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The question of uniqueness of ancient bacteria

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Microorganisms are associated with a variety of ancient geological materials. However, conclusive proof that these organisms are as old as the geological material and not more recent introductions has generally been lacking. Over the years, numerous reports of the isolation of ancient bacteria from geological materials have appeared. Most of these have suffered from the fact that the protocol for the surface sterilization of the sample was either poorly defined, inadequate or rarely included data to validate the overall effectiveness of the sterilization protocol. With proper sterility validation and isolation protocol, a legitimate claim for the isolation of an ancient microbe can be made. Biochemical, physiological, or morphological data indicate that these ancient microbes are not significantly different from modern isolates. As the role (decomposition) of modern and ancient microbes has not changed over time, it is probably unreasonable to expect these organisms to be vastly different. A discussion on the reasons for the homogeneity of ancient and modern microbes is presented.
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Introduction

During the last 5 years, several reports have described the isolation of purportedly ancient bacteria from a variety of geological samples [5,13,17,38]. In each case the authors examined progressively older samples beginning with 25–40 million year old (mya) amber [5] and ending with 250 mya salt crystals [38]. These reports have several commonalities. First, each group isolated bacteria from inside geological materials that were subjected to rigorous surface sterilization procedures. Second, all of these researchers worked inside special containment facilities and were able to provide evidence that contamination problems had been minimized. The reports have also been similar in that each reported the isolation of various spore-forming bacilli and other bacteria that were similar to previously described bacterial species.

These latest reports are not the first to describe the isolation of organisms from noncontemporary materials. Kennedy *et al* [16] described a large number of isolations using everything from 166 years old bottles of Porter beer to ancient (25–40 mya) amber and possibly from Permian aged salt crystals. One of the most striking aspects of all of these studies (and invariably the most controversial) is the fact that most of these reportedly ancient microbes have appeared to be similar to present-day organisms. This single aspect is contested largely because it runs contrary to popular belief in that some microbiologists assume that none of the microbes present on the modern earth could have existed during ancient times. Whether that belief is valid or not is open to conjecture. The purpose of this minireview is to examine the techniques used in these studies on ancient materials and to discuss their various strengths and shortcomings. The review also traces some of the arguments for and against the possibility that the microbes being isolated from so many different sources are in fact ancient or if they are actually recent arrivals.

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The question of sterility

Microbiologists in all areas of the discipline utilize a wide variety of techniques and equipment to "sterilize" the samples with which they work. The idea of something being sterile, and its connotation of extreme safety, has become widespread throughout society. In fact, the very concept of "sterility" (the complete absence of all life forms) as an absolute has achieved a rather mythical position within our common lives. However, microbiologists have long realized that there is no such thing as absolute sterility. At the same time, we do have a useful benchmark that has come from the growth of the pharmaceutical and medical industries. Within these industries, a product or piece of equipment can be listed as "sterile" if the manufacturer can document that each individual item has been treated in a manner that assures a sterility assurance level (SAL) of 1×10^{-6}. In this way, an end user understands that there is only one chance in one million that they will be exposed to an infection from use of the product.

When working with ancient materials, documentation of sample sterility has been one of the hardest obstacles to overcome. We believe that this has occurred for several reasons. First, the earliest workers either said nothing at all or simply "claimed" that their samples were sterile without offering any documentation about the SAL achieved. In one instance, an author simply stated that the methodology was beyond question [9].

Previous authors were certainly not oblivious to the need for sterilizing the outside of their crystal or sample. In fact, they often made great attempts to provide some measure of sterility. Part of their problem lay in the inexactness of the techniques used. Table 1 presents a brief summary of these various techniques. One obvious aspect of the information presented in Table 1 is that each succeeding group has relied upon increasingly stringent sterilization techniques as well as increasing numbers of sterility controls. The importance of being able to provide data on the overall effectiveness of the sterilization techniques being used when attempting to isolate microbes from noncontemporary samples cannot be overstated. Vreeland and Powers [36] considered quantified sterility

Table 1 Sterilization methods used in various articles reporting isolation of ancient microorganisms

Author [Ref.]	Year	Sample type	Sample age ($\times 10^6$ years)	Sterilizing agent	Sterility controls or SAL
Lipman [18,19]	1928, 1931	Coal	650	Peroxide, ethanol and heat	Purposefully contaminated rocks
Dombrowksi [9]	1963	NaCl crystals	250	Ethanol (flamed)	None stated
Reiser and Tasch [30]	1960	NaCl crystals	250	Ethanol soak	None stated
Norton *et al* [23]	1993	NaCl	250	Ethanol soak	Orange pigmented organisms inside crystal; pink organism outside — after ethanol only orange remained
Cano and Borucki [5]	1995	Amber	25–40	2% Glutaraldehyde, 10% chloride bleach, 70% ethanol	Amber pieces in media; solution tests; exposed plates
Lambert *et al* [17]	1998	Amber	25–40	Same as Cano and Borucki [5]	Same as Cano and Borucki [5]
Greenblatt *et al* [13]	1999	Amber	125	Same as Cano and Borucki [5]	Same as Cano and Borucki [5]
Vreeland *et al* [36]	2000	NaCl Crystals	250	10 M NaOH, 10 M HCL	Surface streaks on media and all tools; SAL 1×10^{-9}

assurance levels as one of the three most important criteria needed to defend claims about the isolation of microbes, or biological molecules, from ancient materials.

