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FEMS Microbiology Ecology 19 (1996) 39–45

FEMS MICROBIOLOGY ECOLOGY

The relationship between physical and chemical conditions and low microbial diversity in the Blue Lagoon geothermal lake in Iceland

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Received 28 July 1995; revised 2 October 1995; accepted 6 October 1995

Abstract

The Blue Lagoon in Iceland is a shallow geothermal lake with average temperatures of 37°C, pH 7.5 and about 2.5% salinity. It was formed in 1976 from the effluents of the Svartsengi geothermal power plant and is saturated with silica which constantly precipitates in the lake. It has been colonized by a few types of specialized microorganisms which seem to proliferate in this unusual ecosystem. The average bacterial colony count in the lake was 1.3×10^5 ml^{-1} on plate count agar made with 50% Blue Lagoon fluid but 2.6×10^6 ml^{-1} when determined with the MPN method. A total of 99 isolates were purified and characterized by 54 phenotypic tests and then grouped using Numerical Taxonomy. At similarity values of 80%, one major cluster was formed containing 85% of the isolates. Four representative strains from this cluster were further characterized and all shown to be Gram-negative, obligately aerobic, non-motile rods. They were oxidase positive, catalase negative and grew optimally at 45°C and in 3.5% NaCl with doubling time of about 80 min.

Keywords: Microbial diversity; Blue lagoon; Saline geothermal lake; Iceland; Numerical taxonomy

1. Introduction

The Blue Lagoon was gradually formed from the effluents of the Svartsengi geothermal power plant after it started operation in 1976 [1]. It is located on the highly geothermally active Reykjanes peninsula in south-west Iceland. The Reykjanes peninsula is on the Mid-Atlantic Ridge and built up of very porous lava, thus allowing seawater to enter deep into its aquifers. The fluid in the Svartsengi geothermal aquifer is therefore made out of approximately two thirds seawater and one third freshwater. The chemical composition is modified after interaction with the rocks at 240°C, such that Mg is only 1/1000 and silica is 50 times higher than it would be in seawater at ambient temperatures (about 430 ppm) [2,3]. After the fluid has gone through steam separators and heat exchangers it leaves the plant at about 70°C and enters the lagoon where it cools further and most of the silica precipitates. The lagoon is about 100 m wide and 200 m long with a depth of 1–3 m. The

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SSDI 0168-6496(95)00077-1

effluent flow is about 900 $m^3 \times h^{-1}$, giving an average retention time in the lagoon of about 40 h.

Soon after the formation of the lagoon, the silica precipitate had covered the rough lava bottom with a soft white mud. Since most of the lagoon also had comfortable bathing temperatures it quickly became a popular bathing place. It became especially popular among people with the skin decease psoriasis, who claimed that it had considerable healing effects. In a recent study by Olafsson et al. [4] it has in fact been medically confirmed that regular bathing in the Blue Lagoon results in considerable improvements for these patients. The Blue Lagoon has now been turned into a commercial operation with over 100000 bathers visiting every year. This gives rise to an enormous load of various human-carried bacteria. It was therefore of considerable interest to characterize physical and chemical conditions and the microflora of the lagoon, but no such study of this type of ecosystem has been done before.

Although geothermal brines are used in several places for energy production, they are usually of much higher temperature and/or salinity and often contain high concentrations of toxic compounds like heavy metals [5,6]. In those cases where cooling ponds are formed they are usually saturated with salt and are therefore totally different from the Blue Lagoon [7,8]. In terms of salinity the lagoon can best be compared to brackish ponds. Although many hot springs are saturated with silica, they are much lower in salt and the amount of precipitate formed is much less than in the Blue Lagoon. It therefore appears that this temperature and salinity range, the short retention time, and especially the high silica precipitation, form a unique combination which seems to be found nowhere else.

