Selection of lactic acid bacteria for use in vegetable fermentations*†

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Received 14 August 1984

The fermentation of brined vegetables traditionally has depended upon growth of naturally occurring lactic acid bacteria to metabolize the vegetable sugars to organic acids which, together with added salt, results in preservation. Starter cultures have been used only to a limited extent commercially. However, recent efforts to improve fermentation vessels and to develop controlled fermentation methods for fermented vegetables has resulted in an increased interest in developing cultures suitable for application in such methods. Rapid and dominant growth, type and extent of acid production, salt tolerance, temperature range, CO_2 production, cell sedimentation, bacteriophage resistance, nutritional value, and ability to survive as concentrated cultures are factors to consider in developing lactic acid bacterial cultures for use in controlled fermentation of vegetables. Recent examples of efforts to improve cultures include development of nonmalate-decarboxylating strains of lactobacilli for use in cucumber fermentation and isolation of a new species of lactobacillus that produces only the L-isomer of lactic acid from the glucose for use in sauerkraut fermentation. Further improvements in starter cultures for fermented vegetables are likely when suitable genetic transfer systems are developed for selective incorporation or deletion of specific traits.

Lactic Acid Fermentation of Vegetables

When vegetables are placed in solution of sodium chloride of appropriate concentration, they undergo fermentation by the naturally occurring micro-organisms on the vegetables. The concentration of sodium chloride used depends upon the tendency of the particular vegetable to soften during brine storage and may range from about 1 to 8%. In turn, the sodium chloride concentration greatly influences the types and numbers of micro-organisms active during fermentation (Fleming 1982). Vegetables are not normally washed in commercial brining operations and contain the indigenous microflora when brined. Microbial growth during natural fermentation of vegetables has been categorized into four sequential stages (Fleming 1982): *initiation*, which includes growth of various Gram-positive and Gram-negative

* Paper No. 9431 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina, USA. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable. This paper was prepared by US Government employees as part of their official duties and legally cannot be copyrighted.

† Paper presented at the symposium *Selection Parameters of Micro-organisms for Use in the Fermentation of Plant Foods and Beverages*.

0740–0020/84/040303 + 11 $02.00/0

bacteria present on the vegetable; *primary fermentation,* which includes growth of lactic acid bacteria with or without growth of fermentative yeasts; *secondary fermentation,* which includes growth of fermentative yeasts after growth of lactic acid bacteria has been inhibited by low pH, provided that fermentable carbohydrates remain; and *post-fermentation,* occurring after fermentable carbohydrates have been exhausted and characterized by the absence of microbial growth under anaerobic conditions but showing surface growth of oxidative micro-organisms when the brine surface is exposed to the atmosphere. The naturally occurring lactic acid bacteria proliferate during initiation and primary fermentation depending upon their presence on the raw product and the chemical and environmental conditions under which the brined vegetable is held.

Numerous vegetables have been preserved by brining, which began as a primitive method of preserving vegetables. Cabbage and cucumbers constitute the largest volume of vegetables brined. Certain fruits, such as olives, may be brined also. The brine fermentation of vegetables has been widely researched, and comprehensive reviews are available on the subject for cabbage (Pederson 1960, 1979), cucumbers (Etchells et al. 1975), olives (Vaughn 1975), and in general (Fleming 1982, Vaughn 1982).

Maintenance of structural integrity of tissue is important in the fermentation of vegetables, hence the need for sodium chloride to prevent tissue softening as noted above. In addition, brined cucumbers are affected by the problem of gaseous spoilage known as bloated damage (hollow cucumbers). This arises from a buildup of CO_2 in the brine during fermentation, but can be prevented by purging of CO_2 from the brined cucumbers with nitrogen gas during fermentation (Etchells et al. 1973).

The method of brining varies among vegetable or fruit commodities, depending on properties of the commodity and on characteristics desired in the final product, as illustrated herein for cucumbers, cabbage, and olives. The fermentation of each commodity is influenced by chemical, nutritional, and physical factors associated with that commodity and the manner in which it is brined.

