J. Microbiol. Biotechnol. (2017), 27(12), 2112–2118
https://doi.org/10.4014/jmb.1708.08050

jmb

Development of Cabbage Juice Medium for Industrial Production of *Leuconostoc mesenteroides* Starter

Eun Ji Jeong[1], Dae Won Moon[1], Joon Suk Oh[1], Jin Seok Moon[2], Hyunbin Seong[2], Kwang Yup Kim[2], and Nam Soo Han[2*]

[1]*TOBICO Inc., Cheonan 31035, Republic of Korea*
[2]*Brain Korea 21 Center for Bio-Resource Development, Division of Animal, Horticultural and Food Sciences, Chungbuk National University, Cheongju 28644, Republic of Korea*

Received: August 23, 2017
Revised: September 15, 2017
Accepted: September 21, 2017

First published online
October 14, 2017

*Corresponding author
Phone: +82-43-261-2567;
Fax: +82-43-271-4412;
E-mail: namsoo@cbnu.ac.kr

pISSN 1017-7825, eISSN 1738-8872

Leuconostoc mesenteroides is used as a starter to produce high-quality kimchi products. In this study, an efficient and economical cabbage juice medium (CJM) was developed by process optimization of cabbage extraction and pasteurization and by compositional supplementation of various lacking nutrients. The pasteurized cabbage juice was determined to be a good medium candidate to cultivate *L. mesenteroides*, showing maximal cell numbers (9.85×10^8 CFU/ml) after 24 h. Addition of sucrose and yeast extract with soy peptone resulted in increment of bacterial cell counts in CJM, showing the supplementing effect of the lacking nutrients. Furthermore, addition of shell powder gave a protective effect on bacterial cells by preventing pH decline and organic acid accumulation in CJM, resulting in a 2-fold increase of bacterial counts. The optimized composition of CJM was 70% cabbage juice diluted with water, 0.5% (w/v) sucrose, 1% (w/v) yeast extract, 1% (w/v) soy peptone, and 1.5% (w/v) ark shell powder. The CJM developed in this study was able to yield a comparable level of bacterial counts with MRS medium and reduced the cost by almost 10-fold.

Keywords: Lactic acid bacteria, *Leuconostoc mesenteroides*, cabbage juice medium, vegetable medium, fermentation, medium optimization

Introduction

Kimchi is a characteristic traditional Korean vegetable fermented food that is getting popular worldwide. There have been several studies on lactic acid bacteria (LAB) involved in the fermentation of kimchi [1, 2], and metabolites of LAB growing during kimchi fermentation are also important for its taste and flavor. Of the LAB growing in the early stage of kimchi fermentation at low temperature, *Leuconostoc mesenteroides* is a dominant species acidifying kimchi and maintaining anaerobic condition to inhibit aerobic bacterial growth. Acid-resistant bacterial species such as *Lactobacillus plantarum* subsequently appear in the later stages [3]. *L. mesenteroides* produces lactate, CO_2, and flavoring substances (ethanol, diacetyl, and mannitol) during fermentation, significantly affecting the quality of kimchi [4]. With the growth of the kimchi market size in Asia, kimchi manufacturers began to use *Leuconostoc* species as a seed starter of fermentation [5–7]. Employment of *Leuconostoc* starter culture in kimchi is known to guarantee an optimal sour taste, a consistent flavor quality, and inhibition of harmful bacterial growth [8, 9].

For cultivation of LAB for laboratory use, the de Man, Rogosa, and Sharpe (MRS) medium is often used, but its use for industrial purpose costs relatively high, and optimization of the medium composition is necessary [10]. For production of *L. mesenteroides* cells for use in industry, vegetable media are advantageous because they can provide environments where vegetable-derived LAB dominantly grow, and they are agricultural products that can be acquired at affordable prices. In addition, the bacterial productivity in the medium should be increased by

optimizing the medium component and its concentrations [11]. Recently, media using vegetables and grains such as carrots or buckwheats were used for culturing vegetable-derived LAB [12]. However, very little effort has been made to optimize these agricultural products as economical media for mass production of bacterial culture [13].

