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ORIGINAL PAPER

Novel halophilic aerobic anoxygenic phototrophs from a Canadian hypersaline spring system

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Abstract The first enumeration of cultivable obligately aerobic phototrophic bacteria from a terrestrial saline spring was accomplished in the East German Creek system (salinity ~6%), near Lake Winnipegosis, Manitoba, Canada. Occurring at densities up to 3.3×10^7 CFU/ml of sample, aerobic phototrophs comprised 15–36% of the total cultivable bacterial population in the diatom- and chlorophyte-dominated aerobic microbial mats. Many of the representative strains isolated for phenotypic characterization and phylogenetic analysis possessed <96% 16S rDNA sequence overlap with published species, including an obligately aerobic phototrophic gammaproteobacterium displaying only 92.9% 16S rDNA sequence similarity to *Congregibacter litoralis*. The springs yielded the most highly halotolerant aerobic anoxygenic phototroph yet recorded, strain EG11, which grew with 26% NaCl.

Keywords Aerobic anoxygenic phototrophs · Phototrophic halophiles · Hypersaline springs · Bacteriochlorophyll · Physiology

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Introduction

The globally abundant obligately aerobic anoxygenic phototrophs (AAP) (comprising up to 10% or more of microbes in illuminated environments; Rathgeber et al. 2004) differ drastically from conventional anoxygenic phototrophic bacteria in requiring oxygen for the assembly and functioning of their bacteriochlorophyll (BChl) *a*-based photosynthetic apparatus (Yurkov 2006). AAP augment chemoheterotrophic catabolism with phototrophic energy generation (Yurkov and Csotonyi 2003). Although they are incapable of photoautotrophy, their light-assisted chemoheterotrophy modulates nutrient turnover more efficiently than does strict chemoheterotrophy, since phototrophic energy production allows a greater fraction of their reduced carbon pool to be shunted to biomass production (Yurkov and Csotonyi, in press). Their large populations, efficient metabolism and ability to utilize solar energy in the extensive aerobic fraction of habitats make them key players in modulating resource availability in illuminated environments in which microbial primary production predominates (Yurkov and Csotonyi, in press). The relatively high resistance of AAP membrane potentials to extremes in temperature and pH (Jiao et al. 2004) suggests that AAP may be particularly competitive in extreme environments, from which more than half of their diversity has been described (Rathgeber et al. 2004; Yurkov and Csotonyi 2003). AAP might therefore exert greater proportional biogeochemical influence in such habitats than in more mesophilic environments because conditions are often prohibitive for substantial plant cover (McKillop et al. 1992). Halotolerant AAP were first isolated from tidal flats and pools (e.g., *Erythrobacter litoralis*, *Roseivivax halodurans*, *Roseivivax halotolerans*) (reviewed by Yurkov and Csotonyi 2003), then from lentic ecosystems such as soda

and meromictic lakes (e.g., *Roseivarius*, *Roseinatronobacter*, *Roseisalinus*, *Roseicyclus*) (reviewed by Yurkov and Csotonyi 2003; Yurkov and Csotonyi, in press). However, this study of a central Canadian saline spring system known as East German Creek (EGC) represents the first enumeration and investigation of physiology and phylogenetic affinity of AAP from saline lotic environments, which uniquely combine high salinity with flowing water.

In many terrestrial saline systems, cyanobacteria produce a scaffold, throughout aerobic regions of which AAP respire along with purple and green nonsulfur bacteria (PNSB and GNSB) (Yurkov 2006). At deeper levels in habitats supplied with reduced sulfur compounds, purple and green sulfur bacteria may form distinctly colored strata at sufficiently anoxic and often sulfide-rich depths, which are still adequately illuminated (Pfennig and Trüper 1992; Trüper and Pfennig 1992). Although AAP have been isolated from marine dinoflagellates, the EGC springs represent the first terrestrial AAP-sampled saline mats dominated by eukaryotes (green algae and diatoms) instead of cyanobacteria (Londry et al. 2005). Hence, a novel suite of AAP could be encountered.

Palynological records (Patterson et al. 1997) suggested that the EGC springs have been active for at least 5,500 years, releasing water laden with salts from subterranean ancient marine deposits (Grasby 2000). The constant terrestrial source of hypersaline water, precluding the need for connection with extraneous water bodies to ensure continuous hydration, should allow highly endemic phototrophic populations to develop. Previous studies of other saline springs have yielded taxonomically and morphologically novel forms of PNSB (Guyoneaud et al. 2002).

Materials and methods

Sampling and physico-chemical analysis

Measurements and sampling were conducted on 18 May 2002, at EGC (52° 45′ 10″ N, 100° 52′ 50″ W), near Lake Winnipegosis, Manitoba (site 16 of McKillop et al. 1992). Samples of water, sediment and microbial mat (1–2 cm^2) were transported on ice to the laboratory for analysis. Water pH was measured on site using a Beckman ϕ255 pH meter, and temperature with a Springfield Duo-Temp digital thermometer. Total dissolved solids (TDS) were determined in the laboratory by weighing 10-ml liquid samples before and after drying at 70°C for 24 h.

Enumeration and cultivation

Bacterial populations were investigated by culturing on media designed for AAP and PNSB. Despite disregarding uncultivable members of the community, this technique allowed subsequent phenotypic investigation of isolated representatives. Although AAP were the focus of investigation, representative anaerobic phototrophs and non-phototrophs were also isolated and phylogenetically characterized. In the laboratory, mat samples were homogenized by vortexing 2-ml portions of suspensions in screw-capped tubes, serially diluted and inoculated onto agar-containing aerobic plates of rich organic Medium A for AAP and anaerobic agar deeps of Medium B (for PNSB) and Medium C (for purple sulfur bacteria). Previously described vitamin (VS) and trace element (TES) solutions were used (Yurkov 2006). Media were altered from published sources (Imhoff 2001, 2003; Yurkov 2006) to approximate the springs' mineral composition, and adjusted to pH 7.0 with 0.5 M HCl or 0.5 M NaOH after autoclaving at pH 5.9. Medium A contained (g/l): $MgSO_4 \cdot 7H_2O$, 3; Na_2SO_4, 1.8; KH_2PO_4, 0.3; NH_4Cl, 0.3; KCl, 0.7; $CaCl_2 \cdot 2H_2O$, 0.05; NaCl, 40; Na-acetate$\cdot 3H_2O$, 1; Difco Yeast Extract, 1; Difco Bactopeptone, 0.5; Casamino Acids, 0.5; TES, 2 ml; VS, 2 ml. Medium B contained (g/l): $MgCl_2 \cdot 6H_2O$, 2.5; Na_2SO_4, 3.5; KH_2PO_4, 0.3; NH_4Cl, 0.5; KCl, 0.7; $CaCl_2 \cdot 2H_2O$, 0.05; NaCl, 40; Na-acetate$\cdot 3H_2O$, 1; Difco Yeast Extract, 0.5; $NaHCO_3$, 1.5; $FeCl_3$, 2.9×10^{-4}; $Na_2S_2O_3 \cdot 5H_2O$, 0.3; TES, 10 ml; VS, 2 ml. Medium C contained (g/l): $MgSO_4 \cdot 7H_2O$, 3; Na_2SO_4, 1.8; KH_2PO_4, 0.3; NH_4Cl, 0.5; KCl, 0.7; $CaCl_2 \cdot 2H_2O$, 0.05; NaCl, 40; $NaHCO_3$, 1.5; $Na_2S \cdot 9H_2O$, 0.36; TES, 10 ml; VS, 2 ml.

