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Fermented Vegetables

Vegetable fermentation by lactic acid bacteria (LAB) in a salt brine began as a way to preserve foods for out-of-season use and for long journeys, especially by sea. It is believed that cucumbers were first pickled 4,500 years ago in Mesopotamia. Although originally used to preserve foods, pickling is frequently done because people enjoy the resulting flavor. The current market for pickled vegetables in the United States is \$2 billion or more and is increasing in size. The two primary retail fermented vegetable products produced in the United States are cucumber pickles and sauerkraut. Currently, pickled cucumbers are the most popular pickled foods. The word "pickle" by itself usually refers to a pickled cucumber. The retail market for cucumber pickles in the United States is dominated by acidified, pasteurized, and refrigerated products, not fermented pickles (40). Commercial sale of hamburger dill pickle slices to the food service industry makes up the majority of the market for fermented cucumber pickles. Fermented cabbage, i.e., sauerkraut, was introduced in the United States by immigrants from Germany and other European countries. Although the popularity of sauerkraut in Europe and the United States continues today, consumption has declined in

the United States. Fermented olives are also produced in the United States, mostly in California, but the majority of olives sold in the United States are produced in southern Spain. There are niche markets for other pickled vegetables, including deli-style, half-sour cucumber pickles, which are fermented only slightly prior to consumption (30), and Korean kimchi (40). Other acidified vegetable products familiar to consumers include peppers, okra, cauliflower, and green tomatoes.

Fermented pickle products in the United States typically have a pH value of about 3.7, with acetic acid added in addition to the lactic acid which is produced during fermentation. Fermented foods are considered acid foods and are defined in the U.S. Code of Federal Regulations (21 CFR part 114) (101) as foods that naturally have a pH below 4.6. Acidified vegetables are defined in 21 CFR part 114 as low-acid (pH above 4.6) foods to which acid or acid food ingredients have been added to attain a pH value of 4.6 or below. Most acidified pickles have a pH of about 3.7 and an acetic acid concentration of 1 to 2% and contain 2 to 4% NaCl. Acidified pickles, which are not fermented, are typically heated (pasteurized) to prevent spoilage by LAB. Some pickled pepper products

are acidified with 2 to 3% acetic acid to reach a pH of 3.3 or below. These products may not be heat processed because the concentration of acetic acid is high enough to prevent growth of bacteria and there is better retention of product texture. The high acid/low pH is sufficient to ensure at least a 5-log reduction of acid-tolerant pathogenic bacteria.

Sweet cucumber pickles are often produced from small (<20 mm in diameter) cucumbers that have been fermented. In these products, the lactic acid and salt from the fermentation are removed by washing the fermented cucumbers with water. The cover liquor of sweet pickles is similar to that of sour pickles, but contains 25 to 30% sugar (from corn syrup). Traditional genuine dill pickles, containing lactic acid (up to 2%) from fermentation and salt (4% or more), are a minor market in the United States. Sauerkraut and green table olives, however, are often packed with the naturally present lactic acid and brine from the fermentation. Typically, sauerkraut sold in the United States contains both lactic acid and acetic acid, which are produced during fermentation (up to 2% acid), and about 2% NaCl, although acid and salt levels may be adjusted prior to packaging.

HISTORY OF VEGETABLE FERMENTATION RESEARCH IN THE UNITED STATES

Prior to the 1920s, research in the United States on pickled vegetables was primarily focused on product surveys (73, 74, 90) and descriptions of brining methods (1, 20, 49). Reports on the microbiology and biochemistry of vegetable fermentations appeared in the literature between 1918 and 1920 (21, 51, 59, 77). Initial reports of the "lactic bacilli" and organic acids in cucumber pickles, sauerkraut, and olives were further advanced by a series of studies done by E. B. Fred at the University of Wisconsin. During the decade of the 1920s, his group studied the sauerkraut fermentation, including the growth of yeasts (46, 85), microbial ecology (92, 93), and chemistry of the fermentation (47, 86).

