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A. R. Stanley

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by A. R. STANLEY

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Physiologic and Serologic Studies of the Soft-Rot And Colon Group of Bacteria

by A. R. STANLEY

Introduction

IN the summer of 1930 a study of the bacterial stalk-rot of sweet corn caused by *Bacterium dissolvens* Rosen was begun. While working with some of the soft-rot bacteria in the fall of 1931 the writer observed the close similarity in morphology and physiology between this group and *Bact. dissolvens,* and it was thought desirable to study the relationship between them. The next spring and summer isolations were made from as many sources as possible and representative cultures of the family *Bacterieae* were added. The present study comprises a comparison of the physiologic and serologic reactions of 120 such cultures.

Literature Review

Since 1899, when Potter *(43)* described and named *Pseudomonas destructans* as the organism causing white-rot of turnips, a great number of soft-rot organisms have been described and named. Some of these are *Bacillus carotovorus,* L. R. Jones, 1900 *(21)*; *B. oleraceae,* F. C. Harrison, 1902 *(12)*; *B. omnivorus,* C. J. J. van Hall, 1902 *(59)*; *B. atrosepticus,* C. J. J. van Hall, 1903 *(60)*; *B. phytophthorus,* O. Appel, 1903 *(1)*; *B. aroideae,* C. O. Townsend, 1904 *(58)*; *B. solanisaprus,* F. C. Harrison, 1906 *(13)*; *B. melonis,* N. J. Giddings, 1910 *(7)*; *B. melanogenes,* Pethybridge and Murphy, 1911 *(42)*; *B. apivorus,* H. Wormald, 1913 *(61)*; *B. dissolvens,* H. R. Rosen, 1926 *(45)*; and *B. papaveris,* C. S. Ram Ayyar, 1927 *(44)*. This last one is from India and the reference was discovered too late to obtain a culture for use in this study.

The first attempt to compare these different "species" was undertaken as a joint project in 1902 by H. A. Harding and W. J. Morse of the New York and Vermont Experiment Stations, respectively. They conducted physiologic studies with *Bacillus carotovorus, Bacillus oleraceae, Bacillus omnivorus, Bacillus aroideae,* and 39 other "strains" of soft-rot bacteria which they collected from Europe and America. In the results of their investigation *(11)*, published in 1909, they concluded that the only distinguishing character was their action upon carbohydrates. A summary of their fermentation tests show that *B. carotovorus, B. oleraceae, B. omnivorus,* and 30 unnamed strains produced both acid and gas from dextrose, lactose, and sucrose; *B. aroideae* and 3 unnamed strains produced acid but no gas from the same sugars, and the

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other six strains were intermediate, producing gas in at least one sugar but not in all three. Referring to this separation they report, "Unfortunately this clearness of classification is more apparent than real, since practically each successive determination of the collection of cultures led to a rearrangement of the representatives of the various groups with a gradual shifting toward the upper groups because of the greater importance placed on a positive result than on a negative one. The final accumulation of 33 cultures in the upper group is largely the expression of the continued action of the law of chance and had the study continued longer this group would undoubtedly have been correspondingly enlarged." They concluded that "Unless later studies of the pathogenicity of these cultures shall offer a basis for subdividing them, there is no apparent reason why they should not all be considered as somewhat variant members of a single botanical species."

A very comprehensive study of the organism causing blackleg of potatoes was made by Jennison *(20)* in 1923. He worked with 15 strains which included authentic cultures of *B. phytophthorus, B. solanisaprus, B. atrosepticus,* and *B. melanogenes.* He concluded as a result of fermentation and carbohydrate utilization studies that there is very little if any difference between the organisms studied.

Paine and Chandliuri *(39)* published a very short article in the same year (1923), in which they assert that *B. atrosepticus* and *B. solanisaprus* are not identical. The characteristics used to differentiate these are their resistance to heat, the odor, color, and pH of potato rots, and the diameter of the rotted area at various temperatures.

The first report of serologic studies with this group of bacteria is that of St. John-Brooks, Nain, and Rhodes *(4)* in 1925. No accurate conclusions can be drawn from their results because the only serum dilution used was 1:100. However, it is interesting to note that cross agglutination was obtained at this dilution between *B. solanisaprus* (Paine strain) and *B. carotovorus* (Smith strain); *B. phytophthorus* (Smith strain) and *B. carotovorus* (Bewley strain); and *B. phytophthorus* (Appel strain) and *B. carotovorus* (Bewley strain). However, *B. carotovorus* (turnip strain) and *B. carotovorus* (Guelph strain) showed no cross agglutination with any of the above named strains.

Lacey *(25)* in 1926 reports on physiologic, pathologic, and serologic studies, using *B. carotovorus, B. phytophthorus,* and *B. solanisaprus.* She concludes that although closely related, these are distinct species. According to her work *B. phytophthorus* can be distinguished from *B. carotovorus* and *B. solanisaprus* by its production of acid and gas in maltose broth, its behavior in Uschinsky's solution, its rapid clearing in Fermi's solution and in saccharose broth, its absence of diastatic action, and its failure to grow at 37°C. *B. carotovorus* and *B. solanisaprus* are more closely allied but may be distinguished culturally by their action on Fermi's and Uschinsky's solution by the acid and alkali production in sugar peptone water.

Lacey also found serologic differences to add weight to her contention. Homologous agglutinations with *B. carotovorus* and *B. phy-*

tophthorus were found to a dilution of 1:8000. *B. carotovorus* in *B. phytophthorus* antiserum agglutinated to a dilution of 1:200 while *B. phytophthorus* in *B. carotovorus* (Bewley) antiserum agglutinated in dilutions as high as 1:4000. However, these same antigens acted differently in *B. carotovorus* (strain 50) antiserum. Here *B. carotovorus* agglutinated only at 1:100 while *B. phytophthorus* showed no agglutination. These cultures of *B. carotovorus* and *B. phytophthorus* agglutinated only at 1:100 in *B. solanisaprus* antiserum. When the author concludes that they are marked with constant differences and can be separated by agglutination tests she evidently disregarded this great variation between the antisera of the two strains of *B. carotovorus*.

Berridge *(2)* working at the same laboratory applied chemical agglutination tests to these same strains of bacteria and drew the same conclusions. *B. carotovorus* agglutinated in lower concentrations of acid, *B. phytophthorus* next, and *B. solanisaprus* showed the greatest resistance. In sulphate, *B. phytophthorus* agglutinated in the lower dilutions, *B. carotovorus* next, and again *B. solanisaprus* was the more resistant. She also found a culture of *B. carotovorus* which gave reactions typical of *B. solanisaprus* but, for some reason, did not take account of it in her conclusions.

Link and Taliaferro *(29)* report in 1928 the results of serologic studies of *B. carotovorus* and *B. aroideae*. They obtained cross agglutination between these but in both cases it was a higher concentration than the homologous agglutination. From this they conclude that these species are distinct, seemingly not realizing that a single culture may not be typical of a species.

This same year Stapp *(55)* in Germany published the results of serologic studies including several of the soft-rot bacteria in which he found that *B. phytophthorus, B. atrosepticus,* and *B. melanogenes* belong to one serologic group, while *B. carotovorus* and *B. solanisaprus* are identical serologically but differ from the first group.

Brierly *(3)* in his paper dealing with physiologic studies of the bacteria causing potato tuber rots leaves the impression that he does not consider *B. carotovorus, B. phytophthorus,* and *B. aroideae* to be identical. In addition to these species he also used *B. mesentericus*. The latter and *B. aroideae* appeared to be high-temperature organisms, whereas *B. carotovorus* and *B. phytophthorus* were most active at storage temperatures.

Matsumoto *(31)* working in Japan reported in 1929 the results of serologic studies with 27 strains of soft-rot bacteria of his own isolation. Some of these were grouped but no relation was established with any type cultures.

Again in 1931 Matsumoto and Somazawa *(34)* published the results of agglutination work carried out with 8 strains of soft-rot bacteria. As usual they found all these strains to be practically identical physiologically but to be distinct serologically. In conclusion they say, "Nevertheless this would by no means warrant using these tests as the basis for classification of the organisms in question, since, as has been

CHART 1—*Organisms Studied*

Number	Name or Habitat	Where Obtained
1	*B. aroideae*	Minnesota
2	Blackleg	Minnesota
3	*B. atrosepticus*	U. S. Dept. of Agri.
4	*B. dissolvens*	U. S. Dept. of Agri.
5	Sweet corn	W. Va. University
6	Sweet corn	W. Va. University
7	Sweet corn	W. Va. University
8	Oriental poppy	W. Va. University
9	Cabbage	W. Va. University
10	Watermelon	W. Va. University
11	Iris	W. Va. University
12	Campanula	W. Va. University
13	Canna	W. Va. University
14	Delphinium	W. Va. University
15	Delphinium	W. Va. University
16	Delphinium	W. Va. University
17	Delphinium	W. Va. University
18	Delphinium	W. Va. University
19	Tomato	W. Va. University
20	Potato	W. Va. University
21	Water	W. Va. University
22	Carrot	W. Va. University
23	Carrot	W. Va. University
24	Carrot	W. Va. University
25	Carrot	W. Va. University
26	Carrot	W. Va. University
27	Carrot	W. Va. University
28	Carrot	W. Va. University
29	Tomato	W. Va. University
30	Tomato	W. Va. University
31	Carrot	W. Va. University
32	Carrot	W. Va. University
33	Cauliflower	W. Va. University
34	Cauliflower	W. Va. University
35	Bearded iris	W. Va. University
36	Parsnip	W. Va. University
37	Water	W. Va. University
38	Turnip	W. Va. University
39	Turnip	W. Va. University
40	Red beet	W. Va. University
41	Red beet	W. Va. University
42	Pe Tsai	Japan
43	Onion	Japan
44	Kotyo-ran	Japan
45	Delphinium	W. Va. University
46	Delphinium	W. Va. University
47	Delphinium	W. Va. University
48	*B. carotovorus*	Chicago
49	*B. tabacum*	Penn State
50	*B. tabacum*	Penn State
51	*B. carotovorus*	Chicago
52	*B. carotovorus*	Chicago
53	*B. carotovorus*	Chicago
54	Dissociant of 2	W. Va. University
55	Dissociant of 2	W. Va. University
56	Dissociant of 2	W. Va. University
57	Dissociant of 26	W. Va. University
58	Dissociant of 26	W. Va. University
59	Dissociant of 26	W. Va. University
60	Dissociant of 26	W. Va. University

