The deep gold mines of South Africa: Windows into the subsurface biosphere.

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ABSTRACT

Recent investigations have identified microorganisms in various crustal environments to 2800 meters below the surface (mbls.). Relatively few deep samples of the continental crust (>800 mbls.) have been collected for microbiological analyses, however, because coring is technically difficult and expensive. The gold mines into the 2.9 Ga Witwatersrand Supergroup in South Africa may provide an alternative means of studying microbial communities at depths up to 3500 meters.

Uranium-rich, Au-bearing, carbonaceous rock and water from a gallery borehole at a mined depth of 3200 mbls. and an ambient temperature of 50°C were collected for microbial analyses. Measures were taken to avoid contamination during mining and sampling. Samples were shipped to the U.S.A. in sterile, anaerobic canisters on ice, processed under sterile anaerobic conditions and distributed to participating labs. Microscopic observations revealed the presence of intact cells including filamentous microorganisms. Phospholipid fatty acid and DNA analyses indicated that the samples contain cyanobacteria, sulfate-reducing bacteria (SRB) and iron-reducing bacteria (IRB). The water sample yielded a strain of *Thermus* (IRB-SA) that is the first reported *Thermus* to reduce Fe(III) and the first facultative, thermophilic Fe(III) reducer.

Keywords: anaerobic thermophilic bacteria, lipids, nucleic acid, gold, uranium, radiolysis, lithotrophy

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1. INTRODUCTION

Studies during the last decade have demonstrated that the biosphere extends at least 2.8 kilometers beneath the Earth's surface, as microbial communities existing in a variety of subsurface terrestrial settings[1]. Conditions in the deep subsurface approach the limits for life and novel "extremophiles" have been isolated from these environments. Extreme conditions encountered in the subsurface include high temperature, high pressure, high salinity, high ambient radiation, low availability of energy sources, and in the case of natural gas reservoirs, low H_2O activity.

To date, thermophilic, gram-positive, metal-reducing and sulfate reducing bacteria have been the most frequently cultured bacteria in samples obtained from the deepest strata (>1000 meters below the surface-mbls.). Just how representative these bacterial types are of the subsurface and how they survive at depths greater than 1000 meters remains highly uncertain due to the difficulty and cost of aseptic drilling. The Au mines of South Africa attain depths where the fluid pressures approach those at ocean ridges and the formation temperatures attain the zone for microbial thermophiles (45-65°C). The Au deposits occur within organic-rich layers interstratified with low porosity, sandstone and shales with varying concentrations of saline formation water. This type of rock environment is similar to those encountered in the Taylorsville[2] and Piceance[3] sedimentary basins, where the deep, subsurface microbial environments have been sampled by drilling. Consequently, the deep Au mines of South Africa could provide a unique "window" into the continental, subsurface biosphere through which the distribution of microbial communities as a function of various environmental parameters, the life-sustaining energy resources available to bacteria and the mineralization processes that are related to microbial respiration can be observed and documented.

1.1 Witwatersrand Basin

The Witwatersrand Supergroup is a 2.9 Ga sequence of hydraulically tight, sandstone and shale with minor volcanic units and conglomerates. The Witwatersrand Supergroup is overlain by the 2.7 Ga. Ventersdorp Supergroup volcanics, which in turn are overlain by the 2.3 Ga Transvaal Supergroup dolomites. The entire sequence is dipping at a steep angle all around the basin margin (Fig. 1). Gold mining activity is concentrated along "reefs" within the Witwatersrand Supergroup and the mines follow these "reefs" from shallow levels to great depths (at Western Deep Levels Ltd. the maximum depth is 3800 mbls.).

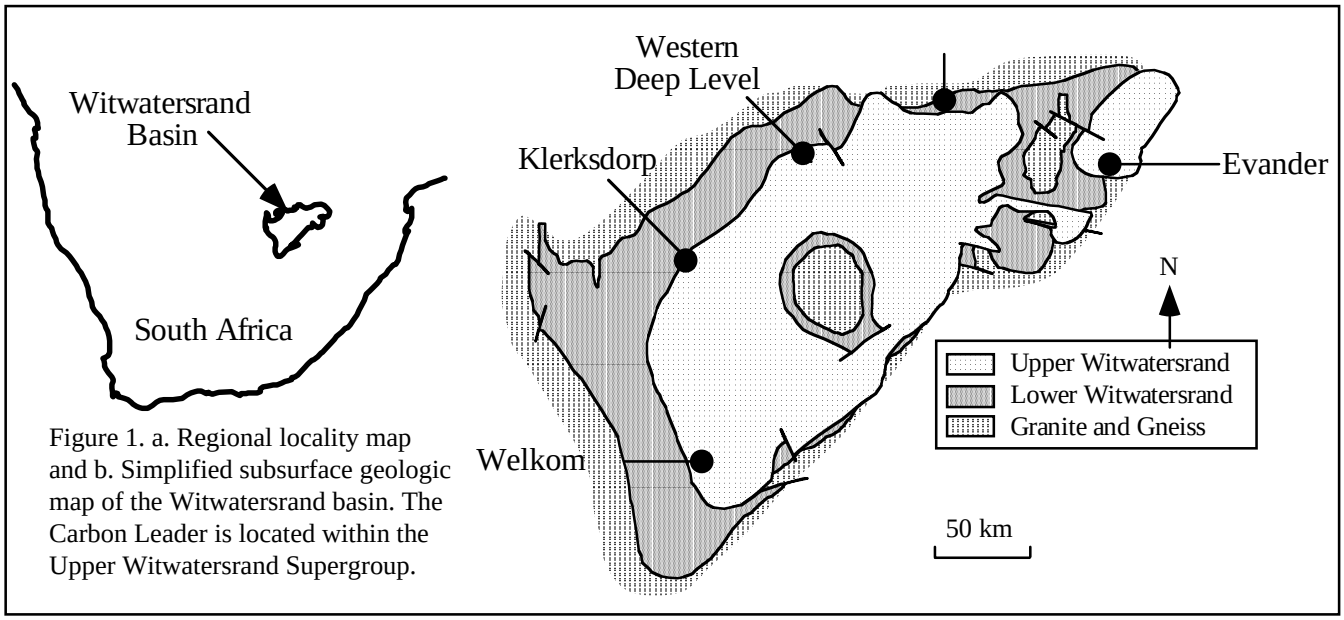

Figure 1. a. Regional locality map and b. Simplified subsurface geologic map of the Witwatersrand basin. The Carbon Leader is located within the Upper Witwatersrand Supergroup.

