Written by: Yang Linxi, Wang Qian

ANALYSIS OF BEER SPOILAGE BACTERIA IN COMMERCIAL AND HOME-MADE DRAUGHT BEER

Wang Qian[1], Yang Linxi[1], Mark Richards[2], Patrick Ng[2]
[1]Nanyang Girls' High School, 2 Linden Drive, Singapore 288683
[2]School of Chemical & Life Sciences, Nanyang Polytechnic,
180 Ang Mo Kio Avenue 8, Singapore 569830

Written by: Yang Linxi, Wang Qian

SUMMARY

Draught beer has become a more popular choice over canned, pasteurized beer among people nowadays because it is truer to the flavours of the ingredients. However, it is also more fragile and susceptible to spoilage compared to canned, pasteurized beer. Thus, this study aims to use a series of molecular biology techniques to identify and analyze various bacteria species that survive and breed in beer, so as to subsequently devise effective ways to prevent the growth of beer spoilage bacteria in draught beer thus generating constructive suggestions on ways to improve the sterilizing system in specific breweries.

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ABSTRACT

Draught beer refers to unpasteurized beer that can be drunk off the keg. It has become a more popular choice over canned, pasteurized beer among people nowadays because it is truer to the flavours of the ingredients [1] . However, as draught beer is almost always unpasteurized, it is more fragile and susceptible to spoilage compared to canned, pasteurized beer. Thus, the goal of this research was to identify beer spoilage bacteria found in commercial (off-the-shelf) and home-brewed draught beer using a series of molecular identification methods. Our findings suggest that the most ubiquitous bacteria in commercial draught beer is *Rhodospirillales,* while *Sphingomonadale, Lactobacillales* and *Burkholderiales* are three predominant bacteria in home-made draught beer. According to past studies, *Lactobacillus* and *Acetobacter* are some of the common beer spoilage bacteria found in beer. The other bacteria types detected in this study could probably be due to other external factors such as the cleanliness of the water used when washing the brewing container. Extending results, the next challenge would be to devise methods to inhibit the growth of beer spoilage bacteria in beer, while at the same time not compromising the authentic taste of beer.

INTRODUCTION

Beer has been recognized as a microbiologically stable medium due to the presence of alcohol (0.5-10% w/w), the high content of carbon dioxide (approx. 0.5% ppm), the low pH (3.8-4.7), and the extremely reduced content of oxygen (<0.1 ppm). However, in spite of these biologically unfavourable conditions, a few micro-organisms, mainly bacteria, still manage to grow in beer. These beer spoilage bacteria often result in an increase in turbidity of the beer, high acidity, and unpleasant sensory changes in terms of smell and taste of beer. Needless to say, these changes can affect negatively the quality of the final product [2].

Some people resort to making their own beer instead, as they feel that home-made beer would be fresher, cleaner and tastier since home-brewing allows individuals to experiment with flavours not available to the mass market consumer. They assume that since they can take control of their own beer and manage how it is made, the beer produced would be able to last for a longer period of time even at room temperature.

In this study, a series of molecular identification techniques such as Polymerase Chain Reaction (PCR), Gene Cloning and Plasmid DNA Purification were employed before sending the samples for DNA sequencing to identify the bacteria present in commercial and home-brewed draught beer. 16S rDNA sequencing was used in this study as this is a standard approach for molecular taxonomy involving bacteria. The 16S rDNA gene is highly conserved between different species of bacteria, with localized variations that enable phylogenetic classification. [4]

METHODS

Genomic DNA Purification

After obtaining the spoiled beer samples, genomic DNA purification was carried out as a means to harvest purified bacteria DNA needed for the experiment. For the purification, we centrifuged each 10ml beer sample for 5min at 4,000rpm (g) and 4°C, then washed the pellet with PBS. The washed pellet was then lysed using lysis buffer (10nM Tris- HCl pH8, 50mM KCl, 1mM EDTA pH8, 0.5% Tween 20) and Proteinase K solution (0.2mg/ml final concentration) followed by incubation at 60°C for 1.5 hours to facilitate digestion of unwanted proteins. It was then subjected to vortexing with glass beads to further enhance lysis. This was then followed by heating at 94°C for 15 minutes and a Phenol-Chloroform Extraction to eliminate Proteinase K, followed by Ethanol Precipitation to obtain pure genomic DNA.

