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The Analysis of the Restriction Site Polymorphism in the Repeated Sequences (rDNA, Subtelomeric Y' Sequences and Retrotransposon Tyl) in the Genomic DNA of the Industrially Used *Saccharomyces cerevisiae* Strains

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Summary: *S. cerevisiae BSP-1, BPS-2, BSP-3 and BSP-4 are industrially used baking strains that have same phenotypes, but claimed to be different strains by the industry due to variations in their fermentation activities in dough. In the present study, we have analyzed the rDNA sequences, subtelomeric Y' sequences and retrotransposon Ty1 that are highly repeated in the genomic DNA of the yeast S. cerevisiae. As these three types of repeats have different sequence arrangements in different strains of S. cerevisiae, the genomic DNA of the industrially used strains utilized in this study were subjected to a series of cleavege reactions by five different restriction endonucleases (Eco RI, Hind III, Bam HI, Bgl II, Xho I) to interpret the sequence arrangements in each strain.*

The rDNA sequence in each strain showed no restriction site polymorphism for the enzymes Eco RI, Hind III and Bgl II. The retrotransposon Ty1, when cut by Xho I, generated a same size fragment in all strains and the Y' subtelomeric sequences also did not indicate any different arrangement when digested with Hind III, Bam HI and Xho I.

The data obtained from this genomic analysis did not support the claims that these strains were different due to variations in fermentation activities in dough, although they have same phenotypes.

Key words : *S. cerevisiae, rDNA, subtelomeric Y' sequence, retrotransposon Ty1, restriction site polymorphism.*

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Sanayide Kullanılan Saccharomyces cerevisiae Suşlarının Genomik DNA'larındaki Yinelenen Dizilerin (rDNA, Suptelomerik Y' Dizileri, Retrotranspozon Ty1) Kesim Noktalarının Polimorfizm Analizi

Özet: *S. cerevisiae BSP-1, BSP-2, BSP-3 ve BSP-4 fenotipik özellikleri aynı olan, fakat hamurda fermentasyon sırasında değişik aktivite göstermelerinden dolayı sanayi tarafından farklı suşlar oldukları iddia edilen maya suşlarıdır. Bu çalışmada S. cerevisiae genomik DNA'sında yinelenen rDNA dizileri, suptelomerik Y' dizileri ve retrotranspozon Ty1 incelenmiştir. Bu yinelenen dizilerin, dizi organizasyonlarının değişik S. cerevisiae suşlarında farklı olmalarından dolayı bu çalışmadaki S. cerevisiae suşlarının genomları beş değişik enzimle kesilerek (Eco RI, Hind III, Bam HI, Bgl II, Xho I) her birindeki yinelenen dizilerin dizi organizasyonları belirlenmeye çalışılmıştır.*

Suşlardaki rDNA dizileri Eco RI, Hind III ve Bgl II kesim noktalarında herhangi bir farklılık göstermemiştir. Retrotranspozon Ty1, Xho I enzimiyle bütün suşlarda aynı DNA kesitini oluşturmuş ve subtelomerik Y' dizilerinin ise Hind III, Bam HI ve Xho I enzimleriyle reaksiyonları sonucunda farklı dizi organizasyonlarına sahip olmadıkları ortaya çıkmıştır.

Bu genomik çalışmadan elde edilen sonuçlar, fenotipleri aynı olmalarına rağmen hamurdaki fermentasyon aktivitelerinin değişiklik göstermelerinden dolayı bu suşların farklı suşlar olma iddialarını desteklememektedir.

Anahtar Kelimeler : *S. cerevisiae, rDNA, suptelomerik Y' dizileri, retrotranspozon Ty1, kesim noktaları polimorfizmi.*

Introduction

Most of the sequences in yeast chromosome are unique (approx. 80 %). Some genes, as for histones, α-tubulin, mating pheromones and ribosomal proteins exists in pairs. Up to 15 % of the nuclear DNA consists of repeated sequences. The most abundant repeated sequence in the yeast genome is the rDNA (ribosomal DNA). The rDNA genes of *S. cerevisiae* are organized in a single tandem array of 9.1kb repeating units on chromosome XII[1-3]. The number of repeats is strain-dependent, and also there is restriction site polymorphism between the repeats. The apparent size of rDNA varies among different yeast strains and even among different isolates of the same strain[4,5].

