COMPARISON OF A V-8 MEDIUM WITH OTHER MEDIA FOR THE DETERMINATION OF ACID-PRODUCING BACTERIA

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COMPARISON OF A V-8 MEDIUM WITH OTHER MEDIA FOR THE DETERMINATION OF ACID-PRODUCING BACTERIA

By

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INTRODUCTION

The lactic acid bacteria, a ubiquitous group of organisms, have been found in the alimentary tract, in soil, and in foods. They are responsible for the fermentation in many kinds of food such as cheese and other fermented milk products, cabbage and cucumbers. They also may cause spoilage in canned foods, in wines and in breweries.

Since these organisms are important, it is necessary to isolate and study them if we are to understand their activity. In order to do this, it is desirable to have a medium capable of detecting and differentiating them from other organisms.

Many media have been suggested for the isolation and enumeration of lactic acid bacteria. It has been felt that the media now in use have not been specific enough for the detection of these organisms. Many allow other microorganisms to grow abundantly, completely overgrowing the lactic acid bacteria in a short time. Some media will support growth of the lactic acid bacteria, but the colonies are often too small to count.

In view of these difficulties, an attempt was made to develop a better medium, to isolate and enumerate lactic acid organisms. This medium consisted of a V-8 vegetable juice as a base plus tryptose, lactose, beef

extract and 0.1 gram per liter of brom cresol green indicator. This medium was used at a pH of 5.7.

The purpose of this study was to compare the above mentioned V-8 medium with other media that have been suggested for the isolation and enumeration of lactic acid bacteria.

REVIEW OF THE LITERATURE

Because of the importance of studying the lactic acid bacteria, many investigators have attempted the preparation of nutrient media for cultivating these organisms.

In 1920, Ayers and Mudge (1) prepared a milk-powder medium for the determination of bacteria in milk. This medium gave the total count and also enumerated the strong and weak acid-producers, the alkali-forming, the inert and the peptonizing bacteria.

Fabian, Bryan and Etchells (9) found the milk-powder agar of Ayers and Mudge (1) to be an excellent medium for growing the bacteria found in fermenting pickle brine and also that it differentiated the bacteria into four main groups as mentioned above.

Rettger and Kulp (20) tried to find a substitute for whey broth and whey agar, because these required exacting technique to obtain a clear medium. They tried galactose agar as a substitute. The galactose agar contained 0.5 to 1 percent of the carbohydrate and although colonies of lactic acid organisms were smaller and did not grow as rapidly as in the whey agar, they were more readily identifiable by their "wooly or hairy appearance". The same authors in a later paper found that casein and Klim digest media were superior for cultivating Lactobacillus acidophilus

and Lactobacillus bulgaricus and that galactose appeared to be the most favorable carbohydrate (15). Kulp (14) found that a tomato juice medium containing peptone, agar, distilled water and adjusted to pH 7.0 gave comparative results with whey-galactose agar and a digest galactose medium and was more easily prepared.

In an attempt to isolate lactobacilli from soil and grain, Hunt and Rettger (12) had difficulty in using an acid enrichment broth containing a fermentable carbohydrate since fungi readily overgrew the lactobacilli. To obtain satisfactory results, they used a medium containing corn flour, peptone, and water with the pH adjusted to 3.5 to 4.0. They inoculated broth tubes with the test material and then put a layer of sterile mineral oil 10 to 15 mm thick over the mash. Plates poured from these tubes seldom contained molds and often contained only lactobacilli in the higher dilutions. In maintaining stock cultures they used a yeast-water broth and agar containing glucose and peptone. They also used the tomato juice broth devised by Kulp (14) and found it to be almost as good as yeast-water for some strains and better for others.

Weiss and Rettger (23) used a tomato broth medium for isolating Lactobacillus bifidus. This was prepared by taking the contents of a large can of tomatoes and filtering the material through coarse filter paper. The filtrate was then diluted with distilled water. To this they added peptonized milk (Difco), peptone (Difco), yeast

extract (Difco), and two-percent granular agar. They found this medium to be particularly good for isolating intestinal lactobacilli, since there was a large amount of carbohydrate present. Thus, enough acid could be produced to lower the pH to 3.6 to 4.0 which could suppress other intestinal organisms.

Barber and Frazier (2), in a study of the dissociants of lactobacilli, used a modification of the carrot liver agar described by Garey, Foster and Frazier (10). They used United States flake agar in place of washed agar and also an infusion of ground beef liver instead of the liver extract.

McLaughin (17) reported a medium which gave much better results than the commercial whey and tomato juice agars. This medium was called "trypticase sugar agar" and contained pancreatic digest of casein, lactose, glucose, sucrose, gelatin and agar. The pH was adjusted to 6.0. He found more and larger colonies on this medium and was also able to count them after 48 hours.

Wade, Smiley, and Smith (22) suggested a method of obtaining the ratio of acid-forming to non-acid-forming bacteria. This medium, a modification of the one suggested by Garey, Rittscheff, Stone, and Boruff (11), consisted of filtered tomato juice, yeast extract, glucose, and a buffer. To this was added $CaCO_3$ and an alcoholic solution of brom cresol purple. Strong acid-producing colonies changed the indicator from purple to yellow and also dissolved the

$CaCO_3$, resulting in a clear zone around the colonies. Weak acid-producers changed the indicator but did not dissolve the $CaCO_3$. Neutral colonies caused no change while the alkali-forming colonies imparted a deeper purple color to the agar.

A medium which reduced the number of common contaminants found when isolating lactobacilli from oral, vaginal and fecal samples has been devised by Rogosa, Mitchell and Wiseman (21). This medium consisted of trypticase, yeast extract, KH_2PO_4, ammonium citrate, a salt solution, glucose, sorbitan mono-oleate, sodium acetate hydrate, acetic acid, agar and distilled water. The final pH was 5.4.

Emard and Vaughn (6) found that sorbic acid was effective in selectively favoring the growth of the catalase negative lactic acid bacteria. Twelve-hundredths of one percent of this acid in liver broth permitted the growth of lactic acid bacteria and clostridia but inhibited the catalase positive actinomycetes, bacteria, molds, and yeasts if the initial pH range of the media was 5.0 to 5.5.

In a study of the chemical and bacteriological changes in dill-pickle brines during fermentation, Jones, Veldhuis and Veerhoff (13) used nutritive caseinate agar (Difco) for the bacterial analysis. The acid-formers showed definite zones of precipitated casein about the colony. They added eight cc of 0.4 percent brom cresol purple indicator as a further aid in identifying the acid-forming bacteria.

McCleskey, Faville, and Barnett (16) in studying characteristics of Leuconostoc mesenteroides isolated from cane juice employed a medium using raw sugar, tryptone, yeast extract, agar, water and adjusted to pH 6.7.

The Committee on the Microbiological Examination of Foods (4) suggested a tryptone-glucose-yeast extract agar for the examination of sauerkraut. This medium had yeast extract substituted for beef extract, and is otherwise the same as tryptone glucose extract agar (Difco).

Murdock, Folinazzo and Troy (18) in evaluating plating media for citrus concentrates employed a number of different media to determine maximum counts of several leuconostoc, lactobacilli, and yeasts. They found that the orange serum agar at pH 5.4 was a suitable differential medium for the leuconostoc, lactobacilli, and yeasts. The orange serum agar consisted of tryptone, yeast extract, dextrose, K_2HPO_4, agar, orange serum and distilled water.

EXPERIMENTAL

The experimental work consisted of a comparison of V-8 medium and different media for plating lactic acid organisms, non-lactic acid bacteria and yeasts. The pour plate technique was used and total counts and colony size were taken. The latter part of the experimental work consisted of plating samples from cucumber and kraut fermentations in six different media.

The following organisms were used in this experiment:

Lactic acid organisms.-- Lactobacillus plantarum (ATCC 8014), Lactobacillus plantarum (NRRL B-227), Lactobacillus fermenti (NRRL B-585), Lactobacillus delbrueckii (NRRL B-445), Lactobacillus casei (NRRL B-442), Lactobacillus casei (NRRL B-441), Lactobacillus casei (ATCC 7469), Streptobacterium dextranicum (NRRL B-1254), Betabacterium vermiforme (NRRL B-1127), Leuconostoc mesenteroides (ATCC 8042), and Streptococcus faecalis.

Yeasts.-- Hansenula subpelliculosa (Etchell's No. RY-135), Torulopsis caroliniana (Etchell's No. RY-147), Torulopsis holmii (Etchell's No. FFL-Y-307), Zygosaccharomyces Sp. A. (Etchell's No. YS-590), Torulaspora rosei (Etchell's No. RX-8).

Non-lactic acid bacteria.-- Sarcina lutea, Micrococcus pyogenes var. aureus, Serratia marcescens, Escherichia coli, Aerobacter cloacae, Bacillus subtilis.

As soon as these cultures were obtained, transfers to be used as stock cultures were made as follows: lactic acid organisms - stab culture in micro assay culture agar (Difco) with the exception of Streptococcus faecalis which was treated like the non-lactic acid bacteria; yeasts - dextrose broth (Difco) plus 0.2 percent yeast extract; non-lactic acid bacteria - nutrient agar slants (Difco).

