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Effect of *kuratsuki Bacillus* and *Priestia* on Taste of Sake

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Abstract: The co-cultivation of sake yeast (AK25, K901, K1401, or K1801 strain) and the *kuratsuki Bacillus* A-10 and/or *Priestia* B-12 strains in *koji* solution was performed to demonstrate the effects of these two *kuratsuki* bacteria on sake taste. The results showed that the Brix and acidity patterns of sake preparations produced with and without these *kuratsuki* bacteria were very similar. This indicated that the addition of these *kuratsuki* bacteria did not inhibit ethanol fermentation or organic acid production by sake yeast. A taste recognition device showed that the effects of these *kuratsuki* bacteria on the saltiness and sourness of sake were greater than those on other taste properties. Astringency stimulation and saltiness of sake produced using the sake yeast K901 were increased by *Bacillus* A-10 and decreased by *Priestia* B-12. Except for these two cases, the taste intensities of sake preparations produced with the *Bacillus* A-10 and *Priestia* B-12 strains were very similar, but differed from those of sake produced with *kuratsuki Kocuria*. These results support our hypothesis that the flavor and taste of sake can be controlled by utilizing the interactions between *kuratsuki* bacteria and sake yeast. For crating the desired sake taste, a combination of *kuratsuki* bacteria and sake yeast should be considered.

Keywords: *kuratsuki* bacteria; microbial interaction; sake making; sake yeast

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1. Introduction

Sake, a traditional Japanese fermented alcoholic beverage, is produced from steamed polished rice, *koji*, and water, using *koji* mold (*Aspergillus oryzae*) and sake yeast (*Saccharomyces cerevisiae*). The *koji* mold and sake yeast convert rice starch into sugar and sugar into ethanol, respectively. Some lactic acid bacteria are used as fermentation starters, and this regards approximately 10% of all sake products. Bacteria other than lactic acid bacteria are not added during the sake production process. DNA analysis of foods and drinks obtained through processes involving fermentation reveals the type of microorganisms participating in the product-making process [1–13]. DNA sequencing of bacteria participating in the sake production processes have shown that many bacteria are involved [14–18]. Most of these bacteria are present by chance; however, some are introduced by necessity and are specific to each sake brewery [19–21]. Bacteria that are introduced in the sake production process by necessity are known as *kuratsuki* bacteria [22]. The Japanese word "*kuratsuki*" corresponds to the term "sake brewery inhabiting".

We isolated and identified the TGY1120_3 and TGY1127_2 strains belonging to the genus *Kocuria* as *kuratsuki* bacteria in sake from the Narimasa Sake Brewery in Toyama, Japan [17,20]. The TGY1120_3 and TGY1127_2 strains differ at the species level [20], corresponding to *Kocuria koreensis* [23] and *K. uropygioeca* [24], respectively. In addition, we isolated and identified the strains A-10 and B-12 belonging to the genus *Bacillus* as *kuratsuki* bacteria in sake from the Shiraki-Tsunesuke Sake Brewery in Gifu, Japan [21]. In 2020, Gupta et al. taxonomically reconsidered the genus *Bacillus* [25], and transferred some *Bacillus* species, such as *Bacillus megaterium*, to the new genus *Priestia*. Therefore, in this study, we sequenced the genomes of the strains A-10 and B-12 and classified them taxonomically.

The compositions of esters and organic acids produced by sake yeast strongly affect the flavor and taste of sake [26–36]. Sake yeasts form a phylogenetic cluster different from that of beer and wine yeasts [37]. In addition, some chemical compounds are involved in interactions among microorganisms during sake production and affect the flavor and taste of sake [38–43]. Lactic acid bacteria used in the *kimoto* method interact with sake yeast, affecting its metabolism [44,45]. Previous reports showed that the interactions among microorganisms in the sake production process affect the flavor and taste of sake. Previously, we used *kuratsuki Kocuria* to study the interactions between *kuratsuki* bacteria and sake yeast during sake production [46–49]. First, co-cultivation experiments were performed using *kuratsuki Kocuria* and sake yeast in artificial media and *koji* solutions [46]. Although the number of viable cells of *kuratsuki Kocuria* gradually decreased, and the cells died during the 2-week culture period owing to ethanol production by sake yeast, the *Kocuria* TGY1127_2 strain survived longer than the TGY1120_3 strain [46].

To confirm the effect of *kuratsuki Kocuria* on sake taste, we used the taste recognition device TS-5000Z (Intelligent Sensor Technology, Inc., Atsugi, Japan), which uses a receptor membrane composed of a lipid, a plasticizer, and polyvinyl chloride to detect taste-producing substances [50,51]. First, the taste recognition device showed that the addition of *kuratsuki Kocuria* to different *koji* solutions altered the taste of sake differently depending on the different *koji* solution [47]. Next, the change in taste with the addition of *kuratsuki Kocuria* differed depending on the sake yeast strain used [48]. Finally, the effect of *kuratsuki Kocuria* on sake taste differed for hydrogen- and non-hydrogen-treated *kuratsuki Kocuria* [49], suggesting that different cell states may induce different microbial interactions. These results showed that when the sake production environment and the cell state of *kuratsuki* bacteria change, the interaction between *kuratsuki* bacteria and sake yeast also changes, affecting sake taste.

A sake test production was performed at an actual sake production company. Sake was produced using the Narimasa *kuratsuki Kocuria* strain TGY1127_2 at the Yoshinotomo Sake Brewery in Toyama, Japan. The Yoshinotomo Sake Brewery does not use *Kocuria* as a *kuratsuki* bacterium, because no *Kocuria* were detected in any samples from the Yoshinotomo Sake Brewery. The addition of *kuratsuki Kocuria* did not change the alcohol concentration, acidity, or amino acid content of sake. However, the taste of sake differed. Notably, 39 out of 41 individuals who were included in the taste test answered that sake produced with the addition of *kuratsuki Kocuria* tasted better than that produced without *kuratsuki Kocuria* [22].

