Bacterial, archaeal and eukaryotic diversity of smooth and pustular microbial mat communities in the hypersaline lagoon of Shark Bay

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ABSTRACT

The bacterial, archaeal and eukaryotic populations of nonlithifying mats with pustular and smooth morphology from Hamelin Pool, Shark Bay were characterised using small subunit rRNA gene analysis and microbial isolation. A highly diverse bacterial population was detected for each mat, with 16S rDNA clones related to Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Gemmatimonas, Planctomycetes, Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Verrucomicrobia and candidate division TM6 present in each mat. Spirochaetes were detected in the smooth mat only, whereas candidate division OP11 was only detected in the pustular mat. Targeting populations with specific primers revealed additional cyanobacterial diversity. The archaeal population of the pustular mat was comprised purely of Halobacteriales, whereas the smooth mat contained 16S rDNA clones from the Halobacteriales, two groups of Euryarchaea with no close characterised matches, and the Thaumarchaea. Nematodes and fungi were present in each mat type, with diatom 18S rDNA clones only obtained from the smooth mat, and tardigrade and microalgae clones only retrieved from the pustular mat. Cultured isolates belonged to the Firmicutes, Gammaproteobacteria, Alphaproteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, and Halobacteriales. The mat populations were significantly more diverse than those previously reported for Hamelin Pool stromatolites, suggesting specific microbial populations may be associated with the nonlithifying and lithifying microbial communities of Hamelin Pool.

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INTRODUCTION

Photosynthetic microbial mats can be found in a diverse range of environments around the world, including the hypersaline setting of Guerrero Negro (Ley *et al.*, 2006), geothermal hot springs at Yellowstone National Park, USA (Walter *et al.*, 1976), the cold dry valleys of Antarctica (Jungblut *et al.*, 2005), and alkaline sulphidic springs of Russia (Namsaraev *et al.*, 2003). At the microbial level, Cyanobacteria and other prokaryotes orient themselves at the sediment/liquid interface according to light and chemical gradients to form biologically stratified layers facilitating diverse metabolic processes. Nutrient cycling, niche differentiation and homeostatic regulation occur within the mat community allowing biological control of the microenvironment and hence greater survival than would be possible for individual species alone (Paerl *et al.*, 2000). This regulation of the microenvironment also directly or indirectly affects trapped or underlying sediments as well as any mineral precipitation or dissolution. Geomorphologically this may result in a range of forms from loose mucilaginous films to smooth, uniformly laminated structures or hard lithified crusts (Golubic, 1976).

When the cumulative record of microbial mat activities becomes lithified, the resulting geological structure is known as a stromatolite. These structures, along with the microfossils, molecular biomarker molecules and mineral isotopic compositions contained within them, represent our earliest evidence of life on Earth (Schopf, 2006). The dominance of stromatolites in the early rock record and up until 600 Ma indicates that benthic microbial communities are a highly persistent mode of life and a significant stage in Earth's evolution. Indeed the oxygenation of the Earth's atmosphere is attributed to the oxygenic photosynthesis and other gas production performed by Archaean stromatolite communities (Gebelein, 1976;

Hoehler *et al.*, 2001). Thus the study of microbial mats is of importance not just for understanding the ecology of present-day environments, but also the history of life on our planet.

From studies of extant Bahamian stromatolites a great deal of information about microbial mat development, lithification and stromatolite formation has been elucidated, such as the role of key metabolic groups (Dupraz & Visscher, 2005), community succession (Reid *et al.*, 2000), extracellular polymeric substances (Visscher *et al.*, 2000; Decho *et al.*, 2005), as well as the effect of the physical environment (Andres & Reid, 2006) and physicochemical parameters (Visscher *et al.*, 1998; Dupraz & Visscher, 2005) on the final geological morphology. Despite this wealth of data, it is also pertinent to investigate extant microbial mats and stromatolites in other regions, such as Hamelin Pool, Australia, in order to more fully understand the breadth of these types of microbial communities and the processes that occur within them.

Living stromatolites with a variety of morphologies were discovered in the hypersaline oligotrophic waters of Hamelin Pool in 1954, where they cover wide areas of the intertidal and subtidal zones along ~100 km of shoreline (Playford, 1990). In addition, nine different types of microbial mat were recognised at Hamelin Pool based on their surface morphology, namely colloform, gelatinous, smooth, pincushion, tufted, pustular, film, reticulate and blister mats (Logan *et al.*, 1974; Playford, 1990). This rich array of both stromatolites and microbial mats forming in a common environment presents a unique opportunity to investigate the biological and physicochemical factors that influence microbial mat composition, morphology and lithification.

The majority of studies of Hamelin Pool to date focused on the stromatolites, particularly the morphology of the geological structures formed (Logan, 1961; Logan *et al.*, 1964; Logan *et al.*, 1974; Reid *et al.*, 2003), and the influence of environmental factors on their formation such as wind, tidal action and sunlight (Playford, 1980; Awramik & Vanyo, 1986). Earlier investigations of the mats have been limited to microscopy of the dominant cyanobacterial groups (Logan *et al.*, 1974) and investigation of total community photosynthesis or sulphate reduction rates (Bauld *et al.*, 1979; Moriarty, 1983; Bauld, 1984; Skyring & Bauld, 1990). Only recently has the microbial diversity of the stromatolites been documented using molecular techniques (Burns *et al.*, 2004; Papineau *et al.*, 2005), whereas the bacterial populations of the mats, and their relationship to the lithified communities in such close proximity, are still largely unknown.

As an early study of Hamelin Pool indicated succession from smooth mat to pustular mat may occur during columnar stromatolite growth (Logan *et al.*, 1974), the smooth and pustular mat microbial communities were selected as the focus of this study. These two mats dominate the intertidal zone of Hamelin Pool (Fig. 1A), and are sometimes found covering the surface of intertidal stromatolites (Fig. 1B). The pustular mat (Fig. 1C) has a dark pigmented surface layer with a crenulated or pustular surface comprised of a cyanobacterium identified as *Entophysalis major* based on morphological studies (Logan *et al.*, 1974). This surface layer ranges from 1 to 8 mm thick through an individual pustule. No clear lamination is visible beneath the surface layer, although patches of green (Cyanobacteria), pink and black (bacterial) colouration indicate that spatial distribution of microorganisms does occur within the medium-grain irregular fenestral fabric of this mat. In contrast, the smooth mat surface is light in colour, with little vertical relief (Fig. 1D). A visible band of Cyanobacteria (*Microcoleus* and other species) is present 1–3 mm beneath the surface. Lamination of subsurface sediments is much more distinct than for the pustular mat, with pink (4–7 mm deep) and black (8–15 mm deep) zones visible in the fine to medium laminoid fenestral fabric (Logan *et al.*, 1974).
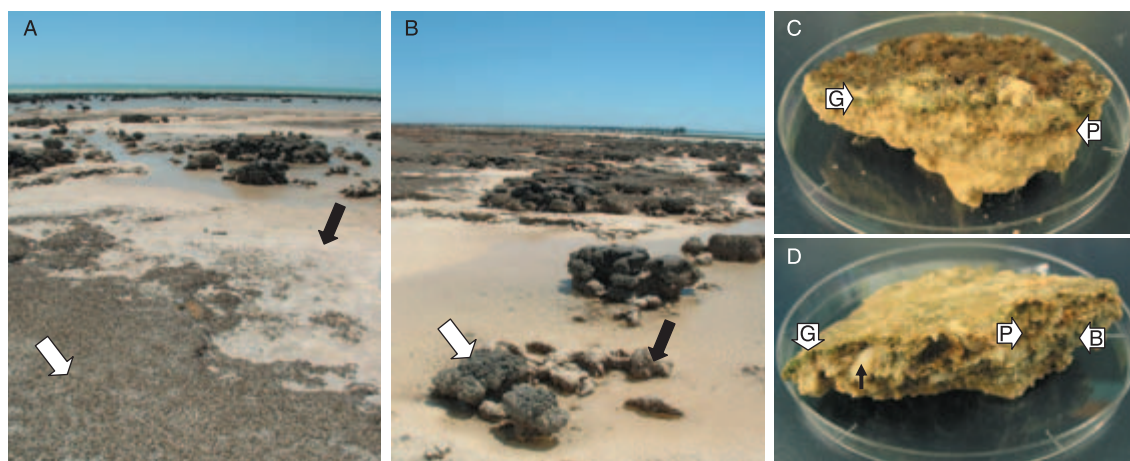
Fig. 1 Photographs of microbial sediment types in the intertidal region of Hamelin Pool, showing (A) pustular mat (white arrow) and smooth mat (black arrow), and (B) stromatolites with pustular mat (white arrow) or smooth mat (black arrow) growing on the surface. Within the pustular mat (C) and smooth mat (D), wide arrows indicate zones of green (G), pink (P) and black (B) colouration, and the small black arrow indicates a *Fragum erugatum* shell trapped in the smooth mat. Petri dish diameters are 11 cm.

