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ORIGINAL PAPER

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# The phylogenetic relationship among Ectothiorhodospiraceae: a reevaluation of their taxonomy on the basis of 16S rDNA analyses

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**Abstract** Sequences of the 16S rRNA gene were determined from all type strains of the recognized *Ectothiorhodospira* species and from a number of additional strains. For the first time, these data resolve the phylogenetic relationships of the Ectothiorhodospiraceae in detail, confirm the established species, and improve the classification of strains of uncertain affiliation. Two major groups that are recognized as separate genera were clearly established. The extremely halophilic species were removed from the genus *Ectothiorhodospira* and reassigned to the new genus *Halorhodospira* gen. nov., to recognize that the most halophilic eubacteria are species of this genus. These species are *Halorhodospira halophila* comb. nov., *Halorhodospira halochloris* comb. nov., and *Halorhodospira abdelmalekii* comb. nov. Among the slightly halophilic *Ectothiorhodospira* species, the classification of strains belonging to *Ectothiorhodospira mobilis* and *Ectothiorhodospira shaposhnikovii* was improved. Several strains that were tentatively identified as *Ectothiorhodospira mobilis* form a separate cluster on the basis of their 16S rDNA sequences and are recognized as two new species: *Ectothiorhodospira haloalkaliphila* sp. nov., which includes the most alkaliphilic strains originating from strongly alkaline soda lakes, and *Ectothiorhodospira marina*, describing isolates from the marine environment.

**Key words** *Ectothiorhodospira* · *Halorhodospira* · Phototrophic purple bacteria · Ribosomal RNA · Phylogeny · DNA sequences · Taxonomy

**Abbreviations** *Ahr. Arhodomonas* · *Chr. Chromatium* · *E. Escherichia* · *Ect. Ectothiorhodospira* · *Hlr. Halorhodospira* · *Rps. Rhodopseudomonas* · *Rsp. Rhodospirillum* · *MK* Menaquinone · *Q* Ubiquinone

Dedicated to Prof. Dr. N. Pfennig on the occasion of his 70th birthday

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## Introduction

The Ectothiorhodospiraceae represent a group of haloalkaliphilic purple sulfur bacteria that has been shown to be separated from, but related to species of the Chromatiaceae according to their ribosomal RNA oligonucleotide catalogues (Stackebrandt et al. 1984). On the basis of physiological and molecular information, the genus *Ectothiorhodospira* was removed from the Chromatiaceae and placed into the new family of the Ectothiorhodospiraceae (Imhoff 1984a), which is at present represented by *Ectothiorhodospira* as the only genus. Both families form distinct groups within the gamma subdivision of the proteobacteria (Fowler et al. 1984; Stackebrandt et al. 1984).

Isolates of *Ectothiorhodospira* have been obtained from marine, hypersaline, and haloalkaline environments and require or prefer saline and alkaline growth conditions. Their pigment content, the performance of anoxygenic photosynthesis, and the formation of elemental sulfur during sulfide oxidation clearly specifies them as purple sulfur bacteria. In contrast to the Chromatiaceae, all Ectothiorhodospiraceae form and deposit elemental sulfur outside their cells (Imhoff 1984a). Among the recognized *Ectothiorhodospira* species are the extremely halophilic species *Ectothiorhodospira halophila* (Raymond and Sistrom 1969), *Ectothiorhodospira halochloris* (Imhoff and Trüper 1977), and *Ectothiorhodospira abdelmalekii* (Imhoff and Trüper 1981), and the slightly halophilic species *Ectothiorhodospira mobilis* (Trüper 1968), *Ectothiorhodospira vacuolata* (Imhoff et al. 1981), *Ectothiorhodospira shaposhnikovii* (Cherni et al. 1969), and *Ectothiorhodospira marismortui* (Oren et al. 1989).

Molecular studies on the systematics of *Ectothiorhodospira* have included 16S rRNA cataloguing (Stackebrandt et al. 1984; Oren et al. 1989), DNA-DNA and rRNA-DNA hybridization (Ivanova et al. 1985), and

analysis of lipopolysaccharides (Zahr et al. 1992), DNA restriction patterns (Ventura et al. 1993), and quinone composition (Imhoff 1984b; Ventura et al. 1993). These studies do not resolve the phylogenetic and taxonomic relationships of *Ectothiorhodospira* because only few strains have been included or because the methods employed do not give the required high resolution.

Although limited information is available from the oligonucleotide analysis of seven strains (including three type strains) of five *Ectothiorhodospira* species (Stackebrandt et al. 1984), these data demonstrate the presence of two major phylogenetically distinct groups within the Ectothiorhodospiraceae. A formal separation into different genera has so far not been proposed.

The aim of the present study was to analyze the complete sequence of the type strains and other representatives of all species of Ectothiorhodospiraceae in order to resolve the phylogenetic relatedness within this group.

