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Most hypogean environments particularly caves, are characterized by a stable temperature, usually in the range of 10±15°C, and high relative humidity. These ecosystems, when an allochthonous input of organic matter is present, seem to favor the growth of heterotrophic bacteria, from which actinomycetes predominate. Streptomyces *species are particularly abundant and, in some cases, can be found as monospecific colonies. The mechanisms involved in colonization and growth are unknown, but microclimate and carbon sources can play an important role.*

Microorganisms, particularly bacteria, inhabit all allowable habitats of the biosphere, including subterranean ones. Subsurface habitats are dark, generally low in organic nutrients, relatively constant in temperature, and relatively large in mineral surface area (Ghiorse 1997).

Literature on hypogean microbial communities is scant and sometimes limited to a few caves. Reports indicate different situations, e.g., that chemotrophs are a possible primary producer in some caves (Peck 1986), that the chemoautotrophic producers play an insignificant role in the cave ecosystem (Caumartin 1963), and that at least five types of chemolitho autotrophic bacteria are supporting large populations of chemoheterotrophic bacteria and many varieties of fungi in caves (Cunninghamet al. 1995). Recently, Sarbu et al. (1996) showed that the Movile Cave (Romania) ecosystem derives its organic carbon from in situ chemoautotrophic production, unlike other limestone cave ecosystems that require allochthonous inputs of organic matter of photosynthetic origin from the surface. Several sulfide-oxidizing chemoautotrophic bacteria were isolated, and *Thiobacillus thioparus* and *Thiosphaera* sp. were identified (Sarbu et al. 1994; Vlasceanu et al. 1997). A similar conclu-sion was reached by Langecker et al. (1996) concerning the Cueva de las Sardinas, Mexico. They considered that the cave ecosystem does not primarily depend on energy input of epigean origin but is principally based on chemolitho autotrophic primary production of bacteria (genus *Beggiatoa*) inside the cave. Chemoautotrophicnitrifying bacteria have also been reported in caves (Fliermans and Schmidt 1977; Pohlman et al. 1997).

Rarely papers dealing with a complete study of the whole range of microorganisms, identification of the most common or abundant species, and study of their role in the

biogeochemical processes of caves can be found (Cunningham et al. 1995). Usually, literature refers to specific aspects, e.g., the occurrence of cyanobacteria and algae in caves with natural openings to sunlightor those with artificial lighting (Abdelahadand Bazzichelli 1988; Aboal et al. 1994;Hernandez-Marineand Canals 1994), fungi (Rutherford and Huang 1994), and so forth.

Actinomycetes have been considered to be mainly confined to soils, where they find their natural habitat (Porter 1971), but despite intensive studies there are still many gaps in our knowledge of the role played by actinomycetes in soil processes (Goodfellow and Williams 1983).

A review of the pertinent literature reveals that little is known concerning the distribution, population dynamics, growth rates, and biogeochemical processes of actinomycetes in hypogean environments, in spite of the fact that they seem to constitute a significant part of the microbial population in these habitats, at least as sampled in the last 2 years (Laiz et al. 1998; Groth et al. 1998). The ecological assessment of environments other than terrestrial soils and marine sediments is important because of the potential value of the latter as a source of actinomycetes that produce novel bioactive substances (Okami 1986). Furthermore, Williams et al. (1993) stated that one approach to the isolation of novel actinomycetes is to concentrate on understudied environments or substrates while using appropriate selective isolation techniques or to investigate habitats in which one or more of the environmental factors (e.g., temperature, pH, aeration, or osmotic stress) are extreme.

This review brie y discusses the presence of actinomycetes in hypogean environments and their involvement in biogeochemical processes.

Hypogean vs Terrestrial Environments

One of the main differences between hypogean and terrestrial environments is climate conditions. Although the hypogean climate is extremely complex and varies a great deal, depending on many different factors, it is colder, more or less stable through the year, and subject to small changes by external conditions, whereas the terrestrial environment is subject to drastic changes not only daily but also seasonally.