When a test was to be made, the organisms were transferred from the stock cultures to the following media: lactic acid organisms - micro inoculum broth (Difco); yeasts - dextrose broth plus 0.2 percent yeast extract; non-lactic acid bacteria - nutrient broth.

The organisms were incubated at the following times and temperatures:

Lactic acid organisms.-- All organisms except Leuconostoc mesenteroides were incubated at 30° C for 48 hours. Leuconostoc mesenteroides was incubated at room temperature for 48 hours.

Yeasts.-- Hansenula subpelliculosa, Torulopsis candidiana and Torulaspora rosei were incubated for 24 hours at 30° C. Torulopsis holmii was incubated for 3 days at 30° C. Brettanomyces versatilis and Zygosaccharomyces Sp. A were incubated for 6 days at 30° C.

Non-lactic acid bacteria.-- These organisms were incubated at 30° C for 24 hours.

Appropriate dilutions of the actively growing cultures were made and plated by the pour plate method. Duplicate plates were poured for all the lactic acid organisms.

All of the organisms were plated in the media listed in Table 1.

An additional medium was used in the yeast study. This was potato dextrose agar acidified with one ml of a 10 percent solution of tartaric acid per 100 ml of medium. This treatment lowered the pH to 3.5 ± 0.1. This was assigned code no. 7A.

The lactic acid organisms after plating were incubated at temperatures described as optimum in Bergey's Manual of Determinative Bacteriology (3). The yeasts and the non-lactic organisms were incubated at 30° C. Colonies were counted on plates showing between 30 and 300 colonies after two, three, and five days. The size of the colony was measured in mm.

In the sauerkraut fermentation, samples were taken according to a method described by Fulde (8). In this manner, samples could be obtained under a nitrogen atmosphere. It was also possible to obtain representative samples from the center of the fermentation.

In the cucumber fermentation, daily samples of the brine were collected from a commercial fermentation of salt-stock pickles by the method of Etchells et al (7).

TABLE 1

LIST OF MEDIA STUDIED

Code no.	Medium	pH of medium	Reference
1	V-8	5.7	8
2	Orange serum agar	5.4	18
3	Lemon serum agar	5.2	18
4	Tryptone glucose extract*	7.0	Difco (5)
5	Nutritive caseinate*	6.6	Difco (5)
6	McCleskey's agar	6.5	16
7	Potato dextrose agar	5.6	Difco (5)
8	Tryptone glucose yeast extract*	6.8	19
9	Tomato juice agar	6.1	Difco (5)
10	Sabouraud's dextrose agar	5.6	Difco (5)
11	Thermoacidurans agar	5.0	Difco (5)

*Media contained 0.04 g brom cresol purple indicator per liter of medium.

Platings of samples from the sauerkraut and cucumbers were made on the following media: V-8, orange serum agar, tryptone glucose extract, nutritive caseinate agar, tomato juice agar, and thermoacidurans agar. The plates were incubated at room temperature for two or three days.

RESULTS AND DISCUSSION

The results are indicated in Tables 2 to 4 and Figures 1 to 5. Table 2 indicates the total count of different organisms as grown by the various media. Table 3 gives the diameter of subsurface colonies.

In consideration of the media studied, it appeared that the V-8 medium was the best. In most cases, after three days incubation, colonies on the V-8 medium were large and easy to count. A colony was produced having the following characteristics:

Size: 0.5 - 1.0 mm in diameter

Color: dark green to black, having a slight fuzzy appearance around the edges of the colony

Halo: characteristic distinct yellow halos appeared around the colonies in most cases. The halo was two to three times the size of the colony

Colonies on the nutritive caseinate, tryptone glucose extract, and tryptone glucose yeast extract agar did not produce a distinct halo in many cases. It appeared as if the acid produced by the organisms could diffuse throughout the medium. This would make it difficult to differentiate acid-producing organisms from non-acid-producing organisms. The tryptone glucose extract and the tryptone glucose yeast extract produced smaller colonies than appeared

on the V-8 medium.

Orange and lemon serum agars as well as thermoacidurans agar could support growth of the test organisms after two days of incubation. However, this might not have been the case if these media contained an indicator. Although some of the test organisms did not grow out as rapidly on V-8 agar (probably due to the inhibitory effect of the brom cresol green indicator), the colony count at the end of three and five days was very high.

It is desirable to have a medium that is inhibitory to organisms that are usually found as active or contaminating organisms in the early part of lactic fermentations. If non-lactic organisms grow out rapidly in a medium, they would be difficult to differentiate from lactic acid-producing organisms. In Table 4 it is shown that the V-8 medium inhibited Sarcina lutea, M. pyogenes var. aureus, and Bacillus subtilis. None of the other media showed this degree of inhibition.

Table 6 shows a comparison of various yeasts which were isolated from cucumber fermentations. No medium proved to be outstanding. Torulopsis caroliniana did not grow on nutritive caseinate agar and Zygosaccharomyces Sp. A did not grow on tryptone glucose extract agar, nutritive caseinate agar, McCleskey's agar, and tryptone glucose yeast extract agar.

The effect of pH of the various media must also be considered. Although no special study of this effect was

made, it was observed that there was a correlation between the pH of the medium and the growth of non-lactic acid bacteria. In general, the lower the pH, the more inhibitory the medium to these organisms.

The medium did not show any special differentiation for the yeasts and lactic acid bacteria as far as pH was concerned. These organisms grew in media possessing both high and low pH values. There is probably an optimum pH for each medium for any one particular organism.

V-8 medium, orange serum agar, tryptone glucose extract agar, nutritive caseinate agar, tomato juice agar and thermoacidurans agar were employed to enumerate organisms of the sauerkraut and cucumber fermentations in order to show how the media would function under practical conditions. The V-8 medium, tryptone glucose extract agar, and nutritive caseinate agar were chosen because they contained an indicator capable of detecting acid-producing organisms. Orange serum, thermoacidurans and tomato juice agars were chosen because they gave relatively high counts of acid-producing organisms in pure culture (See Table 2). Tryptone glucose yeast extract and lemon serum agars were not used because of their similarity to tryptone glucose extract and orange serum agars respectively.

Figure 1 shows a comparison of how these media enumerated total counts in the sauerkraut fermentation. The media showed very little difference in evaluating the total count. Figure 2 indicates the numbers of acid-producing organisms.

acid-producing bacteria. Acid-producing colonies on the V-8 medium were characteristic as in the sauerkraut fermentation. The dark green color and yellow halo greatly facilitated differentiation from other organisms.

TABLE 2

NUMBER OF LACTIC ACID BACTERIA PER ML x 10^7
WHICH DEVELOPED ON THE VARIOUS MEDIA

Media no.	L. plantarum (A)			L. plantarum (N)			L. fermenti		
	Days at 30° C			Days at 30° C			Days at 40° C		
	2	3	5	2	3	5	2	3	5
1	42.0	42.0	42.0	*	*	120.0	18.4	19.2	19.2
2	43.0	43.0	43.0	121.0	126.0	126.0	19.1	20.0	20.0
3	40.0	42.0	42.0	119.0	133.0	133.0	22.0	22.0	22.0
4	*	16.9	32.0	98.0	131.0	134.0	+	+	3.6
5	43.0	43.0	43.0	105.0	129.0	129.0	18.2	19.0	19.0
6	33.0	33.0	33.0	89.0	107.0	120.0	15.3	16.1	16.1
7	39.0	39.0	40.0	+	+	*	9.0	12.2	12.2
8	*	29.8	32.0	*	62.0	116.0	13.4	16.1	16.1
9	39.0	39.0	39.0	*	94.0	102.0	17.0	18.4	20.0
10	37.0	39.0	39.0	*	15.9	35.0	15.0	15.2	15.2
11	44.0	46.0	46.0	85.0	112.0	124.0	23.2	23.2	23.2

* Colonies too small to count
+ No growth in dilutions used
A L. plantarum (ATCC 8014)
N L. plantarum (NRRL B-227)

Media no.	L. delbrueckii			L. casei (N_1)			L. casei (N_2)		
	Days at 40° C			Days at 30° C			Days at 30° C		
	2	3	5	2	3	5	2	3	5
1	*	139	140	*	*	90.0	*	50.0	66.0
2	130	130	130	94.0	99.0	99.0	70.0	72.0	72.0
3	140	140	140	70.0	85.0	92.0	61.0	63.0	63.0
4	145	145	145	75.0	97.0	101.0	30.0	38.0	44.0
5	128	130	131	32.0	40.0	72.0	55.0	62.0	62.0
6	129	138	138	50.0	78.0	86.0	41.0	57.0	62.0
7	*	105	116	*	*	86.0	*	*	30.0
8	125	125	125	35.0	50.0	78.0	50.0	52.0	64.0
9	131	131	131	30.0	54.0	70.0	54.0	55.0	58.0
10	126	126	126	49.0	81.0	86.0	53.0	58.0	58.0
11	132	132	132	79.0	96.0	102.0	58.0	61.0	63.0

N_1 L. casei (NRRL B-442)
N_2 L. casei (NRRL B-441)

TABLE 2 CONT.

