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Reassessment of the succession of lactic acid bacteria in commercial cucumber fermentations and physiological and genomic features associated with their dominance☆

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ABSTRACT

A compositional re-assessment of the microbiota present in commercial cucumber fermentation using culture independent and dependent methods was conducted, with emphasis on lactic acid bacteria (LAB). Two commercial cucumber fermentation tanks were monitored by measuring pH, dissolved oxygen and temperature, and used as sources of samples for microbial plating, genomic DNA extraction and measurement of organic acids and carbohydrates by HPLC. Six additional commercial tanks were included to identify the dominant microorganisms using molecular methods. A comparative analysis of the publically available genome sequences corresponding to the LAB found in cucumber fermentations was completed to gain an understanding of genomic features possibly enabling dominance. Analyses of the microbiota suggest Lactobacillales prevail in cucumber fermentations, including in order of prevalence *Lactobacillus pentosus*, *Lb. plantarum*, *Lb. brevis*, *Weissella* spp., *Pediococcus ethanolidurans*, *Leuconostoc* spp. and *Lactococcus* spp. It was observed that *Lb. pentosus* and *Lb. plantarum* have comparatively larger genomes, higher gene counts, uniquely distribute the ribosomal clusters across the genome as opposed to close to the origin of replication, and possess more predicted amino acids prototrophies and selected biosynthesis related genes. It is theorized that *Lb. pentosus* and *Lb. plantarum* dominance in cucumber fermentations is the result of their genetic make-up.

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1. Introduction

Knowledge of the physiology of industrially relevant lactic acid bacteria (LAB) has increased exponentially as the result of technological advances associated with DNA sequencing, transcriptomics, proteomics, and diverse meta-analyses. Such advances generate innumerable opportunities to expand our understanding of the role of LAB in economically important fermentations. The study presented here focuses on advancing the understanding of the microbiota in modern commercial cucumber fermentations using culture independent techniques and discusses some of the physiological and genomic features possibly influencing the prevalence of selected LAB in such system.

The microbiology of cucumber fermentations has been studied since 1899, shortly after the discovery of yeasts, as active living cells responsible for transforming glucose to alcohol (Nanniga, 2010). Traditional knowledge of the cucumber fermentation microbiota provides evidence for the presence of various Gram-positive and Gram-negative bacteria, yeasts and molds at the outset. The main sources for such diverse microbiota on the cucumber exocarp are irrigation water and the growth supporting soil as well as preprocessing washing water and processing equipment. The

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microbiota originally present in the cucumbers initially compete for dominance, remaining active for several days or weeks depending on factors such as temperature, dissolved oxygen and the salt concentration used in the cover brines (Pérez-Díaz et al., 2014). In the majority of cases LAB dominate the fermentations for a long time, given their ability to generate and tolerate acidic conditions. LAB are known to reach maximum cell densities by the third day of cucumber fermentations brined with 5–10% NaCl (Jones et al., 1940). *Lactobacillus plantarum* was the first LAB associated with cucumber fermentations (Etchells and Jones, 1946; Rosen and Fabian, 1953). This was the result of the characterization of isolates from cover brine samples using carbohydrate fermentation patterns, carbon dioxide production ability and the description of *Lb. plantarum* by Orla-Jensen released in 1938 (Etchells and Jones, 1946; Rosen and Fabian, 1953). It is currently understood that one family and 8 species of LAB may be naturally and actively present in many vegetable fermentations, which in order of dominance are *Enterobacteriacae*, *Enterococcus* spp., *Leuconostoc mesenteroides*, *Weissella* spp., *Pediococcus pentosaceous* (previously known as *P. cerevisiae*), *P. acidilactici*, *Lb. brevis*, *Lb. plantarum* and *Lb. pentosus* (Chen et al., 2012; Costilow et al., 1956; Etchells and Goresline, 1940; Jung et al., 2014; Lee et al., 2015; Paramithiotis et al., 2014; Pederson and Albury, 1950, 1956; Plengvidhya et al., 2007; Vahlteich et al., 1935; Wouters et al., 2013). Generally, *Lc. mesenteroides* survives best in cucumber fermentations at temperatures below 18 °C (Pederson and Albury, 1950), while *P. pentosaceous* ceases to proliferate at the same temperature (Pederson and Albury, 1950). *Lb. plantarum* is more resistant to the acidic pH as compared to *Lc. mesenteroides* (McDonald et al., 1990), which precedes most other LAB in cucumber fermentations (Etchells and Goresline, 1940; Singh and Ramesh, 2008; Vahlteich et al., 1935). Ninety percent of the *P. pentosaceous* isolated from cucumber fermentation cover brines in the 1940s were obtained from samples collected early in the process, while the *Lb. plantarum* and *Lb. brevis* were isolated after the numbers of pediococci started to decline (Fig. 1). In a study conducted in 2008 by Singh and Ramesh, pediococci were detected after 30 h of a cucumber fermentation brined with 2% NaCl (m/v; after equilibration). Such event coincided with a reduction in the number of leuconostoc (Singh and Ramesh, 2008). Generally, cucumber fermentation is defined as an anaerobic process conducted by 2×10^8 CFU/mL of microorganisms, primarily LAB, found in cover brines and able to produce 0.6–1.2% lactic acid (Hamilton and Johnston, 1960).