In this study, to develop an optimized vegetable medium for high cell density culture of *L. mesenteroides*, cabbage was used as a raw material for medium preparation because it is the major ingredient of kimchi and provides rich nutrients to help LAB grow. *L. mesenteroides* KACC91744P isolated from kimchi was inoculated in the cabbage juice medium (CJM), and various nutrients (carbon or nitrogen sources) were added to increase the bacterial productivity. Finally, a natural shell powder containing calcium carbonate was added to reduce the concentration of lactic acid and eventually to prevent the antimicrobial effect of organic acids in CJM.

Materials and Methods

Strain and Chemicals

The strain used in this study was *L. mesenteroides* KACC91744P isolated from kimchi and it was cultured at 30°C for 24 h. CJM, Lactobacillus MRS Broth, or agar (Difco, USA) were used for bacterial culture. To prepare CJM, baechu cabbages (*Brassica campestris* ssp. *pekinensis*) were ground by a blender at room temperature and the cabbage juice (extract) was obtained after removal of the fiber fraction by centrifugation and filtration. To inactivate various enzymes and decontaminate the microbial cells, the juice fractions were pasteurized at 80°C for 1 h (pasteurized CJM) and sterilized at 121°C for 15 min (sterilized CJM) [13, 14]. Fructose (Daejung, Korea), glucose (Daejung, Korea), and sucrose (CJ, Korea) were used as carbon source additives of CJM. Nitrogen source additives were soy peptone (Sempio, Korea), yeast extract (Leiber GmbH, Germany), and fish peptone (Bision, Korea). A natural ark shell powder (Ca Plus; Dream Lime, Korea) containing 36% $CaCO_3$ was used as a food-additive calcium salt.

Medium Composition Analysis

Components and their concentration changes were analyzed against the pasteurized CJM, sterilized CJM, and CJM after cell cultivation. In particular, the CJM after growth of *L. mesenteroides* was centrifuged (SUPRA 22k; Hanil, Korea) at 18,000 ×*g* for 20 min, and the supernatants were filtered using a 0.22 μm filter (Sartolab RF 500; Sartorius, USA). For organic acid analysis, high-pressure liquid chromatography (HPLC; Waters, USA) was used with a Waters 515 pump and Waters 717 automatic sample injection system equipped with a Waters 996 PDA detector. The column was PL Hi-plex H (4.6 × 300 mm; Agilent, USA) and the operation settings were as follows: 5 mM sulfuric acid as the solvent, 10 μl sample injection volume, 0.6 ml/min flow rate, and 210 nm detector wavelength at 55°C column temperature. Vitamin analysis was conducted using HPLC according to the Korean Food Standards Codex [15]. Quantitative analysis of amino acids was performed with the standard solution and sample according to the conditions for amino acids analysis using a GC-FID (6890N Gas Chromatography; Agilent). Amino acids were identified by comparing the retention times for each amino acid peak of standard solutions and the detected peaks in the sample. Mineral element analysis was conducted using an inductively coupled plasma spectroscope (Optima 2100 DV; Perkin Elmer, USA). Free sugar analysis was performed using HPLC (Acme 9000; Younglin, Korea) with an Asahipak NH2P-50 4E column (Shodex, Japan, 4.6 × 250 mm), in which acetonitrile:water = 85:15 (v/v) and 1 ml/min of flow rate were applied. Standards for vitamins (B1, B2, and B3), fructose, glucose, and sucrose were from Sigma (USA), and amino acid standards were from Phenomenex (Amino Acid Standard 1, 2, 3; USA).

Measurement of *L. mesenteroides* Growth Rate

L. mesenteroides KACC91744P was cultured in CJM at 30°C for 24 h and the optical density (OD_{600nm}) value and pH were measured using a spectrophotometer (6300 CM6 3LB; Jenway, UK) and a pH meter (3510 pH meter; Jenway, UK), respectively. For measurement of titratable acidity in the culture medium, 10 ml each of the medium and supernatant after centrifugation (SUPRA 22k; Hanil, Korea) at 18,000 ×*g* for 20 min was adjusted to the equivalence point using 0.1 N NaOH, and then the consumed volume (ml) of NaOH solution was converted to lactic acid content (%) [16]. For bacterial count measurement, the medium was diluted with a sterile saline solution and spread on MRS agar plates to count colonies grown at 30°C for 24 h.

