BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **51(4)**, 271-278, 2016

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

Biotransformation of major ginsenoside Rb1 to pharmacologically active ginsenoside Rg3 through fermentation by *Weissella hellenica* DC06 in newly developed medium

Md. A. Huq[1], S. Akter[2], Yeon-Ju Kim[2], Mohamed El-Agamy Farh[1] and Deok-Chun Yang[1]*

[1]*Graduate School of Biotechnology and Ginseng Bank, College of Life Science, Kyung Hee University, Yongin, 446-701, Republic of Korea*

[2]*Department of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University, Yongin, 446-701, Republic of Korea*

Abstract

The study was conducted to develop an edible and low cost growth medium for cultivation of *Weissella hellenica* DC06, a lactic acid bacteria (LAB) and to study whether, the medium is suitable for bioconversion of major ginsenoside Rb1 into ginsenoside Rg3 through fermentation by *W. hellenica* DC06. Fourteen different media compositions were investigated to cultivate *W. hellenica* DC06. Among these, *W. hellenica* DC06 exhibited the highest growth in media containing 20 g/l radish, 20 g/l glucose, and 10 g/l yeast extract (Medium 3). The optical density of *W. hellenica* DC06 cultivated in medium 3 reached 1.8 (1.066 x 1010 CFU/ml) after 24 h of incubation. Importantly, the optimized medium was approximately four times cheaper compared to MRS medium. In addition to being economical, the new medium was also edible. Also *W. hellenica* DC06 showed strong fermentation ability in newly developed medium regarding on major ginsenoside Rb1 biotransformation. Ginsenoside Rb1 was converted into pharmacologically active ginsenoside Rg3 in new medium. In contrast,*W. hellenica* DC06 showed weak fermentation ability in MRS medium where ginsenoside Rb1 was converted intoginsenoside Rd. The transformation products were analyzed by TLC, and HPLC. Within seven days of fermentation, almost all ginsenoside Rb1 was decomposed and converted into Rg3 in optimized medium. *W. hellenica* DC06 hydrolyzed two glucose moieties attached to the C-20 position of the ginsenoside Rb1aglyconeand synthesized Rg3 in newly developed medium.

Keywords:Economical medium; *Weissellahellenica*; Ginsenoside; Biotransformation.

Introduction

Ginsenoside, a major component of ginseng, has been reported to exhibit various biological effects such as anticancer, anti-obesity,tumor-suppressing, hepatoprotective, neuroprotective effects (Huq *et al.*, 2015a; Lee *et al.,* 2005; Shinkai *et al.*, 1996; Tian *et al.,* 2005). Minor ginsenosides are more active than intact major ginsenosides (Huq *et al.*, 2015a). Therefore, many researchers have attempted to convert major ginsenosides to more active minor ginsenosides (Huq *et al.*, 2014; Huq *et al.,* 2015a,b,c). Researchers also have attempted to use lactic acid bacteria (LAB) for transformation of major ginsenosides into pharmacologically active minor ginsenosides because LAB has many positive effects on human health (Huq *et al*., 2014; Huq *et al.*, 2015c). As LAB cells are fastidious with respect to nutrient requirements, rich medium is required for good growth (Liew *et al*., 2005). The most common media for growth of LAB is Man Rogosa Sharpe medium (MRS) (de Man *et al.*, 1960; Rogosa *et al.,* 1961), which contains 25 g/l of a complex nitrogen source consisting of peptone (protein hydrolysate) and extracts of both meat and yeast. However, the cost of MRS media is prohibitive for use in commercial applications (Zhang and Greasham, 1999). Another important disadvantage of MRS medium is the presence of constituents of bovine origin, which should be avoided in food production because of risks related to the occurrence of bovine spongiform encephalopathy (Wells *et al.,* 1987).

*Corresponding author e-mail: deokchunyang@yahoo.co.kr

Radishes (*Raphanussativus* L.) can be used as an inexpensive source of essential microbial nutrients for a variety of purposes. It is a short duration crop and has very high growth rates. Radishes contain mostly protein, sugar, and vitamins (vitamin C, thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folate), as well as other important nutrients such as sodium, potassium, calcium, magnesium, phosphorus, iron, and zinc (USDA National Nutrient data base).

