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Microbial Characterization of *Jangsu*

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Abstract

Jangsu, a Korean ancient non-alcoholic beverage made by lactic acid fermentation of cooked rice, was prepared and the microbial characteristics were investigated. The periodic removal of fermented product and the addition of newly made cooked rice and cold water as new substrate enhanced the growth of lactic acid forming bacteria but supressed the growth of proteolytic bacteria. The important microorganisms in *jangsu* were *Lactobacillus, Lactococcus, Pediococccus and Leuconostoc species. Lactococcus thermophilus, Lactobacillus coryniformis* and *Leuconostoc mesenteroides* were identified. The isolated strains were cultivated and used as starter culture of *jangsu.* Some useful strains were selected which were able to produce acceptable flavor and sufficient amount of acid lowering the pH to near 4.0.

Key words : *jangsu,* lactic acid bacteria, rice beveraze

Introduction

Jangsu is a Korean ancient non-alcoholic beverage made from lactic acid fermentation of cooked cereals[1]. The use of *jangsu* as a common beverage is recorded in Samkuksaki(History of The Three-Nations Period of Korea) written in 1145(2), and Korydokyung(travel-book of Korea) written in 1123[3]. However, the use of *jangsu* has been disappeared in the records ever since. The term *jang*(漿水) was interpreted as rice tea in Jaryuchusuk, a dictionary written in 1856, and extracts of fruits and cereals in Imwonsibyukiji, an encyclopedia written in 1827[1].

The disappearance of *Jangsu* was probably caused by the introduction of *sikhe*, a rice beverage today, made by the saccharification of cooked rice with malt[4]. The use of *sikhe* as a common beverage was first recorded in Sumunsasul, a cook book written in 1740[5]. The difficulties in keeping *jangsu* starter and the frequent failure by the contamination of putrefactive and harmful microorganisms were probably the reasons for why the people abandoned *jangsu* preparation and preferred *sikhe* making[4].

The unique method of cereal lactic acid fermentation of *jangsu* is interesting, because it implicates the nature of natural fermentation principles. In this study *jangsu* was prepared according to the method written in the old literatures, and the microflora grown in the product were characterized and some of useful microorganism were selected for the starter culture of cereal lactic acid fermentation.

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Materials and Methods

Jangsu preparation

The method adopted in this experiment was that described in Jeminyosul, written in AD 530-550 by Kisahyup of North-Wui[1]. It describes that the newly cooked rice, which is still hot, is placed in an earthen jar and cold water is added. It is kept for 3-4 days in the ambient temperature, and then half of it is taken for the use of drink and new cooked rice and water are added to the jar. *Jangsu* is taken once in every 3-4 days and each time new cooked rice and water are added.

In the present experiment, newly cooked rice, 250 g, was mixed with 1 *l* of tap water in a glass bottle and kept at 25℃. Fig. 1 shows the diagram of *jangsu* production and sampling scheme used in this experiment. Half of the fermented material in the bottle was taken periodically and 100 g of newly cooked rice and 1 *l* of water was added after each sampling.

Microbial tests

One mililiter of *jangsu* sample was homogenized with 9 m*l* of trypton-salt diluent, and 1.0 m*l* of the homogenized diluent was smeared on different agar plates listed below[6]. Yeast glucose Lemco agar with bromocresol purple(1% soln.) and skim milk(10%) was used for total count, acid producing bacteria and proteolytic bacteria, MRS agar for lactic acid bacteria

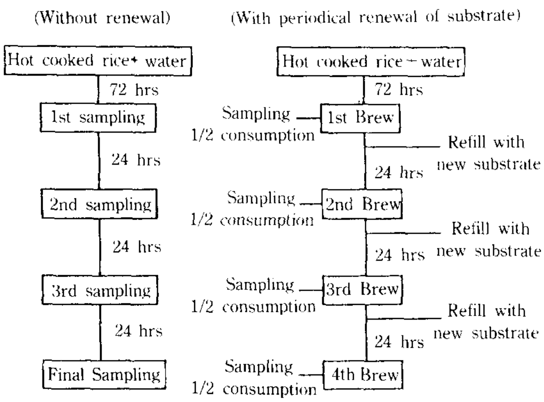

Fig. 1. Experimental scheme for the effect of periodical renewal of substrate on the composition of microbial flora of *jangsu*

