# *ESCHERICHIA AURESCENS* (PARR) COMB. NOV., A PIGMENTED SPECIES

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Chromogenic bacteria from a variety of sources have been found to be similar to members of the coliform group. Probably the earliest known culture of this sort is one received from Professor A. J. Kluyver of Delft, Holland (ECD strain), allegedly obtained from a diseased eye by Stokvie of Amsterdam over 30 years ago. Parr (1937) observed a golden-brown chromogen of the coliform group predominant in the intestinal tract of a single individual. He regarded it as a distinct species and named it *Bacterium aurescens* (strain Ba). Reddish-orange chromogens of the coliform type (strains 219 and 220) were described by Tittsler (1937). Numerous similarly pigmented strains of the coliform group were isolated by Stuart (*personal communication*, 1951) over an extended period of time from unrecorded sources at the Rhode Island Hospital in Providence, Rhode Island (RIH strains). Although the work of Parr and of Tittsler show that these golden-brown to red chromogens and typical *Escherichia* species are much alike, the relationships of these chromogens to each other and to non-chromogenic members of the coliform group are not definitely established.

## MATERIALS AND METHODS

Twelve cultures were used in this study: six "chromogenic coli" and six control cultures. The sources of the chromogenic cultures are indicated above. As controls two *Serratia* and four typical *Escherichia* strains were included.

The media employed for studying the fermentation reactions were prepared by adding the appropriate carbohydrate (Difco), in a concentration of 1 per cent, to Purple Broth Base (Difco); Durham tubes were used for the detection of gas. Simmons Citrate Agar (Difco) was used for all determinations of the utilization of the citrate radical. All cultures were carried on Proteose no. 3 Agar (Difco). A milk agar, prepared according to the method of Chapman (1943), was also used in testing for chromogenesis.

Serological investigations, with special reference to the O antigen distribution among the strains here considered, were carried out according to the methods of Kauffmann (1947). Cultures were incubated at 30 C for 20 hours and then washed and heated for 2½ hours at 100 C to destroy the activity of L and B surface, or envelope, antigens, as well as the flagellar antigens. After heating, the antigens were standardized to a concentration of 1 billion organisms per ml. Rabbits were inoculated on alternate days with quantities of from 0.5 to 1.0 ml. The total course of injections employed about 15 billion organisms. The method of serum production was essentially that used in Salmonella serology.

Macroscopic tube agglutinations were used in all cases, the tests being read after 24 hours in the 37 C water bath. All titers were taken as the last tube showing agglutination and are recorded as their reciprocals.

Homologous and heterologous adsorptions of O immune serum were accomplished with antigens from 20-hour broth cultures concentrated by centrifugation to approximately 10 billion organisms per ml and boiled for one hour. In addition all chromogenic cultures were submitted to the Enteric Bacteriology Laboratories of the Communicable Disease Center at Chamblee, Georgia, for a test of their agglutinability in *E. coli* O group immune serum, which may be taken as evidence of a serological relationship between the chromogenic cultures and typical cultures of *E. coli*.

## RESULTS

The biochemical and cultural investigations of Parr (1937) and Tittsler (1939) on the golden-brown to red chromogenic coliforms have been partially repeated and, as far as carried out, confirmed. Without exception, the IMViC characters of the chromogens were identical with those of typical *E. coli*. All of the strains produced acid from glucose, lactose, maltose, adonitol and mannitol; gas was also produced

TABLE 1

*Results of cross agglutination with somatic antigens*

| Serum Against | Antigen | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ECD | Ba | #219 | #220 | RIH | RIH 17 | *S. marcescens* | *S. indica* | *E. acidilactici* | *E. paragruenthali* | *E. coli* X | *E. coli* Y |
| ECD | **10240** | 80 | 640 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ba | 20 | **1280** | 160 | 0 | 20 | 0 | 0 | 10 | 10 | 0 | 0 | 0 |
| #219 | 20 | 40 | **1280** | 0 | 10 | 10 | 160 | 0 | 10 | 40 | 0 | 0 |
| #220 | 80 | 40 | 10 | **640** | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| RIH | 10 | 80 | 40 | 0 | **640** | 20 | 0 | 10 | 40 | 10 | 0 | 0 |
| RIH 17 | 40 | 20 | 40 | 0 | 0 | **640** | 0 | 0 | 10 | 0 | 0 | 0 |
| *S. marcescens* | 0 | 40 | 80 | 0 | 10 | 0 | **640** | 320 | 0 | 160 | 0 | 10 |
| *S. indica* | 160 | 10 | 160 | 0 | 20 | 0 | 80 | **640** | 0 | 0 | 0 | 40 |
| *E. acidilactici* | 10 | 160 | 80 | 10 | 20 | 20 | 20 | 80 | **5120** | 80 | 20 | 20 |
| *E. paragruenthali* | 10 | 20 | 80 | 0 | 0 | 20 | 80 | 40 | 0 | **1280** | 0 | 40 |
| *E. coli* X | 0 | 40 | 40 | 0 | 10 | 0 | 0 | 20 | 0 | 0 | **320** | 0 |
| *E. coli* Y | 20 | 40 | 20 | 0 | 0 | 0 | 40 | 10 | 0 | 0 | 0 | **1280** |

