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ORIGINAL ARTICLE

Hop resistance and beer-spoilage features of foodborne *Bacillus cereus* newly isolated from filtration-sterilized draft beer

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Abstract To meet consumer demands for fresher and healthier foods, the pasteurization of beer has largely been replaced by membrane filtration methods. Research on beer spoilage caused by hop-resistant bacteria is thus of great interest. In this context, we have isolated a beer-spoilage strain of bacteria from turbid bottled beer treated by membrane filter sterilization, which we identified as *Bacillus cereus* strain 3012. Strain 3012 was able to produce acids and bioamines, resulting in a change in the flavor profile of the beer. Unlike other known strains of *B. cereus*, this isolate was resistant to hop compounds present in the beer and was in an evolutionarily stable state in terms of hop resistance. Our results suggest that this hop-resistant *B. cereus* strain 3012 is a foodborne pathogen with the potential to cause beer-spoilage incidents in the brewing industry.

Keywords *Bacillus cereus* · Hop resistance · Beer spoilage · Isolation · Foodborne

Introduction

Beer has been regarded for hundreds of years as a beverage that is devoid of foodborne pathogens, difficult to spoil and which has a remarkable microbiological stability. These properties are in large part due to the presence of inhibitors, such as hop bitter compounds and alcohol, a limiting pH value and an extremely reduced content of oxygen (Vaughan et al. 2005; Pittet et al. 2011; Wieme et al. 2014). Processes such as membrane filtration, storage at low temperature and pasteurization can also reduce beer contamination. Nevertheless, some microorganisms still manage to grow in beer, leading to turbidity and off-flavors, which is turn lead to in economic damage to the beer brewing companies and loss of consumer confidence (Steiner et al. 2010; Manzano et al. 2011). Among these beer spoilage microbes, both Gram-positive and Gram-negative bacteria possess strong spoilage ability, with both sorts having been frequently isolated from beer and work-in-process products (Bokulich and Bamforth 2013). At the present time, hop-resistant lactic acid bacteria (LAB) are generally considered to be the most problematic beer-spoilage microorganisms (Bokulich and Bamforth 2013). In the last two decades, anaerobic Gram-negative bacteria, such as the *Pectinatus*, *Megasphaera* and *Zymophilus* species, have also gained importance as spoilage bacteria in the brewing industry (Paradh et al. 2011; Matoulkova et al. 2012). Although these microbes produce off-flavors and turbidity, they are typically not harmful if consumed (Pittet et al. 2013).

Bacillus cereus is a widespread food pathogen causing emesis and diarrhea (Cronin and Wilkinson 2010). However, its importance as a pathogen has been truly appreciated only in the last 30–40 years because of the mild and transient symptoms of the illness it causes (Logan 2011) and the diversity of *B. cereus* group strains (Ceuppens et al. 2013). This pathogen causes food spoilage, mainly in milk and dairy products, leading to enormous economic losses to the food industry (De Jonghe et al. 2010; Desriac et al. 2013), while its emergence in alcoholic beverages had only recently been observed (Kim et al. 2014; Jeon et al. 2015).

Nowadays, to meet the present-day consumer's preference for freshness, the brewing industry has increased its

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production of unpasteurized beer using membrane filtration techniques. In this draft beer category, sterile filtration is used to physically remove microorganisms to ensure microbiological stability but without conferral of thermal tainting by heat treatment (Kunze 2004). However, there is the potential risk of possible penetration by comparatively small microorganisms even though the pore size of the membrane filter has been reduced to the recommended level (Back 1992). Therefore, research on beer spoilage caused by hop-resistant bacteria is of great economic interest. A strain of foodborne *B. cereus* was recently isolated from commercially produced turbid beer that had been recalled from the Chinese market by the manufacturer. This strain was able to grow well in the presence of hop compounds and showed a strong beer-spoilage ability. To our knowledge, this was the first report of the beer-spoilage features of hop-resistant *B. cereus*, although they had previously observed in home-brewed beer (Haakensen and Ziola 2008; Jeon et al. 2015). In this paper, we describe the isolation and beer-spoilage characteristics of *B. cereus* strain 3012.

