■ 總 說 ■

The Lactic Acid Bacteria in Connection with the fermentation of vegetables

by **Duk Hyun Cho***

Introduction

The lactic acid bacteria may play the most important role in the preparation and preservation of native Korean vegetable foods. The various seasonal, so called, Kimchis and pickles are all unquestionable lactic acid fermented vegetables. While it may be reasonably assumed that principally the same microbiological events will be involved in these fermentations as in the fermentations of sauerkraut, pickles and olives which have been well studied microbiologically, little has been reported on the microbiology of this particular vegetable foods.

Prior to the study of microbiology of these vegetable foods, therefore, a brief review is intended by the author on those problems of lactic acid bacteria which interested the author in connection with this study.

However this would not be a general historical review which covers every field of lactic acid bacteria of this subject. Effort is made to obtain the orientational ways of thinking and techniques and to obtain the facts which might be a direct help along this line of study.

General taxonomy

The lactic acid bacteria derives its name from its souring of milk by producing lactic acid, as Pasteur discovered in 1867 that souring of milk was caused by an organized ferment and described the organisms which was responsible for the fermentation of sterilized milk.

The conversion of sugar to lactic acid, however, is accomplished by various microorganisms though the amount of lactic acid produced is variable from negligible to almost 98% in conversion of sugars depending on the organisms. It has been shown that the following organisms are capable of producing lactic acid by any amount.

bacteria cocci Some colored micrococci (Hungate 1950)
Streptococcus
Leuconostoc
Pediococcus

rodds *Pseudomonas*(Kluyver 1931)
Coliform bacteria(Scheffer 1928)
Clostridium (Enebo 1954)
Bacillus(Hungate 1950)
Lactobacillus
Propionbacterium
Corynebacterium(de Wolff 1927)

fungi

yeasts

However the conversion of sugar to lactic acid is typically a bacterial process. And only four genera of bacteria are accepted as true lactic acid bacteria on morphological and further biochemical grounds. That is,

cocci *Streptococcus*
Leuconostoc
Pediococcus

rod *Lactobacillus*

The *Escherichia Aerobacter* group is sometimes considered to be on the *borderline* between true lactic acid bacteria and non lactic acid bacteria and referred to as pseudo lactic acid bacteria.

The lactic acid bacteria are usually further subdivided according to the physiological characteristics

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and various viewpoints of investigators, and there remains a considerable space of discussions and arguments.

It is not argued however that the lactic acid bacteria can be divided into two large physiological groups, that is, the homofermentative and heterofermentative lactic acid bacteria according to the types of glucose fermentation. The homofermentatives produce primarily lactic acid, whereas the heterofermentatives produce significant amount of carbon dioxide, acetic acid, ethanol, glycerol, and other substances in addition to the lactic acid.

Rogòsa (1953) even proposed that the hetero *Lactobacillus* should be placed on a separate genus because of differences in morphology observed by electron microscope and in physiology.

Thus our lactic acid bacteria can be grouped as follows.

homo	*Streptococcus*
	Pediococcus
	Lactobacillus
hetero	*Leuconostoc*
	Lactobacillus

There has been a little confusion about the taxonomical position of the *Pediococcus.* Orla Jensen mentioned *tetracoccus*(1919). In the 6th edition of Bergey's Manual of Determinative Bacteriology(1949) they are listed in the appendix of the family *Micococcaceae.* On the other hand Pederson(1949) has proposed that they be placed among the *lactobacteriaceae* beacause of their many similarities to this family of microorganisms. Felton *et al.* (1953), while recognizing the *Pediococci* as a separate genus, however noted that in view of their ability to synthesize catalase under certain conditions serious considerations should be given to retaining this group in the family *Micrococcaceae.*.Shimwell(1949) placed this organism to the genus *Streptococcus* using the term tetrad forming *Streptococcus.* Vaughn(1955), studying the cocci causing the spoilage of California wines, was suspicious whether these cocci belonged to *Streptococcus* or *Pediococcus.* Pederson(1949) from the standpoint that they are readily distinguishable from the species of the genus *Streptococcus* by the tetrad forming and their comparatively high acid production and they produce inactive lactic acid, separated this group from the genus *Streptococcus.* He defined this group as follows:

The genus should include those gram positive, nonmotile, non-spooreforming micrococci that occur in tetrads and sometimes singly or in pairs, show poor surface growth because they are microaerophilic, are high acid homofermentative lactic acid producers and do not reduce nitrates, liquefy gelatin or produce catalase.

