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**Comparative genomic and plasmid analysis of beer-spoiling and
non-beer-spoiling *Lactobacillus brevis* isolates**

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Abstract

Beer-spoilage-related lactic acid bacteria (BSR LAB)¹ belong to multiple genera and species; however, beer-spoilage capacity is isolate-specific and partially acquired via horizontal gene transfer within the brewing environment. Thus, the extent to which genus-, species- or environment- (i.e., brewery-) level genetic variability influences beer-spoilage phenotype is unknown. Publicly available *Lactobacillus brevis* genomes were analyzed via BLAST Diagnostic Gene finder (BADGE) for BSR genes and assessed for pangenomic relationships. Also analyzed were functional coding capacities of plasmids of LAB inhabiting extreme niche environments. Considerable genetic variation was observed in *L. brevis* isolated from clinical samples, whereas 16 candidate genes distinguish BSR and non-BSR *L. brevis* genomes. These genes are related to nutrient scavenging of gluconate/pentoses, mannose, and metabolism of pectin. BSR *L. brevis* isolates also have higher average nucleotide identity and stronger pangenome association to one another, though isolation source (i.e., specific brewery) also appears to influence the plasmid coding capacity of BSR LAB. Finally, it is shown that niche-specific adaptation and phenotype are plasmid-encoded for both BSR and non-BSR LAB. The ultimate combination of plasmid-encoded genes dictates the ability of *L. brevis* to survive in the most extreme beer environment, namely, gassed/pressurized beer.

Keywords: beer-spoilage, genome comparison, lactic acid bacteria, *Lactobacillus brevis*, plasmid analysis

¹ Abbreviations: After initial introduction, bacterial strains are referred to throughout by their isolate designation (as in column 1 of Table 1); ANI, average nucleotide identity; BADGE, BLAST Diagnostic Gene finder; BSR, beer-spoilage- related; DMG, diagnostic marker gene; LAB, lactic acid bacteria; RAST, rapid annotations using subsystems technology.

Background

Discrimination of beer- or food-spoiling lactic acid bacteria (LAB) has focused on finding a *barcode* of genes able to distinguish specific niche-adapted (spoilage) isolates from those that are non-adapted (Behr et al. 2015; O'Sullivan et al. 2009). The discovery of common gene elements in beer-spoilage-related (BSR) LAB is difficult not only because of the considerable genetic variability within LAB at the genus and species levels (Zheng et al. 2015), but also because of the brewery niche, which encourages horizontal gene transfer between organisms. Additionally, the microflora of a given brewery, i.e., what organisms are available at any given time to exchange genetic material, is influenced by raw materials, brewery location, hygiene, workflow, and brewing processes. These potential contributors to genetic variation are appreciated, but exactly how they all work together to influence genetic similarity and/or variability among BSR LAB is simply not understood.

The number of publicly available BSR LAB genomes has increased over recent years, facilitating genomic comparisons that are beginning to indicate true levels of genetic variation in these organisms. An inter-genus comparison of BSR *Lactobacillus* and *Pediococcus* genomes did not prove fruitful for elucidating BSR genetic markers via proteome subtraction (Pittet, 2012). In contrast, intra-genus/inter-species genomic comparison of *Pediococcus damnosus* LMG 28219 (beer-origin), *Pediococcus claussenii* ATCC BAA-344^T (Pc344^T; beer origin), and *Pediococcus pentosaceus* ATCC 25745 (plant origin) revealed that genes involved in *de novo* folate biosynthesis are unique to the strains isolated from beer, and that transcriptional regulators are enriched in the genomes of pediococci capable of growth in beer (Snauwaert et al. 2015). This latter finding fits with transcriptional analysis of *Lactobacillus brevis* BSO 464 (BSO 464)

and Pc344^T which found that transcriptional regulators of multiple gene families are strongly expressed during growth in beer (Bergsveinson et al. 2016; Pittet et al. 2013). Transcriptional regulators were also implicated as important for LAB growth in beer in a study where hybridized genomic array intra-species comparisons were done of BSR vs non-BSR *L. brevis* (Behr et al. 2015). Transcriptional regulators belonging to ArsR, TetR and LysR families were specific to strong-beer-spoilage *L. brevis* strains, and other specific beer-spoilage *lifestyle* genes were identified, including a polygalacturonase, oligogalacturonide transporter, and a specific signal transduction histidine kinase. Finally, a bioinformatics tool called BADGE (BLAST Diagnostic Gene findEr) was used to interrogate beer-spoiling and non-beer-spoiling *P. damnosus* isolates for beer-spoilage diagnostic marker genes (DMGs), revealing that a plasmid-harbored gene *fabZ* could discriminate BSR and non-BSR *P. damnosus* isolates.

In an effort to extend the work of Behr et al. (2015), here we explore the suitability of BADGE for interrogating *L. brevis* isolates for BSR DMGs. We also provide a *L. brevis* pangenomic analyses. Lastly, influence of plasmid-encoded genes on the ability of *L. brevis* to adapt to niche environments was investigated, with emphasis placed on the impact of non-hop tolerance genes on the ability of *L. brevis* to grow in beer.

Methods

L. brevis genomes and plasmid annotation

Genomes of *L. brevis* BSO 464 (Lb464), *L. brevis* BSO 310 (Lb310), and *P. claussenii* ATCC BAA-344^T (Pc344^T) were previously sequenced in-house (Bergsveinson et al. 2015b, 2015c; Pittet et al. 2012). All publicly available *L. brevis* genome assemblies were retrieved from

GenBank on September 30th, 2016 (Table 1). Genomes were annotated via the RAST (rapid annotations using subsystems technology) server, using the *classic RAST* annotation pipeline (Aziz et al. 2008). The RAST server was also used to annotate and parse functionality of protein sequences for plasmids in *L. brevis* KB290 (KB290), BSO 464, and Pc344^T (Pittet et al. 2012).

Beer-spoilage ability assessment

L. brevis ATCC 367^T (ATCC 367^T), *L. brevis* BSO 310 (BSO 310), BSO 464, and KB290 were tested for beer-spoilage ability. Growth in degassed and gassed beer was assessed using plating on agar (Bergsveinson et al. 2015c). Beer-spoilage ability of *L. brevis* TMW 1.313, TMW 1.465 and TMW 1.6 (hereafter referred to by their strain designations; Table 1) was reported in Behr et al. (2015), as determined via a resazurin dye reduction procedure (Preissler et al. 2010).

Diagnostic marker gene (DMG) identification

Files containing RAST-annotated open reading frames for each genome were processed using the RAST2BADGE function of Blast Diagnostic Gene Finder (BADGE) package, then processed using default BADGE/blast settings (megablast_e_value: 1×10^{-15}), but with the “dc_mode” flag set to “true” (dc-megablast search is slower, but more sensitive) (Behr et al. 2016). Genomes were grouped according to the isolate-recovery environment (Table 1), and compared via BADGE in different combinations to reveal potential DMGs for each group. Strains recovered from beer were also compared to one another, grouped according to the geographic location where they were isolated. Sequences of all DMGs annotated as encoding hypothetical proteins were further interrogated to reveal if these sequences were potentially related to non-coding RNAs (ncRNAs) using Rfam (Griffiths-Jones et al. 1991) and/or to

identify specific protein motifs via InterProScan (Jones et al. 2014) (Table 2).

Pan genome interrogation

RAST-annotated open reading frames were parsed using the software package GET_HOMOLOGUES (Contreras-Moreira and Vinuesa, 2013) to assess the *L. brevis* pangenome and relatedness between isolates. The *get_homologues-est.pl* script was run with flags: `-M -c -z -A -t2`; with `-M` proscribing the orthoMCL algorithm (Chen et al. 2006) be utilized and `-t2` to report all clusters containing sequences from at least two taxa. Sequence clusters were compared with GET_HOMOLOGUES package script *compare_clusters.pl*, and *hcluster_martix.sh* was used to generate a pangenome matrix tree.

Results

***L. brevis* genomes and phenotype**

Twenty *L. brevis* genomes were retrieved from NCBI and grouped together based on the common environments they were recovered from (Table 1). This was done to explore how well genomes could be differentiated by genomic properties related to specific environments or niches (Zheng et al. 2015). Two separate genome-sequencing projects exist for *L. brevis* ATCC 14869 (ATCC 14869), with a later NCBI entry of this isolate made under its synonymous identifier DSM 20054 (Table 1). The two separate NCBI entries detail distinct isolation sources (gastrointestinal tract vs feces, respectively) and list different sequencing depths, thus both sequences were included. It was also noted that the Chinese brewing-related *L. brevis* strain BM-LB13908 (BM-LB13908) was notably dissimilar from the other *L. brevis* beer isolates (BSO 310, BSO 464, TMW 1.313, and TMW 1.465), and other food niche-related *L. brevis* isolates,

which share fermentation capacity as a common metabolic activity. The BM-LB13908 genome was found to be phylogenetically ambiguous, with similarities to both *L. brevis* and *Lactobacillus harbinensis* genomes (data not shown). As a test of the validity of the original description of BM-LB13908 as *L. brevis*, and in light of its unconfirmed beer-spoilage capacity of, and status as a viable, but non-culturable isolate from beer (Suzuki et al. 2006), this genome was placed within the *L. brevis* clinical group for analyses.

Of the *L. brevis* isolates listed in Table 1, BSO 464, BSO 310, KB290 and ATCC 367^T were tested in-house for beer-spoilage, with only BSO 464 and BSO310 capable of growth in beer, and BSO464 being further capable of growth in the more extreme environment of gassed/pressurized beer (Bergsveinson et al. 2015c). Isolates TMW 1.313, TMW 1.465, and TMW 1.6 were tested elsewhere for ability to grow in beer, with a different analysis method used (Behr et al. 2015). Thus, it is unknown if the two beer-spoilage strains TMW 1.313 and TMW 1.465 are capable or not of growth in gassed/pressurized beer.

DMG discovery

Genomes within the three environment groupings of *L. brevis* isolates (Beer, Clinical, and Food; Table 1) were compared via the BADGE software. As shown in Fig. 1, certain comparisons yield less than a 100% DMG occurrence rate in a given isolate group, meaning DMGs could not be found within all isolates of the group, as compared to the isolates in the other group. For instance, in the comparison of Clinical vs Beer isolates (Fig. 1), DMGs were not identified in the clinical strains *L. brevis* 15f (15f) and BM-LB13908, meaning DMGs were only determined in 9 of the 11 Clinical isolates (82% occurrence rate). When the Clinical group of isolates was

compared to the Food group, DMGs were found in 7 of 11 isolates (a 64% occurrence rate), but were not identified in *L. brevis* 47f (47f), *L. brevis* 1595 (1595), BM-LB13908, and 15f. Conversely, when the Food isolate group was compared to the Clinical group of isolates, the DMG occurrence rate for Food isolates was below 50%, indicating it was not possible to determine a specific gene found in even half of the Food genomes that could not be found in any of the Clinical isolate genomes.