## Materials and methods

### Bacterial strains and cultivation conditions

All *Ectothiorhodospira* strains used during this study are listed in Table 1, showing their old and new classification, various strain designations, and the accession numbers of their 16S rDNA sequences in the EMBL databank. Most of the strains were isolated by one of us (J. F. Imhoff), maintained in our culture collection in liquid nitrogen, and recultivated from this collection. *Ect. mobilis* DSM 237, DSM 241, *Ect. halophila* DSM 244, and *Ect. marismortui* DSM 4180 were obtained from the German culture collection (DSM; Braunschweig, Germany) *Ect. shaposhnikovii* DSM 243 was obtained from Dr. E. N. Kondratieva (Moscow, Russia), and *Ect. mobilis* DSM 239 was obtained from Dr. H. G. Trüper (Bonn, Germany).

Strains of *Ect. mobilis*, *Ect. vacuolata*, *Ect. shaposhnikovii*, and *Ect. marismortui* were grown in modified Pfennig's medium with 3% NaCl at a pH of 7.6 (Trüper 1970). Other strains were grown in medium for haloalkaliphilic *Ectothiorhodospira* species (Imhoff 1988). Cells were cultivated in 50-ml screw-capped bottles at 37°C and a light intensity of 5,000 lux (tungsten lamp) until the late exponential growth phase.

### Determination of the DNA G+C content

DNA was prepared from cells (2–3 g wet weight) according to the method of Marmur (1961). Impurities were removed by treatment with ribonuclease and proteinase K. The G+C content was determined by thermal denaturation in saline citrate buffer (pH 7.0) according to Mandel and Marmur (1968). DNA from *Escherichia coli* K12 (DSM 498, 51.7 mol% G+C) was used as a reference (Gillis et al. 1970).

### 16S rDNA gene sequences

DNA was extracted and purified from 2 ml of a well-grown culture using the QIAGEN genomic DNA buffer set. Recombinant Taq polymerase was used for PCR (Mullis and Faloona 1987), which was started with the following primers: 5´-GTTTGATCCTGGCTCAG-3´ and 5´-TACCTTGTTACGACTT-3´. Sequences were obtained by cycle sequencing with the SequiTherm sequencing kit (Biozym, Oldendorf, Germany) and the chain termination reaction (Sanger et al. 1977) using an automated laser fluorescence sequencer (Pharmacia, Uppsala, Sweden). Sequences were aligned using the CLUSTAL W program. Distance matrices were calculated on the basis of the algorithm according to Jukes and Cantor (1969) with the DNADIST program within the PHYLIP program package (Felsenstein 1989). The FITCH program in the PHYLIP package fitted a tree to the evolutionary distances. The sequences were deposited with EMBL (see Table 1 for accession numbers).

## Results and discussion

Complete sequences of 16S rDNA from 13 strains of *Ectothiorhodospira* species were determined. Sequences were aligned and compared to those of a related chemoheterotrophic bacterium, *Arhodomonas aquaeolei*, and of *Ectothiorhodospira halochloris*, *Chromatium vinosum*, *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, and *Escherichia coli*, which are available from the EMBL databank. A distance matrix with similarity values and $K_{nuc}$ values and a phylogenetic tree are shown in Table 2 and Fig. 1, respectively.

Compared to previous investigations on the relationships of *Ectothiorhodospira* by 16S rRNA cataloguing

**Table 1** Species and strains used in this study (*Ect. Ectothiorhodospira, Hlr. Halorhodospira*)

| Old classification | New classification | DSM/ATCC | BN number | Other designation | EMBL accession number |
|---|---|---|---|---|---|
| Ect. mobilis | None | DSM 237$^T$ | 9911 | Trüper 8112 | X93481 |
| Ect. mobilis | Ect. shaposhnikovii | DSM 239 | 9912 | Trüper 8115 | X93480 |
| Ect. mobilis | Ect. marina | DSM 241$^T$ | 9914 | Matheron BA1010 | X93476 |
| Ect. mobilis | Ect. haloalkaliphila | ATCC 51935$^T$ | 9903 | Imhoff 51/7 | X93479 |
| Ect. mobilis | Ect. haloalkaliphila | | 9902 | Imhoff C | X93475 |
| Ect. vacuolata | None | DSM 2111$^T$ | 9512 | Imhoff β1 | X93478 |
| Ect. shaposhnikovii | None | DSM 243$^T$ | 9711 | Kondratieva N1 | M59151 |
| Ect. marismortui | None | DSM 4180$^T$ | 9410 | Oren EG-1 | X93482 |
| Ect. halochloris | Hlr. halochloris | DSM 1059$^T$ | 9850 | Imhoff A | M59152 |
| Ect. halochloris | Hlr. halochloris | | 9851 | Imhoff 51/12 | X93483 |
| Ect. abdelmalekii | Hlr. abdelmalekii | DSM 2110$^T$ | 9840 | Imhoff 51/20 | X93477 |
| Ect. halophila | Hlr. halophila | DSM 244$^T$ | 9632 | Raymond SL1 | M26630 |
| Ect. halophila | Hlr. halophila | | 9624 | Imhoff 51/1 | X93474 |
| Ect. halophila | Hlr. halophila | | 9630 | Imhoff 51/3 | X93484 |

