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Process Kinetics of Nisin Production in Batch and Continuous Culture

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회분식 및 연속식 배양시 Nisin의 생산특성

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Fermentation condition of *Streptococcus lactis* IFO 12007 for nisin production was examined. The optimal glucose concentration was 60 g/*l*. The pH and temperature optimum were 6.5 and 37°C, respectively. The maximum nisin activity in batch culture was 2000 IU/m*l*. The fermentation quotients after 7 hours of fermentation in batch culture were; specific glucose uptake rate: 0.59 g/g/h, specific nisin productivity: 34924 IU/g/h, product yield: 5944 IU/g, growth yield: 0.24, biomass: 4.81 g/*l*. The specific growth rate was affected by pH and temperature and the activation energy for growth was 1.35 kcal/mole. pH control was essential for nisin production. Fed-batch culture using 20 g/*l* glucose medium produced 1420 IU/m*l* after 14 hours. The continuous culture could be operated at below 0.38 h^{-1} for nisin production. The steady state nisin concentration and specific nisin productivity were 740 IU/m*l* and 45000 IU/g/h. The growth yield and maintenance energy were 0.144 and 207 mg glucose/g-cell/h.

Bacteriocins are bacteriocidal substances produced by bacteria and active against strains of the same or closely related species (1,2). They are narrow in antimicrobial spectrum and most of activities are limited to Gram-positive bacteria (1,3,4). They are normally inactivated by proteolytic enzyme because most of bacteriocins are of peptides (3,5,6). Out of these peptide antibiotic, Nisin is well known as a prominent preservative in dairy products (7,8), canned products (9,10), alcoholic beverages (11,12), meat products (13,14) and egg products (15). It is very potent against *Clostridium* sp. (16,17), *Bacillus* sp. (17,18), *Lactobacillus* sp. (12) and *Pediococcus* sp. (12). The physicochemical characteristics of Nisin had been so much reported (19,20), however, the process data are not easily available. In this paper, some process kinetics are reported.

Materials and Methods

Culture, medium and culture condition

The bacterium used in this study was *Sc. lactis* IFO 12007. The basal medium contained the following composition per liter of distilled water: glucose (20g), proteose peptone #3 (10g), Lab Lemco powder (10g), yeast extract (5g), Tween 80 (1g), ammonium citrate dibasic (2g), sodium acetate · $3H_2O$ (5g), $MgSO_4 \cdot 7H_2O$ (0.1g), $MnSO_4 \cdot 4H_2O$ (0.05g), Na_2SO_4 (0.05g), Na_2HPO_4 (2g). The culture was performed in 250 m*l* flask (working volume: 50 m*l*, static culture) or in jar fermentor (working volume: 350 m*l*, 1200 m*l*, agitation: 200 rpm, New Brunswick Sci. Inst. Co., U.S.A.), in batch, fed-batch and continuous fermentation without aeration. The glucose was separately autoclaved at 121°C for 15 minutes (15 psi). The pH

Key words: Nisin, *Streptococcus lactis,* batch and continuous culture
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Table 1. Effect of fermentation temperature on the nisin production by *Sc. lactis* IFO 12007 after 7 hours of fermentation in fermentor.

Temp. (°C)	Dry cell weight (g/l)	Activity (IU/ml)	$Y_{x/s}$	$Y_{p/s}$ (IU/ml)	Q_s (g/g/h)	Q_p (IU/g/h)
20	–	–	–	–	–	–
25	0.48	102	0.21	44491	0.68	30354
30	2.39	371	0.22	33423	0.66	22157
33	3.34	830	0.21	51894	0.69	35556
37	3.79	1220	0.19	61337	0.75	46069
40	1.75	241	0.15	20390	0.96	19677

Working volume: 350 ml, carbon source: glucose 20g/l, pH 7.0, $Y_{x/s}$: growth yield, $Y_{p/s}$: product yield, Q_s: specific glucose uptake rate, Q_p: specific nisin productivity

was controlled with 3 N KOH solution by pH controller. The 17 hour-old culture was used as starter.

