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Review

Kuratsuki Bacteria Interactions with Sake Yeast and Effect on Taste

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Abstract: Various microorganisms, referred to as *kuratsuki* microorganisms, inhabit each sake brewery. Previously, *kuratsuki* yeasts had been used for sake production in each sake brewery. *Kuratsuki* lactic acid bacteria have been used to produce *kimoto*, a fermentation starter. *Kuratsuki* non-lactic acid bacteria were examined to evaluate their potential roles and effects in sake production. The addition of *kuratsuki* bacteria to the sake-making process can change the flavor and taste of the sake. This change was observed in both the coculture experiments between sake yeast and *kuratsuki* bacteria and the sake making tests with and without *kuratsuki* bacteria. The comprehensive gene expression analysis of sake yeast cocultured with *kuratsuki* bacteria showed that 1.2% of the yeast genes were upregulated and 1.0% were downregulated following the addition of *kuratsuki* bacteria. This indicates that the change in flavor and taste of sake due to the addition of *kuratsuki* bacteria was caused by the interaction between sake yeast and *kuratsuki* bacteria. To understand the implications of *kuratsuki* bacteria in sake production, it is essential to study the interactions between sake yeast and *kuratsuki* bacteria.

Keywords: flavor and taste; *kuratsuki* microorganisms; microbial interaction; sake making; sake yeast

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1. Introduction

Fermented beverages and foods have been produced all over the world since before the discovery of microorganisms. Japanese sake, a fermented beverage, and sake making maintain a critical position in traditional Japanese culture. Sake has been used not only as a fermented beverage but also as an offering at traditional religious events and festivals in Japan. Sake was granted a geographical indication by the National Tax Agency of Japan in 2015 (https://www.nta.go.jp/publication/pamph/sake/04.pdf, accessed on 1 June 2024). Sake making is generally performed in the winter, spanning October to March. The sake yeast *Saccharomyces cerevisiae* performs ethanol fermentation in a tank maintained at ~15 °C over a period of about one month. Sake making is based on highly advanced technologies, including pasteurization (*hiire* in Japanese; treatment of sake at ~65 °C for ~15 min), which despite reportedly being developed by Louis Pasteur had been practiced in sake making for at least 300 years before Pasteur's invention [1–3].

Koji is produced by growing *koji* mold (*Aspergillus oryzae*) on and in steamed rice. *Moto* is a sake fermentation starter composed of a mixture of *koji*, steamed rice, sake yeast, and water. There are two primary forms of *moto*: *kimoto* and *sokujomoto*. Currently, more than 90% of sake is produced from *sokujomoto*. In *sokujomoto* production, lactic acid is added to a mixture of *koji*, steamed rice, sake yeast, and water to inhibit the growth of microorganisms other than sake yeast. In *kimoto* production, lactic acid bacteria grow in a mixture of *koji*, steamed rice, and water, with sufficient lactic acid production to inhibit the growth of microorganisms. Sake yeast is then added to the mixture. *Sokujomoto* and *kimoto* production requires approximately two and four weeks, respectively, because the growth of lactic acid bacteria needs at least two weeks. Generally, the weight ratio of *moto* ingredients is *koji*:steamed rice:water = 3:7:11 [4].

The process of combining *koji* and *moto* usually takes four days. The first day is called *hatsuzoe* (Figure 1), on which *koji*, steamed rice, and water are added to *moto* according to the following weight ratio: *moto*:*koji*:steamed rice:water = 21:6:14:18 [4]. The second day is called *odori*, on which the *hatsuzoe* mixture is left as it is without adding anything to promote the fermentation of sake yeast. The third day is called *nakazoe* (Figure 1), on which *koji*, steamed rice, and water are added to the *odori* mixture according to the following weight ratio: *odori* mixture:*koji*:steamed rice:water = 59:9:31:48 [4]. The fourth day is termed *tomezoe* (Figure 1), on which *koji*, steamed rice, and water are added to the *nakazoe* mixture according to the following weight ratio: *nakazoe* mixture:*koji*:steamed rice:water = 1470:150:650:1255 [4]. The *tomezoe* mixture is incubated in a tank at 15 °C over approximately one month.