In this study we collected samples of pustular and smooth mat from Hamelin Pool, Shark Bay, Western Australia and for the first time conducted analysis of small subunit (SSU) rDNA genes amplified from the mats, combined with isolation of heterotrophic bacteria, Cyanobacteria and archaea. This allowed us to determine the microbial diversity of the smooth and pustular mats and gain insight into their possible functions and community roles. Although culture-independent molecular studies alone do not allow us to absolutely determine whether sequences represent active stromatolite organisms, we can take advantage of phylogenetic affinity with well-studied species to make predictions about the metabolic contributions of organisms identified. Through phylogenetic methods we were also able to compare and contrast these nonlithifying microbial communities with the microbial consortia associated with the extant stromatolites of Hamelin Pool.

MATERIALS AND METHODS

Sample collection

Sampling was conducted in the intertidal region of Hamelin Pool at Telegraph Station, Shark Bay, Western Australia on 30 November and 1 December 2002. Smooth and pustular mats were collected from the intertidal region using a large spatula, placed into sterile jars or plastic zip-lock bags and maintained at 4 °C during transport back to the laboratory (~48 h). Extractions and organism isolations were initiated immediately upon sample return.

Media and culture conditions

The pustular mat was dissected into top (surface pigmented layer, averaging 2–3 mm thick) and bottom layers (~20 mm), whereas the smooth mat was dissected into top (1–3 mm), middle (4–7 mm deep) and bottom layers (7–20 mm). Dissected portions of each mat type were used to inoculate agar media with a variety of salt and nutrient levels in order to increase the variety of cultured organisms obtained. Three media for heterotrophic bacteria (denoted HP-LB) contained the average salts concentration of Hamelin Pool water (Arp *et al.*, 2001) (per litre of distilled water: NaCl 50.7 g; KCl 1.4 g; $CaCl_2.2H_2O$ 2.7 g; $MgCl_2.6H_2O$ 7.23 g; $MgSO_4.7H_2O$ 13.3 g; adjusted to pH 8.2) and either high, medium or low nutrient levels (per litre of distilled water: yeast extract 5 g and tryptone 10 g for HP-LB100%; yeast extract 2.5 g and tryptone 5 g for HP-LB50%; yeast extract 0.5 g and tryptone 1 g for HP-LB10%). A low salt/high nutrient medium based on Luria Broth (LB15++) (per litre of distilled water: yeast extract 5 g; tryptone 10 g; NaCl 15 g; $CaCl_2.H_2O$ 0.147 g; $MgCl_2.7H_2O$ 0.203 g; adjusted to pH 8.2) was also used. A modified halophilic archaeal medium (Goh *et al.*, 2006) was used for cultivation of archaea. Four media were used for cultivation of Cyanobacteria: BG-11 and ASN-III (Rippka

et al., 1979), ASN-IIIX (ASN-III with an additional 15 g NaCl), and HP-BG11 (BG-11 supplemented with Hamelin Pool salts as listed above for the noncyanobacterial media). Bacterial and archaeal plates were incubated at 30 °C or 37 °C in the dark until colonies were observed, and colonies representative of the observed range of pigmentation, colony height and surface margins observed were re-streaked until pure cultures were obtained. Cyanobacterial media were incubated at RT under natural light cycling (average maximum light intensity 8.1 µmol $m^{-2} s^{-1}$) and single filaments or colonies were transferred to fresh plates until unicyanobacterial cultures were obtained. For long-term storage the pure cultures of bacteria and archaea were stored at –80 °C in 40% glycerol, whereas cyanobacterial strains were stored at –80 °C in 5% DMSO.

DNA extraction from mats and isolates

In order to obtain good-quality DNA from the mats it was necessary to remove carbonate and extracellular polymeric substances from the samples. Mat samples (1–3 cm^3) were ground to a fine paste using a sterile mortar and pestle and transferred to a sterile 50 mL tube using 12 mL of 0.5 M ethylenediaminetetraacetic acid (EDTA). The tubes were incubated with gentle shaking at 37 °C overnight to allow the EDTA to dissolve the carbonate (Wade & Garcia-Pichel, 2003). Samples were then centrifuged at 3000 *g* for 20 min, and the supernatant discarded. For particularly carbonate-rich samples, a second overnight incubation in EDTA was necessary to remove all the carbonate. Once the carbonate-free pellet was obtained, genomic DNA was extracted by the method of Wilson (1990) with additional modifications as follows: five cycles of freezing at –20 °C and thawing at 65 °C were performed after incubation in the lysis buffer to ensure cell lysis had occurred, and the incubation with EPS-binding reagent cetyl trimethyl ammonium bromide was extended to overnight at room temperature to ensure all EPS was removed. We confirmed this method successfully disrupts all cell types by the successful detection of cyanobacterial DNA from species that possess extremely thick sheath material, and by testing on *Halococcus hamelinensis*, a species that is extremely resistant to cell disruption (Leuko *et al.*, 2008). DNA was extracted from cultured isolates using the xanthogenate method (Tillet & Neilan, 2000).

PCR amplification and cloning

Polymerase chain reaction (PCR) amplification and cloning was performed with a GeneAmp PCR system 2400 Thermocycler (Perkin Elmer) using a 20 µL PCR reaction mixture containing 1× reaction buffer, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5–1 µM of each primer, 0.2–1 U of *Taq* polymerase, and 10–100 ng of DNA template. Initial denaturation of template DNA was at 94 °C for 2 min, followed by 30 cycles of 94 °C for 20 s (melting), 50–55 °C for 30 s (annealing) and 72 °C for 45–120 s (extension), with a final extension of 7 min

at 72 °C. The primer sets used were the universal bacterial primers 27F1 (5'-AGAGTTTGATCCTGGCTCAG-3') and 1494Rc (5'-TACGGTTACCTTGTTACGAC-3') (Neilan *et al.*, 1997), 27F1 and 809R (5'-GCTTCGGCACGGCTCGGG-TCGATA-3') (Jungblut *et al.*, 2005) for Cyanobacteria, universal archaeal primers Arch21F (5'-TTCCGGTTGAT-CCYGCCGGA-3') and Arch985R (5'-YCCGGCGTTG-AMTCCAATT-3') (DeLong, 1992), and universal eukarya primers SS3 (5'-GATCCTTCCGCAGGTTCACCTACGGA-AACC-3') and SS5 (5'-GGTTGATCCTGCCAGTAGTC-ATATGCTTG-3') (Rowan & Powers, 1992). SSU rRNA genes amplified from isolates were purified by ethanol precipitation and sequenced directly, whereas the PCR products amplified from the total mat DNA were gel purified using the QIAquick Gel Extraction Kit (Qiagen) and cloned using the pGEM-T Easy Vector (Promega) according to manufacturers instructions.

At least 100 clones were picked for each clone library, and restriction fragment length polymorphism (RFLP) analysis was used to group SSU rRNA clones to avoid sequencing relicates. One digest using *Alu*I (MBI Fermentas) and one digest using *Scr*FI (MBI Fermentas) was set up for each clone according to manufacturer's instructions. Digests were incubated at 37 °C for 4 h then frozen until they could be separated on 3% agarose gels to visualise the RFLP pattern. Patterns were assessed manually and at least one clone from each unique pattern was sequenced.

DNA sequencing

Sequencing reactions contained 1 μL Big Dye (Applied Biosystems Inc.), 3.5 μL 5× sequencing buffer, 3.2 pmol of primer, 20–200 ng of PCR product, and MilliQ water to 20 μL. Amplifications were performed in the same thermocyclers as used for the PCRs using the following conditions: initial denaturation at 96 °C for 3 min, then 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Resulting sequencing template was purified by ethanol precipitation and sequenced on a AB3730 DNA Analyzer (Applied Biosystems) at the Automated Sequencing Facility, University of New South Wales, equipped with DNA Sequencing Analysis Software version 3.4.1 (Applied Biosystems) running on a Macintosh platform. Sequences generated in this study were submitted to the GenBank database (accession numbers DQ058831 – DQ058894 and DQ860865 – DQ861283).

