Microbiol. Biotechnol. Lett. (2015), 43(2), 97–104
http://dx.doi.org/10.4014/mbl.1504.04005
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

# Antimicrobial Efficacy of Fermented Dark Vinegar from Unpolished Rice

Hakjoon Choi[1], Gyeongja Gwak[2], Dabin Choi[1], Jaeyoung Park[1], and Hyeonsook Cheong[1]*

[1]*Department of Life Science & BK21-Plus Research Team for Bioactive Control Technology, Chosun University, Gwangju 501-759, Republic of Korea*
[2]*Department of Complementary and Alternative Medicine, Chosun University, Gwangju 501-759, Republic of Korea*

Received: April 28, 2015 / Revised: May 13, 2015 / Accepted: May 13, 2015

Vinegar is a widely used acidic seasoning and can be manufactured using various methods and bases, including cereals, wheat, and fruits. Most studies on vinegar have been conducted to evaluate its antioxidant activity. In the present study, fermented dark vinegar (FDV) produced from unpolished rice was examined for its antimicrobial activity, biochemical content, including the amounts of sugar, total soluble sugar, organic acid, and free amino acids, and pH and physiological activity. The antimicrobial efficiency of FDV was assessed using the paper disc-agar diffusion method. FDV exhibited strong antimicrobial activity against the pathogenic bacteria and yeast strains that were tested. In fact, the activity of FDV was shown to be higher than that of the commercial antibiotics carbenicillin (50 μg/ml) and tetracycline (50 μg/ml) against *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Lodderomyces elongisporus*. The antioxidant activity of FDV and ascorbic acid was evaluated. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, we found that FDV has the highest activity of the antioxidants. After spreading FDV onto tryptic soy broth and yeast extract-peptone-dextrose agar media, the microbial strains were isolated and characterized through physiological and biochemical analysis. Based on 16S ribosomal DNA sequence analysis, the isolated microorganisms exhibited a close similarity to *Acetobacter papayae*, *Acetobacter pasteurianus*, and *Acetobacter peroxidans*.

**Keywords:** Fermented dark vinegar, antimicrobial activity, antioxidant activity, human pathogenic microorganisms, pathogenic bacteria, 16S rDNA

## Introduction

Unpolished rice (UR) is composed of external thin layers (bran) that enclose the embryo (germ) and endosperm. The nutritional components in UR mainly exist in the germ and bran layers, which are mostly removed as a consequence of the milling or polishing process [3]. For this reason, UR has higher nutritional quality than polished rice. Recently, human and animal studies have shown that consumption of UR reduces the risk of type-2 diabetes, cardiovascular disease, and cancer, and these protective health effects have been linked to the presence of bioactive compounds such as polyphenols, GABA, acylated steryl b-glucoside, and c-oryzanol [3, 5, 7, 11, 17, 29]. Organic acids and their salts have been used as feed additives, functioning as acidifiers of animal feed. Such organic acids, including acetic, butyric, citric, formic, lactic, malic, propionic, and sorbic acids have been shown to improve health and growth performance in livestock and poultry by altering gastrointestinal tract function and energy metabolism, increasing the availability of nutrients and inhibiting the growth of pathogenic bacteria [4]. Vinegar is a widely used acidic seasoning and can be manufactured using various methods and base materials, including cereals, wheat, and fruits [20]. It is produced via a fermentation process carried out by several microorganisms including molds, yeasts, lactic acid bacteria, and acetic acid bacteria (AAB). These organisms produce not only acetic acid, but also various meta-

***Corresponding author**
Tel: +82-62-230-6667, Fax: +82-62-224-6678
E-mail: hscheong@chosun.ac.kr

bolic compounds that modify the taste and flavor of the product. Moreover, some types of vinegar have been shown to contain antioxidants, antitumor compounds, and other bioactive metabolites, which may be responsible for its beneficial health effects [8, 10, 18, 25]. Kurozu, a traditional jar-fermented Japanese black vinegar, is made from unpolished rice and has been reported to inhibit tumor growth, lipid peroxidation, and inflammation [6, 18, 19, 21-24].

In this study, we investigated the antimicrobial activity of fermented dark vinegar (FDV) against various pathogenic bacteria and a yeast strain. Further, the antioxidant activity and the organic components of FDV were analyzed, and culturable microbial analysis was carried out during the fermentation process.

