Report

Antimicrobial properties of Dead Sea black mineral mud

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Our work was supported financially by a grant from the "Dead Sea Research Center", a nonprofit organization recognized by the Israel Ministry of Science, which promotes scientific exploration of the Dead Sea area under the auspices of the Ben Gurion University of the Negev Report.

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Background The unique, black, hypersaline mud mined from the Dead Sea shores is extensively used in mud packs, masks, and topical body and facial treatments in spas surrounding the lake, and in cosmetic preparations marketed worldwide, but little is known about its antimicrobiological properties.

Methods We performed detailed microbial and chemical analysis of Dead Sea mineral mud compounded in dermatological and cosmetic preparations.

Results Using conventional bacteriological media (with or without salt augmentation), we found surprisingly low numbers of colony-forming microorganisms in the mud. The highest counts (up to 20,000 colonies per gram, mostly consisting of endospore-forming bacteria) were obtained on sheep blood agar. Test microorganisms (i.e. *Escherichia coli*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Candida albicans*) rapidly lost their viability when added to the mud. Zones of growth inhibition were observed around discs of Dead Sea mud placed on agar plates inoculated with *Candida* or with *Propionibacterium*, but not with *Staphylococcus* or *Escherichia*. The effect was also found when the mud was sterilized by gamma irradiation. Using ^{35}S-labeled sulfate as a tracer, bacterial dissimilatory sulfate reduction could be demonstrated at a low rate (0.13 ± 0.03 nmol/cm^3.d).

Conclusion The antibacterial properties of Dead Sea mud are probably owing to chemical and/or physical phenomena. Possible modes of antimicrobial action of the mud in relation to its therapeutic properties are discussed.

Introduction

The Dead Sea, located at the lowest point of the Syrio-African Rift Valley, is a unique chemical, microbiological, and climatological environment.[1–4] Its surface is currently 417 m below mean sea level. The water of the lake has a salt concentration of 340 g/l; in order of decreasing concentration, its major cations are Mg^{2+}, Na^+, Ca^{2+}, and K^+, while Cl^- and Br^- are the main anions.

Resorts around the Dead Sea attract patients worldwide, who seek a cure for diseases such as psoriasis,[5–7] atopic dermatitis,[8] and rheumatic disorders.[8,9] Treatments are based on two modalities: (1) bathing in Dead Sea water while the skin is exposed to solar radiation from which harmful short-wavelength UV radiation has been effectively filtered out, and (2) mud packs prepared from the highly saline, sulfide-rich, black mineral mud that is found abundantly in the area.[9,10] Deposits of gray to black mud are located at the mouths of the mostly dry beds of the runoff streams that flow into the Dead Sea (from the Judean Mountains in the west, the Jordanian Moab Mountains in the east, and the Jordan Valley in the north).[11] These mud deposits are impregnated with sulfide-rich, anoxic subsurface salt-rich brines that emerge from groundwater, or with water from sulfide-containing hot springs found at the lake's shores.[12] The mud layers were formed in the Holocene era, less then 10,000 years ago. The mineral mud is also extensively used as an ingredient in cosmetic preparations.[13]

For thousands of years the Dead Sea has been known for its medicinal properties. The healing powers of Dead Sea water are mentioned in the Babylonian Talmud (Tractate Shabbat 108b) by Galen (122-c200) and by other old-world historians.[14–16] The first chemical analysis of Dead Sea water was performed by Lavoisier in 1772 and published 4 years later.[17] It is interesting to note that this was only the second documented quantitative analysis of any natural water source. The authors correctly recognized the predominance of alkaline "earth salts" over NaCl, the high concentration of Cl^-, and the presence of both Ca^{2+} and Mg^{2+}. The elements potassium and

bromine were unknown at that time.[17,18] In spite of extensive use of this gray or black mud in dermatological and cosmetic preparations, there is a paucity of information concerning its chemical composition, indigenous microbiological flora, and effect on pathogenic and nonpathogenic microorganisms. We therefore performed this study to gain a better understanding of the antimicrobiological properties of Dead Sea mineral mud.