Phylogenetic and statistical analysis

Sequence data were viewed and analysed using the AutoAssembler™ program (Version 1.3.0; Applied Biosystems). SSU rRNA gene clone data were checked for chimeric sequences using the programs Bellerophon (Huber *et al.*, 2004) and Pintail (Ashelford *et al.*, 2005) with no chimeras identified. Sequences generated in this study were imported

into ARB (Ludwig *et al.*, 2004) running on a MacBook Pro under OS 10.4.7. Alignment was performed using the ARB Integrated Aligner, with manual verification, against the Greengenes ribosomal RNA database (DeSantis *et al.*, 2006). Phylogenetic trees were created in ARB from selected full-length reference species using a maximum-likelihood algorithim (AxML) and the Lane Mask supplied with ARB to exclude hypervariable regions. The partial 16S rRNA gene sequences generated in this study (600–850 bp) were then added by parsimony analysis without changing the tree topology. Phylogenetic clustering of sequences in the ARB trees correlated with the closest BLAST matches of the sequences from NCBI GenBank. Diversity indices were calculated using EstimateS (Colwell, 2005).

RESULTS AND DISCUSSION

Microbial isolation and SSU rRNA clone library analysis of Hamelin Pool microbial mats

Employing the various culture media, a total of 92 morphologically distinct isolates were obtained. Sixty of these isolates were heterotrophic bacteria displaying a variety of colony morphologies and pigmentation (Table 1). Firmicutes comprised half of the isolates from the pustular mat, and over half of the smooth mat isolates, with two main groups detected – the salt-tolerant *Halobacillus*, *Pontibacillus* and *Virgibacillus* groups, and the nonhalophilic group related to *Bacillus licheniformis*, *Bacillus litoralis* and others. *Bacillus* spp. have many characteristics that would enhance their survival in the Hamelin Pool microbial mat communities including spore formation, secondary metabolite production (Veith *et al.*, 2004), and the ability to grow in hypersaline and/or desiccated environments (Heyrman *et al.*, 2003; Lim *et al.*, 2005). In some strains the ability to precipitate carbonate, which could enhance mat formation, has been documented (Rivadeneyra *et al.*, 2004), whereas other strains can degrade exopolymeric substances (Takeda *et al.*, 2005) and may thus degrade the sticky cyanobacterial sheath material thought to play an integral structural and sediment-trapping role in these microbial mats (Bauld, 1984). The next most abundant group isolated from the pustular and smooth mat was the Gammaproteobacteria. Marine and halophilic genera such as *Vibrio*, *Idiomarina*, *Halomonas* and *Marinobacter* were obtained from both mat types, whereas isolates related to *Pseudoalteromonas* and *Alteromonas* were isolated from the pustular mat only, and isolates related to *Marinomonas* and *Nitrococcus* were isolated from the smooth mat only. In addition, one *Cytophaga*-related isolate was obtained from the pustular mat, and one Actinobacterial isolate and one Alphaproteobacterial isolate was obtained from the smooth mat. No clear correlation was observed between the nutrient level of the isolation media and the types of bacteria isolated. For example, *Idiomarina* isolates were obtained on all HP-LB media regardless of the yeast

