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PRODUCTION POTENTIAL OF ACETIC AND LACTIC ACID OF PROBIOTIC *Bifidobacterium lactis*

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Abstract: *Bifidobacterium lactis* was grown in laboratory to observe the acetic and lactic acid production by the cells. Cells were grown in the laboratory in MRS-IM media where carbohydrate sources were varied. This media was defined by the Christian Hansen, Denmark, who provided the freeze-dried cell sample. A one-liter glass fermenter was used to grow the organisms. Three different carbohydrates source viz. 2% (w/v) glucose, 2% (w/v) glucose + 2% (v/v) soymilk, and 2% (w/v) fructooligosaccharides (FOS) were used. After preparing the product, samples were analyzed in Shimadzu gas chromatograph, where acetic and lactic acid concentration was measured.

Keywords: Bifidobacteria, probiotic, prebiotic, fructooligosaccharides.

Introduction

The beneficial microorganisms that inhabit in human intestine are known as probiotics. These include lactic acid excretors as lactobacilli and bifidobacteria (Zimmer and Gibson, 1998). Bifidobacteria are beneficial lactic acid bacteria that constirutes 25% of the normal gut flora (Mitsuoka,1984; Rowland, 1995 and Scardovi, 1986). The species *Bifidobacterium lactis* has recently been developed (Miele *et al*, 1997) and is now being used in some yogurt production and other commercial purposes (Zimmer and Gibson, 1998). One of the major advantages of the probiotics is to destroy the pathogens in gut (Macfarlane and Gibson, 1998). It has been known that several food materials selectively help to grow the probiotics in the intestine, which are known as prebiotics. Different tuypes of fibrous materials and oligosaccharides are the best known prebiotics (Gibson *et al*., 1996). Using these prebiotics the growth increases with the production of of acids. As glucose is common substrate for all bacteria, FOS that is already proved as prebiotic, soymilk was found to be useful for this organism to grow (Babar, 2001).

Acetic acid and L (+) lactic acid are produced in their metabolic pathway, but no CO_2 is formed. Small amounts of formic acid, ethanol and succinic acid are also produced. There is no production of butyric and propionic acid. Glucose is degraded by the fructose-6-phosphate shunt. In this process fructose-6-phosphoketolase cleaves fructose-6-phosphaate in to acetylphosphate and erythrose-4-phosphate. Through the sequential action of transaldolase, transketolase, xylulose-5-phosphate phosphoketolase, and enzymes of EMP acting on glyceraldehyde-3-phosphate, end products are formed. Except *Bifidobacterium indicum* and *Bifidobacterium asteroids,* all other strains are catalase negative. These two are catalase positive when grown in presence of air with or without added hemin. Among the species, fermentative characteristics vary because of utilization of different kind of carbohydrate sources (Scardovi, 1986).

The present investigation was carried out to show the production of acetic and lactic acid by *Bifidobacterium lactis* with respect to utilization of different substrates as glucose, FOS, soymilk etc.

Materials and Methods

The experiment was conducted in the Bioprocess Technology laboratory of Asian Institute of Technology (AIT), Thailand. Freeze-dried *Bifidobacterium lactis* (Bb-12), which was obtained from Christian Hansen, Denmark through East Asiatic Company, Thailand; was used throughout the investigation.

***Cell Growth*:** Freeze-dried cells were grown in MRS-IM agar plate to isolate the colony in anaerobic jar at 37^{O}C. Distinguished colonies were stored into MRS-IM agar stab and kept in refrigerator for further use. Cells from Agar stab were stepwise cultured in 5 ml test tube, 50 ml flask and then 1000 ml bioreactor. The media was cultured in anaerobic condition and temperature was maintained 37^{O}C. From the bioreactor, samples were collected in centrifuge tubes at every four-hour intervals. Cell density was measured in UV-vis

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spectrophotometer using 600 nano-meter wavelength. These samples were then prepared for gas chromatographic (GC) analysis.

***GC Analysis*:** Samples were centrifuged at 6000 rpm for 10 minutes and supernatant was collected. One ml of the supernatant was taken in a microcentrifuge tube and 60 µl of oxalic acid was added to it. The mixture was then centrifuged at 12500 rpm for 10 minutes. 1 µl of this supernatant was then injected into GC to get the peaks that would indicate acetic and lactic acid production.