In contrast, when the Beer group of isolates was compared to either the Clinical or Food group (Fig. 1), there was a 100% DMG occurrence rate in the Beer isolates, meaning that all 4 brewing-related genomes are distinct enough from the isolates in either of the two other groups to reveal genes specific to all 4 members of the Beer group. The 46 DMGs revealed from the Beer vs Food isolates comparison are listed in Tables 2 and 3. The 2 and 14 DMGs indicated in italics at the top of Table 2 and 3, respectively, are proposed to distinguish Beer *L. brevis* genomes from all other *L. brevis* genomes (i.e., when the Food and Clinical *L. brevis* genomes are grouped together) (Fig. 1 and Table S1²). Of the 46 DMGs of interest in the 4 Beer isolates, 15 were annotated as hypothetical proteins (Table 2). Use of InterProScan resolved the functional identity of 9 of the 15 proteins as being associated with the cellular membrane or performing a regulatory function (Table 2). Of the 19 DMGs indicated in the comparison of Beer vs Clinical isolates (Figure 1 and Table S1), 16 are found among the 46 DMGs produced when Beer vs Food isolate groups are compared (Figure 1 and Table S1). The remaining three DMGs specific to the Beer isolate group when compared to the Clinical group are related to plasmid mobility (Table S2). The annotated DMGs listed in Table 3 indicate that Beer *L. brevis* genomes are

² Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com>

distinguished from genomes of non-beer environment isolates through genes primarily involved in nutrient scavenging/carbohydrate metabolism, degradation of plant material, membrane biogenesis, and specific transcriptional regulation.

Geographic DMGs of brewing-related *L. brevis*

To determine DMGs for brewery isolates recovered from distinct geographic locations, BADGE was used to interrogate and compare the two brewing isolates from the United Kingdom (BSO 310 and BSO 464) and the two isolates from a German culture collection, but unknown recovery locations (TMW 1.313 and TMW 1.465). Twenty DMGs were found to demarcate the United Kingdom isolates from the German/unknown source isolates (Table S3). Apart from a glycosyltransferase gene on the BSO 464 chromosome, the remaining 19 DMGs primarily code for hypothetical proteins and are found on BSO 464 plasmids pLb464-2, -3, and -4, and putative BSO 310 plasmid scaffolds (data not shown). The exception is an ATP-dependent protease ATP-binding subunit Clp coded on plasmid pLb464-8 (Table S3). The reverse comparison found no specific DMGs for the two German/unknown source isolates.

***L. brevis* pangenome analyses**

All *L. brevis* genomes were analyzed to identify their pangenomic relationships (1,120 core genes). It was again observed that inclusion of putative viable, but non-culturable strain BM-LB13908 disrupted the results, with no hits found for this strain in the average nucleotide identity (ANI) or the pangenomic matrix of all strains. Specifically, no genes common to at least two other *L. brevis* genomes were detected in this strain. Thus, BM-LB13908 was confirmed to be mis-identified as *L. brevis* and removed from the pangenome analysis.

The closest genomic relationship exists among *L. brevis* brewing-related genomes, with these isolates clustering together in both the pangenome matrix (Fig. 2) and ANI heatmap (Fig. 3). The German/unknown source isolate TMW 1.313 has greatest distance from the other brewery-related genomes, potentially contributing to why no German/unknown source-specific DMGs could be determined when compared to the two United Kingdom BSR isolates. Food and Clinical *L. brevis* genomes do not exhibit clear groupings in either the ANI heatmap or the pangenome matrix (Fig. 3 and 4), as isolates 15f, 47f, 1595 and *L. brevis* AG48 (AG48) group with other food-related genomes, while *L. brevis* NSP-QW-145 (NSP-QW-145) groups with a large cluster of Clinical isolates. This corresponds to DMGs not being identified for the genomes of isolates 15f, 47f, or 1595 when comparing Clinical vs Food genomes via BADGE.

Functional plasmid analysis

Annotated protein sequences encoded by KB290, BSO 464, and Pc344^T plasmids were assigned to functional categories via RAST and manually curated (Fig. 4). Proteins involved in plasmid function (replication, plasmid partition, copy number control proteins), DNA metabolism and damage-inducible proteins, site-specific recombinases, and a nucleotidyltransferase transcript involved in DNA repair, were encoded by plasmids of all three isolates (Table 4). All three isolates also have plasmids encoding sophisticated Type 1 restriction-modification subunits R, S, and M, and a number of plasmid-based integrases and transposases belonging mostly to the IS30 family. Other shared plasmid-coded functions include an antitoxin of toxin-antitoxin stability systems, ferroxidase, an enzyme likely to be involved in enhancing tolerance to oxidative stress (Serata et al. 2012), the LtrC-like protein (low temperature-requirement C protein), a putative

cold-shock chaperone protein, and multiple copies of electron transfer flavoprotein-ubiquinone oxidoreductase, which can assist in anaerobic respiration (Watmough and Frerman, 2010). Multiple gene copies for the ATP-dependent protease ATP-binding subunit ClpL, which is proposed to provide chaperon activity and degrade mis-folded proteins as part of cellular responses to stress and acid shock (Frees et al. 2007; Wall et al. 2007), were only found in isolates BSO 464 and KB290. Lastly, all three isolates have a number of plasmid-encoded *conserved* hypothetical proteins, i.e., closely related to proteins in other organisms. Each isolate also has a high proportion of *unknown* hypothetical plasmid-encoded proteins, many of which may be isolate-specific.

Relative to the plasmids of the beer-spoiling *L. brevis* isolates BSO 310 and BSO 464, KB290 plasmids differ most notably in the type of membrane transporters they encode; however, all three isolates encode transporters for heavy metal ions. Transporters on KB290 plasmids are primarily involved in ion and/or proton transport, while transporters in BSO 464 and Pc344^T are specific for carbohydrate substrates and hop iso- α -acids. Interestingly, plasmid-encoded cell wall-related enzymes are found in BSO 464 plasmids and not in KB290 or Pc344^T plasmids.

Discussion

BADGE software proved to be a useful tool for interrogating the genetic content of *L. brevis* isolates from distinct environmental niches. As with other means of genomic comparisons, BADGE results are impacted by the number of genomes compared, and characteristics of the genomes themselves (i.e., sequencing quality, isolation source and location). However, even if the number of available genomes for niche environments (such as beer) is limited, BADGE still

provides insight into genetic similarities of the available genomes and provides an effective means of detecting putative genes of interest without need to perform excessive further whole-genome sequencing. The added benefit of BADGE analysis is that it can assist in genome assembly, particularly with identification and assembly of plasmid sequences (Tables 2 and 3).

The advantage of using BADGE for *L. brevis* genomic comparisons is evident when the present results are compared to earlier suggested niche-specific genes for *L. brevis* beer-spoilage. For instance, a comparative genomic hybridization analysis (Behr et al. 2015) suggested that a specific signal transduction histidine kinase gene, a specific ArsR transcriptional regulator, and the competence-damage inducible gene *cinA* indicate beer-spoilage capacity of *L. brevis* strains. When the primers described in Behr et al. (2015) for these three genes were queried against all 20 genomes in the present study using a blastn search (Altschul et al. 1990) for short primer sequences, quality results only correlated with the isolates (TMW 1.313, 1.465 and 1.6) used in the original study (Behr et al. 2015). Notably, the *cinA* primers could not detect *cinA* outside the isolates described in Behr et al. (2015), despite *cinA* being functionally annotated in most *L. brevis* genomes. Thus, these three genes are not suitable as universal *L. brevis* beer-spoilage genetic markers, but rather they may be used as markers for quality control in the specific brewery the isolates were recovered from (Behr et al., 2015). Further, none of the transcriptional regulators indicated in Table 3 for *L. brevis* belong to the ArsR family or is a signal transduction kinase as previously indicated in Behr et al. (2015). Overall, the important implication here is that using a greater number of isolates from unique locations limits the skewing of BSR genes specific for a particular environment.

Brewing-related *L. brevis* DMGs

DMG analysis of the three *L. brevis* niche groups indicates more genetic variability exists in Food and Clinical isolates than in BSR isolates and/or that BSR *L. brevis* genomes are highly specialized. Of the 46 DMGs suggested to resolve BSR strains from Food-related *L. brevis* genomes, only the 16 that differentiate BSR isolates from both Clinical- and Food-related isolates are discussed (Tables 2, 3 and S1). These 16 genes are related to transcriptional regulation, cellular membrane modification, and carbohydrate/nutrient acquisition, which fits with previous transcriptional analysis showing these activities to be critical for growth in beer (Table 3) (Bergsveinson et al. 2016; Pittet et al. 2013).

Interestingly, DMGs related to plant material (pectin) degradation were uncovered, pointing to an important beer niche-specific adaptation for *L. brevis*. For instance, polygalacturonase, previously indicated to be a beer-specific *L. brevis* gene marker (Behr et al. 2015), is an enzyme that breaks down oligogalacturonides in plant cell walls, making carbohydrates available for bacterial cell growth (Ferarri et al. 2013; Ridley et al. 2001). Another DMG, coniferyl aldehyde dehydrogenase (ALDH), participates in a number of metabolic pathways (Table 3) and notably reduces ferulate, a component of plant cell walls (i.e., barley and other cereal grains) (Achterholt et al. 1988). These DMGs further support the theory that BSR LAB are originally associated with plants and become introduced into the brewery via raw materials such as grain. It is notable that these transcripts were not significantly differentially expressed in isolate BSO 464 during growth in lager beer (Bergsveinson et al. 2016); however, this may indicate that their activity is specific to early-log or stationary phase metabolism, or, that these genes are not required for growth in beer, but rather for basic survival at different stages or locations in breweries.

Similarly, carbohydrate metabolism is inferred to be another niche-specific adaptation for BSR *L. brevis* isolates given the DMGs identified for mannose and gluconate phosphotransferase system (PTS) transporter proteins (Table 2). Mannose inhibits flocculation by some brewing yeasts and is a component of yeast cells (Verstrepen et al. 2003), thus bacterial scavenging of this substrate would allow decomposing yeast to be exploited for nutrients. Beyond mannose, these transporters may intake glucose and N-acetylglucosamine, as found in a *Lactobacillus sake* strain (Lauret et al. 1996). Other beer-spoilage DMGs include gluconate-specific PTS genes, and genes for phosphoenolpyruvate-protein phosphotransferase, ribose 5-phosphate isomerase B, 2-dehydro-3-deoxygluconate kinase, mannate dehydratase, and the fructuronate transporter GntP, all involved in intake and metabolism of gluconate and pentoses. Finally, a GntR family transcriptional regulator, which acts as a gluconate operon-repressor in *Bacillus subtilis* (Haydon and Guest, 1991), is a DMG for the *L. brevis* Beer vs Food groups (Table 3).

Gluconate and pentoses are fermented via the gluconate-6-phosphate/phosphoketolase pathway, a component of the pentose phosphate pathway, and are an obvious source of metabolism for BSR LAB, given that brewing yeasts are not capable of metabolizing pentoses (e.g., arabinose, fructose, sorbose) (von Wright and Axelsson, 2004). Interestingly, PTS components specific for gluconate and mannose, and the phosphoenolpyruvate-protein phosphotransferase of PTS system gene demarcate BSR *L. brevis* genomes from all other *L. brevis* genomes (i.e., DMGs above the dashed line in Table 3); while the other genes listed in Table 3 specifically separate BSR and Food *L. brevis* isolates. Genes related to the pentose phosphate pathway have been found to be highly specific niche-adaptations and/or are strain-specific in LAB, however, have been shown

to be unsuitable as taxonomic markers (Zheng et al. 2015). Nevertheless, specific pentose phosphate pathway transcripts appear to serve as *ecotype* identifiers of beer-niche *L. brevis*.