Table 1 Cultured isolates from Hamelin Pool pustular and smooth mat. Isolates from the pustular mat are denoted PB, isolates from the smooth mat are denoted SB
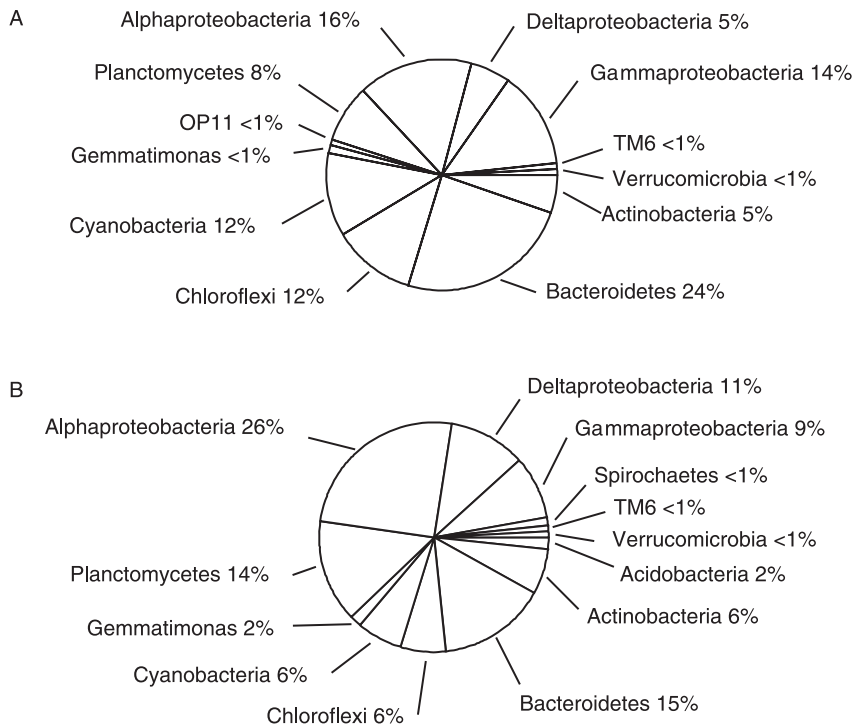
Isolate	Media	Description	Closest BLAST match, GenBank	Accession No.	% identity
PB01	HP-LB	Creamy entire low convex	*Pseudoalteromonas* sp. A25	AF227237	95
PB02	HP-LB	Yellow entire low convex	*Bacillus* sp. m2-6	DQ923218	97
PB03	HP-LB 50%	Spreading translucent flat	*Cytophaga* sp. I-601	AB073568	99
PB04	HP-LB 50%	Yellow convex papillate	*Bacillus* sp. m2-6	DQ923218	96
PB05	HP-LB 50%	Tiny pale dome with yellow centre	*Bacillus* sp. m2-6	DQ923218	96
PB06	HP-LB 50%	White entire low convex	*Salinimonas chungwhensis* strain BH030046	AY553295	96
PB07	HP-LB 50%	Tiny white entire low convex	*Bacillus* sp. CNJ958 PL04	DQ448752	98
PB08	HP-LB 50%	Creamy spreading low convex	*Vibrio* sp. GWS-TZ-H304	AY332206	94
PB09	HP-LB 10%	Orange entire dome	*Bacillus* sp. m2-6	DQ923218	97
PB10	HP-LB 10%	Translucent white dome	*Pseudoalteromonas* sp. SOBB3	AM179825	96
PB11	HP-LB 10%	Creamy entire low convex	*Pseudoalteromonas ruthenica* strain KMM300	AF316891	94
PB12	HP-LB 10%	Creamy with translucent edge domed	*Alteromonas* sp.	AB015135	96
PB13	HP-LB 10%	Golden entire low convex	*Bacillus* sp. m2-6	DQ923218	96
PB14	HP-LB 10%	White entire low convex	*Vibrio* sp. NAP-4	AF064637	97
PB15	HP-LB 10%	Pink entire low convex	*Bacillus* sp. CNJ842 PL04	DQ448790	97
PB16	HP-LB 10%	Pale low convex with brown centre	*Bacillus* sp. MK03	AB062678	97
PB17	HP-DSMmod	Skin coloured entire low convex	*Halobacillus* sp. SB-Hb1	AB198760	99
PB18	HP-DSMmod	Yellow entire flat	*Pontibacillus* sp. CNJ912 PL04	DQ448765	99
PB19	HP-DSMmod	Small white entire low convex	*Marinobacter sedimentalis* R65T	AJ609270	97
PB20	HP-DSMmod	Pale yellow entire low convex	*Halobacillus* sp. SB-Hb1	AB198760	100
PB21	HP-DSMmod	Deep yellow entire domed	*Pontibacillus* sp. CNJ912 PL04	DQ448765	99
PB22	HP-DSMmod	Pale brown entire low convex	*Bacillus* sp. OV1	AY647318	99
PB23	LB15++	Clear flat spreading	*Pseudoalteromonas* sp. Ae30-MC-S	AB267466	99
PB24	LB15++	Creamy spreading low convex	*Halomonas* sp. J21.8	AJ717724	99
PB25	LB15++	Tiny clear domed	*Alcanivorax* sp. DG881	AY258109	99
PB26	LB15++	Translucent creamy spreading	*Halomonas* sp. 3022	AM110977	98
PB27	LB15++	Translucent brown low convex	*Idiomarina* sp. NT N96	AB167019	98
PB28	LB15++	Bright orange entire domed	*Planococcus citreus* strain TF-16	AF500008	99
SB01	LB15++	Creamy entire low convex	*Vibrio* sp. NAP-4	AF064637	99
SB02	HP-LB	Yellow entire low convex	*Idiomarina loihiensis*	AF288370	99
SB03	HP-LB	Large creamy spreading	*Marinomonas* sp. PF1-3	EU651810	97
SB04	HP-LB	Creamy entire low convex	*Roseobacter* sp. CSQ-2	EF512125	95
SB05	HP-LB	Pink entire domed	*Idiomarina* sp. NT N121	AB167036	99
SB06	HP-LB	Clear flat undulate with 'matt' surface	*Halobacillus trueperi* strain GSP062	DQ157162	99
SB07	HP-LB	Pink entire domed	*Bacillus* sp. CNJ842 PL04	DQ448790	96
SB08	HP-LB	Brown centered pale low convex	*Bacillus* sp. YIM C209	EU135718	99
SB09	HP-LB	Orange entire domed	*Idiomarina loihiensis*	AF288370	99
SB10	HP-LB 50%	Large creamy entire with darker centre	*Marinomonas aquimarina* strain 11OM3	AJ843079	96
SB11	HP-LB 50%	Yellow entire lox convex	*Idiomarina loihiensis*	AF288370	99
SB12	HP-LB 50%	Tiny clear entire low convex	*Marinobacter sedimentalis* R65T	AJ609270	98
SB13	HP-LB 50%	Creamy entire with brown centre	*Bacillus* sp. YIM C209	EU135718	98
SB14	HP-LB 50%	Tiny deep red entire low convex	*Bacillus* sp. YIM C209	EU135718	99
SB15	HP-LB 50%	Apricot colour entire low convex	*Bacillus* sp. CNJ842 PL04	DQ448790	99
SB16	HP-LB 10%	White flat irregular with 'matt' surface	*Bacillus* sp. BUS 07-17	AM931043	98
SB17	HP-LB 10%	White crenated, convex papillate	*Bacillus* sp. 19500	AJ315068	98
SB18	HP-LB 10%	Tiny clear entire low convex	*Idiomarina loihiensis*	AF288370	99
SB19	HP-LB 10%	White undulate convex papillate	*Bacillus* sp. BUS 07-17	AM931043	99
SB20	HP-LB 10%	Yellow entire domed	*Cellulomonas* sp. CR1-1	AY205295	99
SB21	HP-LB 10%	White spreading low convex	*Halomonas* sp. 18bAG	AJ640133	99
SB22	HP-LB 10%	Brown spreading low convex	*Bacillus* sp. YIM C209	EU135718	99
SB23	HP-DSMmod	Pale brown entire low convex	*Halobacillus* sp. SB J82	AB167058	98
SB24	HP-DSMmod	White entire low convex	*Nitrococcus mobilis* ATCC 25380	L35510	92
SB25	HP-DSMmod	Brown entire low convex	*Pontibacillus* sp. US13 HS-2008	AM950313	99
SB26	HP-DSMmod	Orange entire low convex	*Halobacillus* sp. YIM-kkny15	AY121438	99
SB27	HP-DSMmod	Deep yellow entire domed colony	*Halobacillus* sp. HS244	DQ458834	99
SB28	HP-DSMmod	Pale pink entire low convex colony	*Halomonas salina*	AM945688	99
SB29	HP-DSMmod	Creamy entire low convex colony	*Halomonas salina*	AM945688	99
SB30	HP-DSMmod	Pale yellow entire low convex colony	*Halobacillus locisalis*	AY190534	99
SB31	HP-DSMmod	Yellow entire low convex colony	*Pontibacillus* sp. CNJ912 PL04	DQ448765	99
SB32	HP-DSMmod	White entire domed	*Virgibacillus* sp. SA-Vb1	AB197851	100
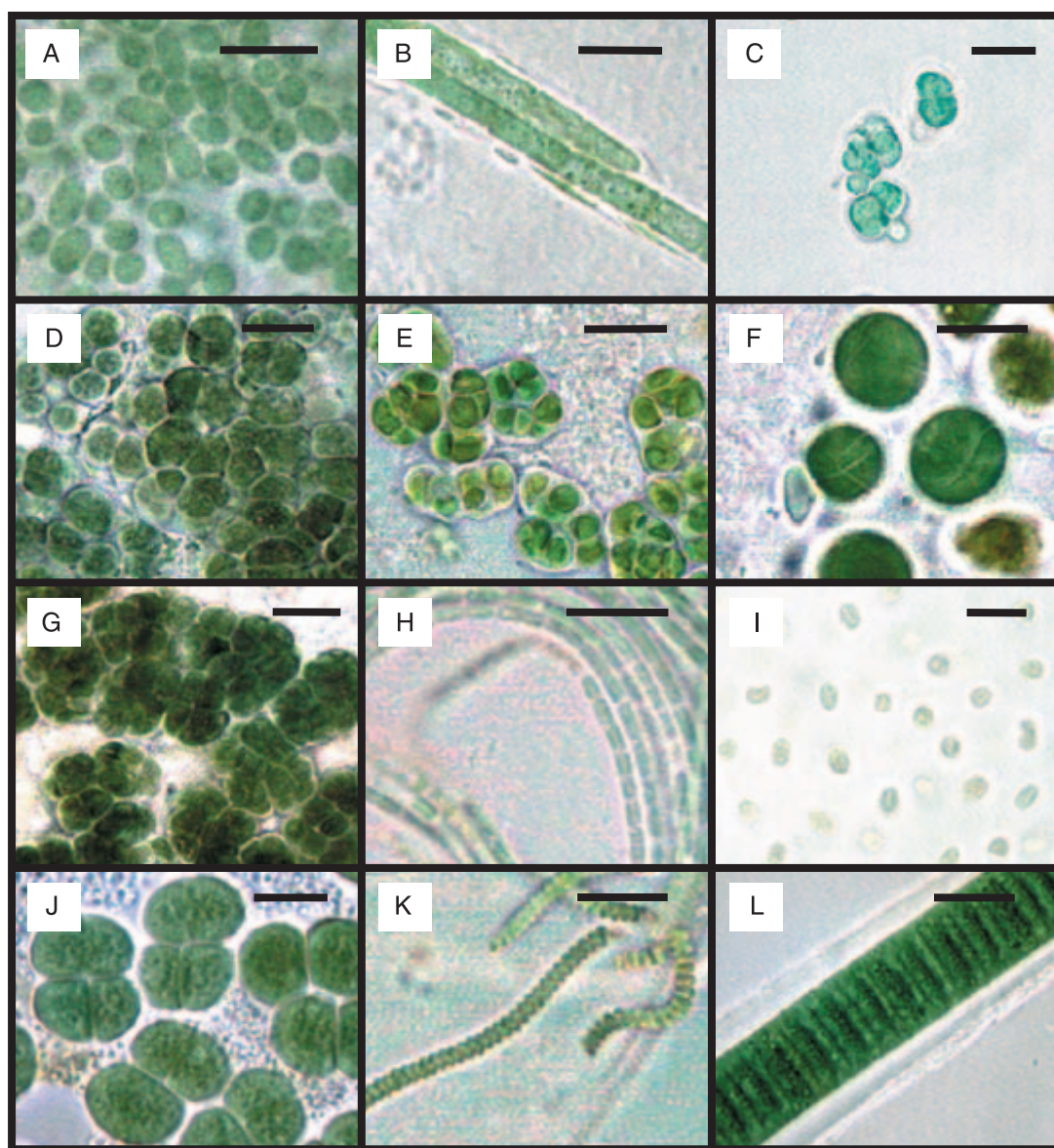
Fig. 2 Light microscopy of selected cyanobacterial isolates from the smooth and pustular mats. (A) *Euhalothece* isolate SCyano4, (B) *Microcoleus* isolate SCyano17, (C) *Cyanothece*-like SCyano22, (D) *Myxosarcina*-like SCyano23, (E) *Stanieria*-like SCyano26, (F) *Chroococcidiopsis*-like SCyano33, (G) *Stanieria*-like SCyano35, (H) *Halomicronema* isolate PCyano40, (I) *Halothece* isolate PCyano42, (J) *Chroococcus*-like PCyano51, (K) *Spirulina*-like SCyano57, (L) *Lyngbya* isolate SCyano73. Scale bar is 10 μm in each image. SCyano, isolated from the smooth mat; PCyano, isolated from the pustular mat.

extract and tryptone concentration, and *Bacillus*-related isolates were obtained on all media used. Neither was there any observable difference between the types of bacteria obtained and the layer of the mat they were isolated from. The majority of isolates showed high sequence similarity (97–100%) to characterised strains in the NCBI GenBank database. Isolate SB24, related to *Nitrococcus mobilis*, was the least similar to sequences in the database, at 92%. Overall, the distribution of bacteria obtained in pure culture from the Hamelin Pool pustular and smooth mats was similar to that observed in other hypersaline environments (Ventosa *et al.*, 1998a,b).

