PRODUCTION OF VARIANTS OF THE COLON AND AEROGENES GROUPS IN DIFFERENT MEDIA

I. Sucrose Medium[1]

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INTRODUCTION

The investigations of Neisser (1906), Massini (1907), Kowalenko (1910), and Baerthlein (1918) did much to establish bacterial variation or dissociation. A comprehensive review of the research on this subject has been given in articles by Hadley (1927), Dulaney (1928), Lewis (1934), and Torrey and Montu (1936); and will not be repeated in this publication.

Most investigators, however, have concerned themselves with the variation in morphological and colonial characteristics, especially in regard to the smooth (S) and rough (R) types. Neisser (1906), in his first work on variation, noted that a non-lactose-fermenting colon-like organism gave daughter colonies which fermented lactose. Burri (1910) observed this same phenomenon in regard to sucrose fermentation, when he studied an organism isolated from fermenting grass.

Many investigators have found no characteristic bio-chemical difference among their variants when the original culture was grown in media containing carbohydrates or higher alcohols, except lactose. The addition of certain chemicals to the media, however, has produced variants with new fermentation reactions. For instances, Dawson (1919) employed fat and Stearn (1923) dyes in media for the growth of *Escherichia coli*, and found that this organism developed the power to ferment sucrose. Smirnow

[1] The material in this communication formed a part of a thesis submitted by the senior author in 1935. Univ. of Colo. Studies, vol. 23, pp. 74–75 (1935).

(1916), on the other hand, found that phenol media caused members of the colon group to lose their power to ferment carbohydrates.

Variation occurs spontaneously under ordinary conditions of cultivation and also with environmental stimuli such as the presence of sugars, dyes, and changes in temperature. We have chosen the presence of sucrose in the medium as the environmental stimulus in this present investigation. The chief object was to study the characteristics of the variants which were produced when separate individual colonies from the mother culture were successively transplanted in sucrose broth for 15 times.

PROCEDURE AND MATERIAL

The cultures used in this investigation were obtained from the laboratory collection which has been kept in the department for over ten years. During this time, fresh slants were made every two or three months, and the cultural and morphological characteristics were checked from one to four times each year.

The sucrose medium was prepared by adding 8 grams of Bactonutrient broth and 5 grams of sucrose to 1 liter of distilled water. The pH was adjusted to 7.0 to 7.1, and the medium was sterilized at 15 pounds pressure for 15 minutes. The eosin-methylene-blue medium was made from the dehydrated preparation prepared by the Difco Laboratories according to the formula of Levine.

A group of 36 sucrose-negative organisms were chosen, 33 of these belonging to the *Escherichia* group and 3 to the *Aerobacter* group. These cultures had been purified by the streak method at least 15 times, and have given during the last 10 years negative reactions after 10 days' incubation in sucrose medium. Eosin-methylene-blue plates were streaked from the stock cultures of these organisms. After incubation, one well-isolated colony was used for restreaking on eosin-methylene-blue agar. Twenty-five colonies were selected from this later plate and each colony was separately transferred to sucrose medium contained in Durham fermentation tubes. Transfers of 10 of the cultures which showed no gas production in 7 days were made every third day to fresh tubes of sucrose medium for 15 times.

EXPERIMENTAL RESULTS

The percentage of gas produced in the Durham tubes of each of the transfers after 7 days' incubation was carefully recorded. In most cases, it was found that all of the 25 colonies first inoculated into sucrose medium were negative to this sugar. In a few cultures, occasional colonies were found which were positive to sucrose. Sherman and Wing (1937) have just reported this same phenomenon. If an organism showed one or more positive sucrose reactions in any of the 25 sub-cultures, this organism was not used for further examination.

Out of the 36 cultures finally tested, 27 (270 transplants) remained negative to sucrose throughout the 15 times of subculturing in sucrose broth. Nine of the cultures showed an acquired ability to ferment sucrose in from 1 to 10 of the transplants. From the cultural reactions, the nine original organisms were named according to Bergey. There were four cultures of *Escherichia coli*; 1 of *Escherichia alba*; 2 of *Escherichia gruenthali*; and 2 of *Aerobacter levans*.

