ISSN: 2582-8118 *Volume 2, Issue 2; March 2022*

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYSIS IN SCIENCE AND ENGINEERING

Web: https://www.iarj.in/index.php/ijrase/index

8. A Comparative Study of The Different Chemical Mutagens on Lactic Acid Fermentation by Lactobacillus Bulgaricus NCIM-2359

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ABSTRACT

Deals lactic acid fermentation by Lactobacillus bulgaricus NCIM-2359 exposed to some chemical mutagens, viz: benzidine, 3,3'-dichlorobenzidine, 1-methyl-3-nitro-1-nitrosoguanidine, N-methyl-N-nitrosoethyl carbamate. Study of the influence of benzidine; 3,3'-dichlorobenzidine; 1-methyl-3-nitro-1-nitrosoguanidine and N-methyl-N-nitrosoethyl carbamate on lactic acid fermentation by ***Lactobacillus bulgaricus NCIM-2359*** *in 6 days of optimum incubation period.*

KEYWORDS:

Lactic Acid, Lactic acid fermentation, Lactobacillus bulgaricus, NCIM- 2359, chemical mutagens.

Introduction:

Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination[1-5]. These duplications are a major source of raw material for evolving new genes, with tens to hundreds of genes duplicated in animal genomes every million years[6]. Most genes belong to larger families of genes of shared ancestry[7]. Novel genes are produced by several methods, commonly through the duplication and mutation of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions[8-9]. Here, domains act as modules, each with a particular and independent function, that can be mixed together to produce genes encoding new proteins with novel properties.[10] For example, the human eye uses four genes to make structures that sense light: three for color vision and one for night vision; all four arose from a single ancestral gene.[11] Another advantage of duplicating a gene (or even an entire genome) is that this increases redundancy;

this allows one gene in the pair to acquire a new function while the other copy performs the original function.[12-13] Other types of mutation occasionally create new genes from previously noncoding DNA.[14-15]

Therefore, in the present investigation the author has made an attempt to study the lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to the following chemical mutagens mentioned below:

1. Benzidine
2. 3,3’-dichlorobenzidine
3. 1-methyl-3-nitro-1-nitrosoguanidine
4. N-methyl-N-nitrosoethyl carbamate

Experimental:

The influence of benzidine on lactic acid fermentation by using the bacterial strain of *Lactobacillus bulgaricus* NCIM-2359.

The composition of the production medium for the production of lactic acid by fermentation was prepared as follows:

Molasses	:	20% (w/v)
Malt Extract	:	0.60 %
Yeast Extract	:	0.60 %
Peptone	:	0.60%
$(NH_4)_2HPO_4$	:	0.60%
$CaCO_3$	:	8.0%
pH	:	6.1
Distilled water	:	To make up 100 ml.

The pH of the medium was adjusted to 6.1 by adding requisite amount of phosphate-buffer solution, and the pH was also ascertained by a pH meter.The above compostion medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation.

Now, the same produciton medium for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 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 benzidine 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 contain no chemical mutagens. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of-benzidine in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below:

	$A \times 10^{-x}M$	$5.0 \times 10^{-5}M$	
i. e.,	$1.0 \times 10^{-5}M$	$6.0 \times 10^{-5}M$	Where A = amount of
	$2.0 \times 10^{-5}M$	$7.0 \times 10^{-5}M$	chemical mutagen
	$3.0 \times 10^{-5}M$	$8.0 \times 10^{-5}M$	in ml,
	$4.0 \times 10^{-5}M$	$9.0 \times 10^{-5}M$	i.e., 1.0 ml to 10ml.
		$10.0 \times 10^{-4}M$	x = molarity of the solution containing Chemical mutagen

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 3, 6 and 9 days for lactic acid formed and molasses sugars left unfermented as described in the experimental portion, i. e., chapter II of this thesis. The experimental procedure for the study of influence of other chemical mutagen were exctly the same as described above with the only difference that in place of M/1000 solution of benzidine other chemical mutagen under trials were added to the lactic acid fermentation medium respectively.

Results and Discussion:

The results obtained in the study of the influence of some chemical mutagens on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 are tabulated in the tables from 1 to 4.