Media no.	L. casei (A_1)			S. dextranicum			B. vermiforme		
	Days at 30° C			Days at 30° C			Days at 30° C		
	2	3	5	2	3	5	2	3	5
1	*	94.0	110.0	*	7.60	9.30	-	3.80	6.20
2	104.0	109.0	109.0	9.20	10.40	10.40	-	5.40	7.00
3	94.0	96.0	96.0	8.40	9.20	9.20	-	5.90	10.00
4	85.0	107.0	107.0	*	6.60	12.00	-	*	7.40
5	108.0	110.0	110.0	0.59	1.00	1.30	-	3.30	6.00
6	107.0	110.0	110.0	9.90	**	**	-	3.80	6.10
7	+	*	52.0	*	10.20	11.30	-	4.20	7.00
8	60.0	99.0	106.0	*	9.70	13.0	-	*	7.20
9	*	97.0	106.0	9.60	10.60	11.20	-	4.80	6.30
10	59.0	97.0	97.0	*	7.50	8.30	-	*	1.02
11	53.0	88.0	88.0	6.70	10.10	11.30	-	8.70	11.30

** Coalescing of colonies
* Colonies too small to count
- No count made
+ No growth in dilutions used
A_1 L. casei (ATCC 7469)

Media no.	L. mesenteroides			Streptococcus faecalis		
	Days at room temp.			Days at 30° C		
	2	3	5	2	3	5
1	22.4	24.0	24.0	6.3	6.8	7.0
2	23.2	24.0	24.0	6.1	6.5	6.9
3	23.3	23.3	23.3	6.3	6.7	6.8
4	16.4	18.2	19.3	8.3	9.0	9.4
5	18.4	19.0	19.0	8.0	8.3	8.4
6	15.2	16.2	16.2	13.0	14.5	15.0
7	*	19.0	21.1	10.5	12.0	12.0
8	*	15.4	18.2	18.0	19.2	20.0
9	20.1	20.1	20.1	6.1	7.5	8.4
10	11.3	14.3	14.3	12.1	13.6	14.2
11	16.6	18.2	18.2	13.2	14.0	15.1

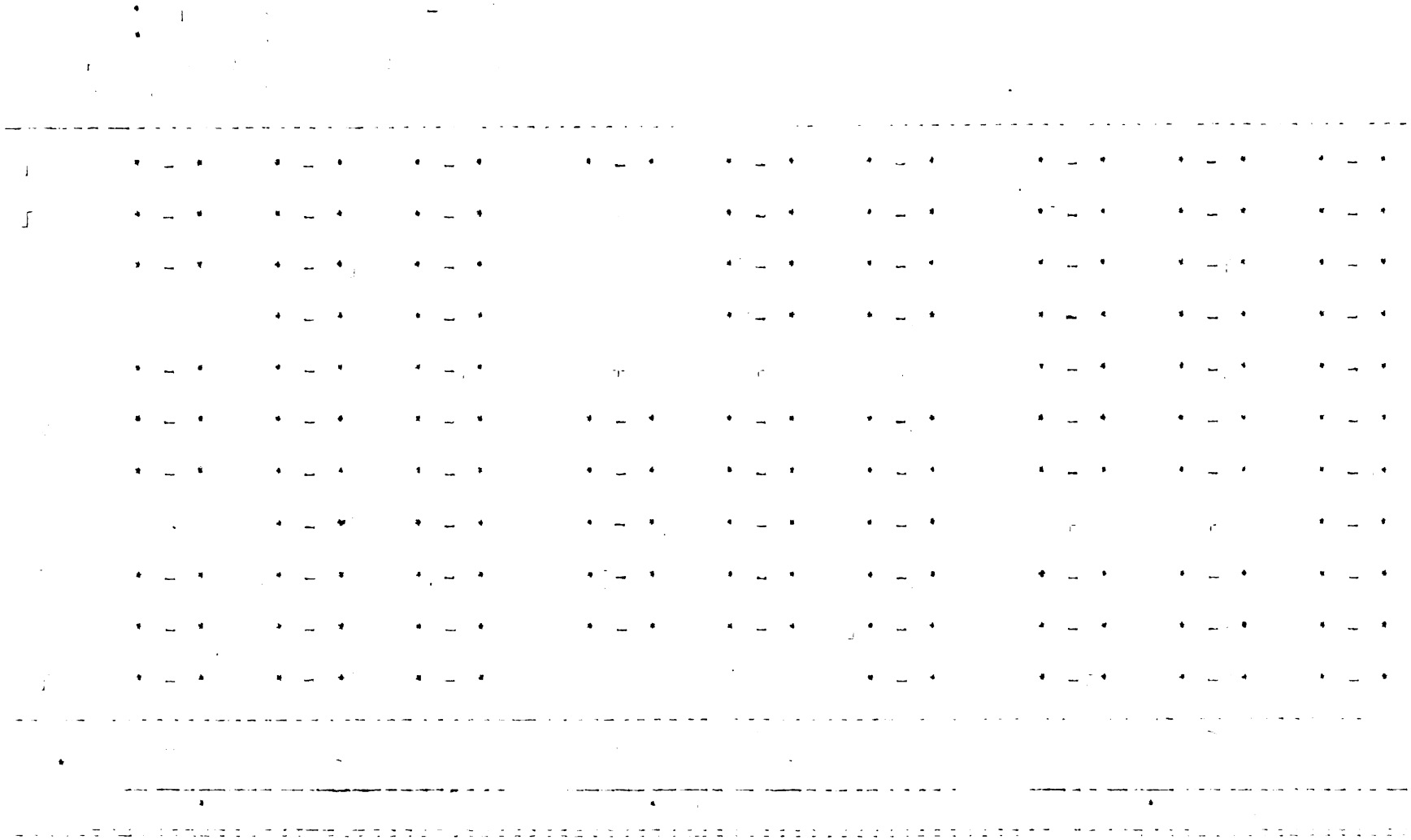


TABLE 3

DIAMETER OF COLONY IN MM OF LACTIC ACID BACTERIA IN VARIOUS MEDIA

Media no.	L. plantarum (A)			L. plantarum (N)			L. fermenti		
	Days at 30° C			Days at 30° C			Days at 40° C		
	2	3	5	2	3	5	2	3	5
1	0.8-1.0	1.0-1.3	1.2-2.0	*	*	0.5-1.1	0.7-1.2	0.8-1.2	0.8-1.2
2	0.8-1.0	1.0-1.2	1.2-1.8	0.2-0.6	0.6-1.2	1.0-1.5	0.5-1.2	0.8-1.5	0.8-1.5
3	0.8-1.0	1.0-1.3	1.5-2.0	0.3-0.5	0.6-1.0	1.0-1.8	0.5-1.0	0.6-1.0	0.6-1.2
4	*	0.1-0.3	0.2-0.5	0.1-0.3	0.3-0.6	0.5-1.0	+	+	0.2-0.5
5	0.6-0.8	0.7-1.0	1.0-1.5	0.2-0.5	0.3-0.6	0.7-1.1	0.3-0.7	0.3-0.7	0.5-0.8
6	0.5-0.7	0.9-1.2	1.2-1.5	0.1-0.3	0.3-0.6	0.8-1.2	0.5-1.0	0.5-1.0	0.6-1.1
7	0.5-0.6	0.7-1.0	0.9-1.2	+	+	*	0.2-0.5	0.2-0.7	0.3-1.0
8	*	0.1-0.3	0.3-0.6	*	0.1-0.2	0.3-0.6	0.2-0.8	0.3-0.8	0.5-1.0
9	0.7-0.8	0.9-1.1	1.0-1.5	*	0.3-0.8	0.8-1.2	0.5-1.0	0.5-1.0	0.6-1.0
10	0.2-0.5	0.4-0.8	0.8-1.3	*	0.1-0.3	0.2-0.5	0.3-0.8	0.4-0.9	0.5-1.0
11	0.5-0.7	0.8-1.0	1.0-1.3	0.2-0.5	0.3-0.8	0.6-1.0	0.5-1.0	0.6-1.0	0.6-1.0

* Colonies too small to measure
\+ No growth in dilutions used
A L. plantarum (ATCC 8014)
N L. plantarum (NRRL B-227)