In table 1 are shown the percentages of gas produced by the variants of cultures of *E. coli*. Table 2 shows the results for the cultures belonging to the species *E. alba*, *E. gruenthali* and *A. levans*.

After the sucrose-positive organisms had been transplanted and grown for 15 times in sucrose medium, streaks were made of each culture. Twenty-five colonies were picked at random and placed in sucrose broth in order to determine whether or not all the sub-cultures were positive to sucrose. The majority of the tubes gave gas production, but in some cases there were a few of the sub-cultures which did not yet produce gas in sucrose broth. Throughout the experimental work, no difference was noticed as to the character of the colonies when the sucrose-positive and sucrose-negative variants were grown on plain agar and eosin-methylene-blue agar. Each variant was tested for motility, indol production, liquefaction of gelatin, for the V.P.—M.R. reaction; and for fermentation of α methyl glucoside, sucrose, salicin, dulcitol, cellose, and raffinose. No cultural reaction with the exception of sucrose fermentation had been changed.

TABLE 1

Production of gas

Sucrose-positive variants

NUMBER OF TRANSPLANT	E. COLI NO. 42								E. COLI NO. 113				E. COLI NO. 28									E. COLI NO. 224			
	Variant																								
	1†	3	5	6	7	8	9	10	1	5	7	9	1	2	3	4	6	7	8	9	10	1	5	6	7
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	10	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
5	10*	10	0	0	0	0	5	10	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	20	20
6	20	10	0	0	0	0	5	10	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30	30
7	40	10	0	0	0	0	10	15	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30	40
8	35	10	0	0	0	0	15	20	0	0	0	0	0	0	0	0	0	0	0	0	10	30	0	30	45
9	30	15	0	0	0	0	30	25	5	0	0	0	0	0	0	0	10	10	0	20	30	35	0	35	50
10	25	15	0	0	0	15	30	30	5	5	0	0	0	0	0	0	25	25	10	30	35	30	10	30	45
11	30	20	10	20	25	20	35	30	5	5	5	5	5	0	0	10	25	35	20	35	35	40	40	50	45
12	85	15	15	30	25	20	40	35	5	5	5	5	10	0	0	10	20	30	20	50	30	35	50	50	55
13	90	25	20	25	20	20	35	30	10	5	5	10	25	20	0	10	25	30	20	50	30	35	45	45	50
14	90	25	30	40	25	15	35	35	15	15	15	15	30	30	15	15	30	25	25	40	35	40	50	50	40
15	90	20	35	50	25	15	40	35	25	20	25	25	40	30	25	30	35	35	30	50	40	55	50	70	40

* Figures are in percentage gas produced in seven days.

† The numbers omitted up to 10 represent the colonies which remain negative to sucrose during the fifteen transplants.

TABLE 2

Production of gas

Sucrose-positive variants

NUMBER OF TRANSPLANT	A. LEVANS NO. 346					A. LEVANS NO. 422										E. ALBA NO. 13					E. GRUENTHALI NO. 184						E. GRUENTHALI NO. 248
	Variant																									
	2†	4	6	7	8	1	2	3	4	5	6	7	8	9	10	1	2	3	5	7	1	2	5	7	8	9	5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	5	0	0	5	0	0	0	0	0	5	0	5	0	0	5	0	0	0	0	0	0	0
4	0	0	5	0	0	30	30	20	30	50	30	40	10	10	20	0	10	0	0	15	0	0	0	0	0	0	0
5	0	0	10	5	5	50	50	25	50	50	30	40	10	15	20	0	10	0	0	15	0	0	0	0	0	0	0
6	0	0	10	5	10	40	50	25	35	50	30	30	40	60	35	0	15	0	0	15	0	0	0	0	0	0	20
7	0	0	15	10	15	40	40	20	30	30	50	50	50	75	40	0	15	0	0	20	20	0	0	0	0	0	55
8	0	0	15	15	20	60	60	20	30	30	40	50	50	80	35	0	20	0	0	25	20	0	0	0	0	0	50
9	0	10	20	15	35	40	70	50	60	65	65	70	80	70	50	0	20	0	0	25	20	0	0	0	0	0	60
10	10*	15	20	20	35	60	65	40	60	60	60	70	75	50	80	5	25	5	0	25	25	5	20	10	0	10	50
11	15	15	25	15	30	70	80	60	70	70	70	50	75	65	70	20	30	30	20	30	30	15	25	30	0	20	50
12	10	15	25	20	30	75	65	50	80	45	45	65	70	50	60	20	30	25	25	50	20	20	30	40	5	20	55
13	10	15	20	20	35	60	50	50	60	40	45	60	60	60	40	25	60	30	60	70	40	20	30	30	10	25	50
14	10	15	25	25	40	60	50	45	60	60	55	65	50	35	60	20	70	70	70	70	30	30	35	35	10	35	60
15	20	25	20	30	40	75	45	55	65	40	45	50	60	40	45	25	80	90	70	80	40	35	35	50	10	35	75

