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Loop-mediated Isothermal Amplification to Detect and Identify Beer Spoilage *Lactobacillus* spp. Bacteria

Primers for a loop-mediated isothermal amplification (LAMP) method to specifically identify beer spoilage caused by *Lactobacillus* spp. including *Lactobacillus brevis*, *Lactobacillus lindneri*, *Lactobacillus backi* and *Lactobacillus paracollinoides* were developed. These LAMP primer sets were designed from target sequences in the 16S rRNA gene or the spacer region. To identify beer spoilage strains in the species, we also developed a LAMP primer set to detect highly specific genetic markers. The LAMP primer sets in this study distinguished the target species or beer spoilage strains from other lactic acid bacteria in 40–60 min. Moreover, using these primer sets in conjunction with the LAMP method enabled detection of approximately 1 × 10^2 cfu/ml lactic acid bacteria from suspensions in distilled water, beer and beer that contained large amounts of bottom-fermenting yeast cells. Thus, the LAMP method may be useful for direct detection and identification of *Lactobacillus* spp. in samples taken during the fermentation process and from final products.

Descriptors: Lactic acid bacteria, beer-spoilage bacteria, rapid detection, loop-mediated isothermal amplification, LAMP, DNA amplification

1 Introduction

Contamination of beer by lactic acid bacteria such as *Lactobacillus* is a major concern in the brewing industry. However, among the numerous *Lactobacillus* species, only a few cause spoilage in beer [1]. While isolates belonging to the aforementioned species have been reported as beer-spoilage bacteria, some strains have resistance to hop-compounds and can grow in beer, whereas others do not have the ability to spoil beer [1].

Lactobacillus brevis is one of the most common contaminants in breweries and beer [2, 3]. *L. brevis* is an obligate heterofermentative bacterium that has beer-spoilage abilities that vary according to its strain and origin. Beer-spoilage strains of *L. brevis* cause clouding, sedimentation and acidification of beer. Another important beer spoilage *Lactobacillus* sp. is *Lactobacillus lindneri*. This organism is highly resistant to bitter compounds from hops, and survives a higher degree of thermal treatment than other lactic acid bacteria found in the brewery environment [4, 5, 6, 7, 8].

Recently, two novel species, *Lactobacillus paracollinoides* and *Lactobacillus backi*, were isolated from brewery environments [9,10]. Comparison of the 16S rRNA gene sequences of these organisms has shown that *L. paracollinoides* is most closely related to *Lactobacillus collinoides*. However, these strains did not show sufficient DNA-DNA homology to be classified as any of the confirmed *Lactobacillus* species, even though they exhibited strong beer-spoilage ability and *L. collinoides* strains are not generally considered to be beer-spoilage bacteria [9]. *L. backi* strains have been isolated from lager, pils and wheat beers from different breweries in Germany and Italy, and are considered to be obligate beer-spoiling bacteria [10]. *L. backi* strains have been found to have a striking morphological resemblance to *Lactobacillus coryniformis* and homofermentative behavior. However, they can be differentiated from *L. coryniformis* on the basis of physiological and biochemical tests and they have been found to show less than 97 % 16S rDNA sequence similarity to any other *Lactobacillus* species [10].

Bitter compounds derived from the hop plant (*Humulus lupulus* L.) have antibacterial properties. However, beer-spoilage strains of these species are resistant to these compounds and are able to grow in beer [11]. Recently, genetic approaches have been used to analyze these strains of beer-spoilage bacteria. Although the mechanisms of resistance towards bitter hop compounds have not yet been completely clarified, some genetic markers that diffe-