A total of 19 cyanobacterial isolates were successfully purified to unicyanobacterial status from the mats – 14 from the smooth mat and 5 from the pustular mat (selected representatives shown in Fig. 2). Isolates with high 16S rDNA similarity to strains in the GenBank database related to *Halomicronema* sp. (strain designations SCyano39 and PCyano40; 99% identity based on BLAST match), *Euhalothece* sp. (SCyano4, SCyano59, PCyano45 and PCyano49; 97–99%) and *Halothece* sp. (SCyano8 and PCyano42; 99%) were obtained from both mat types. An isolate related to *Chroococcus submarinus* (PCyano51; 97%) was unique to the pustular mat,

A

Alphaproteobacteria 16%

Planctomycetes 8%

OP11 <1%

Gemmatimonas <1%

Cyanobacteria 12%

Chloroflexi 12%

Deltaproteobacteria 5%

Gammaproteobacteria 14%

TM6 <1%

Verrucomicrobia <1%

Actinobacteria 5%

Bacteroidetes 24%

B

Alphaproteobacteria 26%

Planctomycetes 14%

Gemmatimonas 2%

Cyanobacteria 6%

Chloroflexi 6%

Deltaproteobacteria 11%

Gammaproteobacteria 9%

Spirochaetes <1%

TM6 <1%

Verrucomicrobia <1%

Acidobacteria 2%

Actinobacteria 6%

Bacteroidetes 15%

Fig. 3 Phylogenetic affiliation of bacterial clones from Hamelin Pool pustular mat (A) and smooth mat (B).

whereas isolates closely related to *Microcoleus chthonoplastes* (SCyano17; 98%) and *Lyngbya* sp. (SCyano73; 97%) were only obtained from the smooth mat. Eight additional isolates from the smooth mat had less than 96% similarity to characterised strains in the GenBank database, and these were affiliated with *Chondrocystis* sp. (SCyano24; 95%), *Spirulina* sp. (SCyano57; 94%), *Myxosarcina* sp. (SCyano23; 93%), *Stanieria* sp. (SCyano35 and SCyano26; 93% and 90%, respectively), *Cyanothece* sp. (SCyano22; 92%), *Halothece* sp. (SCyano58; 90%), and *Chroococcidiopsis* sp. (SCyano33; 90%). Cyanobacteria of the heterocystous order Nostocales or the true-branching order Stigonematales were not observed during microscopic examination of the mats and were not obtained in culture under the experimental conditions used.

Thirteen archaeal isolates were obtained, and the isolates formed two groups based on colony and cell morphology and their closest 16S rRNA gene matches in the GenBank database. After detailed taxonomic characterisation, seven isolates from the smooth mat and one isolate from the pustular mat were found to be members of a novel species, *Haloferax elongans* (Allen *et al.*, 2008), whereas the remaining five isolates, all from the pustular mat, were found to be members of the novel species *Haloferax mucosum* (Allen *et al.*, 2008). Targeted culturing of eukarya was not performed, however diatoms, nematodes and fungi were observed on the cyanobacterial culturing media during the purification procedure.

In addition to microorganism isolation, four clone libraries were constructed and screened for each microbial mat type, in order to assess the bacterial, archaeal and eukaryotic inhabitants of the mats, and to gather in-depth information about the cyanobacterial populations. A total of 111 bacterial clones from each mat were directly sequenced and analysed, as preliminary RFLP of 20 clones from each mat failed to reveal any common patterns. Twelve bacterial divisions were represented by the rDNA clones from the pustular mat, and 13 divisions by clones from the smooth mat (Fig. 3) based on BLAST search results and clustering within phylogenetic trees. The most abundant divisions in the pustular mat were the Bacteroidetes (24%), Alphaproteobacteria (16%), Gammaproteobacteria (14%), Chloroflexi (12%) and Cyanobacteria (12%), whereas the Alphaproteobacteria (25%), Bacteroidetes (15%), Planctomycetes (14%) and Deltaproteobacteria (11%) were the most abundant groups in the smooth mat. The majority of clones from the pustular mat (94 of 111) and smooth mat (104 of 111) had a top BLAST hit with less than 97% similarity to 16S rRNA gene sequences in GenBank, as illustrated in Fig. 4. Both mats had highly diverse bacterial populations, with the Chao1 estimates of total species richness greater than 3000 when operational taxonomic units (OTUs) were grouped at 98% or higher (Table 2). For comparative purposes, the bacterial diversity of one Hamelin Pool intertidal columnar stromatolite was calculated from the data of Burns *et al.* (2004), which yielded a total estimate of 59 ± 14 bacterial species when OTUs were grouped at 100% sequence identity (Table 2). A study of Hamelin Pool stromatolites which used a primer set that detected both bacterial and archaeal species together calculated total estimates of 993–1309 microbial species for a domed stromatolite (HPDOM) and 1500 microbial species for an irregular
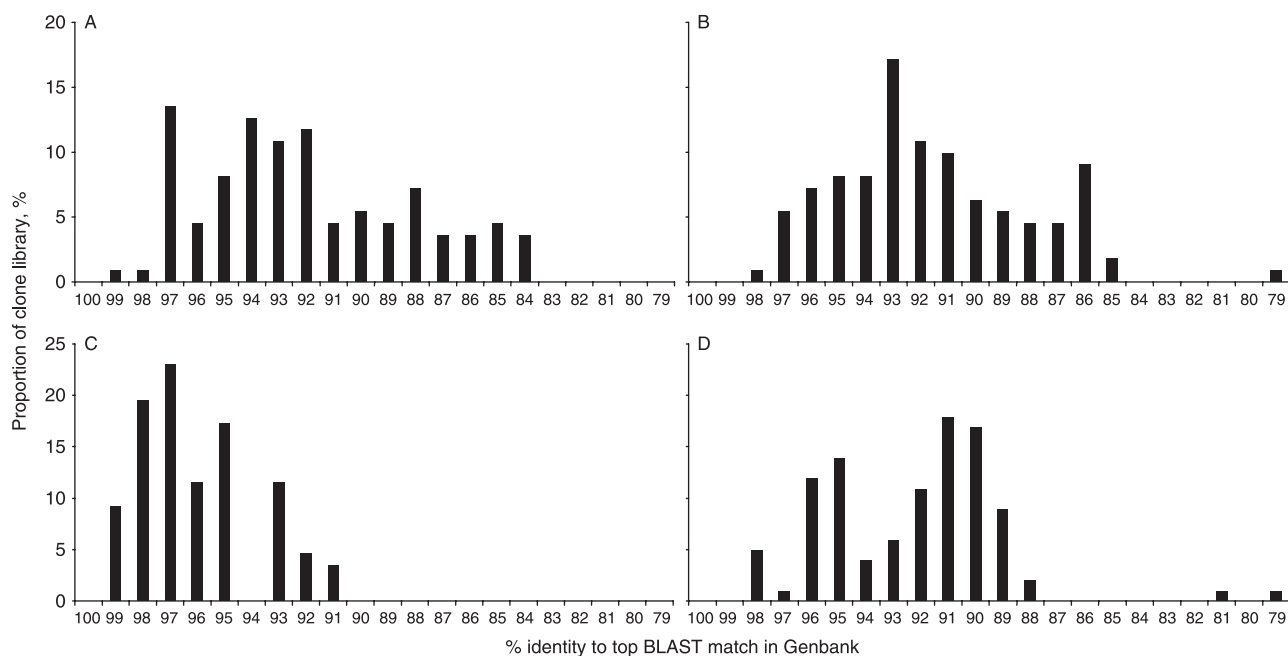
Fig. 4 Distribution of percentage identity of best BLAST matches in GenBank for bacterial clones from the pustular mat (A) and smooth mat (B), and archaeal clones from the pustular mat (C) and smooth mat (D). Best matches (mostly uncultured clones) were required to align over at least 90% of the full length of the query sequence.