In the early 1930s, Fabian began studies on fermented vegetables at Michigan State University. An initial report described the study of softening and spoilage of fermented cucumbers (34). Fabian et al. (35) and Costilow and Fabian (15–17) published extensively in the field of pickled vegetables. Pederson, at Cornell University, studied sauerkraut fermentation from the 1930s to the early 1970s. His group reported on various aspects of the microbiology (78–81) and nutritional and biochemical properties (83, 84, 103) of the sauerkraut fermentation, which culminated in a comprehensive review (82). Stamer and other researchers subsequently published a

series of articles on the microflora of sauerkraut (95, 96), including the development of red or pink discoloration in sauerkraut (95), a continuation of the work done by Fred and Peterson in the early 1920s (46).

Etchells and Veldhuis were the founding scientists of a U.S. Department of Agriculture (USDA) laboratory in Raleigh, N.C., in the mid 1930s, where research on pickled vegetables from 1938 (27, 102) to the present has been ongoing. Included in these studies were the development of pasteurization methods (32, 33, 54), investigations of the yeasts that are responsible for spoilage of cucumber pickle products (28, 31), and a preservation-prediction chart to describe the storage stability of sweet pickles based on salt and sugar concentrations (3). Bell determined the role of pectin-degrading enzymes in cucumber softening in the 1970s (2, 4, 5). Fleming and coworkers, also at the Raleigh USDA laboratory, later developed purging technology for commercial cucumber fermentations (29, 37, 44). Costilow and others have done further research using this technology (14, 18, 19). Other developments include an investigation of the antimicrobial properties of oleuropein in olives and its degradation products (45), the role of malolactic enzymes in the production of carbon dioxide by LAB during cucumber fermentation (72), a *Lactobacillus plantarum* strain that lacks the ability to carry out the malolactic reaction (22), and the use of calcium to improve the texture of pickled vegetables (70, 71).

As we advance into the second century of research on acid and acidified foods, researchers are building on the solid foundation laid by the researchers noted above as well as others. Current research on pickled vegetables includes the genomics of LAB (8), mathematical modeling of bacterial growth and competition (25), the molecular ecology of vegetable fermentations (60, 88), closed-tank fermentation technology to reduce salt waste (38), the use of clays to filter brines for recycling (12), sensory perception of pickled vegetable products (53), and the safety of acidified foods (6, 9).

COMMERCIAL FERMENTATIONS

In the United States, commercial cucumber (*Cucumis sativus*) fermentations are commonly done in 8,000- to 10,000-gallon, open-top, plastic or fiberglass tanks that are located out-of-doors so the brine surface is exposed to sunlight. The UV radiation in sunlight is relied upon to kill aerobic surface yeasts which can metabolize lactic acid produced by the fermentation. Cucumbers are covered with a salt brine and held below the brine surface with wooden headboards. Fermentations are typically carried out in brine equilibrated at about 6% NaCl. Calcium

chloride (0.1 to 0.4%, equilibrated) is added to the cover brine to maintain the firm, crisp texture of the fermented cucumbers during storage (41). Cucumber fermentations typically undergo a homolactic acid fermentation, which does not result in production of carbon dioxide from sugars (glucose and fructose, about 1% each). However, carbon dioxide may be generated from respiration of cucumbers when they are submerged in brine and by the decarboxylation of malate during the fermentation. Some LAB have an inducible malolactic enzyme which converts malate to lactate and carbon dioxide. The malolactic enzyme reaction occurs intracellularly and results in the uptake of a proton, raising internal cell pH. Cucumber fermentations are purged with air during the active fermentation period to remove carbon dioxide from the tank (14). Purging prevents bloating damage in the cucumbers. Potassium sorbate (350 $\mu\text{g}/\text{ml}$) or 0.16% acetic acid can be used to limit the growth of aerobic microorganisms, particularly molds and yeasts, which can grow as a consequence of dissolved oxygen in the fermentation brine resulting from air purging (40, 89). Excessive growth of aerobic microorganisms which can cause spoilage problems (48) is also controlled by stopping air purging for several hours each day. Fermentation is carried out primarily by *Lb. plantarum*, a homofermentative, acid-tolerant LAB. After fermentation, cucumbers may be stored in the fermentation tanks for 1 year or more. In the northern states, the concentration of NaCl is often increased during storage to as high as 10 or 15% to minimize freezing damage and maintain the desirable texture of fermented cucumbers.

