

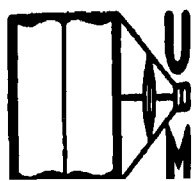
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TITLE THE IMPORTANCE OF BIOTIN, NIACIN
AND PANTOTHENIC ACID IN CUCUMBER
FERMENTATION

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THE IMPORTANCE OF BIOTIN, NIACIN AND
PANTOTHENIC ACID IN CUCUMBER FERMENTATION

By

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GENERAL INTRODUCTION

The cucumber fermentation, as it is carried on in many parts of the world, is uncentrelled. Cucumbers are picked from the field, placed in a vat, covered with brine, and allowed to ferment. The microorganisms involved in the fermentation come from the surface of the cucumbers. Needless to say, a vast variety of organisms are introduced into the fermentation. Many types of microorganisms are prevented from multiplying due to the high salt concentration of the brine. Some of the more important groups of organisms which persist in a cucumber fermentation are the Lactebacilli, Aerebacter and related organisms, and yeasts.

It is a well established fact that the Lactebacilli are the most vital group of microorganisms in a cucumber fermentation. It is also well known that many Lactebacilli are rather fastidious in their vitamin requirements. The purpose of this study was to demonstrate that the Lactebacilli from cucumber fermentations required certain vitamins, and to show how these vitamins were made available for this group of organisms. It was also intended to investigate thoroughly the role of any specific vitamin or vitamins in the cucumber fermentation which appeared to be critical for the Lactebacilli.

PART I

THE MICROORGANISMS OF THE CUCUMBER FERMENTATION

INTRODUCTION

In the fermentation of cucumbers for salt stock, three types of microbial fermentations are generally recognized. These are the lactic fermentation, the hydrogen fermentation, and the carbon dioxide fermentation produced by Lactobacilli, Aerobacter, and yeasts respectively (41). The primary objective of this particular study was to further investigate these groups of organisms.

REVIEW OF THE LITERATURE

Lactic Fermentation. - Aderhold (2), in 1899, and Kessowicz (57), in 1909, were among the first workers to study the flora of active cucumber fermentations. Their work, however, was concerned with spoilage bacteria. Rahn (85), in 1913, reported that at the height of a fermentation, 50,000,000 to 200,000,000 acid-forming bacteria were present. Brown (10), in 1916, found that the acid-forming bacteria were short rods or cocci and facultative anaerobes. Le Fevre (64, 65, 66), in 1919, 1920, and 1922, showed that cucumbers introduced lactic acid-producing bacteria as well as other organisms. He found the optimum temperature for this group of organisms to be 30° C. Fabian et al (42), in 1932, studied the populations of acid-producing organisms in 30° and 40° salometer brines. They found the number of acid-producing organisms to be greater and to reach a maximum sooner in the 30° salometer brine.

The maximum number of acid-producers was 195,000,000 per ml in the 30° salometer brine, which was attained after 6 days. The maximum in the 40° salometer brine was 141,000,000 per ml, which was reached after 13 days. In 1935, Vahlteich, Haurand, and Perry (105) believed that organisms of the Lactebacillus type were the chief acid-producers. However, they indicated that the following organisms might also be present: Leuconestec, yeasts, and other unclassified organisms. Etchells and Jones (38), in 1946, identified all of 49 cultures of acid-producing bacteria isolated from fermentation of salt-stock cucumbers to be Lactebacillus plantarum.

Hydrogen Fermentation. - Veldhuis and Etchells (106), in 1939, demonstrated that hydrogen was evolved from cucumber fermentations. Etchells, Fabian, and Jones (36), in 1945, indicated that Aerebacter developed during the cucumber fermentation and were responsible for the production of hydrogen. They found Aerebacter to occur in 20°, 40° and 60° salometer brines in southern areas of the United States. However, they stipulated that in 20° and 40° brine this fermentation might not occur.

Yeast Fermentation. - Etchells (32), in 1941, showed that yeasts were involved in cucumber fermentations. In 1950, Etchells and Bell (33) found that the predominating genera of yeasts in southern cucumber fermentations were: Terulepsis, Brettanomyces, Hansenula, and Zygesaccharemyces.

They found that Terulepsis and Hansenula appeared in high numbers within twenty days while the other two genera did not appear until late in the fermentation. In the following year Etchells, Costilew and Bell (35) studied yeast populations in northern brining areas. They found that the predominating genera were: Brettanomyces, Terulaspora, Terulepsis, and Hansenula.

Film yeasts on cucumber brines have also been reported. Mrak and Benar (78) and Etchells and Bell (34) have pointed out the predominating genus to be Debaryomyces.

Composite Microbial Population. - Etchells and Jones (37) have shown fermentation trends in 20°, 40°, and 60° salemeter brines. In 20° salemeter brine, the acid-forming bacteria reached a high point in about four days; in 40° salemeter brine the peak was not reached until nine days. The total count was lower in the 40° brine than in the 20° brine. At 60° salemeter, the acid-forming bacteria appeared in low numbers and gradually disappeared.

Aerebacter appeared in similar fashion as the lactic acid organisms. The lower the salt concentration, the quicker the onset of these bacteria and the higher their count. The Aerebacter fermentation was not too active or prolonged at 20° or 40° salemeter. At 60° salemeter, these organisms declined at first and became active after eight days.

Yeasts occurred in fermentations at all levels of salt concentration. A high salt concentration tended to hold

back the rate but not the quantity at which the yeasts developed.

EXPERIMENTAL

Microbiological Analysis of Cucumber Fermentation

Two cucumber fermentations were set up in the laboratory in five-gallon creaks. A bent glass tube was placed in each creak so that a sample of brine could be withdrawn from the approximate geometric center of the creak. The part of the glass tube which extended into the center of the creak was protected by a hollow wooden baffle through which numerous holes had been drilled to permit an interchange of the brine (Figure 1). Number two cucumbers (three to four inches in length) were then placed in the creak and salted according to the 30° salometer schedule. To prevent an excessive accumulation of surface yeasts, an ultraviolet lamp was placed about six inches from the surface of the brine and kept on throughout the fermentation except when samples were being withdrawn. Samples of brine were withdrawn into a Mason jar of one-pint capacity. Smaller samples were then taken from the Mason jar in order to determine the amount and kinds of microorganisms present. Samples were taken every day for the first six days and thereafter every two or three days for twenty days. The brine remaining in the Mason jar was returned to the creak by positive nitrogen pressure.

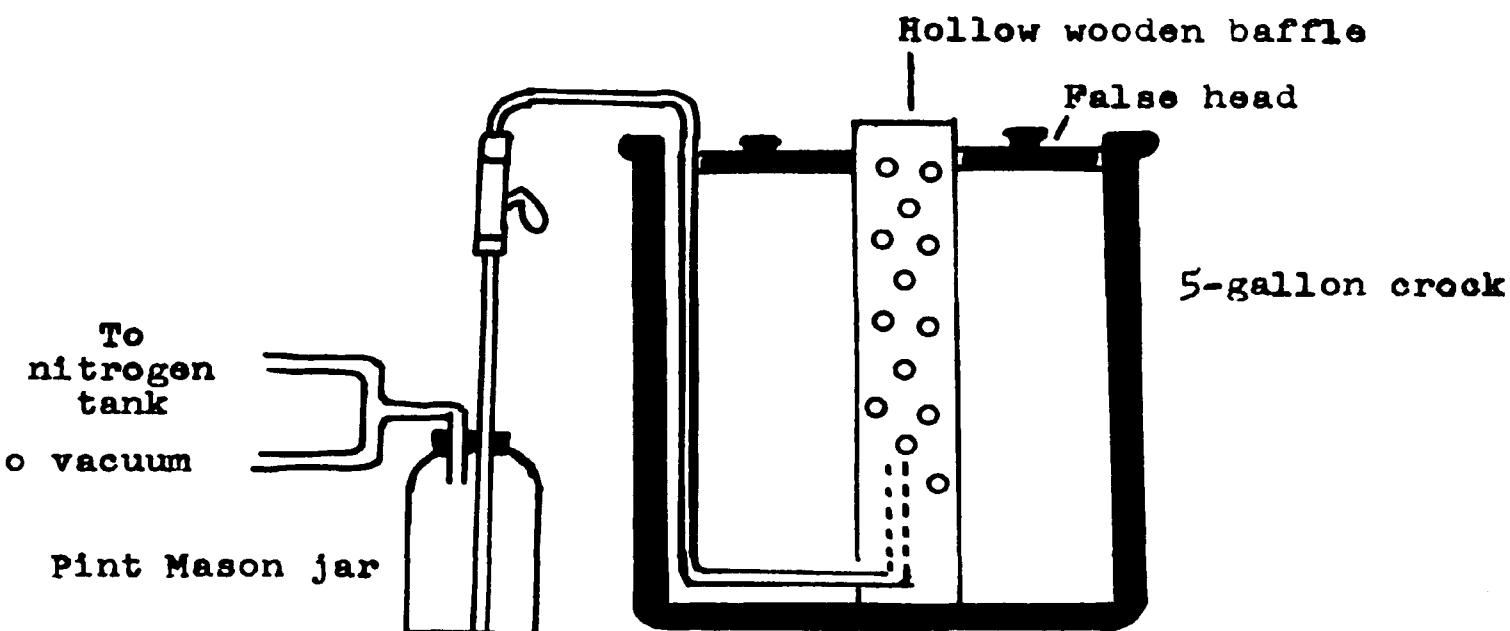


Figure 1. Apparatus used for sampling brine from fermenting cucumbers*

*Adapted from Faville, L. W., and Fabian, F. W., The influence of bacteriophage, antibiotics, and Eh on the lactic fermentation of cucumbers, Tech. Bull. 217, Michigan State College, 1949, and Fulde, R. C., Unpublished thesis.

Total acid, calculated as lactic acid and the pH were determined each time a sample was removed for microbiological analysis.

To isolate and count the Lactobacilli, samples of brine were plated in an agar medium containing Campbells' V-8 vegetable juice as the main constituent. An indicator, brom cresol green, was added to this medium (44). The plates were incubated at room temperature for two to three days and only the acid-producing organisms were counted.

The yeasts were isolated and counted by plating in dextrose agar to which five percent NaCl and three ml of five percent tartaric acid had been added per 100 ml of medium. Counts were made after four days incubation at room temperature.

The isolation and determination of numbers of Aerobacter and related organisms was accomplished in a somewhat different manner. Varying dilutions of the samples of brine were placed in triplicate into tubes containing lauryl sulfate tryptose broth. The tubes were incubated at room temperature and observed for the formation of gas at the end of 48 hours. The most probable number of Aerobacter and related organisms was determined from tables which appear in "Water Bacteriology" by Prescott, Winslow, and McCrady (84). To isolate Aerobacter, material from the highest dilution showing gas in the lauryl sulfate tryptose tubes was streaked onto eosin-methylene blue agar.

The Lactobacilli, yeasts, and Aerobacter organisms were isolated by selecting colonies typical of those groups of organisms. The organisms were purified by repeated platings in their respective isolation media.

The Lactobacilli were maintained on lactose motility medium; the yeasts were maintained on V-8 agar slants; the Aerobacter and related organisms were maintained in lactose broth and on nutrient agar slants.

The numbers of the various groups of microorganisms are presented in Tables 1 and 2 and in Figures 2 and 3.

The Lactobacilli which were subsequently found to belong to the plantarum species could not be detected at first, but grew rapidly for the first few days. They persisted in high numbers for about one week and then there was a gradual decline. The Aerobacter and related organisms were present at the start of the fermentation, grew very well for the first two days, and then declined rapidly. The yeasts were also present initially. Their numbers rose gradually reaching a high point in about eight days. Then there was a gradual decline, but they persisted in comparatively high numbers.

Identification Studies

Cultural, biochemical, and morphological studies were made on these organisms. Each organism studied was assigned a number. Table 3 gives a key to each of the isolates. The day the organism was removed from the fermentation is also

indicated.

TABLE 1

TOTAL ACID, pH, AND ORGANISMS PER ML DURING
CUCUMBER FERMENTATION SALTED AT 30° SALOMETER (CROCK A₁)

Day	Percent acid (Calculated as lactic acid)	pH	<u>Lactobacillus</u> <u>plantarum</u>	<u>Aerobacter</u> <u>and related</u> <u>organisms</u>	Yeast
0	0.007	6.17	0	147,000	2,900
1	0.023	5.81	50,000	430,000	6,000
2	0.12	5.23	1,200,000	430,000	21,400
3	0.46	3.80	145,000,000	220,000	129,000
4	0.77	3.56	41,400,000	920	120,000
5	0.95	3.47	52,000,000	2,500	87,000
6	0.77	3.42	80,000,000	2,500	63,000
8	0.77	3.35	22,200,000	2,500	250,000
10	0.84	3.31	6,800,000	2,500	64,000
12	0.93	3.31	1,310,000	0	44,000
14	1.01	3.30	640,000	0	32,000
17	1.01	3.30	94,000	43,000	85,000
20	1.02	3.29	86,000	14,700	29,000

TABLE 2

TOTAL ACID, pH, AND ORGANISMS PER ML DURING
CUCUMBER FERMENTATION SALTED AT 30° SALOMETER (CROCK B₁)

Day	Percent acid (Calculated as lactic acid)	pH	<u>Laetobacillus</u> <u>plantarum</u>	<u>Aerobacter</u> and related organisms	Yeast
0	0.015	6.61	0	92,000	830
1	0.038	5.55	10,100	920,000	8,300
2	0.112	5.41	880,000	9,200,000	18,400
3	0.20	5.01	5,000,000	920,000	12,000
4	0.31	4.24	21,500,000	92,000	9,400
5	0.31	3.80	26,700,000	2,200	74,000
6	0.40	3.68	53,000,000	25,000	160,000
8	0.54	3.53	40,000,000	2,500	670,000
10	0.66	3.50	20,400,000	240	104,000
12	0.72	3.43	6,500,000	4,300	42,000
14	0.77	3.44	2,090,000	4,300	50,000
17	0.80	3.41	1,900,000	2,500	58,000
20	0.77	3.41	2,270,000	4,300	84,000