Table - 1

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to benzidine

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
Control	3	6.1088532	3.8912463	—
	6	8.1579630	1.8420567	—
(– Mutagen)	9	7.9541847	1.6319838	—
1.0 x 10^{-5} M	3	6.1462275	3.8538720	(+) 0.6118055
	6	8.2852753	1.8141675	(+) 1.5605893
(+Mutagen)	9	8.0433815	1.6148695	(+) 1.1213820
2.0 x 10^{-5} M	3	6.1899791	3.8362261	(+) 1.3280053
	6	8.3784390	1.8098679	(+) 2.7025864
(+Mutagen)	9	8.1293862	1.6081658	(+)2.2026330
3.0 x 10^{-5} M	3	6.2253416	3.8172266	(+)1.9068783
	6	8.4364398	1.7861342	(+) 3.4135580
(+Mutagen)	9	8.1859560	1.5735960	(+) 2.9138284
4.0 x 10^{-5} M	3	6.4276139	3.7638252	(+) 5.2180121
	6	8.7138145	1.6986013	(+) 6.8136065
(+Mutagen)	9	8.4395978	1.5045131	(+) 6.1026128
5.0 x 10^{-5} M	3	6.4693856	3.7281810	(+) 5.9018016
	6	8.8598589	1.6235910	(+) 8.6038132
(+Mutagen)	9	8.5359412	1.4175654	(+)7.3138419
Control	3	6.1088532	3.8912463	—
	6	8.1579630	1.8420567	—
(–Mutagen)	9	7.9541847	1.6319838	—

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
6.0 x 10^{-5} M**	3	6.4948119	3.5051869	(+) 6.3180221
	6	8.9259125***	1.3286950	(+) 9.4134957
(+Mutagen)	9	8.6393446	1.2842671	(+) 8.6138293
7.0 x 10^{-5} M	3	6.4398312	3.5618635	(+) 5.4180054
	6	8.8198581	1.3694263	(+) 8.1134849
(+Mutagen)	9	8.5518485	1.3073741	(+) 7.5138285

Table - 1

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to benzidine

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0 x 10^{-5} M	3	6.3665250	3.6364817	(+) 4.2180060
	6	8.6720246	1.3941366	(+) 6.3013475
(+Mutagen)	9	8.3599582	1.3294110	(+) 5.1013839
9.0 x 10^{-5} M	3	****	–	–
	6	****	–	–
(+Mutagen)	9	****	–	–
10.0 x 10^{-5} M	3	****	–	–
	6	****	–	–
(+Mutagen)	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation ± 2.5 – 3.5%

Table - 2

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 3,3'-dichlorobenzidine

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
Control	3	6.1133671	3.8765326	—
	6	8.2346156	1.7656313	—
(–Mutagen)	9	7.9762581	1.5862016	—
1.0 x 10^{-5} M	3	6.2183945	3.7715049	(+)1.7179959
	6	8.4579612	1.6764853	(+) 2.7122771
(+Mutagen)	9	8.1448594	1.4794268	(+)2.1137894
2.0 x 10^{-5} M	3	6.2428479	3.7434256	(+) 2.1179948
	6	8.5486319	1.6191370	(+)3.8133692
(+Mutagen)	9	8.2246223	1.4272163	(+)3.1137934
3.0 x 10^{-5} M	3	6.3651250	3.6348690	(+) 4.1181544
	6	8.8039142	1.1946537	(+) 6.9134811
(+Mutagen)	9	8.4639198	1.1035361	(+) 6.1139157
4.0 x 10^{-5} M	3	6.4751568	3.5248430	(+) 5.9180103
	6	8.9685928***	1.0314071	(+) 8.9133146
(+Mutagen)	9	8.6162564	1.0259364	(+) 8.0237912
5.0 x 10^{-5} M	3	6.4323620	3.5575624	(+) 5.2179902
	6	8.8368426	1.0398675	(+) 7.3133589
(+Mutagen)	9	8.4958149	1.0298843	(+) 6.5137912
	3	6.1133671	3.8765326	—

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
Control	6	8.2346156	1.7656313	—
(–Mutagen)	9	7.9762581	1.5862016	—
6.0 x 10^{-5} M**	3	6.3956829	3.6043169	(+) 4.6180082
	6	8.1215678	1.2784330	(+) 5.9134782
(+Mutagen)	9	8.3921245	1.2594263	(+) 5.2138031
7.0 x 10^{-5} M	3	6.3396716	3.6385461	(+) 3.7017979
	6	8.6299873	1.3064627	(+) 4.8013376
(+Mutagen)	9	8.3043946	1.2894160	(+) 4.1139152

