Introduction

Biogenic amines (BAs) are widely present in nearly all fermented foods and beverages, and excess consumption can cause adverse health effects. To prepare BA-free Korean black raspberry wine (BRW), four autochthonous starter yeast strains without hazardous BA synthesis activity were selected and their physiological and biochemical properties were examined. The selected strains were identified as *Saccharomyces cerevisiae* based on 26S rDNA sequencing and microsatellite analysis. Molecular fingerprinting revealed that isolates were quite different from commercial wine yeast *S. cerevisiae* (52.4% similarity), but genetically relevant to commercial beer yeasts. The four *S. cerevisiae* strains produced over 10% ethanol during BRW fermentation. In addition, the fermented BRW with these strains showed higher levels of total flavonoids and similar antioxidant activity compared to the control sample. Potentially hazardous BAs that commonly occur in black raspberry extract (BRE) such as cadaverine, histamine, and spermidine were also not detected in the fermented BRW. Thus, we suggest that our strains are promising fermentation tools to ensure high quality and enhanced functionality in the production of BA-free BRW.

Key Words: biogenic amine; black raspberry wine; indigenous yeasts; *Saccharomyces cerevisiae*; technological traits
Black raspberry wine (BRW) is a popular traditional beverage in Korea. BRW fermentation in Korea generally relies on commercial *S. cerevisiae*, which is also commonly used in grape winemaking (Kim and Park, 2015; Lee et al., 2013). Because black raspberry winemaking is usually carried out without post-malolactic fermentation using LAB, yeast is considered a main factor affecting the BA content in BRW. However, evaluation of potential starter cultures for the production of BA-free BRW has not been adequately carried out. Thus, it is desirable to identify and apply safe and functional yeast starter cultures for BRW. The aims of this study were: a) to identify indigenous yeast that degrade a wide range of BAs during alcohol fermentation of black raspberries, b) to evaluate the oenological and technological properties of wine starter culture—specifically, stress tolerance (ethanol, glucose and fructose, sulfur dioxide, temperature), killer yeast, foam/film formation and enzymatic activities, and c) to analyze BA content and functional compounds in wine compared to a commercial strain. To the best of our knowledge, this is the first report to demonstrate that indigenous yeasts possess BA-lowering properties and the first report to present a stepwise strategy to select multifunctional *S. cerevisiae* strains that have adequate fermentative capacity from Korean black raspberries (KBR) harvested in the Muju area. The resultant database enables microbiological control of BAs from wild yeast during winemaking to reduce the potential health risk for consumers.

**Materials and Methods**

**Chemicals.** The BAs tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, serotonin, tryptamine, spermidine, spermine, L-noradrenaline, and dopamine were supplied by Sigma-Aldrich (St. Louis, MO, USA). Dansyl chloride from Sigma-Aldrich was used as the derivatization reagent. All the reagents used were of HPLC grade. HPLC was performed using an Alltech, HPLC-PDA system (Thermo Fisher Scientific Inc., Waltham, MA, USA).

**Isolation of indigenous yeasts.** Black raspberry extracts (BREs) were acquired from barrels, serial diluted with sterilized water, and spread onto agar plates to isolate the microorganisms. Yeasts were isolated on YM agar plates (BD Biosciences, San Jose, CA, USA) containing 100 mg/L penicillin-streptomycin (Sigma-Aldrich) to inhibit bacterial growth, and colonies were counted after incubating at 29°C for 2–3 days. Several yeast isolates were randomly selected and cultivated on YM broth media containing 30% glucose as a substrate. The culture media was centrifuged at 4°C, 8,000 rpm for 15 min and the supernatant was distilled and stored at −20°C until determination of the alcohol concentration using gas chromatography (GC). The commercial strains were purchased from wine yeast suppliers (Supplementary Table S3). Commercial dried yeasts were activated in a sterile rehydration medium for 30 min at 40°C and inoculated on YM agar and broth medium, and then incubated at 24°C for 48 h.

**Black raspberry wine fermentation.** Alcohol fermentation with BRE was performed as described previously (Song et al., 2016). Each yeast strain was cultivated in YM broth at 25°C, 160 rpm for 72 h and 2% of the culture solution was inoculated in 12°Brix of BRE at 25°C, 160 rpm for 48 h and used as a starter solution. The prepared starter solution was used to inoculate the extract (5% by volume, 24°Brix), and the solution was cultivated at 25°C, 160 rpm for 48 h. Alcohol fermentation was performed in the stationary phase at 25°C for 48 h.

**Physicochemical and BA analysis.** The °Brix, pH, total acidity, and ethanol concentration were analyzed as described by Song et al. (2016). Measurement of BA was carried out using the dansyl chloride derivatization reaction (Dugo et al., 2006). After derivatization of the samples, an aliquot was subjected to high pressure liquid chromatography (Agilent Technologies, Santa Clara, CA, USA) equipped with an ultraviolet (UV) detector and CapcellPak C18 Column (4.6 × 250 mm, 5 μm, Shiseido Co., Tokyo, Japan), as described previously (Moon et al., 2010).

