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Towards a phylogeny of phototrophic purple sulfur bacteria – the genus *Ectothiorhodospira*

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Abstract. Seven strains of five species of the genus *Ectothiorhodospira* were characterized by oligonucleotide cataloguing of their 16S rRNA in order to determine the phylogenetic relationship to one another and to other phototrophic purple bacteria. All representatives of *Ectothiorhodospira* are members of that line of descent defined by phototrophic purple sulfur bacteria and relatives, showing a moderate relationship to those phototrophic organisms forming globules of elemental sulfur inside the cell (*Chromatium* and relatives). The 5 *Ectothiorhodospira* species fall into two subgroups. *E. halophila, E. halochloris* and *E. abdelmalekii* form one, *E. mobilis, E. shaposhnikovii* and the unnamed strain BN 9906 form the second subgroup. Within the two subgroups the strains are closely related, while the degree of relatedness found between members of the two subgroups is more distant.

Key words: *Ectothiorhodospira* – *Chromatium* – Taxonomy – Phylogeny – Evolution of sulfur metabolism – 16S rRNA cataloguing

Recent studies on phototrophic purple bacteria, using the 16S ribosomal RNA (Gibson et al. 1979; Woese et al. 1979) and cytochromes of the c-type (Ambler et al. 1979; Dickerson 1980) as probes, have led to significant phylogenetic and systematic conclusions: (i) the high degree of correspondence between the patterns of relationship derived from comparative analysis of each of these two molecules renders it unlikely that lateral gene transfer as a cause of either result has occurred (Dickerson 1980; Woese et al. 1980); (ii) the present division of anoxygenic phototrophic bacteria into two orders, *Rhodospirillales* (Pfennig and Trüper 1971) and *Chlorobiales* (Gibbons and Murray 1978), derived from differences in fine structure and pigments (Trüper and Pfennig 1981) is justified from a phylogenetic point of view; however, members of the families Chloroflexaceae and Chlorobiaceae are as distantly related to each other as is either one to the purple phototrophic bacteria (Fox et al. 1980; Stackebrandt and Woese 1981); (iii) the purple phototrophic bacteria form a major phylogenetic unit within the "urkingdom" of eubacteria (Fox et al. 1980; Stackebrandt and Woese 1981); (iv) these organisms are genealogically intermixed with non-phototrophic Gram-negative bacteria (Gibson et al. 1979; Woese et al. 1982; Seewaldt et al. 1982; Ludwig and Stackebrandt 1983), supporting the conversion hypothesis of the origin of respiring bacteria from phototrophic bacteria (Broda 1971, 1978); (v) within the order *Rhodospirillales* members of Chromatiaceae are phylogenetically separated from those of Rhodospirillaceae; the phylogenetic structure of the latter family, however, does not correlate with the genera into which the family has hitherto been divided on morphological grounds (Pfennig and Trüper 1974; Trüper and Pfennig 1981).

In contrast to the extensively investigated group of purple non-sulfur phototrophic bacteria (Gibson et al. 1979; Woese et al. 1982; Woese, unpublished; Stackebrandt, unpublished) only little information is available on the phylogenetic structure of Chromatiaceae (Gibson et al. 1979). Physiologically the purple sulfur bacteria are separated into those organisms forming globules of elemental sulfur inside the cell (*Chromatium* and related genera) and those depositing sulfur globules outside the cell (*Ectothiorhodospira*), when growing with sulfide as electron donor. The question of whether or not members of *Ectothiorhodospira* show any special phylogenetic relationship to members of *Chromatium* and related genera has not yet been answered. The lack of DNA homology found between a few strains of *Ectothiorhodospira* and *Chromatium* (Turova et al. 1982; Ivanova et al. 1983) excludes a relationship at the species level but gives no indication about a possible relationship at the genus level.

This communication reports a phylogenetic analysis by comparing the 16S rRNA catalogues of 7 strains of 5 described species of *Ectothiorhodospira*.