Weissella hellenica is a lactic acid bacterial species that is usually found in flounder intestine (Cai *et al.*, 1998), kimchi (Kim *et al.,* 2008), meat and meat products, and pickles, as well as some other sources. *W. hellenica* exhibits high antimicrobial activity by producing a bacteriocin called weissellicin that acts against pathogenic bacteria (Masuda *et al.,* 2012). In this study we isolated *W. hellenica* DC06 from a well-known Korean fermented food kimchi that is generally produce from radish and cabbage and developed an economical medium for cultivation of *W. hellenica* by using cheap natural sources. We also checked the major ginsenoside Rb1 fermentation ability of *W. hellenica* that was grown in new medium.

Materials and methods

The *W. hellenica* DC06 strain was isolated from kimchi.This strain has been deposited to the Korean Collection for Type Cultures (KCTC). Deposition number, KCTC 21039. Radish and cabbage were purchased from a local market. Glucose, sucrose, yeast extract, and MRS broth were purchased from Difco (Miller, Becton Dickinson and Co., MD, USA).Ginsenoside Rb1 and all standard ginsenosides were obtained from the Ginseng Genetic Resource Bank (Kyung Hee University, Yongin, Korea).

Preparation of media containing radish and cabbage powder

Radish and cabbage were cut into small pieces and dried in a 50°C oven for 24 h. After drying, radish and cabbage powders were prepared using a blender, mixed with distilled H_2O, and boiled at 100°C for 15 min. All of the nutrients from the radish and cabbage were extracted as a result of the boiling and heating procedures. The dissolved solutions were subjected to filtration after boiling to remove cell debris, which would have inhibited bacteria growth due to its high concentration. Finally, sugars and yeast extract were added to the filtered media and autoclavedat 121°C for 15 min.

Inoculum preparation

For seed culture, *W. hellenica* DC06 was grown on MRS agar plates at 37°C for two days. Single colonies were selected from petri dishes and inoculated into MRS broth (pH 6.5) followed by incubation at 37°C with shaking (160 rpm) overnight.

Culture conditions

Fourteen different media compositions were designed that containing varying amounts of radish, cabbage, sugars, and yeast extract (Table I) for cultivation of *W. hellenica* DC06 as described below. Cultivation was carried out in 100 ml flasks with 50 ml media for 72 h. One ml of seed culture was inoculated in each medium, and the temperature was maintained at 37°C with shaking at 160 rpm. pH was maintained at 6.5 by using NaOH. During incubation, growth was monitored by the measurement of OD (optical density) at 600 nm at 24 h intervals. In addition, growth in optimal medium was compared with that of MRS medium.

Media optimization

For optimization of media composition used different concentration of radish (5 g/l, 10 g/l, 20 g/l, 30g/l), cabbage (5 g/l, 10 g/l, 20 g/l), glucose (10 g/l, 20 g/l) and yeast extract (5 g/l, 10 g/l).

Fermentation of major ginsenoside Rb1 by W. hellenica DC06 in newly developed medium

The fermentation procedure was carried out in a 50 ml screw-cap tube. Single colonies of *W. hellenica* DC06 were selected from petri dishes and inoculated into 10 ml of newly developed medium and then 2 mg ginsenoside Rb1 was inserted into the medium. The mixture was then incubated at 37°C with shaking (160 rpm). Samples were withdrawn at regular intervals (every 24 h), and water-saturated n-butanol was added to each sample to stop the reaction. Subsequently, the butanol fraction was allowed to evaporate completely, and the methanol extract was analyzed by TLC and HPLC.

Table I. Growth rates of *Weissella hellenica* DC06 in different media compositions with the mean ± standard deviation (SD) of replicates g/l.

