RESEARCH ARTICLE

Analysis of the unique geothermal microbial ecosystem of the Blue Lagoon

Solveig K. Petursdottir,[1] Snaedis H. Bjornsdottir,[1,2] Gudmundur O. Hreggvidsson,[1,2] Sigridur Hjorleifsdottir[1] & Jakob K. Kristjansson[3]

[1]Prokaria-Matis Ltd, Reykjavik, Iceland; [2]Institute of Biology, University of Iceland, Reykjavik, Iceland; and [3]Arkea Ltd, Reykajvik, Iceland

Correspondence: Solveig K. Petursdottir, Prokaria-Matis Ltd, Gylfaflöt 5, IS-112, Reykjavik, Iceland. Tel.: +354 858 5123; fax: +354 422 5002; e-mail: solveigp@matis.is

Received 16 October 2008; revised 11 April 2009; accepted 27 July 2009.
Final version published online 26 August 2009.

DOI:10.1111/j.1574-6941.2009.00757.x

Editor: Riks Laanbroek

Keywords
ecosystem; geothermal; biodiversity; *Roseobacter* lineage.

Abstract

Cultivation and culture-independent techniques were used to describe the geothermal ecosystem of the Blue Lagoon in Iceland. The lagoon contains both seawater and freshwater of geothermal origin and is extremely high in silica content. Water samples were collected repeatedly in summer and autumn in 2003 and 2005 and in winter 2006 were analyzed for species composition. The study revealed the typical traits of an extreme ecosystem characterized by dominating species and other species represented in low numbers. A total of 35 taxa were identified. The calculated biodiversity index of the samples was 2.1–2.5. The majority (83%) of analyzed taxa were closely related to bacteria of marine and geothermal origin reflecting a marine character of the ecosystem and the origin of the Blue Lagoon hydrothermal fluid. A high ratio (63%) of analyzed taxa represented putative novel bacterial species. The majority (71%) of analyzed clones were *Alphaproteobacteria*, of which 80% belonged to the *Roseobacter* lineage within the family of *Rhodobacteraceae*. Of seven cultivated species, the two most abundant ones belonged to this lineage. *Silicibacter lacuscaerulensis* was confirmed as a dominating species in the Blue Lagoon. One group of isolates represented a recently identified species within the genus of *Nitratireductor* within *Rhizobiales*. This study implies an annually stable and seasonally dynamic ecosystem in the Blue Lagoon.

Introduction

The Blue Lagoon was gradually formed from the effluents of the Svartsengi geothermal power plant after it started operation in 1976 (Ragnarsdóttir *et al.*, 1984). It is located on the geothermally active Reykjanes peninsula in SW Iceland. The peninsula is a part of the Mid-Atlantic Ridge, which emerges from the sea in Iceland. As the peninsula is built up of very porous lava, it allows seawater to enter deep into its aquifers. The fluid in the geothermal aquifer is made of approximately two-thirds seawater and one-third freshwater. The chemical composition of the fluid is modified after interaction with the rocks at 240 °C, resulting in Mg concentration of 0.6 mg kg^{-1} and SiO_2 of 250 mg kg^{-1} compared with 1295 and 3 mg kg^{-1} in seawater, respectively (Bjarnason, 1988). The fluid is pumped directly from boreholes to the lagoon where most of the silica precipitates. This constant silica precipitation, formerly estimated at a rate of 10 mg L^{-1} h^{-1} in the Blue Lagoon (Pétursdóttir & Kristjánsson, 1996), may add an extreme factor to this environment, which is moderate in other aspects, i.e. in temperature (37 °C), pH (7.5) and salinity (2.7%).

A study of the microbial diversity in the Blue Lagoon fluid was first performed in 1995 and was based on traditional cultivating techniques (Pétursdóttir & Kristjánsson, 1996). The main results showed a viable bacterial count of 1.3×10^5 mL^{-1}, a low microbial diversity and a dominating species comprising 85% of the randomly picked isolates. A study of the dominating species revealed a new genospecies of *Alphaproteobacteria* that was named *Silicibacter lacuscaerulensis* (Pétursdóttir & Kristjánsson, 1997). Another species of the *Silicibacter* genus, *Silicibacter pomeroyi*, was described in 2003 (González *et al.*, 2003). The taxonomical status of this genus is within the *Roseobacter* lineage of the *Rhodobacteraceae*. Species of this lineage comprise up to 25% of marine microbial communities (Wagner-Döbler & Biebl, 2006).

The utilization of culture-independent approaches for describing species composition in various ecosystems has

FEMS MICROBIOLOGY ECOLOGY

increased enormously during the last decades and has changed the view on the level of prokaryotic diversity. By extensive 16S rRNA gene analysis of diverse marine and terrestrial ecosystems, the number of bacterial divisions has increased dramatically in recent years. The species composition of several microbial ecosystems in terrestrial and marine hot springs in Iceland has been investigated to a certain extent (Skirnisdottir *et al.*, 2000; Hjorleifsdottir *et al.*, 2001; Hobel *et al.*, 2005; Kvist *et al.*, 2007). Because the Blue Lagoon is a terrestrial geothermal lake containing fluid of marine origin, it was interesting to estimate whether the microbial community would be more of a terrestrial or a marine nature.

