Arch. Mikrobiol. 80, 38—42 (1971)

Carotenoid Composition in the Genus *Ectothiorhodospira* Pelsh

KARIN SCHMIDT and HANS G. TRÜPER

Institut für Mikrobiologie der Gesellschaft für Strahlen- und Umweltforschung mbH, Göttingen, Germany

Received July 5, 1971

Summary. The three species of the genus *Ectothiorhodospira* have been found to contain the carotenoids of the normal spirilloxanthin series, spirilloxanthin itself being the major component. While the carotenoid composition of *E. mobilis* and *E. shaposhnikovii* is not sufficiently different to allow interspecific differentiation, that of *E. halophila* differs from the two former species by its lack of rhodopin, and its higher amount of spirilloxanthin.

At present, the phototrophic bacterial genus *Ectothiorhodospira* Pelsh comprises the three species *E. mobilis* (Pelsh, 1936; Trüper, 1968), *E. halophila* (Raymond and Sistrom, 1967, 1969) and *E. shaposhnikovii* (Cherni *et al.*, 1969). The most important differentiating properties of these species are their relation to salinity, deoxyribonucleic acid (DNA) base ratios, and certain items of the fine structure.

The purpose of this study was to find out whether the carotenoid composition of the three species could supply additional data useful in their taxonomic differentiation.

Methods

E. mobilis strains *SMG 237* (neotype strain, Pfennig and Trüper, 1971b), *SMG 238*, *SMG 239*, *SMG 240*, and *E. shaposhnikovii* strain *SMG 243* (type strain) were grown in the light in a modification of Pfennig's medium according to Trüper (1970). *E. halophila* strain *SMG 244* (type strain) was grown in the light in medium A of Raymond and Sistrom (1967), supplemented with 22% NaCl, 0.1% $Na_2S_2O_3$ and 0.1% sodium succinate. All strains are kept at the Sammlung für Mikroorganismen, Göttingen. Cells were harvested by centrifugation, washed with water of the same salinity as the respective medium, and stored at $-20°$ C.

For extraction and saponification of the pigments, the frozen cells were thawed, extracted with acetone and finally with a small volume of methanol until no more pigment was dissolved. The combined extracts were dried *in vacuo*, dissolved in ether and saponified with an equal volume of 10% KOH in methanol for about 1 h. The unsaponifiable matter was transferred to ether. The ether solution was dried again *in vacuo* and the pigments were redissolved in petroleum ether for chromatographic purification.

Table 1. *Adsorptive properties of the carotenoids of* Ectothiorhodospira *strains*

Compound	Eluent required from neutral aluminum oxide activity grade 2 (in petroleum ether)	R_f values on Schleicher & Schüll paper No. 288			
		2%[a]	5%	10%	20%
Neurosporene	5% ether	0.79			
Chloroxanthin	8% acetone			0.60	
Lycopene	15—20% ether	0.65	0.82		
Rhodopin	8—10% acetone			0.52	
Rhodovibrin	10% acetone			0.43	
Monodemethyl. spirilloxanthin	20% acetone			0.33	
Spirilloxanthin	5% acetone		0.33	0.65	
Di-OH-lycopene[b]	25% acetone				0.34

[a] Acetone in petroleum ether.
[b] For: 1,1′-dihydroxy-1,2,1′,2′-tetrahydrolycopene.

Chromatography and purification of the pigments: The pigments were first separated on columns filled with neutral aluminum oxide, activity grade 2 (Brockmann and Schodder, 1941; Liaaen-Jensen *et al.*, 1958; Liaaen-Jensen, 1962) and then purified a) on paper with aluminum oxide filler, Schleicher & Schüll No. 288 (Jensen and Liaaen-Jensen, 1959), b) on Silicagel G (Merck AG) thin layer plates.

Identification of the pigments: The purified pigments were identified by their absorption spectra, their adsorptive properties (eluent required on aluminum oxide columns, R_f values: cf. Table 1), their partition coefficients (Petracek and Zechmeister 1956, Liaaen-Jensen, 1962), and by cochromatography with authentic carotenoids. The absorption spectra were recorded on a Unicam spectrophotometer, model Sp 700.