**Table 2** Evolutionary distance matrix and percent similarity of 16S rRNA sequences of *Ectothiorhodospira* species, *Halorhodospira* species, and reference species. The values on the *upper right* are the uncorrected percentages of similarity, and on the lower left are $K_{nuc}$-values corrected for multiple base change by the method of Jukes and Cantor (1969). Reference sequences: *Arhodomonas (Arh.) aquaeolei* (ATCC 49307$^T$) M26631, *Chromatium (Chr.) vinosum* (ATCC 17899$^T$) M26629, *Escherichia (E.) coli* K02555, *Ectothiorhodospira (Ect.) halochloris* (DSM 1059$^T$) M59152, *Rhodospirillum (Rsp.) rubrum* (ATCC 11170$^T$) D30778, *Rhodopseudomonas (Rps.) palustris* (ATCC$^T$) D25312

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 *Hlr. halochloris,* DSM 1059 | | 99.86 | 98.26 | 94.84 | 94.10 | 94.48 | 89.71 | 89.03 | 88.84 | 88.85 | 87.58 | 87.58 | 88.09 | 87.43 | 88.97 | 87.01 | 82.41 | 83.22 | 81.86 |
| 2 *Hlr. halochloris,* BN 9851 | 0.0014 | | 98.22 | 94.57 | 93.91 | 94.35 | 89.63 | 88.77 | 88.65 | 88.66 | 87.34 | 87.36 | 87.83 | 87.19 | 88.75 | 86.84 | 82.31 | 83.08 | 81.44 |
| 3 *Hlr. abdelmalekii,* DSM 2110$^T$ | 0.0176 | 0.0180 | | 93.72 | 93.53 | 93.91 | 89.24 | 89.41 | 89.36 | 88.97 | 87.91 | 87.98 | 88.12 | 86.88 | 89.19 | 86.82 | 82.56 | 83.45 | 81.61 |
| 4 *Hlr. halophila,* DSM 244$^T$ | 0.0534 | 0.0563 | 0.0655 | | 98.71 | 97.96 | 91.88 | 89.31 | 88.91 | 88.86 | 88.03 | 87.75 | 88.85 | 88.42 | 87.91 | 87.50 | 82.33 | 83.03 | 81.48 |
| 5 *Hlr. halophila,* BN 9624 | 0.0614 | 0.0635 | 0.0676 | 0.0130 | | 97.81 | 91.39 | 88.88 | 88.75 | 88.64 | 87.72 | 87.72 | 88.55 | 88.06 | 87.66 | 86.84 | 81.97 | 82.59 | 81.00 |
| 6 *Hlr. halophila,* BN 9630 | 0.0573 | 0.0587 | 0.0635 | 0.0206 | 0.0222 | | 91.16 | 88.95 | 88.88 | 88.83 | 87.72 | 87.77 | 88.62 | 87.98 | 87.38 | 86.91 | 81.88 | 82.75 | 81.11 |
| 7 *Arh. aquaeolei,* ATCC 49307$^T$ | 0.1106 | 0.1115 | 0.1161 | 0.0859 | 0.0914 | 0.0940 | | 88.97 | 88.64 | 88.87 | 88.64 | 88.30 | 89.08 | 88.44 | 87.84 | 88.70 | 83.63 | 83.34 | 81.84 |
| 8 *Ect. shaposhnikovii,* DSM 243$^T$ | 0.1185 | 0.1216 | 0.1141 | 0.1153 | 0.1203 | 0.1195 | 0.1192 | | 99.24 | 98.22 | 95.99 | 96.25 | 96.56 | 95.26 | 95.16 | 90.31 | 84.48 | 83.37 | 82.24 |
| 9 *Ect. shaposhnikovii,* DSM 239 | 0.1208 | 0.1230 | 0.1147 | 0.1199 | 0.1218 | 0.1203 | 0.1231 | 0.0076 | | 98.28 | 95.69 | 95.89 | 96.19 | 94.95 | 94.85 | 89.85 | 84.07 | 83.15 | 82.01 |
| 10 *Ect. vacuolata,* DSM 2111$^T$ | 0.1206 | 0.1229 | 0.1193 | 0.1205 | 0.1231 | 0.1209 | 0.1204 | 0.0180 | 0.0173 | | 95.29 | 95.35 | 96.27 | 95.45 | 95.20 | 89.94 | 84.29 | 83.10 | 82.61 |
| 11 *Ect. haloalkaliphilia,* BN 9902 | 0.1357 | 0.1386 | 0.1318 | 0.1304 | 0.1341 | 0.1341 | 0.1231 | 0.0412 | 0.0443 | 0.0486 | | 98.09 | 98.41 | 95.68 | 94.67 | 89.43 | 83.48 | 82.17 | 81.26 |
| 12 *Ect. marina,* DSM 241$^T$ | 0.1357 | 0.1384 | 0.1309 | 0.1337 | 0.1341 | 0.1334 | 0.1272 | 0.0384 | 0.0422 | 0.0480 | 0.0193 | | 97.93 | 95.46 | 94.89 | 88.86 | 83.25 | 81.87 | 81.10 |
| 13 *Ect. haloalkaliphilia,* ATCC 51935$^T$ | 0.1296 | 0.1327 | 0.1293 | 0.1206 | 0.1242 | 0.1234 | 0.1180 | 0.0352 | 0.0390 | 0.0382 | 0.0160 | 0.0209 | | 96.46 | 95.32 | 89.65 | 84.14 | 82.44 | 81.72 |
| 14 *Ect. marismortui,* DSM 4180$^T$ | 0.1375 | 0.1404 | 0.1442 | 0.1257 | 0.1300 | 0.1309 | 0.1255 | 0.0489 | 0.0522 | 0.0469 | 0.0444 | 0.0468 | 0.0362 | | 95.05 | 89.39 | 84.17 | 82.28 | 81.44 |
| 15 *Ect. mobilis,* DSM 237$^T$ | 0.1193 | 0.1218 | 0.1167 | 0.1318 | 0.1348 | 0.1381 | 0.1326 | 0.0500 | 0.0533 | 0.0495 | 0.0552 | 0.0529 | 0.0483 | 0.0512 | | 89.46 | 83.41 | 82.66 | 81.14 |
| 16 *Chr. vinosum,* ATCC 17899$^T$ | 0.1426 | 0.1447 | 0.1449 | 0.1367 | 0.1447 | 0.1438 | 0.1224 | 0.1037 | 0.1090 | 0.1080 | 0.1139 | 0.1205 | 0.1113 | 0.1143 | 0.1135 | | 85.27 | 83.15 | 80.93 |
| 17 *E. coli* | 0.2004 | 0.2017 | 0.1984 | 0.2014 | 0.2061 | 0.2073 | 0.1846 | 0.1738 | 0.1790 | 0.1762 | 0.1866 | 0.1895 | 0.1781 | 0.1777 | 0.1874 | 0.1639 | | 80.82 | 78.56 |
| 18 *Rsp. rubrum,* ATCC 11170$^T$ | 0.1899 | 0.1917 | 0.1869 | 0.1923 | 0.1981 | 0.1959 | 0.1883 | 0.1879 | 0.1908 | 0.1914 | 0.2035 | 0.2075 | 0.2000 | 0.2021 | 0.1971 | 0.1908 | 0.2214 | | 86.25 |
| 19 *Rps. palustris,* ATCC 17001$^T$ | 0.2076 | 0.2131 | 0.2109 | 0.2126 | 0.2190 | 0.2175 | 0.2079 | 0.2026 | 0.2056 | 0.1978 | 0.2156 | 0.2177 | 0.2094 | 0.2132 | 0.2171 | 0.2200 | 0.2525 | 0.1518 | |