## Materials and Methods

### Preparation of FDV

Mother brew (Mitsul) was prepared as follows: 4 kg polished rice was rinsed 3 times to remove impurities, followed by soaking in water for 4 h until saturation of water adsorption occurred. Excess water was removed, and the soaked rice was then steamed for around 40 min to allow full gelatinization. The steamed rice was cooled to 25°C, then mixed with 2 kg yeast leavening agent (Nuruk powder), and incubated at 32°C for 2–3 days to allow saccharification.

Preparation of steamed rice cakes (Baekseolgi) was as follows: 20 kg UR was rinsed 3 times to remove impurities, followed by soaking in water for 8 h until saturation of water adsorption occurred, and water was the drained for 1 h. For the instant rice cakes, the crushed powder was steamed to Baekseolgi.

Twenty kilograms of Mitsul, 20 kg Baekseolgi, 2 kg Nuruk, and 50 L water were mixed and then incubated at 32°C to reach an alcohol content of 12%. After that the mixture was incubated at 25°C for 1 month to allow fermentation. FDV was then available as the supernatant of the fermented broth.

### Alcohol, pH, and total soluble solid content

One hundred milliliters of the supernatant sample was run through a distiller until around 70 ml had been collected. Distilled water was added to the collected sample to a total volume of 100 ml, and the alcohol content (%) was measured using a vinometer. The alcohol-temperature correction table was used with the sample's alcohol content and a temperature. The pH was measured using an Orion 420A pH meter (Thermo Fisher Scientific, Inc., MA, USA). Total soluble solid content was measured using a Brix Refractometer HI 96811 (Hanna Instruments, RI, USA) [28].

### Free amino acid and componential analysis of FDV

Sugar, organic acid, moisture, ash, and crude protein contents of FDV were determined by proximate composition analysis. The sugars investigated were fructose, glucose, and sucrose. The organic acids investigated were oxalic acid, lactic acid, acetic acid, and propionic acid. Free amino acid analysis was done using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI), operated in selected ion monitoring (SIM) mode. This analysis was performed at Biotechnology Industrialization Center (BIC; Dongshin University, Korea).

### Culture conditions and isolation of microbial strains

To isolate microbial strains from FDV, 100 μl of FDV was spread onto yeast extract-peptone-dextrose (YPD) agar medium (20.0 g/l peptone, 10.0 g/l yeast extract, 20.0 g/l glucose, and 20.0 g/l agar) and tryptic soy broth (TSB) agar medium (BD 211825; Becton, Dickinson and Co., NJ, USA). The plates were incubated at 30°C for 2 days. Single colonies were purified by transferring onto fresh plates, followed by re-incubation. To classify morphological and biochemical characteristics, gram staining was performed using a gram staining kit (Fluka-77730, Sigma-Aldrich, St Louis, MO). Catalase activity was examined by measuring the production of oxygen bubbles in aqueous hydrogen peroxide solution. To identify the carbon sources used by the bacteria, they were grown on basal salt media (BSM) [2] supplemented with maltose, mannitol, cellobiose, D-mannose, D-glucose, lactose, fructose, and D-arabinose, respectively, to a final concentration of 2% as a carbon source [2].

### Polymerase chain reaction (PCR) amplification and sequencing of 16S ribosomal DNA (rDNA)

The 16S rDNA was PCR amplified using the 27F primer 5'-AGAGTTTGATCMTGG-CTCAG-3' and the 1492R primer 5'-TACGGYTACCTTGTTACGACTT-3' [1, 27]. 16S rDNA PCR amplification was performed using an MJ Research Tetrad PTC 225 thermal cycler (Bio-Rad Laboratories, Inc., CA, USA) with the sample in a final volume of 50 μl, con-

http://dx.doi.org/10.4014/mbl.1504.04005

taining 10 mM Tris-HCl pH 7.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 μM of each deoxynucleotide (dNTP), 0.2 μM of each primer, 1.25 U of Taq DNA polymerase (Roche Diagnostics, Basel, Switzerland), and 3 μl of extracted DNA. The PCR product was purified using a QIAquick PCR purification kit (Qiagen, Limburg, Netherlands), according to the manufacturer's instructions. 16S rDNA sequence analysis was performed using an ABI PRISM BigDye Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Inc., CA, USA) and ABI 310 DNA sequencer (Applied Biosystems, Inc.) following the manufacturer's protocol.

