Acta Pharmaceutica Turcica XXXVIII (2) 33-36 (1996)

THE ANALYSIS OF THE RESTRICTION SITE POLYMORPHISM IN THE MITOCHONDRIAL DNA SEQUENCES OF THE INDUSTRIALLY USED *SACCHAROMYCES CEREVISIAE* STRAINS

ENDÜSTRİDE KULLANILAN *SACCHAROMYCES CEREVISIAE* SUŞLARININ MİTEKONDRİ DNA'LARI ÜZERİNDEKİ KESİM NOKTALARINDAKİ POLİMORFİZMİNİN İNCELENMESİ

FATİH İZGÜ[1]*, DEMET ALTINBAY[1], HÜSEYİN GÜN[2]

[1] Middle East Technical University, Dept. of Biology, Faculty of Science and Arts, METU, 06531,
[2] Gülhane Military Medical Academy, Dept. of Microbiology, Ankara, Turkey

The mitochondrial DNAs of the Saccharomyces cerevisiae starter strains that have same phenotypes but claimed to be different strains by the industry due to variations in fermentation activities in dough were subjected to a series of cleavage reactions by different enzymes and analyzed from the point of restriction site polymorphism in their sequences in order to interpret, if exists, the differences among these strains.

Aynı fenotipik özelliklere sahip olmalarına rağmen, hamurda fermentasyon sırasında değişik aktivite göstermelerinden dolayı sanayi tarafından farklı oldukları iddia edilen Saccharomyces cerevisiae suşlarının mitokondri DNA'ları değişik enzimlerle muamele edilerek, bu suşlar arasındaki farklılıkları bulmak için, DNA dizilerindeki kesim noktaları polimorfizmi incelenmiştir.

Keywords: *Saccharomyces cerevisiae; Mitochondrial DNA; Restriction site polymorphism.*

Anahtar kelimeler: *Saccharomyces cerevisiae; Mitokondri DNA; Kesim noktaları polimorfizmi.*

Introduction

The *Saccharomyces cerevisiae* total DNA consists of about 15 % mitochondrial DNA (mt DNA) (1). A normal cell of *S. cerevisiae* contains 20-35 molecules of mt DNA that encodes seven proteins of the oxidative phosphorylation machinery, one protein of the mitochondrial ribosomal subunit, two r RNAs, 24-25 t RNAs and 9S RNA; the vast majority of mitochondrial proteins are encoded by nuclear genes and are imported into the mitochondria from the cytoplasm (2,3).

The genes on the mt DNA are separated by AT-rich spacers of unknown function and in the mitochondrial genome there are some intergenic insertions and deletions accompanied with the presence or absence of introns that leed to great polymorphism and strain-specific variants in the mitochondrial genome (4-6)

S. cerevisiae BSP 1-4 are used as starter strains in baking industry. The phenotypic characteristics of these strains are similar but they possess different fermentation activities in dough that contribute variations to organoleptic tests; thus clamied as different strains by the industry although these strains have similar phenotypes.

Different strains of *S. cerevisiae* exhibit different sequence arrangements in their mitochondrial genome and in the repeated sequences of their chromosomal DNA. In the preceding paper (7) we have shown that, the repeated sequences (rDNA, subtelomeric Y sequence and retrotransposon Ty1) in the chromosomal DNAs of *S.cerevisiae* BSP 1-4 had the same sequence arrangements, that should not be if these strains were different. Further in this study, for a more specific investigation, the mitochondrial genomes that are essential for respiratory metabolism of the above mentioned industrially used *S.cerevisiae* strains were analyzed from the point of restriction site polymorphism in their sequences, in order to interpret, if exists, the differences among these strains.

Materials

Yeast strains:

The *Saccharomyces cerevisiae* strains (BSP1, BSP2, BSP3 and BSP4) were provided from Pak Gıda Inc. as slub cultures.