Materials and methods

Most of the *Ectothiorhodospira* strains investigated were isolated from desert lakes of the Wadi Natrun (Imhoff et al. 1979), *E. shaposhnikovii* was obtained from the German Collection of Microorganisms (DSM) in Göttingen, and *Ectothiorhodospira* strain BN 9906 is the subculture of an original isolate of Dr. Biebl (Braunschweig) from Solar Lake (Sinai). Strain numbers and properties are given in Fig. 1. All strains were grown in a mineral salts medium (Imhoff et al. 1978; Imhoff 1982) at pH 9.0, and supplemented with

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1 mM sodium sulfide, 5 mM sodium thiosulfate, and 5 mM sodium acetate. The total salt content was 25% (w/v) for the *E. halophila* strains, 15% (w/v) for *E. halochloris* and *E. abdelmalekii*, 5% (w/v) for *E. mobilis*, and 3% (Imhoff 1982) for *E. shaposhnikovii* and *Ectothiorhodospira* strain BN 9906. Strains were grown in 1 l screw-cap bottles anaerobically in the light at 2,000–4,000 lux and 30–33° C and harvested in late logarithmic growth phase. For the isolation of 16S rRNA, 3 to 5 g wet weight of cells were resuspended in 5 ml buffer (0.04 M Tris, 0.02 M sodium-acetate, pH 7.2) and opened by passing through a French pressure cell at 138 MPa. The lysate was deproteinized by phenol and the nucleic acids precipitated by addition of 2.5 volumes of ethanol. The precipitate was redissolved in 0.8 to 1 ml buffer (0.04 M Tris, 0.02 M sodium acetate, 0.2% SDS, pH 7.2) and separated on a 2.8% SDS polyacrylamide slab gel as described by Stackebrandt et al. (1981). Further purification steps of the 16S rRNA and the sequence analysis including RNAse T_1 digestion and in vitro labelling with γ-^{32}P-ATP and polynucleotide kinase (both from New England Nuclear) followed published procedures (Stackebrandt et al. 1981, 1982). Labelled oligonucleotides were separated in two dimensions, using high voltage electrophoresis (Savant) and ammonium formate gradient thin layer chromatography. Secondary sequence analysis, including alkaline hydrolysis of isolated oligonucleotides followed by a two-dimensional separation of cleavage products, and tertiary sequence analysis, including determination of 5′ labelled endgroups, one-dimensional separation of oligonucleotides and two-dimensional separation of intermediary fragments, cleaved under controlled conditions with alkali have been described (Stackebrandt et al. 1981, 1982; Fowler et al. 1983). Similarity coefficients (S_{AB}-values) were calculated using a CDC computer.

Results and discussion

The oligonucleotide catalogues of the seven strains of *Ectothiorhodospira* used in this study are shown in Table 1. Table 2 presents the binary association coefficients (S_{AB}) between *Ectothiorhodospira* strains and a variety of purple sulfur and nonsulfur phototrophic bacteria. Figure 1 shows the phylogenetic relationships of *Ectothiorhodospira* strains together with some of their properties and Fig. 2 displays the phylogenetic position of *Ectothiorhodospira* among the purple phototrophic bacteria.

All strains of *Ectothiorhodospira* form a phylogenetically coherent grouping that is clearly separated from all other phototrophic purple bacteria investigated so far. The strains subdivide into two subgroups. Subgroup I contains *Ectothiorhodospira halochloris*, two strains of *E. halophila* and *E. abdelmalekii*, subgroup II embraces *E. mobilis*, *E. shaposhnikovii* and *Ectothiorhodospira* strain BN 9906.

The uniting features of members of subgroup I are the presence of bipolar inserted flagella and high salinity and temperature optima. Despite its high phylogenetic relatedness *E. halochloris* differs markedly from the other two species by a significantly lower G + C content. In addition, both *E. abdelmalekii* and *E. halochloris* have different photosynthetic pigments (bacteriochlorophyll b, rhodopin, and rhodopin derivatives) from all other species of *Ectothiorhodospira*.

