A STATISTICAL CLASSIFICATION OF THE COLON-CLOACAE GROUP

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It is now firmly established that the end products of metabolism, as well as morphological differences, are reliable and convenient indices for differentiation of bacterial species and varieties. In the group of coli-like bacteria, particular attention has been paid to acid and gas production fron various fermentable substances.

Theobald Smith (1893) observed that some *B. coli* ferment sucrose and therefore recognized two forms.

Durham (1901) suggested the name *B. coli-communior* for the sucrose fermenting variety and characterized *B. lactis-aerogenes* as a polysaccharid fractor.

MacConkey (1905 and 1909), whose classification has been most widely employed, subdivided upon gas formation from sucrose and then from dulcitol thus giving 4 main types generally known as the *B. acidi-lactici* type (sucrose negative, dulcitol negative); the *B. communis* type (sucrose negative dulcitol positive); the *B. communior* type (sucrose positive, dulcitol positive), and the *B. aerogenes* type (sucrose positive, dulcitol negative). Under each type are recorded a number of varieties according to gelatin liquefaction, indol production, the Voges-Proskauer reaction, motility, and fermentation of inulin, adonitol, etc.

Very much along the lines of the MacConkey classification is that of Bergey and Deehan (1908). They employed 8 characters —fermentation of sucrose, dulcitol, adonitol, and inulin; gelatin liquefaction, indol production, motility and Voges-Proskauer reaction—and from a consideration of all possible combinations

between these characters recognized the possible existence of 256 varieties of *B. coli*.

The grouping of Jackson (1911) which was accepted by the American Public Health Association and included in the standard methods for 1912, is very similar to that of MacConkey, but here preference is given to dulcitol over sucrose for the primary division. Each of the 4 groups thus formed is subdivided further on raffinose and mannitol, and then on motility, indol, reduction of nitrates, and gelatin liquefaction.

A very serious objection to the classifications of MacConkey, Bergey and Deehan, and Jackson, is their extreme flexibility. As the number of fermentable substances, or other characters observed, increases, the number of "varieties" increases geometrically approaching infinity. The number of "varieties" is given by the formula 2^n where 'n' is the number of characters studied. Thus with 8 characters there are 256 possible combinations; this number rises to 1024 with 10 characters and to 65,536 when 16 characters are observed. The absurdity of regarding each character as of similar and equal differential value is thus evident. In the more recent studies the principle of the correlation of characters has been emphasized.

Howe (1912), from a statistical study of 630 strains of *B. coli* isolated from human feces, concludes that dulcitol, indol production, nitrate reduction, etc., are not correlated with each other nor with vigor of growth, and he therefore recognizes only the sucrose positive *B. communior* and sucrose negative *B. communis*.

Rogers and his associates, (1914–1916) studied a large number of coli-like forms from milk, grains, and bovine feces, and conclude that two distinct groups may be recognized on the basis of the accurately determined gas ratio—the low ratio *B. communis-B. communior* group and the high ratio *B. aerogenes-B. acidi-lactici* group. There is no doubt that *B. communis* and *B. communior* are low ratio strains and *B. aerogenes* of the high ratio group but the inclusion of *B. acidi-lactici* with *B. aerogenes* does not seem justified, and I believe that further studies will place it definitely with the low ratio strains.

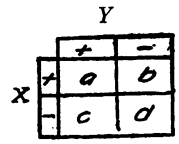
Kligler (1915) suggests that salicin be substituted for dulcitol, in subdividing coli-like bacteria, pointing out that salicin fermentation correlates better with the Voges-Proskauer reaction than does dulcitol decomposition. He thus recognizes a sucrose negative, salicin negative group (*B. acid-lactici*); sucrose negative, salicin positive group (*B. communis*); sucrose positive, salicin negative group (*B. communior*) and sucrose positive, salicin positive (*B. aerogenes*). *B. cloacae* is differentiated from *B. aerogenes* by its inability to ferment glycerol.

The characterization of *B. communior* as salicin negative is probably untenable. The term *B. coli-communior* was first employed by Durham to describe a variety of *B. coli* which fermented sucrose and which was motile. Later Ford recognized it as a species *B. communior*. Such organisms usually ferment salicin as will be shown later in this paper.

Where the principle of correlation has been employed the best correlated character has apparently been picked out by inspection of the data. Inspection is a tedious and difficult procedure, entirely inapplicable where the number of characters considered is large, and it does not permit of a concise statement of the degree of correlation which exists between different reactions. Considerable information in an abstract, concise, and workable, form may however be obtained from a study of the coefficients of correlation.

THE COEFFICIENT OF CORRELATION

Where we are concerned merely with the presence or absence of characters the coefficient of correlation between any two characters may be easily determined. Suppose that it is desired to know if the characters X and Y are correlated and that a study of a number of organisms showed that 'a' cultures are positive for both X and Y; 'b' organisms positive for X but negative for Y, 'c' cultures are negative for X and positive for Y; and 'd' strains are negative for both X and Y. The distribution of the organisms is first tabulated as shown below.



