KOREAN J ORGANIC AGRI
Volume 21, Number 3: 467-476, September 2013
http://dx.doi.org/10.11625/KJOA.2013.21.3.467

ISSN 1229-3571 (Print)
ISSN 2287-819X (Online)

Effects of Different Starter Cultures on the Antibacterial and Antioxidant Activity of Ethanol Extract from Fermented *Chelidonium majus* var. *asiaticum*

Ham, Young-Joo*·Shin, Young-Keun**·Choi, Nag-Jin***·Kang, Sang-Mo****

발효 애기똥풀 주정추출물의 항균 및 항산화활성에 있어 발효 균주의 효과

함영주·신영근·최낙진·강상모

This study was conducted to investigate the effect of fermentation on biological activity of *Chelidonium majus* var *asiaticum* and to screen effective starter culture strains. Antibacterial activity against to *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella gallinarum* and antioxidant activity as free radical scavenging activity by using DPPH were tested. Total six starter culture strains, two of *Lactobacillus brevis*, one of *Lactobacillus plantarum* and three of *Saccharomyces cerevisiae* were used. Plant extract was prepared after fermentation by using ethanol. All strains showed normal growth in viable cell counts of fermented cultures and *L. plantarum* showed the highest cell growth significantly ($p<0.05$). In antibacterial activities of extracts, the activity was found only in the extract from the fermentation using *L. plantarum*. In antioxidant activity, the highest activity was shown in the fermentation using *L. plantarum* significantly ($P<0.05$). Newly produced spots in two of three elution systems on TLC-DPPH test were detected in the fermentation using *L. plantarum*.

Key words: *C. majus var asiaticum, fermentation, antibacterial activity, antioxidant activity, TLC-DPPH*

* 건국대학교 생물공학과
** 주식회사 엠케이생명과학
*** Corresponding authors, 전북대학교 동물자원과학과(E-mail : nagjin@jbnu.ac.kr)
**** Corresponding authors, 건국대학교 생물공학과(E-mail : kangsm@konkuk.ac.kr)

Ⅰ. Introduction

In animal, oxidation is essential for energy acquisition through catabolic process of nutrient. During oxidation, various reactive oxygen species (ROS) are constantly produced, and also these compounds trigger oxidative damages related to cell death and tissue disruption. These reactive and harmful oxygen species are called as free radicals (Lee et al., 2003; Masoko et al., 2007). In normal physiological condition, the body has enough defense systems to protect the body from ROS. However once ROS in the body changed to high levels by elevated energy metabolism such as high intake of energy or infections, the body turns to oxidative stress condition and that can cause various disorders (Masoko et al., 2007). Therefore appropriate and effective control for the reduction of oxidative stress should be performed during intensive feeding period. Particularly during the fattening periods for animal when high energy diet was introduced to animal. Recently, the natural phytochemicals present in various plants such as wild flowers, crops, tea plants, fruit and vegetables have gathered their interests in the development of antibiotics alternatives in animal feed and human food industries (Lee et al., 2003). The most well known antioxidative phytochemicals are polyphenolic and flavonoid compounds because they are an important part of plant's natural defense mechanism against to temperature alteration, UV exposure and pathogenic infections (Chirinos et al., 2007; Faller et al., 2010). And the antioxidant activity of phytochemicals can be improved by the action of micro-organism through fermentation (Hong, 2011; Juan et al., 2010). *C. majus* var *asiaticum* is a biennial plant involved in Papaveraceae. Its aerial part and flower have been used for an ingredient in oriental medicine in Korea and its beneficial effects on various chronic disorders through antioxidant activity was reported (Jung et al., 2011). The present study investigated the effect of fermentation of *C. majus* var *asiaticum* using different starter culture on antioxidant activity.

Ⅱ. Materials and methods

1. Starter strains and culture conditions

Total 6 different starter strains were used. Two *Lactobacillus brevis* were used in treatments A (TrA) and B (TrB). *Lactobacillus plantarum* was used in treatment C (TrC) and three *Saccharomyces cerevisiae* were used in treatments D (TrD), E (TrE) and F (TrF). All micro-organisms were friendly donated from MK-Bioscience company (Suwon, Korea). All starter

strains were maintained on MRS broth (Difco, USA) and incubated in 30℃ with agitation (150 rpm). Seed culture was prepared in same medium and same culture conditions for 24 h of incubation.

