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Cloning of the *HIS3* gene of *Yarrowia lipolytica*

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Abstract

The *HIS3* gene of the yeast *Yarrowia lipolytica* has been cloned from a genomic library by complementation of the *his3* mutation of *Saccharomyces cerevisiae.* The gene was subsequently subcloned in *Escherichia coli* and characterized by restriction enzyme mapping.

Introduction

Yarrowia lipolytica is a yeast of some biotechnological significance, with an ability to secrete a number of potent extracellular hydrolases which permit it to grow on a wide range of substrates (Peters & Nelson 1948; Abdelal et al. 1977; Ogrydziak & Scharf 1982; Ogrydziak et al. 1982; Yamada & Ogrydziak 1983; Cheng & Ogrydziak 1986). Manipulation of this yeast by recombinant DNA techniques will require suitable vectors, and the *Yarrowia* gene isolated in the work described here should prove useful in this context.

Yeast biosynthetic genes were first isolated from genomic libraries of bakers yeast (*Saccharomyces cerevisiae*) by complementation in *Escherichia coli.* This approach was used to clone the *HIS3* (Struhl et al. 1976) and the *LEU2* (Ratzkin & Carbon 1977) genes by complementating the *hisB463* and *leuB6* mutations of *E. coli,* respectively. The development of efficient bakers yeast transformation systems later permitted genes of *S. cerevisiae,* and other yeasts, to be isolated by direct complementation of mutations in *S. cerevisiae* (Beggs 1978; Hinnen et al. 1978).

A *Yarrowia* genomic library was constructed in the *S. cerevisiae* episomal vector Yop1 (a kind gift from D. Rogers) using genomic DNA from *Yarrowia lipolytica* strain NCYC825, and the ability of the library to complement a number of mutations of *S. cerevisiae* was tested. In this way *Yarrowia* sequences complementing the *his3* and also *leu2* mutations of *S. cerevisiae* were cloned. Both *Yarrowia* genes were subcloned and mapped with restriction enzymes (Prodromou 1988). Plasmids containing these cloned genes could be used as integrative vectors for the genetic manipulation of *Y. lipolytica* (Davidow et al. 1985; Gaillardin et al. 1985).

Materials and methods

General methods

Yeast strains used included *Yarrowia lipolytica* strain NCYC825, and *Saccharomyces cerevisiae* strain DBY747 (*leu2*, *his3*, *trp1*, *ura3* and *MAT*a), while the *Eschrichia coli* strains comprised, strain DH1 (*recA, thi1,* and *hsdR17*), strain NCIB12220 (*thr, leuB6, B1, trpc1117,* and *hsdS*) and strain NCIB12454 (*hisB463* and *recA*). The plasmids used were Yop1 (Amp^r, Tet^r and *URA3*, see Fig. 1) and pBR322. Routine recombinant DNA methodology was performed according to Maniatis et al. (1982), and basic yeast methods were described by Sherman et al. (1986) unless stated otherwise. Yeast strains were transformed using the transformation procedure of Ito et al. (1983) and plasmid reisolated as described by Birnboim & Doly (1979). *E. coli* strains were transformed using the $CaCl_2$ transformation procedure of Cohen et al. (1973). Transformed yeast cells were selected on SD (0.17% (W/V) Difco yeast nitrogen base without amino acids or $(NH_4)_2SO_4$, 0.5% (W/V) $(NH_4)_2SO_4$, and 2% (W/V) glucose) containing appropriate amino acids, and *E. coli* transformants selected on LB or M9 minimal medium (Maniatis et al. 1982) containing appropriate amino acids, antibiotics (100 μg/ml) and thiamine (20 μg/ml) as necessary.

Construction of the Y. lipolytica *gene library*

Genomic DNA from *Y. lipolytica* was isolated by the procedure of Cryer et al. (1975) except that proteinase K was added to give final concentration of 1 mg/ml, prior to lysis of the spheroplasts. The lysate was incubated at 37° C for 4–6 h, at 60° C for 2–4 h and finally at room temperature to cool. The lysate was then diluted with an equal volume of TE (10 mM Tris, 1 mM EDTA, pH 8.0). Deproteinization was achieved by the addition of an equal volume of phenol reagent and high molecular weight DNA isolated from the aqueous phase by spooling. The spooled DNA was washed with 70% ethanol before dissolution in 20 ml TE. The dissolved DNA was then respooled and redissolved in 20 ml TE. Finally the DNA was purified further by centrifugation to equilibrium in a cesium chloride density gradient containing ethidium bromide (0.6 mg/ml). The concentration of the purified DNA was determined spectrophotometrically at 260 nm.

The gene library was constructed with partial *Sau*3A1 DNA fragments of *Y. lipolytica* which were fractionated by sucrose density centrifugation. Fractions containing fragments of 5–15 kb were pooled. The cohesive ends of the *Sau*3AI cleaved and fractionated DNA and the *Sal*I cleaved Yop1 plasmid were filled in using Klenow enzyme and appropriate dNTPs to form complementary two base pair overhangs. This technique prevented self ligation by plasmid or genomic DNA.

Isolation of the HIS3 *gene of* Y. lipolytica

S. cerevisiae was transformed with *Y. lipolytica* gene library DNA and plated out onto SD containing leucine, uracil and tryptophan, but lacking histidine. The most frequently occurring *his*$^-$ complementing plasmid (as assessed by restriction enzyme analysis of mini-plasmid preparations from randomly selected transformants) was then isolated and designated Yop12.

Restriction enzyme mapping

Single and double restriction enzyme digests of recombinant plasmids were analyzed by agarose gel-electrophoresis.

Subcloning

Restricted and ligated Yop12 DNA was used to transform *E. coli* strain NCIB12454 and transformants were selected on M9 minimal medium containing ampicillin or tetracyclin (100 μg/ml), thiamine (20 μg/ml) and appropriate amino acids as necessary. A subclone of Yop12, pYL2, was produced in this way.