- *Phenol-Chloroform Extraction*

The purpose of this extraction is to remove the organic impurities present in the samples. The beer samples were mixed with a same volume of phenol-chloroform solution and vortexed till a uniform emulsion was formed. It was then subjected to centrifugation at high speed (13 000 rpm) to separate the organic and aqueous layers. The aqueous layer was then harvested for ethanol precipitation.

- *Ethanol Precipitation*

This is the final step of the genomic DNA purification. To the bacteria sample from the previous step, 0.1 volume of 3M Sodium Acetate pH5.2 solution, 3ul of Glycoblue precipitant (Ambion) and 2 volumes of absolute ethanol solution were added before the mixture was subjected to centrifugation at 12 000rpm.The supernatant was then decanted and the mixture was washed

with 75% ethanol solution before air drying the DNA pellet formed. After this, the pellet was re-suspended in 50μl TE buffer.

Polymerase Chain Reaction with LAC and Universal Primers

The reagents required to prepare the samples were 10μl of 5x PCR Buffer, 3μl of 25mM $MgCl_2$ (1.5mM final concentration), 1μl of 10mM dNTP (0.2mM final dNTP concentration), Forward primer and Reverse Primer 1μl each (0.2mM final primer concentration), 31.75μl of distilled water and 0.25μl of Taq polymerase (Promega; 5U/ul). Lastly, 2μl of the respective DNA was added to each tube before the PCR reaction was carried out.

There were 2 sets of primers used for this experiment. The LAC primers were designed to preferentially pick up *Lactobacillus* bacteria of different species and the Universal primers were used to pick up a spectrum of different bacteria species. PCR using LAC primers (LAC1, 5'- TCCGGATTTATTGGGCGTAAAGCGA -3’ and LAC2, 5'- TCGAATTAAACCACATGCTCCA -3') give amplicons of approximately 400bp. The universal primers (UNIV_1, 5'- GTCGTCAGCTCGTGTCGTGAGA -3' and UNIV_2, 5'- CCCGGGAACGTATTCACCGCG -3') give a product size of approximately 300bp .

The reaction starts by holding the sample at 94°C for 2 minutes to activate the enzyme, followed by repeated cycles of denaturation, primer annealing, and extension. Firstly, the sample was subjected to denaturation at 94°C for 30 seconds and primer annealing at 50°C for another 30 seconds. Extension at 72°C for 1 minute was then carried out to complete the cycle. This cycle was repeated 35 times before final extension at 72°C for 10 minutes. It was then held at 16°C till the agarose gel electrophoresis.

After the completion of the PCR, the completed PCR products are visualized using agarose gel electrophoresis.

- *Agarose Gel Electrophoresis*

This is a process which makes use of electricity to separate DNA fragments by size as they migrate through a gel matrix. [5] The PCR products were loaded on a self-cast gel (Labnet) which consists of 2% agarose with Ethidium Bromide (0.5ug/ml final concentration) added and left to

run at 110V for approximately 40 minutes before they were viewed under the ultraviolet transilluminator (Syngene).

Gene Cloning

Since the primers were designed to amplify a conserved portion of the rDNA gene common to all bacteria (or yeast), a single band shown through the gel electrophoresis may encompass more than one type of microbial strain. Thus, gene cloning aims to facilitate the picking out of desired beer spoilage bacteria species for further identification.

Gene cloning consists of 3 main steps- DNA Ligation, Transformation and Bacteria Solid Phase Culture. It is done using the pGEM-T Easy Vector System (Promega).

The plasmid vector DNA in this cloning system was prepared by Promega by cutting the pGEM®-5Zf(+)with EcoRV and adding a 3´terminal thymidine to both ends. These single 3´-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product (containing 3'-A overhangs) into the plasmids by preventing re-circularization of the vector and providing a compatible overhang for PCR products generated by certain thermo-stable polymerases (1,2). During ligation, the completed PCR products were inserted into the vectors in the presence of T4 DNA Ligase. After this, the vectors now containing the inserted PCR product, will be inserted into E-coli competent cells through heat shock via a process called transformation. During the Bacteria solid phase culture, these cells containing the PCR products of bacterial DNA will then be plated on Luria Bertani (LB) agar plates which contain ampicillin and be allowed to grow. After this, 10 random colonies from the agar plate would be streaked for analysis using PCR to affirm the presence of clones.