CHART 1—*Organisms Studied*—Cont'd

Number	Name or Habitat	Where Obtained
61	Dissociant of 36	W. Va. University
62	Dissociant of 40	W. Va. University
63	Dissociant of 40	W. Va. University
64	*B. coli communis*	W. Va. Medical School
65	*B. coli communior*	W. Va. Medical School
66	*B. coli*	Ohio
67	*B. coli*	U. S. Dept. of Agri.
68	*B. dissolvens*	U. S. Dept. of Agri.
69	Cabbage	W. Va. University
70	Sweet corn	W. Va. University
71	Sweet corn	W. Va. University
72	*Salmonella pullorum*	Yale
73	*Salmonella pullorum*	Yale
74	*Salmonella pullorum*	Yale
75	Feces	W. Va. University
76	Feces	W. Va. University
77	Feces	W. Va. University
78	Dissociant of 2	W. Va. University
79	Dissociant of 2	W. Va. University
80	Dissociant of 2	W. Va. University
81	Dissociant of 2	W. Va. University
82	Dissociant of 2	W. Va. University
83	Dissociant of 2	W. Va. University
84	Dissociant of 2	W. Va. University
85	Dissociant of 2	W. Va. University
86	Dissociant of 2	W. Va. University
87	Dissociant of 2	W. Va. University
88	Dissociant of 2	W. Va. University
89	Dissociant of 18	W. Va. University
90	Dissociant of 18	W. Va. University
91	Dissociant of 18	W. Va. University
92	Dissociant of 18	W. Va. University
93	Dissociant of 18	W. Va. University
94	Dissociant of 18	W. Va. University
95	Dissociant of 18	W. Va. University
96	Dissociant of 18	W. Va. University
97	Dissociant of 26	W. Va. University
98	Dissociant of 26	W. Va. University
99	Dissociant of 26	W. Va. University
100	Dissociant of 26	W. Va. University
101	Dissociant of 26	W. Va. University
102	Dissociant of 26	W. Va. University
103	Dissociant of 26	W. Va. University
104	Dissociant of 26	W. Va. University
105	Dissociant of 26	W. Va. University
106	Dissociant of 26	W. Va. University
107	Dissociant of 26	W. Va. University
108	Dissociant of 36	W. Va. University
109	Dissociant of 36	W. Va. University
110	Dissociant of 36	W. Va. University
111	Dissociant of 36	W. Va. University
112	Dissociant of 36	W. Va. University
113	Dissociant of 36	W. Va. University
114	Dissociant of 40	W. Va. University
115	Dissociant of 40	W. Va. University
116	Dissociant of 40	W. Va. University
117	*B. aerogenes*	W. Va. Medical School
118	*B. cloacae*	W. Va. Medical School
119	Sweet corn	W. Va. University
120	Potato	W. Va. University

shown, the failure of agglutination is not proof of specific differences."

Leach reports in one article *(26)* in 1931 that no consistent morphologic or physiologic difference could be found between any cultures of the "blackleg" pathogens except that *B. solanisaprus* did not liquefy gelatin whereas the others did. In another article *(27)* in the same year he concludes as a result of extensive research that all soft-rot and blackleg cultures are of the same species. He suggested the name *B. carotovorus* and considers as synonyms all of the following: *B. atrosepticus, B. phytophthorus, B. solanisaprus, B. melanogenes, B. oleraceae, B. omnivorus,* and *B. apivorus.* He very ably summed up the literature as follows:

"A careful comparison of the data published by various workers dealing with the various soft-rotting bacteria reveals the following facts:

"1. The differences between the various 'blackleg' pathogens as described by different workers are as great as or greater than those which are supposed to distinguish them from *Bacillus carotovorus.*

"2. The differences found by various workers using, presumably, the same organisms are as great as or greater than those used for distinguishing the blackleg pathogen from *Bacillus carotovorus.*

"3. The differences between the 6 groups of soft-rotting bacteria described by Harding and Morse, of which *Bacillus carotovorus* is the type, are greater than those that are used for distinguishing the blackleg pathogen from them.

"4. The revised description of the blackleg pathogen as given by Jennison agrees in all essential details with the recognized characteristics of *Bacillus carotovorus.*"

Stanley and Orton *(52)* in 1932 pointed out the close similarity of *B. dissolvens* to *B. carotovorus* based on physiologic studies and in 1933 the same authors *(38)* showed that agglutination tests cannot be used to determine relationship between cultures of bacteria in this group. They also assert that the soft-rot bacteria should not be considered as distinct species but rather as variants of the *Bacillus coli* group.

Further physiologic studies on the soft-rot and *B. coli* group were reported by Stanley *(54)* in 1935 while Henry of the same laboratory reported on a pathologic comparison.

It is to be regretted that most of the comparative studies carried out in the past have been confined to a few cultures. Bacteriologists are coming to realize that bacterial culture is not a stable entity. Many strains must be studied comparatively and at intervals over a considerable period of time before an adequate understanding of any related group of bacteria can be reached.

PHYSIOLOGIC STUDIES

Materials and Methods

The cultures used in this comparative study are shown in Chart 1; as can be seen, they were obtained from different sources in the United States and Japan, but a majority of them are from isolations at our own laboratory. Cultures 5, 6, and 7 were isolated from stalk-rot of sweet corn and are identical with *B. dissolvens.* Culture 75 is *B. aerogenes,* while 76 and 77 are *B. coli.*

The Manual of Methods for Pure Culture Study of Bacteria was followed for both physiologic and serologic procedures.

Dunham fermentation tubes were used for all fermentation studies. The medium used was bacto-nutrient broth with 1% sugar added and brom-cresol-purple as an indicator. Brom-thymol-blue was tried as the indicator in the first series, but was not satisfactory because of the difficulty of reading the slight changes. However, brom-cresol-purple has proved very satisfactory. A check of nutrient broth plus indicator but without sugar was run as a control in each series.

A 1% solution of bacto-tryptone in distilled water was used for indole production. Ether and Ehrlich's reagent were the test solutions used. The test was made at 48 hours.

Bacto-M.R.V.P. medium was used for the methyl red and Voges-Proskauer tests. This was also made at 48 hours. One drop of 1% $FeCl_2$ solution was added to each tube for the Voges-Proskauer test to hasten the color change.

Nutrient broth plus 0.1% KNO_3 was the medium in nitrate reduction test. No attempt was made to determine the presence of reduction products other than nitrites. The test was also made at 48 hours using the reagents dissolved in 5 N. acetic acid.

Bacto-Koser's Citrate was used to determine the ability of the organisms to utilize sodium citrate for their carbon supply.

The entire group of organisms was inoculated into all these 9 kinds of media in one day. Inoculations were made from nutrient broth cultures approximately 24 hours old.

Physiologic Results

With but very few exceptions, which are probably of minor importance, the physiological characteristics of the soft-rot bacteria are practically the same. Chart 2 shows their most important characters as compiled from literature, together with a comparison with *B. coli communior.*

As will be seen, this group of bacteria is composed of forms which are gram negative and which produce no pigment on agar. These organisms reduce nitrates to nitrites and produce acid in milk. However, no such agreement is found in connection with such tests as indole production and the hydrolysis of starch; both positive and negative results are recorded on each of these tests for every strain.

CHART 2

	B. coli communior	*B. dissolvens*	*B. carotovorus*	*B. solanisaprus*	*B. phytophthorus*	*B. destructans*	*B. croci*	*B. aroidcae*	*B. melonis*
Gram stain	—	—	—	—	—	—	—	—	—
Color on agar	white	white	white	white	white	white	white	white	white
Nitrates reduced	+	+	+	+	+	+	+	+	+
Indole produced	±	+R —S	+H&M —L	+B —L	+B —L	+	—	+L* —H&M	+
H_2S produced	+	—R +S		+	+		—		+
Starch hydrolized	·	+R —S	+H&M —L	+L —B	+B —L	+		+L —H&M	
Milk curdled	+	+	+	+	+	+	+	+	+
Milk cleared		slight	—	—	—			slight	—
Gelatine liquified	—	—R +S	+	+B —L	+	+	+	+	+
Acid and gas in dextrose	+ +	+ +	+ +	+	+	+ —	+ —	+ —	+ —
Acid and gas in lactose	+ +	+ +	+ +	+ +	+ +	+ ±	+ —	+ —	+ —
Acid and gas in sucrose	+ +	+ +	+ +	+	+	+ —		+ —	+ —
Acid and gas in mannite		+ +	+ +	+ +	+ +			+ —	
Acid and gas in glycerine			+ +	+				+ —	+ —

*R—Rosen L—Leach B—Bergey H&M—Harding and Morse S—Stanley

HART 3—*Fermentation of dextrose*

Number	YEAR					
	1933				1934	
	2-13	3-16	5-13	7-27	1-2	10-24
1	+ —	+ —	+ —	+ —	+ —	+ —
2	+ —	+ —	+ —	+ —	+ —	+ —
3	+ —	+ —	+ —	+ —	+ —	— —
4	+ +	+ +	+ 61	+ 75	+ 62	+ 75
5	+ +	+ +	+ 75	+ 75	+ 59	+ 75
6	+ +	+ +	+ 75	+ 75	+ 75	+ 75
7	+ +	+ +	+ 49	+ 75	+ 75	+ 66
8	+ —	+ —	+ —	+ —	+ —	+ —
9	+ —	+ —	+ —	+ —	+ —	+ —
10	+ —	+ —	+ —	+ —	+ —	+ —
11	+ —	+ —	+ —	+ —	+ —	+ —
12	+ —	+ —	— —	— —	+ —	+ —
13	— —	— —	— —	— —	— —	— —
14	+ —	+ —	+ —	+ —	+ —	+ —
15	+ —	+ —	— —	+ —	+ —	+ —
16	+ —	+ —	+ —	+ —	+ —	+ —
17	+ —	+ —	+ —	+ —	+ —	+ —
18	— —	— —	+ —	+ —	+ —	+ —
19	+ +	+ +	+ 43	+ 39	+ 32	+ 34
20	+ +	+ +	+ 2	+ 4	+ —	+ —
21	+ +	+ +	+ 63	+ 75	+ 65	+ 75
22	+ —	+ —	+ —	+ —	+ —	+ —
23	+ —	+ —	+ —	+ —	+ —	+ —
24	+ —	+ —	— —	+ —	— —	— —
25	+ +	+ +	+ 75	+ 75	+ 54	+ 75
26	+ —	+ —	— —	+ —	+ —	+ —
27	+ —	+ —	+ —	+ —	+ —	+ —
28	+ —	+ —	+ —	+ —	+ —	
29	+ +	+ +	+ 75	+ 75	+ 75	+ 75
30	+ +	+ +	+ 75	+ 75	+ 75	+ 75
31	+ —	+ —	+ —	+ —	+ —	+ —
32	+ —	— —	— —	— —	— —	— —
33	+ —	+ —	+ —	+ —	+ —	+ —
34	+ —	+ —	— —	+ —	+ —	+ —
35	+ +	+ +	+ 17	+ 16	+ —	+ —
36	+ —	+ —	+ —	+ —	+ —	+ —
37	— —	— —	— —	— —	— —	— —
38	+ +	+ +	+ 2	+ 3	+ 3	+ —
39	+ —	+ —	— —	+ —	+ —	— —
40	+ —	— —	— —	— —	— —	— —
41	+ —	+ —	+ —	+ —	+ —	+ —
42	+ —	+ —	+ —	+ —	+ 2	+ —
43	+ +	+ +	+ 15	+ 23	+ 30	+ 16
44	+ +	+ +	+ 15	+ 16	+ 19	— —
45	+ +	+ +	+ 75	+ 75	+ 54	+ 75
46	+ +	+ +	+ 75	+ 46	— —	— —
47	— —	+ —	+ —	+ —	+ —	+ —
48	+ +	+ +	+ 14	+ 20	+ 17	+ 15
49	+ —	— —	+ —	+ —	+ —	+ —
50	+ —	+ —	— —	+ —	+ —	+ —
51	+ +	+ +	+ 14	+ 15	+ 17	+ 15
52	+ +	+ +	+ 14	+ 20	+ 20	+ 16
53	+ +	+ +	+ 10	+ 16	+ 16	+ 13
54	+ —	+ —	+ —	+ —	+ —	+ —
55	+ —	+ —	+ —	+ —	+ —	+ —
56	+ —	+ —	+ —	+ —	+ —	+ —
57	+ +	+ +	+ 15	+ 13	+ 12	+ 9
58	+ —	+ —	+ —	— —	+ —	+ —
59	+ +	+ +	+ 15	+ 23	+ 16	+ 11
60	+ —	+ —	— —	— —	— —	— —