There are a few reviews (e.g., Wefer 1991) and reports on cave microclimatic conditions, the latter dealing mainly with environmental management of tourist caves (Quindos et al. 1987; Cigna 1993; Hoyos and Soler 1993). However, there are some general trends in hypogean environments. In fact, caves are characterized by a very stable temperature throughout the year, usually in the range of 10±15$^{\pm}$C, and high relative humidity (RH). Most of them are closed to the public or have restrictions on visits (VouveÂet al. 1983; Hoyos et al. 1993). Temperatures in caves without control or restrictions for visits, or in tombs and catacombs, range between 19 and 23$^{\pm}$C, and temperature differences between the interior and the galleries near the entrance can be as much as 10$^{\pm}$C (Hoyos and Soler 1993; Pantazidou et al. 1997; ArinoÄ et al. 1997).

There latively low temperatures in some hypogean environmentsis of particular interest, as Williams and Cross stated in 1971 that psychrophilic actinomycetes had not been isolated to date; most species were mesophilic, having their optimun temperatures in the range of 23±40$^{\pm}$C, and species isolated from soil and water grew well at temperatures between 25 and 28$^{\pm}$C. Cold and temperature-stable caves have the possibility to harbor psychrophilic actinomycetes, but the remaining hypogean environments can presumably be colonized by psychrotrophs, which can grow over a wider temperature range.

Recently, Komagata and Nakase (1997) described an obligately psychrophilic actinomycete, and Xu et al. (1996) reported actinomycete populations in cool areas of China, with

average temperatures of 5±C or below 0±C, where *Streptomyces* constituted up to 97% and 83%, respectively, of the total count. Some were psychrophiles, with an optimum growth temperature of 10 to 15±C. Therefore, low temperature is not a limiting factor for actinomycete growth, and it is not strange that psychrophilic or psychrotrophic microorganisms, which are best able to cope with low temperatures, are the most successful colonizers of hypogean environments. Obviously ,these environmentlconditioncould represent one of the factors promoting a divergent colonization of microbial communities in the two ecosystems compared (Russell 1990).

Microbial Communities and Biogeochemical Processes

The study of microbial communities vs biogeochemical processes in hypogean environments is mainly related to the fact that microorganisms affect cultural heritage properties humans wish to protect. Monte and Ferrari (1993) reviewed the mechanisms of biodeterioration in tombs and underground churches. The role of actinomycetes in the deterioration of frescoes in hypogean environments was stressed by Agarossi et al. (1985), Giacobini et al. (1987), and Monte and Ferrari (1993).

Giacobini et al. (1987) described the serious damage suffered by a whole series of famous frescoes in underground environments. Actinomycetes were isolated from these mural paintings located in crypts, caves, and tombs. About 200 strains of actinomycetes were isolated, 60 of which were identified. The most frequent species were *Streptomyces albus, S. cinereoruber, S. griseolus, S. vinaceus*, and some members of the *Nocardia* genus.

Heterotrophic bacteria and actinomycetes were the most abundant microorganisms on the frescoes of Etruscan tombs (Agarossi et al. 1988). Bettini et al. (1977) studied some of the oldest Etruscan tombs (7th to 4th century BC) and found white-grey patinas and spots from streptomycetes in the mural paintings. They related the biodeterioration to the microclimatic conditions (e.g., lower temperatures, high RH). Monte and Ferrari (1993) considered that colonization of the mural paintings was the result of selective, competitive and inhibitory dynamics among different microorganisms under different and changing environmental conditions. The pigments excreted by some actinomycetes were responsible for the violet stains found on the walls of tombs in Tarquinia. Similar stains were observed in one of the Roman tombs, the Circular Mausoleum, the Necropolis of Carmona, Spain, from which a *Streptomyces* sp. producing violet pigments in culture media was isolated (unpublished data).

Pantazidou et al. (1997) found that the micro¯ ora colonizing rock of the Milos Catacomb consisted of fungi characterized by the presence of *Penicillum* and *Fusarium* species, bacteria represented by *Bacillus* and *Streptomyces* species, and cyanobacteria. The *Streptomyces* species were found in mortar samples, remains of the fresco decoration from which only traces have survived, and showed the ability to solubilize calcium carbonate and pro-duce organic acids.