and yeast, malt extract agar for yest and mold. Starch agar was used for amylase producing microorganisms, Tween agar for lipase producing microorganisms, Carr and Passmore medium with bromocresol green(2.2%) solution with ethnol for acetic acid bacteria, and mannitol egg york polymixin agar for the isolation and characterization of *Bacillus cereus*. After 48 hrs of incubation at 25℃, the characteristic colonies were selected for isolation. The lactic acid bacteria were classified by morphological and physiological tests as outlined in Fig. 2. Some interesting strains were further identified by refering to the Bergey's manual[7] with the additional biochemical test results.

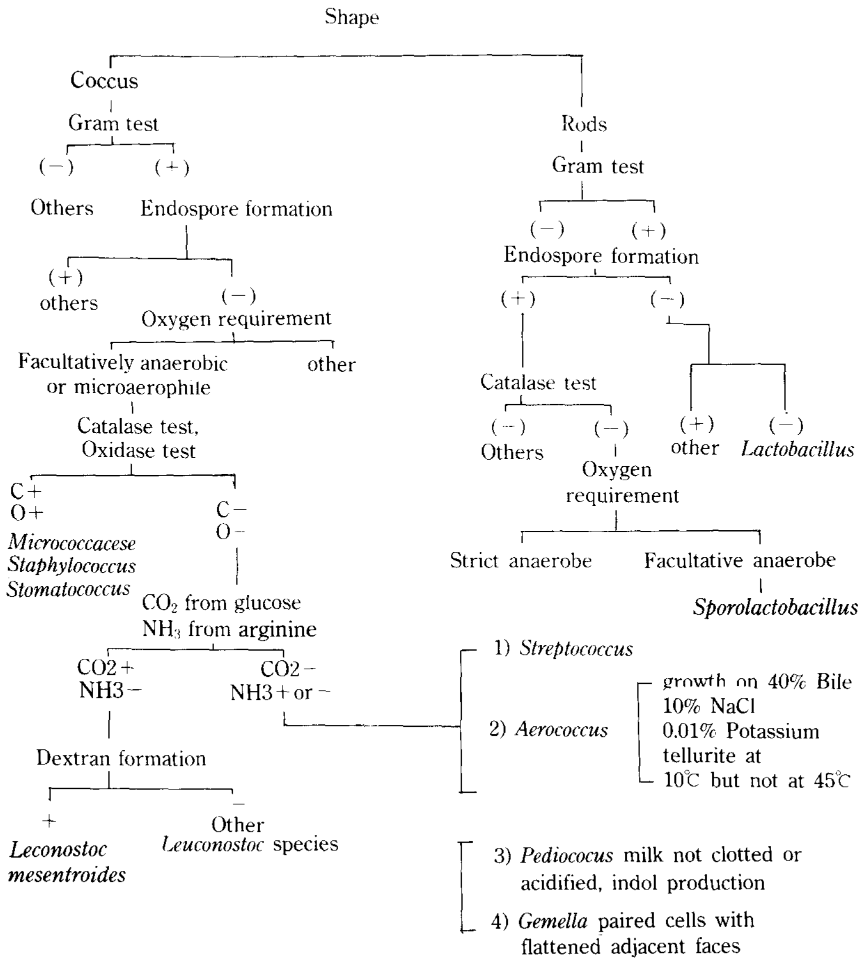

Fig. 2. Presumptive classification scheme of the lactic acid producing bacteria based on characters reported in Bergey's Manual of Systematic Bacteriology