* Figures are in percentage gas produced in seven days.

† The numbers omitted up to 10 represent the colonies which remained negative to sucrose during the fifteen transplants.

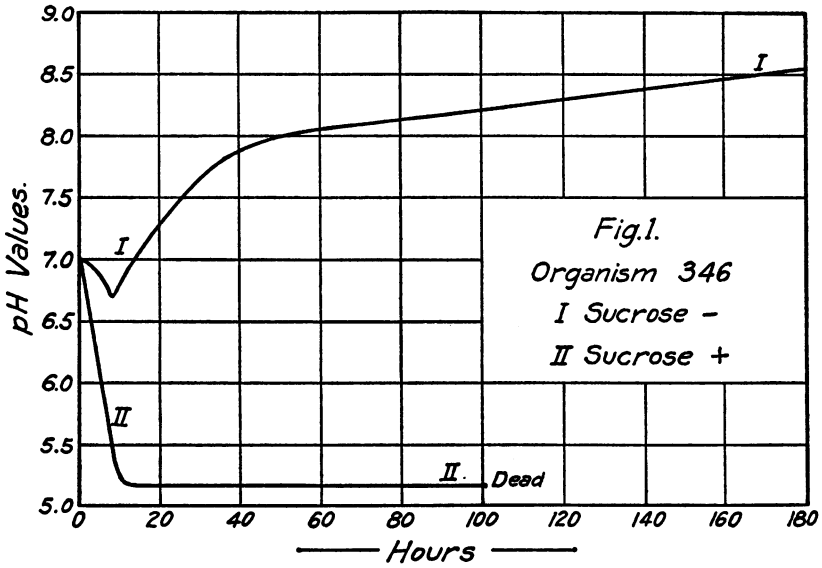

FIG. 1. CURVES SHOWING CHANGES IN pH BY AN ORIGINAL CULTURE OF A. LEVANS AND ONE OF ITS SUCROSE-POSITIVE VARIANTS WHEN EACH IS GROWN IN SUCROSE MEDIUM

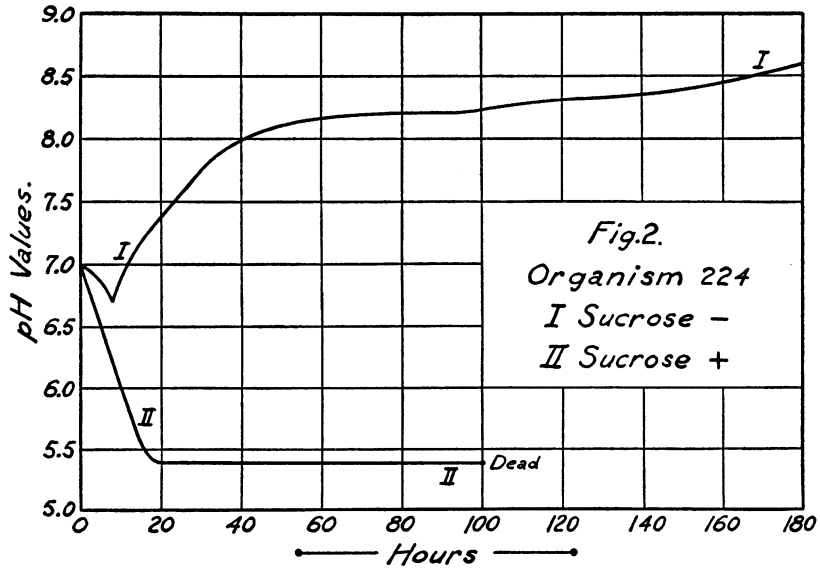

FIG. 2. CURVES SHOWING CHANGES IN pH BY AN ORIGINAL CULTURE OF E. COLI AND ONE OF ITS SUCROSE-POSITIVE VARIANTS WHEN EACH IS GROWN IN SUCROSE MEDIUM