One of the problems with most of the sterilization techniques used by the earliest workers was the fact that they simply assumed that the use of an ethanol flame was an adequate means of sterilization because that is a technique frequently used in microbiological laboratories. There are several problems with the use of ethanol as a sterilizing agent. First, most microbiologists now recognize that ethanol is a better bacteriostatic agent than it is a sterilant [28]. Second, microbiologists generally utilize flaming ethanol in situations where they are working with clean, relatively smooth, nonporous materials such as stainless steel or glass. This is not generally the case with ancient materials, which usually contain small fissures or have attached soil particles. Ethanol was used successfully by Cano and Borucki [5], Lambert *et al* [17] and by Greenblatt *et al* [13] but only following a more rigorous series of sterilants.

Vreeland *et al* [38] and Rosenzweig *et al* [31] have also shown that it is possible to successfully tailor the general sterilization techniques used to the specific sample type being studied to produce a highly defensible and potentially convincing argument for the long-term survival of bacteria inside ancient materials [26]. In this situation, Rosenzweig *et al* [31] made use of the fact that NaCl crystals are insoluble in salt-saturated 10 M NaOH and in 10 M HCl to design a particularly stringent sterilization regimen. Due to the reactivity of NaOH and HCl a similar sterilization protocol may not be suitable for all types of ancient materials. However, in the long run it may be useful for others to investigate additional types of sterilization systems for use with specific samples.

The bottom line is simply that without a clearly effective, preferably quantifiable, sterilization technique it is not possible to defend the origin of any organism isolated from ancient materials regardless of the uniqueness (or lack thereof) of the isolate.

Distribution of microorganisms in ancient materials

There is very little controversy about the distribution of microorganisms around the surface of the Earth. All microbiologists recognize that bacteria can be found in just about every environment. There is considerable interest in determining exactly how many of these ubiquitous microbes we have actually been able to culture and identify. Comparisons between direct observations and viable counts show that current culture techniques recover 0.1% to 10% of the total number of microbes in most environments [2]. Consequently, the majority of bacteria in nature are still to be discovered.

The same thing cannot be said, however, about the distribution of microorganisms in the Earth's ancient sediments and materials. In fact, until the start of the US Department of Energy's (DOE) Deep Subsurface research most microbiologists believed that the viable microbes were limited to growth within the general root zones or, at most, in the top 100 m of the crust [1]. Careful research conducted by the DOE served to change that view, revealing the presence of microbes even in some of the deepest sediments examined [10–12]. In addition to finding microbes in these very deep sediments, the subsurface research also showed that the bacteria represented numerous different microbial groups [11,14]. The current subsurface microorganism collection contains a vast array of isolates representing nearly every genus contained within the domain Bacteria as well as some Archaea [14]. This research also showed that subsurface bacteria are not as homogeneously distributed within subsurface sediments as they are on the Earth's surface. In fact, the DOE researchers documented numerous instances of apparently sterile sedimentary layers sandwiched between other layers that contained large viable populations [3,10,15].

One of the unfortunate aspects of the search for organisms in ancient materials is that there has not been anywhere near as extensive an examination of the distribution and physiological types of microbes within the ancient materials being studied. That is not to suggest that there have been no such studies, only that there have been very few and those have been somewhat incomplete. The first study was conducted in 1919 by Boleslav Namyslowski [22], who examined several underground brine pools located within the Wielcizka mine in Poland. At that time, he found an extensive and prolific population of microbial forms. These organisms ranged from possible protozoa and algae to what were described as various different bacteria. Namyslowski provided detailed descriptions of the microbial population present in every brine sample he studied. Unfortunately, he appears not to have cultured or even attempted to isolate any of the numerous microbes described in the extensive

drawings contained in the manuscript. This early study appears to have been largely ignored during the following years probably because it was written in German and published in an obscure Polish journal.

Aside from the research described in the preceding paragraph, the only distributional study of microorganisms in an ancient formation was conducted by Vreeland *et al* [37]. These researchers had the advantage of having complete access to a single mine to which they could return frequently. This study did not directly address the presence of ancient microorganisms in the salt crystals sampled but did demonstrate that some regions of salt contained detectable microbial populations. However, these small areas were separated by relatively large, apparently sterile beds of salt. These researchers noted that salt brines within the mine invariably contained detectable microbial populations, which appeared to be relatively stable. This microbial distribution is shown in Figures 1 and 2. These data are good indicators of two aspects of research into ancient microorganisms.

First is the difficulty faced by those attempting to reproduce previous research on the isolation of ancient microbes. Many previous studies [9,18,19,22,30] either failed to specify the exact mine locations from which samples were obtained [18,19,22,30], or utilized geological core samples whose locations were poorly described [9]. The distributional data described by Vreeland *et al* [37] and shown in Figures 1 and 2 show that researchers attempting to duplicate such studies might have great difficulty because they would be very likely to sample sterile regions of the same formations. All of these samples were taken within the same geological horizon as determined by their location relative to the main marker bed used to orient the mine. Physical examination of the areas surrounding each set of samples did not reveal any particular differences between any of the sample areas. While chemical analyses were not performed on these samples, extensive analyses of numerous other areas of the mine have shown that a consistent chemical environment exists within all of the salt in the same horizon. This indicates that the salt layers composing each horizon were formed at the same time. It also testifies to the overall stability of the formation and a lack of dissolution/recrystallization by more recent water intrusion. The same statements might not be true if both groups of researchers were examining underground fluids such as brine samples. However, Vreeland and Powers [36] pointed out that moving brines (or even very wet formations) are not suitable for these studies owing to the fact that it is impossible to determine the exact origin of any interformation fluids because these often reach the study site *via* very torturous paths.