Supplementation of CJM with Carbon and Nitrogen Sources

To enhance the bacterial productivity of CJM, various medium components were supplemented, and their effects on the growth of *L. mesenteroides* KACC91744P were evaluated. Each C-source (glucose, fructose, or sucrose) was added to the heat-treated cabbage juice to 0.5%, 1.0%, and 1.5% (w/v). Heat-treated cabbage juice was diluted to 70%, 50%, or 25% to enlarge the total volume, to which 0.5, 1.0, or 1.5% (w/v) of sucrose was added, followed by sterilization and growth measurement. Each nitrogen source, including yeast extract, soy peptone, and fish peptone was added to make 0.5% and 1.0% (w/v) and combined nitrogen, sources were tested: 0.5% yeast extract + 0.5% soy peptone, 0.5% yeast extract + 1% soy peptone, 1% yeast extract + 0.5% soy peptone, and 1% yeast extract + 1% soy peptone. To investigate the protective effect of calcium salt, 1.5% or 2.0% (w/v) ark shell powder was added to CJM.

Results

Monitoring the Growth of *L. mesenteroides* in Cabbage Juice Medium

The time-dependent growth pattern of the *L. mesenteroides*

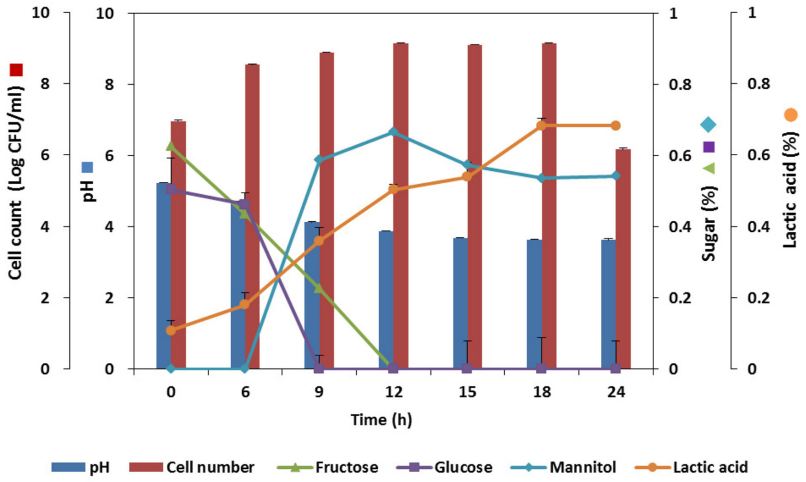

Fig. 1. Cell growth and metabolite production profiles during the cultivation of *L. mesenteroides* in the cabbage juice medium at 30°C for 24 h.

KACC91744P strain in the pasteurized CJM was monitored during cultivation at 30°C for 24 h (Fig. 1). The viable cell count reached the maximal level (1.46×10^9 CFU/ml) after 18 h and thereafter declined owing to the accumulation of organic acids. For the sugar content, glucose (0.50%) and fructose (0.63%) were present in the initial medium, and began to decrease after 6 h of fermentation, resulting in depletion after 9 h for glucose and 12 h for fructose. The lactic acid content continuously increased over time and reached 0.69% during fermentation, whereas the pH decreased to 3.63. Mannitol was produced at 9 h of culture to reach the highest concentration (93.92%) at 12 h, in which the conversion ratio (mannitol production/fructose consumption × 100) was high (86.74%). This is the characteristic property of heterofermentative *L. mesenteroides*, in which fructose in the cabbage medium is converted to mannitol to maintain the cytoplasmic redox potential by mannitol dehydrogenase [17]. The result of this experiment shows that *L. mesenteroides* grows well in the sterilized cabbage juice until sugars are depleted and the low pH condition with acids accumulation suppresses the microbial growth. Based on the results, we made two strategies to improve the efficiency of CJM; supplementing the lacking nutrients in the cabbage juice [18] and protecting the pH changes caused by acid accumulation in the medium [1].