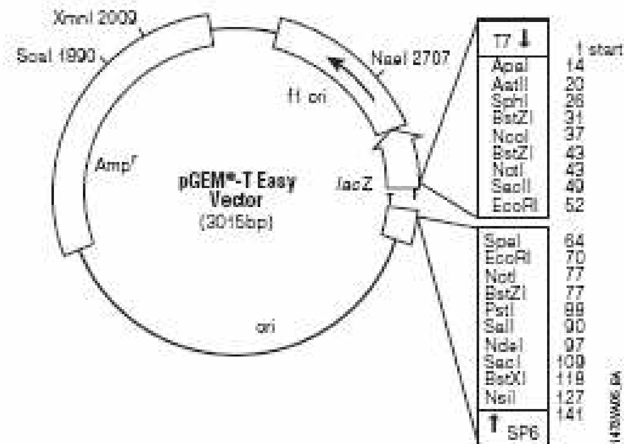

Figure 1: pGEM-T Easy Vector Map

Plasmid Preparation

Plasmid preparation consists of 2 main steps, liquid phase culture and plasmid DNA purification. Liquid phase culture was carried out for the bacteria samples by streaking a colony from each of the agar plates onto a fresh master plate using a sterilized tip, followed by inoculating into 5ml of LB media for overnight shaking. The remaining of the bacteria left on the sterilized tip would then be used to carry out PCR. The samples that show a clear, distinct band after the PCR would then be subjected to plasmid DNA purification using a commercial kit (Qiagen) before being sent for sequencing.

DNA Sequencing

The 16S rDNA PCR products were sent to AIT Biotech Pte Ltd for sequencing results of the first 500 bp of the samples, after which they were compared to the database of all known 16s rDNA DNA sequences in the public domain by BLAST function in the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast) for the identities of the bacteria present in beer.

RESULTS

Gel electrophoresis results for rDNA PCR

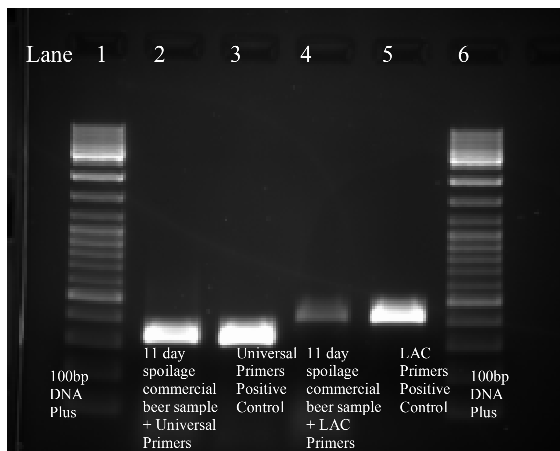

Figure 2: Gel Electrophoresis result of Commercial Draught Beer with Universal and LAC Primers

Figure 3: Gel Electrophoresis result of Home-Brewed Draught Beer with Universal Primers

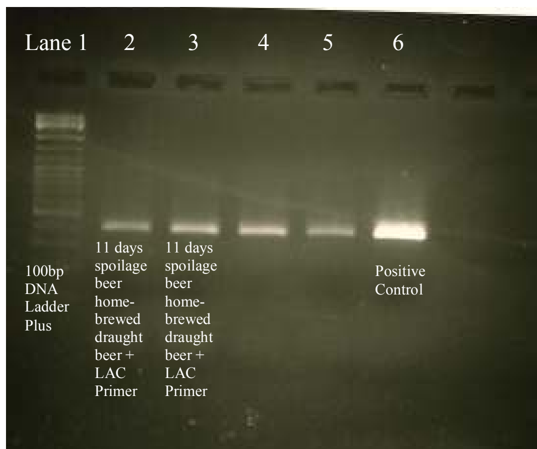

Figure 4: Gel Electrophoresis of Home-Brewed Draught Beer with LAC Primers

From the above figures 2, 3 and 4, clear distinct bands are present, indicating the success of the PCR as desired bands were picked out respectively with the use of the Universal and LAC Primers. However, at this stage it is still not possible to determine the individual bacteria species present in the beer from the above test. After carrying this step of confirmation, gene cloning would then be carried out to further isolate bacterial colonies of bacteria growing in the beer that causes beer spoilage.

