STUDIES ON ORGANISMS OF COLON-AEROGENES GROUP, ISOLATED FROM VARIOUS SOURCES.

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Submitted to the Department of Bacteriology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Degree of Master of Arts.

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The colon group as defined by the Committee on Standard Methods of Water Analysis [1] includes"all non-spore-forming bacilli which ferment lactose with gas formation and grow aerobically on standard solid media". This includes a number of gelatin liquefying organisms which ferment laccose, which were at one time omitted. In 1905 and 1909 MacConkey[2] suggested a classification of the colon group, dividing it into four divisions according to motility, liquifaction of gelatin, Voges Proskauer reaction, and fermentation of saccharose, dulcite, adonite, inulin, and inosit. Jackson[3] in 1911 suggested a modification of MacConkey's classification by dividing his four divisions by their actions on mannite and raffinose. These are the classifications that are largely accepted at the present time. Kligler [4] concludes that the reaction in salicin gives a more logical division than the use of dulcite. It is conceivable however, inasmuch as the members of the B. colon group are very active fermenters of carbohydrates, alcohols, etc, that the number of subdivisions, varieties, etc, are limited only by the number of test substances used. Rogers, Clark, and Davis [5] divided the colon group into two well defined groups according to the ratio of hydrogen to carbon dioxide formed in dextrose broth. Subsequently it was found that the high-ratio group, supposed to consist largely of B. aerogenes, B. cloacae, and closely allied forms, occurred in about 50% of colon organisms isolated from milk[6], and in 90% of colon organisms isolated from grains [7] while it occurred only once in 150 strains from bovine feces.[8]

Thus it is to be seen that in examinationf for the colon group where it is used as an indicator of fecal pollution, the importance lies not so much in the type of the organims found but rather in its source. The means of determining this has been suggested in the Standard Methods of Water Analysis (1) by the reaction to methyl red, the Voges Proskauer reaction, the effect upon gelatin, the absence or presence of indol production and the fermentation of saccharose and adonite.

The following cultures were isolated by the author and others in routine examinations with the idea of determining the source. The results obtained were considered interesting enough to warrant description.

ISOLATION OF CULTURES.

The 60 food strains were isolated from the following substances; milk 14, oysters 22, sausage 18, miscellaneous 6.

A loop-full from positive lactose broth or lactose peptone bile was streaked out on eosin-methylene blue agar plates, incubated 24 hours and characteristic colonies picked. These were transfered to slants, run through dextrose and lactose broth, milk and gelatine, and morphology and gram stains made. All of the 60 food strains and the 37 fecal strains produced acid and gas from dextrose and lactose broth, acid in milk and in most cases coagulation. They were all gram negative rods.

The fecal strains were isolated by Professor N. P. Sherwood and J. B. McNaught of this department, from human feces, by streaking out a suspension of material on eosin-methylene blue agar plates, picking characteristic colonies and culturing as above.

SPECIAL TECHNIC

It does not seem necessary to include a discussion of the general technic of the inocculation of the fermentation tubes, etc. In all fermentation tests meat extract, peptone broth containing 1% of the test

substance, was used, the eosin-methylene blue agar was prepared according to Holt-Harris- and Teague.(9) The only special media used was a peptone gelatin prepared as follows,

12% gelatin.
.5% meat extract
2% peptone (Digestive Ferments Company)

prepared and sterilized as for ordinary gelatin.

While testing a number of cultures in 1% peptone gelatin for liquefaction, it was noted that some of the organisms produced gas bubbles along the line of innoculation.

After some experimentation it was found that the amount of gas was much increased by the use of a 2% peptone instead of a 1%. A higher percentage of peptone produces more gas but is not necessary for differentiation. Sometimes as many as 6--8 large bubbles will form along the stab. These usually begin to appear in 24 hours and are well developed at 48 hours. No cultures were found that produced gas except those beginning within 48 hours.