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Tables and figures see Appendix

rentiate beer-spoilage strains from non-spoilage strains have been identified [1, 12–19]. For example, *Fujii et al.* found that a single loci was highly specific to beer-spoilage strains of *L. brevis* and that the glycosyltransferase family gene in the loci was a good genetic marker for beer-spoilage strains belonging to *L. brevis* and *P. damnosus* [14, 15]. In this study, we designated the glycosyltransferase family gene, which was called "ORF1" in a study conducted by Fujii et al. [15], as *gtbA* (glycosyltransferase family gene of beer-spoilage lactic acid bacteria). The results obtained by Fujii et al. [15] using this genetic marker to assess beer-spoilage strain specificity were found to be better than those obtained using the *hitA*, *horA* and *horC* homologous genes, which were referred to as "ORF9" in the same study conducted by Fujii et al. [15]. Another group also found a highly homologous loci and *gtbA* homologous gene in beer-spoilage strains of *L. brevis*, *L. paracollinoides*, *L. backi* and *P. damnosus* [1, 16, 17, 19]. Conversely, *Haakensen et al.* reported that the putative beer spoilage-associated genes (*hitA*, *horA*, *horC*, etc.) were not statistically correlated with the ability to grow in beer [20]. However, the beers that they used to assess the beer-spoilage ability or some of the sources of the organisms appeared to differ from Japanese and German beer based on their bitter units, pH, etc., and they did not test the *gtbA* gene, which may explain this discrepancy [20].

Detection of beer spoilage bacteria using culture methods [3, 21], conventional taxonomic tests [2] and existing molecular techniques [22–29] is time consuming and/or costly. However, the recently developed loop-mediated isothermal amplification (LAMP) method may overcome some of the problems associated with the aforementioned techniques [30]. The LAMP method is characterized by the use of four different primers (FIP, F3, BIP, B3) specifically designed to recognize six distinct regions on the target gene. In addition, the use of loop primers (either LB or LF) can increase the amplification efficiency [31]. The LAMP method is highly specific, and results in high amplification of the target gene. Furthermore, the use of a DNA polymerase with high strand displacement activity can amplify DNA by tearing off double stranded DNA to yield a single strand. The combination of the DNA polymerase and the primer structure enables amplification of the target DNA at a steady temperature (approximately 65 °C). Therefore, the LAMP method can be conducted using a waterbath or thermal block instead of more specialized and sophisticated equipment such as a thermal cycler. In the LAMP method, the solution becomes white and turbid after the target gene has been amplified successfully as a result of accumulation of magnesium pyrophosphate, an amplification by-product. Because successful amplification is indicated visually by white turbidity in the solution, this method does not require special equipment such as a gel electrophoresis apparatus [30].

Several LAMP primer sets have been developed for identification of the beer-spoilage bacteria, *L. brevis*, *L. lindneri*, *Pediococcus damnosus* and *Pectinatus* spp. [32]. However, specific primers for the identification of *L. paracollinoides* and *L. backi* have not been reported to date, nor have primers that differentiate beer-spoilage strains from non-spoilage strains. Accordingly, in this study, we designed specific primers for use in the LAMP method to detect and identify various *Lactobacillus* beer-spoilage bacteria, including *L. brevis*, *L. lindneri*, *L. backi* and *L. paracollinoides*.

2 Materials and methods

2.1 Bacterial strains

Table 1 shows the strains used in this study. Bacterial strains were obtained from the following culture collections: Japan Collection of Microorganisms (JCM), Saitama, Japan; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Braunschweig, Germany. Isolates from our breweries were also used in this study. The isolates were identified based on sequence analysis of their 16S rRNA genes. All strains were cultivated at 25 °C in MRS (de Man, Rogosa, Sharpes, Oxoid) broth adjusted to pH 5.8 with concentrated HCl, or in m-NBB (modifiziertes Nachweismedium für bierschädliche Bakterien) broth [33].

2.2 Hop bitter compounds.

The HOPSTABIL-ISOHUMULONE 30 % (w/v) solution of iso-α-acids was supplied by Fromm, Mayer-Bass GmbH (München, Germany). This solution was diluted to 25 g/l with 99.5 % (v/v) ethanol and then stored at –20 °C until use. This stock solution was added to sterile broth immediately prior to inoculation of the cells [13].

2.3 Estimation of beer spoilage ability.

Bacterial strains were cultivated in MRS (pH 4.5) supplemented with 15 ppm of iso-α-acids or m-NBB medium at 25 °C for three days. Next, 10^6 cells were added to a bottle of commercial lager beer (pH 4.5, 5.5 % [v/v] ethanol, 21 bitter units [European Brewery Convention 1998]), and the bottle was crowned again [13]. This inoculated beer was incubated at 25 °C for further three months and the turbidity of the beer was observed.