* Correspondance

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 and Hınd III were provided from Boehringer Mannheim (Ger.)

Methods

Identification of the yeast strains:

The identification of the industrially used yeast strains was done with API LAB ID32 C yeast identification system in accordance with the suppliers' instructions (Biomerieux-Fr).

Mitochondrial DNA isolations:

Industrial *S.cerevisiae* (BSP1-4) starter strains were grown until early stationary phase in 1 liter of YEPD broth with aeration on an orbital shaker (200 rpm) at 30°C. The cells were then harvested by centrifugation at 5000 rpm for 10 min. (Jouan MR1812 high speed centrifuge).

The 10-15 grams of cells were resuspended in 100 ml of 50 mM sodium phosphate buffer, 25 mM EDTA, 1% (v/v) β-mercaptoethanol (pH 7.5) and 15 mg of lyticase (Sigma) was added.

The cells were left at 30°C until the medium became viscous. After the cells were lysed, to the medium 100 ml of 0.2 M Tris-HCl, 80 mM EDTA and 1% SDS (pH 9.5) were added and incubated at 65°C. After incubation for 30 minutes 50 ml of 5 M potassium acetate was added and incubated at 0°C for 45 minutes. The cell debris was separated from the DNA solution by centrifugation of the medium at 5000 rpm at 5°C for 10 minutes. To the supernatant 100 ml of isopropanol was added, mixed and kept at 20°C for 1 hour. The solution was then centrifuged at 5000 rpm at 0°C for 15 minutes and to the supernatant 20 ml of 1 mM EDTA, 10 mM Tris-HCl (pH 7.5) was added. After the pellet has been dissolved, it was centrifuged at 13000 rpm for 15 minutes at 4°C.

To each 1 ml of the supernatant 24 µl of 10 mg/ml stock solution of bisbenzimide (Hoechst) and 1.16 g Cs Cl (Boehringer Mannheim) were added. The solution was then centrifuged at 45000 rpm at 15°C in Sorvall ultracentrifuge. At the end of 48 hours centrifugation the bands in the ultracentrifuge tubes (Sorvall 03987) were visualized under UV light (300 nm).

The band containing mitochondrial DNA was harvested by side puncture. The collected mitochondrial DNA was then centrifuged in a Cs-Cl density gradient until equilibrium, and again mitochondrial DNA was harvested by side puncturing and bisbenzimide was extracted for several times by isopropanol that was previously equilibrated with Cs-Cl solution.

The bisbenzimide free solution was mixed with four volume of 0.3 M sodium acetate and 3 volumes of ethanol was added to the mixture. It was then kept at -20°C for 2-4 hours and centrifuged at 10000 rpm at 4°C for 10 minutes.

The pellet was dissolved in 300 µl of 0.3 M sodium acetate and 2 volumes of ethanol was added. It was freezed and centrifuged again. The pellet was redissolved in appropriate volume of 1mM EDTA, 10mM Tris-HCl pH 7.8 (8).

Restriction digest of the mt DNA:

1 µg of DNA was restricted in a 20 µl digestion mix. (2 µl DNA, 2 µl restriction buffer, 5 units of enzyme, 15 µl dd H_2O) for 1 hour at 37°C. After incubation for 1 hour the restriction was stopped by adding 0.5 M EDTA to the reaction mix. (9).

Gel electrophoresis of the DNA molecules:

Agarose gels were prepared by dissolving 0.8 g of agarose (Sigma) in 100 ml of TBE in a boiling water bath until no granules of agarose were visible. After cooling to 60°C, it was poured into UV transparent gel tray and submerged into electrophoresis tank that contains 1 liter of TBE (BRL type 1114) after the agar was solidified. The wells in the solidified agar were then filled with the digestion mix. containing 2 µl of loading buffer (50 mM EDTA, 0.01% SDS, 0.1% bromophenol blue). λ DNA Eco RI/ Hind III double digest was also added into the agarose gel as a standard marker. The electrophoresis was carried out at a constant 75 volts for approximately 5 hours (power supply BRL model 200).