Importantly, the genetic markers described here differ from the markers indicated in analysis of other BSR LAB species, i.e., the *fabZ* operon for BSR *P. damnosus* (Behr et al. 2016) or *de novo* folate synthesis for BSR *pediococci* (Snauwaert et al. 2015). Unfortunately, this strongly suggests that discovery of universal DMGs for beer-spoilage across all LAB is unlikely to occur. Instead, it seems that DMG's for BSR LAB are most readily discernible within a species. If this proves true, then at best, DMGs will resolve beer-spoilage relatedness in closely related (phylogenetically or geographically) species or isolates.

Geographic specific DMGs and pangenome relationships

BADGE comparison of the United Kingdom BSR *L. brevis* isolates (BSO 310, BSO 464) vs the German/unknown BSR isolates (TMW 1.313, TMW 1.465) also fits with the pangenomic analysis in Fig. 2. The TMW 1.313 genome has a large number of frame-shifted (pseudo) genes, potentially explaining why no German/unknown source-specific DMGs were identified and why greater genomic distance exists between isolate TMW 1.313 and the other BSR *L. brevis* isolates, this despite isolate TMW 1.313 grouping in the beer pangenome niche. However, the finding that plasmid-based DMGs are common to the United Kingdom BSR isolates provides further evidence in support of geographic location influencing the acquisition of genetic material on plasmids, likely through use of shared sources of raw materials, water, and bottle recycling.

The *L. brevis* pangenome tree (Fig. 2) and ANI heatmap (Fig. 3) also indicate interesting

relationships between *L. brevis* food and clinical isolates, as neither isolate group clusters within either analysis. This is unexpected, since the phylogenetic position of lactobacilli are purportedly linked to their ecotype (Zheng et al. 2015). Isolates 15f and 47f (Clinical, human feces for which no DMGs could be found when compared against Beer isolates, were found to cluster separately and among the food-related genomes (Fig. 2). Further, the clusters of Beer genomes and the *primarily* Clinical genomes are grouped closer together than the *primarily* Food-related cluster, and diverge at a similar point (Fig. 2). Ultimately, classification as a Food or Clinical isolate based on source may not be sufficiently precise for doing comparisons against isolates from the better-defined brewing environment niche.

Plasmids in LAB from beer and gastrointestinal environments

Maintaining plasmids imposes a substantial energy burden on the bacterial cell (Freter et al. 1983), thus it is presumed that all plasmids present in an isolate impart critical function(s) to the cell for the growth environment involved. Although isolates BSO 464, KB290 and Pc344^T differ in the niche they inhabit (pressurized/gassed beer, stimulated intestinal and gastric juices, and degassed beer, respectively) (Bergsveinson et al. 2015c; Fukao et al. 2013), they each carry a high number of plasmids (Fig. 4). Interestingly, there is no cross-resistance to the different niche environments, since BSO 464 and Pc344^T are unable to survive in intestinal/gastric juices and KB290 cannot survive or grow in even degassed beer (data not shown). This strongly suggests the respective plasmid-encoded gene profiles are tailored to combat specific environmental growth conditions, despite beer and gastric juices imposing some of the same stresses to the cell.

The presence of PemI and PemK in BSO 464 and KB290 provides evidence that the plasmid

profiles of these two isolates are stable despite the extreme, albeit different, environments from which they were isolated (Bergsveinson et al. 2015a; Fukao et al. 2013; Tsuchimoto et al. 1988). Conversely, the absence of these genes in isolate Pc344^T explains why its plasmids are lost during storage or experimental manipulation (Pittet, 2012; Pittet et al. 2013). Furthermore, multiple gene copies of the stress response and acid shock related protein ATP-dependent protease ATP-binding subunit Clp are found in BSO 464 and KB290, but not in Pc344^T, which again correlates with BSO 464 and KB290 being able to handle the higher oxidative stress posed by gassed beer and gastric juice, respectively (Bergsveinson et al. 2015c).

Although similarities exist in BSO 464 and Pc344^T plasmid coding capacity, one notable difference is the presence of plasmid-encoded cell wall-related enzymes in BSO 464 and not in Pc344^T (nor in KB290). The pressurized/gassed beer environment weakens the cellular membrane and increases intracellular oxidative stress (Molina-Gutierrez et al. 2002; Ulmer et al. 2002). These BSO 464 plasmids pLb464-2 and -4, which were both earlier implicated as contributing to the overall beer-spoilage ability of this LAB (Bergsveinson et al. 2015a). That cell-wall modification proteins are coded by BSO 464 plasmids and not Pc344^T plasmids, together with the disparate beer-spoiling virulence of these two isolates (Bergsveinson et al. 2015c), points to the incremental acquisition of the genetics required for growth in beer.

Compared to KB290 plasmids, BSO 464 and Pc344^T plasmids encode a number of ABC type transporters, potentially allowing for greater nutrient scavenging and/or *poison-* (i.e., hops) export. BSO 464 and Pc344^T also contain more phage- and transposon-related products than KB290, and BSO 464 has a large prophage region on plasmid pLb464-4, which at 85 Kb, is also

the largest plasmid in the three isolates. The role of bacteriophages in BSR LAB has been investigated largely from the perspective of phage therapy for controlling the growth of putative beer-spoilage LAB (Kelly et al. 2011; Mahony et al. 2012); however, it is clear that bacteriophages are endemic to BSR LAB isolates.

Relative to the two beer-spoiling isolates, KB290 plasmids pKB290-2 and -3 encode a number of transporters involved in ion and proton transport which likely contribute to efficient proton motive force regulation when growing in the presence of highly concentrated electrolytes of gastric and intestinal juices (Meeroff et al. 1973). Other transporters are involved in the uptake of amino acids or peptides, presumably allowing KB290 to utilize peptides made available by the enzymatic action of pepsin in gastric juices (Fruton, 2002). Interestingly, when plasmid variant analysis was undertaken for KB290, it was found that combined loss of pKB290-1, -2, and -3 from the cell resulted in a significantly decreased survival rate in simulated intestinal/gastric juices and a loss of flocculation phenotype in broth culture (Fukao et al. 2013), with pKB290-1 contributing the most to the stress tolerance and tendency of cells to aggregate. This is likely due to pKB290-1 harboring a cellulose synthase catalytic subunit (UDP forming) that assists in polymerization of cellulose and by extension, flocculation (Table 4; Fukao et al. 2013).

Overall, the comparison of the functional plasmid coding capacity for BSO 464, KB290, and Pc344^T, and past plasmid-curing analysis experiments (Bergsveinson et al. 2015a; Pittet et al. 2013; Fukao et al. 2013) confirm that plasmid acquisition is critical for LAB adaptation to niche-environments. These adaptations often involve catalytic activity, membrane transport, and niche-specific nutrient acquisition. Thus, while plasmid-coding capacity strongly influences isolate

phenotype, adaptation is complex and results from multiple genetic adaptations - both chromosomal and plasmid-encoded - used in combination.

Conclusions

Genomic analysis of *L. brevis* isolates revealed that intra-species comparisons are useful for elucidating specific genetic markers for beer-spoilage, suggesting that selection pressures encountered in the brewery environment influence the genetic content of both plasmids and genomes of these BSR bacteria. Proposed DMGs for *L. brevis* beer-spoilage capacity are not related to hop-tolerance, but instead are involved in the degradation of plant materials and scavenging of carbohydrates not utilized by yeast. Sequence analysis reveals plasmids in BSR LAB share functional coding capacity for anaerobic respiration and oxidative stress responses with LAB adapted to other extreme, non-beer environments. Importantly, BSR LAB also possess genes for cell wall-related enzymes and specific transporters that facilitate growth in beer. While genetic markers can be elucidated for LAB beer-spoilage; thus far the identified markers best fit with BSR isolates of a given species. Robustness of DMGs will be improved as additional BSR and non-BSR LAB from diverse geographic locations have their genomes sequenced and available for beer-spoilage DMG analysis.

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Draft

1 **Table 1. *Lactobacillus brevis* genomes retrieved from NCBI.**

Isolate and Available Reference	Isolation Source	Classification for BADGE	Genome Assembly Level	BioProject ID	Size (MB); # of plasmids *	Proteins †
ATCC 367 ¹ (Makarova et al., 2006)	Sourdough/silage starter culture	Food	Complete	PRJNA404	2.3 ; 2	2174
KB290 (Fukao et al., 2013)	Suguki (fermented vegetable)	Food	Complete	PRJDA64661	2.5 ; 9	2458
NPS-QW-145	Kimchi/Cabbage	Food	Complete	PRJNA318996	2.5 ; 0	2488
D6	Cheese	Food	Contig	PRJNA308170	2.6 ; ?	2630
WK12	Kimchi	Food	Contig	PRJDB3257	2.6 ; ?	2486
BSO 464 (Bergsveinson et al., 2015b)	Spoiled beer; UK	Beer	Complete with gaps	PRJNA203088	2.7 ; 8	2606
BM-LB13908	Beer; China	Beer*Clinical ‡	Scaffold	PRJNA309616	2.9 ; ?	2725
TMW 1.465 (Behr et al., 2015)	Soft-drink/Brewery; Beer; Germany	Beer	Contig	PJNA264952	2.5 ; ?	2104
TMW 1.313 (Behr et al., 2015)	Beer; Germany	Beer	Contig	PRJNA264952	2.7 ; ?	2312
BSO 310 (Bergsveinson et al., 2015c)	Brewery; UK	Beer	Contig	PRJNA288595	2.6 ; ?	2653
ATCC 14869 §	Gastrointestinal tract	Clinical	Scaffold	PRJNA38325	2.4 ; ?	2291
DSM 20054 §	Feces	Clinical	Scaffold	PRJNA222257	2.4 ; ?	2337
15f (Yunes et al., 2015)	Fecal matter of healthy adult	Clinical	Scaffold	PRJNA269010	3.1 ; ?	2256
47f	Fecal matter of healthy adult	Clinical	Contig	PRJNA280953	2.6 ; ?	2449
EW (Kim et al., 2013)	<i>Drosophila</i> intestine	Clinical	Contig	PRJNA209593	2.8 ; ?	2806
DPC 6108	Human fecal matter	Clinical	Contig	PRJNA339712	2.8 ; ?	2933
AG48	Sheep rumen	Clinical	Contig	PRJNA214528	2.5 ; ?	2424
DmCS_003	<i>Drosophila melanogaster</i> gut	Clinical	Contig	PRJNA252105	2.8 ; ?	2758
TMW 1.6 (Behr et al., 2015)	Feces	Clinical	Contig	PRJNA264952	2.5 ; ?	2435
1595	Bovine teat canal	Clinical	Contig	PRJNA284430	2.5 ; ?	2474

2 * For complete genomes, the total genome size includes genome and plasmid sequence; “?” indicates an unknown number of plasmids.