It would appear that the Blue Lagoon should be considered an extreme environment for most living organisms. It is also well known that both saltwater and sunlight is very damaging to enteric bacteria [9]. The silica particles may also have similar reflective effects as salt crystals in brines, by increasing the exposure of bacteria to both UV and visible light [2]. The precipitating silica is, however, an unusual and probably very restrictive environmental factor which makes the conditions unfavourable for most living organisms.

The work reported here was carried out to determine the physical and chemical characteristics of the Blue Lagoon and to investigate the diversity and density of its microflora.

2. Materials and methods

2.1. Study site and sampling

Four sampling stations were selected on each side of the lagoon (Fig. 1). Samples were taken about 1 m from the shore at depths of about 20 cm. Samples were collected in 500-ml sterile bottles, put in a cooler and brought directly to the laboratory and processed within 3 h. Temperature was measured every time at each sampling station. Conductivity and pH were measured at room temperature.

The main study was undertaken in the spring of 1992 when a total of 12 samples was collected at approximately weekly intervals. The first sample was taken on February 27 and the last one on May 13. Four more samples were then collected on July 8 and 27, and on September 9 and 28, the same year. Some data were also available from one previous sampling taken on March 8, 1988.

Samples taken March 22 and June 15 were analysed for concentrations of phosphate and nitrate.

2.2. Enumeration of bacteria

The samples were serially diluted by using autoclaved Blue Lagoon water (BL) as dilution medium and plated onto plate count agar standard (Oxoid, 32.5 g/l) (PCA) made with different proportions of

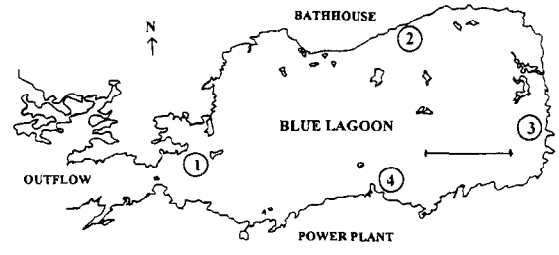

Fig. 1. Overview of the Blue Lagoon and the sampling stations. Station 1: close to the outflow from the lagoon. Station 2: close to the bathhouse. Station 3: the east side with least human activity. Station 4: close to the inflow from the geothermal power plant. Bar: 50 m.

BL (0, 25, 50 and 100%). The plates were incubated at 37°C for 3 days and the colonies counted. The viable count was determined this way in samples taken on March 3, April 10, May 8 and 13, July 27, Septembr 9 and 27. In the sample from March 1988 the viable count had only been determined on PCA with 100% BL. In the samples taken on May 13 the bacteria were also enumerated by using the Most Probable Number (MPN) method and incubating the dilutions in liquid PCA medium with 100% BL.

2.3. Isolation and numerical taxonomy

A total of 99 colonies were picked at random from the plates with 50% BL and purified for phenotypic characterization. For testing growth on single carbon sources a minimal medium with the following composition per l of 50% BL was used: 100 ml mineral base (in g/l): nitrilotriacetic acid, 1.3; $CaSO_4 \cdot 2H_2O$, 0.4; $MgCl_2 \cdot 6H_2O$, 2; 5 ml trace element solution [10], 5 ml iron citrate solution (in g/l, Na_3citrate $\cdot 2H_2O$, 2.94; $FeCl_3 \cdot 6H_2O$, 2.7); 4 ml Vitamin B solution (in mg/l: thiamin-HCL, 200; nicotinamide 200; riboflavin, 200; biotin, 100; pyridoxin-HCL, 200; Ca-panthothenate, 200; cyanocobalamin, 2; p-aminobenzoic acid, 0.5 and folic acid, 0.5) (separately autoclaved); 10 ml phosphate buffer (g/l, KH_2PO_4, 5.44; $Na_2HPO_4 \cdot 2H_2O$, 21.4; pH 7.2) and 4 ml of 13.3% NH_4Cl (autoclaved separately). The final concentration of the carbon sources (autoclaved separately) were 0.4% for carbohydrates and DL-alanine (all other amino acids were pure L-configuration) but 0.2% for all other substrates. Plates were made with 28 g/l of agar (Oxoid no. 1). Growth was tested on the following 34 carbon sources: galactose, histidine, isoleucine, citrate, glucose, acetate, xylose, inositol, arginine, alanine, arabinose, maltose, hydroxy-proline, tartrate, mannitol, lactose, sorbitol, casein, trehalose, sucrose, valine, phenylalanine, glycine, fructose, starch, lysine, lactate, asparagine, threonine, formate, pyruvate, raffinose, ramnose and adonitol. Tests for resistance against 6 antibiotics (Oxoid 6 mm disks: ampicillin 10 μg; bacitracin 10 units; penicillin-G 10 units; streptomycin 10 μg; tetracycline 30 μg; vancomycin 30 μg) and for effects of growth temperature (4, 22, 30, 40, 45 and 50°C) were done on PCA with 50% BL. Salt tolerance of the isolates was tested on PCA containing increasing concentration of NaCl (0, 0.5, 1, 2.5, 4, 6, 8 and 10%).