The results of the co-cultivation and sake test production showed that the addition of *kuratsuki Kocuria* strain TGY1127_2 changed the taste of sake and had a good effect on sake production. From the viewpoint of applied microbiology, the use of *kuratsuki* bacteria in sake production offers unprecedented possibilities for sake production. To the best of our knowledge, no bacteria other than lactic acid bacteria have been used for sake production. Therefore, data are available only for *kuratsuki Kocuria*. To create sake with a new taste using *kuratsuki* bacteria, more *kuratsuki* bacterial strains should be identified and characterized. In this study, we aimed to investigate the effects of the *kuratsuki* bacterial strains A-10 and B-12 on sake taste and found differences with respect to the effects of *kuratsuki Kocuria* strains.

2. Materials and Methods

2.1. Genome Sequencing of the kuratsuki Bacterial Strains A-10 and B-12

The genomes of the strains A-10 and B-12 were determined using both Nanopore (PromethION X5; Oxford, UK) and Illumina (MiSeq; San Diego, CA, USA) DNA sequencers. Hybrid assembly was performed using Trycycler version 0.5.3 [52]. Circulation analysis was performed using Circlator [53]. Illumina short-read mapping was performed using the Burrows–Wheeler aligner (BWA) version 0.7.12 [54], and error correction was performed using Pilon version 1.23 [55].

2.2. Cultivation of the Microorganisms

The sake yeast strains AK25, K901, K1401, and K1801, which are used in Japanese sake breweries [56–58], and *kuratsuki* bacterial strains A-10 and B-12 [21] were used in this study. Each strain was grown in TGY medium (5 g/L tryptone, 1 g/L glucose, and 3 g/L yeast extract) at 25 °C for 12 h prior to cultivation. As a control, 200 mL of water was added to 20 g of *koji* (Miyako koji, Isesou, Tokyo, Japan) and 1 mL of pre-cultivated cell suspensions of AK25 (4.11×10^9 cells/mL), K901 (6.43×10^7 cells/mL), K1401 (1.79×10^8 cells/mL), or K1801 (2.10×10^8 cells/mL). For co-cultivation, 200 mL of water was added to 20 g of *koji*, 1 mL of pre-cultivated yeast suspensions, and 1 mL of pre-cultivated A-10 (1.01×10^9 cells/mL) and/or B-12 (2.19×10^6 cells/mL) cell suspensions. Each mixed solution was incubated at 14 °C for 14 days.

2.3. Measurement of Brix and Acidity

Brix and acidity were measured each day using a PAL-BX/ACID digital refractometer (ATAGO, Tokyo, Japan). Each measurement was repeated three times. The median of the three values was chosen as the representative value.

2.4. Estimation of the Sake Taste

A TS-5000Z (Intelligent Sensor Technology, Inc., Atsugi, Japan) taste recognition device was used. Each taste sensor in this device has a different lipid membrane for taste estimation [50,51]. The strength of each taste is represented by the magnitude of its corresponding current value [50,51]. The taste intensity of sake with *kuratsuki* bacteria (A-10 or B-12 strains) minus that of sake without *kuratsuki* bacteria was calculated. The initial taste in terms of astringent stimulation, bitter miscellaneous taste, saltiness, sourness, and umami was measured using the sensors AE1, CO0, CT0, CA0, and AAE, respectively. The astringency, bitterness, and umami levels were measured using the intensity of the second measurement. The taste intensity for each yeast strain, with and without (control) *kuratsuki* bacteria, was measured. Each measurement was repeated four times. The obtained intensities were subjected to pairwise comparisons (intensity differences) for each sake yeast strain.

2.5. Statistical Analysis

Statistical analyses were performed using R software (R Project for Statistical Computing, http://www.R-project.org/ accessed on 1 January 2023). The Kolmogorov–Smirnov test was performed to compare the Brix and acidity change patterns. Analysis of variance (ANOVA) was performed using Bartlett's test to compare the taste intensity differences with or without *kuratsuki* bacteria among different sake produced using different sake yeasts. Pairwise *t*-tests were performed using Bonferroni correction when the ANOVA showed a p-value < 0.05.

3. Results

3.1. Genome Characteristics

The complete genome sequences and eight contigs of strains A-10 and B-12 were obtained (Table 1). Taxonomic marker genes, such as ribosomal RNA-coding genes, showed that the strains A-10 and B-12 belong to the species *Bacillus safensis* and *Priestia megaterium*, respectively.

Bacillus A-10 contained four insertion sequence (IS) of three family transposases (Table 1). Two of the four transposases were identical. In contrast, the *Priestia* B-12 strain contained 20 transposases belonging to six different families (Table 1). Of the 20 transposases, 7, 6, 3, 2, 1, and 1 belonged to the IS4, IS110, IS21, IS1326, IS3, and IS466 families, respectively. Among the seven IS4 family of transposases, two and five were identical. Among the six IS110 family of transposases, two and four were identical. The IS3 family of transposase of *Priestia* B-12 differed from that of *Bacillus* A-10. Therefore, no transposase genes were exchanged between the *Bacillus* A-10 and *Priestia* B-12 strains.

Table 1. Summary features of the genomic sequences of the strains A-10 and B-12.

Strain A-10	
Total length (bp)	3,749,764
No. of sequences	1
Accession number	BSYL01000001
GC content (%)	41.6
No. of CDSs	3750
No. of rRNA	24
No. of tRNA	81
Coding ratio (%)	87.6
Transposase gene	IS3 family: LOCUS_02590, LOCUS_06730, LOCUS_07270, LOCUS_25590
Strain B-12	
Total length (bp)	5,420,896
No. of sequences	8
Accession number	BSYK01000001-BSYK01000008
GC content (%)	38.2
No. of CDSs	5506
No. of rRNA	39
No. of tRNA	132
Coding ratio (%)	83.3
Transposase gene	IS4 family: LOCUS_34940, LOCUS_52540, LOCUS_52940, LOCUS_54130, LOCUS_54850, LOCUS_54860, LOCUS_55060 IS110 family: LOCUS_05740, LOCUS_10520, LOCUS_31750, LOCUS_32200, LOCUS_42770, LOCUS_54720 IS21 family: LOCUS_52840, LOCUS_53190, LOCUS_53360 IS1326 family: LOCUS_53690, LOCUS_54170 IS3 family: LOCUS_36670 IS466 family: LOCUS_53420

3.2. Brix and Acidity Changes

The Brix and acidity patterns were very similar for sake preparations with and without *kuratsuki* bacteria (Figure 1). A significant difference ($p < 0.05$) was detected in only one case with the Kolmogorov–Smirnov tests. It was detected between the acidity of sake produced using only yeast K901 and that of sake produced using yeast K901, *Bacillus* A-10, and *Priestia* B-12 (Figure 1). This result was different from that obtained with the addition of the *kuratsuki Kocuria* TGY1127_2 strain in the sake production process [48]. The Brix and acidity of sake produced using only yeast AK25 were very different from those of sake produced using yeast AK25 and *Kocuria* TGY1127_2 [48]. However, this difference was not observed in this study.