Cloning of 16s rDNA from putative beer spoilage bacteria

As aforementioned, what appeared to be a single PCR band could actually comprise rDNA from a mixture of different bacterial species. In order to identify each individual species of beer spoilage bacteria, it is necessary to clone and characterize each DNA molecule. After which, it is necessary to carry out bacteria culture of the cloned genes so as to pick out desired colonies for further screening.

- **Commercial Draught Beer Sample**

Figure 5: Bacteria Culture of cloned genes (50µl, with LAC Primers)

Figure 6: Bacteria Culture of cloned genes (100µl, with LAC Primers)

Figure 7: Bacteria Culture of cloned genes (50µl, with UNIV Primers)

Figure 8: Bacteria Culture of cloned genes (100µl, with UNIV Primers)

Figures 5, 6, 7 and 8 show the bacteria culture of different amounts of the cloned genes on agar plates with added ampicillin as labeled above.

Ten random individual bacterial colonies were then picked using a sterile tip and incubated on a master plate, as shown in Figures 9 and 10. The remains of the colonies left on the sterile tips were then used for screening by PCR, prior to DNA sequencing.

Figure 9: Master Plate (LAC Primers)

Figure 10: Master Plate (UNIV Primers)

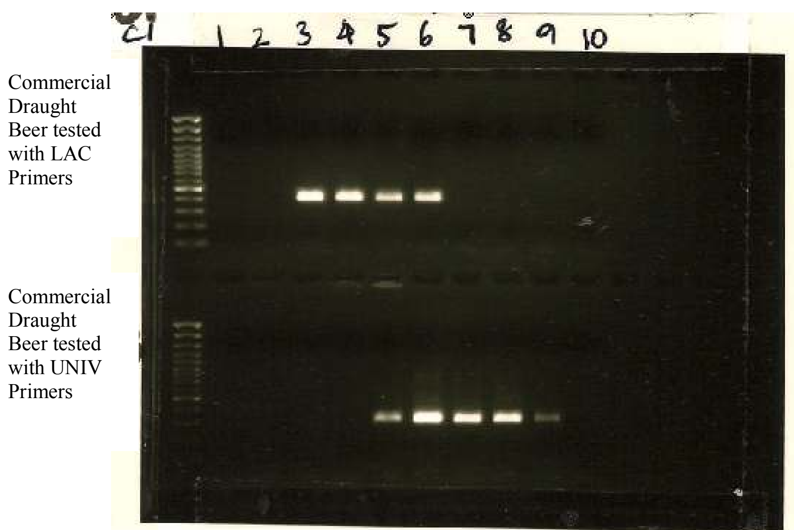

Figure 11: Gel electrophoresis result of the bacteria culture of the gene cloned commercial draught beer spoilage bacteria sample

Figure 11 shows the gel electrophoresis result of PCR screening of individual bacterial colonies transformed with cloned 16s rDNA. The labels 1 to 10 represent 10 random colonies picked from the original agar plate where the bacteria clones were incubated. From the results, it can be concluded that the gene cloning was relatively well done as desired beer spoilage bacteria clones were present in about 4/10 samples (LAC) and 5/10 samples (UNIV).

- **Home- Brewed Draught Beer**

Figure 12: Bacteria Clones on Home Brewed beer sample (1 carbonation drop, 100μl, LAC Primer)

Figure 13: Bacteria Clones on Home Brewed beer sample (2 carbonation drops, 100μl, LAC Primer)

Figure 14: Bacteria Clones on Home Brewed beer sample (1 carbonation drop, 100μl, UNIV Primer)

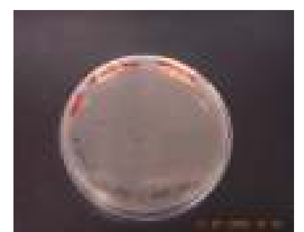

Figure 15: Bacteria Clones on Home Brewed beer sample (2 carbonation drop, 100μl, UNIV Primer)

Figures 12, 13, 14 and 15 show the bacteria culture of the cloned genes of home-brewed beer spoilage bacteria on agar plates with added ampicillin as labeled above.

Figure 16: Master Plate LAC Primer)

Figure 17: Master Plate (UNIV Primer)

Similarly, ten random individual bacterial colonies were then picked using a sterile tip and incubated on a master plate, as shown in Figures 16 and 17. The remains of the colonies left on the sterile tips were then used for screening by PCR, prior to DNA sequencing.