In recent studies of lactic acid fermentation in vegetables by Pederson(1954, 1953) and Costilow (1957) it has been shown that *Pediococci* play a very important role. And it seems proper to handle *Pediococcus* as a separate genus in this discussion.

Many species of lactic acid bacteria have been found in nature, though many controversial arguments still exist in some of their identifications. If we study all of their habitats in nature, probably we may likely be in a position to suspect the existence of two groups of lactic acid bacteria, that is, those which live in animal materials and the others which occur in plant materials. Although this concept becomes indefensible when it is realized that many lactic acid bacteria occur commonly in both material origins, yet there ia a possibility that this idea might be reasonable at least if applied to the first evolutionary stage of lactic acid bacteria. Lactic acid bacteria have a very fastidious nature in the requirements of nutrition. According to Davis (1936) the lactic acid bacteria of milk and fermented vegetable maches and alcoholic drinks may be regarded as artificially produced types of comparatively recent origin, the former arising from dead or living animal matter, the latter from plant material. Hirsch(1952) suggested in his report that the lactic *Streptococci* are comparatively new species of the lactic acid bacteria making adaptations to milk, with the fact that more recently it has been succeeded in isolating strains of *Streptococcus lactis* from vegetable sources.

Orla Jensen(1919) already pointed out that the lactic acid bacteria of milk are introduced into the milk to a greater extent through the cowdung, the bacteria of which, again are to an essential degree

derived from the fodder, and it was therefore necessary to study the lactic acid bacteria both of animal and plant. Anyhow we are presently not in a position to be able to discuss the lactic acid bacteria of vegetables as discernible from the animal origin.

It is clear from the survey of the literature that so far as the beneficial lactic acid fermentations of vegetables are concerned only six species of the lactic acid bacteria have been found important in fermentations of vegetables. That is,

Leuconostoc mesenteroides
Streptococcus faecalis
Pediococcus cerevisiae
Lactobacillus plantarum
Lactobacillus brevis
Lactobacillus fermenti

However, if we consider the versatile intermingling of lactic acid bacteria in nature it can not be neglected to have cautions for the possible flora changes in lactic acid bacteria in a quite different environments.

Enumeration, isolation and cultivation of lactic acid bacteria in samples

There are two general principles governing the methods by which the microbiological population in a given sample may be estimated. One technique is the direct microscopic count. The other procedure widely used is the agar plate count and depends upon the development of colonies(bacterial growth) from single cells on a solid medium.

Essentially two kinds of solid media, the natural and synthetic media have been used to grow lactic acid bacteria. The former is usually for isolation and enumeration of population of bacteria in a given sample, and the latter principally for the physiological chemistry of lactic acid bacteria.

The natural media

A wide varieties of media have been used. According to the survey of Fabian *et al.* (1953) we can list the media as follows.

Ayers and Mudge(1920)	milk powder medium
Rettger and Kulp (1927)	the casein and Klim digest agar
Kulp (1927)	a tomato juice agar
Weiss and Rettger(1934)	a tomato broth medium
Mclaughlin(1940)	tryptase sugar broth
Jones, Veldhuis and Veerhoff (1940)	nutritive casein agar
Garey, Foster and Frazier(1941)	carrot liver agar
Mrak, Phaff and Douglass (1942)	an infusion of four vegetables
The Commitee on the Microbiological Examination of Foods(1943)	trypton glucose yeast
Wade, Smiley and Smith (1946)	filtered tomato juice, yeast extract,glucose and a buffer
Henrici (1946)	V-8 juice and agar
Rogosa, and Mitchel (1951)	a medium for the selective isolation of oral, varginal and fecal *Laotobacillus*
Murdock, Folinazzo and Troy (1952)	orange serum agar
Emard and Vaughn (1952)	sorbic acid as the differential agent
Evans and Niven(1955)	APT medium