Subtelomeric repeat sequences are found immediately proximal to the TEL sequences (Telomers), which are the specialized structure at the ends of linear chromosomal DNA molecules and required both for chromosomal stability and to enable the complete replication of the termini of chromosomes[6,7]. Two subtelomeric repeats have been described in yeast X and Y'. The arrangement of these sequences starting at the end of the chromosome is poly ($C_{1-3}A$) - (Y)n-poly (C_{1-3} A) - (X) n. Y' is a sequence of 6700 bp and X is a family of repeated sequences of between 3000 and 3750 nucleotide pairs[8,9]. Y' repeats have been more extensively studied than the X family. Y' repeats vary in copy number, location and restriction fragment lengths between strains although they exhibit within - strain homogenity [10].

The Transposons (pieces of DNA that are able to insert copies of themselves into many different sites in other DNA sequences within the same cell, without the requirement of DNA homology with the target DNA) of yeast, "Ty elements", have the same sequence organization as retroviruses and like retroviruses transpose by way of RNA intermediates[11,12]. This similarity between the mechanism of Ty transposition and retrovirus replication leads to the term retrotransposons. Among the known yeast transposons "Ty1, Ty2, Ty3, Ty4" Tyl is the most highly repeated Ty element in strains of *S. cerevisiae*. Tyl is 5.9 kb in length and flanked by LTR (Long terminal repeat) of 334-338 bps[13,14]. The restriction map of Tyl element vary at a number of sites both within the coding region and within the LTR sequences [15].

The *S. cerevisiae* strains used in Turkish baking industry showed different fermentation activities in dough that contribute variations to organoleptic tests; thus considered as different strains by the industry, although the phenotypic characteristics of these strains are similar.

Different *S. cerevisiae* strains have different locations of restriction sites for the three classes of the repeated DNA (rDNA, Ty1 elements, telomeric Y' sequences) in their genomic DNA restriction spectra. In the present study we have analyzed the restriction site polymorphism in the repeated DNA sequences to interpret the sequence arrangements for each of the industrially used *S. cerevisiae* strains that are considered as different although they have same phenotypes.

Materials

Yeast Strains:
The *Saccharomyces cerevisiae* strains (BSP-1, BSP-2, BSP-3, BSP-4) were provided from PAK Gıda A.Ş.

Culture Media:
For routine growth of the yeast cells YEPD medium (5% yeast extract, 5 % bactopeptone, 10 % dextrose) was used.

Restriction Endonucleases:
The restriction endonucleases Eco RI, Bam HI, Hind III, Bgl II and Xho I were provided from Boehringer Mannheim.

Methods

Chromosomal DNA Isolation [16]:
Yeast cells were grown for 16 hours at 30°C to a density of $7x10^6$ cells/mL in YEPD. The cell pellet was resuspended in SEP buffer (1,2 mol sorbitol, 25 mM EDTA, 50 mM Tris-HCl, pH 7.5) and spheroplasts were formed by adding lyticase solution (20 µL) (Sigma). The spheroplasts were then pelleted and resuspended in SEP buffer and washed. The spheroplasts were resuspended in saline-EDTA (150 mM NaCl, 100mM EDTA) and 50 µL of 20 % SDS solution was added. After incubation at 37°C for 2 hrs and further

30 min at 60°C, chloroform: isoamyl alcohol and phenol extractions were done. To the aqueous phase 95% ethanol (Merck) was added and left o/n at -20°C. The DNA was pelleted (12 krp, 10 min, 4°C) and resuspended in TE buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA). After resuspension of the DNA, 20 µL of RNase solution (Sigma) was added and phenol extracted. The aqueous phase was left for o/n at -20°C in ethanol. DNA was pelleted and resuspended in 50 µL TE buffer.

Restriction Digest:
Cleavege reactions with restriction endonucleases, ECO RI, Hind III, Bam HI, Bgl II, and Xho I were carried out according to the conditions recommended by the enzyme supplier.

Gel Electrophoresis[17]:
Agarose gels were subjected to electrophoresis in Tris-Borate buffer (54 g Tris, 27,5 g Boric acid, 20 mL 0.5 M EDTA/Lt). Gels of 0.7 % agarose (Sigma) were used to size molecular weights of the fragments. Electrophoresis was carried out in horizontal gel apparatus (Horizon 11-14). Approxiametly 5 µg of yeast DNA and 0.5 µg λ virus DNA -ECO RI/Hind III digest as a standard marker were loaded per slot and electrophoresis was carried out at 50 V (Power Supply model 200.BRL) for 5 hours. The gels were stained in a 0.5 mg/mL Et-Br solution than placed directly on top of a 300 nm transilluminater (Photodyne, Photo perp I) and photographed (Photodyne Poloroid Camera).