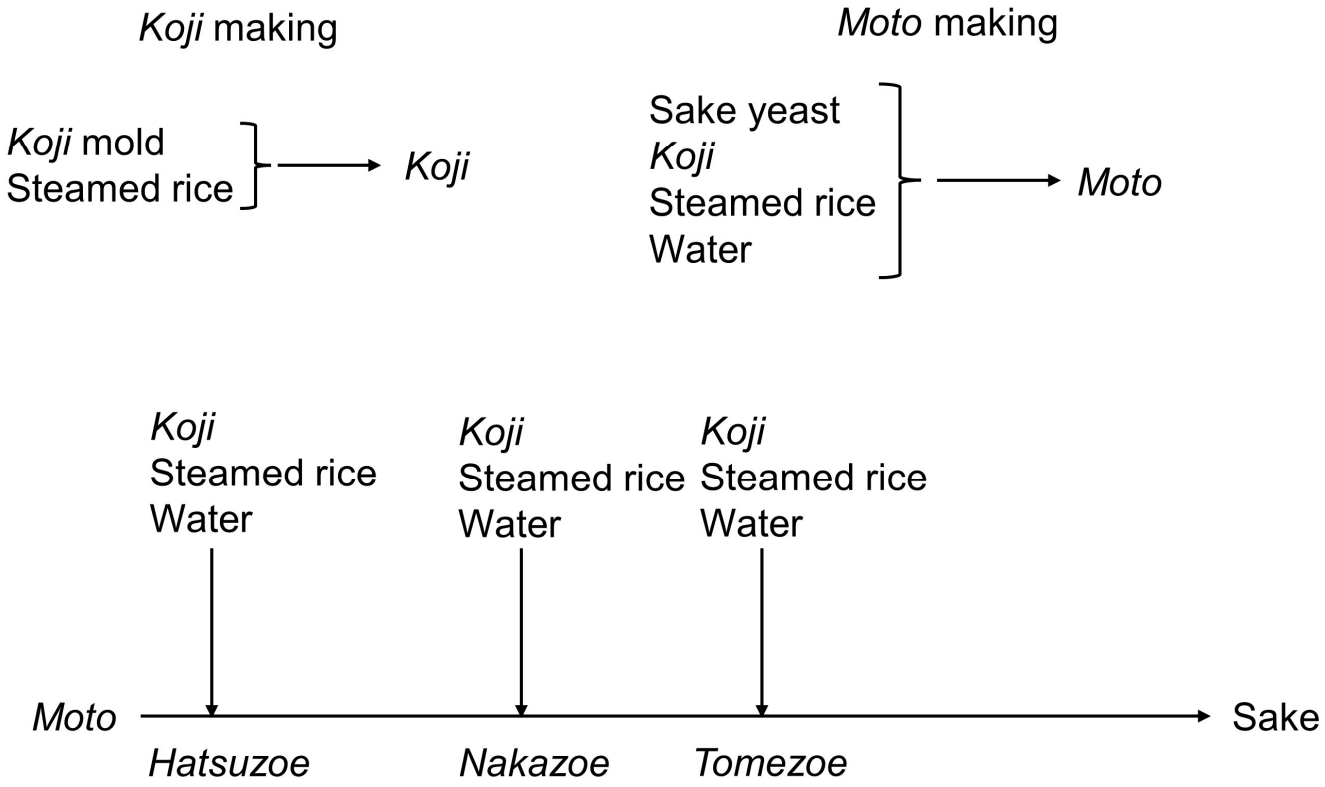

Figure 1. Scheme of sake-making process. *Koji* is made from *koji* mold and steamed rice. *Moto* is made from sake yeast, *koji*, steamed rice, and water. *Koji*, steamed rice and water are added to *moto* in three batches (*hatsuzoe*, *nakazoe*, and *tomezoe*).

Although the ethanol concentration of the *tomezoe* mixture is below 5%, the final ethanol concentration at the end of the sake production process is approximately 20%. Some species of lactic acid bacteria, such as *Fructilactobacillus fructivorans*, can grow at an ethanol concentration of 15% [5,6]. Generally, lactic acid bacteria lack the TCA cycle and convert pyruvate, which is produced during glycolysis, into lactic acid. Therefore, if the ethanol-tolerant lactic acid bacteria are present during the sake-making process, lactic acid increases in the sake and induces changes in the flavor and taste of the sake, which leads to negative perceptions in taste in regular sake drinkers. To prevent the deterioration of sake by lactic acid bacteria, pasteurization is generally performed before storage. Pasteurization not only kills microorganisms but also inactivates the enzymes derived from *koji* mold and thus has a critical effect on the final flavor and taste of the sake.