3 † Includes chromosomal and confirmed plasmid proteins.

4 ‡ Isolate only described as viable, but non-culturable, with no confirmation of beer-spoilage. Analyzed as a part of both the Beer and Clinical groups.

5 § ATCC 14869 = DSM 20054. The same isolate has been sequenced and submitted to NCBI by two different institutions. ATCC 14869 (deposited 2013); DSM 20054 (deposited 2015).

8 **Table 2. Hypothetical protein DMGs for beer-spoilage *L. brevis* identified in Beer vs Food BADGE comparison***

Amino acid length	InterProScan motifs	BSO 310 (scaffold #)	BSO 464	TMW 1.313 (scaffold #)	TMW 1.465 (scaffold#)
49	<i>Cytoplasmic, TM-helix, and transmembrane regions</i>	41	<i>Xsome</i>	2	3
178	<i>DNA-binding HTH domain (TetR-type), regulator Tet-R like, C-terminal</i>	86	<i>Xsome</i>	2	28
60	None	38	Xsome	2	2
61	None	10	Xsome	2	30
91	Mor transcription activator; DNA binding	10	Xsome	2	30
143	Cytoplasmic, TM-helix, and transmembrane regions	10	Xsome	2	30
56	None	49	Xsome	2	3
282	Non-cytoplasmic signal peptide	5	Xsome	2	3
280	Non-cytoplasmic signal peptide	5	Xsome	2	3
305	Predicted Sigma factor regulator N- and C-terminal	57	Xsome	2	28
387	Non-cytoplasmic signal peptide	9	Xsome	2	3
229	Cytoplasmic, TM-helix, and transmembrane region	12	Xsome	2	3
50	None	86	Xsome	2	28
121	None	1	Xsome	2	16
133	None	16	Xsome	2	3

* Entries in italics above the dashed line are DMGs identified in the Beer vs Clinical+Food BADGE comparison.

23 Table 3. DMGs for beer-spoilage *L. brevis* identified in Beer vs Food BADGE comparison*

Annotation	Function	BSO 310 (scaffold #)	BSO 464 [†]	TMW 1.313 (scaffold #)	TMW 1.465 (scaffold#)
<i>Undecaprenyl-phosphate galactosephosphotransferase</i>	<i>Bacterial outer membrane biogenesis</i>	41	<i>Xsome</i>	2	3
<i>Transcriptional regulator TetR family</i>	<i>Transcriptional regulation</i>	53	<i>pLb464-3</i>	10	11
<i>Alcohol dehydrogenase (aryl- or coniferyl- dehydrogenase)</i>	<i>Tyrosine, phenylalanine, biphenyl degradation</i>	86	<i>Xsome</i>	2	28
<i>Phage-related protein</i>	<i>Phage-related</i>	86	<i>Xsome</i>	2	28
<i>Oligogalacturonide transporter</i>	<i>Pectin metabolism</i>	9	<i>Xsome</i>	2	3
<i>Polygalacturonase</i>	<i>Pectin metabolism</i>	9	<i>Xsome</i>	2	3
<i>Putative transcriptional regulator with a sugar kinase domain GntR family</i>	<i>Transcriptional regulation</i>	1	<i>Xsome</i>	2	16
<i>LysR family transcriptional regulator</i>	<i>Transcriptional regulation</i>	16	<i>Xsome</i>	2	3
<i>Phosphoglycerate mutase</i>	<i>Carbohydrate metabolism</i>	16	<i>Xsome</i>	2	3
<i>PTS system gluconate-specific IIC component</i>	<i>Carbohydrate metabolism</i>	16	<i>Xsome</i>	2	3
<i>PTS system mannose-specific IID component</i>	<i>Carbohydrate metabolism</i>	16	<i>Xsome</i>	2	3
<i>PTS system mannose-specific IIA component</i>	<i>Carbohydrate metabolism</i>	16	<i>Xsome</i>	2	3
<i>PTS system gluconate-specific IIB component</i>	<i>Carbohydrate metabolism</i>	16	<i>Xsome</i>	2	3
<i>Phosphoenolpyruvate-protein phosphotransferase of PTS system</i>	<i>Carbohydrate metabolism</i>	16	<i>Xsome</i>	2	3
D-alanyl-D-alanine carboxypeptidase	Bacterial cell wall biosynthesis	49	<i>Xsome</i>	2	3
Muramidase	Cell wall degradation	9	<i>Xsome</i>	2	3
Phage capsid assembly protein	Phage-related	9	<i>Xsome</i>	2	3
Internalin-like protein LPXTG motif	Membrane component	9	<i>Xsome</i>	2	3
Transcriptional regulator GntR	Transcriptional regulation	1	<i>Xsome</i>	2	16
Oligogalacturonide transporter	Pectin metabolism	1	<i>Xsome</i>	2	16
2-dehydro-3-deoxygluconate kinase	Pentose phosphate pathway and pentose and glucuronate inter-	1	<i>Xsome</i>	2	16

conversions					
Fructuronate transporter GntP	Carbohydrate metabolism		Xsome		
Mobile protein element	Not determined	16	Xsome	2	3
Cell wall surface anchor family protein	Integral membrane component/Mg ²⁺ binding	47	Xsome	2	2
RNA polymerase sigma factor ECF subfamily	Transcriptional regulation; extracytoplasmic	57	Xsome	2	8
Transcriptional regulator MerR	Transcriptional regulator	13	Xsome	2	3
Ribose 5-phosphate isomerase B	Pentose phosphate pathway	13	Xome	2	3
Mannonate dehydratase	Pentose and glucuronate interconversions	1	Xsome	2	166
Short chain dehydrogenase	Metabolism/enzymatic function	21	Xsome	2	2
Transcriptional regulator AraC family	Positive transcriptional regulator	21	Xsome	2	2
Transcriptional regulator AraC family	Positive transcriptional regulator	21	Xsome	2	2

* Entries in italics above the dashed line are DMGs identified in the Beer vs Clinical + Food BADGE comparison.

† Xsome = chromosome; pLb464-# indicates the BSO 464 plasmid number.

Table 4. Unique attributes of KB290, BSO 464, and Pc344^T plasmid coding capacities.

Category*	KB290 plasmids [†]	BSO 464 plasmids [†]	Pc344 ^T plasmids [†]
Virulence/ Disease	-5: Antitoxin of toxin-antitoxin stability system -7: Lactococcin A immunity protein	-2: Antitoxin of toxin-antitoxin stability system -4: Universal stress protein, UspA -6: Cd ²⁺ resistance protein; Cd ²⁺ efflux system -8: As ³⁻ efflux pump protein	-3: Antitoxin of toxin-antitoxin stability system -5: As ³⁻ resistance efflux pump As ³⁻ resistance operon repressor -6: Lanthibiotic permease protein Lanthionine biosynthesis protein, LanM -7: Antitoxin of toxin-antitoxin stability system Cu ²⁺ homeostasis transpoter Exopolysaccharide gene, <i>gtf</i> -6: RNA polymerase RpoB
Transcriptional regulator	-2: Two AcrR family MarR family -5: ArsR family TetR family	-2: Hop-tolerance, HorB (TetR family) Two Xre family -3: TetR family -4: Diaglycerol kinase TetR family	
Oxidative stress	-4: Ferroxidase (EC 1.16.3.1) -5: Thioredoxin reductase -6: Ferroxidase (EC 1.16.3.1)	-2: Ferroxidase (EC 1.16.3.1) -8: Ferrodoxin reductase NADH peroxidase, Npx (EC 1.11.1.1)	-3: Ferroxidase (EC 1.16.3.1) Glutathione reductase -5: NADH oxidase
Membrane transport	-2: Kup (K ⁺ uptake) Mg ²⁺ /Co ²⁺ transporter Na ⁺ :H ⁺ antiporter Voltage-gated Cl ⁻ channel family protein -3: Serine transporter; Co ²⁺ /Zn ²⁺ /Cd ²⁺ resistance Two ABC transporters -5: Amino acid ABC transporter -6: Peptide ABC transporter ATP-binding protein, ComA	-1: Hop-tolerance, HorA -2: Hop-tolerance, HorC CrcB protein (ion transport) -3: Hop-tolerance, HitA Mg ²⁺ /Co ²⁺ , CorA -4: Three ABC transporters -8: Two Xyloside transporter, XynT	-4: ABC transporter -6: Two ABC transporters -8: Hop-tolerance, HorA

Catalytic activity	<p>-1: Cellulose synthase catalytic subunit [UDP-forming] (EC 2.4.1.12) Glycosyl transferase</p> <p>-2: Histidinol-phosphatase (EC 3.1.3.15) GCN5-related N-acetyltransferase</p> <p>-3: Long-chain-fatty-acid, CoA ligase Phosphopantetheinyl transferase</p> <p>-5: Aspartate racemase (EC 5.1.1.13) Disulfide isomerase GCN5-related N-acetyltransferase Nicotinate phosphoribosyltransferase</p>	<p>-1: Glycosyl transferase Phospho-beta-glycosidase</p> <p>-2: Enolase (EC 4.2.1.11) Glycosyltransferase</p> <p>-8: SAM-dependent methyltransferase Pyridoxamine-phosphate oxidase-related, FMN-binding D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95)</p>	<p>-8: Two cytosine deaminase (EC 3.5.4.1) Glycosyl transferase Phospho-beta-glycosidase</p>
Cell wall	No related products	<p>-2: Teichoic acid glycosylation protein</p> <p>-4: Lysozyme M1 (1,4-beta-N-acetylmuramidase) N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28)</p>	No related products

* Categories as presented in Figure 4.

† Plasmid numbers for each isolate are denoted as -#: (e.g., pLb464-2 and pKB290-2 are both indicated as -2: in the respective columns of each isolate).

Figure Legends

Fig. 1. Results of BADGE comparisons between *L. brevis* isolate groups. Circles represent pairwise comparisons performed to determine the number of DMGs for each compared group. The (%) occurrence provided in each circle corresponds to the number of isolates within that group for which DMGs could be found based on the given comparison.

Fig. 2. Pangenome matrix tree of *L. brevis* genomes. Hierarchical clustering performed as in (Contreras-Moreira and Vinuesa, 2013) with the Ward D2 method (uses squared Euclidean distances) and Gower's distance to determine dissimilarity scores. The height scale indicates the relatedness between clusters with increasing distance indicating greater dissimilarity.

Fig. 3. Average nucleotide identity heatmap of 19 *L. brevis* genomes. Distances were calculated using Gower's dissimilarity coefficient from an absence-presence table including possible multiple gene counts for each gene. Different shading represents different hierarchical clusters.

Fig. 4. Functional plasmid coding capacity of LAB with multiple plasmids. Cryptic plasmids only have coding capacity for their own replication. Transcripts in the categories of catalytic activity, cell wall, membrane transport, oxidative stress, transcriptional regulators and virulence/disease are further described in Table 4.

Table S1. BADGE output for BSR *L. brevis* genomes vs. Food *L. brevis* g
(Clinical + Food).