**Strain identification and genetic characterization.** Yeasts were subjected to polymerase chain reaction (PCR) amplification using NLI/NL4 primers for the sequencing 26S gene region of rDNA (Song et al., 2016), and the obtained PCR products were sequenced with ABI-Prism 377 Sequencer (Applied Biosystems Japan, Tokyo, Japan) and compared with the DNA Data Bank of Japan (DDBJ) using a BLAST search. The 5.8S rDNA was digested with the restriction enzymes Hinfl, CfoI, and HaeIII (Roche Diagnostics, Mannheim, Germany), according to the supplier’s instructions.

**Molecular genotyping.** For molecular typing, yeast strains isolated from black raspberry were characterized for their allelic variation for 14 microsatellite loci (Legras et al., 2007; Vaudano and Garcia-Moruno, 2008). The distance matrices with the Fingerprinting images were analyzed using the BioNumerics software, version 7.6.2 (Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram was built using the unweighted pair-group mean arithmetic method (UPGMA) for the Dice similarity coefficient, and the cophenetic correlation (percent) is shown at each branch.

**Technological and biochemical characterization.** The foam and film formation of the yeast was observed in YM broth with 12% glucose and cultivated at 29°C for 72 h. The killer behavior was evaluated compared to a sensitive strain (*S. cerevisiae* KCTC 7930) using the seeded agar-plate method. The supernatant of wild yeasts were spotted in the paper disc and killer yeast was identified by a clear zone of inhibition surrounding them. Biotin synthesis was confirmed by PCR analysis using the methods of Fujihara et al. (2014). To determine the environmental tolerance capability of the strains, yeast cells were grown in YM both with various sugar (glucose and fructose mixed 1:1; final concentration 35, 40, 45, and 50%), metabisulfite (200 and 500 ppm), and alcohol (0, 5, 10, 12.5, and 15%) concentrations at 29°C, 180 rpm for 2 days. The absorbance was monitored every 12, 24, and 48 h to assess strain tolerance performance using spectrophotometry at 660 nm (UV-VIS Spectrophotometer, Beckman Coulter, Brea, CA, USA). Also, four selected strains were characterized us-
SONG, LEE, and BAIK

ing the API 20C AUX kit and API-ZYM test (BioMérieux, Lyon, France). Enzyme activity was categorized from 0 (no activity) to 5 (maximum activity) by comparing the color developed within 5 min with the API-ZYM color reaction chart. The results were reported as reactions of low intensity (1), moderate intensity (2–3) and high intensity (4–5).

Statistics. All analyses were performed in triplicate. The data were analyzed by one-way ANOVA using the SPSS, version 19.0 (IBM Corp., Armonk, NY, USA). The analyses were performed in triplicate and are expressed as mean ± standard deviation (SD). The differences among groups were assessed using Duncan’s multiple range tests. Statistical significance was considered at \( p < 0.05 \).

Results

Indigenous yeasts with low BAs from naturally fermented Korean BRW

Among the randomly selected isolates from the naturally fermented BRW, 34 strains produced over 6% of ethanol in synthetic media and were selected and investigated for their ability to metabolize BAs in bench-scale alcohol fermented wine (50 mL each), as shown in Fig. 1. Compared with Korean BRE, which contains not only two major amines—serotonin (69.2 mg/L) and dopamine (7.7 mg/L)—but also three BAs—cadaverine (3.51 mg/L), histamine (2.7 mg/L), and spermidine (1.1 mg/L)—half of the yeast isolates from BRW were capable of synthesizing one or more different BAs including histamine, tyramine, spermidine, spermine, and 2-phenylethylamine. All the isolates could degrade cadaverine and histamine except for two isolates, JBCC-37 and JBCC-199 as shown in Supplementary Table S1, and most of the selected strains showed different BA degradation profiles and ethanol fermentation abilities. To select the most beneficial strains, PCA analysis was performed (Fig. 1B) using six hazardous BAs and ethanol production rates of the selected isolates. Based on PCA analysis, the selected yeasts could be divided into three groups: group A showed production of one or more BA, including cadaverine, tyramine, spermidine, spermine, and 2-phenylethylamine; group B was characterized by the ability to degrade all six BAs in the control sample; and the JBCC-44 strain was clearly located outside of the other two groups, and it showed significantly higher levels of those BAs. Moreover, group B could be further divided into subgroup C: which includes isolates JBCC-21, JBCC-40, JBCC-43, and JBCC-197. As shown in Fig. 1B, high ethanol production properties and spermine, spermidine, and tyramine synthesis of some strains are positively related. A too high an ethanol production activity of yeast strains is not desirable for black raspberry wine fermentation with a low biogenic amine content. The isolates in group C were quite promising because they showed not only BA degradation but also high ethanol production (over 10%, Fig. 1A).

Fig. 1. Ethanol and biogenic amine production by indigenous yeasts.