Agarossi et al. (1985) studied the subterranean Neo-Phytagorean basilica of Porta Maggiore in Rome (1st century AD). The basilica, discovered in 1917, has one of the most relevant collections of decorative stuccoes from the Roman world. The stuccoes were damaged by continuous dripping waters and vibrations from a railway overhead. Also a pronounced biodeterioration of the stucco walls was found, where the most abundant microorganisms were species of *Streptomyces* with white mycelium and *Nocardia*. The authors correlated the high frequency of some groups of microorganisms with the high humidity. In places with a lower moisture level, the actinomycetes counts were lower and the colonizers differed from those found in more humid places. The primary colonizers, the

sulfur-oxidizing bacteria that began the attack on the stucco walls, favored the subsequent development of actinomycetes and fungi, which caused the formation of large unstable layers and white ef¯orescences.

Agarossi et al. (1986) also studied the underground church of St. Clemente in Rome, a basilica composed of two superimposed churches, erected over Roman constructions that are, themselves, on various levels. In the lower basilica and other subterranean places, weak seasonal fluctuations in the climatic parameters and high RH favored the development of microbial populations. In the zones under the in¯uence of artificial lighting a green biofilm of algae developed and considerably higher numbers of actinomycetes and heterotrophic bacteria were found in comparison with zones without the biofilm.

Concerning karstic caves, Hardisson et al. (1982) estimated the colony-forming units of bacteria in Altamira: soil actinomycetes ranging from 10^2 to 2 £ 10^4. Somavilla et al. (1978) listed the bacteria identified in samples of air from Altamira and La Pasiega Caves, none of which was an actinomycete, but in dripping waters *Streptomyces* spp. were obtained in one sample. Gonzalez de los Reyes-Gavilan et al. (1984) did not study the actinomycete ora in Altamira Cave; Arroyo and Arroyo (1996) found actinomycetes in the counts and contact plates from the ¯oor, walls, and ceiling from the same cave but made no reference to a single species. Lefevre (1974) reported the presence of actinomycetes in the Cave of Lascaux.

In the Greek Cave of Perama, artificial illumination since its opening to the public in 1956 has produced an increase of epilithic and endolithic phototrophic microorganisms. The dominant micro¯ ora consist mainly of unicellular, colonial and filamentous cyanobacteria, but examination of the fresh and culture material has also revealed the presence of mosses and moss protonemata as well as a few species of chlorophytes, diatoms, bacteria, fungi, and actinomycetes (Iliopoulou-Georgoudakiet al. 1993).

Groth et al. (1999) investigated the growth of actinomycetes in two Spanish caves with rock art. Approximately 350 actinomycetes were identified by morphological, physiological, and chemotaxonomic methods in Altamira Cave. Most of the actinomycetes growing on the surface of the ceiling and wall rocks were colonies from 1 to 10 mm in diameter, visible with naked eye. Many isolates corresponded to strains obtained directly from the colonies. The genera *Amycolatopsis, Aureobacterium, Brevibacterium, Nocardia, Nocar-dioides, Rhodococcus, Streptomyces*, and the family *Micrococcaceae*were well represented. In Tito Bustillo Cave, the surface of the rock was colonized by a large number of small, yellow or white, round colonies of Ó 1±2 mm. Direct isolates from the colonies were found to be *Streptomyces xanthophaeus* and *Streptomyces* sp.

A study on the bacteria colonizing the stalactites of Grotta dei Cervi, Italy, provided similar genera. In fact, *Agromyces, Amycolatopsis, Arthrobacter, Nocardiopsis, Rhodococcus*, and *Streptomyces* species were isolated (unpublished data). A typical growth pattern on stalactites is shown in Figure 1.

CañaverasÄ et al. (1999) found microbial communities in association with hydromagnesite and needle-fiber aragonite deposits in caves. *Streptomyces* species predominated and showed the ability to precipitate calcium carbonate in laboratory cultures. This suggests that these bacteria might play a role in the formation of moon milk deposits.