Compositional Analyses of Cabbage Juices after Pasteurization, Sterilization, and Cell Cultivation

To investigate the key nutrients in the cabbage juice for growth of *L. mesenteroides* and their changes during heat treatments (pasteurization or sterilization) and cell cultivation at 30°C for 24 h, we analyzed the concentrations of each component in the cabbage juices. As presented in Table 1, the detected components in the cabbage juice were 12 amino acids (alanine, glycine, valine, leucine, isoleucine, serine, proline, asparagine, aspartic acid, phenylalanine, lysine, and tyrosine) with four amino acid derivatives (β-aminobutyric acid, thioproline, 4-hydroxyproline, and ornithine) and two dipeptides (glycine-proline and proline-hydroxyproline), four organic acids (citric acid, malic acid, succinic acid, and acetic acid), four vitamins (B_1, B_2, B_3, and C), and seven minerals (Ca, Fe, K, Mg, Mn, Zn, and P). It was found that the cabbage used in this study has typical compositions as those used for general kimchi production, despite slight variation with that previously reported by Seong *et al.* [19], probably due to seasonal deviation. When the component concentrations were compared between the pasteurized and the sterilized CJM, there were no major changes in the concentrations of amino acids, organic acids, vitamins, and minerals, except two amino acids (valine and asparagine) and two vitamins (B_2 and C) (Table 1). High temperature and pressure conditions during the sterilization process may lead to the hydrolysis of proteins as well as degradation of vitamins B_2 and C.

Meanwhile, when the compositions of the CJM were compared before and after cell cultivation, various changes were observed in the concentrations of amino acids, organic acids, vitamins, and minerals. Among the amino acids present in CJM, the concentrations of alanine, valine, isoleucine, proline, asparagine, phenylalanine, lysine, and tyrosine decreased after cell cultivation. Kim *et al.* [20] reported that valine and isoleucine were essential for

Table 1. Components analysis results of cabbage juice medium (CJM).

	Components	Units	Pasteurized CJM[b]	Sterilized CJM[c]	CJM after cell growth[d]
Amino acids	Alanine	mg/l[a]	359.82	346.55	178.96 $^{---}$
	Glycine		35.64	40.99	41.78
	Valine		56.66	126.34^{+++}	123.91
	β-Aminobutyric acid		43.29	51.04	65.45
	Leucine		49.22	70.14	70.51
	Isoleucine		29.53	34.51	22.94 $^{---}$
	Serine		71.98	85.68	85.47
	Proline		249.37	273.25	250.03
	Asparagine		147.50	324.77^{+++}	210.13 $^{---}$
	Thioproline		163.58	193.68^{+}	217.96^{+}
	Aspartic acid		192.82	153.37 $^{--}$	169.81
	4-Hydroxyproline		160.24	172.50	201.53
	Phenylalanine		37.26	38.44	18.85 $^{--}$
	Ornithine		128.97	154.92	200.19
	Glycine-proline		90.59	107.58	127.82
	Lysine		55.53	66.67	54.72
	Tyrosine		39.72	36.27	25.97
	Proline-hydroxyproline		2,420.98	2,470.32	4,305.95
Organic acids	Citric acid	mg/l	1,350.0	1,365.7	2,065.3
	Malic acid		1,963.4	2,047.0	1,111.7
	Succinic acid		4,096.8	4,103.4	2,537.8
	Acetic acid		5,957.6	5,988.9	6,159.1
	Lactic acid		ND[e]	ND	6,100.0
Vitamins	Vitamin B_1	mg/100 g	0.38	0.37	0.35
	Vitamin B_2	μg RE/100 g	0.13	0.08	0.05
	Vitamin B_3	mg/100 g	0.13	0.12	0.11
	Vitamin C	μg RE/100 g	5.74	5.28	1.77
Minerals	Ca	mg/l	999.78	1,029.19	983.67
	Fe		1.52	1.61	1.49
	K		2,474.67	2,592.88	2,493.51
	Mg		161.23	168.60	161.05
	Mn		1.73	1.75	0.45
	Zn		0.80	0.85	0.82
	P		103.04	107.87	96.78

[a]mg/l, ppm; [b]Pasteurized CJM, a cabbage juice heated at 80°C for 60 min; [c]Sterilized CJM, a cabbage juice heated at 121°C for 15 min; [d]CJM after cell growth, a cabbage juice after growth of *L. mesenteroides* at 30°C for 24 h; [e]ND, not detected.