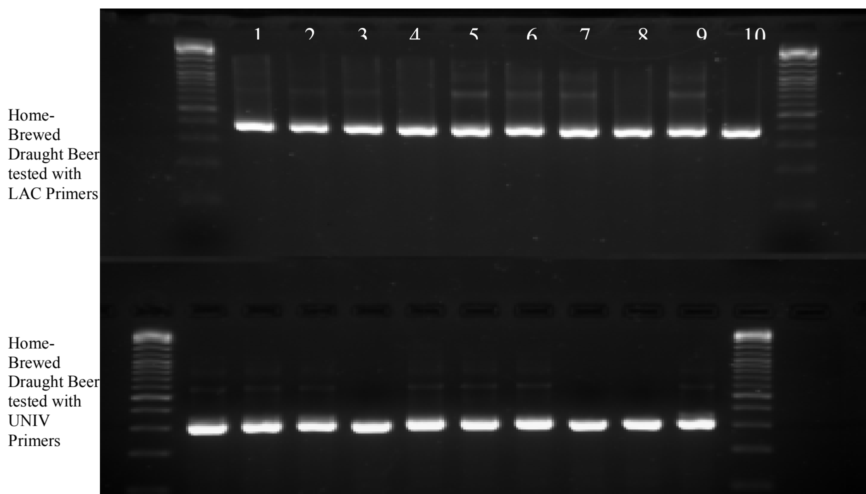

Figure 18: Gel electrophoresis result of the bacteria culture of the gene cloned home-brewed draught beer spoilage bacteria sample.

Figure 18 shows the gel electrophoresis result of PCR screening of individual bacterial colonies transformed with cloned 16s rDNA. The labels 1 to 10 represent 10 random colonies picked from the original agar plate where the bacteria clones were incubated. From the above gel electrophoresis result, it can be concluded that the gene cloning was well done as desired beer spoilage bacteria clones were present in all samples for both LAC and UNIV Primers.

Identification of Beer Spoilage Bacteria in Beer

After sequencing, not all samples produced identified sequences as seen from the many 'N's present. Thus, the following results show bacteria samples with readable sequences, meaning no 'N's found in the sequence.

Sample	Sample Name	Closest Match	Match
Commercial Beer	**C1A6**	*Acetobacter orientalis*	98%

	C1B5	*Acetobacter orientalis*	99%
		Acetobacter pasteurianus	
	C1B8	*Acetobacter orientalis*	99%
		Acetobacter pasteurianus	
Home-Brewed Draught Beer	**HA2**	*Sphingomonas sp.*	99%
		Novosphingobium sp.	
	HA3	*Weissella cibaria*	98%
		Weissella sp.	
		Weissella confusa	
		Weissella kimchii	
		Weissella salipiscis	
		Lactobacillus fermentum	
	HA5	*Sphingomonas sp. PT-4*	99%
		Sphingomonas melonis	
		Sphingomonas sp. dv1	
		Sphingomonas sp. TY	
		Sphingomonas aquatilis	
		Alpha proteobacterium ANRS4-3	
	HA6	*Acinetobacter sp.*	99%
	HA7	*Sphingomonas sp.*	99%
		Sphingomonas echinoides	
		Sphingomonas oligophenolica	
	HA9	*Sphingomonas sp.*	99%
		Sphingomonas oligophenolica	
		Sphingomonas echinoides	
		Alpha proteobacterium	
	HB1	*Sphaerotilus sp. HS*	99%
		Burkholderiales bacterium	
		Aquabacterium	
	HB2	*Sphingomonas sp.*	99%
		Novosphingobium sp.	
	HB3	*Sphaerotilus sp. HS*	99%
		Ideonella sp. K5.1	
		Burkholderiales bacterium	
		Aquabacterium	
	HB5	*Weissella cibaria*	99%
		Weissella sp. PSMS4-4	
		Weissella kimchii	
	HB6	*Weissella cibaria*	99%
		Weissella sp.	
		Weissella kimchii	
	HB7	*Weissella sp.*	99%
		Weissella confusa	
		Lactobacillus confusus	
	HB9	*Sphaerotilus sp.*	98%
		Ideonella sp.	
		Burkholderiales bacterium	
		Aquabacterium	

Table 1: Summary of identification results of beer spoilage bacteria in beer samples

Table 1 shows the matching percentages of the sequencing results obtained for the bacteria present in each of the beer sample by performing the BLAST search. All the matches have the matching percentage of above 98%.