Number	YEAR					
	1933				1934	
	2-13	3-16	5-13	7-27	1-2	10-24
61	+ —	— —	— —	— —	— —	+ —
62	+ +	+ +	+ 14	+ 12	+ 23	+ 15
63	+ +	+ +	+ 13	+ 12	+ 20	+ 13
64	+ +	+ +	+ 20	+ 19	+ 18	+ 21
65	+ +	+ +	+ 20	+ 22	+ 19	+ 19
66	+ +	+ +	+ 12	+ 9	+ 13	+ 9
67	+ +	+ +	+ 14	+ 18	+ 15	+ 12
68	+ +	+ +	+ 75	+ 75	+ 54	+ 70
69	+ +	+ +	+ 5	+ 5	+ 5	+ 5
70	+ +	+ +	+ 75	+ 75	+ 54	+ 71
71	+ +	+ +	+ 75	+ 75	+ 75	+ 65
72	+ +	+ +	+ 6	+ 11	+ 10	+ 10
73	+ +	+ +	+ 8	+ 7	+ 9	+ 9
74	+ +	+ +	+ 6	+ 10	+ 12	+ 6
75		+ +	+ 58	+ 67	+ 57	+ 66
76		+ +	+ 18	+ 15	+ 17	+ 14
77		+ +	+ 17	+ 13	+ 17	+ 12
78		+ —	+ —	+ —	+ —	+ —
79		— —	— —	— —	— —	— —
80		+ —	+ —	+ —	+ —	+ —
81		+ —	+ —	+ —	+ —	+ —
82		+ —	+ —	+ —	+ —	+ —
83		+ —	+ —	+ —	+ —	+ —
84		+ —	+ —	+ —	— —	
85		+ —	+ —			
86		+ —	+ —	+ —	+ —	+ —
87		+ —	+ —	+ —	+ —	+ —
88		— —	— —	— —	— —	— —
89		+ —	+ —			
90		— —	+ —	— —	+ —	+ —
91		— —	— —	— —	— —	— —
92		— —	— —	— —	— —	— —
93		— —	— —	— —	— —	— —
94		— —	± —		— —	— —
95		+ —	± —	— —	— —	— —
96		— —	— —	— —	— —	— —
97		+ —	— —	— —	— —	— —
98		+ +	+ 17	+ 16	+ 23	+ 10
99		+ —	— —	— —	+ —	+ —
100		+ —	— —	— —	— —	— —
101		+ —	— —	— —	— —	— —
102		+ —	+ —	+ —	+ —	+ —
103		+ +	+ 15	+ 19	— —	— —
104		— —	— —	— —	— —	— —
105		+ —	— —	— —	— —	— —
106		+ +	+ 19	+ 25	+ 25	+ 19
107		+ +	+ 13	+ 18	+ 17	— —
108		— —	— —	— —	— —	— —
109		— —	— —	— —	— —	— —
110		— —	— —	— —	— —	— —
111		— —	± —	+ —		
112		+ —	— —	+ —	+ —	+ —
113		+ —	+ —	— —	+ —	+ —
114		+ +	+ 18	— —	— —	— —
115		+ +	+ 15	+ 25	+ 29	+ 8
116		+ +	+ 29	— —	— —	— —
117			+ 34	+ 38	+ 43	+ 39
118			+ 34	+ 34	+ 35	+ 34
119						+ 62
120						+ —

CHART 4—*Fermentation of lactose*

Number	YEAR					
	1933				1934	
	2-13	3-16	5-13	7-27	1-2	10-24
1	+ —	+ —	+ —	+ —	+ —	+ —
2	+ —	+ —	+ —	+ —	+ —	+ —
3	+ —	+ —	+ —	+ —	+ —	— —
4	+ —	+ +	+ 2	+ 6	+ T	+ 4
5	+ —	+ +	+ 5	+ 14	+ 27	+ 15
6	+ +	+ +	+ 6	+ 7	+ 13	+ 8
7	+ —	+ +	+ 5	+ 13	+ 8	+ 8
8	± —	— —	— —	— —	— —	— —
9	± —	+ —	+ —	+ —	+ —	+ —
10	+ —	+ —	+ —	+ —	+ —	+ —
11	± —	— —	— —	— —	+ —	+ —
12	± —	— —	— —	— —	— —	— —
13	— —	— —	— —	— —	— —	— —
14	+ —	+ —	+ —	+ —	+ —	+ —
15	± —	— —	— —	— —	— —	— —
16	+ —	+ —	+ —	+ —	+ —	+ —
17	+ —	+ —	+ —	+ —	+ —	+ —
18	— —	— —	+ —	+ —	+ —	+ —
19	+ +	+ +	+ 23	+ 28	+ 33	+ 30
20	+ +	+ +	+ 5	+ 5	+ 5	+ 2
21	+ +	+ +	+ 7	+ 10	+ 8	+ 8
22	+ —	+ —	+ —	+ —	+ —	+ —
23	+ —	+ —	+ —	+ —	+ —	+ —
24	± —	— —	— —	— —	— —	— —
25	+ +	+ +	+ 16	+ 20	+ 20	+ 12
26	± —	— —	— —	— —	— —	— —
27	+ —	+ —	+ —	+ 3	+ —	+ —
28	+ —	+ —	+ —	+ —	+ —	
29	+ +	+ +	+ 15	+ 19	+ 20	+ 12
30	+ +	+ +	+ 14	+ 23	+ 19	+ 12
31	+ —	+ —	+ —	+ —	+ —	+ —
32	± —	— —	— —	— —	— —	— —
33	+ —	+ —	+ —	+ —	+ —	+ —
34	± —	— —	— —	— —	— —	— —
35	+ —	— —	+ —	+ —	— —	— —
36	— —	— —	— —	— —	— —	— —
37	— —	— —	— —	— —	— —	— —
38	+ +	+ +	+ 4	+ 7	+ 6	+ T
39	± —	— —	— —	— —	— —	— —
40	± —	— —	— —	— —	— —	— —
41	± —	— —	— —	— —	— —	— —
42	+ —	+ —	+ —	+ —	+ —	+ —
43	+ —	+ +	+ 2	+ 6	+ 11	+ 4
44	+ +	+ +	+ 2	+ 5	+ 5	— —
45	+ +	+ +	+ 6	+ 9	+ 10	+ 6
46	+ +	+ +	+ 10	+ 12	— —	— —
47	+ —	— —	+ —	+ —	+ —	+ —
48	+ +	+ +	+ 3	+ 7	+ 7	+ 6
49	± —	— —	— —	— —	— —	— —
50	± —	— —	— —	— —	— —	— —
51	+ +	+ +	+ 6	+ 15	+ 8	+ 4
52	+ +	+ +	+ 5	+ 7	+ 8	+ 5
53	+ +	+ +	+ 9	+ 6	+ 15	+ 14
54	+ —	+ —	+ —	+ —	+ —	+ —
55	+ —	+ —	+ —	+ —	+ —	+ —
56	+ —	+ —	+ —	+ —	+ —	+ —
57	+ +	+ +	+ 2	+ 10	+ 8	+ 5
58	± —	— —	— —	— —	— —	— —
59	+ —	+ +	+ 3	+ 7	+ 15	+ 7
60	± —	— —	— —	— —	— —	— —

Number	YEAR					
	1933				1934	
	2-13	3-16	5-13	7-27	1-2	10-2
61	± —	— —	— —	— —	— —	+ —
62	+ +	+ +	+ 8	+ 13	+ 17	+ 1
63	+ +	+ +	+ 2	+ 10	+ 19	+
64	+ +	+ +	+ 17	+ 18	+ 10	+ 1
65	+ +	+ +	+ 15	+ 17	+ 18	+ 1
66	+ +	+ +	+ 17	+ 16	+ 13	+ 1
67	+ +	+ +	+ 14	+ 13	+ 10	+ 1
68	+ +	+ +	+ 5	+ 10	+ 10	+
69	± —	— —	— —	+ —	— —	— —
70	+ —	+ —	+ 5	+ 14	+ 5	+
71	± —	+ —	+ 2	+ 6	+ 11	+ 3
72	± —	— —	— —	— —	— —	— —
73	± —	— —	— —	— —	— —	— —
74	± —	— —	— —	— —	— —	— —
75		+ +	+ 70	+ 75	+ 56	+ 7
76		+ +	+ 19	+ 21	+ 17	+ 1
77		+ +	+ 20	+ 15	+ 16	+ 1
78		+ —	+ —	+ —	+ —	+ —
79		— —	— —	— —	— —	— —
80		+ —	+ —	+ —	+ —	+ —
81		— —	+ —	+ —	+ —	— —
82		— —	+ —	+ —	+ —	+ —
83		— —	— —	+ —	+ —	+ —
84		— —	— —	— —	— —	
85		— —	— —			
86		— —	+ —	+ —	+ —	— —
87		+ —	+ —	+ —	+ —	+ —
88		— —	— —	— —	— —	— —
89		— —	— —			
90		— —	+ —	+ —	+ —	+ —
91		— —	— —	— —	— —	— —
92		— —	— —	— —	— —	— —
93		— —	— —	— —	— —	— —
94		— —	— —		— —	— —
95		— —	— —	— —	— —	— —
96		— —	— —	— —	— —	— —
97		— —	— —	— —	— —	— —
98		+ +	+ 2	+ 7	+ 18	+
99		— —	— —	— —	— —	— —
100		— —	— —	— —	— —	— —
101		— —	— —	— —	— —	— —
102		— —	— —	— —	— —	— —
103		+ +	— —	+ 8	— —	— —
104		— —	— —	— —	— —	— —
105		— —	— —	— —	— —	— —
106		+ +	+ 2	+ 14	+ 8	+
107		+ +	+ 3	+ 5	+ 13	— —
108		— —	— —	— —	— —	— —
109		— —	— —	— —	— —	— —
110		— —	— —	— —	— —	— —
111		— —	— —	— —		
112		— —	— —	— —	— —	— —
113		— —	— —	— —	— —	— —
114		+ +	+ 2	— —	— —	— —
115		+ +	+ 2	+ 13	+ 7	+ 6
116		+ +	+ 2	— —	— —	— —
117			+ 25	+ 31	+ 31	+ 25
118			+ 24	+ 25	+ 31	+ 24
119						+ 8
120						+ —