Materials and Methods

Dead Sea mud samples

Crude (unprocessed) Dead Sea mud, supplied by Ahava – Dead Sea Laboratories Ltd, a commercial regional company that manufactures Dead Sea cosmetics, was collected at the north-western end of the Dead Sea at a distance of 200–400 m from the shore. Samples were obtained at a depth of 40–60 cm below the surface. In a number of the experiments (as indicated later), the mud was processed by adding unsterilized tap water (18% by weight) to the crude mud; the samples subsequently were homogenized and filtered through a 300-μm sieve. Some of the unprocessed and processed samples were sterilized by gamma irradiation (^{60}Co, 2.5 MRad).

Mineralogical and chemical analyses

The mineralogical composition of the mud was determined by X-ray diffraction (XRD). Elemental analysis (of the water-soluble and water-insoluble fractions) was performed using the inductive coupled plasma (ICP) method as described by Kafri *et al*.[19] Water content was determined by drying 100-g samples of mud for 76 h at 105 °C until a constant weight was achieved. The pH of mud slurries (10 g of mud mixed with 50 ml of distilled water) was measured using a Beckman pH meter (Fulletron, CA, USA). In addition, we measured the pH of the supernatant fraction obtained following centrifugation of the mud for 10 min at 10,000 *g*. Sulfide was assayed by iodometric titration, according to the procedure 4500 – S2–F and 4500 SO32–C Phenanthroline Method,[20] or following distillation of the sulfide with a stream of nitrogen (after weighed portions of mud were acidified),[21] trapping the sulfide in 5 ml of 2% zinc acetate, and quantifying the sulfide colorimetrically using the methylene blue method.[22]

Microbial cultures and growth media

Cultures of *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), and *Propionibacterium acnes* (ATCC 11827), and *Candida albicans* (ATCC 10231) were routinely grown on tryptic soy agar (TSA, Difco, San Jose, CA, USA), aerobically at 30 °C. When indicated, inoculated plates were incubated anaerobically by placing them in sealed plastic bags in the presence of AnaeroGen sachets (Oxoid, Hampshire, UK).

The following growth media were employed for microbiological testing of mud samples, as recommended for the analysis of cosmetic products:[23] (1) Modified Letheen Broth (MLB, Difco), a nonselective medium used to dilute cosmetic products for general counts of microorganisms; (2) modified Letheen Agar (MLA, Difco), a nonselective medium for counting microorganisms in cosmetic products; (3) Oxytetracycline-Glucose-Yeast extract Agar (OGYE Agar, Difco), for counting yeast and fungi in cosmetic products; (4) Tryptic Soy Agar (TSA, Difco), a nonselective medium for general counts; (5) Sheep Blood Agar (SBA; CHAI Laboratories, Rehovot, Israel), which consists of TSA supplemented with 5% sheep blood, a nonselective medium for examination of the hemolytic capacity of microorganisms; (6) Plate Count Agar (Difco); (7) MacConkey Agar (Difco), a selective medium for enteric Gram-negative bacteria; (8) Cetrimide Agar Base (CAB, Difco), a selective medium for pseudomonads; and (9) Baird-Parker Agar (BP, Difco), a selective medium containing egg albumin, tellurite, glycine and pyruvate, designed for isolation of *Staphylococcus aureus* from cosmetic products. In some experiments, TSA, MLA and OGYE media were supplemented with 10% NaCl. Additional media used for microbial enumerations were: (10) yeast extract (2 g/l), amended with different concentrations of NaCl and/or $MgCl_2 \cdot 6H_2O$ (up to 100 g/l of each). At salt concentrations of up to 50 g/l, the agar concentration used was 15 g/l, and at higher salt concentrations this was increased to 20 g/l; (11) Brewer thioglycolate medium (Difco), with or without NaCl or $MgCl_2$ amendment, as indicated. Nine-ml aliquots of this medium were dispensed into screw-capped test tubes; and (12) medium designed for extremely halophilic *Archaea* from the Dead Sea (such as *Halorubrum sodomense*) containing (g/l): NaCl, 125; $MgCl_2 \cdot 6H_2O$, 160; K_2SO_4, 5; $CaCl_2 \cdot 2H_2O$, 0.1; yeast extract, 1; casamino acids, 1; starch, 2; pH 7.0. This medium was used both in liquid form and in solidified form with 20 g/l of agar.