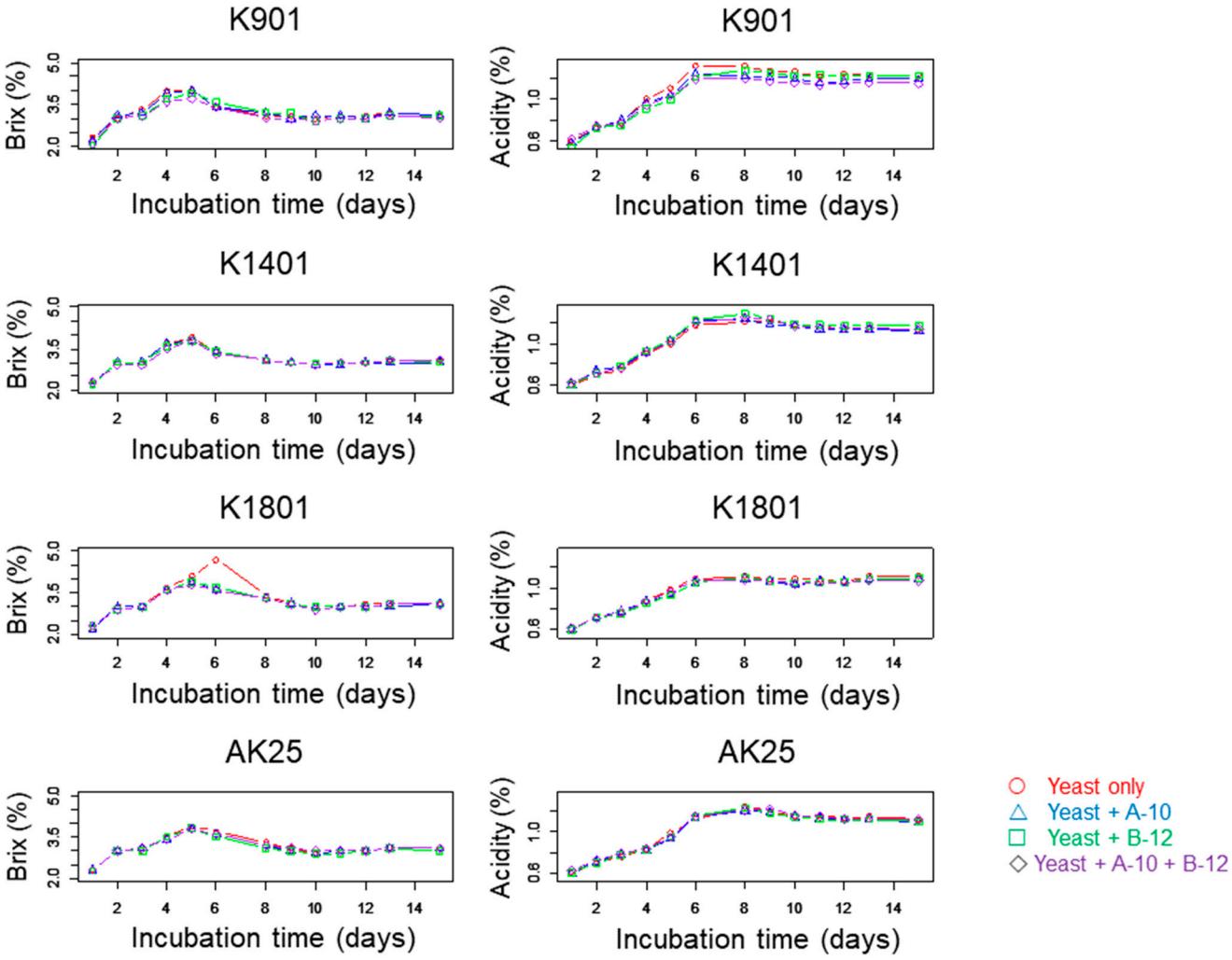

Figure 1. Brix and acidity of sake produced with different strains of sake yeast (AK25, K901, K1401, or K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The acidity of sake obtained using only yeast K901 was significantly ($p < 0.05$) different from that of sake obtained using yeast K901, *Bacillus* A-10, and *Priestia* B in the Kolmogorov–Smirnov tests. For the other sake productions, these values were not significantly different ($p > 0.05$).

3.3. Effects of kuratsuki Bacillus and Priestia on Sake Taste

The effects of *kuratsuki Bacillus* A-10 and *Priestia* B-12 were estimated using the TS-5000Z taste recognition device, and different taste intensities among different sake preparations produced using different sake yeast strains were detected by ANOVA. The ranges of the taste intensity differences were mostly between −1 and +1 (Figures 2–7, S1 and S2), i.e., much lower than those obtained for sake produced using *kuratsuki Kocuria* [48]. The effects of the addition of *kuratsuki Bacillus* A-10 and/or *Priestia* B-12 on saltiness and sourness were more significant than those on other tastes (Figures 4 and 5). The largest taste intensity difference was detected in the sourness of sake produced using yeast K901, *Bacillus* A-10, and *Priestia* B-12 (Figure 5). This result is consistent with the fact that the acidity of sake produced with yeast K901 and that of sake produced with yeast K901, *Bacillus* A-10, and *Priestia* B-12 were significantly different (Figure 1).

