Carotenoids in Yellow-Pigmented Enterococci

RICHARD F. TAYLOR, MIYOSHI IKAWA, AND WILLIAM CHESBRO

Department of Biochemistry and Department of Microbiology, University of New Hampshire, Durham, New Hampshire 03824

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Pigments extracted from three strains of yellow enterococci showed the spectral and solvent partition characteristics of carotenoids. An unusual C25 carotenoid aldehyde appears to predominate.

Yellow, cell-bound pigments are known to occur in the Streptococcaceae only in group D. We are aware of only two studies on the possible identity of these pigments, one (7) suggesting that they are flavanoid in nature, the other (B. J. Cosenza and A. E. Girard, Bacteriol. Proc., p. 45, 1970) indicating that they are carotenoids. We were prompted to investigate the nature of the pigments because of the availability of 80 strains of yellow-pigmented, group D streptococci in a collection of 700 strains, all conforming to Sherman's (10) physiological criteria for enterococci isolated from soil (S. Weinstein, Ph.D. Thesis, Univ. of New Hampshire, Durham, 1969), and of a motile, yellow-pigmented culture originally isolated by J. O. Mundt (Univ. of Tennessee, Knoxville) and designated by him as Streptococcus faecium var. casselilflavus CF21.

The pigmented isolates from soil cluster in three taxonomic patterns: (i) close correspondence, except for pigmentation, to S. faecium (1), (ii) no correspondence to any established species or variety, and (iii) approximate correspondence to S. faecium var. casselilflavus (8), differing primarily in requiring folic acid. Isolate 564, a culture of pattern iii, and the CF21 culture have been examined in greater detail, and a culture of pattern ii, isolate 563, less extensively.

Cells grown in continuous or batch culture (Table 1), after being harvested by centrifugation, were washed once with 0.9% NaCl, washed twice with distilled water, and lyophilized. To saponify cellular lipids, the dried cells were suspended in 4% methanolic KOH and shaken for 24 hr at 40 C. The mixture was centrifuged, and one-half volume of water was added to the yellow supernatant fluid. On shaking the resulting mixture with ether, all of the pigment went into the ether layer. The ether extract was evaporated in vacuo, and the pigment residue was fractionated by silicic acid column chromatography or solvent partition (Table 1).

Spectral curves yielded by the extracts from isolate 564 are shown in Fig. 1. Extracts of an unpigmented strain showed no adsorption. Equivalent fractions of the pigmented cultures showed very similar adsorption spectra with nearly identical maxima and minima. The properties are similar to those of carotenoids characteristic of Xanthomonas (12), Flavobacterium (11), Micrococcus (9, 11), Sarcina (11, 14), Corynebacterium (4), and Cellulomonas (11, 12) species.

Absorption maxima of the variously treated extracts of isolate 564 and CF21 are listed in Table 1. Cosenza and Girard (Bacteriol. Proc., p. 45, 1970) reported detecting a single pigment after chromatography of extracts of a pigmented, group D streptococcus, with absorption maxima at 463, 433, and 408 nm, values very similar to the maxima observed with isolate 564 and CF21 extracts after column chromatography.

The chromatographic fractions are more readily distinguished by degrees of polarity evidenced in their partition characteristics than by their spectral properties. Similar behavior has been reported (4, 9, 15) for pigment families derived from a parent hydrocarbon by increasing hydroxylation. The pigments of Sarcina and Flavobacterium, having the spectral characteristics described here, were shown to be C45 carotenoids (6, 15). The principal pigment of isolate 564 was obtained on development of the silicic acid column with petroleum ether-methanol (4:1; Table 1). It was essentially a single component as judged by thin-layer chromatography, giving an Rf of 0.48 in benzene-methanol-acetic acid (87:11:2) on prepared silica gel plates (Brinkmann). The infrared spectrum (Fig. 2) indicated the presence of a carbonyl group and absence of hydroxyl groups. The hydroxamic acid test was negative, confirming the absence of a carboxylic acid group. The mass spectrum indicated a molecular weight of 449 ± 2. Peaks (m/e) at 43, 59, 69, 73, 83, 109, 133, and M-106 indicated the carotenoid nature of the pigment (3). Specific peaks at M-29 and M-106-29, attributed to loss of carbon monoxide, indicated that the pigment was an aldehyde (3). The absence or small inten-
Table 1. Spectral properties of pigment fractions of yellow-pigmented enterococcus isolates 564 and CF21