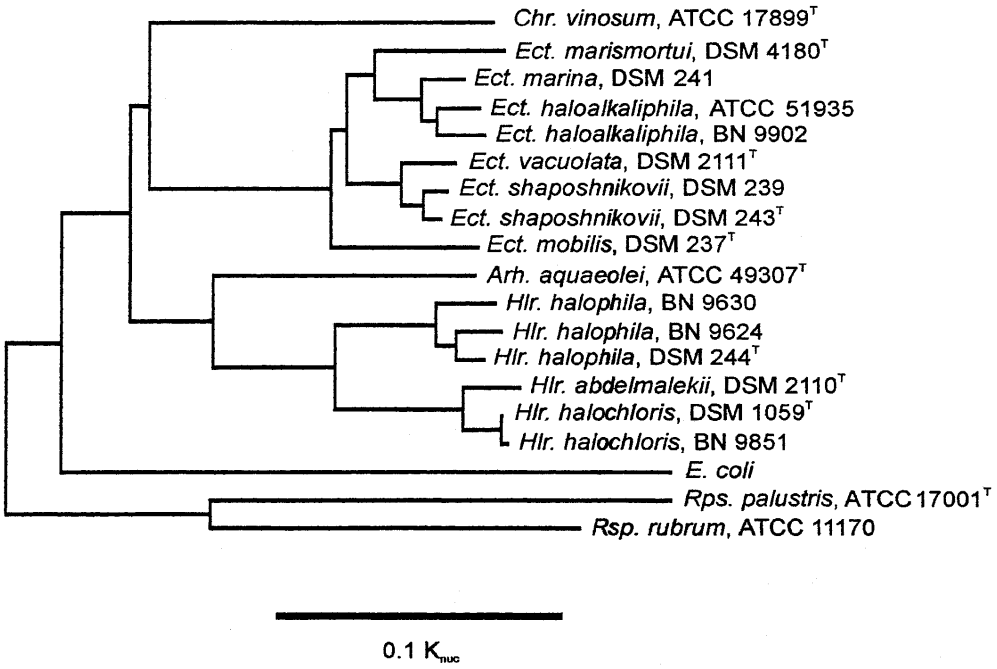


**Fig. 1** Phylogenetic tree of the Ectothiorhodospiraceae derived from the data of the distance matrix calculated as indicated in Materials and methods. For strain designations and deposition numbers, see Table 1

