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Analysis of Bacterial DNA in Skin and Muscle of the Tyrolean Iceman Offers New Insight Into the Mummification Process

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ABSTRACT About 80 sequences (16s ribosomal RNA gene) of bacterial DNA in samples of skin and muscle taken directly from the Tyrolean iceman (3350–3100 years B.C.) or recovered during the 1992 archaeological expedition at the Alpine site were analyzed to obtain clues to the natural mummification process that allowed the corpse of the Neolithic shepherd/hunter to be preserved for more than 5,000 years. The investigation was made more complex by the fact that the surface of the mummy had been swabbed with phenol soon after the discovery (September 19, 1991). Our results show that no trace of microbial DNA is left on the actual surface of the body, while the untreated skin still bears the remains of large numbers of bacteria belonging to the genera *Sphingomonas, Afipia, Curtobacterium, Microbacterium, Agromyces,* and others. Compared to the untreated skin, the iceman's muscle is also very rich in bacterial DNA. However, this DNA comes, with few exceptions, from the species *Clostridium algidicarnis.* The sharp difference in the bacterial DNA composition of skin and muscle suggests that the remains of the original cadaveric microflora of the latter have not disappeared during the iceman's taphonomic history. On the other hand, the massive presence of *C. algidicarnis,* a cold-adapted sporigenous, the DNA of which was previously (Ubaldi et al. [1998] Am. J. Phys. Anthropol. *107:*285–295) found in the soft tissue of a naturally desiccated Andean mummy, indicates that the hypothesis that the iceman's corpse underwent rapid dehydration by the effect of a warm wind (föhn) is no longer plausible. The results best fit with the hypothesis (Bereuter et al. [1997] Chem. Eur. J. 7:1032–1038) that the body was first covered by snow and ice, and then underwent thawing and, finally, desiccation. Am J Phys Anthropol 111:211–219, 2000.

The so-called Tyrolean iceman or "Similaun man" or "Ötzi" is a mummified human corpse found in an Alpine glacier on September 19, 1991 close to the Austrian/Italian border (actually 92.6 m inside Italian territory). The conventional radiocarbon age of the remains is 4,546 ± 17 years B.P. based on nine independent measurements (Prinoth-Fornwagner and Niklaus, 1995; Rollo et al., 1995a). This results in a real age range (calibrated) between 3350–3100 B.C., corresponding to Late Neolithic times. One of the most relevant features of this archaeo-

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logical discovery is the exceptional state of preservation of the mummy and of the clothing and equipment found on and near it. Another feature of extraordinary interest for the anthropologist is that the man, possibly a shepherd/hunter, died accidentally (and alone in all probability) during his journey across the Alps. As a direct consequence, the accompanying goods are not the reflection of any cult ritual, but are fully representative of things in daily use at the time. They include, among many other items, extremely perishable ones such as a large fragment of a cloak made of knotted tufts of grass, a fur hat, and yew bow with arrows and quiver (Spindler, 1995; De Marinis and Brillante, 1998).

The taphonomic history of the iceman has been the object of much speculation. In particular, several hypotheses have been made regarding the process of mummification. Some of them, such as the fantastic claim that the iceman might represent an elaborate hoax in which a pre-Dynastic Egyptian mummy had been planted together with artifacts at the site, were soon abandoned (Bahn and Everett, 1993). Others have been kept and are discussed below.