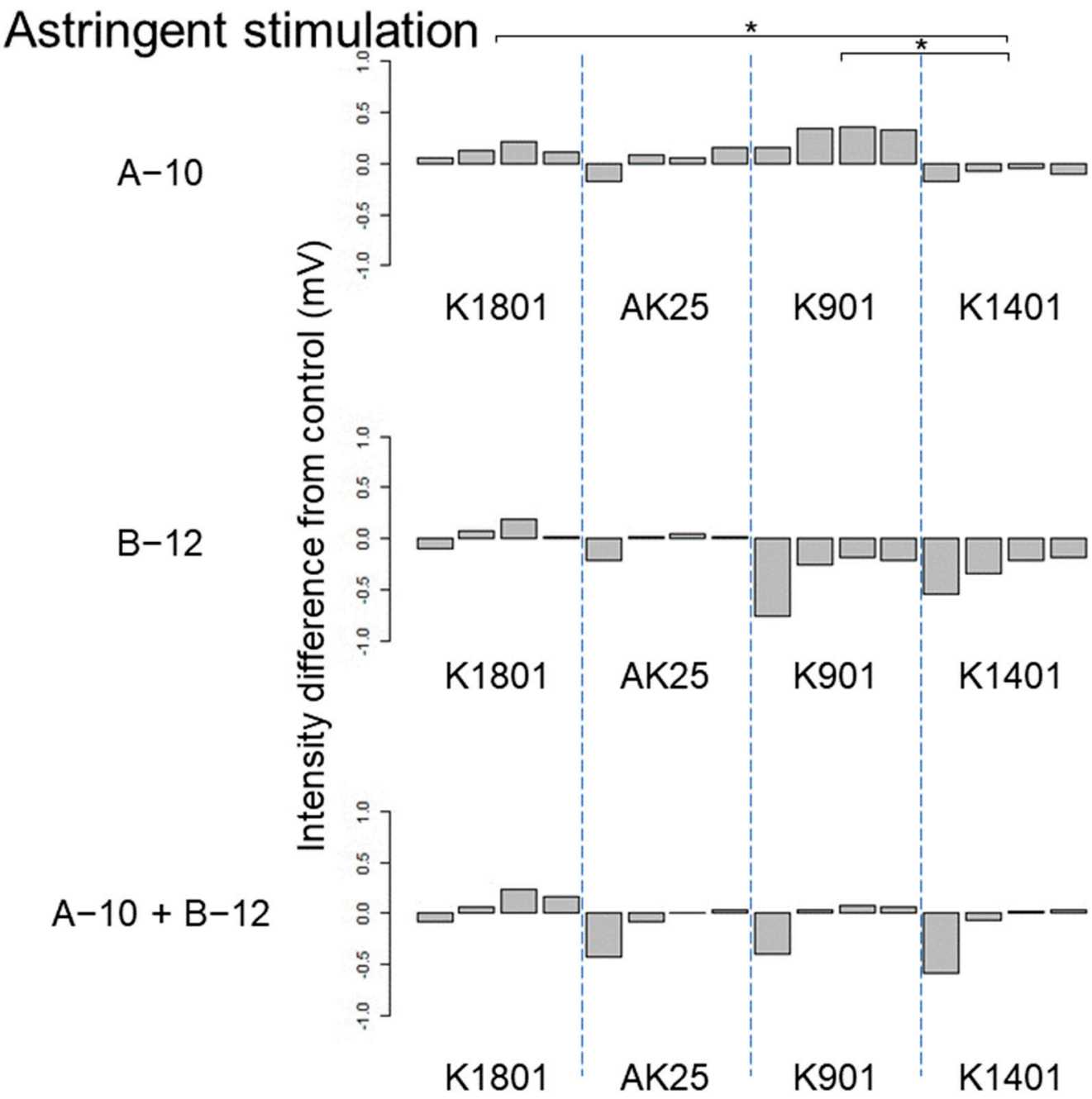

Figure 2. Differences between the astringent taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Asterisk (*) indicates a significant difference ($p < 0.05$) in pairwise *t*-test.