However many of these media are not succesful, because these media do not selectively favor the growth of the lactic acid bacteria, and consequently the other microorganisms present in the sample may outgrow them, which may be mistaken for lactic acid bacteria when plate counts are made.

A few media of recent general usage are only to be discussed.

V-8 medium

According to Fabian, Fulde and Merrick(1953), it has been shown that most of the lactic acid fermenting organsims give rise to a particular characteristic colony that appears to possess a differential-character when cultivated on the V-8 medium. The V-8 medium could differentiate between miscellaneous acid forming bacteria and the true lactic acid types as determined by the typical colony produced on the V-8 medium. Also he said that this media would

grow the acid producing bacteria quickly and to a larger size than any of the media to the exclusion of many other types of bacterial colony formed in association with the acid producing bacteria.

Composition of the V-8 medium

	tryptose	glucose	beef extract	agar	V-8 juice	pH
qualitative purpose	10	5	—	18	125 (filtered)	5.6
comparative purpose	10	5	3	18	500 (filtered adjusted)	5.8

(0.1% of B.C.G. is added as the indicator.)

The appearance of the lactic organisms on the V-8 medium is somewhat variable depending on the particular organism. Most of the *Lactobacilli* are easily characterized by observing on bright yellow halo surrounding a jet black colony. A weaker acid producer such as *Leuconostoc* appear somewhat similar, however the colony is generally green with weaker acid production.

Sorbic acid

Vaughn (1951) reported that sorbic acid selectively favored the growth of strains of *Lactobacillus* and *Leuconostoc* and inhibited generally the catalase positive microorganisms with 0.12% sorbic acid when the pH of the medium was between 5~5.5 and in the absence of phosphate salts. This was confirmed later by Shenman, Costilow (1955) in that 0.1% sorbic acid would effectively preserve sweet cucumber pickles when minimum of 0.5% acetic acid was used. Costilow, Ferguson (1955) extended this research to *Pediococcus* and proved that two strains of *Pediococcus cerevisiae* appeared to be as tolerant to sorbic acid as the catalase negative bacteria. Usually the inhibitory action of sorbic acid has been shown to be enhanced by the addition of salt and acetic acid.

According to Emard, Vaughn (1952) liver infusion media showed to be advantageous for growth of the lactic acid bacteria. And sorbic acid (0.1%) in liver infusion agar was observed to exert to a marked inhibitory effect on most of the catalase positive culture.

Liver infusion broth (Vaughn, 1942)

A pound of finely ground liver is infused(heated) with one liter of tap water at 100C for one hour. On cooling, the liver particles are separated from the infusion liquor by filtration through several thickness of cheesecloth. The infusion is diluted with an equal volume of tap water, and 1% Bacto trypton and 0.1% K_2HPO_4 added. A small portion of dried or freshly infused liver particles are added to each tubes as well as 5 to 8 cc of the infusion liquor. After the tubes are plugged and sterilized in the autoclaves at 15 lbs. pressures for 15 mins. the medium is ready for use.

The same trend in the selectivity of sorbic acid was noted when a glucose yeast extract per liter of water was substituted for the liver infusion.

The concentration of acid required for the suppression of the resistant strains was only 0.07% in the glucose media as compared with 0.12% in the liver infusion media.

The media of present usual usuage is as follows.

Liver sorbate agar

500ml liver infusion
5.0gm tryptone
1.0gm sorbic acid
500ml distilled water
18 gm agar

adjust to pH 5.2 with HCl

There is also a medium particularly suited for detection of *Leuconostoc*.

