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DIFFERENTIATION OF HUMAN AND SOIL STRAINS OF THE AEROGENES SECTION OF THE COLON GROUP

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CONSIDERABLE data have been accumulated during the past decade quite clearly indicating that the aerogenes types of bacteria are relatively rare in the intestinal tract of man, whereas they constitute the predominating colon forms in the soil and grains. The question as to whether the few aerogenes forms encountered in the intestines are identical with the soil strains (a question of some practical sanitary significance) has not as yet been adequately studied. In this paper there are reported observations on 123 strains of the aerogenes section of the colon group, isolated from the soil (76 strains) and human dejecta (47 strains).

The soil specimens were isolated from 24 different samples on eosine methylene blue agar at 30° and 37° C. after preliminary enrichment in milk and in lactose peptone broth at 30° and at 37° C. Fecal strains were obtained from 9 individuals after preliminary enrichment at 37° C. in Koser's citric acid medium or in lactose-peptone broth, containing various quantities of brilliant green to inhibit the coli forms. The citric acid medium of Koser was a particularly successful preliminary enrichment medium.[1] Best results were obtained by suspending the feces in physiological salt solution and letting it stand 3 to 5 days before attempting isolation. Under these conditions direct plating on eosine methylene blue agar could be successfully employed, but preliminary enrichment in brilliant green lactose broth or, better still, the citric acid medium was found to be best.

All strains studied were Gram negative, short rods, did not produce spores, gave a positive Voges Proskauer reaction, and were alkaline to methyl red. Two of the 123 strains observed did not grow in Koser's citric or uric acid mediums. The reactions with respect to indol, motility, gelatin liquefaction (5 weeks), and fermentation of various carbohydrates (acid and gas production in peptone water containing 0.35 to 1.0 per cent of the carbo-

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1. Personal communication.

Sodium chloride	5.0 grams
Magnesium sulphate	0.2 gram
Dipotassium phosphate	1.0 gram
Ammonium phosphate	1.0 gram
Distilled water	1000 c.c.
Citric acid	2.0 grams

Adjust the reaction to pH 6.8

hydrate with Andrade's indicator) are indicated in Table 1 below where all of the groups, which may be recognized by their reactions, are also shown. All strains fermented (with acid and gas production) glucose, lactose, trehalose, mannitol and salicin, and all but one culture attacked sorbitol.

The correlation which has been previously reported by Levine, Kligler and others, between motility, gelatin liquefaction and fermentation of glycerol, was also observed in this series of cultures. Subdivision on any of these characters yields two distinct groups with a small number of intermediate strains as is shown in Figure 1. Two large groups stand out distinctly: (1) a glycerol negative, gelatin positive,[2] motile group, comprising 36 strains, which will be referred to as the cloacae type, and (2) a glycerol positive, gelatin negative, nonmotile[3] group of 78 strains, which will be regarded as the aerogenes type. These differ further in that the cloacae forms do not attack the alcohols dulcitol, inositol, and (with a single exception) adonitol, nor the polysaccharid starch, and do not form indol; whereas the aerogenes type always attacked inositol and (with a single exception) starch, frequently fermented adonitol and dulcitol, and 38.5 per cent were positive for indol.

The third group, which consists of 9 strains all obtained from the soil, is intermediate between the cloacæ and the aerogenes types. They resemble the aerogenes type in their active fermentative characters and the cloacae forms in that they liquefy gelatin and are generally motile. It is possible that these few cultures are not a distinct type but are mixtures of the two main types, for it is now well recognized that the plating method can not be depended upon to always yield pure cultures. We will leave this group in the hope that subsequent work will show whether we are dealing with a true intermediate form or a mixture.

TABLE 1

PER CENT POSITIVE REACTIONS AMONG 123 SOIL AND HUMAN STRAINS OF THE AEROGENES SECTION OF THE COLON GROUP.