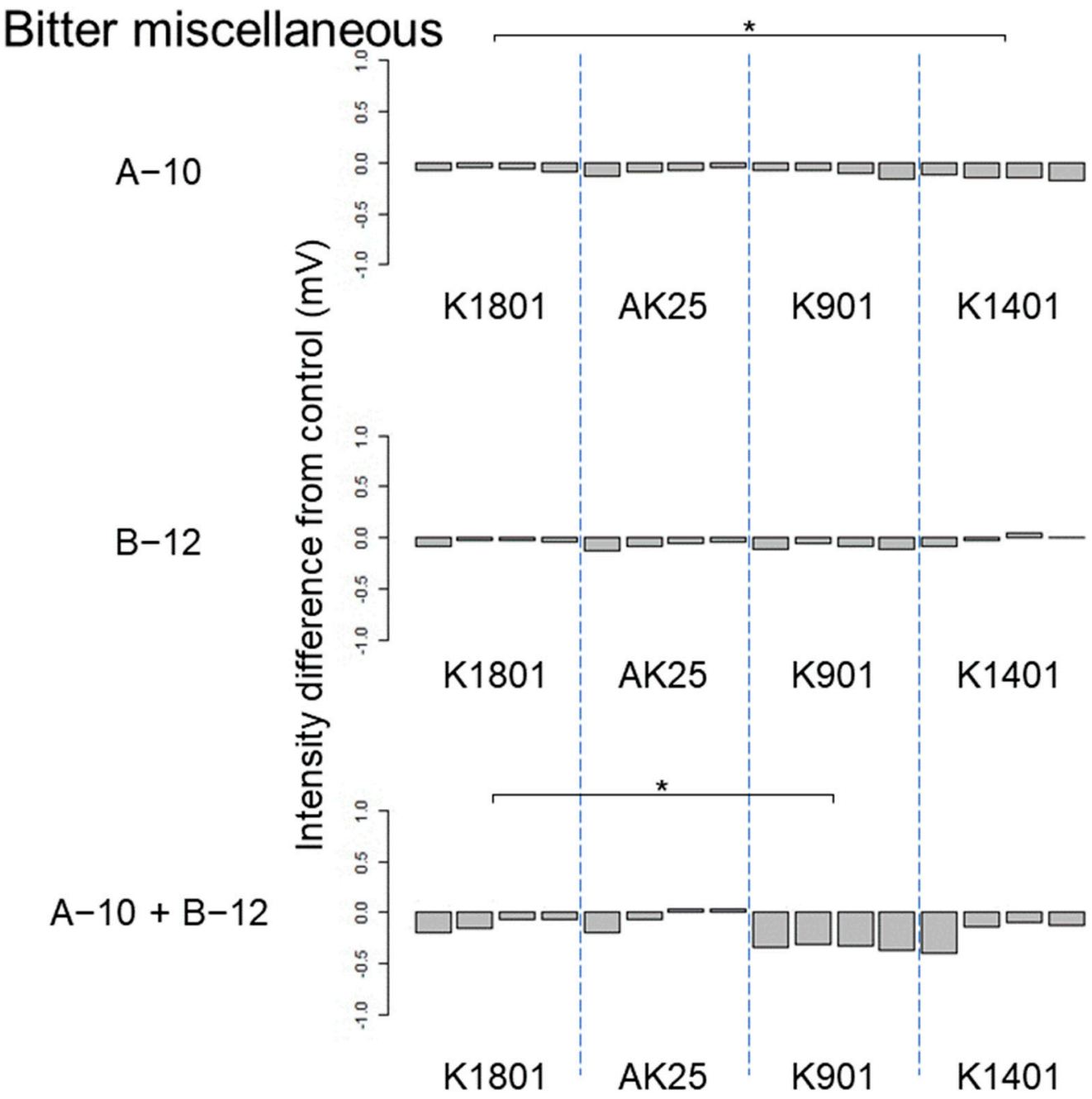

Figure 3. Difference between the bitter miscellaneous taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity of sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Asterisk (*) indicates a significant difference ($p < 0.05$) in pairwise *t*-test.

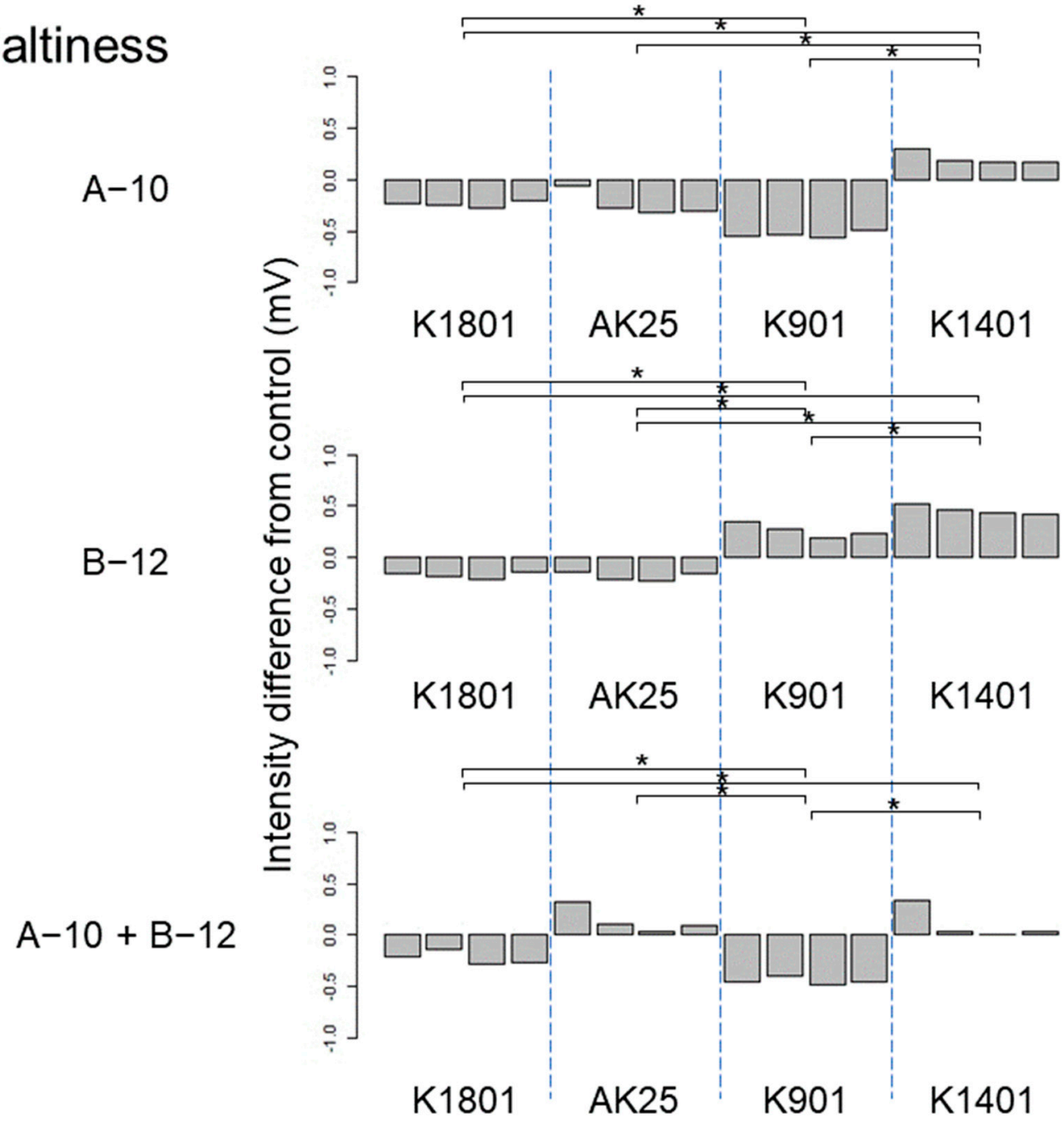

Figure 4. Difference between the saltiness taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Asterisk (*) indicates a significant difference ($p < 0.05$) in pairwise *t*-test.