(Stackebrandt et al. 1984) and by DNA-DNA hybridization (Ivanova et al. 1985), a much larger number of strains was investigated in the present study, including the type strains of all recognized species of *Ectothiorhodospira*. The clear separation of two major groups is now established and supported by a number of signature sequences characteristic for species belonging to either of the two groups (Table 3). Sequences of the extremely halophilic species *Ect. halophila*, *Ect. halochloris*, and *Ect. abdelmalekii* formed one group that was only distantly related ($K_{nuc}$ values of 0.1085–0.1402) to the second group, which was represented by strains designated as *Ect. mobilis*, *Ect. shaposhnikovii*, *Ect. marismortui*, and *Ect. vacuolata*. In the former group, the two species with bacteriochlorophyll *b* are well differentiated by their sequences, and in addition form a subgroup different from *Ect. halophila*. *Ect. halophila* is distinct from these two species on the basis of the 16S rDNA sequence data, which are supported by comparison of oligonucleotide catalogues (Stackebrandt et al. 1984), by DNA-DNA hybridization studies (Ivanova et al. 1985), by DNA restriction pattern analysis (Ventura et al. 1993), and by fatty acid analysis (Thiemann and Imhoff 1995). The sequence data and in particular the similarity between the type strain of *Ect. halophila* DSM 244$^T$ and strain BN 9624 would suggest the recognition of the latter as a strain of *Ect. halophila* and the exclusion of strain BN 9630 from this species. However, close relationships have been found between strain BN 9630 and the type strain DSM 244$^T$ by DNA restriction pattern analysis (Ventura et al. 1993). Two clusters of *Ect. halophila* strains (including 12 different isolates) were clearly differentiated on the basis of their fatty acid composition and salt dependence (Thiemann and Imhoff 1996). The type strain of *Ect. halophila*, according to salt optimum and fatty acid composition, is similar to strain BN 9630, but dissimilar to strain BN 9624. On the

**Table 3** Signature sequences of the 16S rDNA used to differentiate between *Ectothiorhodospira* species and *Halorhodospira* species. Position numbering is according to the *Escherichia coli* sequence

| Position(s) | Ectothiorhodospira | Halorhodospira |
|---|---|---|
| 95–97 | TGA | CGG |
| 137–138 | GG | TT |
| 149–151 | CAA | TAG |
| 167–168 | GC | AT |
| 206–207 | CT | GA |
| 217 | G | A |
| 221–225 | TATCA | CGAAG |
| 234 | T | C |
| 501 | A | G |
| 507 | T | A |
| 512 | C | T |
| 667–668 | AG | GT |
| 736–737 | CT | GC |
| 761 | T | C |
| 868 | G | A |
| 905 | G | A |
| 985 | T | G |
| 1009–1011 | GCA | CTG |
| 1016–1019 | TGCT | CGGA |
| 1188 | T | C |
| 1243 | C | G |
| 1251 | T | A |
| 1292 | G | C |
| 1296 | G | C |
| 1307 | T | C |
| 1328 | A | G |
| 1334–1335 | TC | CA |
| 1354 | T | A |
| 1420 | T | C |
| 1461–1462 | TT | CG |
| 1478–1480 | AAT | GGC |

basis of their oligonucleotide catalogues, high similarities were revealed between strain BN 9628 and strain BN 9624 ($S_{AB}$ value 0.94; Stackebrandt et al. 1984), which according to fatty acid pattern belong to different clusters. Thus, the available data are not congruent, and at present, a differentiation of *Ect. halophila* strains at the species level is not indicated.

Among all chemotrophic eubacteria, *Arhodomonas aquaeolei* is the only known species that shows a reasonable relationship to *Ectothiorhodospira* (Adkins et al. 1993). It is distinct from, but particularly related to the group of extremely halophilic *Ectothiorhodospira* species (Fig. 1).

Those strains with low salt requirement appear to be more heterogeneous according to 16S rDNA sequences than so far recognized and to form several distinct groups. Differences in the 16S rDNA sequences of the type strains of *Ect. mobilis* (DSM 237$^T$) and *Ect. marismortui* (DSM 4180$^T$) from each other and from the other strains of this study support their recognition as different species and indicate that other strains of this study should be excluded from either of the two species.