Table AA1 (Annex A) shows the detailed classification of the bacteria present in each of the beer sample. Most of them belong to the *Sphingomonadale, Lactobacillales, Burkholderiales* and *Rhodospirillales* orders.

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DISCUSSION

Sequencing Results

As seen from the sequencing results, most of the bacteria samples have a 98% matching rate, and there are only few 'N's found, representing the unidentified sequences, in the sequencing results. This shows that 16S rDNA PCR is able to show and identify most of the gene sequences of the isolated bacteria sample.

The most ubiquitous bacteria in commercial draught beer is the *Rhodospirillales,* which consists of *Acetobacter,* a group of acetic acid bacteria characterized by the ability to convert alcohol (ethanol) to acetic acid in the presence of oxygen. They can destroy beer which they infect by producing excessive amounts of acetic acid or ethyl acetate, both of which can render the beer unpalatable.

Sphingomonadale, Lactobacillales and *Burkholderiales* are three predominant bacteria types in home-made draught beer. The *Sphingomonadale* is widely distributed in nature, having been isolated from many different land and water habitats. Some of the *Sphingomonadale* also play a role in human disease, primarily by causing a range of mostly nosocomial, non-life-threatening infections that typically are easily treated by antibiotic therapy. *Lactobacillales* was initially identified as the target group of beer spoilage bacteria in beer since they convert lactose to lactic acid and the production of lactic acid makes beer acidic. The *Burkholderiales* include several pathogenic bacteria.

Previous studies done on beer spoilage bacteria suggested the presence of some lactic acid bacteria such as Lactobacillus brevis, Lactobacillus lindneri and Pediococcus damnosus, and some Gram-negative bacteria such as Pectinatus cerevisiiphilus, Pectinatus frisingensis and Megasphaera cerevisiae [2] in spoilt beer. Comparing the results obtained in this experiment with previous studies done, it can be confirmed that the results obtained were relatively accurate as seen from the sequencing results. However, there is still the presence of other analogous bacteria types in the beer samples. This could be due to other external factors such as unclean water used to clean the beer brewer, among the various possibilities.

Limitations

The choice of draught beer samples was restricted by the limited resources in Singapore. In the commercial draught beer section, only one beer microbrewery was both able and willing to provide draught beer for this experiment. Larger beer factories such as Tiger Beer and Heineken did not produce unpasteurized draught beer and were also unable to provide us with unpasteurized draught beer due to commercial sensitivity. For example, Asia-Pacific Breweries replied to our request for spoiled beer by saying that they did not have spoilage in their production line.

The comprehensiveness of this study was also limited by the environment in which the study was carried out. As the external environment directly affects the spoilage of the beer, it would be more accurate if the study was carried out in various parts of the world simultaneously.

CONCLUSIONS

This study thus shows that bacteria are present in both commercial and home-brewed draught beer. However, they differ in identity. Commercial draught beer mainly contains *Rhodospirillales* order, which includes the *Acetobacter* genus. On the other hand, home-brewed draught beer contains *Sphingomonadale, Lactobacillales* and *Burkholderiales* orders, which include *Weissella, Lactobacillus, Sphingomonas, Ideonella, Burkholderiales* and *Aquabacterium* genera. The bacteria identified are harmful only when present in excessive amounts.

Lactobacillales and Rhodospirillales are known beer spoilage bacteria. However, *Sphingomonadale* and *Burkholderiale,* which are generally present in sludge water, were also identified to be present in the home-brewed beer samples. We speculate that this could be due to the fact that the water used in the brewing procedure may have inadvertently been contaminated.

This study was intended to serve as a stepping stone for further research on methods to prevent the growth of bacteria in draught beer. For example, since *Lactobacillus* ceases to reproduce at a pH of around 3.8, one of the possible methods to inhibit growth of *Lactobacillus* in the beer would possibly be to manufacture beer of a lower pH. Such a low pH would probably cause the beer to taste sour, which may not appeal to most beer lovers, thus another possible method to inhibit the growth of *Lactobacillus* in beer would be to introduce certain hop acids which will slow the growth of most *Lactobacillus* [6] .

However, there is a dilemma between preventing bacterial growth and avoiding alteration of the taste of beer by using additives. Thus, the future challenge is to device methods to prohibit their growth in draught beer so as to produce draught beer with longer shelf life.