Currently, starter cultures of *Lb. plantarum* are rarely used by the industry. Most commercial cucumber fermentations rely upon growth of the epiphytic LAB which occur naturally on the surface of cucumbers. These bacteria effectively control the microbial ecology of the fermentation by consuming the glucose and fructose present, producing lactic acid, and lowering the brine pH. The initial pH of brined cucumbers is about 6.5. In practice, commercial fermentations may use recycled brine or acetic acid may be added to brine solutions, so the initial pH of the fermentation can vary significantly. In addition to lactic acid, LAB produce a variety of metabolites, e.g., bacteriocins, peroxides, and peptides, that can be inhibitory to other bacteria (24). At the end of the fermentation, there may be up to 2% lactic acid, a pH of 3.1 to 3.5, and little or no residual sugar. In this anaerobic, acidic, high-salt environment lacking sugar, very few microorganisms are capable of growing or surviving, effectively preserving the cucumbers.

Commercial production of sauerkraut (41) from cabbage (*Brassica oleracea*) in the United States is also done

in bulk fermentation tanks that may contain 100 tons or more of shredded or chopped cabbage. The cabbage for these fermentations consists of large heads, typically 8 to 10 lb (3.6 to 4.5 kg). The outer leaves and woody core of the cabbage are removed prior to shredding. The shredded cabbage is dry salted on belts as it is conveyed to indoor cement fermentation tanks. This process typically results in an equilibrated NaCl concentration of about 2%. The cabbage is manually distributed in the tanks to create a concave surface and covered with a plastic liner. Water is added on top of the plastic to hold down the liner on the cabbage and allow anaerobic conditions to develop under the plastic. After the first 24 to 48 h, carbon dioxide gas may accumulate under the liner. To prevent heaving of the cabbage, some of the brine formed from the salted cabbage may be drained from the bottom of the tanks during the first week of fermentation. A salt concentration of 2% and temperature of 18°C help ensure a normal fermentation by the naturally present LAB (82). Cabbage typically contains 4 to 5% sugar, consisting of about 2.5% glucose and 2% fructose with smaller amounts of sucrose and other sugars (42). The primary fermentation is initiated by naturally occurring, heterofermentative LAB, primarily *Leuconostoc mesenteroides*, although *Weissella* species are also present and sometimes dominate the initial stage of fermentation (82, 87). The initial heterolactic stage of the fermentation results in production of both lactic and acetic acids. The volatile acetic acid makes an important contribution to the flavor and aroma of the final product. Heterofermentative microorganisms also use fructose as an electron acceptor, converting it to mannitol (68). After about 1 week of fermentation, the heterofermentative LAB, which may reach 9 log CFU/ml or greater, die off. They are replaced by the more acid-tolerant homofermentative LAB. This stage of the fermentation process is usually dominated by *Lb. plantarum*, presumably because it is the most acid-resistant microorganism present (64). The fermentation end products resulting from both stages of the fermentation can include mannitol and acetic acid (about 1% each) and lactic acid, which may exceed 2%, depending on how long the homolactic fermentation is allowed to continue. For most manufacturers in the United States, this may be up to 1 year because sauerkraut is stored in the fermentation tank until it is processed for food service or retail sale. Some European manufacturers package sauerkraut at the end of the heterolactic fermentation stage (about 1 week after the start of fermentation) to produce a product with mild acid flavor (40). Spices, wines, and other ingredients may be added to the sauerkraut to augment flavor.