The degree of association, or the coefficient of correlation, is then expressed, according to Yule, by the formula

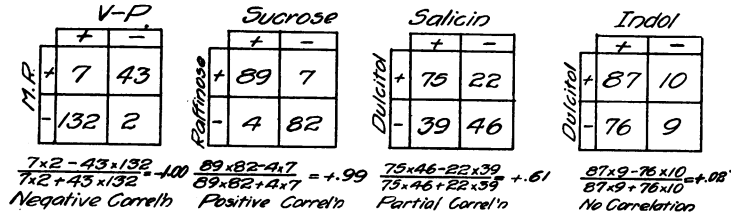
$$\frac{ad - bc}{ad + bc} \tag{1}$$

If 'ad' is equal to 'bc' the coefficient becomes $\frac{0}{ad + bc}$ or 0; which indicates that there is no correlation whatever. If either 'b' or 'c' is zero the formula becomes $\frac{ad}{ad} = 1$; indicating a perfect positive correlation. If 'a' or 'd' is zero then we have $\frac{-bc}{bc} = -1$; showing a perfect negative correlation. It should be observed that an absolute positive correlation exists in reality only if both 'b' and 'c' are zero and an absolute negative correlation when both 'a' and 'd' are zero. In order to avoid coefficients of 1 or -1 where only one group—'a', 'b,' 'c,' or 'd'—is zero. Yule gives the formula

$$\frac{a\,(a+b+c+d) - (a+c)\,(a+b)}{\sqrt{(a+c)\;(b+d)\;(a+b)\;(c+d)}} \tag{2}$$

In practice, however, a few strains are almost always found in each of the four groups and Yule suggests the use of the simpler formula (1). Some caution should therefore be employed in interpreting coefficients of 1 or -1.

For this study it was assumed that if the coefficient between two characters is numerically greater than 0.5 they may be regarded as correlated, but if less than 0.3 there is probably no association. A few examples of correlation coefficients actually obtained in the course of this study are given to illustrate the method of calculation.



The principle of correlation should not be applied indiscriminately to collections of data for systematic purposes. Certain characters and properties have been universally accepted as reliable and appropriate for bacterial differentiation; thus, staining reactions such as the Gram and acid fast stains; spore formation, aerobiosis and anaerobiosis, hardly need to be bolstered up by correlation with other characters to justify their taxonomic value. On the other hand the significance of such characters as motility, indol production, and fermentation of certain substances, is still debatable.

Motility is regarded by many as a highly variable property. Perhaps it is in reality a reliable morphological difference. Certainly if it could be shown that this character goes hand in hand with several others, more reliance and attention should and would be given to motility. The same is true of the indol test. In dealing with gas formation from carbohydrates, alcohols, or polysaccharids, the question naturally arises as to which substance should be given preference for subdivision, or whether all are to be considered of equal taxonomic value. The lack of a criterion for determining the most significant fermentable substances has led to considerable confusion. It has already been pointed out how subdivision on every character studied results in an infinite number of varieties. Where we are dealing with a number of characters each of which is assumed to be of equal taxonomic significance, it would certainly be desirable and advantageous to subdivide on that character which gives the greatest amount of information as to the manner in which the resulting subgroups react with respect to other characters. It is under such circumstances that the principle of correlation of characters may be legitimately, conveniently, and advantage-

ously employed. It may be recalled that the differentiation of the colon-intermediate-typhoid group on glucose and lactose fermentation is strikingly correlated with pathogenicity.

STATISTICAL STUDY

In the following pages is evolved a classification of coli-like bacteria based primarily upon correlated characters. The study is made upon 333 organisms obtained from soil, sewage and the feces of man, horse, sheep, pig and cow.

The characters considered are the methyl-red and Voges-Proskauer reactions, indol production, motility, gelatin liquefaction and gas formation from sucrose, raffinose, dulcitol, glycerol, salicin, dextrin, inulin and corn starch. Other fermentable substances—lactose, maltose, galactose and mannitol—were also observed but as these substances were all attacked with gas formation they need not be considered.

The investigations of Theobald Smith, Hardin, Rogers and others indicate distinctly that the Voges-Proskauer positive or methyl-red negative strains are so different from the Voges-Proskauer negative, or methyl-red positive organisms with respect to the end products of carbohydrate fermentation that subdivision upon these characters seems justified. Two groups are therefore recognized, the methyl-red positive, Voges-Proskauer negative or *B. coli group* and the methyl-red negative, Voges-Proskauer positive or *B. aerogenes-B. cloacae* group.

METHOD OF STUDY

The organisms in each of the two groups are first tabulated as in table 1 in order to facilitate the calculation of the correlation coefficients which are then determined for each pair of characters and recorded as indicated in table 1A. In choosing between any two characters, that one which gives the highest coefficient of correlation with the greatest number of other characters is selected for subdivision. For the resulting subgroups new correlation tables are prepared and subdivision again made as above. A point is very quickly reached where further subdivision upon

correlated characters is no longer feasible. These groups are regarded as species and to each is assigned, as far as possible, the name of the MacConkey variety which it most resembles.

