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Microbial ecology of fermenting plant materials *

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1. SUMMARY

The lactic acid fermentation of plant materials is presented from an ecological perspective emphasizing microbial interactions and their influence on the production of fermented plant foods and silage. The plant lactic acid bacteria are discussed in terms of evolution; epiphytic function; physical distribution within fermented material; substrates and products; microbial sequences in fermentation; interactions among species; pure culture fermentation; and starter culture development.

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2. INTRODUCTION

The preservation of plant material by lactic acid fermentation has been documented as far back as 1000–1500 B.C. by the existence of Egyptian murals depicting the ensilage of grain [1]. It is believed that the Chinese, during the third century B.C., were the first to recognize that vegetables, when placed in a salt brine of suitable concentration, were preserved [2]. In modern times, fermentation as a means of preserving foods has been overshadowed by other preservation methods in developed countries. However, fermentation remains a primary means of preservation in underdeveloped countries and is still important in developed countries because of its low energy requirements and the unique organoleptic properties it imparts to the product.

The lactic acid fermentation of plant material for human and animal consumption constitutes a large volume and diversity. Most vegetables can be fermented; examples include cucumbers, cabbage, olives, carrots, beans, celery, beets, turnips, radishes, and peppers. Silage production in the U.S. on an annual basis is estimated to exceed 150 million tons [3]. A large variety of forage crops is ensiled, including corn, sorghum, ryegrass, alfalfa, and clover. Most, if not all, green plant material probably will undergo a lactic acid fermentation if properly contained. Each particular plant species

provides a unique environment in terms of competing microorganisms; natural plant antagonists; type, availability and concentration of substrate and various physical factors. These conditions allow for the development of a characteristic epiphytic flora, from which arises a population and sequence of fermentation microorganisms when the plant material is harvested and prepared for fermentation.

The intent of this presentation is to review pertinent information concerning the microbial actions and interactions that occur before and during the lactic fermentation of plant material and how these actions influence the processing of plant materials into products of commerce.

3. EPIPHYTIC RELATIONSHIPS

3.1. Evolutionary considerations

Natural environments provide conditions which select for species of lactic acid bacteria that are characteristically found with plants, dairy products or animals. Certain species of lactic acid bacteria are found consistently on a variety of plants, indicating that plants are a natural habitat for lactic acid bacteria.

Taxonomic studies [4–6] indicate that the lactobacilli and pediococci likely have evolved from a common clostridial type of progenitor. It is not known whether these species first appeared before or after the formation of oxygen in the atmosphere ('The Pasteur Point'). However, defenses against oxygen toxicity (primarily the O_2^- radical) evolved and appear to have followed at least three general paths: (1) ancestral clostridia adapted to and occupied niches where oxygen was not present; (2) cells developed enzymatic defenses (superoxide dismutases; (SOD) against toxic oxygen species; (3) cells developed the ability to accumulate Mn(II) intracellularly to use as a defense against endogenous O_2^- ($O_2^{-\cdot} + 2H^+ + Mn^{2+} \rightarrow H_2O_2 + Mn^{2+}$). This last mechanism has only been observed in certain species of the lactic acid bacteria. Upon interpretation of the data of Archibald and Fridovich [7], it appears that lactic acid bacteria associated with plants possess the Mn(II) defense mechanism, i.e., *Lactobacillus plantarum; Lactobacillus fermentum; Lactobacillus casei; Pediococcus pentosaceus; Leuconostoc mesenteroides,* whereas those associated with dairy products (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus lactis*) do not. *L. acidophilus* and *L. bulgaricus* grow poorly aerobically and prefer anaerobiosis. *S. lactis,* along with *Streptococcus faecalis* and *Streptococcus sanguis,* has a SOD rather than Mn(II) oxygen defense mechanisms. Why? Plant material has a high Mn content (Table 1), whereas meat and milk do not. One may speculate that plants serve as a reservoir for the accumulation of Mn by epiphytic lactic acid bacteria. *Lactobacillus plantarum* has a high Mn(II) requirement for growth and can accumulate an intracellular concentration of Mn(II) in excess of 30 mM [8]. Manganese has been recognized as the growth stimulatory component of spices in lactic-acid-fermented sausage [9], and American patents have been issued for using manganese salts in sausage [10] and cucumber [11] fermentations. Lactic acid bacteria associated with dairy products or meat may have evolved or acquired enzymatic defenses (SOD) against oxygen since Mn is present in small amounts.