Enumeration of microorganisms in Dead Sea mud

Portions (1 g) of mud were diluted with 10-ml sterile phosphate buffer (0.01 M KH_2PO_4, sometimes supplemented with 10% NaCl, adjusted to pH 7.0 with KOH) or in MLB, as indicated. Ten-fold dilutions were prepared in buffer, then 0.1-ml aliquots were spread evenly on the surface of the agar by means of a sterile plastic rod. Plates were incubated aerobically at 30 °C for up to 7 days. Anaerobic incubation conditions were obtained by placing the plates in sealed plastic bags in the presence of AnaeroGen sachets (Oxoid, Hampshire, UK). For enumeration of fermentative anaerobes in Brewer thioglycolate medium, tenfold dilutions of mud were made directly in the tubes containing 9 ml of viscous liquid medium, and the results were examined for up to 2 weeks.

To test for survival of known microorganisms, mineral mud samples (20 g) were placed in sterile 100-ml plastic cups and mixed with a 2-ml suspension of the culture to be tested in sterile 0.25 M phosphate buffer, pH 7.0. Suspensions of *E. coli*, *S. aureus* and *P. acnes* were adjusted to an optical density of 0.1 at 550 nm; the suspension of *C. albicans* had an optical density of 1.0. After different incubation times at 30 °C in a moisture-saturated incubator, 1-g samples were withdrawn, 10-fold dilutions in sterile phosphate buffer were prepared as described earlier, and 100-μl aliquots were spread onto TSA plates in four replicates. Plates

were counted after 3 days of incubation at 30 °C. In some of the experiments, plates for the enumeration of *P. acnes* were incubated under anaerobic conditions.

Measurement of bacterial dissimilatory sulfate reduction

While avoiding air contact to the extent possible, six samples of Dead Sea mineral mud were collected in 5-ml syringes. After injection of ^{35}S-sulfate (approximately 100 kBq), the six syringes were closed with butyl rubber stoppers. The syringes were then placed in a large bottle containing anoxic dithionite-reduced water (to make sure that no oxygen was present during incubation) and incubated in the dark at room temperature. Incubation was terminated by quickly transferring the samples to 5 ml of a 5% zinc-acetate solution. Three samples were fixed after 12 h and served as controls, while the remaining three were incubated for a further 56 h. After fixation, the samples were stored at –20 °C. After each sample was centrifuged (10 min, 3000 *g*), the supernatant was removed and the sulfate concentration was measured on a Sykam ion chromatograph system equipped with a polystyrene-based LAC A14 column and a S3111 detector (Sykam, Gewerbering Eresing, Germany). The samples were distilled according to the single-step chromium reduction method of Fossing and Jørgensen.[24] Finally, the amount of radioactivity in the supernatant and the pellet (i.e. the sulfate and the sulfide) was quantified in a liquid scintillation analyzer (Tricarb, Wellesley, MA, USA).

Antimicrobial activity of Dead Sea mud

Antimicrobial activity of Dead Sea mud was tested on TSA plates on which suspensions of test organisms (0.1 ml of *E. coli*, *P. acnes* or *S. aureus* in 0.25 M phosphate buffer, pH 7.0 at OD_{550} of 0.01 or 0.001; OD_{550} of 0.1 for *C. albicans*) were evenly spread. Discs of 0.1 ml of Dead Sea mud were prepared by filling sterile plastic 1-ml syringes with mud, and using the piston to press out 0.1-ml portions; a sterile knife was then used to cut the discs. Three hours after inoculation with the test organism, up to 4–5 discs were placed on the surface of the agar plates and incubated for 36 h at 30 °C, and the appearance of inhibition zones around the discs was recorded.

Results

Chemical analyses

The consistency of Dead Sea mineral mud is that of fine clay. Its mineralogical composition is presented in Table 1. The crude mud was 35.6% water, as determined by measuring the difference in weight of the mud before and after it was dried at 105 °C. The density of the mud was 1.67 g/cm^3, and the pH of the slurry sediment was 6.4. Table 2 shows the results of chemical analysis of crude mineral mud. The concentration of sulfate, as assessed in the course of the sulfate reduction experiments according to procedure 4500 – S2–F and 4500 SO_3^{2-}C Phenanthroline Method,[15] was 3.1 mM. Sulfide was present in crude, processed and irradiated Dead Sea mineral mud in concentrations of approximately 700, 300, and 300 p.p.m., respectively. The content of organic matter was nearly 4%.