The quantitative study of the progressive acid fermentation was conducted on one of the original cultures of *A. levans* and on one of its variants which developed the power to ferment sucrose. These organisms were grown in sucrose medium, and at frequent intervals during 180 hours, the pH values of the medium were determined. The curves representing the results of the change in pH are given in figure 1. Similar determinations were made for a culture of *E. coli* and one of its sucrose-positive variants. These results are shown in figure 2.

A representative number of the sucrose-positive variants were placed on agar slants for permanent cultures. All of these cultures were transferred to sucrose broth after being stored for two years at about 4°C. In every case where the organisms were still alive the reaction to sucrose was still positive.

DISCUSSION OF RESULTS

The production of sucrose-positive variants is easy to accomplish with some members of the *Escherichia* and *Aerobacter* groups. Of the 36 cultures tested, 9 or 25 per cent exhibited the power to produce variants which fermented sucrose. Some strains produced the variants with ease, as was the case with organism number 422. All the 10 sub-cultures of this organism began to ferment sucrose in 4 days. Other organisms acquired the ability with less ease, as may be seen from the results given by organism number 248, which produced only 1 sucrose-positive variant. Of the total of 90 transplants from the 9 organisms, 58 per cent acquired the ability to ferment sucrose.

The ability of bacteria to decompose carbohydrates with acid and gas production has been accepted by most bacteriologists as one of the most valuable means of classifying bacteria. The primary reason for making the investigation reported in this paper was to determine whether or not, in a classification like the one proposed by Bergey, the use of these fermentation reactions for the separation of species could be justified.

The results of the experiment submitted herewith seem to indicate that the fermentation of sucrose is not necessarily a constant characteristic. The acquired ability of the organisms

used in this research to ferment sucrose would, according to Bergey, place the mother colony and the variant in different classifications. The original cultures of *E. coli*—numbers 28 42, 113, and 224—were changed to *Escherichia communior;* those of *A. levans*—numbers 346 and 422—were changed into *Aerobacter hibernicum*; those of *E. gruenthali* to *Escherichia anindolica*; and that of *E. alba* to *Escherichia gastrica.*

In studying the pH curves in figures 1 and 2, it will be discovered that the sucrose-positive variants developed a great capacity to form acid from sucrose. The original cultures showed an acid production for about 7 hours to a minimum pH of about 6.7; and then there was an abrupt rise in the curves until a pH of over 8.5 was reached in 180 hours. The sucrose-positive variants from these cultures, however, showed a rapidly descending curve to a pH value from 5.2 to 5.4 in 10 to 15 hours. The production of acid in the sucrose-positive variants was so great that it caused the death of the organism in most cases.

It is not surprising that an organism fails to retain its stability when it is removed from its natural habitat and placed in an artificial environment. The results in this paper would justify the statement that the use of fermentative reactions, as obtained from sugar media, is not entirely satisfactory as a basis of the subdivision of the *Escherichia* and *Aerobacter* groups of bacteria. Sherman and Wing (1937) have expressed a similar conclusion in their recent publication.

SUMMARY

A study has been made of the variants produced by members of the *Escherichia* and *Aerobacter* groups when different species were serially transplanted in sucrose medium. The results may be summarized as follows:

1. The production of sucrose-positive variants is readily accomplished with some strains.
2. An organism which develops very little acid in sucrose medium may give off variants which produce a large amount of acid.
3. There was no difference in the appearance of the original cultures and the variants on eosin-methylene-blue agar.

4. The sucrose-positive variants in a few cases threw off sucrose-negative variants.

5. The sucrose-positive variants retained the power to ferment sucrose after being stored for two years.

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