2.4 Isolation of genomic DNA

One loop of bacterial cells (ca. 10^8 cells) was harvested from an MRS or m-NBB agar plate and suspended in 1 ml of distilled water. The cells were then centrifuged for 5 min at 2300 x g, after which the supernatant was discarded. Next, the cells were washed with distilled water one more time and then resuspended in 100 µl of PrepMan Ultra reagent (Applied Biosystems). The sample was then incubated at 95 °C for 10 min in a heat block, after which it was centrifuged for 1 min at 2300 x g. The supernatant was used as the genome DNA solution for PCR analysis and/or 16S rRNA sequence analysis to confirm the species of the strains, as well as for the LAMP reaction.

2.5 LAMP primer sets for *Lactobacillus* spp.

The LAMP method requires a set of four primers (B3, F3, BIP and FIP) that recognize a total of six distinct sequences (B1 to B3 and F1 to F3) within the target DNA. Because additional loop primers increase the amplification efficiency [31], one or two loop primers (an LF primer and/or an LB primer) for each species were also designed.

To design the species-specific primer sets, we used the DNA sequences of the 16S rRNA gene of *L. brevis* and *L. lindneri* and

the spacer region between the 23S and 5S rRNA genes of *L. paracollinoides* and *L. backi* (Table 2). We used the DNA sequence of the *gtbA* gene derived from *L. brevis* [14, 15] to distinguish beer-spoilage strains from non spoilage strains.

To determine the species-specific sequences, the nucleotide sequences of the target genes were analyzed using the BLAST sequence similarity search tool provided by the National Center for Biotechnology Information (URL: http://www.ncbi.nlm.nih.gov/BLAST/). The primer sets were designed using the LAMP primer designing software, PrimerExplorer (Fujitsu Limited; URL: http://primerexplorer.jp/e/). The primer sequences are shown in Table 2. Primer solutions were prepared as described previously [34, 35].

2.6 LAMP reactions

The LAMP reactions were performed using a Loopamp DNA amplification kit (Eiken Chemical Co., Tochigi, Japan). LAMP was conducted using reaction mixtures (25 µl) composed of 2.5 µl of genome DNA solution, 2.5 µl of primer mixture, 12.5 µl of 2 x reaction buffer and 1 µl of Bst DNA polymerase and reaction conditions that have been previously described [34, 35]. Amplification was conducted using LA-200 (Teramecs) and LA-320C (Eiken Chemical) real time turbidity meters. A turbidity of 0.1 was taken as the threshold value.

2.7 Detection limits of the LAMP method

To determine the detection limits of the LAMP method, genomic DNA was extracted from lactobacilli cells that had been diluted in distilled water and then evaluated by the LAMP reaction. *L. lindneri* DSM20692, *L. paracollinoides* DSM11969 and *L. backi* BK2 were cultivated on m-NBB agar plates at 25 °C. The cultivated cells (ca. 10^8 cells) were then washed once with distilled water and suspended in 1 ml distilled water. Next, the suspension was serially diluted in distilled water, after which 1 ml aliquots of each diluted suspension were centrifuged for 5 min at 2300 × *g* and the supernatants were discarded. The cells were then re-suspended in 100 µl 0.1 N NaOH and incubated at 95 °C for 10 min in a heat block. Next, the suspension was neutralized with 27 µl of 1 M Tris-HCl (pH 7.0) and centrifuged for 1 min, after which the supernatant was collected for use in the LAMP assays. All LAMP assays were conducted in duplicate and the time required for the turbidity of each sample to exceed the threshold value was defined as the threshold time. The mean of the threshold time was used to draw a calibration curve for quantification of the samples in cfu/ml.