2. Fermentation and extraction

C. majus var *asiaticum* was purchased from medicinal plant market located in Seoul (Kyung-dong market, Seoul) and it was ground by cutter miller (HR2860, Philips). Aliquot 10 g of fine powder of *C. majus* var *asiaticum* was added into 90 mL MRS broth in 250 mL erlenmeyer flask to prepare fermentation culture and then it was autoclaved (121℃, 15 min). Seed culture was inoculated to the prepared fermentation culture at 3% (v/v) rate. The inoculated culture was incubated at 30℃ for 48 h. After incubation, 2 mL of culture broth was taken for the analysis of viable cell counts and the remaining broth was placed on aluminum dish and then dried at 60℃ until completely drying (about 24 h). The dried culture broth was ground using mortar and 2 g of powder was suspended in 99.9% ethyl alcohol for extraction. Then extraction was performed at 30℃ shaking incubator with agitation (150 rpm) for 24 h. The extract was filtered thorough filter paper (Whatman No 1) and the ethanol in filtrate was evaporated using rotary evaporator (N-1110, EYELA, Japan). The concentrated extract was re-dissolved in 2 mL of ethanol and stored in - 20℃ freezer until use.

3. Viable cell count

Viability of inoculated micro-organism was analyzed by viable colony count after serial dilution. Aliquot 1 mL of culture broth was diluted using 9 mL of sterilized 0.8% (w/v) NaCl solution (10 fold dilution rate) and then the diluted sample was serially diluted further more 5 steps to reach 10^{-6} dilutions. Each diluted samples were spread on MRS solid plate and then incubated until countable colony was appeared. The number of colony on the plate was counted and used for the assessment of normal fermentation.

4. Antibacterial activity test

Antibacterial activity of extract was tested by using agar well diffusion method. For pathogenic bacteria, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella gallinarum* were used. Pathogenic bacteria was grown in LB broth at 37℃ shaking incubator for 24 h and 0.1

mL of culture broth was added into 0.8% agar solution which was maintained at 50℃ after autoclaving to prevent solidification. And then the pathogenic bacterial suspension was immediately pour on LB solid plate and cooled at room temperature to be solidified. Aliquot 30 μL of ethanol extract was dropped on paper disk (7 mm in diameter) and the disk was placed on the LB solid plate over-laid pathogenic bacterial suspension. The assay plate was incubated at 37 ℃ incubator and the diameter of appeared clear zone was measured.

5. Antioxidant activity test

Antioxidant activity of extract was examined by measuring free radical scavenging activity using DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl, Sigma, USA) reagent. Briefly, 0.5 mL of diluted extract was mixed with 2.0 mL of DPPH solution (0.2 mM in ethyl alcohol) and then the mixture was incubated at room temperature for 15 min. After incubation, 2.5 mL of distilled water was added and then the observance was measured at 525 nm using spectrophotometer (Optizen, Mecasys, Korea). Antioxidant activity was expressed as EC50, the concentration of extract showing 50% free radical scavenging activity.

6. TLC-DPPH screening assay

Antioxidant compound in extract was screened by thin layer chromatography (TLC) according to Mosoko and Eloff (2007). Aluminum-back TLC silica gel F254 plate was used. The TLC plate was developed on three elutions system: ethyl acetate/methanol/water (EMW, 40:5.4:5, for polar/neutral), chloroform/ethyl acetate/formic acid (CEF, 5:4:1, for intermediate polarity/acidic) and benzene/ethanol/ammonium hydroxide (BEA, 90:10:1, for non-polar/basic). After development, the plate was dried under fume hood and the chromatogram was sprayed by 0.2% DPPH solution (w/v, in methanol).

7. Statistical analysis

Evaluation of significance was performed using general linear model and Duncan's multiple comparison. For statistical software, SPSS (version 18, IBM, USA) program was used.

