B. 15 MUTAGENICITY TESTING AND SCREENING FOR CARCINOGENICITY

GENE MUTATION -*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

A variety of haploid and diploid strains of the yeast *Saccharomyces cerevisiae* can be used to measure the production of gene mutations induced by chemical agents with and without metabolic activation.

Forward mutation systems in haploid strains, such as the measurement of mutation from red, adenine-requiring mutants (*ade*-1 , *ade*-2) to double adenine-requiring white mutants and selective systems such as the induction of resistance to canavnaine and cycloheximide, have been utilized.

The most extensively validated reverse mutation system involves the use of the haploid strain XV 185-14C which carries the ochre nonsense mutations *ade* 2-1, *arg* 4-17, *lys* 1-1 and *trp* 5-48, which are reversible by base substitution mutagens that induce site specific mutations or ochre suppressor mutations. XV 185-14C also carries the *his* 1-7 marker, a missense mutation reverted mainly by second site mutations, and the marker *hom* 3-10 which is reverted by frameshift mutagens.

In diploid strains of *S. cerevisiae* the only extensively used strain is D_7 which is homozygous for *ilv* 1-92.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

Solutions of test chemicals and control should be prepared just prior to testing, using an appropriate vehicle. In the case of organic compounds which are not water soluble, 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 characertistics.

Metabolic activation

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

The most commonly used system 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 haploid strain XV 185-14C and the diploid strain D_7 are the most used in gene mutation studies. Other strains may also be appropriate.

Media

Appropriate culture media are used for the determination of survival and mutant numbers.

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 mutational endpoint.

Exposure concentration

At least five adequately spaced concentrations of the test substance should be used. For toxic substances, the highest concentration tested should not reduce survival below 5 to 10% .Relatively water-insoluble substances should be tested up to their 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.

Incubation conditions

The plates are incubated four to seven days at 28 to 30 °C in the dark.

Spontaneous mutation frequencies

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

Number of replicates

At least three replicate plates should be used per concentration for the assay of prototrophs produced by gene mutation and for cell viability. In the case of experiments using markers such as *hom* 3-10 with a low mutation rate, the number of plates used must be increased to provide statistically relevant data.

Procedure

Treatment of *S. cerevisiae* strains is usually performed in a liquid test procedure involving either stationary or growing cells. Initial experiments should be carried out on growing cells: 1-5 x 10^7 cells/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 an appropriate culture medium. After incubation, plates are scored for survival and the induction of gene mutation.

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.

2. DATA

Data should be presented in tabular form indicating the number of colonies counted, number of mutants, survival and mutant frequency. All results should be confirmed in an independent experiment. The dara should be evaluated using appropriate statistical met hods.

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, compositions of media, incubation temperature and duration, metabolic activat ion system,

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

-number of colonies counted, number of mutants, survival and mutant frequency, dose/response relationship if applicable, statistical evaluation of data,

-discussion of results,

-interpretation of results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES