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Optimization of Food Waste Fermentation for Probiotic Feed Production with Yeast *Kluyveromyces marxianus*

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Abstract

For the probiotic feed production, aerobic liquid fermentation of pulverized food wastes was attempted with a yeast *Kluyveromyces marxianus*. After grinding finely, optimal fermentation conditions of the substrate was investigated by shaking culture. The most active growth of the yeast was shown at solid content of 10%. The proper addition of urea(0.5g/l), o-phosphate(0.4g/l), molasses(4g/l), and yeast extract (1g/l) increased cell growth rate and viable cell count. For optimizing, the nutrients were all added to substrate and fermentation was carried in 2 litre jar fermenter. For the stimulation of hydrolyzing enzyme excretion, mixed culture with *Aspersillus oryzae* was also conducted. In 12 hours of fermentation, viable cell count of the yeast *Kluyveromyces marxianus* amounted to the number of 1.4 X 10^{10}/l in the culture medium.

Key words : fermentation of food wastes, probiotic feed, *Kluyveromyces marxianus*

I. Introduction

In Korea, about 90% of raw materials for feed stuffs have been imported and feed production increased rapidly by the growth of meat consumption. On the contrary, about 30 % of the food prepared were wasted in Korea and the most of the food wastes had been piled in reclamation area, which caused social conflict NIMBY(Not In My Back Yard)by the leakage of highly contaminated water and bad odor to near residents. Moreover, reclamation caused severe contamination of ground water and lakes. For the resolution of both problems, the production of feed stuff from food wastes was proposed and proceeded by the government.

In this study, aerobic liquid fermentation was attempted to increase viable cell count of the yeast *Kluyveromyces marxianus* for the probiotic feed production from food wastes. The yeast was selected by screening procedure and was adapted in food waste substrates, and the optimum substrate concentration was investigated by controlling water content. For optimizing nutritional balance, proper addition of each urea, yeast extract, molasses, and *o*-phosphate was investigated. Especially mixed culture with *Aspergillus oryzae* was also studied, which secretes several enzymes including α-amylase useful for acceleration of the yeast growth.

II. Material and Methods

1. Material

(1) Food waste

Food wastes were taken every day during one week from cafeteria of Hoseo University. After removing moisture by soft hand pressing, they were ground by using homomixer(Oscar DA 502 Dong-A industry. Co) and then stored at -70℃.

For fermentation, they were again homogenized by homogenizer(DIAX 900). Proximate analyses of the food waste is shown in Table 1.

Table 1. Proximate analyses of food waste (dry basis)

Composition	Content
water content(%)	86
pH	4.5
crude protein(%)	19
reducing sugar(g/ℓ)	2.9
crude lipid(%)	8.2
crude fiber(%)	11.63
carbohydrate(%)	41
crude ash(%)	9.03

(2) Microorganisms & media

The yeasts tested for the fermentation of food waste were *Candida rugosa, Candida tropicalis, Kluyvermyces marxianus, Saccharomyces cerevisiae* and mold *Aspgillus oryzae, Geotrichum candidum.*. Among them, thermotolerant strains of *Kluyvermyces marxianus* and *Candida rugosa, Candida tropicalis* were isolated from Africa. The others were purchased from KCTC(Korean Collection for Type Cultures). Seed culture was prepared with a liquid medium of YM(Table 2) and were shaken at 30℃ during two days. As inoculant 5 %(v/v) of precultured microorganism was added to the main substrate. Shaking culture was also adopted for preliminary fermentation test of yeast screening and optimum solid content determination as well as for the determination of proper content of nutrient addition.

Tabel 2. Composition of YM media and fermentation temperature for shaking culture

Media	Composition	incubation temperature	
YM	3 g yeast extract, 3g malt extract, 10 g glucose, 5 g peptone, 15g agar per liter	*Kl. marxianus*	35℃
		Kl. marxianus + *Asp. oryzae*	30℃

2. Conditions for jar fermentation

The main fermentation of liquid food waste was carried with jar fermenter(Korea Fermenter co LTD, 2.5ℓ). Operation conditions for the fermenter was shown in Table 3. As inoculant 5 %(v/v) of precultured microorganism in food waste was added to the main substrate.