RT 5—*Fermentation of sucrose*

YEAR						Number	YEAR					
1933			1934				1933			1934		
2-13	3-16	5-13	7-27	1-2	10-24		2-13	3-16	5-13	7-27	1-2	10-24
+ —	+ —	+ —	+ —	+ —	+ —	61	— —	— —	— —	— —	— —	+ —
+ —	+ —	+ —	+ —	+ —	+ —	62	+ +	+ +	+ 14	+ 12	+ 30	+ 15
+ —	+ +	+ —	+ —	+ —	— —	63	+ +	+ +	+ 18	+ 19	+ 21	+ 12
+ +	+ +	+ 75	+ 75	+ 57	+ 75	64	+ —	— —	— —	— —	— —	— —
+ +	+ +	+ 75	+ 75	+ 53	+ 72	65	+ +	+ +	+ 22	+ 18	+ 20	+ 20
+ +	+ +	+ 75	+ 75	+ 52	+ 69	66	+ —	— —	— —	— —	— —	— —
+ +	+ +	+ 75	+ 75	+ 57	+ 57	67	+ +	+ +	+ 9	+ 10	+ 16	+ 15
± —	— —	— —	— —	— —	— —	68	+ +	+ +	+ 75	+ 75	+ 55	+ 62
+ —	+ —	+ —	+ —	+ —	+ —	69	+ +	+ +	+ 5	+ 5	+ 5	+ —
+ —	+ —	+ —	+ —	+ —	+ —	70	+ +	+ +	+ 75	+ 75	+ 55	+ 75
— —	— —	— —	— —	— —	— —	71	+ +	+ +	+ 75	+ 75	+ 57	+ 68
± —	— —	— —	— —	— —	— —	72	— —	— —	— —	— —	— —	— —
— —	— —	— —	— —	— —	— —	73	+ —	— —	— —	— —	— —	— —
+ —	+ —	+ —	+ —	+ —	+ —	74	— —	— —	— —	— —	— —	— —
± —	— —	— —	— —	— —	— —	75		+ +	+ 75	+ 75	+ 75	+ 70
+ —	+ —	+ —	+ —	+ —	+ —	76		— —	+ —	+ —	+ 4	+ 8
+ —	+ —	+ —	+ —	+ —	+ —	77		— —	— —	+ —	+ ?—	+ 2
— —	— —	+ —	+ —	+ —	+ —	78		+ —	+ —	+ 4	+ —	+ —
+ +	+ +	+ 37	+ 37	+ 53	+ 32	79		— —	— —	— —	— —	— —
+ +	+ —	+ 4	+ 5	+ —	+ —	80		+ —	+ —	+ —	+ —	+ —
+ +	+ +	+ 75	+ 75	+ 60	+ 75	81		+ —	+ —	+ 5	+ —	+ —
+ —	+ —	+ —	+ —	+ —	+ —	82		+ —	+ —	+ —	+ —	+ —
+ —	+ —	+ —	+ —	+ —	+ —	83		+ —	+ —	+ —	+ —	+ —
± —	— —	— —	— —	— —	— —	84		+ —	+ —	+ —	— —	
+ +	+ +	+ 75	+ 75	+ 49	+ 65	85		+ —	+ —			
± —	— —	— —	— —	— —	— —	86		+ —	+ —	+ —	+ —	+ —
+ —	+ —	+ —	+ —	+ 2	+ —	87		+ —	+ —	+ —	+ —	+ —
+ —	+ —	+ —	+ —	+ —		88		— —	— —	— —	— —	— —
+ +	+ +	+ 75	+ 75	+ 57	+ 75	89		+ —	— —			
+ +	+ +	+ 75	+ 75	+ 57	+ 68	90		— —	— —	+ —	— —	+ —
+ —	+ —	+ —	+ —	+ —	+ —	91		— —	— —	— —	— —	— —
± —	— —	— —	— —	— —	— —	92		— —	— —	— —	— —	— —
+ —	+ —	+ —	+ —	+ —	+ —	93		— —	— —	— —	— —	— —
± —	— —	— —	— —	— —	— —	94		+ —	— —		— —	— —
+ —	+ —	+ —	+ —	+ —	+ —	95		+ +	+ +	+ +	+ +	+ +
— —	— —	— —	— —	— —	— —	96		— —	— —	— —	— —	— —
— —	— —	— —	— —	— —	— —	97		— —	— —	— —	— —	— —
+ ±	+ +	+ 2	+ 4	+ 5	+ —	98		+ +	+ 20	+ 20	+ 37	+ 12
± —	— —	— —	— —	— —	— —	99		— —	— —	— —	— —	— —
± —	— —	— —	— —	— —	— —	100		— —	— —	— —	— —	— —
± —	— —	— —	— —	— —	— —	101		— —	— —	— —	— —	— —
+ —	+ —	+ —	+ —	+ —	+ —	102		— —	— —	— —	— —	— —
+ +	+ +	+ 20	+ 20	+ 26	+ 18	103		+ +	+ 12	+ 23	— —	— —
+ +	+ +	+ 22	+ 16	+ 13	— —	104		— —	— —	— —	— —	— —
+ +	+ +	+ 75	+ 75	+ 55	+ 69	105		— —	— —	— —	— —	— —
+ +	+ +	+ 75	+ 75	— —	— —	106		+ +	+ 14	+ 30	+ 28	+ 11
± —	— —	— —	— —	— —	— —	107		+ +	+ 17	+ 14	+ 38	— —
+ +	+ +	+ 18	+ 13	+ 15	+ 14	108		— —	— —	— —	— —	— —
+ —	+ +	— —	— —	+ —	+ —	109		— —	— —	— —	— —	— —
+ —	+ —	— —	— —	+ —	+ —	110		— —	— —	— —	— —	— —
+ +	+ +	+ 14	+ 16	+ 16	+ 10	111		+ —	— —	+ —		
+ +	+ +	+ 10	+ 15	+ 17	+ 10	112		— —	— —	— —	— —	— —
+ +	+ +	+ 14	+ 16	+ 15	+ 11	113		— —	— —	— —	— —	— —
+ —	+ —	+ —	+ —	+ —	+ —	114		+ +	+ 24	— —	— —	— —
+ —	+ —	+ —	+ —	+ —	+ —	115		+ +	+ 20	+ 50	+ 44	+ 14
+ —	+ +	+ —	+ —	+ —	+ —	116		+ +	+ 34	— —	— —	— —
+ +	+ +	+ 13	+ 17	+ 14	+ 9	117			+ 42	+ 51	+ 63	+ 64
± —	— —	— —	— —	— —	— —	118			+ 41	+ 27	+ 43	+ 30
+ +	+ +	+ 25	+ 30	+ 20	+ 10	119						+ 62
— —	— —	— —	— —	— —	— —	120						+ —

The results on the fermentation of dextrose, lactose, and sucrose are shown in Charts 3, 4, and 5. In the first two series no record was made of the amount of gas produced; however, beginning with the third series this datum also was taken. The gas tubes inverted in the broth were 75 mm. long and the amount of gas produced was recorded in millimeters.

The control tubes of nutrient broth without sugar showed no acid or gas except with 3 organisms, #18, 61, and 90. Culture 18 showed an acid reaction in the control tube on the 3rd, 4th, and 6th series. In these same series the same acid reaction was evident in all three sugars. Culture 61 in the 6th series and culture 90 in the 3rd, 4th, 5th, and 6th series showed the same reaction in the controls. However, culture 90 did not always show an acid reaction in all of the sugars when it did in the controls.

After each series of tests the organisms were separated into generic "types" on the basis of their fermentation; Chart 6 shows how this classification varied from series to series. Of the 120 cultures, 38 changed at least once from one generic "type" to another. Altogether, 43 organisms made 67 changes in the 3 sugars but many of these changes were not the kind to cause a change from one generic "type" to another.

One culture, #103, has undergone changes which have placed it in 3 generic types. At times one will make a change and then revert to the original type, as for instance culture #34.

After completing the six series of tests, the organisms were classified into groups according to their ability to produce acid and gas in the sugars, with the following results: 41 cultures produced both acid and gas in all three sugars; 13 produced acid in all the sugars and gas in one or two; 20 produced acid but no gas from all three sugars; 25 produced acid from one or two of the sugars; and 21 cultures produced neither acid nor gas from any of the sugars at any time. Thus in this group of cultures we have all degrees represented from no fermentation to the production of acid and gas from all 3 sugars.

The production of indole gave the most consistent results of any physiologic test used. Only 8 organisms (#62, 64, 65, 67, 69, 76, and 77) have shown the ability to split the indole ring from tryptophane. One of these (#62) did so for some time but has not since July, 1933. All others have been constant. It is of interest to note that culture #62, the only one showing a change, is a dissociant from a non-indole-producing culture, #40. Thus it reverted to the original type on this test. Culture #66, a *B. coli,* does not produce indole, and #69, a soft-rot organism, produces a positive test.

Chart 8 shows the results of the nitrate reduction tests. Only 13 cultures have shown both positive and negative results. Culture #109 has changed each time. Cultures #114 and 116 reduced the nitrates to nitrogen gas on the third series only. Cultures #35 and 95 made three changes each; cultures #61, 103, 114, and 116 made 2 changes, while #3, 14, 49, 95, 108, and 110 showed only 1 change in reaction.