All cultures were incubated at 28°C, AAP in continual darkness, and anaerobic anoxygenic phototrophs in a continuously incandescently illuminated (3,000 lux), temperature-controlled Conviron incubator, model 125L. Colonies were counted and characterized by color and morphology after 7 days. Phototrophs were enumerated by measuring spectra of several subcultured representatives of each pigmented colony morphotype, and calculating the proportion that produced BChl (Yurkov and van Gemerden 1993). Phototrophs were thus differentiated from other pigmented strains.

16S rDNA sequencing and phylogenetic analysis of isolates

Ribosomal DNA from 15 strains of AAP, three anaerobic anoxygenic phototrophs and three aerobic non-phototrophs was sequenced. Extraction of genomic DNA, PCR-mediated amplification of complete 16S rRNA gene sequences and direct sequencing of PCR products were carried out as by Rainey et al. (1996). Sequence reaction mixtures were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems). The 16S sequences were aligned with published sequences obtained from the EMBL

nucleotide sequence database and the Ribosomal Database Project, using the ae2 editor (Maidak et al. 1999), and similarity values were determined. A neighbor-joining dendrogram was reconstructed from a distance matrix using the treeing algorithm of Felsenstein (1993). Bootstrap values were determined according to Felsenstein (1985). Accession numbers obtained for strains are displayed in Fig. 2.

Phenotypic characterization of isolates

Cellular morphology of purified strains and of field samples was assessed with a Zeiss Axioscop 2 phase contrast microscope equipped with a DVC digital camera. Pigmented strains were screened spectrophotometrically (with a Hitachi U-2010 spectrophotometer) at 350–1,100 nm for in vivo diagnostic absorption peaks of BChl (360–1,020 nm), chlorophyll (430–700 nm) and carotenoids (400–550 nm). Absorption spectra of overnight extracts (acetone/methanol, 7:2 v/v) of selected pure cultures were also obtained. Salinity (0–30% NaCl) and pH (4–12) tolerance profiles, and carbon source requirements (compounds listed in Table 3), were acquired at 28°C in continual darkness as described (Yurkov et al. 1999). Capacity for anaerobic photosynthesis was assessed by scoring growth on both plate and liquid illuminated cultures, using Medium B amended with Na-malate (0.3 g/l) and Na-succinate (0.3 g/l) as additional carbon and electron sources, and with and without sulfide (0.36 g/l $Na_2S{\cdot}9H_2O$) as electron donor for liquid cultures. Anaerobic photosynthesis was also assayed on modified CENMED medium designed for *Rhodocista centenaria* (Stadtwald-Demchick et al. 1990), containing (g/l): $MgSO_4{\cdot}6H_2O$, 0.2; KH_2PO_4, 0.6; K_2HPO_4, 0.9; Na_2-EDTA, 0.005; NH_4Cl, 1; $CaCl_2{\cdot}2H_2O$, 0.075; NaCl, 40; Na-pyruvate, 2.2; Difco Yeast Extract, 0.1; $Na_2S_2O_3{\cdot}5H_2O$, 0.5; chelated iron solution, 2 ml; TES, 2 ml; VS, 2 ml.

Results and discussion

Study site

A large salt pan ($\sim$300 m $\times$ 400 m) with few halotolerant plants (e.g., *Salicornia*; McKillop et al. 1992) was stained reddish-orange by extensive deposition of limonite (iron