Materials and methods

Microorganism, materials and culture conditions

The hop-sensitive *B. cereus* AS1.3760 was purchased from the China General Microbiological Culture Collection Centre and used as a reference strain. Turbid beer (670 mg/L dry hops) was obtained from the Gins Beer Co. of China, a brewing company. The beer had been recalled from the Chinese market in 2014 because of turbidity. Commercially available bottled beers (pasteurization, 3.2 % ethanol at pH 4.3) to test for beer-spoilage ability and wort were gifts from the China Resources Snow Breweries, Dalian. The hop extract was purchased from Yasheng Beer Co. Ltd., China.

Bacillus cereus AS1.3760 was incubated on nutrient agar plates (10 g peptone, 3 g beef extract, 5 g NaCl, 1000 ml distilled water, 20 g agar; pH 6.8) at 30 °C. For the evaluation of beer spoilage, the tested strains were incubated statically in the commercially available beers sealed with a sterilized cap at 30 °C. The flasks of beer were gently turned upside down twice every 12 h during the incubation process. Wort agar plates (per liter: 15.1 g glucose, 60.0 g maltose, 23.6 g maltotrisose, 361.5 mg free amino nitrogen content, 20 g agar; pH 5.5) were prepared using the wort from the China Resources Snow Breweries (12.5°P original gravity in all-malt). Hopped wort medium were prepared by supplementing wort agar with 1000 mg/L hop extract before autoclaving. The hop extract was filter-sterilized when supplemented to commercially available beer. Taxonomic properties of *B. cereus* AS1.3760 were characterized by culture on basal medium (per liter: 0.5 g $MgSO_4 \cdot 7H_2O$, 0.7 g KNO_3, 0.5 g NH_4Cl, 1 g NaCl; pH 7.0–7.2).

Isolation and identification of the beer-spoilage isolate

Samples of turbid beer (10 ml) were serially diluted tenfold with sterile saline, spread onto the wort agar plates supplemented with hop extract and incubated aerobically at 30 °C overnight. Colonies were randomly picked and inoculated into commercially available beer samples. After incubation at 30 °C for 7 days, beer turbidity was determined by spectrophotometry at 600 nm to evaluate beer-spoilage ability. Beer samples without inoculation were used as controls.

Unidentified isolates were cultured on the wort agar plates at 30 °C overnight, and cells were examined with a phase-contrast microscope (Leica Microsystems, Wetzlar, Germany) and transmission electron microscope (JEOL Ltd., Tokyo, Japan). Gram staining was performed by the Hucker staining method, and endospore production was analyzed by the Schaeffer–Fulton staining method (Murray et al. 1994). Tests for catalase, oxidase, urease, lecithinase and lipase activities and for nitrate reduction were performed as described previously (Smibert and Krieg 1994). Acid and gas production from carbohydrates was determined in the basal medium supplemented with various carbohydrates, as previously described (Smibert and Krieg 1994). 16S rRNA gene sequencing and phylogenetic analysis were performed as described previously (Chen et al. 2014).

Effects of temperature, pH and ethanol content on beer-spoilage ability

After overnight culture on the wort agar plates at 30 °C, the beer-spoilage isolate [optical density at 600 nm (OD_{600}) = 0.6] was inoculated at 1 % inoculum in commercially available beer and incubated at 4, 10, 20, 30 and 37 °C, respectively, for 7 days. Beer-spoilage ability was determined as the OD_{600}. The optimum pH for beer-spoilage ability in commercially available beer was evaluated by culturing the isolate at different pH and at 30 °C. The effect of ethanol content on beer-spoilage ability was estimated after culturing the isolate in commercially available beer supplemented with different ethanol contents at 30 °C for 7 days.