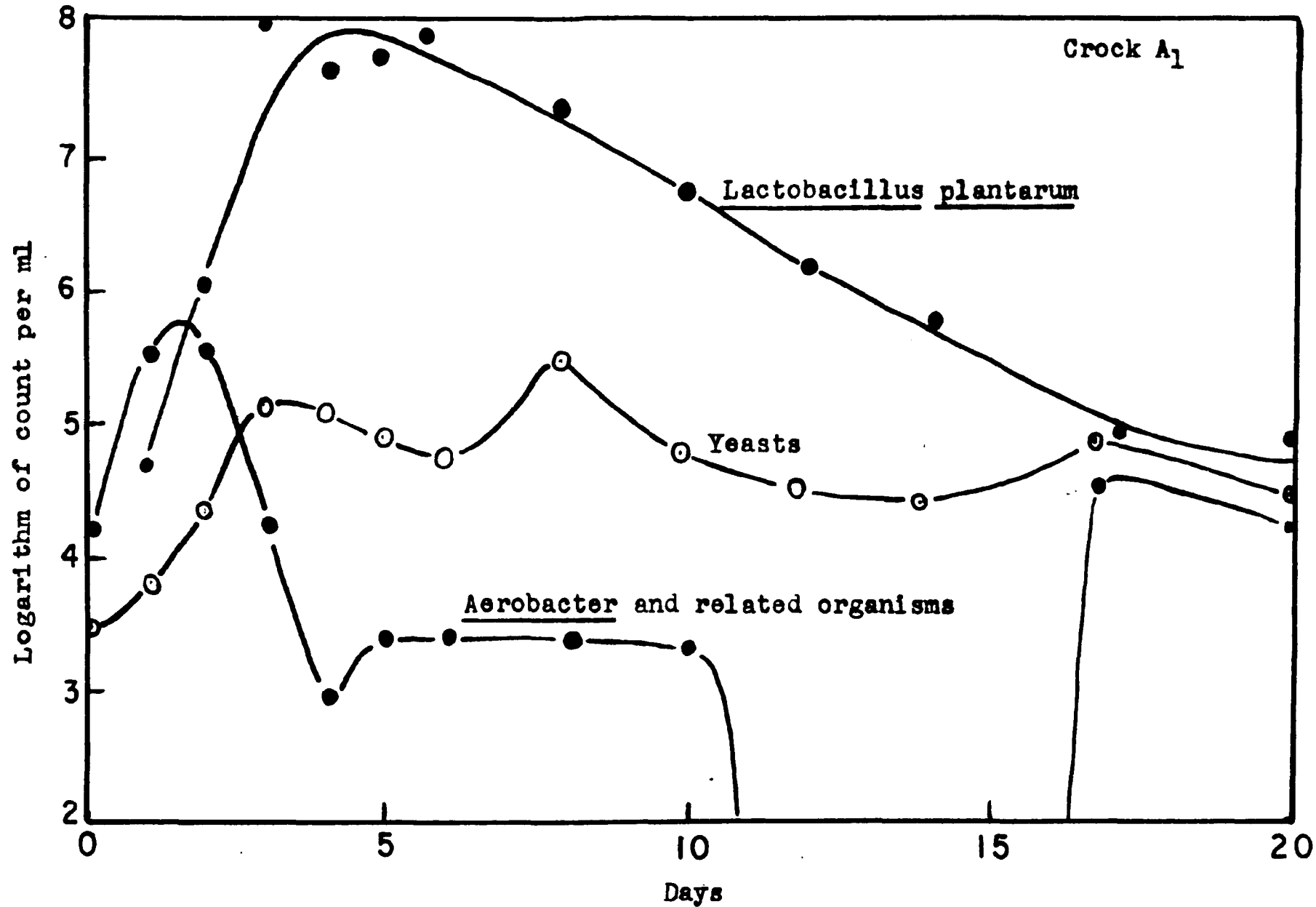


Figure 2. Numbers of organisms per ml in 30° salometer brine

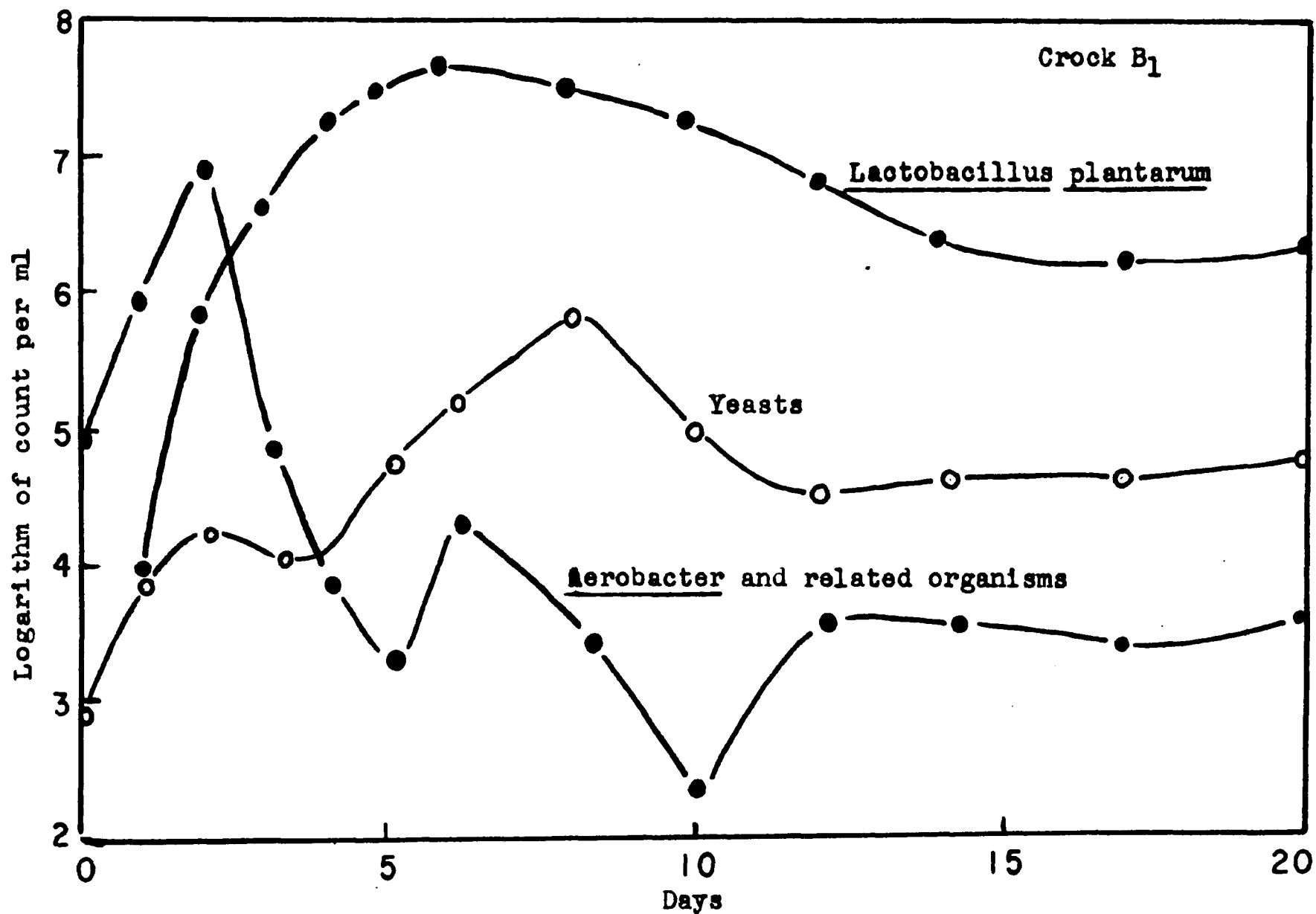


Figure 3. Numbers of organisms per ml in 30° salometer brine

TABLE 3

LIST OF NUMBERED ISOLATES FROM CUCUMBER FERMENTATION

Acid-forming bacteria		Aerobacter and related organisms				Yeasts	
No.	Day isolated	No.	Day isolated	No.	Day isolated	No.	Day isolated
600	2	407	2	439	1	701	12
601	3	408	2	441	2	703	12
602	5	409	2	442	2	704	12
603	5	410	3	443	2	706	0
604	6	411	3	444	3	707	1
605	6	412	3	445	3	709	2
609	3	420	5	446	3	711	3
610	4	421	5	447	4	713	4
611	5	422	5	448	4	714	5
612	6	423	6	449	4	715	8
613	8	424	6	451	5	716	17
614	10	425	6	452	5	717	20
615	12	426	8	454	6	718	0
616	17	427	8	455	6	719	1
617	20	428	8	456	8	720	2
621	4	429	10	457	8	723	6
623	6	430	10	459	8	724	7
624	8	431	10	461	10	725	8
625	9	432	17	462	10	726	9
		433	17	463	10	728	11
		434	17	464	11	729	13
		437	0	465	12		
				470	13		

Identification of Acid-producing Bacteria. - Nineteen isolates of acid-forming bacteria were maintained on lactose motility medium. Transfers were made to sterile cucumber juice diluted 1:2 and incubated for 48 hours at 30° C.

Gram stains were made of the cultures at the end of 24 and 48 hours.

After 48 hours, transfers were made in duplicate to the following media: nutrient broth, nutrient agar slants, litmus milk, and into purple broth base containing the following carbohydrates: arabinose, xylese, dextrose, fructose, galactose, mannose, sucrose, lactose, maltose, raffinose, dextrin, mannitol, and starch. The tubes were incubated at 30° C for one to two weeks.

The Gram stain showed that all nineteen isolates were Gram-positive, short, stubby rods with rounded ends.

Growth in nutrient broth and on nutrient agar slants was sparse. In litmus milk, the organisms produced an acid curd by the end of seven days. All of the isolates produced acid from the carbohydrates tested except starch. There was no change in the tubes containing starch although growth was observed. None of the organisms produced gas in any of the tubes.

According to Bergey's Manual of Determinative Bacteriology, these organisms were identified as Lactobacillus plantarum (9).

Identification of Aerobacter and Related Organisms. -

Forty-five organisms isolated from eosin-methylene blue agar were maintained in lactose broth and on nutrient agar slants.

A Gram stain was made from a 24-hour lactose broth culture incubated at 35° C. Each of the pure cultures was streaked onto eosin-methylene blue agar in order to study further their cultural characteristics. Indol, methyl red, Voges-Proskauer, and citrate tests were performed. Gelatin liquefaction and glycerol fermentation tests were employed for species differentiation.

All of the organisms produced gas in lactose broth. The Gram stain showed all of the 45 isolates to be small Gram-negative rods.

Colonies on eosin-methylene blue agar exhibited the following characteristics after 48 hours of incubation at 35° C:

Size: Two to five mm in diameter.

Color: Light pink to orange to brown.

Centers: Dark centers were observed in many colonies.

Confluence: Colonies tended to run together.

Elevation: Colonies were considerably raised and markedly convex.

Metallic sheen: A metallic sheen was observed on rare occasions.

The results of the indol, methyl red, Voges-Proskauer, citrate, gelatin liquefaction and glycerol fermentation

tests are presented in Table 4.

According to Bergey's Manual of Determinative Bacteriology 29 organisms were identified as belonging to the genus Aerobacter (9). These organisms were indol negative, methyl red negative, Voges-Proskauer positive, and citrate positive. Twenty-one of those 29 organisms liquefied gelatin but did not ferment glycerol. Hence, these organisms were identified as Aerobacter cleaiae. The remaining eight Aerobacter organisms could not definitely be called Aerobacter aerogenes since these liquefied gelatin as well as fermented glycerol. One organism (No. 459) was identified as belonging to the genus Escherichia. The remaining 15 organisms remained unclassified. They could, however, be grouped as intermediate coliforms.

Identification of Yeasts. - Twenty-one yeast isolates were maintained on V-8 agar slants. This medium was prepared according to Etchells and Bell (33). Three-week old cultures from the V-8 agar slants were examined microscopically. Cultural characteristics were studied using dextrose agar and dextrose broth. The yeasts were further studied by determining their ability to ferment the following sugars: dextrose, maltose, galactose, lactose, and sucrose. A nitrate assimilation test as described by Etchells and Bell (33) was also employed.

The morphological, cultural, and biochemical characteristics of the yeasts are presented in Table 5.