In this study, our aim was to obtain a deeper understanding of the Blue Lagoon microbial ecosystem by investigating the variation in its microbial populations. Several methods have been used to estimate the biodiversity of environmental samples. The application of the N_T/N_{max} ratio, where N_T is the total number of 16S rRNA gene clones in a clone library and N_{max} is the total number of clones within the most abundant taxon is quite simple and can be very informative (Curtis *et al.*, 2002). In 1970, the reciprocal of the N_T/N_{max} ratio was proposed as a diversity index in its own right (Berger & Parker, 1970). In this paper, we discuss the nature of the microbial ecosystem of the Blue Lagoon by investigating the origin of the taxa as well as the level of biodiversity. The uniqueness of the ecosystem is assessed by inspection of the novelty of the species found. We suggest the possible roles of the dominating species and discuss the stability of the ecosystem.

Materials and methods

Sampling

Water samples were taken at four different sites in the Blue Lagoon in July and October of 2003. Sampling was repeated in the same months of 2005 and also in February 2006. The samples (10 L) were collected from the top 30 cm surface fluid into sterile plastic containers and were processed on the same day.

Isolation and species determination of cultivated microbial strains

Microbial strains were isolated from the 2003 samples. Water samples were diluted to 10^{-3} and plated onto PCA medium (Atlas, 2004), containing 50% Blue Lagoon water. The plates were incubated at 37, 45, 60 or 65 °C for 3 days. A total of 50 colonies were picked at random from the plates and purified using the same conditions for growth.

DNA was isolated from the purified strains using Dynal magnetic beads as described by the manufacturer (Dynal™). The 16S rRNA gene was amplified by PCR using primers F9 (5′-GAGTTTGATCCTGGCTCAG-3′) and R1544 (5′-AGAAAGGAGGTGATCCA-3′) (Skirnisdottir *et al.*, 2000). The PCR products were sequenced using the BigDye terminator cycle sequencing kit (Applied Biosystems), R805 (5′-GACTACCCGGGTATCTAATCC-3′) sequencing primer internal of the 16S rRNA gene (Skirnisdottir *et al.*, 2000) and the 3730 DNA analyzer (Applied Biosystems). The sequences were processed, edited and classified using the SEQUENCHER 4.0.5 software. A BLAST search in GenBank was used to identify the species of the isolate or its closest relative.

Determination of species composition of uncultured samples

Silica particles in the water samples were allowed to settle overnight at 4 °C after which, the supernatant was centrifuged at 11.325 ***g*** for 15 min. No cells were harvested during a second centrifugation at 12.096 ***g*** for 20 min. The samples could not be filtered due to the remaining silica. The pellet was dissolved in 1 : 1 (v/v) of TE buffer (10 mmol Tris-HCl, pH 8.0) and DNA was extracted as described (Marteinsson *et al.*, 2001). The 16S rRNA gene was amplified using primers F9 and R1544 (Skirnisdottir *et al.*, 2000). The PCR products were purified using GFX™ PCR DNA and Gel Band Purification kit (Amersham Biosciences) as described by the manufacturer and cloned into the TOPO TA® system (Invitrogen). The inserts were sequenced using primer R805 and edited as described above. Operational taxonomy units (OTUs) were established using a 98% sequence similarity level as a cutoff. The 16S rRNA gene sequences determined in this work are available in GenBank under the accession numbers FJ905577–FJ905611.

Environmental origin, novelty and stability

An estimate of environmental origin of the species in the Blue Lagoon, i.e. marine or terrestrial was acquired by examining the origin of the closest relative of each OTU identified by a BLAST search in GenBank. An estimate of the novelty of each OTU was obtained by an inspection of the similarity percentage of the closest relative in GenBank and the ratio of OTUs identified to the generally accepted species level ($\geq$98%) and less than the species level. Stability of the ecosystem was inferred from a comparison of taxa ratios in samples taken in the same season in different years.

Biodiversity and taxonomic studies

The reciprocal of N_T/N_{max} was used as an estimate for biodiversity (N_T, total number of analyzed clones; N_{max}, number of clones within the most abundant OTU) (Curtis *et al.*, 2002).

Representative 16S rRNA gene sequences of OTUs belonging to *Alphaproteobacteria* obtained from uncultured and cultured samples and of reference sequences from GenBank were aligned using the BIOEDIT and CLUSTALW software. A dendrogram based on the neighbor-joining method was calculated in CLUSTALX and edited in NJPLOT.