The most productive "reef" is the "Main Reef" or "Carbon Leader" that occurs at the contact between the underlying Jeppestown shale and the overlying Johannesburg quartzite. The Carbon Leader is a thin organic-rich layer (maximum of 13 mm thick) along which much of the gold and uranium is concentrated. The carbonaceous matter is composed of alkyl-substituted aromatic hydrocarbons, low molecular weight hydrocarbons, aromatic sulfur and aliphatic oxygen compounds[2]. The concentration of organic free radicals is also ten to one hundred times greater than in coal[4]. The aromatic nature and the hydrogen and oxygen content of the carbonaceous matter appear to reflect direct radiolytic effects and interactions with irradiated water[4]. The radiation source is uraninite, which ranges from trace quantities to 20% by volume. FTIR spectra confirm an increase in the concentration of C=O and C=C bonds in the halo surrounding uraninite grains.[5] Amino acids with non-racemic ratios[4] and pentose to hexose ratios indicative of algae[6] are also present.

Arguments persist as to whether the Carbon Leader represents the fossilized remnant of an algal mat[7] or the solidified remains of a migrated, hydrocarbon-bearing post-depositional fluid. Early stable isotope analyses of the carbonaceous matter yielded depleted $\delta^{13}C$ values (-22 to -37$^{o}/_{oo}$ PDB)[4] typical of sedimentary biogenic carbon. Detailed molecular and elemental analyses of the Carbon Leader are consistent with a prokaryotic origin. [4] The presence of delicate circular and annular micro-structures of apparent bacterial or algal morphology[8] suggest a biogenetic origin for the Carbon Leader. The Au in the Carbon Leader commonly occurs as alga-like, filamentous aggregates with filament widths ranging from 1.5 to 2 µm[6]. Siliceous, *Mycelium*-like aggregates of fibers are also present in the carbonaceous reef at Welkom (Fig. 1).[6] Spherical aggregates of framboidal pyrite, some with micron scale laminations of silica and clay occur in the Carbon Leader and adjacent quartzite and conglomerates[9] and have been attributed to *in situ* crystallization.[10]

The preservation of any these delicate filamentous features, if formed *in situ* soon after deposition of the Witwatersrand, could not have survived the observed burial compaction from 2.9 to 2.3 Ga and ensuing metamorphic conditions (300±50°C at 1.5 to 3 kbar).[11,12,13] The nodules of carbonaceous matter that are also present in the quartzite adjacent to the Carbon Leader would have been similarly crushed. Consequently, the Carbon Leader may represent a hydrocarbon-bearing fluid that was emplaced at approximately 2.0 Ga concomittant with the deformation of the Witwatersrand by the Vredefort impact structure. According to this theory the observed filaments are regarded as representing "fiber-vein" growths formed by precipitation of solid hydrocarbons and inorganic compounds in an extensional fracture environment. The sulfide concretions are interpreted to have been silicified into hardened spheres prior to transport and deposition of the Witwatersrand.[9]

2. HYPOTHESES AND RESEARCH GOALS

The presence in the Carbon Leader of non-racemic amino acids may be due to activities of recent or current microbial communities. These communities may have originated by contamination of essentially sterile rock during the mining operations. Alternatively, some of the bacteria may have migrated into the Carbon Leader during post-2.0 Ga ground water infiltration. In the deep Au mines, the bacteria indigenous to the Carbon Leader should be thermophilic, because the formation temperatures range from 50 to 60°C (Fig. 2a); whereas the contaminating communities should be mesophilic. If microbial communities have been present in the Carbon Leader for even tens of millions of years, then their metabolic activity may have led to the precipitation the filamentous mineral textures that appear to have formed *in situ* .

A pilot study was undertaken, therefore, to determine: 1) whether the collection of aseptic, microbial samples from the deep, South African Au mines was logistically feasible; 2) whether these samples contained indigenous, anaerobic, thermophilic microorganisms (i.e., not due to mining contamination); and 3) whether these microrganisms may have played a role in the mineralization observed in the Carbon Leader.

