

Biotransformation of sucrose to 2-hydroxy propanoic acid by *SmF* exposed to methyl methane sulphonate

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Abstract : The efficacy of methyl methane sulphonate on biotransformation of sucrose to 2-hydroxy propanoic acid by *Lactobacillus bulgaricus* BS-18 has been assessed. It has been observed that the bacterial strain *Lactobacillus bulgaricus* BS-18 is most potential and effective for the lactic acid fermentation process. It has been found that the mutagen, i.e., methyl methane sulphonate at concentration 0.3% enhances the biotransformation of sucrose to 2-hydroxypropanoic acid by *Lactobacillus bulgaricus* BS-18 to an extent of 10.544% higher in comparison to control, i.e., 6.961 g/100ml while at concentration 0.4% and onwards concentration of methyl methane sulphonate under trial inhibits and retards the production of lactic acid when experimental parameters are under optimized conditions.

(Key words : Lactic acid fermentation, *Lactobacillus bulgaricus* BS-18 and methyl methane sulphonate)

Introduction

Mutation is the bioprocess, in which genes are permanently alternated under environmental conditions. Factors that lead to mutation are called mutagens and living creatures that have had permanent hereditary changes are called mutants¹⁻⁴. The influence of mutagens in fermentation technology specially lactic acid fermentation has been studied extensively⁵⁻²⁰. Methyl methane sulphonate is one of the few chemical mutagen which has been found detrimental chemical mutagen in fermentations.

The present communication was undertaken for bioconversion of sucrose to 2-hydroxy propanoic acid by *Lactobacillus bulgaricus* BS-18 exposed to methyl methane sulphonate.

Experimental

1.0 g of Methyl methane sulphonate was dissolved in 100ml of pure distilled water. 1.0, 2.0, 3.0, 4.0 and 5.0 ml of this solution was taken and a fresh enriched agar culture medium was prepared in 6-dry and clean sterilized culture tubes as described below :

Now 9.0 ml of fresh enriched agar culture medium and 1.0 of above prepared methyl methane sulphonate solution was taken in the first culture tube, thus making 0.1% methyl methane sulphonate solution in the culture medium. In the same way 2.0, 3.0, 4.0 and 5.0 ml of methyl methane sulphonate were taken into the 2nd, 3rd, 4th and 5th culture tubes respectively. The total volume of the culture medium was made upto 10.00 ml by adding enriched agar culture medium in these tubes, thus making 0.2%, 0.3%, 0.4% and 0.5% methyl methane sulphonate solution in the culture medium respectively. The remaining culture tubes were kept as control and contained only 10ml of enriched agar medium. The total 6 culture tubes were sterilized at 15 lbs. steam pressure for 30 minutes and kept at room temperature in a vertical position overnight.

A 48 hours old wild microbial suspension of *L. bulgaricus* BS-18 was obtained by dipping a sterilized inoculating platinum needle into the culture tube and seeded into the freshly prepared above 6 culture tubes. These culture tubes were then incubated at 48⁰C in an incubator for 48 hours. Thus, under the influence of different concentration of Methyl methane sulphonate in

Table – 1
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Concentration of Mutagen	Incubation period in days	Yield of lactic acid*		Sucrose left unfermented in g/100ml	% of lactic acid increased in 5days
		in g/100 ml	% conversion		
Control (– mutagen)	4	3.989	75.441	4.043	-
	5	6.961		1.273	-
	6	6.339		1.237	-
0.1%	4	4.143	75.844	3.888	-
	5	7.118		1.115	(+) 2.255
	6	6.496		1.080	-
0.2%	4	5.92	76.955	3.440	-
	5	7.567		0.667	(+) 8.7056
	6	6.973		0.631	-
0.3%**	4	4.720	77.243	3.310	-
	5	7.695***		0.538	(+) 10.5444
	6	7.070		0.503	-
0.4%	4	4.618	77.002	3.412	-
	5	7.594		0.638	(+) 9.094
	6	-		-	-
0.5%	4	-	76.463	-	-
	5	7.365		0.868	(+) 5.80376
	6	-		-	-

* Each value represents mean of three trials. ** Optimum concentration of mutagen.

*** Optimum yield of lactic acid (–) Values indicate % decrease in lactic acid production .

Experimental deviation $\pm 2.5 - 3\%$

the above six culture tubes *L. bulgaricus* BS-18 was grown

Now, the 48 hours old *L. bulgaricus* BS-18 from each of the 6-culture tubes were then seeded into 6 freshly prepared flask of culture broth medium separately and were incubated at 48°C for 48 hour. Thus, 6 culture broth medium five containing mutagen treated *L. bulgaricus* BS-18 grown in solid agar-agar culture medium containing different concentrations of methyl methane sulphonate and the remaining one containing wild *L. bulgaricus* BS-18 grown in the agar culture blank medium only were obtained.

An enriched lactic acid culture medium was prepared as follows :

Sucrose : 567g, Malt Extract : 20.25gm, (NH₄)₂HPO₄ : 13.50g, CaCO₃:550g, Distilled water 500 ml, pH 6.2. The pH of the medium was adjusted

to 6.2 by adding requisite amount of phosphate – buffer solution.

The total volume of the medium prepared above was divided into 54 equal parts. Each part was taken in a separate 250ml conical flask. These flask were then arranged in 5- sets, each containing 9-flasks. Each set was then rearranged in 3 sub-sets, each consisting of 3-flasks. The remaining 9 out of 54 flasks were kept as control and these were also arranged in three equal sets.

Now, the total volume of the medium present in each flask was made upto 100ml by adding requisite amount of distilled water. The flasks were then plugged and sterilized. The sterilized flasks were cooled to room temperature. Now, the first set of nine flasks were inoculated with 0.05ml of 48 hours old 0.1% treated *L.*

bulgaricus BS-18. Similarly the second, third, fourth and fifth sets were also inoculated with 48 hour old methyl methane sulphonate respectively lactic acid culture *L. bulgaricus* BS-18. Finally the remaining 9-flasks kept as control were inoculated with the same amount of 48 hrs old lactic culture grown in control culture flasks.

All the above 6-sets each comprising 9-flasks were then incubated at 48°C in an incubator and analysed colorimetrically after 4, 5 and 6 days of incubation period for lactic acid produced and sucrose left unfermented.

Assay methods : Evaluation of lactic acid formed and sucrose left unfermented was made colorimetrically^{21,22}.

Sterilization : The growth and production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for 30 minutes.

Strain : Lactic acid bacteria *L. bulgaricus* BS-18 has been selected and employed in the present

study. The strain was procured from NCL - Pune, India

Age of the inoculum : 48 hours old.

Quantum of the inoculum: 0.5 ml bacterial suspension of *L. bulgaricus* BS-18

Incubation period : 4, 5 and 6 days

Results and Discussion

The influence of methyl methane sulphonate

The data recorded in the table 1 shows that the presence of methyl methane sulphonate has stimulatory effect on the biotransformation of sucrose to lactic acid by *L. bulgaricus* BS-18. It was observed that methyl methane sulphonate has beneficial effect at all the concentrations taken for biotransformation of sucrose to lactic acid. The maximum yield of lactic acid was observed at 0.3% concentration of methyl methane sulphonate, i.e., 7.695g/100 ml in 5 days of optimum incubation period. However, this yield of lactic acid was better in comparison to control .

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