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Evaluation of Br-MMC as a modulator in lactic acid fermentation mediated by LABs

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Abstract: Br-MMC typically refers to 4-Bromo-7-methoxy coumarin, a halogenated derivative of coumarin. Coumarin can regulate the growth, metabolism, or enzyme activity of LABs. Evaluation of Br-MMC as a modulator in lactic acid fermentation mediated by LABs viz. *Lactobacillus rhamnosus* NCIM-1656, *Lactobacillus casei* NCIM-1692, *L. helveticus* NCIM-1661 and *L. pentosus* NCIM-1662 has been assessed. It has been found that the LABs strain of *Lactobacillus casei* NCIM-1962 was much effective and good lactic acid producer. The compound Br-MMC was found as modulator when 25% molasses solution (w/v) was allowed to ferment at pH 5.9, temperature 38^{0}C, and optimum incubation period of 7 days. It has been found that there is a gradual increase in the production of lactic acid with steping of the compound Br- MMC till the maximum yield of lactic acid i.e., 9.925 g/100 ml was obtained at its molar concentration of 5.0×10^{-5}M which is 9.006% higher is comparison to control.

(Keywords : LAF, LABs, Br-MMC, *L. casei* NCIM-1692).

Introduction

The relationship between coumarins and lactic acid fermentation (LAF) technology is emerging as an interesting area of research, coumarins are a group of naturally occurring phenolic compounds found in plants. They are known for antimicrobial, antioxidant, enzyme inhibitory and bioactive properties[1-13]. Certain coumarins can inhibit or modulate microbial growth, depending on their concentration and structure. Coumarins may selectively inhibit spoilage organism while enhancing LABs Viability some coumrins[14-18] dervatives may act as mild inducer are stress agents, potentially boosting metabolite production. High concentrations of coumarins can be toxic to LABs.

The influence of coumarins in fermentation technology especially lactic acid fermentation has been not studied extensively. The compound Br-MMC is one of the few coumarin which has been found insignificant and left effective in lactic acid fermentation technology. The present study was undertaken for the evaluation of Br. MMC as a modulator in lactic acid fermentation mediated by *Lactobacillus casei* NCIM-1692.

Experimental

The influence of Br. MMC on production of lactic acid by *Lactobacillus casei* NCIM - 1692.

The composition of the production medium for the production of lactic acid by *Lactobacillus casei* NCIM - 1692 was prepared as follows :

Molasses : 25% (w/v) , Malt Extract:1.42% Yeast Extract :1.42%, Peptone:1.42%, $(NH_4)_2HPO_4$: 1.42%, $CaCO_3$: 9.50 %, pH: 5.9, Distilled water: To make up 100 ml.

The pH of the medium was adjusted to 5.9 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. Now, the same produciton medium for was prepared for production of lactic acid in 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 Br. MMC 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 coumarin. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of Br. MMC in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x}M to 10.0x10^{-4}M ,

Where

A = amount of coumarin in ml, i.e., 1.0 ml to 10ml.

X = molarity of the solution containing coumarin

The fermentor flasks were then sterilized, cooled, inoculated, incubated and

Table - 1
Evaluation of Br-MMC as a modulator in lactic acid fermentation mediate by LABs