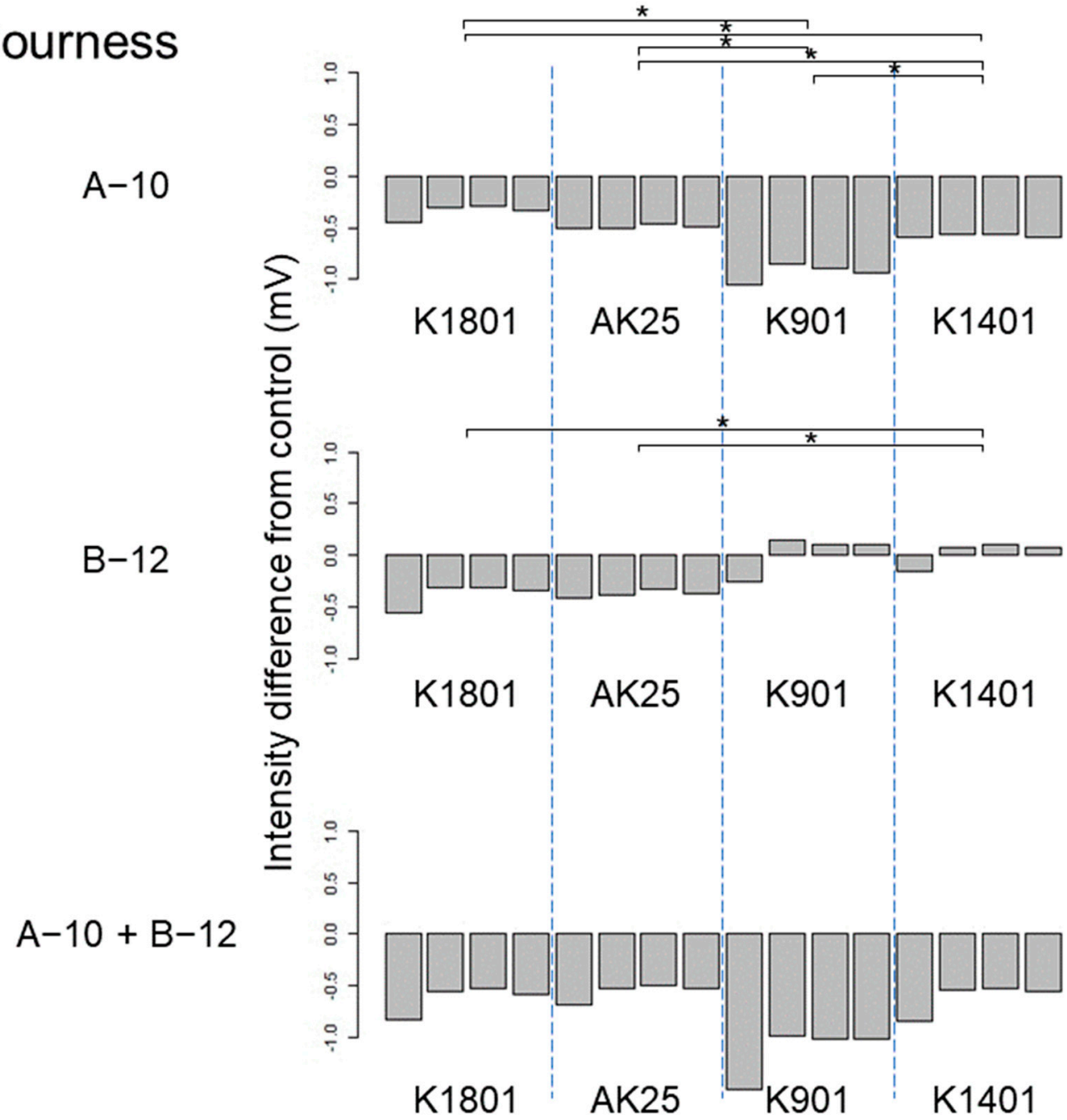

Figure 5. Difference between the sourness taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Asterisk (*) indicates a significant difference ($p < 0.05$) in pairwise *t*-test.