Medium	Radish (g/1)	Cabbage (g/1)	Glucose (g/1)	Sucrose (g/1)	Yeast extract (g/1)	Optical density (OD) at 600nm 24 (h)	48(h)	72(h)
1	20.00	0.00	0.00	0.00	0.00	0.995 ± 0.027	1.097 ± 0.134	1.017 ± 0.179
2	20.00	0.00	10.00	0.00	10.00	1.643 ± 0.042	1.756 ± 0.063	1.470 ± 0.092
3	20.00	0.00	20.00	0.00	10.00	1.805 ± 0.070	1.950 ± 0.098	1.967 ± 0.133
4	20.00	0.00	0.00	20.00	10.00	1.608 ± 0.090	1.676 ± 0.069	1.462 ± 0.057
5	20.00	0.00	20.00	0.00	5.00	1.515 ± 0.119	1.666 ± 0.050	1.314 ± 0.183
6	30.00	0.00	20.00	0.00	10.00	1.753 ± 0.089	1.886 ± 0.083	1.862 ± 0.035
7	10.00	0.00	20.00	0.00	10.00	1.641 ± 0.124	1.783 ± 0.067	1.772 ± 0.093
8	5.00	0.00	20.00	0.00	10.00	1.047 ± 0.212	0.957 ± 0.096	0.892 ± 0.063
9	0.00	5.00	20.00	0.00	10.00	1.496 ± 0.023	1.497 ± 0.011	1.453 ± 0.006
10	0.00	10.00	20.00	0.00	10.00	1.505 ± 0.016	1.719 ± 0.134	1.674 ± 0.063
11	0.00	20.00	20.00	0.00	10.00	1.365 ± 0.093	1.511 ± 0.111	1.620 ± 0.05.7
12	5.00	5.00	20.00	0.00	10.00	1.594 ± 0.074	1.599 ± 0.082	1.572 ± 0.064
13	10.00	10.00	20.00	0.00	10.00	1.728 ± 0.070	1.736 ± 0.060	1.683 ± 0.065
14	10.00	5.00	20.00	0.00	10.00	1.667 ± 0.062	1.683 ± 0.069	1.672 ± 0.064
MRS						2.28 ± 0.093	2459 ± 0.033	2.496 ± 0.032

TLC analysis of ginsenosides

TLC analysis was carried out using silica gel plates (60F254; Merck, Darmstadt, Germany) with a solvent system of $CHCl_3$:CH_3OH:H_2O (65:35:10 v/v/v) as the developer. The spots on the TLC plates were sprayed with 10% (v/v) H_2SO_4 and heated at 110°C for 10 min before detection.

HPLC analysis for biotransformation of ginsenosides

HPLC-grade acetonitrile and water were purchased from SK Chemicals (Ulsan, Korea). The reaction mixture was extracted with *n*-butanol saturated with H_2O, evaporated *in vacuo,* and the residue was dissolved in CH_3OH and applied to the HPLC analysis. HPLC was performed using a C18 (50 × 4.6 mm, Kinetex 2.6u C18 100A) column with acetonitrile (solvent A) and distilled water (solvent B) as the mobile phases with 85% B for 5 min, 79% B for 20 min, 42% B for 55 min, 10% B for 12 min, and 85% B for 18 min, at a flow rate of 1 ml/min. The sample was detected at UV 203 nm.

Statistical analysis

All data are presented as mean ± standard error (S.E.), and all experiments were independently performed three times.

Results and discussion

Determination of an economical medium

The growth activity of *W. hellenica* DC06 in 14 different media compositions varied significantly (Fig. 1). Among the 14 media types, *W. hellenica* DC06 exhibited the highest rate of growth in Medium 3, which contained 20 g/l radish, 20 g/l glucose, and 10 g/l yeast extract. Specifically, after incubation for 24 h *W. hellenica* DC06 had an optical density at 600 nm of 1.805 and 1.066 x 10^{10}CFU/ml. After 48 and 72 h optical density was near constant, reaching 1.950 and 1.967, respectively. With respect to cabbage, we used 5 g/l, 10 g/l and 20 g/l cabbage with 20 g/l glucose and 10 g/l yeast extract; however, none of these composition resulted in better growth compared with Medium 3, which contained 20 g/l radish. Here also used radish and cabbage together with glucose and yeast extract, but they did not exhibit as good of a growth rate as observed for Medium 3 (Fig. 1).

For optimization of radish containing media, four different concentrations of radish was tested with glucose (20g/l) and yeast extract (10g/l). Among these, 20 g/lradish produced maximal growth. Conversely, 30 g/l radish resulted in a slightly lower growth rate (OD, 1.75) after 24 h of incubation. Likewise, growth of *W. hellenica* DC06 in media con-