Since cucumbers are fermented in brine containing salt at a concentration too high to be used in products for consumption, salt is reduced to about 2% prior to packaging for distribution and sale. This results in a waste stream with high concentrations of salt plus a high biological oxygen demand from the lactic acid and other organic components that diffuse out of the cucumbers during the desalting process. The brine from the fermentation tanks is usually recycled and may be used in subsequent fermentations (69). Prior to recycling, fermentation brines may be processed to remove "softening enzymes," primarily polygalacturonases, which can degrade pectic substances in the cucumber cell wall and soften the fruits (12). Waste brine is also generated from sauerkraut manufacture when draining excess brine after the initiation of fermentation.

Other vegetable fermentation processes, including olive fermentation, represent smaller markets in the United States, with Spain being the largest producer and exporter of black olives and green table olives. Commercial production of kimchi, a Korean fermented cabbage product, is now widespread in South Korea. Like cucumber and cabbage fermentation in the United States, both olive and kimchi fermentation practices are based on traditional methods. There are several methods used for processing olives (36). Green table olives are treated with lye (NaOH) and then washed prior to being brined and fermented. Olives may also be aerated during lye treatment to allow blackening (oxidation) of olives for the manufacture of canned black olives (36). Both of these processes can generate NaOH waste, in addition to the salt waste generated from fermentation brines. For commercial production of kimchi, Chinese cabbage (*Brassica campestris* subsp. *pekinensis*) is used. The cabbage is dry salted or brined, and then washed to remove excess salt prior to fermentation and packing with spices (41). Although recycling is used extensively in the vegetable fermentation industry, particularly for cucumber fermentation brine, the need to dispose of NaCl waste is a continuing problem.

BIOCHEMISTRY OF VEGETABLE FERMENTATIONS

There is continuing research interest in fermentation and storage of vegetables, particularly cucumbers, with reduced salt. Chloride waste from vegetable fermentations could be greatly reduced if the salt required for fermentation and storage could be reduced sufficiently to eliminate the need for a desalting step prior to conversion into final products. Lu et al. (61) investigated the effect of replacement of NaCl with different anions and cations on the sugar fermentation in cucumber juice. Interestingly, it

was clear from these experiments that fructose was the preferred sugar for *Lb. plantarum*, since more fructose was fermented in almost every experiment. Sugar utilization decreased as cation or anion concentrations increased with the addition of different salts. Divalent cations (Ca^{2+} and Mg^{2+}) reduced the extent of fermentation at lower concentrations than monovalent cations (Na^+ and K^+).

A number of the volatile components in cucumbers fermented with *Lb. plantarum* in 2% NaCl were identified by Zhou and McFeeters (104). Thirty-seven volatile compounds were identified, though most showed little change as a result of fermentation. The most notable effect of fermentation on cucumber volatiles was the inhibition of production of (E, Z)-2,6-nonadienal and 2-nonenal, the two most important odor impact compounds in fresh cucumbers. Marsili and Miller (62) identified *trans*- and *cis*-4-hexenoic acid as the most potent odorants that define the characteristic brine aroma of cucumbers fermented commercially in about 6% NaCl. Zhou et al. (105) exposed fermented cucumber slurries with 2% NaCl to oxygen and observed nonenzymatic formation of hexanal plus a series of *trans* unsaturated aldehydes with 5 to 8 carbon atoms that correlated with the development of oxidized odor intensity of the fermented cucumber tissue. Calcium disodium EDTA at a concentration of at least 100 $\mu\text{g}/\text{ml}$ protects non-fermented pickles against lipid oxidation and bleaching of pigments in the presence of light (11). However, there was some reduction in firmness retention in pickles when this compound was used.