A total of 54 characters, scored + or −, were analysed by numerical taxonomy by using Simple Matching (Ssm) and Jackard (Sj) coefficients [11]. Clustering was accomplished by unweighted average linkage analysis (UPGMA) using the Numerical Taxonomy computer program developed by Rohlf [12]. The functional evenness index was calculated as an estimate of biological diversity [13].

2.4. Characterization of representative strains

Clusters were formed at 80% similarity level and a total of 5 representative strains were selected from the clusters for further characterization and identification. The additional tests carried out on those strains were as follows: Gram-staining; spore-staining; lipid-granule staining; motility; and optimum salinity, optimum temperature, and anaerobic growth on nitrate, oxidase and catalase.

Tests for optimum salinity was done by measuring the growth rate in liquid PCA medium on microplates with NaCl content of 0, 1, 2, 3, 4, 6, 8 and 10%. An inoculum of 0.5% from a fresh liquid culture was used adding up to a total of 300 μl of medium per well (flat bottom). Absorbances were measured every 2 h for 24 hours. Tests for optimum temperature were done in shake-flasks in 50% BL-PCA medium by incubating at 35, 37, 40, 45 and 50°C. The test for anaerobic growth was done in test tubes with semi-solid (1%) agar with PCA medium with 50% BL. Test for oxidase was done by mixing a drop of a 1:1 (v/v) mixture of 1% α-naphthol in 95% ethanol and of 1% dimethyl-*p*-phenylenediamine with a cell suspension on a glass slide. The catalase test was done the same way with a drop of 3% H_2O_2.

3. Results

3.1. Physical and chemical conditions in the lagoon

The average temperature of the Blue Lagoon calculated from 64 individual measurements (16 times 4 stations) done over a period of 7 months in 1992, was 37°C ± 8.4 (standard deviation). The fluctua-

tions are great, however, as can be seen in Fig. 2. The average temperatures at stations 1, 2, 3 and 4, were (°C): 42 ± 10, 31 ± 6, 34 ± 7 and 40 ± 11, respectively. The lowest and highest recorded temperatures were 15°C and 58°C, both at station 1. Both direct observations and meteorological records indicate that strong wind was the main reason for these great temperature fluctuations, but air temperature or rain had much less effect. Both pH and conductivity showed much less fluctuation during the same period (data not shown). The average pH was 7.5 ± 0.2 and the average conductivity (at 1/100 dilution) was $495\ \mu S \pm 45$, which corresponds to 2.5% salinity of the undiluted water.

Phosphate was found to be 0.48 and 1.4 μM but nitrate was 2.5 and 5.5 μM in the samples taken on March 22 and June 15 respectively.

