Asian Journal of Chemistry Vol. 6, No. 4 (1994), 753–755

Influence of Mutagens on Alcoholic Fermentation by *Saccharomyces cerevisiae* BR-6

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The influence of maleic hydrazide, maganese chloride, hydrazine and 2,4-dinitrophenyl hydrazine were studied on alcoholic fermentation by *S. cerevisiae* BR-6. It has been found that manganese chloride and hydrazine enhances the production of alcohol while maleic hydrazide and 2,4-dinitrophenyl hydrazine retard the yield.

INTRODUCTION

There has been a considerable interest in the chemistry of mutagenic chemicals and related compounds, because of their biological importance in fermentation process.[1–6] Recently[7–9] trials were carried out in which different microbes were treated with a few specific mutagenic compounds to give very interesting results. In the present study an attempt has been made to assess the impact of a few mutagens on alcoholic fermentation.

EXPERIMENTAL

The composition of the production medium for 100 ml flask each was prepared as follows:

Molasses: 25% (w/v), Malt extract: 0.3%, Yeast extract 0.3%:
Peptone: 0.5%, pH: 5.0
Distilled water: To make up 100 ml
(pH was adjusted by adding requisite amount of lactic acid.)

Now, the same production medium was prepared for 99 flasks. These flasks were then arranged in 10 sets each comprising of 9 flasks. Each set was then rearranged in 3 sub-sets, each comprising of 3 flasks. The remaining 9 flasks out of 99 flasks were kept as control and these were also rearranged in 3 sub-sets each comprising of 3 flasks. All the flasks were sterilized and allowed to cool at room temperature.

Now, 1st to 9th set each comprising 3 flasks were added with 1.0 ml to 9.0 ml M/1000 solution of mutagens respectively and were inoculated with 0.5 ml yeast

suspension of *S. cerevisiae* BR-6. The 10th control set was also inoculated with the same inoculum but it contained no mutagen. All the experimental flasks were kept in an incubator maintained at 30°C for 30, 50 and 80 h of incubation period and analysed for the production of alcohol[10]

RESULTS AND DISCUSSION

The results obtained in the study of the influence of different mutagens on alcoholic fermentation for maximum production in 50 h of optimum incubation period only are tabulated in Table 1.

TABLE 1
COMPARATIVE ASSESSMENT OF THE INFLUENCE OF DIFFERENT MUTAGENS ON ALCOHOLIC FERMENTATION BY *S. CEREVISIAE* BR-6 AFTER 50 h, OF OPTIMUM INCUBATION PERIOD

Mutagens	Optimum concentration of mutagens	Max. yield of alcohol* in ml/100 ml in control	Max. yield of alcohol* in presence of different mutagens in ml/100 ml	% Difference in the yield of alcohol (increase(+)/decrease (–) 50 h. of optimum incubation period)
Maleic hydrazide	1.0×10^{-5} M	5.87	5.70	(–)2.8960
Manganese chloride	5.0×10^{-5} M	5.91	6.40	(+)8.2910
Hydrazine	3.0×10^{-5} M	5.95	6.00	(+)0.8403
2,4-dinitro phenyl hydrazine	1.0×10^{-5} M	5.97	5.80	(–)2.8475

* Each value represents mean of three observations.
(±) values indicate % difference in the yield of alcohol
Experimental deviation: 2.5–3.5%

The influence of of maleic hydrazide (vide Table 1) was found to be detrimental for alcoholic fermentation. The yield of alcohol obtained in the control flasks was higher than that obtained from each of the flasks containing maleic hydrazide. The maximum yield of alcohol 5.70 ml/100 ml in presence of maleic hydrazide at 1.0×10^{-5} M concentration was found in 50 h but even this yield was 2.89% less in comparison to control.

The molecule of maleic hydrazide has two —OH groups in the aromatic ring. It is obvious from relation of chemical structure and physiological activity that entrance of hydroxyl groups generally increases the physiological action of the compound and sometimes it becomes much toxic. It may thus be concluded that maleic hydrazide bearing 2 —OH groups may account for detrimental action of the compound for alcoholic fermentation.

Manganese chloride (vide Table 1) enhances the production of alcohol by *S. cerevisiae* BR-6 up to the concentration of 5.0×10^{-5} M and after that there has been a gradual fall in the yield of alcohol. The maximum yield of alcohol 6.40 ml/100 ml was found at 5.0×10^{-5} M concentration of $MnCl_2$ which is

8.2910% higher in comparison to control in 50 h of optimum incubation period. The response of $MnCl_2$ at higher and lower concentrations may be explained on the basis of electrovalent bonding. At lower concentrations of manganese chloride, Mn^{2+} plays the role of micronutrients for the growth and activity of *S. cerevisiae* BR-6. At higher concentration Cl^- ions become sufficient to deactivate the fermentation enzymes and suppress the yield of alcohol.

It is evident from Table 1 that the presence of hydrazine is not significant for the alcoholic production by fermentation. It was found that hydrazine up to the concentration of 2.0×10^{-5} M was slightly stimulating for the fermentation process. The maximum production of alcohol 6.0 ml/100 ml was obtained at 2.0×10^{-5} M concentration of hydrazine in 50 h of optimum incubation period which was just 0.84% higher in comparison to control. Hydrazine has been found to be the most specific mutagen and its appreciable mutagenicity has been reported at its low molar concentration[11]. Singh *et al.*[12, 13] have also reported poor response of hydrazine for citric and lactic acid fermentation.

The data recorded in Table 1 reveals that 2,4-dinitrophenylhydrazine has very much detrimental effect on *S. cerevisiae* BR-6 and fermentative production of alcohol. The maximum yield of alcohol (5.80 ml/100 ml) was observed at initial concentration 1.0×10^{-5} M which was found to be 2.84% less in comparison to control in 50 h of optimum incubation period. The detrimental behaviour of 2,4-dinitrophenylhydrazine is supported by the work of different workers[14,15] using nitro compounds as toxicative agents for different fermentative processess.

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(*Received*: 12 November 1992 *Accepted*: 1 January 1994) AJC-741