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PRODUCTION OF LACTIC ACID BY *Lactobacillus plantarum* IN SEMI SOLID STATE FERMENTATION USING SOME RAW STARCHY MATERIALS.

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ABSTRACT

Lactobacillus plantarum was selected to ferment raw starchy materials of 'rice bran ,wheat bran and potatoes peel " to produce lactic acid and calcium lactate in semi-solid state fermentation.

The noncooked starchy substrates were suspended in diluted solution of sulfuric acid over night not only for the sterilization of the substrate,but also to help the evolution of carbon dioxid upon addition of calcium carbonate(0.5%) .,followed by inoculation of the bacteria($10x10^9$ CFU/10ml).

The selected organism produced lactic acid ranged from 0.49 to 0.75 g/L and the yield of lactate ranged from 6 2 to 78 g /L of substrate.Maximum starch conversion to lactic acid was obtained after 120 h.at pH 6.5.

The addition of small amount of glucoamylase to the fermentation mixture resulted in a significant shortening of the fermentation process by *Lactobacillus plantarum* cultivated at 37 C,without any other additives.

INTRODUCTION

Production of lactic acid by fermentation is an old industrial process (Vick Roy,1985),that has wide range of applications,principally in the dairy and food industries (Aguilar,1991,Buckenhuskes,1993,and Stiles,1996)where the pure L(+)lactic isomer is used in human consumables to avoid health problems (Lipinsky ,1981).

Major use of lactic acid (account to 85% of demand) is still in food and food related applications (Datta et al,1993). Lactic acid and its salts can increase the shelf life of food products by 30 to 50% by inhibiting the growth of food spoilage organisms (Litchfield,1996), because of its ability to produce the wide rang of antimicrobial substances which efficiently contribute in the preservation of the fermentation products (Piard and Desmazeaud, 1992).

Calcium salt is a good dough conditioner, calcium lactate is added to diet drinks and other spirits as mineral application.

In semi solid state fermentation process, the insoluble solid substrate is a solid porous matrix, which absorbs water with a relatively high water activity and also contains available carbohydrates and mineral nutrients.The attraction of this type of culturing comes from its similarity to the natural way of life for many microorganisms (Couto *et al* .2001),and usage of starchy agricultural wastes makes the whole process more economical.It would be attractive to use organisms growing well on raw starch,the present report describes the use of *Lactobacillus plantarum*,an amylolytic organism , for the production of L(+)lactic acid from starch using rice bran ,wheat bran and potatoes as an insoluble solid substrates.

According to the available literature there are no reports on the production of L(+)lactic acid through semi solid fermentations .There for, this study aimed to optimize the conditions namely ,temperature ,concentration of inoculum and other factors which may affect this process to produce lactic acid by batch fermentation using *Lactobacillus plantarum*

MATERIALS AND METHODS

Microorganisms and inoculum preparation

Three homofermentative lactic acid bacterial strains of genus *Lactobacillus* : *Lactobacillus amylophilus* ATCC 19486 *Lactobacillus lactis* ATCC19433 and *Lactobacillus plantarum 10102* (American Type Culture CollectionUSA), were screened for their efficiency for the production of lactic acid .Bacteria were maitained in MRS broth with glycerol at -20 °C,The bacterial strains were plated on MRS agar and incubated at 30°C for 24h. Each single colony was transferred to a new plate and incubated in the same way.The bacteria were maintained in the same medium and subcultured every 30 days. Inoculum size 5% of about 109 cells /ml was obtained by growing the culture containing 1%soluble starch at pH about 6.5 The inoculum was not centrifuged.

Media composition

Prior to this study ,preliminary experiments were carried out to determine the starch contents in the insoluble substrates in order to use equal starch content. Total starch content was determined according to Naveena,2004 . Wheat bran starch content was found to be 44.4% ,rice bran was 46.9% and potatoes peel 52%.We used starch content ranging from 10 to 12 % .

Befor the inculation ,the medium with insoluble substrates were sterilized by autoclaving at 121°C for 15 min.MRS broth medium containing (g/L):peptone 10,yeast extract 5.0, sodium acetate 5.0, tri-ammonium citrate 2.0,NaH2PO4.2H2 2.0, MgSO4. 7H2O 0.1 ,MnSO4.4H20 0.05 and 1ml of Tween 80,corn steep liquor 3.5% at pH 6.5.(Hujanen and linko,1996).The shaking speed 150rev /min.