According to their 16S rDNA sequences, the type strains of *Ect. vacuolata* and *Ect. shaposhnikovii* are most similar, but distinct at a level that could support the recognition of separate species. The great sequence similarity of the type strain of *Ect. shaposhnikovii* and of "*Ect. mobilis*" DSM 239 ($K_{nuc}$ value of 0.0076) demonstrates identity of the two strains at the species level. The particularly high similarity of "*Ect. mobilis*" DSM 239 to *Ect. shaposhnikovii* and the close relationship between *Ect. shaposhnikovii* and *Ect. vacuolata* have also been found by DNA-DNA hybridization (Ivanova et al. 1985) and by fatty acid analysis (Thiemann and Imhoff 1995). We conclude from the available information that strain DSM 239, originally identified as *Ect. mobilis*, is indeed a strain of *Ect. shaposhnikovii*.

Strains of another subgroup have been tentatively assigned to the genus *Ectothiorhodospira* and to *Ect. mobilis*: strain DSM 241 as *Ectothiorhodospira* sp. (Matheron and Baulaigue 1972) and as *Ect. mobilis* (DSM catalog of strains), strain BN 9902 as *Ectothiorhodospira* sp. (Imhoff et al. 1978) and strain ATCC 51935 as *Ect. mobilis* (Imhoff and Riedel 1989; Imhoff et al. 1991; Imhoff and Thiemann 1991; Thiemann and Imhoff 1991; Ditandy and Imhoff 1993). Due to their 16S rDNA sequence, all three strains are significantly different from the type strains of *Ect. mobilis* and other recognized *Ectothiorhodospira* species and form a separate cluster. This is supported by their fatty acid composition (Thiemann and Imhoff 1995). Strains ATCC 51935 and BN 9902 originated from strongly alkaline soda lakes of the Wadi Natrun and represent the most haloalkaliphilic members of this group (Imhoff et al. 1978; J. F. Imhoff, unpublished data). Strain DSM 241 and strain DSM 242 (which is most similar to DSM 241) are of marine origin (Matheron and Baulaigue 1972). Considerable work has been done with strain ATCC 51935 (Imhoff 1984b; Imhoff and Riedel 1989; Imhoff and Thiemann 1991; Imhoff et al. 1991; Zahr et al. 1992; Ventura et al. 1993), which will be the designated type strain of a new species named *Ectothiorhodospira haloalkaliphila* sp. nov., depicting the halophilic nature and considerable salt tolerance of this species and its alkaliphily. The 16S rDNA sequence of strain DSM 241 is significantly different from the designated type strain of *Ect. haloalkaliphila* ATCC 51935 ($K_{nuc}$ value of 0.0209), and we propose the recognition of strain DSM 241 as the type strain of a new species, *Ectothiorhodospira marina*, indicating its origin from the marine environment. Strain BN 9902 shall be regarded as a strain of *Ect. haloalkaliphila*, while the species assignment of strain DSM 242 must await further detailed studies.

## Taxonomic consequences

### *Description of* Halorhodospira *gen. nov.*

Ha.lo.rho.do.spi'ra. Gr.n. halos salt; Gr.n. rhodos the rose; Gr.n. spira the spiral; M.L.fem.n. *Halorhodospira*, the red spiral requiring salt.

Cells are spiral-shaped, may under some conditions appear as rods, 0.5–1.2 μm in diameter, motile by bipolar flagella, and multiply by binary fission. Gram-negative. Internal photosynthetic membranes are present as lamellar stacks that are continuous with the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *a* or *b* and carotenoids.

Growth occurs photoautotrophically under anoxic conditions with reduced sulfur compounds as electron donors, or photoheterotrophically with a limited number of simple organic compounds. Sulfide is oxidized to elemental sulfur, which is deposited outside the cells and may be further oxidized to sulfate.

Growth is dependent on saline and alkaline conditions. A minimum of more than 10% total salts are required by all known species, some of which grow in saturated salt solutions. Growth factors are not required. Storage products are polysaccharides, poly-β-hydroxy-butyrate, and polyphosphate.

*Halorhodospira* species are found in hypersaline and extremely saline environments with slightly to extremely alkaline pH that contain sulfide and that are exposed to light, such as salt flats, salt lakes, soda lakes.

The mol% G+C of the DNA is 50.5–69.7 (Td).

Type species: *Halorhodospira halophila* (*Ectothiorhodospira halophila*, Raymond and Sistrom 1969, 125)

### *Description of Halorhodospira halophila comb. nov. (Ectothiorhodospira halophila, Raymond and Sistrom 1969)*

ha.lo'phi.la. Gr.n. halos salt; Gr.adj. philos loving; M.L.fem.adj. halophila salt-loving.

The description is essentially that of *Ectothiorhodospira halophila* (Raymond and Sistrom 1969; Imhoff 1989).

