15. Achieving pure culture cucumber fermentations: a review*

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SUMMARY

The lactic acid fermentation of vegetables such as cucumbers, cabbage, and olives traditionally has relied upon the activity of the indigenous lactic acid bacteria associated with the fresh vegetable. These natural fermentations usually produce acceptable products; however, inconsistencies in the final product and spoilage do occur. The use of lactic starter cultures could provide more consistent fermentations and products of higher quality. Such starter cultures must possess appropriate traits and be able to predominate over the naturally occurring lactic acid bacteria to be most effective. In this review we focus upon physical, chemical, and biological fermentation control factors that must be considered in the development of starter cultures for use in cucumber fermentations. Specific control factors considered include anaerobic tank technology, gas exchange of the vegetables prior to brining, chemical modification of the brine, and use of bacteriocin-producing strains of lactic acid bacteria.

INTRODUCTION

The brine fermentation of vegetables by lactic acid bacteria is an example of biotechnology which originated long before the term became popular. For centuries it has been recognized that when fresh vegetables were submerged in salt solution a sour, preserved product was formed which was pleasant and safe to consume. Scientific reports on the microbial fermentation of vegetables began to emerge around 1890–1900, following Pasteur's observation of yeast in beer and wine fermentations.

Although freezing and canning have somewhat eclipsed the importance of fermentation as a primary method of vegetable preservation, fermentation still is important because it: (1) results in products with unique organoleptic traits, (2) is an energy-efficient process, and (3) by bulk storage, allows for distribution of labor and equipment needs for the processing of vegetables beyond the growing season.

Fresh vegetables normally harbor an epiphytic microbial population, consisting primarily of aerobic microorganisms, of the order of 1×10^5–1×10^7/g [1]. Lactic acid bacteria constitute less than 1% of this naturally occurring population [23]. When vegetables are brined, the salt serves to suppress the majority of the microorganisms present, and at the same time selects for the salt-tolerant lactic acid bacteria and yeasts.

The sequence of microorganisms that occur during the natural fermentation of brined cucumbers

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Table 1

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

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

[a] From Fleming [16].

is given in Table 1. Fleming [16] discussed in detail the microbial and chemical events that occur in each stage. The successful fermentation of vegetables by naturally occurring lactic acid bacteria is dependent upon adequate numbers of bacteria and suitable temperatures and salt concentrations. Variations in fermentation conditions and methods contribute to the occurrence of spoilage problems. Examples of spoilage with fermented cucumbers include: (1) incomplete conversion of fermentable sugars into acid, which can result in post-processing microbial growth in unpasteurized products, and (2) the development of excessive gas (CO_2) pressure in the cucumber during fermentation which can result in ruptured tissue (bloater damage).

Attempts at achieving pure culture fermentation of cucumbers were first reported by Pederson and Albury [24,25], who found that strains of naturally occurring *Lactobacillus plantarum* completed the fermentation regardless of the species of bacteria used as inoculum. In these experiments, no effort was made to remove or eliminate the naturally occurring lactic acid bacteria present with the cucumbers. Etchells et al. [10,13] were able to obtain pure culture fermentation of cucumbers by hot water blanching or by gamma radiation of the cucumbers prior to culture addition. 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 [11,12], of which certain aspects are used commercially. This procedure sets the environmental conditions to promote rapid growth of the starter culture but does not necessarily result in a pure culture fermentation.

Much of the research on cucumber fermentations has been summarized in recent reviews [14,16,17]. It has become evident that major improvements in the fermentation process will depend on developing cultures of lactic acid bacteria with desirable traits, and establishing conditions for their predominance of fermentations. The objective of this presentation is to review recent applied and basic research in our laboratory to develop controlled fermentation methods for cucumbers and other vegetables.

PHYSICAL AND CHEMICAL APPROACHES TO CONTROLLING MICROBIAL ACTIVITY IN FERMENTATIONS

Brined cucumbers possess unique physical properties that influence the fermentation. The fermentation of whole cucumbers is compartmentalized into two components, the brine and the cucumbers, each possessing its own physical, chemical, and biological characteristics. When cucumbers are brined, each of these characteristics contributes to a dynamic process which, when successful, results in a quality product. *Controlling* the microbial population and subsequent metabolic activities is a key feature for a controlled fermentation strategy.