CHART 6—*Distribution of organisms based on fermentation studies*

Number	YEAR					Number					
	1933			1934			1933			1934	
	3-16	5-13	7-27	1-2	10-24		3-16	5-13	7-27	1-2	10-24
1	2	2	2	2	2	61	3	3	3	3	3
2	2	2	2	2	2	62	1	1	1	1	1
3	2	2	2	2	3	63	1	1	1	1	1
4	1	1	1	1	1	64	1	1	1	1	1
5	1	1	1	1	1	65	1	1	1	1	1
6	1	1	1	1	1	66	1	1	1	1	1
7	1	1	1	1	1	67	1	1	1	1	1
8	2	2	2	2	2	68	1	1	1	1	1
9	2	2	2	2	2	69	4	4	4	4	5
10	2	2	2	2	2	70	4	1	1	1	1
11	2	2	2	2	2	71	4	1	1	1	1
12	2	3	3	2	2	72	5	5	5	5	5
13	3	3	3	3	3	73	5	5	5	5	5
14	2	2	2	2	2	74	5	5	5	5	5
15	2	3	2	2	2	75	1	1	1	1	1
16	2	2	2	2	2	76	1	1	1	1	1
17	2	2	2	2	2	77	1	1	1	1	1
18	3	3	2	2	3	78	2	2	2	2	2
19	1	1	1	1	1	79	3	3	3	3	3
20	1	1	1	2	2	80	2	2	2	2	2
21	1	1	1	1	1	81	2	2	2	2	2
22	2	2	2	2	2	82	2	2	2	2	2
23	2	2	2	2	2	83	2	2	2	2	2
24	2	3	2	3	3	84	2	2	2	3	
25	1	1	1	1	1	85	2	2			
26	2	3	2	2	2	86	2	2	2	2	2
27	2	2	2	2	2	87	2	2	2	2	2
28	2	2	2	2		88	3	3	3	3	3
29	1	1	1	1	1	89	2	2			
30	1	1	1	1	1	90	3	3	3	2	3
31	2	2	2	2	2	91	3	3	3	3	3
32	3	3	3	3	3	92	3	3	2	3	3
33	2	2	2	2	2	93	3	3	3	3	3
34	2	3	2	2	2	94	3	2		3	3
35	5	5	5	2	2	95	2	2	3	3	3
36	2	2	2	2	2	96	3	3	3	3	3
37	3	3	3	3	3	97	2	3	3	3	3
38	1	1	1	1	2	98	1	1	1	1	1
39	2	3	2	2	3	99	2	3	3	2	2
40	3	3	3	3	3	100	2	3	3	3	3
41	2	2	2	2	2	101	2	3	3	3	3
42	2	2	2	4	2	102	2	2	2	2	2
43	1	1	1	1	1	103	1	4	1	3	3
44	1	1	1	1	3	104	3	3	3	3	3
45	1	1	1	1	1	105	2	3	3	3	3
46	1	1	1	3	3	106	1	1	1	1	1
47	2	2	2	2	2	107	1	1	1	1	3
48	1	1	1	1	1	108	3	3	3	3	3
49	3	2	2	2	2	109	3	3	3	3	3
50	2	3	2	2	2	110	3	3	3	3	3
51	1	1	1	1	1	111	3	3	2		
52	1	1	1	1	1	112	2	3	2	2	2
53	1	1	1	1	1	113	2	2	3	2	2
54	2	2	2	2	2	114	1	1	3	3	3
55	2	2	2	2	2	115	1	1	1	1	1
56	2	2	2	2	2	116	1	1	3	3	3
57	1	1	1	1	1	117		1	1	1	1
58	2	2	3	2	2	118		1	1	1	1
59	1	1	1	1	1	119					1
60	2	3	3	3	3	120					2

1—Escherichia and Aerobacter
2—Eberthella and Shigella
3—Alkaligenes
4—Proteus
5—Salmonella

CHART 7—*Production of indole*

Number	YEAR					Number	YEAR				
	1933			1934			1933			1934	
	3-11	5-6	7-15	12-22	10-15		3-11	5-6	7-15	12-22	10-15
1	—	—	—	—	—	61	—	—	—	—	—
2	—	—	—	—	—	62	+	+	+	—	—
3	—	—	—	—	—	63	—	—	—	—	—
4	—	—	—	—	—	64	+	+	+	+	+
5	—	—	—	—	—	65	+	+	+	+	+
6	—	—	—	—	—	66	—	—	—	—	—
7	—	—	—	—	—	67	+	+	+	+	+
8	—	—	—	—	—	68	—	—	—	—	—
9	—	—	—	—	—	69	+	+	+	+	+
10	—	—	—	—	—	70	—	—	—	—	—
11	—	—	—	—	—	71	—	—	—	—	—
12	—	—	—	—	—	72	—	—	—	—	—
13	—	—	—	—	—	73	—	—	—	—	—
14	—	—	—	—	—	74	—	—	—	—	—
15	—	—	—	—	—	75	—	—	—	—	—
16	—	—	—	—	—	76	+	+	+	+	+
17	—	—	—	—	—	77	+	+	+	+	+
18	—	—	—	—	—	78	—	—	—	—	—
19	—	—	—	—	—	79	—	—	—	—	—
20	—	—	—	—	—	80	—	—	—	—	—
21	—	—	—	—	—	81	—	—	—	—	—
22	—	—	—	—	—	82	—	—	—	—	—
23	—	—	—	—	—	83	—	—	—	—	—
24	—	—	—	—	—	84	—	—	—	—	
25	—	—	—	—	—	85	—	—	—		
26	—	—	—	—	—	86	—	—	—	—	—
27	—	—	—	—	—	87	—	—	—	—	—
28	—	—	—	—	—	88	—	—	—	—	—
29	—	—	—	—	—	89	—	—	—		
30	—	—	—	—	—	90	—	—	—	—	—
31	—	—	—	—	—	91	—	—	—	—	—
32	—	—	—	—	—	92	—	—	—	—	—
33	—	—	—	—	—	93	—	—	—	—	—
34	—	—	—	—	—	94	—	—	—	—	—
35	—	—	—	—	—	95	—	—	—	—	—
36	—	—	—	—	—	96	—	—	—	—	—
37	—	—	—	—	—	97	—	—	—	—	—
38	—	—	—	—	—	98	—	—	—	—	—
39	—	—	—	—	—	99	—	—	—	—	—
40	—	—	—	—	—	100	—	—	—	—	—
41	—	—	—	—	—	101	—	—	—	—	—
42	—	—	—	—	—	102	—	—	—	—	—
43	—	—	—	—	—	103	—	—	—	—	—
44	—	—	—	—	—	104	—	—	—	—	—
45	—	—	—	—	—	105	—	—	—	—	—
46	—	—	—	—	—	106	—	—	—	—	—
47	—	—	—	—	—	107	—	—	—	—	—
48	—	—	—	—	—	108	—	—	—	—	—
49	—	—	—	—	—	109	—	—	—	—	—
50	—	—	—	—	—	110	—	—	—	—	—
51	—	—	—	—	—	111	—	—	—	—	—
52	—	—	—	—	—	112	—	—	—	—	—
53	—	—	—	—	—	113	—	—	—	—	—
54	—	—	—	—	—	114	—	—	—	—	—
55	—	—	—	—	—	115	—	—	—	—	—
56	—	—	—	—	—	116	—	—	—	—	—
57	—	—	—	—	—	117		—	—	—	—
58	—	—	—	—	—	118		+	+	+	+
59	—	—	—	—	—	119					—
60	—	—	—	—	—	120					—

CHART 8—*Reduction of nitrates to nitrites*

Number	YEAR					Number	YEAR				
	1933		1934				1934		1933		
	3-11	5-6	7-15	12-22	10-15		3-11	5-6	7-15	12-22	10-15
1	+	+	+	+	+	61	—	—	+	—	—
2	+	+	+	+	+	62	+	+	+	+	+
3	+	+	+	+	—	63	+	+	+	+	+
4	+	+	+	+	+	64	+	+	+	+	+
5	+	+	+	+	+	65	+	+	+	+	+
6	+	+	+	+	+	66	+	+	+	+	+
7	+	+	+	+	+	67	+	+	+	+	+
8	—	—	—	—	—	68	+	+	+	+	+
9	+	+	+	+	+	69	+	+	+	+	+
10	+	+	+	+	+	70	+	+	+	+	+
11	—	—	—	—	—	71	+	+	+	+	+
12	—	—	—	—	—	72	+	+	+	+	+
13	—	—	—	—	—	73	+	+	+	+	+
14	+	+	+	+	—	74	+	+	+	+	+
15	—	—	—	—	—	75	+	+	+	+	+
16	+	+	+	+	+	76	+	+	+	+	+
17	+	+	+	+	+	77	+	+	+	+	+
18	—	—	—	—	—	78	+	+	+	+	+
19	+	+	+	+	+	79	+	+	+	+	+
20	+	+	+	+	+	80	+	+	+	+	+
21	+	+	+	+	+	81	+	+	+	+	+
22	+	+	+	+	+	82	+	+	+	+	+
23	+	+	+	+	+	83	+	+	+	+	+
24	+	+	+	+	+	84	—	—	—	—	
25	+	+	+	+	+	85	—	—			
26	—	—	—	—	—	86	+	+	+	+	+ +
27	+	+	+	+	+	87	+	+	+	+	+
28	+	+	+	+	—	88	+	+	+	+	+
29	+	+	+	+	+	89	—	—			
30	+	+	+	+	+	90	—	—	—	—	—
31	+	+	+	+	+	91	+	+	+	+	+
32	+	+	+	+	+ +	92	+	+	+	+	+ +
33	+	+	+	+	+	93	+	+	+	+	+
34	—	—	—	—	—	94	—	—		+	+
35	—?	+	+	—	+	95	+	—	—	+	—
36	—	—	—	—	—	96	+	+	+	+	+
37	—	—	—	—	—	97	—?	+	+	+	+
38	+	+	+	+	+	98	+	+	+	+	+
39	+	+	+	+	+ +	99	—	—	—	—	—
40	—	—	—	—	—	100	+	+	+	+	+
41	—	—	—	—	—	101	—?	+	+	+	+
42	+	+	+	+	+	102	—	—	—	—	—
43	+	+	+	+	+	103	+	+	+	—	+
44	+	+	+	+	+	104	+	+	+	+	+
45	+	+	+	+	+	105	+	+	+	+	+
46	+	+	+	+	+	106	+	+	+	+	+
47	—	—	—	—	—	107	+	+	+	+	+
48	+	+	+	+	+	108	—	—	—	—	+
49	+	—	—	—	—	109	+	—	+	—	+
50	—	—	—	—	—	110	—?	—	—	+	+
51	+	+	+	+	+	111	—	—	—		
52	+	+	+	+	+	112	—	—	—	—	—
53	+	+	+	+	+	113	—?	—	—	—	—
54	+	+	+	+	+	114	+	+	Gas—	—	—
55	+	+	+	+	+	115	+	+	+	+	+ +
56	+	+	+	+	+	116	+	+	Gas—	+	+
57	+	+	+	+	+	117		+	+	+	+
58	—	—	—	—	—	118		+	+	+	+
59	+	+	+	+	+	119					+
60	+	+	+	+	+	120					—