3.2. Lactic acid bacteria–plant relationship

Plants harbor a numerous and varied microflora. Although the numbers of lactic acid bacteria on plant materials are highly variable, most investigations have shown that their number is very low, often in the range of 10–1000 cells per g

Table 1

Manganese content of selected foods

	μg/100 g or ml
Kale [a]	590000
Beets [a]	575000
Spinach [a]	828000
Milk [b]	2– 3
Beef muscle [c]	15–18
Pork muscle [c]	10–30

[a] Heinz USA [60].
[b] Webb and Johnson [61].
[c] Bechtel [62].

Table 2

Lactic acid bacteria associated with plants

Lactobacillus
L. arabinosus
L. brevis
L. buchneri
L. casei
L. fermentum
L. plantarum
Leuconostoc
L. mesenteroides
Pediococcus
P. acidilactici
P. pentosaceus (including former *P. cerevisiae*)
Streptococcus
S. faecalis
S. faecalis var. *liquefaciens*
S. faecium
S. lactis

[12–17], which represents approx. 0.01–1.0% of the total microbial population. However, the low number but widespread presence of lactic acid bacteria on different plants supports the hypothesis that plants are a natural habitat for some species. On the living plant, the lactic acid bacteria are present on the outer surfaces where several species occur simultaneously [1,2,18]. Among the lactic acid bacteria found on plants (Table 2), there appears to be a predominance of heterofermentative lactic acid bacteria, with *Leuconostoc* being the most frequently occurring genus [15,18].

The numbers of lactic acid bacteria on living plants are restricted by many factors, including UV light, temperature and available nutrients. They multiply where the plant sap is released and are recovered with less frequency from plant parts such as leaves than they are from flowers and fruiting structures. Normally, they compete with the less fastidious, mainly Gram-negative bacteria. The number of lactic acid bacteria increase with the degree of plant maturity and in one study has been reported to be as high as 10^7 per g in fully mature corn plants [19].

Although it appears that lactic acid bacteria coexist with plants, their role on the plant surface is still unknown. Visser et al. [20] presented data suggesting that certain lactic acid bacteria may protect plants from pathogenic microorganisms by producing antagonistic compounds such as acids and bacteriocins. Lactic acid bacteria have been reported [17] to be found in high numbers on damaged parts of plants and, hence, may act to protect the plant against opportunistic phytopathogens.

The microflora of living plant materials is different from that of harvested plants and fermented products. The bacterial flora of living plants is dominated by Gram-negative species and Gram-positive spore formers. Yeasts and molds are also found, but in much smaller numbers [1]. As soon as the plant is harvested, the number of microorganisms increases. This is a result of more nutrients becoming available from the cellular contents of ruptured tissue. Besides the increase in total number, the distribution among different types of microorganisms changes. The dominating, aerobic, Gram-negative bacteria are replaced by facultative and anaerobic, Gram-positive bacteria of the genera *Leuconostoc, Lactobacillus, Pediococcus, Bacillus,* and *Clostridium* [15–17,21, 22]. However, in lactic acid fermentation of plants like alfalfa, ryegrass, cucumbers, carrots, and red beets, it has been shown that Gram-negative bacteria of the family *Enterobacteriaceae* can be present in high numbers and cause problems such as excessive gas in cucumbers [23] and pectinolytic softening in carrots and beets [12]. In silage, clostridia may grow and cause putrefaction if the crops are ensiled too wet or if acidification has not been achieved rapidly [1]. Besides these groups of bacteria, yeasts and molds may cause spoilage problems in fermented vegetable products [1,2,24].

The reasons which determine whether natural plant fermentations are dominated by lactic acid bacteria, by yeast or by spoilage bacteria are not clearly understood. Factors such as initial microbial load, available nutrients, growth rates of certain species, and chemical and physical conditions are undoubtedly important.

The spontaneous lactic acid fermentation is a very complex microbial process in which a very small population of lactic acid bacteria becomes the predominating microbial flora. Knowledge about the ecological events is essential in controlling these fermentation processes. Starter cultures, temperature, pH, and controlled atmosphere are

factors which are used today to improve lactic acid fermentation of plant materials.