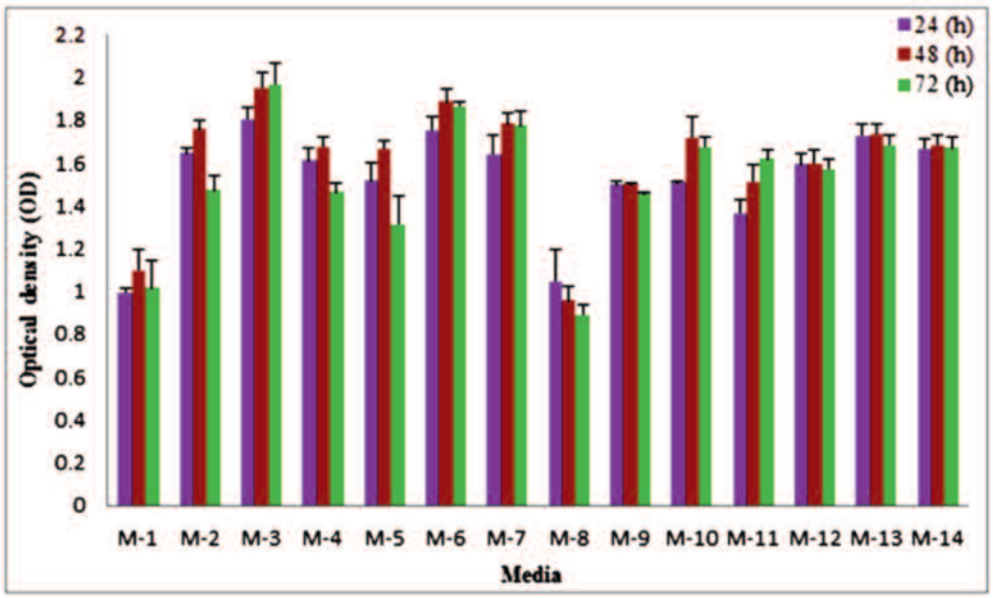

Fig. 1. Growth rates of *W. hellenica* DC06 in fourteen different media compositions with the mean ± standard error.

taining 5 g/l and 10 g/l radish was poor due to insufficient nutrients. For sugar optimization, glucose (10 g/l and 20 g/l) and sucrose (20 g/l) were tested with 20 g/l radish and 10 g/l yeast extract. Glucose (20 g/l) supplementation yielded a higher growth rate than sucrose. With respect to yeast extract, we tested 5 g/land 10 g/lyeast extract with 20 g/l radish and 20 g/l glucose. Between these two compositions, media containing 10 g/l yeast extract resulted in better growth compared with 5 g/l yeast extract (Table I).

Fig. 2 shows a comparison of the growth of the analyzed strain in MRS and optimal media. The optical density of *W. hellenica* DC06 cultivated in optimal medium reached 1.8 (1.066 x 10^{10}CFU/ml) after incubation for 24 h, which was slightly less compared to bacterial growth in MRS medium (optical density, 2.2 and 1.44 x 1010CFU/ml) under the same conditions. Further, in addition to being edible, the new medium was approximately four times cheaper compared to MRS medium.

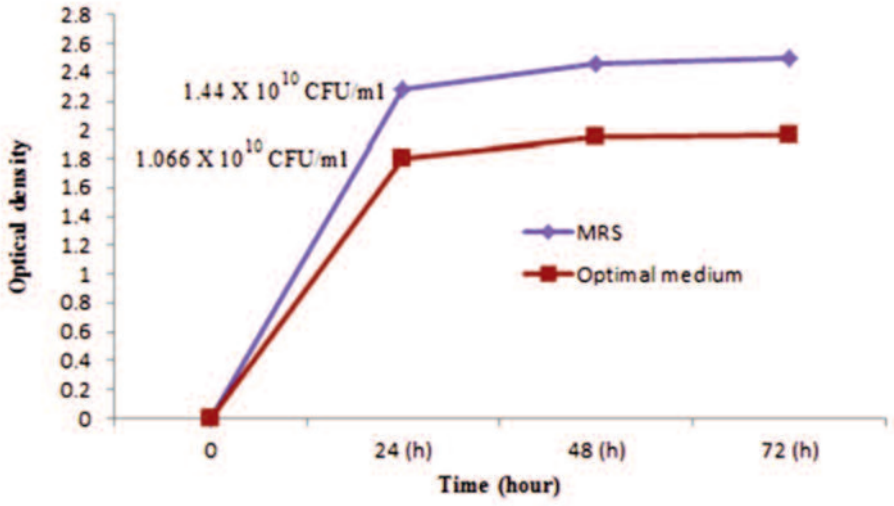

Fig. 2. Growth of *W. hellenica* DC06 in optimal medium (medium-3) compared with MRS medium.