Results

Species of cultivated bacteria

Growth occurred on plates inoculated with samples from the Blue Lagoon, which were incubated at 37 or 45 °C. No growth was observed at 60 or 65 °C. A total of 48 bacterial strains were isolated and purified. Species identification of the isolates by partial sequencing of the 16S rRNA gene and classification of the sequences at the 98% similarity level resulted in seven species, four of which belonged to the *Alphaproteobacteria* as shown in Table 1. The results agree well with previous results because the described dominating species of the Blue Lagoon *S. lacuscaerulensis* (Pétursdóttir & Kristjánsson, 1996, 1997), was found in high ratios in this study and was represented by 17 (35%) of the isolates. Another species cultured at 45 °C was represented by 20 (42%) of the isolates and was also abundant among the uncultured bacteria. These showed 98% rDNA sequence similarity to the closest GenBank relative, 'Bacterium K2-19' (AY345433), found at the Hawaiian archipelago. Six of the isolates belonged to the recently identified species within the genus of *Nitratireductor* within the *Rhizobiales* of the *Alphaproteobacteria*. The remaining four isolates belonged to *Stappia* sp. of the *Alphaproteobacteria*, *Pseudomonas* sp. and *Marinobacter* sp. of the *Gammaproteobacteria* and a species of *Actinobacteria*.

Species composition of uncultured bacteria

A total of 481 clones were analyzed from the Blue Lagoon samples collected in 2003–2006. Of these, 109 were from summer 2003, 152 from autumn 2003, 50 from summer 2005, 96 from autumn 2005 and 74 from winter 2006. An overview of the identified OTUs within each sample is presented in Table 2. The table reveals a strikingly high ratio of *Alphaproteobacteria* or 30 of 35 identified taxa. Other OTUs belong to the γ-subgroup of *Proteobacteria* and other phyla. A closer inspection reveals the abundance of two species. One of them (BL-2) is closely related (99% similarity) to the type strain of *S. lacuscaerulensis*, which was previously shown to dominate in the Blue Lagoon (Pétursdóttir & Kristjánsson, 1997). The other (BL-1) is the photoautotrophic species *Cyanobacterium aponinum* belonging to the *Cyanobacterium* genus within the *Chroococcales*. This organism is dominating in the summer samples, while *S. lacuscaerulensis* dominates in the autumn samples. Another species (BL-11) of the *Rhodobacterales*, is in high proportion in the autumn sample from 2005 and in the winter sample from 2006. This species shows 98% similarity with 'Bacterium K2-19'. This species was also represented in high numbers of cultivated isolates from the lagoon.

Environmental origin and novelty of species in the Blue Lagoon

The environmental origin of the taxa found in the Blue Lagoon was inferred from the recorded habitat of the closest relative (Table 2). From the total of 35 analyzed taxa, 29 (83%) had a closest relative of marine origin, three of which were from hydrothermal vents or marine hot springs. A similar ratio applies for the isolates. This indicates an overall marine origin of the ecosystem in the Blue Lagoon.

Inspection of similarity percentages of the 35 taxa identified in the samples to the closest relatives found in GenBank, revealed that only 13 taxa (37%) were identified to the species level ($\geq$98% similarity). The other 63% showed $<$ 98% similarity to their closest relatives indicating that they represented new species or new genera. The majority (91%) of taxa was identified at or above the 94% similarity level.

Table 1. Identification of bacterial strains isolated from Blue Lagoon water samples

GenBank ID*	Closest relative	Sim (%)†	Phylum/Class	No. of Isolates
U77644	*Silicibacter lacuscaerulensis*	99	*Alphaproteobacteria*	17
AY345433	Bacterium K2-19	98	*Alphaproteobacteria*	20
EU564843	*Nitratireductor* sp.	100	*Alphaproteobacteria*	6
AB111992	Uncultured bacterium clone	98	*Actinobacteria*	1
AF467304	Uncultured *Pseudomonas* sp.	99	*Gammaproteobacteria*	1
AY307927	*Stappia* sp. M8	99	*Alphaproteobacteria*	1
MSU85863	*Marinobacter* sp. ICO 22	98	*Gammaproteobacteria*	2
Total				48

*GenBank ID of the closest relative.

†Sim, similarity % to closest GenBank relative.

