

CHROMOGENIC STRAINS OF *ESCHERICHIA*¹

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Chromogenic strains of the genus *Escherichia*, obtained from human feces and from water, have been described briefly in a preliminary report (1937). Except for the production of a "reddish-orange" pigment, these strains cannot be distinguished from certain well-established non-chromogenic species of *Escherichia*. On the basis of minor differences, they fall into three types. They form gas, produce indol, yield negative Voges-Proskauer reactions, and fail to liquefy gelatin. Thus, they are distinctly different from most of the "yellow" pigment-forming "colon" or "colon-like" organisms which have been isolated from cereals, water, *etc.* by other workers, and which have been mentioned by Parr (1937), and by Lehmann, Neumann and Breed (1931). On the other hand, the present strains may be identical with some of those encountered previously. This point cannot be settled, because the descriptions which have been recorded in the literature are incomplete, except for those of Parr (1937). Two of the present strains seem to be identical with the culture described by him.

The present paper records in detail the characteristics of five strains of chromogenic coliform bacilli and compares them with well-recognized species of *Escherichia*.

MATERIALS AND METHODS

Three of the bacterial strains were isolated in 1932 from eosin methylene-blue agar during the course of routine bacteriological

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examinations of feces and water, and were considered to be typical members of the genus *Escherichia* until they were cultivated on nutrient agar. One strain was obtained in 1936 from the feces of an adult who had suffered for years from chronic constipation. On eosin methylene-blue agar and on Endo's medium, inoculated directly from a fecal suspension, it also appeared to be a typical member of the *Escherichia*, but on blood agar it was distinctly chromogenic. Several isolations were made from each medium and each subculture proved to be chromogenic on nutrient agar. One strain was made available through the courtesy of Dr. R. S. Breed, who had received it from Dr. A. J. Kluyver of Delft, Holland. Originally, it came from Dr. M. W. Beijerinck's collection and "had been carried in the Delft collection for a long time, possibly for as long as 20 years." The original source is not known.

The media and methods employed to determine the various characteristics were the same as those described by Tittsler and Sandholzer (1935). Hemolysis was determined in pork-infusion agar containing rabbits' blood, according to the method of Paulson and Brown (1931).

From 3 to 6 subcultures were isolated from each parent strain, and the cultural characteristics were determined for each subculture. During the course of the present study all of the characteristics were redetermined at least once.

RESULTS

The results of a detailed study of the cultural characteristics of five strains of chromogenic coliform bacilli are recorded, for the most part in tables 1 and 2. All of the strains were found to possess certain features in common. These are shown in table 1. They fermented glucose and lactose with the production of acid and gas; failed to liquefy gelatin; produced indol; gave positive methyl-red tests; and yielded negative Voges-Proskauer reactions. Carbohydrates were fermented rapidly, with the exception of salicin and glycerol, in which acid formation did not occur until the second or third day of incubation.

These cultures differed among themselves in certain char-

acteristics, as shown in table 2. On the basis of these differences, the five strains fell into three types. Strains 219 and 870, from water and from human feces respectively, were alike. Strains 220 and 251 were alike and, evidently, they were identical with the culture described by Parr (1937). Strain 221 differed from the others.

TABLE 1

Characteristics common to all five strains of chromogenic coliform bacilli

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS	CARBOHYDRATES FERMENTED WITH ACID AND GAS	CARBOHYDRATES NOT FERMENTED
Bacilli.....	l-Arabinose	Sucrose
Non-sporogenous.....	d-Galactose	Cellobiose
Gram-negative.....	d-Glucose	Raffinose
Aerobic.....	d-Levulose	α -Methylglucoside
Gelatin not liquefied.....	d-Mannose	Dulcitol
Indol produced.....	d-Xylose	l-Erythritol
Nitrates reduced.....	d-Lactose	l-Inositol
Methyl-red positive.....	Maltose	Inulin
Voges-Proskauer negative.....	Trehalose	Starch
H ₂ S not produced.....	Glycerol	
Citrate not utilized.....	Mannitol	
Milk acidified, coagulated and reduced..	Sorbitol	

TABLE 2

Differential characteristics of five strains of chromogenic coliform bacilli

	221	220	251	219	870
Salicin.....	AG	AG	AG	—	—
Adonitol.....	AG	—	—	AG	AG
Hemolytic type	Beta	Gamma	Gamma	Beta	Beta
Motility.....	+	+	+	—	—
Source.....	Feces	Feces	Unknown	Water	Feces

AG = Acid and gas.