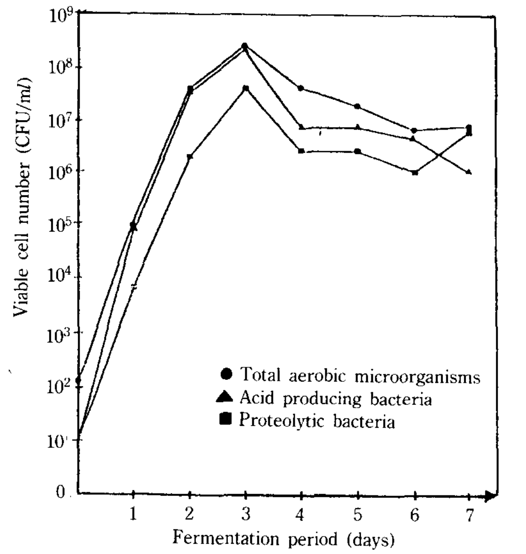

Fig. 3. Changes in number of microflora during *jangsu* fermentation (without renewal of substrate)

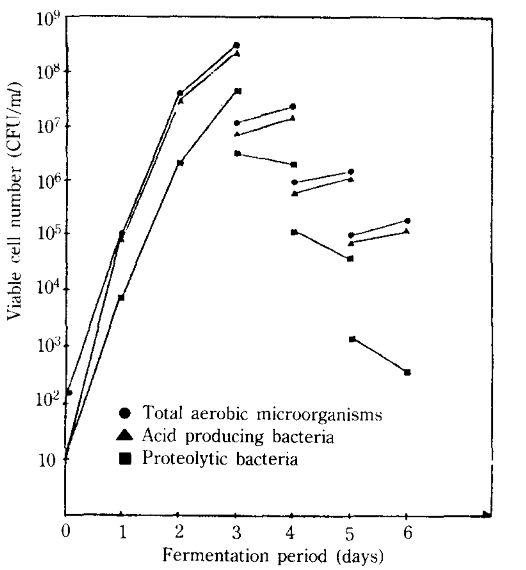

Fig. 4. Changes in microflora during *jangsu* fermentation (with periodical renewal of media)

Preparation of *jangsu* by inoculation

Jangsu was prepared by using starter cultures isolated in this study. The isolated lactic acid bacteria was cultivated in MRS media and inoculated to cooked rice-water suspension homogenized with Waring blendor. It was fermented at 25℃ for 48 hrs and the pH and sensory qualities of the products were evaluated.

Results and Discussion

Effect of *jangsu* process on the microflora pattern

The microflora pattern of *jangsu* was different from that of cooked rice-water mixture kept for several days to ferment naturally. Figs. 3 and 4 compare the microflora pattern of the two systems. When cooked rice-water mixture was incubated at 25℃ the numbers of total cells, acid producing bacteria and proteolytic bacteria grew exponentially for the first 3 days to reach to the maximum number, 3.0×10^8, 2.5×10^8 and 5.0×10^7, respectively, and then they were decreased by the continued incubation. The number of proteolytic bacteria increased after the 6th day of incubation, exceeding the number of acid forming bacteria, which meant putrefaction of the product (Fig. 3).

On the other hand, in *jangsu* process, as shown in Fig. 4, the number of microorganisms decreased by each of the replacement of the half of product with new cooked rice and water. But the reduction rate was far greater with proteolytic bacteria compared to acid forming microorganisms. Consequently, at the 6th day of *jangsu* fermentation, acid producing bacteria became dominant and the number of proteolytic bacteria became insignificant. The old-wisdom cont-

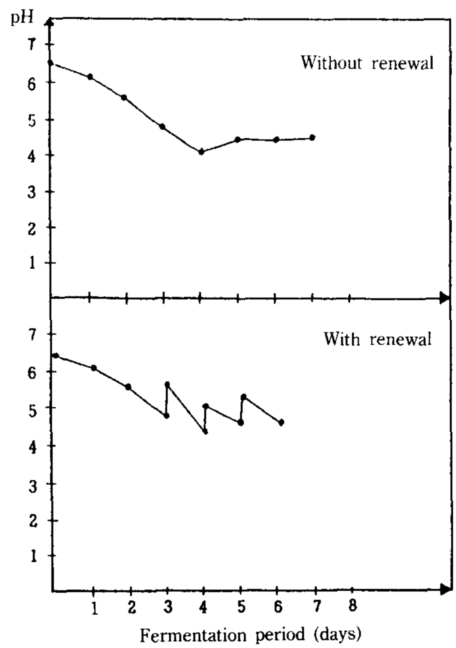

Fig. 5. Changes in pH of *jangsu* with/without renewal of substrate

Table 1. Selection of interesting lactic acid-producing bacteria during *jangsu* fermentation