At the moment of discovery by two German tourists, the corpse lay in a chamber-like depression at 3,213 m above sea level, below a rocky ledge, sheltered from the shearing flow of glacial ice. Presumably, this particular situation had prevented the body from being crushed and then expelled with the regular glacial turnover in the course of centuries, as normally happens to glacier-trapped corpses and artifacts (Meyer, 1992). As concerns the mummification process, one of the first hypotheses was that the corpse had undergone rapid dehydration by a warm wind (an autumn föhn) and had been subsequently covered by snow. A warm windy spell soon followed by a mimetizing snowfall seemed necessary in order to explain how the body could become air-mummified fast enough not to be discovered and attacked by scavengers (vultures or raven-type birds). A wind of that heat at more than 3,000 m of altitude, however, has seemed unlikely to some meteorologists, as the föhn blows at a much lower altitude. An alternative speculation was that the body and equipment became rapidly frozen, and were then covered by a porous layer of snow which allowed the body to slowly desiccate through combined freeze-drying (Spindler, 1995). This reconstruction is mainly based on the glaciological observation that snow can remain air-permeable for several years (Spindler, 1995). Bahn (1996), on the other hand, suggested that the corpse was preserved in the same way as the many frozen carcasses of mammoths and other Ice Age animals in Siberia and Alaska. They were preserved by the buildup of ice in the sediments that enveloped the bodies: the ice layers desiccated the soil and dehydrated the carcasses. Unlike freeze-drying, where the original form remains intact, this process shrivels the body. Fascinating though this model may be, it does not take into account the fact that the iceman's body was not embedded in soil.

The picture is further complicated by a number of features which have been progressively revealed by the investigations carried out independently in several laboratories since the first exploration of the iceman's site. For example, histological and biochemical analyses have shown an almost complete loss of the iceman's epidermis, accompanied by postmortem alterations of skin triacylglycerols, which seems to imply a prolonged permanence in a humid or waterlogged site (Bereuter et al., 1997).

In a previous paper (Ubaldi et al., 1998), we proposed a method based on the analysis of bacterial DNA sequences in soft-tissue samples of a Peruvian mummy as a reliable way of identifying the major bacterial taxa in the intestinal microflora of a person who lived in the Andes about 1,000 years ago. The same method can also be used to determine the composition of the cadaveric flora in corpses and carcasses of any age (Rollo, 1998; Rollo and Marota, 1999).

For the last 8 years, the Tyrolean iceman has been the object of many different types of investigation. In particular, DNA analyses performed by research teams in Munich and Oxford (Handt et al., 1994) have shown that the original DNA of the mummy is, to some extent, preserved. Further evidence on the preservation of genuinely ancient DNA at the iceman's site has come from the analysis of the grass clothing (cloak and

boots) conducted in our laboratory (Rollo et al., 1995a–c).

Practically, we could use specimens of muscle as a potential source of cadaveric flora DNA and samples of skin as a control material to identify the microorganisms of the glacier with which the mummy had been kept in contact through the centuries. Any molecular analysis of the iceman's skin, however, should take into account that this may hardly be representative of the original situation. During the 24 hr that followed the transfer from the glacier to the mortuary at Innsbruck University's Institute of Forensic Medicine, the body was photographed and fingered by members of the local press. The day after (September 24, 1991), Konrad Spindler identified the accompanying metal axe as bronze, and the body was therefore ascribed to the early second millennium B.C. (Bahn and Everett, 1993). Within hours, green bluish spots, perhaps too hastily identified as rapidly growing molds, were noticed all over the corpse; therefore, it was handed over to the Institute of Anatomy, whose staff swabbed the entire surface of the mummy with a sheet daubed with phenol. The pustules were later recognized as being of nonbiological origin: they were actually deposits of vivianite ($Fe_3P_2O_8 + H_2O$) produced by contact of the body with iron-rich sediments (Tiefenbrunner, 1992; Spindler, 1995; De Marinis and Brillante, 1998).

If any trace of the ancient microbial colonization of the skin was left, this has most probably been removed. It was therefore fortunate that, during the very first attempts to pull the mummy out of the ice (September 20, 1991) with the help of a pneumatic chisel (at that time no one suspected the antiquity of the find), the left thigh underwent severe damage with consequent release of skin, muscle, and blood vessel portions into the snow. Several tissue fragments could thus be recovered and identified (Capasso et al., 1995) during the 1992 archaeological expedition.

A fragment of untreated skin from the 1992 survey, together with one of muscular tissue taken directly from the mummy, were used in the present research to reconstruct the exact composition of the original (primary) cadaveric flora of the iceman by isolating and identifying short fragments of bacterial chromosomal DNA.