Fermentation of major ginsenoside Rb1 by *W. hellenica* DC06 in newly developed medium *W. hellenica* DC06 showed strong activity in newly developed medium in terms of ginsenoside biotransformation. Ginsenoside Rb1 was converted into pharmacologically active ginsenoside Rg3 by hydrolysis of two glucose units at the C-20 position of the ginsenosideaglycone. As shown in Fig. 3A, most of the ginsenoside Rb1 was transformed by *W. hellenica* DC06 into Rg3 within 5 days of fermentation and after 7 days of fermentation almost all ginsenoside Rb1 was transformed and converted into Rg3 (Fig. 3A). Ginsenoside Rb1 transformation ability of *W. hellenica* DC06 in MRS medium was compared with that of developed medium.As shown in Fig.3B, transformation activity of *W. hellenica* DC06 was weak, when *W. hellenica* DC06 was cultured in MRS medium with ginsenoside Rb1. After 7 days of fermentation in MRS medium *W. hellenica* transformed most of the ginsenoside Rb1 into Rd by hydrolysis of one glucose units at the C-20 position of the ginsenosideaglycone, but they could not transformed ginsenoside Rb1 to Rg3 in MRS medium. The effect of radish medium for biotransformation of ginsenoside Rb1 was also investigated but there was no effect of radish medium without lactic acid bacteria for Rb1 transformation (Fig. 3C).

The biotransformation of major ginsenoside Rb1 by *W. hellenica* DC06 was confirmed using quantitative HPLC analysis (Fig. 4). The peaks with retention times of 17.47, 21.35, 27.03, 28.6, 30.8, and 31.16 min correspond to ginsenosides Rb1, Rd, F2, Rg3, CK, and Rh2, respectively (Fig. 4A). Fig. 4B shows the control of ginsenoside Rb1. The peak for ginsenosides Rb1 was no longer present within 7 days fermentation and one new peak that had retention time consistent with those of Rg3 was observed (Fig. 4C). Figure 4C showed the metabolite of ginsenoside Rb1 by *W. hellenica* DC06 that was grown in newly developed medium. *W. hellenica* DC06 produced ginsenoside Rg3 from Rb1 by hydrolysis of two glucose units at the C-20 position of the ginsenosideaglycone. The transformation of ginsenosideRb1 by *W. hellenica* DC06 in MRS medium was also confirmed using quantitative HPLC analysis (Fig. 4D). After 7 days of fermentation, ginsenoside Rb1 was decomposed and converted into Rd by *W. hellenica* but they did not produce pharmacologically active minor ginsenoside Rg3 when used MRS medium (Fig. 4D). So, from TLC and HPLC data it is confirmed that *W. hellenica* DC06 showed strong activity in newly developed medium regarding on major ginsenoside Rb1 biotransformation into pharmacologically active minor ginsenoside Rg3.

The growth results showed that growth of *W. hellenica* DC06 with developed media containing 20 g/l radish, 20 g/l glucose, and 10 g/l yeast extract supported sufficient growth that was slightly less than that obtained with the much expensive and non-edible MRS medium. The new medium containing only 10 g/l yeast extract as a protein source compared with MRS medium which contains 25 g/l of complex nitrogen sources. Yeast extract as a protein sourcewas usedin this study because of its relatively cheap cost compared with pro-