Ethanol and total biogenic amine production during pilot-scale black raspberry wine fermentation among indigenous strains (A) and principal component analysis (PCA) for the selection of the most relevant technological isolates (B). Variables: biogenic amine contents (HIS, histamine; CAD, cadaverine; TYR, tyramine; SPM, spermine; SPD, spermidine; PEA, 2-phenylethylamine) and ethanol production (%). Subfigure 1A shows the total six major BAs contents of cadaverine, histamine, tyramine, spermine, and spermidine by four indigenous yeasts which effectively degraded BAs in BRE and No. 7013.
Identification and molecular typing of isolated yeasts

Biochemical characterization using the API 20C AUX kit and 26S rDNA sequencing of the selected yeasts showed 100% homology with *S. cerevisiae* (Fig. 2, Supplementary Table S2). In addition, identification of strains by using the ITS1/ITS4 region including the 5.8S rDNA restriction fragment length polymorphism (RFLP) showed an identical pattern to the *S. cerevisiae* standard strain (data not shown). The multiplex PCR patterns using 12 microsatellite loci were compared with 28 commercial strains: 22 beer yeasts, two dry wine yeasts, and four bread yeasts. The similarity percentage was determined by cluster analysis. As shown in a UPGMA dendrogram in Fig. 3, the molecular diversity was shown not only within the indigenous isolates but also with commercial wine yeasts. The selected four isolates were positioned far from commercial wine strains; *S. cerevisiae* JBCC-21, 40, and 197 belong to the beer yeasts group (63–67% similarity), but *S. cerevisiae* JBCC-43 was more similar to the bread and wine yeasts (67% similarity).
Table 1. Technological characterization and survival rate (%) of the yeast isolates and type strain to various stress factors of sugar, metabisulfite (Na$_2$S$_2$O$_5$) and ethanol.

| Tolerance     | No. 7013 | JBCC-21 | JBCC-40 | JBCC-43 | JBCC-197 |
|---------------|----------|---------|---------|---------|---------|
| Foam formation| low      | medium  | medium  | low     | medium  |
| Film formation| —        | —       | —       | —       | —       |
| Killer yeast  | —        | —       | —       | —       | —       |
| Biotin synthesis| —       | —       | —       | —       | —       |
| Sugar tolerance (%) | 35   | 86.56  | 81.51  | 80.38  | 79.15  | 79.96  |
|                 | 40   | 73.06  | 75.31  | 73.43  | 73.24  | 71.86  |
|                 | 45   | 54.19  | 64.25  | 63.27  | 63.09  | 58.25  |
|                 | 50   | 7.66   | 20.80  | 3.13   | 17.37  | 11.88  |
| Na$_2$S$_2$O$_5$ tolerance (ppm) | 200  | 125.4  | 126.01 | 128.07 | 127.36 | 115.01 |
|                 | 500  | 133.1  | 134.48 | 99.75  | 132.32 | 101.0  |
| Ethanol tolerance (%) | 10   | 89.59  | 82.99  | 86.85  | 88.57  | 85.81  |
|                 | 12.5 | 70.22  | 44.26  | 44.96  | 47.33  | 67.72  |
|                 | 15   | 67.07  | 48.87  | 45.33  | 43.85  | 60.03  |

Growth of yeasts after 48 h of incubation in a laboratory medium (YM broth), the growth was expressed as Survival rate (%) = (Abs$_s$/Abs$_c$) × 100; Abs$_s$ is the absorbance of the samples; Abs$_c$, the absorbance of the positive control (not-modified YM broth inoculated with yeasts and incubated at 29°C).

Table 2. Semi-quantitative extracellular enzyme activities of four selected S. cerevisiae strains and type strain.

| Enzyme                  | Substrate                     | No. 7013 | JBCC-21* | JBCC-40 | JBCC-43 | JBCC-197 |
|------------------------|-------------------------------|----------|----------|---------|---------|---------|
| Alkaline phosphatase    | 2-naphthyl phosphate          | 2        | 1        | 1       | 1       | 1       |
| Esterase (C4)           | 2-naphthyl butyrate           | 3        | 3        | 3       | 3       | 3       |
| Esterase lipase (C8)    | 2-naphthyl caprylate          | 1        | 2        | 2       | 2       | 2       |
| Lipase (C14)            | 2-naphthyl myristate          | 2        | 1        | 1       | 1       | 1       |
| Leucine arylamidase     | t-leucyl-2-naphthylamide      | 5        | 5        | 5       | 5       | 5       |
| Valine arylamidase      | t-valyl-2-naphthlamide        | 3        | 3        | 3       | 3       | 3       |
| Cystine arylamidase     | t-cystyl-2-naphthlamide       | 3        | 3        | 3       | 3       | 2       |
| Trypsin                 | N-benzoyl-tt-arginine-2-naphthylamide | 0   | 1        | 1       | 1       | 1       |
| α-chymotrypsin          | N-glutaryl-phenylalanine-2-naphthlamide | 0   | 1        | 0       | 0       | 0       |
| Acid phosphatase        | 2-naphthyl phosphate          | 5        | 5        | 5       | 5       | 5       |
| Naphthol-AS-BI-phosphohydrolase | Naphthol-AS-BI-phosphate     | 5        | 5        | 5       | 5       | 5       |
| α-galactosidase         | 6-Br-2-naphthyl-α-D-galactopyranoside | 0    | 0        | 0       | 0       | 0       |
| β-galactosidase         | 2-naphthyl-β-D-galactopyranoside | 0    | 0        | 0       | 0       | 0       |
| β-glucuronidase         | Naphthol-AS-BI-β-D-glucuronide | 0    | 0        | 0       | 0       | 0       |
| α-glucosidase           | 2-naphthyl-α-D-glucopyranoside | 3    | 4        | 3       | 3       | 4       |
| β-glucosidase           | 6-Br-2-naphthyl-β-D-glucopyranoside | 1  | 1        | 1       | 1       | 1       |
| N-acetyl-β-glucosaminidase | 1-naphthyl-N-acetyl-β-D-glucosamide | 0    | 0        | 0       | 0       | 0       |
| α-mannosidase           | 6-Br-2-naphthyl-α-D-mannopyranoside | 1    | 1        | 1       | 0       | 0       |
| α-fucosidase            | 2-naphthyl-α-L-fucopyranoside | 0    | 0        | 0       | 0       | 0       |