Specification of the gas chromatography equipment and accessories:

- Shimadzu Gas Chromatograph (GC-14B)with FID Detector, Shimadzu Corp., Japan
- Column : Stainless steel, Length : 2 meters
- O. Diameter : 4 mm, I. Diameter : 3 mm
- Mess range : 100/120, Max Temp : 250°C

Operating Conditions:

- Column materials : 80/120 Carbopack B-DA/4% Carbowax 20M; Packed column.
- Column temperature : 175°C
- Injector temperature : 200°C
- Detector temperature : 200°C
- Air pressure : 50 kPa
- H_2 pressure : 50 kPa
- Carrier (P) pressure : 380 kPa
- Carrier (1) pressure : 225 kPa, 36 ml/min N_2

Before injecting the samples, known concentrations of these acids have been injected in the GC to check the elution patterns of these and for making standard plot. One µl of all samples was injected into the GC for the analysis of the fatty acids (acetic and lactic acid) concentrations. To check the elution time in the sample, the sample was contaminated by concentrated acetic and lactic acids and then observed. The following elution patters have been observed for these two acids present in the samples.

***Elution Time*:** Acetic acid: 3.1 to 3.3 minutes (sharp peak);
Lactic acid: 26.8 to 28.2 minutes(less sharp peak)

Results and Discussion

Products from the growth of *Bifidobacterium lactis* were separated and analyzed in gas chromatography and two main products were obtained. These were acetic acid and lactic acid. From the elution pattern of the chromatogram, the presence of some other acids can also be noticed.

Three experiments have been done to measure these acid concentrations, where three different media were used. Figure 1, 2 and 3 represent the production of these two acids in glucose, soymilk and FOS media respectively. In those figures the patterns of the production of the acids have been reflected. It can be observed that in their initial phase, the acetic acid concentrations were higher than that of lactic acid, but with growth increase, the rate of lactic acid production also increased more than acetic acid production rate in all cases. The higher acetic acid may also because of the conversion of sodium acetate to acetic acid that was present in the substrate.

The bar charts (fig.-4) clearly represent that, for all cases of growth, later part of growth were predominant by the production of lactic acid. The reason is that, end product formations by fermentation of carbohydrates were favorable for lactic acid than acetic acid. Growth in the first two media (media with glucose and media with glucose + 2% dilute soymilk) was higher than growth in FOS medium, which is also reflected in the lactic acid curve. Like other medium, the net increase of lactic acid production was also higher in this medium.

From overall experiments, it has been observed that, growth is highest in the media containing glucose with 2% soymilk. From the acid production curve, it is noticed that lactic acid concentration (7.3 g/L) is also highest for that. Observing the acid production, it can be inferred that, net production of lactic acid by these bacteria is higher than that of acetic acid.

Soymilk has lactose compounds that helps to produce better metabolism and hence higher lactic acid production. Actually, the tendency of this organism is to produce more lactic acid than acetic acid.

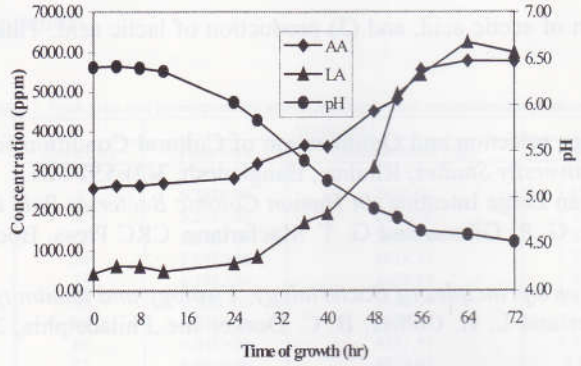

Fig. -1. Production of acetic acid (AA) and lactic acid (LA) by *Bifidobacterium lactis* in glucose containing media.