from these sugars by all of the organisms except ECD. However, it was reported by Breed (*personal communication*, 1949) that tests of ECD made in 1925 showed the production of acid and gas from glucose and lactose, maltose and mannite not having been tested at the time. None of the cultures tested attacked sucrose. The reactions with salicin were variable, one culture, no. 220, producing acid and gas, two cultures, Ba and ECD, producing only acid, and the other three cultures not attacking the carbohydrate.

All of the chromogens produced golden-brown non-water soluble carotenoid pigment at both 37 C and room temperature through weekly transfers on Proteose no. 3 Agar for a period of 7 months. Pigmentation was visible in any medium which was capable of showing it with one exception, which was that the ECD strain was colorless even after two weeks of incubation on Chapman's milk agar although pigmented on other media. Some of the non-pigmented colonies picked from the ECD milk agar plate showed slight chromogenesis when subsequently plated on the same medium. Breed (*personal communication*, 1949) reported that when he grew these same strains on a Bacto-peptone, Liebig's meat extract agar, all developed a red carotenoid pigment, some being a very bright red.

A complete record of the homologous and cross-agglutination reactions of the O antigens of all of the cultures appears in table 1. It is quite evident that none of the organisms thus tested is identical with any of the others.

Furthermore Ewing reported to us (1951, *personal communication*) that, when the six chromogenic cultures were tested for agglutinability in the *E. coli* O-group immune sera of Kauffmann, the Ba strain showed a definite relationship to *E. coli* of O-group 30, the ECD strain exhibited a slight relationship to *E. coli* of O-group 69, and strain no. 219 displayed no serological relationship to any of the *E. coli* O-group 1 to 113. Ewing further found that strains no. 220, RIH and RIH 17 were in the rough state, agglutinating spontaneously upon heating.

## DISCUSSION

The physiological characters of the red chromogens which were studied here (except Serratia controls) are those of typical members of the genus *Escherichia*.

From the serological investigations, it is evident that the cultures studied constitute a rather heterogeneous group.

As mentioned earlier in this paper, the pigmentation of the red-chromogenic coliforms varies with the medium used. When cultured with proteose peptone, (Difco) the growth is golden yellow, becoming darker, but not red, when held for a week; with peptone (Difco) and meat extract media a bright red chromogenesis may develop. The non-water soluble pigment is carotenoid in nature and different from the prodigiosin characteristic of species belonging to the genus *Serratia*.

Clearly these golden-brown to red organisms belong in the genus *Escherichia*, not in *Serratia*. Although these coliforms are, in many other respects, identical with *E. coli* they are here regarded as constituting a distinct species which should bear the name *E. aurescens* (Parr, 1937) comb. nov.

Neish and his associates (*personal communication*, 1954) have used three of these strains (Ba, 219, 220) in a comparative study of the end products produced from glucose by them and by a typical strain of *E. coli*. A report is to be made on their findings (Neish and Ledingham, 1949) which supports the conclusions drawn from our own studies.

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SUMMARY

Six coliform organisms which produce a golden-brown to a red carotenoid pigment have been found to resemble closely members of the genus *Escherichia*, specifically *E. coli*. Although these chromogens are in many other respects indistinguishable from *E. coli*, their ability to produce this characteristic pigment warrants their recognition as a species distinct from *E. coli*. The name *Escherichia aurescens* (Parr) comb. nov. is proposed. The carotenoid nature of the pigment and the biochemical reactions of the organism preclude their inclusion in the genus *Serratia*.

ADDENDUM

At the Würzburg University Medical Clinic Oesterle (1935) isolated from a stool specimen a "yellow" colon bacillus for which he proposed the name *Bacterium coli flavum*. At the time he was interested in the so-called *B. typhi flavum* and he made careful biochemical and serological studies establishing that his organism was not related to the typhoid bacillus. He thought of it as a colon bacillus and mentioned that others had isolated similar yellow-producing bacteria from a variety of sources. His organism differs from *Escherichia aurescens* in the nature of a few fermentation reactions, in pigment coloration and some tests have not been duplicated. It was given a trinomial name not valid in taxonomy but should be remembered as one of the pigmented coliform organisms occurring in the environment occasionally encountered in the human bowel when an extensive survey is made and careful observations carried out.

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