Table 1 Semi-quantitative mineralogical composition of the clay fraction (< 2-μm particle size) of Dead Sea mineral mud (values in percentage by weight)*

Mineral	Percent by weight
Illite–semectite phases	50–70
Kaolinite	10–20
Illite	10–15
Calcite	5–15
Quartz	1–5
Chlorite	< 5
Palygorskite	< 5

*Results are mean values of X-ray diffraction analyses of two separate aliquots of the same sample of crude mineral mud. One was analyzed after washing with distilled water, and the second after treatment with dilute HCl and H_2O_2. Analyses were performed at the Geological Survey of Israel, The Ministry of National Infrastructures, Jerusalem.

The results of our elemental analysis of the interstitial water fraction of the mud are summarized in Table 3. The interstitial water is hypersaline brine, with a salt concentration comparable to that of Dead Sea water, but differing in its ionic composition. Notably, the solution is higher in potassium and lower in calcium than Dead Sea water. Its pH was 5.6–5.7.

Microbial counts and sulfate reduction activity in Dead Sea mineral mud

The numbers of colony-forming microorganisms recovered from the mud are shown in Table 4. The highest counts were consistently obtained on media of low salinity; inclusion of NaCl and/or $MgCl_2{\cdot}6H_2O$, in concentrations of up to 150 g/l, each greatly reduced colony recovery. Endospore-forming bacteria were most commonly encountered; *Bacillus*-type morphologies were found in aerobic media, and *Clostridium*-type cells in Brewer thioglycollate medium. No growth was obtained in a high $MgCl_2$-high NaCl medium designed for *Halorubrum sodomense* and other halophilic *Archaea* found in the Dead Sea. The highest counts were obtained using sheep blood agar (up to 2×10^4 colonies per g of mud with aerobic incubation, 7×10^3 following anaerobic incubation). Other rich, nonselective media yielded only a few hundred colony-forming units per gram of mud. These values are several orders of magnitude lower than counts those found in samples of common soils (results not shown). No growth was recorded on BP and cetrimide agar plates, designed for

Table 2 Dead Sea mineral mud (dry weight 77.5%) was analyzed by inductive coupled plasma (ICP AES and ICP MS) methods and wet analytical methods as described by Kafri *et al.*[14] Analysis was performed at the Geological Survey, Jerusalem, The Ministry of National Infrastructures of The State of Israel, on 23.9.2003 by Dr Olga Yoffe and approved by Dr Ittai Gavrieli, Director of Geochemistry and Environmental Geology Division

Content in percentage	Major minerals
20	SiO_2
15.5	CaO
4.8	Al_2O_3
4.5	MgO
2.8	Fe_2O_3
1.7	Na_2O
1.3	K_2O
0.5	TiO_2
0.4	SO_3
0.3	P_2O_5
6.7	Cl
0.2	Br
Content in PPM	**Trace elements**
550	Sr
250	Mn
200	Ba
75	Cr
60	Zn
60	V
40	Ni
25	Li
20	Cu
8	Co
4	Pb
3	Th
2	As
2	U
2	Mo
1	Sn
< 1	Ag
0.7	Be
0.6	Cd
0.2	Sb

Table 3 Chemical analysis of the interstitial water of Dead Sea mineral mud. Crude Dead Sea mineral mud was centrifuged (10 min, 12,000 g) to extract the liquid phase. The liquid phase was analyzed by at the Geological Survey of Israel, The Ministry of National Infrastructures, Jerusalem, by inductive coupled plasma methods (ICP) as described by Kafri *et al.*[14] The concentrations of major ions in Dead Sea water (mean values for 1996) are provided for comparison

Dead Sea water (1996) (mg/l)	Interstitial water (mg/l)	Macro-elements
230,400	140,000	Cl + Br
45,900	28,250	Mg
36,600	20,200	Na
17,600	9800	Ca
7800	9500	K
	150	Sr
	< 1000	SO_4
	µg/l	Micro-elements
	9800	Mn
	41.08	Ni
	0.69	Co
	2.14	Cu
	16.0	Zn
	0.2251	Y
	0.132	Cd
	0.1968	La
	0.3198	Ce
	0.02716	Pr
	0.11439	Nd
	0.02522	Sm
	0.00836	Eu
	0.00578	Tb
	0.03936	Gd
	0.03567	Dy
	0.00787	Ho
	0.01968	Er
	0.00221	Tm
	0.01292	Yb
	0.00197	Lu
	0.55	Pb
	1.99	U

the selective isolation of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively.