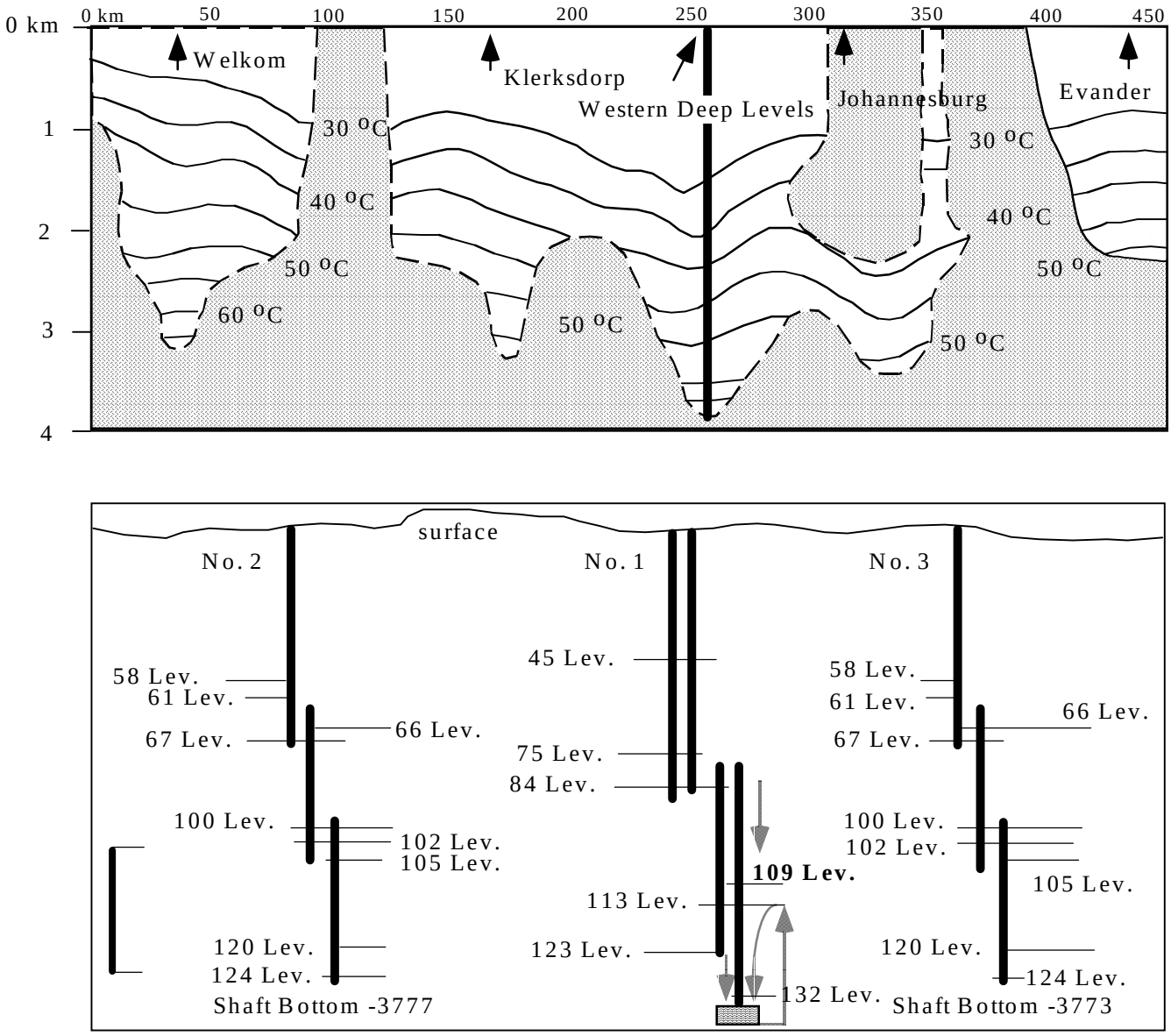

Figure 2. a. Formation rock temperatures in the Witwatersrand gold mining arc (after Jones and Bottomley, 1986) b. Profile of three mines from Western Deep Levels Ltd. located in the Carletonville gold field. Samples examined for this proposal are from mine shaft #1, level 109. Gray arrows schematically indicate water circulation from the base of shaft to level 109.

3. RESULTS

3.1 Sample Collection

Rock and water samples were acquired aseptically from Western Deep Levels, No. 1 (Fig. 2b), where lifts descend to various levels from 2000 to 3500 mbls. Water-chilled air ventilation keeps the active drifts at approximately 35°C and oxygenated. This ventilation is shut off in non-active drifts and in active drifts when miners are not drilling (i.e., during blasting, post-blast settling and during holidays). Without ventilation the temperature increases to formation temperature and the CO_2, CH_4,

NH_3 and other gases build up rapidly. The water utilized for ventilation, drilling, and for post-blasting dust removal is pumped from a repository at the base of No. 1 shaft and drains back to the reservoir from all mine levels (Fig. 2b).
Two samples of the Carbon Leader and adjacent quartzite were collected from a 1 meter high stope at level 109 (3231 mbls.). This level was selected for microbial sampling because: 1) at the time of sampling, level 109 had penetrated the country rock further than any of the adjacent levels at No. 1, thereby eliminating the possibility of mine water contamination from other levels; and 2) the panel was the freshest having been exposed to mine air and water for only 24 hours prior to sampling, thereby reducing the opportunity for surface contamination by microorganisms. The samples were collected from fractured rock face between the locations of dynamite charges that had already been set for the next blast. Two large intact rock samples were pried off the working face, collected in sterile autoclave bags and sealed immediately. A third rock sample exhbiting a superficial salt crust and dark brown stain produced by seepage of water from wall rock fractures was collected from one of the access galleries. Water emanating from a borehole penetrating 121 meters into the formation (3198 mbls.) was also sampled for microorganisms and for chemical and isotopic analyses. The water temperature was 48°C.

3.2 Sample Processing

Upon return to the surface the stope samples were removed from the autoclave bags, their outer surfaces heat-sterilized with an oxy-acetylene torch, wrapped in sterile aluminum foil and placed in flame-sterilized, gas-tight canisters. The canisters were evacuated and filled with Ar several times. The rock sample containing the brown stain and salt crust was placed in a bleach-sterilized, gas-tight canister. The canisters and serum bottles were packed in ice chests and shipped to Pacific Northwest National Laboratories (PNNL).