Table 2. Identified OTUs in five samples from the Blue Lagoon

OTU no.	Closest GenBank match*	Sim (%)†	No. of clones	Phylum/class	In no. of samples‡	Habitat§
BL1	*Cyanobacterium aponinum* (AM238427)	99	105	*Cyanobacteria*	4/5	Thermal spring
BL2	*Silicibacter lacuscaerulensis* (U77644)	99	159	*Alphaproteobacteria*	5/5	Blue lagoon
BL3	Uncultured organism clone (DQ396098)	97	24	*Alphaproteobacteria*	3/5	Marine
BL4	Alphaproteobacterium (AB302371)	97	14	*Alphaproteobacteria*	2/5	Marine
BL5	Uncultured alphaproteobacterium (DQ200564)	98	16	*Alphaproteobacteria*	2/5	Marine
BL6	Uncultured *Erythrobacteraceae* (FJ516850)	99	3	*Alphaproteobacteria*	2/5	Wetland
BL7	*Thiobacillus hydrothermalis* (M90662)	99	17	*Gammaproteobacteria*	3/5	Marine
BL8	*Albidodovulum inexpectatum* (AF465833)	97	9	*Alphaproteobacteria*	3/5	Marine hot spring
BL9	*Rhodovulum* sp. CP-10 (AB079682)	94	3	*Alphaproteobacteria*	2/5	Marine
BL10	Alphaproteobacterium B27 (AB302385)	95	8	*Alphaproteobacteria*	2/5	Marine
BL11	Uncultured marine bacterium (FJ594814)	96	47	*Alphaproteobacteria*	3/5	Marine
BL12	Alphaproteobacterium ML6 (AJ315682)	97	19	*Alphaproteobacteria*	2/5	Meromictic lake
BL13	Uncultured marine bacterium (FJ594800)	92	18	*Alphaproteobacteria*	1/5	Marine
BL14	Uncultured bacterium clone (EF582536)	99	2	*Firmicutes*	1/5	Salt marsh sediment
BL15	Uncultured bacterium clone (FJ656497)	99	11	*Gammaproteobacteria*	1/5	Marine
BL17	*Rhodobacteraceae* bacterium (EU070404)	96	2	*Alphaproteobacteria*	1/5	Marine
BL18	*Rhodobacteraceae* bacterium (AM990693)	93	2	*Alphaproteobacteria*	1/5	Marine
BL19	Uncultured organism clone (DQ396053)	97	1	*Alphaproteobacteria*	1/5	Marine
BL20	*Rhodobacteraceae* bacterium (EF587965)	96	1	*Alphaproteobacteria*	1/5	Marine
BL21	*Pseudoruegeria* sp. (EU564840)	96	1	*Alphaproteobacteria*	1/5	Marine
BL22	Uncultured alphaproteobacterium (FM242450)	99	2	*Alphaproteobacteria*	1/5	Marine
BL23	Uncultured alphaproteobacterium (FM253684)	95	1	*Alphaproteobacteria*	1/5	Rock
BL24	*Rhodobacteraceae* bacterium (FJ265707)	96	1	*Alphaproteobacteria*	1/5	Marine
BL25	Iodide-oxidizing bacterium (AB159209)	96	1	*Alphaproteobacteria*	1/5	Marine
BL26	Alphaproteobacterium (AY162090)	97	1	*Alphaproteobacteria*	1/5	Marine
BL27	Uncultured *Caulobacterales* (EU361354)	97	1	*Alphaproteobacteria*	1/5	Marine
BL28	Uncultured bacterium clone (EU799426)	95	1	*Alphaproteobacteria*	1/5	Marine
BL29	Uncultured alphaproteobacterium (FM242321)	98	1	*Alphaproteobacteria*	1/5	Marine
BL30	Uncultured bacterium clone (FJ792406)	98	1	*Alphaproteobacteria*	1/5	Hydrothermal field
BL31	Uncultured alphaproteobacterium (EU780271)	94	1	*Alphaproteobacteria*	1/5	Marine
BL32	*Rhodobacteraceae* bacterium (EU070404)	91	1	*Alphaproteobacteria*	1/5	Marine
BL33	*Thermus thermophilus* (DQ974208)	100	1	Deinoc.-Thermus	1/5	Marine hot spring
BL34	Uncultured bacterium (EU488289)	98	3	*Alphaproteobacteria*	1/5	Marine
BL35	Uncultured marine bacterium (FJ594814)	95	2	*Alphaproteobacteria*	1/5	Marine
BL36	Uncultured bacterium clone (EU735674)	96	1	*Alphaproteobacteria*	1/5	Oil field pristine soil

*The GenBank accession numbers of closest relatives are given in parentheses after the closest GenBank match.
†Sim, similarity % to the closest GenBank relative.
‡The values show in how many samples the taxon was found in the total of five samples.
§Habitat from which the closest relative in GenBank was isolated.

Stability of the ecosystem

A summary of the taxa distribution from Table 2 is presented in Table 3. The dominating species, *C. aponinum* and *S. lacuscaerulensis* are shown individually. Several clones belong to *Gammaproteobacteria* and very few to other phyla. The ratios of the two dominating bacteria appear quite similar in samples collected in the same season in 2003 and 2005. The combined ratio of *Alphaproteobacteria* is 81% in both autumn samples. In the winter samples, the ratio of *Alphaproteobacteria* reaches 95% of analyzed clones and the *Cyanobacterium* species has disappeared. The ecosystem of the Blue Lagoon, therefore, appears to be dynamic between seasons while showing stable annual patterns.