Analysis

Dry cell weight was determined by turbidometry (21). Glucose was analyzed by glucose oxidase (22). Antibiotic activity was assayed by agar diffusion method (23). Total activity was determined after adjusting pH to 2.0, boiling in water bath for 5 minutes and separating the cell at 3000 rpm (24). Extracellular activity was determined by using the supernatant after centrifuging the culture broth. Agar plate for assay was prepared by using *Lactobacillus plantarum* ATCC 8014 as indicator organism. The standard nisin was from Applin & Barett Co. (U.K., 10^6 IU/g)

Results and Discussion

Cultivation temperature

Cultivation temperature was examined in jar fermentor containing glucose medium (pH 7.0) (Table 1). The cell growth was drastically retarded at below 33°C and above 37°C. The antibacterial activity was in similar pattern. The optimum temperature was 37°C. The total activity, dry cell weight, growth yield ($Y_{x/s}$) and product yield ($Y_{p/s}$) at the temperature were 1,220 IU/ml, 3.79g/l, 0.19 and 61337 IU/g after 7 hours. The specific glucose uptake rate was not much affected by temperature, however, the specific rate of nisin production was sensitively influenced. The glucose uptake rate (Q_s)

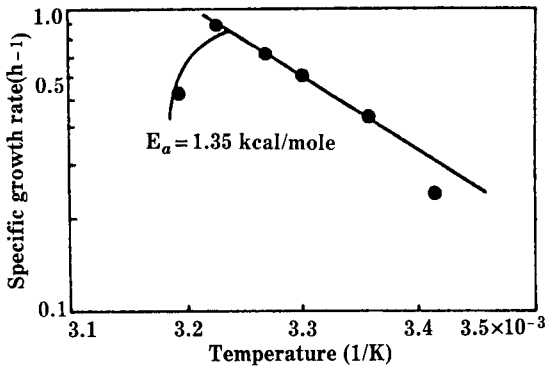

Fig. 1. Arrhenius plot of specific growth rates of *Sc. lactis* IFO 12007.

Table 2. Effect of pH on the nisin production by *Streptococcus lactis* IFO 12007 at 37°C after 7 hours of fermentation in fermenator.

pH	Dry cell weight (g/l)	Activity (IU/ml)	$Y_{x/s}$	$Y_{p/s}$ (IU/g)	Q_s (g/g/h)	Q_p (IU/g/h)	μ (h^{-1})
5.0	0.09	–	–	–	–	–	–
5.5	0.42	69	0.21	34500	0.69	23638	0.22
6.0	2.00	413	0.19	38962	0.76	29485	0.49
6.5	5.01	1195	0.25	59750	0.57	34061	0.88
7.0	3.79	1178	0.19	59196	0.75	44461	0.88
7.5	2.65	371	0.27	38247	0.52	19977	0.56
8.0	1.30	101	0.13	9806	1.12	11073	0.47
8.3	0.07	–	–	–	–	–	–

Carbon source: glucose 20 g/l, working volume: 350 ml, μ: specific growth rate

and specific nisin productivity (Q_p) at 37°C was 0.75g/g-cell/h and 46069 IU/g-cell/h. Berridge *et al.* (25) and Willimowska-Pelc *et al.* (26) reported that a single preparation of nisin consisted of a number of closely related polypeptides, the ratio of which could be altered by a variety of conditions. Berridge (27) noted that antimicrobial spectrum of nisin was affected by the cultivation temperature. Fig. 1. shows the Arrhenius plot of specific growth rate (μ). The maximum specific growth rate was 0.8818 h^{-1} and doubling time was 68 minutes. The activation energy for growth was 1.35 kcal/mole.

pH

Table 2 shows the pH on the nisin production in jar fermentor. *Sc. lactis* IFO 12007 did not grow at the pH below 5.5 and above 8.3. The optimum pH

Table 3. Effect of sugar sources on the nisin production by *Sc. lactis* IFO 12007 at 37°C after 20 hours of fermentation.

