OECD GUIDELINE FOR TESTING OF CHEMICALS

481

Adopted
23 Oct 1986

"Genetic Toxicology: *Saccharomyces cerevisiae*, Mitotic Recombination Assay"

1. INTRODUCTORY INFORMATION

• Prerequisites

– Solid, liquid, vapour or gaseous test substance
– Chemical identification of test substance
– Purity (impurities) of test substance
– Solubility characteristics
– Melting point/boiling point
– pH (where appropriate)
– Vapour pressure data (if available)

• Standard documents

There are no relevant international standards.

2. METHOD

A. INTRODUCTION

This assay may be used to measure mitotic recombination (gene conversion or crossing-over) in yeast, a eukaryotic micro-organism. Strains of *Saccharomyces cerevisiae* have been developed which detect such events by the production of homozygous alleles from heterozygous alleles. These recombinations are essentially DNA exchanges between segments of homologous chromatids and this assay gives an indication of non-specific DNA damage.

• Definitions

Mitotic crossing-over is the exchange of segments of DNA between genes (or more generally between a gene and its centromere), resulting in reciprocal products.

Mitotic gene conversion is the unilateral transfer of DNA sequence information within a gene, resulting most frequently in non-reciprocal products.

• Principle of the test method

Mitotic crossing-over and mitotic gene conversion can be detected in *Saccharomyces cerevisiae*.

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 detection of mitotic gene conversion are D_4 (heteroallelic at ade 2 and trp 5), BZ_{34} (heteroallelic at arg 4), D_7 (heteroallelic at trp 5) and JD_1 (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.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

Test substance

Solutions of the test substance and positive control substances should be prepared just prior to testing, using, when necessary, an appropriate vehicle. The final concentration of the vehicle should not significantly affect cell viability and growth characteristics.

Test strains

The most frequently used strains are the diploids D_4, D_5, D_7 and JD_1. 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.

Metabolic activation

Cells should be exposed to the test substance both in the presence and absence of an appropriate exogenous mammalian metabolic activation system.

The most commonly used system is a cofactor supplemented post-mitochondrial fraction from the livers of rodents pretreated with enzyme inducing agents. The use of other species, tissues, post-mitochondrial fractions, or procedures may also be appropriate.

"Genetic Toxicology: *Saccharomyces cerevisiae*, Mitotic Recombination Assay"

- Test conditions

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 cell viability. For freely water-soluble, non-toxic chemicals, the highest test concentration should be determined on a case-by-case basis. For toxic chemicals the highest concentration tested should not reduce survival below 5-10 per cent. Poorly water-soluble chemicals should be tested up to the limits of solubility using appropriate procedures.

Spontaneous mitotic recombination frequencies

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

Number of replicates

A minimum of 3 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 crossing-over, the number of replicate plates should be increased to provide an adequate number of colonies.

Controls

Positive controls, using both a direct acting compound and a compound requiring metabolic activation, should be included in each study. A vehicle control should be used. The following are examples of substances which might be used as positive controls:

– methylmethanesulphonate, ethylmethanesulphonate, 4-nitroquinoline-N-oxide (direct acting compounds);

– cyclophosphamide (indirect acting compound).

- Performance of the test

Treatment of *Saccharomyces cerevisiae* is usually performed in a liquid test procedure involving either stationary or growing cells. Initial experiments should be done on growing cells. 1-5x10^7 cells/ml are exposed to the test substance for up to 18 hours at 28°-37° C with shaking. For experiments with metabolic activation, an adequate amount of mammalian metabolic activation system is added during treatment. At the end of the treatment, cells are centrifuged, washed and seeded upon appropriate culture medium.

After incubation for 4-7 days at 28°-30°C in the dark, plates are scored for survival and the induction of mitotic recombination. Plates used for the assay of red and pink homozygous sectors produced by mitotic crossing-over should be kept in a refrigerator (4°C) for a further 1-2 days before scoring to allow for the development of the appropriate pigmented colonies.

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 appropriate independent experiment.

3. DATA AND REPORTING

- Treatment of results

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

The data should be evaluated using appropriate statistical methods.

- Evaluation of results

There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of recombinants.

Another criterion may be based upon the detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

A test substance producing neither a statistically significant dose-related increase in the number of recombinants, nor a statistically significant and reproducible positive response at any one of the test substance concentrations is considered not to have produced DNA recombination in this test system.

Both biological and statistical significance should be considered together in this evaluation.

- Test report

The test report should include the following information:

– strain used;
– test conditions: stationary phase or growing cells, composition of the media, incubation temperature and duration, metabolic activation systems;

- treatment conditions: exposure concentrations, 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 results;

- interpretation of results.

4. L I T E R A T U R E

P.J. Davies, W.J. Evans and J.M. Parry, *Mutation Res. 29*, 301-314 (1975).

R. Fahrig, *Mutation Res. 31*, 381-394 (1975).

G.R. Fink and R. Lowenstein, *J. Bacteriol. 100*, 1126-1127 (1969).

D. Kelly and J.M. Parry, *Mutation Res. 108*, 147-159 (1983).

B.A. Kunz, B.J. Barcley and R.H. Haynes, *Mutation Res. 73*, 215-20 (1980).

K.K. Mortimer and T.R. Manney, in *Chemical Mutagens. Principles and Methods for their Detection*, Vol. 1 (edited by A. Hollaender), pp. 289-310, Plenum Press, New York (1971).

M.S.S. Murthy, *Mutation Res. 64*, 1-17 (1979).

E.M. Parry and J.M. Parry, The assay of genotoxicity of chemicals using the budding yeast *Saccharomyces cerevisiae*, in *Mutagenicity testing, a practical approach* (edited by S. Venitt and J.M. Parry), pp. 119-148, IRL Press, Oxford (1985).

D.C. Sharp and J.M Parry, in *Evaluation of Short-Term Tests for Carcinogens* (edited by F.J. de Serres and J. Ashby), pp. 502-626, Elsevier/North Holland, New York (1981).

F.K. Zimmermann, R. Kern and H. Rosenberger, *Mutation Res. 28*, 381-388 (1975).

F.K. Zimmermann, in *Handbook of Mutagenicity Test Procedures*, 2nd edition (edited by B.J. Kilbey, M. Legator, W. Nichols, and C. Ramel), pp. 215-238, Elsevier Scientific, Amsterdam (1984).

F.K. Zimmermann and I. Scheel, in *Evaluation of Short-Term Tests for Carcinogens* (edited by F.J. de Serres and J. Ashby), pp. 481-490, Elsevier/North Holland, New York (1981).

"Genetic Toxicology: *Saccharomyces cerevisiae*, Mitotic Recombination Assay"

F.K. Zimmermann, V.M. Mayer and J.M. Parry, *J. Appl. Toxicol. 2*, 1-10 (1982).

F.K. Zimmermann, R.C. von Borstel, E.S. von Halle, J.M Parry, D. Siebert, G. Zetterberg, R. Barale and N. Loprieno, Testing of chemicals for genetic activity with *Saccharomyces cerevisiae*: a report of the US Environmental Protection Agency Gene-Tox Program, *Mutation Res. 133*, 199-244 (1984).