TABLE 1

Showing correlation of characters among 151 strains of the Aerogenes-cloacae group

		Gelatin		Motility		Indol		Sucrose		Raffinose		Dulcitol		Glycerol		Salicin		Dextrin		Inulin*		Starch	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Gelatin	+	83		81	2	13	70	83		79	4	11	72	7	76	82	1	27	56	4	79	5	78
	−		68	8	60	33	35	65	3	66	2	34	34	62	6	67	1	63	5	18	41	60	8
Motility	+	81	8	89		15	74	86	3	83	6	14	75	8	81	87	2	28	61	4	84	4	85
	−	2	60		62	31	31	62		62		31	31	61	1	62		62		18	36	61	1
Indol	+	13	33	15	31	46		46		46		24	22	37	9	46		39	7	9	31	33	13
	−	70	35	74	31		103	102	3	99	6	21	84	32	73	103	2	51	34	13	89	32	73
Sucrose	+	83	65	86	62	46	102	148		144	4	44	104	69	79	146	2	90	58	22	117	65	83
	−		3	3			3		3	1	2	1	2		3	3			3		3		3
Raffinose	+	79	66	83	62	46	99	144	1	145		44	101	68	77	143	2	88	57	21	115	65	80
	−	4	2	6			6	4	2		6	1	5	1	5	6		2	4	1	5		6
Dulcitol	+	11	34	14	31	24	21	44	1	44	1	45		38	7	45		37	8	11	28	33	12
	−	72	34	75	31	22	84	104	2	101	5		106	31	75	104	2	53	53	11	89	32	74
Glycerol	+	7	62	8	61	37	32	69		68	1	38	31	69		69		66	3	20	41	63	6
	−	76	6	81	1	9	73	79	3	77	5	7	75		82	80	2	24	58	2	79	2	80
Salicin	+	82	67	87	62	46	103	146	3	143	6	45	104	69	80	149		89	60	22	119	65	84
	−	1	1	2			2	2		2			2		2		2	1	1		1		2
Dextrin	+	27	63	28	62	39	51	90		88	2	37	53	66	24	89	1	90		22	60	63	25
	−	56	5	61		7	54	58	3	57	4	8	53	3	58	60	1		61		60		61
Inulin*	+	4	18	4	18	9	13	22		21	1	10	11	20	2	22		22		22		21	1
	−	79	41	84	36	31	89	117	3	115	5	22	89	41	79	119	1	60	60		120	36	84
Starch	+	5	60	4	61	33	32	65		65		33	32	63	2	65		65		21	36	65	
	−	78	8	85	1	13	73	83	3	80	6	12	74	6	80	84	2	25	61	1	84		86

* Nine strains not tested in inulin.

THE AEROGENES-CLOACAE GROUP

In the *B. aerogenes-B. cloacae* group are included all strains which gave the Voges-Proskauer reaction—(practically always alkaline to methyl-red) and 10 cultures which fermented starch with gas formation but did not react typically for the Voges-Proskauer nor methyl-red tests. There are 151 organisms in the group, 9 of which were obtained from sewage and the rest, 142, from soil.

The distributions of the strains with respect to gelatin liquefaction, motility, indol and gas formation from sucrose, raffinose, dulcitol, glycerol, salicin, dextrin, inulin and starch are shown in table 1. Mannitol, maltose, lactose and galactose were always fermented and are therefore not included.

Gelatin was liquefied by 83 (55 per cent) (observed for thirty-four days at 20°C.); 89 (59 per cent) were motile; 46 (30.5 per

TABLE 1A

Coefficients of correlation for each pair of characters in table 1

	Gelatin	Motility	Indol	Dulcitol	Glycerol	Dextrin	Inulin	Starch
Gelatin		+.99	-.67	-.74	-.98	-.93	-.79	-.98
Motility	+.99		-.66	-.69	-.99	-1.00	-.83	-1.00
Indol	-67	-.66		+.63	+.80	+.71	+.33	+.71
Dulcitol	-.74	-.69	+.63		+.86	+.65	+.62	+.73
Glycerol	-.98	-.99	+.80	+.86		+.97	+.90	+.99
Dextrin	-.93	-1.00	+.71	+.65	+.97		+1.00	+1.00
Inulin	-.79	-.83	+.33	+.62	+.90	+1.00		+.96
Starch	-.98	-1.00	+.71	+.73	+.99	+1.00	+.96	

cent) formed indol from Witte's peptone, and gas was formed as follows: sucrose 148 (98.2 per cent); raffinose 145 (96.2 per cent); dulcitol 45 (29.8 per cent); glycerol 69 (45.6 per cent); salicin 149 (98.8 per cent); dextrin 90 (59.5 per cent); inulin 22 (14.6 per cent) and starch 65 (43 per cent). It is evident from table 1 that sucrose, raffinose and salicin, because of their extreme availability, cannot be employed for differentiation within the group. The coefficients of correlation for each pair of remaining characters are given in table 1A.

Mere inspection of table 1A shows that gelatin liquefaction is almost perfectly correlated with motility and fermentation of glycerol, dextrin, and starch; the association being positive with motility and negative with the others. Similarly motility is

correlated with glycerol, dextrin, starch and gelatin. Each of these characters is correlated with each other. Under these circumstances any of these reactions may be selected for subdivision; the choice depending upon which were employed in an investigation and to some extent on the personal preference of the investigator. The characterization of *B. aerogenes* by Durham as a starch fermenter; the differentiation of *B. aerogenes* from *B. cloacae* by MacConkey on gelatin liquefaction and motility, and by Kligler on glycerol fermentation are all correct; the apparent confusion being the inevitable result of separation upon single characters.

