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Carotenoids of *Staphylococcus citreus.*

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The pigments produced by chromogenic staphylococci were first investigated by Chargaff[1] who detected zeaxanthin in *Staphylococcus aureus.* Later, δ-carotene and rubixanthin were detected in every strain of *Staph. aureus* examined by Sobin and Stahly,[2] and lutein and lycopene were found by Ohta[3] in a strain of the same species, but these authors did not detect zeaxanthin. Recently, Steuer[4] reported that zeaxanthin alone was detected in three strains of *Staph. aureus* examined by him.

The present authors now examined carotenoids present in *Staphylococcus citreus* in the interests of investigation on the pigment formation of micrococci. When chromatographed on alumina, the carotenoid pigments of *Staphylococcus citreus* are separated into two bands. The carotenoid eluted from the upper chromatogram behaves as a xanthophyll (A) and the lower one as a hydrocarbon (B) in the partition test. Both carotenoids show almost the same ultraviolet spectral maxima as listed in Table I.

Table I. Absorption Spectral Maxima (mμ)

Solvent / Carotenoid	Benzine (b.p. 70～80°)	EtOH	$CHCl_3$	CS_2
A (xanthophyll from *Staph. citreus*)	417, 439, 470	417, 439, 469	424, 448, 478	438, 468, 498
B (carotene hydrocarbon from *Staph. citreus*)	416, 440, 470		424, 448, 478	439, 467, 499
Sarcinaxanthin[5]	415, 440, 469	415, 441, 469.5	423, 451, 480	436, 466.5, 499
Sarcinaxanthin[7]		417, 439, 469	422, 448, 476	436, 467, 497
Sarcinene[1]	415, 440, 469	416, 438, 468		
Neoxanthin[7]		415, 437, 467	422, 447, 478	433, 458, 489
Corynexanthin[7]		416, 440, 469	423, 447, 478	435, 466, 497

From these properties, (A) is probably identical with sarcinaxanthin, first isolated by Takeda and Ohta[5] in crystalline state from *Sarcina lutea* and recently detected in *Flavobacterium marinotypicum* by Courington and Goodwin,[6] because it differs slightly from corynexanthin and neoxanthin[7] in the absorption spectral maxima. On the other hand, (B) is almost certainly sarcinene first detected by Chargaff and Dieryck[1] in *Sarcina lutea.*

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Experimental

Culture of *Staphylococcus citreus*—The culture was carried out on the nutrient agar plates, which consisted of Mikuni meat extract (5.0 g.), Polypeptone (Takeda) (10.0 g.), agar (30.0 g.), and water (950 cc.), for 4 days at 37° after inoculation of a strain of *Staphylococcus citreus*. The bacterial cells were collected, sterilized by steam for 5 mins., and dried, first at room temperature and then in a vacuum desiccator over $CaCl_2$.

Extraction and Detection of Pigments—The dried bacterial mass was ground in the ball-mill, and to 58.4 g. of this, 540 cc. of a mixture of benzene-acetone (1:2) was added. The whole was allowed to stand over night in an ice box with occasional shaking. The lemon-colored benzene-acetone extract was freed from the solvent under a reduced pressure and the residue was saponified with 10% MeOH-KOH (10 cc.), warming at 40° for 1 hr. After standing over night, the carotenoid pigments were liberated into benzine (b.p. 70~80°) from the unsaponifiable fraction. The benzine solution was washed thoroughly with water and dried over $CaCl_2$. This was chromatographed through a column (0.7×9 cm.) of Al_2O_3 and developed with benzene to give two separate bands. The upper orange-colored band (2 mm.) and the lower lemon-colored band (9 mm.) were eluted with benzine containing EtOH, and each fraction was freed from the solvent in vacuum. Both eluates gave positive Carr-Price test. The carotenoid from the lower band was completely epiphasic and that from the upper band was hypophasic in the partition test between benzine and 95% MeOH. These pigments were identified by measurement of their absorption spectra using Shimadzu photoelectric spectrophotometer type QB 50, because both fractions were an oily liquid and very small in quantity. Consequently, the conclusion was drawn that the carotenoid from the upper band was sarcinaxanthin and that from the lower band was sarcinene (cf. Table I). When the living bacterial cells were extracted with acetone, the foregoing carotenoids were also detected.

Summary

The presence of sarcinene and sarcinaxanthin was postulated in a *Staphylococcus citreus* strain.

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