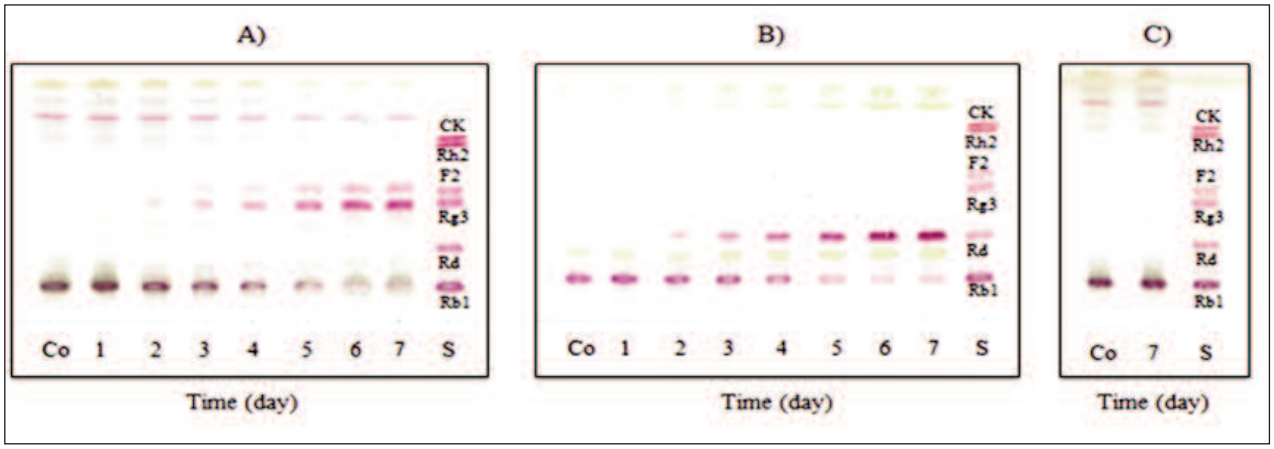

Fig. 3. TLC profile of transformation of major ginsenoside Rb1 by *W. hellenica* DC06.A), fermentation of ginsenoside Rb1 in new medium; B), fermentation of ginsenoside Rb1 in MRS medium; C), fermentation of ginsenoside Rb1 in new medium without *W. hellenica* DC06 (Co = control; and S =saponin standards).

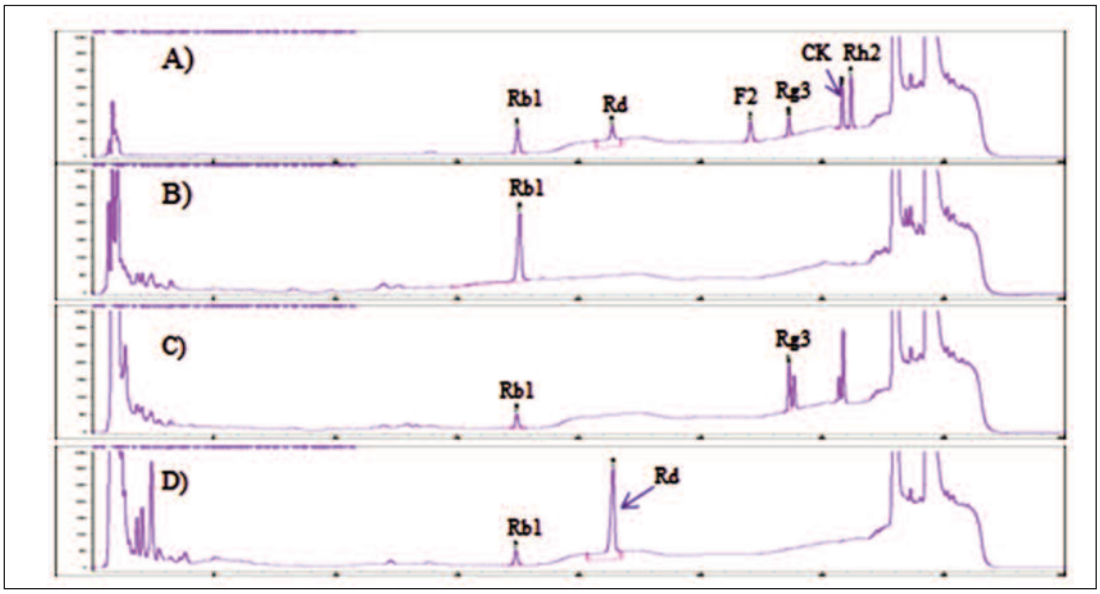

Fig. 4. HPLC profile of transformation of major ginsenoside Rb1 by *W. hellenica* DC06 in newly developed medium.A) Ginsenoside standards; B) ginsenoside Rb1 control; C) fermentation of ginsenoside Rb1 by *W. hellenica* in new medium and D) fermentation of ginsenoside Rb1 by *W. hellenica* in MRS medium.

tein sources used for MRS media, namely, peptospecial and beef extract. In addition, yeast extract is known to be a rich source of vitamins. Radishes also contain sufficiently high levels of protein at 680 mg per 100 g of fresh root (USDA National Nutrient data base). Thus, radish also served as a protein source in the optimized medium.

While MRS medium contains several different types of supplemented micronutrients, no such micronutrientswere directly added to the new medium, and it only contained radish with carbon and protein sources. Thus, it was assumed that radish supplied necessary micronutrients because it contains sufficient amounts of sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, and different vitamins (USDA National Nutrient data base), all of which may have contributed to its suitability for growing LAB.