Sucrose agar

0.5% tryptone
0.3% yeast extract
0.12% sorbic acid
10.0% sucrose
1.8% agar

APT medium

Evans and Niven (1955) used a laboratory medium that would support rapid and vigorous growth of these organisms(heterofermentative *Lactobacillus*) isolated from naturally occuring outbreaks of surface discoloration of a variety of cured meat products.

APT medium

tryptophane	10 g
yeast extract	5

sodium chloride	5
K_2HPO_4	5
$2Na_3C_6H_5O_7 11H_2O$	5
glucose	10
sorbitan monoacetate	1
$MgSO_4 \cdot 7H_2O$	0.8
$MnCl_2 \cdot 4H_2O$	0.14
$FeSO_4 \cdot 7H_2O$	0.04(in one liter of water)
agar	1.5% for plate

The synthetic media

See "Physiology".

Physiology

Sugar fermentation

The ability of an organism to ferment various sugars and its related compounds is one of the important characters used for its classification. This is true also in the lactic acid bacteria. As it was already mentioned, the lactic acid bacteria are divided into two large physiological groups, that is, to the homofermentatives and the heterofermentatives according to the manner by which they break down the sugars to lactic acid and the other substances. However these characters are effected by various external circumstances. Orla Jensen(1919) reported that many of the heterofermentative bacteria after long-continual cultivation in laboratory media fermented hexose in the same way as did the homofermentative organisms. Also this is very dependent upon pH. Alkali media favors the heterofermentation. When the homofermentative lactic acid bacteria are put on the alkali media they act just as the heterofermentative bacteria.(Gunsalus and Niven, 1942)

Vaughn reported(1955) that the sugar fermentation largely depended on pH. *Lactobacillus hilgardia* that he isolated from spoiled California wine fermented glucose at pH 5.0 to 5.5, but did not ferment glucose at pH 6.5 to 7.0. Same was observed when he examined the utilization of sucrose and citric acid by *Leuconostoc mesenteroides*.

Fornachon(1940) reported that the optimum pH for growth did not differ greatly for metabolism of carbon compounds for the tested lactic acid bacteria, both being greatest in the range of pH 5.0 to 6.0.

According to Orla Jensen(1919) in lactic acid formation *Leuconostoc* is very little affected by the concentration of sugar and produced the acid even at greater concentration than 15% of sugar. *Streptococcus* is also little affected by the concentration of sugar until 15% while the optimum concentration is 1/2~2%. The other Lactic acid bacteria are varied in their optimum and tolerant concentration of sugar from 2~10%.

pH

According to Orla Jensen(1916) the optimum pH of the cocci were pH 6.5, limiting pH 4.0, while the rods were 6.2 and 3.5 for each. Fornachon (1940, 1943, 1949) reported the optimum pH of the lactic acid bacteria generally as 5.0 to 6.0 and the limiting pH of *Lactobacillus trichodes* in appetizer and dessert wine as 3.5. Rushing, Veldhuis and Senn(1956) studied the lactic acid bacteria in frozen orange juice and found that *Leuconostoc* species were more effected by changing pH than was *Lactobacillus*. The negative growth rates were observed at pH 3.4 in 12 and 18 degree Brix of orange juice whereas 3.8 was the limiting in 42 degree Brix.

Tests by Orla Jensen(1943) indicates that the final pH of the hemofermentative *Lactobacillus* is significantly different in glucose from heterofermentatives. Generally the pH produced by the homofermentative strain was at least 0.5 unit lower than that for the heterofermentative species. It has generally been recognized that the homolactobacillus predominates in the final stage of various vegetable fermentation.

Pediococcus produces high acidity than *Streptococcus*. Pederson(1949) observed 0.5~0.9% of acid production from hexoses with the final pH of 3.25 3.4% in *Pediococcus* while final pH produced by *Streptococcus* was 3.7~3.9 and the homofermentative *Lactobacillus* 3.05~3.25.