At PNNL, the samples were processed in an anaerobic glove bag with sterile tools. The two carbon leader samples were prepared by separating the organic rich layer (C-leader 1 and C-leader 2) from the quartzite (Bulk R 1B and Bulk R 2B). The sample bearing the surface Fe stain and salt crust was subdivided and designated as "Rock Slime". All samples were placed in sterile, whirl-pak bags and packed into canning jars with palladium pellets and filled with Ar-H_2. These jars were packed on ice and shipped to T.J. Phelps (Oak Ridge National Laboratory), David Balkwill (Florida State University) and David Boone (Oregon Graduate Institute) for enrichments. Frozen rock and water samples were shipped to M.F. DeFlaun of Envirogen, Inc. for acridine orange direct coutns (AODC) and DNA extraction for sequencing, to Anna-Louise Reysenbach of Rutgers University for *in situ* rRNA probe analyses and to D.C. White of the University of Tennessee for phospholipid fatty acid analyses (PLFA). Samples were retained at PNNL for select enrichments and nucleic acid-based analyses.

3.3 Microscopic Counts

To avoid possible contamination, all reusable supplies, the filters and the crushers were rinsed with a bleach solution and autoclaved prior to use and the outside surfaces of the rock fragments were heat-sterlized with a propane torch to the point that the hydrocarbons in the Carbon Leader ignited. A range of rock to water dilutions was used and standard protocols for AODC were followed. The cell density associated with the rock samples was very high (Table 1), four orders of magnitude higher

Table 1. Biomass estimates of microbial samples

Sample	AODC	PLFA*	Units
C-Leader 1	6.7×10^8	3.1×10^7	cells/g
C-Leader 2	3.4×10^8	1.6×10^5	cells/g
Bulk R 1B	n.d.	8.6×10^6	cells/g
Bulk R 2B	5.4×10^8	1.1×10^6	cells/g
Rock Slime	n.d.	$>3.8 \times 10^7$	cells/cm^2
Water	5.3×10^4	$6.5\text{-}13.5 \times 10^4$	cells/ml

* Cell densities are converted from total phospholipid fatty acid concentrations by multiplying by 5 x 10^5 cells/pmol[39].

than typical for pristine deep subsurface samples. The water sample yielded counts consistent with subsurface environments[14].

3.4 Enrichments

Enrichments of the water and rock samples were set up using a wide range of aerobic and anaerobic media within one to two weeks after samples were collected. Enrichments for aerobic and anaerobic, mesophilic and thermophilic bacteria with various electron acceptors (sulfate, ferric iron, manganese, CO_2) and donors (glucose, acetate, methane, H_2) were established. After approximately six weeks of incubation, a thermophilic, facultative metal-reducing bacterium (strain IRB-SA = dissimilatory iron reducing bacteria from South Africa[@]) was cultured from the water sample on a Fe(III) acceptor/H_2 donor medium. Growth was also detected in the Carbon Leader sample in a Fe(III) acceptor/H_2 donor medium. Growth was not detected in the other rock samples under anaerobic conditions. Approximately 300 mesophilic aerobic heterotrophic bacteria and fungi were isolated from the rock samples using direct contact agar plates.

The 16S rRNA sequence of strain IRB-SA indicated that it is closely related (>96% sequence similarity) to several well-characterized strains of *Thermus*. Cell morphology of IRB-SA (filaments, rotund bodies) is consistent with other *Thermus* sp. However, IRB-SA differs from previously described *Thermus* strains in being able to reduce Fe(III), Co(III)-EDTA, and a humic acid analog, anthraquinone disulfonate, in addition to growing aerobically and anaerobically with NO_3^-. The only other previously described genus with species demonstrating such versatility in electron acceptor usage is *Shewanella*. *Thermus* sp. IRB-SA is unique in its ability to reduce a wide range of electron acceptors at high temperature.

3.5 DNA Analyses

Using procedures developed at PNNL for rock cores,[15] DNA was extracted, purified, amplified by polymerase chain reaction (PCR), sequenced, and the preliminary phylogenetic relationships obtained by maximum likelihood analysis (Fig. 3). The Rock Slime contained a predominant clone type, a *Mycobacterium*, that accounted for 22 out of the 36 randomly selected clones. Several of the other Rock Slime clones aligned to known thermophiles including *Thermus* and *Acidiphilum.* In Bulk R 2B two clones (2B11 and 2B7) are phylogenetically similar to the photosynthetic (and heterotrophic) bacteria *Rhodoferax* and *Rhodobacter* (Fig. 3a), the latter is purportedly capable of dissimilatory iron reduction.[16] Two other sequences (2B29 and 2B5; Fig. 3a) are similar to iron and sulfate reducing bacteria (SRB). One clone aligned to *Leptospirillum*, a known iron-oxidizer. Clones from the C-leader were characterized by two dominant types that accounted for 61% of the randomly selected clones. One type was closely associated with *Caulobacter* (CLB in Fig. 3b) and the second type aligned with cyanobacteria (CL-A). Since the occurrence of cyanobacteria was unexpected, the complete clone was sequenced. Phylogenetic analysis and secondary structure mapping of this sequence to sulfate-reducing and cyanobacterial sequences confirmed that CL-A was most closely related to cyanobacteria (Fig. 3b). No particular clone type dominated Bulk R 1B and 2B; *Pseudomonas*, *Acinetobacter*, and *Arthrobacter* were all common.

3.6 PLFA Analyses

The neutral, glycol, and polar lipids were extracted from the water sample and powdered rock samples using the procedures developed at the Center of Environmental Biotechnology.[17] Rock Slime yielded the highest biomass (Fig. 4; Table 1) and contained PLFA indicative of gram positive and gram negative bacteria and SRB. The biomarker for *Desulfobacter sp.* (10me16:0) represented 6% of the total PLFA of Rock Slime, suggesting that it dominated the community. PLFA for other genera of sulfur reducing bacteria (SRB), *Desulfovibrio*, was detected in this sample (i17:1w7c). The predominant fatty acids of *Thermus aquaticus* (i15:0, i17:0 and a17:0)[18] were also detected in Rock Slime. The presence of *Actinomycetes* and

[@] IRB-SA has been deposited in the Subsurface Microbial Culture Collection/West at the Oregon Graduate Institute with an accession number of 654.