*Four selected S. cerevisiae strains isolated in this study (S. cerevisiae JBCC-21A, 40B, 43B and 197B).
Enzyme activity was expressed on a scale from 0 (no activity) to 5 (maximum activity).

Physicochemical characterization of the selected indigenous S. cerevisiae strains
As shown in Table 1, four selected S. cerevisiae strains JBCC-21, JBCC-40, JBCC-43, and JBCC-197 showed adequate sugar tolerance, as BRE for wine fermentation is usually approximately 20–25°Brix (Song et al., 2016). Moreover, those strains had a broad sugar metabolizing capacity, strong metabisulfite tolerance, and ethanol tolerance up to 10%, although the growth rate was slightly slower at 12.5% and 15% ethanol. These physicochemical properties clearly indicate that all S. cerevisiae strains were useful as wine fermentation starter cultures. Based on the physiological results reported in Fig. 1, the selected four isolates were evaluated for enzymatic activities using the API-ZYM test (Table 2). Although only a few strains of S. cerevisiae possess β-glucosidase activity, which impart special characteristics to wine, all four of the isolates displayed similar activity to the commercial strain, No. 7013.
Biogenic amine degrading native yeasts

The four BRWs were prepared by selecting indigenous S. cerevisiae starter cultures as described in Section “Materials and Methods”, and fermentation properties were analyzed. As shown in Table 3, the prepared four BRWs fermented by S. cerevisiae—JBCC-21, JBCC-40, JBCC-43, and JBCC-197—had a pH of 3.39–3.46, which is similar to typical red wine (pH 3.2–3.6). Because too high a pH (>3.5) and high sulfite levels promote LAB growth, which is an important factor in wine spoilage, our selected indigenous S. cerevisiae starter culture is promising for BRE fermentation (Edwards et al., 1993). The fermented wine with commercial strain of S. cerevisiae No. 7013 had a significantly (p < 0.05) lower pH and a higher acidity (0.85 ± 0.01%) than those of other BRWs (0.61–0.67%), but within the typical red wine value. During wine fermentation by indigenous S. cerevisiae, viable cells were log 5.5–5.8 CFU/mL at the end of alcohol fermentation, and there were no significant differences among the strains including commercial yeast (p > 0.05). The final ethanol concentration was achieved at 10–13% using 25∞Brix of BRE at 25°C for six days accompanied with a soluble solids (∞Brix) decrease to 10.3–10.8∞Brix. Specifically, ethanol production of the indigenous S. cerevisiae JBCC-21 was comparable with the widely used commercial Saccharomyces strain, No. 7013. In addition, the antioxidant activity and total flavonoids fermented by the selected yeasts was superior to those of the control wine (p < 0.05), but the total polyphenol content was lower than No. 7013. When the selected four indigenous S. cerevisiae starters that showed a high fermentative capacity and a low BA production were applied to a pilot-scale BRW fermentation, the concentrations of the main BAs in the non-inoculated black raspberry must were different depending on the S. cerevisiae starter strain, as shown in Table 4. When commercial starter S. cerevisiae No. 7013 was applied to the pilot scale fermentation process, several BAs