BSR DMG_ID	% occurrence in "Beer"	dc_blast_hit: no/yes if a DMG has a remaining dc-megablast hit with quality values below BADGE within the 'opposed' group (vs group B) [8]		
		Beer vs Food	Beer vs Clinical	Beer vs Clinical + Food
BSR DMG_1	100	yes	Not DMG	Not DMG
BSR DMG_2	100	no	Not DMG	Not DMG
BSR DMG_3	100	no	Not DMG	Not DMG
BSR DMG_4	100	no	Not DMG	Not DMG
BSR DMG_5	100	no	no	no
BSR DMG_6	100	no	no	no
BSR DMG_7	100	no	Not DMG	Not DMG
BSR DMG_8	100	no	Not DMG	Not DMG

BSR DMG_9	100	yes	Not DMG	Not DMG
BSR DMG_10	100	no	Not DMG	Not DMG
BSR DMG_11	100	no	Not DMG	Not DMG
BSR DMG_12	100	no	no	yes
BSR DMG_13	100	no	Not DMG	Not DMG
BSR DMG_14	100	no	Not DMG	Not DMG
BSR DMG_15	100	no	no	no
BSR DMG_16	100	yes	yes	yes
BSR DMG_17	100	no	Not DMG	Not DMG

BSR DMG_18	100	no	no	no
BSR DMG_19	100	no	Not DMG	Not DMG
BSR DMG_20	100	yes	Not DMG	Not DMG
BSR DMG_21	100	no	Not DMG	Not DMG
BSR DMG_22	100	no	Not DMG	Not DMG
BSR DMG_23	100	no	no	no
BSR DMG_24	100	no	no	no
BSR DMG_25	100	no	Not DMG	Not DMG
BSR DMG_26	100	no	Not DMG	Not DMG
BSR DMG_27	100	no	Not DMG	Not DMG

BSR DMG_28	100	yes	Not DMG	Not DMG
BSR DMG_29	100	no	Not DMG	Not DMG
BSR DMG_30	100	no	Not DMG	Not DMG
BSR DMG_31	100	no	Not DMG	Not DMG
BSR DMG_32	100	yes	Not DMG	Not DMG
BSR DMG_33	100	no	yes	yes
BSR DMG_34	100	no	Not DMG	Not DMG
BSR DMG_35	100	no	no	no
BSR DMG_36	100	no	no	no

BSR DMG_37	100	no	no	no
BSR DMG_38	100	no	no	no
BSR DMG_39	100	no	no	no
BSR DMG_40	100	no	no	no
BSR DMG_41	100	yes	yes	yes
BSR DMG_42	100	no	Not DMG	Not DMG
BSR DMG_43	100	no	Not DMG	Not DMG
BSR DMG_44	100	no	Not DMG	Not DMG
BSR DMG_45	100	no	Not DMG	Not DMG
BSR DMG_46	100	no	Not DMG	Not DMG

Draft

enomes. 46 DMGs were produced when comparing Beer vs. Food groups. The occurrence of a

max_blastn_hit_length: maximum length of a blastn hit produced by a DMG in the 'opposed' group (vs group B) [8]			ORF_ID	
Beer vs Food	Beer vs Clinical	Beer vs Clinical + Food		Beer vs Food
45	Not DMG	Not DMG	Lb310	183
			Lb464	183
			TMW 1.313	183
			TMW 1.465	183
21	Not DMG	Not DMG	Lb310	186
			Lb464	186
			TMW 1.313	186
			TMW 1.465	186
25	Not DMG	Not DMG	Lb310	276
			Lb464	276
			TMW 1.313	276
			TMW 1.465	276
21	Not DMG	Not DMG	Lb310	144
			Lb464	144
			TMW 1.313	144
			TMW 1.465	144
22	29	29	Lb310	150
			Lb464	150
			TMW 1.313	150
			TMW 1.465	150
30	32	32	Lb310	495
			Lb464	495
			TMW 1.313	495
			TMW 1.465	495
38	Not DMG	Not DMG	Lb310	1947
			Lb464	1947
			TMW 1.313	1947
			TMW 1.465	1947
32	Not DMG	Not DMG	Lb310	945
			Lb464	945

			TMW 1.313	945
			TMW 1.465	945
171	Not DMG	Not DMG	Lb310	171
			Lb464	171
			TMW 1.313	171
			TMW 1.465	171
33	Not DMG	Not DMG	Lb310	849
			Lb464	849
			TMW 1.313	849
			TMW 1.465	849
25	Not DMG	Not DMG	Lb310	843
			Lb464	843
			TMW 1.313	843
			TMW 1.465	843
31	194	194	Lb310	645
			Lb464	645
			TMW 1.313	645
			TMW 1.465	645
21	Not DMG	Not DMG	Lb310	918
			Lb464	918
			TMW 1.313	918
			TMW 1.465	918
33	Not DMG	Not DMG	Lb310	483
			Lb464	483
			TMW 1.313	483
			TMW 1.465	483
45	45	45	Lb310	537
			Lb464	537
			TMW 1.313	537
			TMW 1.465	537
41	80	80	Lb310	1116
			Lb464	1116
			TMW 1.313	1116
			TMW 1.465	1116
24	Not DMG	Not DMG	Lb310	153
			Lb464	153
			TMW 1.313	153
			TMW 1.465	153

30	30	30	Lb310	399
			Lb464	399
			TMW 1.313	399
			TMW 1.465	399
25	Not DMG	Not DMG	Lb310	2274
			Lb464	2208
			TMW 1.313	2208
			TMW 1.465	2208
315	Not DMG	Not DMG	Lb310	1785
			Lb464	1785
			TMW 1.313	1785
			TMW 1.465	1785
49	Not DMG	Not DMG	Lb310	2433
			Lb464	2469
			TMW 1.313	2382
			TMW 1.465	2433
28	Not DMG	Not DMG	Lb310	1164
			Lb464	1164
			TMW 1.313	1164
			TMW 1.465	1164
32	32	32	Lb310	1206
			Lb464	1206
			TMW 1.313	1152
			TMW 1.465	1206
25	24	25	Lb310	1302
			Lb464	1302
			TMW 1.313	1302
			TMW 1.465	1302
29	Not DMG	Not DMG	Lb310	690
			Lb464	690
			TMW 1.313	690
			TMW 1.465	690
43	Not DMG	Not DMG	Lb310	483
			Lb464	483
			TMW 1.313	483
			TMW 1.465	483
19	Not DMG	Not DMG	Lb310	480
			Lb464	480

			TMW 1.313	480
			TMW 1.465	480
1066	Not DMG	Not DMG	Lb310	1074
			Lb464	1074
			TMW 1.313	1089
			TMW 1.465	1074
34	Not DMG	Not DMG	Lb310	666
			Lb464	666
			TMW 1.313	666
			TMW 1.465	666
31	Not DMG	Not DMG	Lb310	1566
			Lb464	1566
			TMW 1.313	1566
			TMW 1.465	1566
30	Not DMG	Not DMG	Lb310	366
			Lb464	366
			TMW 1.313	366
			TMW 1.465	366
937	Not DMG	Not DMG	Lb310	963
			Lb464	963
			TMW 1.313	963
			TMW 1.465	963
38	153	153	Lb310	1074
			Lb464	1074
			TMW 1.313	1074
			TMW 1.465	1074
22	Not DMG	Not DMG	Lb310	1338
			Lb464	1338
			TMW 1.313	1338
			TMW 1.465	1338
36	36	36	Lb310	897
			Lb464	897
			TMW 1.313	897
			TMW 1.465	897
25	33	33	Lb310	651
			Lb464	651
			TMW 1.313	651
			TMW 1.465	651

34	25	25	Lb310	771
			Lb464	771
			TMW 1.313	771
			TMW 1.465	771
33	25	25	Lb310	894
			Lb464	894
			TMW 1.313	894
			TMW 1.465	894
21	24	25	Lb310	423
			Lb464	423
			TMW 1.313	423
			TMW 1.465	423
21	27	27	Lb310	483
			Lb464	483
			TMW 1.313	483
			TMW 1.465	483
1308	1309	1309	Lb310	1728
			Lb464	1728
			TMW 1.313	1728
			TMW 1.465	1728
29	Not DMG	Not DMG	Lb310	402
			Lb464	402
			TMW 1.313	402
			TMW 1.465	402
228	Not DMG	Not DMG	Lb310	297
			Lb464	297
			TMW 1.313	297
			TMW 1.465	297
36	Not DMG	Not DMG	Lb310	813
			Lb464	813
			TMW 1.313	813
			TMW 1.465	813
24	Not DMG	Not DMG	Lb310	789
			Lb464	798
			TMW 1.313	798
			TMW 1.465	798
36	Not DMG	Not DMG	Lb310	147
			Lb464	147

			TMW 1.313	147
			TMW 1.465	147

Draft

given DMG in Beer vs. Clinical and Beer vs. C

ORF_length	
Beer vs Clinical	Beer vs Clinical + Food
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
150	150
495	495
	Undecaprenyl-phosp
	Undecaprer
	Undecapren
Not DMG	Not DMG
Not DMG	Not DMG
	D-alanyl-D-a

	D-2
	D-2
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
645	645
Not DMG	Not DMG
Not DMG	Not DMG
537	537
1116	1116
	Alc
Not DMG	Not DMG

399	399
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
1206	1206
"	"
1152	1152
1206	1206
1302	1302
	Pol
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
	Ribose 5

Draft

Not DMG	Not DMG
	Man
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
	2-dehydro-3
	2-de
	2-c
1074	1074
	Pr
Not DMG	Not DMG
897	897
651	651

771	771
	PTS system glucc
	PTS syst
	PTS syst
894	894
423	423
	PTS system man
	PTS syst
	PTS syst
483	483
	PTS system glucc
	PTS syst
	PTS syst
1728	1728
	Phosphoenolpyruvate-pro
	Phosphoenolpyru
	Phosphoenolpyru
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG
Not DMG	Not DMG