A second difficulty is the fact that even when specific sampling locations are known, areas that contained detectable bacterial populations on one occasion frequently appear sterile in a subsequent sampling. This results directly from the requirements of normal mining operations and maintenance. As mining operations progress, they invariably weaken the surrounding rock, creating an area known as the disturbed rock zone (DRZ). This zone has been found to extend as much as 9 m from the mine workings in all directions [4]. Over time, the disturbed rock zones slowly move (creep) toward the open mine areas. To maintain safe

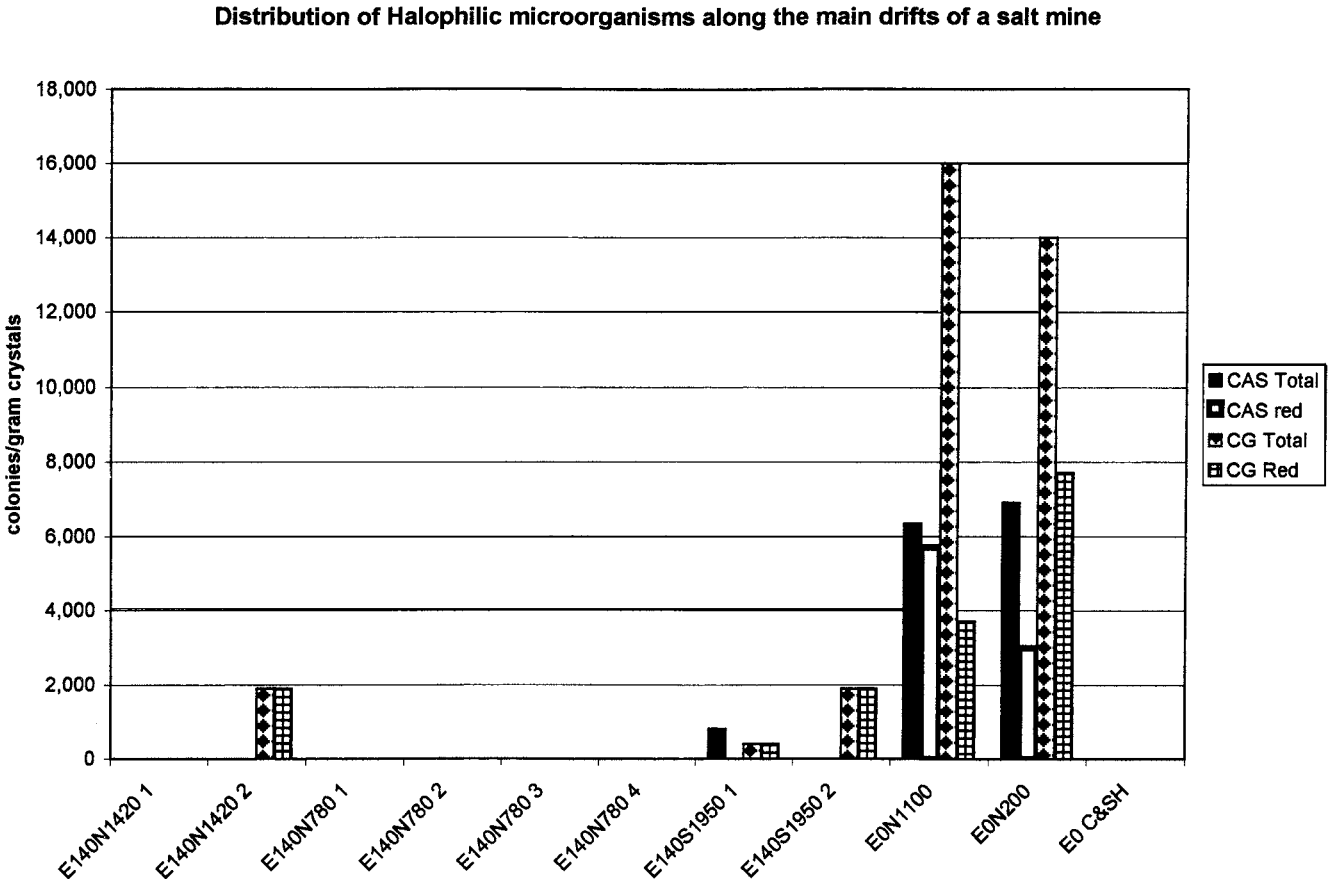


Figure 1 Distribution of viable halophilic bacteria along a single corridor (drift) of a modern salt mine. Data plotted from that of Vreeland *et al* [37].