Microorganisms other than ethanol-tolerant lactic acid bacteria cannot survive at an ethanol concentration of ~20% in sake; thus, these microorganisms do not typically produce chemical compounds involved in the flavor and taste of sake. Conversely, sake yeasts play a central role in the flavor and taste of sake. During the sake-making process, sake yeasts

produce chemical compounds such as esters and organic acids, the composition of which affects the flavor and taste of sake [7–16]. Thus, if microorganisms interact with sake yeast, this interaction may alter the metabolism of sake yeast and change the composition of chemical compounds in sake. Different bacterial species have different ethanol tolerances and therefore different durations of interaction with sake yeast.

The functions and effects of lactic acid bacteria on sake production have been studied and found to induce both positive and negative effects. For example, a positive effect was found in *kimoto* making, wherein bacteria produce lactic acid to inhibit the growth of microorganisms, except sake yeast [17–21]. Furthermore, the interaction between sake yeast and lactic acid bacteria during *kimoto* production produces compounds that contribute to the flavor and taste of sake. Interestingly, lactic acid bacteria are also used in sour beer production and wine malolactic fermentation [22,23]. Negative effects of ethanol-tolerant lactic acid bacteria in the sake production process have been reported, and interactions between yeast and lactic acid bacteria have been reported in other beverage production processes in addition to sake production [24,25].

The interaction between sake yeast and bacteria and the resultant effects of the interaction on the sake flavor and taste have garnered significant research attention. As the sake-making process progresses, bacterial growth weakens until the bacteria die, except for ethanol-tolerant lactic acid bacteria. Thus, it is unlikely that the compounds produced by the bacteria directly affect the flavor or taste of sake. However, it is likely that sake yeasts interact with bacteria to change their metabolism. The composition of these compounds affects the flavor and taste of sake. If so, these bacteria can be applied in the sake production process to produce novel flavors and tastes. To date, *kuratsuki* bacteria other than lactic acid bacteria have never been used in the sake-making process. This review summarizes the effectiveness of *kuratsuki* bacteria in sake making.

2. Detection of Bacteria during Sake Making Based on Their DNA Sequences

The DNA sequences of the 16S rRNA gene are the largest in the international DNA database. Therefore, this gene nucleotide sequence has been widely used for microbial identification and phylogeny [26–28]. The 16S rRNA gene contains a mosaic of regions whose nucleotide sequences are commonly conserved among different bacterial species, and variable regions whose nucleotide sequences differ among different bacterial species. To study bacterial flora, a portion of the bacterial 16S rRNA gene between different conserved regions is amplified using PCR primers, and the amplified products are subjected to comprehensive DNA sequencing. This approach is considered highly effective for identifying bacteria present in the sake-making process [29–36].

Bokulich et al. [29] showed that *Bacillus* and *Staphylococcus* bacteria were mainly detected in *koji*, whereas *Bacillus*, *Klebsiella*, *Lactococcus*, *Staphylococcus*, Lactobacillaceae, and Planococcaceae bacteria were detected in *kimoto*. *Koji* is used for *moto* making (Figure 1); therefore, *koji* strongly affects the bacterial flora during the early stages of *kimoto* production [29]. The proportion of bacteria belonging to the Lactobacillaceae family increases over the course of *kimoto* production and eventually becomes dominant. Tsuji et al. [33] showed that *Lactobacillus* increased drastically and became dominant in *yamahai–moto*, a type of *kimoto*. Ito et al. [34] showed that *Luconoctoc* was detected, but not Lactobacillaceae, in *kimoto* and that different lactic acid bacteria are found in different sake breweries. In contrast, lactic acid bacteria were not detected in sake or in sake-making processes using *sokujomoto* [31]. During *sokujomoto* production, lactic acid is added directly before the growth of lactic acid bacteria.