MATERIALS AND METHODS

The iceman's soft-tissue specimens

One sample of muscular tissue and one of skin were personally taken from the mummy by F.R. at the Institute of Anatomy, University of Innsbruck, in 1992, under the supervision of a staff member (Othmar Gaber). The skin comes from the coccygeal region, and the muscular tissue from the left thigh (Gaber et al., 1992). Both regions were damaged following the first attempt to pull the body out of the ice.

Another skin sample (specimen no. 183) was recovered during the 1992 archaeological exploration of the iceman site conducted by an Austro-Italian interdisciplinary research team lead by Andreas Lippert from the University of Vienna. The ice was melted off gradually in the rock gully by the help of steam jet blowers. The melting water was filtered in order to trace small objects. About 400 different fragments of artificial, anatomical, and botanical origin were discovered (Bagolini et al., 1995). Six anatomical specimens of interest, including a fingernail (specimen no. 168), two fragments of skin (specimen nos. 183 and 187), and remains of hair (specimen nos. 158, 189, and 324), were accurately identified and described by Capasso et al. (1995).

Since 1992, all the samples have been constantly kept frozen at −20°C.

Bacterial DNA analysis

DNA extraction. The details of the phenol-based DNA extraction procedure have been reported previously (Ubaldi et al., 1998). The samples were left overnight in an extraction medium composed of sodium dodecyl sulfate (SDS), Na_2EDTA, Tris-HCl (pH 8.0), and phenol. Each tissue sample (approximately 100 mg) was then milled in a mortar with a pestle in the presence of the extraction medium. The homogenate was then extracted stepwise using phenol, phenol/chloroform, chloroform, and ether, and the DNA was finally precipitated using ethanol at −20°C. The DNA was further purified by a MerMaid kit (BIO 101, Vista, CA) to

eliminate polymerase chain reaction (PCR) inhibitors.

PCR amplification of DNA. Enzymatic amplifications by PCR were performed in 50 ml of a reaction mixture containing 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, 200 mM (each) dNTPs, 300 ng of each oligonucleotide primer, 2.5 units Taq polymerase, and 1 µl DNA. The 338f/531r primer-pair (338f, 5′-AACTGAGACACGGTCCAGAC-3′; 531r, 5′-ACGCTTGCACCCTCCGTATT-3′) behaves as "universal" primer-pair for bacterial 16s ribosomal RNA genes. Following an initial step at 94°C for 5 min, the thermal cycler was set as follows: 94°C for 60 sec (denaturation); 56°C for 30 sec (annealing); and 72°C for 60 sec (elongation). Forty amplification cycles were performed. To eliminate small amounts of *E. coli* DNA possibly present in the commercial enzyme preparations, and, in general, any types of contaminant DNA, the Taq polymerase and the reaction components were pretreated with 3 units of DNase I (from bovine pancreas) for 30 min at room temperature. After incubation, the DNase I was inactivated by boiling for 10 min at 94°C.

Precautions to avoid contamination. In the course of DNA extraction and PCR amplification steps, we routinely employed the standard precautions for ancient DNA work (e.g., wearing of sterile gloves, pretreatment of mortars, pestles, and homogenizers with HCl, use of ultraviolet (UV)-irradiated safety cabinets, and dedicated gel trays and tanks). We also implemented the different steps in physically separated laboratories and used a DNA-free chamber.

Traditional contamination controls are represented by mock extractions and blank amplification reactions. We routinely perform both types of control. In our laboratories, however, monitoring of contaminant DNA does not stop at this point, as we clone amplified DNA and sequence large numbers of bacterial amplicons. In this way we are able to detect low copy number contaminant DNA species which might escape detection by other means (Ubaldi et al., 1998). Finally, it must be pointed out that the contamination problems are not relevant if the amplification target is a very high copy number. This is precisely the case of the low molecular-weight bacterial chromosomal DNA in the iceman's bioptic samples.