Media no.	L. delbrueckii			L. casei (N_1)			L. casei (N_2)		
	Days at 40° C			Days at 30° C			Days at 30° C		
	2	3	5	2	3	5	2	3	5
1	*	0.5-0.8	0.6-1.0	+	*	0.8-1.0	*	0.2-0.5	0.3-0.8
2	0.7-1.3	0.8-1.5	1.0-2.0	0.3-0.6	1.0-1.3	1.5-2.0	0.5-0.8	0.8-1.0	1.0-1.5
3	0.8-1.2	0.8-1.5	1.0-1.8	0.2-0.5	0.8-1.1	1.0-2.0	0.6-1.0	0.8-1.2	1.0-1.8
4	0.5-1.0	0.8-1.2	0.9-1.2	0.1-0.4	0.3-0.7	0.4-1.0	0.2-0.4	0.2-0.5	0.3-0.8
5	0.3-0.8	0.8-1.3	1.0-1.5	0.2-0.5	0.5-1.0	0.5-1.0	0.3-0.8	0.6-1.0	1.0-1.5
6	0.5-1.0	0.8-1.2	0.9-1.5	0.2-0.5	0.5-0.8	0.5-1.2	0.2-0.4	0.2-0.6	0.5-1.0
7	*	0.2-0.5	0.3-0.6	*	*	0.1-0.3	+	*	0.1-0.3
8	0.3-0.8	0.5-1.0	0.8-1.3	0.1-0.2	0.2-0.4	0.4-0.8	0.1-0.3	0.2-0.5	0.5-0.8
9	1.0-1.3	1.0-1.5	1.0-1.8	0.1-0.3	0.3-0.8	0.8-1.2	0.3-0.6	0.3-1.0	0.5-1.2
10	0.5-0.8	0.5-1.0	0.8-1.2	0.1-0.3	0.3-0.8	0.6-1.2	0.1-0.3	0.3-0.5	0.3-0.6
11	0.2-0.6	0.6-1.0	0.8-1.5	0.1-0.3	0.4-0.9	0.6-1.2	0.3-0.5	0.4-0.8	0.5-0.8

N_1 L. casei (NRRL B-442)
N_2 L. casei (NRRL B-441)

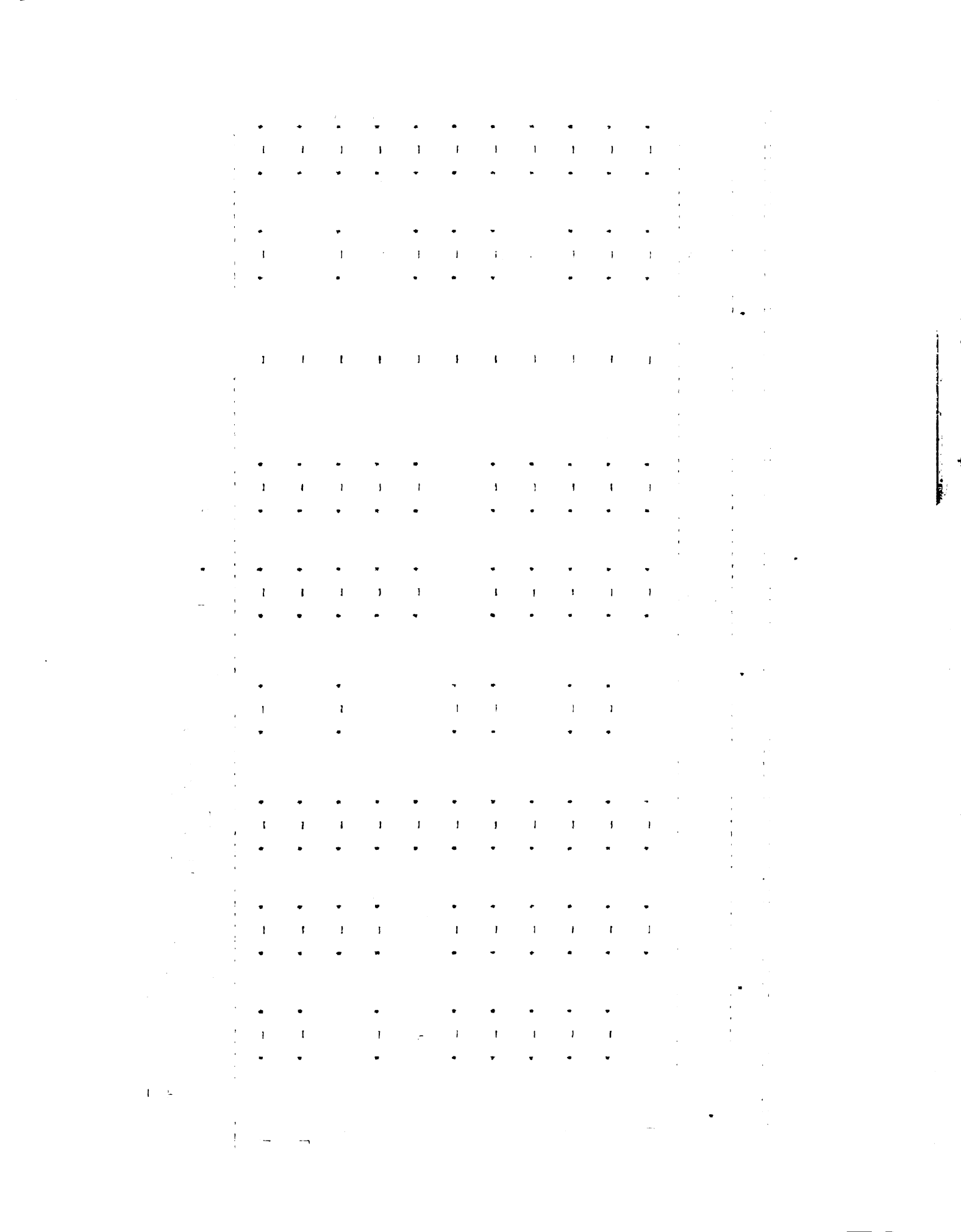


TABLE 3 CONT.

Media no.	L. casei (A_1)			S. dextranicum			B. vermiforme		
	Days at 30° C			Days at 30° C			Days at 30° C		
	2	3	5	2	3	5	2	3	5
1	*	0.3-0.8	0.6-1.0	*	0.2-0.6	0.8-1.2	-	0.5-1.0	0.5-2.0
2	0.5-1.0	1.2-1.8	1.3-2.0	1.0-3.0	2.0-4.0	3.0-6.0	-	0.3-0.8	0.5-2.0
3	0.3-0.8	1.0-1.8	1.3-2.0	0.2-0.4	0.5-0.8	0.8-1.0	-	0.2-0.8	0.3-1.2
4	0.2-0.5	0.5-0.8	0.8-1.1	*	0.1-0.4	0.1-0.5	-	*	0.1-0.3
5	0.3-0.5	0.8-1.2	1.0-1.5	0.1-0.3	0.2-0.5	0.3-0.6	-	0.1-0.2	0.1-0.3
6	0.2-0.5	0.6-0.9	0.9-1.2	1.0-2.0	**	**	-	0.1-0.3	0.1-0.8
7	+	*	0.3-0.8	*	0.2-0.4	0.3-0.5	-	0.1-0.3	0.2-0.7
8	0.1-0.2	0.2-0.3	0.3-0.8	*	0.1-0.3	0.2-0.5	-	*	0.1-0.3
9	*	0.3-0.8	1.0-1.5	0.1-0.5	0.4-0.7	0.8-1.0	-	0.2-1.0	0.5-1.3
10	0.1-0.3	0.5-0.8	0.8-1.3	*	0.2-0.4	0.3-0.6	-	*	0.1-0.2
11	0.1-0.3	0.5-1.0	0.8-1.2	0.1-0.3	0.3-0.5	0.4-0.6	-	0.3-0.5	0.6-1.2

** Coalescing of colonies
* Colonies too small to measure
+ No growth in dilutions used
- No count made

A_1 L. casei (ATCC 7469)

Media no.	L. mesenteroides			Streptococcus faecalis		
	Days at room temperature			Days at 30° C		
	2	3	5	2	3	5
1	0.5-0.9	0.8-1.0	0.8-1.0	0.3-1.0	0.5-1.0	1.0-2.0
2	0.6-1.0	0.8-1.2	0.8-1.2	0.3-1.0	0.5-1.0	1.0-2.0
3	0.3-0.8	0.8-1.0	0.9-1.1	0.5-1.5	0.5-1.5	1.5-2.5
4	0.1-0.3	0.2-0.5	0.3-0.6	0.5-1.5	0.5-1.5	0.5-2.0
5	0.3-0.5	0.5-0.8	0.7-0.9	0.5-1.0	0.6-1.0	1.0-2.0
6	0.2-0.4	0.5-0.8	0.8-1.0	0.8-1.0	1.0-1.2	2.0-3.0
7	*	0.1-0.2	0.2-0.3	0.1-0.2	0.1-0.2	0.2-0.5
8	*	0.1-0.3	0.2-0.5	0.1-0.2	0.8-1.0	1.0-2.0
9	0.6-1.0	0.8-1.2	1.0-1.3	0.3-0.8	0.3-0.8	0.5-1.0
10	0.1-0.3	0.2-0.4	0.3-0.6	0.3-0.5	0.5-1.0	1.0-1.5
11	0.3-0.5	0.5-0.8	0.6-0.9	0.3-1.0	0.5-1.0	0.8-1.0