Radishes contain significant amount of carbohydrates; however, glucose was used as a supplemental carbon source in the optimized medium because it produced better growth compared with sucrose. We also tested cabbage during development of the optimized medium (Table I); however, it facilitated less growth compared with radish.

Most LAB strains have similar growth requirements. So, new medium can be a good candidate for cultivation of other LABs. Moreover, the new medium was less expensive compared to MRS media: 1 liter of the new medium cost approximately 3 USD while 1 liter of MRS broth purchased from Sigma-Aldrich costs more than 10 USD. Another less using medium for cultivation of *Lactobacillus* is Tomato Juice Broth but it is around 10 times more expensive than new medium and 3 times more expensive than MRS medium according to price of DIFCO.Another important feature of the new medium is that it can be consumed by humans, and thus may be used by the food industry for cultivation of LAB strains. Along these same lines, there have been several efforts reported to date to develop an economical medium for LAB growth, including *Lactobacillus fermentum* (Gao *et al.*, 2009), *Lactobacillus rhamnosus* (Berecka *et al*., 2010) and *Lactobacillus plantarum* (Horn *et al.,* 2005). However, all of these media types are also more expensive than the newly described optimized medium.

Ginsenoside, a major component of ginseng, has been reported to exhibit various biological effects (Cho *et al*., 2006; Huq *et al.*, 2015a). Minor ginsenosides are more active than intact major ginsenosides(Huq *et al*., 2015a). Therefore, many researchers have attempted to convert major ginsenosides to more active minor ginsenosides (Huq *et al.*, 2014; Huq *et al.,* 2015a,b). Minor ginsenoside Rg3 has strong anti-metastatic (Mochizuki *et al.,* 1995), tumor-suppressing (Shinkai *et al.,* 1996), hepatoprotective (Lee *et al*., 2005), neuroprotective (Tian *et al.,* 2005) and vasodilating

effects (Kim *et al.*, 2003). This pharmacologically active ginsenoside Rg3 is not available in ginseng plant. In this study newly developed medium was used to produce available ginsenoside Rg3 from major ginsenoside Rb1 by *W. hellenica* DC06. Fermentation of ginsenoside Rb1 by *W. hellenica* DC06 using newly developed media produced Rg3 by hydrolysis of two glucose units at the C-20 position of the ginsenoside Rb1 aglycone, while used MRS medium for fermentation of ginsenoside Rb1 by *W. hellenica* DC06, the activity of biotransformation was decreased and *W. hellenica* DC06 produced Rd by hydrolysis of one glucose unit at the C-20 position of the ginsenosideaglycone. The new medium may be enhanced the production of beta-glucosidase enzyme by *W. hellenica* DC06 that can easily convert the major ginsenoside Rb1 into pharmacologically active minor ginsenoside Rg3.

In conclusion, the results of this study demonstrated that media containing radishes may provide a good alternative to MRS for growing *W. hellenica* DC06 and other LAB strains with similar nutritional requirements. In addition, the new optimized medium may be potentially useful for industrial cultivation of *W. hellenica* DC06 and other lactic acid bacteria, especially in the food industry and for the synthesis of pharmacological active ginsenoside Rg3.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) (#20110015122), and is also supported by a grant from the Korea Institute of Planning and Evaluations for the Technology of Food, Agriculture, Forestry, and Fisheries (#111035-03-1-SB010).

References

Berecka MP, Wasko A, Wiater MK, Podlesny M, Targonski Z and Komar AK (2010), Optimization of medium composition for enhancing growth of *Lactobacillus rhamnosus* PEN using response surface methodology, *Polish journal of Microbiol*. **59**:113-118.

Cai Y, Benno Y, Nakase T and Oh TK (1998), Specific probiotic characterization of *Weissellahellenica* DS-12 isolated from flounder intestine, *J. Gen. Appl. Microbiol.* **44:** 311-316.

Cho W, Chung W, Lee S, Leung A, Cheng C and Yue K (2006), Ginsenoside Re of *Panax ginseng* possesses significant antioxidant andanti-hyperlipidemic efficacies in streptozotocin-induced diabeticrats, *Eur. J. Pharmacol.* **550:** 173-179.

De Man JC, Rogosa M and Sharpe ME (1960), A medium for the cultivation of lactobacilli, *J. Appl. Bacteriol.* **23:** 130-135.

Gao X, Qiao SY and Lu WQ (2009), Determination of an economical medium for growth of *Lactobacillus fermentum* using response surface methodology, *Lett. Appl. Microbiol.* **49**: 556-561.