$^{+}$, $^{++}$, $^{+++}$, Concentrations increased ≤20%, ≤50%, and >50%, respectively, after each treatment.; $^{-}$, $^{--}$, $^{---}$, Concentrations decreased ≤20%, ≤50%, and >50%, respectively, after each treatment.

growth of *L. mesenteroides* ATCC8293, and proline and phenylalanine were necessary to stimulate the cell growth in the minimal medium, and thereby they could be metabolized during the cell cultivation in CJM. Notably, the levels of β-aminobutyric acid, thioproline, aspartic acid, 4-hydroxyproline, ornithine, glycine-proline, and proline-hydroxyproline (highest up to 1,800 mg/l) increased, and were speculated to be produced by hydrolysis of proteins

by a *L. mesenteroides* protease [21] or via metabolic reactions during fermentation. In the case of organic acids, the levels of malic acid and succinic acid decreased while acetic acid and lactic acid increased after cell cultivation, and this change was probably via heterolactic fermentation and/or malolactic fermentation of *Leuconostoc* spp. Vitamin analysis showed that high amounts of Vit. C > Vit. B_1 > Vit. B_3 > Vit. B_2 exist in CJM, and their levels varied a little except Vit. C, which was regarded as a stimulating compound for growth of *L. mesenteroides* ATCC 8293 [20]. Mineral analysis showed that K > Ca > Mg > P > Mn > Fe > Zn were present in CJM, and their levels decreased after cultivation owing to microbial consumption during their cell propagation. These results revealed that cabbage juice is rich in essential nutrients such as sugars, amino acids, vitamins, and minerals, and some of those key nutrients (sugars, essential amino acids, and Vit. C) were consumed during the cell growth of *L. mesenteroides*. Therefore, supplementing those key nutrients in CJM by addition of complex medium sources was considered effective to enhance the biomass productivity of CJM.

Growth Rate in CJM with Addition of Carbon Sources

Analysis of microbial growth in CJM demonstrated that carbon sources were consumed soon after 8 h during the cultivation of *L. mesenteroides*, and this depletion resulted in inhibition of further bacterial growth. To complement it, different concentrations (0.5%, 1.0%, or 1.5% (w/v)) of carbon sources (glucose, fructose, and sucrose) were added to CJM, and the OD value and organic acid concentrations were measured during the cell cultivation (Fig. 2A). When sucrose (0.5%, 1.0%, or 1.5% (w/v)) was added to 100% CJM, the viable cell count of *L. mesenteroides* increased in a concentration-dependant pattern (Fig. 2B); the viable cell counts of the control (no sucrose) and the sucrose media (0.5%, 1.0%, and 1.5%) were 1.27×10^9 CFU/ml < 1.47×10^9 CFU/ml < 1.55×10^9 CFU/ml < 1.77×10^9 CFU/ml, respectively. The OD and organic acid levels also increased in proportion to the bacterial count. The addition of sucrose to diluted CJM (70%, 50%, or 25% dilution) also facilitated bacterial growth.

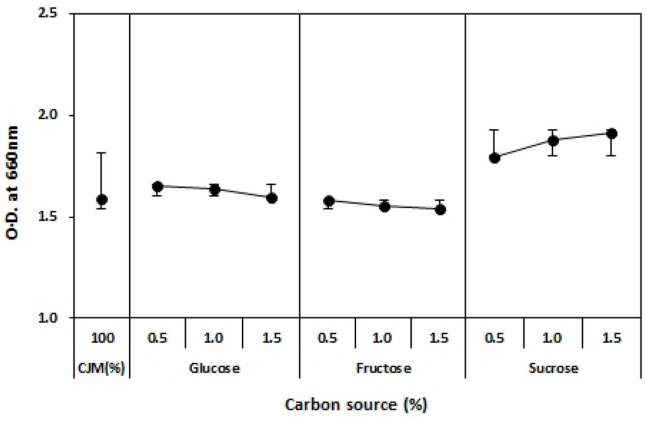

Fig. 2. Cell growth of *L. mesenteroides* in the cabbage juice medium (CJM) containing 0.5%, 1.0%, and 1.5% of glucose, fructose, and sucrose at 30°C for 24 h.
Values represent the mean of data.