Results

Table 2 shows that all investigated *Ectothiorhodospira* strains belong to the spirilloxanthin series, group 1, according to Schmidt *et al.* (1965). Spirilloxanthin is the main product in the carotenoid biosynthesis of these organisms (61—88% of total carotenoid content). Except in *E. halophila*, rhodopin is synthesized in relatively large amounts (10—20%) while all other intermediates of the biosynthetic path of spirilloxanthin are found in remarkably smaller amounts (below 10%). Anhydro-rhodovibrin was below the limits of detection.

Discussion

The carotenoid composition of the *Ectothiorhodospira* strains tested (Table 2) reveals a rather uniform picture. An earlier determination of the carotenoids in *E. shaposhnikovii* (Kondratieva and Malofeeva, 1964;

Table 2. *Composition of carotenoids of* Ectothiorhodospira *strains*

Species	strain	Carotenoid in % of total								
		neurosporene	chloroxanthin	lycopene	rhodopin	anhydro-rhodovibrin	rhodovibrin	monodemethylated spirilloxanthin	spirilloxanthin	di-OH-lycopene[a]
E. mobilis	*SMG237*[b]	(0.5)	1	9	19	below detection	4	5	62	
	SMG237[c]			6	19		9	5	61	
	SMG238			4	13		6	4	73	
	SMG239	(0.5)	1	5	19		2	(0.5)	72	
	SMG240	(0.5)	1	5	10		2	6	76	
E. halophila	*SMG244*[d]	(0.5)	1	(0.5)			1	4	88	(0.5)
E. shaposhnikovii	*SMG243*			7	18		3	2	70	(0.5)

[a] See Table 1 [b].
[b] Batch, 1968.
[c] Batch, 1971.
[d] The missing percentage is due to denaturation products that occurred during extraction.

then, the species was still called the "autotrophic *Rhodopseudomonas* spec.") had shown a carotenoid composition of 6% lycopene, 21.2% rhodopin, 24.4% P-481, 8.5% rhodovibrin, 12.2% monodemethylated spirilloxanthin, and 27.7% spirilloxanthin. The differentiation between anhydro-rhodovibrin (P-481) and *cis*-isomers of spirilloxanthin is, however, difficult (Raymond and Sistrom, 1967). During the present investigation we always found carotenoid fractions with the absorption maxima 458, 483, and 518 nm in petroleum ether in amounts of about 10—25%. These fractions were isolated and subjected to iodine catalysis (Liaaen-Jensen, 1962). The resulting stereoisomeric set was typical for spirilloxanthin. Therefore, we believe that the amount of P-481 found by Kondratieva and Malofeeva (1964) has to be added to the 27.7% spirilloxanthin, thus arriving at values close to those given for spirilloxanthin in Table 2.

A comparison with the other genera of the *Chromatiaceae* (synonym *Thiorhodaceae*), that belong to group 1 (the normal spirilloxanthin series), indicates that the *Ectothiorhodospira* strains tested have to be grouped into subgroup 1 A (Schmidt *et al.*, 1965). This subgroup comprises

those species, whose major carotenoid is spirilloxanthin. These are *Amoebobacter roseus* (synonym *Rhodothece conspicua*, Pfennig and Trüper, 1971 a), *A. pendens* (synonym *Rhodothece pendens*), and *Thiocapsa roseopersicina* (synonym *T. floridana*; also *Thiopedia* sp. strain *Lascelles* belongs to this species).

Within subgroup 1 A the two species *E. mobilis* and *E. shaposhnikovii* are unique in possessing a rhodopin content as high as 10—20% *E. halophila*, lacking rhodopin, has a carotenoid composition similar to that of *Thiocapsa roseopersicina*, also with respect to the relatively higher spirilloxanthin content.