Table 2 Observed operational taxonomic units (OTU), estimated coverage and estimated richness of Hamelin Pool bacterial and archaeal populations

OTU (%)‡	Smooth Mat*				Pustular mat*				Columnar Intertidal stromatolite†			
	Obs	C_{ACE}	Chao1 ± SD§	D§	Obs	C_{ACE}	Chao1 ± SD§	D§	Obs	C_{ACE}	Chao1 ± SD§	D§
Bacterial clone libraries												
100	111	0	6216 ± 1164	4.71	111	0	6216 ± 1164	4.71	35	0.69	58.7 ± 14.4	3.23
99	111	0	6216 ± 1165	4.71	110	0.02	3053 ± 586	4.7	34	0.72	44.8 ± 7.6	3.06
98	111	0	6216 ± 1166	4.71	110	0.02	3053 ± 586	4.7	34	0.72	45.2 ± 9.8	2.89
97	109	0.04	1999 ± 379	4.68	110	0.02	3053 ± 586	4.69	33	0.73	46.2 ± 10.9	2.99
96	105	0.09	1788 ± 348	4.62	108	0.05	2891 ± 562	4.66	32	0.77	41.8 ± 9.7	2.93
95	103	0.12	1291 ± 249	4.59	103	0.12	1054 ± 199	4.58	nd	nd	nd	nd
Archaeal clone libraries												
100	64	0.46	235.5 ± 82.3	3.90	42	0.68	69.6 ± 12.8	3.44	36	0.70	116.5 ± 88.3	2.54
99	58	0.57	142.5 ± 40.1	3.80	31	0.82	38.8 ± 5.3	3.06	31	0.77	46.0 ± 17.0	2.24
98	48	0.71	81.8 ± 18.0	3.61	23	0.89	29.1 ± 6.1	2.81	29	0.80	30.0 ± 7.1	2.2
97	37	0.70	81.1 ± 28.4	3.11	15	0.88	17.7 ± 3.5	2.20	27	0.81	31.0 ± 14.6	2.24
96	34	0.75	58.1 ± 16.0	3.02	12	0.88	14.3 ± 3.4	1.95	26	0.83	32.8 ± 17.8	2.15
95	31	0.80	46.17 ± 9.4	2.95	12	0.88	14.3 ± 3.4	1.95	nd	nd	nd	nd

*Data from this study; †data calculated from the clone libraries of Burns *et al.* (2004); ‡OTUs represent groups of sequences related by greater than or equal to the % sequence identity indicated; Obs, number of lineages observed; C_{ACE}, Coverage as calculated by the method of Chao *et al.* (1993); Chao1 ± SD, Chao1 estimate of total number of lineages ± standard deviation; D, Shannon-Wiener index of diversity; §diversity indices calculated using EstimateS (Colwell, 2005); nd, not determined.

'knobby pustular' stromatolite (HPIRR) (Papineau *et al.*, 2005; figures for OTUs grouped at 100% sequence identity reported). The extremely high diversity of bacterial sequences obtained from the mats was reflected in the Shannon-Weaver index of diversity, but also meant that the clone library cover-age was very low, and this under-sampling precluded further statistical comparison of the mat libraries with the libraries from the stromatolites.

Archaeal rDNA clones from libraries constructed for each mat type were grouped by RFLP and sequenced. The 87

Table 3 Comparison of cyanobacterial genera detected in the pustular and smooth mat by culturing and 16S rDNA clone libraries. For clone libraries, the number of clones affiliated with each species is indicated, followed by their % identity (BLAST search, 20 June 2008). The % identity is also shown for the isolates

Closest characterised match from Genbank	Pustular mat			Smooth mat		
	Clone library			Clone library		
	Cyanobacterial-specific	Universal bacterial	Isolate	Cyanobacterial-specific	Universal bacterial	Isolate
Arthrospira sp.	1 (92%)			2 (91%, 90%)		
Chondrocystis sp.						95%
Chroococcidiopsis sp.	30 (95%)	1 (95%)		5 (94%)		90%
Chroococcus sp.			97%	1 (97%)		
Cyanothece sp.	6 (92%, 91%)			5 (93%, 92%, 91%)	1 (90%)	92%
Euhalothece sp.	5 (98%, 94%)		99%, 97%	17 (99%)	1 (95%)	99%, 98%
Gloeocapsa sp.	16 (91%, 90%)					
Gloeothece sp.				4 (91%)		
Halomicronema sp.	1 (92%)	4 (95%, 93%, 92%)	99%			99%
Halothece sp.	15 (89%, 86%)	1 (92%)	99%	15 (99%, 90%)	1 (91%)	99%, 90%
Leptolyngbya sp.	7 (95%, 94%, 93%, 90%)	1 (94%)		9 (94%, 93%, 90%)		
Lyngbya sp.						97%
Microcoleus sp.	12 (98%)	2 (94%, 92%)		24 (97%)		98%
Myxosarcina sp.						93%
Phormidium sp.	4 (93%)	1 (95%)			4 (96%, 95%)	
Pleurocapsa sp.		1 (93%)				
Spirulina sp.	5 (94%, 90%)			1 (90%)		94%
Stanieria sp.	4 (91%)			7 (93%)		93%, 90%
Synechococcus sp.		1 (91%)				
Synechocystis sp.				2 (99%)		
Xenococcus sp.	1 (91%)	1 (95%)		1 (96%)		

clones from the pustular mat were all members of the Halobacteriales, with closest cultured relatives including *Halobacterium cutirubrum*, *Haloferax volcanii* and *Natronomonas pharaonis*, and the low-salt tolerant Haloarchaea represented by isolates Haloarchaeon 10AH and Haloarchaeon 14AHG (Purdy *et al.*, 2004). The 101 clones from the smooth mat included three groups of clones which are postulated to belong to the newly created archaeal phylum Thaumarchaeota (Brochier-Armanet *et al.*, 2008), previously designated as the mesophilic Crenarchaea (Fig. 5), and a similar range of haloarchaeal clones to that found in the pustular mat. In addition, a group of 18 euryarchaeal clones with no cultured relative available in their top 100 BLAST hits was identified, as well as two clones with very distant matches to the methanogenic archaea (77–78% sequence identity over 840 bp). However, these two clones did not cluster with known methanogens during phylogenetic tree construction. In contrast, sequences closely related to methanogens were found in both Hamelin Pool stromatolite studies (Burns *et al.*, 2004; Papineau *et al.*, 2005), along with a variety of haloarchaeal and thaumarchaeal clones. The finding of potential methanogens here and in these other studies is intriguing, as it has been suggested that high-sulphate environments such as that found in Shark Bay may not support most forms of methanogenesis (Burns *et al.*, 2004). Whereas 52% of the pustular mat clones (45 of 87) were closely related to sequences in GenBank (≥ 97% sequence

identity), the majority of clones from the smooth mat are only distantly related to sequences in the databases (Fig. 4). Clone library coverage was good, and the total estimate of archaeal species richness was 236 ± 82 for the smooth mat, and 70 ± 13 for the pustular mat when OTUs were grouped at 100% sequence identity (Table 2). The Chao1 estimate of total species richness for an intertidal columnar stromatolite was intermediate to the two mat types (117 ± 88, Table 3) (calculated from the data of Burns *et al.*, 2004).

