High-Throughput Isolation of Bacteriocin-Producing Lactic Acid Bacteria, with Potential Application in the Brewing Industry

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ABSTRACT

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Lactic acid bacteria (LAB) were isolated from malted cereals by means of a high-throughput screening approach and investigated for antimicrobial activity against a range of beer-spoiling bacteria. Putative bacteriocin-producing strains were identified by 16S rRNA analysis and the inhibitory compounds were partially characterized. Following determination of the inhibitory spectra of the strains, an unspeciated *Lactobacillus* sp. UCC128, with inhibitory activity against a range of beer-spoiling strains was subjected to further characterization. A bacteriocin was purified from this strain and analyzed by mass spectrometry to determine the weight of the protein. The result indicated that the bacteriocin was highly similar to pediocin AcH/PA-1 from *Pediococcus acidilactici.* The bacteriocin-producers identified in this study have the potential to be used in the brewing industry to enhance the microbiological stability of beer in conjunction with hurdles already in place in the brewing process.

Key words: Bacteriocin, beer-spoiler, lactic acid bacteria, high throughput screening.

INTRODUCTION

Lactic acid bacteria (LAB) are ubiquitous in nature and as a consequence are present as natural contaminants on a variety of foods. LAB are defined as Gram positive, catalase-negative, non-sporulating, aerotolerant, fermentative organisms that produce lactic acid as the major end-product of carbohydrate metabolism[1]. Genera belonging to the LAB family include *Lactococcus, Lactobacillus, Leuconostoc, Weissella* and *Pediococcus,* as well as *Streptococcus* and *Enterococcus.* They are responsible for the contribution of a variety of the organoleptic properties characteristic of fermented foods such as meat[14,26,42], vegetables[35,43] and dairy-products[23,27]. LAB are commonly exploited for the bio-preservation of various foods, feed and beverages, and this ability is attributed to the production of fermentation end-products such as lactic acid, diacetyl, acetaldehyde and hydrogen peroxide, which possess the ability to eliminate or retard the growth of many spoilage organisms[22]. In addition, some LAB exhibit potent antimicrobial activities in the form of small, heat-stable, ribosomally synthesized antimicrobial peptides called bacteriocins[36,38,39]. These have a bacteriocidal or bacteriostatic mode of action, generally inhibiting micro-organisms that are closely related to the producing strain. It is thought that the ability to produce these antimicrobial agents gives bacteria a competitive advantage over their non-producing counterparts. The bacteriocins produced by LAB have been classified into three categories according to size, mode of action and structural characteristics[7,9,10]. Class I, the lantibiotics, contain unusual amino acids, such as lanthionine and methyl-lanthionine, and are post-translationally modified[15,25]. Class II consist of non-lanthionine containing bacteriocins, which are ribosomally synthesized as prepeptides[30]. This latter class can be further sub-divided into three subclasses: Class IIa are the pediocin-like bacteriocins which have antilisterial activity, Class IIb are two-peptide bacteriocins, e.g. Lacticin F and Lactococcin G, while Class IIc include other peptide bacteriocins secreted by a sec-dependent mechanism[34]. The group formerly known as Class III bacteriocins is now referred to as non-bacteriocin lytic proteins, e.g. lysostaphin. Bacteriocinogenic LAB have previously been isolated from various environments, prompting serious interest in their potential as food and beverage preservatives. Bacteriocin-producing strains have been discovered in kimchi[6], sourdough[20], fish[41], meat[48], wine[9], dairy products[4] and malting and brewing environments[32,46].

A high-throughput screening study was undertaken to isolate and characterize interesting/novel antimicrobial-producing LAB from malted cereals directed against beer-spoiling bacteria, in particular those with bactericidal activity against *Lactobacillus casei* BSH1, which was isolated as a persistent contaminant of a commercial brewing facility.

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Table I. Isolation of putative bacteriocin-producing LAB from malted cereals.