CHART 9—*Voges-Proskauer reaction*

Number	YEAR					Number	YEAR				
	1933			1934			1933			1934	
	3-21	5-13	7-21	12-20	10-15		3-21	5-13	7-21	12-20	10-15
1	+?	+	+	+	—	61	—	—	—	—	—
2	—	—	—	—	—	62	+	+?	—	+?	—
3	—	—	—	—	—	63	—	—	—	—	—
4	+	++	+	+	+	64	—	—	—	—	—
5	+	++	+	+	+	65	—	—	—	—	—
6	—	++	+	+	+	66	—	—	—	—	—
7	+	++	+	+	+	67	—	—	—	—	—
8	—	—	—	—	—	68	+	++	+	+	+
9	+	+	+	—	—	69	—	—	—	—	—
10	+	++	+	+?	+	70	+	++	+	+	+
11	—	—	—	—	—	71	—	++	+	+	+
12	—	—	—	—	—	72	—	—	—	—	—
13	—	—	—	—	—	73	—	—	—	—	—
14	+	++	+	+	+	74	—	—	—	—	—
15	—	—	—	—	—	75	+	++	—	+	+
16	+	++	+	+	+	76	—	—	—	—	—
17	+	++	+	+	+	77	—	—	—	—	—
18	—	—	—	—	—	78	—	—	—	—	—
19	+	++	+	+	+	79	—	—	—	—	—
20	—	+?	+?	—	—	80	—	—	—	—	—
21	+	++	+	+?	+	81	—	—	—	—	
22	—	+	+?	—	—	82	—	—	—	—	—
23	—	—	+?	—	—	83	—	—	—	—	—
24	—	—	—	—	—	84	—	—	—	—	
25	+	++	+	+	+	85	—	—	—		
26	—	—	—	—	—	86	—	—	—	—	—
27	+?	+?	+	—	—	87	—	—	—	—	—
28	+	+	+	—		88	—	—	—	—	—
29	+	++	+	+	+	89	—	—	—		
30	+	+	+		+	90	—	—	—	—	—
31	+?	+?	+?	—	—	91	—	—	—	—	—
32	—	—	—	—	—	92	—	—	—	—	—
33	+	++	+	+	+	93	—	—	—	—	—
34	—	—	—	—	—	94	—	—	—	—	—
35	—	—	—	—	—	95	—	—	—	—	—
36	—	—	—	—	—	96	—	—	—	—	—
37	—	—	—	—	—	97	—	—	—	—	—
38	—	—	—	—	+?	98	+	+?	+	+	+
39	—	—	—	—	—	99	—	—	—	—	—
40	—	—	—	—	—	100	—	—	—	—	—
41	—	—	—	—	—	101	—	—	—	—	—
42	—	—	—	—	—	102	—	—	—	—	—
43	+?	+?	+?	+	+?	103	+?	+	—	—	—
44	+?	+	—	+	—	104	—	—	—	—	—
45	+	+	+	+	+	105	—	—	—	—	—
46	+	++	+	—	—	106	+?	+?	—	+	+
47	—	—	—	—	—	107	+	+	+	+	—
48	+	++	+	+	+	108	—	—	—	—	—
49	—	—	—	—	—	109	—	—	—	—	—
50	—	—	—	—	—	110	—	—	—	—	—
51	+	+?	+?	+	+	111	—	—	—		
52	+	++	+	+?	+	112	—	—	—	—	—
53	+	++	+	+	+	113	—	—	—	—	—
54	—	—	—	—	—	114	+?	+?	—	—	—
55	—	—	—	—	—	115	+	+	—	—	—
56	+	—	—	—	—	116	+?	+?	—	—	—
57	—	+	—	—	—	117		+	+?	—	+?
58	—	—	—	—	—	118		++	+	+	+
59	+?	+	—	+?	+	119					+
60	—	—	—	—	—	120					—

CHART 10—*Methyl red test*

Number	YEAR					Number	YEAR				
	1933			1934			1933			1934	
	3-21	5-13	7-21	12-20	10-13		3-21	5-13	7-21	12-20	10-13
1	+?	—	—	+	—	61	—	—	—	—	—
2	—	+	+	—	+	62	—	—	—	+?	—
3	+	+	+	+	—	63	+?	—	—	—	
4	—	—	—	—	—	64	++	++	++	+	++
5	—	—	—	—	—	65	++	++	++	+	++
6	—	—	—	—	—	66	++	++	++	+	++
7	—	—	—	—	—	67	++	+	++	+	++
8	—	—	—	—	—	68	—	—	—	—	—
9	—	+	+	+?	+	69	+	+	+	+	+
10	—	—	—	—	—	70	—	—	—	—	—
11	—	—	—	—	—	71		—	—	—	—
12	—	—	—	—	—	72	—	+	+	+?	—
13	—	—	—	—	—	73	—	+	+	+?	—
14	—	—	—	—	—	74	—	+?	+	+?	—
15	—	—	—	—	—	75	—	—	—	—	—
16	—	—	—	—	—	76	++	++	++	+	++
17	—	—	—	—	—	77	++	++	++	+	++
18	—	—	—	—	—	78	—	+	+	—	+
19	—	—	—	—	—	79	—	—	—	—	—
20	+	+	+	+	+	80	—	+	+	—	—
21	—	—	—	—	—	81	—	—	—	—	
22	+	+	+	+	+	82	—	+	+	—	—
23	+	+	+	+	+	83	—	+	+	—	—
24	—	—	—	—	—	84	—	—	—	—	
25	—	—	—	—	—	85	—	—	—		
26	—	—	—	—	—	86	—	+	+	—	—
27	+	+	+	+	+	87	—	+	+	+?	—
28	—	—	—	+		88	—	—	—	—	—
29	—	—	—	—	—	89	—	—	—		
30	—	—	—	—	—	90	—	—	—	—	—
31	+	+	+	+	+	91	—	—	—	—	—
32	—	—	—	—	—	92	—	—	—	—	—
33	—	—	—	—	—	93	—	—	—	—	—
34	—	—	—	—	—	94	—	—	—	—	—
35	+	+	+	—	—	95	—	—	—	—	—
36	—	—	—	—	—	96	—	—	—	—	—
37	—	—	—	—	—	97	—	—	—	—	—
38	—	—	—	—	+	98	+?	—	+	—	+
39	—	—	—	—	—	99	—	—	—	—	—
40	—	—	—	—	—	100	—	—	—	—	—
41	—	—	—	—	—	101	—	—	—	—	—
42	+	+	+	+	+	102	—	—	—	—	—
43	—	—	—	—	—	103	+?	—	—	—	—
44	—	—	—	+	—	104	—	—	—	—	—
45	—	—	—	—	—	105	—	—	—	—	—
46	—	—	—	—	—	106	+?	—	—	—	+
47	—	—	—	—	—	107	+	—	—	+?	—
48	+?	+?	+	+	+	108	—	—	—	—	—
49	+?	—	—	—	—	109	—	—	—	—	—
50	—	—	—	—	+	110	—	—	—	—	—
51	—	—	+?	+?	+	111	—	—	—		
52	+	+	—	+?	+	112	—	—	—	—	—
53	—	+?	—	+	+	113	—	—	—	—	—
54	—	+	+	+	+	114	+?	—	—	—	—
55	—	+	+	+?	+	115	+?	—	—	—	—
56	—	+?	—	+?	+	116	—	—	—	—	—
57	+	—	—	+	+	117		—	—	—	+?
58	—	—	—	—	—	118		—	—	—	+
59	+?	—	—	+	—	119					+
60	—	—	—	—	—	120					—

CHART 11—*Growth in Koser's citrate solution*

Number	YEAR				
	1933			1934	
	3-21	5-13	7-21	12-20	11-3
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	—	—	—
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	+	+	+	+	+
11	+	+	+	+	+
12	+	+	+	+	+
13	+	+?	+	+	—
14	+	+	+	+	+
15	+	+	+	+	+
16	+	+	+	+	+
17	+	+	+	+	+
18	—	+	+	+	—
19	+	+	+	+	+
20	+	+	+	+	+
21	+	+	+	+	+
22	+	+	+	+	+
23	+	+	+	+	+
24	+	+	+	+	+
25	+	+	+	+	+
26	+	+	+	+	+
27	+	+	+	+	+
28	+	+	+	+	+
29	+	+	+	+	+
30	+	+	+	+	+
31	+	+	+	+	+
32	+	+	+	+	+
33	+	+	+	+	+
34	+	+	+	+	+
35	+	+	+	+	+
36	+	+	+	+	+
37	+	+	+	+	+
38	+	+	+	+	+
39	+	+	+	+	+
40	+	+	+	+	+
41	+	+	+	+	+
42	+	+	+	+	+
43	+	+	+	+	+
44	+	+	+	+	—
45	+	+	+	+	+
46	+	+	+	+	—
47	+	+	+	+	+
48	+	+	+	+	+
49	+	+	+	+	+
50	+	+	+	+	+
51	+	+	+	+	+
52	+	+	+	+	+
53	+	+	+	+	+
54	+	+	+	+	+
55	+	+	+	+	+
56	+	+	+	+	+
57	+	+	+	+	+
58	+	+	+	+	+
59	+	+	+	+	+
60	+	+	+	+	—

Number	YEAR				
	1933			1934	
	3-21	5-13	7-21	12-20	11-3
61	+	+	+	+	—
62	+	+	+	+	+
63	+	+	+	+	+
64	—	—	—	—	—
65	—	—	—	—	—
66	—	—	—	—	—
67	—	—	—	—	—
68	+	+	+	+	+
69	—	—	+	+	—
70	+	+	+	+	+
71	+	+	+	+	+
72	—	—	—	—	—
73	+	+	+	+	+
74	—	—	—	—	—
75	+	+	+	+	+
76	—	—	—	—	—
77	—	—	—	+	—
78	+	+	+	+	+
79	—	—	—	+	—
80	+	+	+	+	+
81	+	+	+	+	+
82	+		+	+	+
83	+	+	+	+	+
84	—	—	—	—	
85	—	—	—		
86	+	+	+	+	+
87	+	+	+	+	+
88	—	+?	—		—
89	—	—	—		
90	—	+	+	+	+
91	—	+?	—	+	—
92	+	+	+	+	+
93	+	—	—	+	+
94	—	—	—	+	—
95	+	+	+	+	+
96	+	+?	+	+	+
97	+	+	+	+	+
98	+	+	+	+	+
99	+	+	+	+	+
100	+	+	+	+	—
101	+	+	+	+	—
102	+	+	+	+	+
103	+	+	+	+	+
104	—	+?	+	+	—
105	+	+	+	+	—
106	+	+	+	+	+
107	+	+	+	+	—
108	+	+	+	+	—
109	+	+	+	+	+
110	+	+	+	+	—
111	—	—	—		
112	+	+	+	+	+
113	+	+	+	+	+
114	+	+	+	+	+
115	+	+	+	+	+
116	+	+	+	+	—
117		+	+	+	+
118		+	+	+	+
119					+
120					+

Seventeen cultures varied in the Voges-Proskauer reaction as seen in Chart 9. Culture #117, which is *B. aerogenes,* reacts negatively in the 4th series and culture #75, which is also *B. aerogenes,* reacts negatively in the third series. It seems that most of this change consists of a loss in the ability to produce acetyl-methyl-carbinol. Whether or not this is a result of culturing is not known. It is very possible that this ability would be regained again as in the cases of cultures #59, 75, and 106.