Retention of firmness is a key quality issue in the fermentation and storage of cucumbers and peppers. It has not been possible to ensure the retention, in cucumbers fermented in reduced salt, of firmness equivalent to that which can be achieved by fermenting in 6% NaCl and storage in 6% or greater NaCl concentrations (39). However, in the past several years there has been some increased understanding of the cucumber tissue softening. Fleming et al. (43) showed that calcium is beneficial in maintaining the firmness of fermented cucumbers. Non-enzymatic softening of blanched, acidified cucumber tissue was found to follow first-order kinetics (70). This kinetic behavior made it possible to determine the entropy and enthalpy of activation for nonenzymatic softening of cucumbers, even though the chemical reactions responsible for softening were not known. Both the enthalpy and entropy of activation were high at pH 3.0 in the presence of 1.5 M NaCl. Calcium inhibited cucumber softening because it reduced the entropy of activation so much that the overall free energy of activation was reduced (71). This thermodynamic behavior is more like that which occurs when polymers change conformation, such as occurs in

protein denaturation. It is very different from the characteristics observed for acid hydrolysis of pectin (58). Krall and McFeeters (58) found that the rate of acid hydrolysis of pectin was too slow to be the cause of nonenzymatic cucumber tissue softening. McFeeters et al. (66) determined the combined effects of temperature, salt, and calcium concentration on the rate of softening of fermented cucumber tissue. The kinetics of softening for fermented cucumbers did not follow a simple first-order reaction.

As with many other plant tissues, cucumbers contain enzymes that can degrade components of the plant cell walls, which may result in changes in texture. Pectinesterase (5), exopolysaccharuronase (91), and endopolysaccharuronase (67) activities have been found in cucumbers. Pectinesterase removes methyl groups from pectin when cucumbers are fermented or acidified (52, 65, 98). However, it has not been determined if enzymatic hydrolysis of pectin by cucumber polygalacturonases is a significant factor in softening of fermented cucumbers. Commercially important enzymatic softening of fermented cucumbers has been associated with the introduction of fungal polygalacturonases into fermentation tanks, particularly on flowers attached to small cucumbers (4). Buescher and Burgin (10) developed a sensitive diffusion plate assay to measure polygalacturonase activity in fermentation brines and determined that an aluminosilicate clay can adsorb and remove polygalacturonase activity from fermentation brines that are recycled.

In addition to enzymes that degrade pectin, enzymes that may degrade other cell wall polysaccharides in cucumbers have been investigated to a very limited extent. Meurer and Gierschner (75) reported endo- β -1,4-gluconase activity in the cucumber that is inactivated below pH 4.8 and an endoglucomanan-splitting enzyme that retains activity down to pH 4.0 but is inactivated during fermentation. They detected six enzymes in fresh cucumbers, which hydrolyze *p*-nitrophenylglycosides of α -D-galactose, α -D-galactose, α -D-glucose, β -D-xylose, α -D-mannose, and α -L-arabinose, which were inactivated during fermentation. Enzymes capable of hydrolyzing these synthetic substrates are common in plants, e.g., most of the same enzymatic activities have been found in pears (26), olives (50), and Semillon grapes (97). Maruvada (63) observed the same *p*-nitrophenylglycosidases observed by Meurer and Gierschner (75) in cucumbers. She found that the concentration of all of these enzymes declined to nondetectable levels during the first week of fermentation in 2% NaCl brines. Fleming et al. (39) combined blanching of fresh cucumbers to partially inactivate enzymes, calcium addition, and rapid fermentation with a malolactic-negative *Lb. plantarum* strain in order to ferment cucumbers and

maintain desirable texture with the NaCl concentration reduced to 4%.