Draft

Clinical + Food are indicated. Bold rows indicate a DMG demarcating "Beer" genomes from all others

annotation
hypothetical protein scaffold38_1 4804-4986
hypothetical protein gi_741039890_gb_CP005977_1_ 622457-622639
hypothetical protein JXUF01000002_1 2342417-2342599
hypothetical protein JXUG01000002_1 90634-90816
hypothetical protein scaffold10_1 53221-53406
hypothetical protein gi_741039890_gb_CP005977_1_ 395316-395501
hypothetical protein JXUF01000002_1 1865605-1865790
hypothetical protein JXUG01000030_1 11125-11310
hypothetical protein scaffold10_1 53570-53845
hypothetical protein gi_741039890_gb_CP005977_1_ 395665-395940
hypothetical protein JXUF01000002_1 1865954-1866229
hypothetical protein JXUG01000030_1 11474-11749
hypothetical protein scaffold10_1 59748-59891
hypothetical protein gi_741039890_gb_CP005977_1_ 403060-403203
hypothetical protein JXUF01000002_1 1873181-1873324
hypothetical protein JXUG01000030_1 17860-18003
hypothetical protein scaffold41_1 19588-19737
hypothetical protein gi_741039890_gb_CP005977_1_ 1174409-1174558
hypothetical protein JXUF01000002_1 129210-129359
hypothetical protein JXUG01000003_1 861085-861234
phenyl-phosphate galactosephosphotransferase_EC 2_7_8_6;Ontology_term_KEGG_ENZYME_2_7_8_6 scaffold41_1 1977
phenyl-phosphate galactosephosphotransferase_EC 2_7_8_6;Ontology_term_KEGG_ENZYME_2_7_8_6 gi_741039890_gb_CP005977_1_ 622457-622639
phenyl-phosphate galactosephosphotransferase_EC 2_7_8_6;Ontology_term_KEGG_ENZYME_2_7_8_6 JXUF01000002_1 128
phenyl-phosphate galactosephosphotransferase_EC 2_7_8_6;Ontology_term_KEGG_ENZYME_2_7_8_6 JXUG01000003_1 861
cell wall surface anchor family protein scaffold47_1 883-2829
cell wall surface anchor family protein gi_741039890_gb_CP005977_1_ 564303-566249
Collagen adhesion protein JXUF01000002_1 2403661-2405607
cell wall surface anchor family protein JXUG01000002_1 26880-28826
D-alanyl-D-alanine carboxypeptidase_EC 3_4_16_4;Ontology_term_KEGG_ENZYME_3_4_16_4 scaffold49_1 307-1251
D-alanyl-D-alanine carboxypeptidase_EC 3_4_16_4;Ontology_term_KEGG_ENZYME_3_4_16_4 gi_741039890_gb_CP005977_1_ 24638

alanyl-D-alanine carboxypeptidase_EC 3_4_16_4;Ontology_term_KEGG_ENZYME_3_4_16_4 JXUF01000002_1 845884-8468
alanyl-D-alanine carboxypeptidase_EC 3_4_16_4;Ontology_term_KEGG_ENZYME_3_4_16_4 JXUG01000003_1 152645-1535
hypothetical protein scaffold49_1 1809-1979
hypothetical protein gi_741039890_gb_CP005977_1_ 2465314-2465484
hypothetical protein JXUF01000002_1 847386-847556
hypothetical protein JXUG01000003_1 151917-152087
hypothetical protein scaffold5_1 63702-64550
hypothetical protein gi_741039890_gb_CP005977_1_ 2247689-2248537
hypothetical protein JXUF01000002_1 620578-621426
hypothetical protein JXUG01000003_1 378973-379821
hypothetical protein scaffold5_1 64580-65422
hypothetical protein gi_741039890_gb_CP005977_1_ 2246817-2247659
hypothetical protein JXUF01000002_1 619706-620548
hypothetical protein JXUG01000003_1 379851-380693
Transcriptional regulator TetR family scaffold53_1 2641-3285
Transcriptional regulator TetR family gi_741042308_gb_CP005980_1_ 3529-4173
Transcriptional regulator TetR family JXUF01000010_1 15838-16482
Transcriptional regulator TetR family JXUG01000011_1 6173-6817
hypothetical protein scaffold57_1 7611-8528
hypothetical protein gi_741039890_gb_CP005977_1_ 272817-273734
hypothetical protein JXUF01000002_1 1728498-1729415
hypothetical protein JXUG01000028_1 36827-37744
RNA polymerase sigma factor ECF subfamily scaffold57_1 8521-9003
RNA polymerase sigma factor ECF subfamily gi_741039890_gb_CP005977_1_ 272342-272824
RNA polymerase sigma factor ECF subfamily JXUF01000002_1 1728023-1728505
RNA polymerase sigma factor ECF subfamily JXUG01000028_1 37737-38219
hypothetical protein scaffold86_1 714-1250
hypothetical protein gi_741039890_gb_CP005977_1_ 283339-283875
hypothetical protein JXUF01000002_1 1739365-1739901
hypothetical protein JXUG01000028_1 26341-26877
Alcohol dehydrogenase_EC 1_1_1_1;Ontology_term_KEGG_ENZYME_1_1_1_1 scaffold86_1 1337-2452
alcohol dehydrogenase_EC 1_1_1_1;Ontology_term_KEGG_ENZYME_1_1_1_1 gi_741039890_gb_CP005977_1_ 283962-2850
Alcohol dehydrogenase_EC 1_1_1_1;Ontology_term_KEGG_ENZYME_1_1_1_1 JXUF01000002_1 1739988-1741103
Alcohol dehydrogenase_EC 1_1_1_1;Ontology_term_KEGG_ENZYME_1_1_1_1 JXUG01000028_1 25139-26254
hypothetical protein scaffold86_1 3306-3458
hypothetical protein gi_741039890_gb_CP005977_1_ 285931-286083
hypothetical protein JXUF01000002_1 1744095-1744247
hypothetical protein JXUG01000028_1 24133-24285

Phage-related protein scaffold86_1 3512-3910
Phage-related protein gi_741039890_gb_CP005977_1_286137-286535
Phage-related protein JXUF01000002_1 1744301-1744699
Phage-related protein JXUG01000028_1 23681-24079
muramidase scaffold9_1 2622-4895
FIG00752971_ hypothetical protein gi_741039890_gb_CP005977_1_2461544-2463751
FIG00752971_ hypothetical protein JXUF01000002_1 842398-844605
FIG00752971_ hypothetical protein JXUG01000003_1 154866-157073
Phage capsid assembly protein scaffold9_1 5669-7453
FIG00752971_ hypothetical protein gi_741039890_gb_CP005977_1_2458920-2460704
FIG00752971_ hypothetical protein JXUF01000002_1 839778-841562
FIG00752971_ hypothetical protein JXUG01000003_1 157912-159696
Internalin-like protein _LPXTG motif_ Lmo2026 homolog scaffold9_1 54438-56870
Internalin-like protein _LPXTG motif_ Lmo2026 homolog gi_741039890_gb_CP005977_1_2408174-2410642
Internalin-like protein _LPXTG motif_ Lmo2026 homolog JXUF01000002_1 790105-792486
Internalin-like protein _LPXTG motif_ Lmo2026 homolog JXUG01000003_1 206547-208979
hypothetical protein scaffold9_1 62527-63690
hypothetical protein gi_741039890_gb_CP005977_1_2401354-2402517
hypothetical protein JXUF01000002_1 783255-784418
hypothetical protein JXUG01000003_1 214636-215799
Oligogalacturonide transporter scaffold9_1 72493-73698
Oligogalacturonide transporter gi_741039890_gb_CP005977_1_2391430-2392635
Oligogalacturonide transporter JXUF01000002_1 774953-776104
Oligogalacturonide transporter JXUG01000003_1 223158-224363
Polygalacturonase _EC 3_2_1_15;Ontology_term_KEGG_ENZYME_3_2_1_15 scaffold9_1 74047-75348
Polygalacturonase _EC 3_2_1_15;Ontology_term_KEGG_ENZYME_3_2_1_15 gi_741039890_gb_CP005977_1_2389780-2391430
Polygalacturonase _EC 3_2_1_15;Ontology_term_KEGG_ENZYME_3_2_1_15 JXUF01000002_1 773249-774550
Polygalacturonase _EC 3_2_1_15;Ontology_term_KEGG_ENZYME_3_2_1_15 JXUG01000003_1 224712-226013
hypothetical protein scaffold12_1 5214-5903
hypothetical protein gi_741039890_gb_CP005977_1_1973886-1974575
hypothetical protein JXUF01000002_1 382415-383104
hypothetical protein JXUG01000003_1 612932-613621
Transcriptional regulator MerR family scaffold13_1 56552-57034
Transcriptional regulator MerR family gi_741039890_gb_CP005977_1_2151597-2152079
Transcriptional regulator MerR family JXUF01000002_1 524516-524998
Transcriptional regulator MerR family JXUG01000003_1 474737-475219
Ribose 5-phosphate isomerase B _EC 5_3_1_6;Ontology_term_KEGG_ENZYME_5_3_1_6 scaffold13_1 57334-57813
Ribose 5-phosphate isomerase B _EC 5_3_1_6;Ontology_term_KEGG_ENZYME_5_3_1_6 gi_741039890_gb_CP005977_1_2150818-2150818

Ribose 5-phosphate isomerase B_EC 5_3_1_6;Ontology_term_KEGG_ENZYME_5_3_1_6 JXUF01000002_1 523738-524217
Ribose 5-phosphate isomerase B_EC 5_3_1_6;Ontology_term_KEGG_ENZYME_5_3_1_6 JXUG01000003_1 475519-475998
Mannonate dehydratase_EC 4_2_1_8;Ontology_term_KEGG_ENZYME_4_2_1_8 scaffold1_1 49024-50097
nonate dehydratase_EC 4_2_1_8;Ontology_term_KEGG_ENZYME_4_2_1_8 gi_741039890_gb_CP005977_1_ 1869845-1871
Mannonate dehydratase_EC 4_2_1_8;Ontology_term_KEGG_ENZYME_4_2_1_8 JXUF01000002_1 1074275-1075363
Mannonate dehydratase_EC 4_2_1_8;Ontology_term_KEGG_ENZYME_4_2_1_8 JXUG01000016_1 452657-453730
Transcriptional regulator GntR family scaffold1_1 50446-51111
Transcriptional regulator GntR family gi_741039890_gb_CP005977_1_ 1871267-1871932
Transcriptional regulator GntR family JXUF01000002_1 1073261-1073926
Transcriptional regulator GntR family JXUG01000016_1 454079-454744
Oligogalacturonide transporter scaffold1_1 51340-52905
Oligogalacturonide transporter gi_741039890_gb_CP005977_1_ 1872161-1873726
Oligogalacturonide transporter JXUF01000002_1 1071467-1073032
Oligogalacturonide transporter JXUG01000016_1 454973-456538
hypothetical protein scaffold1_1 52913-53278
hypothetical protein gi_741039890_gb_CP005977_1_ 1873734-1874099
hypothetical protein JXUF01000002_1 1071094-1071459
hypothetical protein JXUG01000016_1 456546-456911
2-dehydro-3-deoxygluconate kinase_EC 2_7_1_45;Ontology_term_KEGG_ENZYME_2_7_1_45 scaffold1_1 54783-55745
3-deoxygluconate kinase_EC 2_7_1_45;Ontology_term_KEGG_ENZYME_2_7_1_45 gi_741039890_gb_CP005977_1_ 187560
2-dehydro-3-deoxygluconate kinase_EC 2_7_1_45;Ontology_term_KEGG_ENZYME_2_7_1_45 JXUF01000002_1 1068631-1069
2-dehydro-3-deoxygluconate kinase_EC 2_7_1_45;Ontology_term_KEGG_ENZYME_2_7_1_45 JXUG01000016_1 458416-4593
Putative transcriptional regulator with a sugar kinase domain GntR family scaffold16_1 276-1349
Putative transcriptional regulator with a sugar kinase domain GntR family gi_741039890_gb_CP005977_1_ 2387377-238845
Putative transcriptional regulator with a sugar kinase domain GntR family JXUF01000002_1 771411-772484
Putative transcriptional regulator with a sugar kinase domain GntR family JXUG01000003_1 227119-228192
Fructuronate transporter GntP scaffold1_1 56607-57944
Fructuronate transporter GntP gi_741039890_gb_CP005977_1_ 1877428-1878765
Fructuronate transporter GntP JXUF01000002_1 1066432-1067769
Fructuronate transporter GntP JXUG01000016_1 460240-461577
LysR family transcriptional regulator HdfR scaffold16_1 14277-15173
LysR family transcriptional regulator HdfR gi_741039890_gb_CP005977_1_ 2372528-2373424
LysR family transcriptional regulator HdfR JXUF01000002_1 757182-758078
LysR family transcriptional regulator HdfR JXUG01000003_1 241119-242015
Phosphoglycerate mutase family scaffold16_1 15199-15849
Phosphoglycerate mutase family gi_741039890_gb_CP005977_1_ 2371852-2372502
Phosphoglycerate mutase family JXUF01000002_1 756506-757156
Phosphoglycerate mutase family JXUG01000003_1 242041-242691

