Isolation and Identification of Lactic Acid Bacteria from Spent Mushroom Substrate for Silage Making and Determination of Optimal Medium Conditions for Growth

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ABSTRACT

This study was conducted to isolate and identify the lactic acid bacteria (LAB) from spent mushroom substrates (SMS) for the effective anaerobic fermentation to utilize SMS as an animal feed and to determine the optimal medium conditions for their growth. At first, a total of 23 strains were isolated from the ensiled SMS based on the LAB counts and pH tested. Then, a total of 16 strains which rapidly produce lactate and decreased the pH, were selected for a screening test. The optical density (OD), pH, and yellow clear zone were tested for the selected 16 strains. Among the strains, KU5 strain had wider yellow clear zone and lower pH and KU13 strain had higher OD at 24 hr of incubation and wider yellow clear zone compared to other strains and control strain (*Lactobacillus plantarum* KCCM 12116). Accordingly, KU5 and KU13 strains were finally selected. The KU5 and KU13 were identified as *Lactobacillus plantarum* by the 16S rRNA sequencing. The KU5 strain was named as *Lactobacillus plantarum* KU5, and the KU13 strain was named as *Lactobacillus plantarum* KU13. *Lactobacillus plantarum* KU5 and *Lactobacillus plantarum* KU13 were registered at the National Center for Biotechnology Information (NCBI). Access number of *Lactobacillus plantarum* KU5 was HQ542227 and that of *Lactobacillus plantarum* KU13 was HQ542228. The optimal medium conditions for growth of KU5 and KU13 were soybean meal 2% and formulated feed 2%, respectively.

(Key words : Spent mushroom substrate, Spent mushroom compost, Lactic acid bacteria, Fermentation, Silage)

INTRODUCTION

Fresh spent mushroom substrates (SMS) are used as an animal feed in many Asian countries (Suzuki et al., 1995; Zhang et al., 1995; Kakkar and Dhandu, 1998; Lee et al., 2010). The SMS is released on a large scale from commercial mushroom farms. The released wet SMS putrefy rapidly in 3~4 days when exposed to air (Kwak et al., 2008). Thus, an effective storage technique must be developed. In general, anaerobic fermentation storage can be a desirable method among various storage methods.

Lactic acid starter bacteria are commonly used as inoculants by food fermentation industries to produce a variety of fermented foods. The fermentative process not only creates a different food product but also adds other attributes to the food such as preservation of the food and amends the food with desirable microbes. Also, the addition of microbial inoculants with or without enzymes has been reported to improve ensiling characteristics of by-product

feeds such as wet brewer's grains (Schneider et al., 1995) and food waste (Yang et al., 2006). Lactic acid, which was produced during the anaerobic fermentation by lactic acid bacteria (LAB), inhibited both the growth of undesirable epiphytic microorganisms and the activity of endogenous plant catabolic enzymes, thus maximizing nutrient preservation (Bolsen et al., 1992). Kim et al. (2007a) isolated and identified the highly cellulolytic bacteria (*Enterobacter ludwigii* KU201-3 and *Bacillus cereus* KU206-3) from stacked SMS in order to improve fiber digestibility of SMS. Fiber and mineral digestibilities were enhanced when SMS was treated with the above microbes (Kim et al., 2007b). It was expected that if the powerful LAB was isolated from SMS and applied to SMS fermentation, the SMS released on a large scale could be effectively utilized as a fermented feed.

Based on the above characteristics of SMS and the earlier reported results, this study was conducted to isolate and identify the LAB from SMS for the effective anaerobic fermentation of SMS used as silage for animals and to

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determine the optimal medium conditions for their growth.

MATERIALS AND METHODS

1. Isolation of lactic acid bacteria from SMS

Fresh SMS was obtained from a mushroom farm located in Chungju-city and then anaerobically ensiled. LAB strains were isolated from the ensiled SMS. A 10g sample of SMS was added to 200 ml of sterilized water and then it was blended (HR 2870, PHILIPS, China) for two minutes. The blended solution was serially diluted, then 0.1 ml of serially diluted solution was inoculated to MRS agar plate, and afterwards incubated (HK-IB157, Korea) at 30°C for 24 hr. Strains were isolated from the incubated MRS agar plate. Each LAB strain was isolated and inoculated to the new MRS agar plate and given a temporary identification number. A total of 23 strains were isolated.