Lactobacillaceae DNA became dominant in a mixture of *koji* and *kimoto* [29]. In this process, ethanol and lactic acid in *moto* may inhibit *koji* bacterial growth. The role of lactic acid bacteria in sake production is not limited to the inhibition of microorganism growth, but it has also been reported that they affect the flavor and taste of sake by interacting with sake yeast [21,37–40].

Variations in conditions across sake-making processes and sake breweries produce different flavors and tastes. The variation in bacteria that entered in the sake production process of each sake brewery was studied. After a portion of the bacterial 16S rRNA gene of DNA in sake was sequenced, the bacterial flora of each sake sample was analyzed. As a result, 1611 different bacterial nucleotide sequences were obtained from 47 sakes and 11 sake lees [32]. The nucleotide sequences were divided into 168 genera. Among the 168 genera, the most frequently detected genus was *Pseudomonas*, which was detected in 40 of the 58 samples [32]. Cluster analysis was performed based on the bacterial flora at the genus level. Consequently, the components of bacterial genera formed three major clusters [32]. The bacterial diversity was so great that no producing area specificity was found in the bacterial flora. In many cases, the bacterial flora differed significantly across the sakes produced, even when sampling those from the same sake brewery. This strongly suggests that most of the detected bacteria accidentally entered the sake production process. It is very difficult to identify the bacteria that inhabit a sake brewery and inevitably enter the sake production process when evaluating bacterial DNA diversity.

Except for a few ethanol-tolerant lactic acid bacteria, no bacteria are isolated from sake products. Due to the high percentage of ethanol in sake, bacteria die during sake production. Thus, bacterial DNA in sake comes from dead bacteria that enter and grow temporarily in the sake-making process. Bacterial DNA sequence analysis showed that bacterial contamination in sake making was much more diverse than previously imagined [32]. To identify the bacteria that inevitably enter the sake production process and the interactions among microorganisms during the sake-making process, live bacteria should be isolated from the sake production process.

3. *Kuratsuki* Microorganisms

Microorganisms that inhabit a sake brewery are called *kuratsuki* microorganisms [41,42]. The Japanese words "*kura*" and "*tsuki*" correspond to "sake brewery" and "inhabiting", respectively. *Kuratsuki* microorganisms inevitably enter the sake-making process. In the past, different sake breweries have used different yeast strains for sake making, which inhabited each sake brewery and entered the *moto*-making process. These sake yeasts are known as *kuratsuki* yeasts. *Kuratsuki* yeasts belong to *Saccharomyces cerevisiae*, most of which used to be called *Saccharomyces sake* [10]. The differences among *kuratsuki* yeasts are at the strain level. Before people recognized the existence and function of the *kuratsuki* yeast, it entered the sake-making process and facilitated ethanol fermentation. At present, almost all sake breweries buy and use specific strains of sake yeast yielding superior fermentation that are managed, maintained, and sold by the Brewery Society of Japan [10]. These strains have been established in a limited number of sake breweries [10]. Thus, most sake breweries use *kuratsuki* yeast strains obtained from a limited number of sake breweries. Interestingly, a sake brewery's *kuratsuki* yeast entered the sake-making process using the specific yeast strains [43].

Using strains of sake yeast producing superior fermentation, many sake breweries have been able to produce sake with a consistent flavor and taste. However, even when the same strain of sake yeast is used, the flavor and taste of sake may still vary depending on the sake brewery. It is thought that the diversity of sake yeasts currently in use is lower than that of *kuratsuki* yeasts used in sake making. Therefore, some sake breweries produce sake using yeast strains isolated from nature, such as flower nectar, or using wine yeast, which differs from the sake yeast lineage [44]. Considering the rapid progress of global climate change, it is important to confirm the current state of *kuratsuki* yeasts in each sake brewery and to collect and maintain *kuratsuki* yeasts.

Yeasts and other microorganisms are known to inhabit sake breweries. For example, a sake brewery discovered and reported that the wooden barrels used in sake making were inhabited by lactic acid bacteria used in the *kimoto*-making process [45]. Bacteria seem to be nonessential in sake making, with the exception of lactic acid bacteria in *kimoto* making. As previously mentioned, identifying bacteria that inhabit a sake brewery and inevitably enter the sake production process is difficult, inferred from the bacterial DNA diversity. Thus,

bacterial strains were isolated and identified from *hatsuzoe* mixtures as such bacteria die after this stage due to the increasing ethanol concentration. Different *hatsuzoe* mixtures were used from each sake brewery. *Kuratsuki* bacterial strains were detected from the different *hatsuzoe* mixtures.