system gluconate-specific IIC component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 scaffold16_1 16019-16
onate-specific IIC component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 gi_741039890_gb_CP005977_1_2
tem gluconate-specific IIC component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 JXUF01000002_1 755567-
em gluconate-specific IIC component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 JXUG01000003_1 242861-
system mannose-specific IID component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 scaffold16_1 16804-17
PTS system mannose-specific IID component gi_741039890_gb_CP005977_1_2370004-2370897
PTS system mannose-specific IID component JXUF01000002_1 754659-755552
PTS system mannose-specific IID component JXUG01000003_1 243646-244539
system mannose-specific IIA component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 scaffold16_1 17712-18
nose-specific IIA component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 gi_741039890_gb_CP005977_1_2
tem mannose-specific IIA component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 JXUF01000002_1 754222-
tem mannose-specific IIA component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 JXUG01000003_1 244554-
system gluconate-specific IIB component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 scaffold16_1 18156-18
onate-specific IIB component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 gi_741039890_gb_CP005977_1_2
tem gluconate-specific IIB component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 JXUF01000002_1 753718-
em gluconate-specific IIB component_EC 2_7_1_69;Ontology_term_KEGG_ENZYME_2_7_1_69 JXUG01000003_1 244998
pyruvate-protein phosphotransferase of PTS system_EC 2_7_3_9;Ontology_term_KEGG_ENZYME_2_7_3_9 scaffold16_1
rotein phosphotransferase of PTS system_EC 2_7_3_9;Ontology_term_KEGG_ENZYME_2_7_3_9 gi_741039890_gb_CP005
uvate-protein phosphotransferase of PTS system_EC 2_7_3_9;Ontology_term_KEGG_ENZYME_2_7_3_9 JXUF01000002_1
uvate-protein phosphotransferase of PTS system_EC 2_7_3_9;Ontology_term_KEGG_ENZYME_2_7_3_9 JXUG01000003_
hypothetical protein scaffold16_1 22587-22988
hypothetical protein gi_741039890_gb_CP005977_1_2363519-2363920
hypothetical protein JXUF01000002_1 749165-749566
hypothetical protein JXUG01000003_1 249430-249831
Mobile element protein scaffold16_1 23735-24031
Mobile element protein gi_741039890_gb_CP005977_1_2362476-2362772
Mobile element protein JXUF01000002_1 748123-748419
Mobile element protein JXUG01000003_1 250578-250874
short chain dehydrogenase scaffold21_1 35280-36092
short chain dehydrogenase gi_741039890_gb_CP005977_1_698563-699375
short chain dehydrogenase JXUF01000002_1 2266181-2266993
short chain dehydrogenase JXUG01000002_1 164624-165436
Transcriptional regulator AraC family scaffold21_1 36118-36906
Transcriptional regulator AraC family gi_741039890_gb_CP005977_1_699392-700189
Transcriptional regulator AraC family JXUF01000002_1 2265367-2266164
Transcriptional regulator AraC family JXUG01000002_1 165453-166250
Transcriptional regulator AraC family scaffold21_1 36941-37087
Transcriptional regulator AraC family gi_741039890_gb_CP005977_1_700224-700370

Transcriptional regulator AraC family JXUF01000002_1 2265186-2265332
Transcriptional regulator AraC family JXUG01000002_1 166285-166431

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contig	start	stop
scaffold38_1	4804	4986
gi_741039890_gb_CP005977_1_	622457	622639
JXUF01000002_1	2342599	2342417
JXUG01000002_1	90634	90816
scaffold10_1	53406	53221
gi_741039890_gb_CP005977_1_	395501	395316
JXUF01000002_1	1865790	1865605
JXUG01000030_1	11310	11125
scaffold10_1	53570	53845
gi_741039890_gb_CP005977_1_	395665	395940
JXUF01000002_1	1865954	1866229
JXUG01000030_1	11474	11749
scaffold10_1	59748	59891
gi_741039890_gb_CP005977_1_	403060	403203
JXUF01000002_1	1873181	1873324
JXUG01000030_1	17860	18003
scaffold41_1	19588	19737
gi_741039890_gb_CP005977_1_	1174558	1174409
JXUF01000002_1	129359	129210
JXUG01000003_1	861085	861234
scaffold41_1	19773	20267
gi_741039890_gb_CP005977_1_	1174373	1173879
JXUF01000002_1	129174	128680
JXUG01000003_1	861270	861764
scaffold47_1	2829	883
gi_741039890_gb_CP005977_1_	564303	566249
JXUF01000002_1	2405607	2403661
JXUG01000002_1	26880	28826
scaffold49_1	1251	307
gi_741039890_gb_CP005977_1_	2464756	2463812

JXUF01000002_1	846828	845884
JXUG01000003_1	152645	153589
scaffold49_1	1809	1979
gi_741039890_gb_CP005977_1_	2465314	2465484
JXUF01000002_1	847386	847556
JXUG01000003_1	152087	151917
scaffold5_1	64550	63702
gi_741039890_gb_CP005977_1_	2247689	2248537
JXUF01000002_1	620578	621426
JXUG01000003_1	379821	378973
scaffold5_1	65422	64580
gi_741039890_gb_CP005977_1_	2246817	2247659
JXUF01000002_1	619706	620548
JXUG01000003_1	380693	379851
scaffold53_1	3285	2641
gi_741042308_gb_CP005980_1_	3529	4173
JXUF01000010_1	15838	16482
JXUG01000011_1	6817	6173
scaffold57_1	8528	7611
gi_741039890_gb_CP005977_1_	272817	273734
JXUF01000002_1	1728498	1729415
JXUG01000028_1	37744	36827
scaffold57_1	9003	8521
gi_741039890_gb_CP005977_1_	272342	272824
JXUF01000002_1	1728023	1728505
JXUG01000028_1	38219	37737
scaffold86_1	714	1250
gi_741039890_gb_CP005977_1_	283339	283875
JXUF01000002_1	1739365	1739901
JXUG01000028_1	26877	26341
scaffold86_1	1337	2452
gi_741039890_gb_CP005977_1_	283962	285077
JXUF01000002_1	1739988	1741103
JXUG01000028_1	26254	25139
scaffold86_1	3306	3458
gi_741039890_gb_CP005977_1_	285931	286083
JXUF01000002_1	1744095	1744247
JXUG01000028_1	24285	24133

scaffold86_1	3910	3512
gi_741039890_gb_CP005977_1_	286535	286137
JXUF01000002_1	1744699	1744301
JXUG01000028_1	23681	24079
scaffold9_1	4895	2622
gi_741039890_gb_CP005977_1_	2461544	2463751
JXUF01000002_1	842398	844605
JXUG01000003_1	157073	154866
scaffold9_1	7453	5669
gi_741039890_gb_CP005977_1_	2458920	2460704
JXUF01000002_1	839778	841562
JXUG01000003_1	159696	157912
scaffold9_1	54438	56870
gi_741039890_gb_CP005977_1_	2410642	2408174
JXUF01000002_1	792486	790105
JXUG01000003_1	206547	208979
scaffold9_1	63690	62527
gi_741039890_gb_CP005977_1_	2401354	2402517
JXUF01000002_1	783255	784418
JXUG01000003_1	215799	214636
scaffold9_1	73698	72493
gi_741039890_gb_CP005977_1_	2391430	2392635
JXUF01000002_1	774953	776104
JXUG01000003_1	224363	223158
scaffold9_1	75348	74047
gi_741039890_gb_CP005977_1_	2389780	2391081
JXUF01000002_1	773249	774550
JXUG01000003_1	226013	224712
scaffold12_1	5214	5903
gi_741039890_gb_CP005977_1_	1973886	1974575
JXUF01000002_1	382415	383104
JXUG01000003_1	613621	612932
scaffold13_1	57034	56552
gi_741039890_gb_CP005977_1_	2151597	2152079
JXUF01000002_1	524516	524998
JXUG01000003_1	475219	474737
scaffold13_1	57334	57813
gi_741039890_gb_CP005977_1_	2151297	2150818

JXUF01000002_1	524217	523738
JXUG01000003_1	475519	475998
scaffold1_1	50097	49024
gi_741039890_gb_CP005977_1_	1870918	1869845
JXUF01000002_1	1074275	1075363
JXUG01000016_1	453730	452657
scaffold1_1	50446	51111
gi_741039890_gb_CP005977_1_	1871267	1871932
JXUF01000002_1	1073926	1073261
JXUG01000016_1	454079	454744
scaffold1_1	51340	52905
gi_741039890_gb_CP005977_1_	1872161	1873726
JXUF01000002_1	1073032	1071467
JXUG01000016_1	454973	456538
scaffold1_1	52913	53278
gi_741039890_gb_CP005977_1_	1873734	1874099
JXUF01000002_1	1071459	1071094
JXUG01000016_1	456546	456911
scaffold1_1	54783	55745
gi_741039890_gb_CP005977_1_	1875604	1876566
JXUF01000002_1	1069593	1068631
JXUG01000016_1	458416	459378
scaffold16_1	1349	276
gi_741039890_gb_CP005977_1_	2387377	2388450
JXUF01000002_1	771411	772484
JXUG01000003_1	228192	227119
scaffold1_1	56607	57944
gi_741039890_gb_CP005977_1_	1877428	1878765
JXUF01000002_1	1067769	1066432
JXUG01000016_1	460240	461577
scaffold16_1	15173	14277
gi_741039890_gb_CP005977_1_	2372528	2373424
JXUF01000002_1	757182	758078
JXUG01000003_1	242015	241119
scaffold16_1	15849	15199
gi_741039890_gb_CP005977_1_	2371852	2372502
JXUF01000002_1	756506	757156
JXUG01000003_1	242691	242041

scaffold16_1	16019	16789
gi_741039890_gb_CP005977_1_	2371682	2370912
JXUF01000002_1	756337	755567
JXUG01000003_1	242861	243631
scaffold16_1	16804	17697
gi_741039890_gb_CP005977_1_	2370897	2370004
JXUF01000002_1	755552	754659
JXUG01000003_1	243646	244539
scaffold16_1	17712	18134
gi_741039890_gb_CP005977_1_	2369989	2369567
JXUF01000002_1	754644	754222
JXUG01000003_1	244554	244976
scaffold16_1	18156	18638
gi_741039890_gb_CP005977_1_	2369545	2369063
JXUF01000002_1	754200	753718
JXUG01000003_1	244998	245480
scaffold16_1	19228	20955
gi_741039890_gb_CP005977_1_	2367279	2365552
JXUF01000002_1	753128	751401
JXUG01000003_1	246070	247797
scaffold16_1	22587	22988
gi_741039890_gb_CP005977_1_	2363920	2363519
JXUF01000002_1	749566	749165
JXUG01000003_1	249430	249831
scaffold16_1	24031	23735
gi_741039890_gb_CP005977_1_	2362476	2362772
JXUF01000002_1	748123	748419
JXUG01000003_1	250874	250578
scaffold21_1	35280	36092
gi_741039890_gb_CP005977_1_	698563	699375
JXUF01000002_1	2266993	2266181
JXUG01000002_1	164624	165436
scaffold21_1	36118	36906
gi_741039890_gb_CP005977_1_	699392	700189
JXUF01000002_1	2266164	2265367
JXUG01000002_1	165453	166250
scaffold21_1	36941	37087
gi_741039890_gb_CP005977_1_	700224	700370