Temperature

Orla Jensen observed that the greatest quantity of acid was formed somewhat below the temperature at which liveliest growth takes place. For the optimum temperatures of various lactic acid bacteria see the table (Summary of the lactic acid bacteia

by Thimann). Faville, Hill and Parush (1951) observed the bacteria in frozen orange juice stored at −17.8 degree C., large of which were *Leuconostoc* and died off rapidly. Niven, Buettner and Evans (1954) studied the heat tolerance of heteroferment-ative *Lactobacilli* isolated from various types of greening of cured meat products and obtained the result that some of them were capable of withstanding as much as 45 mins. at 155 Fahrenheit degrees.

Vaughn(1955) observed that different strains of the same species have different optimum tempera-tures. The ordinary *Lactobacillus plantarum* has an optimum temperature at 30~35 degree C., while a strain of which that fermented the tartarate had its optimum temperature at 25~30.

Salt tolerance

Only a very few of the *Streptococcus* are affected by the presence of 2.5% of salt (Orla Jensen, 1919) The maximum salt tolerance of homofermentative *Lactobacillus* and *Leuconostoc* and *Pediococcus* are more than 10%. The hetero *Lactobacillus* is tolerant from 5 to 6%(Vaughn 1955, Pederson 1949).

However care should be paid to the fact that the salt tolerance of lactic acid bacteria are different when in a laboratory medium than in actual natural conditions. Etchells (1946) reported that *Lactobacil-lus plantarum* isolated from actively fermenting brines at 10 to 12% salt concentration did not show growth in liquid media plus salt much above one half the original isolated concentration.

Alcohol tolerance

Leuconostoc is strongly inhibited by 10% alcohol by volume (Olson, 1948). The hetero *Lactobacillus* has a greatest resistence to alcohol. All can grow in 15% of alcohol. Some can grow even in 18%. *Lacto-bacillus trichodes* can grow even in 20%(Fornachon 1949) (Vaughn *et al.*, 1949). Homofermentative *Lac-tobacillus* are less alcohol tolerant and all, markedly limited their growth when in 10%(Vaughn 1949).

Optical rotation of lactic acid

Optical rotation is the characteristic of organism. The rotation has nothing to do with the rotation of the sugar used as substrates, but depend on the en-zymes in the cell.

Kitahara and Obayashi (1955) proposes that the lactic acid bacteria can be classified into two groups, namely D-formers and L-formers, and each of these can be further subdivided on the basis of presence or absence of racemiase.

D-former racemiase—D-formers
racemiase+D+DL—DL formers

Variation in the vitamins requirements of different lactic acid bacteria

culture	benzoic acid	vitamins required in medium for growth* biotin	folic acid	nicotinic acid	pantothenic acid	riboflavin	thiamin	pyridoxal pyridoxiamine
L. arbinosus 17—5	±′	+″	−	+	+	−	−	+
L. casei	−	+	+″	+	+	+	−	+
L. delbruckii″		+	+	+	+	+	−	+
L. fermenti 36		+	−	+	+	−	+	−
Leu. mesenteroides P—60	±	+	−	+	+	−	−	−
Leu. mesenteroides		−	−	+	+	−	+	+
S. faecalis R		+	+	+	+	−	−	+
S. faecalis		+	−	+	+	+	−	+
S. lactis		+	−	+	+	+	+	−

* + indicates the vitamin is required for growth, −that it is not

′ Apparently only certain culture of this organism require amino-benzoic acid when the medium is otherwise com-plete, and the requirement for it can be readily demonstrated.

″ Whether or not this and some other lactic acid bacteria require the vitamin for growth is markedly dependent upon the composition of the medium.