<table>
<thead>
<tr>
<th>Culture mode</th>
<th>Fractionation method</th>
<th>Fraction or phase</th>
<th>Isolate 564 (nm)</th>
<th>CF21 (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>Column chromatography$^a$</td>
<td>Petroleum ether</td>
<td>(411), 434, 463</td>
<td>(413), 433, 463</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Petroleum ether-methanol (4:1)</td>
<td>411, 434, 463</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>414, 434, 465</td>
<td>413, 435, 465</td>
</tr>
<tr>
<td>$^*$ Solvent partition$^c$</td>
<td>Petroleum ether</td>
<td>(411), 434, 463</td>
<td>(410), 434, 463</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>90% Methanol</td>
<td>415, 438, 466</td>
<td>414, 437, 466</td>
</tr>
<tr>
<td>Continuous</td>
<td>Solvent partition$^d$</td>
<td>Petroleum ether</td>
<td>400, 426, 463</td>
<td>400, 424, 463</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90% Methanol</td>
<td>415, 438, 467</td>
<td>417, 439, 468</td>
</tr>
</tbody>
</table>

$^a$ Continuous cultures were grown at 35°C and at a specific dilution rate of 0.1/hr in the slightly modified, completely synthetic medium of Stonehill and Hutchison (13), by using valine limitation. Batch cultures were grown at 35°C to early stationary phase in the same medium with 2.0% acid hydrolyzed casein, plus 0.1% cysteine and 0.02% tryptophan, substituted for the amino acid mixture.

$^b$ All spectra were determined in absolute methanol on a Cary model 15 recording spectrophotometer scanning from 210 to 800 nm.

$^c$ Pigment mixtures were placed on columns of silicic acid (silicAR CC-7, 100 to 200 mesh, Mallinckrodt) and successively eluted with petroleum ether, petroleum ether-methanol (4:1), and methanol; 5-ml fractions were collected.

$^d$ Wavelengths in brackets are derived from shoulders in the spectral curves.

$^e$ The absorbance of this fraction was too low for determination of maxima.

$^f$ Pigment mixtures in 90% methanol were shaken with petroleum ether.

$^g$ Insufficient amounts of continuous culture cells were available.

![Fig. 1](http://jb.asm.org/)
sivity of the M-92 region as compared to the M-106 peak indicated that the pigment was acyclic (2). It is therefore concluded that the pigment is probably a C₂₈ acyclic carotenoid aldehyde and not a C₃₀ or C₃₂ carotenoid.

The solvent partition fractions of the two organisms cultivated continuously in the completely synthetic medium also have the spectral characteristics of carotenoids, eliminating the possibility that these compounds arose as contaminants from the medium, but two peaks of the petroleum ether residue are displaced, in comparison with the analogous batch fraction, about 10 nm toward shorter wavelengths.

Extracts of isolate 563 grown in continuous culture gave spectra closely resembling those of the other two enterococci. Thus, pigmentation, unlike the single characteristic identifying S. faecalis varieties liquefaciens and zymogenes, seems to assort with considerable independence of other taxonomic characteristics, making a varietal epithet based on pigmentation (7) of dubious value.

Since carotenoid pigments may protect membrane-bound functions from photodynamic damage (5), it is reasonable to suppose that they have survival value to those enterococci which are most often found in soil, insects (Cosenza and Girard, Bacteriol. Proc., p. 45, 1970), and as plant epiphytes (8).

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LITERATURE CITED