Growth Rate in CJM with Addition of Nitrogen Sources

To supplement amino acids required for growth of *L. mesenteroides* KACC91744P, nitrogen sources (yeast extract, soy peptone, or fish peptone) were added at 0.5% and 1.0% (w/v) concentration. Fig. 3A shows that viable cell counts increased in all diluted media supplemented with nitrogen

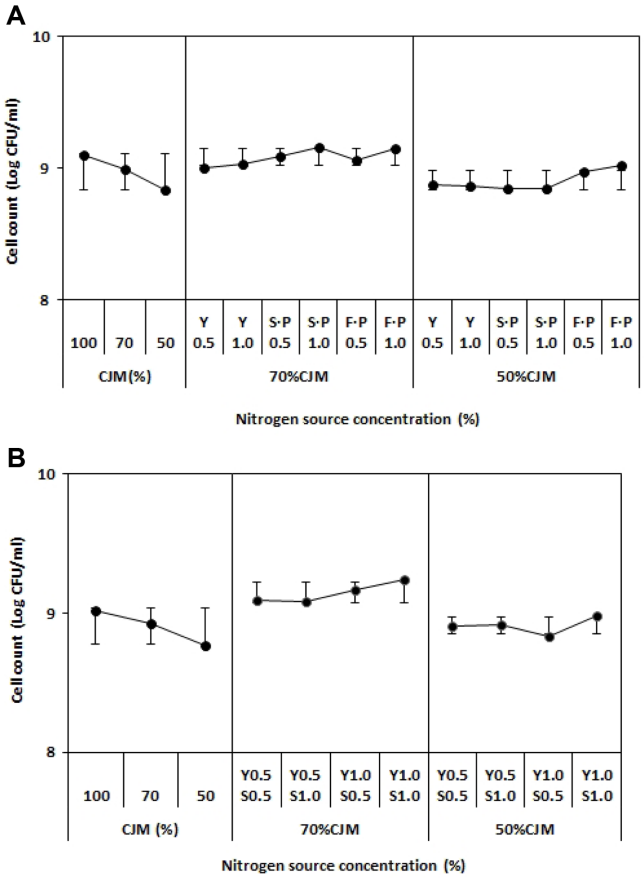

Fig. 3. Viable cell counts (CFU/ml) of *L. mesenteroides* in different concentrations of cabbage juice medium (CJM) containing nitrogen sources (yeast extract, soy peptone, and fish peptone) after cultivation at 30°C for 24 h.
Y: Yeast extract; S·P: Soy peptone; F·P: Fish peptone.

sources. The soy peptone in 70% CJM gave 1.45 × 10^9 CFU/ml, which was higher than the bacterial count (9.85 × 10^8 CFU/ml) in the existing 70% CJM without the supplemented nitrogen source. When mixtures of yeast extract and soy peptone were added (0.5% and 1.0% (w/v)) to investigate the synergistic effect of nitrogen sources, the combined nitrogen sources resulted in increase in viable cell counts (Fig. 3B). In particular, the bacterial count (1.20 × 10^9 CFU/ml) of diluted 70% CJM with combined nitrogen sources was higher than that (1.05 × 10^9 CFU/ml) of 100% CJM. This result indicated that addition of combined nitrogen sources enabled the volume expansion of CJM to reduce the cost. The highest viable cell count was found in CJM with 1% yeast extract + 1% soy peptone (Fig. 3B).

Growth Rate in CJM with Addition of Ark Shell Powder

The rapid pH reduction in CJM observed in the experiment of Fig. 1 was attributed to organic acids synthesized during the growth of *L. mesenteroides*. The antibacterial activity of organic acids has been known to be caused by the decline of cytoplasmic pH by ionization of acids that were not dissociated and by the permeability changes of the cell membrane, which affects the membrane transport mechanisms of cells [18, 22]. Therefore, a natural shell powder containing 36% $CaCO_3$ was used to reduce the soluble organic acid levels in the CJM during microbial growth. As shown in Fig. 4, when 1.5% or 2.0% (w/v) of shell powder was added in CJM, the lactic acid concentration in CJM decreased from 0.50% (blank) to 0.04% (in 1.5% sample), resulting in increment of the viable cell count up to 1.61 × 10^9 CFU/ml in CJM. It was postulated that the lactic acid produced during fermentation was converted into insoluble calcium salt through reaction with the calcium ion dissolved from shell powder [23].