2. Screening of lactic acid bacteria

For counting the isolated 23 LAB strains, they were inoculated to MRS broth in 50 ml conical tube. The conical tubes were placed in a shaking incubator (HK-SIL25C, Korea Total Instrument, Korea) at 30°C and 150 rpm for 24 hr. During incubation, the LAB was counted at 12 and 24 hr, and pH was measured at 24 hr by a pH meter (HI 9321, Hana Instrument, Portugal). The LAB colony was counted by a serial dilution method. The inoculated MRS agar plate was incubated (HK-IB157, Korea total Instrument, Korea) at 30°C for 24 hr, then the colony was counted. Based on the LAB count and pH, 16 strains were selected. The LAB count, optical density (OD), and pH according to the incubation periods (0, 3, 6, 9, 12, 15, and 24 hr) were measured for selected 16 strains. The yellow clear zone was measured at the 24 hr of incubation. *Lactobacillus plantarum* KCCM 12116 was used as a control strain. The selected 16 strains were preliminarily incubated on MRS broth for 12 hr, and then 0.1 ml of initially incubated culture solution was inoculated to 200 ml MRS broth, then incubated in shaking incubator (HK-SIL25c, Korean instrument) at 30°C and 150 rpm for 24 hr. During the incubation, LAB counts, pH, and OD were measured according to incubation periods (0, 3, 6, 9, 12, 15 and 24 hr).

For OD measurement, 3 ml of culture solution was transferred to a cuvette and the OD was measured by a

visible UV spectrophotometer (S-1100, Scinco, Korea) at 600 nm. For the yellow clear zone measurement at 24 hr of incubation, 0.01 ml of culture solution was inoculated to the paper disk placed on top of MRS agar plate containing 1.5% bromocresol green, then it was incubated in an incubator (HK-IB157, Korea total Instrument, Korea) at 36°C for 24 hr. The yellow clear zone was defined as a diameter from the edge of the paper disk to the edge of the discolored MRS agar plate.

3. Identification

Finally selected strains were identified by Bergey's manual of systematic bacteriology (Claus and Berkeley, 1986). Biochemical characteristics of strains were tested by an API (50CHB) kit (Bio-Merieux, France). The 16S rRNA sequence was tested using the method of Thompson et al. (1994) and then compared with ribosomal RNA sequence of GENBANK by BLAST algorithm program. The homogeneity of strains were compared and analyzed by Clustal X and Mega 2 program.

4. Optimization of medium conditions for the LAB growth

The optimal medium condition for growth was tested for finally selected strains. Cheap and easily available feedstuffs (soybean meal, wheat bran, rice bran, and formulated concentrate mix) were used as substrates. Chemical composition of substrates is presented in Table 1. The CP of dehulled soybean meal was 50.2% which was highest. The ether extract of rice bran was 15.3%. The substrates were ground with a Wiley mill (Thomas Scientific, Model 4, New Jersey, USA). Each substrate, of which particle size was lower than 1 mm, was added to 200 ml shake flask containing 100 ml distilled water, then sterilized at 121°C for 15 minutes. The concentration of substrates was 0.5, 1, and 2% in this experiment. The finally selected strains were incubated on MRS broth at 30°C and 150 rpm for 12 hr. The 0.1 ml of incubated culture solution was inoculated in a sterilized 200 ml shake flask containing 100 ml sterilized water and incubated in a shaking incubator (HK-SIL25c, Korea total Instrument, Korea) at 30°C and 150 rpm for 24 hr. After the incubation, the total LAB count was measured by a serial dilution method.

Table 1. Chemical composition of substrates

	Concentrates ¹⁾	Soybean meal	Rice bran	Wheat bran
	 % (DM basis)			
Dry matter	90.1	91.2	90.9	88.1
Crude protein	12.4	50.2	15.2	15.5
Ether extract	3.3	1.5	15.3	3.0
Crude ash	6.7	7.3	10.8	4.9
Neutral detergent fiber	25.2	29.5	29.5	44.1
Acid detergent fiber	13.3	13.2	15.8	13.1
Hemicellulose	11.9	16.3	13.7	31.0
Non-fibrous carbohydrate	52.4	11.5	29.2	32.5

¹⁾ Formulated concentrate mix for growing Hanwoo.