Mycobacterium is suggested by the tuberculostearic acid (10me18:0). Gram positive bacteria, such as *Micrococcus* and *Bacillus* sp., were probably present in the biofilm given the occurrence of terminally branched fatty acids (Fig. 4).

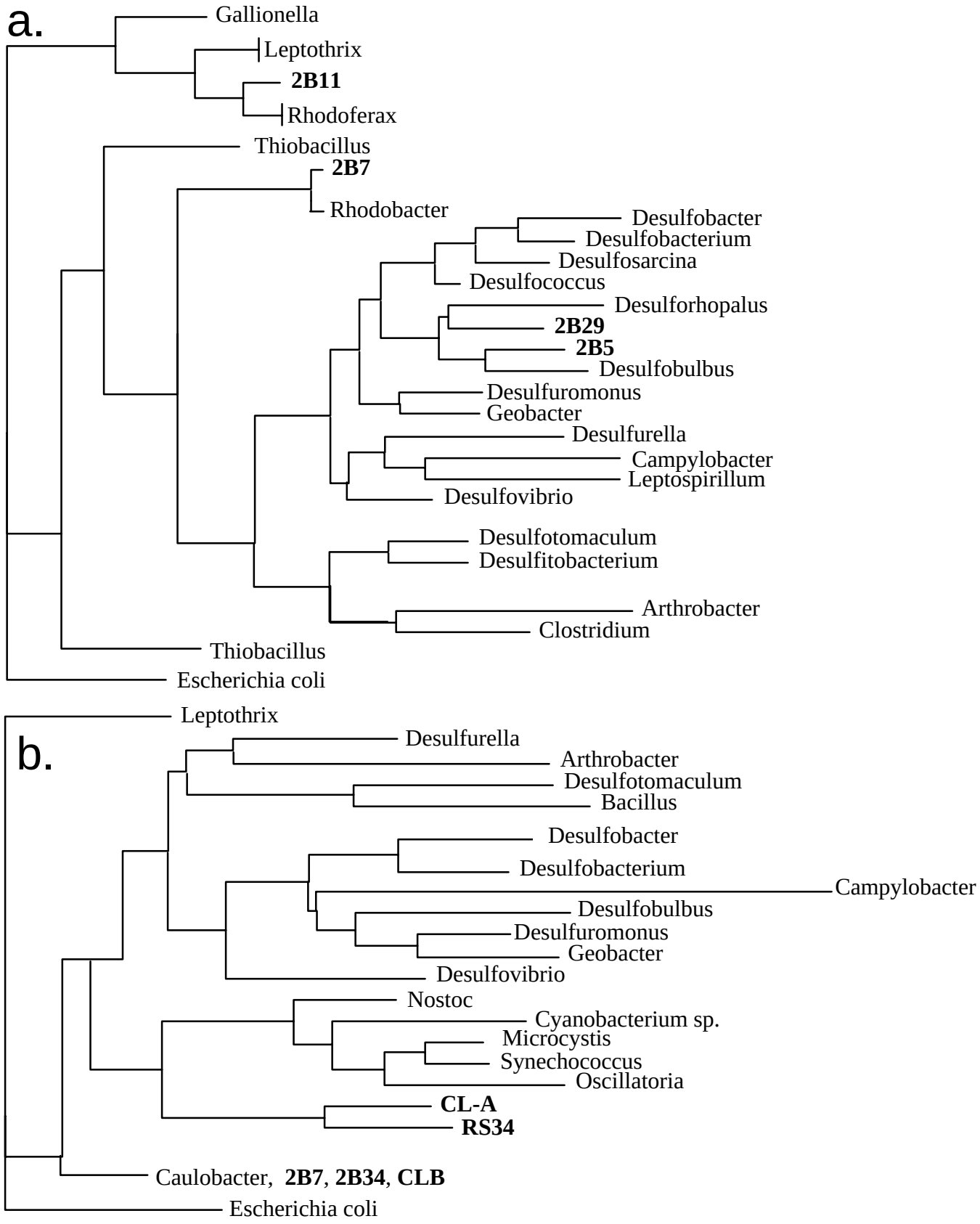

Figure 3. Maximum likelihood trees based on full 16S rRNA gene sequencing of clone material. (a) Tree showing sample Bulk R 2B clones corresponding to sulfate-reducing and other mixotrophic bacteria that can reduce iron. (b) Tree showing gene sequence of clones from the Carbon Leader A (CL-A) and B (CL-B) samples.

