Chromogenesis by Variants of Staphylococcus aureus

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Received for publication 11 July 1966

Chromogenesis in Staphylococcus aureus has received considerable attention, although today it is generally accepted that pigmentation in this organism is of little taxonomic value. Numerous carotenoids from this organism have been characterized (B. Sobin and G. L. Stahly, J. Bacteriol. 44:265, 1942). The purpose of this investigation was to determine whether the variations in pigmentation of a parent strain of S. aureus and chromogenic variants were due to either quantitative or qualitative differences in their production of carotenoids.

The parent strain, S. aureus 6, and four spontaneous chromogenic variants (Table 1), all phage-typing 80/81, were isolated and grown on milk-agar as reported by J. Parisi (J. Bacteriol. 92:589, 1966), Growth from milk-agar was suspended in physiological saline, washed three times by sedimentation in a centrifuge from saline suspensions, and the suspensions were adjusted to the same optical density with a Bausch & Lomb Spectronic-20 colorimeter. The standardized suspensions were then sedimented by centrifugation, and the supernatant fluids were discarded. The extraction and partition of carotenoid pigments into hydrocarbons, alcohols, esters, and acids were according to the method of B. Sobin and G. L. Stahly (J. Bacteriol. 44:265, 1942), as modified by M. F. Starr and W. L. Stephens (J. Bacteriol. 87:293, 1964). Purification was by column chromatography with alumina used as the adsorbent for carotenoid hydrocarbons and calcium carbonate for carotenoid alcohols and esters. Petroleum ether (PE) was the solvent, PE containing 2% acetone was the developer, and PE containing 10% acetone was the eluent for the pigments during column chromatography. The absorption spectra of the purified pigments were determined in carbon disulfide with a Bausch & Lomb Spectronic-505 recording spectrophotometer.

None of the strains contained detectable carotenoid acids, but all five strains contained a yellow-orange carotenoid hydrocarbon with absorption maxima similar to those of delta carotene (Table 1). Quantitative determinations showed that the amounts of this pigment produced by these strains were in the following order: 6 > 6C > 6A > 6B > 6D (Table 1). Interestingly, only 6C and 6D contained a yellow-green carotenoid ester with absorption maxima identical to those of sarcinaxanthin, which was isolated from Sarcina lutea by Nakamura (see P. Karrer and E. Jucker, Carotenoids, Elsevier Publishing Co., Amsterdam, The Netherlands, 1950). As shown in Table 1, this pigment was present in greater concentration in 6D, and minute amounts of this pigment were detected as a freed carotenoid alcohol in 6C only. The presence of sarcinaxanthin in 6C could account for its more intense chromogenesis as compared with the parent, and its presence in considerable quantities in 6D could account for the yellow-green color of this organism on milk-agar. No other carotenoid pigments were found upon chromatography of the five extracts. Although delta carotene has been isolated from S. aureus, to our knowledge sarcinaxanthin has not. These studies show both quantitative and qualitative differences in the

### Table 1. Production of carotenoids by parent and variant strains of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Strain</th>
<th>Color on milk-agar</th>
<th>Units of absorbance of Delta carotene</th>
<th>Sarcinaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Golden-yellow</td>
<td>71</td>
<td>2</td>
</tr>
<tr>
<td>6A</td>
<td>Light-yellow</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>White</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>6C</td>
<td>Golden-yellow*</td>
<td>68</td>
<td>24, 2</td>
</tr>
<tr>
<td>6D</td>
<td>Yellow-green</td>
<td>6</td>
<td>41*</td>
</tr>
</tbody>
</table>

* At 488 mμ.
* At 460 mμ.
* More intense than 6.
* Ester.
* Alcohol.

1 Taken in part from a thesis submitted by R. J. Douglas in partial fulfillment of the requirements for the M.S. degree from Duquesne University.


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Vol. 92, No. 5
Printed in U.S.A.

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biosynthesis of carotenoids in the parent and variants studied. Interestingly, differences were also observed in the production of virulence factors by these variants (J. T. Parisi, J. Bacteriol. 92:589, 1966), and chromogenesis has been useful in epidemiological studies of staphylococcal infections (G. C. Turner and A. T. Willis, J. Pathol. Bacteriol. 84:349, 1962).