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Research Article

Microbiological Characteristics of Wild Yeast Strain *Pichia anomala* Y197-13 for Brewing *Makgeolli*

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Abstract *Makgeolli* is a traditional cloudy-white Korean rice wine with an alcohol content of 6~7%. The present study investigated the morphological characteristics, carbon-utilizing ability, fatty acid composition, alcohol resistance, glucose tolerance, and flocculence of *Saccharomyces cerevisiae* Y98-5 and *Pichia anomala* Y197-13, non-*S. cerevisiae* isolated from *Nuruk*, which is used in brewing *Makgeolli*. Similar morphological characteristics were observed for both isolated wild yeast strains; and the carbon source assimilation of Y197-13 differed from that of other *P. anomala* strains. Strain Y197-13 was negative for D-trehalose, mannitol, arbutin, l-erythritol, and succinic acid. The major cellular fatty acids of strain Y197-13 included C18:2n6c (33.94%), C18:1n9c (26.97%) and C16:0 (20.57%). Strain Y197-13 was Crabtree-negative, with 60% cell viability at 12% (v/v) ethanol. The flocculation level of strain Y197-13 was 8.38%, resulting in its classification as a non-flocculent yeast.

Keywords *Makgeolli*, *Nuruk*, *Pichia anomala*, Y197-13

The taste of *Makgeolli* comes from components such as free sugars, amino acids, and organic acids, which are produced via degradation of raw materials by the microorganisms present in *Nuruk*, and from volatile flavor compounds produced by alcohol fermentation mediated by yeast [1]. Recent popularity of *Makgeolli* in Korea and Japan has led to increased production. However, the yeasts that are essential for *Makgeolli* fermentation are not as diverse as those used for production of Japanese sake, European wines, or European distilled liquors. Domestic universities and research centers have studied the fermentation of microorganisms related to brewing and the fermentation process, although most studies have focused on the *Aspergillus* genus of fungi isolated from Japanese sake. In addition, most studies on the microorganisms in traditional *Nuruk* have focused on fungi [2] and *Saccharomyces cerevisiae* [3].

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Therefore, in order to improve the quality of traditional Korean alcoholic beverages, study of the microorganisms present in *Nuruk* is needed. Accordingly, the previous study [4] was conducted in order to identify novel yeasts that can be used in brewing *Makgeolli*. Unlike previous studies that identified yeasts that yield a high alcohol content [5], we isolated the wild yeast strain, *Pichia anomala* Y197-13, from *Nuruk*, which has characteristics similar to those of *Makgeolli* brewed with *S. cerevisiae* Y98-5, despite their low alcohol production.

Because traditional Korean *Nuruk* is produced naturally, aflatoxins B1, B2, G1, and G2, which are secondary metabolites of fungi (e.g., *Aspergillus flavus* and *A. parasiticus*) may be produced [6]. According to the US Department of Agriculture (USDA) Agricultural Research Service (ARS), the yeast *P. anomala* competes with *A. flavus*, a fungus that causes rotting and growth inhibition of nuts. In addition, *P. anomala* improves the taste, texture, yield, and safety of agricultural products by outcompeting more undesirable fungi, which has brought it to the attention of researchers working in environmental, industrial, and medical fields. *P. anomala* varies in terms of its environmental habitat, growth pattern, metabolism, stress tolerance, and anti-microbial characteristics. It is isolated from plant seeds, fruit skins, human tissue, milk products, baked products, salted foods, waste water, tree secretions, and marine environments [7]. Although the level of sugar fermentation by *P. anomala* varies depending on the species, it is usually weak. *P. anomala* is a film-forming yeast. Despite some reports of pathogenic infections by *P. anomala*, it was recently granted "qualified presumption of safety (QPS)" status by the European

Food Safety Authority (EFSA) and is considered to be beneficial to food biotechnology [8].

Of particular interest, strain Y197-13 did not form a biofilm in *Koji* extract medium and brewed *Makgeolli* was expected to have an appropriate flavor in contrast to the plain flavor of conventional *Makgeolli* [4]. In this study, the morphological characteristics, including flocculation, carbon source assimilation, cellular fatty acid composition, alcohol resistance, and glucose tolerance characteristics of strain Y197-13 that could potentially be used in brewing of *Makgeolli* were investigated.