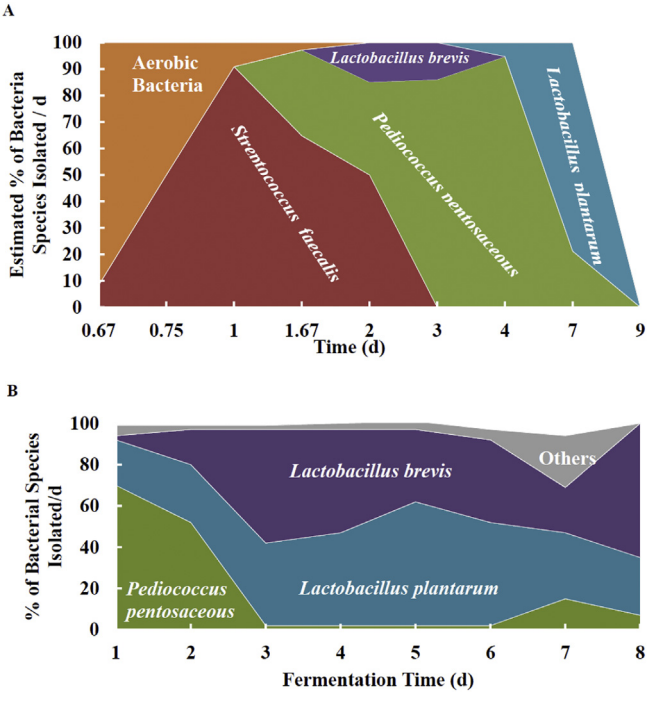

Fig. 1. Microbial succession in cucumber fermentations brined with 5% NaCl (m/v) at 30 °C (86 °F) as described by Pederson and Albury in 1950 (a) or with 5–10% NaCl (m/v) at 15–28 °C as described by Costilow et al., in 1956 for a sample size of 84 commercial fermentations (b).

Current commercial cucumber fermentation practices differ significantly from those in the 1940s, when most of the studies described above were completed. Modern commercial cucumber fermentations are carried out in 40,000 L open top tanks containing 50–70% whole cucumbers or pre-cut pieces of the vegetable, and 50-30% cover brine solution containing acetic acid, added as concentrated vinegar, and NaCl to achieve average equilibrated concentrations of 25 mM and 1.03 M (6%), respectively. Cucumbers are packed in fiberglass tanks and immediately covered with wooden boards to prevent them from floating until an equilibrium between the vegetables and cover brine solution components is achieved. Air purging is applied in combination with vinegar supplementation to reduce the incidence of bloating; a defect involving the entrapment of carbon dioxide produced during the fermentation in the whole cucumber tissue, forming hollow cavities, similar to those desired in Swiss cheese (Costilow et al., 1977; Fleming and Pharr, 1980). The decrease in the concentration of the sodium chloride used for modern cucumber fermentation as compared to the traditional process containing up to 17% NaCl (m/v), translates into a reduction of the amount of chlorides discharged to the environment. The current practice to reuse fermentation cover brines provides for the reclamation of salt and reduces the amount of water needed to process cucumbers (McFeeters et al., 1977). The wooden fermenting vessels used in the 1940s have been replaced with fiberglass tanks to reduce the cost of maintenance and loss of salty cover brines that would end up leaking into the ground.

It is an objective of this study to apply current microbial identification techniques to review and define the microbiota of modern cucumber fermentations at the commercial scale. Two commercial cucumber fermentation tanks located in the eastern and northern parts of the USA and exposed to slightly different climates were monitored to characterize the microbial ecology of cover brine samples using culture and non-culture based methods. The fermentation biochemistry was evaluated using HPLC. Other physical parameters such as pH, dissolved oxygen and temperature were also monitored. An additional 6 commercial tanks located across the USA from Texas to Michigan were included in the study to characterize the dominant microorganisms present in cover brine samples during the peak of the fermentation, using molecular biology methods.

The second objective of this study was to review the current knowledge of the genomics of LAB to gain an insight into the genetic basis for the dominance of particular LAB in cucumber fermentations. Significant progress has been made in the comparative genomics of LAB and the evolutionary patterns associated with this group of industrially relevant bacteria. A comparison of the publically available genome sequences corresponding to the LAB prevailing in cucumber fermentations was completed.

Comprehensively, this study provides an update of the microbiota in commercial cucumber fermentations and describes some of the known physiological and genomic features that allow *Lb. pentosus*, *Lb. plantarum* and *Lb. brevis* to prevail in such system.