3.2. Silica precipitation rates

The silica precipitation rate can be estimated from the available chemical analysis [3]. The average concentration (as SiO_2) is 430 mg/kg of well fluid. After steam removal it is concentrated to about 620 mg/kg as calculated from the chloride concentration factor of about 1.45 (the Cl^- goes from 12.8 g/kg in the well fluid to 18.5 g/kg in the Blue Lagoon water [3]). The SiO_2 in the Blue Lagoon water was 137 mg/kg. The difference of 483 mg/kg had therefore precipitated. With flow rates of 900 m^3/h this gives total silica precipitation of 435 kg/h and if the total volume is 4×10^7 l it means about 10 mg/l/h.

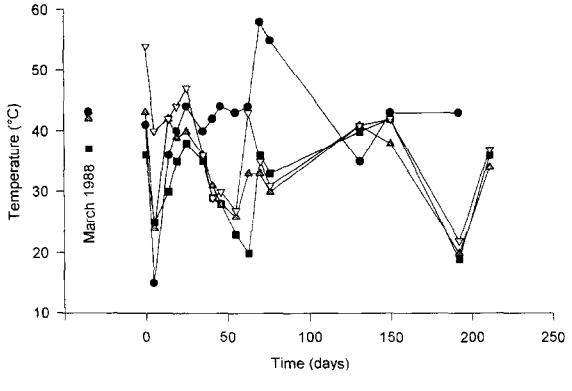

Fig. 2. Temperature profile of the Blue Lagoon at four sampling stations (Fig. 1) in 1992. Station 1, ●; Station 2, ■; Station 3, ▲; Station 4, ▼. The first measurement was taken on February 27 (day 0) and the last on September 28 (day 211). Measurements from March 1988 were done at stations 1–3.

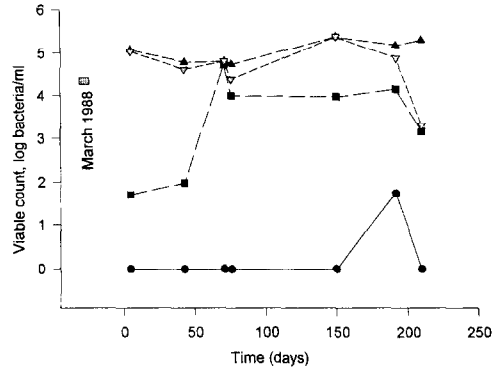

Fig. 3. Viable bacterial count in the Blue Lagoon in 1992. The sampling times were as in Fig. 2. Plate count agar (PCA) containing increasing proportions of Blue Lagoon fluid (BL). PCA made with distilled water, ●; PCA made with 20% BL, ■; PCA made with 50% BL, ▲; PCA made with 100% BL, ▼. The viable count from March 1988 was on PCA with 100% BL. Each point is an average of separate determinations done at all four sampling stations.

3.3. Viable counts

The viable count was very similar for all sampling stations in the lagoon at a given sampling date and therefore an average viable count for the lagoon was calculated from the values obtained at the four stations. Also, only minor fluctuations were seen over the whole study period as can be seen in Fig. 3. No bacteria grew on normal PCA medium, except in one case when the count was 55 ml^{-1}. Also the count on the medium with 20% BL showed the greatest fluctuations and was always lower than at 50% BL, which was similar or slightly higher then on 100% BL. The low counts on normal PCA indicated that common human-borne or soil bacteria did not grow, or survive long, in the lagoon.

The average viable count for the Blue Lagoon, over the whole period, based on the colony count on 50% BL was 1.3×10^5 ml^{-1} and 8×10^4 ml^{-1} on 100% BL. This is in good agreement with the value from March 8 1988, which was 2.7×10^4 ml^{-1} on 100% BL. When determined with the MPN method the count was however found to be 2.6×10^6 ml^{-1}.

The viable counts are remarkably stable over time (Fig. 3) and they do not seem to be significantly higher in the summer, during the high bathing activity and number of visitors.