Two species are evidently present, the *B. aerogenes* which rarely, if ever, liquefies gelatin; is non-motile; and forms gas from glycerol and starch; and the *B. cloacae* which liquefies gelatin (often very slowly); is motile; and does not form gas from glycerol nor starch. As gelatin liquefaction is an inconvenient character the organisms are subdivided for further study upon motility into the non-motile *B. aerogenes* and the motile *B. cloacae*. Glycerol or starch would do just as well. Whichever character is selected, a few strains are present in each of the resulting groups which possess some of the salient characteristics of the other. Thus of 89 motile strains 8 did not liquefy gelatin, 8 formed gas from glycerol and 4 from starch, while of 62 non-motile strains, 2 liquefied gelatin, and glycerol and starch were attacked by one.

The presence of a few supposedly non-liquefiers among the motile strains may as probably—and even more probably—be an indication of the inaccuracy and unreliability of the gelatin liquefaction test than of the presence of true intermediate organisms, for the number of gelatin liquefiers recognized increases with the period of incubation. Again is it not reasonable to explain the presence of several glycerol and starch fermenters among the motile strains as due to mixed cultures? Picking off a colony from a plate, even after several replatings, is no absolute criterion that a pure culture was obtained. Some species stick tenaciously together.

One of the motile starch fermenting strains referred to above

was plated out on brilliant green agar. Ten colonies were fished into motility agar and starch; three were non-motile starch fermenters, three were motile and did not attack starch, while four were both motile and starch fermenters thus indicating that, in this instance at least, the presumably overlapping or intermediate

TABLE 2

Showing correlation of indol, dulcitol, and inulin for 62 strains of B. aerogenes

		Indol		Dulcitol		Inulin *	
		+	−	+	−	+	−
Indol	+	31		20	11	7	18
	−		31	14	17	11	18
Dulcitol	+	20	14	34		8	20
	−	11	17		28	10	16
Inulin*	+	7	11	8	10	18	
	−	18	18	20	16		36

* Eight cultures not tested in inulin.

TABLE 2A

Coefficient of correlation for each pair of characters in table 2

	Indol	Dulcitol	Inulin
Indol		+.39	-.22
Dulcitol	+.39		-.22
Inulin	-.22	-.22	

strains are in all probability merely mixed cultures. It is not contended that intermediate strains do not occur; they undoubtedly do; but it is desired to point out that these have been over emphasized in the past and that the plating method cannot always be relied upon to yield pure cultures.

B. cloacae may be defined as a gram negative short rod which ferments lactose weakly; forms acetylmethylcarbinol from glucose; is alkaline to methyl-red; motile; rarely forms indol; prac-

tically always forms gas from sucrose, raffinose, mannitol and salicin; and occasionally from dextrin; gelatin is typically liquefied; and glycerol, inulin and starch are not fermented. As noted above, the few glycerol, inulin and starch fermenters are probably due to mixed cultures and may be dismissed.

The three sucrose negative cultures (also raffinose negative) may be regarded as a variety corresponding to the *B. levans* which MacConkey records as very rare.

The dextrin fermenters probably also constitute a variety of *B. cloacae* but as the composition of dextrin is so variable we hesitate to employ it for differential purposes for the present.

B. aerogenes resembles *B. cloacae* in several respects. It forms acetylmethylcarbinol from glucose; is alkaline to methyl-red; and ferments sucrose, raffinose, mannitol, and salicin with gas formation. On the other hand lactose is more vigorously attacked; gelatin is typically not liquefied; the organisms are non-motile; while glycerol and starch are fermented with gas formation.

Indol was formed by 31 (50 per cent); gas from dulcitol was formed by 31 (50 per cent); and from inulin by 18 ($33\frac{1}{3}$ per cent) of the *B. aerogenes* strains (8 cultures were not tested with inulin). From tables 2 and 2A which show the distribution with respect to indol, inulin and dulcitol, and the correlation coefficients for these reactions, it is evident that the characters are not associated. They may be of significance for separation of varieties. The utter lack of correlation necessitates the employment of all of these characters, which would lead to the formation of eight varieties. It is deemed unwise to establish such varieties until more extensive collections are studied.

THE COLI GROUP

In the *B. coli* group are included 182 strains quite evenly distributed between the different animal sources, sewage and soil. The group differs sharply from the *B. aerogenes-B. cloacae* series in that the Voges-Proskauer reaction is negative and the methyl-red reaction positive. Starch is not attacked by any of the strains. It has been shown by the author that the Voges-Pros-

kauer negative strains attack the monosaccharids more vigorously, but the disaccharids, trisaccharid, and glucoside no less vigorously than the Voges-Proskauer positive strains.

In table 3 is shown the correlation between the various reactions. As all strains attacked galactose, lactose, maltose and

TABLE 3

Showing correlation of characters among 182 strains of the coli group

		Motility		Indol		Sucrose		Raffinose		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−
Motility	+	130		114	16	77	53	77	53	80	50	92	37	89	41
	−		52	49	3	16	36	19	33	17	35	33	19	25	27
Indol	+	114	49	163		84	79	86	77	87	76	110	52	109	54
	−	16	3		19	9	10	10	9	10	9	15	4	5	14
Sucrose	+	77	16	84	9	93		89	4	64	29	56	36	63	30
	−	53	36	79	10		89	7	82	33	56	69	20	31	38
Raffinose	+	77	19	86	10	89	7	96		65	31	60	35	66	30
	−	53	33	77	9	4	82		86	32	54	65	21	48	38
Dulcitol	+	80	17	87	10	64	33	65	32	97		63	34	75	22
	−	50	35	76	9	29	56	31	54		85	62	22	39	46
Glycerol	+	92	33	110	15	56	69	60	65	63	62	125		89	36
	−	37	19	52	4	36	20	35	21	34	22		56	25	32
Salicin	+	89	25	109	5	63	51	66	48	75	39	89	25	114	
	−	41	27	54	14	30	38	30	38	22	46	36	32		68

mannitol with gas formation and failed to attack the polysaccharids, dextrin, inulin and starch, or to liquefy gelatin, these substances are not included in the table.