MATERIALS AND METHODS

Strains and media. The wild yeast strains Y98-5 (*Saccharomyces cerevisiae*) and Y197-13 (*Pichia anomala*) used in this study were isolated from stock *Nuruk* samples collected from Chungnam Gongju and Chungbuk Jeungpyeong province of South Korea. Type strain *S. cerevisiae* ATCC 24858 (KFRI 1017) was obtained from the Korea Food Research Institute. Potato dextrose agar and potato dextrose broth (PDB) were purchased from Becton Dickinson (Sparks, MD, USA). Unless otherwise specified, all analytical chemicals used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA) and were of the highest grade available.

Morphological characteristics. The morphological characteristics of the isolated yeasts were examined after growth in 5% malt extract media (Difco, Detroit, MI, USA) at 25°C for three days, as previously described [9]. An OLYMPUS BX43 microscope (Olympus, Tokyo, Japan) and Motic Images plus 2.0 ML (Motic China Group Co., Ltd., Xiamen, China) software were used for observation.

Phylogenetic analysis. The 18S rRNA gene sequence of strain Y197-13 was amplified from genomic DNA using the universal primers ITS1 and ITS4 [10] and the purified PCR products were sequenced by Macrogen (Seoul, Korea) according to the previous study [4]. The sequences (ITS1, 1,258 bp; ITS4, 1,401 bp) of the 18S rRNA genes were assembled using SeqManII software version 5.0 (DNAStar, Inc., Madison, WI, USA). The 18S rRNA gene sequences of related taxa were obtained from the GenBank database and gaps were edited using the BioEdit program [11]. Multiple alignments of the sequences were performed using the ClustalX program [12]. A phylogenetic tree was constructed using the neighbor-joining method using the MEGA4 program [13]. The 18S rRNA gene sequence of strain Y197-13 was deposited in the GenBank database under accession number JX566694.

Flocculation. For measurement of flocculation, the yeast strains were incubated with shaking at 150 rpm on a shake incubator for two days at 25°C in a PDB (Difco) liquid culture. Grown cultures were divided, 10 mL each, and added to two tubes (A and B). Tube A was centrifuged at 3,000 rpm for 2.5 min, and the supernatant was discarded. Tube A was re-suspended in distilled water/0.5 M EDTA solution (pH 7.0). The solution was vortexed for 15 sec, then diluted 10-fold with distilled water and the absorbance measured at 600 nm. Tube B was centrifuged at 3,000 rpm for 2.5 min, and discarded the supernatant. Tube B was resuspended in a 3.75 mM $CaSO_4$ washing solution, centrifuged at 3,000 rpm for 2.5 min again, and the supernatant was discarded. Tube B was re-suspended in $CaSO_4$ suspension solution (0.051% $CaSO_4$, 0.68% CH_3COONa, and 0.4% CH_3COOH, pH 4.5). The solution was vortexed for 15 sec, and left to settle for exactly 6 min, then diluted 10-fold with distilled water and the absorbance was measured at 600 nm [14]. Flocculence was calculated as a percentage, as follows: $\% = (A - B) \times 100/A$.

Carbon source assimilation. The carbon source assimilation of the isolated yeasts was examined in 96-well MicroPlate coated with 95 different carbon sources (BIOLOG, Hayward, CA, USA) and analyzed using a MicroLog System (BIOLOG). Yeasts were cultured on BUY medium (BIOLOG) for 48 hr at 26°C. The inoculating fluid was made at a concentration of 47% turbidity in sterilized water, and transferred to a YT MicroPlate (100 µL per well). The yeasts were then cultured at 26°C for 48 or 72 hr; the optical density was then determined using a microstation reader (BIOLOG) at a wavelength of 590 nm.