The methyl-red test shows more variation than any other physiologic test, with 30 organisms showing changes. This is to be expected, however, since it is merely a pH determination, a definite positive reaction being about pH 4.4, and a negative reaction about pH 6.0. The cultures of *B. coli* were very strongly positive in this test and seemed to be more acid in reaction than any of the other cultures. Results of this test are shown in Chart 10.

Growth in Koser's Citrate solution is shown in Chart 11, where 24 organisms manifested a variation. This medium is used to test the ability of an organism to utilize sodium citrate for its carbon supply and is a differential test for *B. coli,* which should be negative, and *B. aerogenes,* which is able to grow in the medium. Culture #77, which is a *B. coli* type of fecal origin, reacted typically in all but the 4th series, when it grew in the citrate; this is a characteristic of *B. aerogenes.*

CHART 12—*B. aroideae (1) antiserum*

CULTURE NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
1	— settled	++++	++++	++++	++++	++++	++++
2	— settled	—	—	—	—	—	—
3	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—
9	—	++	+	—	—	—	—
10	—	±	—	—	—	—	—
15	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—
42	—	+	±	—	—	—	—
43	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—
49	—	++	+	±	—	—	—
50	—	+	±	—	—	—	—
51	—	—	—	—	—	—	—
52	—	—	—	—	—	—	—
53	—	—	—	—	—	—	—
64	—	+	±	±?	—?	—	—
65	—	±?	±??	—?	—	—	—
68	—	—	—	—	—	—	—
69	—	—	—	—	—	—	—
70	—	—	—	—	—	—	—
71	—	—	—	—	—	—	—

Culture #69, the soft-rot organism which produced indole, a characteristic of a typical *B. coli*, has reacted as a *B. coli* in this test only 3 out of the 5 times, but there was no corresponding change in the indole reaction.

Agglutination Studies

This is a continuation of a study previously reported *(53)* and is carried out with the same antisera and by the same methods. These are in agreement with the methods set forth in Manual of Methods for the Pure Culture Study of Bacteria.

Charts 12, 13, 14, and 15 summarize the agglutination experiments conducted in 1932 and 1933.

As will be seen in Chart 12, *B. aroideae* antiserum agglutinates *B. tabacum*, a leaf-spotting bacterium; *B. coli communis* shows a trace of agglutination with *B. coli communior*, with a culture from Chinese cabbage, Pe Tsai, and possibly with organisms of watermelon rots, while many other organisms isolated from various soft-rots were not agglutinated.

B. phytophthorus (potato blackleg) antiserum (Chart 13) agglutinates *B. aroideae* slightly and possibly organisms from sweet corn and the Pe Tsai.

CHART 13—*Blackleg (2) antiserum*

CULTURE NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
1	— settled	+	±	—	—	—	—
2	—	++++	++++	++++	++++	++++	++++
3	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—
6	—	±	±	—	—	—	—
7	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—
42	—	±	—	—	—	—	—
43	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—
51	—	—	—	—	—	—	—
52	—	—	—	—	—	—	—
53	—	—	—	—	—	—	—
64	—	—	—	—	—	—	—
65	—	—	—	—	—	—	—
68	—	—	—	—	—	—	—
69	—	—	—	—	—	—	—
70	—	—	—	—	—	—	—
71	—	—	—	—	—	—	—

CHART 14—*C. 2 (5) antiserum*

CULTURE NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
1	—	—	—	—	—	—	—
	settled						
2	—	—	—	—	—	—	—
	settled						
3	—	++++	++++	+++	+	±	—?
4	—	++++	++++	++++	++++	++++	+++
5	—	++++	++++	++++	++++	++++	+++
6	—	—	—	—	—	—	—
7	—	++++	++++	++++	++++	++++	+++
9	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—
42	—	++	+	+	±	—	—
43	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—
51	—	—	—	—	—	—	—
52	—	—	—	—	—	—	—
53	—	—	—	—	—	—	—
64	—	++	++	+	±	—?	—
65	—	—	—	—	—	—	—
68	—	++	+	+	+	+	±
69	—	—	—	—	—	—	—
70	—	++++	++++	++++	++++	++++	+++
71	—	++++	++++	++++	++++	++++	+++

CHART 15—*L. 6. (9) antiserum*

CULTURE NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—
9	—	++++	+++	++	++	+	±
10	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—
42	—	++	+	±	—	—	—
43	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—
51	—	—	—	—	—	—	—
52	—	—	—	—	—	—	—
53	—	—	—	—	—	—	—
68	—	—	—	—	—	—	—
69	—	—	—	—	—	—	—
70	—	—	—	—	—	—	—
71	—	—	—	—	—	—	—

In Chart 14, cultures #4, 5, 7, 70, and 71 appear to be identical serologically. All these except #4 are the writer's own isolations from stalk rots of sweet corn, #4 being a type culture of *B. dissolvens* sent by Dr. H. R. Rosen to the United States Department of Agriculture and furnished by them to the writer. It is interesting to note that another culture of *B. dissolvens,* #68, of the same source, agglutinates to a much less extent, while another of my isolants from stalk-rot of sweet corn, #6, shows no sign of cross agglutination with culture #5 antiserum. *B. coli communis* agglutinates in this antiserum whereas *B. coli communior* does not. Agglutination is also secured with an organism (#42) isolated from a rot of Pe Tsai.

The organism (#42) from Pe Tsai is the only one in Chart 15 which agglutinated in the antiserum from culture #9, except, of course, the homologous antigen.

It appears that culture #42 has at least a slight antigenic relationship with all four of the cultures used to produce antisera.

Charts 16, 17, and 18 give the results of agglutination carried out in the year 1934-35.

Culture #18 seemed to show agglutination in all the checks but not in the serum dilutions.

Culture #22 from carrot settled out in all the tubes but showed slight agglutination in #1 (*B. aroideae*) antiserum.

CHART 16—*1 (B. aroideae antiserum)*

ANTIGEN NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
1	—	++++	++++	++++	++++	++++	+++
8	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—
18	+?	—	—	—	—	—	—
20	—	—	—	—	—	—	—
	settled	+?	±				
22	—	settled	settled	—	—	—	—
23	—	++++	++++	++++	++++	++++	++
24	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—
66	—	—	—	—	—	—	—
67	—	—	—	—	—	—	—
72	—	—	—	—	—	—	—
73	—	—	—	—	—	—	—
74	—	—	—	—	—	—	—
75	—	—	—	—	—	—	—
76	—	—	—	—	—	—	—
77	—	—	—	—	—	—	—
117	—	—	—	—	—	—	—
118	—	—	—	—	—	—	—
119	—	—	—	—	—	—	—
120	—	—	—	—	—	—	—

The most striking results shown in these charts are the agglutination of culture #23 to the same degree in all three antisera.

These results did not seem to be explainable, especially in view of the fact that in Chart 8 antiserum #23 agglutinated much more than the homologous organism, unless it was a general serum-protein reaction.

To determine this point, another experiment was set up with human serum in addition to #1, 2, and 9. A small amount of #23 antigen, left over from the previous work, was used with the human serum and labeled "old antigen". All the rest of the antigens were made up anew. The results are shown in Chart 20. The fact that there was no agglutination in the human serum shows that it was not a general serum-protein reaction. Also #23 did not agglutinate in #9 antiserum in this test. The reason for this difference is not known. Culture #23 agglutinated again quite strongly in both #1 and #2 antisera though less strongly in the former.

In order to determine more fully the antigenic relationships between these 3 organisms, #1, 2, and 23, an agglutinin adsorption experiment was carried out. Separate tubes of antiserum #1 were adsorbed by antigen #1 and 23, while antiserum #2 was adsorbed by antigen #2 and 23. The antisera were adsorbed twice with quite dense suspensions of the antigens before making the agglutination tests. As a result the lowest antiserum dilution was 1:320 and it was carried up

CHART 17—*2 (Blackleg antiserum)*

ANTIGEN NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
2	—	++++	++++	++++	+++	++	+
8	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—
18	+?	—	—	—	—	—	—
20	—	—	—	—	—	—	—
	settled	settled	settled	settled	settled	settled	settled
22	—	—	—	—	—	—	—
23	—	++++	++++	++++	++++	+++	++
24	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—
66	—	—	—	—	—	—	—
67	—	—	—	—	—	—	—
72	—	—	—	—	—	—	—
73	—	—	—	—	—	—	—
74	—	—	—	—	—	—	—
75	—	—	—	—	—	—	—
76	—	—	—	—	—	—	—
77	—	—	—	—	—	—	—
117	—	—	—	—	—	—	—
118	—	—	—	—	—	—	—
119	—	—	—	—	—	—	—
120	—	—	—	—	—	—	—

CHART 18—*9 (L. 6. antiserum)*

ANTIGEN NO.	Check	Antiserum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
9	—	+++	++	+	—	—	—
8	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—
18	+?	—	—	—	—	—	—
20	—	—	—	—	—	—	—
	settled	settled	settled	settled	settled	settled	settled
22	—	—	—	—	—	—	—
23	—	++++	++++	++++	++++	+++	++
24	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—
66	—	—	—	—	—	—	—
67	—	—	—	—	—	—	—
72	—	—	—	—	—	—	—
73	—	—	—	—	—	—	—
74	—	—	—	—	—	—	—
75	—	—	—	—	—	—	—
76	—	—	—	—	—	—	—
77	—	—	—	—	—	—	—
117	—	—	—	—	—	—	—
118	—	—	—	—	—	—	—
119	—	—	—	—	—	—	—
129	—	—	—	—	—	—	—

CHART 19—*B. aroideae (1) antiserum*

CULTURE NO.	Check	Serum dilutions					
		1:80	1:160	1:320	1:640	1:1280	1:2560
1	—	++++	++++	++++	++++	++++	+++
	settled						
23	—	++++	++++	++++	+++	+	+
Blackleg (2) antiserum							
2	—	++++	++++	++++	++++	+++	++
	settled						
23	—	++++	++++	++++	++++	++++	+++
L. 6. (9) antiserum							
9	—	+++	++	+	—	—	—
	settled	settled	settled	settled	settled	settled	settled
23	—	—	—	—	—	—	—
Human serum (not an antiserum)							
	settled	settled	settled	settled	settled	settled	settled
23	—	—	—	—	—	—	—
*23	—	—	—	—	—	—	—

*Antigen 3 days old.

o 1:5120. Agglutination in unadsorbed antisera was used as a control. The results are shown in Chart 20.

The adsorption of agglutinins by antigen #23 does not affect the agglutination of culture #1 in antiserum #1. These organisms are evidently quite different antigenically.

The adsorption of antiserum #2 by antigen #2 indicates that at least part of the agglutinins for #23 are not removed by #2. It would seem that culture #2 has changed antigenically since being used to produce this antiserum, since if it were the same it should have removed all agglutinins present in the antiserum.