Following are the cultural characteristics, of all the cultures studied, for the sake of brevity the reactions in milk, dextrose, and lactose are omitted, as stated before all the cultures reacted typically in the above media.

Probably the fact that the cultures were obtained by plating from enrichment broth instead of from the material direct, explains why no atypical forms were isolated.

After obtaining the pure cultures they were streaked back on eosin-methylene blue agar plates and their appearance noted. This is discussed later.

Cultures No.1916-A17-A 22-A 28-A 29-A 38-and A 51 were lost before their characteristics were obtained and are omitted from the tables.

TABLE # 1

No.	Source	Gelatin Liquefaction	Fermentation of Gas	Saccharose	Salicen	Dulcite	Adonite	Glycerine	Voges and Proskauer	Indol	Classified according to Jackson	Kligler
1	Feces #	-	-	+	-	+	-	A	-	+	B.Communior	B.Communior
2	"	-	-	-	+	A	-	A	-	+	B.Acidi lactici	B.Communis
3	"	-	-	-	-	-	-	+	-	+	"	B.Acidi lactici
4	"	-	-	+	+	+	-	+	-	+	B.Communior	B.Aerogenes
5	"	-	-	+	A	+	-	+	-	+	"	"
6	"	-	-	-	-	+	-	+	-	+	B.Communis	B.Acidi lactici
7	"	-	-	-	-	-	-	+	-	+	B.Acidi lactici	"
8	"	-	-	-	+	-	-	+	-	+	"	B.Communis
9	"	-	-	+	-	+	-	+	-	+	B.Communior	B.Communior
10	"	-	±	-	-	-	-	A	-	?	B.Acidi lactici	B.Acidi lactici
11	"	-	-	A	-	+	-	+	-	+	B.Communior	B.Communior
12	"	-	-	+	-	-	-	A	-	+	B.Aerogenes	"
13	"	-	-	-	+	+	-	+	-	+	B.Communis	B.Communis
14	"	-	-	+	-	-	-	+	-	?	B.Aerogenes	B.Communior
15	"	-	-	A	-	+	-	+	-	+	B.Communior	"
16	"	-	-	-	-	+	-	+	-	+	B.Communis	B.Acidi lactici
17	"	-	-	-	+	-	-	-	-	+	B.Acidi lactici	B.Communis
18	"	-	-	+	-	-	-	+	-	?	B.Aerogenes	B.Communior
19	"	-	-	-	+	+	-	A	-	+	B.Communis	B.Communis
20	"	-	-	-	+	-	-	A	-	+	B.Acidi lactici	"
21	"	-	-	-	+	+	-	+	-	+	B.Communis	"
22	"	-	-	-	+	-	-	A	-	+	B.Acidi lactici	"
23	"	-	-	-	-	+	-	+	-	+	B.Communis	B.Acidi lactici
24	"	-	-	+	+	+	-	A	-	+	B.Communior	B.Aerogenes
25	"	-	-	-	-	+	-	+	-	+	B. Communis	B.Acidi lactici
26	"	-	-	-	+	+	-	A	-	+	B.Communis	B.Communis
27	"	+	-	+	A	+	-	+	-	?	?	?
28	"	-	-	-	-	+	-	A	-	+	B.Communis	B.Acidi lactici
29	"	-	-	-	-	-	+	A	-	+	B.Acidi lactici	"
30	"	-	-	-	-	+	-	A	-	+	B.Communis	"
31	"	-	-	+	-	+	-	+	-	+	B.Communior	B.Communior
32	"	-	-	+	-	+	-	+	-	+	"	"
33	"	-	-	-	-	+	-	+	-	+	B.Communis	B.Acidi lactici
34	"	-	-	+	A	+	-	+	-	+	B.Communior	B.Aerogenes
35	"	-	-	+	+	+	-	A	-	+	"	"
36	"	-	-	+	-	-	-	A	-	+	B.Aerogenes	B.Communior
37	"	-	-	-	-	+	-	+	-	+	B.Communis	B.Acidi lactici

In this and the following tables: + in fermentation indicates acid and gas production; A acid production only; + in liquefaction of gelatin indicates liquefaction in 14 days at 20°C; - indicates no liquefaction in that time; + in gelatin gas, indicates gas produced in peptone gelatin in 48 hours; + in Voges Proskauer and Indol need no explanation.