DNA cloning and sequencing. Amplification products were cloned using a pMOSBlue T-vector kit (Amersham, Arlington Heights, IL) according to the manufacturer's instructions. Plasmid DNA was extracted using an alkaline lysis based method followed by silica gel purification (Plasmix, Talent, Trieste, Italy). Sequencing was performed by the use of a Dye Terminator Cycle Sequencing Kit/AmpliTaq FS (PE Applied Biosystems, Foster City, CA). Cycle sequencing products were subsequently purified by Centri-sep spin columns (Princeton Separations, Adelphia, NJ) and analyzed using an ABI-PRISM 310 Sequencer (Perkin-Elmer, Oak Brook, IL).

Analysis of DNA sequences. Chimeric sequences were detected using the Check Chimera program available from the Ribosomal Database Project (Maidak et al., 1997). Comparisons with reference sequences in the National Center for Biotechnology Information (NCBI) data library were performed using the BLAST (Altschul et al., 1990) search program.

Quantitative PCR test. To quantify the number of bacterial chromosomal DNA molecules in the iceman's tissue (skin and muscle) extracts, we employed a competitive PCR (cPCR) system. The assay was performed by mixing an equal volume (1 µl) of the iceman's DNA with a stated copy number (1,000) of a competitor (a 232-bp-long fragment of *Variovorax paradoxus* 16s rDNA which had previously been PCR-amplified using the 338f/531r primer-pair and cloned into a pMOSBlue T-vector) and by submitting the mixture to enzymatic amplification, using the 338f/531r primer pair (Ubaldi et al., 1998).

RESULTS

Bacterial DNA in the iceman's soft-tissue specimens

DNA preparations obtained from the iceman's soft-tissue specimens (treated and

untreated skin, and muscle) were initially checked for the presence and copy number of bacterial DNA, using a competitive PCR system designed to amplify a short (196-bp) fragment of the prokaryotic 16s ribosomal RNA gene (16s rDNA). The oligonucleotide primers (338f/531r) employed bind to a broad range of bacterial DNAs ("universal" primers).

The result is shown in Figure 1. When DNA extracts from the iceman's tissues are PCR-amplified in the presence of a 232-bp-long competitor, virtually no amplification signal is produced by the skin sample taken directly from the phenol-swabbed mummy, thus showing the absence of remnants of bacteria. Conversely, the signal produced by the muscle extract is equivalent to approximately 2,000 copies of bacterial DNA; this, in turn, corresponds to about 100,000 copies of bacterial DNA per milligram of desiccated muscle. The extract obtained from the skin sample collected at the site in 1992 (untreated skin) contains bacterial DNA at an even higher copy number than that in the muscle, as witnessed by the disappearance of the competitor (232-bp) band.

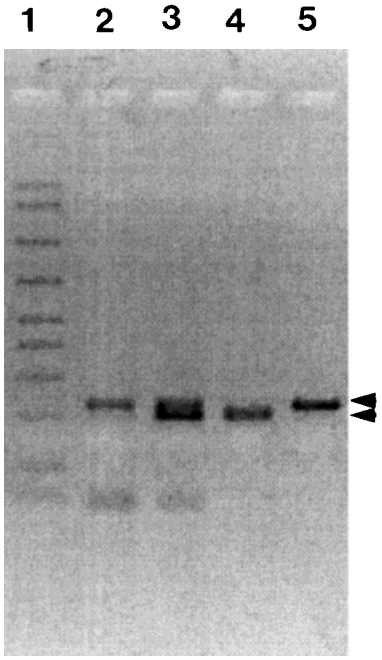

Fig. 1. Competitive PCR test (negative picture of a 3% agarose gel) designed to quantify the bacterial DNA fragments (196 bp, lower arrowhead) in preparations of the iceman's phenol-treated and untreated skin, and muscle. **Lane 1,** molecular weight standard (50–2,000-bp ladder, Bio-Rad, Richmond, CA); **lane 2,** phenol-treated skin; **lane 3,** muscle; **lane 4,** untreated skin; **lane 5,** negative control (no DNA). A competitor (232 bp, upper arrowhead) at a multiplicity of 1,000 copies was added to each reaction mixture.