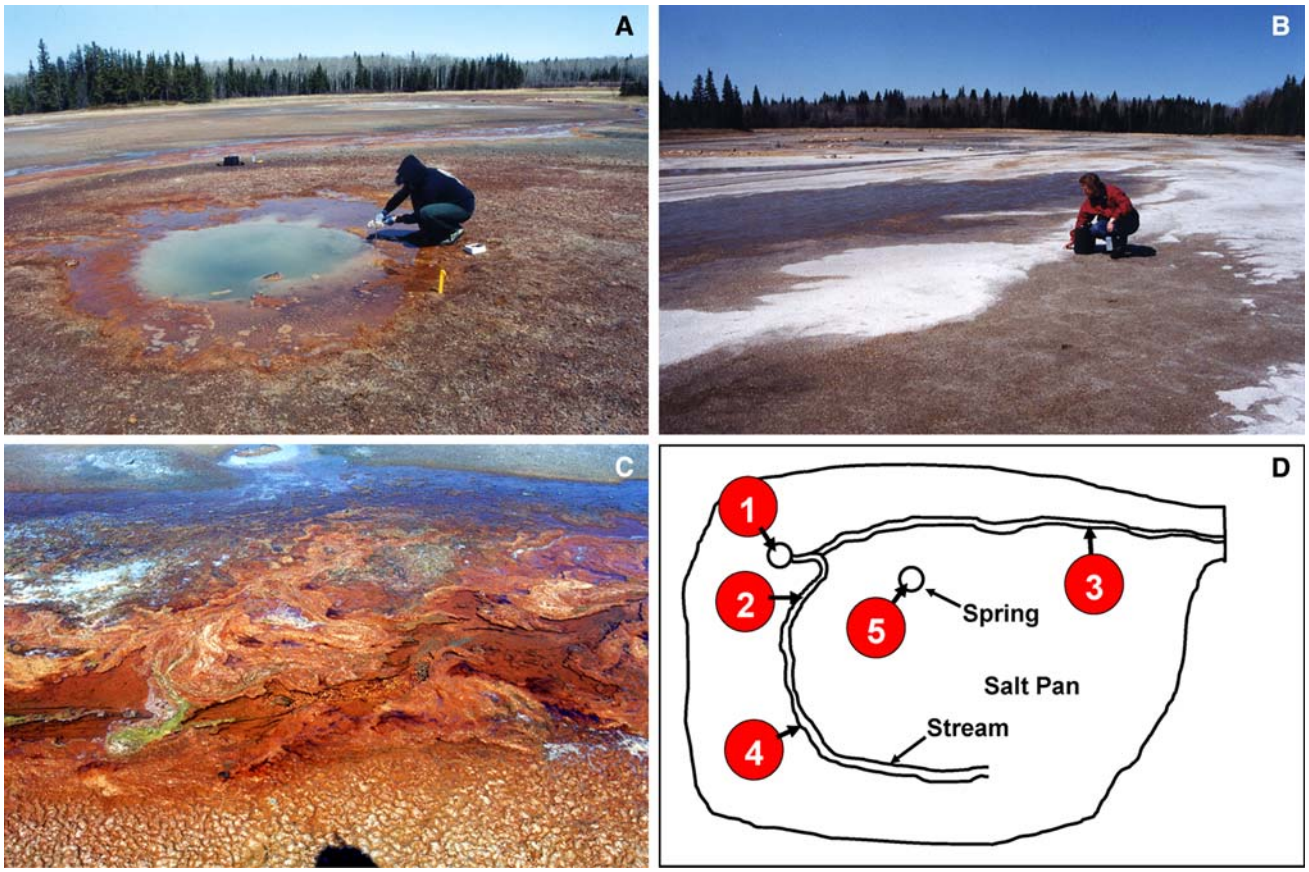

Fig. 1 East German Creek brine springs. **a** Spring pool, with effluent stream and limonite-stained salt pan in background. **b** White evaporate deposits of NaCl. **c** Effluent stream (site 2) from main spring (site 1), demonstrating a thick floating microbial mat. **d** Map of EGC showing positions of sampling sites 1 to 5 (*red dots*)

oxyhydroxide) (Fig. 1a). Widespread patches of white, indicating heavy salt precipitation, signified the extremely hypersaline conditions surrounding the pools and streams (Fig. 1b). Black subterranean sediments emitted the odor of hydrogen sulfide. Water discharging at up to 4,800 l/h (McKillop et al. 1992) from crater-like springs (15–100 cm deep by up to 2 m wide, with shallow peripheral shelves 5 cm deep) (Fig. 1a) atop raised mounds of sinter generated shallow streams (Fig. 1c) tens or hundreds of meters long.

East German Creek was sampled at five sites (Fig. 1d). Sites 1 and 5 were springs lined with fine gray benthic sediments and (at site 1) a large floating green microbial community. Sites 2 and 4 were in an outflow channel 5 and 100 m downstream of its parent spring, respectively, the former bearing both a several-cm-thick orange and green floating microbial community (Fig. 1c) and a 1-mm-thin olive green and brown benthic mat, the latter possessing only the benthic mat. Site 3, 100 m downstream of a different spring, resembled site 4, but had numerous stones and a more yellowish green benthic mat.

Physico-chemical analysis

pH was approximately neutral, but rose by 0.7 units from springs to streams (Table 1), possibly due to loss of CO_2 upon atmospheric exposure (McKillop et al. 1992). TDS values (56.7–67.3‰, w/v) (Table 1) bracketed the value (61.6‰) reported by McKillop et al. (1992), with variation likely due to evaporation or dilution by intersection with subterranean fresh water conduits.

Microscopic survey of natural samples

Although AAP could not be distinguished microscopically, identifiable dominant organisms reflected the aerobic nature of the sampled microhabitats. Most of the biomass composing the thick floating mat-like growth at site 2 was the North American/European filamentous coastal marine green alga *Percursaria percursa* (Chlorophyta: Ulvaceae), of which EGC and surrounding spring systems constitute the only interior North American account (Londry et al. 2005). Filamentous cyanobacteria were rarely observed (a few filaments in only two samples). Pennate diatoms dominated all benthic samples from sites 1, 3 and 4 (diatoms were the only algae observed in site 3), and co-dominated floating mats from site 2 with *Percursaria*. Sulfur-like intracellular inclusions were present in rare filamentous *Thiothrix*-like cells from sites 2 and 5, but sulfur-inclusion-filled short rods typical of purple sulfur bacteria were absent. These observations are consistent with the fact that none of the sample profiles possessed the purple color banding typical of anaerobic phototrophs in laminated mats.

Table 1 Physico-chemical properties of sampling stations at EGC

Site	Description	Measurement source	pH	TDS (‰, w/v)
1	Spring pool (2 m × 1 m)[a]	Pool center	7.05	66.3
		Periphery	7.03	NA
2	Stream (30 cm × 5 cm)[a]	Central channel	6.90	65.9
3	Stream (30 cm × 5 cm)[a]	Central channel	7.22	NA
4	Stream (30 cm × 5 cm)[a]	Central channel	7.51	67.3
5	Spring pool (1.5 m × 0.2 m)[a]	Pool center	6.80	56.7
		Periphery	7.08	NA

NA not applicable

[a] Measurements are given as width × depth

Table 2 Enumeration of bacteria cultured from aerobic microhabitats at four of five EGC sampling sites

Culture conditions and organism type	CFU/ml × 10^6 (total %)			
	Site 1 (thin brown pool mat)	Site 2 (thick orange stream mat)	Site 3 (thin yellow stream mat)	Site 4 (thin brown stream mat)
Aerobic plates (Medium A)				
Pigmented	2.08 (29.9)	7.14 (63.4)	74.25 (81.4)	1.58 (20.6)
Anoxygenic phototrophs	1.09 (15.7)	2.37 (21.1)	33.18 (36.4)	2.48 (32.4)
Total aerobic	6.96	11.26	91.25	7.66
Anaerobic agar deeps (Medium B)				
Total pigmented	0.01 (2.4)	0.02 (0.3)	0.16 (5.1)	0.35 (3.8)
Anoxygenic phototrophs	0 (0)	0.0002 (0.003)	0.04 (1.3)	0.0001 (0.001)
Total anaerobic	0.41	5.72	3.16	9.15

Enumeration

About 15–32% of the 6.96–11.26 × 10^6 CFU/ml aerobic bacteria of sites 1, 2 and 4 were anoxygenic phototrophs (Table 2), in accord with the 0.3–3.6 × 10^7 CFU/ml of AAP reported by Yurkov and van Gemerden (1993) for a Dutch supralittoral cyanobacterial microbial mat. Paucity of anoxygenic phototrophs in anaerobic EGC enumerations, and the fact that no PNSB were recovered from aerobic plates (even though the PNSB EG17 subsequently grew on Medium A), implies that AAP constituted the vast majority of this population, reflecting its O_2-rich algal environment. The AAP fraction was greatest (~36%) in the most diatom-rich site 3 sample. This value markedly exceeds the 23% BChl-containing fraction (AAP and PNSB) of all surface bacteria cultivated on Na_2SO_4-amended (15 g/l) aerobic media in meromictic Mahoney Lake (Yurkova et al. 2002). Although diatom-dominated EGC mats are the first such communities screened for AAP, these observations suggest that eutrophic diatomaceous mats may be especially replete with AAP. By comparison, AAP usually form <10% of oligotrophic marine bacterial populations, with the highest relative abundance (up to 18.74%) in temperate nutrient-rich estuaries (reviewed by Yurkov and Csotonyi, in press).

