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PRODUCTION OF LACTIC ACID FROM MILK WHEY BY *LACTOBACILLUS ACIDOPHILUS*

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Growth medium of milk whey for the production of lactic acid by *Lactobacillus acidophilus* was developed and different conditions were optimized for lactic acid production. Maximum yield of lactic acid (4.4 %) was recovered from shake medium in the presence 01'0.1 % NaCI. 0.1 % $MgS0_4.7Hp$. 0.1 % $NH_4H_2P0_4$. 2% molasses. 0.1 % yeast extract and 2.0% corn steep liquor after 48 hours incubation at 35 C and pH 5.5 on orbital shaker.

Key words: lactic acid, milk whey

INTRODUCTION

A great deal of effort is being made by biotechnologists for utilizing agro-industrial wastes for fermentative production of various metabolires of commercial value. Crude lactic acid is used as raw material for preparing esters such as methyl. ethyl and n-butyl lactates which are useful solvents and modifiers in food industry. Lactate is also used in animal feed and pharmaceuticals as a remedy for gastric problems and in chemical industries as an acidulant. In leather industry lactic acid is used for soaking and decalcification of hides and in the textile industry as an aid for dying and priming. It is also an auxiliary material in the plastic industry (Anonymous. 1952).

Lactobacillus acidophilus acts as an antagonist to other microbes due to its acidic environment and production of certain toxins and hence is a useful organism for the production of pure grade lactic acid (Garret, 1930). It finds considerable use in food and agriculture for preservation and improvement of t1avour and texture of foods (Ehteshamuddin and Baig, 1991).

Whey from dairy industry although low in sugar content constitutes an economical raw material for lactic acid production (Anonymous. 1952). Different experiments were therefore planned for optimization of fermentation period. concentration of NaCI, $MgS0_4.7H_2O$. $NH_4H_2P0_4$. molasses (cane). yeast extract and corn steep liquor for maximum production of lactic acid by *Lactobacillus acidophilus.*

MATERIALS AND METHODS

Substrate: Milk whey obtained from a big sweet shop in Faisalabad city was stored in a freezer and used as substrate in different experiments.

Growth Medium: *Lactobacillus acidophilus* procured from the Department of Vet. Microbiology, University of Agriculture, Faisalabad was maintained on growth medium of milk whey (Table I) at 35C and pH 5.5 (Hanson and Mocquot , 1970).

Inoculum Medium: The spores of bacterium from growth medium were transferred aseptically to an autoclaved 500 ml 11ask containing 200 ml inoculum medium of milk whey (Table I). pH of inoculum medium was adjusted at 5.5 and the flask was incubated for 72 hours at 35 C.

Culture Cultivation: The Erlenmeyer f1asks (500 ml) containing 100 m1 of milk whey were plugged with cotton and autoclaved at 15 lb/irr' pressure for 30 minutes. pH of sterilized medium was adjusted aseptically at 5.5 with N NaOH or N HC\. Spore inocula (l %) were added aseptically to each flask with the help of a sterilized pipette. The f1asks were incubated on orbital shaker working at a rate of 100-150 rpm at 35C. Duplicate f1asks were harvested by steaming for 5 minutes and the fermented mash was then analysed for lactic acid content.

Optimum Conditions: The growth media of milk whey for lactic acid production were developed and various conditions such as fermentation period (by fermenting for 24, 48 and 72 hours), inorganic ion concentrations like NaCI, $MgS0_4.7Hp$, $NH_4H_2P0_4$,and molasses (cane), yeast extract and corn steep liquor concentrations (using different concentrations in each case) were optimized for the maximum production of lactic acid.

Analytical Methods: Lactic acid in the fermented mash was estimated by the procedure of Clerk (1958) and data thus collected were analysed using analysis of variance technique (Steel and Torrie, 1980).'

RESULTS AND DISCUSSION

Optimum Conditions: The results of different experiments regarding optimization of different conditions for lactic acid production have been presented in Table 2.

Fermentation Period: Three different time periods (24. 48 and 72 hours) were tested for maximum lactic acid production. It was observed that maximum lactic acid (0.78 %) was recovered from the medium containing only milk whey after 48 hours. Fermentation in the subsequent experiments was therefore carried out for 48 hours.

NaCl: Addition of 0.01,, 0.05. 0.1 and 0.15% NaCI in the medium yielded 0.95. 0.96. 0.99 and 0.92% lactic acid respectively after 48 hours. The medium containing 0.1 %

Table I. Percentage composition of growth and inoculum medium

Materials	Growth medium (per lOO ml)	Inoculum medium (per :200 ml)
Milk whey unl)	80.00	I SO.0
'\-ta,1 extract. Ig)	:2.0	40
NaCI (g)	05	OA
Nll)IJ·().lg)	05	04
MgSO,.7I-LO (gl	0.5	0.4
Agar (g)	200	-