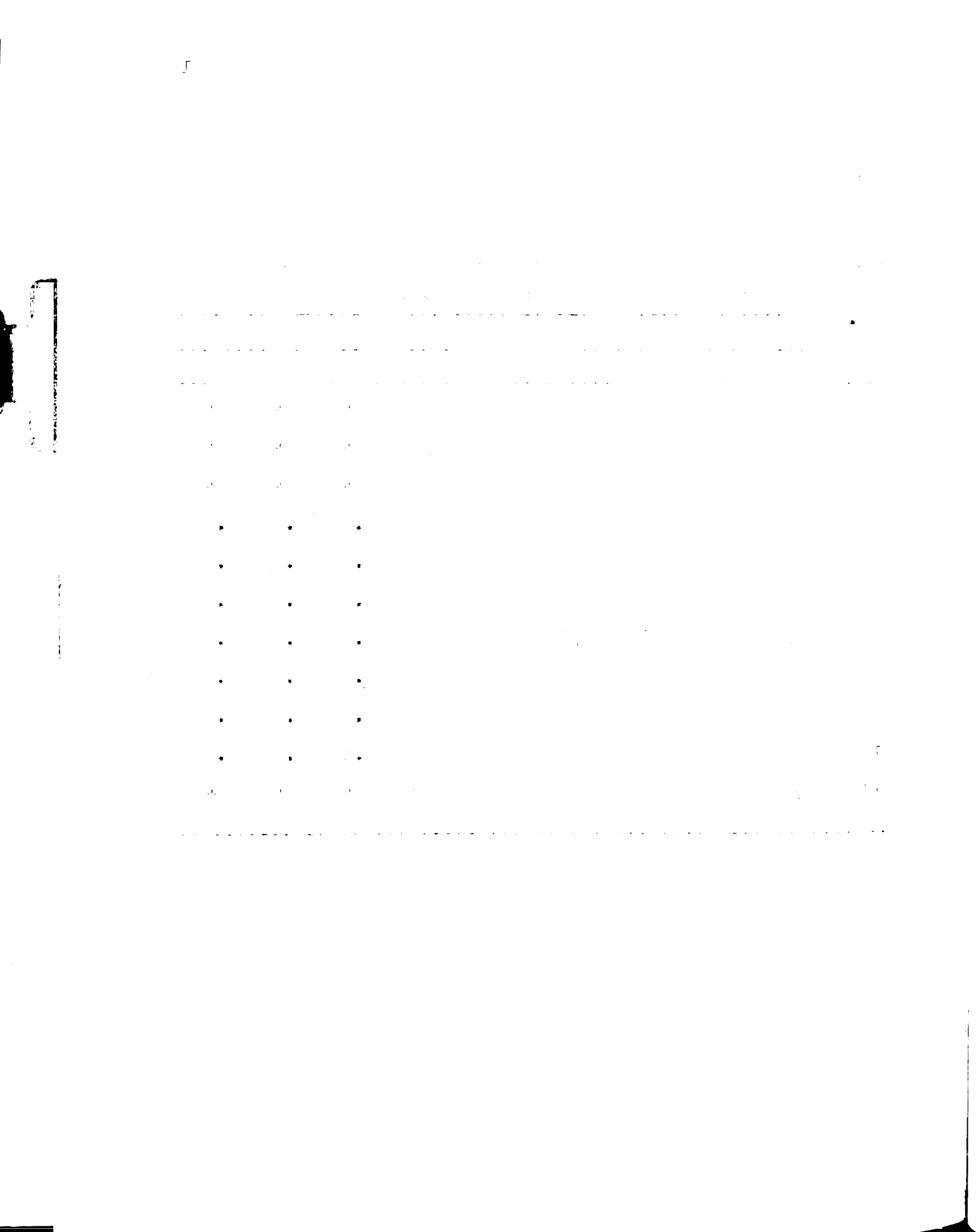
TABLE 4

NUMBER OF NON-LACTIC ACID BACTERIA PER ML x 10^6
WHICH DEVELOPED ON THE VARIOUS MEDIA

Media no.	Sarcina lutea			M. pyogenes var. aureus			Serratia marcescens		
	Days at 30° C								
	2	3	5	2	3	5	2	3	5
1	+	+	+	+	+	+	27.8	30.0	31.0
2	+	+	+	100.0	101.0	103.0	35.0	38.0	40.0
3	+	+	+	110.0	114.0	115.0	37.0	40.0	41.0
4	8.8	9.0	12.0	149.0	155.0	158.0	42.0	45.0	48.0
5	+	+	*	70.0	77.0	85.0	34.0	36.0	36.0
6	10.4	10.5	10.7	130.0	137.0	139.0	44.0	47.0	47.0
7	+	+	+	46.0	56.0	65.0	40.0	44.0	47.0
8	7.0	7.2	7.2	54.0	62.0	77.0	35.0	38.0	40.0
9	+	+	+	80.0	62.0	77.0	60.0	68.0	71.0
10	+	+	+	131.0	143.0	160.0	42.0	42.0	44.0
11	+	+	+	99.0	114.0	120.0	40.0	43.0	45.0

+ No growth in dilutions used
* Colonies too small to count

Media no.	Escherichia coli			Aerobacter cloacae			Bacillus subtilis		
	Days at 30° C								
	2	3	5	2	3	5	2	3	5
1	60	61	64	530	540	540	+	+	+
2	71	78	84	510	530	530	+	+	+
3	47	49	53	800	820	840	+	+	+
4	197	201	210	690	700	720	4.90	5.40	5.50
5	169	175	189	700	700	730	0.55	0.63	0.76
6	30	35	38	710	750	790	3.80	4.10	5.00
7	41	49	56	760	790	800	0.10	0.17	0.17
8	57	64	74	750	770	800	5.20	5.70	6.00
9	34	42	50	690	700	740	0.61	0.70	0.70
10	200	205	208	590	630	690	0.176	0.178	0.18
11	30	36	40	750	780	780	+	+	+