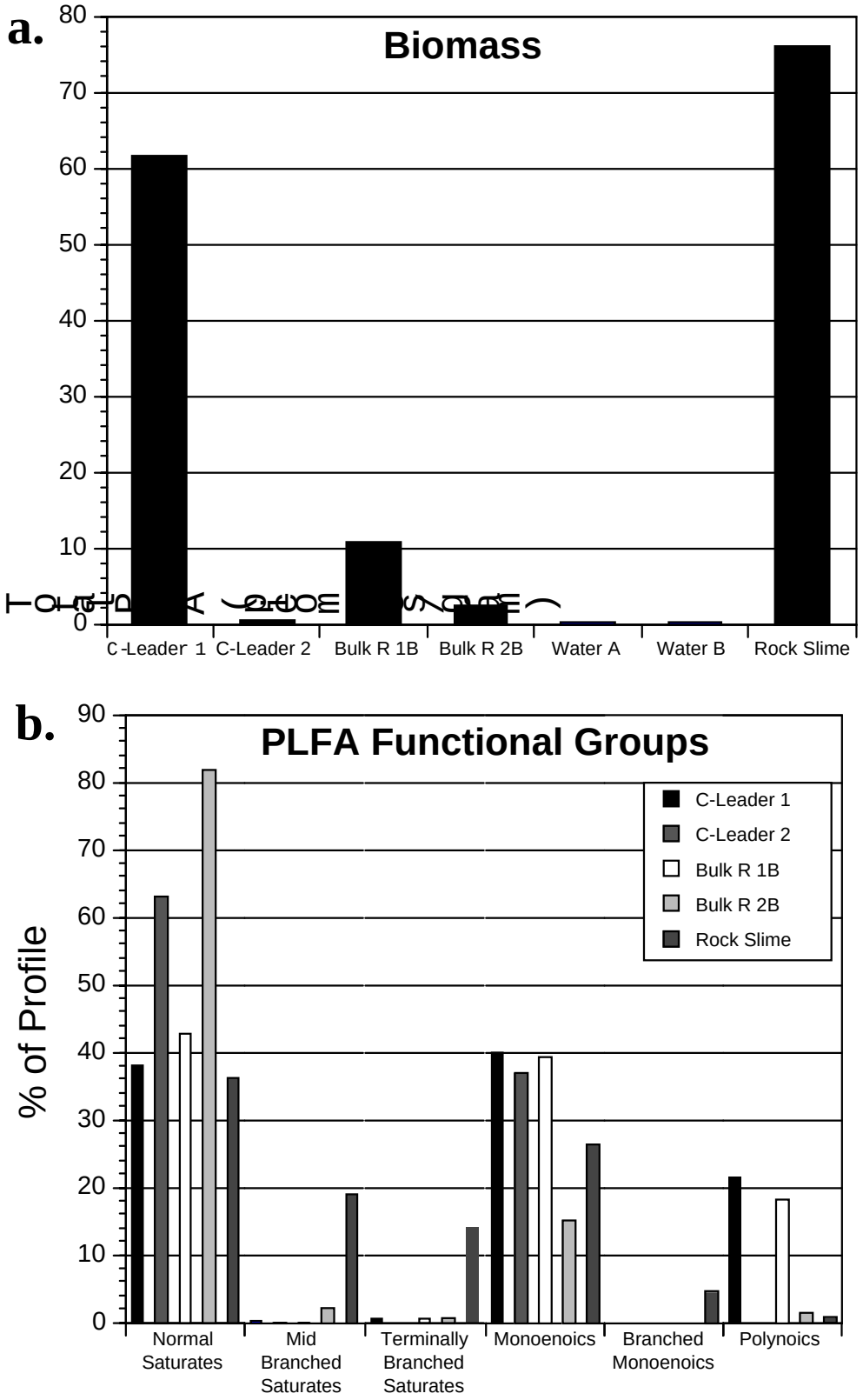

Fig. 4. (a) Total PLFA concentration for rock and water samples. (b) Relative molar abundance of PLFA functional groups in rock and water samples.

C-Leader 1 and the Bulk R 1B exhibited very high mole percentage (18-20%) of 18:2w6 (Fig. 4), which is attributed to fungal/algal membrane fatty acid. This functional group was also observed in Bulk R 2B and the Rock Slime. This biomarker can also be associated with cyanobacteria, and the PLFA profile most closely resembles *Tolypothrix* sp. The cellular density equals 10^7 cells/gm (Table 1). C-leader 2 exhibited a lower biomass (10^5 cells/gm) containing normal saturates and monoenoics indicative of gram negative bacteria (Fig. 4). Bulk R 1B and 2B exhibited high biomass (1-5 x 10^7 cells/gm) composed of PLFA indicative of gram negative bacteria and a biomarker for the *Desulfobacter* sp. (10me16:0). The water sample exhibited the lowest biomass of all samples (<10^5 cells/gm) with no diagnostic PLFA detected (Table 1).

3.7 Chemistry/Mineralogy

3.7.1 Rock Samples

The C-Leader contained authigenic Au, quartz, clay and minor calcite.[19] Both allogenic (anhedral) and authigenic (euhedral) pyrite crystals were observed in polished thin sections. Carbonaceous matter in the C-Leader occured as grain coatings of pyrite crystals with brownish color and as columnar masses. FEG-SEM imaging revealed nodular filaments in a pore within one the carbonaceous masses (Fig. 5). Bulk R 1B was a tightly cemented quartzite with pyrite and hydrocarbon bearing fluid inclusions. The He porosity of Bulk R 1B was 0.02%. The Hg porosimetry data indicate that the pore radii ranged from 0.004 to 0.4 µm with a median at 0.02 µm (Fig. 6). The air permeability estimated from the Hg-Porosimetry data is 1.57 x 10^{-7} mD.

3.7.2 Water Sample

The water sample had a pH of 8.9 and an Eh of 59 mV. The composition was primarily Na-Cl of moderate salinity with minor carbonate, sulfate, and sulfide (Table 2). The inorganic carbon isotope ratio is -13 $^{o}/_{oo}$ PDB, which is intermediate between that of the organic carbon in the Carbon Leader, -22 to -37 $^{o}/_{oo}$ PDB, and that of typical marine inorganic carbon (±0 $^{o}/_{oo}$). The water is saturated with respect to calcite, muscovite and chlorite. The $\delta^{18}O$ and δD values fall on the meteoric water line for recent, local precipitation (Fig. 7).

Table 2. Chemical and isotopic composition of water sample from Western Deep Levels, Inc. 109

CATIONS							ANIONS					ISOTOPES		
Na	Ca	Mg	K	Fe	Si	Al	Cl	F	Br	SO_4	CO_3	$\delta^{13}C$	$\delta^{18}O$	δD
204.3	47.4	0.1	2.0	0.6	15.5	.1	433.6	4.6	2.4	10.4	1.5	-13.42	-5.25	-27.4

All concentrations are in mg/L. The $\delta^{13}C$ are in units of $^{o}/_{oo}$ PDB and the $\delta^{18}O$ and δD are in units of $^{o}/_{oo}$ SMOW.