Strains of subgroup II show a higher similarity in phenotypic properties. The peaks of their salt optima are all

Table 1. Oligonucleotide catalogues for the 16S rRNAs of various members of *Ectothiorhodospira*. The sequences occur in organisms as listed by number, as follows: 1, *E. halophila* BN 9624; 2, *E. halophila* BN 9628; 3, *E. halochloris* [T] BN 9850; 4, *E. abdelmalekii* BN 9840; 5, *E. mobilis* BN 9903; 6, Ectothiorhodospira strain BN 9906; 7, *E. shaposhnikovii* [T] BN 9711; [T] indicates type strain

6-mers		CAUCUG	4–7	CAUUUCG	6
AAACCG	2, 3, 5, 7	CCCUUG	1–3, 5–7	CCAUCAG	3, 4
AAACUG	1, 2, 6, 7	CCUUCG	1–7	CCCCAAG	7
AACAUG	6	CUAACG	1–7	CCCUACG	1–6
AACUAG	4–7	CUACCG	6	CCCUCUG	3
AAUACG	1–7	CUCACG	1–4	CCCUUUG	2, 4
AAUCCG	4	CUCUUG	4	CCUUUCG	5
AAUCUG	3, 5–7	UAAACG	1–7	CUAUCAG	5–7
AAUUUG	4	UAACAG	3	CUCCUAG	5
ACAAUG	1–7	UAACUG	5–7	UAACAAG	1–7
ACACUG	5–7	UAAUCG	1–7	UAAUACG	1–7
ACAUAG	5	UCAAUG	5–7	UACCACG	1–4
ACCAAG	4	UCCACG	1–7	UCAACCG	4
ACCUCG	5–7	UCCUAG	1–3	UCCCUAG	1, 2, 4–7
ACUCCG	3, 4	UCUUAG	4	UCCUCAG	3
AUACUG	1, 2, 4	UUCCCG	1–7	UCCUUAG	3
AUAUCG	1–7	*7-mers*		UUAAUCG	1–7
AUCACG	6	AAACCCG	3, 4	UUACCCG	1, 2
AUCCAG	1, 3, –5, 7	AAACUCG	1, 2, 5–7	UUCUCCG	1, 4–7
AUCCUG	1–7	AAUAUUG	4–7	UUCUCUG	2
AUUAAG	4	AAUCCUG	5–7	*8-mers*	
CAAACG	7	AUCACCG	5–7	AACACCAG	1–7
CACAAG	1–7	AUCUUCG	6	AACUCAUG	1, 2
CACCAG	1–7	AUUCAAG	3	AACUCUAG	5–7
CACUCG	3	CAAACAG	1–7	AAUCACUG	2–4
CAUACG	1, 2, 4–7	CAACUCG	1–7	AAUUACUG	5–7
CAUCCG	1, 2	CAUUACG	1–3	AAUUCCCG	1, 3, 4

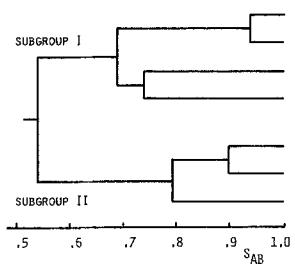
Table 1 (continued)