**Free radical scavenging activity of FDV**

The overall antioxidant activity of the prepared sample was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [12, 13]. DPPH is a stable free radical that shows decreased absorbance at 517 nm when it is reduced by antioxidants. DPPH stock was prepared by dissolving 0.4 mM of DPPH in 100 ml absolute ethanol. Twenty microliters of FDV sample was added to 180 μl of DPPH solution. The mixture was shaken vigorously, and the absorbance was measured at 517 nm using a microplate spectrophotometer (Eon, BioTek, VT, USA) for 30 min. As a positive control, ascorbic acid (1 mg/ml, 5 mM) was used. The percentage of inhibition, which represents the scavenging ability of the sample were DPPH radicals, was calculated as follows [13]:

$$\text{Antioxidant Activity (AOA)} = 100 - [(\text{absorbance increase of sample} / \text{absorbance increase of control}) \times 100]$$

**Antimicrobial activity of FDV**

Determination of FDV antimicrobial activity was performed using the agar diffusion method [14, 16] on solid media, employing common pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Lactobacillus casei*, *Gluconacetobacter intermedius*, and *Lodderomyces elongisporus*. *Lodderomyces elongisporus* was plated onto a YPD agar plate and all other test strains were plated onto TSB agar plates. Paper discs (8 mm diameter) were impregnated with FDV or four organic acids (latic acid, oxalic acid, acetic acid and propionic acid; 25 μg/ml, Sigma-Aldrich, St Louis, MO) and were placed onto the surface of the inoculated agar plates. These were then incubated at 37°C for 18 h. The total diameter (mm) of the inhibition zone was measured for each test microorganism [16]. Tetracycline (50 μg/ml) and carbenicillin (50 μg/ml) were used as positive controls. The antimicrobial activity of 3-year FDV was further measured according to changes in its acidity (pH). As a control, 100 mM KCl buffer (pH 3.0) was used.

## Results

**Alcohol, pH, and total soluble solid content, and componential analysis of FDV**

Values for the alcohol, pH, and total soluble solid content of FDV increased during fermentation, while moisture content decreased. Componential analysis of FDV was carried out for three sugars and four organic acids. The three sugars were fructose, glucose, and sucrose. Fructose was not present in FDV; however, glucose and sucrose content increased as fermentation progressed. The four organic acids were oxalic, lactic, acetic, and propionic acid. Lactic acid levels did not increase during fermentation, but oxalic acid, acetic acid, and propionic acid content increased as the fermentation process went on. These results are shown in Table 1.

**Analysis of the free amino acid content of FDV**

The free amino acid contents of FDV were analyzed and the results are shown in Table 2. A total of 18 amino acids were assessed, of which 15 amino acids showed increas-

**Table 1. Biochemical component analysis of fermented dark vinegar (FDV)**

| | | Contents (mg/l) | |
|---|---|---|---|
| | | 1 year - FDV | 3 year - FDV |
| pH | | 3.2 | 4.7 |
| Sugar Content (Brix) | | 5.0 | 7.1 |
| Moisture (%) | | 97.49 ± 0.07 | 95.79 ± 0.02 |
| Ash (%) | | 0.26 ± 0.02 | 0.35 ± 0.0 |
| Crude Protein (%) | | 2.29 ± 0.19 | 4.41 ± 0.03 |
| Alcohol (%) | | 0 | 0 |
| Sugar (mg/l) | Fructose | - | - |
| | Glucose | 0.582 ± 0.052 | 0.628 ± 0.047 |
| | Sucrose | 0.052 ± 0.046 | 0.552 ± 0.080 |
| Organic acid (mg/l) | Oxalic acid | 0.034 ± 0.004 | 0.100 ± 0.011 |
| | Lactic acid | 4.775 ± 0.122 | 3.826 ± 0.047 |
| | Acetic acid | 42.253 ± 0.048 | 49.577 ± 0.035 |
| | Propionic acid | 7.391 ± 0.046 | 9.443 ± 0.025 |

[a]FDV fermented for 1 year, [b]FDV fermented for 3 years.

ing levels as fermentation progressed. The total free amino acid content for the 1-year-FDV was 1398 ± 409.1 mg/l and one for 3-year-FDV was 1841.1 ± 202.4 mg/l. These results are higher than those reported by Kim *et al.* [10]. In particular, alanine, valine, threonine, leucine, isoleucine, arginine, tyrosine, and GABA were present at higher levels in FDV than by Kim *et al.* [10].