4. IMPLICATIONS

4.1 Thermophilic Microorganisms

The recovery of the *Thermus* strain IRB-SA demonstrates that viable, thermophilic microorganisms are present in the hot, alkaline, ground water within the Witwatersrand Au mines. *Thermus* sp. are have also been reported from hot, artesian wells within the Great Artesian Basin of Australia[20] suggesting that *Thermus* sp. may be a common subsurface dweller. IRB-SA, however, represents the first *Thermus* species discovered to reduce Fe(III) [21, 22] and is the first facultative, thermophilic IRB. The only other thermophilic metal-reducing bacteria are *Bacillus infernus* ,[23] *Thermoanaerobacterium* sp.,[24] and an IRB related to the *Desulfotomaculum*.[6] All were isolated from sedimentary rock cores collected from depths exceeding 850 mbls.

4.2 Mining Contamination vs. Indigenous Communities

One of the most intriguing results is the presence of DNA from cyanobacteria and *Caulobacter* in the C-leader samples. Cyanobacteria are predominant organisms in many mat communities and *Caulobacter* are often found in low-nutrient aquatic environments associated with algae. Although the Carbon Leader has often been considered to represent a relict, Archean

microbial mat, the survival of microbial mat organisms or their DNA during the peak metamorphic conditions of 300±50°C seems unlikely.

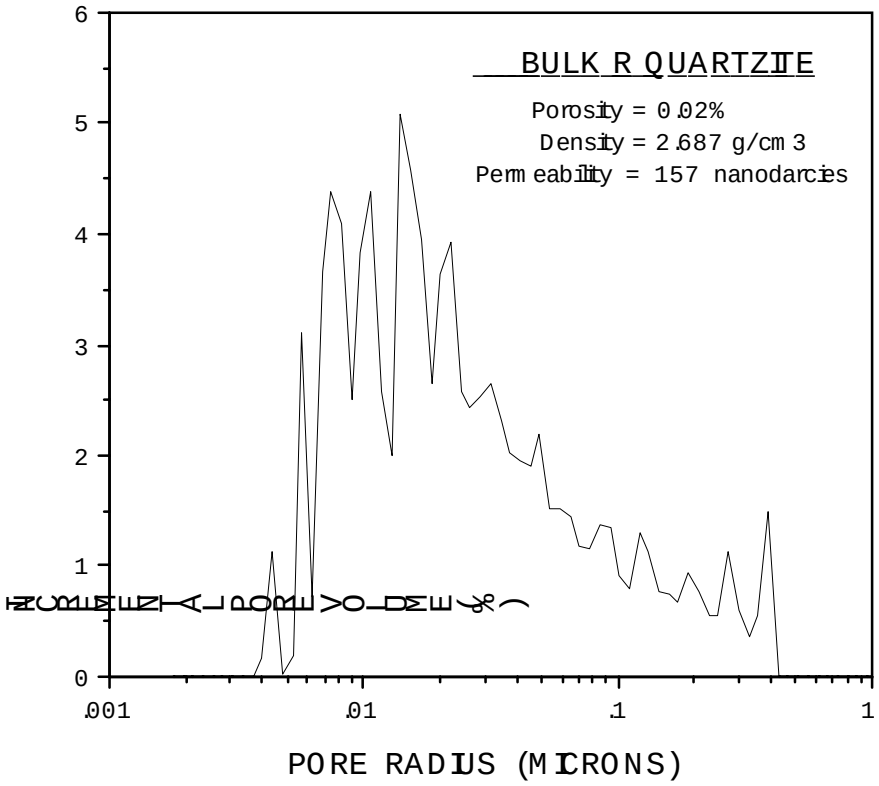

Fig. 6. Petrophysical data for Bulk R quartzite sample. Porosity is derived from He saturation and the pore radius distribution is determined by Hg injection.

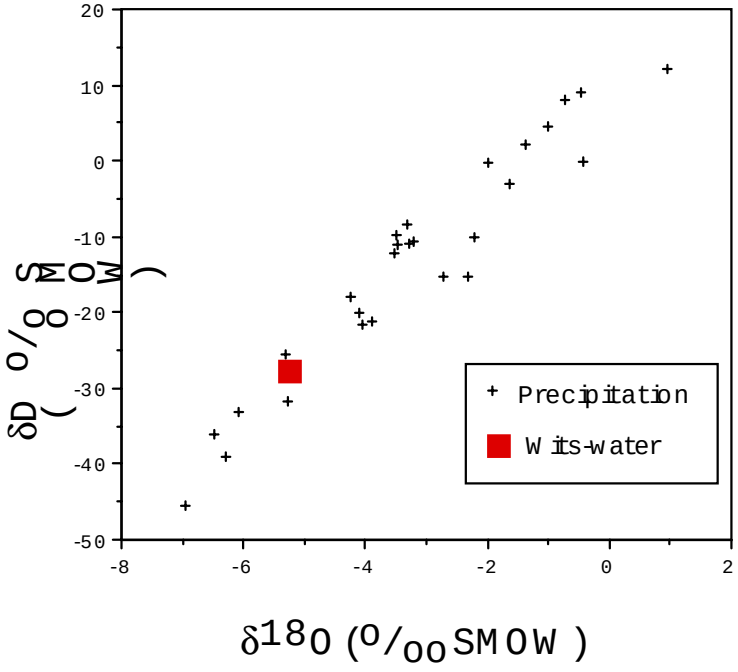

Fig. 7. The dD and d18O of the water sample collected at level 109 in Western Deep Levels mine shaft #1 compared to that of recent precipitation as recorded in Pretoria, South Africa and Harare, Zimbabwe.