Diversity

Rarefaction curves showing the sample coverage are shown in Fig. 1. The curves representing the summer samples of 2003 and 2005 reach a plateau indicating a sufficient number of analyzed clones. The curves representing the autumn samples of 2003 and 2005 approach, but do not reach the plateau. The seasonal curves from the two different years are almost identical. Table 4 shows the estimated biodiversity within the samples based on the biodiversity index (N_T/N_{max}) as described by Curtis *et al.* (2002). The values of N_T/N_{max} are in the range of 2.1–2.5. For comparison, similar data from a hot spring sample (Yi & Chun, 2006), a sample from Grimsey marine vent field and from a

pond sediment sample (S. Petursdottir, unpublished data) are included (Table 4).

Taxonomy of *Alphaproteobacteria* in the Blue Lagoon

Of the total of 481 clones analyzed in this study, 341 or 71% belong to *Alphaproteobacteria* (Table 2). The most abundant taxon in the Blue Lagoon is the *Roseobacter* lineage within the family of *Rhodobacteraceae*. Around 60% or 290 clone sequences belong to this lineage or 18 out of 35 identified OTUs. Of 48 isolated bacterial strains, 44 or 92% belong to *Alphaproteobacteria*, of which 37 (77%) belong to two species within the *Roseobacter* lineage of *Rhodobacteraceae*, i.e. to *S. lacuscaerulensis* and 'Bacterium K2-19'. Figure 2 shows a dendrogram of partial (593 bp) 16S rRNA gene sequences from OTUs of the majority of *Alphaproteobacteria* from the Blue Lagoon and reference sequences from GenBank. The identified sequences belong to three orders within the *Alphaproteobacteria*, i.e. *Sphingomonadales*, *Rhodobacterales* and *Rhizobiales*.

Table 3. Taxa distribution of analyzed clones from the Blue Lagoon

	Summer		Autumn		Winter	
	No.	%	No.	%	No.	%
Taxa 2003						
Cyanobacterium aponinum	53	48.6	19	12.5	ND	
Silicibacter lacuscaerulensis	9	8.3	71	46.7	ND	
Other *Alphaproteobacteria*	31	28.4	52	34.2	ND	
Gammaproteobacteria	11	10.1	9	5.9	ND	
Other phyla	5	4.6	1	0.7	ND	
Total	109	100	152	100		
Taxa 2005						
Cyanobacterium aponinum	20	40	13	13.5	0	0
Silicibacter lacuscaerulensis	7	14	46	47.9	26	35.1
Other *Alphaproteobacteria*	23	46	32	33.3	44	59.5
Gammaproteobacteria	0	0	4	4.2	4	5.4
Other phyla	0	0	1	1.1	0	0
Total	50	100	96	100	74	100

The numbers and ratios of clones within taxa are shown.
ND, not determined.

Discussion

The results of this study show that the Blue Lagoon geothermal lake is a dynamic ecosystem, which is mainly characterized by two organisms: firstly, the photoautotrophic *Cyanobacterium*, which represents the primary producers, using light as the energy source, gradually dominating the ecosystem as the brightness increases during the summer and creating organic matter for the consumers, and secondly, *S. lacuscaerulensis* (Pétursdóttir & Kristjánsson, 1997), the heterotrophic alphaproteobacterium, which is dominant during autumn and winter and represents the main heterotroph of the ecosystem. These two species, one

Table 4. Biodiversity parameters for samples taken at different seasons in the Blue Lagoon

Sample	N_T	S	n1	N_{max}	N_T/N_{max}
Summer 03	109	7	0	53	2.1
Autumn 03	152	20	8	71	2.1
Summer 05	50	7	2	20	2.5
Autumn 05	96	14	9	46	2.1
Winter 06	74	10	4	31	2.4
Hot Spring	68	5	2	59	1.15
Marine Vent Field	219	51	26	44	4.97
Pond sediment	150	81	66	13	9.85

N_T, the total number of analyzed clones; S, the number of species or taxonomic units in the sample; n1, the number of species containing only one sequence; N_{max}, the number of sequences within the most abundant species; N_T/N_{max}, the index of biodiversity (Curtis *et al*., 2002). The three bottom lines show data from other habitats for comparison.

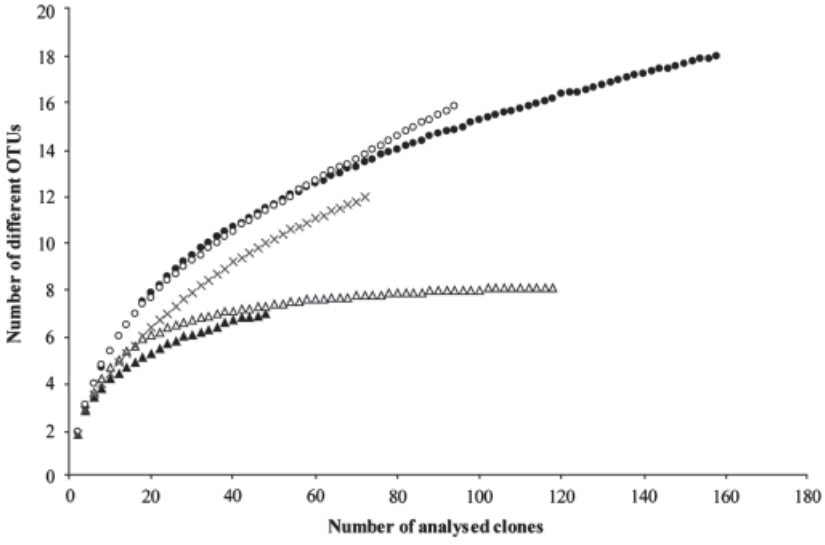

Fig. 1. Rarefaction curves for results obtained from the community analysis of the Blue Lagoon using culture-independent techniques. Symbols: △, summer 2003; ▲, summer 2005; •, autumn 2003; ○, autumn 2005; ×, winter 2006.