| Table 3. Physicochemical properties of fermented wines using indigenous yeasts and type strain. |
|---------------------------------------------------------------|--------|--------|--------|--------|--------|
| Log CFU/mL                                                   | 6.2 ± 0.15<sub>a</sub> | 5.78 ± 0.16<sub>b</sub> | 5.52 ± 0.38<sub>a</sub> | 5.79 ± 0.25<sub>b</sub> | 5.61 ± 0.64<sub>b</sub> |
| Soluble solids (∞Brix)                                      | 10.5 ± 0.01<sub>a</sub> | 10.3 ± 0.01<sub>a</sub> | 10 ± 0.00<sub>a</sub> | 10.7 ± 0.01<sub>a</sub> | 10.8 ± 0.00<sub>b</sub> |
| pH                                                          | 3.20 ± 0.05<sub>a</sub> | 3.46 ± 0.02<sub>a</sub> | 3.44 ± 0.02<sub>a</sub> | 3.40 ± 0.01<sub>a</sub> | 3.39 ± 0.02<sub>b</sub> |
| Ethanol (%)                                                  | 13.22 ± 0.21<sub>a</sub> | 13.31 ± 1.50<sub>a</sub> | 10.64 ± 1.29<sub>a</sub> | 9.75 ± 2.50<sub>a</sub> | 11.68 ± 0.78<sub>b</sub> |
| Acidity (%)                                                 | 0.05 ± 0.01<sub>a</sub> | 0.04 ± 0.01<sub>a</sub> | 0.01 ± 0.01<sub>a</sub> | 0.07 ± 0.02<sub>a</sub> | 0.01 ± 0.01<sub>b</sub> |
| Antioxidant activity (AEAC, %)                              | 83.85 ± 1.10<sub>a</sub> | 82.91 ± 2.41<sub>a</sub> | 83.47 ± 0.53<sub>a</sub> | 81.70 ± 0.50<sub>a</sub> | 83.66 ± 1.29<sub>b</sub> |
| Total polyphenol (g GAE/L)                                  | 1.09 ± 0.12<sub>a</sub> | 0.71 ± 0.01<sub>a</sub> | 0.71 ± 0.00<sub>a</sub> | 0.72 ± 0.00<sub>a</sub> | 0.72 ± 0.01<sub>b</sub> |
| Total flavonoid (g CE/L)                                    | 1.55 ± 0.06<sub>a</sub> | 1.83 ± 0.14<sub>a</sub> | 1.77 ± 0.16<sub>a</sub> | 2.28 ± 0.29<sub>a</sub> | 2.05 ± 0.22<sub>b</sub> |

Total polyphenol expressed by gallic acid equivalent (GAE); total flavonoid expressed by catechin equivalent (CE); antioxidant activity expressed by ascorbic acid equivalent antioxidant capacity (AEAC) with DPPH radical scavenging activity (%).<sup>a,b</sup>Small letters in the same column indicate significant differences of values between two samples by student’s t-test (p < 0.05).

Data is expressed as mean ± standard deviation (n = 3).

| Table 4. Biogenic amine contents in black raspberry wine fermented by different S. cerevisiae strains. |
|---------------------------------------------------------------|--------|--------|--------|--------|--------|
| Biogenic amine (mg/L)                                        | Control<sup>1</sup> | no.7013 | JBCC-21 | JBCC-40 | JBCC-43 | JBCC-197 |
| Putescine                                                   | nd     | nd     | nd     | nd     | nd     | nd     |
| Cadaverine                                                  | 1.87 ± 0.03 | 1.90 ± 0.08 | nd     | nd     | nd     | nd     |
| Histamine                                                   | 2.35 ± 0.02 | 5.24 ± 0.06 | nd     | nd     | nd     | nd     |
| Tyramine                                                    | nd     | nd     | nd     | nd     | nd     | nd     |
| 2-phenylethylamine                                          | nd     | nd     | nd     | nd     | nd     | nd     |
| Spermidine                                                  | 0.60 ± 0.01 | 4.10 ± 0.07 | nd     | nd     | nd     | nd     |
| Spermine                                                    | nd     | nd     | nd     | nd     | nd     | nd     |
| Serotonin                                                   | 47.56 ± 0.12 | 25.54 ± 0.30 | 14.10 ± 1.10 | 14.59 ± 0.91 | 14.16 ± 0.52 | 8.26 ± 0.31 |
| Dopamine                                                    | 3.90 ± 0.08 | 1.28 ± 0.05 | nd     | nd     | nd     | nd     |
| Tryptamine                                                  | nd     | nd     | nd     | nd     | nd     | nd     |
| L-noradrenaline                                             | nd     | nd     | nd     | nd     | nd     | nd     |

<sup>1</sup>Control: black raspberry extract without starter culture inoculation. nd: not detected.
presented in the non-inoculated black raspberry solution including histamine, spermidine, and cadaverine, which increased 1, 2.2, and 7-fold, respectively, in the final wine. However, those BAs were completely degraded in the fermented BRW by indigenous *S. cerevisiae* JBCC-21, JBCC-40, JBCC-43, and JBCC-197.