The proportion of positive reactions for all strains of the *B. coli* group is as follows: motility 130 (71.5 per cent); indol 163 (89.6 per cent); sucrose 93 (51.1 per cent); raffinose 96 (52.7 per cent); dulcitol 97 (53.3 per cent); glycerol 125 (68.8 per cent) and salicin 114 (62.7 per cent).

The correlation coefficients for each pair of characters is given in table 3A.

The highest coefficient obtained for motility is 0.53 with both

sucrose and dulcitol. Its correlation with other characters is therefore not very marked.

Indol seems to be correlated with salicin, but the small proportion of indol negative strains (10.4 per cent) makes the association of questionable value, and as the coefficients with other substances are extremely low, indol may be eliminated.

Glycerol correlates somewhat with salicin (coefficient 0.52) but

TABLE 3A

Coefficients of correlation for each pair of characters in table 3

	Motility	Indol	Sucrose	Raffinose	Dulcitol	Glycerol	Salicin
Motility		-.39	+.53	+.43	+.53	+.18	+.40
Indol	-.39		+.08	+.00	+.02	-.28	+.76
Sucrose	+.53	+.08		+.99	+.58	-.38	+.20
Raffinose	+.43	+.00	+.99		+.58	-.29	+.27
Dulcitol	+.53	+.02	+.58	+.58		-.21	+.60
Glycerol	+.18	-.28	-.38	-.29	-.21		+.52
Salicin	+.40	+.76	+.20	+.27	+.60	+.52	

with no other character and is therefore not considered desirable for subdivision at this point.

The choice of a differential character is thus narrowed down to sucrose, raffinose, dulcitol and salicin. Sucrose and raffinose are almost perfectly associated (coefficient of correlation 0.99). Consideration of either therefore suffices for both and as the former is slightly better correlated with other characters, sucrose is selected for further discussion.

A comparison of salicin with dulcitol indicates that the alcohol is to be preferred. Salicin correlates better with glycerol and indol (the latter relation of questionable value), while dulcitol has higher coefficients with motility, sucrose and raffinose. A similar consideration leads to the choice of sucrose over salicin.

Sucrose and dulcitol therefore remain. These are the two characters in regard to which there is considerable difference of opinion among students of the *B. coli* group. MacConkey gives preference to sucrose, and in this selection is supported by many investigators (Howe 1912, Kligler 1915, Rogers 1915, etc.); while Jackson (1911) and more recently Giltner (1916) subdivide first on dulcitol. It is quite interesting, therefore, that on the basis of the correlation coefficients there is really little choice between the two. Both are equally well correlated with motility (coefficient 0.53); partially with each other (coefficient 0.58) and not associated with indol. Dulcitol correlates partially with salicin (coefficient 0.60), while sucrose does not (coefficient 0.20). On the other hand, sucrose is almost perfectly correlated with raffinose (coefficient 0.99), whereas salicin is only partially (coefficient 0.58). Although neither can be regarded as associated with glycerol, the coefficient with sucrose (-0.38) is greater than with dulcitol (-0.21).

If our selection is to be guided entirely by correlation, the choice between dulcitol and sucrose is a toss up. Sucrose was finally selected for the primary division because it is more widely distributed in nature, more available to students for investigational purposes, more widely accepted by bacteriologists, and its fermentation better correlated with the source than is dulcitol decomposition. Differentiation on sucrose yields a sucrose positive group of 93 strains, and a sucrose negative group of 89 strains.

THE SUCROSE NEGATIVE STRAINS OF THE COLI GROUP

Of the 89 strains which did not form gas from sucrose, 33 (37.1 per cent) were positive in dulcitol; 69 (77.6 per cent) positive in glycerol; and 51 (57.3 per cent) gave gas in salicin; 53 (59.6 per cent) were motile; only 10 (11.3 per cent) failed to form indol.

The distribution of the organisms, with regard to motility, dulcitol, glycerol, salicin and indol is given in table 4, and the coefficient of correlation for each pair of reactions is given in table 4A. For these strains motility is not distinctly correlated with

any other character. Dulcitol and glycerol are not correlated with each other, nor with indol and motility, but each has a high coefficient of association with salicin. The coefficient for dulcitol

TABLE 4

Showing correlation of characters among 89 sucrose negative strains of the coli group

		Motility		Indol		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−	+	−
Motility	+	53		45	8	22	31	43	10	33	20
	−		36	34	2	11	25	26	10	18	18
Indol	+	45	34	79		29	50	61	18	51	28
	−	8	2		10	4	6	8	2		10
Dulcitol	+	22	11	29	4	33		27	6	28	5
	−	31	25	50	6		56	42	14	23	33
Glycerol	+	43	26	61	8	27	42	69		48	21
	−	10	10	18	2	6	14		20	3	17
Salicin	+	33	18	31		28	23	48	3	51	
	−	20	18	28	10	5	33	21	17		38

TABLE 4A

Coefficients of correlation for each pair of characters in table 4

	Motility	Indol	Dulcitol	Glycerol	Salicin
Motility		−.50	+.22	+.25	+.25
Indol	−.50		−.07	−.08	+1.00
Dulcitol	+.22	−.07		+.20	+.78
Glycerol	+.25	−.08	+.20		+.86
Salicin	+.25	+1.00	+.78	+.86	

with salicin is 0.78 and for glycerol with salicin is 0.86. Indol is also correlated with salicin; all of the 10 indol negative strains are also salicin negative. Differentiation is therefore made upon salicin which gives a sucrose negative, salicin positive subgroup

of 51 strains, and a sucrose negative, salicin negative subgroup of 38 organisms.