TABLE # 2

No.	Source	Liquefaction	Gas	Saccharose	Salicen	Dulcite	Adonite	Glycerine	Voges and Proskauer	Indol	Classified according to Jackson	Classified according to Kligler
199a	Milk	-	-	-	-	+	-	+	-	-	B.Communis	B.Acidi lactici
199b	"	-	+	+	+	-	+	+	-	-	B.Aerogenes	B.Aerogenes
1910a	"	-	+	+	+	+	-	+	+	+	B.Communior	B.Aerogenes
1910b	"	-	+	+	+	-	+	+	+	+	B.Aerogenes	"
1911a	Cream	-	+	+	+	+	-	+	-	-	B.Communior	"
1911b	"	-	+	+	+	+	+	+	+	+	"	"
1912	Milk	-	-	-	-	-	-	A	-	+	B.Acidi lactici	B.Acidi lactici
1914	"	-	-	+	+	-	-	-	+	-	B.Aerogenes	B.Aerogenes
1917a	"	-	+	+	+	-	+	+	-	-	"	"
1917b	"	-	-	-	-	+	-	+	-	-	B.Communis	B.Acidi lactici
1920	"	-	+	+	+	-	+	+	-	-	B.Aerogenes	B.Aerogenes
1922	"	-	-	+	+	-	-	+	-	+	"	"
1924a	"	-	-	-	-	-	-	+	-	-	B.Acidi lactici	B.Acidi lactici
1924b	"	-	-	-	-	+	-	A	-	+	B.Communis	"

TABLE # 3

A 1	Oysters	-	+	+	+	-	+	+	±	-	B.Aerogenes	B.Aerogenes
A 2	"	-	+	+	+	-	+	+	±+	-	"	"
A 3	"	-	+	+	+	-	+	+	+	-	"	"
A 8	"	+	-	+	+	-	+	+	±	±	B.Cloacae	B.Cloacae
A 9	"	+	-	+	+	-	-	-	-	-	"	"
A 14	"	+	-	+	+	-	-	-	-	-	"	"
A 15	"	-	+	+	+	-	+	+	±	-	B.Aerogenes	B.Aerogenes
A 16	"	-	+	+	+	-	A	+	+	-	"	"
A 18	"	-	+	+	+	-	+	+	±	-	"	"
A 19	"	-	-	-	-	+	+	+	+	-	B.Communis	B.Acidi lactici
A 20	"	-	-	-	+	-	-	+	-	+	B.Acidi lactici	B.Communis
A 21	"	-	-	-	+	-	-	A	-	+	"	"
A 23	"	-	+	+	+	-	+	+	+	-	B.Aerogenes	B.Aerogenes
A 24	"	+	+	+	+	-	-	-	-	-	B.Cloacae	B.Cloacae
A 25	"	-	+	+	+	-	?	+	±	-	B.Aerogenes	B Aerogenes
A 26	"	-	+	+	+	-	+	+	±	-	"	"
A 34	"	-	+?	-	+	-	-	-	-	-	B.Acidi lactici	B.Communis
A 35	"	-	-	+	+	-	-	-	....	-	B.Aerogenes	B.Aerogenes
A 36	"	+	-	-	+	-	-	A	±	-	?	?
A 37	"	-	-	-	+	-	-	A	-	-	B.Acidi lactici	B.Communis
A 45	"	+	-	-	-	-	-	+	±	-	"	B.Acidi lactici
A 53	"	-	-	-	-	-	-	+	-	-	"	"