Estimation of the Chromosomal DNA Restriction Fragment Sizes[17]:
The measurement of fragment sizes was done by using the mobilities of the λ ECO RI/Hind III marker digest fragments to construct a calibration curve; the sizes of the unknown fragments were determined from the distance they have migrated.

Results and Discussion

For the analysis of the three classes of repeated DNA rDNA, Y' sequences, Ty1 elements) genomic DNA solations were done from each of the industrially sed baking strains of *Saccharomyces cerevisiae* BSP-1, SP-2, BSP-3, BSP-4 (Fig. 1a, 1b). The purified chromosomal DNAs of the strains were then subjected to a series of cleavage reactions with five different restriction endonucleases (Eco RI, Hind III, Bam HI, Bgl II, Xho I) to detect the restriction site polymorphism in repeated DNA, which are the origins of most bands seen in the restriction spectrum of the genomic DNA in yeast.

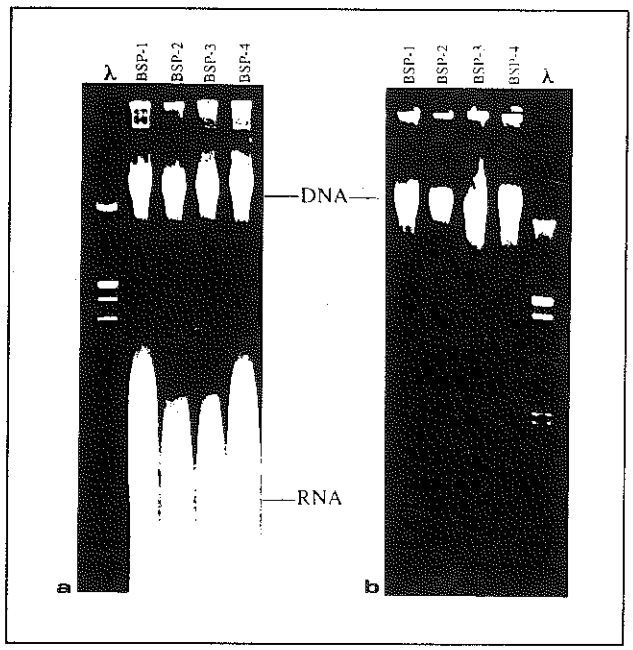

Figure 1. Seperation of *S. cerevisiae* nucleic acids by electrophoresis in 0.7% agarose gels. (a) before RNase treatment (b) purified DNA after RNase treatment.

In the yeast genome, rDNA is the most abundant repeated sequence that is organized in a single tendom array of 9,1 kb repeat units on chromosome XII (Fig. 2a)[3]. The Eco RI spectrum of the genomic DNA of each strain utilized in this study generated five visible rDNA fragments of different sizes. DNA size analysis showed that these fragments were 2.8, 2.4, 2.0, 0.7 and 0.6 kb respectively in all strains (Fig. 3). In the Hind III spectrum 2 strong rDNA fragments appeared and the sizes of these fragments in every strain were calculated as 6.4 and 2.7 kb (Fig. 4). Bgl II cleavege reactions of the genomic DNA of the *S. cerevisiae* strains generated one band and the size of this band in each strain was found to be 4.5 kb which contain two rDNA fragments equal in size (Fig. 5).

The second repeated sequence in the genomic DNA of yeast is the retrotransposon Ty1, which is 5,9 kb in size and has restriction site polymorphism for the enzyme Xho I (Fig. 2b)[15]. The genomic DNA cleavage