Table - 2

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 3,3'-dichlorobenzidine

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0 x 10^{-5} M	3	6.2611986	3.7387915	(+) 2.418682
	6	8.5568659	1.3984163	(+) 3.9133617
(+Mutagen)	9	8.2325983	1.3670139	(+) 3.2137901
9.0 x 10^{-5} M	3	****	–	–
	6	****	–	–
(+Mutagen)	9	****	–	–
10.0 x 10^{-5} M	3	****	–	–
	6	****	–	–
(+Mutagen)	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation $\pm$ 2.5 – 3.5%

Table - 3

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 1-methyl-3-nitro-1-nitrogoguanidine

Concentration of AOM used A x 10^{-x} M	Incubation period in hours	Yield of lactic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 3, 6, 9 days of incubation pd.
Control (–Mutagen)	3	6.1241869	3.8658131	—
	6	8.3540970	1.6458714	—
	9	7.9936560	1.4371863	—
1.0 x 10^{-5} M (+Mutagen)	3	6.1619431	3.8281426	(+) 0.6165095
	6	8.4638112	1.5364260	(+) 1.3132981
	9	8.0587053	1.3681056	(+) 0.8137615
2.0 x 10^{-5} M (+Mutagen)	3	6.2355321	3.7643810	(+) 1.8181221
	6	8.6799598	1.3200801	(+) 3.9006346
	9	8.2095958	1.2081634	(+) 2.7013897
3.0 x 10^{-5} M (+Mutagen)	3	6.2845253	3.7154760	(+) 2.6181173
	6	8.8397356	1.1698126	(+) 5.8131788
	9	8.3994679	1.1354929	(+) 4.7013769
4.0 x 10^{-5} M (+Mutagen)	3	6.3753799	3.6260127	(+) 4.1016547
	6	9.0068271	1.1280480	(+) 7.8132932
	9	8.4983764	1.1041457	(+) 6.3140120
	3	6.3953645	3.6046145	(+) 4.4279772
	6	9.0318789	1.1079371	(+) 8.1131677

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
5.0 x 10^{-5} M (+Mutagen)	9	8.5383462	1.0841826	(+) 6.8140310
Control (–Mutagen)	3	6.1241869	3.8658131	—
	6	8.3540970	1.6458714	—
	9	7.9936560	1.4371863	—
6.0 x 10^{-5} M** (+Mutagen)	3	6.5591256	3.4418073	(+) 7.1019827
	6	9.1321390	1.1008136	(+) 9.3132986
	9	8.6822312	1.0183692	(+) 8.6140209
7.0 x 10^{-5} M (+Mutagen)	3	6.6825889	3.3117400	(+) 9.1179777
	6	9.4245315***	1.1006312	(+) 12.8132878
	9	8.8181266	1.0135296	(+) 10.3140615

Table - 3

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 1-methyl-3-nitro-1-nitrogoguanidine

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0 x 10^{-5} M (+Mutagen)	3	6.5233691	3.4765308	(+) 6.5181256
	6	9.2081612	1.1013620	(+) 10.2232976
	9	8.6582395	1.0156311	(+) 8.3138866
9.0 x 10^{-5} M (+Mutagen)	3	6.4376315	3.5623612	(+) 5.1181422
	6	9.0911939	1.1018602	(+) 8.8231786
	9	8.4983583	1.0194756	(+) 6.3137855
10.0 x 10^{-5} M (+Mutagen)	3	****	–	–
	6	****	–	–
	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation ± 2.5 – 3.5%

Table - 4

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to exposed to N-methyl-N-nitrosoethyl carbamate

Concentration of AOM used A x 10^{-x} M	Incubation period in hours	Yield of lactic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 3, 6, 9 days of incubation pd.
Control	3	6.0816379	3.9163692	—
	6	8.1273413	1.8726590	—
(–Mutagen)	9	7.9381629	1.6342065	—
1.0 x 10^{-5} M	3	6.1253194	3.8727269	(+) 0.7182522
	6	8.2259786	1.77402210	(+) 1.2136478
(+Mutagen)	9	8.0097163	1.5418279	(+) 0.9013848
2.0 x 10^{-5} M	3	6.1374736	3.8625259	(+) 0.9181029
	6	8.3062629	1.6937313	(+) 2.2014776
(+Mutagen)	9	8.0424691	1.5303681	(+) 1.3139841
3.0 x 10^{-5} M	3	6.2378983	3.7621054	(+) 2.5693801
	6	8.3829346	1.6169543	(+) 3.1448574
(+Mutagen)	9	8.1694769	1.5084927	(+) 2.9139827