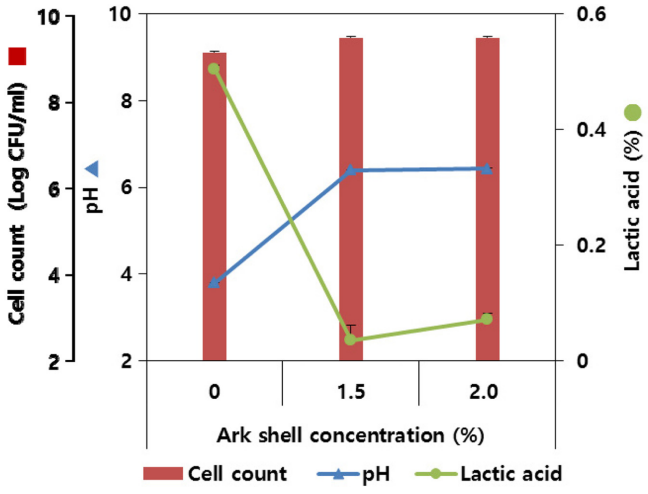

Fig. 4. Viable cell counts (CFU/ml) of *L. mesenteroides*, pH, and lactic acid concentration in the cabbage juice medium after cultivation at 30°C for 24 h.

Discussion

Recently, plant-derived LAB have gained attention due to their different physiological attributes, like better growth in harsh environments, compared with milk-derived LAB [24, 25]. For the effective growth of plant-derived LAB, a plant-derived medium would be necessary. Therefore, a vegetable juice medium has been developed to cultivate various LAB such as *Lactobacillus plantarum* and *Pediococcus acidilactici* by using vegetables (gourd, cabbage, carrot, cucumber, celery, green olives, *etc.*) [26]. The CJM developed in this study can also be used for industrial high-density production of various plant-derived LAB, after a proper modification of its composition depending on bacterial strains. Furthermore, a vegetable juice medium is necessary for the laboratory investigation of fermented vegetables such as kimchi and sauerkraut; a liquid type of sterilized medium simulating the fermentation environment is required for the reproducible monitoring of microbial growth and metabolite synthesis. Therefore, the CJM developed in this study can be used as an efficient vegetable juice medium that contains similar nutritional compositions of kimchi.

In conclusion, this study optimized a vegetable juice medium using cabbage extract for high-density cultivation of *L. mesenteroides* to be used as starter cultures for kimchi fermentation. For the nitrogen sources, 1% soy peptone in 70% CJM and a mixture of 1% yeast extract and 1% soy peptone were effective. Furthermore, an ark shell powder containing 36.0% $CaCO_3$ was determined as an effective additive to reduce the soluble lactic acid concentration and thereby to increase the viable cell counts. Therefore, we were able to develop an optimized, economical medium that produces comparable levels of *L. mesenteroides* KACC91744P as the MRS medium and that reduces the cost by almost 5-fold. This CJM can be used to cultivate various plant-derived LAB for industrial purposes after some modifications of its composition.

Acknowledgments

This work was supported by a research grant of Chungbuk National University in 2014 and the fund of the

strategic initiative for microbiomes in agriculture and food, Korean Ministry of Agriculture, Food and Rural Affairs (914002041SB).

Conflict of Interest

Eun Ji Jeong, Dae Won Moon, and Joon Suk Oh are employed in TOBICO that produces the *L. mesenteroides* starter in Korea.