TABLE 4

BIOCHEMICAL REACTIONS OF AEROBACTER AND RELATED ORGANISMS

Isolate no.	Indol	Methyl red	Voges- Proskauer	Citrate	Gelatin	Glycerol
401	-	-	+	-	+	+
408	-	-	+	-	-	+
409	-	+	-	+	-	+
410	-	-	+	+	+	+
411	-	+	-	+	+	-
412	-	+	-	-	-	+
420	-	+	+	+	+	+
421	-	+	-	+	+	+
422	-	+	+	+	+	+
423	-	-	+	+	+	+
424	-	-	+	+	+	+
425	-	+	+	+	+	+
426	-	-	+	+	+	-
427	-	-	+	+	+	-
428	-	-	+	+	+	-
429	-	-	+	+	+	-
430	-	+	+	+	+	+
431	-	-	+	+	+	+
432	-	-	+	+	+	-
433	-	-	+	+	+	-
434	-	-	+	+	+	-
437	-	+	+	+	+	-
439	-	-	+	+	+	-
441	-	-	+	+	+	-
442	-	-	+	+	+	-
443	-	+	-	-	+	+
444	-	+	+	+	+	-
445	-	-	+	+	+	-
446	-	-	+	+	+	-
447	-	+	-	-	+	+
448	-	-	+	+	+	-
449	-	-	+	+	+	-
451	-	-	+	+	+	-
452	-	-	+	+	+	+
454	-	-	+	+	+	+
455	-	-	+	+	+	+
456	-	-	+	+	+	-
457	-	-	+	+	+	-
459	+	+	-	-	-	+
461	-	-	+	+	+	+
462	-	-	+	+	+	-
463	-	-	+	+	+	-
464	-	-	+	+	+	-
465	-	-	+	+	+	-
470	-	-	+	+	+	+

- = negative test; + = positive test

TABLE 5

MORPHOLOGICAL, CULTURAL, AND BIOCHEMICAL CHARACTERISTICS OF YEASTS
ISOLATED DURING CUCUMBER FERMENTATION

Isolate no.	Microscopic picture	Dextrose agar growth	Dextrose breth growth
701	Rough spores	White, rough, dull, abundant	Heavy film
703	Cepulation tube visible	White, smooth, glistening, abundant	Ring
704	Large, pleomorphic cells, non-sperulating	White, smooth, glistening, abundant	Ring
706	Rough spores	White, rough, dull, abundant	Film
707	Hat-shaped spores	White, smooth, glistening, abundant	Ring
709	Round cells	Red, smooth, glistening, abundant	Red ring
711	Round cells	Red, smooth, glistening, abundant	Red ring
713	Hat-shaped spores	White, smooth, glistening, abundant	Ring
714	Round cells	Red, smooth, glistening abundant	Red ring
715	Small, pleomorphic cells, non-sperulating	White, smooth, dull, abundant	Ring
716	Small, pleomorphic cells, non-sperulating	White, smooth, dull, abundant	Ring
717	Cells and fragments, non-sperulating	White, smooth, glistening, abundant	Ring
718	Hat-shaped spores	White, smooth, glistening, abundant	Ring
719	Hat-shaped spores	White, smooth, glistening, abundant	Ring
720	Hat-shaped spores	White, smooth, glistening, abundant	Ring
723	Hat-shaped spores and fragments containing hat-shaped spores	White, smooth, glistening, abundant	Ring
724	Same as 723	White, smooth, glistening, abundant	Ring
725	Small, pleomorphic cells, non-sperulating	White, smooth, glistening, abundant	Ring
726	Small, oval cells, non- sperulating	White, smooth, glistening, abundant	No film, ne ring
728	Same as 723	White, smooth, glistening, abundant	Ring
729	Hat-shaped spores	White, smooth, glistening, abundant	Ring

Isolate no.	Sugars fermented					Nitrate assimilation	Genus and species
	Dextrose	Maltose	Galactose	Lactose	Sucrose		
701	+	-	-	-	+	-	<u>Debaryomyces</u> sp.
703	+	-	-	-	+	-	<u>T. rosei</u>
704	+	-	-	-	-	-	*
706	+	-	-	-	+	-	<u>Debaryomyces</u> sp.
707	+	+	-	-	+	+	<u>H. subpelliculosa</u>
709	-	-	-	-	-	+	<u>Rhodeterula</u> sp.
711	-	-	-	-	-	+	<u>Rhodeterula</u> sp.
713	+	+	+	-	+	+	<u>Hansenula</u> sp.
714	-	-	-	-	-	+	<u>Rhodeterula</u> sp.
715	-	-	-	-	-	-	*
716	-	-	-	-	-	-	*
717	+	-	-	-	+	-	*
718	+	+	-	-	+	-	<u>H. subpelliculosa</u>
719	+	-	-	-	+	-	<u>H. subpelliculosa</u>
720	+	+	-	-	+	+	<u>H. subpelliculosa</u>
723	+	-	-	-	+	-	*
724	+	+	+	-	+	+	<u>Hansenula</u> sp.
725	+	+	+	-	+	+	*
726	+	-	+	-	+	-	<u>T. holmii</u>
728	+	+	+	-	+	+	<u>Hansenula</u> sp.
729	+	+	-	-	+	+	<u>H. subpelliculosa</u>

+ = positive test

- = negative test

* = organism not classified

According to Etchells and Bell (33), Lodder (70), and Stelling Dekker (99), the yeasts were classified as indicated in Table 5. Of the 21 isolates, eight were placed in the genus Hansenula. The remaining isolates were classified as follows: Rhodoterula (3 isolates), Debaryomyces (2 isolates), Torulospora resei (1 isolate), and Torulopsis holmii (1 isolate). Six isolates could not be classified.

DISCUSSION

Although scientists are readily tempted to look for a constant set of data, one must be extremely cautious in analyzing data obtained from a cucumber fermentation. The following six points are suggested as uncontrollable factors which may lead to different results.

1. The microorganisms introduced into the fermentation vary according to the type of soil and weather conditions under which the cucumbers were grown.

2. During fermentation there may be a large variation in temperature. Even in the same area, fermentations started a few days apart may have a great temperature difference affecting the various stages of the fermentation.

3. A tank may be contaminated with chemicals, scum, filth, etc.

4. The salt concentration could easily vary from one to two percent. This variation would affect the numbers and kinds of microorganisms present during a fermentation.

5. The method of filling a tank varies with different salters. Some may take several days to fill a tank whereas others may fill their tank in one day.

6. The variety of cucumber introduced into the fermentation may have different skins, nutrient materials, water content, etc.

In spite of the difficulty of dealing with an uncontrolled fermentation certain trends were noted.

The acid-producing organisms apparently belong to one species, viz., Lactobacillus plantarum. They appeared in very high numbers and reached the peak of their activity between three and eight days.

The Aerebacter fermentation was significant in that it disappeared as quickly as it appeared. The peak of this fermentation preceded that of the lactic fermentation. It seemed that the acid produced by L. plantarum could decrease the number of Aerebacter. Perhaps these organisms benefited the fermentation by creating conditions suitable for L. plantarum. They might have created lower oxygen tension conditions or synthesized vital nutrient substances.

The identification of many of these organisms as Aerebacter cleacae confirmed the work of Etchells, Fabian, and Jones (36).

The yeasts appeared in slightly lower numbers in these cucumber fermentations than reported previously. This may have been due to the fact that the incubation period of four days was not long enough to detect the tiny yeast, Terulepsis caroliniana.

It should be pointed out that no quantitative characterization of the different yeasts was attempted. Isolated colonies were picked at random. The fact that Hansenula appeared so frequently in Table 5 is not an indication that this yeast occurred to that extent during the actual cucumber fermentation.

PART II

A VITAMIN STUDY OF THE CUCUMBER FERMENTATION

INTRODUCTION

During the initial stages of a cucumber fermentation, the acid-producing bacteria appear in great numbers in spite of the fact that only a few of these organisms are introduced into the fermentation. Obviously the environmental conditions must permit these organisms to dominate the early part of the fermentation.

It has been explained that the acid-producing bacteria tolerate the high salt concentration which is inhibitory to many other types of organisms. It has also been said that the pH of the brine is optimum for the acid-producers.

It was the purpose of this particular work to study another possible reason for the early appearance of acid-producing organisms. This study was concerned with the nutrition of these bacteria. Since it has been reported that many Lactobacilli require certain vitamins, it was first decided to establish a few vitamin requirements for L. plantarum isolated from cucumber fermentations. Biotin, niacin, and pantothenic acid were the vitamins selected for study since it has been shown that these vitamins are needed as growth factors for many lactic acid bacteria.

After determining the vitamin requirements for this organism, cucumber juice would be analyzed for these vitamins.

The effect of non-acid-producing organisms isolated from cucumber fermentations on the vitamin content of cucumber juice would also be studied.

The vitamin content during an actual fermentation would also be determined which would demonstrate the net amount of vitamins available for microorganisms.

REVIEW OF THE LITERATURE

Vitamin Requirements of Lactic Acid Bacteria. - There is confusion in the literature concerning the nomenclature of lactic acid bacteria. Bergey's Manual of Determinative Bacteriology (9) lists many lactic acid bacteria to be synonomous. The literature concerned with the nutrition of these organisms discusses them as individual species and indicates different nutritional requirements for organisms listed as identical in Bergey. In fact, Campbell and Hucker (14) found that cultures of Lactobacillus plantarum obtained from various sources differed markedly in their riboflavin requirements. Some cultures grew well in the absence of riboflavin, while others failed to grow regardless of the amount of riboflavin added to the medium. One strain, L. plantarum var. rudensis would not grow in a riboflavin-free medium but produced increasing amounts of acid with additional amounts of riboflavin.

The confusion in nomenclature of lactic acid organisms has not stepped the enormous progress made in their nutritional studies. Sandford (87) has indicated that the earliest nutritional studies of microorganisms were made with yeasts. It was noted that yeasts failed to grow when small inocula were used. It was stated that Wildiers

believed the ability of yeasts to grow to be governed by the presence of water soluble nutrient material occurring in large inocula. Thiamine was perhaps the first pure vitamin to be shown to be required by microorganisms. This was shown in 1935 with the mold Phycomyces blakesleamus. Earlier in 1930, Williams and Reehm (110) had demonstrated that antineuritic vitamin preparations would stimulate the growth of yeasts.

A few years later work was begun on the vitamin requirements of lactic acid bacteria. In 1938, Snell and Strong (95) found that lactic acid bacteria may or may not require riboflavin, depending on the species tested. In 1938 and 1939, Snell, Strong, and Petersen (96, 97) reported that pantothenic and nicotinic acid were required by certain lactic acid bacteria. The following year, Snell and Peterson (93) confirmed the work of Mueller that pyridoxine was required. In 1940, thiamine was shown to be required by certain heterofermentative lactic acid bacteria by Wood et al (116). In 1941, Mitchell et al (77) using a purified preparation of folic acid from spinach demonstrated that this vitamin stimulated Lactobacillus casei, Lactobacillus delbrueckii, and rats. Chattaway et al (15), in 1942, noted that p-aminobenzoic acid would stimulate L. casei. Biotin was found to be required for Lactobacillus casei, in 1943, by Shull et al (90).

At this time, studies were broadened to determine if substances resembling the vitamins in chemical structure

influenced microbiological activity. Koser (55), in 1941, showed that nicotinic acid and nicotinamide varied in their effect on various microorganisms. Snell and Rannefeld (94), in 1945, found that pyridexamine and pyridexal were more effective than pyridexine for most microorganisms tested.

Studies were completed by several workers on numerous species of lactic acid bacteria. In 1942, Behones et al (7) found many lactic acid bacteria to require pyridexine. They also showed that other lactic acid organisms did not require this vitamin. In 1945, Cheldelin et al (16) listed 33 strains of lactic acid bacteria requiring pantethenic acid.

In 1945, Snell (92) summarized the vitamin requirements of certain lactic acid bacteria. These requirements may be seen in Table 6.

TABLE 6

VITAMIN REQUIREMENTS OF VARIOUS SPECIES
OF LACTIC ACID BACTERIA (AFTER SNELL)

Vitamin	L. casei	L. del-brueckii 3	L. fermentum 36	L. arabinosus 17-5	Leuconostoc mesenteroides P-60
Riboflavin	+	+	-	-	-
Pantethenic acid	+	+	+	+	+
Nicotinic acid	+	+	+	+	+
Biotin	+	+	+	+	+
Pyridexine	+	+		-	-
Thiamine	-	-	+	-	-
Folic acid	+	+		-	-

+ = vitamin required; - = vitamin not required

In 1945, Petersen and Petersen (81) stated that biotin, nicotinic acid, pantothenic acid, and riboflavin were the most frequently reported vitamins required by microorganisms, each being required by about 50 different organisms.

There is a constant search for additional nutritional factors. In 1946, Guirard et al (46) showed that acetate and certain lipids could stimulate certain lactic acid bacteria. It was suggested by these workers that acetate might play an important role in lipid metabolism.

In 1947, Shankman et al (88) indicated that many vitamins considered essential would not be required if the organisms were incubated over a longer period of time.

Biotin, Niacin and Pantothenic Acid Content of Cucumbers, Pickles and Cabbage. - There is a paucity of information on the vitamin content of cucumbers and pickles. Slightly more data is available on the vitamin content of cabbage. For this reason and the fact that the fermentation of cabbage is similar to that of cucumbers, a literature search was made on the vitamin content of both vegetables.

The research staff of the H. J. Heinz Company (47) reported less than one mg nicotinic acid per 100 g for cucumbers and cabbage.

Cheldelin and Williams (17) gave the vitamin content of cabbage as follows:

Biotin	0.0024 mg/100 g cabbage
Niacin	0.21 mg/100 g cabbage
Pantothenic acid	0.18 mg/100 g cabbage

Elvehjem (30) reported the vitamin content of cabbage to be 0.3 mg niacin per 100 g and 0.18 mg pantothenic acid per 100 g.

Camillo, Hoppert, and Fabian (13), in a study of fresh and fermented cucumbers shewed that the type of soil in which cucumbers were cultivated had little effect on their vitamin content. They also showed that certain water soluble vitamins (ascorbic acid, thiamine, and riboflavin) in pickles decreased after a fermentation, whereas the vitamin A value increased.

Lampen et al (60), in a study of free and bound biotin found that vegetables contained a water extractable form of biotin whereas yeasts, animal products, seeds, and nuts had most of their biotin in bound form. This is particularly significant in food fermentations because one may more readily understand how vitamins are made available for bacteria during a fermentation.

Microbial Synthesis and Destruction of Vitamins. -

The vast majority of publications is concerned with vitamin synthesis by microorganisms. However, in this study, vitamin destruction by bacteria and yeasts was of primary interest.

In 1930, McElroy and Gross (61) reported that riboflavin, pyridoxine, thiamine, and pantothenic acid formed

in the rumina of sheep and cows fed diets low in those vitamins. In 1933, Williams et al (109) reported that Aspergillus niger could synthesize pantothenic acid. In 1938, Almquist et al (4) showed that vitamin K was produced by organisms isolated from fish meal. In the same year it was demonstrated by Abdel-Salaam and Leong (1) that thiamine was synthesized by bacteria isolated from the intestine of the rat. In 1939, Silverman and Werkman (91) proved that propionic acid bacteria could be adapted to synthesize thiamine. Evans et al (39), in the same year, indicated that certain strains of Corynebacterium diphtheriae would synthesize pantothenic acid if nicotinic acid, pimelic acid and beta-alanine were present in the medium. In 1940, it was shown by McElroy and Jukes (72) that biotin was produced in the rumen of the cow. The following year, it was demonstrated by Landy and Dicken (62) that many microorganisms would synthesize biotin in an amino acid-glucose medium. In 1942, Eakin and Eakin (27) reported that biotin was produced by Aspergillus niger in a medium containing pimelic acid. In the same year, Nielson et al (79) pointed out that biotin could be synthesized in the intestines of rats. Mitchell and Isbell (76), in 1942, showed that intestinal bacteria were capable of synthesizing many B vitamins especially biotin and pyridoxine. Nicotinic and pantothenic acids were synthesized slightly. They also pointed out that a diet containing lactose, favoring the production of acid, had a marked influence on the amounts of vitamins synthesized. An acid-forming diet made pantothenic acid more

available while pyridoxine and thiamine were made more available by a non-acid-forming diet. In 1942, Burkholder and McVeigh (12) showed that biotin, niacin, riboflavin, and thiamine were synthesized by Bacterium aerogenes and Escherichia coli. In 1942, Thompson (103) demonstrated that many bacteria including Aerobacter aerogenes were capable of synthesizing biotin, niacin, pantothenic acid, and other B vitamins. This worker found that the vitamin content of the bacterial cell remained constant. Any excess vitamin produced was "excreted" into the medium. In 1943, Rogosa (86) indicated that riboflavin was synthesized by certain lactose fermenting yeasts. In 1944, Miller (74) reported that Escherichia coli could synthesize folic acid and biotin. In the same year, Lewis et al (67) found that Torulepsis utilis was able to convert fermentable substrates into many B vitamins except biotin.

