-4B.

Thus, by a combined analysis of the karyotypes of genetically different strains of *S. fibuligera*, with a study of phenotypic differences of intergeneric hybrids and their mitotic segregants and the patterns of hybridization signals for specific genes, a more discriminative means of assessing the phenotypic differences of the yeast strains studied was possible. Of special interest is the possibility of using the weakly amylolytic strain, S5, as a recipient in transformation experiments which employ plasmids carrying the glucoamylase gene, *GLU1*, and the 26S ribosomal DNA sequence of *S. fibuligera*. Preliminary results indicate that integration of the linearized 8.94 kb plasmid, pYLN1826GLU1, into the corresponding rDNA locus of S5, produces strongly amylolytic transformants.

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