B. 16 MITOTIC RECOMBINATION - *SACCHAROMYCES CEREVISIAE*

1. METHOD

1.1. Introduction

See General Introduction Part B.

1.2. Definition

See General Introduction Part B.

1.3. Reference substances

None.

1.4. Principle of the test method

Mitotic recombination in *Saccharomyces cerevisiae* can be detected between genes (or more generally between a gene and its centromere) and within genes. The former event is called mitotic crossing-over and generates reciprocal products whereas the latter event is most frequently non-reciprocal and is called gene conversion. Crossing-over is generally assayed by the production of recessive homozygous colonies or sectors produced in a heterozygous strain, whereas gene conversion is assayed by the production of prototrophic revertants produced in an auxotrophic heteroallelic strain carrying two different defective alleles of the same gene. The most commonly used strains for the detection of mitotic gene conversion are D_4 (heteroallelic at *ade* 2 and *trp* 5) D_7 (heteroallelic at *trp* 5) BZ_{34} (heteroallelic at *arg* 4) and JDl (heteroallelic at *his* 4 and *trp* 5). Mitotic crossing-over producing red and pink homozygous sectors can be assayed in D_5 or in D_7 (which also measures mitotic gene conversion and reverse mutation at *ilv* 1-92) both strains being heteroallelic for complementing alleles of *ade* 2.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

Solutions of test chemicals and control or reference compounds should be prepared just prior to testing, using an appropriate vehicle. With organic compounds that are water insoluble not more than a 2% solution v/v of organic solvents such as ethanol, acetone or dimethylsulphoxide (DMSO) should be used. The final concentration of the vehicle should not significantly affect cell viability and growth characteristics.

Metabolic activation

Cells should be exposed to test chemicals both in the presence and absence of an appropriate exogenous metabolic activation system. The system most commonly used is a co-factor supplemented post-mitochondrial fraction from the livers of rodents pre-treated with enzyme inducing agents. The use of other species, tissues, post-mitochondrial fractions, or procedures may also be appropriate for metabolic activation.

Test conditions

Tester strains

The most frequently used strains are the diploids D_4, D_5, D_7 and JD1. The use of other strains may be appropriate.

Media

Appropriate culture media are used for the determination of survival and the frequency of mitotic recombination.

Use of negative and positive controls

Positive, untreated and solvent controls should be performed concurrently. Appropriate positive control chemicals should be used for each specific recombination endpoint.

Exposure concentrations

At least five adequately spaced concentrations of the test substance should be used. Among the factors to be taken into consideration are cytotoxicity and solubility. The lowest concentration must have no effect on celf viability. For toxic chemicals, the highest concentration tested should not reduce survival below 5 to 10% .Relatively water-insoluble chemicals should be tested up to the limit of solubility using appropriate procedures. For freely water-soluble non-toxic substances the upper concentration should be determined on a case by case basis.

Cells may be exposed to test chemicals in either the stationary phase or during growth for periods of up to 18 hours. However, for long treatment times cultures should be microscopically inspected for spore formation, the presence of which invalidates the test.

Incubation conditions

The plates are incubated in the dark for four to seven days at 28 to 30 °C. Plates used for the assay of red and pink homozygous sectors produced by mitotic crossing-over should be kept in a refrigerator (about 4 °C) for a further one to two days before scoring to allow for the development of the appropriate pigmented colonies.

Spontaneous mitotic recombination frequencies

Sub-cultures should be used with spontaneous mitotic recombination mutation frequencies within the accepted normal range.

Number of replicates

A minimum of three replicate plates should be used per concentration for the assay of prototrophs produced by mitotic gene conversion and for viability. In the case of the assay of recessive homozygosis produced by mitotic corssing-over, the plate number should be increased to provide an adequate number of colonies.

Procedures

Treatment of *S. cerevisiae* strains is usually performed in a liquid test procedure involving either stationary or growing cells. Initial experiments should be done: on growing cells. 1-5 x 10^7 celles/ml are exposed to the test chemical for up to 18 hours at 28 to 37 °C with shaking; an adequate amount of metabolic activation system is added during treatment when appropriate.

At the end of the treatment, cells are centrifuged, washed and seeded upon appropriate culutre medium. After incubation plates are scored for survival and the induction of mitotic recombination.

If the first experiment is negative, then a second experiment should be carried out using stationary phase cells. If the first experiment is positive it is confirmed in an independent experiment.

2. DATA

Data should be presented in tabular form indicating the number of colonies counted, the number of recombinants, survival and the frequency of recombinants.

Results should be confirmed in an independent experiment.

The data should be evaluated using appropriate statistical methods.

3. REPORTING

3.1. Test report

The test report shall, if possible, contain the following information:

-strain used,

-test conditions: stationary phase or growing cells, composition of media, incubation temperature and duration, metabolic activation system,

-treatment conditions: exposure concentration, procedure and duration of treatment, treatment temperature, positive and negative controls,

-number of colonies counted, number of recombinants; survival and recombination frequency, dose/response relationship if applicable, statistical evaluation of data,

-discussion of the results,

-interpretation of the results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.