Chemical and nutritional factors

The concentration of salt used for fermentation of cucumbers and olives is 5–8%, at equilibrium, the concentration in the initial cover brine being considerably higher in order to compensate for water contained within the vegetable. Alternatively, dry salt may be added on head boards of the tanks and allowed to dissolve slowly. Cabbage is dry salted so as to contain usually less than 2·5% sodium chloride at equilibrium. The difference in salt concentration between that used for sauerkraut and cucumber pickles probably accounts for much of the difference in types of lactic acid bacteria that grow in each.

Buffering capacity of the vegetable influences the extent to which lactic acid bacteria can ferment the natural sugars before being inhibited by low pH. Sodium acetate (Etchells et al. 1973) and calcium acetate (Fleming et al, 1978) have been used as buffering agents to assure complete sugar utilization during primary fermentation of cucumbers. Calcium acetate serves a dual function since the calcium ion also improves firmness retention of brined cucumbers (Fleming et al. 1978, Buescher et al. 1979).

Cucumbers and olives contain mainly glucose and fructose as fermentable sugars, while cabbage contains sizeable amounts of sucrose in addition to these sugars. Cucumbers actually contain

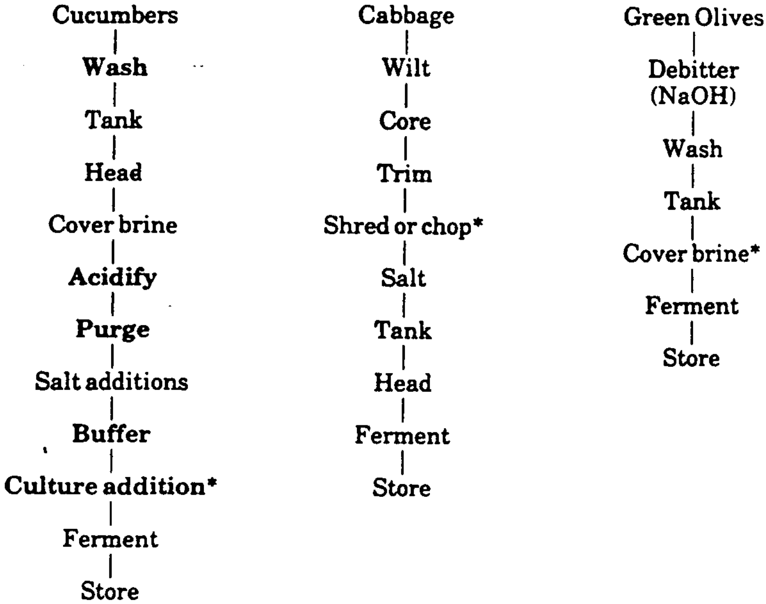

Fig. 1. Flow chart for the fermentation of produce. For cucumbers, steps that have been added to the natural fermentation procedure to obtain controlled fermentation are indicated in bold face. For cabbage and olives, steps are those involved in natural fermentation. * Pure culture, added. From Fleming et al. (1984).

more sugar than is desired to produce the acid required for preservation. Screening efforts have been made to find cucumber cultivars with lower amounts of sugars than the 2–2·5% commonly found in pickling cucumbers (McCreight et al. 1978). The problem is reversed with Spanish-style green olives, however, because natural sugars are removed during the leaching process required to remove excess alkali after the debittering process (Fig. 1). It is common practice among some briners of Spanish-style green olives to add fermentable sugar (dextrose) to assure adequate acid formation. Cabbage may contain a great excess of fermentable sugars resulting in the problem of excess acid production. The practical solution to this problem at present is to can and heat process the sauerkraut when the desired concentration of acid is achieved, or to leach part of the acid from the product.

Lactic acid bacteria are nutritionally fastidious and require many vitamins and amino acids. Cabbage, cucumbers, and olives used for brining apparently contain all of the essential nutrients for growth of lactic acid bacteria normally associated with fermentation of these commodities. Spanish-style green olives subjected to improper alkali and leaching treatments could provide an exception.