The sucrose-negative, salicin-positive subgroup (B. coli). The distribution of the 51 sucrose negative salicin positive strains on motility, dulcitol, and glycerol is indicated in table 5. 33 (64.9 per cent) are motile; 28 (54.9 per cent) form gas from dulcitol, and 48 (94.3 per cent) from glycerol. The extremely small proportion of glycerol negative strains (5.7 per cent) eliminates

TABLE 5

Showing correlation of characters among 51 sucrose negative—salicin positive strains of the coli group. (B. coli)

		Motility		Dulcitol		Glycerol	
		+	−	+	−	+	−
Motility	+	33		18	15	30	3
	−		18	10	8	18	
Dulcitol	+	18	10	28		25	3
	−	15	8		23	23	
Glycerol	+	30	18	25	23	48	
	−	3		3			3

* Coefficient of correlation for motility and dulcitol = 0.02.

this alcohol from further statistical consideration. From table 5 it is seen that motility and dulcitol are not correlated. Further subdivision on correlated characters is not feasible. This entire group then is regarded as the species *B. coli* and two varieties may be formed on motility—the motile *B. coli-communis* and the non-motile *B. coli-immobilis.*

The sucrose negative, salicin negative subgroup (B. acidi-lactici). Of the 38 organisms which were negative for both sucrose and salicin, 20 (52.7 per cent) were motile, 28 (73.7 per cent) formed indol, 21 (55.3 per cent) were positive with glycerol, while only 5 (13.2 per cent) formed gas from dulcitol as shown in table 6. From table 6A it appears that motility is correlated with dulcitol fermentation and indol, and has a slightly higher coefficient with

glycerol than has dulcitol. Indol has a slightly higher coefficient with dulcitol than motility, but the number of dulcitol positive strains is so small, that the coefficients observed cannot be relied upon. Indol and motility seem to be correlated (coefficient

TABLE 6

Showing correlation of characters among 38 sucrose negative, salicin negative strains of the coli group. (B. acidi-lactici)

		Indol		Motility		Dulcitol		Glycerol	
		+	−	+	−	+	−	+	−
Indol	+	28		12	16	2	26	14	14
	−		10	8	2	3	7	7	3
Motility	+	12	8	20		4	16	13	7
	−	16	2		18	1	17	8	10
Dulcitol	+	2	3	4	1	5		2	3
	−	26	7	16	17		33	19	14
Glycerol	+	14	7	13	8	2	19	21	
	−	14	3	7	10	3	14		17

TABLE 6A

Coefficients of correlation for each pair of characters in table 6

	Indol	Motility	Dulcitol	Glycerol
Indol		+.68	+.69	+.40
Motility	+.68		+.62	+.40
Dulcitol	+.69	+.62		+.34
Glycerol	+.40	+.40	+.34	

0.68), and their coefficients with glycerol are identical (0.40). In a preliminary report subdivision was made upon motility into the motile species *B. Gruenthal*, and non-motile *B. acidi-lactici*. It seems best, until more extensive collections are studied, that

all of the sucrose-negative, salicin-negative strains be included in the species *B. acidi-lactici* in which may be recognized two varieties, the motile *B. acidi-lactici* var. *Gruenthali* and the non-motile *B. acidi-lactici* var. *immobili.*

TABLE 7

Showing correlation of characters among 93 sucrose positive strains in the coli group

		Motility		Indol		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−	+	−
Motility	+	77		69	8	38	19	49	27	56	21
	−		16	15	1	6	10	7	9	7	9
Indol	+	69	15	84		58	26	49	34	58	26
	−	8	1		9	6	3	7	2	5	4
Dulcitol	+	58	6	58	6	64		36	28	47	17
	−	19	10	26	3		29	20	8	16	13
Glycerol	+	49	7	49	7	36	20	56		41	15
	−	27	9	34	2	28	8		36	21	15
Salicin	+	56	7	58	5	47	16	41	21	63	
	−	21	9	26	4	17	13	15	15		30

TABLE 7A

Coefficients of correlation for each pair of characters in table 7

	Motility	Indol	Dulcitol	Glycerol	Salicin
Motility		−.27	+.67	+.40	+.54
Indol	−.27		+.05	−.42	+.28
Dulcitol	+.67	+.05		−.32	+.39
Glycerol	+.40	−.42	−.32		+.32
Salicin	+.54	+.28	+.39	+.32	

THE SUCROSE POSITIVE STRAINS OF THE COLI GROUP

Of the 93 organisms which fermented sucrose with gas formation, 77 (82.8 per cent) were motile; 84 (90.4 per cent) formed indol; and positive gas reactions were obtained as follows: dulcitol 64 (72.1 per cent); glycerol 56 (60.2 per cent) and salicin