2.8 Detection of *Lactobacillus* sp. from beer and beer containing bottom-fermenting yeast

L. backi BK2 was cultured on an m-NBB agar plate at 25 °C and the cultivated cells (ca. 10^8 cells) were then suspended in 1 ml degassed commercial lager beer (pH 4.3, 5.5 % (v/v) ethanol). The suspension was then serially diluted with beer. In addition, the bottom-fermenting yeast BFY70 was cultured on a malt agar plate and the cells were suspended in the same beer. An aliquot (900 µl) of the bottom-fermenting yeast suspension (10^7 cells/ml) was then mixed with 100 µl of the bacteria suspension. Mixtures of 900 µl of the beer and 100 µl of the bacteria suspension were also prepared. These suspensions were then centrifuged for 5 min at 2300 × *g* and the supernatants were discarded. The cells were then washed once with distilled water. The genomic DNA was isolated with 0.1 N NaOH and then used in the LAMP assay as described above.

3 Results and discussion

3.1 Specificity of LAMP primer sets for *Lactobacillus* spp.

The specificity of the developed LAMP primer sets was tested using genomic DNA isolated from several strains of lactic acid bacteria. The LAMP primer sets for *L. brevis*, *L. lindneri* and *L. backi* distinguished the target species from other lactic acid bacteria in approximately 40 min (Table 3). However, in some cases (twice in four times), it took approximately 60 min for amplification of *L. paracollinoides* JCM 11969 to occur using the LBR1LB1LF1 primers. The sequences of the 16S rRNA gene of *L. brevis* and *L. paracollinoides* are similar; therefore, cross-reaction may have occurred in these cases. Our results suggest that a combination of the LAMP primer sets for *L. brevis* and *L. paracollinoides* is the most effective method to differentiate these two bacteria. For example, if a strain is positive for the primer set LBR1LB1LF1 and negative for the primer set LCN3LF1, the strain can be identified as *L. brevis*. Conversely, a strain that is positive for both primer sets LBR1LB1LF1 and LCN3LF1 can be identified as *L. paracollinoides*.

The LAMP primer set DMS1LB1 was capable of differentiating beer-spoilage strains of *L. brevis*, *P. damnosus*, *L. paracollinoides* and *L. backi* from the non-spoilage strains of these species, as previously reported [14, 15]. Additionally, highly specific PCR primers were found to detect the beer-spoilage strains of *L. brevis* and *P. damnosus* [14, 15]. In our previously conducted studies, the LAMP results were strongly correlated with the PCR results. Specifically, the results obtained using the LAMP and PCR methods matched for 42 lactobacilli strains [36]. Moreover, another group found that the *gtbA* gene was highly homologous among the beer-spoilage strains of *L. brevis*, *L. paracollinoides*, *L. backi* and *P. damnosus* [1, 16, 17, 19]. However, we used a limited number of strains in this study; therefore, further analysis using a larger number of isolates should be conducted to assess the specificity of this genetic marker to beer-spoilage strains, especially *L. paracollinoides* and *L. backi*.

The LAMP primer set LCN3LF1 could not differentiate between *L. collinoides* and *L. paracollinoides* because the sequences of their rRNA genes are very similar. However, the beer-spoilage strain of *L. paracollinoides* could be differentiated from *L. collinoides* and a non-spoilage strain of *L. paracollinoides* using a combination of LCN3LF1 and DMS1LB1. For quality control in breweries, it is important to identify strains that have the ability to affect the quality of beer. Therefore, we assumed that it was not necessary to differentiate between non-spoilage strains of *L. collinoides* and *L. paracollinoides*. Additionally, based on these results, we consider

the combination of LCN3LF1 and DMS1LB1 to be suitable for specific detection of its target strain, which was the beer-spoilage strain of *L. paracollinoides*.