Identification of bacterial DNA

To identify the bacteria, the extracts from untreated skin and muscle were PCR-amplified with the 338f/531r primers, and the products cloned into a plasmid vector as described in Materials and Methods. Subsequently, we determined the nucleotide sequence of about 40 amplicons from each collection, and the sequences were used to scan the NCBI data library. The results are shown in Tables 1 and 2.

The skin microflora is mainly composed of microorganisms belonging to five genera: *Sphingomonas, Afipia, Curtobacterium, Microbacterium,* and *Agromyces.* The members of the genus *Sphingomonas* are ubiquitous; they can be found in soil, water, and sediments. *Afipia* genosp. is also a soil microorganism. *Curtobacterium luteum* is a plant pathogen related to *Clavibacter michiganensis,* and to *Corynebacterium aquaticum* (Komagata and Suzuki, 1986; Funke et al., 1994). The Coryneform bacteria group includes a vast range of microorganisms which are animal or plant pathogens or grow saprobically on decaying organic matter. Within the iceman's skin microflora, the genus *Microbacterium* (Collins et al., 1983) is represented by the species *M. lacticum.* These microbacteria are thin, irregular rods capable of growing at temperatures up to 40°C in raw milk, cheese, and other dairy products. *Agromyces cerinus* and *A. ramosum* are soil-dwellers. Other species, such as *Lentzea albidocapillata* and *Arthrobacter* sp., are represented by a single amplicon each. The first is a recently discovered, rare species of the order *Actinomycetales,* isolated from clinical specimens. The members of the genus *Arthrobacter,* on the other hand, are inhabitants of the soil (Hagedorn and Holt, 1975). Psychrophilic strains of *Arthrobacter* have been isolated from glacier sediments and Arctic cavern muds (Gounot, 1968).

Contrary to that of the skin, the muscle microflora is dominated by a single species,

TABLE 1. Identification of 16s rDNA amplicons from iceman's untreated skin[1]

Amplicon	BLAST identification	NCBI accession no.	Similarity (%)
183S-11	*Sphingomonas mali*	Y09638	100
183S-20	*Sphingomonas mali*	Y09638	100
183S-21	*Sphingomonas mali*	Y09638	100
183S-25	*Sphingomonas mali*	Y09638	100
183S-28	*Sphingomonas mali*	Y09638	100
183S-29	*Sphingomonas mali*	Y09638	100
183S-37	*Sphingomonas mali*	Y09638	100
183S-44	*Sphingomonas mali*	Y09638	100
183S-47	*Sphingomonas mali*	Y09638	100
183S-27	*Sphingomonas mali*	Y09638	100
183S-37	*Afipia* genosp.	U87782	100
183S-39	*Afipia* genosp.	U87782	100
183S-48	*Afipia* genosp.	U87782	98
183S-61	*Afipia* genosp.	U87782	100
183S-65	*Afipia* genosp.	U87782	100
183S-30	*Curtobacterium luteum*	X77437	99
183S-35	*Curtobacterium luteum*	X77437	99
183S-38	*Curtobacterium luteum*	X77437	98
183S-49	*Curtobacterium luteum*	X77437	100
183S-3	*Microbacterium lacticum*	D21343	98
183S-35	*Microbacterium lacticum*	D21343	98
183S-52	*Microbacterium lacticum*	D21343	98
183S-71	*Microbacterium lacticum*	D21343	98
183S-31	*Agromyces cerinus*	X72723	92
183S-40	*Agromyces cerinus*	X72723	96
183S-42	*Agromyces cerinus*	X72723	96
183S-43	*Agromyces cerinus*	X72723	96
183S-24	*Agromyces ramosum*	X77447	96
183S-23	*Rathaybacter iranicus*	U96184	96
183S-32	*Corynebacterium aquaticum*	X77450	99
183S-45	*Lentzea albidocapillata*	X84321	98
183S-58	*Arthrobacter* sp.	MB90	99

[1] Not included are the chimeric (Wang and Wang, 1996) amplicons 183S-46, 183S-60, and 183S-66.