Analysis of fatty acids. Fatty acids were extracted using the modified Folch extraction method [15]. Briefly, a freeze dried sample (3 g) was placed in a vial followed by addition of 18 mL of extraction solvent (chloroform : methanol = 2 : 1, v/v). The internal standard (200 ppm [v/v] heptadecanoic acid) was added to the sample vial, which was then sealed and sonicated at 30°C for 30 min. The vial was then vortexed and the solution was re-sonicated at 30°C for 30 min. Four milliliters of a 0.88% NaCl solution were then added to the vial. The vial was vortexed again and then centrifuged at 2,000 rpm for 10 min. The lower phase of the resulting solution was collected using sodium sulfate and transferred to a new tube; it was then blown up using N_2 gas. Twenty five milligrams of the extracted fat were placed in a capped tube. Following addition of 1.5 mL of 0.5 N methanolic NaOH, the tube was heated at 100°C for 5 min and then cooled in a water bath (30°C) for 1 min. For esterification, 2 mL of BF_3-methanol (Sigma-Aldrich) was added to the tube. The tube was then heated in a heat block (100°C) for 2 min and cooled in a water bath (30°C) for 1 min. The solution in the tube was extracted using 2 mL hexane followed by addition of 1 mL saturated NaCl solution. The extract was gently shaken, and left to stand for 2 min. The upper layer of the extract was then collected and dehydrated using anhydrous sodium sulfate [16]. One microliter of the extracted fatty acids was loaded onto a gas chromatograph (GC; Agilent 6890; Agilent, Palo

Alto, CA, USA) equipped with a HP88 capillary column (100 m length × 0.25 mm i.d. × 0.2 μm film thickness; Agilent) for analysis. The oven temperature of the GC was held at 140°C for 5 min and then set to rise to 240°C at a rate of 4°C/min. The conditions were: injector temp, 250°C; detector temp, 260°C; split, 10 : 1. Helium was used as the carrier gas at a flow rate of 2 mL/min.

Ethanol resistance and glucose tolerance. To determine the ethanol resistance of each yeast strain, anhydrous ethanol (zero, 8, 12, 16, or 20% [v/v]) was added to the liquid YPD culture (2% glucose, 0.5% yeast extract, 1% bacto-peptone; Sigma-Aldrich) immediately after injection of yeast. The absorbance at 660 nm was determined after incubation for 72 hr at 20°C. Glucose tolerance was determined by measuring the absorbance at 660 nm after incubating the yeast strains for 48 hr at 20°C in liquid YPD culture containing 2%, 30%, 40%, 50%, or 60% glucose [17].

RESULTS AND DISCUSSION

Morphological characteristics. The morphological characteristics of *S. cerevisiae* ATCC 24858 and the isolated strains Y98-5 and Y197-13 are shown in Table 1. Because most yeast have a similar cell shape identification of different yeast species is impossible based on morphological characteristics alone. All of the yeast cells examined had a spheroidal-to-ovoid shape. *S. cerevisiae* ATCC 24858 measured 3.0~6.5 (width) × 5~8.5 (length) μm. Y98-5 and Y197-3 measured 3.5~6.5 × 5~8.5 μm and 3.0~5.0 × 3.5~6.5 μm, respectively. Colonies of all strains on 5% malt agar were circular convex and light cream-colored with three days of growth at 25°C. All strains had one to four ascospores, which were globose-to-short ellipsoidal in shape.

Phylogenetic analysis. The 18S rRNA gene sequence analysis indicated the strain Y197-13 (GenBank: JX566694, 1 ctgcngaagg nncatnatag tattctattg ccagcgctta attgcgcggc gataaacctt 61 acacacattg tctagttttt ttgaactttg ctttgggtgc atcagcctag ctgcgtgccc 121 aaaggtctaa acacattttt ttaatgttaa aacctttaac caatagtcat gaaaattttt 181 aacaaaaatt aaaatcttca aaactttcaa caacggatct cttggttctc gcaacgatga 241 agaacgcagc gaaatgcgat acgtattgtg aattgcagat tttcgtgaat catcgaatct 301 ttgaacgcac attgcaccct ctggtattcc agagggtatg cctgtttgag cgtcatttct 361 ctctcaaacc ttcgggtttg gtattgagtg atactctgtc aagggttaac