TABLE # 4

No.	Source	Gelatin Liquefaction	Fermentation of Gas	Saccharose	Salicen	Dulcite	Adonite	Glycerine	Voges and Proskauer	Indol	Classified according to Jackson	Classified according to Kligler
A 4	Sausage	-	+	+	+	-	+	+	-	-	B.Aerogenes	B.Aerogenes
A 5	"	-	-	+	-	-	-	A	-	+	"	B.Communior
A 6	"	-	+	+	+	-	+	+	-	-	"	B.Aerogenes
A 7	"	-	-	+	-	-	-	+	-	+	"	B.Communior
A 10	"	-	-	+	-	-	-	+	-	+	"	"
A 11	"	-	-	+	-	-	-	A	-	+	"	"
A 12	"	-	+	+	+	-	+	+	-	-	"	B.Aerogenes
A 13	"	-	+	+	+	-	+	+	-	-	"	"
A 30	"	-	-	-	-	+	-	+	-	-	B.Communis	B.Acidi lactici
A 31	"	-	-	+	-	+	-	+	-	+	B.Communior	B.Communior
A 32	"	-	-	-	-	+	-	+	-	-	B.Communis	B.Acidi lactici
A 33	"	-	-	+	-	-	-	+	-	+	B.Aerogenes	B.Communior
A 46	"	-	+	+	+	+	+	+	-	+	B.Communior	B.Aerogenes
A 47	"	-	+	+	+	-	+	+	-	+	B.Aerogenes	"
A 48	"	-	+	+	+	+	+	+	±	+	B.Communior	"
A 49	"	-	+	+	+	+	+	+	-	+	"	"
A 50	"	-	+	+	+	+	+	+	+	-	"	"
A 52	"	+	-	+	+	-	-	+	±	-	B.Cloacae	B.Cloacae

TABLE # 5

A 39	Misc.	+	-	+	+	-	-	-	-	-	B.Cloacae	B.Cloacae
A 40	"	+	-	+	+	-	-	-	-	+	"	"
A 41	"	-	-	-	+	-	-	A	-	+	B.Acidi lactici	B.Communis
A 42	"	-	-	-	+	-	-	A	-	+	"	"
A 43	"	-	+	+	+	-	+	+	-	-	B.Aerogenes	B.Aerogenes
A 44	"	-	+	+	+	-	+	+	+	-	"	"

The classification according to Jackson [3] and Kligler [4] were included in the above tables for the purpose of comparison only. It is evident that there are many discrepancies, especially between the B. communis and acidi lactici groups, and between the B.aerogenes and communior groups. This is caused by the differences in the ability to ferment dulcity and salicin, saccharose being common to both classifications. This is shown in tabular form below.

TABLE # 6

PERCENTAGE OF ORGANISMS IN JACKSON'S FOUR GROUPS THAT FERMENT SALICIN.

Group	Number of Cultures	Sal. -	Sal. +
Saccharose negative-dulcite negative	19	42	58
Saccharose negative-dulcite positive	18	77.7	22.3
Saccharose positive-dulcite positive	19	36.8	63.8
Saccharose positive-dulcite negative	32	28	72

As seen from the above table 58% of Jackson's B. acidi lactici would be called B. communis by Kligler. 77.7% of Jackson's B.communis would be called B. acidi lactici by Kligler. 63.8% B. communior (Jackson) would be called B. aerogenes (Kligler), and 28% of B. aerogenes (Jackson) would be called B. communior (Kligler). The gelatin liquefying organisms are omitted from the above table although most of them are saccharose positive--dulcite negative.

Of the 37 feces strains studied all gave a negative Voges Proskauer and all produced well isolated colonies on eosin-methylene agar, blue to purple in color having a darker center and having a heavy metallic

sheen by reflected light, with the exception of # 10 which persistently gave small clear discrete colonies. (Will be discussed later).

Of 27 food strains which developed blue or purple colonies with a pronounced sheen, 25 or 92.6% gave a negative Voges Proskauer reaction. These colonies, however, were larger and tended more to become confluent than the fecal strains.