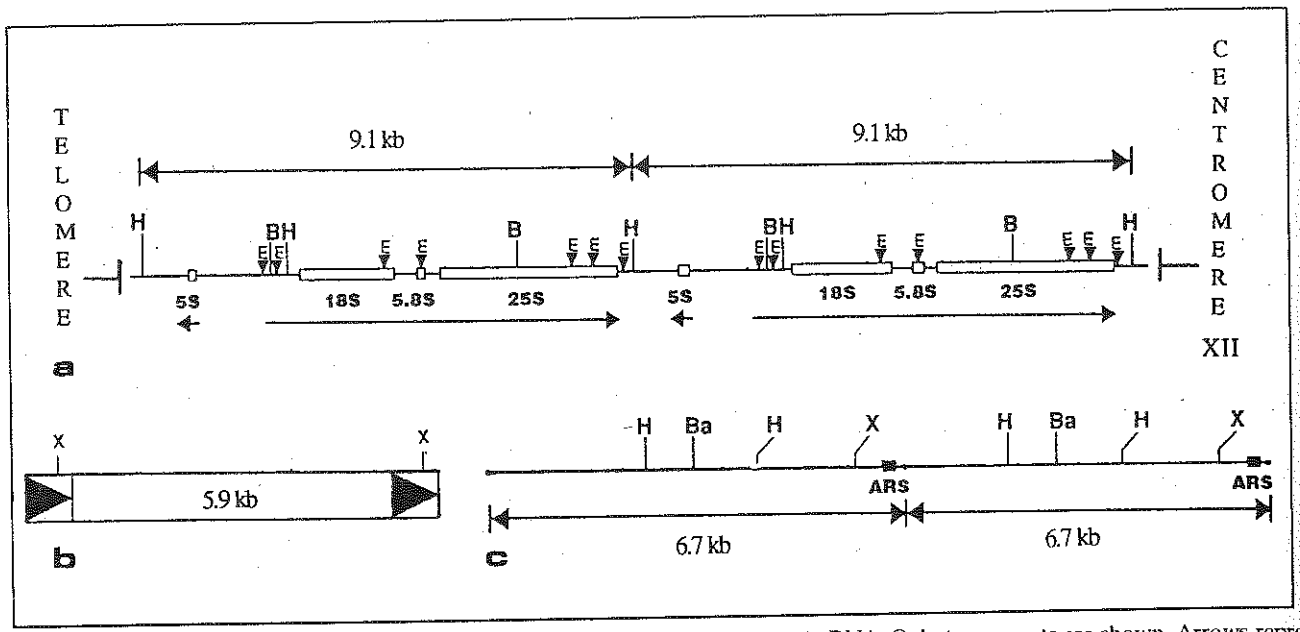

Figure 2. Structures and restriction maps of repeated DNA sequences in yeast (a) rDNA: Only two repeats are shown. Arrows represent the direction of transcription for the 35 S precursor rRNA and the 5 S rRNA. (E for Eco RI, H for Hind III, B for Bgl III) (Woolford and Warner, 1991)
(b) Retrotransposon Tyl: Shaded boxes indicate LTR sequences (X for Xho I) (Boeke and Sandmeyer, 1991)
(c) Y' Repeats: Only two repeats are shown. The black boxes are the autonomously replicating sequences (Ba for Bam HI). (Chan and Tye, 1983)

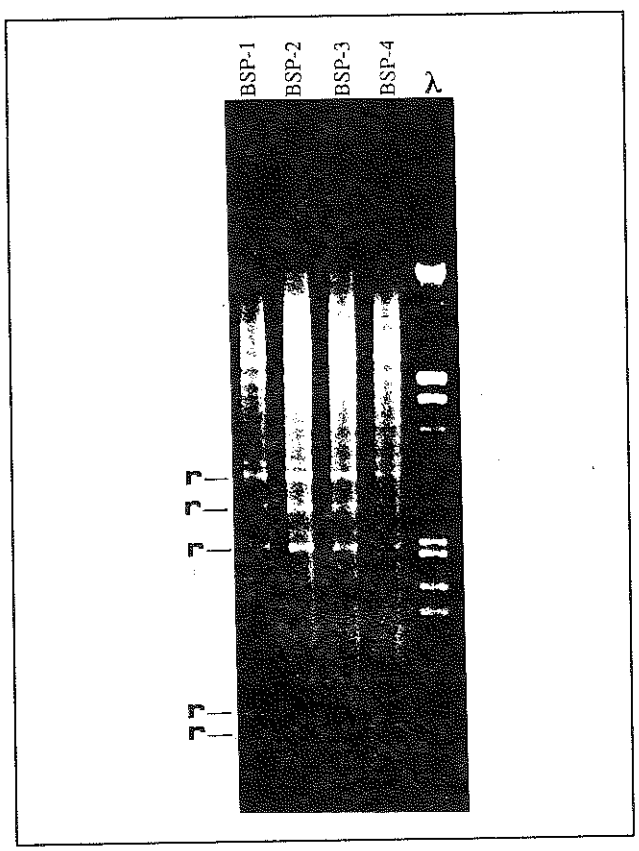

Figure 3. Eco RI digest of the genomic DNA of the *S. cerevisiae* strains. (r for rDNA fragments)