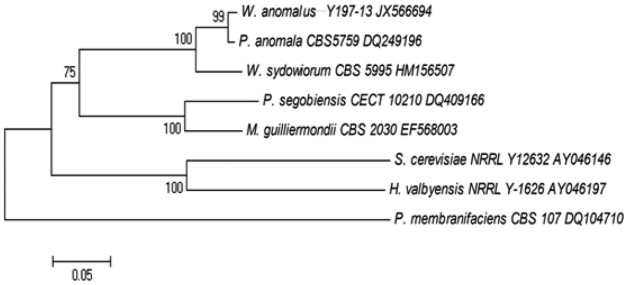

Fig. 1. Phylogenetic tree based on 18S rRNA gene sequences showing the positions of strain Y197-13 with other *Pichia* species. Bootstrap values > 50% based on 500 replications are shown at branching points. Bar, 0.05 substitutions per nucleotide position. *W.*, *Wickerhamomyces*; *M.*, *Meyerozyma*; *S.*, *Saccharomyces*; *H.*, *Hanseniaspora*.

ttgaaatatt 421 gacttagcaa gagtgtacta ataagcagtc tttctgaaat aatgtattag gttcttccaa 481 ctcgttatat cagctaggca ggtttagaag tattttaggc tcggcttaac aacaataaac 541 taaaagtttg acctcaaatc aggtaggact acccnntgnn cttaagca) is most closely related to the *Wickerhamomyces anomalus* with similarity levels of 98% (match 522 bp/total 530 bp) [4]; *Wickerhamomyces anomalus,* with the synonym *Pichia anomala*. Based on the phylogenetic tree, these reference strains include *P. anomala* CBS 5759^{T} and *W. sydowiorum* CBS 5995^{T}, were the phylogenetically closest taxa (Fig. 1).

Flocculation. Yeast flocculation is of critical importance to the brewer as very flocculent yeast provide a positive effect; separation of yeast cells from beer at the end of fermentation is a simple process [18]. On the contrary, non-flocculent yeast may be suitable for brewing *Makgeolli* while still providing its characteristic cloudiness. The flocculation value for *S. cerevisiae* ATCC 24858 was 25.27% and that of strain Y98-5 was 21.75%, indicating that they belonged to the moderately flocculent group of yeasts (20~60%). However, strain Y197-13 showed flocculation of 8.38%, resulting in its classification as a non-flocculent yeast (< 20%).

Carbon source assimilation. BIOLOG kit accurately identifies microorganisms by producing a characteristic pattern or metabolic fingerprint from discrete test reactions performed in 96-well microplate. Ha *et al.* [19], who isolated starch-utilizing yeasts from *Nuruk,* reported that,

Table 1. Morphological characteristics of strains Y98-5 and Y197-13

Strain	Cell form			Ascospores	
	Size (width × length, μm)	Color	Shape	Number	Shape
1	3.0~6.5 × 5.0~8.5	Light cream	Spheroidal, ovoid	1-4	Globose, ellipsoidal
2	3.5~6.5 × 5.0~8.5	Light cream	Spheroidal, ovoid	1-4	Globose, ellipsoidal
3	3.0~5.0 × 3.5~6.5	Light cream	Spheroidal, ovoid	1-4	Globose, ellipsoidal

Strains: 1, *Saccharomyces cerevisiae* ATCC 24858; 2, *S. cerevisiae* Y98-5; 3, *Pichia anomala* Y197-13.

among the six isolated *H. anomala* species, raffinose could not be utilized. Strain Y197-13 was positive for D-raffinose. Unlike previously reported characteristics of *P. anomala* [9], strain Y197-13 was negative for D-trehalose. D-Xylose,