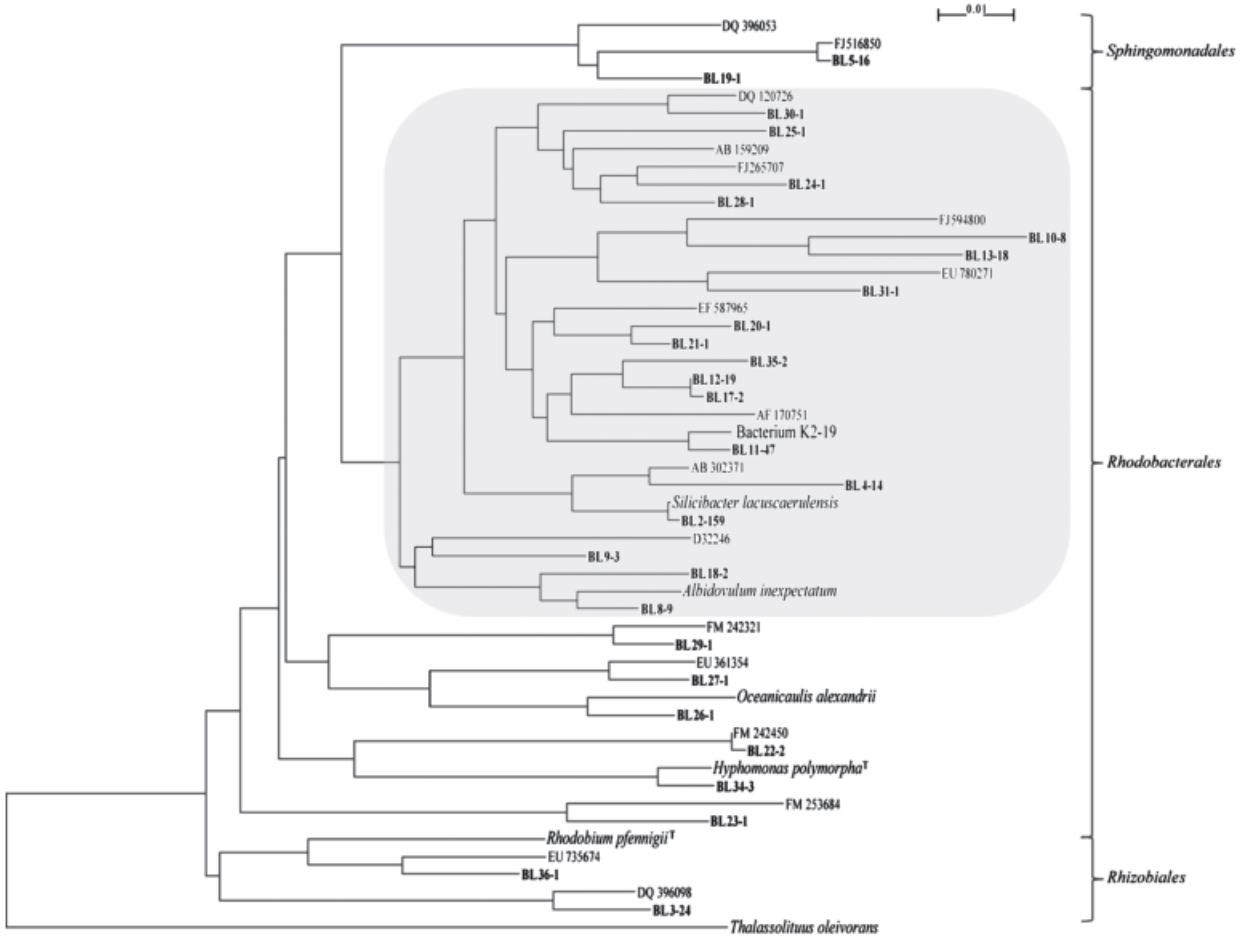

Fig. 2. A dendrogram of *Alphaproteobacteria* based on alignments of the majority of the partial 16S rRNA gene sequences (593 bp) of OTUs from the Blue Lagoon and reference sequences from GenBank. The sequences belong to three orders within the *Alphaproteobacteria*, i.e. *Sphingomonadales*, *Rhodobacterales* and *Rhizobiales*. The majority of the identified *Alphaproteobacteria* clones were located within the *Roseobacter* lineage of the *Rhodobacteraceae* (shaded). The gammaproteobacterium *Thalassolituus oleivorans* is used as an outgroup. The number of clones within each OTU is indicated by the last number of the OTU. Reference sequences from GenBank used in the dendrogram: uncultured organism clone (DQ396053); uncultured *Erythrobacteraceae* bacterium (FJ516850); *Roseobacter* sp. 812 (DQ120726); iodide-oxidizing bacterium (AB159209); *Rhodobacteraceae* bacterium ISL-46 (FJ265707); uncultured marine bacterium clone (FJ594800); uncultured alphaproteobacterium clone (EU780271); *Rhodobacteraceae* bacterium UST061013-021 (EF587965); *Roseobacter* sp. QSSC9-8 (AF170751); Bacterium K2-19 (AY345433); alphaproteobacterium (AB302371); *Silicibacter lacuscaerulensis* (U77644); *Rhodovulum* sp. (D32246); *Albidovulum inexpectatum* (AF465833); uncultured alphaproteobacterium clone (FM242321); uncultured *Caulobacterales* (EU361354); *Oceanicaulis alexandrii* (AJ309862); unculturedalphaproteobacterium clone (FM242450); *Hyphomonas polymorpha*^{T} (AJ227813); uncultured alphaproteobacterium clone (FM253684); *Rhodobium pfennigii*^{T} (AJ510235); uncultured bacterium clone (EU735674); uncultured organism clone (DQ396098); *Thalassolituus oleivorans* (AM279755).

photoautotrophic and the other heterotrophic, show regular seasonal dominance shifts in the Blue Lagoon water. Because other environmental conditions do not change at regular time intervals these shifts are clearly dependent on the amount of light available and thus, seasonal. The seasonal shifts contribute also to the stability of the ecosystem, resulting in its regeneration every year. Fluctuations in environmental factors create a community in a dynamic equilibrium. The almost unchanged ratio of species/taxa identity of analyzed clones in samples taken at an interval of 2 years in this study supports this stability and equilibrium.

The 16S rRNA gene analysis of water samples from the Blue Lagoon revealed another characteristic of this ecosystem, i.e. the extremely high ratio of species belonging to the *Roseobacter* lineage of the *Rhodobacteraceae* family, because 18 or almost 50% of the 35 identified OTUs belonged to this lineage. The lineage is a phylogenetically coherent, but a physiologically heterogenous group comprising up to 25% of marine microbial communities especially in coastal and polar oceans (Wagner-Döbler & Biebl, 2006). Species within the *Roseobacter* lineage are aerobic, but physiologically quite diverse, some containing bchl a and thus capable of