**Table 2. Amino acid analysis of fermented dark vinegar (FDV).**

| | Contents (mg/l) | |
|---|---|---|
| | 1 year - FDV | 3 year - FDV |
| Glycine | 83.9 ± 18.7 | 80.5 ± 18.8 |
| Alanine | 148.8 ± 47.8 | 164.1 ± 52.8 |
| Serine | 70.2 ± 11.3 | 93.6 ± 12.3 |
| Proline | 73.2 ± 6.3 | 82.5 ± 5.0 |
| Valine | 111.7 ± 15.1 | 146.3 ± 17.7 |
| Threonine | 138.1 ± 177.2 | 43.2 ± 3.9 |
| Leucine | 127.8 ± 30.7 | 163.1 ± 15.6 |
| Isoleucine | 146.9 ± 71.1 | 303.8 ± 32.0 |
| Aspartic acid | 32.2 ± 3.8 | 46.1 ± 4.6 |
| Lysine | 15.8 ± 1.0 | 49.4 ± 2.9 |
| Glutamic acid | 68.4 ± 6.2 | 92.4 ± 7.1 |
| Methionine | 10.9 ± 1.4 | 2.1 ± 0.4 |
| Histidine | 33.1 ± 2.5 | 69.2 ± 4.6 |
| Phenylalanine | 21.5 ± 1.4 | 35.3 ± 2.3 |
| Arginine | 105.3 ± 6.9 | 138.2 ± 7.5 |
| Tyrosine | 64.4 ± 4.0 | 142.7 ± 8.2 |
| Cystine | 1.8 ± 0.2 | 4.6 ± 0.6 |
| γ-aminobutyric acid | 144.0 ± 3.5 | 184.0 ± 6.1 |

[a]FDV fermented for 1 year, [b]FDV fermented for 3 years.

### Identification of microorganisms isolated from FDV

The reason for isolated the microorganisms of FDV was to investigate the influence of strains appearing during fermentation to vinegar and standardization of FDV. The three microorganisms isolated from FDV were named as fermented dark vinegar strain (FDVS)-1, -2, and -3. All strains were gram-negative and did not sporulate. Peptidoglycan typing and 16S rDNA sequence analysis revealed that FDVS-1 was 98% homologous to *Acetobacter pasteurianus*, FDVS-2 was 100% homologous to *Acetobacter peroxidans*, and FDVS-3 was 96% homologous to *Acetobacter senegalensis* (Fig. 1). All strains FDVS-1, -2, and -3 represent the names of each identified bacterial strain. Carbon source usage tests for the three strains were conducted using BSM (Table 3).