The rate of dissimilatory sulfate reduction, measured using ^{35}S-labeled sulfate as a tracer, was 0.13 ± 0.03 nmol/cm^3.d, indicating that some type of prokaryotic activity persisted in the mud.

Bactericidal effect of Dead Sea mud

When suspensions of *E. coli*, *P. acnes* or *C. albicans* were mixed with Dead Sea mud, the number of colonies that could be recovered declined rapidly. After 3 days of incubation, limited numbers of *C. albicans* could be recovered, while the numbers of *E. coli* and *P. acnes* had declined by 3–4 orders of magnitude (Fig. 1). In processed (diluted, filtered) mud, with or without radiation induced sterilization, the decline was less pronounced. In another experiment, comparing the decline in viable numbers in unprocessed mud with or without radiation-induced sterilization, no significant differences were observed between the survival of organisms in sterilized vs. unsterilized mud (results not shown). There was only a slight decline in colony-forming numbers of *S. aureus* following exposure to Dead Sea mud.

One possible explanation for the differences observed in survival between crude and processed mud is the difference in salinity. However, another potentially significant difference between the two types of mud is their concentrations of

Table 4 Numbers of colony-forming bacteria recovered per gram of crude Dead Sea mineral mud on different growth media incubated under different conditions. Portions (0.1 ml) of dilutions of Dead Sea mud in 10 mM phosphate buffer, pH 7.0, were streaked on agar plates, and incubated at 30 °C. Colonies were counted after 3 days. Numbers of colony-forming units are presented as mean values ± standard deviation (n = 3–4)

	Incubation conditions		
Medium	**Aerobic**	**Anaerobic**	**Comments**
TSA	375 ± 150	325 ± 50	Sporeformers
TSA + 10% NaCl	< 100	50 ± 57	
MLA	400 ± 182	150 ± 57	
MLA + 10% NaCl	< 100	< 100	
OGYE	< 100	< 100	
OGYE + 10% NaCl	< 100	< 100	
CAB	< 100	NT	
MacConkey	< 100	NT	
BP	150 ± 130	NT	Not *Staphylococcus aureus*
PCA	125 ± 50	50 ± 57	
SBA	20,750 ± 3400	6900 ± 1840	Sporeformers

TSA = tryptic Soy Agar; MLA = modified letheen agar; OGYE = oxytetracycline-glucose-yeast extract agar; CAB = cetrimide agar base; BP = Baird-Parker agar; PCA = plate count agar; SBA = sheep blood agar; NT = not tested.

sulfide. Measured iodometrically, the sulfide concentration was 700 p.p.m. for crude mud, while diluted and irradiated mud contained only 300 p.p.m. sulfide (Fig. 1). The difference was owing to oxidation of sulfide by oxygen in the dilution water and in the air to which the mud had been exposed during the manipulations. To test whether the difference in sulfide concentration may explain the differences in observed survival, we examined the sensitivity of the test organisms to sulfide exposure. Tubes with 5 ml of TSB containing different sulfide concentrations (added as $Na_2S{\cdot}9H_2O$), and controls without sulfide, were inoculated in triplicate with the test organisms; growth was recorded after incubation at 30 °C for 5 days. The minimal inhibitory concentrations of sulfide for *C. albicans*, *A. niger*, *E. coli*, *P. acnes*, and *S. aureus* were 64, 256, 1, 1, and 2 p.p.m., respectively. Although direct extrapolation of these results to the conditions prevailing in Dead Sea mud is not feasible (most sulfide there is bound and not freely dissolved), the importance of sulfide as a factor in the death of microorganisms in Dead Sea mud is strongly suggested by a correlation between the decline in viability of the different organisms (Fig. 1) and their sensitivity to sulfide. To further examine the nature of the antibacterial activity of Dead Sea mud, disks of 0.1 ml of mud or radiation-sterilized mud were placed on TSA plates inoculated evenly with suspensions of the test organisms. Inhibition zones were obtained around the discs on plates inoculated with *A. niger*, *C. albicans*, *E. coli* and *P. acnes*, but not with *S. aureus* (Table 5). The inhibition was probably not primarily owing to diffusion of salt from the discs into the agar. When the discs were replaced with filter paper (5 mm in diameter) onto which solid NaCl (14 mg) or $MgCl_2{\cdot}6H_2O$ (27 mg) was added (in quantities calculated to be equivalent to those of salt present in the mud discs), no inhibition zones were observed. The effect is thus probably owing to the diffusion of toxic substances, such as sulfide, from the mud into the agar.