Clostridium algidicarnis. This Gram-positive, anaerobic, spore-forming rod was isolated for the first time from chilled, vacuum-packed raw beef (Cato et al., 1986; Lawson et al., 1994). In the laboratory, this microorganism grows down to at least 4°C. Fragments of 16s rDNA of *C. algidicarnis* have also been obtained, as the prevailing bacterial DNA, following PCR amplification, using the universal 338f/531r primer pair, of the DNA isolated from the colon of an Andean mummy (Ubaldi et al., 1998). To our knowledge, these are the first demonstrations of the presence of this species of *Clostridium* in corpses. The composition of the cadaveric flora is still largerly unknown. In the initial stages of decay of a corpse, the bacteria are commonly assumed to come from the intestines.

DISCUSSION

The iceman's corpse shows no major signs of putrefaction (e.g., eyeballs and dermis are preserved), though his epidermis, hair, and nails are absent (Spindler, 1995; De Marinis and Brillante, 1998). Molecular analyses performed in the past few years have shown that the soft tissues still contain traces of the original human genetic material (Handt et al., 1994). Actually, the quantitation (Handt et al., 1996) of mitochondrial DNA (mtDNA) fragments showed that 1 mg dry soft tissue contained approximately 2,000 fragments of 103 bp in length; the same test indicated that longer (189-bp) fragments were also present, though at a much lower copy number (10 copies/mg). Lipid analysis of skin samples recovered during the 1992 archaeological expedition, performed by Bereuter et al. (1997), on the other hand, demonstrated a declining gradient of triacylglycerols and unsaturated fatty acids from the outer to the inner side. This is in agreement with adipocere formation, which begins in the subcutaneous fat deposits and proceeds upwards to the neighboring tissues (Mayer et al., 1996). Transformation of human fat by microbes into adipocere usually

TABLE 2. Identification of 16s rDNA amplicons from iceman's muscle[1]

Amplicon	BLAST identification	NCBI accession no.	Similarity (%)
FS-6	*Clostridium algidicarnis*	X77676	100
FS-7	*Clostridium algidicarnis*	X77676	100
FS-9	*Clostridium algidicarnis*	X77676	96
FS-13	*Clostridium algidicarnis*	X77676	100
FS-14	*Clostridium algidicarnis*	X77676	100
FS-15	*Clostridium algidicarnis*	X77676	100
FS-17	*Clostridium algidicarnis*	X77676	100
FS-18	*Clostridium algidicarnis*	X77676	100
FS-22	*Clostridium algidicarnis*	X77676	100
FS-25	*Clostridium algidicarnis*	X77676	99
FS-40	*Clostridium algidicarnis*	X77676	95
FS-42	*Clostridium algidicarnis*	X77676	100
FS-46	*Clostridium algidicarnis*	X77676	100
FS-47	*Clostridium algidicarnis*	X77676	100
FS-49	*Clostridium algidicarnis*	X77676	100
FS-51	*Clostridium algidicarnis*	X77676	100
FS-55	*Clostridium algidicarnis*	X77676	99
FS-57	*Clostridium algidicarnis*	X77676	100
FS-58	*Clostridium algidicarnis*	X77676	100
FS-62	*Clostridium algidicarnis*	X77676	100
FS-64	*Clostridium algidicarnis*	X77676	100
FS-65	*Clostridium algidicarnis*	X77676	99
FS-76	*Clostridium algidicarnis*	X77676	100
FS-77	*Clostridium algidicarnis*	X77676	100
FS-80	*Clostridium algidicarnis*	X77676	100
FS-81	*Clostridium algidicarnis*	X77676	100
FS-20	*Clostridium paraputrificum*	X73445	96
FS-50	*Clostridium paraputrificum*	X73445	95
FS-53	*Clostridium paraputrificum*	X73445	96
FS-71	*Clostridium paraputrificum*	X73445	96
FS-75	*Clostridium paraputrificum*	X73445	95
FS-78	*Clostridium paraputrificum*	X73445	96
FS-82	*Clostridium paraputrificum*	X73445	96
FS-66	*Clostridium* sp.	X95274	99

[1] Not included are the chimeric (Wang and Wang, 1996) amplicons FS-2, FS-3, FS-21, FS-29, FS-35, FS-38, FS-63, FS-65, and FS-84.

takes several months and requires humid conditions as well as temperatures above freezing.