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Annex A

	Sample	Class	Order	Family	Genus	Species
Commercial Draught Beer	**C1A6**	*Alpha Proteobacteria*	*Rhodospirillales*	*Acetobacteraceae*	*Acetobacter*	*Acetobacter Orientalis*
	C1B5	*Alpha Proteobacteria*	*Rhodospirillales*	*Acetobacteraceae*	*Acetobacter*	*Acetobacter Orientalis*
		Alpha Proteobacteria	*Rhodospirillales*	*Acetobacteraceae*	*Acetobacter*	*Acetobacter pasteurianus*
	C1B8	*Alpha Proteobacteria*	*Rhodospirillales*	*Acetobacteraceae*	*Acetobacter*	*Acetobacter Orientalis*
		Alpha Proteobacteria	*Rhodospirillales*	*Acetobacteraceae*	*Acetobacter*	*Acetobacter pasteurianus*
Home-Brewed draught Beer	**HA2**	*Alpha Proteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-
		Alpha Proteobacteria	*Sphingomonadales*	*Flavobacteriaceae*	*Novosphingobium*	-
	HA3	*Bacilli*	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella cibaria*
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	-
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella confusa*
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella kimchii*
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella salipiscis*
		Bacilli	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*Lactobacillus fermentum*
	HA5	*Alpha Proteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-
		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-
		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-
		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-

		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonas aquatilis*
		Alpha proteobacterium	-	-	-	-
	HA6	*Gamma proteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	-
	HA7	*Alpha Proteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-
		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonas echinoides*
		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonadale s bacterium*
	HA9	*Alpha Proteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonas echinoides*
		Alpha Proteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	*Sphingomonas oligophenolica*
		Alpha proteobacterium	-	-	-	-
	HB1	*Chlamydiae*	*Chlamydiales*	*Chlamydiaceae*	*Sphaerotilus*	-
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Burkholderiales*	*Burkholderiales bacterium*
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Aquabacterium*	-
	HB2	*Alpha Proteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*	-
		Alpha Proteobacteria	*Sphingomonadales*	*Flavobacteriaceae*	*Novosphingobium*	-
	HB3	*Chlamydiae*	*Chlamydiales*	*Chlamydiaceae*	*Sphaerotilus*	-
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Ideonella*	-
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Burkholderiales*	*Burkholderiales bacterium*
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Aquabacterium*	-
	HB5	*Bacilli*	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella cibaria*
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	-
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella kimchii*
	HB6	*Bacilli*	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella cibaria*
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	-
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella kimchii*
	HB7	*Bacilli*	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	-
		Bacilli	*Lactobacillales*	*Leuconostocaceae*	*Weissella*	*Weissella confusa*
		Bacilli	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*Lactobacillus confusus*
	HB9	*Chlamydiae*	*Chlamydiales*	*Chlamydiaceae*	*Sphaerotilus*	-
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Ideonella*	-
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Burkholderiales*	*Burkholderiales bacterium*
		Beta Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Aquabacterium*	-

Table AA1: Detailed classification of the bacteria present in each of the beer sample

Annex C

Identification of Bacteria Species

<u>Key</u>
C1: Commercial Draught Beer
H: Home-Brewed Beer
A: LAC Primer
B: UNIV Primer