Table 2. Phenotypic characteristics of strains Y98-5 and Y197-13

Carbon source	Strain			
	1	2	3	4
Dextrin	w	v	−	v
Inulin	w	−	w	−
Cellobiose	−	−	w	+
Gentiobiose	w	−	w	v
D-Melezitose*	+	v	+	+
D-Melibiose	−	v	−	−
Stachyose	+	+	w	v
D-Trehalose	+	+	−	+
N-Acetyl-D-glucosamine	w	−	−	−
D-Galactose*	+	v	+	v
D-Psicose	w	v	w	v
Salicin	−	−	−	+
D-Mannitol	w	−	−	+
D-Sorbitol	−	−	w	v
Xylitol	w	−	−	−
Glycerol	−	−	w	+
α-Methyl-D-glucoside	+	v	w	+
Amygdain	+	−	−	−
Arbutin	w	−	−	+
Maltitol	+	+	w	v
I-Erythritol	−	−	−	+
L-Arabinose	−	−	w	v
D-Arabinose	w	−	−	−
D-Ribose	w	-	-	v
D-Xylose	w	−	−	v
N-Acetyl-L-glutamic acid + D-xylose	w	−	w	−
Quinicacid + D-xylose	w	−	−	v
D-Glucuronicacid + D-xylose	w	−	−	−
α-D-Lactose + D-xylose	w	−	−	−
D-Galactose + D-xylose	+	+	w	v
m-Inositol + D-xylose	w	−	−	−
1,2-Propanediol + D-xylose	w	−	−	−
Acetic acid	−	−	w	−
Propionic acid	−	−	w	−
Succinic acid	−	v	−	+
D-Gluconic acid	−	v	−	v

Strains: 1, *Saccharomyces cerevisiae* Y98-5; 2, *S. cerevisiae* CBS 1171; 3, *Pichia anomala* Y197-13; 4, *P. anomala* CBS 5759. Phenotypic characteristics of yeast strains 2 and 4 are indicated by the result reported by Kurtzman and Fell [9] and included in the BIOLOG database. In the BIOLOG kit, all strains were positive for maltose*, maltotriose*, D-raffinose*, sucrose* and D-glucose* but negative for L-sorbose, D-arabitol, tween 80, D-glucosamine, L-rhamnose, β-methyl-D-glucoside, adonitol, acetoin + D-xylose, formic acid, L-aspartic acid, L-glutamic acid, L-proline, fumaric acid, L-malic acid, methyl succinate, bromosuccinic acid, γ-amino butyric acid, α-ketoglutaric acid and 2-keto-D-gluconic acid. +, positive; −, negative; w, weak positive; v, valuable; *, fermentable carbon sources of Y197-13.

a component of plant cell wall hemicellulose, is not assimilated by *S. cerevisiae*, however, it is assimilated by *P. anomala* [9]. Findings of the current study showed that D-xylose was weakly assimilated by Y98-5 but was not assimilated by Y197-13. The isolated strainsY98-5 and Y197-13 showed weak positivity for inulin. *S. cerevisiae* cannot utilize cellobiose, however, *P. anomala* can [9]. According to our findings, Y98-5 was negative for cellobiose, but Y197-13 was weakly positive. One study reported that *S. cerevisiae* cannot assimilate mannitol, but *P. anomala* can [9]. Strain Y98-5 showed weak positivity for mannitol, but Y197-13 was negative. In addition, previous reports have suggested that *P. anomala* can assimilate I-erythritol [19]; however, in this study, Y197-13 did not assimilate it. α-Methyl-D-glucoside may be assimilated by yeast depending on the circumstances [9]. Shin *et al.* [3] reported that isolated yeasts from *sake* were negative for α-methyl-D-glucoside. *P. anomala* was reported to assimilate α-methyl-D-glucoside [9]. Strain Y98-5 was positive for α-methyl-D-glucoside, which was weakly assimilated by Y197-13. Strain Y98-5 was negative for most of the acids examined, including succinic acid. Unlike previously reported characteristics of *P. anomala* [9], Y197-13 was negative for succinic acid, whereas Y197-13 showed weak positivity for acetic acid and propionic acid (Table 2).