Phylogenetic analysis of isolates

All sequenced strains from this cold continental hypersaline community were related to either marine (11 strains) or meromictic lake (10 strains) species (Fig. 2). Representative non-phototrophs and anaerobic anoxygenic phototrophs were sequenced to briefly describe the non-AAP community. Non-phototrophic bacteria included relatives of psychrotolerant and halophilic Antarctic and marine chemotrophs such as *Psychroflexus gondwanensis* (EG26, 97.7% sequence similarity) (Bowman et al. 1998) and *Pseudoalteromonas carrageenovora* (EG27, 99.7% sequence similarity) (Gauthier et al. 1995), the latter being an exclusively marine genus. Delivery may have been mediated by the transpolar migratory arctic tern (*Sterna paradisaea*), a seasonal resident of Manitoba waters. Obligately anaerobic phototrophs (e.g., EG16, EG18) were phylogenetically most similar to halophilic *Roseospira marina* (98.1%) and *Halochromatium glycolicum* (95.6%), the former also described from a hypersaline spring system

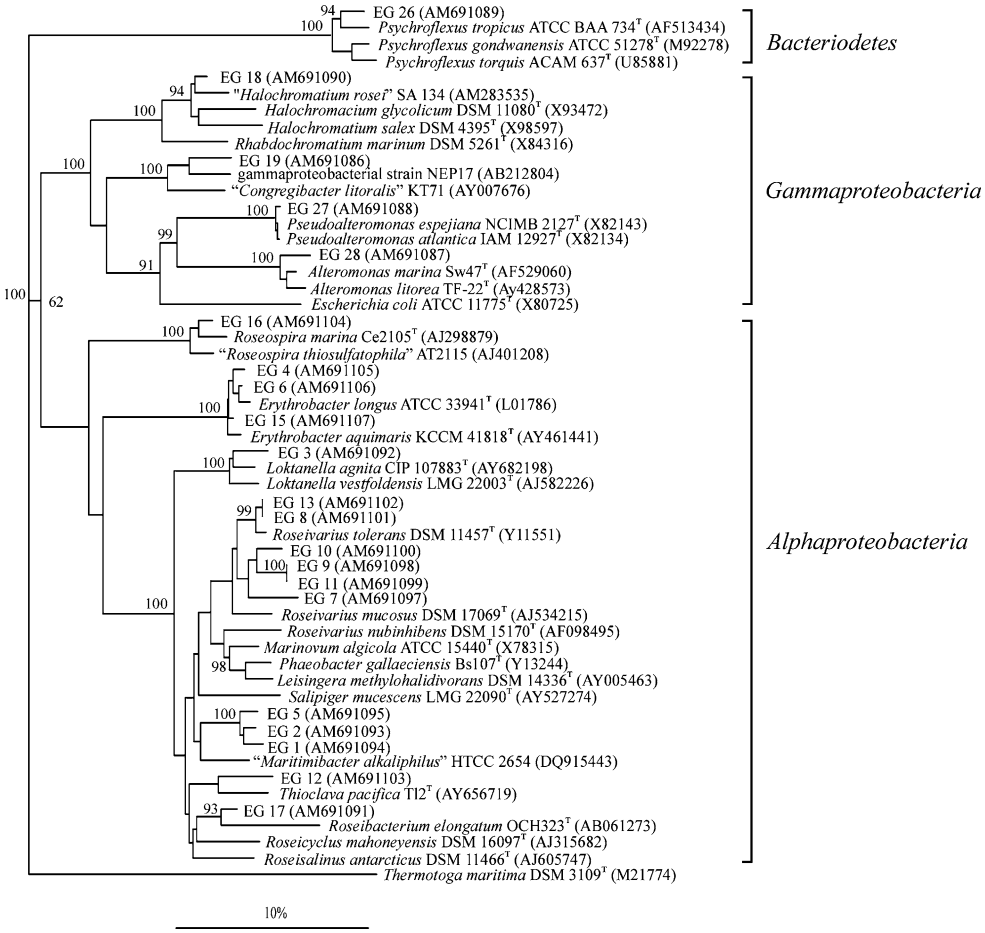

Fig. 2 Neighbor-joining phylogenetic tree showing relatedness of 21 EGC isolates based on 16S rDNA sequences more than 1,400 nucleotides long. Accession numbers follow strain names. *Bootstrap values* are indicated at branch points. *Quotation marks* around Latin names indicate species that have not yet been validated

(Guyoneaud et al. 2002). A facultatively anaerobic PNSB, EG17, was related to halotolerant AAP taxa such as *Roseisalinus antarcticus* (95.4%) (Labrenz et al. 2005).

Aerobic anoxygenic phototrophs exhibited marked novelty, with 9 of the 15 sequenced strains bearing 96% or less 16S rDNA sequence similarity to taxonomically characterized species (Fig. 2). Only a few strains (e.g., *Erythrobacter* relatives, EG4, EG6, EG15; >98.5% sequence similarity to *Erythrobacter* species and to each other) formed tight phylogenetic clusters. The considerable heterogeneity displayed by the *Roseivarius tolerans*-related cluster (EG7–EG11, EG13; 95.5–98.6% sequence similarity with *Roseivarius tolerans*) was probably due to either numerous delivery events from multiple sources or extensive local evolutionary divergence. Eleven of 15 AAP were members of the α-3 proteobacterial *Roseobacter* clade, many of which hail from hypersaline environments (Yurkov and Csotonyi 2003). For several AAP (EG1, EG2, EG5, 92.7–95.2% similar to *Maritimibacter alkaliphilus*; EG3, 96.7% similar to *Loktanella vestfoldensis*; EG12, 94.5% similar to *Thioclava pacifica*), the closest taxonomically described relatives were non-phototrophs (Lee et al. 2007; Sorokin et al. 2005; Van Trappen et al. 2004), reflecting the deep phylogenetic interspersion of AAP with non-phototrophs among the *Proteobacteria*, and probably granting these strains novel genus status on the basis of a significant metabolic distinction and phylogenetic distance.

Of greatest phylogenetic significance, strain EG19 was a member of a recently uncultivable clade (NOR5/OM60), and is only the second reported gammaproteobacterial AAP (92.9% 16S rDNA sequence similarity to *Congregibacter litoralis*). Until the description of gammaproteobacterial *C. litoralis* (Fuchs et al. 2007), AAP encompassed 29 alphaproteobacterial genera and one betaproteobacterial genus (Yurkov and Csotonyi 2003; Yurkov and Csotonyi, in press). *Congregibacter* represents a marine clade previously known only from Monterey Bay BAC clones (Fuchs et al. 2007). Cultivating such previously unaccessible groups is tremendously beneficial, as these organisms can thereafter be investigated in greater detail than could their environmental gene sequences alone.