RESULTS AND DISCUSSION

1. Isolation and screening of LAB

The LAB counts and pH according to incubation time of the selected 23 strains are presented in Table 2. The pH ranged from 3.9 to 6.8, and LAB count at 24 hr of incubation ranged from 7.9 to 9.7 log₁₀ cfu/ml. For the LAB counts at 12 hr of incubation, KU4, KU5, KU6, KU26, and KU28 were higher than others. Thus, strains KU1, KU11, KU17, KU18, KU21, KU24, and KU25 were removed from the selection because they showed low LAB counts at 24 hr of incubation. As a result, 16 strains were selected.

The OD, pH and yellow clear zone were tested for selected 16 strains. The OD according to the incubation time is shown in Table 3. Yellow clear zone at 24 hr of incubation is shown in Table 4. Among strains, the KU5 strain had the widest yellow clear zone and lowest pH at incubation 15 and 24 hr. Thus, it was considered that the KU5 strain most rapidly dropped the pH. Also, the KU13 strain had the highest OD at 24 hr of incubation and the widest yellow clear zone. Thus, it was considered that the active growth of KU13 was highest. Furthermore, the pH and yellow clear zone of KU5 strain and pH and OD of KU13 were higher than those of control strain (*Lactobacillus plantarum* KCCM 12116). Accordingly, KU5 strain and KU13 strain were finally selected.

2. Identification and characteristics of LAB

The characteristics of carbohydrate utilization for KU5 and KU13 are shown in Table 5. KU5 strain could utilize melezitose, but KU13 could not utilize it. KU13 strain could

Table 2. Total lactic acid bacteria count according to the incubation time and pH¹⁾

Strain	Incubation (hr)		pH
	12	24	
	 log ₁₀ cfu/ml		
KU1	6.3	7.9	6.8
KU4	9.0	9.7	3.9
KU5	9.3	9.6	3.9
KU6	9.2	9.6	3.9
KU7	8.5	9.7	4.0
KU8	8.8	9.7	4.0
KU9	7.6	9.6	4.0
KU10	8.9	9.7	4.0
KU11	6.7	9.1	4.3
KU12	8.7	9.5	4.0
KU13	8.2	9.6	4.0
KU14	7.6	9.6	3.9
KU16	8.6	9.6	4.0
KU17	9.0	9.0	4.0
KU18	7.8	8.7	4.0
KU19	8.9	9.5	4.0
KU20	8.7	9.7	4.0
KU21	8.2	9.0	3.9
KU22	8.7	9.6	4.0
KU24	8.8	8.9	4.1
KU25	8.8	8.4	4.0
KU26	9.4	9.5	4.0
KU28	9.1	9.5	4.0

¹⁾ Incubated in MRS broth at 30°C.

utilize L-arabinose, but KU5 strain could not utilize it. Because both KU5 strain and KU13 strain could utilize cellobiose, but could not utilize L-xylose and D-xylose, it was considered that KU5 and KU13 strains could decompose

Table 3. Optical density according to the incubation time¹⁾

Strain	Incubation (hr)						
	0	3	6	9	12	15	24
KU4	0.1	0.1	0.7	2.9	6.8	14.0	16.2
KU5	0.1	0.1	0.8	2.9	5.3	11.8	15.7
KU6	0.1	0.2	0.9	2.8	5.9	14.2	14.6
KU7	0.1	0.2	2.8	3.3	6.6	13.4	16.6
KU8	0.1	0.1	0.5	2.1	5.6	13.0	17.9
KU9	0.1	0.4	3.1	3.5	10.3	13.7	15.8
KU10	0.1	0.2	2.2	–	11.4	15.5	19.0
KU12	0.1	0.1	0.4	2.1	7.8	13.0	12.1
KU13	0.1	0.3	2.8	–	7.7	13.1	20.6
KU14	0.1	0.2	2.1	4.0	8.3	13.3	18.6
KU16	0.1	0.1	0.4	1.6	6.6	12.2	17.9
KU19	0.1	0.4	3.0	2.9	13.5	14.3	19.2
KU20	0.1	0.1	0.2	0.7	4.2	8.1	14.5
KU22	0.1	0.1	0.9	3.0	8.7	14.2	15.6
KU26	0.1	0.2	3.1	3.1	9.9	12.4	16.0
KU28	0.1	0.1	0.4	2.0	7.9	11.9	17.8
<i>L. plantarum</i>	0.1	0.1	0.4	2.6	6.8	8.3	19.3

‘–’: missing value

¹⁾ Incubated in MRS broth at 30°C.Table 4. pH¹⁾ and effect zone²⁾ according to the incubation time