3.2 Detection of *Lactobacillus* spp. using the LAMP method

The ability of LAMP to detect *Lactobacillus* spp. in a suspension of distilled water was evaluated. The LL12, LCN3LF1 and HM6LB1LF1 primer sets amplified target sequences in samples containing 10^6–10^2 cfu/ml of *Lactobacillus* sp. (Table 4). In addition, a power approximation revealed that the threshold time of LAMP was strongly correlated with the log of colony-forming units per ml (R^2=0.96–0.99; Fig. 1, Table 4). Previously [34, 35], we found a good correlation between the threshold time and the log of cfu/ml using power approximation. Additionally, the LAMP method revealed that there was a good correlation with linear approximation between the threshold time and copy number of DNA [37]. It is important to note that the time required for detection may be affected by the matrix of the DNA extract solution when there is a low cell concentration. The reaction times at the detection limit of the LL12, LCN3LF1 and HM6LB1LF1 primer sets were approximately 80 min, 40 min and 60 min, respectively. However, it is recommended that the reaction time be prolonged when it is assumed that the target strain exists in very small concentrations in the sample.

In the production process, yeasts used for fermentation are dominant when compared with contaminating bacteria. Therefore, we tested the ability of LAMP to detect *L. backi* in mixtures containing 10^7 cells/ml of bottom-fermenting yeast in beer. The detection limit of *L. backi* was 10^2 cfu/ml, which was the same detection limit obtained using a distilled water suspension (Table 4, 5). The detection limit of *L. backi* was the same when bottom-fermenting yeast were omitted from the beer. Furthermore, the reaction times required for detection using a distilled water suspension, beer and beer with bottom-fermenting yeast were nearly identical. These findings suggest that the presence of a large amount of additional yeast and beer did not affect detection of contaminating organisms using the LAMP method. However, the approximate formulas vary depending on the matrix of the suspensions. Therefore, quantification using the LAMP method should be tested using a matrix similar to that of the samples. The quantification of beer-spoilage bacteria in samples will make it possible to assess the risk of accidents that decrease the quality of beer.

In previous reports, approximately 10^3 cells of lactobacilli were detected using PCR [22], and 50-100 cfu of *Pediococcus claussenii* per 100 ml of beer were detected using real-time PCR [25]. The LAMP method was assumed to have a lower detection limit than that of the standard PCR method and a higher detection limit than that of real-time PCR [34, 35, 38]. Haakensen et al. [25] used a membrane filter to collect the cells from beer and a DNA purification kit when determining the detection limit. However, in the present study, we employed a method that used an alkaline solution for extraction of genomic DNA when determining the detection limit. The method used in the present study requires only a few procedures and inexpensive reagents, to simulate use in small to mid-scale breweries. Modification of the methods used for cell collection and DNA extraction may improve the detection limit of the LAMP method.

4 Conclusion

A LAMP method that specifically detects target *Lactobacillus* spp. and strains responsible for beer spoilage was developed. The method successfully detected lactic acid bacteria at a concentration of approximately 10^2 cfu/ml in a distilled water suspension, beer and in beer containing large amounts of bottom-fermenting yeast cells. The LAMP method has many advantages; it is highly sensitive, rapid, low-cost and has few operational steps. This LAMP method for the detection and identification of *Lactobacillus* spp. will be very useful for quality control in breweries.

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Appendix

Table 1 Bacterial strains used in this study

Strain	Strain No.	Source
Lactobacillus brevis	JCM1059 (T)	Human faeces
Lactobacillus lindneri	DSM 20690 (T)	Spoilt beer
L. lindneri	DSM 20691	Spoilt beer
L. lindneri	DSM 20692	Spoilt beer
Lactobacillus collinoides	JCM 1123 (T)	Fermenting apple juice
Lactobacillus paracollinoides	JCM 11969 (T)	Brewery envrionment
L. paracollinoides	JCM 1113	Beer
Lactobacillus backi	DSM18080 (T)	Lager beer
Lactobacillus buchneri	JCM 1115 (T)	Tomato pulp
Lactobacillus coryniformis subsp. *coryniformis*	JCM 1164 (T)	Silage
Lactobacillus casei	JCM 1134 (T)	Cheese
Lactobacillus plantarum subsp. *plantarum*	JCM 1149 (T)	Pickled cabbage

(T): type strain

Isolates from brewery

Strain	Strain No.
L. brevis	L40
L. brevis	L50
L. brevis	L164
L. brevis	H4
L. brevis	H5
L. backi	BK1
L. backi	BK2
L. lindneri	606
Lactobacillus paracasei	U5
Pediococcus damnosus	B11
P. damnosus	B15
P. damnosus	B20
P. damnosus	B27
Pediococcus inopinatus	PI-1

JCM, Japan Collection of Microorganisms, Saitama, Japan; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.
Isolates were identified based on sequence analysis of the 16S rRNA gene.
The species "*Lactobacillus backi*" is not a validly published species; thus, the strains should be '*Lactobacillus backi*' or 'Candidatus *Lactobacillus backi*'. In this study, the strains were simply referred as *Lactobacillus backi*.

