

Facile biotic production of lactic acid exposed to mutagens

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Abstract : The impact of diphenyl amine on lactic acid fermentation by some lactic acid bacteria such as *Lactobacillus amylophilus* R-88, *Lactobacillus amylovorus* R-95, *Lactobacillus lactis* R-83 and *Lactobacillus helveticus* R-79 was assessed. It was found that the strain of *Lactobacillus helveticus* R-79 was much effective and good lactic acid producer. The compound diphenyl amine was found stimulatory when 18.50% (w/v) molasses solution was allowed to ferment at pH 6.2, temperature 37.0°C and optimum incubation period of 6 days. It has been found that there is a gradual increase in the production of lactic acid with stepping up of the compound diphenyl amine till the maximum yield of lactic acid, i.e., 9.445 g/100 ml was obtained at its molar concentration of 7.0×10^{-4} M which is 20.165% higher in comparison to control fermentor flasks.

(Keywords: Lactic acid fermentation, molasses, mutagen, diphenylamine, *Lactobacillus helveticus* R-79)

Introduction

A mutagen is a chemical or physical agent capable of inducing change in DNA called mutagen. Examples of mutagens include tobacco product, radio active substances, X-rays, ultraviolet radiation and a wide variety of chemical.

Chemical mutagens are standard tools for mutagenesis in a variety of organisms, and they are a primary means of creating mutations in phenotype-based screens in most genetic systems. Chemical mutagens have also been used successfully in the mutagenesis.

Chemical mutagens are compounds

that increase the frequency of some types of mutation.¹⁻⁷ Mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens.⁸⁻¹⁵ Many chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals.¹⁶ Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. While many researchers found chemical mutagens are to be more effective than physical ones¹⁷⁻¹⁸ and many others researchers found the reverse case¹⁹.

A number of workers²⁰⁻²² have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants²³. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes²⁴⁻⁴⁵. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manipulation and exploitation specially for lactic acid fermentation and in view of this the author has studied the influence of diphenylamine on production of lactic acid by *Lactobacillus helveticus* R-79.

Experimental

The impact of diphenylamine on lactic acid fermentation by *Lactobacillus helveticus* R-79. The composition of the production medium

Table - 1
Facile biotic production of lactic acid exposed to mutagens

Concentration of mutagens used A × 1/10000 × 10M	Yield of lactic acid* in gm/100 ml			%Diff. in the yield of lactic acid in 6 days of optimum incubation pd.
	4 days	6 days	8 days	
Control	3.501	7.860	5.540	—
1×1/10000×10M	3.536	8.016	5.595	+ 1.984%
2×1/10000×10M	3.677	8.190	5.760	+ 4.198%
3×1/10000×10M	3.781	8.490	5.983	+ 8.015%
4×1/10000×10M	3.850	8.658	6.094	+ 10.152%
5×1/10000×10M	3.991	8.985	6.315	+ 14.312%
6×1/10000×10M	4.096	9.236	6.480	+ 17.506%
7×1/10000×10M**	4.201	9.445***	6.648	+ 20.165%
8×1/10000×10M	4.026	9.056	6.370	+ 15.216%
9×1/10000×10M	3.780	8.490	5.983	+ 8.015%
10×1/10000×10M	3.570	8.042	5.650	+ 2.315%

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

*** Optimum yield of lactic acid in 6 days (+) Values indicate % increase in the yield of lactic acid after 6 days. Experimental deviation (±) 1.5-3%.

for the lactic acid fermentation by *Lactobacillus helveticus* R-79 was prepared as follows : Molasses : 18.5% (w/v), Malt Extract : 2.5% Yeast Extract : 2.5%, Peptone:2.5% (NH₄)₂HPO₄ : 1.8%, CaCO₃ : 10.00%, pH : 6.2 Distilled water To make up 100 ml.

The pH of the medium was adjusted to 6.2 by adding requisite amount of phosphate-buffer solution and the pH was also ascertained by a pH meter. The above composition medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation by *Lactobacillus helveticus* R-79. Now, the same produciton medium for production of lactic acid by *Lactobacillus helveticus* R-79 was prepared for 99 fermentor flasks, i. e., each fermentor flask containg '100 ml' of production medium.

The above fermentor flasks were then

arranged in ten sets, each comprising 9 fermentor flask. Each set was again rearranged in three subsets, each comprising of 3 fermentor flasks. The remaining nine fermentor flasks out of 99 fermentor flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now M/1000 solution/suspension of diphenylamine was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml of this solution was added to the fermentor flasks of 1st to 10th sets respectively. The control fermentor flasks contained no mutagen i.e., diphenylamine Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of diphenylamine in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 1/10000×10M

1.0 x 1/10000×10M to 10.0 x 1/10000×10M

Where A = amount of mutagen,
i.e. diphenylamine in ml,

$1/10000 \times 10M$ = molarity of the solution.

The above fermentation flasks were then sterilized cooled, inoculated, incubated at 37°C and analysed after 4,6 and 8 days for lactic acid⁴⁶ formed.

Results and Discussion

The influence of the chemical mutagen, i.e., diphenylamine on fermentative production of lactic acid by *Lactobacillus helveticus* R-79

The maximum yield of lactic acid, i.e., 9.445 mL/100 ml in the presence of diphenylamine was observed at 7.0×10^{-5} M molar concentration in 6 days of optimum incubation period which is 20.165% higher in comparison to control flat water fermentor flasks, i.e; 7.860 ml/100ml in the same times course and some other same lab experimental parameters.

The data given in the table-1 shows that the chemical mutagen diphenylamine has been found quite stimulatory for the production of lactic

acid by *Lactobacillus helveticus* R-79. From the data given in the table-1 it is clear that the chemical mutagen, i.e., diphenylamine influences the lactic acid fermentation bioprocess in different phases. The main well characteristics of the chemical mutagen, i.e, diphenylamine is as follows:

- (i) Diphenylamine is quite stimulatory at its all molar concentrations used during the course of the lactic acid fermentation bioprocess, i.e; from $1.0 \times 1/10000 \times 10$ M to $10.0 \times 1/10000 \times 10$ M.
- (ii) The molar concentration 1.0×10^{-5} M, 2.0×10^{-5} M and $7.0 \times 1/10000 \times 10$ M of chemical mutagen, i.e., diphenylamine influences the yield of lactic acid in an approximately regular doubling order after each stage; i.e., 1.984%, 4.198%, 2.015%, 10.152%, 4.198%, 8.015%, 10.152%, 14.312%, 17.506% and 20.165%.
- (iii) The higher molar concentrations, i.e.; $9.0 \times 1/10000 \times 10$ M to $10.0 \times 1/10000 \times 10$ M of chemical mutagen, i.e., diphenylamine influences the yield of lactic acid is as follows :8.015% and 2.315% respectively.

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