Subgroup	No. of Orig.	Motility	Gelatin	Indol	Glycerol	Adonitol	Dulcitol	Melezitose	Sucrose	Esculin	Starch	Inulin	Glycogen	Sorbitol	Inositol	Source
I	4	100	50	0	0	0	0	0	0	100	0	0	0	75	0	Soil
II	14	100	100	0	0	0	0	0	100	100	0	0	0	100	0	Soil
III	18	100	100	0	0	5.5	0	0	100	0	0	11.1	0	100	0	Soil & human*
IV	9	66.6	100	22.2	100	100	66.6	0	100	100	33.3	44.4	0	100	100	Soil
V	11	0	0	0	100	0	100	0	100	100	100	0	0	100	100	Soil
VI	6	0	0	100	100	100	0	100	100	100	100	0	0	100	100	Soil
VII	4	0	0	100	100	100	100	100	100	100	100	0	0	100	100	Human
VIII	3	0	0	100	100	100	0	0	100	100	66.7	100	100	100	100	Soil
IX	3	0	0	100	100	100	100	0	100	100	100	66.7	100	100	100	Human
X	14	7.1	0	92.9	100	100	100	0	100	100	100	42.9	0	100	100	Soil
XI	21	0	0	9.5	100	100	100	0	100	100	100	0	0	100	100	Human
XII	16	0	0	0	100	100	0	0	100	100	100	31.3	0	100	100	Human
All strains†	123	34.2	34.9	26	70.7	62.6	39.9	8.1	96.9	84.8	65	17.9	7.3	98.6	70.7	

* These fecal strains may be differentiated on rate of lactose fermentation and appearance of colony on E.M.B. agar.

† All strains fermented glucose, lactose, trehalose, and salicin with acid and gas.

CORRELATION OF CHARACTERS WITH SOURCE

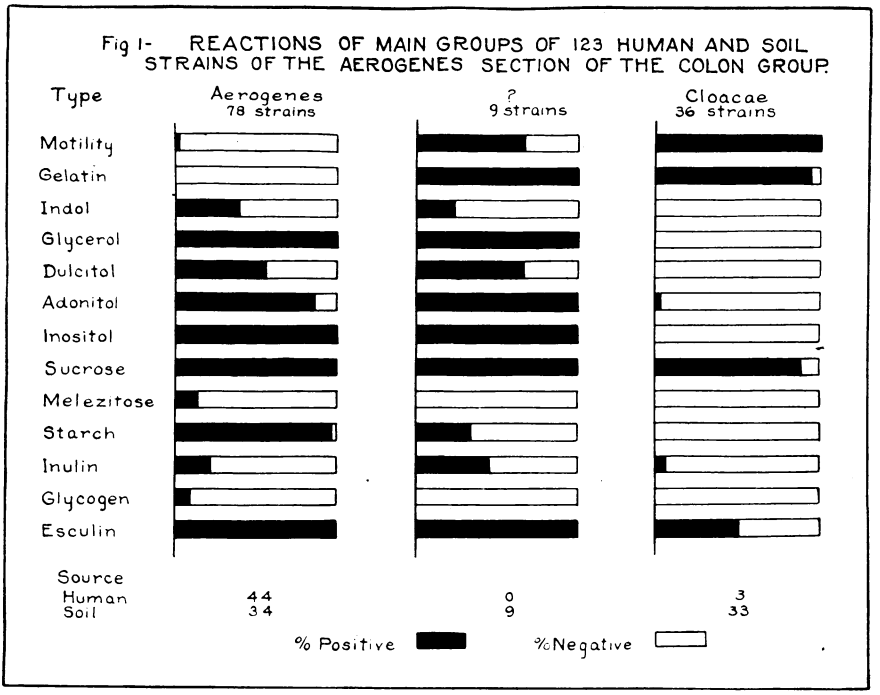
Of the 36 cloacae forms, 3 were of human and 33 of soil origin. Four strains failed to attack sucrose and thereby correspond to the *B. levans*. These strains fermented lactose rapidly and were all isolated from soil.

Of the remaining 32 strains, 14 fermented esculin and 18, including the 3 human types, did not attack this glucoside.