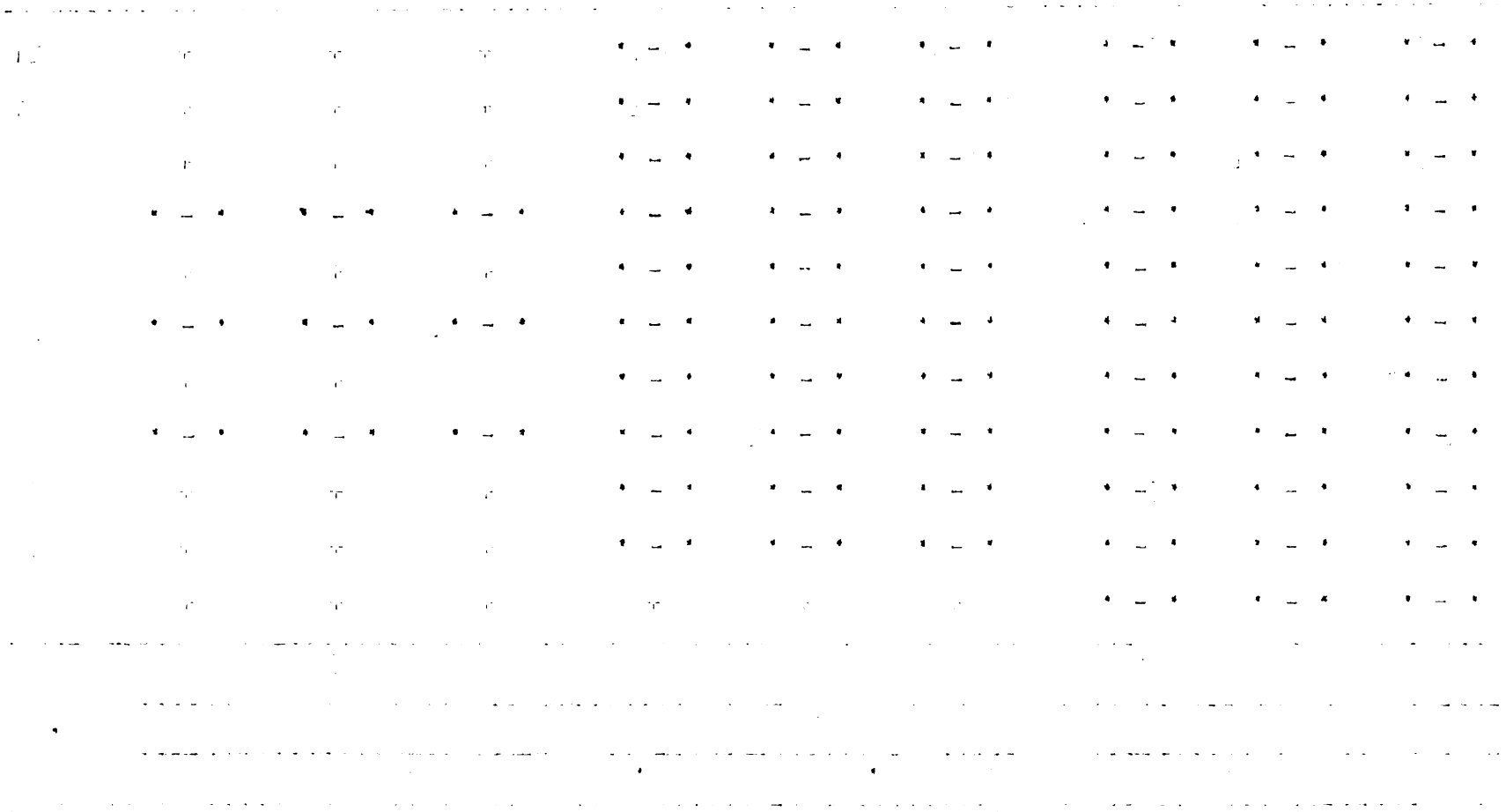


TABLE 5

DIAMETER OF COLONY IN MM OF NON-LACTIC ACID BACTERIA IN VARIOUS MEDIA

Media no.	Sarcina lutea			M. pyogenes var. aureus			Serratia marcescens		
	Days at 30° C								
	2	3	5	2	3	5	2	3	5
1	+	+	+	+	+	+	0.3-3.0	1.0-4.0	1.0-6.0
2	+	+	+	0.1-0.2	0.2-0.5	0.5-1.0	0.8-4.0	1.0-5.0	1.8-7.0
3	+	+	+	0.1-0.2	0.2-0.5	0.8-1.5	0.8-3.0	1.2-5.0	1.8-7.0
4	0.5-1.0	0.8-1.5	2.0-4.0	0.5-1.0	1.0-2.0	1.0-3.0	1.0-3.0	1.5-5.0	2.0-10.0
5	+	+	*	0.2-0.8	0.3-1.0	0.6-2.5	0.5-2.5	0.5-6.0	1.0-8.0
6	0.1-0.2	0.2-0.3	1.5-4.0	0.2-0.5	0.3-1.0	0.5-2.0	0.5-3.0	0.5-5.0	1.0-8.0
7	+	+	+	0.1-0.2	0.1-0.2	0.1-0.3	0.5-1.5	1.5-5.0	1.5-7.0
8	0.3-0.5	0.4-0.8	2.0-6.0	0.3-1.0	0.5-1.5	0.8-2.5	1.0-3.0	1.0-6.0	2.0-7.0
9	+	+	+	0.1-0.2	0.1-0.3	0.5-1.0	0.5-3.0	1.0-5.0	2.0-6.0
10	+	+	+	0.1-0.3	0.3-1.0	0.8-2.0	0.5-1.0	0.5-1.5	0.8-2.0
11	+	+	+	0.1-0.2	0.2-0.5	0.5-1.5	0.2-3.0	0.3-4.0	0.3-5.0

+ No growth in dilutions used
* Colonies too small to measure

Media no.	Escherichia coli			Aerobacter cloacae			Bacillus subtilis		
	Days at 30° C								
	2	3	5	2	3	5	2	3	5
1	0.5-1.0	1.0-3.0	1.0-3.0	2.0-6.0	3.0-8.0	3.0-8.0	+	+	+
2	0.2-0.5	0.5-1.0	0.8-1.5	1.5-6.0	3.0-8.0	3.0-8.0	+	+	+
3	0.8-1.0	1.0-2.0	1.5-3.0	1.0-5.0	2.0-5.0	2.0-5.0	+	+	+
4	0.5-1.5	1.0-3.0	1.0-4.0	0.3-5.0	0.5-5.0	1.0-5.0	1.0-5.0	2.5-12.0	12.0-25.0
5	0.5-2.5	1.0-3.0	1.0-4.0	0.5-2.0	1.0-4.0	2.1-5.0	0.5-2.0	1.0- 4.0	1.0- 7.0
6	0.5-2.0	1.0-4.0	1.0-4.0	0.5-1.5	1.0-4.0	1.5-5.0	1.0-3.0	1.0- 7.0	5.0-10.0
7	0.5-0.8	0.8-1.0	0.8-1.0	2.0-5.0	2.5-5.0	2.5-5.0	0.5-1.5	1.0- 3.0	1.0- 4.0
8	0.8-1.0	0.8-1.0	1.0-1.2	1.5-4.0	2.0-5.0	2.0-5.0	1.0-4.0	2.0- 7.0	5.0-15.0
9	0.3-1.0	0.5-1.0	0.5-1.0	0.5-3.0	1.0-4.0	1.0-5.0	0.5-4.0	1.0- 9.0	4.0-12.0
10	0.5-1.5	1.0-2.5	1.0-3.0	2.0-5.0	3.0-6.0	3.0-6.0	1.0-3.0	1.0- 7.0	1.0- 8.0
11	0.5-0.8	0.6-0.9	0.8-1.5	3.0-5.0	3.0-6.0	3.0-6.0	+	+	+

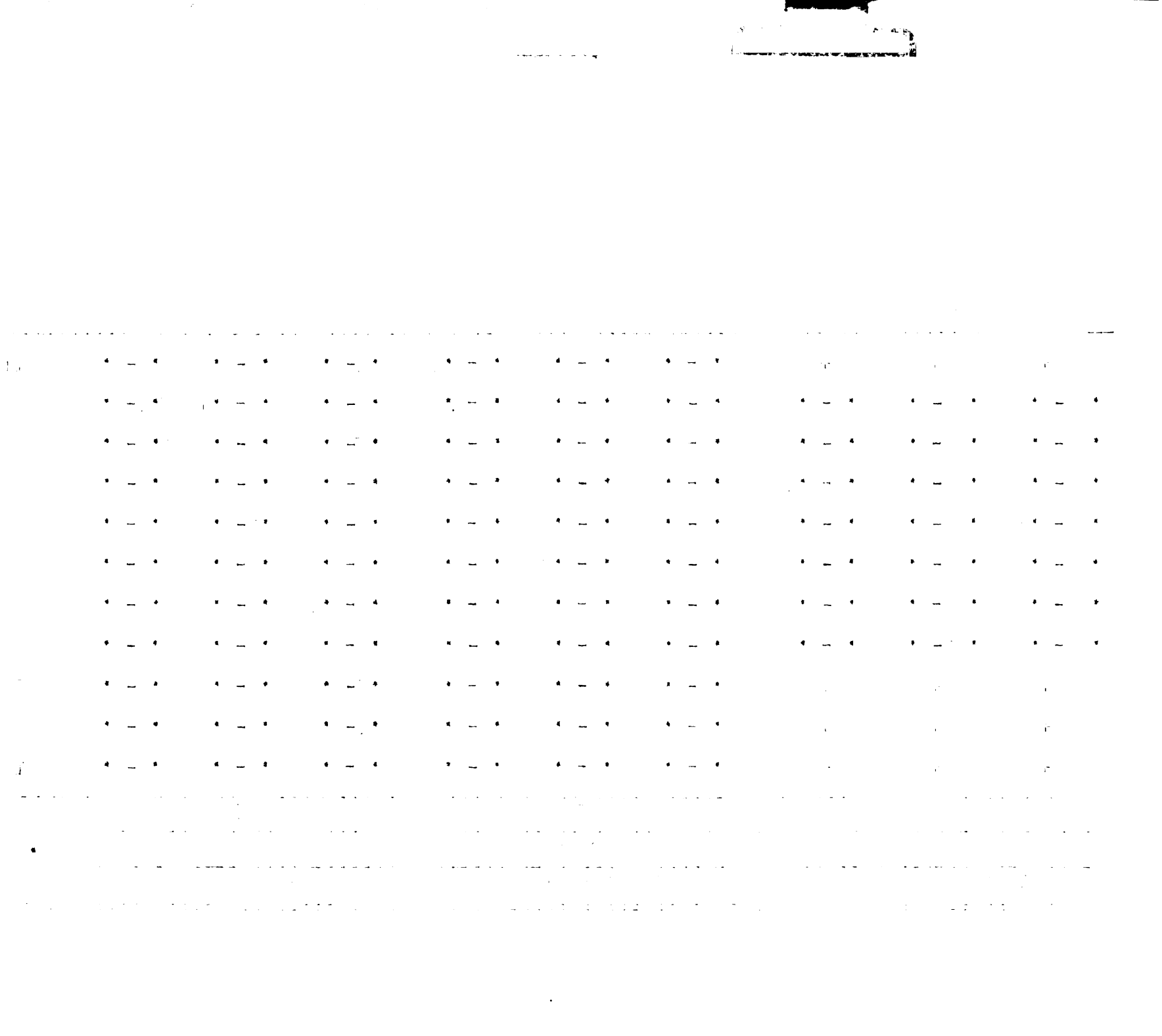


TABLE 6

YEASTS PER ML x 10^6 AS ENUMERATED BY VARIOUS MEDIA

Total count at 3 days

Yeast	Media numbers											
	1	2	3	4	5	6	7	7A	8	9	10	11
Hansenula subpelliculosa	9.8	10.5	10.0	9.0	9.5	9.2	11.1	10.0	8.8	9.5	9.8	9.0
Torulopsis careliniana	33.0	31.0	35.0	31.0	+	37.0	34.0	32.0	30.0	37.0	37.0	36.0
Zygosaccharomyces Sp. A	200.0	206.0	226.0	+	+	+	192.0	185.0	+	203.0	206.0	195.0
Torulopsis holmii	30.0	30.0	33.0	32.0	36.0	33.0	34.0	30.0	30.0	34.0	30.0	33.0
Torulaspora rosei	34.0	33.0	30.0	25.6	34.0	40.0	35.0	30.0	25.8	30.0	30.0	28.2