Table 5 Antimicrobial effect of discs of Dead Sea mud and radiation-sterilized Dead Sea mud on different microorganisms. Mud discs were placed on TSA plates inoculated evenly with suspensions of different microorganisms. After incubation for 2 days at 30 °C, the diameter of the inhibition zones around the discs was measured. Data presented are mean values of eight independent determinations

	Inhibition zone (mm)	
Organism	**Crude Dead Sea mud**	**Irradiated Dead Sea mud**
Aspergillus niger	10 ± 1	10 ± 1
Candida albicans	10 ± 1	8 ± 1
Eschericia coli	7 ± 1	7 ± 1
Propionibacterium acnes	7 ± 1	7 ± 1
Staphylococcus aureus	None	None

Discussion

Our chemical analyses of Dead Sea mud show a few prominent features. First, the mud is hypersaline, with the salt concentration of interstitial waters approaching that of Dead Sea water. However, the ionic composition of mud differs somewhat from that of Dead Sea brines. Other important features are a high content of organic matter (almost 2–3% by weight) supporting prokaryotic metabolism in the form of sulfate reduction, and the presence of both soluble and insoluble sulfur compounds. The latter finding is not surprising, considering that brine emerging from hot springs and groundwater on the Dead Sea beach and the lake's bottom contain sulfides. These sulfides were believed to have been produced by the activity of sulfate-reducing bacteria.[12] However, we now have demonstrated that bacterial sulfate reduction still occurs within the mud at a very low rate (0.13 ± 0.03 nmol/cm^3.d), a value several orders of magnitude less than found to occur in most marine sediments, but still significantly greater than the detection level of the method. Until quite recently, there have been indications that bacterial sulfate reduction also takes place in the bottom sediments of the Dead Sea,[2,5] but currently there is little if any sulfate reduction activity in Dead Sea water.[2,3] Little is known about the process of dissimilatory