### Free radical scavenging activity of FDV

Antioxidants reduce the oxidative stress in cells and are

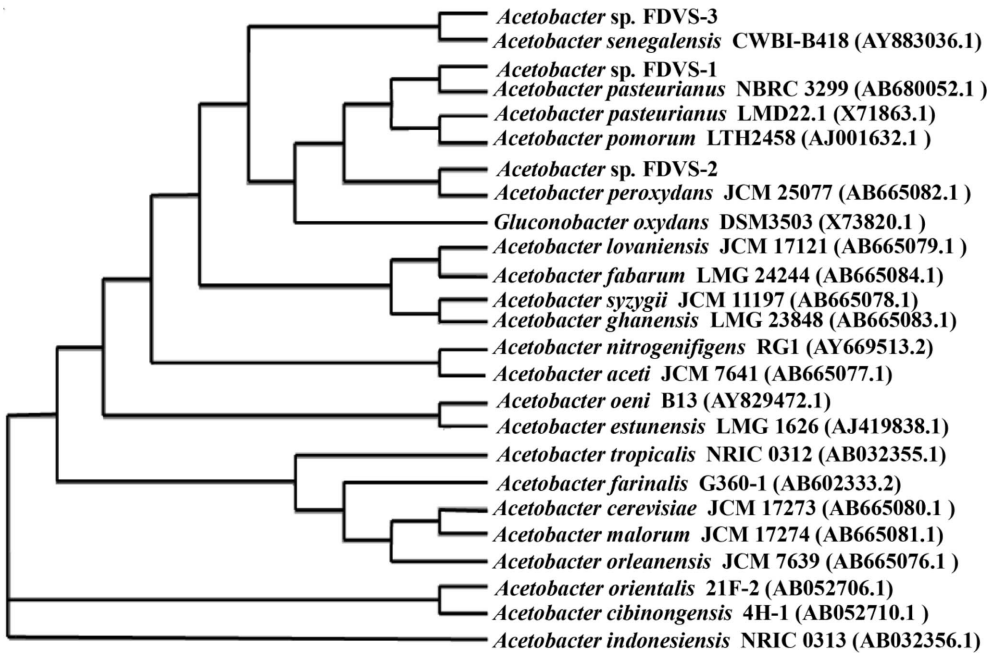


**Fig. 1. Phylogenic tree based on 16S ribosomal RNA (rRNA) gene sequences of three microorganisms isolated from fermented dark vinegar (FDV).** The tree was based on an alignment of 1,318 bp of 16S rRNA gene sequences, and constructed by the neighbor-joining method, *Acetobacter* sp. FDVS-1, -2, and -3; fermented dark vinegar strains.

http://dx.doi.org/10.4014/mbl.1504.04005

**Table 3. Carbon source usage of three microorganisms isolated from fermented dark vinegar (FDV).**

| | Maltose | Fluctose | Lactose | Arabinose | Cellobiose | Mannose | Mannitol | Glucose |
|---|---|---|---|---|---|---|---|---|
| *Acetobacter* sp. FDVS-1[b] | -[a] | - | + | - | - | - | - | - |
| *Acetobacter* sp. FDVS-2 | - | - | - | - | - | - | - | - |
| *Acetobacter* sp. FDVS-3 | - | - | - | - | - | - | - | - |

Carbon source usage tests were carried out using basal salt media.
[a]+, growth; -, no growth.

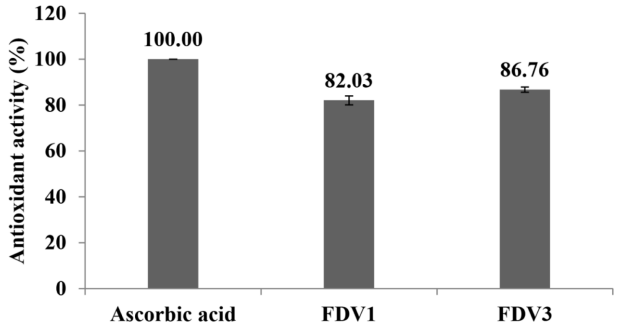


**Fig. 2. Antioxidant activity of fermented dark vinegar (FDV).** The overall antioxidant activity of FDV was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Ascorbic acid (1 mg/ml, 5 mM) was used as a positive control. FDV1, FDV fermented for 1 year; FDV3, FDV fermented for 3 years.

therefore useful in the treatment of human diseases. Plant-derived antioxidants have been widely studied due to their relative safety for consumption compared to synthetic antioxidants [18]. The radical scavenging activity of FDV was determined using DPPH systems. The activities of FDV after 1 year- and 3 years of fermentation were found to be 82.07 ± 1.90% and 86.76 ± 1.14%, respectively. The antioxidant activities of FDV determined by the DPPH method were similar to those of ascorbic acid (1 mg/ml) (Fig. 2).

### Antimicrobial activity of FDV

The antibacterial activity of the organic acids studied and FDV was examined using the paper disc diffusion method against three strains of gram-positive bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Lactobacillus casei*), six gram-negative bacterial strains (*Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Gluconacetobacter intermedius*), and one strain of fungus (*Lodderomyces elongisporus)*. The results are shown in Table 4. FDV effectively inhibited the growth of all three gram-positive strains, five gram-negative bacterial strains (*Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Gluconacetobacter intermedius*), and the fungus strain. In these cases, FDV produced a zone of inhibition ranging from 12 to 22 mm in diameter.