+ No growth in dilutions used

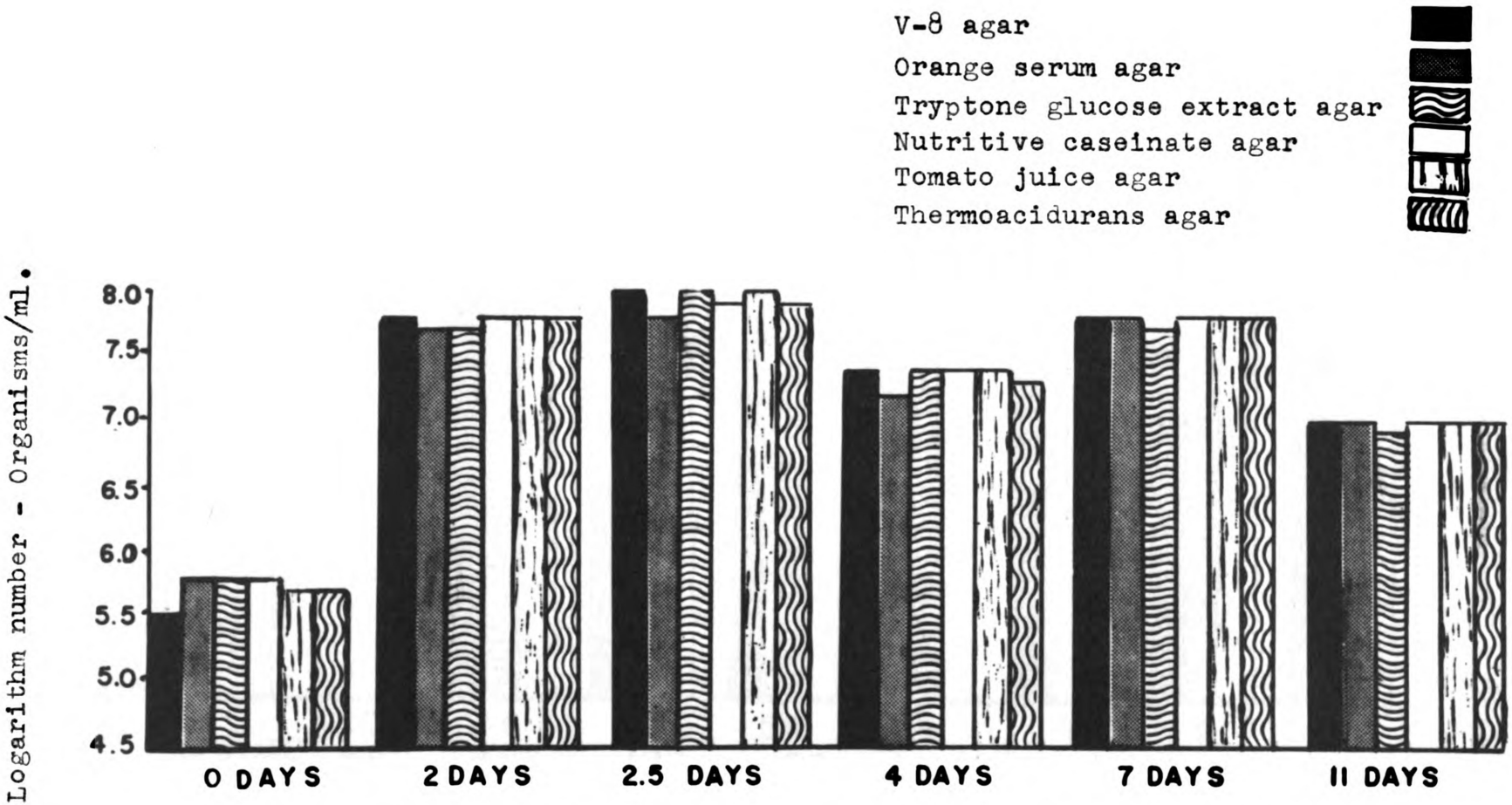


Figure 1. Evaluation of media to determine total organisms of sauerkraut fermentation

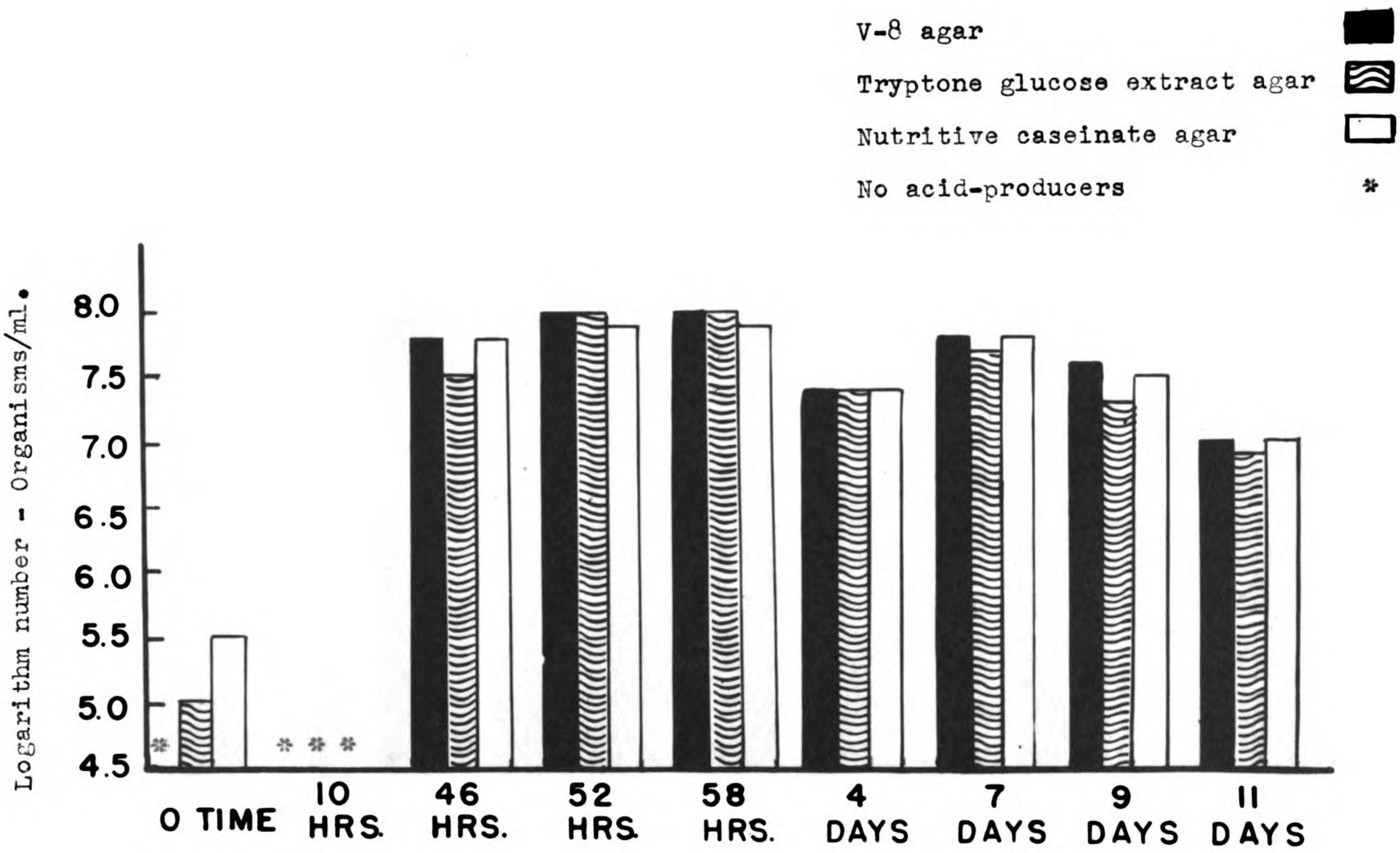


Figure 2. Evaluation of media to determine acid-producers of sauerkraut fermentation