References

1. Atrih A, Rekhif N, Michel M, Lefebvre G. 1993. Detection of bacteriocins produced by *Lactobacillus plantarum* strains isolated from different foods. *Can. J. Microbiol.* **75:** 117-140.
2. Cheigh HC, Park KY, Lee CY. 2009. Biochemical, microbiological, and nutritional aspects of kimchi. *Crit. Rev. Food Sci. Nutr.* **34:** 175-203.
3. Rhee JS, Lee JE, Lee CH. 2011. Importance of lactic acid bacteria in Asian fermented foods. *Microb. Cell Fact.* **10:** S5.
4. Li L, Shin SY, Lee KW, Han NS. 2014. Production of natural antimicrobial compound D-phenyllactic acid using *Leuconostoc mesenteroides* ATCC 8293 whole cells involving highly active D-lactate dehydrogenase. *Lett. Appl. Microbiol.* **59:** 404-411.
5. Fernández MF, Boris S, Barbés C. 2003. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J. Appl. Microbiol.* **94:** 449-455.
6. Jung JY, Lee SH, Jeon CY. 2014. Kimchi microflora: history, current status, and perspectives for industrial kimchi production. *Appl. Microbiol.* **98:** 2385-2393.
7. Kastner S, Perreten V, Bleuler H, Hugenschmidt G, Lacroix C, Meile L. 2006. Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria use in food. *Syst. Appl. Microbiol.* **19:** 145-155.
8. Chang JY, Chang HC. 2011. Growth inhibition of foodborne pathogens by kimchi prepared with bacteriocin-producing starter culture. *J. Food Sci.* **76:** 72-78.
9. Choi IK, Jung SH, Kim BJ, Park SY, Kim J, Han HU. 2003. Novel *Leuconostoc citreum* starter culture system for the fermentation of kimchi, a fermented cabbage product. *Antonie Van Leeuwenhoek* **84:** 247-253.
10. Jang YM, Shin WS, Lee DH, Kim SY, Park CH, Jeong YS, *et al.* 2009. Statistical optimization of production medium for enhanced production of itaconic acid biosynthesized by fungal cells of *Aspergills terreus*. *KSBB J.* **24:** 30-40.
11. Choi IJ, Nam HS, Lee HJ, Shin ZI, Oh TK. 1997. Medium optimization for production of thermostable akaline proteases from *Bacillus licheniformis* NS70. *Korean J. Appl. Microbiol. Biotechnol.* **25:** 207-211.
12. Zhao Y, Wang Y, Song Z, Shan C, Zhu R, Liu F. 2016. Development of a simple, low-cost and eurytopic medium based on *Pleurotus eryngii* for lactic acid bacteria. *AMB Express* **6:** 65-72.
13. Park HG, Jeon IC. 2004. Culture medium for lactic acid bacteria prepared from Chinese cabbage wastes. Korea Patent 10-2004-0062743.
14. Saguir FM, de Nadra CM. 2007. Improvement of a chemically defined medium for the sustained growth of *Lactobacillus plantarum*: nutritional requirement. *Curr. Microbiol.* **54:** 414-418.
15. Korea Food & Drug Administration. 2017. *Korean Food Standards Codex* **9:** 1.2.2.2-1.2.2.5.
16. Bae MS, Lee SC. 2008. Preparation and characteristics of kimchi with added *Styela clava*. *Korean J. Food Cookery Sci.* **24:** 573-579.
17. Otgonbayar GE, Eom HJ, Kim BS, Ko JH, Han NS. 2011. Mannitol production by *Leuconostoc citreum* KACC 91348P isolated from kimchi. *J. Microbiol. Biotechnol.* **21:** 968-971.
18. Ahn YS, Shin DH. 1999. Antimicrobial effects of organic acids and ethanol on several foodborne microoganisms. *Korean J. Food Sci. Technol.* **31:** 1315-1323.
19. Seong JH, Park EM, Kim HS, Kim DS. 2006. Contents of chemical constituents in organic Korea cabbage. *Korean J. Food Preserv.* **13:** 655-660.
20. Kim YJ, Eom HJ, Seo EY, Lee DY, Kim JH, Han NS. 2012. Development of a chemically defined minimal medium for the exponential growth of *Leuconostoc mesenteroides* ATCC8293. *J. Microbiol. Biotechnol.* **22:** 1518-1522.
21. Jeong JK, Zheng Y, Choi HS, Han GJ, Park KY. 2010. Catabolic enzyme activities and physiological functionalities of lactic acid bacteria isolated from Korean traditional meju. *J. Korean Soc. Food Sci. Nutr.* **39:** 1854-1859.
22. Kang KJ, Song HH, Kim YB, Chung DH, Lee C. 2004. Effect of adipic acid on growth of psychrotrophic kimchi lactic acid bacteria and its effect on mulkimchi fermentation. *J. Korean Soc. Food Sci. Nutr.* **33:** 857-863.
23. Kim MJ, Kim MH, Kim SD. 2003. Effect of water extracts of shellfish shell on fermentation and calcium content of kimchi. *J. Korean Soc. Food Sci. Nutr.* **32:** 161-166.
24. Cho YH, Park SN, Jeong SH. 2009. A study on the physiological activity and industrial prospects of plant-origin lactic acid bacteria. *Korean J. Dairy Sci. Technol.* **27:** 53-57.
25. Igarashi T. 2007. Development of beverage and food using plant origin lactic acid bacterium. *Bioindustry* **24:** 32-39.
26. Gardner NJ, Savard T, Obermeier P, Caldwell G, Champagne CP. 2001. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int. J. Food Microbiol.* **64:** 261-275.