Revista CENIC Ciencias Biológicas, 22, 1-2, 1991

RESPIRATORY DEFICIENT MUTANTS OF THE YEAST CANDIDA TROPICALIS

M. de los A. Martínez, A. Alonso, D. Higginson and J. Benítez

National Center for Scientific Research, Havana City, Cuba

Received: 1st January, 1989

ABSTRACT. Ethidium bromide was found to be effective in the induction of respiratory deficient (RD) mutants in the yeast **Candida tropicalis.** Houever, under conditions leading to 100 % conversion to petites in **S. cerevisiae,** only 23 % of **C. tropicalis** cells were converted to respiratory deficients when compared on viable count basis. RD mutants of **C. tropicalis** were non-revertable and defective in cytochrome b and a + a3. The isolation of these RD mutants is of importance for the genetic improvement and the study of erobic metabolism in this yeast.

RESUMEN. Se ha visto que el bromuro de etidio es efectivo en la inducción de mutantes deficientes respiratorios (DR) en la levadura **Candida tropicalis.** Sin embargo, bajo condiciones de 100 % de conversión a petites en **S. cerevisiae,** sólo el 23 % de células de **C. tropicalis** son convertidas a deficientes respiratorios cuando se comparan en un conteo de viables. Los mutantes DR en **C. tropicalis** resultaron no revertibles y defectivos en los citocromos b y a + a3. El aislamiento de estos mutantes DR es importante para el mejoramiento genético y el estudio del metabolismo aeróbico de la levadura.

INTRODUCTION

Candida tropicalis could play a role in the development of Biotechnology since it has been shown to be active in ethanol production from D-xylose.[1] However, several factors can influence the performance of yeast in alcoholic fermentation. One of these factors is the capacity of yeast to oxidize ethanol via respiratory chain to CO_2 and water. RD mutants are expected to show an improved yield of alcohol in fermentation, depending on their capacity to oxidize ethanol in the mitochondrion. In addition, mitochondrial mutations has been shown to be useful as genetic markers in diagnosis of hybridization of yeasts of industrial interest.[2,3]

Finally, the different classes of non-mendelian petite mutations have proved to be an important tool for studying the structure and function of the mitochondrial genome in **S. cerevisiae**.[4] Yeasts of the Genus **Candida** are believed to be petite-negative. However, a RD deficient mutant of **C. utilis** has been isolated.[5]

In the present paper it was described the isolation of RD mutants of **C. tropicalis** using the mutagen ethidium bromide which is specific for **S. cerevisiae** mitochondrial genome. This result open new possibilities in the study of aercbic metabolism, replication and structure of a **petite negative** yeast mitochondrial DNA.

MATERIALS AND METHODS

Organisms and media

The organisms used were a lys$^-$ade$^-$ mutant from strain CBS-644 **C. tropicalis** and strain 196-2 (a his 6 Rho$^+$) **S. cerevisiae** from the collection of M. Luzzati (Orsay, Paris). Yeasts were grown aerobically at 30 °C in YPG (Yeast extract 10 g/L, Bactopectone 10 g/L, glucose 20 g/L) medium. Glucose was replaced by glycerol (20 g/L) or ethanol (20 g/L) for RD diagnosis. For solid media, 20 g/L Oxoid agar No. 1 was added.

Ethidium bromide mutagenesis

Both strains were treated with 12,5 to 100 μg/mL of ethidium bromide for 16 h at 30 °C in YPG medium with agitation. After treatment, cells were plated in YPD medium and RD mutants detected by the RD colony production making use of the 2,3,5 triphenyltetrazolium chloride overlay technique[6] and by replica plating in YPG medium with glycerol or ethanol as a sole carbon source.

Manometric techniques

Oxygen consumption and carbon dioxide production were assayed manometrically in a Warburg apparatus.[7]

Low temperature cytochrome spectra

Cytochrome spectra of intact yeast cells was performed at liquid nitrogen temperature.[8]

RESULTS

In contrast to **S. cerevisiae** strain 196-2 no spontaneous RD mutants could be recovered from **C. tropicalis** strain CBS-644. For **C. tropicalis** the highest yield of RD mutants was obtained with 75 μg/mL of ethidium bromide. Under this condition the frecuency of petite colony formation was 25 % for **C. tropicalis** and 100 % for **S. cerevisiae.** The growth rate of **C. tropicalis** in YPG medium was 0,76 h^{-1}. The growth rate of its RD mutants ranged from 0,04 to 0,4 h^{-1}. As expected, the RD mutants cannot grow on the non fermentable carbon sources such as glycerol or ethanol. For all RD mutants tested respiratory competent revertants could not be detected after plating 10^9 RD cells in YPG medium containing glycerol as a sole carbon source.

As shown in Table I oxygen consumption was drastically reduced in RD mutants derived from CBS-644. It was selected two RD mutants showing very low oxygen consumption in order to study their cytochrome spectra at liquid nitrogen temperature. As shown in figure 1, RD mutants EB-8 and EB-10 are defective in cytochrome a + a3.

