A Culture-Independent Survey of the Bacterial Community in a Radon Hot Spring

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ABSTRACT

Paralana is an active, radon-containing hot spring situated in a region of South

originated in a hydrothermal environment like that found at deep-sea vents (Woese, 1987; Weigel and Adams, 1998).

Investigations of the microbiota inhabiting modern hydrothermal systems are also important for interpreting presumed biomarkers (microfossils, lipids, isotopes, etc.) found in ancient systems on Earth (Walter and Des Marais, 1993; Reysenbach and Cady, 2001). Finally, since it is highly probable that thermal springs were present for a large part of Martian history, both modern and ancient hydrothermal systems can provide information relevant to the search for extant or extinct microbial life on Mars (Brakenridge, 1990; Walter and Des Marais, 1993; Christensen et al., 2000). Despite

of sample, was processed as above to test for laboratory contamination.

16S rRNA polymerase chain reaction (PCR) and recombinant libraries

Community 16S rRNA genes were PCR-amplified from each DNA sample with Bacteria domain-specific PCR primers PB36 (5ʹ-AGR GTT TGA TCM TGG CTC AG-3ʹ) and PB38 (5ʹ-GKT ACC TTG TTA CGA CTT-3ʹ) (Saul et al., 1993). PB36 and PB38 bind to, respectively, positions 8–27 and 1,492–1,509 of the 16S rRNA gene [Escherichia coli gene numbering (Blattner et al., 1997)]. Sample DNA (~100 ng) was amplified using the following cycling conditions: 94°C for 3 min (1 cycle); 94°C for 45 s, 55°C for 30 s, 72°C for 90 s (25 cycles); 72°C for 7 min (1 cycle). PCR products were purified using a High Pure PCR Product Purification Kit (Roche). A library of the purified 16S rRNA genes was prepared for each Paralana site using the pGEM-T Easy Vector System (Promega) and subsequent transformation into Max Efficiency DH5a competent E. coli cells (Invitrogen). For each library (A-I), 95 recombinant (i.e., white) E. coli colonies were selected and grown overnight (37°C) in 150 mL of Luria broth containing 100 mg/mL ampicillin. Cultured cells were lysed (94°C for 5 min), and debris was pelleted by centrifugation (5 min). Recombinant plasmid 16S rRNA gene inserts were reamplified from 2 mL of the lysate (supernatant) with pGEM-T vector-specific PCR primers (PGEMF, 5ʹ-GCC GCG GGA ATT CGA TT-3ʹ; PGEMR,