Brief phenotypic characterization of isolates

Reflecting their phylogenetic variety, AAP from EGC demonstrated wide phenotypic diversity. Although no novel morphologies were observed, cell shape spanned the gamut from coccoid or ovoid cells (EG2, EG5) (Fig. 3a) to short rods (EG3, EG10, EG15) (Fig. 3b, c), curved rods (EG19) (Fig. 3d) or very long rods (EG6) (Fig. 3e), some with tapered ends (EG12) (Fig. 3f).

Spectrophotometric characteristics

In vivo spectrophotometry revealed that most AAP possessed a photosynthetic apparatus whose core elements were the typical *Erythrobacter* type (EG4, EG6, EG15): a single light harvesting complex (λ_{max} ~800, 870 nm) and reaction center (λ_{max} ~800 nm) (Yurkov 2006). Several phylogenetically novel AAP, such as the *Maritimibacter*-related cluster (EG1, EG2, EG5) and gammaproteobacterial EG19 (Fig. 4a) also shared this property. These organisms were principally distinguished by their carotenoid profiles (Table 3).

However, three phylogenetic/spectral classes were particularly intriguing either in BChl spectral properties or in levels and conditions of expression of their photosynthetic apparatus. First, although *Thioclava*-related EG12 incorporated BChl into an *Erythrobacter*-like photosynthetic apparatus (λ_{max} 803 and 869 nm), the extracted BChl (acetone/methanol 7:2, v/v) was blue-shifted 18 nm (λ_{max} 752 nm) from the typical 770 nm λ_{max} of Mg-ligated BChl *a* (Fig. 4b). This component may be bacteriopheophytin resulting from an easily metal-stripped form of BChl *a*, or it may be Zn-chelated BChl *a*, thus far found only in acidophilic AAP, *Acidiphillium* and *Acidisphaera* (Hiraishi and Shimada 2001). Detailed BChl structural analysis will be required to discriminate between competing explanations.

Second, the *Loktanella*-related EG3 produced no BChl when cultured at 28°C in the dark for 12 days, (conditions under which most AAP produce BChl), but a near-infrared BChl peak (λ_{max} 862 nm) was detectable after 9 months at 7°C in the dark (Fig. 4c). Thus it resembled several strains of *Roseivarius tolerans*, which synthesized BChl (λ_{max} 877 nm) only after long-term cold storage (Labrenz et al. 1999), implicating environmental cues in the induction of photosynthesis genes. A satisfactory understanding of the regulation of photosynthetic expression in AAP is far from complete, and because research with *Dinoroseobacter shibae*, *Hoeflea phototrophica* and *Labrenzia alexandrii* suggests that several factors (e.g., illumination regime and nutritional status) can interact in complex and species-specific ways (Biebl and Wagner-Döbler 2006), investigation of new species with fastidious BChl expression, such as EG3, will help to complete the picture.

Third, in surprising contrast to the pale beige EG3, the intensely pigmented reddish-purple *Roseivarius*-related cluster (EG7-EG11, EG13) produced uncharacteristically prodigious BChl. In vivo peak height ratios of BChl:carotenoids (1.33:1) (Fig. 4d) rivaled anaerobic values in PSB (EG18, 1.51:1) or PNSB (EG16, 1.59:1). Most AAP exhibit values around 1:8 to 1:10 (Yurkov and Csotonyi 2003); comparable AAP ratios occur only in Australian strains OCh 245 (1.40:1) and OCh 303 (1.31:1)