Kuratsuki bacterial isolates were obtained from the Narimasa Sake Brewery in Toyama, Japan, and the Shiraki-Tsunesuke Sake Brewery in Gifu, Japan. The Narimasa Sake Brewery has *kuratsuki* bacteria belonging to the genus *Kocuria*, which existed in different *hatsuzoe* mixtures of the Narimasa Sake Brewery [46]. These strains of *Kocuria* were divided into two groups. One group (major group) was isolated from all six of the different *hatsuzoe* mixtures used in that study. The other group (minor group) was isolated from three of the six *hatsuzoe* mixtures. The Shiraki-Tsunesuke Sake Brewery has *kuratsuki* bacteria belonging to the genera *Bacillus* and *Priestia*, which existed in different *hatsuzoe* mixtures of the Shiraki-Tsunesuke Sake Brewery [47]. The strains of *Bacillus* were isolated from all four of the *hatsuzoe* mixtures used in that study. Those of *Priestia* were isolated from three of the four *hatsuzoe* mixtures. Among the 92 isolates from the Shiraki-Tsunesuke Sake Brewery, bacteria belonging to *Kocuria* were not found [47].

The genus *Kocuria* belongs to the phylum Actinobacteria [48]. The genera *Bacillus* and *Priestia* belong to the phylum Firmicutes [49]. Thus, *kuratsuki* bacteria are more diverse than *kuratsuki* yeasts and differed at the phylum level. These *kuratsuki* bacteria are not lactic acid bacteria. The *kuratsuki* bacteria inevitably enter the sake-making process as well as *kuratsuki* yeasts. While many sake brewers understand the importance of *kuratsuki* yeasts and maintain some high-quality yeast strains for sake production, they do not consider the usefulness of *kuratsuki* bacteria. Brewers still typically believe that bacteria diminish sake quality [5,50]. Research on *kuratsuki* bacteria is lacking, and the diversity and distribution of these bacteria are not yet clear.

It is uncertain where *kuratsuki* bacteria come from or when they began influencing sake production. The bacterial flora of the isolates from the environments around the Narimasa Sake Brewery differed completely from those of the isolates found in the sake production process [42]. No strains belonging to the genus *Kocuria* were isolated, but strains belonging to the genera *Achromobacter*, *Agrococcus*, *Burkholderia*, *Microbacterium*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas* were isolated from the environments around the Narimasa Sake Brewery [42]. This strongly suggests that *kuratsuki* bacteria do not originate from the environment around sake breweries.

Sake is produced under conditions that are not completely sterile. Therefore, many microorganisms enter by chance, including *kuratsuki* microorganisms, which are inevitably present in the sake-making process. Thus, *kuratsuki* microorganisms are constantly exposed to the sake-making environment and are thought to adapt to that environment [42]. The history of adaptation to the environment has been recorded as genomic modifications.

The genomes of two *kuratsuki* bacterial strains of *Kocuria* from the Narimasa Sake Brewery, TGY1120_3 (belonging to the minor group) and TGY1127_2 (belonging to the major group), were sequenced [46]. A complete 16S rRNA gene sequence comparison indicated that these two isolates differed at the species level. Strains TGY1120_3 and TGY1127_2 belonged to *Kocuria koreensis* and *Kocuria uropygioeca*, respectively [46]. These two strains are strongly suggested to enable adaptation to the environment of sake production, which is characterized by high acidity, high ethanol concentration, and low temperature. Stackebrandt et al. [48] suggested that the growth temperature for the genus *Kocuria* is between 22 °C and 37 °C. However, these two isolates optimally grow at 15 °C, the temperature at which sake is made [51]. This strongly suggests that genome alteration of the *kuratsuki* bacterial strains facilitated adaptation to the sake-making environment.

Adaptation of the *kuratsuki* strains of *Kocuria* to the sake-making environment is believed to be incomplete and still in the developmental stage because these strains cannot grow under 7% and more of ethanol conditions [52]. In a coculture of sake yeast and *kuratsuki* bacteria of *Kocuria*, viable cells of the *kuratsuki* strains gradually decreased and finally died [51].