Code number	Isolation condition			Presumed identification
	Media	Brew number	CFU/m*l*	
J9001	STA	1	1.2×10^6	Not identified
J9002	YGLA	2	6.0×10^4	*Pediococcus* spp
J9003	MRSA	2	1.2×10^6	*Streptococcus* spp
J9004	STA	2	1.2×10^6	*Lactobacillus* spp
J9005	MRSA	4	2.8×10^5	*Lactococccus*
J9006	YGLA	4	1.0×10^6	Not identified
J9007	MRSA	5	1.2×10^5	*Pediococcus* spp
J9008	YGLA	5	7.0×10^5	Not identified
J9009	YGLA	5	1.1×10^5	*Leuconostoc mesenteroides*
J9013	YGLA	1	6.0×10^7	*Lactobacillus*

Table 2. Identification of important lactic acid bacteria isolated from *jangsu*

Characters	J9005	J9013	J9009
Shape	Coccus	Irregular rod	Coccus
Gram test	+	+	+
Capsule	+ (Pseudo)	−	+(Pseudo)
Endospore formation	−	−	−
Motility	−	−	−
Oxygen requirement			
Catalase	−	−	−
Cytochrome oxidase	−	−	−
Arginine hydrolysis	−	+	−
Starch hydrolysis	+++	−	−
Esculin hydrolysis	−	−	−
CO_2 from glucose	−	+	+
Dextran formation	−	−	+
Citrate utilization	−	+	−
Lipolytic activity	−	−	−
Proteolytic activity	−	−	−
Coagulation of milk	+	+	−
Coagulation of milk with			
0.1% methylenblue	−	+	−
0.3% methylenblue	−	+	−
Litmus milk reaction			
Growth on media with			
0.01% potassium tellurite	−	−	−
0.04% potassium tellurite	−	−	−
40% Bile agar	−	−	+
4.0% NaCl	+	+	+
6.5% NaCl	−	+	+
10% NaCl	−	−	−
Growth in broth at			
5℃	−	−	−
35℃	+	+	+
40℃	+	+	+
45℃	+	±	−
47℃	+	−	−
With initial pH of			
4.2	+	+	+
4.8	+	+	+
6.5	+	+	+
7.5	+	+	+

Table 2. *continued*

Characters	J9005	J9013	J9009
8.5	+	+	+
9.6	−	+	−
Acid production from			
melibiose	+	+	+
mannose	±	+	+
rhamnose	−	±	+
cellobiose	−	±	−
inulin	−	−	−
fructose	+	+	+
xylose	−	±	±
raffinose	+	±	+
arabinose	±	±	±
sorbitol	−	+	−
galactose	±	±	−
mannitol	+	−	−
lactose	±	±	−
sucrose	+	+	+
dextrose	+	+	+
maltose	+	+	+
Acid production from			
starch	+	−	−
dextrin	+	−	+
sorbose	−	−	±
amygdalin	−	−	−
trehalose	−	−	−
Presumptive identification	Similar to *Lactococcus thermophilus* except for its ability to grow on 4% NaCl medium and starch hydrolysis	Similar to *Lactobacillus coryniformis* except for acid production from mannitol	Similar to *Leuconostoc mesenteroides* subsp. *mesenteroides* except for the ability to grow at pH 4.2 and 4.8 and non fermentation of trehalose

rolling the undesirable microorganisms in natural fermentation was demonstrated in this experiment.

Fig. 5 shows the changes in pH during the fermentation of cooked rice. The pH decreased down to 4.0 for the first 4 days and then increased indicating the putrefaction of the product without substrate renewal. In *jangsu* process the pH was lowered by each replacement with new substrate.