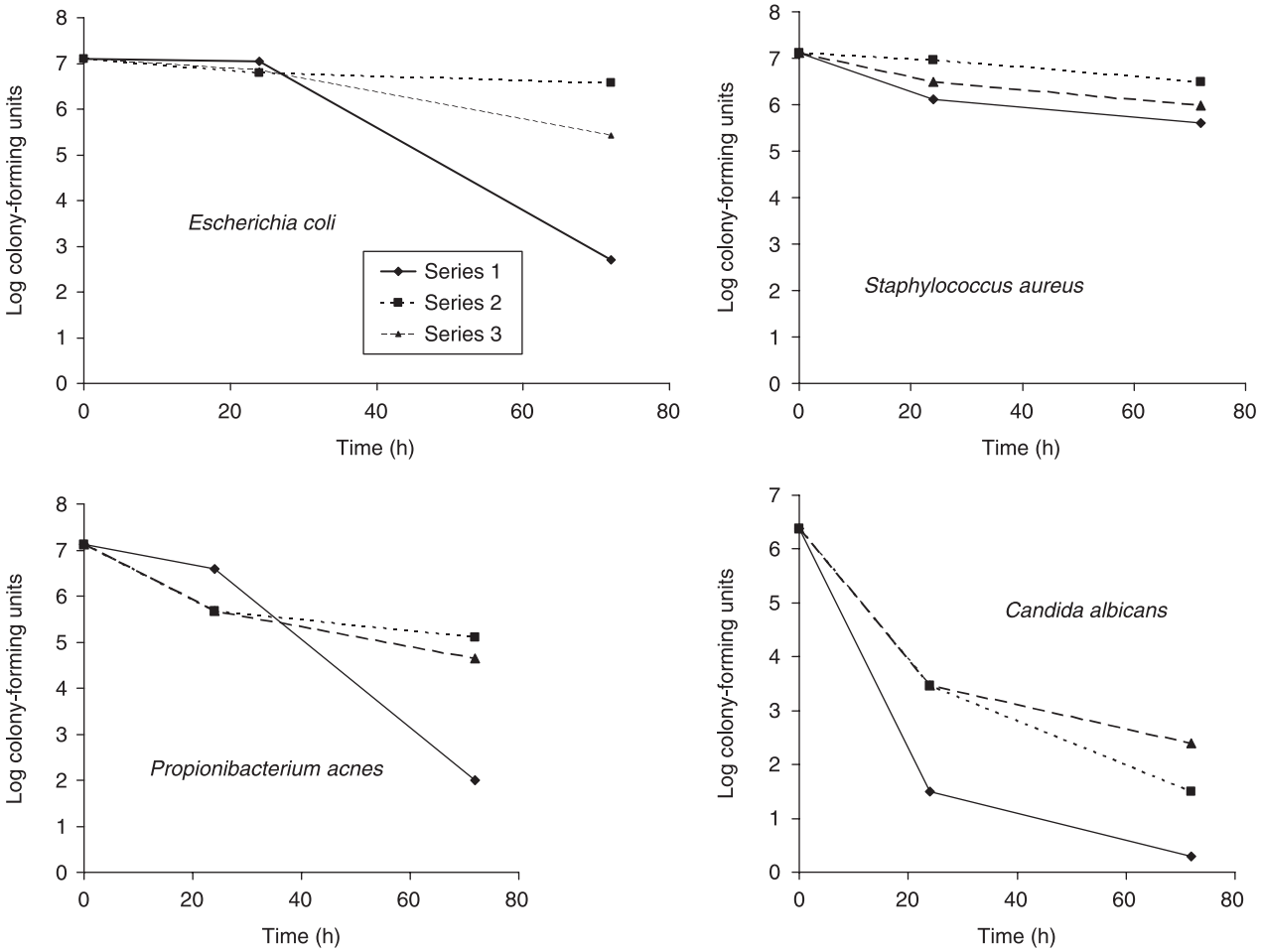

Figure 1 Survival of different microorganisms introduced to Dead Sea mineral mud. Suspensions of *Escherichia coli*, *Staphylococcus aureus*, *Propionibacterium acnes*, and *Candida albicans* (2 ml) were mixed with 20 ml of crude mud (◆), mud processed by addition of 18% water and filtration (■), and mud processed by addition of 18% water and filtration plus irradiation with 2.5 MRad of ^{60}Co (▲). After different periods of incubation at 30 °C, samples were withdrawn, tenfold dilutions were prepared using saline, and the numbers of colony-forming units were determined on TSB agar plates

sulfate reduction in the bottom sediments of the Dead Sea. Prior to the 1979 overturn of the water column, the hypolimnion of the meromictic Dead Sea was anaerobic and contained sulfide. Stable isotope analyses showed that the sulfide was enriched in light sulfur isotopes relative to the sulfate, which points to bacterial sulfate reduction as the source of the sulfide.[25,26] The microorganisms responsible for formation of the sulfide have not yet been isolated, and we still do not know of any bacterium that is capable of performing dissimilatory sulfate reduction at the level of salinity encountered in the Dead Sea.[26] Attempts to quantify sulfate reduction in Dead Sea sediments by following the formation of $H_2{}^{35}S$ from $^{35}SO_4^{2-}$ have never yielded conclusive evidence about the process (A. Oren, unpublished observations, 1980–1990). Whether sulfate reduction has a direct impact on the therapeutic effect of this mud is unknown.

Discrepancy between the relatively large colony counts obtained on SBA and the significantly lower counts on MLA, TSA and the other media may be attributed to the presence of a dormant microbial population that does not form colonies even on rich, nonselective media, but can be activated by sheep blood. As most of the colonies that developed on SBA were endospore-forming bacilli, the dormancy could be related to a low efficiency of germination of bacterial endospores on the other media rather than to the impaired multiplication capacity of vegetative forms.[27]

A variety of nonpathogenic filamentous fungi have recently been isolated from Dead Sea water, but we elected not to study fungi. Some species may survive in the hypersaline brine for extended time periods, probably as spores.[28]

Dead Sea black mineral mud is an anaerobic environment. We therefore tested for the presence of halophilic and

nonhalophilic anaerobic microorganisms in the mud. While sediments of the Dead Sea have been shown to contain specialized halophilic anaerobic bacteria, such as *Halobacteroides halobius*,[29] *Sporohalobacter lortetii* (formerly *Clostridium lortetii*),[30] and *Orenia marismortui* (formerly *Sporohalobacter lortetii*),[31] we did not find large quantities of such anaerobes. Counts in Brewer thioglycolate medium were a few hundred cells per gram of mud. Increasing the salt concentration of the medium further decreased the recovery of micoorganisms, indicating that the organisms grown in the enrichments were not active in the mud *in situ*. Rather, they were present as spores and were activated by the shift to lower salinity in the media. In this respect, it should be remembered that the first microorganisms isolated from the Dead Sea in the last decade of the 19th century were tetanus and gas gangrene-causing bacteria which had survived in the mud as resistant endospores.[32]