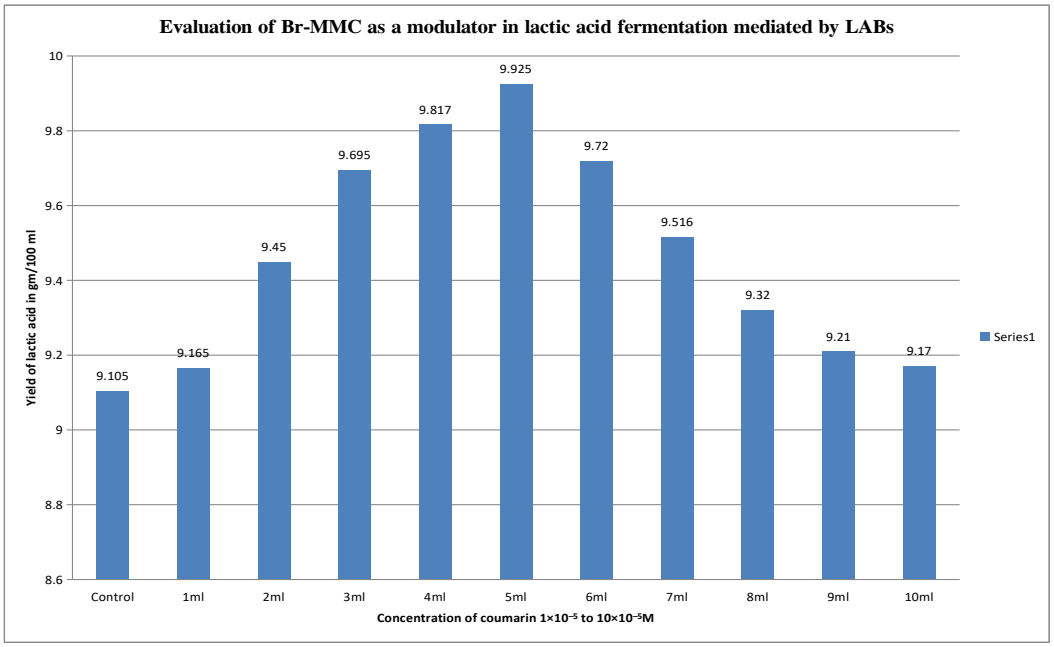
Concentation of coumarin used a x 10^{-x} M	Incubation period in hours	Yield of lactic acid in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 7 days of incubation pd.
Control	3	5.975	4.286	—
(– Coumarin)	7	9.105	1.159	—
	10	6.716	1.113	—
1.0 x 10^{-5} M	3	6.016	4.269	—
(+ Coumarin)	7	9.165	1.133	(+) 0.658
	10	6.756	1.115	—
2.0 x 10^{-5} M	3	6.196	4.163	—
(+ Coumarin)	7	9.450	1.118	(+) 3.789
	10	6.964	1.093	—
3.0 x 10^{-5} M	3	6.356	3.918	—
(+ Coumarin)	7	9.695	1.086	(+) 6.479
	10	7.145	1.050	—
4.0 x 10^{-5} M	3	6.441	3.820	—
(+ Coumarin)	7	9.817	1.059	(+) 7.819
	10	7.239	1.030	—
5.0 x 10^{-5} M**	3	6.512	3.749	—
(+ Coumarin)	7	9.925***	1.030	(+) 9.006
	10	7.320	1.016	—

Contd.....

Table - 1
Evaluation of Br-MMC as a modulator in lactic acid fermentation mediate by LABs

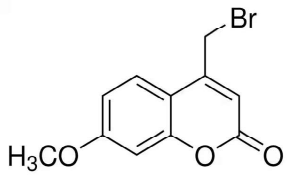
Concentation of coumarin used a x 10^{-x} M	Incubation period in hours	Yield of lactic acid in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 7 days of incubation pd.
6.0×10^{-5} M	3	6.375	3.889	—
(+ Coumarin)	7	9.720	1.188	(+) 6.754
	10	7.165	1.163	—
7.0×10^{-5} M	3	6.243	4.116	—
(+ Coumarin)	7	9.516	1.199	(+) 4.514
	10	7.018	1.80	—
8.0×10^{-5} M	3	6.112	4.159	—
(+ Coumarin)	7	9.320	2.085	(+) 2.361
	10	6.870	2.039	—
9.0×10^{-5} M	3	6.040	4.220	—
(+ Coumarin)	7	9.210	2.188	(+) 1.153
	10	6.789	2.139	—
10.0×10^{-5} M	3	6.016	4.266	—
(+ Coumarin)	7	9.170	2.215	(+) 0.713
	10	6.763	2.190	—

* Each value represents mean of three trials. ** Optimum concentration of coumarin
*** Optimum yield of lactic acid (+) Values indicate % increase in the yield of lactic acid
Experimental deviation $\pm$ 2.5 – 3.5%



analysed after 3, 7 and 10 days for lactic acid formed[19] and molasses sugars[20] left unfermented.

Results and Discussion



4-bromomethyl-7-methoxycoumarin

The data recorded in the table-1 shows that 4-bromomethyl-7-methoxycoumarin was found to be increasing up to its molar concentration from 1.0×10^{-5} M to 5.0×10^{-5} M. It has been observed that gradual addition of coumarin, i.e., 4-bromomethyl-7-methoxy - coumarin to the fermentation medium gradually increases the production of lactic acid by *Lactobacillus casei* NCIM - 1692.

It has been observed that higher concentrations of 4-bromomethyl-7-methoxycoumarin, i.e.; on 5.0×10^{-5}M and onwards has retarded the production of lactic acid by *Lactobacillus casei* NCIM - 1692.

The maximum yield of lactic acid has been recorded at 5.0×10^{-5} M concentration of coumarin 4-bromomethyl-7-methoxycoumarin, i.e., 9.925g/100 ml in 7 days of optimum incubation period which is 9.006% higher in comparison to the control fermentor flasks, i.e.,9.105g/100 ml in the same set of experimental parameters for production of lactic acid by *Lactobacillus casei* NCIM - 1692

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