Company	Source(s) of malt	No. of malt varieties screened	No. of colonies picked	No. of potential bacteriocin-producers
Brewery A	Scotland	6	2,976	0
Brewery B	England	5	1,920	0
Brewery C	Canada	2	3,840	0
Brewery D	Egypt	2	2,362	1
Brewery E	Ireland	1	436	1
Brewery F	Belgium/France/Czech Rep.	16	25,728	31
Distillery A	France/Luxembourg	9	11,484	5
Malt Co. A	Australia	25	3,840	0
Malt Co. B	England/Scotland	15	21,597	141
Malt Co. C	Ireland	4	3,610	0

MATERIALS AND METHODS

Bacterial cultures and strains

All bacterial strains used in this study were maintained as frozen stocks at –80°C in 1% DMSO and propagated once in broth for 16 h before use. LAB isolated from malt were cultivated at 30°C for 48 h on Bacteriocin Screening Medium (BSM)[44], which contains the same ingredients as MRS but is buffered at pH 6.5. The two indicator cultures, *Lb. sakei* LMG2313 and *Lb. casei* BSH1, were grown overnight in MRS broth (Oxoid, Basingstoke, Hampshire, UK) at 30°C. *Lb sakei* LMG2313 was used because it appears to be particularly sensitive to bacteriocins produced by LAB. *Lb. casei* BSH1 is a beer-spoiler, originally isolated as a contaminant from a commercial brewing facility. Solid media was prepared by the addition of 1.5% agar to the appropriate broth, or 0.75% agar in the case of overlay media. Anaerobic conditions were established using Anaerocult A gas packs (Merck). Suspensions of the malt samples and serial dilutions were prepared using sterile quarter strength Ringers solution (Merck). Pronase and catalase (Sigma Chemical Company) enzyme solutions were used at a final concentration of 10 mg mL^{-1} in distilled water and 20 mM sodium phosphate buffer (pH 7), respectively.

High-throughput isolation of inhibitor-producing LAB

Breweries and malting companies in various countries from different continents (see Table I) were invited to donate malt samples. LAB cultures were isolated by aseptically adding 5 g of the test cereal into 50 mL of sterile quarter strength Ringers solution followed by incubation at 30°C for 4 h with constant agitation. Serial dilutions of the samples were prepared in Ringers solution and 750 µL of appropriate dilutions were spread plated using glass beads on Q-trays (Genetix, Hampshire, UK) containing solid BSM with added cycloheximide (Oxoid, Basingstoke, Hampshire, UK) at a final concentration of 100 µg mL^{-1} to inhibit fungal growth and assist in the selection of LAB.

For the purpose of this screening effort, the MegaPix2 robot (Genetix, Hampshire, UK) was used to mechanically pick assumed LAB colonies from the Q-trays and transfer them to wells of 96-well plates (Genetix), which contained 100 µL of BSM broth. The microtitre plates were incubated anaerobically overnight at 30°C. Following this incubation, cultures were replicated robotically and in duplicate onto BSM agar contained in Q-trays. The replicate plates were incubated overnight at 30°C. Following this the colonies were overlaid with either of two indicators, the beer-spoiling strain *Lb. casei* BSH1 or the bacteriocin-sensitive strain, *Lb. sakei* 2313, and incubated anaerobically overnight at 30°C. Colonies shown to inhibit either or both of the indicator strains were traced back to the malt source and company from which they were isolated and recorded for future reference. The potential inhibitors were removed from their corresponding well on the 96-well plate, inoculated into BSM and grown overnight at 30°C. Following this, the cultures were restocked in glycerol and stored at –20°C.

Characterisation of antimicrobial activity

Once restocked at –20°C, the potential bacteriocin-producers were re-tested for inhibitory activity against the indicators using the agar spot test[24]. The inhibitory agents produced by LAB cultures were tested for sensitivity to a proteolytic enzyme or to catalase. Cell-free supernatant (CFS) was prepared from overnight cultures, which were centrifuged at 12,000 rpm for 6 min. The supernatant was transferred to clean eppendorf tubes and heated to 95°C for 5 min to eliminate any remaining cells. Following this, the CFS of each culture was tested against the two indicators strains by the agar spot assay once again.

Detection and verification of nisin-producing LAB

In order to detect the presence of the *nisA* gene in the bacteriocin-producing bacteria, a polymerase chain reaction (PCR)-based test was performed using the primers, NisAF (5′-AATAAATTATAAGGAGGCACTCAA-3′) and NisAR (5′-ACAAAATACTATCCTTTGATTTGG-3′) which were based on the sequence of the nisin structural gene, *nisA* (Accession No: AY526091). Conditions for PCR were as follows: 30 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 54°C and extension for 30 sec at 72°C, followed by a final elongation step at 72°C for 8 min. PCR products were separated by electrophoresis on a 2% (w/v) agarose gel. *Lactococcus lactis* 8614, a nisin-producing strain was used as a positive control and *Lactococcus lactis* LMG1363, a non-nisin producing strain acted as the negative control.