Physical factors

The general handling and brining procedures for cucumbers, cabbage, and olives vary as indicated in Fig. 1. Cucumbers and olives are normally brined whole in a solution of sodium chloride, olives are lye-treated to destroy the bitter principle, oleuropein, and then are washed to remove the alkali before brining, white cabbage is normally chopped or shredded and dry salt then added. A relatively small quantity of cabbage is brined as whole heads. Availability of nutrients in the brine surrounding the vegetable, and movement of salt into the vegetable are influenced by the manner in which the product is treated before brining. Growth of the natural microflora also is influenced by the prebrining treatment. For example, initiation in the fermentation of shredded cabbage is rapid compared to that of cucumbers and olives, probably because of more rapid availability of nutrients as well as the lower concentration of salt present in cabbage fermentation.

The temperature for fermentation is usually dependent on ambient conditions at the time the vegetables are harvested. Cucumbers and olives normally are fermented in tanks that are held out-of-doors and are subject to wide temperature fluctuations. Cabbage is normally fermented indoors and is less subject to extreme temperature fluctuations.

Fermentation tanks vary from less than 1000 to over 10 000 l and may be made of wood, concrete, fiberglass, or plastic. The surface of sauerkraut tanks is covered with plastic sheeting, weighted down with water or brine, to maintain anaerobiosis. The brine surface of cucumber tanks is exposed to sunlight in the out-of-doors tanks to prevent surface growth of undesired micro-organisms. Efforts are underway, however, to develop anaerobic tanks for the cucumber pickle industry which will eliminate problems of surface growth and other problems associated with open tanks (Fleming et al. 1983). Also, such tanks will provide more suitable conditions for use of starter cultures and controlled fermentation methods such as that suggested by Etchells et al. (1973) and as outlined in Fig. 1 (steps in bold face) for cucumbers. Anaerobic tanks were introduced into the olive industry over 10 years ago and are replacing open tanks in the USA and Spain.

Characteristics desired in cultures and efforts for their development

Four species of lactic acid bacteria have been associated with vegetable fermentations: *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Pediococcus pentosaceus* (formerly called *Pediococcus cerevisiae*), and *Lactobacillus plantarum* (Pederson 1960). These species appear during fermentations, in the approximate order listed, for sauerkraut (Pederson 1960). The latter three also have been reported to occur in cucumber (Etchells et al. 1975) and olive (Vaughn 1975) fermentations. Properties of the above four species are summarized in Table 1. Various attempts have been made to use pure cultures of these species for fermentation of vegetables (Cruess 1937, Etchells et al. 1964, 1966, Pederson 1960, Pederson and Albury 1956, 1961). At present, however, pure cultures are used on only a limited commercial basis.

Fleming et al. (1984) listed the following factors which may account for the lack of widespread commercial use of cultures.

(1) Cucumbers, olives, and cabbage undergo natural fermentation by lactic acid bacteria if the product is properly handled and held at salt concentrations that have been established for each particular product.

(2) Brine from one natural fermentation can be used to inoculate other containers.

(3) Heat is the only effective and acceptable means known for ridding vegetables of the natural lactic acid bacteria but is expensive, and changes the flavor and other characteristics of the product.

(4) The fermentation vessels and general handling procedures to date are not compatible with pure culture fermentations.

Table 1. Characteristics of lactic acid bacteria associated with vegetable fermentations.

Property	*Lactobacillus Plantarum*	*Lactobacillus brevis*	*Pediococcus pentosaceus*	*Leuconostoc mesenteroides*
Morphology	Short to medium rods usually singly	Short rods, occurring singly or in short chains	Cocci occurring singly, in pairs and in tetrads	Cocco or bacilli usually in pairs
Optimum temperature	30–35	30	35	20–30
Growth at 45°C	No	No	Yes	No
Growth in 8% NaCl	Yes	No	Yes	No
Glucose metabolism	(Homo-fermenter) Lactic acid	(Hetero-fermenter) Lactic and acetic acids, ethanol, CO_2	(Homo-fermenter) Lactic acid	(Hetero-fermenter) Lactic and acetic acids, ethanol, CO_2
Lactic acid produced From glucose	DL	DL	DL	D

(5) No sufficiently unique strains of lactic acid bacteria have been revealed to date that have mandated their use as starter cultures.