63 (67.8 per cent). The distribution with respect to these characters and the correlation coefficients are shown in tables 7 and 7A respectively, where it is seen that motility correlates better with dulcitol than does any other of the characters. It also correlates best with salicin. Motility is the best correlated

TABLE 8

Showing correlation of characters among 77 sucrose positive motile strains of the coli group. (B. communior)

		Indol		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−
Indol	+	69		53	16	43	25	51	18
	−		8	5	3	6	2	5	3
Dulcitol	+	53	5	58		32	26	41	17
	−	16	3		19	17	1	15	4
Glycerol	+	43	6	32	17	49		37	13
	−	25	2	26	1		27	19	8
Salicin	+	51	5	41	15	37	19	56	
	−	18	3	17	4	13	8		21

TABLE 8A

Coefficients of correlation for each pair of characters in table 8

	Indol	Dulcitol	Glycerol	Salicin
Indol		+.33	−.27	+.26
Dulcitol	+.33		−.87	−.22
Glycerol	−.27	−.87		+.09
Salicin	+.26	−.22	+.09	

character. There are thus two subgroups, a sucrose-positive, motile subgroup of 77 strains and a sucrose positive non-motile subgroup comprising 16 strains.

The sucrose positive motile subgroup (B. communior). Inspection of tables 8 and 8A shows that among the sucrose positive motile strains, neither indol production nor salicin fermentation is correlated with other characters. Gas formation from dulcitol

and glycerol shows a strong negative association. Those strains which failed to attack glycerol practically always fermented dulcitol. Thus 26 of 27 glycerol negative are dulcitol positive while 17 of 18 dulcitol non-fermenters, tested, formed gas from glycerol. To put it another way, inability to attack either of the alcohols is accompanied by fermentation of the other. Fermentation of either, however, yields but little information as to

TABLE 9

Showing correlation of characters among 16 sucrose positive, non-motile strains, of the coli group

		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−
Dulcitol	+	6		4	2	6	
	−		10	3	7	1	9
Glycerol	+	4	3	7		5	2
	−	2	7		9	2	7
Salicin	+	6	1	5	2	7	
	−		9	2	7		9

TABLE 9A

Coefficients of correlation for each pair of characters in table 9

	Dulcitol	Glycerol	Salicin
Dulcitol		+.65	+1.00
Glycerol	+.65		+.80
Salicin	+1.00	+.80	

how the other will react. The desirability of subdividing on either glycerol or dulcitol to form two species is questioned. For the present the entire group of sucrose fermenting motile forms is designated as *B. communior* and two varieties may be formed on glycerol or dulcitol.

The sucrose-positive non-motile subgroup. Only 16 of the sucrose fermenters were non-motile, and only one of these failed to produce indol. Although the number of organisms is small, it is quite surprising that they should be so evenly divided with respect to gas formation from the test substances. Thus 6

(37.5 per cent) are positive with dulcitol, 7 (43.7 per cent) with glycerol, and 7 (43.7 per cent) with salicin. From tables 9 and 9A it is seen that salicin is the best correlated character. Of the seven salicin positive strains, 6 (85.8 per cent) attack dulcitol and 5 (71.5 per cent) glycerol. The characteristics of this group

TABLE 10

Distribution of organisms from different sources among the various species and varieties

		B. cloacae	B. aerogenes	B. communior	B. neapolitanus	B. coscoroba	B. coli communis	B. coli immobilis	B. acidi-lactici Gruenthali	B. acidi-lactici immobili	Total
Soil	No	88	54	26	0	0	2	0	7	0	177
	%	49.7	30.5	14.7			1.1		4.0		
Horse	No	0	0	15	0	0	4	0	0	0	19
	%			79.0			21.0				
Sheep	No	0	0	16	0	5	1	0	0	0	22
	%			72.8		22.7	4.5				
Cow	No	0	0	6	4	0	9	0	1	0	20
	%			30.0	20.0		45.0		5.0		
Pig	No	0	0	9	0	1	11	1	9	0	31
	%			29.0		3.2	35.6	3.2	29.0		
Sewage	No	1	8	3	3	2	1	12	2	7	39
	%	2.6	20.5	7.7	7.7	5.1	2.6	30.8	5.1	17.9	
Man	No	0	0	2	0	1	5	5	1	11	25
	%			8.0		4.0	20.0	20.0	4.0	44.0	
Total		89	62	77	7	9	33	18	20	18	333

therefore resemble the *B. neapolitanus* of MacConkey's varieties. On the other hand, 7 (77.8 per cent) of the 9 salicin negative strains are negative for glycerol while all failed to ferment dulcitol. These are therefore the *B. coscoroba* of MacConkey's classification.

RELATION OF SPECIES TO SOURCE

Table 10 shows the distribution of the organisms from different sources among the various species and varieties. Species or varieties and habitat seem to be somewhat related.

B. aerogenes and *B. cloacae* were obtained only from soil and sewage and were not isolated from any of the animals tested. *B. cloacae* constituted 49.7 per cent of the soil and 2.6 per cent

of the sewage strains, while 30.5 per cent from soil and 20.5 per cent from sewage were *B. aerogenes.*

B. communior was isolated from all sources as follows: soil 14.7 per cent; horse 79 per cent; sheep 72.8 per cent; cow 30 per cent; pig 29 per cent; sewage 7.7 per cent and man 8 per cent. The relative abundance of *B. communior* among the lower animals and scarcity in man and sewage, may well be investigated further.