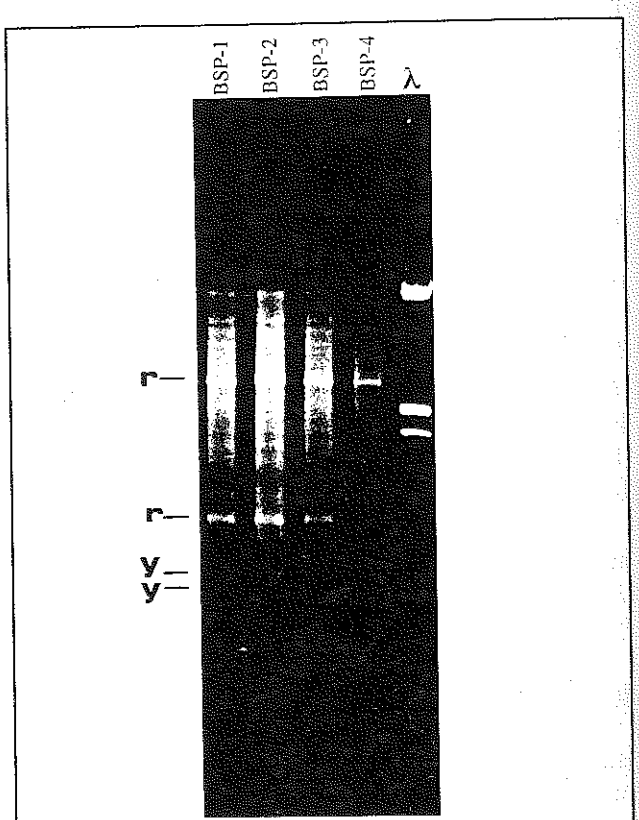

Figure 4. Hind III digest of the genomic DNA of the*S. cerevisiae* strains (r for rDNA fragments, y for Y' repeated sequence fragments)

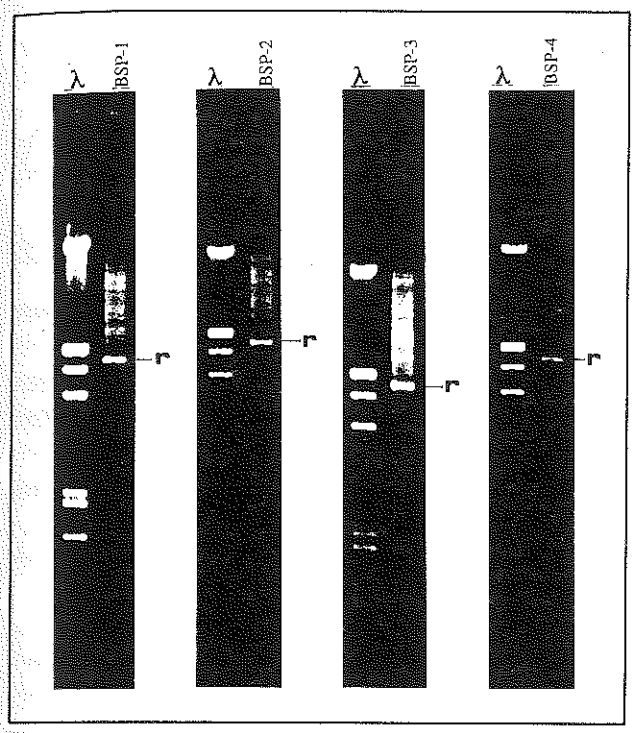

Figure 5. Bgl II digest of the genomic DNA of the *S. cerevisiae* strains (r for rDNA band).