Actinomycetes are well known for their abilityto grow on very poor media (Lechevalier and Lechevalier1967),and streptomycetes exist for extended periods as resting arthrospores that germinate in the occasional presence of exogenous nutrients (Goodfellow and Williams 1983). Cave communities usually rely on allochthonous input of organic matter trans-ported from the surface. The main transport pathway is water. Dissolved organic carbon content in dripping waters from some caves is highly variable, with carbon <5 mg/L in winter to Ó 2200 mg/L in late spring (Laiz et al. 1999); composition is also variable,

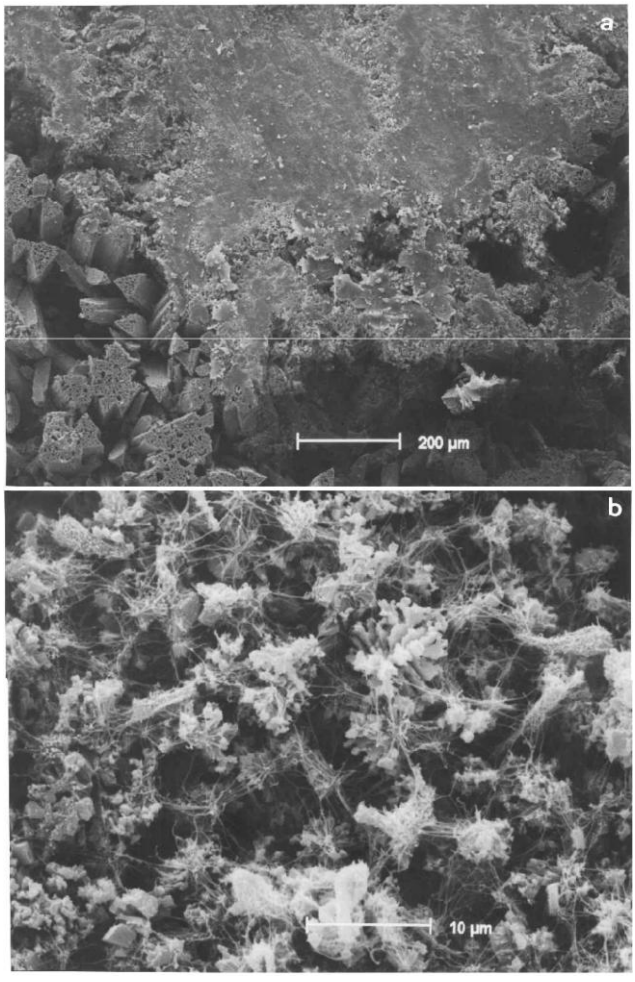

FIGURE 1 Scanning electron micrographs from a stalactite (Grotta dei Cervi, Italy). (a) Stalactite surface with an actinomycete colony. The colony forms a dense mat or biofilm coating the surface. In some cases detaching layers can be observed. (b) The biofilm is composed of a variety of structures connected by radiating hyphae.

with aliphatic organic acids and lignin phenolic compounds (Saiz-Jimenez and Hermosin 1998).

Guggenberger and Zech (1994) reported that dissolved organic matter from soil, which is the origin of the organic carbon in the dripping waters, is composed of polymeric water-soluble lignocellulose degradation products. Both lignocellulose and humic materials are almost selectively degraded by actinomycetes (Crawford et al. 1983; McCarthy 1987; Ball et al. 1989, 1990; Kontchou and Blondeau 1992; Dari et al. 1995), and humic acid is even used in an actinomycete isolation medium (Hayakawa and Nonomura 1987).

Concluding Remarks

Caves are characterized by a stable temperature, usually in the range of 10±15$^{\pm}$C, and high RH, around 90±100%. Although closed or seldom-visited caves are relatively stable,

those opened to visitors, particularly when the number of visitors is considerable, can increase air temperatures by as much as 8±C and decrease RH by 60%. Other hypogean environments (tombs and catacombs) present even higher variations, owing to the influence of external conditions, which usually increase the air temperature by 5 to 12±C and promotes greater changes in RH.

The existence of abundant colonies of *Streptomyces* species in caves with temperatures of 12±14±C throughout the year favors the hypothesis that growth of actinomycetes in hypogean environments may result from the association of two factors: low temperatures and high RH. These environmental conditions, together with nutrient availability and nature of the organic matter, are recognized to be important factors controlling the activity of actinomycetes in caves. At stable low temperatures the microorganisms with more psychrophilic traits are likely to be the more important in nutrient-cyclin iin caves. In addition, the mechanisms involved in colonization and growth are possibly related to the particular chemical structure of the dissolved organic matter (lignocellulose residues, humic substances) in dripping waters. Russell (1990) has hypothesized that it is not necessary for a microbe to function at optimal rates as long as it can compete effectively in its particular environment. It may be quite advantageous for cave bacteria to metabolize submaximally and have long generation times in nutrient-poor environments.