Cabbage contains a group of glucosinolates which have received considerable attention in recent years due to potential health benefits of some of the degradation products formed during the processing of cabbage. A recent report indicates that a high intake of sauerkraut correlates with a reduced incidence of breast cancer in women (94). Daxenbichler et al. (23), who were concerned about potentially toxic compounds derived from glucosinolates, did an excellent study of glucosinolates and their degradation products in three cabbage cultivars that were fermented and stored for up to 28 weeks. They reported the presence of 11 glucosinolates in fresh cabbage and found that all glucosinolates disappeared within 2 weeks after the start of fermentation. Tolonen et al. (99) found isothiocyanates and allyl cyanide to be the predominant degradation products of glucosinolates in sauerkraut fermented with and without a starter culture. Only minor amounts of goitrin, a toxic compound, and the beneficial phytochemical sulforaphane nitrile were found in sauerkraut. Tolonen et al. (100) found greater amounts of glucosinolate degradation products in sauerkraut fermented with *Lactobacillus sakei* than in sauerkraut made with starter cultures consisting of other LAB. Ciska and Pathak (13) reported that ascorbigen, a compound formed from the reaction of a degradation product of indole glucosinolate (glucobrassicin) and ascorbic acid, is the dominant glucosinolate degradation product in sauerkraut. A large fraction of glucoraphinin present in fresh cabbage was converted to sulforaphane during fermentation, though sulforaphane was only a minor glucosinolate degradation product in fermented cabbage.

There is some concern about the formation of biogenic amines in sauerkraut. Kalač et al. (55) reported that tyramine was the major biogenic amine formed in sauerkraut stored for up to 12 months. Only trace levels of histamine, tryptamine, and spermine were detected. These results were confirmed in a survey of vegetables (76) showing that the content of tyramine was 4.9 mg/100 g in canned sauerkraut, virtually the same concentration reported by Kalač et al. (55). These biogenic amine levels would not represent a health risk, with the possible exception of individuals taking medications containing monamine oxidase inhibitors.

GENOMICS RESEARCH ON LAB IN VEGETABLE FERMENTATIONS

Bolotin et al. (7) reported the first genome sequence for a lactic acid bacterium, *Lactococcus lactis* IL1403. They observed that the metabolic potential of this microorganism is more extensive than previously considered. The

L. lactis genome sequence contains putative genes for unexpected functionalities, including aerobic respiration, and biosynthesis pathways for all amino acids. Release of the *L. lactis* genome sequence was followed by sequencing of several other LAB genomes (55). Among these genome sequences are those belonging to the predominant bacteria present in fermented vegetables, *Lb. plantarum*, *L. mesenteroides*, *Pediococcus pentosaceus*, and *Lactobacillus brevis*. The *Lb. plantarum* WCFS1 genome sequence was published in 2002 by Kleerebezem et al. (57). Genome sequencing efforts by the Joint Genome Institute (Walnut Creek, Calif.) and the Lactic Acid Bacteria Genome Consortium (LABGC, USA) have contributed draft sequences for 10 genomes. These genomes include those for *L. mesenteroides* ATCC 8293, *P. pentosaceus* ATCC 25745, and *Lb. brevis* ATCC 367.

The genome sequences for the *L. mesenteroides*, *Lb. brevis*, and *P. pentosaceus* consist of approximately 2,000,000 bp. The *Lb. plantarum* genome consists of approximately 3,000,000 bp. The 2,000,000- or

3,000,000-bp genomes have approximately 2,000 or 3,000 open reading frames (ORFs), respectively. About 75% of the ORFs in the *L. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus* sequences have been assigned functions in metabolic pathways based on BLAST (Basic Local Alignment Search Tool) predicted protein sequence similarities. These ORFs have been classified according to clusters of orthologous groups (COG) categories. Each COG represents proteins or sets of paralogs from a representative number of lineages or an ancient conserved domain. Table 36.1 shows a comparison of COG categories among the four LAB genome sequences noted above. About 50% of the ORFs have been assigned functions relating to the metabolism or transport of amino acids, carbohydrates, and inorganic ion, the proteins involved in transcription and translation, including ribosomal structures and biogenesis, replication, recombination, and repair, and cell wall and membrane biogenesis. Only about 7% of these ORFs are dedicated to energy production by fermentation of sugars.