4. FERMENTATION

4.1. Physical factors

Lactic acid fermentations involving liquid materials such as milk and soy sauce usually exhibit 'batch culture' kinetics, where the growth rate is not limited by the concentration of readily available substrate. The lactic acid fermentation of brined plant material such a cucumbers, cabbage and olives consists of solids in a liquid environment; hence, the microbial growth rate is influenced by solute (nutrient) movement from the plant material into the surrounding liquid. However, the fermentation of brined vegetables by lactic acid bacteria does not occur exclusively in the brine as once thought. Daeschel and Fleming [25] observed that lactic acid bacteria can enter and grow within cucumbers after they are brined (Fig. 1). They concluded that stomata of the cucumber skin are likely ports of entry. Further studies clarified the density and distribution of the stomata [26], the routes of liquid entry and presumably bacterial entry into the cucumber. Later studies [27,28] enumerated the numbers of lactic acid bacteria distributed between the brine and cucumbers and how different pre-brining treatments altered this ratio. From this same study came information that yeasts, when added to cucumber fermentations, are unable to enter the cucumbers through stomata presumably because of their larger size.

These observations point to the fact that the anatomical structure of the plant material may influence the rate of fermentation as well as the type of microorganisms that may be involved. Silage is also particulate in nature, however, it does not have a liquid enveloping it as with the brine of cucumbers or olives. Nutrients diffuse from solid vegetables into the brine where they are accessible to microorganisms, whereas with silage the nutrients are released by mechanical chopping of the crop. Cucumbers and olives are fermented whole and hence retain physical integrity. Brine also serves to distribute the microorganisms present throughout the fermentation vessel, whereas in silage the microbial distribution would be more dependent on natural epiphytic distributions of individual plants.

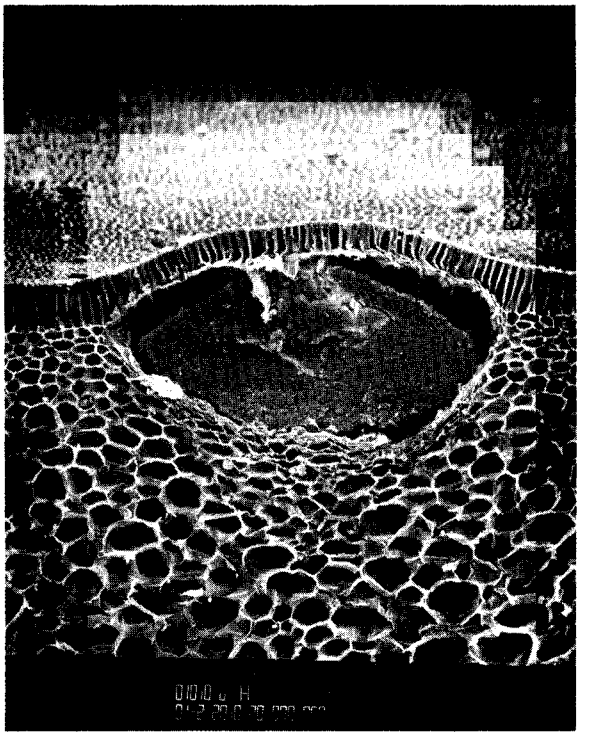

Fig. 1. Scanning electron micrograph of a bacterial (*Pediococcus pentosaceus*) colony directly below the epidermal cell layer of a fermented cucumber.

4.2. Microbial sequences

The fermentation of plant materials results from activities of the lactic microflora which eventually predominate from a large and varied epiphytic flora. The lactic acid fermentations of plant materials are distinguished from those of other raw materials such as meat and pasteurized milk in that the latter initially have a comparatively lower number of microorganisms of less diversity. The diversity of microorganisms on plants provides for several microbial interactions, which influence the activity of certain lactic acid bacteria at different stages and are of great importance for product quality. The key to a successful fermen-

Table 3

Sequence of microbial types during natural fermentation of brined vegetables [a]

Stage	Prevalent microorganisms
Initiation	Various Gram-positive and Gram-negative bacteria
Primary fermentation	Lactic acid bacteria, yeasts
Secondary fermentation	Yeasts
Post-fermentation	Aerobic: surface growth of oxidative yeasts, molds and bacteria Anaerobic: none

[a] From Fleming [22].

tation is to enhance the activity of the desired fermentation microorganisms and to suppress the growth of pathogenic and spoilage microorganisms.