Batch cultures were conducted aerobically at 30°C in 250 ml Erlenmeyer flasks with 100 ml of medium .Fermentation at regulated pH was maintained at 6.5 with 5 NaOH .Inoculation at 5%(v/v) was performed with a 20h pre-culture in the same medium.

A different temperatures were used at 30.35 ,37°C and 40 C,at pH value 6.5.When potatoes peel were used for the fermentation,during the sterilization they were treated by acid phosphatase ,because the phosphates combined with glucose residues of potato starch inhibit the activity of a-amylase produced by *Lactobacillus plantarum.*

The noncooked starchy substrates were suspended in dilute solution of sulfuric acid (pH 1.5—2.0)over night not only for sterilization of substrate,but also for causing evolution of carbon dioxid upon addition of calcium carbonate(0.5%).

The cultivation conditions and medium were the same for all tested strains.

Estimations:

Lactic acid produced after fermintation was extracted into the 50ml medium, in which solid substrates were dispensed by squeezing the fermented substrate using cheesecloth.The extracts were centrifuged (8000 rpm for 20 min) and the supernatant was taken to estimate lactic acid according to modified method by Taylor ,(1996.)

Total starch content was determined colorimetrically at 620 nm by adding 0.1ml of sample to 2.4 ml of an iodine solution containing 1ml distilled water (starch solution diluted to 4%).(Giraud *et al* 1994).

Calculated parametares

$$\text{Productivity/L/h} = \frac{\text{lactic acid concentration (g/L)}}{\text{Fermentation (h)}}$$

RESULTS

Three homofermentative strains were screened for lactic acid production at 37°C.In this screening the best lactic acid producing strain was *Lactobacillus plantarum* with 100% yield ,also the strain *Lactobacillus amylophilus* yield 95% *Lactobacillus lactis* yield 87% showed lactic acid potential at 37°C. and *Lactobacillus plantarum* was selected for further research because of their excllent lactic acid production potential.

Effect of temperature:

Lactobacillus plantarum strain was adapted to four different temperatures during five culture generation. Fig 1 shows the time course of the production of lactic acid ,it can be seen,the maximum acid production by *Lactobacillus plantarum* was at 37C,which the highest lactic acid concentrations were 77 g/L, 72g/L/h. ,and 67 g/L/ for starch substrates (rice bran ,wheat bran and potatoes) ,respectively.

Table (1): Effect of different temperatures on lactic acid production (as lactate,g/L) by *Lactobacillus plantarum* grown on (a) Rice bran, (b)Wheate bran and (c)Potatoes media.

(a)

Fermentation time (h)	Fermentation Temperature °C			
	30C	35C	37C	40C
20	5	21	22	11
40	12	33	32	15
60	25	54	40	33
80	28	59	55	41
100	30	62	73	49
120	31	71	75	55
140	31	71	71	51

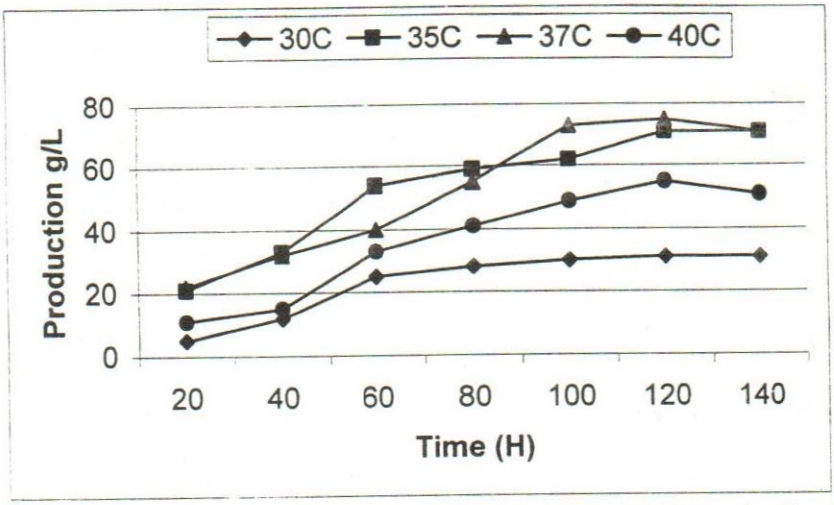

Fig. (1): Effect of different temperatures on lactic acid production(as lactate,g/L) by *Lactobacillus plantarum* grown on rice bran.