Table 3. Conditions for liquid fermentation of food waste

Condition / microorganism	Temperature (℃)	Speed of agitation(r.p.m)	Aeration rate (v.v.m)
Kl. marxianus	35	900	1.5
Kl. marxianus + *Asp. oryzae*	30	900	1.5

v.v.m: volume per volume & minutes

3. Analyses

(1) Proxiamte analyses

Water content was determined by oven drying at 105℃ during 24 hours. Crude

protein was determined by Kjeldahl method and crude lipid by Soxhlet method. Crude ash was determined by oven at 600℃ until constant weight. The totoal carbohydrate was determined by Anthron method, and reducing sugar by Dinitrosalicylic acid method(DNS). Organic acid content was determinated by titration with 0.1N-NaOH to pH 8.4. and expressed as lactic acid.

(2) Determination of viable cell count

After proper diluting 1 mℓ sample, 0.1 mℓ was taken and poured on YM media. The composition of YM media and incubation temperature was shown in Table 2. After 2 days incubation the number of colony was counted.

(3) Determination of α-amylase activity

For the test of enzyme activity, 10ml sample was taken out and mixed with 200ml distilled water. After stirred during 4 hours, the diluted sample was centrifuged at 1,000g, filtrated with filter paper(Whatman No 41.).

For the test of α-amylase acitivity, 1% starch solution(0.02M phosphate buffer, pH 6.9) was used as substrate. 1 mℓ Enzyme solution was added to the substrate, and was incubated at 40℃ for 30 minutes. 10 mℓ of 1 M acetate were put to stop the reaction. For color formation, 50μℓ of dissolved 0.05N iodine in 3 % KI was added to the prepared sample and the absorbance was determined at 660nm. 1 Unit was defined which decreased 10 % of OD(Optical Density) value of the blank, and was expressed for 1g sample.

Ⅲ. Results and Discussion

1. Screening of yeasts for the aerobic fermentation of pulverized liquid food waste

Until 12 hours of fermentation, the yeasts grew with almost same growth rate, but *C. tropicalis* grew slightly faster(Fig. 1). After 18 hours, most yeasts showed decreased viable cell count, and then again increased, which showing typical diauxic growth in complex media. In the last stage of the fermentation, the viable cell count of them was close to 1.4×10^{9}/mℓ. Among them, *Geotrichum Candidum* showed highest viable cell count. *Kl. marxianus* was also shown to grow faster than the other yeasts.

For the test of preservation capability of fermented products, they were incubated open at room temperature during 2 weeks. Even after four days, *Kl. marxianus* did not cause any bad odor, but sweet alcohol smell was generated during four weeks. The other cultures caused to bring slightly bad odor, and after two weeks they were all fouled. *Kl. marxianus* produced alcohol during the fermentation and it enhanced the preservation capability of the fermented food waste. As the results, *Kl. marxianus* was choosen for the proper strain of the aerobic liquid fermentation of the food waste. Since it showed high viable cell count and its fermented product was very stable against deterioration in the room temperature.

2. Determination of optimal solid content of substrate

For the determination of optimal solid content of liquid substrate in the fermentation, the solid content of the substrate was controlled to 5, 10 and 15% by addition of distilled waster. After controlling solid content, 5% inoculum of *Kl. marxianus*(v/v) was added to the fermenter and the viable cell count was observed during 32 hours(Fig.2).

During first 8 hours, all the yeasts did not grow significantly, but after 16 hours, they increased fast. At 24 hours, the observed viable cell count was maximum as about 1.0×10^{9}/ml, and thereafter decreased slowly. The viable cell count of the substrate sample with 10% solid content was more than 3 times higher compared to that with 5% solid content. But in the case of the sample with 15% solid content, it was lower than that of the sample with 10% solid content. In the substrate with lower solid content, substrate restriction inhibited the growth of yeasts, and the viable cell count was

remained lower level too. In case of the substrate with high solid content of 15%, high osmotic pressure and restriction of oxygen transfer might inhibit the yeast growth as was reported by Shon et al.