The initial stages in the fermentation are important in establishing a strong lactic acid bacteria population which will carry out a rapid fermentation. In the fermentation of cucumbers, root vegetables, cabbage, and ensiled plant material, the desired fermentation microorganisms are lactic acid bacteria. In the fermentation of cocoa, yeasts and acetic acid bacteria, in addition to lactic acid bacteria are desirable. In soy sauce production, molds and yeasts are also important in the fermentation.

The natural fermentation of cucumbers has been categorized [29] into four distinct stages: initiation, primary fermentation, secondary fermentation, and post-fermentation (Table 3). Once the cucumbers have been put into the brine, there is a rapid growth of Gram-positive and Gram-negative bacteria and yeasts. An initial pH of about 5.5 and the fermentable sugars present (glucose and fructose) are factors which make cucumbers a suitable substrate for lactic acid bacteria. The addition of NaCl helps attain the establishment of a lactic acid bacteria flora. During the fermentation of brined cucumbers, *L. mesenteroides, P. pentosaceus* and *L. plantarum* generally appear in the order listed, reflecting increasing total acid production during fermentation. Initial load, growth rates, salt- and acid-tolerances are factors which are responsible for the sequence within the lactic acid bacteria. Growth of the heterofermenter, *L. mesenteroides,* is undesirable from a product viewpoint since the CO_2 produced contributes [30,31] to gaseous spoilage. Although *L. mesenteroides* is not as acid-tolerant as the other species, it generally initiates the fermentation because it is present at an initially higher number than the other lactic acid bacteria associated with the fresh cucumbers [16].

In the production of sauerkraut, the fermentation is initiated by *L. mesenteroides,* and to some extent *S. faecalis,* and terminates with *L. plantarum* [2,24].

Fermentation of silage is a solid-state fermentation where conditions are not as uniform as when a brine is used. Fluctuations in conditions influence the availability of nutrients and diffusion of metabolic products. The buffer capacity is also much greater in a silage fermentation, which affects the rate of acidification and might be advantageous to less acid-tolerant lactic acid bacteria such as *S. faecalis.* A rapid decrease in pH is the key to a successful silage fermentation primarily to preclude the growth of clostridia. Silage, more than any other lactic-acid-fermented commodity, is subject to the inclusion of chemical additives to enhance fermentation [1]. In fermentation of silage, *S. faecalis* and *L. mesenteroides* usually initiate the fermentation. These are in turn replaced by more acid-tolerant species such as *L. brevis, L. plantarum* and *Lactobacillus buchneri.*

The fermentation of cocoa is mediated by yeasts, acetic acid bacteria and lactic acid bacteria. Two main stages are recognized, an early anaerobic yeast fermentation and a secondary bacterial fermentation. The reason why the yeasts become active first is not understood. However, the ethanol produced is then oxidized by acetic acid bacteria to acetic acid, which is important to aroma development in the cocoa fermentation. Lactic acid bacteria also participate [32] in the fermentation, but are viewed as undesirable because the nonvolatile lactic acid contributes to unwanted acidity in processed cocoa [21].

The above-described fermentations suggest the complexities in the microbial ecology of naturally fermented plant materials. Each type of microorganism in one way or another contributes to the final characteristic properties of the products.

From an industrial point of view, it is necessary to know how to control such a complex fermentation so that the desirable microorganisms develop and to ensure that the products are of high quality and consistency. For these reasons, the use of starter cultures is an attractive technique, especially where a rapid decrease in pH is the most critical feature. Commercial starter cultures are available for cucumber and silage fermentations. However, in other lactic acid fermentations like sauerkraut, the several species and the particular sequence in which they evolve create a characteristic identity (primarily flavor) for the product. It might be difficult to repeat such a natural fermentation by using starter cultures without changing the character of the final product.