Identification and lactic acid bacteria in *jangsu*

Some interesting lactic acid bacteria in *jangsu* were isolated. The criteria for selection were the abundance of colony types, smell of colonies, starch utilization, citrate utilization and dextran formation. Table 1 shows the list of colonies selected and identified species according to the scheme of Fig. 2. The important microorganims in *jangsu* were *lactobacillus, Lactococcus, Pediococcus* and *Leuconostoc spp.* Further identification for the strain was carried out for

Table 3. pH and sensory quality of *jangsu* fermented at 25°C for 48 hrs with selected strains of lactic acid bacteria

Strains	Final pH	Sensory characteristics
J9001	5.5	Not sour, rice flavor
J9004	3.4	Sour, *sikhe* flavor
J9005	4.0	Sour, no flavor no taste, no smell
J9006	5.9	Not sour
J9007	5.9	Not sour, dark grey, good flavor
J9008	3.9	Sour, strong cooked rice flavor
J9009	4.0	Sour, no specific flavor
J9010	6.1	Mildly sour, rice flavor
J9012	6.4	Not sour, putrid smell

Table 4. pH and sensory quality of *jangsu* fermented with mixed culture of lactic acid bacteria for 48 hrs at 25℃

Strains	Final pH	pH change	Sensory characteristics
J9005	4.1	−1.3	Tastes of cooked rice, bitter, astringent
J9004	4.0	−2.6	Sweet taste, good flavor
J9007	4.5	−1.6	Not sour, good flavor
J9005+J9007	4.8	−0.7	Not sour, good flavor
J9004+J9005	4.1	−1.1	Mildly sour, sikhe flavor and taste
J9004+J9007	4.2	−1.2	Taste of cooked rice, good flavor

three microorganisms, J9005, J9013, and J9009. On the basis of the physiological and biochemical tests as shown in Table 2, they were tentatively identified as *Lactococcus thermophilus, Lactobacillus corynifomis,* and *Leuconostoc mesenteroides* subsp. mesenteroides respectively. The *Lactococcus thermophilus* could grow in 4% NaCl medium and hydrolize starch. The *Lactobacillus coryniformis* could produce acid from mannitol, and *Lauconostoc mesenteroides* could grow at initial pH of 4.2.

Quality of *jangsu* made by starter culture inoculation

Table 3 shows the final pH and sensory quality of *jangsu* prepared by the inoculation of different starter microorganisms isolated from traditional *jangsu*. J9004(*Lactobacillus*), J9005(*Lactococcus thermophilus*) produced sufficient acid(pH below 4.0) and acceptable flavor. J9007(*Pediococcus* spp.) produced good flavor but the acid production was not sufficient.

Table 4 shows the results of mixed culture inoculation. Combination of J9004(*Lactobacillus* spp.) with either J9005(*Lactococcus thermophilus*) or J9007(*Pediococcus* spp.) resulted in sufficiently low pH of final product and acceptable flavor of *jangsu*.

Acknowledgement

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장수 발효의 미생물학적 연구

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우리나라 신라시대를 전후하여 일반적인 청량음료로 사용되었던 것으로 알려진 장수(漿水)의 제조방법을 제민요술에 근거하여 재현하고 그 발효학적 특징을 규명하려 하였다. 새로지은 뜨거운 쌀밥을 항아리에 담고 찬물을 부어 3일간 상온에서 발효한 후 윗물과 밥 절반을 떠낸 후 다시 새로지은 뜨거운밥과 찬물을 채워 넣는 일을 주기적으로 반복하면서 미생물균총의 조성을 관찰하였다. 밥과 물을 혼합하여 실온에 방치하면 3일 동안 총균수, 단백질 분해균, 산생성균수가 지수적으로 증가하나 그 이후부터 다소 감소하며 6일 후에는 단백질 분해균수가 증가하여 부패현상을 나타낸다. 그러나 장수 제조법에 의하면 단백질 분해균이 크게 감소하고 산생성균이 지배균총이 된다. 장수에서 분리한 주요 산생성균은 *Lactobacillus, Lactococcus, Pediococcus, Leuconostoc spp.*였으며 이중 주요균으로 *Lactococcus thermophilus, Lactobacillus coryniformis* 및 *Leuconostoc mesenterioides*를 잠정 동정하였다. 분리한 유산균 들을 starter로 사용하여 장수를 제조한 결과 산생산력이 크고 풍미가 우수한 제품을 얻을 수 있었다.