(b)

Fermentation time (h)	Fermentation Temperature °C			
	30 C	35C	37C	40C
20	11	21	25	5
40	12	33	32	15
60	25	44	36	33
80	28	55	51	41
100	30	60	73	45
120	30	69	72	51
140	30	71	71	51

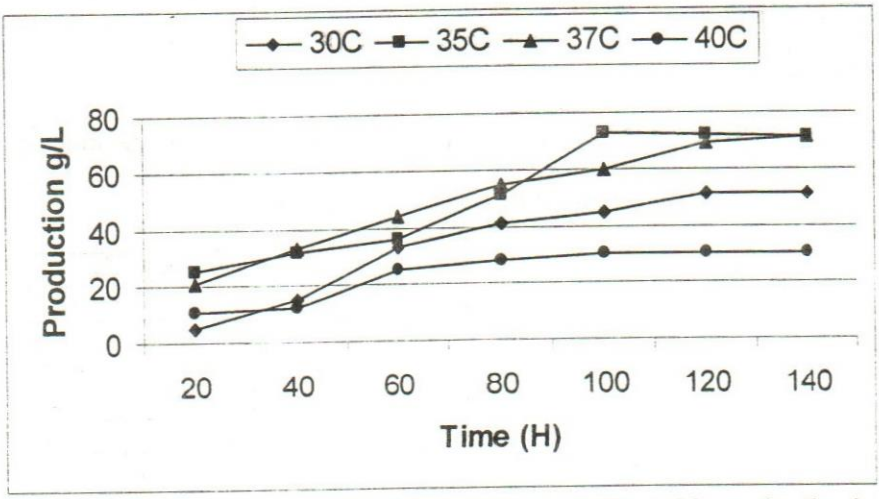

Fig. (2):Effect of different temperatures on lactic acid production(as lactate,g/L) by *Lactobacillus plantarum* grown on wheat bran.

(c)

Fermentation time (h)	Fermentation Temperature °C			
	30 C	35C	37C	40C
20	11	18	15	5
40	12	33	25	15
60	25	41	36	23
80	28	55	51	31
100	30	60	61	42
120	30	60	62	50
140	32	61	60	50

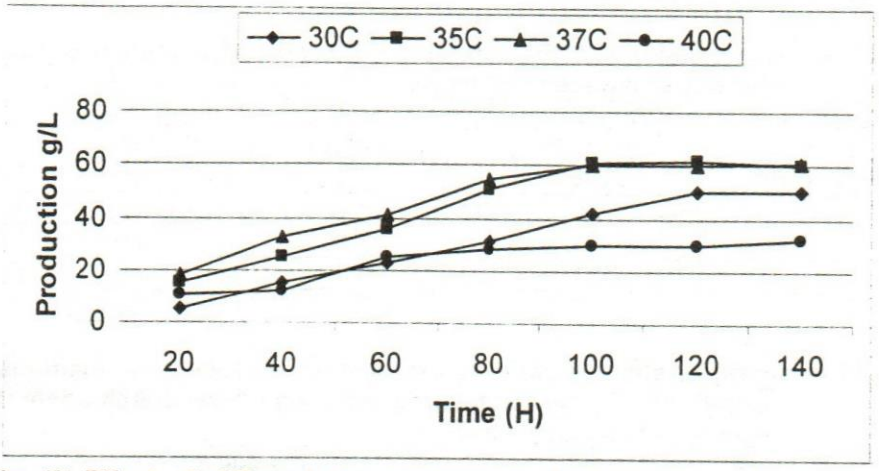

Fig. (3):Effect of different temperatures on lactic acid production(as lactate,g/L) by *Lactobacillus plantarum* grown on potatoes media.

The influence of different temperatures on the fermentation process was estimated,it was established that the optimal temperature for lactic acid production and the maximum rate of lactate production was obtained at 37C These results were confirmed with those obtained by Hujanen and Linko (1996)and Naveena *et al*(2004).

To obtaine the optimal inoculum size of *Lactobacillus plantarum* for lactic acid fermentation with various initial inoculum concentrations were added to the culture media containing equal starch content (Table2).(Initial concentration of starch in the fermentation media was 10%) Results showed that the ratio of 5- 10% of inoculate was to be more economically preferable.