Horn SJ, Aspmo SI and Eijsink VGH (2005), Growth of *Lactobacillus plantarum* in media containing hydrolysates of fish viscera, *J. Appl. Microbiol.* **99**: 1082-1089.

Huq MA, Kim YJ, Min JW, Bae KS and Yang DC (2014), Use of *Lactobacillus rossiae* DC05 for bioconversion of the major ginsenosides Rb1 and Re into the pharmacologically active ginsenosides CK and Rg2, *Food Sci. Biotechnol*. **23**: 1561-1567.

Huq MA, Siraj FM, Kim YJ and Yang DC (2015a), Enzymatic transformation of ginseng leaf saponin by recombinant β-glucosidase (bgp1) and its efficacy in an adipocyte cell line, *Biotechnology and Applied Biochemistry* DOI: 10.1002/bab.1400.

Huq MA, Kim YJ, Hoang VA, Siddiqi MZ and Yang DC(2015b), *Paenibacillusginsengiterrae* sp. nov., a ginsenoside-hydrolyzing bacteria isolated from soil of ginseng field, *Archives of Microbiology* **197**: 389-396.

Huq MA, Kim YJ, Min JW, Siraj FM, Siddiqi MZ and Yang DC (2015c), Enzymatic transformation of the major ginsenoside Rb1 to compound K by *Weissella hellenica* DC06, *Indian Journal of Biotechnology* **14:** 270-275.

Kim MJ, Seo HN, Hwang TS, Lee SH and Park DH (2008), Characterization of exopolysaccharide (EPS) produced by *Weissella hellenica* SKkimchi3 isolated from kimchi, *J. Microbiology* **46**: 535-541.

Kim ND, Kim EM, Kang KW, Cho MK, Choi SY and Kim SG (2003), Ginsenoside Rg3 inhibits phenylephrine-

induced vascular contraction through induction of nitric oxide synthase, *Br. J. Pharmacol*. **140:** 661-670.

Lee HU, Bae EA, Han MJ and Kim DH (2005), Hepatoprotective effect of 20(S)-ginsenosides Rg3 and its metabolite 20(S)-ginsenoside Rh2 on tert-butyl hydroperoxide-induced liver injury, *Biol. Pharm. Bull.* **28**: 1992-1994.

Liew SL, Ariff AB, Raha AR and Ho YW (2005), Optimization of medium composition for the production of a probiotic microorganism, *Lactobacillus rhamnosus,* using response surface methodology, *Int. J. Food Microbiol*. **102:** 137-142.

Masuda Y, Zendo T, Sawa N, Perez RH, Nakayama J and Sonomoto K (2012), Characterization and identification of weissellicin Y and weissellicin M, novel bacteriocins produced by *Weissella hellenica* QU 13. *J. Appl. Microbiol.* **112**: 99-108.

Mochizuki M, Yoo YC and Matsuzawa K (1995), Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside Rb2, 20(R)- and 20(S)-ginsenoside Rg3, of red ginseng, *Biol. Pharm. Bull*. **18**: 1197-1202.

Rogosa M, Franklin JG and Perry KD (1961), Correlation of vitamin requirements with cultural and biochemical characters of *Lactobacillus sub, Appl. Microbiol. Biotechnol. **24:** 473-482.*

Shinkai K, Akedo H, Mukai M, Imamura F, Isoai A, Kobayashi M and Kitagawa I (1996), Inhibition of in vitro tumor cell invasion by ginsenoside Rg3. *Japan J. Cancer Res*. **87**: 357-362.

Tian JW, Fu FH, Geng MY, Jiang YT, Yang JX, Jiang WL, Wang CY and Liu K (2005), Neuroprotective effect of 20(S)-ginsenoside Rg3 on cerebral ischemia in rats, *Neuroscience Letters* **374**: 92-97.

USDA National Nutrient data base, Radish (*Raphanussativus*), Fresh, raw, Nutrition Value per 100 g.

Wells GAH, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M and Bradley R (1987), A novel progressive spongiform encephalopathy in cattle, *Vet. Re.* **121**: 419-420.

Zhang J and Greasham R (1999), Chemically defined media for commercial fermentations, *Appl. Microbiol. Biotechnol.* **51**: 407-421.

Received: 26 April 2016; Revised:06 June 2016; Accepted: 28 July 2016.