Thus, the carotenoid compositions of the three *Ectothiorhodospira* species, in spite of their basic similarity, allow specific differentiation of *E. halophila*. As a consequence, the most typical specific properties now are:

E. mobilis. Salinity requirement between 2 and 14% NaCl; a special textured outer layer of the cell wall; a polar tuft of flagella; a DNA base ratio of 67.3—69.9 moles % guanine plus cytosine (Trüper, 1968; Remsen *et al.*, 1968); a rhodopin content of 10—20% of total carotenoids.

E. halophila. Salinity requirement between 14 and 22% NaCl; a single sheathed flagellum (Raymond and Sistrom, 1967); a DNA base ratio of 68.4 moles % guanine plus cytosine; a rhodopin content below 1% of total carotenoids.

E. shaposhnikovii. No salinity requirement; a smooth cell wall (S. W. Watson, C. C. Remsen, H. G. Trüper, unpublished); a DNA base ratio lower than that of the other two species, namely of 61.2—62.8 moles % guanine plus cytosine (R. Matheron, personal communication); a rhodopin content of 10—20% of total carotenoids.

References

Brockmann, H., Schodder, H.: Aluminiumoxyd mit abgestuftem Adsorptionsvermögen zur chromatographischen Adsorption. Chem. Ber. **74**, 73 (1941).

Cherni, N. E., Solovjeva, J. V., Fedorov, V. D., Kondratieva, E. N.: Ultrastructure of two species of purple sulfur bacteria. Mikrobiologiya **38**, 479—484 (1969).

Jensen, A., Liaaen-Jensen, S.: Quantitative paper chromatography of carotenoids. Acta chem. scand. **13**, 1863—1868 (1959).

Kondratieva, E. N., Malofeeva, I. V.: A contribution to the carotenoids of purple sulphur bacteria. Mikrobiologiya **33**, 758—762 (1964).

Liaaen-Jensen, S.: The constitution of some bacterial carotenoids and their bearing on biosynthetic problems. Kgl. Norske Videnskab. Selskabs Skrifter No. 8 (1962).

— Cohen-Bazire, G., Nakayama, T. O. M., Stanier, R. Y.: The path of carotenoid synthesis in a photosynthetic bacterium. Biochim. biophys. Acta (Amst.) **299**, 477—498 (1958).

Pelsh, A. D.: Hydrobiology of Karabugaz. Trudy Solyan. Lab., Akad. Nauk USSR, Moscow **5**, 49—80 (1936).

Petracek, F. J., Zechmeister, L.: Determination of partition coefficients of carotenoids as a tool in pigment analysis. Analyt. Chem. **28**, 1484—1485 (1956).
Pfennig, N., Trüper, H. G.: New nomenclatural combinations in the phototrophic sulfur bacteria. Int. J. System. Bact. **21**, 11—14 (1971a).
— — Type and neotype strains of the species of phototrophic bacteria maintained in pure culture. Int. J. System. Bact. **21**, 19—24 (1971b).
Raymond, J. C., Sistrom, W. R.: The isolation and preliminary characterization of a halophilic photosynthetic bacterium. Arch. Mikrobiol. **59**, 255—268 (1967).
— — *Ectothiorhodospira halophila:* A new species of the genus *Ectothiorhodospira*. Arch. Mikrobiol. **69**, 121—126 (1969).
Remsen, C. C., Watson, S. W., Waterbury, J. B., Trüper, H. G.: Fine structure of *Ectothiorhodospira mobilis* Pelsh. J. Bact. **95**, 2374—2392 (1968).
Schmidt, K., Pfennig, N., Liaaen-Jensen, S.: Carotenoids of *Thiorhodaceae*. IV. The carotenoid composition of 25 pure isolates. Arch. Mikrobiol. **52**, 132—146 (1965).
Trüper, H. G.: *Ectothiorhodospira mobilis* Pelsh, a photosynthetic sulfur bacterium depositing sulfur outside the cells. J. Bact. **95**, 1910—1920 (1968).
— Culture and isolation of phototrophic sulfur bacteria from the marine environment. Helgoländer wiss. Meeresunters. **20**, 6—16 (1970).

Dr. Karin Schmidt
Dr. Hans G. Trüper
Institut für Mikrobiologie der GSF
D-3400 Göttingen
Grisebachstraße 8
Germany