Hop-resistant stability and minimum inhibitory concentration of *B. cereus* strain 3012

Bacillus cereus strain 3012 was subcultured for more than 20 times in the wort medium not supplemented with hop compounds, following which the latest generation of the strain was incubated in commercially available beer supplemented with 0–1000 mg/L hop extract at 30 °C for 7 days. The culture fluid was then sampled and spread on the wort agar plates for the colony counting assay. Cell growth of wild-type isolate 3012 in commercially available beer was used as the control. The hop-resistant yield was defined as 1 − (colony number of wild

strains − colony number of subcultured strains)/colony number of wild strains.

Overnight cultured strain 3012 (OD_{600} = 0.6) was inoculated at 1 % inoculum in commercially available beer containing 0–3000 mg/ml hop extract and incubated at 30 °C for 7 days. The minimum inhibitory concentrations (MICs) were determined from the extent of cell growth in commercially available beer by the colony counting assay (Zhao et al. 2016). The hop-sensitive *B. cereus* strain AS1.3760 was cultured in commercially available beer as the reference.

Sample preparation for fermentation performance assays

Overnight cultured strain 3012 was inoculated at 1 % inoculum in commercially available beer and incubated at 30 °C for 7 days. The cultured beer was ultrasonicated for 30 min to remove CO_2 in the beer and centrifuged at 12,000 rpm for 10 min. The supernatant was used for detecting the component profile of organic acids, bioamines and flavors. Commercially available beer without inoculation was incubated at 30 °C as the control.

Evaluation of beer-spoilage ability

The turbidity was used as an indicator of beer spoilage by microbes and determined at 600 nm as the optical density (OD_{600}).

Colony counting assay

Six tenfold serial dilutions of the bacterial suspension were made. A 200-μl volume of the resultant suspension was uniformly spread onto overnight-dried wort agar plats with a sterile spatula. After cultivation at 30 °C for 24 h, all colonies were enumerated, and the mean values and maximal scatter in colony-forming units were determined.

Analysis of organic acids

Beer cultures were first filtered through a 0.22-μm membrane, and then the organic acid composition was determined by high performance liquid chromatography (HPLC) on an Agilent 1260 Infinity LC system (Agilent Technologies, Santa Clara, CA) equipped with a UV detector (Shui and Leong 2002). The mobile phase was composed of 0.01 M $(NH_4)_2HPO_4$ buffer (pH 2.5) and acetonitrile. The programmed gradient was set as: 100 % phosphate buffer for 3 min, from 100 % to 96 % phosphate buffer for the following 10 min, 96 % phosphate buffer for 10 min, from 96 % to 100 % phosphate buffer for the following 10 min and 100 % phosphate buffer for 5 min. A nonpolar C18 column (4.6 × 250 mm; Dalian Elite Analytical Instruments Co., Ltd. Dalain, China) filled with 5-μm SinaChrom ODS-BP support was used at 30 °C at a flow rate of 0.8 ml/min. The injection volume was 10 μl and the wavelength was set as 215 nm. The identified peaks were all confirmed by comparison with organic acid standards that had been added to the samples. The integrated peak area was used for quantification.

Bioamine assay

The bioamine assay was performed as described by Tang et al. (2009). Briefly, a 10-ml aliquot of beer sample was mixed with 3 g NaCl and adjusted to pH 12 with 2 M NaOH. The sample (1 ml) was mixed with an equal volume of normal butanol and chloroform solution (v/v, 1:1) and votexed for 5 min. After centrifugation at 6000 *g* for 10 min, the upper layer was collected and the lower layer was extracted again. The combined extract was then acidified with 1 M HCl and dried by N_2 at 40 °C. The dried extract was then dissolved in 1 ml of 0.1 M HCl and mixed with 1.5 ml saturated $NaHCO_3$ and 1 ml dansyl chloride at 60 °C for 30 min; 100 μl sodium glutamate was then supplemented to the reaction solution, and after 15 min of incubation the mixture was mixed with 1 ml pure water and dried to 3 ml by N_2 at 40 °C. Extraction was carried out with an equal volume of ether, and the upper layer was collected. The lower layer was extracted repeatedly, and the combined extract was dried by N_2. The dried extract was then dissolved in 1 ml methanol and the solution passed through a 0.22-μm filter; 50-μl samples were injected into the HPLC system for the bioamine assay. A Zorbax SB-C18 column (4.6 × 150 mm; Agilent, Technologies) was used at 35 °C at a flow rate of 1.0 ml/min. The mobile phase consisted of pure water (Buffer A) and methanol (Buffer B). The gradient elution program is shown in Table 1. The wavelength was set at 254 nm. The identified peaks were all confirmed by comparison with a standard bioamine that had been added to the samples. The integrated peak area was used for quantification.