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
4.0 x 10^{-5} M (+Mutagen)	3	6.2399853	3.7501398	(+) 2.6036966
	6	8.4657369***	1.5342529	(+) 4.1636691
	9	8.2399240	1.4794266	(+) 3.8016971
5.0 x 10^{-5} M (+Mutagen)	3	6.2095639	3.8011260	(+) 2.1034793
	6	8.3794898	1.5968261	(+) 3.1024721
	9	8.1535942	1.4998254	(+) 2.7138684
Control (–Mutagen)	3	6.0816379	3.9163692	—
	6	8.1273413	1.8726590	—
	9	7.9381629	1.6342065	—
6.0 x 10^{-5} M** (+Mutagen)	3	6.1923185	3.8076851	+ 1.819943
	6	8.3641352	1.6358639	+ 2.9135468
	9	8.0899886	1.5276195	+ 1.9126049
7.0 x 10^{-5} M (+Mutagen)	3	6.1799562	3.8201398	+ 1.6166417
	6	8.3072438	1.6978501	+ 2.2135467
	9	8.0582453	1.5764261	+ 1.5127227

Table - 4

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to exposed to N-methyl-N-nitrosoethyl carbamate

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0 x 10^{-5} M (+Mutagen)	3	****	–	–
	6	****	–	–
	9	****	–	–
	3	****	–	–

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
9.0 x 10^{-5} M (+Mutagen)	6	****	–	–
	9	****	–	–
10.0 x 10^{-5} M (+Mutagen)	3	****	–	–
	6	****	–	–
	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

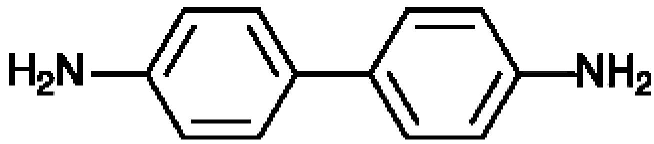
**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation ± 2.5 – 3.5%

DISCUSSION

The data recorded in the table 1 shows that **benzidine** has stimulatory effect on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359



Benzidine

The maximum yield of lactic acid, i.e; 8.9259125g/100 ml in the presence of benzidine was observed at 6.0 x10^{-5}M molar concentration in 9 days of optimum incubation period which is 9.4134957% higher in comparison to control fermentor flasks, ie; 8.1579630/100 ml in the same times course and other same experimental parameters.

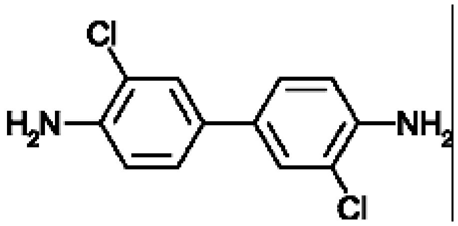
The higher molar concentrations of benzidine were not much favourable for the lactic acid by *Lactobacillus bulgaricus* NCIM-2359. So, the gradual addition of the mutagen benzidine after certain concentrations were not beneficial for the lactic acid fermentation process.

It has been observed that molar concentration of the mutagen, i.e., benzidine from 1.0 x 10^{-5}M to 6.0 x 10^{-5}M enhances the yield of lactic acid to a certain order being 1.5605893%, 2.7025864%, 3.4135580%, 6.8136065%, 8.6038132 and 9.4134957% higher in comparison to control flasks but at 7.0 x 10^{-5}M and 8.0 x10^{-5}M the yield of lactic acid shifted to be 8.1134849% and 6.3013475% higher in comparison to previous concentrations of benzidine taken into experimental trials.

It has been observed further that after optimum concentration, i.e., 6.0 x 10^{-5}M, the addition of the same mutagen to the production medium causes fall in the yield of lactic acid gradually and at 9.0 x 10^{-5}M and 10.0 x 10^{-5}M.; the production of lactic acid has been found insignificant. However, at all the experimental concentrations of mutagen used the yield of lactic acid by submerged fermentation has been found higher in comparison to control fermentor flasks.