C1A3

GGNNTGACCTCGATGTAGAGGGNGCATTTGTGGGCCTNAACTGNTTGAGANGANNCGGANANCCCTTTTAANAGG
GTNGAAGGAAGGGTGCNACANGAAGAGTNCCNTTAANNNGNGGGCCAGCGGTACTGTTTGNAANNAATATCCCG
GATGANGGAGAGNAGCNGAGGNCNCCCCGGNNGCCCCCGCGNCTGAGGGAATGGTTACNTGTTNGGNANAAATA
ATGGGGNGNNTGGCTTTAACAANNNCGNGGTGNTNGGNANCNNGGTNCNNGGGCNTGATAACNNGTGCNTNGTG
TNGAGAAGTGTAGGANAACNTCNNCACATGTGCNTNTNNGANNANANNNATNNGGNAAGNGGGANNANNGGNG
GANNTCGNAATTAAATAGTAGNTTGACGNTTGATNNTNNNTNNGGGTAGGGTGTGGGNGAGNCNTNNNTNNNNN
NNGNAAATTATGANTNNNAGTNGTGGGNNNTTATTNNNNGANTGTCGNGTATTAANTTNNTGNAGTNNGGNCNGG
CTGCGTNGTTNTGNGTNGANTGATATTGANAGNGNTTGANGTGANNNTNNNTTNTTTTNGTNGANGTTTTNTNNAT
AACGNGCNGNAANATNTNNNNACTCTNNNNGGGACNNGGNGNNGGTGNTNTTTNNTGNANNTNNNNATNGNCTG
GANCGNNACANTTGTNNGNNGNGAAANNTTNNGAAANANGGNTTTANGNTTNTGTANGNGGNTGNCNNANTTAN
TGGNATNNGGNTNNATGTNNNCTNGATAGTGTNANAATGTCCANTTGTNATTGNTTTTNAAATTNNAGNATGNNN
CTTNANTNTNGGGANNTTNNNNNNTNTNTTTATNNAAGGGTGANNGCNCNNAAATCTNGCNNNTNNGGTNTAGAG
NATTGTTNNTNAGNNTTNCNATTGTNNNAATTNTANTCNNTGGCCANGNTNGGNCTTCATAGTNGNNNANATNNT
ATNTAATAGAATCTTTNTNTTNNATGNNATGNNTNNANTTNCGNGGAANATNNTTTTNNGCGNNAAAGTTCNANA
NNGATTNNTNANNTTGNTANTAGAAAGTAGNTTNNNTTGNTATGTATTANAAGNTCTNATCGAAATCANGTGNAA
AGNNNTGNTGGCANTNGGTGTNTGATCANNTACTNTNATNATGNNTTTTCNNAGNGTNTNTANTGNNTTNT

C1A4

ANNTCGATGTAGAGGNTGCATTTANTNGGCCNGGACTGCTTGGAGAAACNGNATNNCCCTTNAANANNGGGNAA
GGAAGTGGTGCGNTNNGGTAGANTCCNCNTTNGAANGNGGGCCNGCGTACTGTTTCAAAACAGCATCCGCGGATT

GTGGCAGAAGGTANNNGNTGGNGNCTCCCGGAGNGCCTCCCTCCGCANNGGGGAATGGTTACGTAATGGGNACC
NGTNNTGGGGAGACTANNTNGANNATCNGTAGGAGGTGNTNGGNNAGTGGGGAAGGTGGNCGTGANAATNTGCG
CGGGGGGAGAGNGNGNGGATATCANNGTGACATGTTTGTTTTTAANAATAGTGTAATGNAAAGCGGGAAGNANG
GTGGNGTNGTTCGNTNANTGAGGTGGATGATGTGNNTNTNTATNTGTGTNGGGTGTTNGAGACGNNTAAGNAAGN
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GNGAGNGNGTGATATTGNGAGTGGANGNNGTNAGAANTGTNNNNTTTTNGGNTAGGGTTAGNTGGGTTTTNNGGT
GTATGGCNNGAATGTTANGATATNGGANNGGTGGNGNTNTNGGAGTATTTNGTNCTGTNATTANNTTNTNTNANA
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C1A5

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GNAGGANGNNAGGATTTNTG

C1A6

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CTCCCTTCGGGAAGCGTGGC

C1B5

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GTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTC
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GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGGACCGCTGGCGCC

C1B6

GGGGGGTGTCGCAGTNCGCACCCCTATCTTTAGGTTGNCAGCATGCCCTGNGNGGGACCTGTNCANAGNGTGCCC
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TTN

C1B7

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GTGNNNTNTATATATNTN

C1B8

GGGGTTCTAGTGCTTCGCACGAGCGCACCCCTATCTTTAGTTGCCAGCATGTTTGGGTGGGCACTCTAGAGAGACT
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TACAATGGCGGTGACAGTGGGAAGCTAGATGGCGACATCGTGCTGATCTCTAAAAACCGTCTCAGTTCGGATTGTA
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ACCCTATTGTTA

C1B9

GNANGGGGAANTCTCCATATAAANCCNGANANCACGCGTGGNGTTTTGNCGCCCTCTTCATCGACATCCGACANT
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HA2

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GGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
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HA3

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HA4

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GGNNANTTGCNCNGG

HA5

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HA6

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TGT

HA7

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HA8

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HA9

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HA10

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TNNN

HB1

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HB2

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HB3

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HB4

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GG

HB5

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HB6

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HB7

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HB8

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HB9

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HB10

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