L-former racemiase+ DL-DL+L formers
racemiase- L-formers

Accessory growth factors

It is well known that the nutrition of the *Lactobacilli* is complex. Orla Jensen(1919, 1936) recognized this early. These organisms belong to that group of heterotrophs which have lost the ability to synthesize many of the specific chemical compounds required for their growth, especially vitamins, amino acids and other nitrogen sources. Not all strains classified as a given species will show the same requirements for vitamins and amino acids. Different combinations of them are required for growth of different organisms. Because of the ease with which they can be grown, various lactic acid bacteria are now widely used for the bioassay of vitamins and amino acids. The next tables will show the requirements of vitamins and amino acids required by various lactic acid bacteria and an example of synthetic media that shows the feature of nutrition of lactic acid bacteria (data cited from Snell, 1948).

Amino acid requirements of 23 lactic acid bacteria

Amino acid	No. of organisms for which the amino acid is		
	essential	stimulatory	non-essential
Glutamic acid	23	0	0
Valine	23	0	0
Isoleucine	20	3	0
Leucine	18	2	3
Tryptophane	13	7	3
Cystine	13	7	3
Methionine	10	13	0
Arginine	10	8	4
Histidine	9	11	4
Threonine	6	11	5
Phenylalanine	7	12	4
Tyrosine	8	7	8
Glycine	4	10	8
Aspartic	4	12	5
Lysine	4	10	9
Serine	2	13	8
Alanine	3	10	8

Composition of a complete synthetic medium for several lactic acid bacteria

component	amount per 100ml.	component	amount per 100ml.
Glucose	4.0g	DL-alnine	200mg
Na-citrate	4.0	DL-aspartic acid	200
Na-acetate	0.2	L-glutamic acid	200
NH_4Cl	0.6	L-arginine hydrochloride	40
K_2HPO_4	1.0	L-lysine hydrochloride	40
$MgSO7H_2O$	160mg	histidine(L or D)	20 or 40"
Mn SO_44H_2O	32	isoleucine(L or D)	20 or 40
$FeSO_47H_2O$	8	methionine (L or DL)	20 or 40
NaCl	8	phenylalanine (L or DL)	20 or 40
Adenine sulfate	2	proline(L or DL)	20 or 40
Guanine hydrochloride	2	threonine(L orDL)	20 or 40
Xanthine	2	tyrosine(L or DL)	20 or 40
Uracil	2	valine(L or DL)	20 or 40
Thiamine chloride	200	tryprophane (L or DL)	20 or 40
Riboflavine	200	cystine(L or DL)	20 or 40
Pyridoxal	40	serine(L or DL)	20 or 40
Ca-pantothenate	200	glycine	20
Nicotinic acid	200		
P-aminobenzoic acid	200		
Biotin	2		
Folic acid	2		

* The composition give in twice the final concentration used.
" 20mg of the L-amino acid or 40mg of the DL amino acid. Addition of other amino acid, e.g., hydroxyl-L-proline is permissible if desired.

The importance of inorganic compound(phosphates, Fe, Mg, Mn), citrates and acetate has been demonstrated by Evans and Niven(1951). The requirement of bases for lactic acid bacteria is also clear (Heathcots, 1949). Not only they are required for their growth but the proportions are often critical; for example, cystine in rather low concentrations may inhibit growth(Heathcots, 1949).

Recently Tamura (1956) discovered the identity of a new growth factor indispensable for the growth of the Hiochi bacteria(L. homohiochi and L. hetero-

hiochi). It is a saturated beta-hydroxy-delta-lactone, mevalonic acid.

Microfloral change in the lactic acid fermentation of vegetables

Whatever the vegetable products are used in the manufacture of lactic fermented foods, the vegetables are placed in brine and allowed to undergo spontaneous lactic fermentations. The salt restricts the activity of undesirable organisms but permits the development of the lactic acid bacteria and a few other organisms, which convert the natural sugars in the vegetable to lactic acid. The lactic acid produced again prevents the growth of other microorganisms if the foods are preserved in a favorable condition.

According to Etchells(1946) and other investigators. marked microfloral changes are not to be ex pected, provided different materials are brined or salted at similar concentrations and under similar conditions and also contain sufficient amounts of readily fermentable carbohydrates. So far until present only following microorganisms have been found as worthy of discussion in connection with the lactic acid fermentation of vegetables (cucumber, sauerkraut and olives).