Analysis of flavor compound in beer

Flavor profiles in the beer samples were measured by gas chromatography on an Agilent 6850 system (Agilent Technologies) equipped with headspace sampler, flame

Table 1 Gradient elution program for the bioamine assay

Buffer	Time (min)								
	0	10	15	20	27	30	35	40	45
Buffer A (pure water) (%)	35	35	25	20	15	10	5	35	35
Buffer B (methanol) (%)	65	65	75	80	85	90	95	65	65

The bioamine assay comprised high-performance liquid chromatography, the mobile phase of which was consisted of pure water (Buffer A) and methanol (Buffer B)

ionization detector and DB-FFAP column (0.25 mm × 30 m × 0.25 μm) (Li et al. 2012). The operating conditions were: vial equilibration, 30 min; injection duration, 0.5 min; injector and detector temperature of 250 °C; injection mode, split; split ratio: 2:1; carrier gas: N_2 (10 ml/min), H_2 (30 ml/min) and air (300 ml/min) at a flow rate of 1.6 ml/min; column oven temperature profile: kept at 50 °C for 2 min, increased to 190 °C at a rate of 5 °C/min, kept at 190 °C for 1 min, increased to 230 °C at a rate of 10 °C/min, kept at 230 °C for 10 min. The identified peaks were all confirmed by comparison with flavor compound standards added to the samples. The integrated peak area was used for quantification.

Statistical analysis

All tests were performed in triplicate, and the results were expressed as the mean ± standard deviation . Analysis of variance and significant differences among means were tested by the independent-sample *T* test ($p < 0.05$) using SPSS software (ver. 17.0; IBM Corp., Armonk, NY).

Results

Isolation and identification of beer-spoilage bacterium

Fifty 50 colonies which grew on the hopped wort agar plates were randomly picked out and cultured in commercially available beer; of these 49 colonies were able to grow in commercially available beer, of which 16 colonies showed strong beer-spoilage ability. Endospore formation was observed in five colonies. An NCBI BLAST comparison for 16S rRNA sequence (Johnson et al. 2008) revealed that these five endospore-forming colonies matched best with the genus *Bacillus*. As endospore-forming strains are not considered to be important beer-spoilage microbes (Bokulich and Bamforth 2013), we selected these five endospore-forming colonies for further study.

The 16S rRNA gene sequences from all five endospore-forming colonies were identical. When compared with those available in the GenBank/EMBL databases (Johnson et al. 2008), the sequence close to those endospore-forming colonies was that from the type strain of *B. cereus* which shared 100 % similarity of 16S rRNA gene sequence. Thus, we chose one colony with maximal beer-spoilage ability from those endospore-forming bacteria for further study and designated as it as strain 3012.

Strain 3012 was Gram-positive, facultatively anaerobic and straight rod-shaped. Endospore formation was observed. The strain tested positive for catalase, urease, nitrate reductase and lecithinase activities, but negative for oxidase and lipase activities. Acid was produced from glucose and maltose, but not from mannose, galactose and manitol. The isolate could not produce gas from glucose.

Effects of temperature, pH and ethanol content on beer-spoilage ability of isolate 3012

The beer-spoilage ability of isolate 3012 in commercially available beer was determined after incubation at different temperatures and pH. As shown in Fig. 1, a good growth was obtained at all tested temperatures except at 4 °C. Beer spoilage by isolate 3012 was observed in the pH range of 3.5–7.5 (Fig. 1), while relatively lower turbidity was detected at pH 3.5 than at the other pH values.