Although bacteria are most frequently given credit for synthesis of vitamins, a few workers have pointed out that bacteria are also capable of destroying vitamins. Kendall and Chinn (50), in 1938, and Esselen and Fuller (31), in 1939, showed that ascorbic acid was oxidized by many intestinal bacteria. In 1943, Allison (3) demonstrated that certain soil bacteria were capable of destroying nicotinic acid. In the same year, Mirick (75) found that p-aminobenzoic acid was oxidized to carbon dioxide, water and ammonia by soil organisms. In 1944, Foster (43) discovered that Pseudomonas oxidized riboflavin to lumi-

chrome. In 1944, Koser and Baird (54) found that Pseudo-
monas and Serratia species when grown in a medium using
only nicotinic acid as a carbon source, would decrease the
content of this vitamin. If nicotinic acid was replaced by
glucose, these organisms grew and synthesized this vitamin.

EXPERIMENTAL

Vitamin Requirements of Lactobacillus plantarum Isolated from Cucumber Fermentation

Ten organisms isolated from different fermentations
were selected for study. The well known assay organism,
Lactobacillus arabinosus 17-5, was run concurrently with
the other Lactobacilli. Since this organism was known to
require biotin, niacin, and pantothenic acid, it served as
a check for the materials and methods of the determination.

The media used to determine the vitamin requirements
of these organisms were the dehydrated assay media manu-
factured by Difco Laboratories, Inc.

The inoculum was prepared by inoculating 10 ml of
sterile, centrifuged cucumber juice diluted 1:2 with dis-
tilled water. The cucumber juice was contained in small
centrifuge tubes. After inoculation, the tubes were in-
cubated for 48 hours at room temperature. They were then
centrifuged in order to separate the cells from the nutrient
medium. The supernatant was poured off and the cells were
resuspended in 100 ml of sterile physiological saline.

One drop per tube of this suspension was used as the inoculum.

Varying amounts of biotin, niacin and pantothenic acid were dispensed into test tubes. The concentrations used were suggested by a paper of Krueger and Peterson (58), who determined what these vitamin requirements were for a strain of Lactobacillus pentosus. The respective assay medium was added to the tubes containing the vitamin solutions. Each tube contained a final volume of 10 ml. The tubes were sterilized for only eight minutes at 15 pounds pressure. Incubation was carried out for 72 hours at 28° C. The amount of growth was determined by titrating with 0.1 N NaOH.

The results are presented in Tables 7, 8, and 9 and are summarized in Figure 4.

As expected, L. arabinosus 17-5, represented by the solid line in Figure 4, responded to increasing amounts of vitamins. The Lactobacilli isolated from cucumber fermentations responded in a similar fashion. The top dotted line represents the most active, whereas the bottom dotted line represents the least active of the ten isolates tested.

These data definitely indicated that Lactobacilli of the cucumber fermentation required biotin, niacin, and pantothenic acid when the determination was made by the previously described method.

BIOTIN REQUIREMENTS OF L. PLANTARUM AND L. ARABINOSUS 17-5

Biotin (mcg/10 ml)	ml 0.1 N acid produced										
	L. arabinosus 17-5 (control)	Numbered isolates of L. plantarum									
		600	602	604	609	611	613	615	617	621	625
0	1.0	0.5	0.3	0.8	0.1	0.8	0.4	0.7	0.2	0.5	0.9
0.2	6.3	3.8	4.6	4.6	4.7	4.1	4.3	4.4	4.5	5.4	4.0
0.4	8.2	5.9	6.7	6.6	6.3	6.2	6.5	6.3	6.8	7.8	6.0
0.6	9.9	7.0	8.0	7.6	8.8	7.7	8.0	7.8	8.3	9.2	6.9
0.8	11.1	7.7	8.9	8.9	9.5	8.6	9.0	8.6	8.5	10.5	7.9
1.0	12.1	8.6	10.0	10.3	10.1	9.4	9.2	9.2	10.4	11.3	8.4
1.2	13.0	9.7	10.7	10.9	11.2	10.3	10.2	10.3	11.4	12.4	9.3
1.4	13.2	10.1	11.7	11.1	12.9	11.2	12.1	•	11.3	13.4	9.8
1.6	14.5	10.8	12.0	12.2	12.7	12.7	12.5	12.3	12.3	13.6	10.2

• = determination not made

TABLE 8

NIACIN REQUIREMENTS OF L. PLANTARUM AND L. ARABINOSUS 17-5

Niacin (mcg/10 ml)	Ml 0.1 N acid produced										
	L. arabinosus 17-5 (control)	Numbered isolates of L. plantarum									
		600	602	604	609	611	613	615	617	621	625
0	0.6	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4
0.2	5.4	3.0	3.6	3.0	3.7	3.4	3.1	3.2	3.9	5.4	3.7
0.4	7.9	5.3	6.6	6.1	7.2	5.8	5.9	6.0	6.5	8.9	5.8
0.6	10.3	8.4	8.0	8.7	8.8	8.3	8.7	7.0	9.3	10.9	8.0
0.8	11.4	9.9	9.7	10.4	10.4	10.2	10.1	10.4	11.1	12.1	9.6
1.0	12.1	11.3	11.2	11.3	12.3	11.6	11.2	11.4	12.0	12.4	10.4
1.2	12.3	12.0	12.3	11.8	13.1	12.3	12.6	12.2	12.9	12.8	11.0
1.4	13.3	12.7	12.3	12.4	13.4	12.9	12.9	12.4	13.8	13.3	11.2
1.6	13.2	12.7	13.2	12.5	13.1	12.6	13.5	12.3	13.4	13.3	11.2

TABLE 9

PANTOTHENIC ACID REQUIREMENTS OF L. PLANTARUM AND L. ARABINOSUS 17-5

Pantothenic acid (mcg/10 ml)	Ml 0.1 N acid produced										
	L. arabinosus 17-5 (control)	Numbered isolates of L. plantarum									
		600	602	604	609	611	613	615	617	621	625
0	0.4	0.3	0.3	0.3	0.1	0.2	0.4	0.4	0.2	0.3	0.3
0.05	8.9	1.4	4.5	3.4	5.8	5.6	5.4	5.3	3.2	5.2	5.0
0.10	11.8	4.0	7.4	8.4	7.5	7.3	7.7	7.2	8.2	8.6	5.5
0.15	13.2	4.7	10.3	10.4	10.2	9.7	10.3	9.5	9.8	10.4	7.5
0.20	14.3	7.4	11.7	11.7	11.5	11.9	11.9	11.4	11.2	12.2	9.6
0.25	14.7	11.4	12.7	12.3	12.2	12.4	12.7	12.7	12.2	13.4	12.3
0.30	16.0	13.5	13.3	12.6	13.1	13.6	13.2	13.8	13.8	14.0	13.0
0.35	15.8	14.7	14.4	14.0	13.8	14.7	14.4	14.2	14.9	14.6	13.3
0.40	16.5	14.9	14.9	14.1	14.1	15.1	14.8	14.8	14.6	15.0	13.5

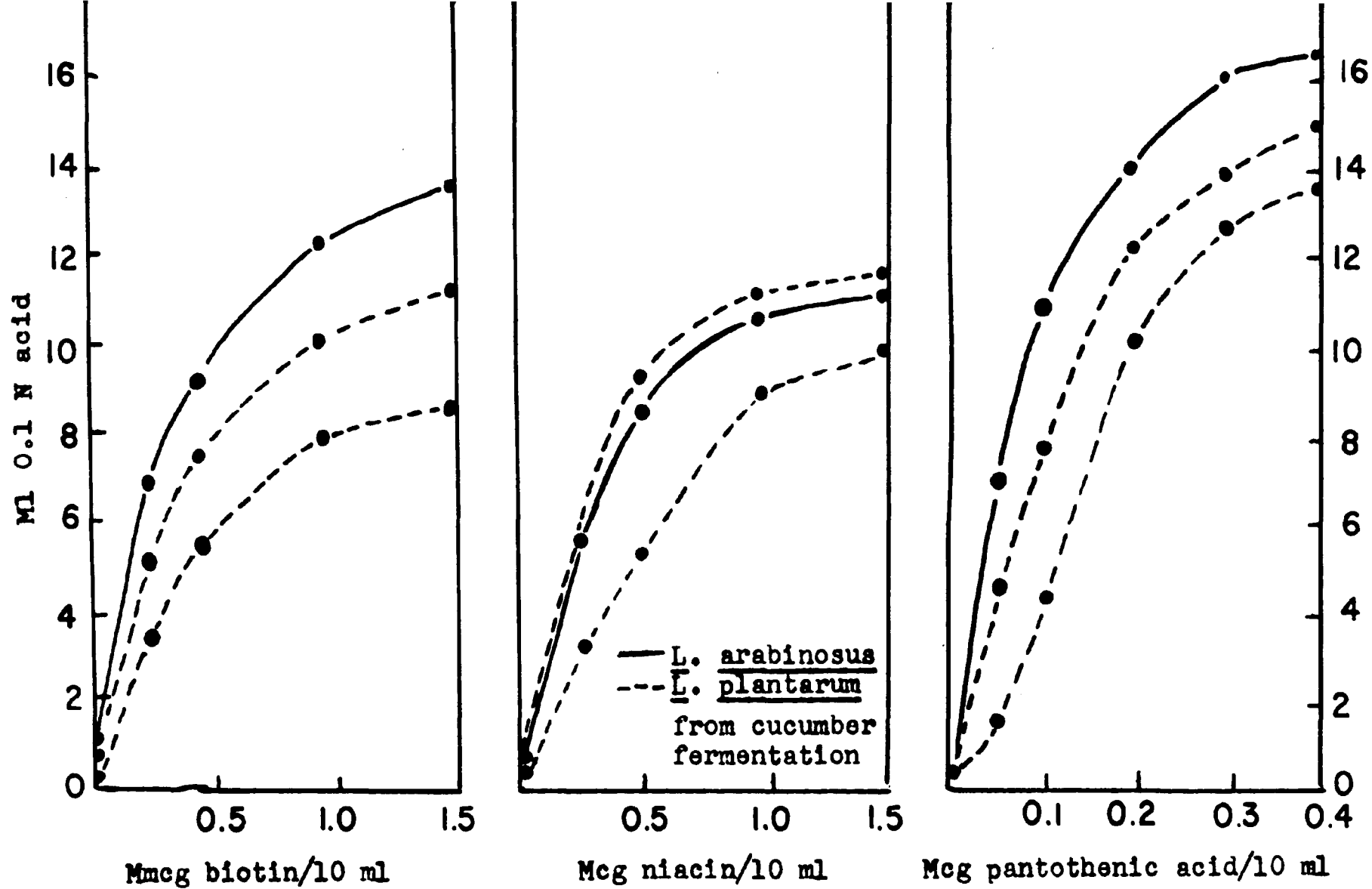


Figure 4. Vitamin responses of Lactobacilli isolated from cucumber fermentation compared with Lactobacillus arabinosus 17-5

Determination of Biotin, Niacin, and Pantothenic
Acid in Cucumber Juice

It was then decided to determine the content of these vitamins in cucumber juice. The assay was made by microbiological means. The assay organism, L. arabinosus 17-5, was used for the assay of all three vitamins. The media used were the same as those used for the determination of the vitamin requirements, viz., Difco's dehydrated assay media. The method of assay was similar to that described in "Methods of Vitamin Assay" (5).

Several varieties of cucumbers obtained from different sources were analyzed.

The cucumber juice was extracted by macerating the cucumbers in a Waring Blendor. The ground cucumbers and accumulated liquid were then pressed through cheesecloth. Appropriate dilutions of this preparation were made and assayed.

Since it was desired to determine the vitamin content that would be available for microorganisms, no attempt was made to free any combined vitamins. It was not necessary to filter or adjust the pH of the cucumber juice since only 0.01 ml of cucumber juice could bring about a response corresponding to the standard curves. The results are given in Table 10.

It may be noted that all of the cucumbers tested contained a rich supply of the three vitamins studied. The extreme values for the vitamin content of cucumber juice

TABLE 10

VITAMIN CONTENT PER ML OF CUCUMBER JUICE OF DIFFERENT
VARIETIES OF CUCUMBERS OBTAINED FROM VARIOUS SOURCES

Variety of cucumber	Source	Vitamin content		
		Biotin (mmcg)	Niacin (mcg)	Pantethenic acid (mcg)
MR-17	a	17.3	5.05	2.42
MR-17	b	33.0	4.62	2.13
MR-17	c	16.2	2.90	2.24
Producer	a ⁺	5.20	1.83	2.05
Producer	a ⁺	8.85	2.80	1.45
Producer	b	14.6	2.61	1.05
SR-6	a	6.65	3.37	1.45
Model	a	12.2	2.96	1.70
Davis	a	10.8	2.63	1.84
Yorkstate	b	9.13	3.05	1.61
Large ripe cucumber*	a	7.00	3.04	1.85
Large long green slicer	d	6.75	2.26	2.85

*Variety not known

+Obtained on different days

a H. V. Madison Pickle Co., Mason, Mich.

b Dept. of Horticulture, Michigan State College

c Feed Stores, Michigan State College

d Market Basket, East Lansing, Mich.

were as follows:

Biotin	5.20 to 33.0 mcg/ml
Niacin	1.83 to 5.05 mcg/ml
Pantethenic acid	1.05 to 2.42 mcg/ml

In general, there was no difference in the vitamin content of the different varieties of cucumbers. However, MR-17 cucumbers, obtained from three different sources, contained more biotin than any of the other varieties tested.