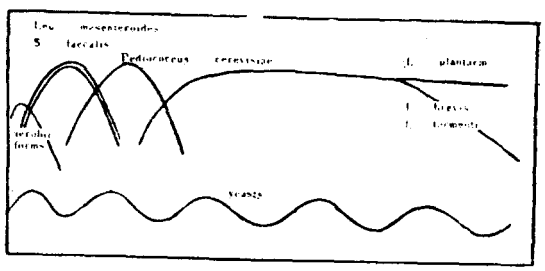
Aerobic coliforms and *Bacillus*
Streptococcus faecalis
Pediococcus cerevisiae
Leuconostoc mesenteroides
Lactobacillus brevis
Lactobacillus fermenti
Lactobacillus plantarum

From survey of the literature, the microfloral sequece of all above organisms may be easily found for the case if they are supposed to be active altogether in one fermentation process. Author proposes the following scheme as the probable microbiological sequence of natural lactic acid fermentations in vegetables.

It is not predictable however if there may be any such actual condition where all of these organisms are active in the fermentation. Nor even a single data is available in which all of the organisms listed above were acting. This is probably because many ecological conditions are different, that is, the concentration of salt, the temperature of brine and the other miscellaneous factors (for example the experimental condition).

Also for any reason during the first stage, if the fermentation does not proceed in a normal fashion and any of the undesirable microorganism may predominate and the normal seqence of microbiological events are disturbed, this may contribute to undesirable deterioration of the vegetable products (Vaughn, 1954).

The microfloral change of natural lactic acid fermentaion



This scheme is derived according to the data obtained by Pederson(1953), Costilow and Fabian (1953), Pederson(1954), Vaughn(1954) and Costilow (1957).

According to Pederson(1953, 1954) in cucumber fermentation the best results are secured in a fermentation started by the heterofermentative lactic acid, alcohol and carbon dioxide producing species *Leuconostoc mesenteroides*. The poorest cucumber stocks were obtained from the pure homofermentative fermentations. Sauerkraut fermentation with high salt concentration or at high temperature were found to be poor in color, flavor and texture.

The coliform bacteria and others may produce some latic acid during the initial stage of fermentation. However no significant activity have been noted (Costilow, 1953).

The yeasts, the presence of which was studied in cucumber fermentation(Costilow 1953, Niven 1952) and olives(Mark, Vaughn, Miller and Phaff 1956) is considered to do no beneficial activity but the harmful action to oxidize the lactic acid.

The effect of salt on flora

The salt concentration of the brine has a marked effect on the total numbers and types of microorganisms found in the fermenting vegetables. Vaughn (1954) noted in the study of olive fermentation that the Manzanillo veriety, which have been customarily fermented in the presence of 7 to 8% salt have a different bacterial population than the Sevillano olives, which are fermented in the presence of from 3 to about 5% salt. Whereas both homofermentative and heterofermentative species of *Lactobacillus* have been always recovered from the brines of Sevillano olives, only the homofermentative species *Lactobacillus plantarum* has been recovered from Manzanillo brines. Pederson(1953) observed in his study of cucumber fermentation that when the concentration of brine was 7.5% *Streptococcus faecalis*, coliforms had no opportunity to predominate contrary to the case of 3%. In sauerkraut fermentation Pederson(1954) also observed the following facts. In the lowest salt content sauerkraut of 1.0%, the heterofermentative species *Leuconostoc mesenteroides* and *Lactobacillus brevis* were favored. In the higher salt content sauerkraut of 3.5%, fermentaion was retarted and the species *Lactobacillus brevis* was almost entirely suppressed. Instead, strains of the species *Pediococcus cerevisiae* were obtained more frequently than from lower salt content sauerkrauts, and in conjunction with the species *Lactobacillus plantarum* created more of a homofermentation than occurred in the sauerkrauts of lower salt content.