4.3. Substrates and products

The lactic acid bacteria are broadly classified as being homofermentative, heterofermentative or facultatively heterofermentative depending on the presence or absence of aldolase or phosphoketolase. The reader is referred to Kandler [33,34] for definitive descriptions of carbohydrate metabolism in lactic acid bacteria. Glucose, fructose and sucrose are the primary substrates in plant material that are fermented by the lactic acid bacteria. Pentoses, citric acid and malic acid are also present in most plant material, but in small amounts and can also be metabolized by the lactic acid bacteria. Mannitol is present in some plants such as mushrooms. Mannitol can be formed during fermentation by the reduction of fructose. This commonly occurs during the heterofermentative part of the sauerkraut fermentation when fructose is used as an electron acceptor by such species as *L. brevis* and *L. mesenteroides*. The mannitol can then serve as a substrate for homofermentative species which occur later. Dextrans can be formed from sucrose by *L. mesenteroides* in sauerkraut fermentations. A ropy viscosity is seen sometimes during the early phases of sauerkraut fermentation due to dextran production, but disappears later on. Presumably the dextrans serve as a substrate for other species of lactic acid bacteria.

Free pentoses are normally not present in living plants but are liberated after harvest as a result of hydrolysis of hemicellulose [35]. Many lactic acid bacteria, but very few yeasts, are able to ferment pentoses. The lactic acid bacteria usually found in plant fermentation are either heterofermentative or facultatively heterofermentative, hence they possess phosphoketolase for the dissimilation of pentoses. The strictly homofermentative lactobacilli do not have phosphoketolase and are rarely isolated from plant fermentations.

Malic and citric acids are the predominating organic acids in fruits, vegetables, grains, and grasses and can be metabolized into several different products by the lactic acid bacteria during fermentation [1,36,37]. Of special importance is the malolactic reaction whereby malate is dissimilated into lactate and CO_2. This reaction is desirable in some wines since it reduces the acidity. However, in cucumber fermentations, the CO_2 produced by this reaction can contribute to cucumber bloater damage [38,39].

In the fermentation of silage, the buffering capacities of citrate and malate have a significant influence on pH. When the acids are metabolized by lactic acid bacteria, acidity is reduced with subsequent retardation in the rate of pH decrease. Dissimilation of organic acids also results in undesirable losses in dry matter, primarily from decarboxylation.

The amount and type of acids produced during fermentation influence subsequent microbial activity in the fermented material. In addition to low pH, the type of acid formed has influence. Acetic acid, for example, is more antagonistic against yeasts compared to lactic acid. Among the lactic acid bacteria, there is a difference in sensitivity to acetate, a factor that may influence the microbial sequence in a lactic acid fermentation. It should also be pointed out that oxidative yeasts are able to utilize organic acids as a carbon and energy source and, thus, can cause spoilage through deacidification in fermented plant material.

Lactic acid bacteria from plants are in general regarded as having weak or no proteolytic activity, and their capability to degrade amino acids is limited compared to many other microorganisms. Lactic acid bacteria have been shown to deaminate arginine and serine, yielding ornithine and acetoin, respectively [40].

4.4. Ecological observations

Many different types of ecological interactions have been observed to occur among the microbial populations that exist in fermenting plant materials. Particular strains can possess a competitive advantage that may be manifested in many ways such as faster inherent growth rates, diverse substrate utilization and increased tolerance to high acid concentrations and low pH. An interaction which is favorable to two or more populations, but not necessarily obligatory, is readily observed in the 'sour dough fermentation,' which is mediated by yeast and lactic acid bacteria. The bacterium *Lactobacillus sanfrancisco* ferments maltose, but not glucose. Some glucose is provided by the action of the maltose phosphorylase pathway which is then fermented by the acid-tolerant yeast, *Saccharomyces exiguus,* which cannot use maltose. The yeast in turn provides growth stimulants for the bacterium. One such stimulant was identified to be a small peptide of $M_r \approx 1065$ [41]. This association appears to be very stable and has apparently been propagated and maintained in nonaseptically handled 'starter sponges' for many years.

Ecological interactions which result in a benefit to one population without benefit or detriment to another are probably the most common interaction present in plant fermentations. Examples include: (1) the fermentation of sugars in cocoa by yeast to ethanol which will then support a population of acetic acid bacteria; (2) in cucumber fermentations, the lactic acid bacteria lower the pH to such an extent (pH 3.1) that they and all other microorganisms cannot grow except for certain fermentative yeasts which will then ferment residual sugars. This last example is an interaction that is discouraged in the fermentation because the CO_2 produced by the yeast can contribute to product spoilage. The addition of buffers to the fermentation prevents this interaction by allowing the lactic acid bacteria to ferment all sugars present [29]. Another interaction that processors deem undesirable is the oxidation of the preserving organic acids produced by the lactic acid bacteria by oxidative yeasts. This results in a rise in pH and a loss of preservative action and of dry matter. This interaction is inhibited by either maintaining anaerobiosis or using antimicrobials such as potassium sorbate.