Brined cucumbers are traditionally fermented and contained in large wooden, fiberglass, or polyethylene tanks that are open to the atmosphere (Fig. 1A). The open top allows UV rays of the sunlight to strike the brine surface and thus suppress growth of acid-oxidizing yeasts and molds. This feature, however, allows entrance of oxygen, rainwater and foreign material. Salt is added in excess as insurance against problems associated with the tank being open to the atmosphere. Wooden tanks are also subject to leaking, necessitating further

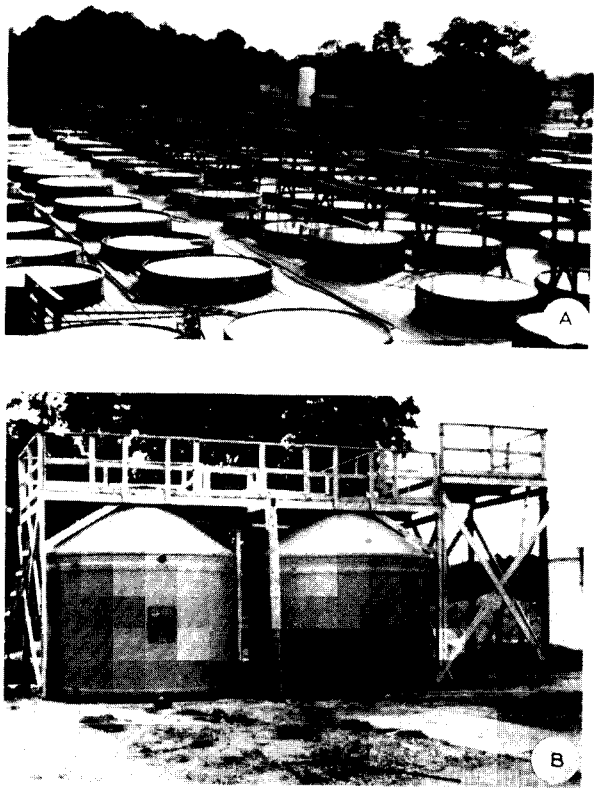

Fig. 1. (A) Conventional brine yard with open-top wooden tank. (B) Prototype closed-top, anaerobic tanks for vegetable fermentations.

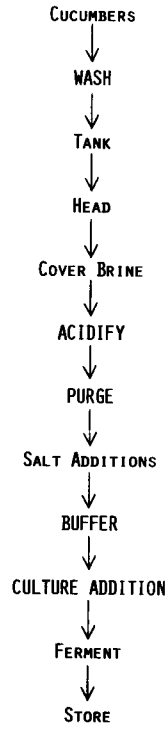

Fig. 2. Controlled fermentation flow chart.

brine addition. These features are not conducive to controlling microbial fermentation activity. The use of fiberglass or polyethylene tanks has eliminated the leaking problem; however, it is apparent that the open-top tank is a deterrent to implementation of pure culture fermentation methodology. A closed-top anaerobic tank [19]. (Fig. 1B) for brining cucumbers is being developed currently and promises to provide a closely controlled environment for fermentation. This tank design permits the inclusion of several fermentation control features, including gas composition, pH, and salt concentration, which heretofore were subject to the vagaries of the open-top design.

Essential features of the controlled fermentation procedure of Etchells et al. [11,12] for brined cucumbers are summarized in Fig. 2. This procedure does not result in a pure culture fermentation, but serves to set the environment to favor growth by the starter culture rather than the naturally occurring lactic acid bacteria. The washing step removes the majority of bacteria adhering to the fresh cucumber (Table 2), including naturally occurring lactic acid bacteria that would compete with a starter culture after its addition.