Sugar source	Dry cell weight (g/l)	Activity (IU/ml)	$Y_{x/s}$*	$Y_{p/s}$* (IU/g)	Q_p (IU/g/h)
Glucose	0.93	295	0.05	14750	15860
Fructose	1.07	159	0.05	7950	7430
Galactose	0.31	52	0.02	2600	8387
Mannose	0.90	224	0.01	11200	12444
Xylose	0.23	49	0.01	2450	10515
Sucrose	0.36	146	0.02	7300	20357
Lactose	0.42	174	0.02	8700	20719
Maltose	0.75	235	0.04	11750	15751

Sugar concentration: 20 g/l, culture was grown in 250 ml Erlenmyer flask (50 ml working volume) under static condition, initial pH 6.5, *: calculation based on the sugar added.

was 6.5-7.0. The nisin production after 7 hours was near 1200 IU/ml. $Y_{x/s}$ and $Y_{p/s}$ were 0.25 and 59750 IU/g. Q_s and Q_p were 0.57 g/g-cell/h and 34061 IU/g-cell/h. The pH seemed to affect not only the growth but also the nisin production because the nisin production was small at pH of 7.5 despite of 2.7g of cell production. The pH effect was noted by Hirsch (28) and White and Hurst (24). The specific growth rate was so sensitive to pH that it decreased at below and above the pH optimum.

Carbon source

Flask culture was undertaken in static condition to find optimum carbon source without pH control (Table 3). Glucose was found to be most proper. The dry cell weight, nisin activity, $Y_{x/s}$, $Y_{p/s}$ and Q_p in glucose medium were 0.93g/l, 295 IU/ml, 0.05, 14750 IU/g and 15860 IU/g/h. Maltose and mannose were also utilizable for nisin production. The low activity is due to the growth inhibition by lowered pH of medium. Joseph *et al.* (2) reported that xylose and arabinose were stimulating the bacteriocin production by *Rhizobium trifolii*. Baranova and Egorov (29) recommended sucrose, fuctose and glucose for *Sc. lactis*.

Glucose concentration

Table 4. shows the effect of glucose conentration. The dry cell weight and nisin production were

Table 4. Effect of glucose concentration on the nisin production by *Sc. lactis* IFO 12007 at 37°C after 7 hours of fermentation in fermentor.

Conc. (g/l)	Dry cell weight (g/l)	Activity (IU/ml)	$Y_{x/s}$	$Y_{p/s}$ (IU/g)	Q_s (g/g/h)	Q_p (IU/g/h)	μ (h^{-1})
5	1.18	117	0.24	23400	0.61	14160	0.78
10	2.35	293	0.24	29300	0.61	17803	0.80
20	4.46	1026	0.23	52081	0.64	32850	0.90
40	4.38	1080	0.19	46753	0.75	35201	0.80
50	4.80	1096	0.22	50741	0.64	32596	0.81
60	4.81	1177	0.24	59444	0.59	34924	0.93
80	4.79	1257	0.23	59292	0.63	37472	0.96

Working volume: 350 ml, pH 6.5, μ: specific growth rate

Table 5. Effect inoculum size on the nisin production by *Sc. lactis* IFO 12007 at 37°C after 7 hours of fermentation.

Inoculum size (%)	Dry cell weight (g/l)	Activity (IU/ml)	Q_p (IU/g/h)	Q_s (g/g/h)	μ (h^{-1})
1.0	3.79(6.10)	705(1636)	26574	0.42	0.79
3.0	3.88(6.96)	1143(2125)	42084	0.38	0.80
5.0	4.82(8.50)	1177(2043)	34924	0.59	0.92

Working volume: 350 ml, glucose 60 g/l, pH 6.5, μ: specific growth rate, ( ): 11 hour-data showing maximum activity

drastically enhanced with the increase of concentration when less than 20g/l of intial glucose was used. But they are slightly increased when more than 20g/l of glucose was used. The dry cell weight and nisin activity stayed in the level of more than 4.0g/l and 1000 IU/ml, respectively, when using more than 20g of glucose. However, $Y_{p/s}$ linearly increased until 60g/l of glucose was used. The $Y_{p/s}$ at the concentration was 59444 IU/g. The specific growth rate was not so much affected by the concentration. Therefore, 60g/l of glucose was used for further experiment. Baranova and Egorov (29) found that the increase of glucose concentration from 10g/l to 25g/l was not effective for bacteriocin production by *Sc. lactis*.