Of 23 food strains which developed large, confluent colonies with brown centers and no sheen, 17 or 73.9% were Voges Proskauer + or ±

B. cloacae was found to produce small clear, discrete colonies resembling smoewhat those of B. typhosus and B. dysentery, etc.

RELATION OF GAS PRODUCTION IN 2% PEPTONE GELATINE TO FECAL AND NON--FECAL STRAINS.

In table # 1 it may be noted that all the cultures are negative Voges Proskauer, all are negative in adonite except one (# 29), all fail to produce gas in peptone gelatin except one (# 10) which at first produced gas bubbles but upon second inoculation failed to do so. It is also atypical in respect to indol production and colonies on eosin-methylene blue agar.

The relation to the Voges Prosiauer group can best be studied by means of the following tables.

TABLE # 7.

PERCENTAGE POSITIVE IN VOGES PROSKAUER, ADONITE, AND GAS IN PEPTONE GELATIN OF THE B. AEROGENES GROUP.

Group	V.P.+	Adonite	Gas in peptone gelatin
Gelatin- Saccharose + Dulcite - (32) #	37.5 (12)	62.5 (20)	62.5 (20)

The first numbers in columns refer to percentage and the second to number of cultures.

TABLE # 8.

PERCENTAGE POSITIVE IN ADONITE AND GAS IN PEPTONE GELATIN OF THE VOGES PROSKAUER GROUP.

Group	Adonite	Gas in peptone gelatin
Gelatin- Saccharose + V.P. + (17)	82.3 (14)	88.2 (15)

TABLE # 9.

RELATION OF GAS FORMATION IN PEPTONE GELATINE TO FERMENTATION OF ADONITE IN TABLE # 8.

Group	Gas +	Gas -
Gelatin- Saccharose + V.P.+ adonite+ (14)	100 (14)	0

The above tables need very little discussion as they are self-explanatory. It may be noted particularly however, that in Table # 7 of the Gelatin- Saccharose + Dulcite- group only 37.5% are positive Voges Proskauer, which seems rather low considering the fact that with the exception of four strains, all of this group was isolated from foods. Also that there is a correlation between the fermentation of adonite and the gas production in peptone gelatin.

From Table # 8 it may be seen that of the Gelatin- Saccharose + V.P. + group, 82.3% ferment adonite and according to the Standard Methods of Water Analysis would be classified as B. aerogenes of fecal origin. From the same group 88.2% produce gas in peptone gelatin showing again a very close correlation to the fermentation of adonite. The peptone gelatin however, gives 5.9% more positive results than adonite. Considering the small number of cultures that fall in this group, this does not seem large,

also considering the fact that the whole proceedure of determining fecal and non-fecal strains, is relative having been derived from data obtained from a large number of cultures of various sources, and compiled on a percentage basis.

Table # 9 shows that of the 14 cultures in table # 8 that fermented adonite, every one produced gas in peptone gelatin.

From the study of the above tables attention is called to the fact that lack of correlation between fermentation of adonite and formation of gas in peptone gelatin occurs in 97 cultures only once where it would prove confusing.

At an early date the author intends to check these results on a large series of cultures realiging that the cultures studied are comparatively few in number. However, attention is directed to the fact that they represent a number of sources and were isolated under ordinary routine conditions and he believe that from the foregoing, the following conclusions are justified.

CONCLUSIONS.

1. That inasmuch as gas formation in 2% peptone gelatin so closely correlates the fermentation of adonite, it may be substituted for the latter in the determination of fecal and non-fecal strains of the colon group.

2. That unmodified eosin-methylene blue agar may be used for differentiating between colon and aerogenes like organisms, and

3. That classifications such as those of Jackson and Kligler serve only to confuse when the source of the organisms is sought.

Opportunity is here taken to express the appreciation of the author for the valuable aid and advice given by Professor N.P. Sherwood of the Bacteriology Department of the University of Kansas.

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