To verify PCR results, cross-sensitivity tests were performed with the nisin-producing culture, *L. lactis* 8614. Each test culture was spotted on BSM agar and overlaid with *L. lactis* 8614 and vice versa. The plates were incubated at 30°C anaerobically overnight and examined for zones of inhibition the following day

Species identification of inhibitor-producing LAB

Bacteriocin-producing isolates were speciated using 16S rRNA analysis. Genomic DNA was extracted from the potential bacteriocin-producers and used as a template for amplification for approximately 1.4 kb of the 16S rRNA gene by PCR as described by Corsetti et al.[8] The PCR products were subjected to sequence analysis and the obtained sequences were used to search for homologous sequences in the non-redundant DNA sequence database present at the NCBI website located at http://www.ncbi.nlm.nih.gov/BLAST.

Inhibitory spectrum

To determine the inhibitory spectrum of some of the antibacterial-producing LAB, cultures were tested for activity against a range of beer-spoiling LAB, using the agar spot test.

Bacteriocin purification and characterisation of the purified peptide

The inhibitor-producing strain *Lactobacillus* sp. UCC128 was grown for 16 h in 1 litre of BSM broth at 30°C, from a 1% inoculum. The culture was centrifuged for 10 min at 8,000 g to remove all cells. The inhibitory activities were precipitated from the CFS with the addition of ammonium sulphate (40% saturation). The precipitated proteins were retrieved through centrifugation at 8,000 g for 30 min and resuspended in 20 mM sodium phosphate buffer (pH 6). This suspension was fractionated using three successive chromatography steps: cation exchange, hydrophobic interaction and reversed phase chromatography[31]. Reversed phase chromatography was performed using a Beckman Ultrasphere ODS (5 µm, 4.6 mm × 25 mm) C_{18} column on a HPLC system (Beckman). Bacteriocin was eluted with an increasing linear gradient (10 to 100%) of acetonitrile in 0.1% aqueous trifluoroacetic acid solution. Absorption was measured at 280 nm to detect protein peaks and 1 mL fractions were collected and assayed for bacteriocin activity against the indicator *Lb. sakei* 2313. The active fraction was lyophilised and sent for mass spectrometry, which was performed with a PE Sciex API 1 electrospray mass spectrometer and a MALDI-TOF (Voyager-DE™ RP, Perspective Biosystems, CA, USA).

Identification of the UCC128 bacteriocin

Primers Pap1F (5′-GAAGAAGGAGATTTTTGTG-AT-3′) and Pap1R (5′-CTAGCATTTATGATTACCTTG-ATG-3′) were designed to detect the pediocin AcH/PA-1 structural gene (204 bp) (Accession no: U02482) and were used for PCR with chromosomal DNA isolated from *Lb.* sp. UCC128 as a template. Chromosomal DNA from *Pediococcus acidilactici* was used as a positive control, while DNA preparations from *Lb. curvatus, L. lactis, Lb. plantarum* and *Lb. mali* acted as negative controls. The PCR products were subjected to sequence analysis and the obtained sequences were used to search for homologous sequences in the non-redundant DNA sequence database present at the NCBI website located at http://www.ncbi.nlm.nih.gov/BLAST. Primers Pap2F (5′-GAGTGGGAA-CTAGAATAAGCGCG-3′) and Pap2R (5′-GAATTAAC-CGTGCATAATACCC-3′) were designed to detect the pediocin gene cluster, *papABCD* (3431 bp) (Accession no: U02482) and PCR was performed with DNA from the same strains and controls as outlined above.

RESULTS

Isolation of bacteriocin-producing LAB

LAB were isolated using the agar spot assay, on the basis of their ability to inhibit either *Lb. sakei* 2313 or *Lb. casei* BSH1. Some of the cereals could not be tested due to excessive fungal growth, while other samples appeared to be devoid of LAB. In total, 77,793 presumed LAB isolates were screened for antimicrobial activity. Table I illustrates the number of different malt varieties investigated in this study and the locations from which they were sourced. It also represents the number of colonies isolated and the number of bacteriocin-producers uncovered. In total, 179 potential bacteriocin-producing isolates were found (0.23%) in this study. All of these were active against the bacteriocin-sensitive strain *Lb. sakei* 2313, while only 29 of these had the ability to inhibit the beer-spoiler *Lb. casei* BSH1. No isolates were found to exclusively inhibit *Lb. casei* BSH1.