**Table 4** Differentiation of *Ectothiorhodospira* species and *Halorhodospira* species. Selected properties of the type strains and additional strains of this study are summarized [*EM* standard medium for *Ectothiorhodospira* according to Imhoff (1988) with indication of salt concentrations, *mPF* modified Pfennig's medium according to Trüper (1970), *MK* menaquinone, *Q* ubiquinone]

| Species and strains | Cell size (μm) | Growth medium | Fatty acid cluster[a] | Quinones[b] | mol% G+C |
|---|---|---|---|---|---|
| *Halorhodospira* (bipolar flagella) | | | | | |
| *Hlr. halophila* DSM 244$^T$ | 0.6–0.9 | EM15%, 25% | II | MK8, Q8 (MK4/5) | 68.4[c] |
| *Hlr. halophila* BN 9630 | nd | EM15%, 25% | II | MK8, Q8 (MK4/5) | 66.5 |
| *Hlr. halophila* BN 9624 | nd | EM15%, 25% | I | MK8, Q8 (MK4/5) | 69.4 |
| *Hlr. abdelmalekii* DSM 2110$^T$ | 0.9–1.2 | EM15%, 25% | III | MK4/5 (Q8/MK8) | 63.8 |
| *Hlr. halochloris* DSM 1059$^T$ | 0.5–0.6 | EM15%, 25% | IV | MK4/5 (Q8/MK8) | 52.9 |
| *Hlr. halochloris* BN 9851 | nd | EM15%, 25% | IV | MK4/5 (Q8/MK8) | 50.5 |
| *Ectothiorhodospira* (polar tuft of flagella) | | | | | |
| *Ect. mobilis* DSM 237$^T$ | 0.7–1.0 | mPF | V | MK7, Q8 | 67.3[c] |
| *Ect. marismortui* DSM 4180$^T$ | 0.9–1.3 | mPF | V | MK7, Q8 | 65.0[d] |
| *Ect. marina* DSM 241$^T$ | 0.8–1.2 | mPF | VII | MK7, Q8 | 62.8[e] |
| *Ect. haloalkaliphila* ATCC 51935$^T$ | 0.7–1.2 | mPF/EM5% | VII | MK7, Q8 | 63.5 |
| *Ect. haloalkaliphila* BN 9902 | nd | mPF/EM5% | VII | | 62.2 |
| *Ect. vacuolata* DSM 2111$^T$ | 1.5 | mPF/EM5% | VI | MK7, Q7 | 63.2 |
| *Ect. shaposhnikovii* DSM 243$^T$ | 0.8–0.9 | mPF/EM5% | VI | MK7, Q7 | 62.3 |
| *Ect. shaposhnikovii* DSM 239 | nd | mPF/EM5% | VI | MK7, Q7 | 62.9 |

[a] Fatty acid clusters are from Thiemann and Imhoff (1995)
[b] Major quinone components are taken from Imhoff (1984b) and Ventura et al. (1993)
The G+C content was determined as indicated in Materials and methods by Dr. H. Hippe (DSM, Braunschweig, Germany) or was taken from [c] Mandel et al. (1971), [d] Oren et al. (1989), and [e] Matheron (1976)

*Description of Halorhodospira halochloris comb. nov. (Ectothiorhodospira halochloris, Imhoff and Trüper 1977)*

ha.lo.chlo'ris. Gr.n. halos salt; Gr.adj. chloros green; M.L.adj. halochloris green-colored and salt-loving.

The description is essentially that of *Ectothiorhodospira halochloris* (Imhoff and Trüper 1977; Imhoff 1989).

*Description of Halorhodospira abdelmalekii comb. nov. (Ectothiorhodospira abdelmalekii, Imhoff and Trüper 1981)*

abd.el.ma.lek'i.i. M.L.gen.n. abdelmalekii of Abd-El-Malek; named for Yousef Abd-El-Malek, an Egyptian microbiologist.

The description is essentially that of *Ectothiorhodospira abdelmalekii* (Imhoff and Trüper 1981; Imhoff 1989).

*Differentiation between Halorhodospira and Ectothiorhodospira*

Differentiation of *Halorhodospira* and *Ectothiorhodospira* species is possible on the basis of molecular and physiological properties (Table 4). *Ectothiorhodospira* species contain menaquinone MK-7 and either ubiquinone Q-7 or Q-8 as major components, whereas *Halorhodospira* species do not contain significant proportions of homologs with seven isoprenoid units, but have MK-8 and Q-8 together with a short chain menaquinone component as major components (Imhoff 1984b; Ventura et al. 1993). Both genera also form separate groups according to their fatty acid composition (Thiemann and Imhoff 1995). They are significantly different according to their 16S rDNA sequence, which is depicted in a number of signature sequences and sequence similarities from 87.2–89.9%.