Most of the organic acids tested showed no inhibitory activity against any strains. However, propionic acid showed reasonably strong activity against all strains (15-28 mm zones of inhibition). At basic pH ranges, FDV did

**Table 4. Screening for antimicrobial activity of fermented dark vinegar (FDV) and variety of other antibiotics and control substance by paper disc assay.**

| | KCl buffer pH 3 | Carbenicillin (50 μg/ml) | Tetracycline (50 μg/ml) | 1 year - FDV | 3 year - FDV | Propionic acid |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | - | 11 | 14 | 15 | 15 | 15 |
| *Escherichia coli* | - | 11 | 13 | 13 | 13 | 17 |
| *Listeria monocytogenes* | - | 11 | 12 | 12 | 13 | 19 |
| *Pseudomonas aeruginosa* | - | 11 | 14 | 12 | 17 | 17 |
| *Salmonella typhimurium* | - | 12 | 20 | 21 | 21 | 22 |
| *Yersinia enterocolitica* | - | 10 | 20 | 21 | 22 | 28 |
| *Lactobacillus casei* | - | - | 12 | 14 | 17 | ND |
| *Gluconacetobacter intermedius* | - | 11 | 16 | 14 | 15 | ND |
| Lodderomyces *elongisporus* | - | 11 | 13 | 14 | 15 | ND |

Loaded with each sample (50 μl) was placed on the agar plate which was seeded with each test microorganism. Tetracycline was used as a positive control. K; KCl Buffer pH 3, C; Carbenicillin (50 μg/ml), T; Tetracycline (50 μg/ml), F1; FDV 1 year, F3; FDV 3 year.

**Table 5. Screening for antimicrobial activity according to the acidity of 3-year fermented dark vinegar using paper disc assay.**

| | pH 3 | pH 4 | pH 5 | pH 6 | pH 7 |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | 15 | 12 | 10 | - | - |
| *Escherichia coli* | 13 | 11 | 10 | - | - |
| *Listeria monocytogenes* | 13 | 12 | 11 | - | - |
| *Pseudomonas aeruginosa* | 17 | 13 | 11 | - | - |
| *Salmonella typhimurium* | 21 | 16 | 10 | - | - |
| *Yersinia enterocolitica* | 22 | 16 | 13 | - | - |

Acidity was adjusted using 0.1 N NaOH. Units: mm, values represent diameter of the inhibition zone.

showed no antimicrobial activity, as shown in Table 5.

## Discussion

The purpose of this study was to evaluate the antimicrobial activity of FDV and to determine its antioxidant activity, pH-, sugar-, total soluble solid-, total acid-, and free amino acid content. Three-year-FDV had an organic acid content of 62.946 ± 0.245 mg/l, free amino acid content of 1841.1 ± 202.4 mg/l, and sugar content of 1.18 ± 0.127 mg/l. These results represent the highest organic acid and free amino acid contents compared to previous report [10]. And the organic acid and total free amino acid content of FDV increased during the fermentation processes. Three strains of AAB, *Acetobacter* sp. FDVS-1, -2, and -3, were isolated from FDV. AAB are an important group of bacteria in the food and beverage industry, mainly due to their ability to oxidize ethanol to acetic acid. These bacteria represent the key microorganism in vinegar production [15].

In the DPPH system, the radical scavenging activity of FDV in the 1-year and 3-year fermented vinegar was found to be 82.07 ± 1.90% and 86.76 ± 1.14%, respectively. FDV showed antioxidant activity comparable to that of ascorbic acid.

Regarding its antimicrobial activity, FDV inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Lodderomyces elongisporus*. This inhibitory activity of FDV was greater than that of the commercial antibiotics carbenicillin (50 μg/ml) and tetracycline (50 μg/ml). In particular, FDV strongly inhibited the growth of *Salmonella typhimurium* and *Yersinia enterocolitica*. *Salmonella typhimurium,* which infects a variety of animals including humans, is widely distributed throughout the world and causes enteritis and septicemia [9]. Yersiniosis is the third most reported food-borne bacterial zoonoses in humans, and *Yersinia enterocolitica* is the most commonly reported bacterial species to cause this disease in humans [26]. But, propionic acid in the FDV may have affected the antimicrobial activity. In conclusion, FDV exhibits strong antioxidant and antimicrobial activity against human pathogenic microorganisms.

## Acknowledgments

This research was supported by the IPET (Korea Institue of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries) funded by the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Thechnology Commercialization) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2012R1A1A3019667).