Table 2 Sequences of LAMP primer sets used in this study

LBR1LB1LF1 (for detection of *L. brevis*) Target gene: 16S rRNA gene

FIP: TGGGCAGATTTCCCACGTGTTAGTGCTTGCACTGATTTCAACAA
F3: AACGAGCTTCCGTTGAATGA
BIP: AGCAGGGGATAACACTTGGAAACACCGAAGCCACCTTTCAAACA
B3: TAATACGCCGCGGGATCA
LF: ACCAGTTCGCCACTCGC
LB: CCGTATAACAACAAAATCCG

LL12 (for detection of *L. lindneri*) Target gene: 16S rRNA gene

FIP: GGTCAGATCTATCGTCAAGCTGATGGCCTAAATAACATGCAAGTCGA
F3: TCAGGACGAACGTTGGC
BIP: AACCTGCCCAGAAGAAGGGGTGGTTCTCGTTGTTATACGGT
B3: GAAGCGATAGCATAAAAGCCA

LCN3LF1 (for detection of *L. paracollinoides*) Target gene: 23S–5S spacer region

FIP: CCGTTTTCTTACTTGGTTAAGTCCTCGGGAAGTCCAGTGATGGATGG
F3: TGATCAGGTAGATAGGCTAGAAGT
BIP: GGTAGGAAAACGATATTATCTAGTTTTGAGAGATCCTTCAGGCTATCGCCA
B3: TTCGGCATGGGAACAGGT
LF: ACCGATTAGTACTAGTCCGCT

HM6LB1LF1 (for detection of *L. backi*) Target gene: 23S–5S spacer region

FIP: ACGGCACTTCCACTTCCAACTTCCTTTATGGAAGTAAGACCCCT
F3: CCCGCAAGATTAGATTTCCCA
BIP: GTCGGAAACAATGTTTCCGCGTAGCTCTCAAAACTGGACCTTCT
B3: CCACACCACTATCTGAGAACTT
LF: CTATCTACCTGATCATCTCTC
LB: GTGTTCAAAGGTCAAAGAAAAT

DMS1LB1 (for differentiation of beer-spoilage strains from non-spoilage strains)
Target gene: *gtbA* gene

FIP: TTCACGACTTCACTCATGCCGGCGACGGACTGCGTTTTTCCCG
F3: CGAAAAACTGCACGCCATG
BIP: CCGACCTCCAAGATGATCCCGAGGACGCCGTAGACGATGT
B3: CGGTTTCCCGGTTGTTCC
LB: TGGTGGATGCCTATGCGGAT

Sequence accession numbers: *L. brevis* 16S rRNA gene (AF515219, AF515220), *L. lindneri* 16S rRNA gene (D37784), *L. paracollinoides* 23S–5S spacer region (AB514872), *L. backi* 23S–5S spacer region (AB514873) and *L. brevis gtbA* gene (glycosyltransferase family gene of beer spoilage lactic acid bacteria, AB182366).

Table 3 Specificities of LAMP primer sets for individual species of lactic acid bacteria