This last point forms the basis for the remainder of this paper. Improvements in bulk fermentation vessels, better environmental controls during fermentation, and development of appropriate controlled fermentation methods may provide the impetus for use of pure cultures of lactic acid bacteria in some, if not all, fermented vegetables. Some parameters on which to base a culture development program and recent attempts to improve cultures for fermented vegetables are given below.

Rapid and predominant growth

Commercial fermentation of vegetables is by naturally occurring micro-organisms, as previously discussed. Predominance of growth by a species of lactic acid bacteria is influenced by the chemical and physical environment under which it must compete. When heat was used experimentally to inactivate the natural microbial flora, pure culture fermentations of sauerkraut (Engelland 1962), cucumbers (Etchells et al. 1964), and olives (Etchells et al. 1966) resulted. However, heat inactivation of undesired micro-organisms has not been considered to be of practical use in vegetable fermentations because of energy requirements and changes in product quality.

For predominance, therefore, the added culture must be highly competitive under the chemical and physical conditions under which the product is held. Salt concentration and temperature are major factors that influence the course of natural fermentations. Direct, mild acidification has been used to eliminate growth by many undesirable bacteria during controlled fermentation of cucumbers (Fig. 1). Acidification does not prevent growth of other acid-tolerant lactic acid bacteria and yeasts, however.

Lactobacillus plantarum characteristically predominates in the later period of vegetable fermentation, apparently because of its greater acid tolerance. It has been found to complete cucumber fermentations regardless of the species of lactic acid bacteria added (Pederson and Albury 1961). Because of its acid tolerance and tendency to terminate fermentations, *Lac. plantarum* seems a likely choice for adaptation and/or modification for use when homolactic fermentation is desired.

Bacteriocins have been demonstrated in many lactic acid bacteria and may have value in achieving a pure culture fermentation if the starter culture is a producing strain. Etchells et al. (1964) observed in pure culture fermentation of pasteurized cucumbers inoculated with *Lac. plantarum* and *P. pentosaceus* that the *Pediococcus* inhibited the growth of the *Lactobacillus*. In later studies, Fleming et al. (1975) demonstrated bacteriocin-like activity in the strain of *Pediococcus* that Etchells observed to be antagonistic to *L. planatrum*. This inhibitory property may be useful if possessed by a strain with superior fermentation characteristics in order to achieve dominance over the competing natural flora. This approach has been used in the development of yeast cultures for sake and wine (Hara et al. 1980). A wild strain of yeast containing the 'killer factor' was mated with starter strains to obtain hybrids containing desirable fermentation characteristics as well as the inhibitory killer factor that is active against contaminating wild yeasts.

Acid production

Rapid acid production is essential for lowering of pH and, thus, inhibiting the growth of undesirable bacteria during the initiation stage of fermentation. Acid production and the added salt thus serve to preserve the vegetable. Lactic acid is the principal acid formed by homofermentative lactic acid bacteria and also accounts for half or more of the acid formed by heterofermentative lactic acid bacteria. While exclusively homofermentative bacteria might be preferred in cucumber fermentations because of the reduced amount of CO_2 produced, such is not the case with sauerkraut. Volatile acid (acetic) and ethanol formed by heterofermentative species, particularly *L. mesenteroides*, are preferred in sauerkraut to impart the desired flavor.

Chen et al. (1983), using the heterofermentative *Lactobacillus cellobiosus* to ferment green beans, produced a mild acid product which was microbiologically stable. Daeschel et al. (1982), using yeasts with lactic acid bacteria as mixed starter cultures, were able to ferment cucumber juice to different degrees of final pH and acidity by manipulating the time of inoculation of each species.

The FAO/WHO Expert Committee on Food Additives (WHO, 1974) made recommendations that the consumption of D (−) lactic acid, in large amounts, should be avoided. Since most lactic acid bacteria associated with vegetable fermentation produce both the D (−) and L (+) forms of lactic acid, efforts were made to isolate strains that produce only L (+) lactic acid for use as starter cultures. Stetter and Stetter (1980) isolated a new species, *Lactobacillus bavaricus*, from sauerkraut which produces only the L (+) isomer. The use of *Lac. bavaricus* as a starter culture for sauerkraut resulted in only L (+) lactic acid being produced (Eden-Warren 1976). *Lactobacillus bavaricus* also has the added benefits of being salt-tolerant (5%) and possessing the ability to grow at very low temperatures (5°C) (Stetter 1974).