TABLE I

Oxygen consumption and carbon dioxide production in ethidium bromide induced RD mutants of Candida tropicalis

Strain	Oxygen consumption	CO_2 production	Oxygen consumption/ CO_2 production
CBS-644 lys$^-$ade$^-$	230,88	66,26	3,48
EB6	144,76	1,09	132,80
EB7	192,90	3,92	49,20
EB8	145,62	0,67	217,34
EB9	157,75	1,37	115,14
EB10	144,19	0,57	252,96
EB11	148,64	1,05	141,56
EB12	165,62	0,60	276,03
EB15	214,70	2,20	97,91

1 μmol min^{-1} · g dry weight

DISCUSSION

RD mutants of the petite negative yeast **C. utilis** unable to grow on non-fermentable carbon sources have been isolated[5] after ethidium bromide mutagenesis. However, both the nature of the gene products affected and nuclear or cytoplasmic localization of the mutation remain unknown. It has been recently observed that the RD mutation of **C. utilis** can be complemented by protoplast

fusion with **S. cerevisiae** respiratory competent cells.[9] For there more it has been suggested that the RD mutation of **C. utilis** might be of mitochondrial origin.[9]

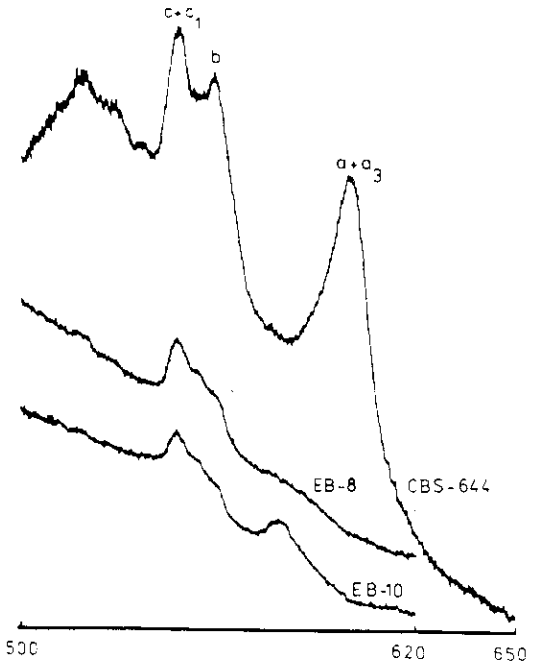

Fig. 1. Low temperature cytochrome spectra of respiratory competent and RD mutants of **C. tropicalis**

The present paper reports the isolation of ethidium bromide induced RD mutants of **C. tropicalis** defective in cytochrome b and cytochrome a + a3. In **S. cerevisiae** subunits II, III and I of cytochrome oxidase are encoded by the mitochondrial oxi1, oxi2 and oxi3 genes respectively and cytochrome b by the mitochondrial cox-box gene.[4] The RD mutants of **C. tropicalis** might be of mitochondrial origin although a nuclear mutation cannot be ruled out. The genetic stability of these mutants suggests that they may be deletions. Preliminary analysis of Eco RI digests of the mitochondrial DNA of respiratory competet cells and RD mutants of **C. tropicalis** suggest that these RD mutants may harbour a gross deletion of the mitochondrial genome (unpublished results). Any way RD mutation might be an important tool for metabolic research in **C. tropicalis** as well for increasing ethanol production in this yeast.

CONCLUSIONS

Rd mutants of non-revertables and defectives in cytochrome b and cytochrome a + a3 were isolated using the mutagen ethidium bromide. The stability and preliminary analysis of ECORI digests of the DNA suggests that these mutants might be deletions. These mutants are useful in the study of aerobic metabolism and genetic in this yeast.

ACKNOWLEDGEMENTS

We are grateful to Dr. A. Putrament (Institute of Biochemestry & Biophysics, Polish Academy of Sciences) and to Dr. P.P. Puglisi (Institute of Genetics, University of Parma, Italy) for helpful discussions.

BIBLIOGRAPHY

1. Fein J., Tallin S. and Lawford G. **Can. J. Microbiol., 30,** 682, 1984.
2. Spencer J. and Spencer D. **Curr. Genet., 4,** 177, 1981.
3. Spencer J., Land P. and Spencer D. **Molec. Gen. Genet., 178,** 651, 1980.
4. Dujon B. The Molecular Biology of the yeast Saccharomyces, Life Cycle and Inheritance, 505-520 (ed. Strathern J., Jones E. and Broach J.) Cold Spring Harbor Laboratory, New York, 1981.
5. Richard M., Figueroa L. and de van Broock M. **Curr. Mirobiol., 8,** 201, 1983.
6. Ogur M., St. John R. and Nagai S. **Science, 125,** 928, 1957.
7. Umbreit W., Borris R. and Sauffer J. Manometric Techniques, 61-90, Burgess Publishing Co., Minneapolis, 1964.
8. Sherman F., Fink G. and Hicks J. Methods in Yeast Genetics, 31-50, Cold Spring Harbor Laboratory, 1982.
9. Richard M. and de van Broock M. **Curr. Microbiol., 10,** 117, 1984.

Este equipo resulta de gran utilidad en el trabajo de laboratorio clínico, principalmente, en aquellos casos que involucran procesos extractivos que son realizados en tubos de ensayo, erlenmeyers, vasos de precipitado y otros útiles de laboratorio, o que estén dirigidos específicamente, a la extracción de triglicéridos, catecolaminas, lípidos totales a partir de órganos, ácidos grasos, así como de sustancias estimulantes (dopping) contenidas en la orina, etcétera.

DATOS TECNICOS
- *Voltaje: 110 / 220 V (CA)*
- *Frecuencia: 60 Hz*
- *Consumo: 300 W*
- *Velocidad: 0 a 200 r/ min*
- *Desplazamiento: 30 mm*

ZARANDA DE MESA

Centro Nacional de Investigaciones Científicas
Avenida 25 y calle 158, Cubanacán, Playa
Ciudad de La Habana, Cuba
Apartados Postales 6880 y 6990
Teléfono: 21 8066 Télex: 51 1582 CNIC CU

PRODUCIDO Y EXPORTADO POR:®
PRODUCED AND EXPORTED BY:
CNIC