Characterisation of antimicrobial agents

The 179 inhibitor-producing LAB were re-assayed for antimicrobial activity and divided into two groups: the 29 strains with activity against *Lb. casei* BSH1 (and *Lb. sakei* 2313) and the remaining 150 strains with activity solely against *Lb. sakei* 2313. Of the *Lb. casei* BSH1 inhibitors, only 10 isolates were shown to produce the antimicrobial compound(s) upon re-testing. In the case of the *Lb. sakei* 2313 inhibitors, 41 of the 150 inhibitor-producing strains showed reproducible inhibition of the indicator. Of the 10 remaining *Lb. casei* BSH1 inhibitors, eight were shown to release the inhibitory agents into the CFS, while of the 41 LAB with inhibitory activity against *Lb. sakei* 2313, only 10 strains were capable of producing the compounds into the CFS under the conditions used.

The antimicrobial compounds were tested for their sensitivity to catalase and pronase. The inhibitory activity produced by the 10 *Lb. casei* BSH1 inhibitors and the remaining 10 *Lb. sakei* 2313 inhibitors was not affected by the addition of catalase indicating that the inhibition was not due to the production of hydrogen peroxide. In contrast, all inhibitory compounds produced by the 20 LAB strains were confirmed to be proteinaceous in nature as they were all shown to be sensitive to pronase. Taken together these results show that these inhibitory activities possess typical characteristics of bacteriocins (as will be assumed for the remainder of the text).

Detection and verification of nisin-producing LAB

The remaining 20 bacteriocin-producing strains were subjected to PCR with primers designed specifically to detect the presence of the nisin structural gene, *nisA*.

Table II. Identity of non-nisin bacteriocin-producing LAB.

Origin	Indicator	Identity
Scotland/England	*Lb. casei* BSH1	*Lc. pseudomesenteroides* UCC1
Scotland/England	*Lb. casei* BSH1	*Lc. pseudomesenteroides* UCC6
Scotland/England	*Lb. sake* 2313	*Weissella cibaria* UCC32
Scotland/England	*Lb. sake* 2313	*Lc. pseudomesenteroides* UCC66
Scotland/England	*Lb. sake* 2313	*Lc. pseudomesenteroides* UCC69
France/Belgium/Czech Rep	*Lb. sake* 2313	*Pediococcus acidilactici* UCC119
France/Belgium/Czech Rep	*Lb. sake* 2313	*Enterococcus faecalis* UCC122
France/Belgium/Czech Rep	*Lb. sake* 2313	*P. pentosaceus* UCC124
France/Belgium/Czech Rep	*Lb. sake* 2313	*Lactobacillus* sp. UCC125
France/Belgium/Czech Rep	*Lb. sake* 2313	*Lactobacillus* sp. UCC126
France/Belgium/Czech Rep	*Lb. sake* 2313	*Lactobacillus* sp. UCC127
France/Belgium/Czech Rep	*Lb. sake* 2313	*Lactobacillus* sp. UCC128

Eight of the 10 isolates active against *Lb. casei* BSH1 were positive for *nisA*. Cross-sensitivity tests were performed against a nisin-producing culture, *L. lactis* 8614. No inhibition of the eight test strains was seen by *L. lactis* 8614 or vice versa. This indicates that the eight bacteriocin producers are all insensitive to nisin, which is consistent with the assumption that these strains were nisin-producing LAB. The two strains which were negative for *nisA* but which were active against *Lb. casei* BSH1 had previously been identified as strains of *Leuconostoc pseudomesenteroides.* These results indicate that their inhibitory activity was not as a result of nisin production, but of another bacteriocin.

Of the 10 bacteriocin-producers active against *Lb. sakei* 2313 (and producing this activity in the CFS), none were observed to possess *nisA,* indicating that these LAB were producing bacteriocins other than nisin.

Species identification of bacteriocin-producing LAB

Table II lists the derived identities of 12 "non-nisin" bacteriocin producers, only two of whom are active against *Lb. casei* BSH1. The eight *nisA*-containing isolates that had originally been isolated as inhibitors of *Lb. casei* BSH1 were also speciated by 16S rRNA sequencing and were all shown to be strains of *L. lactis* (results not shown).