Table 36.1 Distribution of ORFs among COG functional categories^a

COG categories	<i>Lb. brevis</i> ATCC 367	<i>L. mesenteroides</i> ATCC 8293	<i>P. pentosaceus</i> ATCC 25745	<i>Lb. plantarum</i> WCFS1
Energy production and conversion	6.8	6.0	6.7	8.8
Cell cycle control, cell division, chromosome partitioning	2.4	2.2	1.8	2.2
Amino acid transport and metabolism	13.6	17.3	10.4	16.1
Nucleotide transport and metabolism	7.2	8.3	8.8	7.5
Carbohydrate transport and metabolism	14.4	12.8	17.0	21.9
Coenzyme transport and metabolism	5.5	7.1	4.9	7.8
Lipid transport and metabolism	3.8	3.9	3.9	4.3
Translation, ribosomal structure, and biogenesis	17.4	15.9	17.5	15.4
Transcription	15.7	11.3	14.0	20.2
Replication, recombination, and repair	16.2	11.7	13.2	12.8
Cell wall/membrane/envelope biogenesis	10.8	9.4	11.8	13.2
Cell motility	0.2	0.1	0.3	0.3
Posttranslational modification, protein turnover, chaperones	5.4	5.5	5.2	4.9
Inorganic ion transport and metabolism	10.6	9.0	8.5	11.2
Secondary metabolite biosynthesis, transport, and catabolism	3.1	2.3	1.4	2.6
General function prediction only	32.2	26.9	28.4	38.6
Function unknown	20.5	16.4	18.1	20.0
Signal transduction mechanisms	7.8	6.2	6.5	8.4
Intracellular trafficking, secretion, and vesicular transport	2.4	2.2	2.9	2.1
Defense mechanisms	4.1	3.0	4.1	5.4
Extracellular structures	0.0	0.1	0.1	0.3
Other ORF without a category	0.0	0.0	0.0	0.0

^a Values are in percentages.

An average of 16% of the ORFs identified in the four genome sequences have putative functions related to carbohydrate transport and metabolism. The *Lb. plantarum* sequence contains the largest number of putative phosphotransferase systems (PTS) genes, which may encode 25 complete complexes and several incomplete complexes (57). *P. pentosaceus* has a slightly reduced number of putative PTS genes compared to *Lb. plantarum*. *Leuconostoc mesenteroides* contains at least five putative phosphotransferase transport systems and several incomplete systems. *Lb. brevis* seems to be at a disadvantage regarding PTSs. There are only six putative PTS-related genes in the *Lb. brevis* genome, which may potentially encode two complete PTSs at the most. Although PTS genes are evidently present in the *L. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus* genomes, genes for phospho- β -galactosidase have not been found; nonetheless, β -galactosidase genes are apparently present in all four genomes.

Intracellular glucose may be converted to pyruvic acid via glycolysis or to pentoses via the pentose phosphate or phosphogluconate pathway. Putative genes coding for phosphofructokinase are present in *Lb. plantarum* and *P. pentosaceus*. As expected, phosphofructokinase genes have not been found in the sequences for the heterofermentative bacteria *Lb. brevis* and *L. mesenteroides*. All four bacteria, *L. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus*, have predicted genes for enzymes involved in catabolic functions, including glucose-6-phosphate dehydrogenase, lactonase, 6-phosphogluconate dehydrogenase, ribose-5-phosphate isomerase, ribulose-phosphate 3-epimerase, and phosphopentose

isomerase. Putative genes for phosphoketolases, which catalyze the initial step in the conversion of D-xylulose-5-phosphate to ethanol and lactic acid, have been found in *Lb. plantarum*, *L. mesenteroides*, and *P. pentosaceus*. These genes have assigned functions based on sequence similarity; therefore, further research will be needed to determine which genes are expressed.

Predicted gene products related to pyruvic acid catabolism in *L. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus* are noted in Table 36.2. These data suggest that *Lb. plantarum* and *L. mesenteroides* contain more pyruvate-dissipating enzymes than *Lb. brevis* and *P. pentosaceus*. The absence of an oxaloacetate decarboxylase in these four LAB was unexpected. Putative genes for lactate dehydrogenases are present in all four bacteria.