If the cyanobacteria represent contamination, then their presence in the low permeability matrix of Bulk R 1B must be explained. The cyanobacteria could be growing on surfaces near the well-lit elevator entrances and could drain to the base of the shafts into the water reservoir (Fig. 2b). The mining water and cyanobacteria could then have been pumped to the mined panels and hydraulically injected into the rock during the percussion drilling for dynamite charges. If the microbial communities in the mining water are dominated by cyanobacteria, then they provide a readily detectable, field contamination tracer[25] in future studies. The C-Leader and Bulk R samples yielded distinctly different colonies of aerobic microorganisms and some fungi, indicating that not all of bacteria could have originated from a single source of contamination.

The best candidates for indigenous microorganisms are the apparent SRB and IRB clones 2B29 and 2B5 from Bulk R 2B. This would be consistent with the detection of sulfate and sulfide in the water sample and of SRB in the Rock Slime. Indigenous SRB could also explain the occurrence of spherical aggregates of framboidal pyrite in the rock. IRB can precipitate Au and U[26], and metal-reducing bacteria are believed to play a key role in roll-type uranium deposits.[27, 28] The filamentous structure of the Au in the Carbon Leader[4, 7] would be consistent with precipitation around bacteria with sheath-like membranes,[29] such as *Thermus filiformus* .[30] Additional sampling to minimize contamination through fractures in conjunction with the use of tracers and controls are needed to distinguish indigenous subsurface bacteria from contaminants

4.3 Hypothetical mechanisms supporting long term, subsurface microbial activity

If the filamentous mineralization in the Carbon Leader is associated with active bacterial processes, then what would be the ecological nature of this hypothetical, indigenous microbial community? How would such a community arise in low permeability rock given that the 300±50°C maximum paleotemperature exceeds the known limit for microbial life (120°C)?

4.3.1 Radiotrophic Subsurface Microbial Communities

Any microbial community residing within the Carbon Leader would have to be resistant to the gamma radiation resulting from K, U and Th decay. The ambient gamma radiation flux in the Carbon Leader can attain values as high as 10^6 rads/gm-yr (10^4 Gy/gm-yr). *Deinococcus radiodurans* can survive this dosage (15,000 Gy without lethality or mutation), because of its efficient DNA repair machinery[31]. *Thermus* is phylogenetically related to *Deinococcus* and the *Thermus* sp. isolated from the water sample may exhibit similar radiation tolerance (research in progress).

If the radiation flux is not at a lethal dosage for the bacteria, then radiation may provide an important energy resource. This energy is transferred to the bacteria, primarily by the radiolytic conversion of water in the pores, fractures, and clays to H_2, O_2, formate and H_2O_2.[32] H_2O_2 is converted to O_2 by catalases or abiotically by the oxidation of Fe(II) in the clay or water. O_2 can also be converted to sulfate by the abundant pyrite present in the Carbon Leader. A redox gradient may result around the Carbon Leader that supports a spatially zoned microbial community. Depending upon radiolytic production rates, the community could be microaerophilic at its center, with Fe-reduction occurring where Fe(II) interacts with H_2O_2 to produce Fe(III) and sulfate diffusing outward to be reduced to sulfide by SRB. This may explain the high cell counts and PLFA concentrations. At the distal margins of the community, the outward diffusion of H_2 and CO_2 could stimulate autotrophic activity. H_2-based lithoautotrophic microbial communities have been reported for the confined aquifer of the Columbia River Basalt in the NW USA where one possible source of H_2 is the alteration of the basalt.[33] We hypothesize that radiolysis of water is another potential reaction that can contribute to lithoautotrophy in the subsurface. Interestingly, IRB-SA was initially enriched in medium with H_2 as the only exogenous energy source. Studies are currently underway to determine if IRB-SA can grow autotrophically with H_2 coupled to Fe(III) reduction.

The low permeability of the adjacent quartzite and the thickness of the Carbon Leader suggest that if such a community exists, it occurs on the scale of tens of centimeters. This type of subsurface microbial environment may be common in Archean age sedimentary deposits, which are frequently rich in U. The ambient temperatures in the deep mines at Western Deep Levels is

also comparable to that predicted for the exterior of high level radioactive waste containers[14]. Consequently, many of the biogeochemical processes postulated to occur around buried, radioactive waste canisters, may be taking place where the Carbon Leader is highly radioactive.

4.3.2 Geological Origin of the Subsurface Microbial Macrocosm

Fluid inclusion data indicate that large scale fluid migration did occur during the waning stages of the last thermal/tectonic episode at 2.0 Ga,[19, 34, 35, 36] probably as a result of the formation of the Vredefort impact. Substantial hydrocarbon transport occurred at this time leading to redistribution of the organic matter in the basin and perhaps to the formation of the Carbon Leader.[5] The homogenization temperatures of primary fluid inclusions in authigenic quartz associated with the authigenic Au and calcite in the "basal reef" at Welkom (Fig. 1) range as low as 120°C.[19] If the temperatures continued to decrease, then the Carbon Leader would have been "habitable" very soon after 2.0 Ga. Colonization most probably occurred at this time, because microbial transport would have been enhanced by topographically generated meteoric water flow and by fracture generated permeability.[37, 38]

5. CONCLUSIONS

Rock and water samples collected at 3200 mbls. in a South African Au mine contained viable, thermophilic bacteria. One isolate, IRB-SA, is the first Thermus known to reduce Fe(III) and is the first reported thermophilic facultative Fe(III) reducer. The dominant DNA signature extracted from the Au and U-bearing Carbon Leader is closely related to cyanobacteria. The extent to which these bacteria represent contamination during mining has not yet been quantified. If the bacteria are indigenous to the rock formations, then they may be involved in the precipitation of the Au and could be deriving energy from radiolytic reactions in the water.

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