The sequencing results identified some of the antimicrobial LAB as species that have previously been associated with bacteriocin-production, i.e. *Enterococcus faecium*[3], *Pediococcus acidilactici*[5] and *Pediococcus pentosaceus*[45]. However, the remainder were identified as strains, which to our knowledge, have not been associated with bacteriocin-production. Strains of *Leuconostoc pseudomesenteroides* with bactericidal activity were isolated in this study. Unfortunately, these strains failed to produce bacteriocin into the CFS, unlike the other strains isolated.

The 16S rRNA sequencing results showed that the four bacteriocin-producing strains named as *Lactobacillus* sp. UCC125-128 (Accession no: EU074850), isolated in this study shared 98% sequence similarity with an un-speciated *Lactobacillus* sp. 88[37]. They were also found to have 97% sequence similarity with *Lactobacillus mali*[2]. This would indicate that these bacteriocin-producing strains represent a new species in the genus of *Lactobacillus.*

Inhibitory spectrum

The inhibitory spectra of a subsection of the bacteriocin-producing LAB were investigated by testing against

Table III. Inhibitory spectrum of bacteriocin-producing LAB.

Beer-spoilers	UCC1	UCC12†	UCC32	UCC66	UCC119	UCC122	UCC124	UCC128
Enterococcus faecalis 109	++	+++	+	++	++	+	++	+
Lactobacillus collinoides 110	–	+++	–	–	–	–	+	++
Lactobacillus brevis 111	–	++	–	++	–	+	+	+
Lactobacillus brevis 112	–	++	–	–	–	–	–	+
Pediococcus damnosus 113	+	++	–	–	–	–	–	+
Pediococcus inopinatus 1011	+	+++	–	+	++	+	++	++
Lactobacillus casei BSH1	+	++	–	–	–	–	–	–
Lactobacillus brevis BSH2	–	++	–	–	–	–	–	–
Lactobacillus brevis BSH4	++	++	–	++	–	–	–	–
Lactobacillus brevis BSH5	–	++	–	–	–	–	–	–
Lactobacillus brevis BSH7	++	+++	++	++	–	–	–	–
Lactobacillus brevis BSH 9	++	++	++	++	–	–	–	–
Lactobacillus brevis BSH11	+	++	–	+	–	–	–	–
Lactobacillus brevis RAP51	–	++	+	–	–	–	–	+
Lactobacillus brevis RAP77	++	++	++	–	–	–	–	+
Lactobacillus brevis RAP42	+	++	+	–	–	–	–	–
Lactobacillus brevis MB95	++	+++	++	–	–	–	+	+
Lactobacillus brevis MB94	–	+++	–	++	–	–	+	+

*+++ >10 mm zone of inhibition
++ 6–10 mm zone of inhibition
+ 1–5 mm zone of inhibition
† UCC12-nisin-producing *L. lactis*

Table IV. Purification stages of the UCC128 bacteriocin.

Step	Volume (mL)	AU/mL	AU	% Recovery	Purification Factor
CFS	1000	1,600	1,600,000	100	1
AS ppt	200	1,600	320,000	20	1
Sepharose	50	1,600	80,000	5	1
Octyl	10	6,400	64,000	4	4
HPLC	1	12,800	12,800	0.8	8

a wide range of Gram positive beer-spoiling LAB. The range of indicators and their sensitivity to the producing cultures is shown in Table III.

Purification and identification of the antimicrobial peptide produced by *Lactobacillus* sp. UCC128

The inhibitory activities of *Lactobacillus* sp. UCC128 were purified to homogeneity from the CFS using three consecutive chromatography steps. Following partial purification by ion-exchange, the protein was subjected to a final purification treatment using HPLC (Table IV). Mass spectrometry determined the molecular mass of the UCC128 bacteriocin to be 4614 Da which is very similar to that of pediocin AcH (4628 Da) produced by *Pediococcus acidilactici* H[28].

PCR analysis confirmed that *Lactobacillus* sp. UCC128 contained the pediocin structural gene, as did a positive control represented by *Pediococcus acidilactici.* In contrast, the negative control strains *L. lactis, Lb. curvatus, Lb. plantarum* and *Lb. mali* failed to produce an amplification product (data not shown). Sequencing of the PCR products showed that the *papA* gene from *Lb.* sp. UCC128 had an identical nucleotide sequence to that of the original *papA* gene from *P. acidilactici.* This leads to the conclusion that *Lb.* sp. UCC128 is indeed producing pediocin AcH/PA-1. An amplification product was also obtained when primers were used that had been designed to detect the entire pediocin gene cluster, *papABCD,* in contrast to the negative control.

