Lactobacillus plantarum - beyond great flavor and efficient acidification

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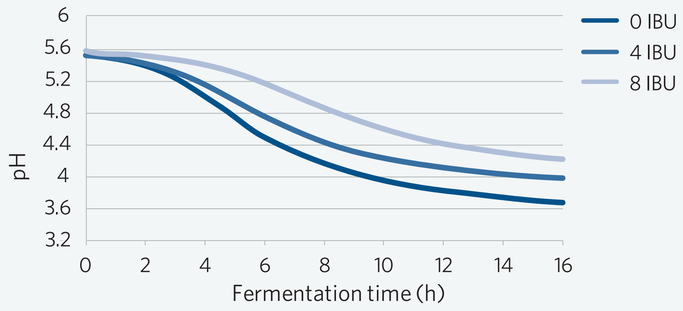
Lactobacillus plantarum cultures have long been the workhorses for the production of Berliner Weisse and Gose style beers. Over time, several practices have been utilized for acidifying wort: from inoculation with spontaneously generated starter cultures to adding yogurt or small bottles of probiotics solutions. However, for most breweries, speed and consistency of kettle souring is paramount as the brew kettle is one of the rate-limiting steps in brewing operations. Chr. Hansen offers the *L. plantarum* strain Harvest LB-1 for fast acidification of the wort, occupying the brew kettle for no more than 16 hours (without pre-acidification needed). In the development of this strain, Chr. Hansen has leveraged our bacterial expertise which we have developed over the last 140 years in the dairy and wine industry. As food safety is of the utmost importance to us, we ensure that all our strains have been verified from being free of antibiotic markers, as well as unable to produce biogenic amine and ethyl carbamate precursor. Here we present Chr. Hansen's *L. plantarum* solution for kettle souring together with our quality control and quality insurance programs towards ensuring safe, and reliable performance for wort acidification. We hope that this level of transparency will help to increase the overall awareness of food safety standards for bacteria applied in brewing and hereby ensure that the consumer can enjoy great sour beer products that are safe to consume.

Harvest LB-1 main features

- Direct inoculation: 1 sachet for 8-10 BBL
- Freeze-dried; can be stored at <0⁰ F
- Dairy free, high cell count & activity
- Fermentation temperature 85 ⁰F (70 – 100 ⁰F)
- Rapidly acidifies wort to pH 3.2
- No pre-acidification required

Souring time

Fastest souring is achieved at low IBU levels. Harvest LB-1 tolerates 8 IBU, which minimizes risk of the cross contamination in the brewery.



Fermentation conditions:
Wort (12°Plato), 30°C

Inoculation:
1 pouch/10hl

Origin, species identity and QPS status

The *L. plantarum* Harvest LB-1 strain originates from a Spanish Winery (fermenting Bobal grapes). Using molecular methods for species identification, the strain has been unambiguously identified as *Lactobacillus plantaru*m.
The species *L. plantarum* has been evaluated by the EFSA Panel on Biological Hazards (BIOHAZ) and based on the long history of safe use found to be suited for the QPS (Qualified Presumption of Safety) status since the start in 2007 (EFSA, 2007; EFSA BIOHAZ Panel, 2020).

Antibiotic susceptibility

Minimum inhibitory concentrations (MICs) of 9 antibiotics were determined for Harvest LB-1 according to the ISO 10932 | IDF 223 international standard (Table 1). These MICs were compared with the cut-off values established for *Lactobacillus plantarum/pentosus* group by the European Food Safety Authority (EFSA, 2018). The Harvest LB-1 strain is sensitive to most of the antibiotics tested with MIC values that are less than or equal to EFSA 2018 cut-off values for *Lactobacillus plantarum/pentosus* group.
The resistance to vancomycin is intrinsic to many *Lactobacillus species* including *L. plantarum* due to the structure of their cell wall (Billot-Klein et al. 1994, Journal of Bacteriology, 176:2398; Kirtzalidou et al. 2011, Anaerobe, 17:440; Solieri et al. 2014, Food Microbiology, 38:240).

To identify genes with high identity to previously published antibiotic resistance genes, the genome of Harvest LB-1 was analyzed against a curated database of antibiotic resistance genes. The database focus on acquired antibiotic resistance genes from the scientific literature and covers both Gram-positive and Gram-negative bacteria including pathogenic species. The analysis did not detect any antibiotic resistance gene in line with the strain being sensitive to relevant antibiotics the strain was tested.

Table 1: MIC values for the Harvest LB-1 strain

Antibiotic type	Antibiotic	MIC IN µg/ML	EFSA CUT-OFF VALUES IN µg/MLA
Aminoglycoside	Gentamicin	2	16
	Kanamycin	32	64
	Streptomycin	32	n.r.
Tetracycline	Tetracycline	32	32
Macrolide	Erythromycin	0.25	1
Lincosamide	Clindamycin	1	4
Chloramphenicol	Chloramphenicol	8	8
β-lactam	Ampicillin	0.5	2
Glycopeptide	Vancomycin	>128	n.r.

n.r.: not required to be tested by EFSA
a: EFSA cut-off values for the *Lactobacillus plantarum/pentosus* group as listed in 'Guidance on the characterisation of microorganisms used as feed additives or as production organisms', EFSA Journal 2018,16:5206

Biogenic amines and ethyl carbamate

Lactic acid bacteria are amongst the most prevalent biogenic amine producers in fermented foods. They produce these molecules from decarboxylation of amino acids, e.g. histidine -> histamine, arginine -> putrescine, and tyrosine -> tyramine are the most common ones found for *L. plantarum*. As all precursor amino acids of the biogenic amines can be found in wort, it is important to ensure that the applied strains produce little to no biogenic amines. As biogenic amine production is strain-dependent, Chr. Hansen test all strains for their biogenic amine production potential. The Harvest LB-1 strain was tested for the production of histamine, tyramine, cadaverine, and putrescine using an in-house procedure based on published methods. No production of the four biogenic amines was detected.

When working with lactic acid bacteria for food production it is also important to be alert towards the production of the carcinogenic compound ethyl carbamate. Ethyl carbamate can be formed by the spontaneous reaction between ethanol produced by brewers' yeasts and the citrulline produced from arginine by lactic acid bacteria. This is typically an issue in wine fermentations that can be caused by citrulline production during malolactic fermentation. However, with increasing ethanol concentrations and with the application of an increased number of less characterized lactic acid bacteria for sour beer production, this could also become a food-safety issue in sour beers. The genome of *L. plantarum*, Harvest LB-1, was analyzed for the presence of relevant genes involved in the conversion of the arginine to citrulline and the enzymes were not detected.

Table 2: Biogenic amine production of the Harvest LB-1 strain.

Biogenic amine compound	Monoamines		Polyamines	
	Histamine	Tyramine	Cadaverine	Putrescine
Harvest LB-1	Not detected	Not detected	Not detected	Not detected

The threshold for reporting is set to 5 mg/l corresponding to approximately 0.04 mM.

Statements

Upon request, Chr. Hansen A/S can supply statements confirming the results mentioned in this poster. Furthermore, allergen information, safety data sheet, Halal and Kosher certificates are available.

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