Strain	Incubation (hr)							Effect zone
	0	3	6	9	12	15	24	
KU4	6.4	6.6	6.3	5.1	4.3	3.9	3.8	cm
KU5	6.4	6.5	6.1	5.1	4.3	3.7	3.6	3.8
KU6	6.4	6.5	6.0	5.0	4.3	3.7	3.6	4.5
KU7	6.4	6.7	5.9	5.0	4.4	4.1	4.0	3.9
KU8	6.4	6.7	6.4	5.4	4.4	4.0	3.8	3.8
KU9	6.4	6.5	5.8	5.1	4.4	4.2	4.1	4.2
KU10	6.4	6.7	6.0	5.1	4.4	4.1	4.1	3.4
KU12	6.4	6.7	6.5	5.4	4.5	4.1	4.0	4.3
KU13	6.4	6.6	6.0	5.2	4.5	4.2	4.1	3.8
KU14	6.4	6.6	6.1	5.0	4.3	4.1	4.0	4.3
KU16	6.4	6.4	6.4	5.5	4.5	4.3	4.0	4.0
KU19	6.4	6.5	5.9	5.2	4.5	4.2	4.2	3.1
KU20	6.4	6.6	6.7	6.2	5.0	4.4	4.0	4.1
KU22	6.4	6.6	6.2	5.0	4.2	4.0	3.9	2.9
KU26	6.4	6.7	5.9	5.5	4.3	4.2	4.1	3.5
KU28	6.4	6.8	6.5	5.5	4.5	4.2	3.8	3.6
<i>L. plantarum</i>	6.4	6.9	6.8	5.8	4.9	4.4	4.3	3.4

¹⁾ Incubated in MRS broth at 30°C.²⁾ Incubated in MRS agar + BCG 1.5% at 30°C for 24 hr.

cellulose to some degree. The KU5 strain was identified as *Lactobacillus paracasei* (97.5%) and identified as *Lactobacillus lactis* (76.9%) by the API 50 CHB kit.

The 16S rRNA sequencing of KU5 and KU13 is shown in Fig. 1. Both KU5 and KU13 were identified finally as *Lactobacillus plantarum* by 16S rRNA sequencing. The KU5

Table 5. Biochemical (carbohydrates) characteristics of lactic acid bacteria isolated from spent mushroom substrates

Characteristics	Strain		Characteristics	Strain	
	KU5	KU13		KU5	KU13
Glycerol	–	–	Salicine	+	+
Erythritol	–	–	Cellobiose	+	+
D-Arabinose	–	–	Maltose	+	+
L-Arabinose	–	+	Lactose	+	+
Ribose	+	+	Melibiose	–	–
D-xylose	–	–	Saccharose	+	+
L-xylose	–	–	Trehalose	+	+
Adonitol	–	–	Inuline	–	–
β Methyl-xyloside	–	–	Melezitose	+	–
Galactose	+	+	D-Raffinose	–	–
D-Glucose	+	+	Amidon	–	–
D-Fructose	+	+	Glycogen	–	–
D-Mannose	+	+	Xylitol	–	–
L-sorbose	–	–	β Gentiobiose	+	+
Rhamnose	–	–	D-Turanose	–	–
Dulcitol	–	–	D-Lyxose	–	–
Inositol	–	–	D-Tagatose	–	–
Mannitol	+	+	D-Fucose	–	–
Sorbitol	+	+	L-Fucose	–	–
α Methyl-D-mannoside	–	–	D-Arabitol	–	–
α Methyl-D-glucoside	–	–	L-Arabitol	–	–
N Acetyl glucosamine	+	+	Gluconate	–	–
Amygdaline	+	+	2 ceto-gluconate	–	–
Arbutine	+	+	5 ceto-gluconate	–	–
Esculine	+	+			

strain was named as *Lactobacillus plantarum* KU5 and the KU13 strain named as *Lactobacillus plantarum* KU13. Phylogenetic tree by 16S rRNA sequencing analysis of *Lactobacillus plantarum* KU5 and *Lactobacillus plantarum* KU13 are shown in Fig. 2 and Fig. 3, respectively. *L. plantarum* KU5 and *L. plantarum* KU13 were phylogenetically similar to *L. plantarum*, *L. paraplantarum* and *L. pentosus*. The 16S rRNA sequencing of *L. plantarum* KU5 and *L. plantarum* KU13 were registered at the National Center for Biotechnology Information (NCBI). Access number of *L. plantarum* KU5 was HQ542227 and that of *L. plantarum* KU13 was HQ542228.