In comparison to the universal bacterial and universal archaeal clone libraries, obtaining eukaryotic 18S rDNA clones from the mats was difficult, with a high level of junk sequences obtained. In total only 11 positive clones were obtained from the pustular mat, and 10 from the smooth mat. Nematode sequences were most numerous, comprising eight sequences from the pustular mat (seven related to *Rhabdolaimus* cf. *terrestris* (98–97% sequence identity) and one to *Achromadora* cf. *terricola* (89%)) and six sequences from the smooth mat (two related to *Rhabdolaimus* cf. *terrestris* (98%), two to *Diplolaimelloides meyli* (97%), and one each related to *Prismatolaimus intermedius* (92%) and *Leptolaimus* sp. (94%)). One clone related to the mitosporic fungus *Engyodontium album* (99%) was obtained from each mat. Three diatom sequences were unique to the smooth mat (two related to *Pseudogomphonema* sp. (98%) and one to *Navicula cryptotenella* (98%)). One microalgal sequence *Pinguiococcus pyrenoidosus* (96%)
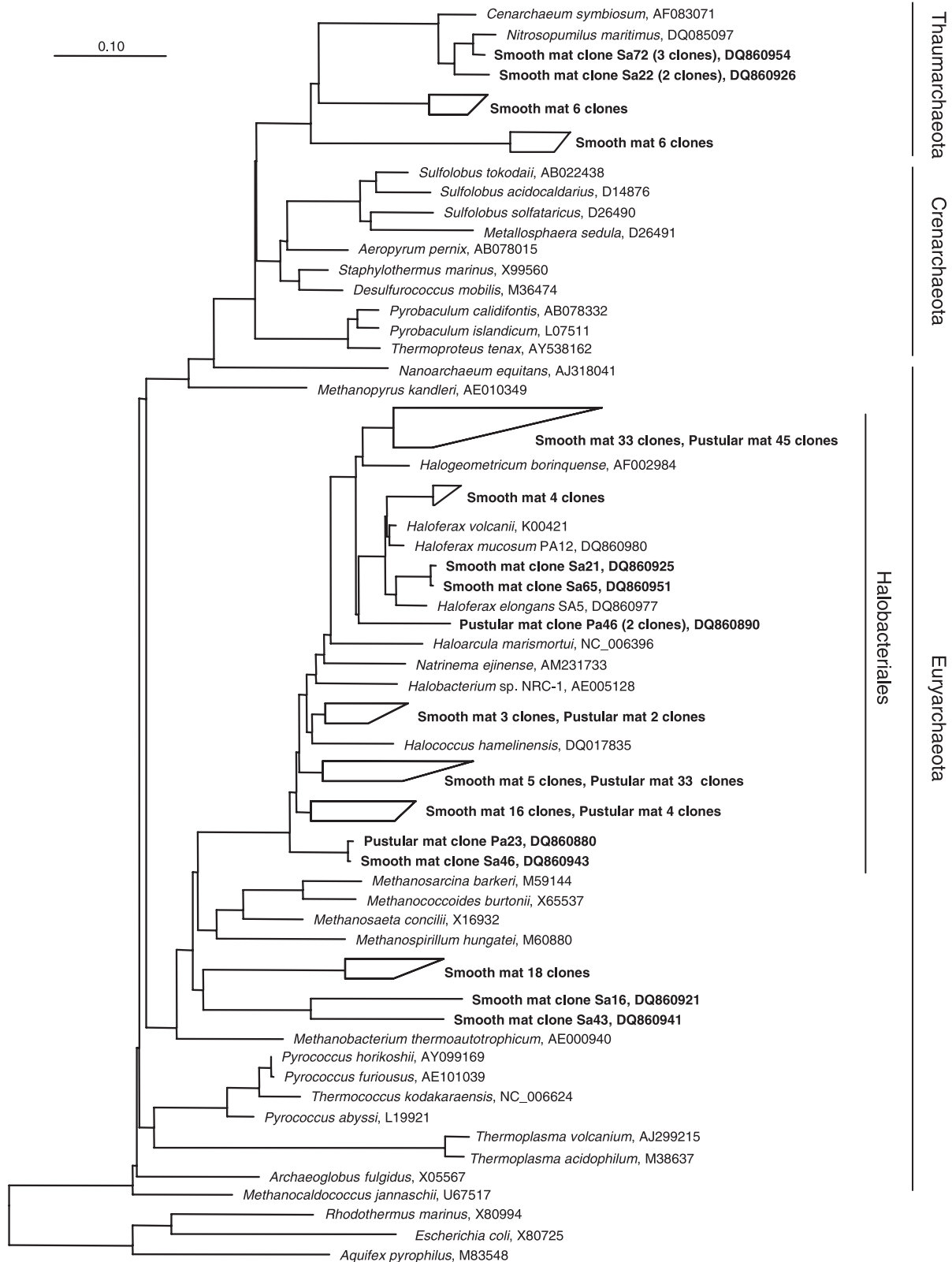
Fig. 5 Maximum likelihood tree constructed in ARB from full-length reference sequences, with archaeal clones from the smooth and pustular mat (bold) added without changing the tree topology. The outgroup contains the bacterial species *Escherichia coli*, *Aquifex pyrophilus* and *Rhodothermus marinus*. Scale bar indicates 0.1 nucleotide changes per site.

and one tardigrade sequence (*Pseudechiniscus islandicus* 88%) were unique to the pustular mat. Although only a few eukaryotic sequences were obtained, the types of organisms detected correlated with our observations during culturing and microscopy of the mats, and with a recent report from a hypersaline microbial mat in Guerrero Negro in which the sparse eukaryal diversity was dominated by nematode sequences (Feazel *et al.*, 2008).

rDNA clones belonging to the cyanobacterial orders Oscillatoriales, Chroococcales and Pleurocapsales were detected in each clone library constructed with Cyanobacteria-specific PCR primers (Table 3). No Nostocales or Stigonematales clones were obtained, which was consistant with the results from the universal bacterial 16S rRNA gene analysis and from organism isolation. *Microcoleus* sp. were the dominant clones obtained from the smooth mat (24 of 93 clones), which corresponds with its reported dominance in this mat type (Bauld, 1984) and with our microscopic observations (data not shown). Other halophilic genera such as *Euhalothece* sp. (17 clones) and *Halothece* sp. (15 clones) were also abundant in the smooth mat. *Entophysalis major* was previously identified as the dominant species in the pustular mat by microscopy analyses (Bauld, 1984); however, no 16S rDNA sequence data is currently available for *Entophysalis major* or other members of this genera, and culturing studies were unable to isolate this organism. The dominant clone in the pustular mat library was most closely related to *Chroococcidiopsis* sp. PCC6712 (95% sequence identity) (30 of 107 clones). Although morphology-based taxonomy of Cyanobacteria broadly correlates with their 16S rDNA-based phylogenetic relationships, members of the Oscillatoriales, Chroococales and Pleurocapsales do not form tight phylogenetic clades and are instead dispersed throughout the cyanobacterial phylogenetic tree (Wilmotte & Herdman, 2001). The taxonomy of unicellular Cyanobacteria is further complicated by a lack of validly published strains, and conflicting nomenclature used by botanists and bacteriologists (See Oren, 2004 for a discussion). As such, it is possible that the abundant *Chroococcidiopsis*-related clones observed in the pustular mat may represent *Entophysalis major*. Other abundant species from the pustular mat were related to *Gloeocapsa* sp. (16 clones) and *Halothece* sp. (15 clones).

When OTUs were grouped at 98% sequence identity, the coverage (C_{ACE}) of the cyanobacterial libraries was calculated as 71% and 78% for pustular and smooth mat, respectively. Chao1 estimates indicated a total of 33 ± 6 species for the pustular mat and 32 ± 9 species for the smooth mat if the libraries were sampled to completion. There was good correspondence in the present study between the cyanobacterial populations detected using microbial isolation, universal bacterial primer PCR and cyanobacterial-specific primer PCR (Table 3). All the genera obtained in culture were also detected in at least one clone library, with the exception of the *Lyngbya*, *Chondrocystis* and *Myxosarcina* isolates. Most of the cyanobacterial types detected in the pustular mat universal bacterial library

were also detected in the cyanobacterial-specific library (10 of 13 clones); however, as anticipated the cyanobacterial-specific libraries did reveal a greater diversity of cyanobacterial genera, with an additional six species in the pustular mat and ten in the smooth mat detected. *Halothece* sp. were the only organisms to be detected in all four clone libraries and by culturing from each mat. Interestingly, there appears to be two subpopulations present in Hamelin Pool: one with high similarity to characterised *Halothece* spp. (~99%) and one with low similarity to the characterised strains (~90%). The majority of the cyanobacterial species identified in this study had not been previously reported from the Hamelin Pool mats, including members of the recently identified halophilic groups *Euhalothece*, *Halothece* (Garcia-Pichel *et al.*, 1998), and *Halomicronema* (Abed *et al.*, 2002).