The color of the pigment produced by the parent cultures was designated by the writer as "reddish-orange" (1937). When classified according to Ridgway's Color Standards (1912), however, it is xanthine orange. Parr characterized the pigment produced by his strain as "a distinct golden-brown" and "practically the same color as that observed for a typical *Staphylococcus*

aureus." When strains 219 and 221 were plated on nutrient agar, they usually yielded a few colonies which had very little color, but subcultures from such colonies formed as much pigment as did the parent strains. On the other hand, strains 220 and 870 yielded some colonies which were practically non-chromogenic and subcultures from them developed but little pigment. When these slightly chromogenic substrains were plated and isolations made from the least chromogenic colonies, cultures were obtained which develop no detectable pigment in colonies and very little in mass growths. Otherwise, the slightly chromogenic strains did not differ from the most chromogenic substrains of the same parent culture. All of the parent strains have retained their chromogenicity constantly during repeated cultivation on nutrient agar, for as long as six years.

DISCUSSION

The results of a detailed study of the cultural characteristics of five chromogenic strains of coliform bacilli show that they are more closely related to members of the genus *Escherichia* than to those of any other genus. On the basis of chromogenesis alone, they might be allocated to either of the genera, *Flavobacterium* or *Serratia*. This designation would seem to be illogical, however, because their failure to liquefy gelatin, their fermentation of numerous carbohydrates with the formation of gas, and their production of indol, are different from those recorded for members of either of these genera. When the characteristics of the present strains are compared in detail with those recorded in Bergey's Manual (1934) for certain members of the genus *Escherichia*, one finds a close agreement, except for the production of pigment. Thus, strains 219 and 870 differ from *Escherichia acidilactici*, and strains 220 and 251 from *Escherichia paragrauenthali*, only in their inability to ferment raffinose. Strain 221 differs from *Escherichia paragrauenthali* in its ability to ferment adonitol and in its inability to ferment raffinose. The writer, however, has found that raffinose is not fermented by members of the *Escherichia* genus which do not ferment sucrose, *i.e.*, not by members of the *coli-communis* group. Thus,

except for chromogenesis, strains 220, 221 and 251 would be classified as *Escherichia paragruenthami* and strains 219 and 870 as *Escherichia acidilactici*. Strains 220 and 251 are identical with the strain described by Parr (1937) and for which he proposed the name *Bacterium aurescens*, n.s., because it differed from *Escherichia paragruenthami* "not only in being chromogenic but in failing to ferment raffinose." Whether this proposal be acceptable or not depends upon the point of view. If strains like 220 and 251 are given a specific name, the argument can be raised that strains 219 and 870, and 221 deserve similar consideration on the basis of differences in the fermentation of salicin and adonitol and in hemolysis. The writer does not favor the segregation of closely related strains into new species, unless the procedure serves a useful purpose or cannot be avoided. Accordingly, no additional specific names are proposed for the present chromogenic strains. It seems sufficient to consider them as chromogenic varieties of either *Escherichia paragruenthami* or *Escherichia acidilactici*, depending upon their fermentation of salicin.

SUMMARY

A detailed study of the cultural characteristics of five strains of chromogenic coliform bacilli, isolated from water and human feces, is presented. On the basis of differences in the fermentation of salicin and adonitol and in hemolysis, these strains fall into three distinct types. Attention is directed to the relationship of these strains to *Escherichia paragruenthami* and *Escherichia acidilactici*, and the opinion is expressed that they can conveniently be considered as chromogenic varieties of these recognized species, thus avoiding the necessity for the creation of new species.

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