Inoculum size

The inoculum size is an important factor in determining the facility and efficiency of operation.

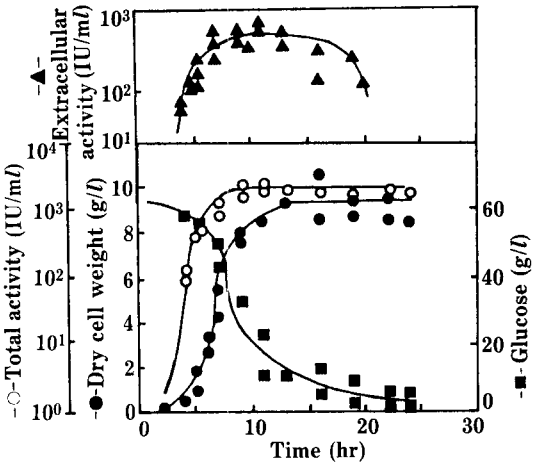

Fig. 2. Nisin production in batch culture by *Sc. lactis* IFO 12007.

Therefore, the inoculum size for nisin production was examined (Table 5). The higher dry cell weight and nisin activity were observed when more inoculum was used. However, more than 3% of inoculum was not necessary for enhancing the maximum nisin production, whereas it accelerated the specific growth rate.

Fermentation profile

Fermentation profile was examined in batch culture using 60 g/*l* of glucose. (Fig. 2). Three hours of growth lag was observed and the stationary growth appeared around 10 hours. The dry cell weight after 24 hours was 8.5 g/*l*. Most of glucose was consumed within 7-9 hours of fermentation time and exhausted after 22 hours. Nisin synthesis seemed to commence when the bacterium showed a logarithmic growth and mostly completed between 5-7 hours. The nisin activity was 2000 IU/m*l* at 9-13 hours of fermentation and then decreased with the elapsed time. The activity after 24 hours of was 1800 IU /m*l*. Hurst (30) reported that nisin production started in a late logarithmic phase and reached maximum at 9 hours of fermentation time and it could be decomposed by an inactivation system. The extracellular activity was also examined. The activity increased during the logarithmic phase, reached maximum in the early stationary phase and then decreased after 13 hours. The activity was not detected after 24 hours and observed in cell boundform. The fermentation without pH control was tried, however, only 300 IU/m*l* was produced and cell growth was very much inhibited (result not shown).

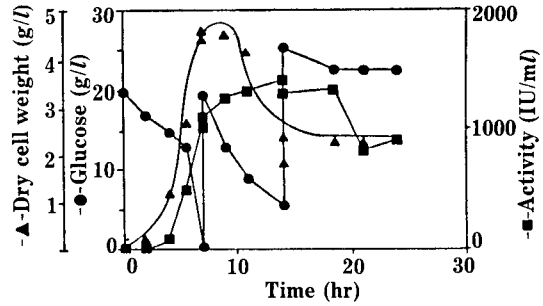

Fig. 3. Nisin production in fed-batch culture by *Sc. lactis* IFO 12007.

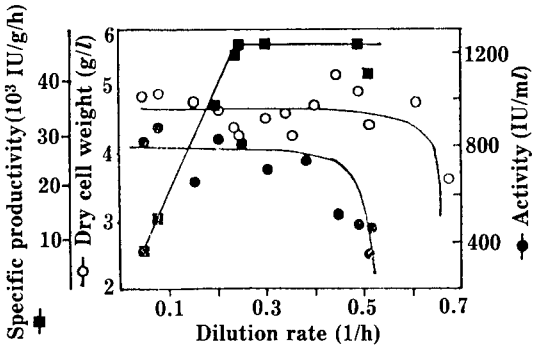

Fig. 4. Nisin production in continuous culture by *Sc. lactis* IFO 12007.