Homo-fermentative LAB has been known to reduce nutrient losses in ensiled forages by increasing the rate of lactate production and acidification (Weinberg and Muck, 1996). However, the use of such inoculum could predispose silages to aerobic deterioration because they produced relatively greater levels of residual water soluble carbohydrates and lactate, which were used as growth substrates by spoilage-causing yeasts and molds (Weinberg and Muck, 1996). In contrast, inoculum containing hetero-fermentative

Table 6. Total lactic acid bacteria count according to the substrates and concentrations¹⁾

Items	Strains		
	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>
	KU5	KU13	(KCCM 12116)
.....		log ₁₀ cfu/ml	
Concentrates ²⁾			
0.5%	8.0	8.5	8.3
1.0%	—	8.8	8.6
2.0%	8.8	9.1	8.7
Rice bran			
0.5%	8.7	8.6	8.7
1.0%	8.8	8.6	8.8
2.0%	8.9	9.1	9.1
Wheat bran			
0.5%	8.5	8.2	8.6
1.0%	8.1	8.3	8.6
2.0%	8.9	8.7	9.2
Soybean meal			
0.5%	8.7	8.6	8.3
1.0%	8.9	9.1	8.7
2.0%	9.3	9.1	9.2

¹⁾ Incubated at 30 °C for 24 hr.

²⁾ Formulated concentrate mix for growing Hanwoo.

CTATACATGCAGTCGAACGAACCTCTGGTATTGATTGGTGCTTGCATCAT
 GATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACC
 TGCCAGAAAGCGGGGATAACACCTGGAACAGATGCTAATACCGCATA
 ACAACTTGGACCGCATGGTCCGAGTTTGAAGATGGCTTCGGCTATCAC
 TTTTGGATGGTCCCGCGCGTATTAGCTAGATGGTGGGTAACGGCTCA
 CCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGG
 GACTGAGACACGGCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTT
 CCACAATGGACGAAAGTCTGATGGAGCAACGCCGTGAGTGAAGAAGG
 GTTTCGGCTCGTAAACTCTGTTGTTAAAGAAGACATATCTGAGAGTA
 ACTGTTCAAGTATTGACGGTATTTAACGAGAAAGACACGGCTAACTACG
 TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTTGCCGGATTTAT
 TGGGCGTAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAGCCT
 TCGGCTCAACCGAAGAGTGATCGGAACTGGGAACTTGAGTGCAGA
 AGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGG
 AAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAG
 GCTCGAAAGTATGGGTAGCAACAGGATTAGATACCCTGGTAGTCCATA
 CCGTAAACGATGAATGCTAAGTGTGGAGGGTTTCCGCCCTTCAAGTGT
 GCAGCTAACGCAATTAAGCATTCCGCTGGGAGTACGGCCGCAAGGCTG
 AAACCTCAAAGGAATTGACGGGGGCCGACAAAGCGGTGGAGCATGTGGT
 TTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGC
 AAATCTAAGAGATTAGAGCTTCCCTTCGGGACATGGATACAGGTGGTG
 CATGGTTGTGCTGAGCTCGTGTGCTGAGATGTTGGGTTAAGTCCCGCAA
 CGAGCGCAACCCCTTATTATCAGTTGCCAGCATTAAAGTGGGCACTCTGG
 TGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCA
 TCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACA
 ACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCA
 GTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGT
 AATCGCGGATCAGCATGCCGCGTGAATACGTTCCCGGGCTTGTACAC
 ACCGCCGTCACACCATGAGAGTTTGTAAACCCAAAGTCGGTGGGGTA
 ACCTTTTAGGAACGACCGCCTAAGGGTG