+ : amplification in 40min,
– : no amplification in 40 min

Strains	Beer spoilage ability	LBR1 LB1LF1	LL12	LCN3 LF1	HM6 LB1LF1	DMS1 LB1
Lactobacillus brevis L40	+	+	–	–	–	+
L. brevis L50	+	+	–	–	–	+
L. brevis L164	–	+	–	–	–	–
L. brevis H4	+	+	–	–	–	+
L. brevis H5	+	+	–	–	–	+
L. brevis JCM1059	–	+	–	–	–	–
L. lindneri DSM 20690	+	–	+	–	–	–
L. lindneri DSM 20691	+	–	+	–	–	–
L. lindneri DSM 20692	+	–	+	–	–	–
L. lindneri 606	+	–	+	–	–	–
L. collinoides JCM 1123	–	–	–	+	–	–
L. paracollinoides JCM 1113	–	–	–	+	–	–
L. paracollinoides JCM 11969	+	–	–	+	–	+
L. backi DSM18080	+	–	–	+	–	+
L. backi BK1	+	–	–	–	+	+
L. backi BK2	–	–	–	–	+	–
L. buchneri JCM 1115	–	–	–	–	–	–
L. coryniformis JCM 1164	–	–	–	–	–	–
L. casei JCM 1134	–	–	–	–	–	–
L. paracasei U5	–	–	–	–	–	–
L. plantarum JCM 1149	–	–	–	–	–	–
Pediococcus damnosus B11	+	–	–	–	–	+
P. damnosus B15	–	–	–	–	–	–
P. damnosus B20	+	–	–	–	–	+
P. damnosus B27	–	–	–	–	–	–
P. inopinatus PI-1	–	–	–	–	–	–

Table 4 Detection limit and quantification of *Lactobacillus* spp. in distilled water using the LAMP method

Primer set	Strain	Detection limit 10^2 cfu/ml	Tt with	Approximate formula	R^2
LCN3LF1	*L. paracollinoides* DSM11969	3.4x102 cfu/ml	45 min	$y = 19792x^{-2.3525}$	0,956
LL12	*L. lindneri* DSM20692	$3.3x10^2$ cfu/ml	79 min	$y = 97125x^{-2.4127}$	0,996
HM6LB1LF1	*L. backi* BK2	$1.2x10^2$ cfuml	57 min	$y = 1026.2x^{-1.5426}$	0,990

Tt: threshold time, y = log cfu/ml, x = threshold time.

Table 5 **Detection limit of *L. backi* BK2 using the HM6LB1LF1 primers**

Suspension	Detection limit	Tt with 10^2 cfu/ml	Approximate formula	R^2
Beer	$2.1x10^2$ cfu/ml	53 min	$y = 7531.8x^{-2.0322}$	0,993
Beer + bottom fermenting yeast (1 x 10^7 cells)	$2.1x10^2$ cfu/ml	54 min	$y = 6409.4x^{-1.9554}$	0,961

Tt: threshold time, y = log cfu/ml, x = threshold time

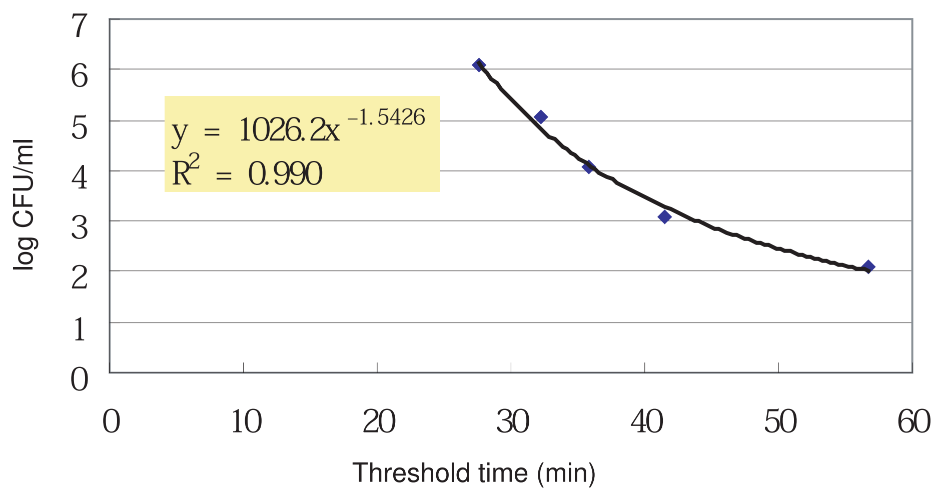

Fig. 1 **Calibration curve for quantification of *L. backi* BK2 using the HM6LB1LF1 primer set**