**Discussion**

Among the yeast strains isolated from grapes and wines, the highest concentration of total BAs was reported by *Brettanomyces bruxellensis*, followed by *S. cerevisiae* during grape must fermentation (Caruso et al., 2002). In wine, *S. cerevisiae* synthesizes ethanolamine, agmatine, and, in particular, cadaverine, and putrescine (Caruso et al., 2002; Granchi et al., 2005; Romano et al., 2007; Tristezza et al., 2013); however, tyramine, spermidine, and spermine production by *S. cerevisiae* has never been reported. Unexpectedly, the commercial wine fermentation strain in our study, *S. cerevisiae* No. 7013, produced higher spermidine than the control (over 4-fold), whereas selected *S. cerevisiae* JBCC-21 and *S. cerevisiae* JBCC-197 did not produce spermidine. Also, the other selected *S. cerevisiae* JBCC-40, and *S. cerevisiae* JBCC-43, effectively degraded spermidine during wine fermentation. Moreover, the selected *S. cerevisiae* strains did not produce histamine and cadaverine, even though the commercial *S. cerevisiae* No. 7013 yeast produced up to 5.24 ± 0.06 mg/L, 1.90 ± 0.08 mg/L, respectively. Although BA levels are lower than the significant toxicological threshold, causing adverse health problems, continuous exposure of those BAs might cause allergic reactions, tissue damage, or hypertension, especially in BA-intolerant people (Ladero et al., 2010; Linares et al., 2016). Because the individual toxicological threshold of BAs has not been established (except for histidine), it is important to reduce or minimize BAs using microbiological control (Caruso et al., 2002; Gardini et al., 2016). Cadaverine and putrescine are known to enhance histamine toxicity by inhibiting histamine metabolizing enzymes (Lehane and Olley, 2000). However, none of selected strains produced cadaverine, which is the most abundant compound in wines followed by histamine and tyramine (Beneduce et al., 2010; Costantini et al., 2009). Moreover, the selected four isolates in our study were also able to efficiently degrade three hazardous amines (cadaverine, histamine, and spermidine), which were originally derived from BRE. indicating that the selected *S. cerevisiae* strains might be useful as BA scavengers during fermentation. Specifically, the highest degradation capacity was evidenced by *S. cerevisiae* JBCC-197 in BRW; potentially risky histamine, cadaverine, spermidine, spermine, and 2-phenylethylamine were not detected after 3 days of BRW fermentation. Marcobal et al. (2006) reported that no remarkable change in the concentration of BA could be observed during alcoholic fermentation. Also, Landete et al. (2007) observed several yeast genera including *S. cerevisiae* did not appear to be responsible for the control of most amines found in synthetic medium as well as grape must. However, in our study, indigenous yeasts isolated from BRW effectively reduced BAs and controlled levels of BAs including histamine, cadaverine, and spermidine in BRW. Only a few studies have been conducted on the degradation of BAs by yeasts. Bäumlisberger et al. (2015) found that another potential starter culture *D. hansenii* H525, which was able to degrade a broad spectrum of BAs, had a low ethanol tolerance and possibly generated off flavors. Some *S. cerevisiae* are involved in the degradation of putrescine during alcoholic fermentation (Granchi et al., 2005), but yeast isolates herein were able to degrade a broader range of BAs; particularly, all strains degraded cadaverine, which is the major BA in BRE. Cadaverine and putrescine, which are not toxic themselves, increase adverse effects from BAs by interfering with enzymes that metabolize histamine, tyramine, and phenylethylamine (Ten Brink et al., 1990). Thus, BRWs inoculated with selected isolates that degrade both histamine and cadaverine, which already exist in black raspberry must, have a lower potential risk of adverse effects. However, those BAs were increased in BRW inoculated *S. cerevisiae* No. 7013, because No. 7013 had the ability to synthesize histamine and that resulted in an increased toxicity of cadaverine. Degradation of BA by indigenous yeast could be attributed to amino acid consumption and enzyme activity. Consumption of precursor amino acids varies depending on the inoculated yeasts during fermentation (Torrea and Ancin, 2002), or enzyme activity, such as amine oxidase and the presence or absence of amino acid decarboxylase genes (e.g., histidine decarboxylase, tyrosine decarboxylase, ornithine decarboxylase) in the yeast. Although there are some discrepancies due to the limit of analytical techniques, further analysis is needed to gain insight into the correlation between genotypic and phenotypic BA-degrading characters of selected isolates.

Unlike commercial wine yeast, local strains easily dominate raw materials and provide typical characteristics representative of each wine area. Moreover, some authors highlight that native strains are genetically diverse depending on the viticulture region and offer important oenological features (Tofalo et al., 2014). Thus, discriminating indigenous yeasts from commercial strains using molecular techniques is needed. Recently, microsatellite (or simple sequence repeat) analysis has been employed for the characterization of *S. cerevisiae*, according to the diversity of polymorphisms by allelic variation (Börlin et al., 2016; Marongiu et al., 2015). An analysis comparing microsatellites can clearly identify a yeast strain, and the genetic discrimination is so powerful that microsatellites are extensively used for paternity exclusion tests (Helminen et al., 1988) and forensic medicine (Hagelberg et al., 1991). Indeed, microsatellites were used to study the pathogen yeast *Candida albicans* (Brengne et al., 1997), and for successful identification of closely related yeast species (Hennequin et al., 2001; Richards et al., 2009). In our study, we examined microsatellites to differentiate the selected *S. cerevisiae* strains. Generally, yeast strain clustering is linked to technological use (Legras et al., 2007; Marongiu et al., 2015). The commercial beer strains partially gather and are divided into three clusters, bread strains are scattered but are positioned near the beer strains. Despite the same BRW origin, the four isolates were positioned far from commercial wine strains;
S. cerevisiae JBCC-21, 40, and 197 belong to the beer yeasts group (63–67% similarity), but S. cerevisiae JBCC-43 was close to bread and wine yeasts (67% similarity). Interestingly, the similarities between wine yeasts were even lower (52.4%). Thus, the isolates in this study were genetically distinct from the widely used commercial wine yeasts but were useful for BRW fermentation.