According to the genome information, *K. uropygioeca* TGY1127_2 has a urease gene cluster consisting of nine genes. Ureases may be associated with acid resistance [53–56]. Sake yeast produces urea using arginases [57]. The reduction of urea is important for sake production because ethyl carbamate is produced from urea and ethanol [58,59]. In addition, *K. koreensis* TGY1120_3 and *K. uropygioeca* TGY1127_2 have four and seven genes, respectively, encoding alcohol dehydrogenase. Some of these alcohol dehydrogenases may be associated with ethanol resistance [60–63]. *K. uropygioeca* TGY1127_2 has two alcohol dehydrogenases that are similar to those of the ethanol-tolerant lactic acid bacterium *Fructilactobacillus fructivorans*. In contrast, *K. koreensis* TGY1120_3 does not contain this alcohol dehydrogenase. However, it remains unclear which alcohol dehydrogenase of *F. fructivorans* is involved in its ethanol tolerance.

K. koreensis TGY1120_3 and *K. uropygioeca* TGY1127_2 have three plasmids and one plasmid, respectively, which exhibit highly similar nucleotide sequences [46]. This suggests that horizontal gene transfer may have occurred between the two strains via the plasmids. In addition, an identical nucleotide sequence (1308 nucleotides) of the ISL3 family transposase ISAar30 was found on the chromosome and one plasmid of *K. koreensis* TGY1120_3 and on the plasmid of *K. uropygioeca* TGY1127_2 [46]. This suggested that genomic modifications may have occurred through these transposons. These genomic changes are thought to be caused by the sake-making environment.

The genomes of *kuratsuki* bacterial strains of *Bacillus* and *Priestia* from Shiraki-Tsunesuke Sake Brewery, A-10 and B-12, respectively, were sequenced [64]. Complete 16S rRNA gene sequence comparison indicated that strains A-10 and B-12 belonged to *Bacillus safensis* and *Priestia megaterium*, respectively. These genomic sequences were deposited in an international DNA database and are available for viewing. The accession number of *B. safensis* A-10 (complete genome) is BSYL01000001 and those of *P. megaterium* B-12 (eight contigs) are BSYK01000001-BSYK01000008. The two strains did not contain plasmids. *B. safensis* A-10 and *P. megaterium* B-12 have 8 and 35 transposase-coding genes, respectively, suggesting that genomic modification may occur through these transposons. Song et al. [65] showed that transposable elements increase before the genome reduction in bacteria. *B. safensis* A-10 has a urease gene cluster, whereas *P. megaterium* B-12 does not. *B. safensis* A-10 has six alcohol dehydrogenase genes and *P. megaterium* B-12 has eight alcohol dehydrogenase genes.

Therefore, *kuratsuki* bacteria are more diverse than *kuratsuki* yeasts. This strongly suggests that if *kuratsuki* bacteria function in the production process of sake making, each *kuratsuki* bacterium has a different function. If we could apply the functions of *kuratsuki* bacteria to the sake production process to control the flavor and taste of sake, it would be possible to make sake unprecedented. To achieve this, the interaction between sake yeast and *kuratsuki* bacteria and its effects on sake quality should be studied.

4. *Kuratsuki* Bacteria Affect Sake Taste

Saccharomyces cerevisiae cannot digest starch. Therefore, the *koji* mold *Aspergillus oryzae* has been used as a starch decomposer in sake production. The *koji* mold converts rice starch into sugar, which is then used for ethanol fermentation by sake yeast. The *koji* mold cannot produce ethanol. Therefore, only sake yeast produces ethanol during the sake-making process. Sake yeast produces not only ethanol but also other chemical compounds that affect the flavor and taste of sake. For example, sake yeast produces isoamyl acetate and ethyl caproate, which yield a fruity aroma similar to that of apples or bananas [7,8,66,67]. The ester composition affects the flavor of sake. Sake contains five major organic acids: acetic acid, citric acid, lactic acid, malic acid, and succinic acid, which are mainly produced by sake yeast. Lactic acid, malic acid, and succinic acid account for 80% of all organic acids in sake [68]. The organic acid composition affects the taste of sake.