## References

1. Amalfi M, Yombiyeni P, Decock C. 2010. Fomitiporia in sub-Saharan Africa: morphology and multigene phylogenetic analysis support three new species from the Guineo-Congolian rainforest. *Mycologia* **102**: 1303-1317.
2. Banat IM, Samarah N, Murad M, Horne R, Banerjee S. 1991. Biosurfactant production and use in oil tank clean-up. *World J. Microbiol. Biotechnol.* **7**: 80-88.
3. Caceres PJ, Martinez-Villaluenga C, Amigo L, Frias J. 2014. Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions. *Food Chem.* **152**: 407-414.
4. Castillo S, Rosales M, Pohlenz C, Gatlin DM. 2014. Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum Sciaenops ocellatus. *Aquaculture* **433**: 6-12.
5. Diana M, Rafecas M, Quílez J. 2014. Free amino acids, acrylamide and biogenic amines in gamma-aminobutyric acid enriched sourdough and commercial breads. *J. Cereal Sci.* **60**: 639-644.

6. Fukuyama N, Jujo S, Ito I, Shizuma T, Myojin K, Ishiwata K, *et al.* 2007. Kurozu moromimatsu inhibits tumor growth of Lovo cells in a mouse model in vivo. *Nutrition* **23**: 81-86.
7. Goffman FD, Bergman CJ. 2004. Rice kernel phenolic content and its relationship with antiradical efficiency. *J. Sci. Food Agr.* **84**: 1235-1240.
8. Hashimoto M, Obara K, Ozono M, Furuyashiki M, Ikeda T, Suda Y, *et al.* 2013. Separation and characterization of the immunostimulatory components in unpolished rice black vinegar (kurozu). *J. Biosci. Bioeng.* **116**: 688-696.
9. Kim J-H, Kim S-G, Kim S-S, Kim J-H, Park S-H, Nam K-H, *et al*. 2013. Analysis of the antibiotic resistance gene in Salmonella Typhimurium isolates from diseased pigs in Gyeongbuk province. *Korean J. Vet. Serv.* **36**: 73-78.
10. Kim S-H, Cho H-K, Shin H-S. 2012. Physicochemical properties and antioxidant activities of commercial vinegar drinks in Korea. *Food Sci. Biotechnol.* **21**: 1729-1734.
11. Kim SP, Kang MY, Nam SH, Friedman M. 2012. Dietary rice bran component gamma-oryzanol inhibits tumor growth in tumor-bearing mice. *Molecul. Nutr. Food Res.* **56**: 935-944.
12. Kitagaki H, Tsugawa M. 1999. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging ability of sake during storage. *J. Biosci. Bioeng.* **87**: 328-332.
13. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis* : *PCA* **13**: 8-17.
14. Lee SB, Cha KH, Kim SN, Altantsetseg S, Shatar S, Sarangerel O, *et al.* 2007. The antimicrobial activity of essential oil from Dracocephalum foetidum against pathogenic microorganisms. *J. Microbiol.* **45**: 53-57.
15. Mateo E, Torija MJ, Mas A, Bartowsky EJ. 2014. Acetic acid bacteria isolated from grapes of South Australian vineyards. *Int. J. Food Microbiol.* **178**: 98-106.
16. Mehni AM, Ketabchi S, Bonjar, GHS. 2014. Antibacterial activity and polyphenolic content of Citrullus colocynthesis. *Int. J. Biosci.* 190-196.
17. Monks JLF, Vanier NL, Casaril J, Berto RM, de Oliveira M, Gomes CB, *et al.* 2013. Effects of milling on proximate composition, folic acid, fatty acids and technological properties of rice. *J. Food Compos. Anal.* **30**: 73-79.
18. Nishidai S, Nakamura Y, Torikai K, Yamamoto M, Ishihara N, Mori H, *et al*. 2000. Kurosu, a traditional vinegar produced from unpolished rice, suppresses lipid peroxidation in vitro and in mouse skin. *Biosci. Biotechnol. Biochem.* **64**: 1909-1914.
19. Seki T, Morimura S, Tabata S, Tang Y, Shigematsu T, Kida K. 2008. Antioxidant activity of vinegar produced from distilled residues of the Japanese liquor shochu. *J. Agric. Food Chem.* **56**: 3785-3790.
20. Shimoji Y, Tamura Y, Nakamura Y, Nanda K, Nishidai S, Nishikawa Y, *et al.* 2002. Isolation and identification of DPPH radical scavenging compounds in Kurosu (Japanese unpolished rice vinegar). *J. Agric. Food Chem.* **50**: 6501-6503.
21. Shimoji Y, Kohno H, Nanda K, Nishikawa Y, Ohigashi H, Uenakai K, Tanaka T, *et al.* 2004. Extract of Kurosu, a vinegar from unpolished rice, inhibits azoxymethane-induced colon carcinogenesis in male F344 rats. *Nutr. Cancer.* **49**: 170-173.
22. Shizuma T, Nagano M, Fujii A, Mori H, Fukuyama N. 2011. Therapeutic effects of four molecular-weight fractions of Kurozu against dextran sulfate sodium-induced experimental colitis. *Turk J. Gastroenterol.* **22**: 376-381.
23. Shizuma T, Ishiwata K, Nagano M, Mori H, Fukuyama N. 2011. Protective effects of Kurozu and Kurozu Moromimatsu on dextran sulfate sodium-induced experimental colitis. *Digest. Dis. Sci.* **56**: 1387-1392.
24. Tong LT, Katakura Y, Kawamura S, Baba S, Tanaka Y, Udono M, *et al.* 2010. Effects of Kurozu concentrated liquid on adipocyte size in rats. *Lipids in Health and Disease* **9**: 134.
25. Verzelloni E, Tagliazucchi D, Conte A. 2007. Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsamic vinegar. *Food Chem.* **105**: 564-571.
26. Vilar MJ, Virtanen S, Laukkanen-Ninios R, Korkeala H. 2015. Bayesian modelling to identify the risk factors for Yersinia enterocolitica contamination of pork carcasses and pluck sets in slaughterhouses. *Int. J. Food Microbiol.* **197**: 53-57.
27. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**: 697-703.
28. Woo K-S, Ko J-Y, Song S-B, Lee J-S, Oh B-G, Kang J-R, *et al.* 2010. Physicochemical characteristics of Korean traditional wines prepared by addition of Sorghum (Sorghum bicolor L. Moench) using different nuruks. *J. Korean Soc. Food Sci. Nutr.* **39**: 548-553.
29. Zhang G, Malik VS, Pan A, Kumar S, Holmes MD, Spiegelman D, *et al.* 2010. Substituting brown rice for white rice to lower diabetes risk: a focus-group study in Chinese adults. *J. Am. Diet. Assoc.* **110**: 1216-1221.