Different enzymatic activities during fermentation may impact the physicochemical stability, and sensorial profile of the wine (Strauss et al., 2001). Glycosidases, especially β-glucosidase, are responsible for the hydrolysis of glycosidic monoterpenes (terpenyl glucoside), converting them from their precursors in grapes into free aroma compounds, which contribute to the aromatic character of wine (Padilla et al., 2016). In our study, higher α-glucosidase activity (level 4) was detected for S. cerevisiae JBCC-21 and JBCC-197 compared to S. cerevisiae No. 7013 (level 3). β-glucosidase activity of S. cerevisiae is strain-dependent; Elmaci et al. (2014) reported that none of the strains isolated from Turkish wine had β-glucosidase activity, and the reference strain showed only slight β-glucosidase activity (level 1) and α-glucosidase activity. Aponé and Blaiotta (2016) reported yeast strains isolated from Moscato di Saracena wine exhibited negative or moderate (level 2–3) activities of β-glucosidase. Even some non-S. cerevisiae sp. exhibited β-glucosidase activity. β-glucosidase activity may be inhibited by acid or high ethanol concentrations, so these properties and enzymatic activities of must and wine should be considered collectively. Furthermore, yeast esterase activity also plays an important role in the varietal and fermentative flavor of wine (Elmaci et al., 2014). The four yeast isolates in this study exhibited moderate (level 2) esterase lipase (C8) activity that was slightly higher than the type strain (level 1). Enzymatic hydrolysis of haze-forming proteins is important for wine stabilization and clarification (Moreno-Arribas and Polo, 2005). These strains possessed positive levels (2–5) of leucine, valine, and cysteine arylamidase activity. The commercial strain did not show endopeptidase, trypsin, or α-chymotrypsin activities, while weak trypsin activity (level 1) was recorded for all isolates and only S. cerevisiae JBCC-21 exhibited a weak (level 1) α-chymotrypsin activity. Overall, the isolates in this study showed different enzyme activities than the commercial strain.

This study describes the development of four specific indigenous S. cerevisiae strains based on their BA-degrading capacity and suggests that our strains are promising tools for low-BA BRW. Additionally, the strains’ physiological and biochemical properties on BRW fermentation ensures high quality and functionality with good ethanolation production during fermentation. This is the first report of efficient BA reduction in BRW by indigenous black raspberry-originated yeasts. BA-free or low-BA BRW should be produced to avoid possible harmful effects of BAs and enhance the safety of wine. The oenological and physicochemical traits of wines obtained with indigenous starters showed better characteristics compared with those obtained by commercial and type strains. Future work should assess these newly isolated yeast strains from BRE as starter cultures in various berry wine fermentation.

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Supplementary Materials

Supplementary tables are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/ggam).
Gardini, F., Özogul, Y., Suzzi, G., Tabanelli, G., and Özogul, F. (2016) Technological factors affecting biogenic amine content in foods: a review. *Front. Microbiol.*, 7, 1218.

Granchi, L., Romano, P., Mangani, S., Guerrini, S., and Vincenzini, M. (2005) Production of biogenic amines by wine microorganisms. *Bull. O.I.V.*, 78, 595–610.

Hagelberg, E., Gray, I. C., and Jeffreys, A. J. (1991) Identification of the skeletal remains of a murder victim by DNA analysis. *Nature*, 352, 427–429.

Helminen, P., Lokki, M. L., Ehnholm, C., Jeffreys, A., and Peltonen, L. (1988) APPLICATION OF DNA. *Lancet*, 331, 574–576.

Hennequin, C., Thierry, A., Richard, G. F., Lecointre, G., Nguyen, H. V. et al. (2001) Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. *J. Clin. Microbiol.*, 39, 551–559.

Kim, B. H. and Park, S. K. (2015) Volatile aroma and sensory analysis of black raspberry wines fermented by different yeast strains. *J. Inst. Brew.*, 121, 87–94.

Ladero, V., Calles-Enríquez, M., Fernández, M., and A Alvarez, M. (2010) Toxicological effects of dietary biogenic amines. *Curr. Nutr.* Food Sci., 6, 145–156.

Landete, J. M., Ferrer, S., and Pardo, I. (2005) Which are the lactic acid bacteria responsible of histamine production in wine? *J. Appl. Microbiol.*, 99, 580–586.