It seems logical to assume that the mummification process responsible for the preservation of the human DNA, irrespective of the way this process took place, allowed preservation of the DNA of the bacteria (cadaveric microflora) that grew within the corpse for a certain time span following death. In view of this, we employed a PCR primer pair designed to bind to fragments of bacterial chromosomal 16s rDNA, comparable to the longest mtDNA fragments detected by Handt et al. (1996), i.e., about 196 bp. Of course, as the body lay in close contact with ice and sediments for most of its taphonomic history, we can conceive that, through the millennia, a certain number of microorganisms of the environment seeped through the mummified tissues, with the result of masking and erasing any traces of the primitive cadaveric flora. Operatively, we can put forward the two following working hypotheses: 1) the flow of microorganisms from the outer environment has been massive; 2) it has been negligible compared to the extent of primary proliferation.

If the first hypothesis were true, we should find a substantial uniformity in the bacterial DNA composition of skin (which has been constantly in contact with the outer environment) and muscle. Conversely, if the second were true, we would expect to find a significant difference between the bacterial DNA composition of muscle and skin.

The result of the sequence analysis of bacterial amplicons shown in Tables 1 and 2 clearly speaks in favor of the second hypothesis. This conclusion, i.e., negligible or very low secondary penetration of environmental microorganisms, is further strengthened by the observation that small fragments of grass clothing recovered from the glacier contain only very tiny amounts of bacterial DNA (Cano et al., 1999). A further element of

interest is the observation that, actually, skin and muscle share no common species. The composition and eco-physiological characteristics of the skin bacterial flora suggest a history of close contact with aerated sediments and plant debris at relatively mild temperatures. The muscle, conversely, contains the DNA of the anaerobic psychrotroph *Clostridium algidicarnis* at a very high copy number, thus demonstrating that this microorganism once found the appropriate conditions for its growth.

Regarding the above two hypotheses on the mummification process, the proliferation of *C. algidicarnis* within the iceman's tissues does not seem compatible with the hypothesis of rapid desiccation of the body by effect of a warm wind shortly after death. On the other hand, one is struck by the similarity of the bacterial DNA composition of the iceman's muscle with that of the soft tissue of a natural mummy from Cuzco, i.e., 54% of the PCR amplicons identified as *C. algidicarnis* (Ubaldi et al., 1998). The Cuzco mummy, as many other Andean mummies, is a corpse that desiccated by effect of the dry cold air of the heights.

The presence of adipocere, however, indicates that the mummification process of the iceman cannot be so simple. According to Bereuter et al. (1997), "Shortly after his death at the site of discovery in that prehistoric early autumn, iceman's corpse was covered by snow and ice. At some later stage, perhaps during Roman times, his sheltered corpse thawed and became immersed in the water collecting within the depression where it lay. During this brief warm period, most of the physical damage incurred by the corpse, as well as the formation of adipocere and loss of epidermis took place. In the light of its excellent preservation, it would seem likely that desiccation of the corpse occurred shortly after its immersion in water." Our results fit rather well with this picture. In particular, the composition of the skin microflora, as inferred from the analysis of bacterial DNA, is best explained if we accept the hypothesis of a prolonged permanence of the body in a humid site at a relatively mild temperature. The absence of identifiable representatives of the normal skin microflora is probably the result of the loss of epidermis. The growth of *C. algidicarnis* may have taken place before the warm period, shortly after it, or in both periods. Incidentally, the spoiling activity of this microorganism may be responsible for the marked degradation and loss of human DNA observed by Handt et al. (1994).

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