DISCUSSION

The objective of this study was to isolate bacteriocin-producing LAB from the brewing environment, specifically those with bactericidal activity against beer-spoilage bacteria. This work represents one of the most elaborate screening projects undertaken in the quest for bacteriocin-producing LAB to date, with respect to the diversity and number of samples investigated, as well as the number of LAB tested for bactericidal activity. Other studies have been undertaken using malted and raw cereals to discover LAB with antimicrobial activity[16,32,46]. However, this study was performed on a much larger scale, to increase the probability of discovering a wide variety of malt-derived bacteriocin-producing LAB. In order to improve the chances of isolating novel/interesting bacteriocin-producing LAB, the cereals for this study were sourced from vastly different locations. From 85 malt samples obtained from 11 commercial malting companies and brewing facilities, 77,793 LAB colonies were picked, replicated and tested for bactericidal activity against the beer-spoiling strain *Lb. casei* BSH1 and the bacteriocin-sensitive strain, *Lb. sakei* 2313. From an initial count of 179 potential bacteriocin-producers, only 51 of these LAB cultures retained inhibitory activity when re-tested. It is not unusual for LAB cultures to switch off their bacteriocin producing mechanisms. Bacteriocin biosynthesis is often regulated by quorum sensing[11,18] and it is has been shown that some bacteriocins have a cell density-dependent on-off mechanism[21]. It has been suggested that unfavourable growth conditions stimulate bacteriocin production. Factors such as pH and temperature, as well as the composition of the growth medium have an effect on the ability of a culture to produce bacteriocins[17].

Following preliminary characterisation of inihibitor-producing strains, only 12 LAB isolates were taken for further investigation. These remaining 12 strains were identified to be several different species of LAB, including known bacteriocin-producers *P. acidilactici* and *E. faecium.* Strains of *Lc. pseudomesenteroides* were also isolated in this study, which had not been the case in previous screening projects from cereals[16,32] and, to the best of our knowledge, this species has not previously been associated with bacteriocin-production.

Four bacteriocinogenic isolates belonging to an unknown species of *Lactobacillus* were obtained from the same malt sample. These four unspeciated isolates appear to be identical and therefore, only one, *Lactobacillus* sp. UCC128, was chosen for further characterisation. The bacteriocin produced by the isolate UCC128 was shown to be heat stable and was shown to be more active in the acidic pH range (Results not shown).

The bacteriocin produced by *Lactobacillus* sp. UCC128 was observed to have a narrow inhibitory spectrum, but was still capable of inhibiting several beer-spoiling LAB including heterofermentative species of *Pediococcus* and *Lactobacillus,* which together account for up to 70% of beer-spoilage incidents in breweries[40]. The other important feature of this bacteriocin is that it exhibits anti-listerial activity, indicating that it belongs to the Class IIa, pediocin-like group of bacteriocins[12].

Mass spectrometry results showed the UCC128 bacteriocin to be very similar in size to pediocin AcH (also known as pediocin PA-1) produced by *Pediococcus acidilactici* H[28]. Our bacteriocin-producing isolate *Lactobacillus* sp. UCC128 was also found to possess *papA,* the pediocin structural gene, which had an identical nucleotide sequence to that of pediocin AcH/PA-1. *Lb. sp.* UCC128 also possessed the entire pediocin gene cluster, *papABCD.* Collectively, these results are convincing evidence to prove that this isolate produces pediocin AcH.

The fact that we profess to have a strain of *Lactobacillus* producing a bacteriocin characteristic of the genera

Pediococcus is unusual, but not without precedent. A strain of *Lactobacillus plantarum* producing pediocin AcH was previously isolated from Munster cheese[13].

We propose that *Lactobacillus* sp. UCC128, as well as the other bacteriocin-producing LAB isolated in this study, could be applied to enhance the microbiological stability of the brewing process and beer. They have antimicrobial activity against several beer-spoiling bacteria and have the potential to be used, singly or in combination, for wort acidification to reduce the pH of wort, while conferring an added preservative effect through bacteriocin production. The potential also exists to use these bacteriocin-producing isolates as starters in the malting process as described by Laitila et al.[19] Purified bacteriocins have the potential to be used in conjunction with other hurdles, i.e. pasteurisation and filtration, and added to finished beer[47].

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