It is necessary to mention that only about seventy percent of starch was consumed during the fermentation (Table 3)This may caused due to the specificity of amylolytic activity of *Lactobacillus plantarum* ,its amylases action belongs to the type of saccharogenic α-amylases.This type of enzymes provides a higher reducing ability,than liquefying or dextrinizing amylases .By hydrolyzing a-1,4-glucoside bonds of starch. It first these amylases produce simple and branched sugars as end products from the fermantation process

of oligosaccarides .Secondly this type of amylases can hydrolyze maltotriose molecule into glucose and maltose.Thus it can be predicted,that the remainig non converted sugars are represented by branched olygosaccharides,which cannot be hydrolyzed by α -amylases of of *Lactobacillus plantarum.*

The investigations were carried out to solve the problems of intensificatin of lactate fermentation and to increase the starch conversion rate.

To enhance the saccharification of substrates starch to achieve complete conversion into lactat to solve this problem using commercial preparation of glucoamylase ,this enzyme is capable to hydrolyse glucoside bond yielding glucose as end product .

Table 2 :The influence of the amount of introduced inoculum on the intensity of lactate production (g/L).

Inoculate amount % (v/v)	Rice bran medium g/L	Wheat bran medium g/L	Potatoes medium g/L
3	34	31	24
5	63	44	46
10	71	68	66
15	73	71	68

Table 3 :characteristics of lactic acid fermentation processes of various substrates by *Lactobacillus plantarum* (intial starch content in the medium was 10%)

Characteristics	Starchy substrates		
	Rice bran	Wheat bran	Potatoes beels
Yield of lactate g/L	75	72	62
Fermentation period ,hrs	115	120	125
Productivity/L	0.7	0.6	0.49
Concentration of remaining sugars g/L	23	27	33
Conversion coefficient g/g	0.75	0.72	0.62

Glucosamylase preparation in a ratio of 0.05% towards starch content and calcium carbonate were added to different media content 10-12% starch,

The results of this experiment are presented in the Table 4.The addition of glucoamylase up to 0.05 % to starch substrate (Rice bran ,as a substrate sample),is valuably decreasing the fermentation period,the addition of more amount of glucoamylase did not result in further acceleration.

The addition of glucoamylase reduced the fermentation period for about 30 -40% by using 0.05% enzyme and 10-12 % srarch ,as a result increasing the efficincy of the processes ,as well promoting complete conversion of starch and decrease of the content of remaining sugars in the culture .

Finally, it could be concluded that the lactate formation process can be achieved by growing *Lactobacillus plantarum* on these starchy substrate successfully

Table 4: The influence of addition of glucoamylase (Rhizopus glucoamylase)on the lactic acid fermentation of rice bran using *Lactobacillus plantarum.*

Intial concentration of starch %	Glucoamylase added to media Mg/g of starch	Fermentation period without glucoamylase,hrs	Fermentation period with glucoamylase,hrs
8	0.02	134	100
8	0.04	120	88
8	0.06	115	85
10	0.02	140	100
10	0.04	124	88
10	0.06	115	80
12	0.02	140	100
12	0.04	116	71
12	0.06	103	60

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إنتاج حامض اللاكتيك بواسطة لاكتوباسيللس بلانتارم في تخمر نصف صلب باستعمال بعض المواد النشوية الخام.

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تم اختيار لاكتوباسيللس بلانتارم لتخمير المواد النشوية وهي ردة الأرز و ردة القمح و قشور البطاطس وذلك لإنتاج حامض اللاكتيك و لاكتات الكالسيوم في تخمر نصف صلب.

تم تعليق المواد النشوية غير المطبوخة في محلول مخفف من حامض كبريتيك تراوحت قيمة ال pH له من١,٥-٢ وذلك طوال الليل وذلك لتعقيم المواد المستعملة وكذلك لإطلاق ثاني أكسيد الكربون عند إضافة كربونات الكالسيوم (٠,٥%) وتم عقب ذلك إضافة اللقاح البكتيري (١٠x١٠^{٩} وحدة خلوية مكونة للمستعمرات لكل ١٠٠ ملي) .وقد قامت السلالة المستخدمة بإنتاج حامض اللاكتيك بمعدل يتراوح من ٠,٤٩إلي٠,٧٥ جرام /لتر بينما تراوحت كمية اللاكتات ما بين ٦٢-٧٨ جرام /لتر. وقد بلغ أقصي معدل تحول للنشا إلي حامض اللاكتيك في فترة زمنية بلغت ١٢٠ ساعة وذلك تحت قيمة pH مقدراها ٦,٥ .

وقد أدت إضافة كميات صغيرة من الجلوكوأميليز في خليط التخمير إلي تخفيض جوهري في زمن عملية التخمر لسلالة لاكتوباسيللس بلانتارم علي درجة حرارة ٣٧°م دون إضافات أخري.