Effect of *Aerobacter cleacae* and Various Yeasts on the Vitamin Content of Cucumber Juice

Since a review of the literature and data presented earlier in this study indicated that *A. cleacae* and various yeasts were present during the cucumber fermentation, it was decided to determine what effect these organisms had on the vitamin content of cucumber juice. The vitamins selected for study were biotin, niacin, and pantethenic acid because in a previous experiment these vitamins were proven to be essential for *L. plantarum* isolated from cucumber fermentations. Nine isolates* of *Aerobacter cleacae* were selected for study. Three yeast isolates** identified as *Hansenula subpelliculosa* as well as the following yeasts obtained from Northern Regional Research Laboratory were also tested: *Hansenula subpell-*

*Isolate Nos. 426, 427, 428, 429, 432, 433, 434, 449, 457

**Isolate Nos. 707, 718, 729

culosa RY-135, Terulopsis careliniana RY-147, Terulopsis rosei RY-8, Brettanomyces versatilis Y-146, Zygesaccharomyces sp. A YS-590, and Terulopsis holmii FFL-Y-307. These yeasts were isolated by Etchells from commercial cucumber brines.

The test organisms were inoculated into 10 ml of cucumber juice diluted 1:2. Two tubes containing only cucumber juice were used as a control in each determination. After 30 hours incubation, the microbiological activity in the cucumber juice was stopped by autoclaving. Appropriate dilutions were made and the vitamin assay was done as previously described. The results are given in Tables 11, 12, and 13, and are presented graphically in Figures 5, 6, and 7.

TABLE 11

EFFECT OF AEROBACTER CLOACAE ON THE
VITAMIN CONTENT OF CUCUMBER JUICE

Vitamin	Trial No. 1		Trial No. 2	
	Average of nine isolates		Average of two isolates	
	Uninoculated	Inoculated	Uninoculated	Inoculated
	(control)		(control)	
Biotin (mcg/ml)	5.6	2.0	3.50	1.02
Niacin (mcg/ml)	1.3	1.7	1.55	1.50
Pantothenic acid (mcg/ml)	0.87	0.95	0.85	0.76

TABLE 12

EFFECT OF HANSENULA SUBPELLICULOSA ISOLATED FROM
LABORATORY CUCUMBER FERMENTATIONS ON THE VITAMIN
CONTENT OF CUCUMBER JUICE

Vitamin	Trial No. 1		Trial No. 2	
	Average of three isolates (Nos. 707, 718, 729)		Average of two isolates (Nos. 707, 718)	
	Uninoculated (control)	Inoculated	Uninoculated (control)	Inoculated
Biotin (mmcg/ml)	4.56	2.57	3.5	2.80
Niacin (mcg/ml)	1.85	1.75	1.55	1.53
Pantethenic acid (mcg/ml)	0.97	1.19	0.85	1.07

TABLE 13

EFFECT OF VARIOUS YEASTS ISOLATED FROM COMMERCIAL
CUCUMBER FERMENTATIONS ON THE VITAMIN CONTENT
OF CUCUMBER JUICE

Yeast	Vitamin		
	Biotin (mmcg/ml)	Niacin (mcg/ml)	Pantethenic acid (mcg/ml)
Control (uninoculated cucumber juice)	3.95	1.61	1.17
<u>Hansenula subpelliculosa</u>	2.80	1.37	1.39
<u>Terulopsis caroliniana</u>	1.90	1.15	1.11
<u>Terulepsis rosei</u>	1.35	1.28	1.14
<u>Brettanomyces versatilis</u>	3.90	1.30	1.36
<u>Zygosaccharemyces</u> sp. A	3.70	1.67	1.07
<u>Terulepsis holmii</u>	1.16	1.01	1.32

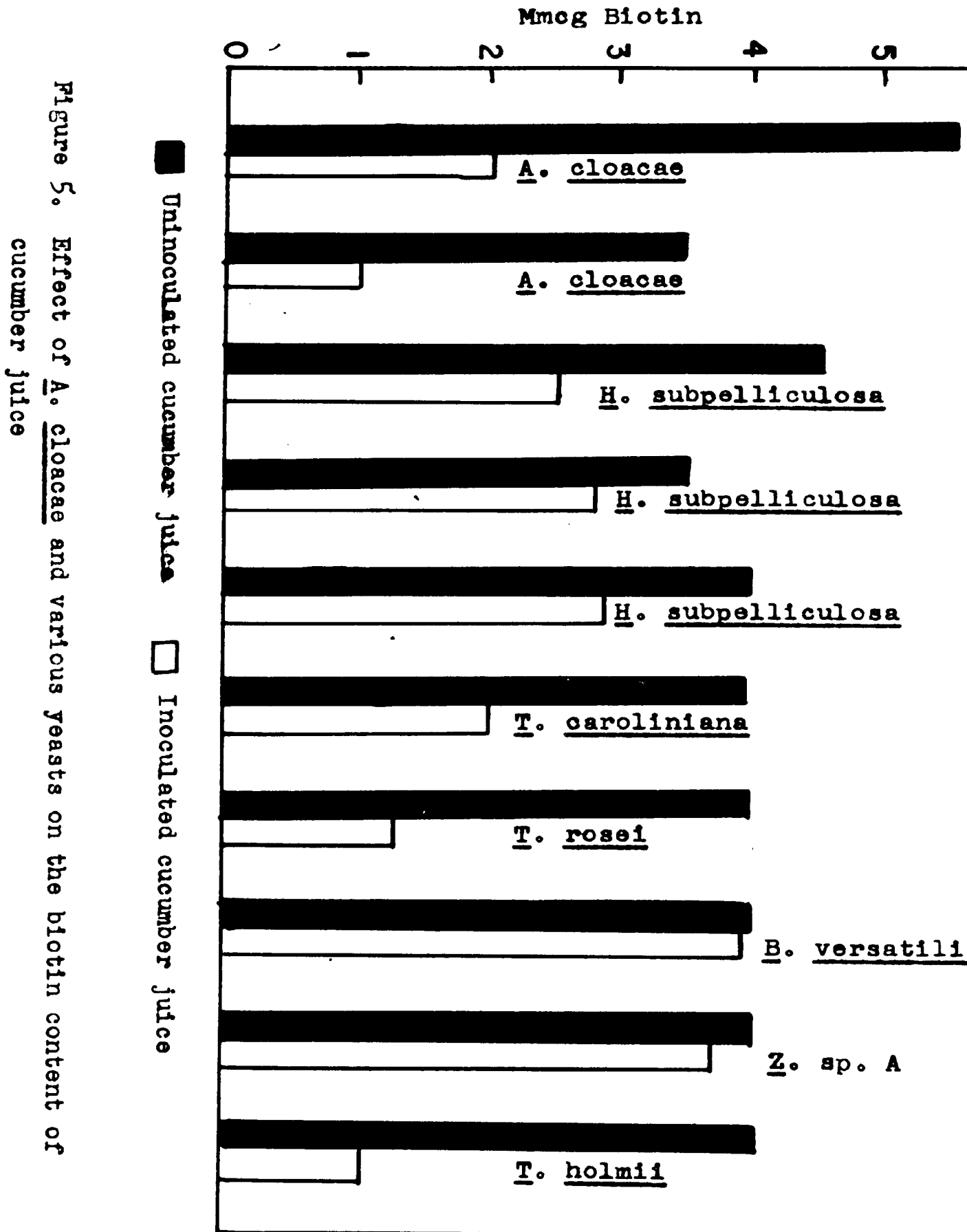


Figure 5. Effect of *A. cloacae* and various yeasts on the biotin content of cucumber juice

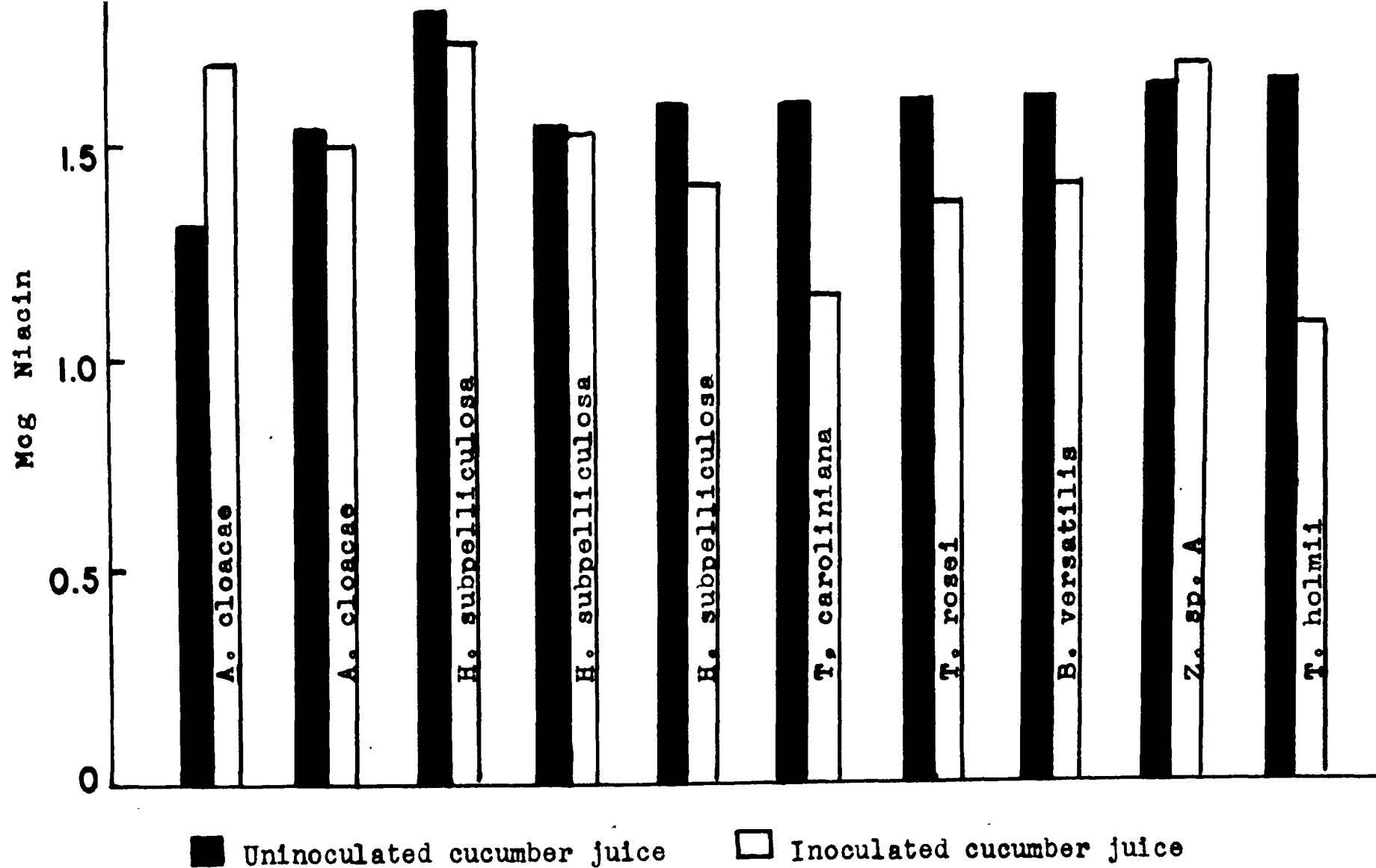


Figure 6. Effect of *A. cloacae* and various yeasts on the niacin content of cucumber juice

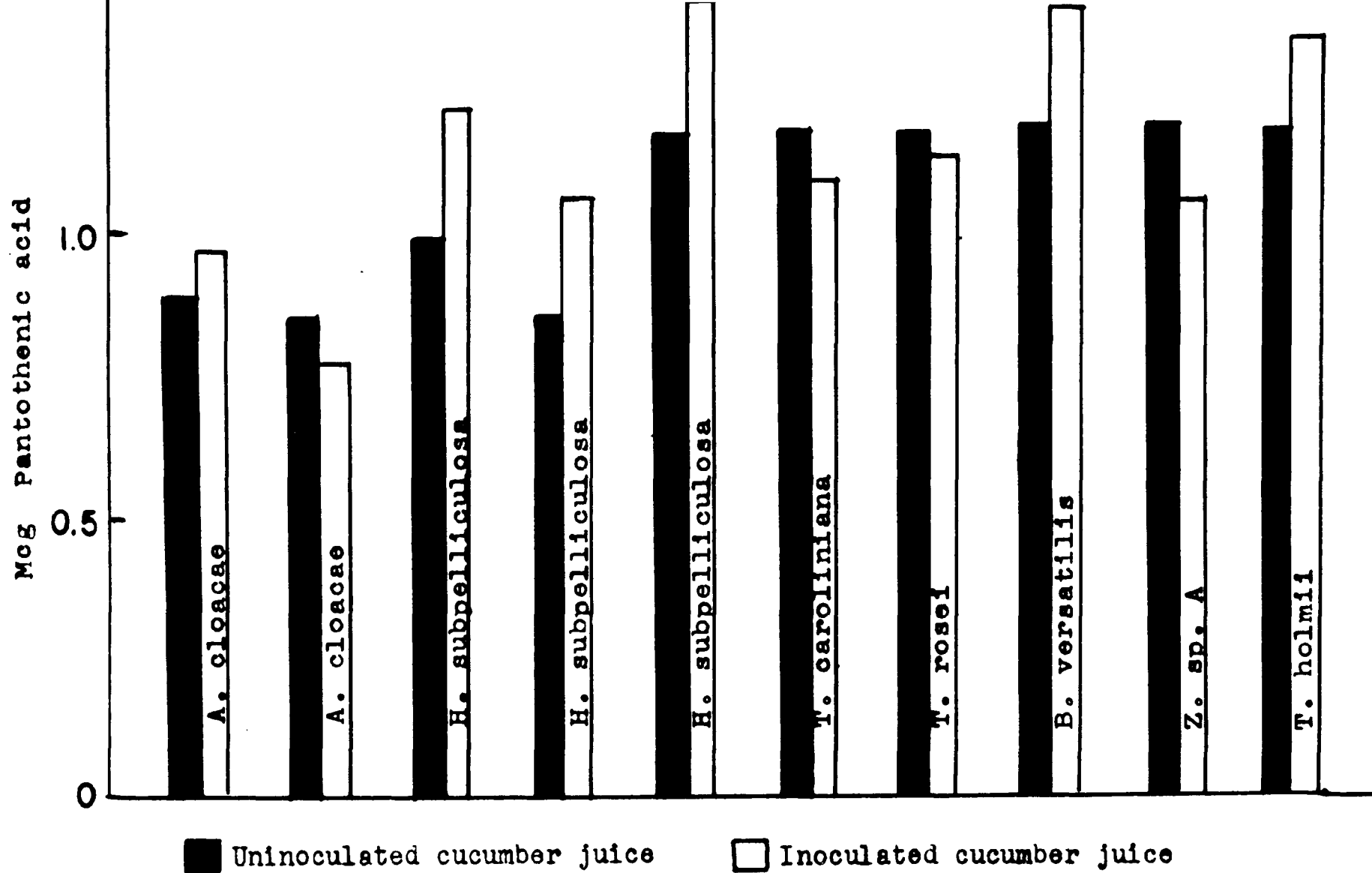


Figure 7. Effect of A. cloacae and various yeasts on the pantothenic acid content of cucumber juice

From Figure 5 it may be seen that most of the yeasts and A. cleaceae markedly lowered the biotin content of cucumber juice. Figure 6 reveals that the niacin content was not altered appreciably by A. cleaceae and the various yeasts. Figure 7 illustrates that pantothenic acid was barely utilized by any of the organisms. In fact, most of the yeasts appeared to synthesize this vitamin.