Of the human strains, 2 fermented inulin and 1 adonitol, all the soil strains

2. Two of the 36 strains did not liquefy gelatin in 5 weeks.

3. One of the 78 strains was motile.

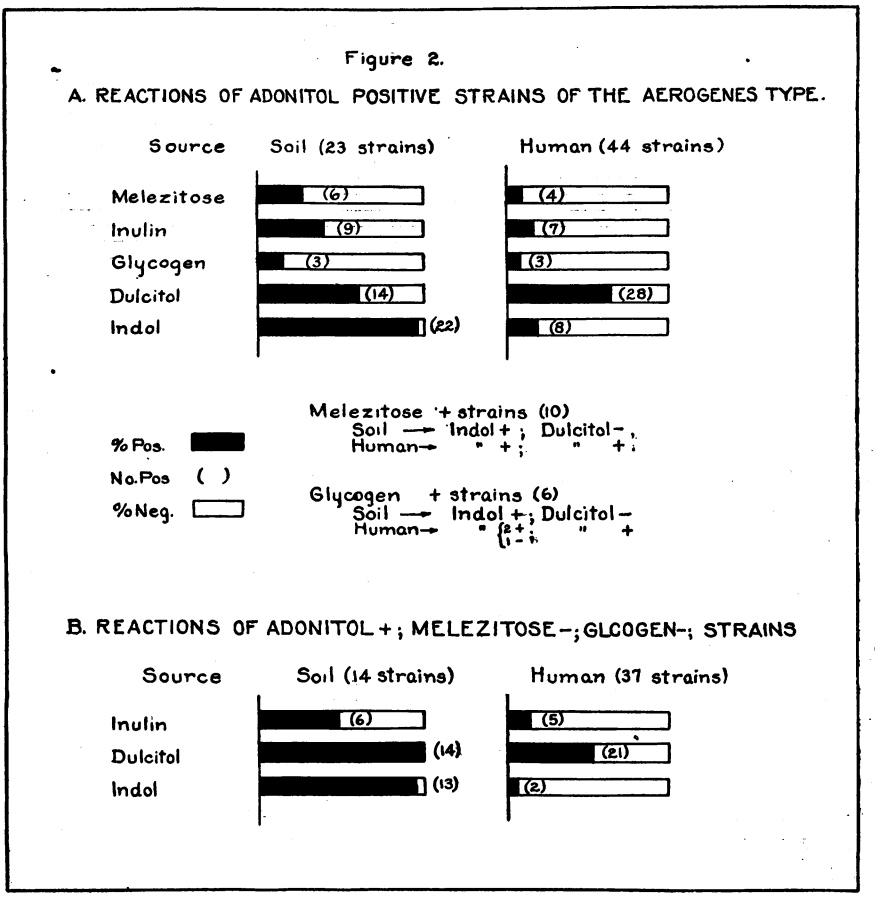


being negative for these characters. These differences, however, must not be overemphasized because the inulin fermentation was slow, and therefore an undesirable criterion, and adonitol was fermented by but a single culture. The human types fermented lactose in 24 hours with the production of acid and 20 to 40 per cent gas, whereas, of the soil strains, only 6 (20.7 per cent) showed gas in 48 hours and the remaining 23 (79.3 per cent) did not give evidence of lactose fermentation until the fourth day, when there was produced 15 to 40 per cent gas as well as an acid reaction. Another rather striking difference between the soil and the fecal types of cloacae was the appearance of the colonies on eosine methylene blue agar. The colonies of the soil types of *B. cloacae* were about 1½ mm. in diameter, slightly raised with a flat surface, no apparent tendency to coalesce, and no evidence of metallic sheen. The central darker portion was about one-third the diameter of the colony and appeared bluish by reflected light and pink by transmitted light. Of 26 colonies observed, 23 were of this type. Of the 3 fecal cloacae cultures, 1 was a large colony about 4 mm. in diameter with a convex surface, confluent, and with a distinct greenish metallic sheen; the other 2 produced colonies 2 mm. in diameter with a flat surface, a slight green sheen in the center, and slightly confluescent. The central portion of the colonies were almost black by transmitted light and dark brown by reflected light.

The aerogenes type includes 78 strains, 44 of human and 34 of soil origin. Inspection of Figure 1 shows that the characters which are available for subdivision within the group are indol, dulcitol, adonitol, melezitose, inulin and glycogen. The adonitol negative strains were all of soil origin. There were 11 cultures in this category. That adonitol might be of significance in differentiating fecal and nonfecal types of aerogenes was suggested by Rogers and in the Standard Methods of Water Analysis for 1920 the adonitol nonfermenting type is listed as probably not of fecal origin. Rogers

found that of 46 human strains, all fermented adonitol, whereas of 111 strains isolated from grains, only 12.6 per cent were adonitol fermenters. Other investigators, Winslow and Cohen, Chen and Rettger and Monfort, point out that soil strains and water forms from presumably nonpolluted sources are frequently adonitol fermenters and are therefore inclined is in confirmation of the contention of Rogers, that the adonitol negative forms are probably not of human origin.