Both the concentration and chemical composition of the brine play a governing role in the sequence of microorganisms that occur in natural cucumber fermentations. Proper salt concentration exerts a selective effect on natural flora, resulting in the growth of lactic acid bacteria. Too little salt ($<5\%$) favors the growth of Enterobacteriaceae along with the lactic acid bacteria. Too much salt ($>15\%$) suppresses the lactic acid bacteria and allows the development of halotolerant fermentative yeast [15]. The acidification step (acetic acid) in the controlled fermentation procedure effectively suppresses the growth of the natural microbial flora that occurs during the initiation stage of natural fermentations. After 1 day, the brine is buffered

Table 2

Removal of microorganisms from cucumbers by washing[a]

Washing treatment	Sample No.	Microorganisms/g cucumbers			
		Total aerobes	Enterobacteriaceae	Yeasts and molds	Lactic acid bacteria
Not washed	1	870 000	20 000	2	1 800
	3	560 000	30 000	6	2 300
	5	530 000	18 000	3	1 300
	7	1 000 000	61 000	52	6 200
	Average:	750 000	32 000	16	2 900
Washed	2	68 000	7 800	<1	75
	4	130 000	5 500	<1	60
	6	140 000	21 000	<1	91
	8	400 000	110 000	<1	19
	Average:	180 000	35 000	<1	61
Washed/unwashed		0.26	1.13	–	0.02

[a] Adapted from Fleming et al. [19].

with sodium acetate or the acetic acid is neutralized to form acetate. Buffering serves to eliminate secondary fermentation by yeasts by allowing the starter culture to ferment all the sugars. With the pH at about 4.7, the brine is inoculated with either *L. plantarum* or *Pediococcus pentosaceus*, or a combination of these organisms for a total cell count of 1–4 billion cells per gallon of brined cucumbers.

By setting the appropriate chemical environment in the fermentation, we may be able to: (1) eliminate or suppress microorganisms competing for the same substrate as the starter culture, (2) provide conditions for rapid growth of the starter culture, and (3) ensure that all fermentable substrate is metabolized by the starter culture, hence eliminating a nutrient source for competing and contaminating microorganisms.

The closed-top tank design may also influence microbial activities by regulating the amount of oxygen present. The present design concept of the tank incorporates a N_2-containing headspace. This is a critical feature since, previously, the suppression of oxidative microorganisms depended on the microbiocidal activity of UV rays from sunlight. The N_2 headspace suppresses the growth of oxidative microorganisms whose growth could spoil or reduce the quality of the product and also compete with the starter culture.

Fleming et al. [22] found that exchange of the internal gases of fresh cucumbers with pure oxygen immediately before brining caused the brined cucumbers to acquire the translucent appearance of fully cured pickles within hours, as compared to several months for nonexchanged cucumbers. When the cucumbers are covered with brine, respiration in the cucumber tissue consumes O_2 with subsequent accumulation of CO_2, leaving only N_2 and CO_2 in the cucumber. CO_2 is about 80-times more soluble than O_2, so much of the CO_2 formed dissolves in the liquid of the cucumber tissues. This causes the gas pressure in the air spaces to decrease and form a vacuum which reaches a maximum after about 1 h [2]. The vacuum causes brine to be taken into the tissue. The vacuum within the cucumber also causes bacteria that may be present in the brine or on the fruit to be drawn into the tissue, where

they may grow [4]. Previous studies [6,8] have shown that the percentage of bacteria located within the cucumbers can be 8–51% of the total, depending upon: (1) gas exchange treatment of the cucumbers before brining and (2) time of inoculation with starter cultures after brining.

It has been proposed that a rate-limiting factor in the fermentation in cucumbers is the diffusion rate of soluble sugars from the cucumber into the brine [26]. Using oxygen-exchange methodology, the starter culture bacteria can be brought into immediate contact with cucumber nutrients, with a likely increase in fermentation rate and rapid growth of the desired bacterial strain over the natural lactic acid bacteria present. This approach must be tempered with a note of caution. Undesirable members of the natural flora may also be taken into the cucumber during the oxygen exchange procedure, where they may be more likely to cause spoilage. For example, heterofermentative lactic acid bacteria, because of their ability to produce CO_2 during glucose fermentation, may produce enough CO_2 to cause bloater damage if they grow within the cucumbers. Bloater damage is usually sufficiently controlled by purging the fermentation brine with N_2 to sweep out CO_2 accumulation in the brine. However, purging is *not* effective if large amounts of CO_2 are produced by microbial activity *within* the cucumbers [3]. Nonetheless, it may be possible to exploit the oxygen exchange technology to help promote the pure culture fermentation strategy as long as we are aware that complications may arise.