Metabolic properties such as utilization of carbon, sulfur, and nitrogen sources are very similar among the species of both genera. Whereas several species of the genus *Ectothiorhodospira* are able to grow under chemotrophic conditions in the dark, all *Halorhodospira* species are obligately phototrophic. They are extremely halophilic bacteria and do not grow below 10% total salts. *Ectothiorhodospira* species, on the other hand, have growth optima well below 10% total salts and only strains of *Ect. haloalkaliphila* tolerate and grow with up to 15% total salts.

*Emended description of the genus Ectothiorhodospira Pelsh 1936, 120AL*

Ec.to.thi.o.rho.do.spi'ra. Gr.prep. ectos outside; Gr.n. rhodos the rose; Gr.n. spira the spiral. M.L. fem.n., *Ectothiorhodospira* the rose spiral with sulfur outside.

Cells are rod-shaped or vibroid, also appearing as true spirals, 0.7–1.5 μm in diameter, motile by a polar tuft of flagella, and multiply by binary fission. May contain gas vesicles. Gram-negative. Internal photosynthetic membranes are present as lamellar stacks that are continuous

with the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series.

Growth occurs photoautotrophically under anoxic conditions with reduced sulfur compounds or hydrogen as electron donors, or photoheterotrophically with a limited number of simple organic compounds. Sulfide is oxidized to elemental sulfur, which is deposited outside the cells and can be further oxidized to sulfate. Some species are able to grow microaerobically to aerobically in the dark. Growth is dependent on saline and alkaline conditions. Growth factors are not required, but vitamin $B_{12}$ enhances growth of some strains. Storage products are polysaccharides, poly-β-hydroxybutyrate, and polyphosphate.

*Ectothiorhodospira* species live in marine and extremely saline environments with slightly to extremely alkaline pH that contain sulfide and are exposed to light, such as estuaries, salt flats, and even salt lakes and soda lakes; occasionally they may be found in soil.

The mol% G+C of the DNA is 61.4–65.0 (Td) or 62.3–69.9 (Bd).

Type species: *Ectothiorhodospira mobilis* Pelsh 1936, 120

*Description of Ectothiorhodospira marina, sp. nov.*

ma.ri'na. L.fem.adj. marinus marine

Cells are curved, sometimes slightly bent rods, 0.8–1.2 μm wide, and 1.5–4.0 μm long. They are motile by means of polar tufts of flagella and are gram-negative. Internal photosynthetic membranes are present as lamellar stacks. Color of cell suspensions that are free of polysulfides and elemental sulfur is red. Absorption spectra of living cells show characteristic absorption bands at 379, 483, 513, 552, 593, 798, 826, 853, and 893 nm. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol) and carotenoids of the spirilloxanthin series with spirilloxanthin as the major component.

Cells grow preferably anaerobically in the light under photoautotrophic and photoheterotrophic conditions. Reduced sulfur compounds or organic carbon sources can serve as electron donors. Photoautotrophic growth is possible with sulfide, thiosulfate, elemental sulfur, and sulfite. Acetate, pyruvate, lactate, propionate, malate, succinate, and fumarate are used as organic carbon sources and electron donors. Ketoglutarate and peptone also support growth. Ammonia, dinitrogen, and glutamine are used as nitrogen sources. Sulfate can be used as sole sulfur source. Optimal growth is at 30–40°C and pH 7.5–8.5. Growth occurs between 0.5% and more than 10% salts, with an optimum at 2–6% NaCl.

Major quinone components are Q-8 (Q-7) and MK-7.

The mol% G+C of the DNA of the type strain is 62.8 (Bd).

Type strain: DSM 241 (Matheron BA 1010).

*Description of Ectothiorhodospira haloalkaliphila, sp. nov.*

ha.lo.al.ka.li'phi.la. Gr.n. halos salt; arab.n. al kali potash; Gr.adj. philos loving; M.L.fem.adj. haloalkaliphila loving salt and alkaline conditions

Cells are vibrio-shaped or curved in a short spiral, 0.7–1.2 μm wide, and 2.0–3.0 μm long. They are motile by means of polar tufts of flagella and are gram-negative. Internal photosynthetic membranes are present as lamellar stacks. Color of cell suspensions that are free of polysulfides and elemental sulfur is red. Absorption spectra of living cells show maxima at 379, 488, 513, 552, 593, 798, 826, 851, and 892 nm. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol) and carotenoids of the spirilloxanthin series with spirilloxanthin as the major component.

Cells grow preferably anaerobically in the light with reduced sulfur compounds or organic carbon sources as electron donors. Photoautotrophic growth is possible with sulfide, thiosulfate, and elemental sulfur. Acetate, pyruvate, malate, succinate, and fumarate are used as organic carbon sources and electron donors. Ammonia and some amino acids are used as nitrogen sources. Sulfate can be used as sole sulfur source. Optimal development is at 26–40°C and pH 8.5–10. Growth occurs between 2.5–15% salts, with an optimum at 5%.

Major quinone components are Q-8 (Q-7) and MK-7. The mol% G+C of the DNA of the type strain is 63.5 (Td).

Type strain: ATCC 51935 (BN 9903).

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