

Bacterial Variations in Salicin Medium.

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Most investigators have concerned themselves with the variation in morphological and colonial characteristics, especially in regard to the smooth (S) and rough (R) types. Neisser,¹ in his first work on variation, noted that a nonlactose-fermenting, colon-like organism gave daughter colonies which fermented lactose. Burri² observed this same phenomenon in regard to sucrose fermentation, when he studied an organism isolated from fermenting grass.

Many investigators have found no characteristic biochemical difference among their variants when the original culture was grown in media containing carbohydrates or higher alcohols except lactose.

Variation occurs spontaneously under ordinary conditions of cultivation, and also with environmental stimuli such as the presence of sugars, dyes, or changes in temperature. The presence of salicin in the medium as the environmental stimulus was chosen 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 salicin broth for 15 times.

A group of 36 salicin-negative organisms were selected, 31 of these belonging to the *Escherichia* genus and 5 to the *Aerobacter* genus. Eosin-methylene blue plates were streaked from these cultures, and 10 colonies which showed no gas production in salicin medium were transferred every third day to fresh tubes of salicin medium for at least 15 times. The percentages of gas produced were recorded after 7 days incubation at 37°C. As an example, a record of the acquired ability of culture *E. anindolica* G. to ferment salicin is shown in Table I.

¹ Neisser, M., *Centralbl. f. Bakt., Abt. I*, Ref., 1906, **38**, 98.

² Burri, R., *Centralbl. f. Bakt., Abt. II*, 1910, **28**, 321.

Of the 36 organisms used, 22 (220 sub-cultures) remained negative to salicin throughout the 15 transplants in salicin broth. Of the other 14 organisms (140 sub-cultures), there were from 1 to 10 of the sub-cultures of each organism which acquired the ability to ferment salicin after from 1 to 12 transplants. Seventy-six of these 140 sub-cultures fermented salicin and 64 remained negative. Ten of the 64 salicin-negative sub-cultures were streaked on eosine methylene blue agar plates. Ten colonies were selected from each plate and placed in salicin medium. Positive variants were obtained from 7 of the salicin-negative daughter colonies. In no instance, however, did more than one tube out of 10 representing a given culture produce more than 10% gas. In many instances, it was discovered that the salicin-negative and salicin-positive variants from the same culture gave different forms of colonies on agar plates.

A representative number of the salicin-positive variants were placed on agar slants for permanent cultures. All of these cultures were transferred to salicin broth after being stored for 3 years at about 4°C. In every instance, where the organisms were still alive, the reaction to salicin was still positive. Streaks of 20 of the cultures were made on eosin-methylene blue agar. Ten of the individual colonies from each culture were grown in salicin medium for 7 days. The majority of the sub-cultures produced gas in salicin medium.

The quantitative study of the progressive acid fermentation was conducted on the original culture of *Aerobacter cloacae* and on one of its variants which developed the power to ferment salicin. These 2 organisms were grown in salicin medium, and at frequent intervals during 180 hours, the pH values of the medium were determined. Similar determinations were made for a culture of *Escherichia formica* and one of its salicin-

TABLE 1.
Production of Gas in Salicin Medium of Variants of *E. anindolica* G.

No. of transplant	Daughter colonies									
	1	2	3	4	5	6	7	8	9	10
1	0*	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	5	10	0	20	10	0	0	0	0	5
7	10	10	5	25	15	0	0	0	0	15
8	10	10	5	25	30	0	0	0	20	10
9	20	20	15	25	25	0	20	0	25	20
10	20	25	25	20	15	5	15	0	20	15
11	20	25	35	20	15	20	25	0	20	30
12	30	20	30	20	15	20	20	20	20	35
13	35	35	30	25	15	20	20	30	20	40
14	35	35	20	25	15	30	25	35	20	40
15	35	35	30	30	25	30	30	35	35	40

* Figures are in percentages of gas produced in 7 days.

positive variants.

It was discovered that the salicin-positive variants developed a greater capacity to form acid from salicin. The original cultures (salicin-negative) showed an acid production for about 10 hours to a minimum pH of about 6.7, after which there was an abrupt rise in pH to over 8.5 during 180 hours. The salicin-positive variants from these cultures reduced the pH value of the medium to a value around 5 in 10 to 30 hours. The acid production was so great that the or-

ganisms were killed in most instances.

The results of this investigation indicate that, the fermentation of a given substance, such as salicin, is not necessarily a constant characteristic. Because of the acquired ability to ferment this glucoside, a number of organisms changed from one species to another according to the Bergey classification. Five cultures change their classification from *E. anindolica* to *E. communior*, 3 from *E. formica* to *E. coli*, and 3 from *E. gruenthali* to *E. paragruenthali*.

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High Molecular Weight Polyglycols as Reagents in the Gravimetric Determination of Inorganic Phosphate.

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Despite the availability of the simpler, direct colorimetric methods for the determination of small amounts of inorganic phosphate in biological materials, gravimetric methods, by reason of their greater precision and accuracy, have continued to find some application. Two precipitates that have been employed for quantities of phosphate too small for the magnesium ammonium phosphate method are the ammonium and strychnine salts of phospho-

molybdic acid. The strychnine phosphomolybdate appears to have gained wider preference; its advantages over the ammonium salts have been summarized by Peters and Van Slyke¹ as follows: (1) the weight of the precipitate is greater, for per 1 mg of P the

¹ Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, Methods, pp. 849, 873, Baltimore, The Williams and Wilkins Co., 1932.