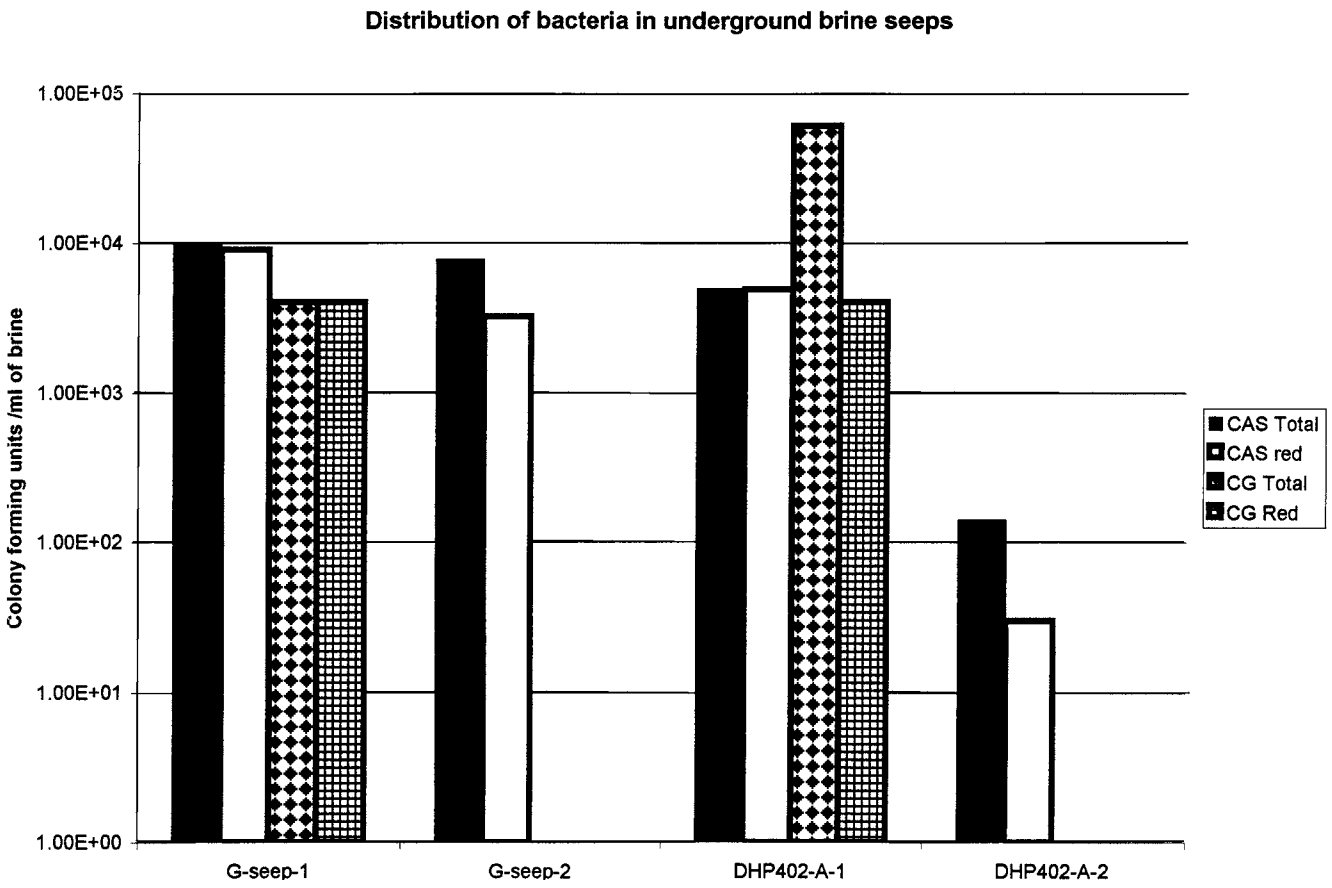


Figure 2 Distribution of viable halophilic bacteria in two small brine seeps of a modern salt mine. Data plotted from that of Vreeland *et al* [37].

working conditions, these disturbed zones, which become increasingly fragmented as they continue to close in on the mine, must be removed from the walls, ceilings, and floors of the mine. Naturally, as the DRZ creeps toward the mine additional DRZ is created behind it. Consequently, the samples taken from a mine in two consecutive samplings have actually come from different regions of the same sedimentary layers simply due to normal maintenance. If the mine is being operated for profit, working faces may move hundreds of meters further into the rock regions between two different samplings.

Assuming that the heterogeneity described by Vreeland *et al* [37] (Figure 1) is indicative of the distribution in many ancient formations, it is easy to see why competent researchers might have great difficulty reproducing work on ancient materials. At present, there is no mechanism able to detect crystals or salt regions likely to harbor viable bacterial populations. This means that even experienced researchers are forced to sample large amounts of material to find the rare samples that have protected trapped microbes.

Biological ages of bacteria

Very few trained biologists will dispute the idea that the macroscopic biota inhabiting planet Earth has changed greatly over the period discernible from the fossil record. While the exact point at which metazoan life forms first appeared on Earth is a matter of some controversy, the fact that present-day animal and plant life bears little true resemblance to earlier forms is not a hot scientific argument. Nor is there a great debate about the fact that microorganisms were probably the first true biological systems, appearing sometime between 3.5 and 3.8 billion years ago [8,39]. Beyond this general agreement, the evolutionary situation becomes far more clouded.

Much of our understanding of the changes that have occurred in Earth's macrofauna over time comes from the extensive instances in which the hard tissues of these organisms (bones, teeth, eggs, etc) have been preserved through fossilization reactions. Having these records has allowed us to develop basic phylogenetic trees in which we can show the progression of groups of organisms as they slowly evolved into other types of life. At the same time, we have been able to use this record to describe and document the development of various groups of dominant organisms. Thus, we know that domination of the Earth's biosphere by such things as insects, dinosaurs and mammalian forms is something that has happened relatively recently. Because of the extent of the fossil records, we can even make similar statements about the plant life that accompanied and supported these various animal groups. Despite the great variety of animals and plants that have populated the Earth, the dominant (and only) life forms present for most of the Earth's 4.5 billion year history were microbes [33].

Unfortunately, the same thing cannot be said about microorganisms or soft tissues or macromolecules, which generally do not leave hard fossils in the manner of bones, teeth and shells. Consequently, our overall view of the evolution of microbes is somewhat biased by our knowledge of what has happened in the

realm of the larger life forms. This was particularly true before the seminal discoveries of Woese and colleagues [40–42] in relation to their studies on small subunit (ssu) rRNA molecules. The volumes of data that have been produced on bacterial ssu rRNA have certainly allowed microbiologists to develop a clearer view of bacterial phylogeny. At the same time, these data have not been particularly useful in determining the timing of this evolution or the biological state of microorganisms during the ancient past. One reason for this has been that we can only speculate on how microorganisms may or may not have changed through time. Unlike fossil-producing organisms, microbes have left an ethereal trail that must somehow be deciphered from the current biochemical and molecular status of microorganisms. Because there are no datable events at which we can unequivocally say that a group of specific microorganisms appeared for the first time or were present for the last time, we really have very little hard information about bacterial development.

