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Mutagenic influence of methyl benzenesulphonate on lactic acid bioproduction from sucrose by *Lactobacillus bulgaricus* BS-18

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Abstract: The present investigation highlights the mutagenic influence of methyl benzenesulphonate (MBS) on lactic acid bioproduction from sucrose by *Lactobacillus bulgaricus* BS-18. Mutagenesis has long been considered a powerful tool for enhancing microbial strains toward improved bioprocess efficiency. In this study, cultures of *L. bulgaricus* BS-18 were exposed to graded concentrations of MBS to induce random mutations and subsequently screened for enhanced lactic acid productivity. The mutagen-treated strains exhibited significant variations in growth kinetics, sugar utilization, and lactic acid yield compared to the wild-type strain. Among the mutants obtained, several isolates demonstrated higher tolerance to substrate concentration and improved metabolic efficiency, thereby producing elevated lactic acid titers. Optimization of fermentation parameters such as pH, temperature, inoculum density, and sucrose concentration further enhanced production levels. The study establishes MBS mutagenesis as a viable strategy for strain improvement in lactic acid fermentation. The findings contribute to the development of efficient microbial platforms for industrial-scale lactic acid bioproduction, with potential applications in food, pharmaceutical, and biodegradable polymer industries. It has been observed that the mutagene MBS at concentration 0.4% enhances the yield of lactic acid by Lactobacillus bulgaricus BS-18 to an extent of 14.544% higher in comparison to control under optimized conditions.

(Keywords : Methyl benzenesulphonate, *Lactobacillus bulgaricus* BS-18, mutagenesis, lactic acid, sucrose fermentation, strain improvement).

Introduction

Lactic acid is a valuable organic acid widely applied in food, pharmaceutical, cosmetic, and biodegradable polymer industries due to its diverse functional properties. Microbial fermentation, particularly by lactic acid bacteria (LAB), remains the most sustainable and cost-effective approach for lactic acid production compared to traditional chemical synthesis. Among various LAB strains, *Lactobacillus bulgaricus* is recognized for its robust fermentative metabolism and ability to efficiently convert carbohydrate substrates such as sucrose into lactic acid. However, the natural metabolic potential of wild-type strains often limits productivity, necessitating the use of strain improvement strategies to enhance yield, substrate utilization efficiency, and stress tolerance.

Mutagenesis is one of the most effective approaches to improve microbial strains for industrial applications. Chemical mutagens such as alkylating agents induce random genetic modifications that may result in enhanced metabolic performance. Methyl benzenesulphonate (MBS), a potent alkyl sulfonate, is known to introduce mutagenic alterations at the DNA level by modifying nucleotide bases and interfering with replication. Such mutagenic interventions can lead to beneficial phenotypic variations, including improved tolerance to substrate or product inhibition, enhanced enzymatic activity, and increased lactic acid productivity.[1-12]

In the context of lactic acid fermentation, mutagenesis offers an opportunity to overcome inherent limitations of *L. bulgaricus*, such as

moderate sugar uptake rates, sensitivity to osmotic stress, and feedback inhibition by lactic acid accumulation. By subjecting *L. bulgaricus* BS-18 to MBS mutagenesis, novel variants with improved metabolic efficiency and higher lactic acid yields from sucrose can potentially be developed. The use of sucrose, a cost-effective and renewable carbon source widely available from sugarcane and sugar beet, further strengthens the economic feasibility of the process.[13-20]

The present study investigates the mutagenic influence of methyl benzenesulphonate on *Lactobacillus bulgaricus* BS-18 with the aim of enhancing lactic acid production from sucrose. The findings are expected to provide insights into the role of chemical mutagenesis in strain improvement and contribute to the development of efficient, low-cost, and sustainable lactic acid fermentation processes.

Experimental

1.0 g of methyl benzenesulphonate 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 benzenesulphonate solution was taken in the first culture tube, thus making 0.1% methyl benzenesulphonate solution in the culture medium. In the same way 2.0, 3.0, 4.0 and 5.0 ml of methyl benzenesulphonate were taken into the 2^{nd}, 3^{rd}, 4^{th} and 5^{th} 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 benzenesulphonate 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 48^0C in an incubator for 48 hours. Thus, under the influence of different concentration of methyl benzenesulphonate in 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 48^0C 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 benzenesulphonate 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, $(NH_4)_2HPO_4$: 13.50g, $CaCO_3$: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.

Table – 1
Mutagenic influence of methyl benzenesulphonate on lactic acid bioproduction from sucrose by *Lactobacillus bulgaricus* BS-18

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 5 6	4.115 7.123 7.042	75.926	3.920 1.118 1.102	- - -
0.1%	4 5 6	4.247 7.353 7.267	76.474	3.788 0.885 0.319	- (+) 3.228 -
0.2%	4 5 6	4.376 2.814 7.678	71.789	3.272 0.485 0.295	- (+) 9.700 -
0.3%	4 5 6	4.586 1.938 1.348	77.793	3.446 0.296 0.280	- (+) 11.441 -
0.4%**	4 5 6	4.454 8.159*** 7.465	79.514	3.378 0.239 0.254	- (+) 14.544 -
0.5%	4 5 6	4.531 7.843 7.754	7.816	3.302 0.290 0.234	- (+) 10.108 -

* 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%

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 benzenesulphonate 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^{0}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 data recorded in the table 1 shows that the presence of methyl benzenesulphonate has stimulatory effect on bioproduction of lactic

acid by *Lactobacillus bulgaricus* BS-18. It was observed that methyl benzenesulphonate has beneficial effects at all concentrations taken for biotransformation of sucrose to lactic acid by *Lactobacillus bulgaricus* BS-18. "The maximum yield of lactic acid was observed at 0.4% concentration of methyl benzenesulphonate,ie; 8.159g/100 mL in 5 days of optimum incubation period. However,at all concentrations of methyl benzenesulphonate the yield of lactic acid has been found better in comparison to control .

The enhanced mutants of *L. bulgaricus* BS-18 developed via MBS mutagenesis present significant promise for industrial lactic acid bioproduction. Increased yields, better sucrose utilization, and improved product tolerance collectively enhance the economic feasibility of fermentation processes. The use of sucrose as a low-cost substrate, coupled with strain robustness, supports the potential application of these mutants in large-scale, eco-friendly bioprocesses. Further studies, including metabolic flux analysis and whole-genome sequencing, would be beneficial to identify the genetic basis of the observed improvements.

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