Costilow, Coughlin and Robach(1957) in the study of laboratory fermentation of cucumber made a comparison of the type of lactic acid bacteria isolated from 20 degree and 40 degree salometer brines. No effect of brining was noted. However the lactic acid bacteria involved in this fermentation were only *L. plantarum*, *L. brevis* and *Pediococcus cerevisiae*.

Temperature of the brine

According to the work of Pederson(1930, 1931) on sauerkraut, brine temperature of about 30 degree C would not encourage rapid development of members of the *Leuconostoc* as compared with that of acid formers such as *Lactobacillus plantarum*. Etchells (1946) reported that when cucumber were put down at brine temperature of about 20 degree C the predominating flora of organisms were members of the *Leuconostoc*. He observed that at 7.5 degree C fermentation did not progress sufficiently in several months to cure the sauerkraut. By contrast, at 32 degree C and 37 degree C a high acidity was attained in less than two weeks with the greater acidity attained at 32 degree C.

The other factors

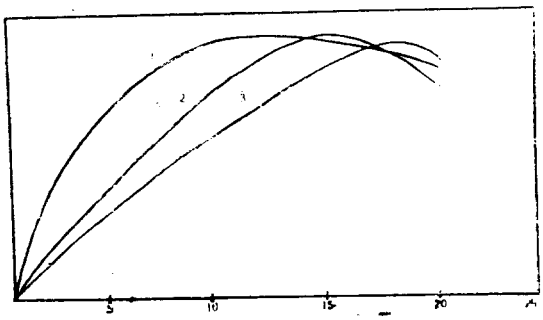
Undoubtedly seasonal natural nature play an important role in fermentation(Pederson,1953). Costilow *et al.* (1957) has shown that three species of lactic acid bacteria are active in cucumber fermentation in Michigan. *L. plantarum*, *L. brevis* and *Pediococcus cerevisiae*. These results are not in agreement with some of Pederson *et al.* He supposed this difference as due to differences in conditions. The fermentaion studied were in glass containers of the laboratory in the case of Pederson's experiments while those in his work were in barrel and tank lots under commercial conditions. Costilow(1953) demonstrated that the microbiological activity in laboratory fermentations may not be comparable with commercial tanks. Costilow(1957) also supposed the possibility that his method of selecting culture eliminated minor species.

Pederson(1953) in his study of inoculated cucumber fermentation could not recover any yeasts and explained this fact as due to the action of carbon dioxide replacing the air and producing a more anaerobic condition, only when inoculated with the homofermentative bacteria.

Costilow and Fabian(1953) studied the availability of essential vitamins and amino acids for *Lactobacillus plantarum* in cucumber fermentations and obtained the following results. Raghab and Fabian (1957) studied the effect of addition of sugar, cystine and tryptophane in cucumber fermentation. It was supposed that the two amino acids might be defficient for *Lactobacillus plantarum* during cucumber fermentation because of the activity of the coliforms and yeasts. However no effect on total titratable acidity and microbiologic activity of cucumber fermentation were observed except the rapid increase in yeast population which was noted when sugar was added in the 7th day. In contrary to this work,

Lopez and Pratt(1954) on sauerkraut studies showed that the effect of certain added nutrients increased the final total acidity of sauerkraut. Dextrose, sodium acetate, a mixture of inorganic salts and the vitamin combination were used alone or in combinations.

Availability of essential vitamins and amino acids for *Lactobacillus plantarum* in cucumber fermentation



days

Summarized from the data obtained by Costilow and Fabian(1953). The maximum points of the curves, 1, 2 and 3 correspond to

curve 1	niacin	2.5mu g/ml
	pantothenic acid	2.5mu g/ml
	biotin	20mu g/ml
curve 3	leucine	350mu g/ml
	isoleucine	200mu g/ml
	valine	240mu g/ml
curve 2	tryptophane	35mu g/ml
	glutamic acid	350mu g/ml
	cystine	20mu g/ml

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