Antagonistic interactions have also been well documented in plant fermentations. In fact, it is the production of antagonistic compounds (lactic and acetic acids) which forms the basis of preservation by fermentation. The production of these compounds directly determines the numbers, types and sequences of microbial populations that exist in plant fermentations. Noda et al. [42] demonstrated that acetic acid was the compound involved in antagonism by osmophilic lactic acid bacteria towards yeasts in brine fermentation of soy sauce. However, yeasts have also been shown to be antagonistic toward lactic acid bacteria [43] mainly due to the ethanol produced, but also by sulfur dioxide that can be produced by some yeasts. This becomes important when considering the use of lactic acid bacteria to bring about a malolactic fermentation in high-acid wines to reduce the acidity.

True antibiosis in lactic fermentation is most clearly seen with strains that produce bactericidal proteins known as bacteriocins. A hint that bacteriocins may play a role in plant fermentations came when Etchells et al. [44] observed in pure culture fermentation of pasteurized cucumbers inoculated with *L. plantarum* and *P. pentosaceus* that the pediococcus inhibited the growth of *L. plantarum.* In later studies, Fleming et al. [45] demonstrated bacteriocin-like activity in the strain of pediococcus that Etchells observed to be antagonistic to *L. plantarum.* Recently [46], evidence was presented that suggested that the bacteriocin produced by the pediococcus and immunity to the bacteriocin were associated with plasmid DNA. There has been much recent interest in bacteriocins from lactic acid bacteria in respect to their genetics and biochemistry [47–50]. Bacteriocins are presumed to provide a competitive advantage to producer strains by inhibiting competing strains.

Plant fermentation at the microbial level is abundant in examples of ecological interactions which are the basis of fermentation. These interactions are what man attempts to control, enhance and inhibit in order to obtain products of con-

sistent high quality. The next section of this presentation deals with these aspirations.

5. FERMENTATION CONTROL

5.1. Pure culture fermentation

Man continually tries to improve his environment by controlling, directing and selecting for favorable interactions. In plant fermentations, control procedures are necessary in order to prevent spoilage and to provide products of consistent high quality. However, the extent and sophistication of such measures is governed by the economics of the particular product. Sterilization and aseptic handling of plant material prior to culture inoculation is currently considered to be economically impractical.

The fermentation of plant materials is difficult to control primarily because of their shapes, the large number of naturally occurring microorganisms and the variability in nutrient content. Silage, more than any other commodity, is subject to a large number of pre-fermentation treatments [1]. These treatments fall into several general categories: (1) acidulants, (2) fermentation inhibitors, (3) fermentation stimulants, (4) antimicrobials, and (5) nutrient additives.

Pure culture fermentation realistically cannot be achieved with plant materials since it would require a sterilization step which would be redundant with the primary objective of fermentation, that being one of preservation. The most economical and practical approach would be to have a controlled fermentation where the fermentation is conducted by one or more cultures of known species that possess desirable fermentation characteristics and that the fermentation is the result of the metabolic activities of those species. Growth of microorganisms other than the desired species may occur, but their effect on the final product should be inconsequential.

Attempts at achieving the fermentation of cucumbers by addition of pure cultures were first reported by Pederson and Albury [51,52], who found that strains of naturally occurring *L. plantarum* completed the fermentation regardless of the species of bacteria used. In these experiments, no effort was made to remove or eliminate the naturally occurring lactic acid bacteria present with the cucumbers. Etchells et al. [30,44] were able to obtain pure culture fermentation of cucumbers by hot-water blanching or by γ-radiation of the cucumbers prior to inoculation. These procedures, while feasible for experimental purposes, have been considered to be economically and technically impractical for commercial use. From these studies evolved a controlled fermentation procedure for cucumbers [53,54] which has gained limited commercial acceptance. This procedure sets the environmental conditions to promote the rapid growth of the starter culture, but does not necessarily preclude the growth of naturally occurring lactic acid bacteria.