The influence of 3,3'-dichlorobenzidine

The data recorded in the table-2 shows that 3,3'-dichlorobenzidine **also** has stimulatory effect on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359



3,3'-dichlorobenzidine

The data (vide table-2) reveals that the chemical mutagen 3,3'-dichlorobenzidine stimulates the lactic acid fermentation process and enhances the yield of lactic acid upto its 3,3'-dichlorobenzidine concentrations from 1.0 x 10^{-5} to 4.0 x 10^{-5} M. The effect of 3,3'-dichlorobenzidine on the productivity (yield) of lactic acid was gradually in increasing order and attains its best role at 4.0 x 10^{-5}M where maximum yield of lactic acid, i.e., 8.9685928 g/100 ml is given in 9 days of optimum incubation period which is 8.9133146 % higher in comparison to control fermentor flask, i.e., 8.2346156 g/100 ml.

In the second phase of mutagenic chemical's effect the molar concentration, i.e., from 5.0 x 10^{-5}M to 10 x 10^{-5}M the production of lacic acid has been enhanced but the order of lactic acid productivity is reverse in respect to increasing molar concentrations of 3,3'-dichlorobenzidine.

However lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 under the influence of each concentration of 3,3'-dichlorobenzidine used has been stimulating and the yield of lactic acid has been found greater than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of lactic acid formed is as below:

Phase- I

Concentration of **3,3'-dichlorobenzidine** from 1.0 x 10^{-5}M to 4.0 x 10^{-5}M.

Productivity of lactic acid

2.7122771%, 3.8133692%, 6.9134811% and 8.9133146%

Phase - II

Concentration of **3,3'-dichlorobenzidine** from 5.0 x 10^{-5} M to 10.0 x 10^{-5}M.

Productivity of lactic acid.

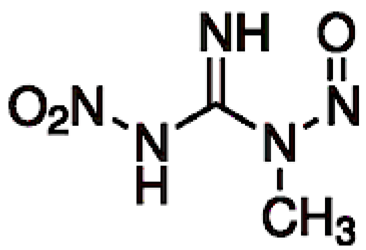
7.3133589%, 5.9134782%, 4.8013376%, 3.9133617%, insignificant and insignificant

Exposure of bacterial strain to 3,3'-dichlorobenzidine may produce a variety of effects. Depending upon the concentration of 3,3'-dichlorobenzidine to which bacterial strain *Lactobacillus bulgaricus* NCIM-2359 were exposed may influence disruption of cells, precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells. Although the special mode of action is not very clear, there is a consensus that the lethal effect is associated with physical damage of the membrane structure of the cell surface, which initiates further deterioration.

Thus, it is concluded that *Lactobacillus bulgaricus* NCIM-2359 at lower concentrations is stimulatory and at higher concentrations is deterioratory for the lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359

The influence of 1-methyl-3-nitro-1-nitrosoguanidine

The data given in the table 3 shows that the mutagen 1-methyl-3-nitro-1-nitrosoguanidine has been found stimulatory for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359. From the data given in the table it is obvious that 1-methyl-3-nitro-1-nitrosoguanidine influences the lactic acid fermentation process in different phases. The main characteristics of the 1-methyl-3-nitro-1-nitrosoguanidine is as follows:



1-methyl-3-nitro-1-nitrosoguanidine

(i) 1-methyl-3-nitro-1-nitrosoguanidine is stimulatory at its all molar concentrations used during course of the lactic acid fermentation, i.e. from 1.0 x10^{-5}M to 10.0 x 10^{-5}M.

(ii) The molar concentration 1.0x10^{-5}M, 2.0 x10^{-5} M and 3.0 x 10^{-5} of 1-methyl-3-nitro-1-nitrosoguanidine influence the yield of lactic acid in a approximately regular doubling order after each state i.e. 1.3132981%, 3.9006346% and 5.8131788%.