As shown in Fig. 2, there was no significant difference in the beer-spoilage ability when the final concentration of ethanol was less than 5.7 % in commercially available beer. Nevertheless, a sharp decrease in the turbidity was observed when the ethanol content was above 6.7 %.

Hop-resistant stability and MIC of isolate 3012

The reduction in hop resistance was determined according to the method of Suzuki et al. (2002) for *Lactobacillus brevis*. Accordingly, isolate 3012 was serially subcultured 20 times in the wort medium without hop compounds, following which cell growth of the isolate was determined in commercially available beer supplemented with hop extract. It was observed that the subcultured isolate could still grow well in beer supplemented with hop extract in the range of 0–1000 mg/L. Moreover, there was no significant difference ($p < 0.05$) in hop-resistant yield among beers with different concentrations of hop extract (Fig. 3). The subcultured strain in commercially available beer with hop extract showed similar cell growth to that of the original strain 3012, suggesting that isolate 3012 is stable in terms of hop resistance.

The MIC of isolate 3012 was determined, revealing that this isolate could grow even at a hop extract concentration of 3000 mg/L, suggesting that hop compounds could not inhibit

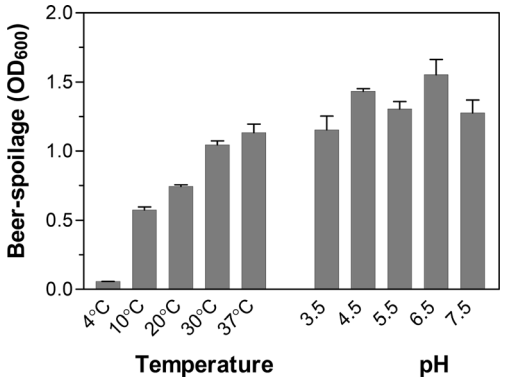

Fig. 1 Cell growth of *Bacillus cereus* strain 3012 in commercially available beer at different temperatures and at different initial pH values at 30 °C

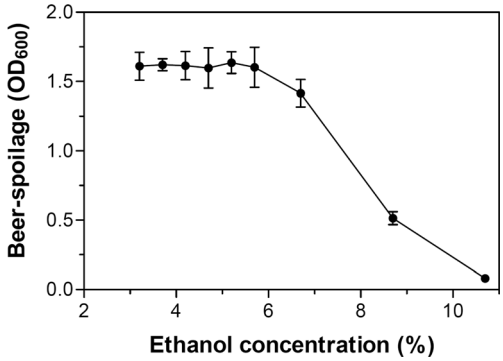

Fig. 2 Effect of ethanol concentration on cell growth when *B. cereus* strain 3012 was incubated in commercially available beer supplemented with different ethanol concentrations

the cell growth of isolate 3012. In contrast, the reference strain of *B. cereus* AS1.3760 could not grow well in commercially available beer even though the beer was not supplemented with hop extract, indicating that the reference strain was sensitive to hop compounds.

Changes in organic acids, bioamines and flavor compounds in beer when incubated with isolate 3012

The cell growth of beer-spoilage strains can cause changes in beer components in addition to beer turbidity (Sakamoto and Konings 2003). We therefore identified changes in characteristic components when isolate 3012 was cultured in commercially available beer. The content of three organic acids, namely, glyoxylic acid, pyruvic acid and lactic acid, changed markedly, i.e., between 5 and 15 %, in the inoculated beer. The content of the other organic acids did not change to any great exten (Table 2).