Ⅲ. Results and Discussion

1. Cell growth

Microbial cell growth in the MRS medium containing 10% (w/v) *C. majus* var *asiaticum* was summarized in Figure 1. All strains showed cell growth over 108 CFU/mL and the significantly highest cell growth was found in TrC (*Lactobacillus plantarum*, $p<0.05$). From cell growth results, all strains used in this experiment were found to be suitable for the fermentation of *C. majus* var *asiaticum*. In this experiment, 3% (v/v) of inoculation rate was used and the cell content in seed culture was about 108 CFU/mL. So the counts for cell growth in fermentation broth could be supposed to be 100 times higher than initial cell counts.

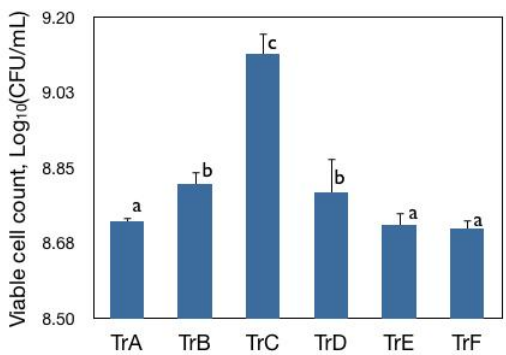

Fig. 1. Cell growth of starter culture strains in MRS medium containing 10% (w/v) *C. majus* var *asiaticum*. In treatment, TrA and TrB were *Lactobacillus brevis*, TrC was *Lactobacillus plantarum* and TrD, TrE and TrF were *Saccharomyces cerevisiae*

2. Antibacterial activity

Antibacterial activity is one of important features of plant extract and that can determine the value of plant extract in industrial application. Antibacterial activities of extracts were represented in Table 1. The activity was found only in TrC where *Lactobacillus plantarum* was used as starter culture. Interestingly no the antibacterial activity was detected in control, without inoculation, whereas fermentation using *Lactobacillus plantarum* showed antibacterial activity against to all tested pathogenic bacteria. *Lactobacillus plantarum* is a frequently isolated mesophilic lactic acid bacteria (LAB) and it is widely present in nature and one of famous LAB in industry

for food processing. It was reported that some of *Lactobacillus plantarum* have shown an ability reduce and suppress pathogenic bacteria and improve antioxidant activity (Chang et al., 2010; Tallon et al., 2003).

Table 1. Antibacterial activity against *Staph. aureus*, *L. monocytogenes* and *Sal. gallinarum* of ethanol extract of fermented *C. majus* var *asiaticum* using different starter culture strains

Pathogenic bacteria	Treatment						
	CON	TrA	TrB	TrC	TrD	TrE	TrF
	clear zone, mm						
Staph. aureus	ND	ND	ND	16	ND	ND	ND
L. monocytogenes	ND	ND	ND	12	ND	ND	ND
S. gallinarum	ND	ND	ND	10	ND	ND	ND

Treatment : CON, not fermented; TrA and TrB were *Lactobacillus brevis*; TrC, *Lactobacillus plantarum*; TrD, TrE and TrF were *Saccharomyces cerevisiae*

ND, not detected

3. Antioxidant activity

Free radical scavenging activity test using DPPH is based on the feature of electron-donating activity of compound in extract. Electrons or hydrogen can be donated by this activity of compound to DPPH and then the color of DPPH will change from purple to yellow. The differences of absorbance between purple and yellow induced by free radical scavenging activity of compound is used for the calculation of antioxidant activity (Masoko et al., 2007; Naik et al., 2003). Antioxidant activities of extracts were summarized in Figure 2. The activity was presented as EC50, the concentration of extract showing 50% of free radical scavenging activity. So lower EC50 value was better activity as antioxidant (Masoko et al., 2007). In our assay system, 0.2 mM of ascorbic acid was detected as EC50. The lowest EC50 value was found in TrC (*Lactobacillus plantarum*) significantly ($p<0.05$). Other treatments showed their EC50 from 0.7 mg/mL to 1.3 mg/mL. Particularly, the activity in TrC (0.55 mg/mL) was about 2 times higher than that in control (1.05 mg/mL). Those improvements could be regarded as the action of used starter culture strain during fermentation.