Vitamin Analysis During Cucumber Fermentation

Cucumber fermentations were set up in the laboratory as described earlier in this study. Samples were taken for total acid, microbial numbers and vitamin content. The microbiological analysis was carried out as previously described. The vitamin analysis was accomplished by pipetting samples of brine into volumetric flasks. Enough brine was added so that when the contents of the flask were brought to volume, there would be a 1:5 dilution of the brine. As soon as the brine was pipetted about three times as much distilled water was added to the flasks. The flasks were then autoclaved for 15 minutes at 121° C. When an assay was to be made the contents of the flask were brought to volume. Appropriate dilutions were made and the assay was accomplished as described in previous sections of this study.

The cucumbers were analyzed for vitamins at the beginning of the fermentation and after 20 days. The volume of the cucumbers and brine were measured at the end of the

fermentation in order to determine whether vitamins were synthesized or utilized. The vitamins determined were biotin, niacin and pantothenic acid.

The cucumbers in crock A₂ were of the Producer variety whereas those in crock B₂ were of the MR-17 variety. The results are presented in Tables 14 to 18.

Tables 14 and 15 illustrate the rapid entry of biotin, niacin and pantothenic acid from the cucumbers into the brine during a fermentation. Table 17 indicates that the cucumbers furnished much of the vitamins for the fermentation. However, the vitamin content of the cucumbers after 20 days was not depleted. From Table 18 the microbiological effect on vitamins during a fermentation may be noted. The pantothenic acid content was more than doubled in both crocks. The niacin content remained unchanged. There was a slight increase in biotin in crock A₂ and a marked decrease in this vitamin in crock B₂.

DISCUSSION

The need for biotin, niacin and pantothenic acid was clearly established for lactic acid bacteria isolated from cucumber fermentations (Figure 4). The vitamin content of cucumbers (Table 10) indicated that sufficient biotin, niacin and pantothenic acid was supplied for these organisms (compare with Figure 4). Although most of the non-acid-producing organisms of the cucumber fermentation

TABLE 14

TOTAL ACID, ORGANISMS PER ML, AND VITAMIN CONTENT PER ML BRINE OF
CUCUMBER FERMENTATION (30° S) (CROCK A₂)

Day	Percent acid (as lactic acid)	Organisms per ml			Vitamin per ml brine		
		L. plantarum	Aerobacter	Yeasts	Biotin (mmcg)	Niacin (mcg)	Pantothenic acid (mcg)
0	-	0	25,000	0	0.330	-	0.0835
1	0.017	1,400	290,000	35	-	0.324	-
2	0.020	15,000	2,290,000	0	2.69	0.650	0.645
3	0.061	7,700,000	29,000	0	3.17	0.820	1.00
4	0.070	9,000,000	430	0	3.66	0.840	1.22
5	0.13	12,400,000	4,300	0	4.27	0.670	0.800
6	0.20	28,700,000	1,470	4,000	3.69	1.06	0.991
8	0.28	8,500,000	43,000	80,000	3.38	1.12	0.963
10	0.34	3,600,000	4,300	230,000	3.93	1.11	0.813
12	0.38	1,800,000	1,470	70,000	4.63	0.983	1.08
14	0.43	730,000	920	220,000	4.50	0.923	1.17
17	0.54	300,000	430	300,000	4.30	0.980	0.987
20	0.69	300,000	1,470	800,000	2.89	0.938	1.27

- = determination not made

TOTAL ACID, ORGANISMS PER ML, AND VITAMIN CONTENT PER ML BRINE OF
CUCUMBER FERMENTATION (30° S) (CROCK B₂)

Day	Percent acid (as lactic acid)	Organisms per ml			Vitamin per ml brine		
		L. plantarum	Aerobacter	Yeasts	Biotin (mmcg)	Niacin (mcg)	Pantothenic acid (mcg)
0	-	0	14,700	90	0.071	-	0.0184
1	0.018	40,000	220,000	6,000	3.12	0.217	0.356
2	0.038	12,000,000	430,000	25,000	5.30	0.523	0.880
3	0.15	45,000,000	920,000	1,210,000	5.75	0.631	0.870
4	0.28	33,000,000	36,000	850,000	4.57	0.946	1.52
5	0.37	30,000,000	430,000	760,000	5.94	0.525	1.14
6	0.44	28,700,000	250,000	600,000	6.57	0.442	1.08
8	0.54	1,000,000	250,000	110,000	6.35	0.475	1.47
10	0.54	450,000	74,000	65,000	6.10	0.508	1.29
12	0.54	300,000	2,500	550,000	5.88	0.520	1.47
14	0.46	200,000	430	700,000	5.78	0.600	1.43
17	0.43	45,000	920	460,000	4.40	0.828	1.37
20	0.43	60,000	240	120,000	3.97	0.683	1.60

- = determination not made

TABLE 16

DILUTION FACTORS FOR CUCUMBERS FERMENTING IN CROCKS A₂ AND B₂

	Crock A ₂	Crock B ₂
Total volume (cucumbers + brine)	11,900 ml	12,600 ml
Volume of brine	<u>5,300 ml</u>	<u>5,500 ml</u>
Volume of cucumbers	6,600 ml	7,100 ml
Dilution factor for cucumbers	$\frac{11,900}{6,600} = 1.80^*$	$\frac{12,600}{7,100} = 1.78^*$

*The vitamin content of the cucumbers, when divided by this factor, represented the potential vitamin content per ml of brine. These values were taken as the actual vitamin content at the beginning of the fermentation. These values are given in Table 18.

TABLE 17

VITAMIN CONTENT PER ML OF CUCUMBER JUICE OF CUCUMBERS AT THE BEGINNING OF A FERMENTATION AND AFTER 20 DAYS

	Crock A ₂		Crock B ₂	
	Beginning	After 20 days	Beginning	After 20 days
Biotin (mmcg)	8.85	2.72	16.20	2.00
Niacin (mcg)	2.80	0.750	2.90	0.683
Pantothenic acid (mcg)	2.24	1.33	2.24	1.54

TABLE 18

VITAMIN CONTENT PER ML OF CUCUMBERS AND BRINE AT THE
BEGINNING OF A FERMENTATION AND AFTER 20 DAYS

	Crock A ₂		Crock B ₂	
	Beginning	After 20 days	Beginning	After 20 days
Biotin (mmcg)	4.90	5.61	9.10	5.97
Niacin (mcg)	1.50	1.69	1.63	1.51
Pantothenic acid (mcg)	1.24	2.60	1.26	3.14

reduced the biotin level (Figure 5) it appeared that enough biotin remained to assure adequate growth of the Lactobacilli. The other two vitamins did not appear to be critical since their level remained unchanged or was increased (Figures 6 and 7). The action of the yeasts may easily be explained. Since most yeasts require biotin, one would expect a reduction of this vitamin by these organisms. However, the fact that A. cloacae reduced the biotin content of cucumber juice was rather surprising (Figure 5). This finding was studied further and the results are presented in the following section of this study.

Regardless of the microbiological activity, there seemed to be in the brine an abundant quantity of biotin, niacin, and pantothenic acid within 24 hours after brining (Tables 14 and 15). The fact that pantothenic acid was synthesized (Table 18) during a cucumber fermentation was to be expected when one considers that brine yeasts syn-

thesized this vitamin (Figure 7). The fact that biotin was synthesized in one crock, but reduced in another (Table 18) was difficult to explain. It may be that where the biotin content was initially low, one may expect an increase due to some organism of the cucumber fermentation which has not yet been isolated. It is conceivable that there may have been types of microorganisms which appeared in one fermentation and not in another depending on the availability of nutrients in the brine.

PART III

ROLE OF BIOTIN IN CUCUMBER FERMENTATION

INTRODUCTION

The previous sections of this study indicated that biotin could be a critical vitamin during a cucumber fermentation. This vitamin was markedly reduced by most of the organisms occurring in a cucumber fermentation. In one fermentation studied, biotin was largely reduced, whereas the other vitamins remained constant or were synthesized. It was noted that biotin exhibited the largest variation in concentration among the different varieties of cucumbers studied.

This particular study attempted to demonstrate the importance of biotin during a cucumber fermentation.

REVIEW OF THE LITERATURE

History of Biotin. - Eddy (29) has given an account of the recognition of this vitamin. The first indication of the existence of biotin was given by Wildiers in 1901 who stated that bios contained a multiplicity of growth factors for yeast. In 1927, Boas described an injury to rats caused by feeding raw egg white. This injury could be prevented by feeding rats other foods. In 1939, Georgy called the factor capable of preventing raw egg white injury, vitamin H. In 1933, Allison reported a growth factor for Rhizobium which he called coenzyme R. In 1935, the bios I Ib fraction of Wildiers was crystallized from egg yolk by Kogl which he called biotin. Minute quantities

of this substance were stimulatory for yeasts. In 1940, Georgy and co-workers demonstrated that vitamin H, coenzyme R, and Kogl's biotin were identical.

Biotin Requirements for Microorganisms. - The biotin requirements of lactic acid bacteria have been discussed in a previous section of this study. Burkholder (11), Kogl and Tennis (53), and Lockhead and Landerkin (69), have indicated the biotin requirements of yeasts. Burkholder showed that of 38 yeasts tested, 36 required biotin. West and Wilson (107) and Wilson and Wilson (114) have demonstrated that many strains of Rhizobia require biotin. Peterson et al (80), Lampen and Peterson (61), and Snell and Williams (98) have tested several species of Clostridia and found that they required biotin. Du Vigneaud et al (26) have proven biotin to stimulate the diphtheria bacillus. Hottle et al (49) indicated the need of hemolytic streptococci for this vitamin. Bohonos and Subba-Row (8) tested 33 strains of pneumococci and found that they required biotin. Requirements of a variety of other microorganisms are given in a review by Knight (51).

Antibiotins. - The antibiotins include avidin and a list of compounds related in chemical structure to biotin. In 1940, Eakin, Snell, and Williams (28) reported the isolation of avidin from raw egg white. Avidin was described as a protein capable of inactivating biotin. Landy et al (63) demonstrated that avidin was an antibiotin to micro-

organisms which required biotin but would be ineffective in preventing growth of organisms that did not require biotin. An excessive amount of biotin added to the medium could overcome the effect of avidin.

In 1944, Dittmer and du Vigneaud (20) reported that desthiobiotin, biotin sulfone, and other compounds related structurally to biotin possessed antibiotin activity. Lilly and Leonian (68) showed that the antibiotin effects of desthiobiotin could be overcome by additional amounts of biotin. Williams et al (108) have published an up to date list of antibiotins.

Substitutes for Biotin. - Many vitamers of biotin are listed by Williams et al (108). Desthiobiotin, previously discussed as an antibiotin was found to stimulate certain organisms. Melville et al (73) found this compound to be active for certain yeasts and some bacteria. They also noted that this compound was inactivated by avidin. Stokes and Gunness (100) found biotin methyl ester to be active for Lactobacillus casei. These workers in a later publication (101) showed that synthetic biotin gave responses identical to those of natural biotin. They believed that d-biotin was the active form of biotin. Although they found that large amounts of l-biotin had biological activity, they believed that this isomer was contaminated with d-biotin. Many workers have shown oxybiotin (o-heterobiotin) to be active for microorganisms and animals. Pilgrim et al

(82), Duschinsky et al (25), Krueger and Peterson (59), and Winnick et al (115) have shown this compound to be active for various microorganisms. The latter workers have demonstrated that this compound is inhibited by avidin. Hofmann et al (48) proved oxybiotin to have biotin activity for the rat and chick.