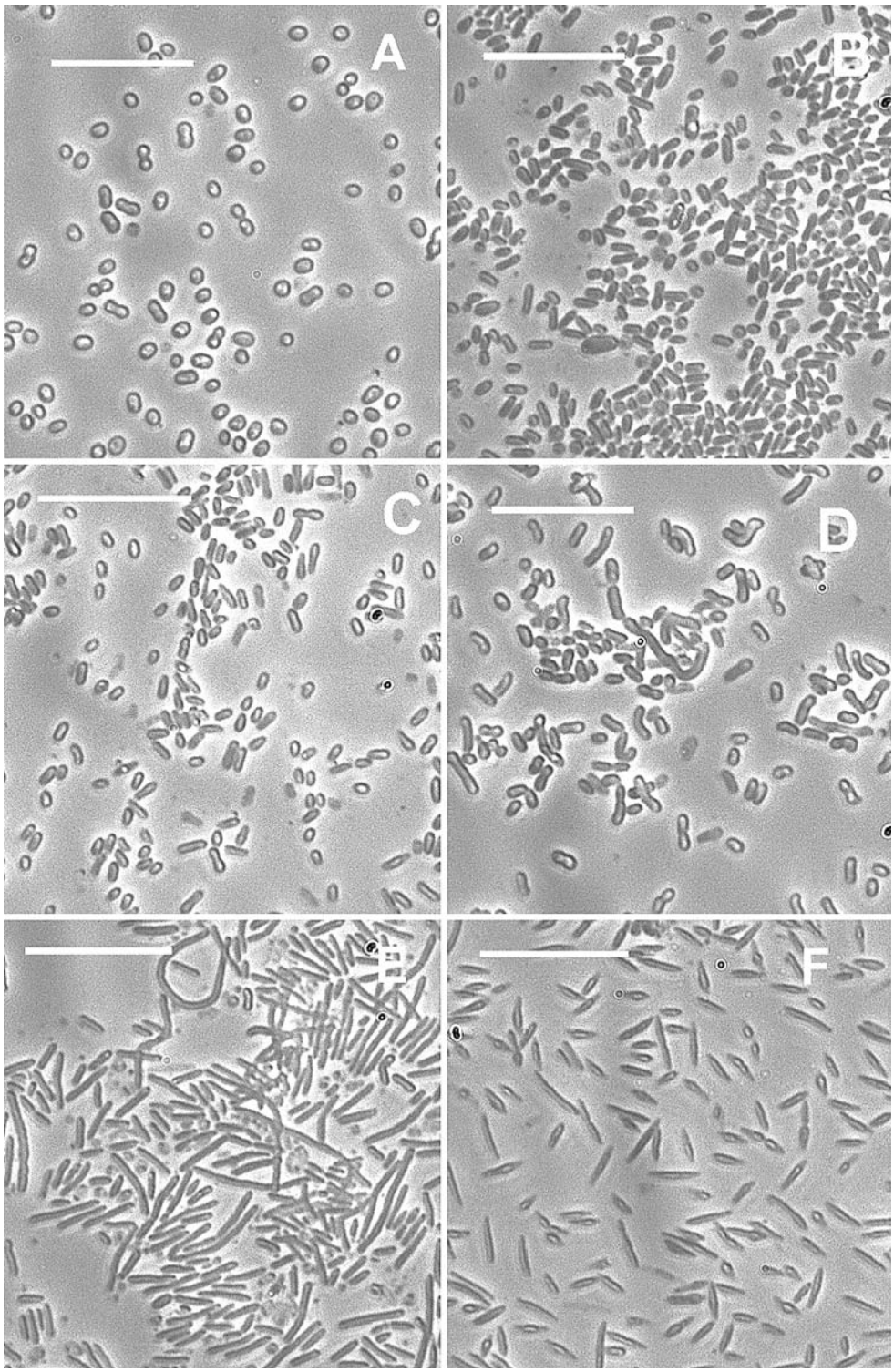

Fig. 3 Phase contrast microscopy of strains. **a** EG2, pale pink *Maritimibacter* relative. **b** EG3, beige *Loktanella* relative. **c** EG10, bright pink *Roseivarius* relative. **d** EG19, pinkish-orange gammaproteobacterium. **e** EG6, reddish-orange *Erythrobacter* relative. **f** EG12, pale pink *Thioclava* relative. *Scale bars*: 10 μm

(Shiba et al. 1991). Although unusual PNSB such as *Rhodocista centenaria* exist in which O_2 does not inhibit BChl production (Stadtwald-Demchick et al. 1990), our strains could not grow anoxically on either Medium B or modified CENMED medium, designed especially for *Rhodocista centenaria*. Unusually plentiful BChl suggests a more extensive reliance on the photosynthetic apparatus than in most AAP. Even more unprecedented for AAP, in which light usually completely inhibits BChl synthesis (Yurkov 2006), was the relatively uninhibited BChl synthesis in illuminated EG13 cultures (46% of the maximal dark value was produced under 500 lux illumination, the

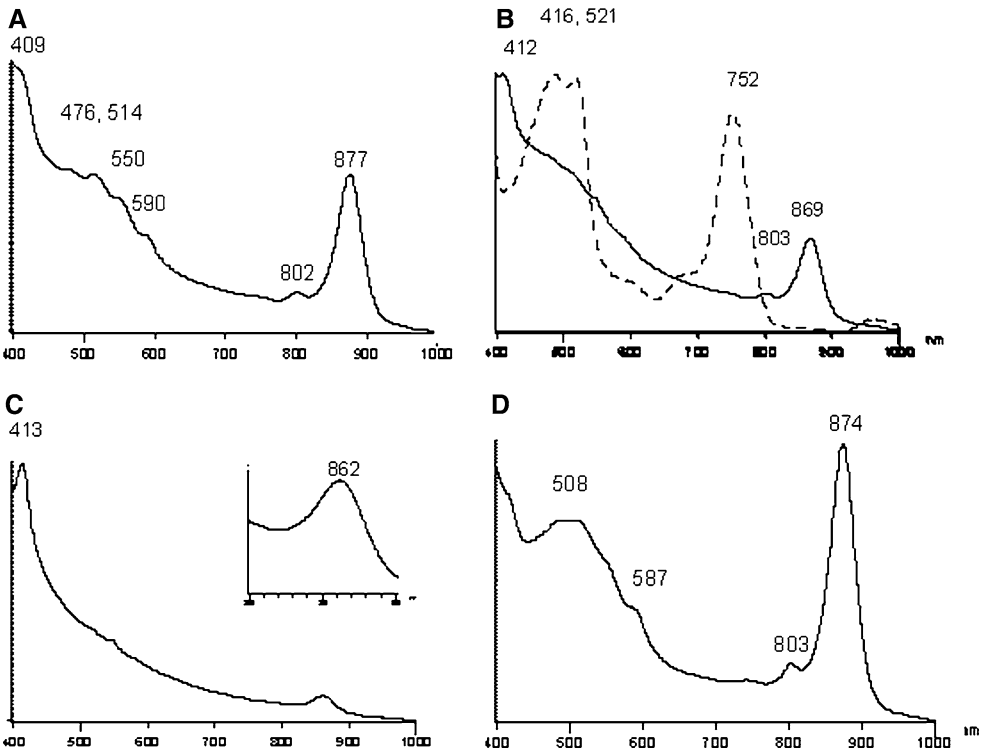

Fig. 4 Absorption spectra of phototrophic strains. **a** EG19, pinkish-orange gammaproteobacterium; in vivo spectrum. **b** EG12, pale pink *Thioclava* relative; in vivo spectrum (*solid line*) and organic extract in acetone/methanol (7:2 v/v) extract (*dotted line*). **c** EG3, pale brown *Loktanella* relative, with inset highlighting BChl peak; in vivo spectrum. **d** EG9, reddish-purple *Roseivarius* relative; in vivo spectrum. *Numerals above spectra* denote λ_{max} values

highest value for any AAP yet described). Alleviation of light-inhibited BChl synthesis would minimize diurnal BChl dilution in rapidly dividing eutrophic populations. Species of AAP producing copious BChl are invaluable to studies of the intriguing biophysics of light-mediated energy transduction in AAP.

Salinity and pH tolerance

Most AAP preferred neutral to weakly basic pH for growth, similar to their native habitat pH (Table 1), but EG8, EG10 and EG19 tolerated pH 12 (Table 3), making them well adapted to the elevated pH generated diurnally in microbial mats by photosynthesis. EGC also yielded strains with the highest known halotolerance in AAP. All tested isolates tolerated NaCl concentrations of at least 6%. Some exhibited optimal growth at the highest salinity (14 or 18% NaCl; EG13, EG11) yet reported for AAP (Table 3). Others tolerated the greatest salinity range (2–26%, EG11) and the highest upper salinity limit for growth (22 or 26% NaCl; EG1, EG8, EG11). The previous maximum NaCl concentration endured was 20% by *Roseivivax halodurans*, *Roseivivax halotolerans* and *S. guttiformis* (reviewed by Yurkov and Csotonyi 2003). Most saline environments screened for AAP are salt, soda or meromictic lakes, but the few investigated marine tidal salt flats have yielded considerably halotolerant AAP, such as *E. litoralis* (0.5–9.6% NaCl) and *Roseibium denhamense* (0–10% NaCl) (reviewed by Yurkov and Csotonyi 2003). However, presence of a diverse halotolerant microbial community 600 km from the ocean (Hudson Bay) is noteworthy. McKillop et al. (1992) speculated on waterfowl-mediated delivery of marine invertebrates to the springs, which may also explain rapid colonization of the EGC region by marine foraminifera from the Gulf of Mexico during the Holocene Hypsithermal, about 5,500 years ago (Patterson et al. 1997).