panel). The water then flows around a large rock and through sediment to form a larger pool (main pool, ~10 3 5 m; Fig. 1, lower panel); Paralana Creek
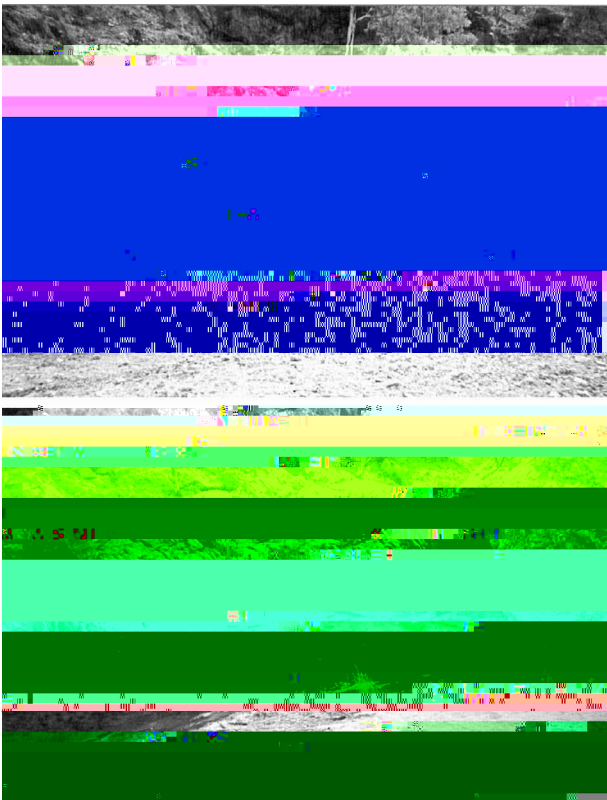
FIG. 1. The Paralana hot spring. Upper panel: The smaller source pool, from which the spring water emerges. **Lower panel:** The larger main pool forms from water flowing around and under the large **bou**lder seen in both
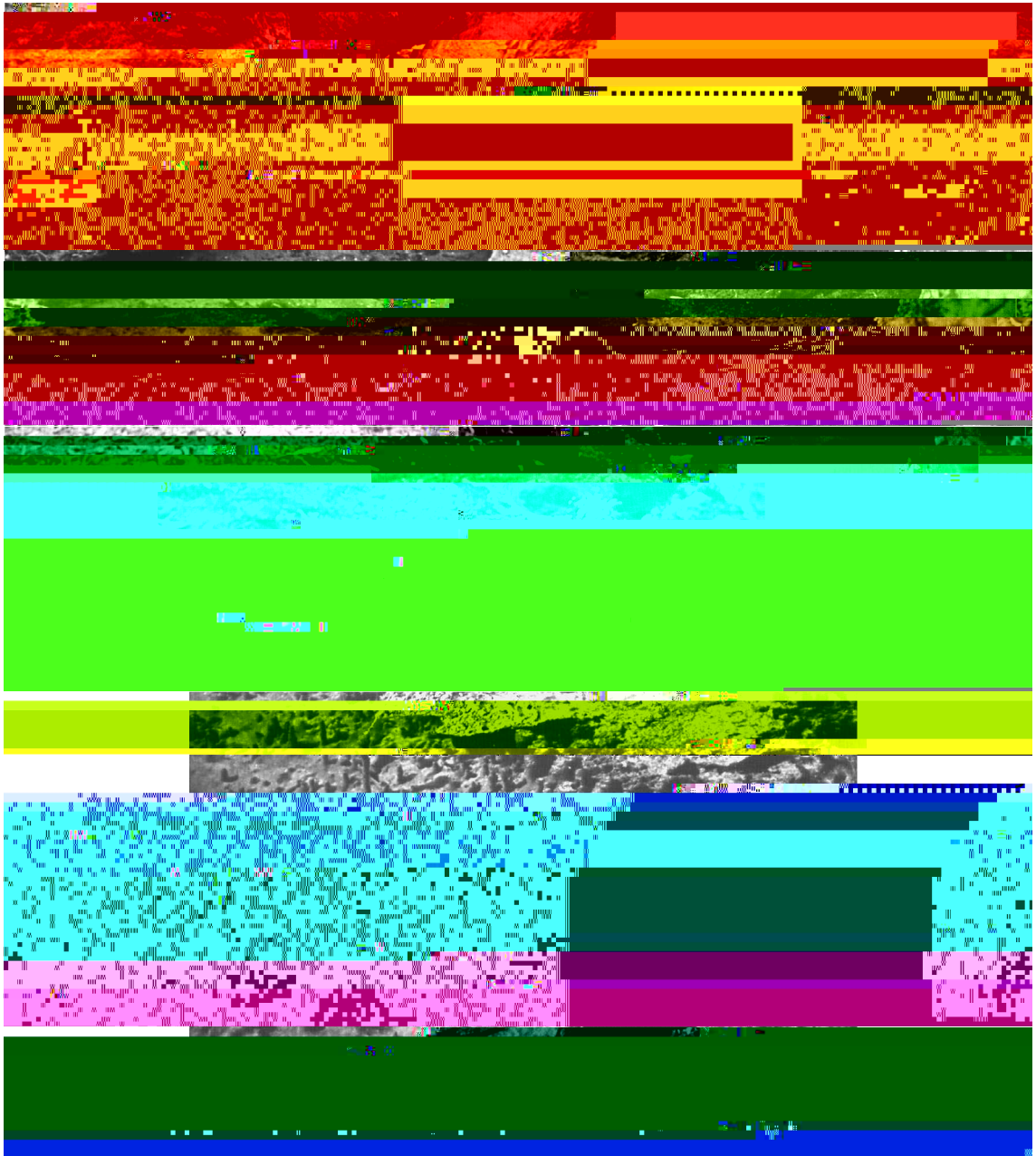
Samples A and B (60–63°C). Located at the source, these sites were the hottest sampled. They differed in vertical, but not horizontal, position. Sample B, a coarse, sandy sediment with some small, brown biomaterial, was taken 2–3 cm below sample A, a benthic brown biofilm that coated a finer sandy sediment and leaves. The 16S rRNA libraries A and B displayed a wide, yet generally similar range of division-level diversity that encompassed six and five bacterial divisions, respectively (Table 3). The affiliation of two sequences was uncertain. Sample A was dominated