JXUF01000002_1	2265332	2265186
JXUG01000002_1	166285	166431

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Table S2 - Three Beer <i>L. brevis</i> DMGs found specific to the Beer vs Clinical comparison.															
Beer vs. Clinical DMG_ID	% occurrence in beer	dc_blast_hit	max_blastn_hit_length	ORF_ID	ORF_length	annotation	contig	start	stop						
DMG_1	100	no	26	Lb310	192	hypothetical protein scaffold4_1 42625-42816	scaffold4_1	42625	42816						
				Lb464	192	hypothetical protein gi_741039890_gb_CP005977_1_874261-874452	gi_741039890_gb_CP005977_1	874452	874261						
				TMW 1.313	192	hypothetical protein JXUF01000002_1 2084117-2084308	JXUF01000002_1	2E+06	2E+06						
				TMW 1.465	192	hypothetical protein JXUG01000007_1 41634-41825	JXUG01000007_1	41634	41825						
DMG_2	100	yes	340	Lb310	900	mosome_plasmid_partitioning protein ParA_Sporulation initiation inhibitor protein Soj scaffold90_1 2467	scaffold90_1	2467	3366						
				Lb464	900	Chromosome_plasmid_partitioning protein ParA gi_741042308_gb_CP005980_1_8720-9619	gi_741042308_gb_CP005980_1	8720	9619						
				TMW 1.313	900	Chromosome_plasmid_partitioning protein ParA JXUF01000010_1 21028-21927	JXUF01000010_1	21028	21927						
				TMW 1.465	900	Chromosome_plasmid_partitioning protein ParA JXUG01000011_1 728-1627	JXUG01000011_1	1627	728						
DMG_3	100	yes	349	Lb310	1047	Mobile element protein scaffold1_1 108198-109244	scaffold1_1	109244	108198						
				Lb464	1047	Mobile element protein gi_741039890_gb_CP005977_1_2167963-2169009	gi_741039890_gb_CP005977_1	293867	294913						
				Lb464	1047	Mobile element protein gi_741039890_gb_CP005977_1_293867-294913	gi_741039890_gb_CP005977_1	710896	711942						
				Lb464	1047	Mobile element protein gi_741039890_gb_CP005977_1_710896-711942	gi_741039890_gb_CP005977_1	2E+06	2E+06						
				TMW 1.313	1047	Mobile element protein JXUF01000002_1 569637-570683	JXUF01000002_1	569637	570683						
				TMW 1.465	1047	Mobile element protein JXUG01000016_1 512616-513662	JXUG01000016_1	513662	512616						
dc_blast_hit: no/yes if a DMG has a remaining dc-megablast hit with quality values below BADGE within the 'opposed' group (vs group B) (Behr et al., 2016)															
max_blastn_hit_length: maximum length of a blastn hit produced by a DMG in the 'opposed' group (vs group B) (Behr et al., 2016)															

Table S3. BADGE output for BSR *L. brevis* isolates from UK (Lb464, Lb310) vs. BSR *L. brevis* isolates from German/Unknown (TMW 1.131, TMW 1.465).

UK DMG_ID	% occurrence in beer	dc_blast_hit	max_blastn_hit_length	ORF ID	ORF_length	annotation	contig	start	stop	Lb464 plasmid/location
UK DMG_1	100	no	18	Lb310	348	hypothetical protein scaffold42_1_8383-8730	scaffold42_1	8730	8383	
				Lb464	348	hypothetical protein gi_741042329_gb_CP005981_1_70077-70424	gi_741042329_gb_CP005981_1	70077	70424	plb464-4
UK DMG_2	100	no	43	Lb310	801	plasmid partition protein2 putative scaffold42_1_8732-9532	scaffold42_1	9532	8732	
				Lb464	801	Chromosome_plasmid_partitioning protein Para gi_741042329_gb_CP005981_1_69275-70075	gi_741042329_gb_CP005981_1	69275	70075	plb464-4
UK DMG_3	100	no	24	Lb310	738	Glycosyltransferase scaffold101_1_1350-2087	scaffold101_1	1350	2087	
				Lb464	738	Glycosyltransferase gi_741039890_gb_CP005977_1_1168652-1169389	gi_741039890_gb_CP005977_1	1169389	1168652	chromosome
UK DMG_4	100	no	16	Lb310	216	FIG00743137_hypothetical protein scaffold53_1_13551-13766	scaffold53_1	13551	13766	
				Lb464	216	FIG00743137_hypothetical protein gi_741042308_gb_CP005980_1_15452-15667	gi_741042308_gb_CP005980_1	15667	15452	plb464-3
UK DMG_5	100	no	20	Lb310	159	hypothetical protein scaffold85_1_1022-1180	scaffold85_1	1180	1022	
				Lb464	159	hypothetical protein gi_741042272_gb_CP005979_1_24886-25044	gi_741042272_gb_CP005979_1	24886	25044	plb464-2
UK DMG_6	100	no	34	Lb310	588	Mobile element protein gi_741042444_gb_CP005982_1_426-1013	scaffold85_1	2179	1592	
				Lb464	588	Mobile element protein gi_741042444_gb_CP005982_1_426-1013	gi_741042444_gb_CP005982_1	1013	426	plb464-5
UK DMG_7	100	no	35	Lb310	279	hypothetical protein scaffold85_1_2283-2561	scaffold85_1	2283	2561	
				Lb464	279	hypothetical protein gi_741042444_gb_CP005982_1_1117-1395	gi_741042444_gb_CP005982_1	1117	1395	plb464-5
UK DMG_8	100	no	23	Lb310	303	hypothetical protein scaffold85_1_2551-2853	scaffold85_1	2551	2853	
				Lb464	303	hypothetical protein gi_741042444_gb_CP005982_1_1385-1687	gi_741042444_gb_CP005982_1	1385	1687	plb464-5
UK DMG_9	100	yes	65	Lb310	849	DegV family protein scaffold91_1_2688-3536	scaffold91_1	2688	3536	
				Lb464	849	DegV family protein gi_741042308_gb_CP005980_1_11233-12081	gi_741042308_gb_CP005980_1	12081	11233	plb464-3
UK DMG_10	100	no	19	Lb310	153	hypothetical protein scaffold91_1_3564-3716	scaffold91_1	3564	3716	
				Lb464	153	hypothetical protein gi_741042308_gb_CP005980_1_11053-11205	gi_741042308_gb_CP005980_1	11205	11053	plb464-3
UK DMG_11	100	no	26	Lb310	924	Plasmid replication initiation protein scaffold94_1_2105-3028	scaffold94_1	3028	2105	
				Lb464	924	Plasmid replication initiation protein gi_741042454_gb_CP005983_1_2482-3405	gi_741042454_gb_CP005983_1	2482	3405	plb464-6
UK DMG_12	100	yes	329	Lb310	2115	ATP-dependent protease ATP-binding subunit clpt scaffold96_1_508-2622	scaffold96_1	2622	508	
				Lb464	2115	ATP-dependent protease ATP-binding subunit clpt gi_741042463_gb_CP005985_1_211-2325	gi_741042463_gb_CP005985_1	211	2325	plb464-8
UK DMG_13	100	no	44	Lb310	678	Phage repressor scaffold19_1_2850-3527	scaffold19_1	3527	2850	
				Lb464	678	Phage repressor gi_741042329_gb_CP005981_1_63570-64247	gi_741042329_gb_CP005981_1	63570	64247	plb464-4
UK DMG_14	100	no	21	Lb310	168	hypothetical protein scaffold19_1_4694-4861	scaffold19_1	4694	4861	
				Lb464	168	hypothetical protein gi_741042329_gb_CP005981_1_61768-61935	gi_741042329_gb_CP005981_1	61935	61768	plb464-4
UK DMG_15	100	no	18	Lb310	204	hypothetical protein scaffold19_1_5303-5506	scaffold19_1	5303	5506	
				Lb464	204	hypothetical protein gi_741042329_gb_CP005981_1_60033-60236	gi_741042329_gb_CP005981_1	60236	60033	plb464-4
UK DMG_16	100	no	27	Lb310	684	hypothetical protein scaffold19_1_6611-7294	scaffold19_1	6611	7294	
				Lb464	684	Phage-associated recombinase gi_741042329_gb_CP005981_1_58494-59177	gi_741042329_gb_CP005981_1	59177	58494	plb464-4
UK DMG_17	100	no	29	Lb310	771	DNA replication protein scaffold19_1_9133-9903	scaffold19_1	9133	9903	
				Lb464	771	Helicase loader DnaI gi_741042329_gb_CP005981_1_54899-55669	gi_741042329_gb_CP005981_1	55669	54899	plb464-4
UK DMG_18	100	no	31	Lb310	324	hypothetical protein scaffold19_1_11236-11559	scaffold19_1	11236	11559	
				Lb464	324	hypothetical protein gi_741042329_gb_CP005981_1_52601-52924	gi_741042329_gb_CP005981_1	52924	52601	plb464-4
UK DMG_19	100	no	28	Lb310	192	hypothetical protein scaffold19_1_11989-12180	scaffold19_1	11989	12180	
				Lb464	195	hypothetical protein gi_741042329_gb_CP005981_1_51315-51509	gi_741042329_gb_CP005981_1	51509	51315	plb464-4
UK DMG_20	100	no	28	Lb310	789	Chromosome_plasmid_partitioning protein Para_Sporulation initiation inhibitor protein Sji scaffold19_1	scaffold19_1	42201	41413	
				Lb464	789	Chromosome_plasmid_partitioning protein Para gi_741042329_gb_CP005981_1_23691-24677	gi_741042329_gb_CP005981_1	23691	24477	plb464-4

dc_blast_hit: no/yes if a DMG has a remaining dc-megablast hit with quality values below BADGE within the 'opposed' group (vs group B) (Behr et al., 2016)

max_blastn_hit_length: maximum length of a blastn hit produced by a DMG in the 'opposed' group (vs group B) (Behr et al., 2016)