Salt tolerance

Greater salt tolerance is required for bacteria used in cucumber and olive fermentations than in sauerkraut fermentation. The lower concentration of salt in sauerkraut probably accounts for the prominence of *L. mesenteroides* in sauerkraut compared to cucumber or olive fermentations. If the salt and acid tolerance of *L. mesenteroides* could be raised, or the salt concentration required for texture retention could be reduced, this species might offer the possibility of improved flavor in cucumbers and olives.

Temperature range

The preferred temperature for sauerkraut fermentation is 18°C or lower, for the best flavored product. *Leuconostoc mesenteroides* grows optimally at a lower temperature than the homofermentative *Lac. plantarum*, presumably resulting in a higher ratio of volatile: nonvolatile acids than at higher temperatures.

Homofermentative lactic acid bacteria with growth over a wide temperature range are required for cucumber fermentation. This is true because of ambient, out-of-door storage of brined cucumbers and the diverse geographic locations where cucumbers are brined. In southern and southwestern parts of the United States, brine temperatures of 27–32°C are typical, while in northwestern and northern regions, temperatures of 16–21°C are typical.

Cultures of *P. pentosaceus* for cucumber fermentation reportedly have been developed that grow well at temperatures down to 18°C (Raccach 1982) and higher than 35°C (Microlife Technics). Cultures of *P. pentosaceus* and/or *Lac. plantarum*, capable of rapid growth at 18°C, could be especially useful for cucumber fermentations in cooler climates or in late fall.

Completeness of fermentation

For cucumbers, it is important that all fermentable sugars be removed during the primary lactic acid fermentation. If residual sugars remain, secondary fermentation by yeasts can result in production of a high concentration of CO_2 with consequent bloater formation. Although purging can be continued until all fermentable sugars are metabolized, for economic reasons it is desirable to complete the fermentation as soon as possible. In the case of olives, it is important to remove all fermentable sugars before packaging, to prevent additional microbial growth which will result in cloudiness in the consumer jar. Fermented olives are not heat processed, and so residual sugar must not be allowed in the final product in quantities sufficient to cause cloudiness.

Carbon dioxide production

Minimal CO_2 production is desired from the lactic acid bacteria used for fermentation of cucumbers, because of the problem of bloater formation. Thus, homofermentative rather than heterofermentative species are desired for cucumbers. Carbon dioxide production contributes to buoyancy pressure created in sauerkraut fermentations which may cause difficulty in keeping the product submerged. Otherwise, CO_2 is not a serious problem and has been ascribed a beneficial role in that it helps maintain anaerobiosis in sauerkraut (Pederson 1960). Heterofermentative species are preferred for sauerkraut fermentation because of their production of volatile acid, a factor that would override any undesirable effects associated with CO_2 production by these species. Fish-eye spoilage in olives is a result of gas-producing microorganisms, but probably not lactic acid bacteria, for Enterobacteriaceae normally are associated with the problem, and proliferate in improperly brined olives.

Decarboxylation of amino acids is undesired in all fermented vegetables because of resultant production of biogenic amines such as histamine and tyramine. Such compounds have been reported in sauerkraut (Mayer 1976). Decarboxylation of malic acid is undesired in cucumber fermentations because the CO_2 produced can contribute to bloater damage. Malic acid, a naturally occurring constituent of pickling cucumbers (McFeeters et al. 1982a) can be decarboxylated by lactic acid bacteria to lactic acid and CO_2. Both homofermentative and heterofermentative species are capable of this activity. McFeeters et al. (1982b) demonstrated that malate decarboxylation can account for most of the CO_2 produced during fermentation of cucumber juice by *L. plantarum*. It was also observed (McFeeters et al. 1984) that bloater damage can be reduced without the necessity for purging in laboratory cucumber fermentations inoculated with a strain of *Lactobacillus* species that is unable to decarboxylate malate. Recently, Daeschel et al. (1984a) developed a differential medium for distinguishing between malate-decarboxylating and nondecarboxylating strains of lactic acid bacteria.