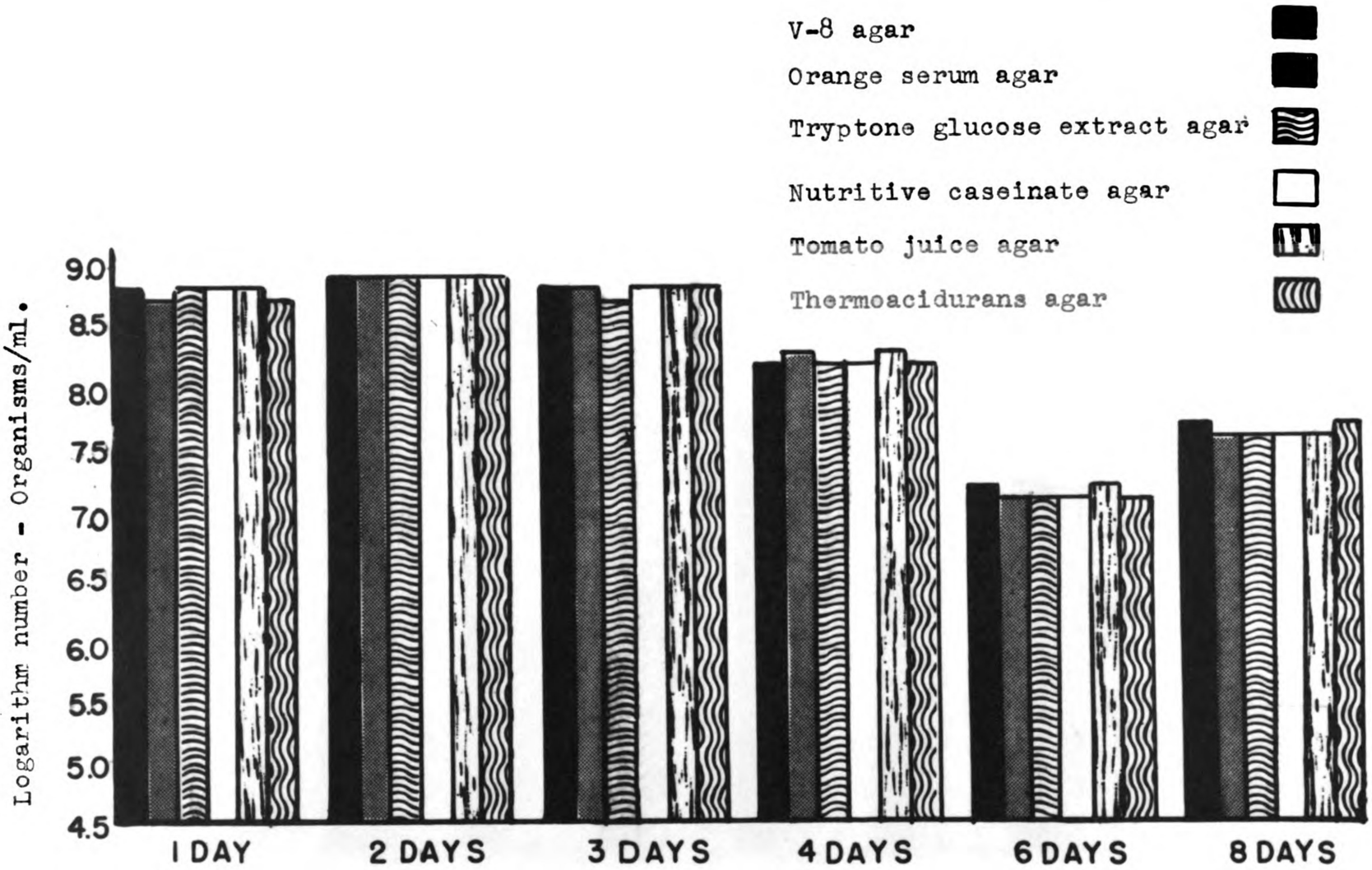


Figure 3. Evaluation of media to determine total organisms of cucumber fermentation

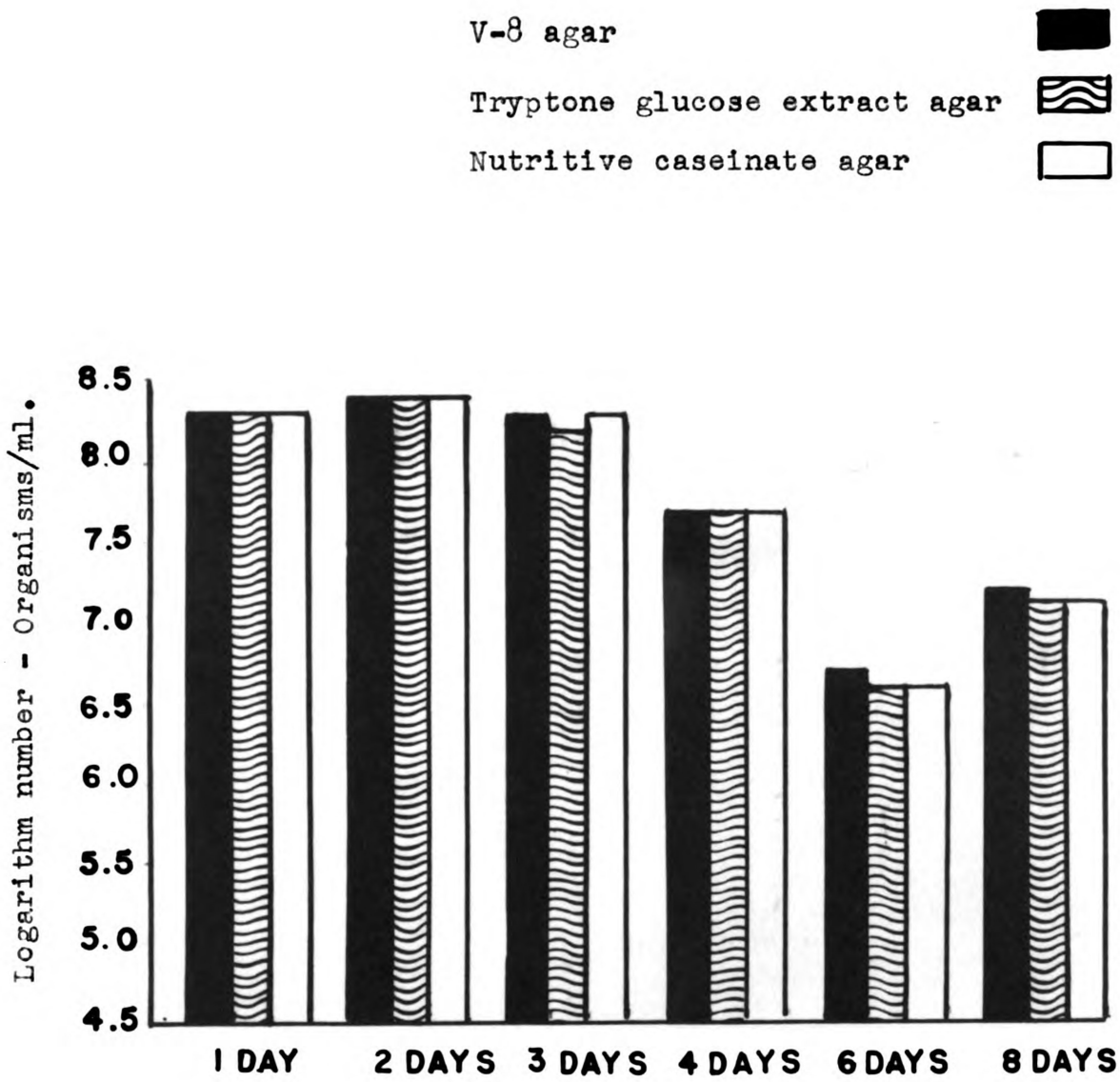
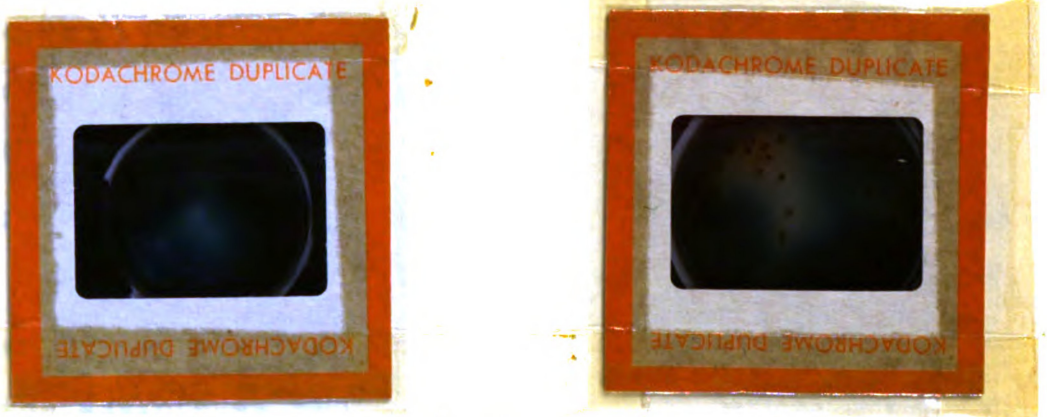


Figure 4. Evaluation of media to determine acid-producers of cucumber fermentation



24 hours

48 hours

Figure 5. Colonies of Lactobacillus plantarum on V-8 agar

SUMMARY

A new medium for the detection of lactic acid bacteria has been compared with ten other media used for the detection of these organisms. This medium contained V-8 vegetable juice as the main constituent and brom cresol green as an indicator. The V-8 medium compared favorably with the other media. Although in some cases growth was not as rapid in this medium as in some of the others, it had the advantages that the acid-producing bacteria gave characteristic colonies with distinct yellow halos and inhibited certain non-lactic acid bacteria.

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