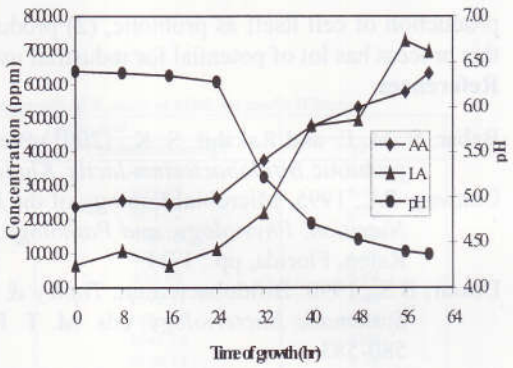

Fig.-2. Acetic acid (AA) and lactic acid (LA) production By *Bifidobacterium lactis* using in glucose and 2% soymilk containing media

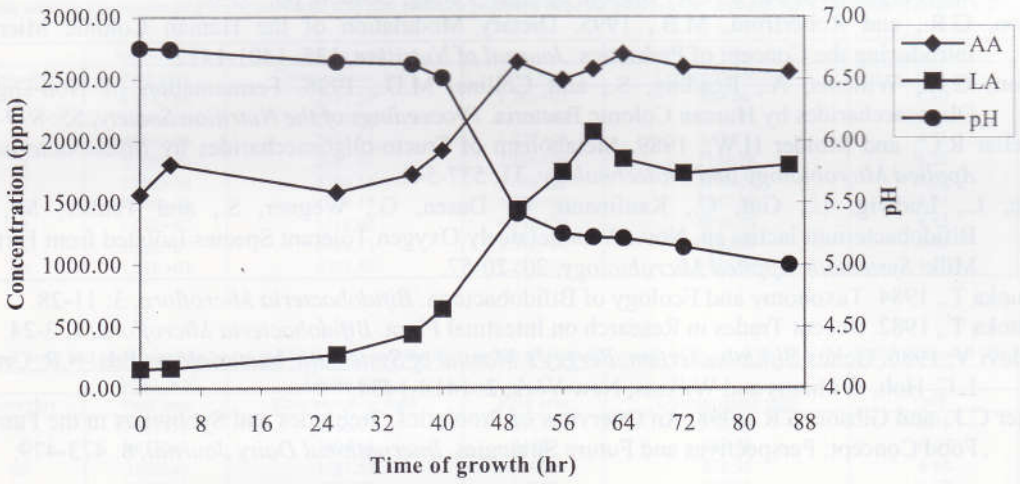

Fig.-.3. Production of acetic acid (AA) and lactic acid (LA) by *Bifidobacterium lactis* in 2% FOS media

As there was increase in growth, the rate of lactic acid production also increased compared to acetic acid. This was observed in all cases.

Referring to manual of the eluting curve from the gas chromatographic column producer, it can be said that, the organisms also produce some other acids like either or combination of propionic, pyruvic, isobutyric, butyric acid. These compounds might add and the inhibitory effect of bifidobacteria on other organisms that might be affected by them.

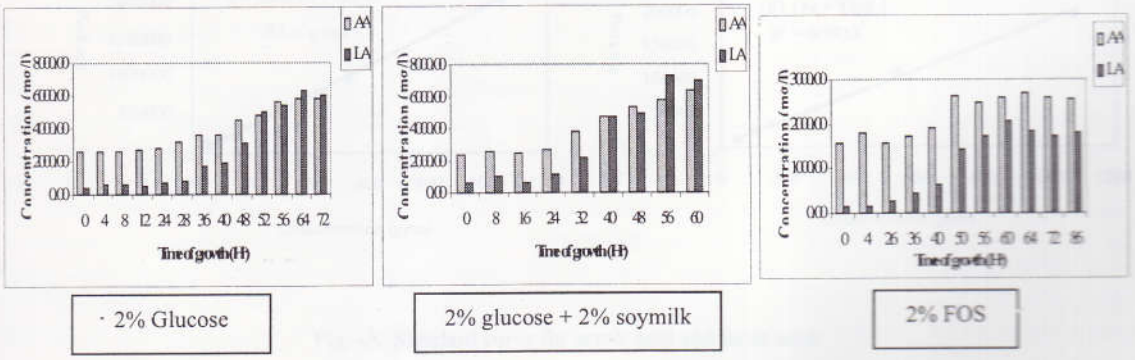

Fig.-4. Comparison of acetic and lactic acid production with growth

Comparing to the existing commercial acid producing system, this fermentation process produce less acid. But, by using this process many commercial purposes can be served simultaneously. These are (1)

production of cell itself as probiotic, (2) production of acetic acid, and (3) production of lactic acid. Thus, this process has lot of potential for industrial use.