From a practical standpoint, the primary function of microorganisms in Earth's biosphere is simply a constant recycling effort. Microbial systems using various oxidation–reduction couples are able to establish biogeochemical cycles for virtually every material found on the planet [45]. In addition to establishing simple cycles for elements such as sulfur and phosphorous, microbes mediate the complex cycles for nitrogen and carbon. This ongoing, continuous process is recognized as one of the most important aspects for maintaining life on planet Earth. When considering the basic aspects of these cycles it becomes obvious that neither animals nor plants would have the ability to completely mineralize all of the components of a biological system. The need for microbes to keep such cycles turning is therefore obvious. Considering the long history of life on Earth and the rise and fall of the numerous groups over thousands of millennia, one can recognize that microorganisms have undoubtedly played their role as mineralizers for many, many years. The point here is to recognize that while the specific sequences, or structures of the proteins, lipids etc. of animals throughout history may have differed to some degree, these materials were still fundamentally the same and would have been mineralized by microbes using common pathways.

Wyckoff [43] and Paabo *et al* [25] studied a wide variety of fossils that appear to have preserved organic fractions. Wyckoff [43] recognizes that while the structural features that give a protein its function are rather labile and are therefore lost rapidly following the death of the organism, the amino acids that composed the molecule are stable and tend to remain for long periods of time. The data in Table 2 illustrate the amino acid composition of a variety of ancient eggshell proteins compared to similar modern materials. These data illustrate quite clearly that microorganisms degrading such materials in the distant past would have dealt with essentially the same amino acids present today.

A similar argument holds for both carbohydrates and lipid materials. The data presented by Wyckoff [43] illustrate that the primary shell material of invertebrates was most likely typical chitin [43]. Further, most of the available data point to the presence of palmitic, stearic, palmitoleic, myristic, lauric and oleic acids in decreasing concentrations in fossilized materials. Thus, the ancient organisms at the biochemical level probably resembled modern day fauna [43]. Consequently, the catabolic pathways that were useful to microbes in the Silurian (ca 500 mya) or even earlier have probably remained useful into the present. This would argue that microbes isolated from ancient materials might present very similar biochemical profiles compared to their modern day relatives.

Table 2 Amino acid comparison of various modern and fossil egg shells

Amino acid	Recent ratite birds	Fossil ratites		Reptiles	
		Aepyomis	Ornitholithus	Modern	Dinosaurs
Asp	9.2	10.6	11.6	8.7	8.4
Thr	4.9	3.5	8.7	6.9	6.3
Ser	8.1	3.4	6.2	7.7	8.2
Glu	10.4	13.7	10.9	8.0	11.5
Pro	7.6	3.7	6.7	10.2	5.9
Gly	9.5	8.2	9.8	7.6	13.3
Ala	8.1	10.7	13.3	6.3	11.2
Val	4.0	7.4	7.8	6.5	7.9
1/2 Cys	3.3		0.2	6.1	1.4
Met	0.4	0.6	0.8	1.1	1.4
lleu	3.5	5.5	4.3	3.9	4.8
Leu	8.4	13.1	9.4	5.8	9.3
Tyr	4.1	4.1	1.9	6.7	2.7
Phe	4.0	5.8	3.4	3.2	3.9
Orn	0.4		0.3	0.0	0.6
His	2.7	0.9		3.0	1.5
Lys	4.2	4.2	4.2	3.7	3.2
Arg	7.6	6.6		4.6	5.1
B-Ala	0.1			0.0	0.0

Averages calculated from data presented by Wyckoff, 1972 [43] (values in mole percent).

In recent years, more data comparing ancient and modern DNA sequences of mitochondria and other materials have become available. As described by Paabo *et al* [25], these short DNA pieces are far from being complete or unaltered. At the same time, however, many do retain sufficient integrity so that short sequences can be amplified for analyses. These DNA pieces show that while differences exist, there is widespread homology between the mitochondrial DNA of both extinct and extant vertebrates [25]. While the existence of these short sequences is useful for studying evolution within larger organisms, a similar situation does not exist for microbes. Further, the molecular archeologists studying amplified DNA sequences have the additional advantage of working with accepted ages within the fossils. Consequently, molecular archeologists may ultimately be able to provide a reasonable calibration for the molecular clocks of higher life forms. Again, this is not the case for microorganisms, due mostly to the fact that there is no way to guess at the DNA or RNA sequences of microorganisms from very ancient materials.

Perhaps the most pervasive of all modern techniques being used to characterize bacteria is phylogenetic analyses using 16S rRNA sequences. This molecule has the advantage of being present in all organisms, is relatively easy to handle, has sufficient size to provide detailed information and has portions whose sequence is highly conserved even in the most distant of organisms. At the same time, this molecule has several regions that appear to change [29]. This aspect has led to the development of a conceptualized molecular clock postulating a 1% change in the sequence of the ssu rRNA gene every 50 mya [24]. One problem may be that these calculations are based upon the sequence divergence existing between related species of the endosymbionts of insects. While this molecular chronometer may be a useful idea, because it would allow for the determination of divergence times between organisms, it may not hold for all microbes. This would be particularly true for free-living bacteria

that subsist within highly oligotrophic soils and waters of Earth. Due to the very low nutrient content found in most of the natural environments of Earth, free-living microbes quite literally exist in a state of almost continuous starvation. Morita [20] and Phelps *et al* [27] presented evidence indicating that the average doubling times for bacteria in nature may be on the order of centuries rather than the hours normally calculated in the laboratory. If this is true, it would mean that estimates of sequence divergence-based endosymbionts living in the high-nutrient environment of a eukaryote could be off by orders of magnitude.