Organic carbon requirements

As for Mahoney Lake (Yurkova et al. 2002), AAP isolates fell into three clusters based on organic carbon utilization profiles (Table 3). Phylogenetically diverse cluster A (EG1, EG2, EG3, EG8, EG10, EG11, EG13) grew on most (11–14) carbon sources tested. Cluster B (*Erythrobacter* relatives, EG4, EG6 and EG15, with representatives recovered from every site sampled) utilized six to seven substrates. Most interestingly, Cluster C (*Thioclava* relative, EG12) used only complex carbon sources (Bactopeptone, casamino acids and yeast extract) and could not be cultured on defined media, reflecting a likely adaptation to niches providing a reliable source of a single nutrient.

Conclusions

Aquatic environments that are geographically discontinuous with each other, the ocean or a parent river system

Table 3 Phenotypic characteristics of representative AAP isolates

Strain (source)	Color	Cell shape and size (μm)	Infrared BChl absorption peaks (nm)	Carotenoid and other absorption peaks (nm)	% NaCl tolerance, full range (optimum)	pH tolerance, full range (optimum)	Carbon sources used for growth[a]
Erythrobacter-related							
EG4 (site 1 peripheral mat)	R-O	Long rod (1.5–6.3 × 0.5)	803, 867	422, 476	0–14 (4)	6–10 (7–8)	1, 2, 5, 13, 18, 20
EG6 (site 1 peripheral mat)	R-O	Long rod (2.0–14.5 × 0.5)	805, 865	417, 465, 482	2–10 (6)	7–10 (7–9)	1, 2, 5, 18–20
EG15 (site 5 peripheral mat)	B-R	Rod (1.2–4.2 × 0.6)	806, 866	418, 480	2–12 (4)	7–11 (7–9)	2, 5, 7, 10, 18–20
Maritimibacter-related							
EG1 (site 1 peripheral mat)	P-Bg	Ovoid to rod (1–2.2 × 0.7)	801, 867	411, 509, 547	0–22 (12)	7–10 (7–8)	1, 5–14, 18–20
EG2 (site 1 peripheral mat)	Pale P	Coccoid (0.9–1.2 ∅)	802, 867	416, 481, 514, 549	2–14 (10)	NA	1, 3, 5–14, 16, 19, 20
EG5 (site 1 peripheral mat)	R-Pr	Ovoid (1.0–1.7 × 0.8)	803, 867	406, 477, 510, 550	NA	7–11 (7–9)	NA
Loktanella-related							
EG3 (site 1 peripheral mat)	Bg	Rod (1.2–2.0 × 0.7)	862	413, 548	0–12 (4)	NA	2, 4, 7, 8, 10–14, 18–20
Roseivarius-related							
EG7 (site 2 benthos)	R-Pr	NA	802, 879	409, 481, 504, 548	NA	7–10 (7)	NA
EG8 (site 2 floating mat)	P-O	Rod (1.2–2.0 × 0.6)	801, 870	412, 473, 506, 550	0–22 (2)	7–12 (7–8)	1, 2, 5–11, 14, 16, 18–20
EG9 (site 3 diatom mat)	R-Pr	Rod (1.5–2.8 × 0.7)	803, 874	414, 489, 508, 551	NA	7–11 (8)	NA
EG10 (site 3 diatom mat)	Bright P	Rod (1.1–2.2 × 0.7)	801, 870	415, 475, 509, 549	0–22 (4)	7–12 (7–9)	1, 2, 5, 7–10, 15, 16, 18–20
EG11 (site 3 diatom mat)	R-Pr	Rod (1.5–3.0 × 0.5)	801, 874	414, 479, 509, 548	2–26 (18)	7–11 (7–8)	1, 2, 5, 7–11, 16, 19, 20
EG13 (site 4 benthic mat)	P-O	Rod (1.7–2.8 × 0.5)	801, 870	411, 479, 511, 549, 642	0–22 (14)	7–11 (7–8)	1, 5–11, 16, 18–20
Thioclava-related							
EG12 (site 4 benthic mat)	Pale P	Tapered rod (2–3.8 × 0.5)	803, 869	412, 476, 512, 548	0–18 (6)	7–11 (7–8)	18–20
Gammaproteobacteria							
EG19 (site 3 diatom mat)	P-O	Rod to spirilloid (1.5–3 × 0.7)	802, 877	409, 476, 514, 550	NA	7–12 (7)	NA

R-O reddish-orange, *B-R* brownish-red, *P-Bg* pinkish-beige, *P* pink, *Bg* beige, *R-Pr* reddish-purple, *P-O* pinkish-orange, *Ø* diameter, *NA* not applicable

[a] 1, Acetate; 2, butyrate; 3, citrate; 4, formate; 5, glutamate; 6, glycolate; 7, lactate; 8, malate; 9, propionate; 10, pyruvate; 11, succinate; 12, fructose; 13, glucose; 14, sucrose; 15, ethanol; 16, glycerol; 17, methanol; 18, bactopeptone; 19, casamino acids; 20, yeast extract

resemble ecological island habitats in which endemism is expected to be high (MacArthur and Wilson 1967). The physically extreme conditions of saline environments such as meromictic lakes and the EGC saline springs further isolate them biologically from their surroundings. Surveys of their biodiversity are hence not only invaluable to establishing proper environmental protection policies for rare habitats, but especially constructive to our understanding of the phylogenetic extent, evolution, and physiology of groups such as the AAP, whose great relative global abundance, and thus biogeochemical influence, are only now being recognized (Yurkov and Csotonyi, in press).

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References

Biebl H, Wagner-Döbler I (2006) Growth and bacteriochlorophyll *a* formation in taxonomically diverse aerobic anoxygenic phototrophic bacteria in chemostat culture: influence of light regimen and starvation. Process Biochem 41:2153–2159

Bowman JP, McCammon SA, Lewis T, Skerratt JH, Brown JL, Nichols DS, McMeekin TA (1998) *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. Microbiology 144:1601–1609

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Felsenstein J (1993) PHYLIP (phylogenetic inference package), version 3.5.1. Department of Genetics, University of Washington, Seattle

Fuchs BM, Spring S, Teeling H, Quast C, Wulf J, Schattenhofer M, Yan S, Ferriera S, Johnson J, Glockner FO, Amann R (2007) Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. Proc Natl Acad Sci USA 104:2891–2896

Gauthier G, Gauthier M, Christen R (1995) Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. Int J Syst Bacteriol 45:755–761

Grasby SE (2000) Saline spring geochemistry, west-central Manitoba. In: Report of activities 2000. Manitoba Industry, Trade and Mines, Manitoba Geological Survey, Winnipeg, pp 214–216