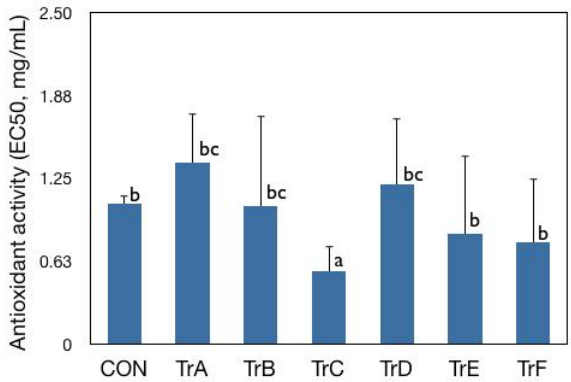

Fig. 2. Antioxidant activities of the extracts from fermented *C. majus* var *asiaticum* using different starter culture strains. In treatment, TrA and TrB were *Lactobacillus brevis*, TrC was *Lactobacillus plantarum* and TrD, TrE and TrF were *Saccharomyces cerevisiae*

4. TLC-DPPH assay

In this experiment, three different elution systems for development of compounds in extracts were used. And the chromatogram was stained by using DPPH solution to detect constituents relevant to antioxidant activity and the their changes by used starter culture strains. This TLC-DPPH screening method can indicate the presence of antioxidant compounds conveniently (Masoko et al., 2007). In EMW elution system, TrC treatment showed newly produced band at Rf 0.35 as shown in Figure 3. In CEF system, a new spot relevant to antioxidant activity was found at Rf 0.5 in TrC also (Figure 4). In BEA system, no new and different spots were found in all treatments (Figure 5). Various alkaloid compounds such as coptisine, chelidonine and berbenine were reported to present in *C. majus* var *asiaticum* and anti-inflammatory, antibacterial and antioxidant activities were also reported as those compound's representative features (Kim et al., 2000; Lenfeld et al., 1981).

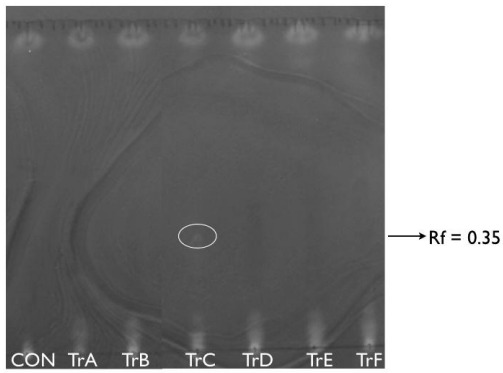

Fig. 3. Antioxidant constituents developed in TLC plate using elution solvent composed of ethyl acetate/methanol/water (40:5.4:5). The developed chromatogram was reacted with 0.2% DPPH solution and the bright spot represented as the compound involved in antioxidant activity. In treatment, TrA and TrB were *Lactobacillus brevis*, TrC was *Lactobacillus plantarum* and TrD, TrE and TrF were *Saccharomyces cerevisiae*

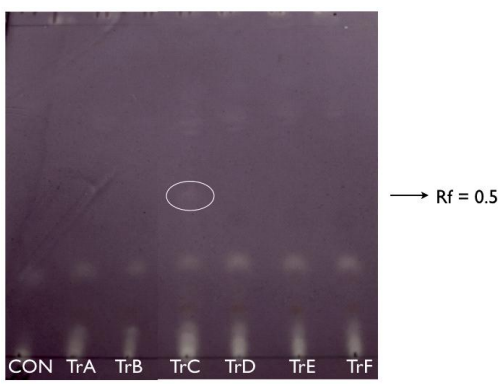

Fig. 4. Antioxidant constituents developed in TLC plate using elution solvent composed of chloroform/ethyl acetate/formic acid (5:4:1). The developed chromatogram was reacted with 0.2% DPPH solution and the bright spot represented as the compound involved in antioxidant activity. In treatment, TrA and TrB were *Lactobacillus brevis*, TrC was *Lactobacillus plantarum* and TrD, TrE and TrF were *Saccharomyces cerevisiae*