AAUUUCCG	5–7	CAACCCUUG	1–7	UUACCCAAAG	3, 4
ACAAACCG	1–7	CACUUUCAG	5–7	UUACCCACAG	7
ACAACCCG	5–7	CCCACCAAG	7	*11-mers*	
ACUCCAUG	5–7	CCUACCAAG	1–5	AACCUUACCUG	5–7
ACUCUCUG	5–7	CUAACUACG	5–7	CACUUUCAUCG	1, 2
AUAAACUG	6	CUAAUACCG	7	CACUUUCAUUG	3, 4
AUACCCUG	1–7	CUAAUACUG	5, 6	CCAACACUUUG	7
AUCCAUAG	5, 6	CUACACACG	1–7	CCUAACACAUG	1–6
CAACCCUG	5, 6	CUCAACCUG	3, 7	CCUCUCUUAUG	1, 2
CACUCUCG	3, 4	CUUAACCUG	4	CUUAACACAUG	7
CCAAUCCG	1, 2	CUUAAUACG	5, 6	UCUAACCUUCG	1, 2, 5–7
CCACACUG	1–7	CUUACCAAG	6	UUAAUACCCUG	1, 2
CCACCCAG	2	UACACACCG	1–7	*12-mers*	
CCCUUAUG	1–7	UUCACCACG	5, 6	ACAUCACCCACG	5, 6
CUACAAUG	1–7	UUUAAUUCG	1–7	$AUCACCUCCUUU_{OH}$	1–7 (3′terminus)
CUCUCAUG	4	UUUACCACG	7	UCUAACCCUUCG	3
CUUCCCUG	1, 2	*10-mers*		*13-mers*	
UCAUCAUG	1–7	AAACUCAAAG	5–7	AAUUUACCCUUCG	1, 2
UUAAACUG	5	AACCUUACCG	1–4	CUAAAACUCAAAG	1–4
9-mers		ACUAAAACUG	5	UUUAACCUCUUAG	7
ACAUCCUCG	1–7	AUUAAUACCG	1–4	*14-mers*	
ACCUUCUAG	4	CUAAUCCCAG	4	AAUUAACCCCUUCG	5, 6
ACUCCUACG	1–7	CUAAUCCCUG	1, 2	CUAAUCCCAAAAAG	5–7
CAAACUCCG	4	CUCAACCUAG	1, 2	CUAAUCUCUUACAG	3
CAAACUUCG	1–3	UCACACCAUG	1–7		

Table 2. Binary comparisons among the 16S rRNA catalogues of *Ectothiorhodospira* strains and various Gram-negative reference organisms

	Ectothiorhodospira						
	E. halophila BN 9624	*E. halophila* BN 9628	*E. halochloris* BN 9850	*E. abdelmalekii* BN 9840	*E. mobilis* BN 9903	*E. strain* BN 9906	*E. shaposhnikovii* BN 9711
Ectothiorhodospira halophila BN 9624[a]	–	0.94	0.70	0.70	0.56	0.56	0.55
E. halophila BN 9628[a]	0.94	–	0.69	0.68	0.54	0.54	0.53
E. halochloris BN 9850[a]	0.70	0.69	–	0.74	0.52	0.49	0.51
E. abdelmalekii BN 9840[a]	0.70	0.68	0.74	–	0.57	0.54	0.54
E. mobilis BN 9903[a]	0.56	0.54	0.52	0.57	–	0.90	0.80
Ectothiorhodospira strain BN 9906[a]	0.56	0.54	0.49	0.54	0.90	–	0.79
E. shaposhnikovii BN 9711[a]	0.55	0.53	0.51	0.54	0.80	0.79	–
Chromatium vinosum strain D[c]	0.49	0.49	0.49	0.48	0.48	0.48	0.55
Amoebobacter roseus Pf 6611[b]	0.50	0.49	0.49	0.49	0.51	0.50	0.55
Thiocapsa roseopersicina Pf 1711[b]	0.50	0.50	0.50	0.50	0.50	0.50	0.55
Rhodopseudomonas sphaeroides NCIB 8253[c]	0.35	0.35	0.34	0.35	0.30	0.32	0.30
Rhodopseudomonas capsulata strain St. Louis[c]	0.34	0.35	0.33	0.35	0.30	0.32	0.29
Rhodopseudomonas palustris ATCC 11168[c]	0.29	0.29	0.31	0.30	0.26	0.26	0.31
Rhodomicrobium vannielii[c]	0.31	0.32	0.32	0.32	0.32	0.31	0.35
Rhodospirillum rubrum ATCC 11170[c]	0.37	0.36	0.35	0.38	0.35	0.38	0.37
Rhodopseudomonas gelatinosa strain TG9[c]	0.41	0.39	0.43	0.40	0.39	0.38	0.42
Rhodospirillum tenue Pf 3760[c]	0.35	0.33	0.35	0.32	0.31	0.31	0.36

[a] BN = Strain collection of the Department of Microbiology, University Bonn, FRG
[b] Pf = strain collection of the Department of Microbiology, Konstanz, FRG
[c] The catalogues of rhodopseudomonads and relatives and the similarity coefficients derived therefrom have been published previously (Gibson et al. 1979)