3.4. Numerical taxonomy analysis

Out of the total of 54 characters tested, 12 were invariable for all 99 strains so only 42 were used for calculating similarity and making the dendrogram (Fig. 4). None of the strains would grow on glycine, starch, rhamnose, formate, raffinose and adonitol. All were sensitive to tetracycline, no strain grew at 4°C but all of them grew at 22, 30, 40 and 45°C.

At the similarity level of 80%, two main clusters are formed with 85% of the strains in cluster A and 6% in cluster B. The other branches only have 1 or 2 strains. The strains in clusters A and B each formed a homogenous group as can be seen by the results summarized below and in Table 1.

The main differences between clusters A and B are therefore the carbon sources utilized and the optimum salt concentrations for growth on PCA. A total of 13 carbon sources were utilized by > 80% of the strains in cluster B whereas only 2 (arginine and lysine) were used to same extent by cluster A.

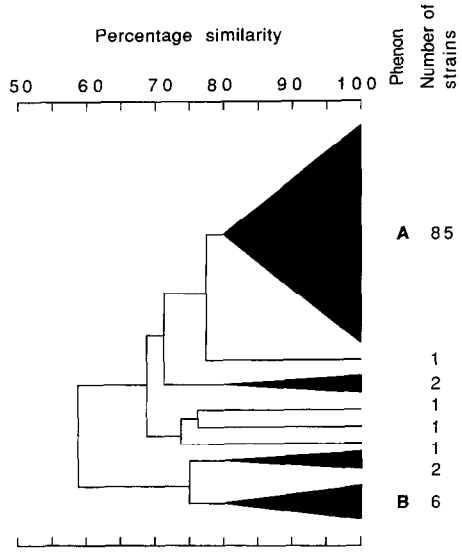

Fig. 4. A simplified dendrogram showing the clustering of the strains into two main groups based on the S_{SM} coefficient and UPGMA clustering analysis.

Table 1
Main characters of the bacteria in clusters A and B

	Cluster	
	A	B
Colony colour	Tan	Yellow
Cell morphology	Rods	Coccus, tetrads
Gram reaction	−	+
Motility	+	−
Opt. temp., °C	45	35
Opt. NaCl, %	3.5	1
Growth at NaCl:		
0%	−	+
8%	+	+
10%	−	−
Oxidase	+	−
Catalase	−	+
Anaerobic growth on nitrate	−	−
Lipid granules	+	−
Growth on:		
Arginine	+	+
Glucose	−	v
Lysine	+	+
Casein hydrolysis	−	+
Starch hydrolysis	−	−
Resistance to:		
Penicillin	−	−
Streptomycin	+	+
Vancomycin	+	−

−: > 80% of strains negative; +: > 80% of strains positive; v: 20–80% of strains positive.

The majority of the carbon sources, or 25 out of 34 tested, were only utilized by < 10% of the strains in cluster A. About 15% of the cluster A strains grew at 50°C on plates but none of the cluster B strains. The growth pattern of cluster A on PCA with different salt concentrations is probably due to the fact that the bacteria grew poorly on medium without Blue Lagoon fluid (data not shown).

The functional evenness index [13] was calculated for the 99 isolates and found to be 0.033 which is very low when compared to estimates that have been done for other bacterial populations [13,14].

3.5. Distinguishing characters of the bacteria

To characterize the bacteria further, a total of 4 representative strains (numbers 1, 4, 57 and 69) were selected from cluster A and one (number 89) from cluster B. All 4 strains from cluster A gave very

similar results, but they were significantly different from strain 89 of cluster B (see Table 1). The strains in cluster A were Gram-negative rods, whereas strain 89 of cluster B was a Gram-positive coccus, arranged in tetrads. Other distinguishing characters are the oxidase and catalase reactions, the hydrolysis of casein and the optimum growth conditions. Strains A showed optimal growth at 3.5–4% NaCl but no growth without added salt (in liquid culture). They gave clear growth at 8% NaCl but not at 10%. Strain 89 (B) had optimal growth at 1% NaCl and grew well without added salt. It showed growth up to 8% NaCl but not at 10%. The effect of temperature on growth rate was similar for all of the four A strains, with an optimum at 45°C and growing even at 50°C but not at 55°C. The doubling time of the four strains A was about 80 min at 45°C. The optimum for strain 89 was at 35°C and it grew at 45°C but not at 50°C.