As expected, none of the predominant LAB in fermented vegetables have predicted genes for a complete citric acid cycle. However, putative genes coding for enzymes involved in the citric acid cycle reductive route are present in most of the available genomes. Additionally, all four microorganisms have putative genes for the malolactic enzyme.

The genome sequences of *L. mesenteroides*, *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus* contain multiple copies of the rRNA clusters, which display minimal or no intracellular polymorphism. The 16S, 5S, and 23S rRNA sequence copies in each bacterium have 99 or 100% identity over their entire length. The *L. mesenteroides* genome sequence contains four putative rRNA clusters, which are located close to the origin of replication. The *Lb. plantarum*, *Lb. brevis*, and *P. pentosaceus* genome

Table 36.2 Pyruvic-acid-dissipating enzymes present in the predominant LAB in fermented vegetables^a

Pyruvate catabolism-related enzymes	<i>Lb. brevis</i> ATCC 367	<i>L. mesenteroides</i> ATCC 8293	<i>P. pentosaceus</i> ATCC 25745	<i>Lb. plantarum</i> WCFS1
Pyruvate oxidase	X	X	X	X
Pyruvate dehydrogenase	X	X	X	X
Pyruvate formate lyase		X	X	X
Acetolactate synthase	X	X		X
Lactate dehydrogenases	X	X	X	X
Hydroxyisocaproate dehydrogenase		X		X
Malic enzyme (E.C. 1.1.1.38)				X
Malate dehydrogenase (E.C. 1.1.1.40)	X	X	X	
Pyruvate kinase	X	X	X	X
Oxaloacetate decarboxylate				
Pyruvate carboxylase		X	X	X

^a X, enzyme is present.

sequences contain five putative rRNA clusters distributed around the genome. The *Lb. brevis*, *Lb. plantarum*, and *P. pentosaceus* 16S, 5S, and 23S rRNA sequences are at least 95% identical to each other.

With the exception of the cysteine tRNA, most tRNAs are present in the four bacterial genomes in multiple copies. Ten amino acids (asparagine, aspartate, cysteine, histidine, isoleucine, methionine, phenylalanine, tryptophan, tyrosine, and valine) seem to be uniquely encoded by a single codon in these bacteria. The strains analyzed contained several plasmids. *Lb. plantarum* WCFS1 contains two small plasmids of approximately 2,000 bp and a larger plasmid of about 36,000 bp (57). Functions assigned to the ORFs in these plasmids include conjugal plasmid transfer. *L. mesenteroides* ATCC 8293 contains a plasmid of approximately 37,000 bp, which codes for several mobile genetic elements. Although plasmids encoding bacteriocin production, lactose utilization, and citric acid utilization related genes have been isolated from several *Leuconostoc* species, none of these functions appear to be present in the sequenced plasmid. Only the *P. pentosaceus* genome sequence data do not reveal the presence of plasmids. The overall G+C content of these plasmids tends to be lower than the 40% chromosomal G+C content. Most of the ORFs identified in these plasmids have no assigned functions.

The recent advances in genomics, molecular microbial ecology, analytical biochemistry, plant breeding, and fermentation technology suggest a bright future for vegetable fermentation science and applications, enhancements of existing products, and new processing techniques. Principal areas for the application of this technology may include the development of commercial low-salt fermentations to reduce salt wastes. Current industrial fermentation practices are in large part based on traditional practices, which have been adapted to a larger scale. The development of low-salt fermentations and storage of fermented vegetables for commercial use present significant technological hurdles, including the potential need for starter cultures (and the impact of bacteriophage on starter cultures) and for new product handling equipment. Future products may have nutritional properties superior to those of current products and incorporate nontraditional vegetables. The reasons for developing these products will be the same as they have been for centuries. Fermented vegetable products are microbiologically safe and nutritious. They have appealing sensory characteristics and can be conveniently stored for extended periods without refrigeration.

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