At the end of the gel electrophoresis the agarose gel was submerged into the distilled water containing 0.5 µg/ml of Et-Br and the DNAs were stained for 30 minutes by occasional agitation. Then the agarose gel was rinsed in distilled water for 10 minutes and placed directly on a 300 nm transilluminator (Fotodyne type, FotoPrep I) and photographed (Fotodyne poloroid camera type, PCR-10). The fragment size analysis of the mtDNA molecules in the agarose gels 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 mitochondrial DNA fragments were determined from the distance that they have migrated (10).

Results and Discussion

Prior to mt DNA restriction analysis the yeast strains obtained from Pak Gıda Inc. were subjected to API LAB ID32 C yeast identification system which is the most reliable indentification system for yeasts that is currently referred to.

According to the test results that were interpreted with API LAB ID32 C computer program, all of the four yeast strains were

identified as *S.cerevisiae* with an accuracy of over 99%.

Mitochondrial genome of *S.cerevisiae* is extremely rich in A+T (80%) and low in G+C content; thus show abnormal properties as very low melting point. Being A+T rich and having the ability to be denatured easily it is not possible to isolate and detect this DNA by routinely used total DNA isolation methods. Although it is a time consuming process the mt DNA can only be separated by density gradient centrifugation in the presence of dyes which bind to (A+T)-rich DNA that is free of chromosomal DNA, 2 μ plasmid and r DNA contamination (11). mt DNA isolated from yeast consists of many random fragments and thus electrophoretic gels have a background smear (12). For this reason it is not always possible to detect small fragments out of this background smear. As the endonucleases Eco RI and Hind III generate relatively large fragments in the mitochondrial genomes of yeast strains (3,5), we preferred to digest the mt DNAs with these enzymes.

The Eco RI spectrum of the mt DNAs generated seven visible fragments of different sizes DNA size analyses showed that these fragments were 14.2, 11.9, 10.6, 7.0, 3.8, 2.6 and 1.8 kb in all strains (Fig.1).

Hind III digest of the mt DNAs generated ten intense bands of 22.0, 7.6, 6.8, 3.7, 3.0, 2.75, 2.1, 1.95 and 1.2 kb respectively in all strains (Fig.2).

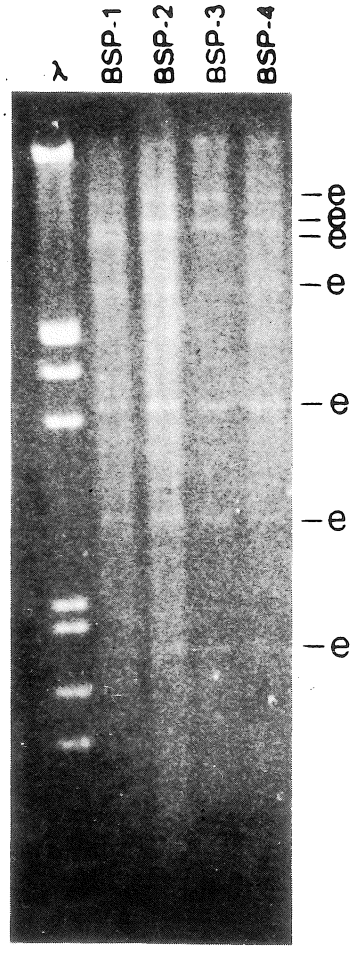

Fig.1. Restriction digest of the mitochondrial genome of *Saccharomyces cerevisiae* (BSP 1-4) strains with Eco RI (e for Eco RI fragments).