	PROPERTIES								
	G+C CONTENT (MOL%)	ISOPRENOID QUINONE COMPOSITION	CELL DIAMETER (µM)	FLAGELLA	COLOUR OF SUSPENSION	MAIN CHLOROPHYLL	CAROTENOID	OPTIMAL SALINITY (%(W/V)	OPTIMAL TEMPERATURE (°C)
E. HALOPHILA BN 9624	69.4		0.8-0.9	BIPOLAR	RED	A	SPIRILLOXANTHIN	18-34	32-40
E. HALOPHILA BN 9628	N.D.	Q-8	0.8-0.9	BIPOLAR	RED	A	SPIRILLOXANTHIN	18-34	32-40
E. HALOCHLORIS BN 9850(DSM 1059)	52.9	MK-8	0.5-0.6	BIPOLAR	GREEN	B	RHODOPIN	14-27	33-44
E. ABDELMALEKII BN 9840(DSM 2110)	63.8		0.9-1.2	BIPOLAR	GREEN	B	RHODOPIN	12-18	30-40
E. MOBILIS BN 9903	63.5[1]	Q-7	0.7-1.0	POLAR TUFT	RED	A	SPIRILLOXANTHIN	2-17	25-40
E. SPECIES BN 9906	N.D.	MK-7	0.7-1.0	POLAR TUFT	RED	A	SPIRILLOXANTHIN	3-6	33-40
E. SHAPOSHNIKOVII BN 9711(DSM 243)	62.0[1]		0.8-0.9	POLAR TUFT	RED	A	SPIRILLOXANTHIN	1-7	30-35

Fig. 1. Dendrogram of relationships for members of the genus *Ectothiorhodospira* and distribution of some of their properties. Data are from Trüper and Imhoff (1981), Imhoff and Trüper (1981) and Imhoff (unpublished). [1] H. Hippe (unpublished)

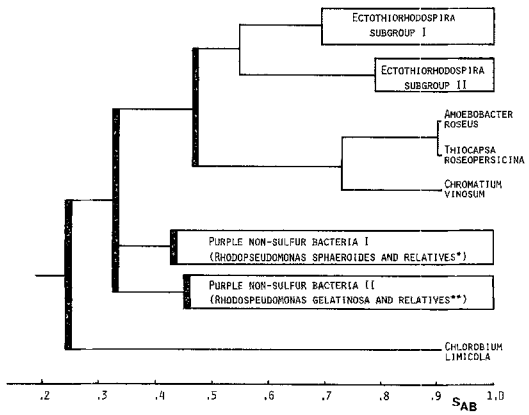


Fig. 2. Dendrogram of relationships showing the phylogenetic position of the genus *Ectothiorhodospira* among other purple sulfur and purple non-sulfur phototrophic bacteria

* Group I of purple non-sulfur bacteria contains among others *Rhodopseudomonas sphaeroides, R. capsulata, R. viridis, R. palustris, Rhodomicrobium vannielii, Rhodospirillum rubrum, Paracoccus denitrificans, Rhizobium leguminosarum, Nitrobacter winogradskyi* and *Aquaspirillum itersonii*

** Group of purple non-sulfur bacteria contains among others *Rhodopseudomonas gelatinosa, Rhodospirillum tenue, Sphaerotilus natans, Alcaligenes faecalis, Spirillum volutans* and *Aquaspirillum gracile*

near 5%; because the salt concentrations given in Fig. 1 are for 75% optimal growth, the upper value for *E. mobilis* BN 9903 is very high as this strain is very salt tolerant. In addition, the DNA base composition of all strains of subgroup II are rather close. Five strains of *E. mobilis, E. shaposhnikovii* DSM 243, and *E. vacuolata* DSM 2111 all have a G+C content of 61.2–64.0 mol% (Imhoff et al. 1981; Mandel et al. 1971; H. Hippe, unpublished). The independently determined G+C values of *E. shaposhnikovii* are well in agreement (62.0, 62.3, and 64.0 mol% H. Hippe, unpublished; Mandel et al. 1971) while the values determined by Mandel et al. (1971) for the same strains of *E. mobilis* are significantly higher than those of H. Hippe (unpublished). *Ectothiorhodospira vacuolata*, which has not been included in the present study, is in all probability a member of subgroup II, since it has quinones and menaquinones of the Q-7 and MK-7 types, respectively, and shares certain phenotypic properties with members of this subgroup (Imhoff et al. 1981).