Cellular fatty acids composition. According to an analysis of fatty acids extracted from *S. cerevisiae* isolated at a beer-brewing facility, *S. cerevisiae* mainly contains unsaturated fatty acids such as C16:1 and C18:1n9c at a 1:1 ratio; then, C16:0, a saturated fatty acid, accounted for 5.6~10.7% of the total fatty acids [20, 21]. Consistent with this result, fatty acids extracted from *S. cerevisiae* Y98-5 in the present current comprised mainly C16:1 and C18:1n9c at 3:1 ratio (Table 3). Compared with the findings reported by Thomas *et al.* [22], showing that ethanol

Table 3. Fatty acid profiles of strains Y98-5 and Y197-13

No.	Fatty acids	Y98-5	Y197-13
1	C10:0	1.03 ± 0.18	ND
2	C12:0	1.94 ± 0.14	ND
3	C14:0	1.42 ± 0.01	ND
4	C14:1	1.45 ± 0.11	ND
5	C15:1	1.00 ± 0.12	ND
6	C16:0	5.98 ± 0.22	20.57 ± 0.43
7	C16:1	63.17 ± 0.09	6.19 ± 0.29
8	C17:1	ND	1.15 ± 0.01
9	C18:0	1.48 ± 0.07	1.60 ± 0.16
10	C18:1n9c	21.16 ± 0.53	26.97 ± 0.01
11	C18:2n6c	ND	33.94 ± 0.23
12	C18:3n3	ND	9.59 ± 0.22
13	C23:0	1.36 ± 0.04	ND
	Total	100	100

Values are percentages (mean ± SD, n = 3) of the total fatty acid methyl esters.
ND, not detected.

tolerance increases with palmitoleic acid content, the aforementioned ratio is consistent with the finding that the viability of *S. cerevisiae* Y98-5 was 60% in an environment containing 20% (v/v) ethanol. In addition, C10:0 and C12:0, which both saturated medium-chain fatty acids, accounted for 1~1.94%, and C16:0 accounted for 5.98%.

Fatty acids extracted from *P. anomala* were mainly unsaturated and included C18:1n9c + C18:3n3, and saturated fatty acids such as C16:0. The ratios were as follows: C18:1n9c + C18:3n3 (40~59%) > C18:2n6c (18~38%) > C16:0 (8~18%) [22, 23]. The major cellular fatty acids of strain Y197-13 were C18:2n6c, C18:1n9c, C16:0, C18:3n3, and C16:1.

Ethanol resistance and glucose tolerance. Ethanol resistance of the isolated strains and reference strain are shown in Fig. 2. Strains Y98-5 and ATCC 24858 showed high cell viability (90%) in 8% (v/v) ethanol, whereas Y197-13 showed viability of 80%. At 12% (v/v) ethanol, strains Y98-5 and ATCC 24858 showed high cell viability: 80.4% and 77.9%, respectively. Strain Y197-13 showed viability of 60%, and is therefore unsuitable for production of liquors with high alcohol content. Inhibition of growth of the *sake* yeast is reported to occur at 12% (v/v) ethanol [24]. At 16% (v/v) ethanol, ATCC 24858 showed the same viability as in 12% (v/v) ethanol, whereas Y98-5 showed viability of 71.9%. Strain Y197-13 showed viability of 54.5%. At the maximum ethanol content of 20% (v/v), the cell viability of Y98-5, ATCC 24858, and Y197-13 was 63.96%, 56.11%, and 39.6%, respectively. The levels of resistance to 20% (v/v) ethanol reported herein are similar to, or higher than, those reported by Kim and Koh [25] and Lee *et al.* [26].