FIG. 3. An example of the different ribotypes obtained from the Paralana hot spring. The 16S rRNA
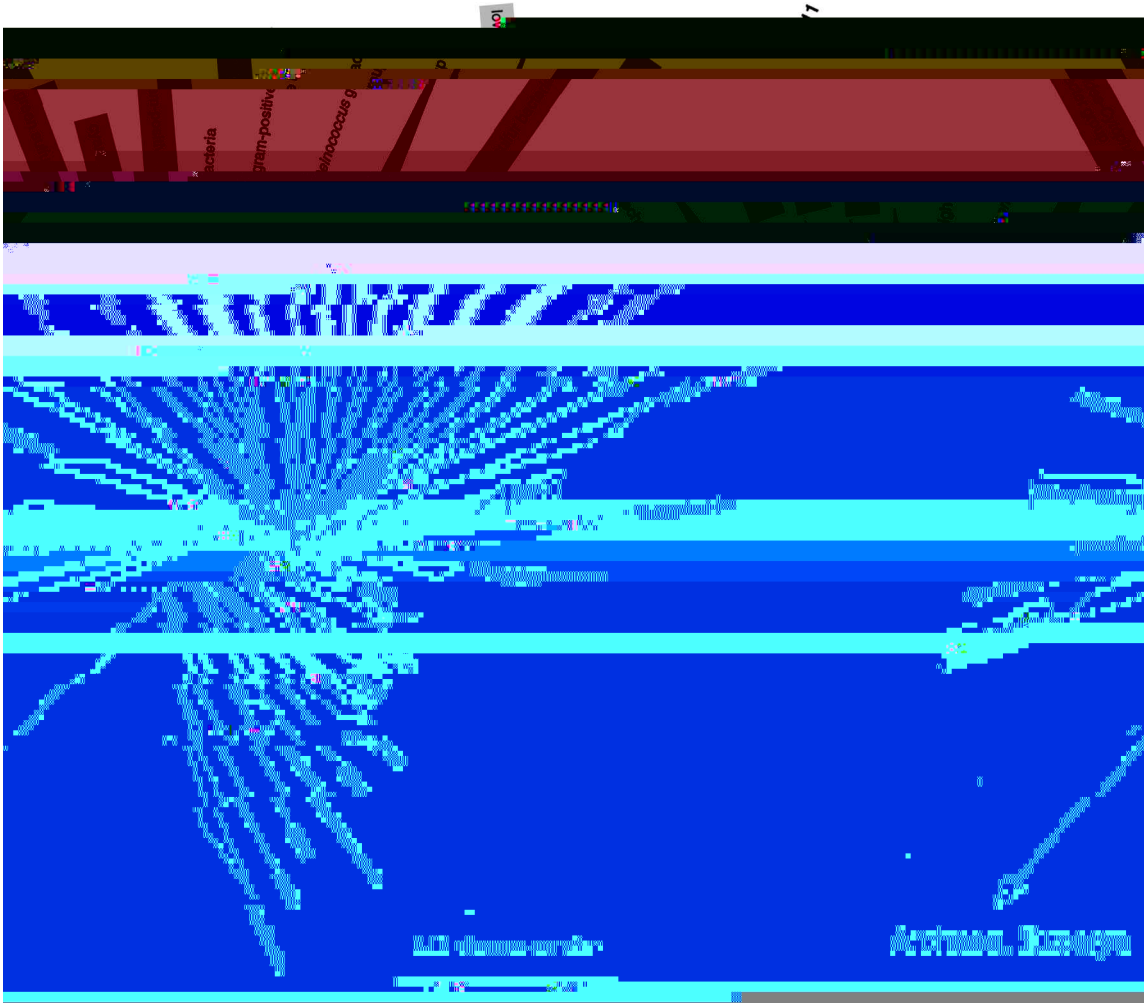
subdivision. Both samples A and B contained small numbers of sequences displaying homology (84–99%) with members of Nitrospira, the gr39 2805 5ira27ospira,

G5C, 95% with Thermodesulfovibrio icelandicus, an Icelandic hot spring isolate (Sonne-Hansen and Ahring, 1999)], and OP12 clone OPB54 (95–97% homology). Lower sequence homologies were observed with members of the Cytophaga–Flexibacter–Bacteroides (CFB) group, the d-Proteobacteria, green non-sulfur bacteria, another Cyanobacteria, and an isolate of uncertain affiliation (Table 3).