The close genetic relationship of *E. shaposhnikovii* and *E. mobilis*, as seen from 90–100% DNA homology (Ivanova et al. 1983) could not be confirmed in our studies. However, the type strain of *E. mobilis*, included in the hybridization studies, was not included in the present study. A broader DNA homology survey is certainly necessary.

At a lower level of relationship *Ectothiorhodospira* is related to those members of Chromatiaceae depositing sulfur globules inside their cells [*Chromatium, Amoebobacter, Lamprocystis, Thiocapsa, Thiocystis* and *Thiospirillum* (Fowler, Widdel, Pfennig, Stackebrandt, unpublished)] and their non-phototrophic relatives in this subline of the purple phototrophic eubacteria (Group III, according to Gibson et al. 1979), which, among others, harbours enterobacteria, vibrios and the fluorescent pseudomonads. No specific relationship can be detected between *Ectothiorhodospira* strains and purple non-sulfur bacteria of the two groups defined by *Rhodopseudomonas sphaeroides* and relatives and by *Rhodopseudomonas gelatinosa* and relatives (Group I and II, respectively, Gibson et al. 1979), some of which are also capable of using sulfide as the photosynthetic electron donor (Hansen and Veldkamp 1973).

Even more distant is the relationship to *Chlorobium limicola*, which, though resembling *Ectothiorhodospira* strains in its ability to deposite sulphur extracellularly, represents a major division of eubacteria (Fox et al. 1980; Stackebrandt and Woese 1981). The finding that those eubacteria using sulfide as an electron donor are found in various major lines of descent (*Chlorobium, Chloroflexus,* cyanobacteria, purple sulfur and some purple non-sulfur bacteria) indicates that this property has to be considered an ancient character (Trüper 1982). Broda (1978), from the use of organic compounds as electron donors in the light, considered the purple non-sulfur bacteria to be direct descendants of the most ancient heterotrophic eubacterial ancestor. However, the 16S rRNA data indicate that this is not very likely. It rather seems that the purple nonsulfur bacteria evolved from the sulfide oxidizing phototrophic bacteria at a later stage. The finding that *Rhodopseudomonas capsulata, R. palustris, R. sphaeroides* and *R. sulfidophila* actually oxidize sulfide, with *R. capsulata* SMG 155 and *R. sphaeroides* being able to convert sulfide into extracellularly deposited sulfur (Hansen and Gemerden

1972; Hansen and Veldkamp 1973) may be taken as a supporting argument for this hypothesis.

Since *Ectothiorhodospira* is phylogenetically older than *Chromatium* [as seen from the S_{AB} values, separating *Ectothiorhodospira* species at lower S_{AB} values than representatives of *Chromatium* and related taxa (Fowler, Widdel, Pfennig and Stackebrandt, in preparation)] it seems that the ability to form elemental sulfur globules outside the cells is a more ancient feature than the intracellular deposition of sulfur. Trüper (1982) has already pointed out that the latter property may perhaps have some advantage over extracellular deposition with respect to the accessibility of sulfur.

The taxonomic interpretation of the phylogenetic data of *Ectothiorhodospira* strains allows two alternatives.

Firstly as in the present classification (see Trüper and Pfennig 1981) *Ectothiorhodospira* and *Chromatium* and related genera could be members of one family Chromatiaceae, since all genera enclosed show a distinct relationship. Alternatively, the deep branching separating the two lines of descent leading to *Ectothiorhodospira* and to *Chromatium* and related taxa, respectively, together with the differences in metabolism, could allow the exclusion of *Ectothiorhodospira* from Chromatiaceae, followed by the description of a family Ectothiorhodospiraceae with *Ectothiorhodospira* as the only recognized genus so far. Chromatiaceae and Ectothiorhodospiraceae would then comprise one order (yet to be described) equivalent to the order Rhodospirillales, which consequently would lose the family Chromatiaceae.

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