Glucose tolerance of the isolated strains and reference strain are shown in Fig. 3. At 30% (w/v) glucose, Y98-5 showed high cell viability (97.68%), 13% higher than that for ATCC 24858 (84.12%), while strain Y197-13 showed lower viability (69.86%). These values are higher than those reported by Kim and Koh [25] at 20% (w/v) glucose. At 40% (w/v) glucose, the viability of ATCC 24858, Y98-5, and Y197-13 was 81.87%, 78.59%, and 58.05%, respectively. At 50% (w/v) glucose, the viability of Y98-5 was 7.6% higher than that of ATCC 24858. Strain Y197-13 showed 59.35% viability, similar to that in 40% (w/v) glucose. These values are higher than those for the 18 yeasts reported by Lee *et al.* [26] at 50% (w/v) glucose, and similar to those for *S. cerevisiae, P. anomala,* and *W. anomalus* strains that were identified as having resistance to high osmotic pressure. At 60% (w/v) glucose, the cell viability of ATCC 24858 and Y98-5 showed a marked decrease (to 47%). This result is similar to those reported by Yang *et al.* [27]. The cell viability of Y197-13 at 60% (w/v) glucose was similar to that at 50% (w/v), suggesting that Y197-13 is not affected by the high sugar concentration. In contrast to *S. cerevisiae,* which is popular as a brewing yeast, *P. anomala* has a different mechanism for central carbon metabolism (Crabtree-negative). They do not show mitochondrial repression by glucose, but do show high sensitivity to changes in oxygen concentration [28]. Thus, the decrease in cell viability of Y197-13 up to 40% (w/v) glucose was likely due to decreased oxygen concentrations. However, the consistent viability up to 60% glucose was likely due to its tolerance of high sugar concentrations.

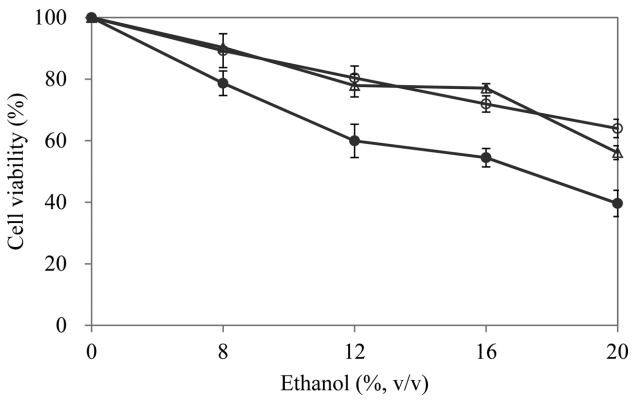

Fig. 2. Ethanol resistance of the type strain ATCC24858 and the wild yeast strains Y98-5 and Y197-13. Cells were cultured at 20°C for 72 hr in YPD broth containing 2, 8, 12, 16, and 20% ethanol. Y98-5 (open circles), Y197-13 (closed circles), ATCC 24858 (open triangles). Each data point represents the mean ± SD (n = 3).

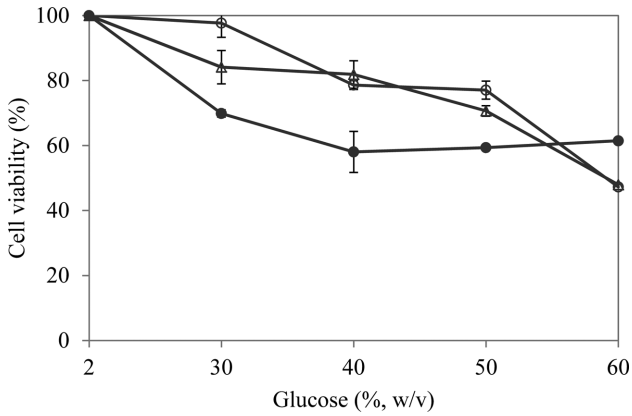

Fig. 3. Glucose tolerance of wild yeast strains Y98-5 and Y197-13. Cells were cultured at 20°C for 48 hr in YPD broth containing 2%, 30%, 40%, 50% and 60% glucose. Y98-5 (open circles), Y197-13 (closed circles), ATCC 24858 (open triangles). Each data point represents the mean ± SD (n = 3).

In conclusion, the morphological characteristics of strain Y197-13 were similar to those of *Saccharomyces cerevisiae*. The wild yeast strain Y197-13 showed very low flocculation. Carbon source assimilation was negative for D-trehalose, mannitol, arbutin I-erythritol, and succinic acid but weakly positive for inulin, acetic acid, and propionic acid. The major cellular fatty acids included C18:2n6c, C18:1n9c, and C16:0. At 12% (v/v) ethanol or 60% (w/v) glucose, cell viability was 60%. Strain Y197-13 (JX566694) was isolated from *Nuruk* for brewing *Makgeolli*.

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