The hostility of Dead Sea mud toward nonhalophilic microorganisms was shown by the rapid decline in the number of colony-forming units when *E. coli*, *P. acnes*, *S. aureus* and *C. albicans* were mixed with the black mud. All our test organisms were facultative anaerobic or aerotolerant fermentative microorganisms. Therefore, this phenomenon cannot be explained by the anaerobic conditions prevailing in the mud. No significant difference was found in the killing of added organisms between radiation-sterilized and untreated mud. Hence, the possibility that biological factors (protozoa, bacteriophages) are responsible for the decline can thus be ruled out. The apparent bactericidal effect of Dead Sea mud may be owing to its high salt concentration combined with its special ionic composition. Exposure to Dead Sea water rapidly kills *E. coli*.[33] A 17% solution of Dead Sea salts ("bath salts") used as a substitute to bathing in the Dead Sea is also bactericidal to *E. coli* (Z. Ma'or, unpublished observations, 1985–1990). Another possibly important factor is the low pH (approximately 5.6) measured in the interstitial waters of the mud, which is less than that (pH 6.0) of the Dead Sea proper. The somewhat higher pH value of mud-distilled water slurries may be attributed to shifts in the carbonate system; it has been documented that the pH of Dead Sea water increases sharply upon dilution with distilled water.[25] Bacteria such as *E. coli* also survive poorly in normal seawater (35 g/l of salt), probably owing to a variety of factors, including abiotic factors (light, salinity, pH, nutrient deprivation, and temperature) as well as biotic factors (grazing and competition, bacteriophages, antibiotics and toxins).[34,35] The factor that may be most important is the presence of high concentrations of sulfide. The mud is rich in sulfide (derived from bacterial sulfate reduction that took place in the distant geologic past,[12] as well as from sulfate reduction activity within the mud itself), as shown in this study. All test organisms were sensitive to sulfide; all minimal inhibitory concentrations measured were less than the actual sulfide concentrations measured in the mud. Although a comparison of these parameters is not straightforward, because most of the sulfide present in the mud is bound rather that freely dissolved, the immediate decrease in the total sulfide pool upon exposure to oxygen shows the presence of at least some highly reactive sulfide.

The appearance of inhibition zones around discs of mud placed on a lawn of different test organisms was probably not owing to the presence of high salt concentrations in the mud, because addition of equivalent amounts of NaCl or $MgCl_2$ failed to cause the same effect. It is possible that diffusion of toxic sulfide was responsible for the formation of inhibition zones. No such zones were obtained when *S. aureus* was used as the test organism. As shown in Table 5, *S. aureus* is the least sulfide-sensitive of all organisms tested. It would be difficult to quantify the effect of sulfide using more controlled experiments, because sulfide binds to components within the mud and is rapidly oxidized when exposed to molecular oxygen.

We conclude that Dead Sea black mud contains only very low numbers of viable microorganisms, and that most of these are endospore-forming nonhalophilic bacteria. The mud has pronounced antimicrobiocidal action, which is probably owing to the combination of high salt and sulfide concentrations plus low pH. This anitimicrobiocidal capacity, including the inhibitory effect on potential skin pathogens, might partially explain the therapeutic properties of the mud, and may explain the antiacne effect attributed to facial Dead Sea mud masks (Z. Ma'or, unpublished observations, 1998). Plant extracts such as *Clinopodium vulgare* L. and the essential oils of flora, such as *Eucalyptus pauciflora* and *Octimum gratissimum*, as well as metabolites extracted from marine sponges, are included in a list of antimicrobial therapies.[36] Catechol derivatives, caffeic acid, flavonoids and chlorogenic acid are among hundreds of natural originated compounds reported to have antimicrobial properties.[37] Further research is needed to fully understand the antimicrobial activities of Dead Sea mineral mud.

Acknowledgments

We thank Shlomit Mimran, Lily Mana and Rahel Elevi for their help with the experiments, and Ittai Gavrieli and his staff at the Geological Survey of Israel for the chemical analyses. This study was supported by a grant from The Dead Sea Research Center, Israel.

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