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Appendix

Table 1. Peak area and corresponding acetic and lactic acid concentration produced from growth of *B. lactis* in MRS-IM media (Glucose)

	Acetic acid		Lactic acid		
Time (hr)	Peak area	Concentration mg/l	Peak area	Concentration mg/l	pH
0	5.11E+06	2553.78	8.31E04	429.58	6.41
4	5.28E+06	2636.64	1.19E+05	622.75	6.42
8	5.27E+06	2632.36	1.15E+05	603.52	6.40
12	5.39E+06	2693.84	9.27E+04	481.78	6.37
24	5.64E+06	2818.44	1.29E+05	680.72	6.03
28	6.38E+06	3188.81	1.58E+05	839.09	5.84
36	7.30E+06	3648.04	3.51E+05	1698.15	5.40
40	7.24E+06	3616.74	3.57E+05	1926.97	5.18
48	9.04E+06	4551.91	5.71E+05	3091.14	4088
52	9.65E+06	4819.61	9.11E05	4950.13	4.78
56	1.11E+07	5566.74	9.99E+05	5430.30	4.64
64	1.16E+07	5781.74	1.15E+06	6262.75	4.60
72	1.16E+07	5772.82	1.10E+06	6001.42	.4.52

Table 2. Peak areas and corresponding acetic and lactic acid concentration produced from the growth of *B. lactis* in MRS-IM media (Glucose + 2% soymilk).

	Acetic acid	Lactic acid			
Time (hr)	Peak area	Concentration mg/l	Peak area	Concentration mg/l	pH
0	4.75E+06	2371.23	1.26E+05	664.66	6.40
8	5.16E+06	2575.26	2.02E+05	1076.91	6.8
16	4.87E+06	2434.34	1.22E+05	643.71	6.35
24	5.30E+06	2647.43	2.10E+05	1124.78	6.28
32	7.50E+06	3744.39	4.05E+05	2186.14	5.22
40	9.54E+06	4764.47	8.78E+05	4767.80	4.71
48	1.07E+07	5318.80	9.13E+05	4958.75	4.53
56	1.16E+07	8784.03	1.34E+06	7305.18	4.40
60	1.27E+07	6328.46	1.28E+06	6975.65	4.37

Table 2. Peak areas and corresponding acetic and lactic acid concentration produced from the growth of *B. lactis* in MRS-IM media (Glucose + 2% FOS).

	Acetic acid	Lactic acid			
Time (hr)	Peak area	Concentration mg/l	Peak area	Concentration mg/l	pH
0	3133451	1565.17	33138	156054	6.75
4	3613012	1804.64	34409	163.48	6.74
26	3166249	1581.55	54640	273.95	6.64
36	3455002	1725.73	84547	437.25	6.62
40	3835398	1915.69	121460	638.81	6.51
50	5280725	2663.41	267894	1438.38	5.41
56	4989265	2491.87	323490	1741.95	5.25
60	5167190	2580.72	383710	2070.77	5.22
64	5413460	2703.69	343648	1852.02	5.21
72	5203885	2599.04	322165	1734.72	5.14
86	5125815	2560.06	334688	1803.10	5.00

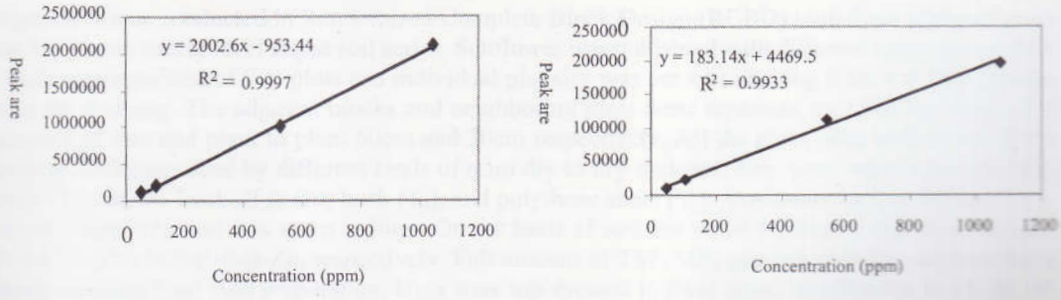

Fig. -A. Standard curve for acetic acid and lactic acid.