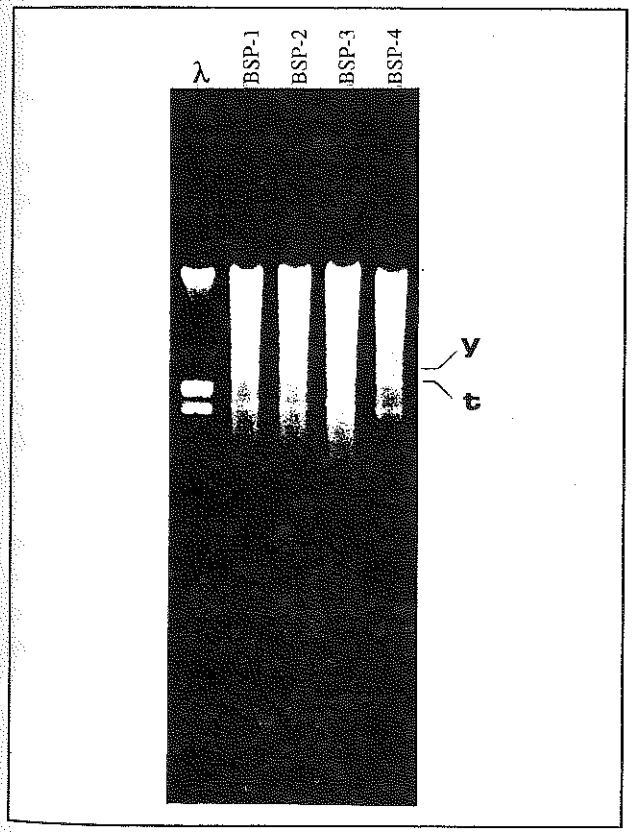

Figure 6. Xho I digest of the genomic DNA of *S. cerevisiae* strains (t for Tyl fragment, y for Y' fragment)

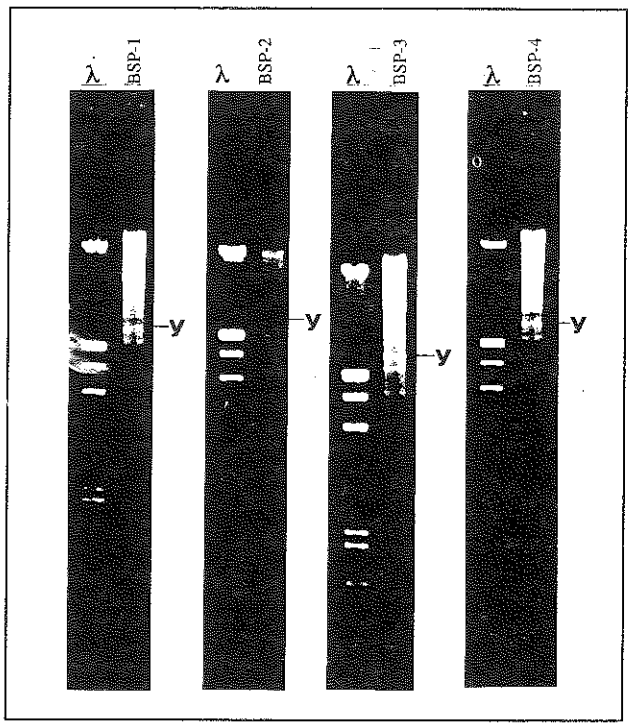

Figure 7. Bam HI digest of the genomic DNA of *S. cerevisiae* strains (y for Y' fragment)

reactions of the strains BSP-1, BSP-2, BSP-3, BSP-4 for the analysis of the Tyl element with the restriction endonuclease Xho I (Fig. 6) created only a fragment of 5.6 kb in each strain.

The Y' sequence, that we have studied as the third repeated sequence found in the genomes of the yeast strains, are about 6,7 kb and organized in single or tendomly at telomers (Fig. 2c)[8]. For the analysis of the restriction site polymorphism of the Y' repeated sequences in *S. cerevisiae* strains BSP-1, BSP-2, BSP-3, BSP-4, we have examined the Hind III, Bam HI and Xho I spectrums of the genomic DNAs. In every strain Hind III spectrum showed two Y' fragments of 2.2 and 2.01 kb (Fig. 4). Xho I (Fig. 6) and Bam HI (Fig. 7) digests generated same fragments of 6.7 kb as they both cleave the Y' sequence only once. The size of this fragment in all strains was same.

A few clearly visible bands were left unassigned in the genomic DNA spectrums. These originate from the 2 μ plasmids that are found naturally in the yeast genome and from the mitochondrial DNA, which is fragmented during genomic DNA isolations.