The remaining strains (23 soil and 48 human cultures) all of which fermented adonitol, are shown in Figure 2 with respect to the frequency of fermentation of melezitose, inulin, glycogen and dulcitol, and production of indol. Six of the



to disregard the value of adonitol for differentiation of fecal and nonfecal forms. Evidence is quite clear, however, that an aerogenes form, which does not ferment adonitol, is probably not of human origin, at least we have not, as yet, come across a record of a human strain which failed to ferment this alcohol. The fermentation of this carbohydrate, on the other hand, is no evidence of the fecal origin of the strain. Our work therefore

soil and 4 of the human strains fermented the trisaccharid melezitose. The soil strains were, however, all dulcitol negative, whereas the human strains were all dulcitol positive.

Three of the human and 3 of the soil cultures decomposed glycogen, and here again we find that these soil strains differed from the human strains in their inability to decompose the alcohol dulcitol.

Only 8 of the 44 human strains pro-

duced indol and in these 8 are included the 4 melezitose positive and 2 of the glycogen positive strains. There remains for consideration 14 soil strains and 37 human strains to be differentiated on the three characters—fermentation of inulin and dulcitol, and production of indol. The distribution of the human and soil strains with respect to these characters is also shown in Figure 2. The soil forms are characterized by their more frequent fermentation and production of indol. Thus 47.9 per cent, 100 per cent and 92.9 per cent of the soil strains are positive for inulin and dulcitol fermentation and production of indol,[4] respectively, as compared with 13.5 per cent, 51.8 per cent and 5.4 per cent for the human strains. Inulin fermentation was slow and therefore will not be stressed here as a differential character, but the dulcitol and indol tests were very distinct and clear cut. With these two characters the human and soil strains in the group under consideration were readily distinguishable. Of the 17 soil strains, 1 was indol negative and dulcitol positive and therefore was not distinguishable from similar organisms of human origin. Of 37 human strains, 2 were indol positive and dulcitol positive, thereby resembling the soil forms. With these three exceptions, the soil and human types were distinguishable by means of the characters employed.

SUMMARY

A collection of 123 cultures of the aerogenes section of the colon group, comprising 47 strains isolated from human dejecta and 76 strains from soil, were studied as to their morphological, cultural and biochemical characters.

Two distinct types were evident when subdivision was made on glycerol fermentation, motility or gelatin liquefaction: (1) a group which is considered as the cloacae type (comprising 36 strains) did not ferment glycerol, was actively motile, and practically always liquefied gelatin; other carbohydrates (particularly the alcohols) were very rarely attacked; (2) a group which is representative of the aerogenes type of organism (comprising 78 strains) which attacked glycerol vigorously, did not liquefy gelatin, and was nonmotile; other carbohydrates were frequently attacked. Besides these two main types, 9 cultures, which were intermediate in their characters, but which may be mixtures rather than a distinct type, were encountered.

Adonitol nonfermenting strains of the aerogenes type were found only in the soil.

Of the adonitol positive strains of the aerogenes type, which included 23 from soil and 44 from human sources, the soil and human strains could be differentiated in all but 3 instances on the following characters: melezitose, inulin, glycogen and dulcitol fermentation, and the production of indol.

In the cloacae group 4 strains of soil origin did not ferment sucrose, thereby resembling *B. levans*. In the remaining 32 strains there are included 3 of human origin which differed from the others in their more rapid fermentation of lactose and the appearance of their colonies on eosine methylene blue agar.

4. The tests for indol were made after 5 days incubation at 37° C. in Witte's peptone in the following manner: A piece of absorbent cotton was moistened with a few drops of paradimethylamonibenzaldehyde and potassium persulphate and inserted into the tube about 1 inch from the surface of the medium. The tube was then placed in a boiling water bath for 15 to 20 minutes. The formation of a red color on the bottom of the cotton plug indicates the presence of indol. This test, which was suggested by Mr. B. H. Butcher of our laboratories, is very delicate as it restricts the reaction to volatile substances.

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