Yeast strains have been bred, maintained, and distributed for sake production in response to market demands. In addition, *kuratsuki* bacteria affect the metabolism of sake yeast, influencing the flavor and taste of the sake. For example, a coculture of sake yeast and *kuratsuki* bacteria showed that *kuratsuki* bacteria activated ethanol production in sake

yeast [52]. As previously mentioned, research on *kuratsuki* bacteria other than lactic acid bacteria is relatively new, and many questions remain to be answered.

Coculture experiments are essential for elucidating the interactions between sake yeast and *kuratsuki* bacteria. Both artificial media and *koji* solution have been used for the coculture of sake yeast and *kuratsuki* bacteria. The strains *Saccharomyces cerevisiae* AK25, K901, and K1801 were selected as sake yeasts [64,69]. *Bacillus safensis* A-10, *Kocuria uropygioeca* TGY1127_2, and *Priestia megaterium* B-12 were selected as *kuratsuki* bacteria [64,69]. The following eight tastes were estimated using a taste recognition device TS-5000Z (Intelligent Sensor Technology, Inc., Atsugi, Japan) [70,71], astringent, astringent stimulation, bitter, bitter miscellaneous, saltiness, sourness, umami, and umami richness. Among these tastes, bitter miscellaneous, sourness, umami, and umami richness were significantly ($p < 0.05$) different among the different sake yeast strains according to the analysis of variance (ANOVA) (Figure 2). Astringent stimulation, saltiness, sourness, umami, and umami richness differed significantly among the different *kuratsuki* bacteria (Figure 2). Thus, sourness, umami, and umami richness were affected by both sake yeast and *kuratsuki* bacteria.