Landete, J. M., Ferrer, S., and Pardo, I. (2007) Biogenic amine production by lactic acid bacteria, acetic bacteria and yeast isolated from wine. *Food Control*, 18, 1569–1574.

Lee, Y., Kim, J. C., Hwang, K. T., Kim, D. H., and Jung, C. M. (2013) Quality characteristics of black raspberry wine fermented with different yeasts. *J. Korean Soc. Food Sci. Nutr.*, 42, 784–791.

Legras, J. L., Merdinoğlu, D., Cornuet, J., and Karst, F. (2007) Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. *Mol. Ecol.*, 16, 2091–2102.

Lehane, L. and Olley, J. (2000) Histamine fish poisoning revisited. *Int. J. Food Microbiol.*, 58, 1–37.

Linares, D. M., del Rio, B., Redruello, B., Ladero, V., Martin, M. C. et al. (2016) Comparative analysis of the in vitro cytotoxicity of the dietary biogenic amines tyramine and histamine. *Food Chem.*, 197, 658–663.

Lonvau-Funel, A. (2001) Biogenic amines in wines: Role of lactic acid bacteria. *FEMS Microbiol. Lett.*, 199, 9–13.

Marcobal, A., Martin-Alvarez, P. J., Polo, M. C., Muñoz, R., and Moreno-Arribas, M. V. (2006) Formation of biogenic amines throughout the industrial manufacture of red wine. *J. Food Prot.*, 69, 397–404.

Marongiu, A., Zara, G., Legras, J. L., Del Caro, A., Mascia, I. et al. (2015) Novel starters for old processes: use of *Saccharomyces cerevisiae* strains isolated from artisanal sourdough for craft beer production at a brewery scale. *J. Ind. Microbiol. Biotechnol.*, 42, 85–92.

Moon, J. S., Kim, Y., Jang, K. I., Cho, K. J., Yang, S. J. et al. (2010) Analysis of biogenic amines in fermented fish products consumed in Korea. *Food Sci. Biotechnol.*, 19, 1689–1692.

Moreno-Arribas, M. V. and Polo, M. C. (2005) Winemaking biochemistry and microbiology: current knowledge and future trends. *Crit. Rev. Food Sci. Nutr.*, 45, 265–286.

Padilla, B., Gil, J. V., and Manzanares, P. (2016) Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.*, 7, 411.

Richards, K. D., Goddard, M. R., and Gardner, R. C. (2009) A database of microsatellite genotypes for *Saccharomyces cerevisiae*. *Antonie Van Leeuwenhoek*, 96, 355–359.

Romano, P., Capece, A., and Poeta, C. (2007) Biogenic amine formation in alcoholic fermentation. *Bull. O.I.V.*, 80, 251–262.

Rosi, I., Nannelli, F., and Giovani, G. (2009) Biogenic amine production by *Oenococcus oeni* during malolactic fermentation of wines obtained using different strains of *Saccharomyces cerevisiae*. *LWT- Food Sci. Technol.*, 42, 525–530.

Song, N. E., Cho, H. S., and Baik, S. H. (2016) Bacteria isolated from Korean black raspberry vinegar with low biogenic amine production in wine. *Brac. J. Microbiol.*, 47, 452–460.

Spano, G., Russo, P., Lonvau-Funel, A., Lucas, P., Alexandre, H. et al. (2010) Biogenic amines in fermented food. *Eur. J. Clin. Nutr.*, 64, S95–S100.

Strauss, M. L. A., Jolly, N. P., Lambrechts, M. G., and Van Rensburg, P. (2001) Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. *J. Appl. Microbiol.*, 91, 182–190.

Ten Brink, B., Damink, C., and Joosten, H. M. L. J. (1990) Occurrence and formation of biologically active amines in foods. *Int. J. Food Microbiol.*, 11, 73–84.

Tofalo, R., Perpetuini, G., Fasoli, G., Schirone, M., Corsetti, A., et al. (2014) Biodiversity study of wine yeasts belonging to the “terroir” of Montepulciano d´Abruzzo “Colline Teramane” revealed *Saccharomyces cerevisiae* strains exhibiting atypical and unique 5.8 S-ITS restriction patterns. *Food Microbiol.*, 39, 7–12.

Torrea, D. and Ancin, C. (2002) Content of biogenic amines in a Chardonnay wine obtained through spontaneous and inoculated fermentations. *J. Agric. Food Chem.*, 50, 4895–4899.

Tristezza, M., Vetrano, C., Bleve, G., Spano, G., Capozzi, V. et al. (2013) Biodiversity and safety aspects of yeast strains characterized from vineyards and spontaneous fermentations in the Apulia Region, Italy. *Food Microbiol.*, 36, 335–342.

Vaudano, E. and Garcia-Moruno, E. (2008) Discrimination of *Saccharomyces cerevisiae* wine strains using microsatellite multiplex PCR and band pattern analysis. *Food Microbiol.*, 25, 56–64.