phototrophic living by anoxygenic photosynthesis (Allgaier *et al*., 2003), others capable of lithoheterotrophy using inorganic compounds such as CO and H_2S (Moran *et al*., 2004), and still others capable of reducing nitrogen compounds (Yi & Chun, 2006). A study by González *et al*. (2003) showed that some species of this lineage, among others, *S. lacuscaerulensis*, are able to degrade dimethylsulfoniopropionate (DMSP) produced by marine algae as well as other sulfur compounds related to DMSP that are cycled in marine environments. The majority, or 60% of the analyzed clones in this study belonged to this lineage. Therefore, conditions in the Blue Lagoon seem to create a suitable habitat for a number of species of the *Roseobacter* lineage. The last group of species found in the samples belonged to the *Gammaproteobacteria*. This group is in a ratio of up to 10% of analyzed samples, the main species being *Thiobacillus hydrothermalis*, for which the type species is a mesophilic obligately chemolithotrophic bacterium capable of thiosulfate oxidation (Durand *et al*., 1993). No human-borne bacteria were found in the samples from the Blue Lagoon, in spite of a high number of bathing guests every year. This is in agreement with results from regular sampling by the Icelandic Health Authorities. The traditional explanations of the absence of contaminants in the lagoon are a short retention time of the geothermal water as well as the salinity and the continuous silica precipitation. Other unknown factors may add to the inhibition of bacterial growth.

This study indicates that the microbial communities of the Blue Lagoon ecosystem is composed primarily of members of marine character. An examination of the 16S rRNA gene sequences of the closest relatives in GenBank revealed that the majority of taxa are most probably of marine origin and some of these are of hydrothermal origin. The Blue Lagoon shows the typical traits of an extreme ecosystem characterized by dominating species and several other species represented in low numbers. Relatively few phyla were represented in the samples, indicating a low diversity in the lagoon water. The calculated biodiversity (N_T/N_{max}) of the samples was in the range of 2.1–2.5, which is typical of samples from extreme environments. Similar results from diverse marine and terrestrial hot springs in Iceland showed N_T/N_{max} ratios of 1.1–3.0 (Petursdottir *et al*., 2006).