Guyoneaud R, Moune S, Eatock C, Bothorel V, Hirschler-Rea A, Willison J, Duran R, Liesack W, Herbert R, Matheron R, Caumette P (2002) Characterization of three spiral-shaped purple nonsulfur bacteria isolated from coastal lagoon sediments, saline sulfur springs, and microbial mats: emended description of the genus *Roseospira* and description of *Roseospira marina* sp. nov., *Roseospira navarrensis* sp. nov., and *Roseospira thiosulfatophila* sp. nov. Arch Microbiol 178:315–324

Hiraishi A, Shimada K (2001) Aerobic anoxygenic photosynthetic bacteria with zinc-bacteriochlorophyll. J Gen Appl Microbiol 47:161–180

Imhoff JF (2001) The phototrophic alpha-*Proteobacteria*. In: Dworkin M et al. (eds) The prokaryotes: an evolving electronic resource for the microbiological community, 3rd edn, release 3.6, June 22, 2001. Springer-Verlag, New York. http://link.springer-ny.com/link/service/books/10125

Imhoff JF (2003) The Chromatiaceae. In: Dworkin M et al (eds) The prokaryotes: an evolving electronic resource for the microbiological community, release 3.13, May 12, 2003. Springer-Verlag, New York. http://link.springer-ny.com/link/service/books/10125

Jiao N, Yang Y, Luo T (2004) Membrane potential based characterization by flow cytometry of physiological states in an aerobic anoxygenic phototrophic bacterium. Aquat Microb Ecol 37:149–158

Labrenz M, Collins MD, Lawson PA, Tindall BJ, Schumann P, Hirsch P (1999) *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. Int J Syst Bacteriol 49:137–147

Labrenz M, Lawson PA, Tindall BJ, Collins MD, Hirsch P (2005) *Roseisalinus antarcticus* gen. nov., sp. nov., a novel aerobic bacteriochlorophyll *a*-producing α-proteobacterium isolated from hypersaline Ekho Lake, Antarctica. Int J Syst Evol Microbiol 55:41–47

Lee K, Choo Y-J, Giovannoni SJ, Cho JC (2007) *Maritimibacter alkaliphilus* gen. nov., sp. nov., a genome-sequenced marine bacterium of the *Roseobacter* clade in the order *Rhodobacterales*. Int J Syst Evol Microbiol 57:1653–1658

Londry KL, Badiou PH, Grasby SE (2005) Identification of a marine green alga *Percursaria percursa* from hypersaline springs in the middle of the North American continent. Can Field Nat 119:82–87

MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton

Maidak BL, Cole JR, Parker CT Jr, Garrity GM, Larsen N, Li B, Lilburn TG, McCaughey MJ, Olsen GJ, Overbeek R, Pramanik S, Schmidt TM, Tiedje JM, Woese CR (1999) A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res 27:171–173

McKillop WB, Patterson RT, Delorme LD, Nogrady T (1992) The origin, physico-chemistry and biotics of sodium chloride dominated saline waters on the western shore of Lake Winnipegosis, Manitoba. Can Field Nat 106:454–473

Patterson RT, McKillop WB, Kroker S, Nielson E, Reinhardt EG (1997) Evidence for rapid avian-mediated foraminiferal colonization of Lake Winnipegosis, Manitoba, during the Holocene Hypsithermal. J Paleolimnol 18:131–143

Pfennig N, Trüper HG (1992) The family Chromatiaceae. In: Balow A, Trüper HG, Dworkin M, Harder W, Schleifer K-Z (eds) The prokaryotes, 2nd edn. Springer-Verlag, Berlin, pp 3200–3221

Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. Int J Syst Bacteriol 46:1088–1092

Rathgeber C, Beatty JT, Yurkov V (2004) Aerobic phototrophic bacteria: new evidence for the diversity, ecological importance and applied potential of this previously overlooked group. Photosynth Res 81:113–128

Shiba T, Shioi Y, Takamiya K, Sutton DC, Wilkinson CR (1991) Distribution and physiology of aerobic bacteria containing bacteriochlorophyll *a* on the east and west coasts of Australia. Appl Environ Microbiol 57:295–300

Sorokin DY, Tourova TP, Spiridonova EM, Rainey FA, Muyzer G (2005) *Thioclava pacifica* gen. nov., sp. nov., a novel facultatively autotrophic, marine, sulfur-oxidizing bacterium from a near-shore sulfidic hydrothermal area. Int J Syst Evol Microbiol 55:1069–1075

Stadtwald-Demchick R, Turner FR, Gest H (1990) Physiological properties of the thermotolerant photosynthetic bacterium, *Rhodospirillum centenum*. FEMS Microbiol Lett 67:139–144

Trüper HG, Pfennig N (1992) The family Chlorobiaceae. In: Balow A, Trüper HG, Dworkin M, Harder W, Schleifer K-Z (eds) The prokaryotes, 2nd edn. Springer-Verlag, Berlin, pp 3583–3592

Van Trappen S, Mergaert J, Swings J (2004) *Loktanella salsilacus* gen. nov., sp. nov., *Loktanella fryxellensis* sp. nov. and *Loktanella vestfoldensis* sp. nov., new members of the *Rhodobacter* group, isolated from microbial mats in Antarctic lakes. Int J Syst Evol Microbiol 54:1263–1269

Yurkov V (2006) Aerobic phototrophic proteobacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) Prokaryotes, 3rd edn. Springer, Berlin, pp 562–584

Yurkov VV, Csotonyi JT (2003) Aerobic anoxygenic phototrophs and heavy metalloid reducers from extreme environments. In: Pandalai SG (ed) Recent research developments in bacteriology, vol 1. Transworld Research Network, Trivandrum, pp 247–300

Yurkov V, Csotonyi JT (in press) New light on aerobic anoxygenic phototrophs. In: Govindjee (ed) Advances in photosynthesis and respiration. Springer, Berlin

Yurkov V, van Gemerden H (1993) Abundance and salt tolerance of obligately aerobic, phototrophic bacteria in a marine microbial mat. Neth J Sea Res 31:57–62

Yurkov V, Krieger S, Stackebrandt E, Beatty JT (1999) *Citromicrobium bathyomarinum*, a novel aerobic bacterium isolated from deep-sea hydrothermal vent plume waters that contains photosynthetic pigment-protein complexes. J Bacteriol 181:4517–4525

Yurkova N, Rathgeber C, Swiderski J, Stackebrandt E, Beatty JT, Hall KJ, Yurkov V (2002) Diversity, distribution and physiology of the aerobic phototrophic bacteria in the mixolimnion of a meromictic lake. FEMS Microbiol Ecol 40:191–204