Recently, much emphasis has been placed on the substitution of lipids for biotin. Perhaps the first indication that lipids might substitute for biotin was in 1932 when Evans and Lepkovsky (40) noted that rats could utilize certain unsaturated fatty acids in place of vitamin B. In 1940, Cohen and Mueller (18) found that oleic acid could stimulate colony development of Corynebacterium diphtheriae. In 1945, Dubos (21) began a series of experiments in an attempt to obtain a better medium for the growth of tubercle bacilli. He first noted that phospholipids and long chain fatty acid esters of polyhydric alcohols (e.g., Tween 80) permitted submerged and rapid growth of tubercle bacilli. In 1946, Dubos (22) found that fatty acids in themselves were toxic whereas esterified fatty acids were non-toxic. In the same year, Davis and Dubos (19) found Tween 80 to be toxic to small inocula, but they were able to show that this substance contained small amounts of unesterified fatty acids. The first explanation for this activity of Tween 80 was given in 1946 by Dubos and Davis (24). They claimed that the wetting action of the Tweens prevented pellicle formation

and that the nutrients of the medium were in closer contact with the bacterial cell. In 1947, Dubos (23) also suggested that fatty acids, supplied by certain Tweens, functioned in the metabolism of the tubercle bacillus. He also suggested that serum albumin be added to the medium to offset the toxic effect of unesterified fatty acids. Kodicek and Worden (52) have shown that fatty acids inhibited the growth of Lactobacillus helveticus, while the methyl esters of these fatty acids did not show any inhibitory effect. In 1946, Williams and Fieger (111) found that oleic acid would stimulate the growth of Lactobacillus casei in a biotin-free medium. In 1947, Trager (104) discovered a fat-soluble material from plasma having the biological activities of biotin. He showed that this material was not inactivated by avidin. He did not believe this material to be a fatty acid fraction of plasma since this fraction was non-saponifiable and insoluble in acetone. In 1947, Williams and Fieger (112) found that eleates, especially Tween 80, were excellent substitutes for biotin in stimulating growth of L. casei. In 1947, Axelrod et al (6) demonstrated that vaccenic acid had biotin activity for higher animals. In 1947, Williams et al (113) showed that Tween 80 was better than oleic acid as a biotin substitute for several lactic acid bacteria. They suggested that biotin catalyzes the synthesis of oleic acid because certain organisms require this fatty acid even in the presence of biotin.

Functions of Biotin. - It has already been indicated that biotin prevents egg white injury to animals and serves as a growth factor for many microorganisms. It has been postulated by Williams et al (113) that one function of biotin is to catalyze the formation of oleic acid. This was given further support by Gavin and McHenry (45) who demonstrated that excessive biotin fed to rats would cause fatty livers and an increase in body fat to that animal. Petter and Elvehjem (83) believed biotin to have three functions:

1. Aid in synthesis of oleic acid.
2. Aid in synthesis of aspartic acid.
3. Catalyze the beta carboxylation of pyruvic acid to aspartate.

Several other workers have indicated an interrelationship between biotin and aspartic acid. Koser et al (56), in 1942, found that aspartic acid served as a partial substitute for biotin for Torula cremoris. Stokes et al (102), in 1947, showed that several lactic acid bacteria when supplied with biotin would synthesize aspartic acid. There have been indications that biotin may also serve as part of an enzyme system which functions in the carboxylation of pyruvic acid. Shive and Rogers (89) have demonstrated that biotin is involved in the biosynthesis of oxalacetic and alpha-ketoglutaric acid from pyruvic acid.

EXPERIMENTAL

Biotin Utilization by *L. plantarum* in Cucumber Juice

Since it was established that *L. plantarum* required biotin, it was believed that *L. plantarum* could reduce the biotin content of cucumber juice.

The procedure for this experiment was the same as that used for the effect of *Aerobacter* and yeasts on the vitamin content of cucumber juice. Three isolates (Nos. 602, 609, 611) of *L. plantarum* were selected for study. Since it was difficult to visually detect growth of *L. plantarum* in cucumber juice, the number of organisms was determined by plating on V-8 agar. The values for the effect of all three isolates of *L. plantarum* on the biotin content of cucumber juice were very close. An average of those values is given in Figure 8.

A very slight reduction of the biotin content was observed. This was rather surprising in view of the fact that this organism required biotin. It was therefore decided to determine whether *L. plantarum* could diminish the biotin content in a medium which contained pure biotin.

Biotin Utilization by *L. plantarum* in Biotin Assay Medium

This experiment was set up in a similar fashion to the previous experiment except that measured amounts of biotin added to its respective assay medium were used instead of cucumber juice. Each time the experiment was performed,

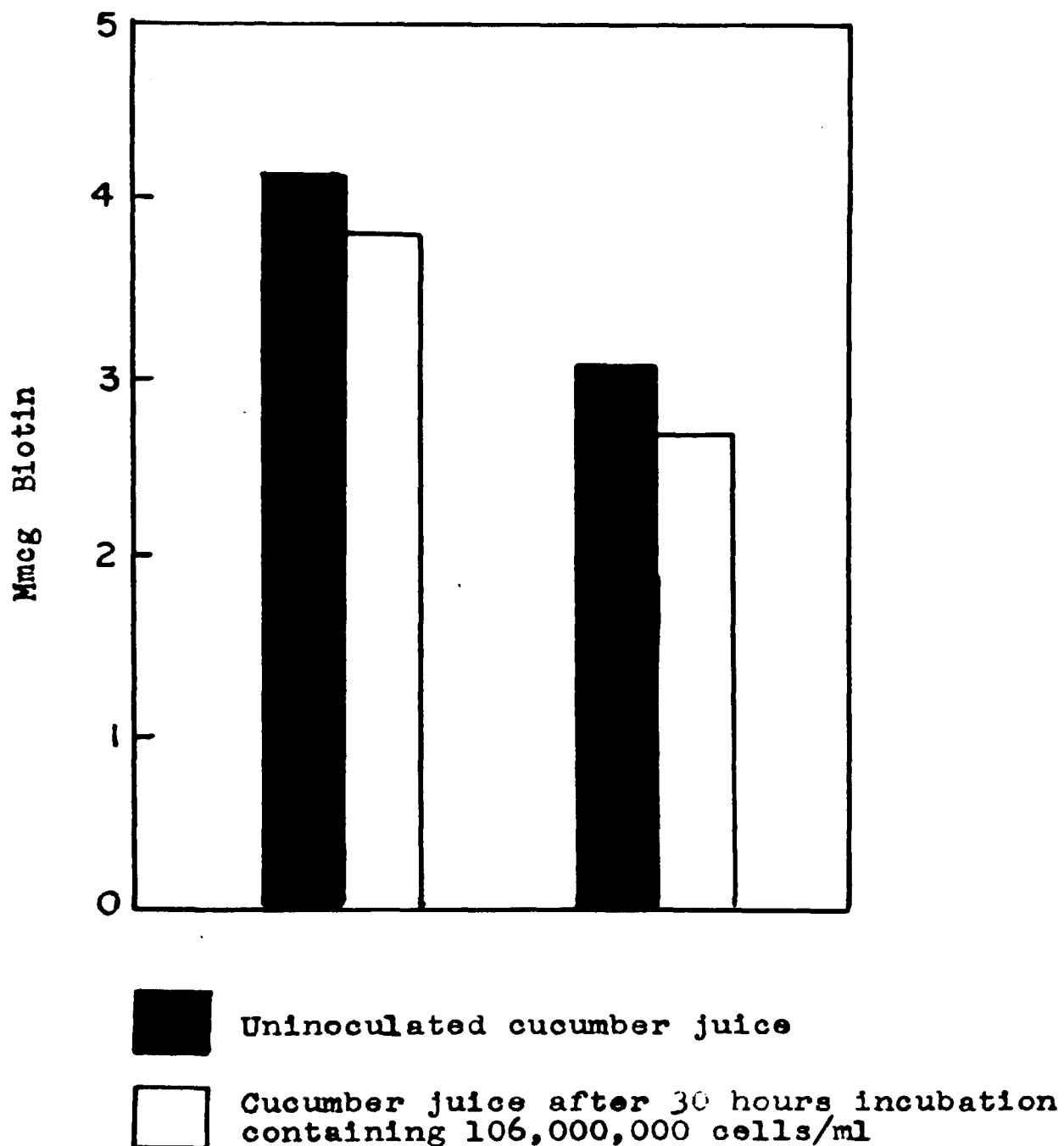


Figure 8. Effect of L. plantarum on the biotin content of cucumber juice

the biotin from the control tube was assayed by the usual procedure. The results are given in Table 19.

TABLE 19
EFFECT OF L. PLANTARUM ON THE BIOTIN CONTENT
IN BIOTIN ASSAY MEDIUM

Biotin in uninoculated assay medium (mmcg/ml)	Biotin in inoculated assay medium (mmcg/ml)
4.42	0.065
3.60	0.070
0.153	0.0070

Unlike cucumber juice, the biotin in the assay medium, which contained only biotin as a "biotin-active" material, was reduced considerably. This led to the opinion that the biotin activity of cucumber juice might be due to a variety of substances.

Tween 80 As a Biotin Active Material for L. plantarum

Tween 80 has been reported to be an excellent biotin active material (112, 113). To prove that this substance could stimulate the growth of L. plantarum varying amounts were added to biotin assay medium. The experiment was first done using one isolate of L. plantarum and L. arabinosus 17-5. The experiment was repeated using three isolates of L. plantarum and L. arabinosus 17-5.

The tubes were incubated at 30° C for 72 hours. Growth

was determined by titrating for the amount of acid produced. The results appear in Table 20 and show clearly that Tween 80 could serve as an excellent biotin substitute for L. plantarum.

TABLE 20
EFFECT OF TWEEN 80 AS A BIOTIN ACTIVE MATERIAL
FOR L. PLANTARUM AND L. ARABINOSUS 17-5

Mg Tween 80/ 10 ml	Ml 0.1 N acid produced per 10 ml medium					
	Trial 1			Trial 2		
	L. arabinosus 17-5 (control)	L. plantarum 611	L. arabinosus 17-5 (control)	<u>L. plantarum</u>		
				602	609	611
0	2.5	1.2	1.6	0.5	0.5	0.3
0.1	4.4	4.5	3.4	3.3	2.3	3.1
0.5	9.6	9.2	8.3	5.7	3.9	5.8
1.0	12.8	10.0	12.0	7.5	5.1	7.7
5.0	15.4	9.7	14.3	8.5	5.9	9.0
10.0	16.7	10.7	16.3	8.1	5.2	9.5

Utilization of the Biotin Active Fraction of
Tween 80 by L. plantarum

An attempt was made to explain the fact that L. plan-
tarum would markedly deplete the biotin in the assay medium,
but would hardly diminish the biotin content in cucumber
juice. It was believed that Tween 80 could support the
growth of L. plantarum without much of its biotin active
substance being utilized.

The procedure for this determination was the same as that used in previous experiments of this study. Tween 80 was added to biotin assay medium in concentrations of 0.5 mg and 0.05 mg per ml. These amounts were subsequently found to be equivalent to 0.303 mmcg and 0.035 mmcg of biotin respectively.

The results are given in Figure 9 and it may be noted that when Tween 80 was used in the biotin assay medium, the medium still possessed considerable biotin activity after 30 hours incubation with L. plantarum.

Effect of L. plantarum on the Biotin Content of
Assay Medium Containing Only Biotin and a Combination
of Biotin and Tween 80

It was still possible that the biotin was not lowered by Lactobacilli in cucumber juice because these organisms did not grow as well in this medium as they did in the biotin assay medium.

It has been shown that Tween 80 "resists" reduction of its biotin active fraction (Figure 9). However, Tween 80 did not appear to support growth as well as biotin as judged by titration values given in Tables 7 and 20. It was, therefore, decided to culture L. plantarum in the assay medium containing biotin and in the assay medium containing a combination of biotin and Tween 80. Growth was determined by measuring the acid produced. The tubes were incubated for 30 hours at 30° C. The results are given in Table 21.

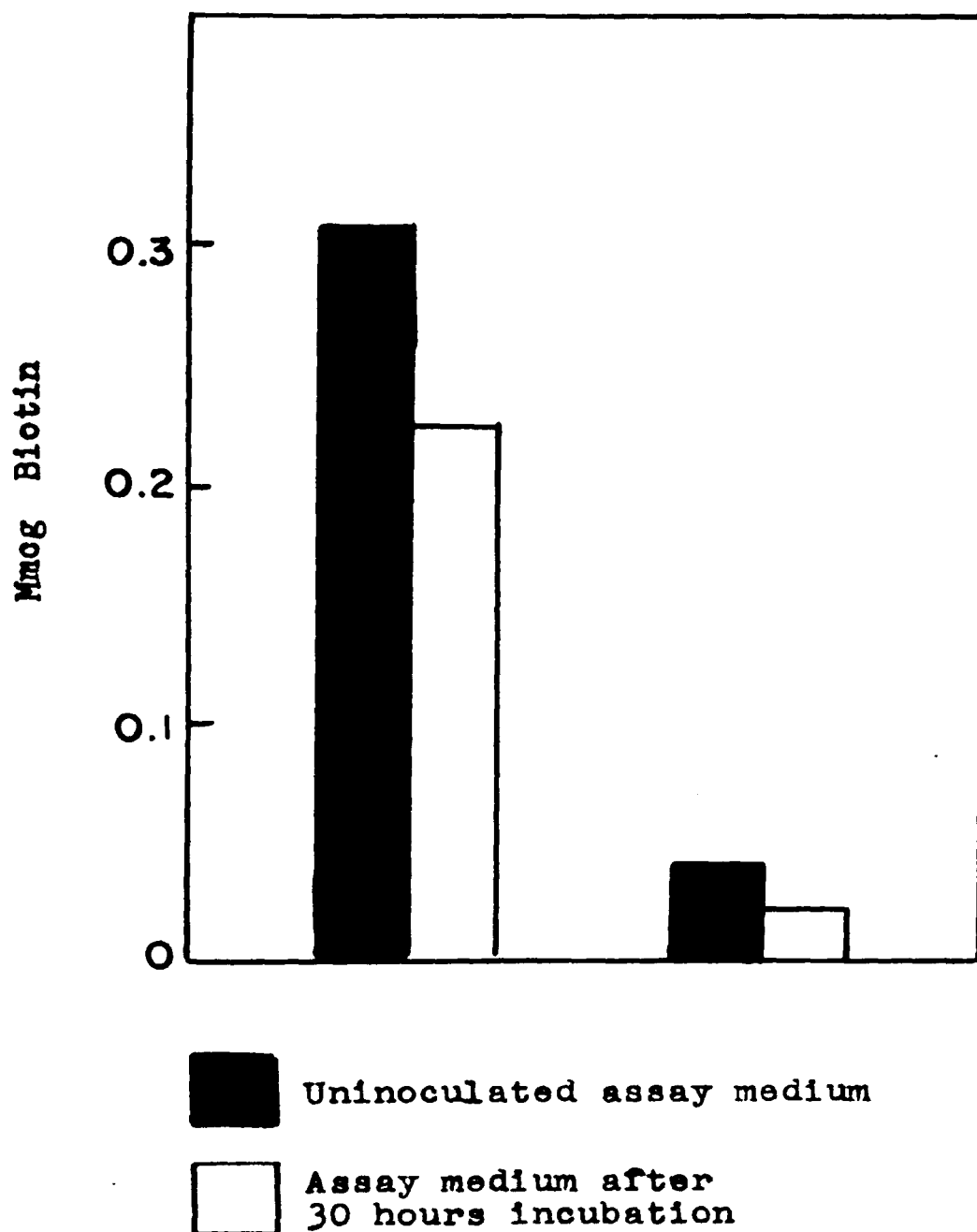


Figure 9. Effect of L. plantarum on the content of the biotin active fraction of Tween 80 in biotin assay medium

TABLE 21

UTILIZATION OF BIOTIN BY L. PLANTARUM FROM ASSAY MEDIUM
CONTAINING ONLY BIOTIN AND A COMBINATION OF
BIOTIN AND TWEEN 80

	Ml acid produced per 10 ml medium	Uninoculated. Biotin (mmcg/ml)	Inoculated. Biotin (mmcg/ml)
Biotin in assay medium	17.9	3.60	0.070
Biotin and Tween 80 (0.1 mg/ml) in assay medium	18.5	2.73	0.80
Biotin and Tween 80 (0.1 mg/ml) in assay medium	18.4	2.45	0.75

Thus, it was indicated that when pure biotin was available for L. plantarum, it was markedly reduced. When Tween 80 was available as the biotin active material for this organism there was not as sharp a reduction in the biotin content. This simulated the results obtained in Figure 8 where it was shown that L. plantarum hardly affected the biotin content of cucumber juice.