When antiserum #2 was adsorbed by antigen #23 the agglutinins for culture #2 were practically unaffected, whereas those for culture #23 were almost entirely removed. Thus we see that cultures #2 and 23, although antigenically related, are nevertheless distinct. We would also suggest that cultures #2 and 23 are more closely related antigenically than #1 and 23, as culture #23 agglutinated much stronger in antiserum #2 than in antiserum #1.

Antisera were produced for cultures #2, 42, 51, 64, 69, and 117,

T 20—*Antiserum 1 unabsorbed*

TI- :N).	Check	Serum dilutions						
		1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
	—	++++	++++	++++	++++	++++	+++	
	—	±	±	—	—	—	—	
	settled							
	—	+	+	±	±	—	—	
Antiserum 2 unabsorbed								
	—	++++	++++	++++	++++	++++	++++	
	—	+++	++	+	±	—	—	
	settled							
	—	++++	++++	++++	++++	++++	+++	
Antiserum 1 absorbed by antigen 1								
1	—			++	++	+	+	±
2	settled			—	—	—	—	—
3	—			—	—	—	—	—
Antiserum 1 absorbed by antigen 23								
1	—			++++	++++	++++	++++	+++
2	—							
	settled							
3	—			—	—	—	—	—
Antiserum 2 absorbed by antigen 2								
2	—			+	±	±?	—	—
1	—			—	—	—	—	—
	settled							
3	—			++	++—	+	+—	±
Antiserum 2 absorbed by antigen 23								
2	—			++++	++++	+++	+	±
1	—							
	settled			+	+ —?	—	—	—
23	—			—				

and agglutinations were carried out using cultures #1-18, 19-27, 29-42, 43-53, 64, 66-69, 71-75, 77, and 117-120; or 67 cultures in all against each of the six antisera.

Charts 21 to 26 show the organisms giving evidence of cross agglutination.

It will be noted that culture #31 from carrot was questionable in all six antisera; therefore it has been discarded.

Chart 21 links very closely cultures #2 and 23 (which are identical physiologically as far as studied), thus bringing together, antigenically, a culture of the organism causing blackleg of potato and one from a soft-rot of carrot.

Chart 22 with an antiserum of a culture of *B. aroideae*, isolated from a soft-rot of Pe Tsai in Japan, shows an antigenic relationship between cultures isolated from sweet corn, carrots, potatoes, and Pe Tsai

CHART 21—*Culture 2 antiserum*

ANTIGEN NO.	Culture name or source	Check	Antiserum dilutions					
			1:80	1:160	1:320	1:640	1:1280	1:
2	Blackleg	—	++++	++++	++++	+++	++	
23	Carrot	—	++++	++++	++++	+++	+	

CHART 22—*Culture 42 antiserum*

42	Pe Tsai *B. aroideae*	—	++++	++++	++++	+++	++	
4	*B. dissolvens*	—	++++	+++	++	+	—	
5	Sweet corn	—	++++	+++	++	+	—	
7	Sweet corn	—	++++	+++	++	+	—	
20	Potato	—	++++	++	++	+	±	
22	Carrot	settled	++++	++++	+++	+++	++	
71	Sweet corn	—	+++	+++	+++	++	++	
120	Potato	—	++++	++++	++++	+++	++	

CHART 23—*Culture 51 antiserum*

51	*B. carotovorus*	—	++++	+++	++	++	+	
9	Cabbage	—	±	±	±	±	±	
10	Watermelon	—	+	+	+	±	—	
13	Canna	—	++	+	±	—	—	
53	*B. carotovorus*	—	++	+	+	±	—	
64	*B. coli communis*	—	±	—	—	—	—	

CHART 24—*Culture 64 antiserum*

64	*B. coli communis*	—	++++	++++	++++	++++	++++	+++
66	*B. coli*	—	++++	++++	+++	++	+	
74	*Salmonella pullorum*	—	+	+	+	±	—	

CHART 25—*Culture 69 antiserum*

69	Cabbage	—	++++	++++	+++	++	+	

CHART 26—*Culture 117 antiserum*

117	*B. aerogenes*	—	++++	++++	+++	++	+	
75	Feces	—	+	±	—	—	—	

and links them with a type culture of *B. dissolvens* from sweet corn. These fall into four types physiologically: (1) Cultures #4, 5, 7, and 71 (all from sweet corn) produced acid and gas in dextrose, lactose, and sucrose and were V.P. positive and M.R. negative. (2) Cultures #22 and 42 (from carrot and Pe Tsai) produced acid but no gas from the sugars and were V.P. negative and M.R. positive. (3) Culture #20 (from potato) produced acid in all sugars; gas was constant in lactose but variable in dextrose and sucrose and was V.P. negative and M.R. positive. (4) Culture #120 (from potato) produced acid but no gas from sugars and was negative in both the M.R. and V.P. tests. This culture also gave a negative nitrite test, whereas the other seven cultures were positive.

Chart 23 shows the results obtained with the antiserum of *B. carotovorus*. In addition to showing the antigenic distinction between two cultures of *B. carotovorus* (#51 and 53) it also shows an antigenic relationship between cultures from various sources and #51.

Chart 24 shows a slight agglutination of *Salmonella pullorum* in a *B. coli communis* antiserum.

DISCUSSION AND INTERPRETATION

The group of organisms studied in this work includes 43 cultures isolated from soft rots at this laboratory. Of this group 24, or 55.8%, can be identified on the basis of physiological tests as belonging to a certain group of so-called "species". Fourteen of these would fall in the *B. carotovorus* group, which produces both acid and gas from dextrose, lactose, and sucrose, while ten belong to the *B. aroideae* group which produces acid but no gas from these sugars.

The remaining 19 cultures, or 44.2% of the total number of isolated cultures, cannot be classified in either group. One of these would belong to the *B. aroideae* group except that it does not reduce nitrates. Three of them produce acid in all sugars and gas only in one or two, and so constitute an intermediate type. Eleven cultures produce acid in only one or two sugars, and 4 cultures do not produce acid or gas in any of the three sugars.

This inability to identify all isolated cultures is not an unusual difficulty. Harding and Morse in 1909 *(11)* found that of their 43 cultures 6 were intermediates. Demeter and Sauer *(6)* in a study of 115 cultures of colon-aerogenes type organisms from milk found that on the basis of results from indole, methyl red, Voges-Proskauer, and citrate tests, the cultures were grouped as follows: 15.2% typical *Escherichia coli*, 10.1% atypical *E. coli*, 10.1% typical *Aerobacter aerogenes*, 9.1% atypical *A. aerogenes*, and 55.0% were intermediate forms.

Ryti *(46)* divided 304 cultures of true colon bacilli into 14 groups on the basis of fermentation of sugars, production of indole, and hemolysis of blood agar, concluding that the greater the number of characteristics used, the greater the number of groups.

That the group is a variable one is evident from the results reported in this paper. This, however, is characteristic not alone of this group, but of the entire Tribe *Bacterieae*. Nungester and Anderson *(36)* reported on a series of variants of a *B. coli*-like organism from a case of empyema of the gall bladder. They state that the change from lactose-fermenting forms to non-lactose-fermenting forms is effected with difficulty but the reverse change is brought about readily.

Others reporting variability in the typhoid-dysentery-enteritidis group are Hoder and Sinck *(14)*, Hoder and Singer *(15)*, Hoder and Kiyoski *(16)*, Latze *(26)*, and Seligmann *(48)*.

Grumbach *(10)* disagrees with the interpretation placed on many of these physiological changes. He says that he has "observed nothing in their material to justify acceptance of variability, especially in the bacteria in the typhoid-paratyphoid group . . . Loss of hemolytic action or of the property of splitting sugars or liquefying gelatine permit in no way the effacing of border species. Before we speak of actual variation we must be clear as to which attributes of bacteria can be considered as characteristic of a species."

It is obvious from this study that agglutination may not be relied upon in all instances to express strain relationship. This is very evident when culture #6 showed no agglutination in antiserum #5, whereas cultures #4, 7, 70, and 71 all agglutinated to the same extent as the homologous organism. These cultures are all from stalk rot of sweet corn and indistinguishable physiologically. Culture #4 is the type culture of *B. dissolvens*.

In contrast to this we find that *B. coli communis* and *B. atrosepticus*, two physiologically distinct types, are distinctly agglutinated by #5 antiserum. Also another type culture of *B. dissolvens* agglutinated only to a moderate degree.

Chart 22 presents further evidence that agglutination does not show strain relationship in these organisms, by linking agglutinatively organisms falling into four physiologic groups.

Cross agglutination between *B. coli communis* and *B. dysenteriae* is reported by Kligler (23), and between *B. coli mutabile* and paradysentery Y by Silberstein (50).

Meyer (35) in speaking of the agglutination of *B. coli* says, "serologically, its strains exhibit the most diverse reactions."

As a result of this study it is obvious that these strains are not sufficiently fixed physiologically, serologically, and possibly biologically to make possible a satisfactory classification, at the present time.

Undoubtedly the soft-rot bacteria belong to the colon-typhoid-dysentery group of organisms and could rightfully be referred to as *B. coli*, as the term is commonly implied in the broader sense. Though *B. coli* is commonly nonpathogenic, it is very often known to become definitely pathogenic to animals. Hence there would appear no reason to assume that *B. coli* may not as easily become a plant pathogen when conditions are suitable.

If this be true, there is no reason for classifying plant pathogens in a separate Tribe (Erwineae) as in Bergey's Manual.

The final classification and naming of these organisms can come only after a more complete study of the entire group of colon-typhoid-dysentery bacteria, and a better understanding of the species limiting characters as applied to this group.

Until such study has been made it might appear most suitable to place these organisms in the colon-typhoid-dysentery group without committing oneself definitely to any fixed terminology as at present used or implied.

SUMMARY

1. A group of 120 cultures of soft-rot and colon bacteria were studied physiologically over a period of two years. Fifty-eight of these were also studied serologically.
2. Forty-three organisms made 67 changes in the fermentation of dextrose, lactose, and sucrose. Thirty-eight cultures changed at least once from one generic "type" to another.
3. Only eight organisms were able to produce indole. One of these lost this ability in 1933. In the nitrate reduction tests, 13 organisms have changed in their reaction. Seventeen cultures showed variation in the Voges-Proskauer tests, while 30 changed their reaction in the methyl red medium. Twenty-four organisms varied in their ability to grow in Koser's Citrate solution.
4. Of 43 cultures isolated from soft-rots only 24, or 55.8%, were identifiable, while 19 cultures, or 44.2%, were intermediate types.
5. Seven cultures of bacteria from stalk rot of sweet corn were tested serologically in an antiserum from one of the number. Five of the seven proved to be identical serologically. Though the entire group is physiologically identical, one showed only a moderate agglutination, while another showed no agglutination whatever.
6. *B. coli communis* agglutinated in an antiserum of an organism causing stalk-rot, while *B. coli communior* did not. *B. atrosepticus* also agglutinated in this same antiserum.
7. The soft-rot bacteria undoubtedly belong to the colon-typhoid-dysentery group of bacteria, but their final classification can come only after a complete study of this group of organisms.

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