The current study reaffirms the status of the Blue Lagoon as an unusual ecosystem. Its moderate, mesophilic characteristics of 37 °C, pH 7.5 would be expected to result in a high microbial diversity. However, this study of both cultivated and noncultivated microorganisms from the Blue Lagoon water shows relatively low diversity with only a few dominant players exhibiting seasonal fluctuations. The explanation for the low biodiversity is probably the salinity of 2.5%, the unusual source and chemical composition of the dilute geothermal sea water and the high silica content underlining the extreme characteristics of this unique environment.

Acknowledgements

We thank the Blue Lagoon Geothermal Spa staff, especially Dr Med Grimur Sæmundssen, and the Blue Lagoon R&D scientists Asa Brynjolfsdottir and Sigurbjorn Einarsson for supporting this work and for their interest, collaboration and valuable inputs. We also thank Steinunn Magnusdottir and Solveig Olafsdottir for their contribution to this project.

References

Allgaier M, Uphoff H, Felske A & Wagner-Döbler I (2003) Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microb* **69**: 5051–5059.

Atlas RM (2004) *Handbook of Microbiological Media*. CRC Press, London, p. 1390.

Berger W & Parker FL (1970) Diversity of planktonic *Foraminifera* in deep sea sediments. *Science* **168**: 1345–1347.

Bjarnason JO (1988) Svartsengi. Chemical monitoring 1980–1987. National Energy Authority of Iceland Report OS-88001/JHD-01:98 (in Icelandic with English summary).

Curtis TP, Sloan WT & Scannell JW (2002) Estimating prokaryotic diversity and its limits. *P Natl Acad Sci USA* **99**: 10494–10499.

Durand P, Reysenbach AL, Prieur D & Pace N (1993) Isolation and characterization of *Thiobacillus hydrothermalis* sp. nov., a mesophilic obligately chemolithotrophic bacterium isolated from a deep-sea hydrothermal vent in Fiji basin. *Arch Microbiol* **159**: 39–44.

González JM, Covert JS, Whitman WB *et al*. (2003) *Silicibacer pomeroyi* sp. nov. and *Roseovarius nubinhibens* sp. nov., dimethylsulfoniopropionate demethylating bacteria from marine environments. *Int J Syst Evol Micr* **523**: 1261–1269.

Hjorleifsdottir S, Skirnisdottir S, Hreggvidsson GO, Holst O & Kristjansson JK (2001) Species composition of cultivated and noncultivated bacteria from short filaments in an Icelandic hot spring at 88 °C. *Microb Ecol* **42**: 117–125.

Hobel CFV, Marteinsson V, Hreggvidsson GO & Kristjánsson JK (2005) Investigation of the microbial ecology of intertidal hot springs by using diversity analysis of 16S rRNA and chitinase genes. *Appl Environ Microb* **71**: 2771–2776.

Kvist T, Ahring BK & Westermann P (2007) Archaeal diversity in Icelandic hot springs. *FEMS Microbiol Ecol* **59**: 71–80.

Marteinsson VT, Kristjansson JK, Kristmannsdottir H, Dahlkvist M, Sæmundsson K, Hannington M, Petursdottir SK, Geptner A & Stoffers P (2001) Discovery and description of giant submarine smectite cones on the seafloor in Eyjafjordur, Northern Iceland, and a novel thermal microbial habitat. *Appl Environ Microb* **67**: 827–833.

Moran MA, Buchan A, Gonzalez JM *et al.* (2004) Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. *Nature* **432**: 910–913.

Pétursdóttir SK & Kristjánsson JK (1996) The relationship between physical and chemical conditions and low microbial diversity in the Blue Lagoon geothermal lake in Iceland. *FEMS Microbiol Ecol* **19**: 39–45.

Pétursdóttir SK & Kristjánsson JK (1997) *Silicibacter lacuscaerulensis* gen. nov., sp. nov., a mesophilic moderately halophilic bacterium characteristic of the Blue Lagoon geothermal lake in Iceland. *Extremophiles* **1**: 94–99.

Petursdottir SK, Thordarson T, Magnusdottir S & Hreggvidsson GO (2006) Environmental assessment of the effect of geothermal powerplants on microbial life in hot springs in Olkelduhals and Hverahlid. A report for the Power Distribution Center in Reykjavik. Report in Icelandic.

Ragnarsdóttir KV, Walther JW & Arnorsson S (1984) Description and interpretation of the composition of fluid and alteration minerology in the geothermal system, at Svartsengi, Iceland. *Geochim Cosmochim Ac* **48**: 1535–1553.

Skirnisdottir S, Hreggvidsson GO, Hjörleifsdottir S, Marteinsson VT, Petursdottir SK, Holst O & Kristjansson JK (2000) Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats. *Appl Environ Microb* **66**: 2835–2841.

Wagner-Döbler I & Biebl H (2006) Environmental biology of the marine *Roseobacter* lineage. *Annu Rev Microbiol* **60**: 255–280.

Yi H & Chun J (2006) *Thalassobius aestuarii* sp. nov., isolated from tidal flat sediment. *J Microbiol* **44**: 171–176.