The majority of organisms in an environment are not recovered by culturing studies (for example, Amann *et al.*, 1995), and although a range of isolates spanning five bacterial phyla and one archaeal phylum were readily obtained in this study, the diversity of isolates was still far less than the range of organisms revealed using culturing-independent SSU rRNA gene analysis. However, when one particular group, the Cyanobacteria, was targeted with media specifically designed for its isolation, the range of isolates more closely resembled the diversity obtained by culture-independent methods, with 12 of the 21 cyanobacterial genera detected in the mats now having a cultured isolate available. We anticipate further diverse species of interest could be successfully obtained in culture by the use of tailored media and isolation conditions (Davis *et al.*, 2005). The array of mat isolates now available as pure cultures will facilitate testing of our hypotheses regarding the roles of heterotrophs, Cyanobacteria and Haloarchaea in the Hamelin Pool environment.

Comparison of the microbial diversity of Hamelin Pool microbial mats and stromatolites

The most abundant bacterial phyla identified in the present study were the Actinobacteria, Alphaproteobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deltaproteobacteria, Gammaproteobacteria and Planctomycetes, and bacteria of these divisions are also abundant in the hypersaline mat systems of Guerrero Negro, Mexico (Ley *et al.*, 2006); Eilat, Israel (Sørensen *et al.*, 2005); and Salin-de-Giraud, France (Mouné *et al.*, 2003).

The abundance of the major phylogenetic divisions in the two mats was compared with the phylogenetic groups previously observed in bacterial 16S rDNA clone libraries from Hamelin Pool stromatolites of differing morphology (Burns *et al.*, 2004; Papineau *et al.*, 2005). Alphaproteobacteria were the only division with consistently high abundance across all Hamelin Pool samples. The bacterial population from the intertidal columnar stromatolite of Burns and coworkers was dominated by Alphaproteobacteria (30%) and Actinobacteria (21%) (Burns *et al.*, 2004), whereas HPDOM was dominated

by Planctomycetes (22%) and Alphaproteobacteria (18%), and HPIRR by Actinobacteria (32%) and Alphaproteobacteria (27%) (Papineau *et al.*, 2005). Bacteroidetes, Cyanobacteria, Deltaproteobacteria and Gammaproteobacteria were all more abundant in the smooth and pustular mats examined here than in the stromatolites studied by Papineau *et al.* (2005). Although they have similar surface morphology, the relative proportions of the major bacterial divisions in the Hamelin Pool sediments did not suggest any simple correlation between the smooth mat and the smooth-surfaced domed stromatolite HPDOM, or between the pustular mat and the irregular-surfaced stromatolite HPIRR. The overall mat populations were also distinct from the columnar stromatolite studied by Burns *et al.* (2004), for which no clones from the Cyanobacteria or Bacteroidetes were detected when universal bacterial PCR primers were employed. Several candidate bacterial divisions (as described by Hugenholz *et al.*, 1998) detected in the Hamelin Pool stromatolites were not found in the mats, namely OP1, OP5, OP9, OS-K, SBR1093 and WS3. Although the pustular and smooth mat bacterial clones all clustered with known bacterial divisions and no novel division-level diversity was observed, the majority of the clones had low sequence similarity to those in GenBank (Fig. 4). This suggests that these mats are a reservoir of untapped bacterial diversity at the family, genus and species level.

The high microbial diversity detected in the Hamelin Pool mats mirrors results from a hypersaline microbial mat from Guerrero Negro (Ley *et al.*, 2006) and the microbial community associated with a sulphide-rich spring (Tanner *et al.*, 2000). Although microbial mats can be considered ideal simple ecosystems to use as analogues of early life on Earth, data from this study and other recent studies indicates they can contain very diverse bacterial communities. This is attributed to the multitude of potential environmental niches present (Ley *et al.*, 2006), and the large diel fluctuations of key geochemical gradients such as pH, oxygen and sulphide, which may select for a highly diverse microbial assemblage capable of withstanding these extremes. Thus, the lower diversity observed in the stromatolite communities studied by Burns *et al.* (2004) and Papineau *et al.* (2005) may indicate a more stable environment with fewer environmental niches, or a more specialised population and thus less biodiversity.

Putative role of microorganisms within the Hamelin Pool microbial mats

Based on the phylogenetic divisons that were observed in this study, a diverse range of metabolisms are likely to be present in the pustular and smooth mats. As the majority of clones from the pustular and smooth mat have only low sequence identity with their closest cultured relative (Fig. 4), caution in attributing functional roles within the mat community to particular species or guilds is necessary. Nonetheless, the following metabolisms common to microbial mats (Des

Marais, 2003; Dupraz & Visscher, 2005) are proposed to be present in the Hamelin Pool mats on the basis of rDNA clone phylogeny: carbon cycling by oxygenic phototrophy (Cyanobacteria), anoxygenic phototrophy (Chloroflexi, some Cyanobacteria, Alphaproteobacteria such as *Roseobacter*, *Rhodobium* and others) and heterotrophy (many organisms); sulphate-reduction (Deltaproteobacteria such as *Desulfatibacillum* sp.) and sulphur-oxdiation (Gammaproteobacterium *Thialkalivibrio* sp. and others); and nitrogen-metabolism (some actinobacteria such as *Frankia* sp., Cyanobacteria such as *Microcoleus* sp., and others).

Interestingly, Deltaproteobacterial clones are twice as abundant in the smooth mat than in the pustular mat (Fig. 3). This greater abundance of potential sulphate-reducers in the smooth mat correlated with the presence of a visible black sulphidic layer in the smooth mat but not in the pustular mat (Fig. 1C,D), and with a previous report that sulphate-reduction could be detected in the smooth mat but not in the pustular mat (Bauld *et al.*, 1979). Conversely, the relative abundance of Cyanobacteria in the pustular mat universal bacterial clone library is twice that observed in the smooth mat. The uncoupling of sulphate reduction and carbon fixation is known to be a key factor in the precipitation of calcium carbonate in microbial communities (Visscher *et al.*, 1998; Dupraz & Visscher, 2005) – thus it appears that the relative abundance of these bacterial divisions may be significant for the type of mat morphology formed, and whether or not the mat will progress to become lithified (i.e. a stromatolite). The possible interaction of methanogenic archaea with sulphate reducers in the stromatolites is also of interest, and investigation of the carbon fixation, sulphate reduction and any methanogenesis rates in these sediments, along with direct enumeration of these organisms via fluorescence *in situ* hybridisation, are areas of ongoing research.

Although distinct from each other in morphology, lamination (Fig. 1), community function (Bauld *et al.*, 1979; Skyring, 1984) and the relative abundance of various bacterial and archaeal phyla, the high microbial diversity revealed in this study indicates the smooth and pustular mat communities are more similar to each other than the stromatolites they superficially resemble. The process of lithified (stromatolite) rather than nonlithified microbial mat formation is of great interest, not least because of the prominence of stromatolites on early Earth and the associated environmental and astrobiological implications (Des Marais, 2003; Schopf, 2006). Cyanobacteria dominate the biomass of Hamelin Pool microbial mats and stromatolites, and in Bahamian stromatolites cyanobacterial sediment trapping and microboring are known to be crucial processes for mat lithification (Macintyre *et al.*, 2000; Reid *et al.*, 2000). However, as yet, the role and significance of specific cyanobacterial genera in mat and stromatolite formation at Hamelin Pool is not well understood. As such, the detailed identification of the particular cyanobacterial genera present, along with identification of sulphate-reducing bacteria and

gammaproteobacterial populations whose physiology and metabolism may play a role in the differentiation of the nonlithified and lithifying community types in Hamelin Pool, provides a platform for further functional studies. The intriguing question of the role of the Thaumarchaeota and the Euryarchaeal species with no cultured relatives in the smooth mat remains to be addressed, whereas the eukaryal population is likely to contribute to top-down control of the microbiota through bactivorous grazing.

CONCLUDING REMARKS

In conclusion, data obtained from molecular and statistical analysis of the Hamelin Pool smooth and pustular mats indicated they shared many bacterial, archaeal and eukaryotic phyla even though they have strikingly different morphologies. Both mats also contained abundant bacterial and archaeal phylotypes with low similarity to known species, indicating the presence of novel and as-yet undescribed lineages in Hamelin Pool. To complement this finding, a total of 92 isolates were cultured from the mats and are now available for further biogeochemical characterisation. With this improved understanding of the smooth and pustular mat populations and their relationship to stromatolite microbial communities of Hamelin Pool, further investigation of the function and structure of these fascinating and evolutionarily significant communities can be undertaken.

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