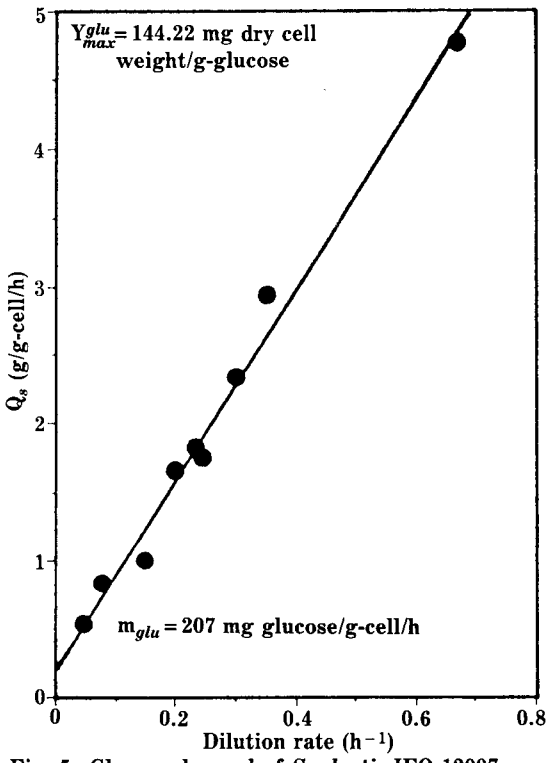

Fig. 5. Glucose demand of *Sc. lactis* IFO 12007.

Fed-batch culture was also undertaken in 20 g/*l* glucose medium (Fig. 3). The glucose concentration was readjusted to 20 g/*l* after 7 hours of elapsed time when glucose was exhausted. 1420 IU/m*l* was producted after 14 hours even if cell growth started to deteriorate just after first run. However, it was not possible to operate third running and the activity was rather decreased.

Nisin production in continuous culture

Continuous culture was tried by feeding 60 g/*l* glucose medium (Fig. 4). Steady state data were obtained after operating 5 residence time. It was possible to operate at 0.6 h^{-1}, however, nisin activity was detected at only below 0.38 h^{-1}. The steady state dry cell weight and nisin activity were 4.7 g/*l* and 740 IU/m*l*. Q_p increased linearly at below 0.23 h^{-1} of dilution rate and stayed in the constant level of 45000 IU/g/h, which could be observed in secondary metabolite production (20,31). Fig. 5. shows the plotting of specific glucose uptake rate versus dilution rate. The parameter estimation was by the least square method. The glucose demand for maintenance was 207 mg glucose/g-cell/h and growth yield was 0.144.

요 약

Streptococcus lactis IFO 12007의 nisin 생산을 위한 발효조건을 검토하였다. Nisin 생성을 위한 포도당의 농도는 60g/*l*이며 pH와 온도는 각각 6.5와 30℃이었다. 이 조건에서 최대 2,000 IU/m*l*의 생산량을 보이며 이 때 specific glucose uptake rate는 0.59g/g/h, specific nisin productivity는 34924 iu/g/h, growth yield는 0.24, 7시간 후 균체 생산량은 4.81g/*l*이었다. 비성장속도는 온도와 pH에 의하여 영향을 많이 받으며 증식활성화 에너지는 1.35 Kcal/mole 이었다. 유가배양에 의하여 1420 IU/m*l*의 nisin을 생산하며 연속배양은 0.38 h^{-1}까지 가능하고 이 때 nisin 농도는 740 IU/m*l*, specific nisin productivity는 45000 IU/g/h, true growth yield는 0.144, maintenance energy는 207 mg glucose/g-cell/h 이었다.

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