PIGMENT PRODUCTION BY HAEMOPHILUS PARAPERTUSSIS

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The brownish-red discoloration caused by *Haemophilus parapertussis* growing on various media was reported by Bradford and Slavin (1937), Eldering and Kendrick (1938), and others. Bradford and Slavin (1937) extracted agar slants containing this discoloration with various solvents, and compared these extracts with various iron containing solutions. Their conclusion was that the conversion of iron from the organic to the inorganic form was responsible for this pigment.

In the present work, strains of *Haemophilus parapertussis* and *Haemophilus pertussis* were studied for their ability to produce pigment in the absence of any iron. A rapid method for the differentiation between these two organisms also is presented.

MATERIALS AND METHODS

All strains were donated graciously by Drs. Kendrick and Eldering at the Michigan State Department of Health; except the Boling strain of *H. pertussis*, which was obtained as a fresh isolate from the Indiana University Medical Center.

Those cultures received in lyophile form were carried on charcoal agar (Powell *et al.*, 1951) until they were inoculated on the media described below.

EXPERIMENTAL RESULTS

A dark brown pigment was observed in four to five days when *H. parapertussis* was grown on Bordet-Gengou agar with or without blood. However, when 0.1 per cent L-tyrosine was added, *H. parapertussis* rapidly discolored the medium. Within eighteen hours there was a deep red-brown color running the length of the slant about 7 mm deep, which spread within forty-eight hours throughout the entire agar butt. The color gradually turned to a very dark brown-black pigment when the tube was kept at 37 C.

Altogether, eight strains of *H. parapertussis* (strains 21-353, 21-838, 21-851, 23-054, 23-141, 23-144, 23-991, and 24-040) were used with the same results.

Strains S26 and S33 of *H. pertussis*, known rough strains, did not discolor Bordet-tyrosine agar within ten days: neither did *Brucella bronchiseptica*, strain 32067.

Experiments were performed to find out whether iron was essential for the pigment production of *H. parapertussis*. Consequently, the medium proposed by Cohen and Wheeler (1946) was modified in order to eliminate as much iron as possible. Instead of Difco Casamino Acids, Technical, individual amino acids were added according to the Difco analyses of Casamino Acids, Tech (*personal communication*). $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and KH_2PO_4 were left out of the media, as they would interfere with quantitative iron determinations. To this medium made with triple glass-distilled water, were added 2.0 per cent agar and 0.1 per cent L-tyrosine. Using the methods of Sandell (1950) and Woiwood (1947), no iron was found in this medium. Results were read with a Coleman, Jr. spectrophotometer using a 508 $m\mu$ filter.

When grown on the above agar all eight strains of *H. parapertussis* produced the red-brown pigment. The strains then were carried for eight subcultures on this medium with the same pigment production throughout the eight transfers. *H. pertussis* and *B. bronchiseptica* gave no pigment.

The medium described above also was made without L-tyrosine, but with the addition of $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and KH_2PO_4 to the medium. None of the eight strains of *H. parapertussis* produced pigment on this medium within ten days.

DISCUSSION

Differentiation between *H. parapertussis* and *H. pertussis* on the basis of the ability of the former to grow on ordinary media is inadequate because some rough strains of *H. pertussis* (for example, strains S26 and S33) grow well on

many ordinary media. The two organisms, however, may be separated by a simple and quick method involving growth on Bordet agar with the addition of 0.1 per cent L-tyrosine. *H. parapertussis* produces a red-brown discoloration in twenty-four hours, whereas *H. pertussis* does not change this medium within ten days. It is recognized that Bordet agar already contains some tyrosine; however, without added tyrosine, discoloration is very slow.

H. parapertussis pigment is formed in the absence of any iron. Thus, we are not in agreement with the conclusions of Bradford and Slavin (1937) that iron is essential for the formation of this pigment.

Instead, it was observed that L-tyrosine is essential for this pigment production. It can be postulated that *H. parapertussis* contains a tyrosinase that converts tyrosine into a melanin-like pigment.

SUMMARY

A rapid method for differentiation between *Haemophilus pertussis* and *Haemophilus parapertussis* on the basis of pigment production has been presented.

Haemophilus parapertussis produced the pigment in the absence of iron but not in the absence of tyrosine, indicating that a tyrosinase is associated with the organism.

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