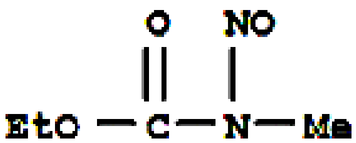
(iii) The molor concentration 4.0 x 10^{-5}M, 5.0 x 10^{-5}M and 6.0 x10^{-5} of 1-methyl-3-nitro-1-nitrosoguanidine now influence the productivity of lactic acid in a regular manner enhancing the yield from X to 1 + X and 2+ X approx. respectively where X is the % increase in the yield of lactic acid in comparison to control. The % increase in the yield of lactic acid at respective molar concentration of 1-methyl-3-nitro-1-nitrosoguanidine has been found to be as follows: 7.8132932%, 8.01131677%, and 9.3132986 (X, 1 + X, and 2 + X)

(iv) The molar concentrations, i.e., 8.0 x 10^{-5}M and 9.0 x 10^{-5} of 1-methyl- 3-nitro-1-nitrosoguanidine influences the yield of lactic acid in decreasing order and therefore, the % difference in the yield of lactic acid has been found to be in the order as mentioned below: 10.2232976%, 8.8231786% and insignificant respectively.

(v) The higher molar concentrations, i.e 10 x 10^{-5}M of 1-methyl-3-nitro-1-nitrosoguanidine decreases the yield of lactic acid and the result is very much insignificant.

The influence of N-methyl-N-nitrosoethyl carbamate

The data recorded in the table-4 shows that the chemical mutagen N-methyl-N-nitrosoethyl carbamate at higher concentration has insignificant effect on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359. The yields of lactic acid obtained at concentration of mutagens i.e. 1.0×10^{-5} to 4.0×10^{-5}M has been found in increasing order and slight better in comparison to control fermenter flasks.



N-methyl-N-nitrosoethyl carbamate

The maximum yield of lactic acid, i.e., 8.4657369 g/100 ml. in the presence of N-methyl-N-nitrosoethyl carbamate, i.e., at 4.0 x 10^{-5} M was found in 6 days of optimum incubation period and this optimum yield has been found to be 4.1636691% higher in comparison to control fermentor flasks, i.e., 8.1273413 g/100ml.

However, at higher concentrations, i.e., 8.0 × 10^{-5} M and onwards of the chemical mutagen, i.e., N-methyl-N-nitrosoethyl carbamate the production of lactic acid was found almost negligible and insignificant. Thus, it is obvious from the results that the chemical mutagen N-methyl-N-nitrosoethyl carbamate under trial at higher concentrations is much detrimental and inhibitory for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359.

Conclusion:

A comparative assessment of the different chemical mutagens on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 an be had from the table-5 given below.

Table - 5

Study of the influence of benzidine; 3,3'-dichlorobenzidine; 1-methyl-3-nitro-1-nitrosoguanidine and N-methyl-N-nitrosoethyl carbamate on lactic acid fermentation by **Lactobacillus *bulgaricus* NCIM-2359** in 6 days of optimum incubation period

Chemical Mutagens used	Optimum concentration of the mutagens used.	Max. yield of lactic acid* in control flasks in g/100ml	Max. yield of lactic acid* in the presence of mutagens	% of lactic acid increase in 6 days of incubation pd.
1	6.0 x 10^{-5}M	8.1579630	8.9259145	(+) 9.4134957
2	4.0 x 10^{-5}M	8.2346156	8.9685928	(+) 8.9133146
3	7.0 x 10^{-5}M	8.3540970	9.4245315	(+)12.813283
4	4.0 x 10^{-5}M	8.1273413	8.4657369	(+) 4.1636691

* Each value represents mean of three observations

(+) Values indicates % increase in the yield of lactic acid.

Experimental deviation ($\pm$) 2.5 to 3.5%.

1. Benzidine

2. 3,3'-dichlorobenzidine

3. 1-methyl-3-nitro-1-nitrosoguanidine

4. N-methyl-N-nitrosoethyl carbamate

Thus, it may be summarised that benzidine; 3,3'-dichlorobenzidine and 1-methyl-3-nitro-1-nitrosoguanidine stimulates and enhances the lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359. It has been observed that 1-methyl-3-nitro-1-nitrosoguanidine has influenced the production of lactic acid significantly to a great extent while benzidine and

3,3'-dichlorobenzidine were approximately equally effective for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 and could increase the lactic acid production nearly in the range of 9.4143957 and 8.9133146 in comparison to control.

However, the N-methyl-N-nitrosoethyl carbamate was found to be inhibitory at higher concentration which deactivates lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359.

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