The use of physical and chemical control mechanisms for fermentation will only be effective if the starter culture employed is optimal for the system. Vegetable fermentations represent a dynamic ecosystem where the principles of natural selection can easily be applied. The succession of microorganisms seen in natural fermentation reflects the changing chemical and physical environment of the fermentation as it proceeds. From the start to the end of fermentation, large changes occur in solute concentration of sugars, salt and acids, with a decrease in pH from 5.5 to 3.2 and a change from an aerobic to an anaerobic atmosphere. In addition, there exist two distinct environments (cucumber and brine) within the fermentation. It may not be feasible to develop a single lactic acid bacterium that would grow optimally and competitively throughout the fermentation. More than one species or strain of bacteria may be required for control during the fermentation, each optimized for a specific phase. This would mimic the succession found in natural fermentations, but with optimized strains. Thus, 'mixed' rather than 'pure' cultures may be preferable for vegetable fermentations. In either case, the culture(s) should possess desirable traits and be able to predominate over naturally occurring bacteria to be most effective.

BIOLOGICAL CONTROL: BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA

Bacteriocins have been demonstrated in many lactic acid bacteria and may have value in achieving pure culture fermentation. Etchells et al. [13] observed in pure culture fermentation of pasteurized cucumbers inoculated with *L. plantarum* and *P. pentosaceus* that the *Pediococcus* inhibited the growth of the *Lactobacillus*. In later studies, Fleming et al. [18] demonstrated bacteriocin-like activity in the strain of *Pediococcus* that Etchells observed to be antagonistic to *L. plantarum*. This inhibitory property may be useful, if possessed by a strain with superior fermentation characteristics, in achieving dominance over the competing natural flora.

One drawback to this approach is that nearly all described bacteriocins from lactic acid bacteria have a relatively narrow spectrum of activity in terms of species inhibited. One exception is the bacteriocin, Pediocin A, from *P. pentosaceus*, originally described by Fleming et al. [18] as being active against all gram-positive bacteria tested, but not against gram-negative bacteria or yeast. Daeschel and Klaenhammer [7] extended this study and found that many food-poisoning bacteria were also inhibited (Table 3). This same study also presented physical evidence that bacteriocin production and immunity were associated with a plasmid DNA element. This observation may allow one to consider

Table 3

Bacterial strains and their sensitivity to bacteriocins produced by *P. pentosaceus* L7230 and FBB61[a]

Species	Source[b]	Sensitivity to strain:	
		L7230	FBB61
Clostridium spp.			
C. botulinum 62A (type B)	P.M. Foegeding, NCSU	+	+
C. botulinum 213B (type B)	P.M. Foegeding, NCSU	+	+
C. botulinum 12885A (type A)	P.M. Foegeding, NCSU	+	+
C. perfringens NCTC8798	P.M. Foegeding, NCSU	+	+
C. sporogenes	University of Tennessee	+	+
Lactobacillus spp.			
L. brevis LB50	Our culture collection	+	+
L. plantarum WSO	Our culture collection	+	+
Pediococcus spp.			
P. acidilactici 33314	ATCC	+	+
P. dextrinicus 33087	ATCC	+	+
P. pentosaceus L7230	Our culture collection	−	−
P. pentosaceus FBB61	Our culture collection	−	−
P. pentosaceus FBB61-2	Our culture collection	+	+
P. pentosaceus FBB61-8	Our culture collection	+	+
P. pentosaceus PC39	Our culture collection	+	+
P. pentosaceus B1325	NRRL	+	+
P. pentosaceus B11465	NRRL	+	+
P. pentosaceus 25745	ATCC	+	+
P. pentosaceus 33316	ATCC	+	+
Strain 'lactocel 75'	Microlife Technics, Sarasota, FL	+	+
Staphylococcus spp.			
S. aureus MD9	University of Tennessee	+	+
S. aureus 138CPS	ABC	+	+
S. aureus 146CPS	ABC	+	+
S. aureus 153CPS	ABC	+	+
S. lactis 11454[c]	ATCC	+	+

[a] From Daeschel and Klaenhammer [7].