Another aspect of microbes that would alter the perceived rate of molecular clocks is the ability to exchange genetic material with other microbes. This would be especially true of endosymbionts that live within close proximity to other microbes. Schierup and Hein [32] recently showed that even small amounts of DNA recombination within viruses invalidate the typical likelihood ratio tests used to test for the existence of molecular clocks.

Yousten and Rippere [44] completed an analysis of the DNA similarity existing between the *Bacillus sphaericus* strain isolated from amber [5], and the type strains of the other members of this genospecies. These analyses demonstrated that an 80% similarity existed between the DNA of the amber strain and group II of the *B. sphaericus* complex. At the same time, the DNA similarity of the amber isolate to several of the other DNA groups in the genospecies was higher than that of the type strain to those same groups. Yousten and Rippere [44] concluded that while their results supported the interpretation that the amber isolate was indeed *B. sphaericus*, their data could neither confirm nor disprove the ancient origin of this bacterium. They further concluded that there appears to be no definitive biological test that could do so, stating that such claims can only be based upon the adequacy of the precautions taken during the original isolation. Finally Cournoyer and Lavire [6] showed that evolutionary questions about the divergence of strains of a single species cannot be adequately resolved using a data set based upon sequences of a single gene. In the study conducted by these authors, the divergence of infective strains of *Frankia* species infecting different plant hosts could not be determined from differences within the 16S rRNA molecule alone. Rather, the resolution required a combined data set using other genes.

Should we expect unique microbes?

As is probably obvious by this point, the main thesis of this discussion has been that ancient materials appear to contain bacteria that are similar to organisms present on the modern surface of the planet. While this goes against what might be considered as basic current theory, the previous sections within this paper have presented several reasons why it might be time to rethink this argument. At the same time, there are other data available that would begin to indicate the basic validity of this statement.

The beginning of this paper presented a brief discussion of the historical aspects of previous research that, following their publication, had been generally discounted for reasons involving sterilization protocols. At the same time, an interesting aspect that has come out of the numerous subsequent studies is that the primary reason these studies were ultimately rejected was based largely upon the fact that the researchers simply found microbes that were similar to "modern" organisms. Yet, even though the sterilization techniques and the supporting geochemical data have become more sophisticated or defensible, researchers have continued to find microbes similar to those present on Earth's surface.

Therefore, the real question must be whether or not uniqueness should be a prerequisite for acceptance of a particular microorganism as being ancient. First, we must look briefly at the available data starting with what might be considered the first experiments in which the claim was that the isolated microbes had been trapped within the sample and were therefore as old as the original rock [9].

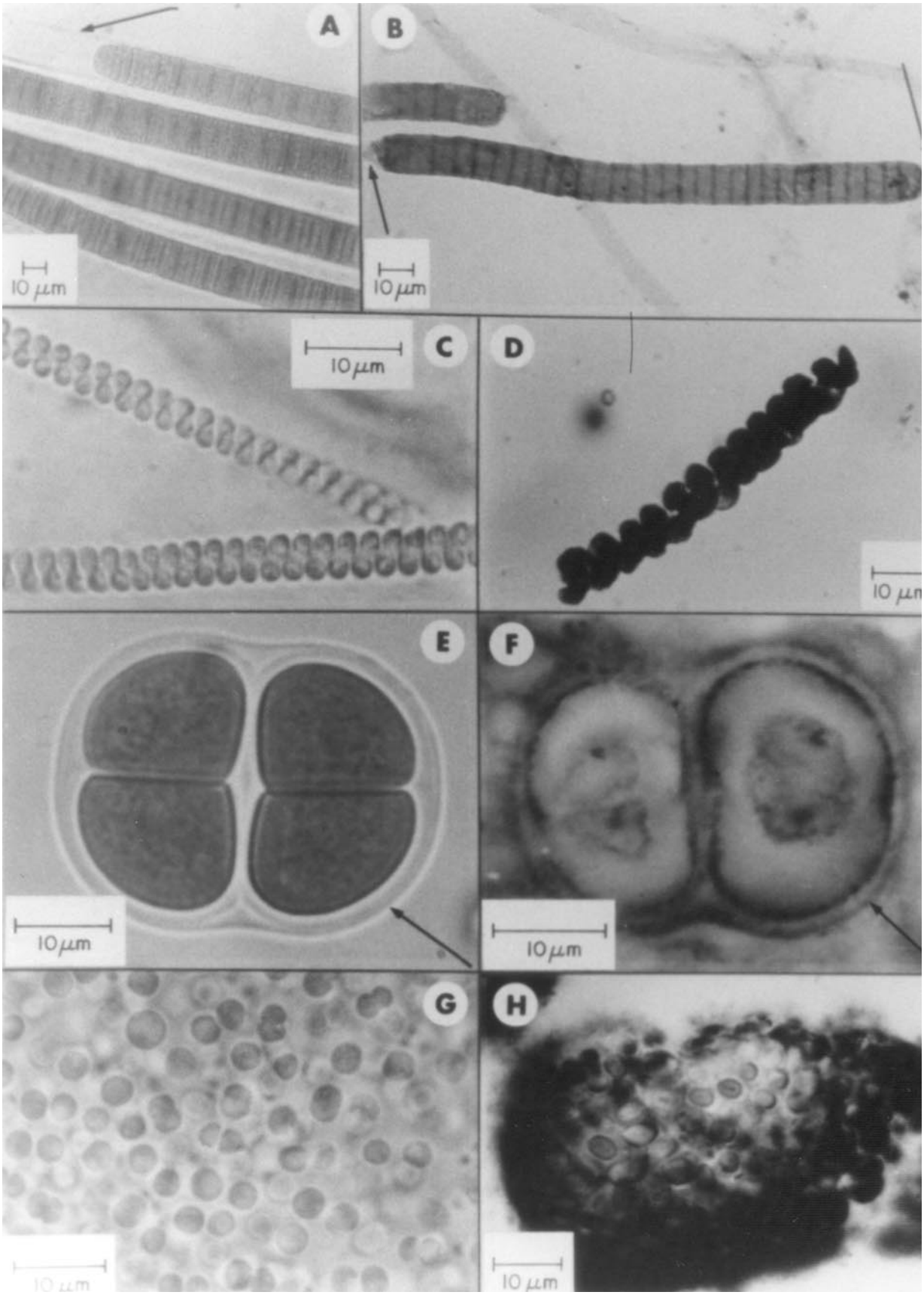
In choosing this particular article as the first real claim, we have discounted the work described by Lipman [18,19] for several reasons. First, the sterilization techniques described in this 1931 article were so impossibly extreme [autoclaving the sample for 12 h, followed by heating it in a bunsen burner] it is difficult to see how any microbe could have survived. Second, within the entire manuscript the author presented no data or statistics. He simply claimed that the details and data were too extensive to be discussed. Finally, the author never provided even a rudimentary description of the organisms found.