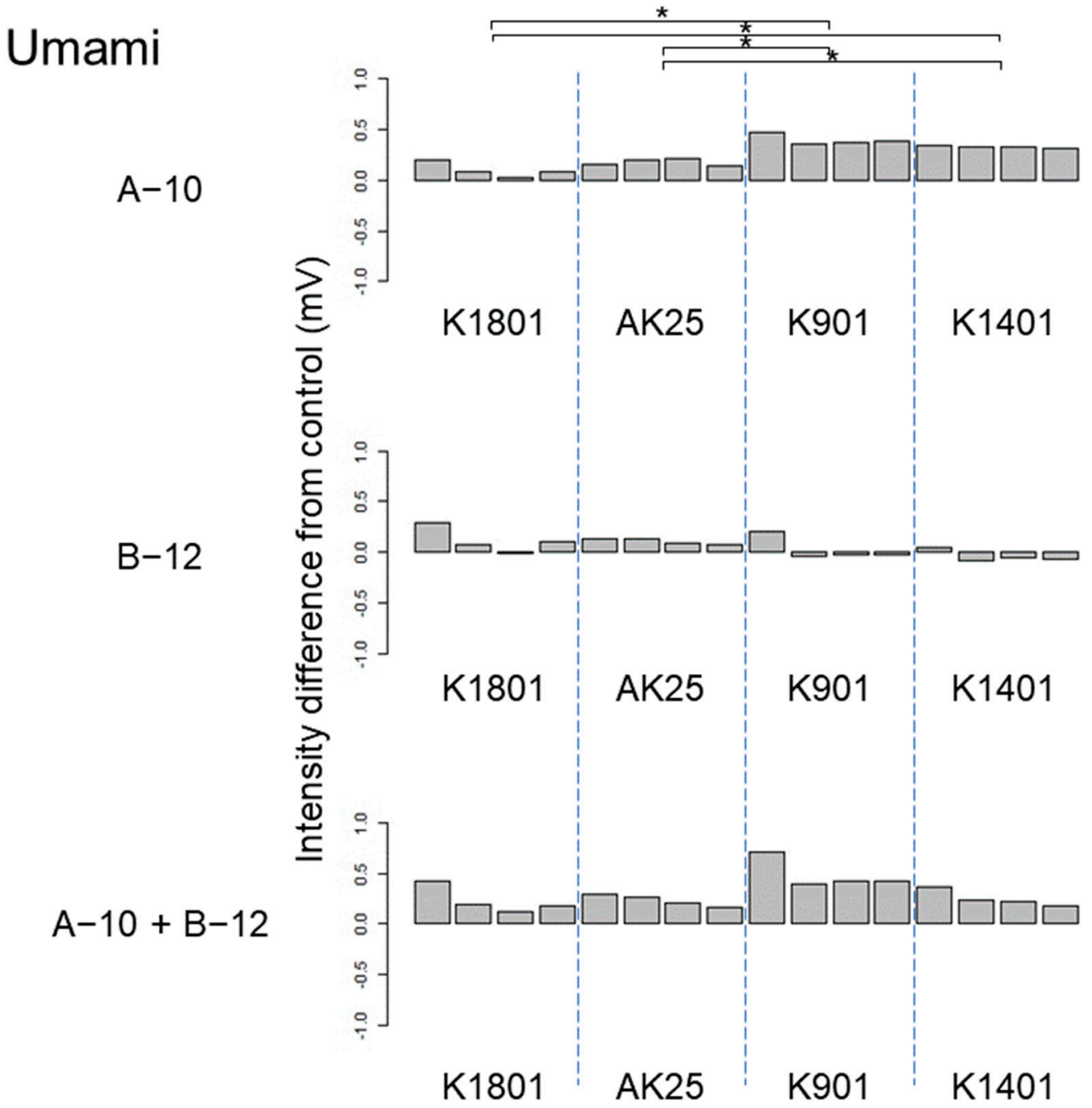

Figure 6. Difference between the umami taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Asterisk (*) indicates a significant difference ($p < 0.05$) in pairwise *t*-test.

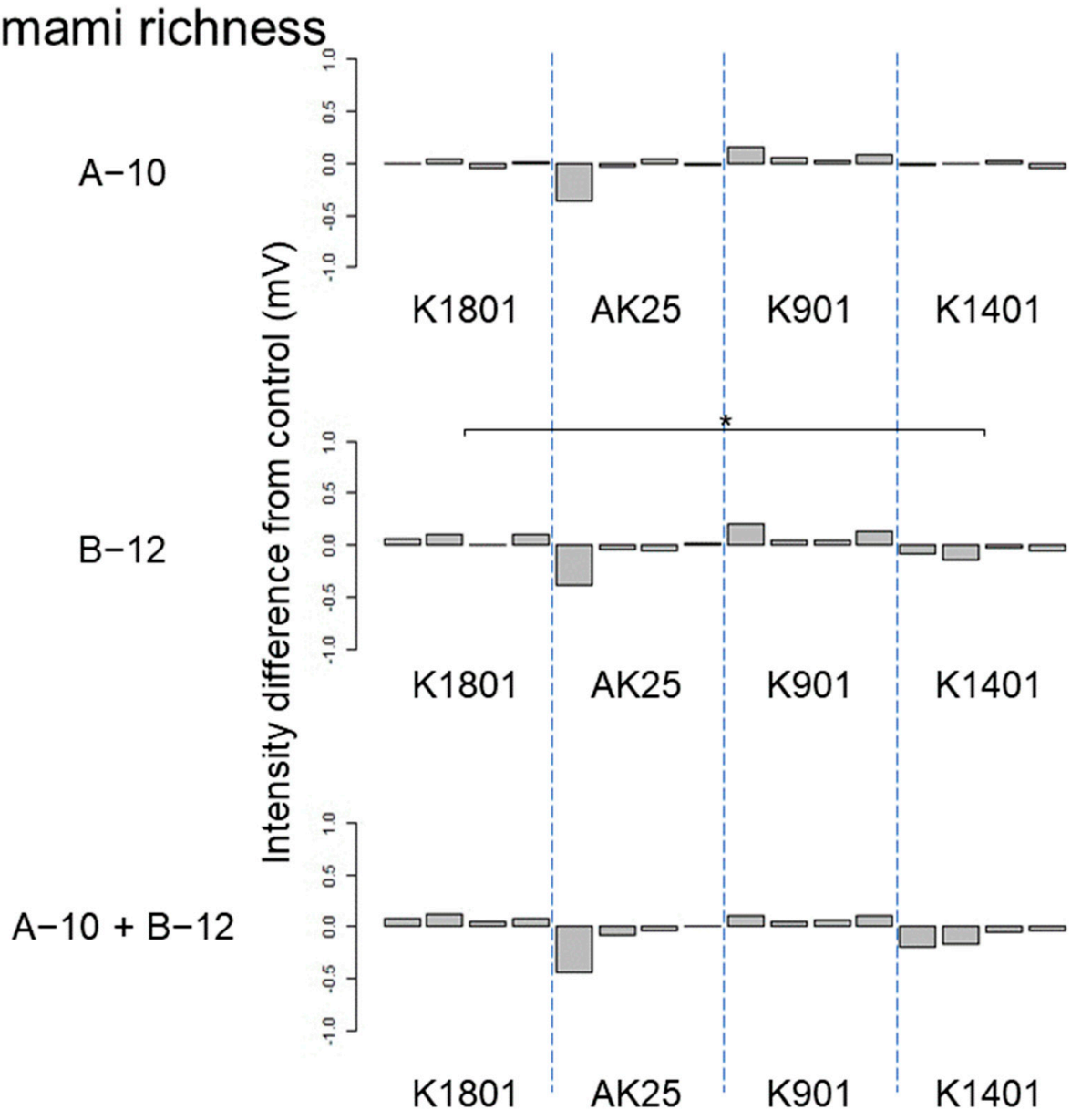

Figure 7. Difference between the umami taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Asterisk (*) indicates a significant difference ($p < 0.05$) in pairwise *t*-test.

The results of ANOVA showed that the taste difference among four sake produced using different sake yeast strains was not significant ($p > 0.05$) in the following seven cases: astringency of sake produced using *Bacillus* A-10, *Priestia* B-12, and both *Bacillus* A-10 and *Priestia* B-12; astringent stimulation of sake produced using both *Bacillus* A-10 and *Priestia* B-12; bitter miscellaneous taste of sake produced using *Bacillus* B-12; umami taste of sake produced using *Priestia* B-12; and umami richness of sake produced using *Bacillus* A-10. Although differences were detected by ANOVA, different pairs were not identified by pairwise *t*-test in the following seven cases: astringent stimulation of sake produced using *Priestia* B-12; bitterness of sake produced using *Bacillus* A-10, *Priestia* B-12, and both *Bacillus*

A-10 and *Priestia* B-12; sourness of sake produced using both *Bacillus* A-10 and *Priestia* B-12; umami taste of sake produced using both *Bacillus* A-10 and *Priestia* B-12; and umami richness of sake produced using both *Bacillus* A-10 and *Priestia* B-12. Therefore, the effects of the *kuratsuki Bacillus* A-10 and *Priestia* B-12 strains on sake taste were smaller than those of *kuratsuki Kocuria* TGY1127_2 strain [48].