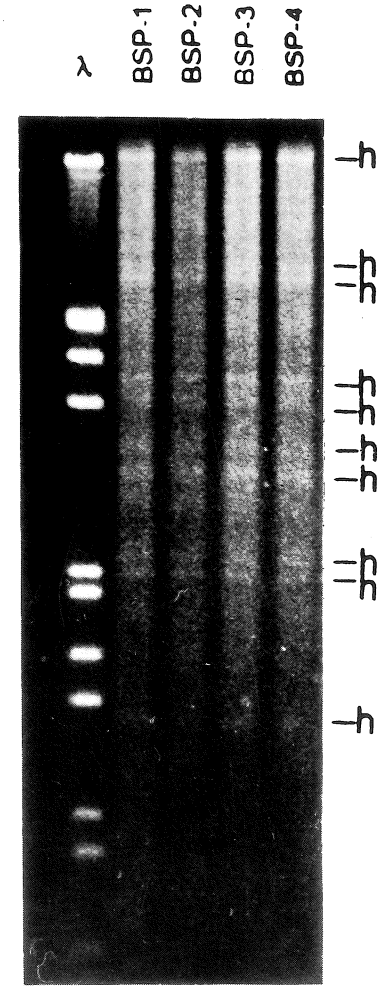

Fig.2. Restriction digest of the mitochondrial genome of *Saccharomyces cerevisiae* (BSP 1-4) strains with Hind III (h for Hind III fragments).

The restriction spectrums of the mitochondrial DNAs did not indicate any restriction site polymorphism for each of the enzymes Eco RI and Hind III, and thus showed no different sequence arrangements.

As a consequence, the data that we have obtained from this genomic analysis did not support the claims that these strains were different due to variations in fermentation activities in dough.

Acknowledgements

This work was supported by Pak Gıda Inc.

References

1. Fangman, W.L., Zakian, V.A.: Genome structure and replication. In: Strathern, J.N., Jones, E.W Broach, J.H. (Eds). Molecular Biology of the Yeast *Saccharomyces*, pp. 27-58. Cold Spring Harbour Laboratory Press, New York, 1981
2. de Zamaroczy, M., Bernardi, G.: Gene. 37, 1 (1985)
3. Pon, L., Schatz, G.: Biogenesis of yeast mitochondria. In: Pringle, J., Jones, E. (Eds): The Molecular and Cellular Biology of the Yeast *Saccharomyces*, pp. 33-406. Cold Spring Harbour Laboratory Press, New York, 1991
4. de Zamaroczy, M., Faugeron-Fonty, G.: Gene 32, 439 (1984)
5. Faugeron-Fonty, G., Kim., C.V., de Zamoroczy, M.: Gene 32, 459 (1984)
6. Colin, Y., Baldacci, G., Bernardi, G.: Gene 36, 1 (1983)
7. İzgü, F., Altınbay, D.: FABAD J. Pharm. Sci. 20, 49 (1995)
8. Rickwood, D., Dujon, B., Darley-Usmar, V.M.: Yeast Mitochondria. In : Camphell, I., Duffus, J.H. (Eds). Yeast, pp. 185-254, IRL Press, Oxford, 1991
9. Manniatis, T., Fritsch, E.F., Sambrook, J.: Molecular Clonning . A Laboratory Manual, Cold Spring Harbour Laboratory Press, New York, 1989
10. Sealey, P.G., Southern, M.: Gel Electrophoresis of DNA. In: Rickwood, D., Hames, B.D. (Eds), Gel Electrophoresis of Nucleic Acids, pp. 39-75, IRL Press, Oxford, 1994
11. Wach, A., Pick, H., Philippsen, P.: Procedures for Isolating Yeast DNA for Different Purposes. In: Jonston, J.R. (Ed.), Molecular Genetics of Yeast, pp. 1-17, IRL Press, Oxford, 1994
12. Fox, T.D., Folley, L.S., Mulero, J.J.: Analysis and Manipulation of Yeast Mitochondrial Genes. In: Guthrie, C., Fink, G.R. (Eds), Methods in Enzymology (194), pp., 149-165, Academic Press, London, 1991

Accepted: 12.02.1996