In his reports, Dombrowski [9] claimed to have isolated a new species of *Pseudomonas* (*Pseudomonas halocrenea*). Following the publication of Dombrowski's work, numerous groups began publishing information refuting his claims. Many of these refutations were based upon molecular comparisons of *P. halocrenea* and what were deemed modern pseudomonads. When *P. halocrenea* proved to be similar to other pseudomonads, Dombrowski's claims were criticized as being nothing more than contamination. The reality is that the sterilization and other methods used by Dombrowski [9] were not sufficiently documented to allow him to defend his data. The basic truth is that none of the data provided by the critics actually prove that *P. halocrenea* is a modern organism. All their data show is that *P. halocrenea* was similar to organisms present on the Earth's surface.

In more recent research Stan-Lotter *et al* [34,35], while not claiming antiquity for their isolate, provided a detailed comparison of the ATPases of *Halorubrum saccharovorum* and a halophilic strain (54R) they isolated from British rock salt that had been soaked in ethanol for several hours. These authors showed that these enzymes, while having some differences, are in fact very similar to one another in size, amino acid sequence and in activity. In this study, the primary differences were in the levels of sensitivity to inhibitors, and the slightly higher number of acidic amino acid residues in the ATPase from strain 54R. Otherwise, these enzymes were identical.

In a separate study, Stan-Lotter *et al* [35] compared the strains of the halophilic coccus *Halococcus salifodinae* isolated from salt formations in Germany and England. Once again, while these authors did not claim that these isolates were actually as old as the salt formations, they were able to show that the different strains were virtually identical in molecular, chemical and physiological characteristics. A similar, more expansive, physiological comparison conducted by Vreeland *et al* [37] showed that underground salt formations do in fact contain a wide variety of halophilic organisms. Based on physiological tests, Vreeland *et al* [37] demonstrated that some of these microbes were different from other halophilic organisms, but the large majority of these bacteria were actually similar to halophiles isolated from surface sources.

This is true of the bacteria that have now been isolated from several different samples of amber. A variety of bacteria have been isolated from different amber sources since the first announcement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by Cano and Borucki [5]. While a few newly recognized species have been found, the organisms isolated from inside these samples have invariably been shown to belong to commonly recognized genera including *Staphylococcus, Bacillus, Clostridium* and others [5,13,17]. In short, based upon all of the available information from very careful studies, it would appear that the bacteria that were present on Earth in ancient times are nearly identical to some of the organisms around today.

It might be best to end this particular argument by briefly playing "devil's advocate." Let us suppose that in one of these studies the researchers were actually able to isolate a truly unique microorganism from within an ancient sample. This isolation could occur either from the use of different media or primers or even from just plain luck. Nevertheless, let us assume that this bacterium is sufficiently different from presently identified microorganisms to fit the difference criterion. Once this information is published, it is inevitable that other researchers will adopt either the media, probes or techniques used to find this new bacterium. They will then begin to search other environments. This would of course be encouraged, but suppose a similar organism is found in some other surface environment? Would that mean that now the first researcher, no matter how careful they had been would have to retract all of their work? Would we decide that because the organism had first been found in an ancient material we would then classify the surface isolate as a modern relative, or an evolutionary throwback? Would we decide that the new isolate was some other organism that had begun to experience a backward evolution? The reality is that we should do none of these things. We should look carefully at all of the techniques and data presented. We should accept or reject the age of each isolate based on the strength of the supporting data and techniques used in each of the studies and not upon the idea that the unique organism has now been found in the modern world so it must therefore have been an unrecognized contaminant.

Reasons supporting possible similarities

The reality is that there are many reasons why organisms on Earth's current surface might be direct descendents, and very similar to those present in ancient times.

First and foremost is evolution itself. The basic tenet of all evolutionary discussion revolves around the simple fact that organisms change in response to stress from natural systems. As should be obvious from the foregoing discussion, there is good reason to assume that aside from some temperature changes, the chemical world of the microbe has changed very little over the eons. Microbes, unlike larger fauna, would not be greatly affected by the types of upheavals that split continents or raised mountains. Those changes would certainly force evolution of the larger fauna, but as long as the things upon which nonsymbiotic microorganisms rely remained constant, there would be no real reason to expect changes in free-living microbes. Naturally, this might not be true for pathogens or symbionts whose lives are inextricably tied to the larger forms. These organisms might be expected to change along with the evolution of their host species. The calculated molecular clock [24] is largely based upon just such bacteria.