A summary of the results presented in this section is given in Table 22.

Biotin Requirements of Aerobacter cloacae

Earlier in this study it was shown that A. cloacae decreased the biotin content of cucumber juice (Figure 5). It was therefore assumed that this organism might require biotin. This was unusual since similar organisms have been

TABLE 22

SUMMARIZED DATA FOR THE UTILIZATION OF BIOTIN ACTIVE COMPONENTS FROM VARIOUS SOURCES BY L. PLANTARUM

Substances containing biotin active materials	Uninoculated. Biotin (mmcg/ml)	Inoculated. Biotin (mmcg/ml)
Cucumber juice	4.10	3.79
Cucumber juice	3.10	2.84
Biotin in assay medium	4.42	0.065
Biotin in assay medium	3.60	0.070
Biotin in assay medium	0.153	0.0070
Tween 80 (0.5 mg/ml) in assay medium	0.303	0.225
Tween 80 (0.05 mg/ml) in assay medium	0.035	0.019
Biotin and Tween 80 (0.1 mg/ml) in assay medium	2.73	0.80
Biotin and Tween 80 (0.1 mg/ml) in assay medium	2.45	0.75

reported to synthesize this vitamin (81, 103). A simple experiment was performed in order to determine if this organism needed biotin. Two isolates (Nos. 449 and 457) were selected for study. The organisms were inoculated into biotin assay medium containing no biotin and varying concentrations of biotin. Growth was observed visually and the results are indicated in Table 23.

Obviously, this organism did not require biotin. On

TABLE 23
BIOTIN REQUIREMENTS OF AEROBACTER CLOACAE

Biotin (mmcg/ml)	Isolate Nos. of <u>Aerobacter cloacae</u>	
	<u>449</u>	<u>457</u>
Ø (uninoculated)	-	-
0	++++	++++
0.10	++++	++++
1.0	++++	++++

- = no growth; ++++ = heavy, turbid, frothy growth

the contrary, it could probably synthesize biotin from this medium.

Effect of Aerobacter cloacae on the Biotin Activity of Biotin Assay Medium Containing Varying Concentrations of Biotin and Tween 80

Although A. cloacae depleted the biotin content of cucumber juice (Figure 5), the preceding experiment indicated that this organism might synthesize this vitamin from the assay medium. The organism was grown in biotin assay medium containing varying concentrations of biotin and Tween 80 and in the assay medium containing no added nutrient. The tubes were incubated for 30 hours at 30° C and assays were made as previously described. The results are given in Table 24.

The results indicated that A. cloacae was capable of synthesizing biotin when no biotin or very small amounts

TABLE 24

UTILIZATION OF BIOTIN ACTIVE SUBSTANCES BY A. CLOACAE
IN BIOTIN ASSAY MEDIUM CONTAINING BIOTIN AND TWEEN 80

	Uninoculated. Biotin (mmcg/ml)	Inoculated. Biotin (mmcg/ml)
Biotin	0	0.169
Biotin	0.064	0.134
Biotin	3.35	1.72
Tween 80 (0.05 mg/ml)	0.035	0.101
Tween 80 (0.5 mg/ml)	0.303	0.382

of this nutrilitate was present. When a large amount of biotin was present, an amount equal to a 1:2 dilution of cucumber juice, a partial reduction was evident.

Effect of A. cloacae on the Biotin Active Substances
in Varying Dilutions of Cucumber Juice

The preceding experiment illustrated that A. cloacae was capable of synthesizing biotin in the assay medium if no biotin or if small amounts of biotin active substances were present. Therefore, it was considered possible that if cucumber juice were diluted so that only a small amount of biotin was present, synthesis of this vitamin might occur. This experiment was performed using 1:2 and 1:20 dilutions of cucumber juice. The procedure was the same as that used in preceding sections. The results are presented in Table 25.

TABLE 25

UTILIZATION OF BIOTIN ACTIVE SUBSTANCES BY
A. CLOACAE IN VARYING DILUTIONS OF CUCUMBER JUICE

Dilution of cucumber juice	Uninoculated. Biotin (mmcg/ml)	Inoculated. Biotin (mmcg/ml)
1:2	3.84	1.27
1:20	0.384	0.070

No synthesis of biotin occurred at either dilution of cucumber juice. At a lower concentration of cucumber juice, a greater percentage of biotin was diminished.

DISCUSSION

It was believed that biotin might be a critical factor for L. plantarum during a cucumber fermentation. However, it was shown that when L. plantarum grew in cucumber juice, the biotin content was barely diminished (Figure 8). When L. plantarum grew in an assay medium containing pure biotin, the content of this vitamin was markedly depleted (Table 19). Cucumber juice must therefore contain compounds capable of substituting for biotin instead of or in addition to this vitamin. The biological activity of this substance or substances was hardly affected by the growth of L. plantarum, an organism requiring biotin. It was found that Tween 80 had biotin activity (Table 20) and when L. plantarum grew in the assay medium containing Tween 80, there was only a moderate

reduction of the biotin activity (Figure 9). This behavior might have been explained that when pure biotin was used, growth would be greater, and consequently more biotin would be utilized. However, from Table 21 it may be noted that acid production was the same in tubes containing biotin, and in tubes containing a combination of biotin and Tween 80. The biotin activity in the tube containing only biotin was reduced about 50 fold. The biotin activity in the tubes containing the combination of biotin and Tween 80 was reduced less than four fold. Thus Tween 80 seemed to not only have the effect of being a biotin substitute, but was also capable of resisting changes to its biotin active fraction when subjected to bacterial growth. An explanation for this behavior may be in the supposition that the constituents of Tween 80 and not biotin are required for the growth of this organism. Tween 80 is a polyoxyethylene derivative of sorbitan mono-oleate. It is suggested that L. plantarum is capable of producing a lipase to split off oleic acid from Tween 80. It is believed that oleic acid is the biotin active fraction of this compound (111, 113) but if not presented to the organism in an esterified form (as in Tween 80) it is somewhat toxic (22, 23, 52).

It has been suggested that biotin serves as a catalyst for the production of essential fatty acids (113). Therefore, it is expected that if biotin were present and not the essential fatty acids, that the biotin might be utilized in the formation of these substances, and thus, the

biotin level would be markedly decreased. On the other hand, if an organism is supplied with its essential nutrients in a different form it is possible that proportionately less of those nutrients are utilized. Hence, when Tween 80 was used as a biotin substitute, the organism might have to produce a lipase as it was multiplying. Growth would be dependent on the amount of Tween 80 available and the capability of the organism to utilize this compound.

It would be of interest to show more conclusively that oleic acid is synthesized when biotin is present. It is suggested that lactic acid organisms be grown in an assay medium containing biotin. The oleic acid content would be determined before and after growth. This could be done roughly at first by determining the iodine number. If the iodine number increased, the experiment would be repeated on a larger scale and oleic acid would be isolated, identified and its concentration determined.

The role of Aerobacter cloacae and its effect on biotin levels in cucumber juice and in the assay medium proved to be of interest. It was observed that A. cloacae reduced the biotin level of cucumber juice (Figure 5). However, it was shown that this organism did not require biotin (Table 23). In fact, this organism was capable of synthesizing this vitamin from an assay medium containing little or no biotin (Table 24). If biotin was added in an amount equal to that in a 1:2 dilution of cucumber juice,

the biotin content was lowered. The reason for this decrease may be due to the production of antibiotics which this organism may produce from biotin. Many substances similar in structure to biotin exert an antibiotic effect (20, 108).

In dilute cucumber juice, A. cloacae did not synthesize biotin. On the contrary, a greater percentage of this vitamin was reduced more in 1:20 cucumber juice than in 1:2 cucumber juice. The synthesis of this vitamin did not occur in dilute cucumber juice probably because the available materials from which this synthesis might occur was also diluted. The fact that a greater utilization of this vitamin occurred in the more dilute cucumber juice indicated that materials having biotin activity may have served as a source of energy for this organism.

GENERAL DISCUSSION

The curing of salt stock cucumbers is a complicated process. Cucumbers are placed in a vat, covered with brine, and in a few weeks many biological and chemical changes have taken place. Lactic acid and other products have been formed as the result of microbial activity in the brine. Besides lactic acid bacteria, Aerobacter and yeasts have left their influence on the fermentation. In addition to these groups of organisms, many other types of biological activity may have taken place. The most complicated group of microorganisms studied so far appear to be the yeasts. Many genera of these organisms appeared during the fermentation. The Aerobacter, common soil-type bacteria, appeared only at the beginning of a 30° salometer fermentation. Although these organisms may have an important bearing on the lactic acid bacteria, they probably do not have any effect on the curing process.

It is extremely interesting that L. plantarum was able to assume a dominant role in the fermentation since this organism was present in small numbers at the beginning of the fermentation. Many factors probably contributed to the growth of this organism. The high salt concentration, the low pH and the moderate temperature are probably optimum for the lactic acid bacteria when in competition with other

microorganisms. It was demonstrated that this organism required biotin, niacin, and pantothenic acid. Cucumbers were shown to contain a large quantity of these vitamins. These vitamins were available quickly due to a withdrawal of the water soluble nutrients of the cucumbers when the brine was added. The vitamin content was high enough within 24 hours to support excellent growth. The content remained high for at least 20 days. At this point the important lactic fermentation was in its last stages. Therefore, it appeared as if cucumbers were able to furnish some of the essential nutrients required by L. plantarum.

An important consideration may be drawn at this point. In this study, three common growth factors (biotin, niacin, and pantothenic acid) were selected for study. Many other growth factors are probably required by L. plantarum which undoubtedly are contained in cucumber juice. Many accessory growth factors have been isolated from foods. Since L. plantarum probably requires many vitamins it is suggested that cucumbers may contain vitamins which to date have not been characterized.

Further evidence of the probability that cucumber juice contains a variety of growth factors was indicated by the behavior of L. plantarum in cucumber juice. It was found that this organism hardly depleted the biotin content of cucumber juice although it required this vitamin. This suggested that cucumber juice contained other factors which could substitute for biotin.

SUMMARY

1. A study was made of the kinds and numbers of microorganisms present during the cucumber fermentation when salted at 30° salometer. All of the acid-producing bacteria studied were identified as Lactobacillus plantarum. These organisms were found in great numbers; the peak of their activity was between three and eight days after the fermentation began. Aerobacter were isolated and many were identified as A. cloacae. Their activity lasted for a brief period, and the peak of activity preceded that of the lactic fermentation. Varieties of yeasts were found and their numbers increased slowly and steadily and subsequently declined gradually.

2. A vitamin study of the cucumber fermentation was made. It was found that L. plantarum required biotin, niacin, and pantothenic acid. Cucumber juice of various varieties of cucumbers was analyzed for these vitamins and found to contain the following amounts:

Biotin	5.20 to 33.0 mmcg/ml
Niacin	1.83 to 5.05 mcg/ml
Pantathenic acid	1.05 to 2.42 mcg/ml

The effect of non-acid-producing organisms isolated from cucumber fermentations on the vitamin content of cucumber juice was studied. It was found that the niacin content remained unchanged, the biotin content was decreased, and

the pantothenic acid content was increased. The vitamin content of a cucumber fermentation was followed. It was demonstrated that an abundant supply of biotin, niacin, and pantothenic acid was available within 24 hours after brining. After fermenting 20 days, the vitamin content of cucumbers was decreased. However, the vitamin content of the pickles and brine remained unchanged for niacin, increased for pantothenic acid, and results were variable for the change in biotin content.

3. The role of biotin in the nutrition of L. plantarum and A. cloacae was further studied. It was found that when L. plantarum grew in cucumber juice, the biotin content was reduced slightly. When L. plantarum grew in an assay medium containing biotin, the content of this vitamin was markedly depleted. Tween 80 was found to serve as a biotin substitute and appeared to "resist" reduction of biotin when subjected to bacterial activity.

It was demonstrated that A. cloacae did not require biotin. This organism was shown to synthesize this vitamin if grown in an assay medium containing small amounts of biotin or no biotin. This organism lowered the biotin level when this vitamin was present in high concentrations. When grown in cucumber juice, A. cloacae reduced the biotin content.

CONCLUSIONS

1. The lactic, hydrogen, and carbon dioxide fermentations produced by L. plantarum, Aerobacter and yeasts respectively were of importance in a 30° salometer cucumber fermentation under laboratory conditions.

2. Biotin, niacin, and pantothenic acid, shown to be required by L. plantarum, were not considered critical vitamins in a cucumber fermentation because cucumbers were found to contain an adequate amount of these vitamins, which were made available within 24 hours after brining.

3. Studies on the biotin nutrition of L. plantarum and A. cloacae indicated that cucumber juice probably contains one or more compounds capable of substituting for biotin which may be lipoidal in nature.

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