QUALITATIVE DETECTION OF PENICILLINASE PRODUCED BY YEAST STRAINS CARRYING CHIMERIC YEAST-COLI PLASMIDS

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1. Introduction

A procedure for transformation of yeast strains by chimeric plasmids made of the 2 μm DNA plasmid from yeast (in toto or as *Eco* RI fragments) ligated to *Escherichia coli* plasmids has been described ([1,2] F. Lacroute, in preparation). Nonmendelian heredity is typical of cells transformed by such hybrid plasmids. Such chimeric plasmids made by the ligation of the *Eco* RI D fragment of the yeast 2 μm DNA into the *Eco* RI site of clone 6 DNA [3] were obtained in our laboratory. Clone 6 DNA is composed of *E. coli* pBR 322 plasmid containing a DNA sequence coding for penicillinase plus the yeast gene *URA 3* inserted into the *Hin*dIII restriction site of pBR 322 [4,5]. Yeast cells carrying two mutations at the *URA 3* locus have been transformed with such chimeric plasmids and selected on the basis of uracil prototrophy. M. Crabeel and F. Messenguy have shown that yeast strains carrying an Amp^R gene on a plasmid express this gene and produce penicillinase (personal communication). It was therefore interesting to develop a rapid test for the detection of this character on agar plates.

2. Method

This test was set up starting from the method developed for bacteria [6,7]. The basis of the test is that penicillinase hydrolyses penicillin giving a reducing compound, penicilloic acid. The reducing action of penicilloic acid is visualized by the decoloration of a deep blue iodine–starch complex incorporated into the solid medium. Thus a white halo appears around the penicillinase producing strains.

Test medium: Yeast nitrogen base (Difco) 0.65%; glucose 0.1%; soluble starch 0.2%; agar 2%, buffered with 0.02 M phosphate at pH 6–7.

Soft agar test medium: same as before except agar 1%.

Reagent: 3 mg/ml I_2; 15 mg/ml KI; 0.02 M phosphate bufffer pH 7; 3 mg/ml ampicillin. The ampicillin is added just before use, the I_2/KI mixture can be kept in the dark for several weeks.

Plates containing the test medium are inoculated with a loopful of each strain to be tested. They are incubated at 30°C for 12–36 h. A mixture of 4 ml melted soft agar test medium plus 1.5 ml reagent is prepared. The addition of I_2 to the starch containing medium produces a deep blue colour. The mixture is stirred and gently poured on the plates so as to avoid spreading of the cells. The plates which are now deep blue are then left for ~1 h at 30°C and thereafter put at 6°C.

After about 24 h, strains carrying plasmids coding for ampicillin resistance show a well-defined white halo whereas control strains without plasmid show only a very slight and limited decoloration. This background is easily distinguished from the positive reaction (fig.1).

The size of the clear halo increases during further incubation in the refrigerator. Eventually all the blue colour disappears due to complete reduction of the iodine–starch complex. This occurs as a result of

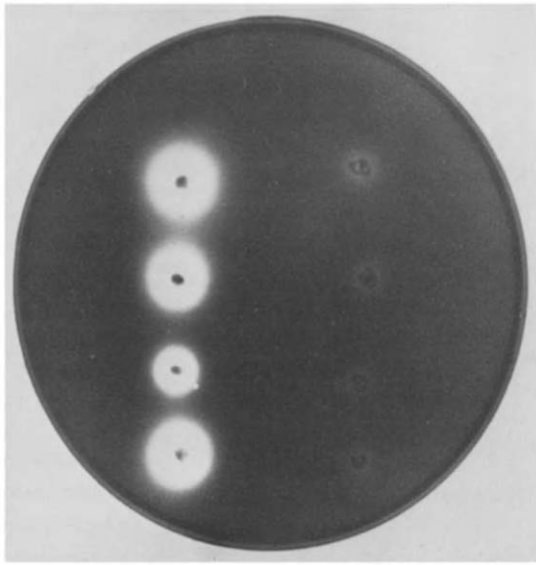

Fig.1. Example of penicillinase producing yeast clones. Left: 4 clones carrying the Amp^R gene. Right: Control wild-type clones.

spontaneous hydrolysis of ampicillin to penicilloic acid. If plates are left at 30°C instead of being transferred to 6°C the complete decoloration is faster and the production of the halo around the penicillinase producing strains can be missed.

The addition of zymolyase (50 μg/ml) to the reagent agar mixture helps the development of the halo, but viability of the cells tested is greatly reduced.

We obtained similar results with plates buffered at pH 6.1, 6.5 and 7.1. Higher pH values gave a quicker answer but also promoted faster overall decoloration, probably due to higher spontaneous rate of hydrolysis of ampicillin at these pH values.

With more glucose in the plates, non-specific decoloration around the negative clones was enhanced. However, distinction between penicillinase producing and non-producing strains remained clear-cut. Casein hydrolysat at 0.1% in the medium did not affect the test.

3. Results

We found that 44 strains independently transformed and carrying different kinds of chimeric plasmids were all positive in the test. But in 26 independent tests made with the same positive strain, three were negative. The only obvious reason for this negative result was a smaller inoculum. It is therefore important to test inocula of different sizes and to repeat the test if negative.

As an example, this test was used in constructing a pool of new chimeric plasmids bearing pBR 322 (AMP^R – *URA* 3^+ – 2 μm. The (*ura* 3^-) yeast strain was transformed by a mixture of potential plasmids and the URA^+ clones selected on minimal medium. We then checked for the presence of the Amp^R gene in the URA^+ yeast cells by the penicillinase test. All strains gave a clear-cut positive answer. Seven of these URA^+ clones were streaked out on complete medium, then tested for uracil auxotrophy. Each clone showed some instability of the phenotype. Two URA^+ and two ura^- subclones of each strains were tested for the presence of the Amp^R gene. All the URA^+ strains synthetized penicillinase, and all the ura^- did not. This clearly shows the genetic linkage between the *URA* 3^+ and the Amp^R genes on the plasmid.

4. Discussion

In yeast, this test is extremely useful to rapidly ascertain the presence of an *E. coli* plasmid carrying the gene for penicillinase production. We assume that the plasmid is maintained in multiple copies, allowing a high level of penicillinase production. It would be interesting to test whether strains having only one chromosomally integrated Amp^R gene [8] will produce enough penicillinase (if any) to be detected by this test.

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