S. cerevisiae strains BSP-1, BSP-2, BSP-3, BSP-4 utilized in this study are industrial baking strains. The phenotypic characteristics of these strains are similar but they possess different fermentation activities in dough. Because of the variations in fermentation activities they are claimed to be different strains by the industry. It is obvious that in different strains of *S. cerevisiae* the sequence arrangements of the repeated sequences (rDNA, Tyl element, Telomeric Y' sequence) are not similar, as they exhibit restriction site polymorphism[4,5,10,15]. All of the four baking strains of *S. cerevisiae* (BSP-1, BSP-2, BSP-3, BSP-4) showed no restriction site polymorphism in their rDNA sequences for neither Hind III nor Eco RI and Bgl II.

The Ty1 sequence in each strain presented the same sequence arrangement, as they did not generate different DNA fragment for the enzyme Xho I.

Also the subtelomeric Y' sequence in each strain did not indicate restriction site polymorphism for each of the enzymes Hind III, Bam HI and Xho I.

These data have led us to the fact that each type of repeated sequence (rDNA, Tyl, Y') has the same sequence arrangement in each strain that should not be if these strains were different as has been claimed by the industry.

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References

1. Planta, R. J., Raue, H. A., "Control of Ribosome Biogenesis in Yeast" *Trends Genetic*, 4, 64-68, 1988.
2. Warner, J. R., "Synthesis of Ribosomes in Saccharomyces Cerevisiae", *Microbial. Rev.* 53, 256-271, 1989.
3. Woolford, S. L. and Warner, J. R., "The Ribosome and its Synthesis" in Broach, J. R., Pringle, J. R. and Jones, E. W. (eds), *The Molecular and Cellular Biology of The Yeast Saccharomyces*, Cold Spring Harbor, CSHL Press, Vol. 1, p. 587, 1991.
4. Petes, T. D., "Unequal Meiotic Recombination Within Tandem Arrays of Yeast Ribosomal DNA Genes" Cell, 19, 765-774, 1980.
5. Petes, T. D. and Botstein, D. "Simple Mendelian Inheritance of the Reiterated Ribosomal DNA of Yeast". *Proc. Natl. Acad. Sci.*, USA 74, 5091-5095, 1977.
6. Blackburn, E. H., "Telomers; Structure and Synthesis". *J. Biol. Chem.*, 265, 5919-5921, 1990.
7. Walmsley, R. M. "Yeast Telomers" *Yeast*, 3, 139-148, 1987.
8. Chan, S. C. M. and Tye, B. K. "Organization of DNA Sequences and Replication Origins at Yeast Telomers" *Cell*, 33, 563-573, 1983.
9. Chan, C. S. M. and Tye, B. K., "A Family of Saccharomyces Cerevisiae Repetitive Autonomously Replicating Sequences That Have Very Similar Genetic Envirements". *J. Mol. Biol*. 168, 505-523, 1983.
10. Haber, J. E. and Louis, E. J., "Mitotic Recombination Among Subtelomeric Y' Repeats in S. cerevisiae". *Genetics*, 124, 547-559, 1990.
11. Cameron, J. R., Loh. E. Y. and Davis, R. W., "Evidence for Transposition of Despersed Repetetive DNA Families in Yeast" *Cell*, 16, 739-751, 1979.
12. Boeke, J. D. and Garfinkel, D. J., "Yeast Ty Elements as Retroviruses" in Leibowitz, M. J. and Koltın, Y. (eds), "*Viruses of Fungi and Lower Eukaryotes*" New York, Marcel Dekker, Inc., p. 15, 1988.
13. Farabaugh, P. J., and Fink, G. R., "Lusertion of the Eukaryotic Transposable Elemen Tyl Creates a 5 bp Duplication." *Nature*, 286, 414-418, 1980.
14. Gafner, J., and Philippsen, P., "The Yeast Transposon Tyl Generates Duplication of Target DNA on Insertion." *Nature* 286, 352-356, 1980.
15. Boeke, J. D. and Sandmeyer, S. B. "Yeast Transposable Elements" in Broach, J. R., Pringle, J. R. and Jones, E. W. (eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces*, Cold Spring Harbor, CSHL Press, Vol. I, p. 193, 1991.
16. Cryer, D. R., Eccleshall, R. and Marmur, J. "Isolation of Yeast DNA", *Meth. Cell. Biol.*, 12, 39-44, 1975.
17. Maniatis, T., Fritsch. E. F., Sambraok, J. (eds.) "*Molecular Cloning: A Laboratory Manual*"., Cold Spring Harbor, CSHL Press, 1989.