4. Discussion

In this paper we report for the first time the main physical, chemical and biological characteristics of the unusual Blue Lagoon geothermal lake in Iceland. The chemical composition of the hydrothermal fluid entering the Blue Lagoon has been determined [15] but this is the first time that the lagoon itself has been characterized. Most of the parameters determined here (i.e. temperature, pH and salinity) would indicate that the Blue Lagoon should be a favourable environment for a number of living organisms. That is not the case, however, since the only organisms that we found were a few types of prokaryotes, that is the bacteria described herein and some cyanobacteria. The cyanobacteria have been known to grow abundantly in the lagoon, often forming green slime layers on the rocks in the lagoon. These layers seem to be primarily composed of a single species of a filamentous, sheethed cyanobacterium, tentatively identified by Dr. A. Couté as *Leptolyngbya erebi*, var. *thermalis* (personal communication).

This low diversity of organisms present shows that the Blue Lagoon can be considered an extreme environment for most life forms. The high silica precipitation rate in the lagoon is probably the main reason for the low observed diversity. The calculated silica precipitation rate of about 10 mg/l/h would give about 400 mg/l overall if the retention time is 40 h. This is consistent with visible precipitate forming in the sampling bottles after standing overnight.

The Blue Lagoon is now very popular for bathing and is run as a commercial health spa with over 100 000 visitors every year. With no artificial disinfecting taking place, it would be expected that various environmental contaminants, like enteric bacteria would be in high numbers. That, however, is not the case. Over the whole study period did we only once get any growth on normal PCA (Fig. 3). This indicates an almost total absence of any human-borne or common soil bacteria. This is also confirmed by the monitoring regularly done by the health authorities (data not available).

In spite of the apparently harsh environment, our results show that the organisms which are adapted to these conditions can really proliferate in high numbers. This is very typical for organisms adapted to extreme environments [16]. The observed bacterial count of 10^5–10^6 ml^{-1}, indicates that at least the endogenous bacteria are not efficiently removed by the silica precipitate.

The simple dendrogram obtained by the numerical taxonomy analysis and the low functional evenness index (E) of 0.033 show that the bacterial flora obtained by plate cultivation is very homogenous and is apparently composed of only one or two types which seem to be optimally adapted to these unusual conditions. The isolates in cluster B have many morphological similarities to *Micrococcus* but at this point we cannot conclude anything about where the bacteria in cluster A might be classified. They are moderately halophilic, mesophiles but with rather high optimum growth temperature. This relatively wide temperature growth range is, however, a favorable property for organisms that grow in the Blue Lagoon, where great temperature fluctuations frequently happen (Fig. 2).

The bacteria, especially in cluster A, showed some requirements for having BL in the medium. This can be seen by the fact that if BL was replaced by added NaCl the viable counts were much lower (data not shown) and the purified isolates would not grow as well on such medium, even though the final salinity was the same as in corresponding BL medium. The isolates also had poor viability on plates and were easily lost upon storage.

The characterization presented in Table 1 is not sufficient to positively identify the isolated bacteria. Further characterization is needed before the bacteria can unequivocally be identified or possibly described as a new species.

Acknowledgements

This work was supported by the Blue Lagoon Committee (Ministry of Health) and The Icelandic Science Foundation (grant no. 94-L-105). We thank Grimur Saemundsen and Ása Brynjólfsdóttir of the Health Company by the Blue Lagoon for their cooperation.

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