[b] Abbreviations: NCSU – North Carolina State University; ATCC – American Type Culture Collection (Rockville, MD); NRRL – Northern Regional Research Laboratory (USDA, Peoria, IL); ABC – ABC Research Corporation (Gainesville, FL).

[c] Whereas *S. lactis* 11454 was sensitive to *P. pentosaceus* FBB61 and L7230 bacteriocins, the latter strains were sensitive to *S. lactis* 11454 (nisin producer). This indicated that the pediocin was not nisin.

genetically transferring the bacteriocin-producing ability and immunity to superior fermentation strains. This trait may prove to be very stable once transferred, since spontaneous loss of the plasmid would result in a loss of immunity, thus making any 'cured' variants subject to the lethal action of the bacteriocin. We have recently described the characteristics of a bacteriocin (plantaricin A) produced from a strain of *L. plantarum* that was isolated from cucumber fermentation liquid [9]. Like most other bacteriocins described, plantaricin A is active only against other closely related lactic acid bacteria.

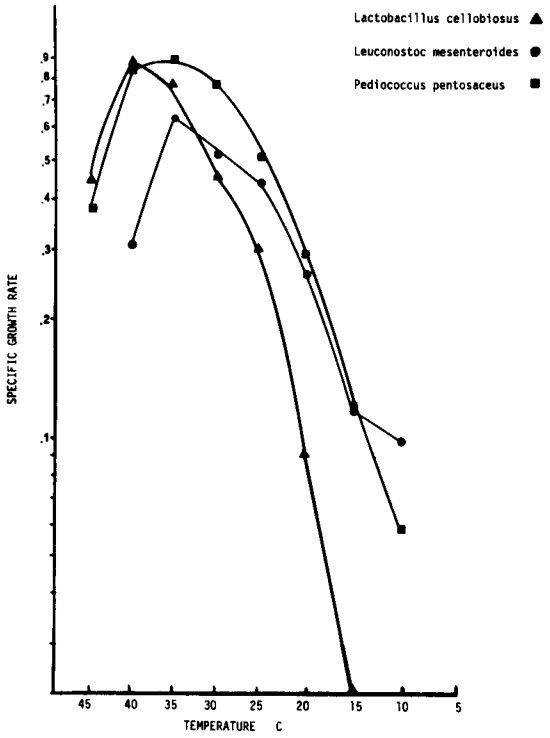

Fig. 3. Effect of temperature on the growth rate of lactic acid bacteria cultivated in cucumber juice.

However, this strain possesses satisfactory fermentation characteristics and would probably only have to compete with other naturally occurring *L. plantarum* strains.

DEVELOPMENT OF SUITABLE STRAINS

This topic has recently been the subject of several reviews, and the reader wishing more detailed information is referred to them [5,9,20,21]. Briefly, rapid and dominant growth, type and extent of acid production, salt tolerance, inability to decarboxylate malic acid, temperature range, 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. All of these traits, no matter how effectively they are selected for or engineered into a strain, will only be relevant if the culture possesses rapid and dominant growth under the physical and chemical conditions of the fermentation. This is illustrated in Fig. 3, where it is shown that temperature can influence the growth rates and presumably the dominance of certain strains during fermentation. Cucumber fermentation temperatures vary significantly depending on geographical location and time of year. There exists a need for starter cultures which can rapidly ferment at lower temperatures (15–25°C).

CONCLUSIONS

The development and implementation of practical methodology for achieving pure culture fermentation of vegetables brined in bulk will depend on the proper integration of various physical, chemical, and microbiological control factors. Recent efforts in developing cucumber starter cultures and anaerobic tanking procedures have provided further impetus for developing pure culture fermentation strategies. Pure culture fermentation promises to provide products of consistently high quality with potentially greater market value. Pure culture fermentation will also allow us to begin to look ahead and take advantage of the promise of recombinant DNA technology to develop starter cultures with enhanced fermentation characteristics.

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