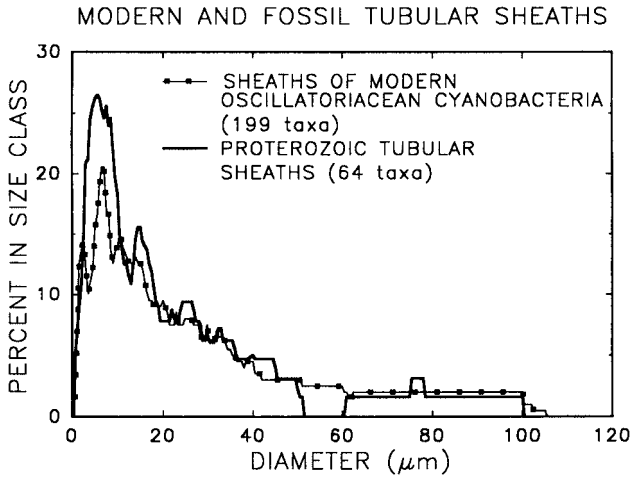

Figure 4 Comparison of the size of the tubular sheaths of living oscillatoriacean cyanobacteria with those in the Precambrian fossil record Reprinted with permission from J. Schopf and Princeton University Press [33].

A second reason would involve the Earth itself. This planet supports life because the planet has an active water cycle, and tectonic movement. The water cycle acts to carry things from one area of the Earth to another. At the same time, the combined action of water and tectonic movement continually erodes old surfaces and exposes new ones. This process happens along coastlines, in the mountains and even below ground. Materials relatively easy to dissolve in water (i.e., salt crystals) are brought to the surface by normal artesian flow where any newly released microbes may be disseminated. Even materials that are not soluble in water may be penetrated by water, or may be brought to the surface by other geologic forces, each time exposing and releasing trapped organisms to the modern surface. In reality, this process would simply carry microbes back to an environment that for many of them would be nearly identical to the one they left.

Crowley and North [7] and Schopf [33] have thoroughly reviewed information about the Earth's paleoclimate. These authors show that over the last 500 million years concentrations of major gases, especially oxygen and carbon dioxide, varied around their present levels. Despite this variation the levels of these two gases were never so different that they would have placed great stress on free-living microorganisms. In addition, chemical analyses of several ancient materials has indicated that simulated January temperatures on most of the Pangean continent were between -10 and 35°C with apparent fluctuations of no more than 10°C except in the extreme southern regions of the continent. Most predictions also indicate that even during the very distant Precambrian eras (a time when only prokaryotes existed) surface temperatures hovered between 60 and 70°C [7]. Perhaps the most telling information relative to this argument is not from chemical analyses or any type of predictive science.

Figure 3 Currently living cyanobacteria from norther Mexico (A, C, E, and G) compared to Precambrian fossils (B, D, F, H), (B) from the 950-mya-old Lakhanda Formation and (D) from the 850-mya Miroedikha Formation, both of Siberia, (F) from the 1550-mya Satka Formation of Bashkiria, (G) from the 2100-mya Supergroup of Canada. (A) Lyngbya sp. compared with (B) Paleolyngbya. (C) Spirulina compared to (D) Heliconema. (E) Gleocapsa compared to (F) Gleodinopsis. (G) Entophysalis compared to (H) Eoentophysalis (scale bars = 10 μm). Reprinted with permission from J. Schopf and Princeton University Press [33].

Rather it is from the fossil record itself. Schopf [33] examined morphological similarities between several modern cyanobacteria and fossilized remnants dating from 2150 to 850 mya (Figures 3 and 4). These ancient fossils have proven to be virtually indistinguishable from their modern day relatives (Figure 3). This includes all aspects of the basic cell including the cell shape, size, division planes and presence of capsules (Figure 3E–H). These and other fossils also show that the size range and distribution of tubular sheaths in living and fossilized cyanobacteria are identical, as are those of trichome fossils compared to present-day trichome-forming bacteria (Figure 4) [33]. Definitive morphological and physiological similarities of microbes are certainly not a guarantee of genetic identity. However, such high degrees of similarity cannot be taken as supporting tremendous genetic changes within critical cellular functions.

The third reason for the possibility of homogenization of ancient and modern microbes is simply the action of man. In the several thousand years of human endeavors, our species has carried all manner of minerals, gems and metals from deep within the Earth to the surface. Industrial processes, or simple use of specific materials ultimately leaves behind wastes that we have disposed upon the Earth's surface. In terms of salt alone, hundreds of millions of tons were removed from beneath Europe and were spread around the globe [21]. In fact, during the 2000/2001 winter season, southeastern Pennsylvania (including Philadelphia and West Chester) planned to spread 84,000 tons of salt on local roadways! Because we now know that the deep Earth sediments contain living microbes, there is simply no way that all of this activity could fail to bring ancient bacteria to the modern surface. Once these organisms reach the surface, they would generally find it is still a hospitable environment and could easily join with their more modern brothers and sisters creating an eclectic mix wherein the ancient bacteria would be biologically indistinguishable from those that have been present on the surface all along.

The implications of the growing number of discoveries of live microorganisms trapped inside ancient geological materials is profound and extends far beyond a debate about evolution or the environment of ancient Earth. As described by Parkes [26], the possibility of bacterial immortality provides a means by which Earth or some other unknown planet could act as a seed for the dissemination of life to other portions of the solar system or universe. It could also provide a means of cross-pollination between living things arising independently on several planets or solar systems. If such events were to occur we could expect that searching a young planet with conditions similar to that of ancient Earth might reveal the presence of microorganisms similar to those known here. However, if we only interpret similarity to mean contamination, the universe could appear to be a rather lonely place.

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