B. neapolitanus was present only in bovine feces and sewage, comprising 20 per cent of the bovine and 7.7 per cent of the sewage strains.

Of the 9 *B. coscoroba*, 5 were from sheep, 1 from pig, 2 from sewage, and 1 from man. 22.7 per cent of sheep, 3.2 per cent of pig, 5.1 per cent of sewage and 4 per cent of human strains fall in this species.

B. coli, like *B. communior* was isolated from all of the sources tested, but a rather distinct correlation with the source is observed with the varieties *B. coli-communis* and *B. coli-immobilis*. The former comprise 1.1 per cent of soil; 21 per cent of horse; 4.5 per cent of sheep; 45 per cent of cow; 35.6 per cent of pig; 2.6 per cent of sewage; and 20 per cent of human strains. *B. coli-immobilis* was not obtained from the soil, horse, sheep or cow, but it made up 3.2 per cent of the pig, 30.8 per cent of the sewage, and 20 per cent of the human strains.

B. acidi-lactici was not obtained from the horse nor sheep, and only rarely from the cow (5. per cent) or soil (4 per cent). The motile variety *B. acidi-lactici var. Gruenthali* was particularly abundant among the pig cultures (29 per cent) and rare in sewage (5.1 per cent) and man (4 per cent). The non-motile *B. acidi-lactici var. immobili* was restricted to man and sewage entirely, comprising 44 per cent of the human and 17.9 per cent of the sewage strains.

If subsequent and more extensive studies confirm these results the determination of species and varieties would have some bearing on the interpretation of the colon test.

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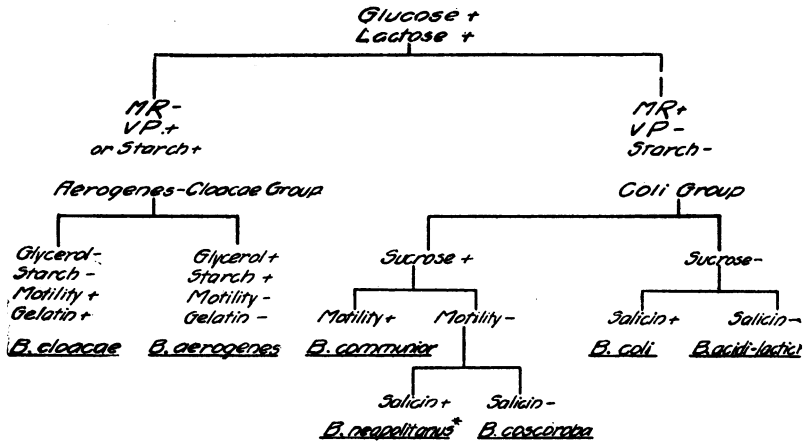
SUMMARY

From a statistical study of 333 coli-like bacteria isolated from soil, sewage, and the feces of various animals, the following classification is suggested:



* Designation as species questionable. Probably preferable to regard it as a variety of *B. communior*.

TABLE 11

Per cent of positive reactions

	V.P.	Indol	Gelatin	Motility	Starch	Inulin	Dextrin	Salicin	Raffinose	Sucrose	Dulcitol	Glycerol	No. of Strains
B. cloacae	100.0	16.8	91.0	100.0	4.5	4.5	30.4	98.0	93.3	96.7	15.7	9.0	89
B. aerogenes	100.0*	50.0	3.2	0.0	98.5	29.1	100.0	100.0	100.0	100.0	50.0	98.5	62
B. communior	0.0	89.6	0.0	100.0	0.0	0.0	0.0	72.8	94.8	100.0	75.4	63.7	77
B. neapolitanus	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	85.8	71.5	7
B. coscoroba	0.0	89.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	22.2	9
B. coli	0.0	100.0	0.0	64.7	0.0	0.0	0.0	100.0	11.8	0.0	55.2	94.3	51
B. acidi lactici	0.0	64.0	0.0	52.7	0.0	0.0	0.0	0.0	2.6	0.0	13.2	55.2	38

* Ten questionable reactions included.

The per cent of positive reactions of the different species is indicated in table 11. *B. neapolitanus* differs from *B. communior*

only with respect to motility, and it may therefore be well to regard it as a variety of *B. communior*. However *B. coscoroba* is so distinctly different from the other sucrose fermenters that its designation as a species seems justified. It should be borne in mind however, that the differentiation in this instance is based on only 9 individual cultures so that the correlations observed must not be over emphasized.

CONCLUSIONS

To treat all characters as of equal taxonomic significance leads to an infinite number of unstable varieties; a condition to be avoided.

Subdivision on correlated characters results in a small number of groups which possess considerable stability.

The species described are quite strikingly correlated with the source, and, if more extensive investigations confirm these observations, recognition of the various species may be of sanitary significance.

It is not supposed that the classification presented is the last word in the differentiation of coli-like bacteria, but it is hoped that if subdivision is to be made upon correlated characters—and there is much to commend such a procedure—the method described in this paper for the determination of the best correlated character, by a study of the coefficients of correlation, will be an aid to later investigators.

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