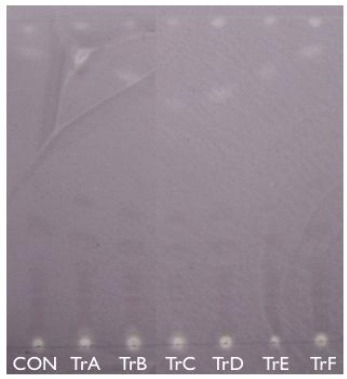

Fig. 5. Antioxidant constituents developed in TLC plate using elution solvent composed of benzene/ethanol/ammonium hydroxide (90:10:1). The developed chromatogram was reacted with 0.2% DPPH solution and the bright spot represented as the compound involved in antioxidant activity. In treatment, TrA and TrB were *Lactobacillus brevis*, TrC was *Lactobacillus plantarum* and TrD, TrE and TrF were *Saccharomyces cerevisiae*

Ⅳ. Conclusion

The present study conducted to ferment *C. majus* var *asiaticum* using three lactic acid bacteria and three yeasts. And the changes by fermentation in antibacterial activity and antioxidant activity were investigated. Antibacterial activity was found only in *Lactobacillus plantarum* (TrC) and the strain showed the highest antioxidant activity among treatments. The high antioxidant activity found inTrC could be explained with the newly synthesized spot on TLC-DPPH test. This study suggested that fermentation of *C. majus* var *asiaticum* using specific lactic acid bacteria could improve biological activity of plant.

Ⅴ. Acknowledgement

This work was supported by funds from IPET (Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), Ministry of Food Agriculture, Forestry and Fisheries.

[논문접수일 : 2013. 8. 13. 논문수정일 : 2013. 8. 19. 최종논문접수일 : 2013. 8. 20.]

References

1. Chang, W.-K., S.-B. Cho, D.-W. Kim, S.-S. Lee, and S.-K. Kim. 2010. Cell Growth and Antioxidant Activity on Onion Juice Fermentation by Using *Lactobacillus plantarum* as Animal Probiotics. J. Life Sci. 20: 1729-1737.
2. Chirinos, R., H. Rogez, D. Campos, R. Pedreschi, and Y. Larondelle. 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz and Pavón) tubers. Sep. Purif. Technol. 55: 217-225.
3. Faller, A. L. K. and E. Fialho. 2010. Polyphenol content and antioxidant capacity in organic and conventional plant foods. J. Food Compos. Anal. 23: 561-568.
4. Hong, K.-P. 2011. Optimum Conditions for Production of Fermented Grapefruit Extract using Leuconostoc mesenteroides KCTC3505. J. East Asian Soc. Diet. Life 21: 661-668.
5. Juan, M.-Y. and C.-C. Chou. 2010. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC 14715. Food microbiol. 27: 586-591.
6. Jung, H. M., S. J. Seo, J. B. Kim, N. W. Kim, and E. Y. Joo. 2011. The Study of Physiological Activities from *Chelidonium majus* var. *asiaticum* Extract. J. Invest. Cosm. 7: 359-366.
7. Kim, M. S., B. Y. Hwang, S. G. Choe, M. K. Lee, J. S. Ro, and K. S. Lee. 2000. Alkalodial components of chelidonii fructus. Kor. J. Pharmacogn. 31: 390-392.
8. Lenfeld, J., M. Kroutil, E. Marsalea, J. Slavik, and V. Preininger. 1981. Anti-inflammatory activity of quatemary benzophenanthridine alkaloids from *Chelidonium majus*. Planta Med. 43: 161-165.
9. Masoko, P. and J. N. Eloff. 2007. Screening of Twenty-Four South African Combretum Species and Six Terminalia Species (Combretaceae) for Antioxidant Activities. Afr. J. Trad. Cam. 4: 1-9.
10. Naik, G. H., K. I. Priyadarisini, J. G. Satav, M. M. Banavalikar, D. P. Sohoni, M. K. Biyani, and H. Mohan. 2003. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 63: 97-104.
11. Tallon, R., P. Bressollier, and M. C. Urdaci. 2003. Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. Res. Microbiol. 154: 705-712.