Using the derivatization method, we were able to identify six bioamines in the beer incubated with isolate 3012. Compared to the control group (beer incubated without strain 3012), the levels of tryptamine, cadaverine and spermine in the beer incubated with isolate 3012 were significantly decreased by up to 25 %, putrescine was increased by about

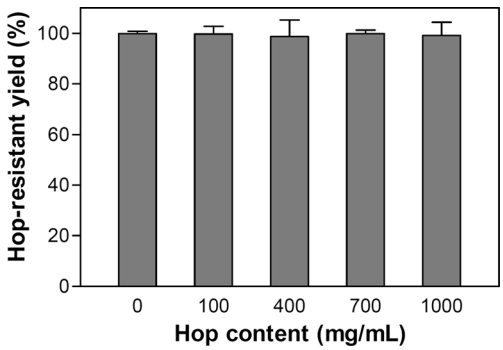

Fig. 3 Hop-resistant stability of *B. cereus* strain 3012 in commercially available beer supplemented with hop compounds in the range of 0–1000 mg/ml after serially subculturing in the wort medium without supplementation of hop extract

Table 2 Changes in flavor compounds, organic acids and bioamines in bottled beer following incubation with *Bacillus cereus* strain 3012 at 30 °C for 7 days

Components	Control group[a]	Strain 3012 group
Organic acids		
Glyoxylic acid	506.11 ± 3.93	534.25 ± 2.80*
Pyruvic acid	703.40 ± 12.40	596.20 ± 11.82*
Lactic acid	208.87 ± 6.22	230.92 ± 4.83*
α-Ketoglutaric acid	205.13 ± 8.42	220.98 ± 18.38
Citric acid	655.99 ± 37.45	657.76 ± 3.36
Malic acid	325.28 ± 127.62	559.45 ± 7.87
Bioamines		
Tryptamine	1108.46 ± 2.51	1027.77 ± 2.00*
Putrescine	83.10 ± 0.68	100.78 ± 0.60*
Cadaverine	36.84 ± 0.54	28.53 ± 0.48*
Tyramine	164.50 ± 1.68	161.52 ± 2.70
Spermidine	10.52 ± 0.66	22.08 ± 0.82*
Spermine	120.76 ± 0.49	106.79 ± 1.64*
Flavor compound		
Ethyl formate	11.24 ± 0.15	12.99 ± 0.74*
Ethyl acetate	575.49 ± 4.99	1193.88 ± 5.02*
Isobutyl acetate	11.69 ± 0.35	12.71 ± 0.23*
n-Propanol	98.86 ± 2.10	101.92 ± 2.02
Isobutanol	232.97 ± 4.33	235.00 ± 6.72
Isoamyl acetate	162.73 ± 7.84	153.58 ± 5.88
1-Butanol	4.56 ± 0.07	4.49 ± 0.14
Isopentanol	1439.47 ± 28.03	1424.41 ± 58.53
Ethyl caprylate	28.324 ± 0.56	23.58 ± 2.28*
2,3-Butanediol	1.892 ± 0.41	1.30 ± 0.06
Phenylethyl alcohol	53.71 ± 0.68	54.76 ± 0.47

*Significant difference between control group and Strain 3012 group at $p < 0.05$

Data in table are presented as the average ± standard deviation($n = 3$)

[a] The control group is beer incubated without strain 3012

20 % and spermidine was increased by more than 100 %. The level of yyramine was not remarkably changed (Table 2).

Among the low abundant flavor substances, ethyl formate and isobutyl acetate were significantly increased after incubation with isolate 3012, while ethyl caprylate was significantly decreased (Table 2). Ethyl acetate is found in high abundance in beer, and in beer incubated with isolate 3012 the peak area was double that of the control. The other detected flavor substances were not significantly affected by the presence of isolate 3012.

Discussion

Although LAB are the cause of most microbial beer-spoilage incidents, some other microbes have also been identified as

spoilage factors in beer (Matoulkova et al. 2012; Bokulich and Bamforth 2013). When the hopped wort agar plate was used to isolate beer-spoilage bacteria in this study, endospore-forming strains were obtained from the turbid beers. Based on characterization of the 16S rRNA sequence, these endospore-forming isolates matched best with the type strain of *B. cereus* (Johnson et al. 2008). Their phenotypic properties were found to be consistent with the key characteristics of the genus *Bacillus*, including Gram-positive straight rods, formation of endospores, facultatively anaerobic and production of catalase (Claus and Berkeley 1986). Therefore, these endospore-forming isolates should belong to the strain of *B. cereus*, and one isolate with the maximal beer-spoilage ability was designated as strain 3012 of *B. cereus* for this study.