Using the medium allowed rapid screening and isolation of N-methyl-N′-nitro-N-nitrosoguanidine-mutagenized cells of *Lac. plantarum* that had lost the ability to produce CO_2 from malate. The mutants did not produce significant amounts of CO_2 when fermenting cucumber juice that contained native malate or additional malate (Daeschel et al. 1984b). These mutants may be useful as starter cultures for the fermentation of cucumbers by reducing the need for purging of CO_2 from fermenting brines to prevent bloating. To our knowledge, this is the first instance where a lactic acid bacterium has been genetically altered to obtain a specific metabolic trait desired in starter cultures for fermented vegetables.

Cell sedimentation and filterability

An inherent advantage to low salt brining is that the processor may be able to directly pack the product with the fermentation brine, hence eliminating the cumbersome desalting step. In such a system, efficient and rapid removal of the cells would be necessary.

To facilitate cell removal, the cells should possess the ability to rapidly sediment after fermentation and to be amenable to filtration. The brewing industry has developed strains of yeast which have improved flocculation and sedimentation characteristics, hence simplifying cell removal (Johnston and Reader 1983). Many types of filtration systems are used by this industry and may be applicable to brines of fermented vegetables.

Bacteriophage resistance

Bacteriophage infection has not been found to be a problem in vegetable fermentations, perhaps because pure culture fermentation procedures have not been used extensively. Although apparently rare, bacteriophage has been demonstrated in strains of *lac. plantarum* (Sozzi et al. 1981). If and when pure cultures are used extensively for vegetable fermentations, bacteriophage infection could become a problem, as has occurred in cheese fermentations (Lawrence 1978). Bacteriophage infection of malolactic cultures used in wines has recently been recognized (Sozzi et al. 1982).

Nutritive value

Costilow and Fabian (1953) conducted studies on changes in certain nutrients during lactic fermentation of cucumber juice. Losses of 10–30% occurred for pantothenic acid and five amino acids. The possibility for development and use of lactic starter cultures that actually increase the nutritive value of fermented vegetables is worthy of consideration.

Fresh vegetables do not contain vitamin B_{12}, an essential nutrient in human nutrition. Inoculation of fermented foods with vitamin B_{12}-producing bacteria is one approach to increase the nutritive value of food. Ro et al. (1979) inoculated kimchi (Korean-style, fermented Chinese cabbage) with *Propionibacterium freudenreichii*, a bacterium that is a producer of vitamin B_{12}. After a 1 week fermentation, the amount of vitamin B_{12} in the kimchi approximately doubled from 47 to 102 ng $(100\ g)^{-1}$.

Suitable for use as concentrated cultures

Strains of *P. pentosaceus* and *Lac. plantarum* are produced commercially in the USA in concentrated cultures containing 5×10^9 to 10^{10} cells g^{-1} or higher, for use in cucumber fermentations (Porubcan and Sellars 1979). The cultures have been successfully stored in liquid nitrogen and shipped on dry ice. Recently, commercial manufacturers have reverted to storage and shipment of lyophilized cultures. Improved methods of lyophilization have resulted in improved viability of cultures (Porubcan and Sellars 1975). New cultures intended for commercial use must be amenable to the conditions necessary for growth, concentration and storage and the concentrated culture must grow vigorously when inoculated into brined vegetables. An otherwise superior culture could be judged unacceptable if it cannot be grown, concentrated and stored according to commercial requirements.

New Horizons

Fermentation has been viewed as a fundamental method for preservation of vegetables. The production of desirable flavors and other organoleptic qualities has been achieved by manipulation of environmental factors such as salt concentration and temperature to favor growth of preferred and disfavor growth of undesired micro-organisms.

Development of strains of lactic acid bacteria capable of improving the nutritional and organoleptic properties of fermented vegetables seems to be a reasonable goal. Methods are available for the engineering and selection of micro-organisms that already are being applied to other food-related micro-organisms. Suitable controlled fermentation methods and fermentation vessels will be required for the improved culture(s) to be effective. Hopefully, the essential factors necessary for controlled fermentation of vegetables on a practical basis will be realized within the near future. At such time, the vegetable fermentation industry will be in a position for considerable technological advancement.

Acknowledgments

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, Illinois, USA.

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