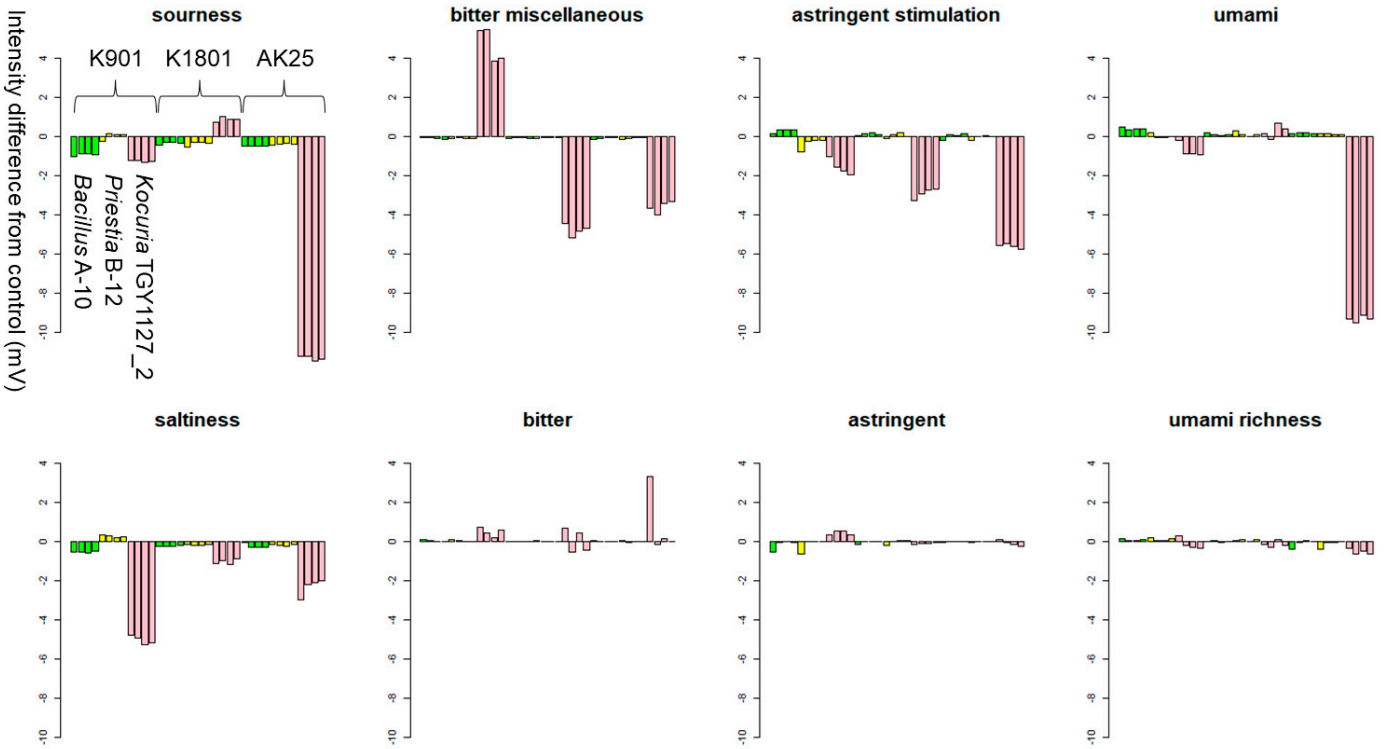

Figure 2. Differences between each taste intensity of sake produced using different sake yeast strains (AK25, K901, and K1801) with or without *kuratsuki* bacterial strains (green, *Bacillus safensis* A-10; pink, *Kocuria uropygioeca* TGY1127_2; and yellow, *Priestia megaterium* B-12). The data shown here are a combination of the results of Kobayashi and Nishida [64] and Yazaki and Nishida [69].

In addition to laboratory experiments, sake making tests using *kuratsuki* bacteria in sake breweries were performed. Although sake breweries other than the Narimasa Sake Brewery refused to add *kuratsuki* bacteria of *Kocuria* to the sake-making process, the Yoshinotomo Sake Brewery in Toyama, Japan, allowed the addition of Narimasa's *kuratsuki* strain into the sake-making process. No *Kocuria* strains were isolated from the Yoshinotomo Sake Brewery. *Kocuria uropygioeca* TGY1127_2 was added to a *hatsuzoe* mixture during sake production. Sakes with and without *K. uropygioeca* TGY1127_2 were produced completely. The taste sensor TS-5000Z showed that the saltiness and umami intensities in sake with the *kuratsuki* bacterial strain were higher than those in sake without the strain [42]. The intensity of bitter miscellaneous in sake with the *kuratsuki* strain was lower than that of sake without the strain [42]. In taste testing, 39 of 41 individuals reported that sake with the

strain was better than that without the strain [42]. These results indicate that the *kuratsuki* bacterial strain may positively affect the sake-making process.

Yeast gene expression patterns change during sake production [72,73]. Recently, comprehensive gene expression analyses of sake yeast cocultured with and without the *kuratsuki* bacterial strain were performed [74]. Sake yeast K1401 and *kuratsuki* bacterium *Kocuria uropygioeca* TGY1127_2 were used in the coculture. After incubation at 14 °C for seven days, RNA was isolated from monoculture of *S. cerevisiae* K1401 and coculture of *S. cerevisiae* K1401 and *K. uropygioeca* TGY1127_2. A comparison between the RNA expression in monoculture and coculture showed that among 5922 genes of *S. cerevisiae*, 71 and 61 genes were upregulated by more than 2-fold and downregulated by less than 0.5-fold, respectively, in coculture experiments [74]. Replication- and sporulation-related genes were upregulated. Gene expression changes caused by the addition of *kuratsuki K. uropygioeca* TGY1127_2 differ from those caused by the addition of lactic acid bacteria [75,76]. These differences in gene expression may be due to differences in incubation time; however, the findings strongly suggest that yeast gene expression differs depending on the surrounding bacteria.

Although the Brix (sugar content) change was similar between the coculture and monoculture, the expression of some genes involved in metabolism tended to be suppressed [74]. For example, the expression levels of three genes, *TDH1*, *TDH2*, and *TDH3* encoding glyceraldehyde-3-phosphate dehydrogenase, which is involved in one step of glycolysis, were reduced by less than half. These genes are upregulated at the early stage of alcoholic fermentation and then are downregulated in yeasts [77,78]. This strongly suggests that the alcoholic fermentation was more advanced in the coculture than in the monoculture. This finding was consistent with that relating to ethanol concentration of sake using a coculture of sake yeast and *kuratsuki* bacteria, which was higher than that of sake with a monoculture of sake yeast [52].

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