KU5

TGCTATACATGCAGTCGAACGAACCTCTGGTATTGATTGGTGCTTGCATCATGA
 TTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCA
 GAAGCGGGGATAACACCTGGAACAGATGCTAATACCGCATAACAACTTGA
 CCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTTGGATGGTCCC
 GCGCGTATTAGCTAGATGGTGGGTAACGGCTCACCATGGCAATGATACGTA
 GCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCAAACTC
 CTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG
 CAACGCCGCGTGAAGTGAAGAAGGGTTTCGGCTCGTAAACTCTGTTGTTAAAG
 AAGAACATATCTGAGAGTAAGTGTTCAGGTATTGACGGTATTTAACGAGAAAG
 CCACGGCTAACTACGTGCCAGCAGCCGCGGTAAACGTAGGTGGCAAGCGTTG
 TCCGGATTTATTGGGCGTAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTG
 AAAGCCTTCGGCTCAACCGAAGAGTGATCGGAACTGGGAACTTGAGTGC
 AGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGA
 AGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAGGCTCG
 AAAGTATGGGTAGCAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACG
 ATGAATGCTAAGTGTGGAGGGTTTCCGCCCTTCAAGTGTGACGTAAACGAT
 TAAGCATTCCGCCGCGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA
 CGGGGGCCCGACAAGCGGTGGAGCATGTGGTTAATTCGAAGCTACGCGAAG
 AACCTTACCAGGTCTTGACATACTATGCAAACTAAGAGATTAGACGTTCCCT
 TCGGGGACATGGATACAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGAGA
 TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCATT
 AAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGAGGAAGGTGGGGAT
 GACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGG
 ATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATT
 CTCAGTTCCGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGT
 AATCGCGGATCAGCATGCCGCGTGAATACGTTCCCGGGCTTGTACACACCG
 CCGGTACACCATGAGAGTTTGTAAACCCAAAGTCGGTGGGGTAACCTTTTA
 GGAACGACCGCCTA

KU13

Fig. 1. 16s rRNA sequence of lactic acid bacteria isolated from spent mushroom substrates.

Fig. 2. Phylogenetic tree of strain KU5 (*Lactobacillus plantarum*).

LAB (*Lactobacillus buchneri*) alone typically improved the aerobic stability of silages, but often slightly increased DM losses and pH (Driehuis et al., 2001; Adesogan et al., 2003; Kleinschmit and Kung, 2006).

3. Optimal medium conditions for the LAB growth

Counts of finally selected LAB strains, KU5 and KU13 according to the substrates and their concentrations are shown in Table 6. The counts of KU5 and KU13 strains

Fig. 3. Phylogenetic tree of strain KU13 (*Lactobacillus plantarum*).Table 7. Total LAB count according to incubation time¹⁾

Strain	Substrates	Incubation time (hr)							
		0	3	6	9	12	15	24	72
		log ₁₀ cfu/ml							
KU5	Soybean meal 2%	6.3	6.7	7.1	8.7	8.4	8.4	8.6	9.3
KU13	Concentrates ²⁾ 2%	6.0	6.7	6.4	7.4	7.6	8.4	9.0	8.6
<i>L. plantarum</i>	Soybean meal 2%	5.7	6.8	6.7	8.4	8.5	8.6	8.8	9.3

¹⁾ Incubated at 36°C.²⁾ Formulated concentrate mix for growing Hanwoo.

were above 8 log₁₀ cfu/ml. The count of KU5 strain was highest for 2% soybean meal. The count of KU13 strain in 2% formulated concentrate mix, 2% rice bran, 1% and 2% soybean meal were the same (9.1 log₁₀ cfu/ml) and highest. If the price and convenience of supply and demand were considered, the formulated concentrate mix was most suitable for substrates of KU13. It is well known that the convenience of supply and demand as well as the effect on growth rate is an important factor for substrates selection (Yang et al., 2001; Jung et al., 2003). As a result, the optimal medium conditions of KU5 and KU13 for growth were 2% soybean meal and 2% formulated concentrate mix, respectively.

The LAB counts in the optimal medium condition for growth according to the incubation time are shown in Table 7. The growth rate reached to peak at 72h of incubation for KU5 and at 24 hr of incubation for KU13. When KU5 and control strain were incubated in the same substrates condition (2% soybean meal), KU5 and control strain appeared to show a similar growth pattern.

In conclusion, the KU5 strain and the KU13 strain, which were finally selected on the basis of wide yellow clear zone, low pH, high OD and rapid growth rate, were identified as

Lactobacillus plantarum and grew well in the medium containing 2% soybean meal and 2% formulated feed, respectively. Further studies are required to determine the effect of LAB addition on fermentation parameters of SMS silage in the field situation and to evaluate its feeding effect to animals.

IMPLICATION

The LAB developed in this study can be used for the effective anaerobic storage of SMS released on a large scale from mushroom farms and for the rapid and improved anaerobic fermentation of SMS used as silage for animals. The optimal medium conditions for the LAB may be helpful for the manufacture of a commercial LAB additive.

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