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SHORT COMMUNICATION

A COMPARISON OF THE CAROTENOIDS FROM FIVE BACTERIAL SOURCES

R. H. C. STRANG

Department of General Microbiology, College of Agriculture, Kings Buildings, West Mains Road, Edinburgh, 9

and

D. THIRKELL

Department of Biochemistry, University of St. Andrews, Bute Medical Buildings, St. Andrews, Fife

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Abstract—The carotenoids purified from *Sarcina flava, S. lutea, Micrococcus lysodeikticus*, and a *Coryneform* species were very similar, but those of *S. aurantiaca* bore no resemblance; pigmentation cannot, therefore, be used as a means of classification in the Sarcina group. The bacteria other than *S. aurantiaca* may synthesize C_{50} carotenoids.

INTRODUCTION

IT HAS been shown that *Sarcina flava* and *S. lutea* synthesize similar carotenoids,[1] and those of *Micrococcus lysodeikticus* have been shown to have a similar chromophore.[2] There also appear to be some similarities between the pigments produced by the above bacteria and those present in the *Coryneform* species.[3] A further comparison of the carotenoids from these bacteria was undertaken, and *S. aurantiaca* was included as another *Sarcina* although the carotenoids which it has been reported to synthesize appear to be different.[4–6]

RESULTS AND DISCUSSION

Comparisons of the carotenoids from the various sources were made with those of *Sarcina flava* by TLC on 250 μ layers of silica gel G (Merck) using (*a*) benzene/methanol/acetic acid (87/11/2; v/v)[2] or (*b*) chloroform/methanol (9/1; v/v) as solvent. The plates were pre-washed with solvent before use. Solvent (*a*) gave a much better resolution than solvent (*b*), but little difference, if any, was seen between the carotenoids extracted from *S. flava, S. lutea, Micrococcus lysodeikticus* and the *Coryneform* species when the extracts were run adjacent to one another or when they were co-chromatographed.

[1] D. THIRKELL and R. H. C. STRANG, *J. Gen. Microbiol.* **49**, 53 (1957).
[2] G. H. ROTHBLAT, D. S. ELLIS and D. KRITCHEVSKY, *Biochim. Biophys. Acta* **84**, 340 (1964).
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[4] V. READER, *Biochem. J.* **19**, 1039 (1925).
[5] E. CHARGAFF, *Compt. Rend.* **197**, 946 (1933).
[6] B. SOBIN and G. L. STAHLY, *J. Bacteriol.* **44**, 265 (1942).

The carotenoids isolated from *S. flava* and *S. lutea* were examined spectrophotometrically (Table 1) and their partition ratios were determined.[7]

If the carotenoids from the various sources are identical, the present authors' findings for *M. lysodeikticus* differ from those of Rothblat *et al.*[2] in that three fractions were found to be *cis* isomers and that fraction 1 was shown to be a carotene. For the *Coryneform* species, the results recorded here agree with those of Hodgkiss *et al.*[3] except that the least polar xanthophyll which was reported by them as neoxanthin, co-chromatographed with, and has the same chromophore as, the monohydroxy C_{50} carotenoid of *S. flava*.[9] Since Curl[10] suggested that neoxanthin and foliaxanthin are identical, and since Cholnoky, *et al.*[11] showed that foliaxanthin is an epoxy carotenoid, Hodgkiss *et al.*[3] must have been mistaken in their assumption that their compound was neoxanthin as they also stated that no epoxy carotenoids were present. Jensen[12] has also reported that epoxy carotenoids are unknown in non-photosynthetic bacteria. Corynexanthin appears to be identical with the most polar xanthophyll of *S. flava*.

TABLE 1. SPECTRAL CHARACTERISTICS OF THE CAROTENOIDS OF *Sarcina flava* AND *S. lutea*

Pigment	λ_{max} in methanol (nm)	λ_{max} in hexane (nm)	Comment
1	—, 415, 439, 469	—, 415, 437, 466	—
2	—, 415, 439, 469	—, 415, 437, 466	—
3	—, 415, 439, 469	—, 415, 437, 466	—
4	331, 413, 436, 466	331, 412, 435, 465	*cis* isomer[8,*]
5	331, 413, 436, 466	Insoluble	*cis* isomer[8,*]
6	—, 415, 439, 469	Insoluble	—
—	331, 413, 436, 466	Insoluble	*cis* isomer[8,*]

*Not necessarily naturally occurring.

Since Thirkell *et al.*[9] showed that at least two of the xanthophylls of *S. flava* were C_{50} carotenoids, the possibility now exists that a number of other bacteria may also synthesize this type of carotenoid.

S. aurantiaca was shown to synthesize a different series of carotenoids, two of which were identified as β-carotene and zeaxanthin.

EXPERIMENTAL

The carotenoids were extracted from quantities of the following bacteria:

1. *Sarcina flava*—strain 7503, NCTC, London.
2. *Sarcina lutea*—strain 196, NCTC, London.
3. *Micrococcus lysodeikticus*—strain 2665, NCTC, London.
4. *Sarcina aurantiaca*—American Type Culture Collection, Rockville, Maryland.
5. *Coryneform* species—strain 1032, Torry Research Station, Aberdeen.

[7] F. J. PETRECEK and L. ZECHMEISTER, *Analyt. Chem.* **28**, 1484 (1956).
[8] L. ZECHMEISTER and A. POLGÁR, *J. Am. Chem. Soc.* **65**, 1522 (1943).
[9] D. THIRKELL, R. H. C. STRANG and J. R. CHAPMAN, *J. Gen. Microbiol.* **49**, 157 (1967).
[10] A. L. CURL, *J. Food Sci.* **30**, 426 (1965).
[11] L. CHOLNOKY, K. GYÖRGYFY, J. SZABOLCS and B. C. L. WEEDON, *Chem. Commun.* **13**, 404 (1966).
[12] S. L. JENSEN, *Ann. Rev. Microbiol.* **19**, 48 (1965).

Bacteria 1–4 were grown on nutrient agar (Oxoid Ltd.) and number 5 on blood agar. All bacteria were grown at 34° with the exception of the *Coryneform* species which was grown at 15°. *S. aurantiaca* and the *Coryneform* species were harvested at 72 hr, the others at 48 hr.

The carotenoids were extracted by method (*a*) of Thirkell and Strang[1] and purified by the lipid precipitation technique of Blessin[13] followed by saponification in 10% (w/v) KOH in methanol overnight, in the dark, at room temperature in N_2. The unsaponifiable material was extracted in the usual way, concentrated and stored under N_2 in the dark at $-10°$ until used.

[13] C. W. Blessin, *Cereal Chem.* **39,** 236 (1962).