5.2. Starter culture development

The lactic acid fermentation of vegetables and silage has traditionally relied upon the activity of the indigenous lactic acid bacteria. The commercial use of lactic starter cultures has found application primarily with cucumbers and silage. Increasing the use of lactic starter cultures could provide more consistent fermentations and products of higher quality. Such starter cultures must possess appropriate traits and, most importantly, be able to predominate over the naturally occurring lactic acid bacteria to be effective. We recently have reviewed the critical physical, chemical and biological fermentation control factors that must be considered in the development of starter cultures for use in cucumber fermentations [55]. Specific control factors include anaerobic tank technology, gas exchange of vegetables prior to brining, chemical modification of the brine, and the use of bacteriocin-producing strains of lactic acid bacteria as starter cultures. The development and implementation of such control factors will provide further impetus for starter culture improvement. Desirable starter culture traits have been proposed for fermented cucumbers [56] and silage [57]. These traits are summarized and compared in Table 4. Bacteriophage resistance is an important trait for dairy starters, but has not been demonstrated to be such for silage or vegetable

Table 4

Traits cited as being desirable in starter cultures

Traits	Cucumbers [a]	Silage [b]
Rapid and predominant growth	Yes	Yes
Homofermentative metabolism	Yes	Yes
Temperature range	15–35 °C	Up to 50 °C
Inability to metabolize organic acids	Yes	Yes
Salt tolerance	Yes	NA
Acid production and tolerance	Yes	Yes
Do not produce dextrans	NA [c]	Yes
Do not reduce fructose to mannitol	Yes	Yes
Ability to ferment: glucose	Yes	Yes
fructose	Yes	Yes
sucrose	Not present	Yes
fructosans	Not present	Yes
pentosans	Not present	Yes

[a] Daeschel and Fleming [27].
[b] Whittenbury [57].
[c] NA, not applicable.

fermentations. This may be explained by the probability that pure culture fermentations are not occurring. In the event a starter is infected with phage, members of the naturally occurring lactic flora would quickly predominate the fermentation. Phage infection would also be less of a problem in plant fermentations, particularly silage, because of its particulate nature. A phage infection would likely be localized since the absence of liquid would preclude its dissemination. Efforts in our own laboratory have focused on developing bacteriocin-producing starter cultures to enhance the ability of the strain to predominate the fermentation. We have identified and characterized bacteriocins from *P. pentosaceus* [46] and *L. plantarum* [58] and are evaluating their potential in controlling the natural microflora in cucumber fermentations.

6. FUTURE DIRECTIONS

A further understanding about the interaction of lactic acid bacteria and plant material is necessary to improve the use of lactic acid fermentation as a process to preserve and produce food and feedstuffs. Such understanding will aid in the further development of existing fermentation processes and open possibilities for the fermentation of other plant materials.

Use of starter cultures is an attractive technique for production of desirable, fermented products. Selection, adaptation and genetic engineering offer great possibilities in the development of strains with respect to nutritional, organoleptic and preservative properties. Further genetic knowledge about the lactic acid bacteria, particularly genetic transfer systems, is needed. Development of such systems would be an important step in the development of predictable fermentations.

One limiting factor in utilizing lactic acid fermentation as a preservation technique for plant materials over all is an incomplete fermentation of sugars resulting in possible growth of spoilage microorganisms. To eliminate this risk, the combination of lactic acid bacteria and yeasts may be useful to increase sugar utilization [59]. Another approach to improve the storage stability of fermented products is to use starter cultures which produce antimicrobial compounds with antagonistic activities against yeasts and spoilage bacteria, including spores. Further research is needed in these areas.

Most lactic acid-fermented plant products have a characteristic organoleptic quality which probably is the most important factor for consumers. However, few data are available about how fermentation can influence the flavor of the products and how this can be improved by the lactic acid bacteria or other microorganisms. Further basic understanding about the formation of flavor compounds in fermented plant materials is necessary to maintain existing quality and in developing improved controlled fermentation procedures.

Vegetables are considered to be nutritious, wholesome and essential in balanced diets. However, too little is known about how a lactic acid fermentation process influences the nutritional values of plant materials, whether it be in a beneficial or detrimental manner. Nutritional research of lactic acid-fermented plant materials is, therefore, another area of interest.

Hopefully, further research will allow us the understanding to control the fermentation

processes of many different plant materials toward a common goal of high and consistent product quality in the future, and help to enhance the lactic acid fermentation as an attractive process for food and animal feed production.

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