Although the Shiraki-Tunesuke *kuratsuki* bacteria were different at the genus level, the effects of their addition to the sake production process on sake taste were surprisingly similar. The difference between the effects of *Bacillus* A-10 and *Priestia* B-12 on sake taste was limited. The astringency stimulation of sake produced with sake yeast K901 was increased by *Bacillus* A-10 (Figure 2). However, *Priestia* B-12 decreased it (Figure 2). A similar pattern was detected for sake saltiness using yeast K901 (Figure 4). Except for these two cases, the changes in taste intensity induced by the strains A-10 and B-12 were similar. Therefore, sake yeast K901 interacted differently with the *kuratsuki Bacillus* A-10 and *Priestia* B-12 strains.

4. Discussion

Horizontal transfer of genetic information has been strongly suggested between different *kuratsuki Kocuria* strains (TGY1120_3 and TGY1127_2), because similar plasmids encoding identical transposase-coding gene were detected in the different *Kocuria* strains TGY1120_3 and TGY1127_2 [20]. In contrast, no plasmids were detected in the genomes of the *kuratsuki Bacillus* A-10 and *Priestia* B-12 strains. The *Kocuria* strains were *kuratsuki* bacteria from Narimasa but not *kuratsuki* bacteria from Shiraki-Tsunesuke. The *Bacillus* A-10 and *Priestia* B-21 strains were *kuratsuki* bacteria from Shiraki-Tsunesuke but not from Narimasa. This suggests that different sake production environments may influence differently genome evolution in *kuratsuki* bacteria.

The addition of *Bacillus* A-10 and/or *Priestia* B-12 to the sake production process using the sake yeasts AK25, K901, K1401, and K1801 did not significantly affect the Brix and acidity patterns (Figure 1). These results indicated that neither the *kuratsuki Bacillus* strain nor the *Priestia* B-12 strain inhibited ethanol fermentation and organic acid production by all four sake yeasts used in this study. Such characteristics can be expected for all *kuratsuki* bacteria used in sake production. If *kuratsuki* bacteria inhibit the metabolism of sake yeast, the characteristic flavor and taste of sake yeast may be lost.

Furthermore, the effects of *Bacillus* A-10 and/or *Priestia* B-12 addition to the sake production process on sake taste were limited (Figures 2–7, S1 and S2). The ranges of the taste intensity differences in this study were much smaller than those found for sake produced using *kuratsuki Kocuria* [48]. This suggests that *kuratsuki Kocuria* interacts more strongly with sake yeast than *kuratsuki Bacillus* or *Priestia*. The effects of *kuratsuki Bacillus* A-10 on sake taste were similar to those of *kuratsuki Priestia* B-12 in sake produced using the sake yeasts AK25, K1401, and K1801 (Figures 2–7, S1 and S2). However, the effects of these *kuratsuki* bacterial strains differed for sake produced using yeast K901 (Figures 2 and 4). This indicated that a combination of *kuratsuki* bacteria and sake yeast should be considered when modifying sake taste. From the viewpoint of geographical indication, an increasing interest is recognized in autochthonous microorganisms related to fermented foods and drinks [12,59,60]. It is necessary to perform additional research to investigate the interaction between different *kuratsuki* bacteria and sake yeasts in order to modify sake flavor and taste profiles.

When the effects of *kuratsuki* bacteria on sake taste were compared among sake preparations produced with only *Bacillus* A-10, only *Priestia* B-12, and both A-10 and B-12, the taste intensity of sake obtained with A-10 and B-12 was different from that of sake obtained with A-10 and that of sake obtained with B-12, in most cases (Figures 2–7, S1 and S2). This strongly suggests that the interaction between *kuratsuki Bacillus* A-10 and sake yeast is not independent of the interaction between *kuratsuki Priestia* B-12 and sake yeast. Therefore, microbial interactions may also occur between the *Bacillus* A-10 and *Priestia* B-12 strains.

5. Conclusions

Our aim was to use *kuratsuki* bacteria for sake production. The flavor and taste of sake can be controlled by utilizing the interactions between sake yeast and *kuratsuki* bacteria. To achieve this goal, *kuratsuki* bacteria should be collected from various sake breweries and characterized. To the best of our knowledge, this is the first study using *kuratsuki* bacteria for sake production. Currently, we have collected only four strains of *kuratsuki* bacteria from two different sake breweries and characterized only two strains, *Kocuria* TGY1120_3 and TGY1127_2, from one sake brewery.

In this study, we characterized the *kuratsuki Bacillus* A-10 and *Priestia* B-12 strains. This study elucidated differences in the effects of *kuratsuki* bacteria on sake taste. We were able to quantitatively show differences in the taste of sake and clearly show differences in the effects of different *kuratsuki* bacteria. Although all sake yeasts are *Saccharomyces cerevisiae*, *kuratsuki* bacteria are diverse and differ at the genus level. Surprisingly, the *kuratsuki* bacterial strains A-10 and B-12 differ at the genus level, although the taste intensity differences among sake preparations produced using the sake yeasts AK25, K1401, and K1801 and either *kuratsuki Bacillus* A-10 or *kuratsuki Priestia* B-12 were very small. To demonstrate *kuratsuki* bacterial diversification, additional *kuratsuki* bacteria should be collected and characterized.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/applmicrobiol4010011/s1. Figure S1: Difference between the astringency taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain. Figure S2: Difference between the bitter taste intensities of sake produced using different sake yeasts (AK25, K901, K1401, and K1801) with or without *kuratsuki Bacillus* A-10 and/or *Priestia* B-12. The taste intensity for each yeast strain with and without the *kuratsuki* bacterium (control) was measured. Each measurement was repeated four times. Thus, four values of taste intensity for sake with *kuratsuki* bacterium and four values of taste intensity for sake without *kuratsuki* bacterium (control) were obtained and subjected to pairwise comparisons (intensity differences) for each sake yeast strain.

Author Contributions: Conceptualization, H.N.; methodology, H.N.; formal analysis, K.K.; writing—original draft preparation, H.N.; supervision, H.N.; project administration, H.N.; funding acquisition, H.N. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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