3. Mixed culture of *Kluyvermyces marxianus* with *Aspersillus oryzae* and α-amylase activity

For accelerating the growth rate and high amount of cell biomass, mixed culture of *Kluyvermyces marxianus* with *Aspersillus oryzae* was attempted. By the ability of several enzyme excretion for hydrolizing biopolymers like carbohydrates and proteins, *Asp. oryzae* was adopted for the mixed culture with *Kl. marxianus*. Fermentation were conducted in 10% substrate for both mixed culture, and single culture only with *Kl. marxianus*. Viable cells was counted for each sample taken every 6 hours. In case of mixed culture, yeast cells grew faster than in single culture(Fig.3). But viable cell count was not much higher than expected. During fermentation α-amylase activity of the mixed culture showed higher level than in single culture(Fig. 4).

4. Optimization of nutritional valance as yeast substrate

For the stimulation of cell growth and the effective utilization of food waste for substrate, some nutrients were added for enhancing nutritional valance according to the preliminary test results by shaking culture. Urea(1g/l), o-phospate(0.4g/l), molasse(4g/l), and yeast extract(1g/l) were added to food waste substrate and sterilized for the fermentation in 2 liter jar fermenter. Initial cell concentration of inoculum was 1.0 X 10^8 for both single and mixed culture. The yeasts grew fast and the maximum viable cell count of mixed culture could reach 1.2 X 10^{10}in 12 hours, and single culture 1.0 X 10^{10} under optimized fermentation condition(Fig. 5). With enough oxygen supply and optimized substrate in jar fermenter, the growth rate of the yeast was much higher than in shaking culture. There were no distinct difference in viable cell count of the final culture stage between mixed and single culture. But mixed culture showed some higher cell growth rate. As results, the waste food can be used as a very chief and good source for the production of valuable probiotic feed.

Ⅳ. References

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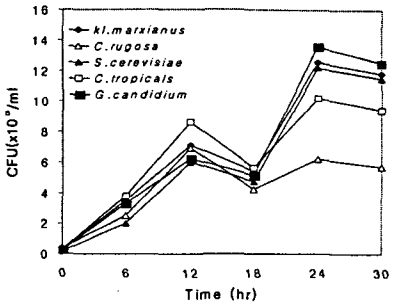

Figure 1. Change of viable cell count during the aerobic fermentation of food waste in 10% of solid content at 35□ □

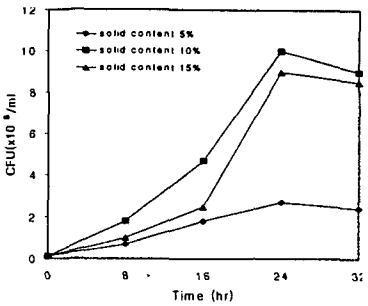

Figure 2. Change of viable cell count of *kl. marxianus* during the aerobic liquid fermentation of food waste in according to solid content.

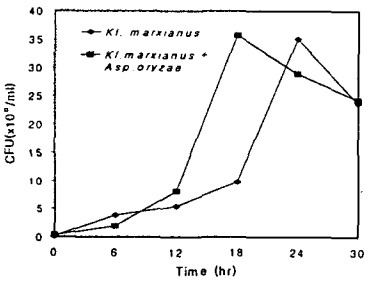

Figure 3. Change of viable cell count of *kl.*

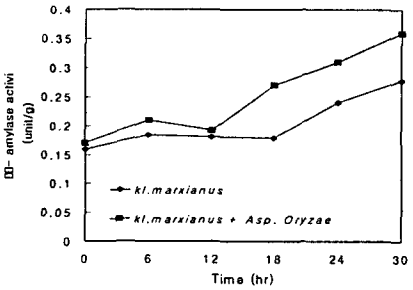

Figure 4. Changes of □ 8 amylase activity during the liquid fermentation of food waste at the agitation speed of 500rpm.

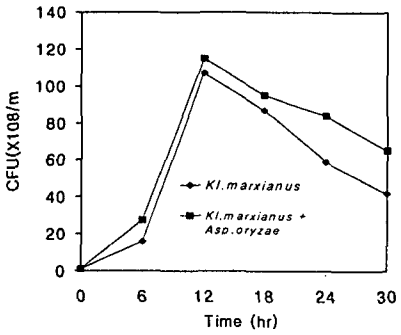

Figure 5. Change of the viable cell count of yeast *Kl.marxianus* during the aerobic jar cultivation of pulverized food waste by nutritionary optimized condition