## 국문초록

**현미 발효 흑초의 항균활성**

최학준[1], 곽경자[2], 최다빈[1], 박재영[1], 정현숙[1]*

[1]*조선대학교 자연과학대학 생명과학과*

[2]*조선대학교 보완대체의학과*

식초는 세계적으로 사용되는 조미료로 밀, 과일, 곡물 등을 원료로하여 다양한 방법으로 제조된다. 지금까지 식초에 대한 대부분의 연구들은 항산화활성에 한정된 연구였다. 본 연구에서는 현미를 이용하여 만든 현미 발효 식초의 이화학적 특성과 항균활성에 대해 시험하였으며, 현미발효식초의 항균활성은 paper disc-agar diffusion 방법을 이용하여 조사하였 때, 병원성 박테리아와 효모에 대해 강한 항균활성을 나타내었다. 특히 *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Lodderomyces elongisporus*의 균주에 대해서는 상용되는 항생제인 카베니실린과 테트라사이클린보다 더 높은 항균활성을 보였다. 항산화활성은 2,2-diphenyl-1-picrylhydrazyl (DPPH) 라디칼 소거능을 이용하여 측정하였고, 대표되는 항산화제인 아스코르빅 산과 비슷한 활성을 나타내었다. 현미발효흑초의 발효중에 나타나는 균주를 동정하기 위해 TSB 고체배지와 YPD 고체배지에 현미발효흑초를 도포하였을 때, 분리된 콜로니를 16S rDNA sequence 분석을 통하여, FDVS-1, 2, 3 세가지 균주를 분리하였으며, phylogenic tree 분석법을 이용하여 조사하였을 때, 각각 *Acetobacter papayae*, *Acetobacter pasteuranus*, *Acetobacter peroxidans*와 유사하였다.

http://dx.doi.org/10.4014/mbl.1504.04005