Table 2. le ttcc ı "J tcrmcut.uion period. NaCI. MgSO,. 711,O.NII,I1.l'O, . 11lolaSSl'S. ye~ISI extract. · ami corn sleep liquor 01'1 till' pioduc non ot Iacuc aCid trom milk whcv hy *l.t'l{(l/!{/c;/Al/l* .. *lIll//O/Jll;/l/.l* at:;5 *C* :uul pl I 5.5

l'IIl'II- men:	l-crmcut.ruou pel'l<ld	NaCl	MgSO,.711,O	NII,II.I'O,	Molasses	Yeast cxuac:	Corn 'ICCp liquor	l.acuc ,'IJU alter -'IX hours
l	2-l Il	-	-	-	-	-	-	0.67
	='IX l,l	-	-	-	-	-	-	0.78
	7'1_ 1l	-	-	-	-	-	-	IUS
2	='181l,	o.ut	-	-	-	-	-	0.'15
		0.05	-	-	-	-	-	0.%
		0.10	-	-	-	-	-	0.'l'l
		015	-	-	-	-	-	092
3	='IX l,l	O. I	0.0 I	-	-	-	-	US
		0.1	U.05	-	-	-	-	1.6:;
		0.1	O. 10	-	-	-	-	1.7-'l
		U.I	U.15	-	-	-	-	1.6lJ
4	='181l,	U.I	0.1	0.01	-	-	-	1.8-'l
		O. I	0.1	OU5	-	-	-	1.%
		U. I	(l.!	U. 10	-	-	-	1.'18
		U.I	0.1	0 .)5		-	-	1.70
5	='IX l,l	U. l	Ul.	U. l	[0	-	-	2.85
		0. l	0.1	U. I	2.0	-	-	, . 10
		11. l	Ul	01	:;.0	-	-	2.86
		U. l	U.I	U. I	='I.U	-	-	2.h7
6	='IX l,l	01	Ul	l) . I	2.0	IU) I	-	:;. ()O
		0.1	0.1	0.1	2.0	0.0S	-	_,.10
		U . I	0.1	0.1	2.0	0.10	-	3.:;lJ
		ll.!	0.1	01	2.0	O. 15	-	:;.26
7	=IX l,l	U . l	0.1	0.1	2.0	0.1	0.5	:;5U
		U. l	Ul.	ill	2U	ll.!	1.0	J.70
		U. l	U. I	il.1	2.0	O. I	J)5	.1'10
		il. l	ill	ill	20	U. I	2.11	.UO

N;ICI yieldl:d nn\III'HII1I ı lactic acid ~11II1 it was considered as the optimum level.

'IgS( )4' 7H!() : An addition of 0 . I 'X ivlgSO,. 7H.0 along with 0.1 '1 NaC! ga\e the maxiuuun \'ll'ld 1I . 74',:) of lactic acid ami was consi(!ercd ~IS its opunuuu conccnrrarion. Aksu and Kutsal (jl)Sh) observed an increase ııı lactic acid production by *LW/O/)({lil/ll.l* *:leitnukii* with the addition of MgSO,.7HJ)

with other nutrients using molasses as a substrate.

NH.HzPO.: Along with the optimum levels of NaCI and MgSO".7H,O. the addition of 0.01 %.0.05%.0.1 % and 015% NH,H,PO. gave 1,84.1.96.1.98 and 1.70% lactic ac Id after 48 hours respectively. The maximum production ol lactic acid (1.98 '7,)) was noted in the presence of 0.1 % NH"H.PO" along with other nutrients. Gamal and Gamal (1984)' observed that *Lactobacillus casei* RA. 9867 showed maximum lactic acid production (3.67 %) after incubation for 6 days in the presence of 0.1 % (NH)~HPO. along with other nutrients.

Molasses: Fermentation medium with the optimum levels of NaCl. MgSO•.7H,O and NH.H,PO". yielded 2.85. 3.10. 286 and *2.67'Yt* lactic acid respectively hy adding 1.2.3 and 4 'l, molasses after 48 hours. Addition of molasses resulted in a significant increase in lactic acid production and 2% molasses gave the maximum yield (3.10%). El-Sherbiny *et* {(I(1986) produced lactic acid from molasses using *Lactobacillus delbrukii* and reported the highest yield (626%) at 45 C and pH 5.0 after 8 days.

Yeast Extract: Addition of 0.01.0.05.0.1 and 0.15% yeast extract. along with optimum concentration of NaCI, MgSO,.7Hp. NH.H:PO, and molasses yielded 3.00. 3.10. 3.39 and 3.26% lactic acid respectively. The maximum lactic acid (3.39%) was recorded in the presence 01'0.1% yeast extract. Gamal and Gamal (1984) used molasses and cheese whey as carbon source for lactic acid bacteria and observed that addition of 0.5 % yeast extract caused a significant increase in lactic acid yield.

Corn Steep Liquor: In the last experiment. milk whey was fermented with 0.5, 1.0. 1.5 and 2.0% corn steep liquor along with the optimum NaCI (0.1 %). MgSO•.7H,O (0.1 %). NH,H,PO" (0.1 %). molasses (2%), and yeast extract (0.1 %) levels. Maximum lactic acid (4.4 %) was recovered with 2.0% supplementation of corn steep liquor and it caused a significantly higher lactic acid production. Martinez *et al.* (1988) produced lactic acid from molasses. sugar cane juices. corn Sleep liquor and corn cooking water using *Lactobacillus delbrukii* and observed the highest lactic acid conversion using corn steep liquor with volumetric productivity of 2.78 g/l/hour.

Conclusion: The final yield of lactic acid was 4.4% when milk whey was fermented for 48 hours at 35 C and pH 55 in the presence of 0.1 % NaCl.. 0.1 % MgSO".7H,O. 0.1 % NH"H~PO", 2% molasses, 0.1 'X, yeast extract and 2% corn steep liquor. Microbial lactic acid production leads to an economical utilization of milk whey for a valuable industrial product..

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