Sample H (53°C) and sample I (48°C). When examined in situ, sampling sites H (a green-yellow/green floating mat in the source pool) and I (green-yellow and emerald green benthic mat in the main pool) appeared to be predominantly cyanobacterial. After vortex-mixing, sample H also contained small amounts of reddish-brown biofilm. Sample I had some rust-colored "slime." Cyanobacterial sequences dominated both samples, with their respective ribotypes representing 63% and 79% of recombinants analyzed. Four cyanobacterial sequences were identified in sample H, with three dominating (Table 3). The most prevalent, as in sample C, was O. amphigranulata (recombinant I1G). The other dominant sequences belonged to F. muscicola (recombinant H11A), which is a heterocystous branching member of the Stigonematales, and Lyngbya spp. [recombinant H9E (U. Nuebel, 1997, unpublished GenBank entry)]. Two minor ribotype sequences were homologous with recomb0 1 1988

(Paralana recombinant I9H). Database matches suggested that this latter recombinant may be a member of the purple non-sulfur bacteria (Rhodocyclus spp.).

The community profile of sample I was very similar to that of H, except that F. muscicola sequences were not identified, and an OP12 representative was present (Table 3). Over half of the ribotypes identified in sample I were O. amphigranulata (recombinant I1G). The other dominant sequence type present was Lyngbya spp., also identified in sample H (recombinant H9E). Based on its H9 value of 1.73, sample I was the second least diverse Paralana sample analyzed.

DISCUSSION

We have conducted a qualitative, culture-independent 16S rRNA survey of the bacterial composition of the Paralana hot spring in order to characterize the microbial biota. There are no previous reports of comprehensive DNA- or culture-based studies of an Australian hot spring, and no such description of a radon hot spring anywhere in the world. Other researchers have concentrated on the hot springs of Yellowstone National Park in the United States and, in particular, Octopus Spring and Obsidian Pool (for example, Hugenholtz et al., 1998a; Ferris et al., 2001). As a result, these two

the main pool was not examined). Experiments aimed at obtaining a more quantitative indication of mat composition (for example, fluorescent in situ hybridization with 16S rRNA probes or flow cytometry) are required. The columnar/pinnacle-shaped structures in the benthic mat of the main pool were similar in appearance to structures containing Phormidium and Synechococcus cyanobacterial species in some Yellowstone hot springs (Walter et al., 1976; Brock, 1978). The Yellowstone structures are mineralized by the deposition of silica (Walter et al., 1972), but this is not the case with those in Paralana.

The majority of the Paralana 16S rRNA sequences show a high level of sequence similarity to those of Bacteria previously identified in hot springs by either culture- or DNA-based (culture-independent) techniques. For example, the closest database relatives of Paralana recombinants G10C, G11D, B5H, B12C, A3D, and E12H were found in Yellowstone National Park hot springs (Weller et al., 1992; Hugenholtz et al., 1998a; G.T. Bonheyo et al., 2001, unpublished GenBank entry). Paralana supports other phototrophic microbes in addition to Cyanobacteria, viz., members of the green sulfur and green non-sulfur bacterial divisions. Proteobacteria were ubiquitous in the Paralana hot spring samples, reflecting the large size and diversity of this

tified in sample B. This was an unexpected observation considering its close physical proximity and similar temperature to sample A. One possible explanation is that one of the minor sample B ribotypes we did not sequence represented a related OP8 species.

The Paralana water contains high levels of radon, measured at between ~2,000 and 5,800 Bq/L (Grant, 1938; Beverley Environmental Impact Statement, 1998). The value for the gas was ~29,000 Bq/L (Grant, 1938), which is ~2×10^6 times greater than the average background level for outdoor air; it is also ~2×10^5 fold above the "indoor air action level" at which it is recommended that remedial mitigation measures be taken (U.S. Environmental Protection Agency, 2001a,b). Whilst radon has been detected in some Yellowstone hot springs, this was not in the context of microbial communities, and radon levels were much lower than those present at Paralana (Clark and Turekian, 1990). With one exception (recombinant B5D, see below), none of the Paralana 16S rRNA sequences represents Bacteria previously identified in ionizing radiation environments. In particular, it was somewhat surprising that there was no evidence in Paralana of the extremely radiation-resistant Deinococcus genus, thermophilic members of which have been cultured from a hot spring in Italy (Ferreira et al., 1997). The 16S rRNA sequence of Paralana recombinant B5D, only seen in sample B, was 93% homologous to a sequence originally identified in an ionizing radiation environment—that of clone GR-WP33–30, detected in the anaerobic drain waters of

and Mars. It is clear that Paralana will add to the rapidly growing knowledge base of modern and ancient hydrothermal systems on Earth.

ACKNOWLEDGMENTS

We would like to thank Mark Pirlo for providing the latitude and longitude co-ordinates of the Paralana hot spring.

ABBREVIATIONS

CFB, Cytophaga–Flexibacter–Bacteroides; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; rRNA, ribosomal RNA; SSU, small 16S subunit.

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