As shown in Fig. 1, strain 3012 grew well at 30 °C and hardly grew at 4 °C, which coincides with storage practices in China where beer is generally stored at room temperature but not at a refrigerated temperature. In contrast to known strains of *B. cereus* growing at above pH 5.0 (Okanlawon et al. 2010), strain 3012 could grow at acidic pH (Fig. 1). It has been reported that traces of nutritive substances, such as glucose, maltose and maltotriose, do not support the better growth of *B. cereus* (Vaughan et al. 2005). However, *B. cereus* strain 3012 showed good growth in commercially available beer. It is possible that the residual sugars in commercially available beer can also not be detected after incubation with isolate 3012 (data not shown). The yeast metabolite ethanol is also known to reduce the growth rate of *B. cereus*, eventually causing a decline in the final levels of cell growth (Daifas et al . 2003). In contrast, the beer-spoilage ability of *B. cereus* strain 3012 in commercially available beer was not inhibited by ethanol until the concentration of ethanol was higher than 6 % (Fig. 2). In general, the ethanol content in beer is not more than 6 % in China. Therefore, it can be concluded that ethanol in beer does not affect beer spoilage caused by isolate 3012.

Hop resistance is a prerequisite for microbes growing in beer (Sakamoto and Konings 2003). Strain 3012 was able to grow and survive in a wide range of hop concentrations, suggesting that *B. cereus* strain 3012 is hop-resistant. In contrast, the reference strain of *B. cereus* AS1.3760 could not grow in commercially available beer with or without the addition of hop extract. Richards and Macrae (1964) reported that in their study hop resistance decreased upon prolonged serial subculture in the absence of hop compounds. However, we detected no attenuation in hop resistance in isolate 3012 (Fig. 3), leading us to suggest that the acquired hop-resistance ability of isolate 3012 is of a very stable nature. This is different from that of *L. brevis*, the hop resistance of which decreased when it was serially subcultured in the absence of hop compounds (Suzuki et al. 2002).

Consistent with the feature of acid production caused by beer-spoilage (Sakamoto and Konings 2003), the acid profile of all detected organic acids increased after incubation with isolate 3012, with the exception of pyruvic acid. The levels of three acids were significantly increased. This result suggests that isolate 3012 is likely to produce organic acids.

Bioamines are generally used as spoilage indicators (De Borba and Rohrer 2007) and, therefore, changes in bioamine levels are normally measured in commercially available beer. In our study, the levels of a number of bioamines, such as putrescine and spermidine, increased remarkably after incubation with strain 3012. In contrast, tryptamine, cadaverine and spermine levels were distinctly decreased.

Flavors, including isobutanol and isoamyl acetate, changed during incubation of the beer with isolate 3012 when compared with fresh beer (data not shown), but such changes are consistent with that of beer incubated without *B. cereus* and were presumably due to beer aging (Vanderhaegen et al. 2006). Similar to *L. brevis*, *B. cereus* strain 3012 could also metabolize some flavor substances in beer (Sakamoto and Konings 2003), such as causing an increase in ethyl acetate, ethyl formate and isobutyl acetate and a decrease in ethyl caprylate.

Conclusion

Bacillus cereus strain 3012 was isolated from turbid beers commercially manufactured by bottling after membrane filter sterilization. This strain showed strong growth in beer supplemented with different hop concentrations and possessed the beer-spoilage characteristics, such as rendering beer turbid and producing acids and bioamines. It could also metabolize the produced flavor substances in beer, resulting in changes in beer flavor. Therefore, we conclude that *B. cereus* strain 3012 is a foodborne pathogen with strong beer-spoilage ability.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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