The biodeteriorative role of microbial communities colonizing hypogean environments with rock art or mural paintings, particularly the role of actinomycetes, has been emphasized by many authors. The appearance of whitish powdery patinas, scattered colored spots, production of unstable layers, and scaling disfigures the paintings and represents a threat for the conservation of these cultural properties.

Finally, the sampling of microorganisms by traditional approaches always reveals only a minor fraction of the existing biodiversity in the special ecosystem (Amann et al. 1995). Taking into account that growing on the isolation plates are only the bacteria that are adapted to the conditions used, one can assume a much greater bacterial diversity than that shown in caves. Innovative molecular techniques now offer possibilitiesto overcome this problem and have to be applied in further studies. In some cases, however, investigators have found that the isolation of the bacterium responsible of the colonization and growth on the calcite formations is possible without further need for molecular techniques.

References

AbdelahadN, BazzichelliG. 1988. *Geitleria calcarea* Friedmann, Cyanophycee cavernicole nouvelle pour l' Italie. Nova Hedwigia 46:265±270.

Aboal M, Asencio AD, Prefasi M. 1994. Studies on cave cyanophytes from southeastern Spain: *Scytonema julianum* (Meneghini ex Franck) Richter. Algol Stud 75:31±36.

Agarossi G, Ferrari R, Monte M. 1985. Microbial biodeteriorationin the hypogea: the subterranean Neo-Phytagoreanbasilica of Porta Maggiore in Rome. In: VIth International Congress on Deterioration and Conservation of Stone, Lausanne; Vol 2, p 597±605.

Agarossi G, Ferrari R, Monte M. 1986. The basilica of St. Clemente in Rome: studies on biodeterioration. In: Scienti®c methodologies applied to work of art. Montedison Progetto Cultura; p 52±56.

Agarossi G, Ferrari R, Monte M, Gugliandolo C, Maugeri T. 1988. Changes of microbial system in an Etruscan tomb after biocidal treatment. In: VIth International Congress on Deterioration and Conservation of Stone. Torun: Nicholas Copernicus University; Supplement, p 82± 91.

Amann RI, Ludwig W, Schleifer KH. 1995. Phylogenetic identification and in situ detection

of individual microbial cells without cultivation. Microbiol. Rev 59:143±169.

Arino Ä X, Hernandez-Marine M, Saiz-Jimenez C. 1997. Colonization of Roman tombs by calcifying cyanobacteria. Phycologia 36:366±373.

Arroyo I, Arroyo G. 1996. Annual microbiological analysis of Altamira cave (Santillana del Mar), Spain. In: Riederer J, editor. 8th International Congress on Deterioration and Conservation of Stone; p 601±608.

Ball AS, Betts WB, McCarthy AJ. 1989. Degradation of lignin related compounds by actinomycetes. Appl Environ Microbiol 55:1642±1644.

Ball AS, Godden B, Helvenstein P, Penninckx MJ, McCarthy AJ. 1990. Lignocarbohydrate solubilization from straw by actinomycetes. Appl Environ Microbiol 56:3017±3022.

Bettini C, Agarossi G, Ferrari R, Monte M. 1977. Fenomeni di biodeterioramentoin ambienti ipogei: esperienze sul controlo di alcune specie microbiche. In: Proceedings 2nd International Conference on Nondestructive Testing, Microanalytical Methods and Environmental Evaluation for Study and Conservation of Work of Art. Rome: Istituto Centrale del Restauro; p 4.1±4.14.

CanaverasÄ JC, Hoyos M, Sanchez-Moral S, Sanz-Rubio E, Bedoya J, Soler V, Groth I, Schumann P, Laiz L, Gonzalez I, Saiz-Jimenez C. 1999. Microbial communities associated with hydromag-nesite and needle-®ber aragonite deposits in a karstic cave (Altamira, Northern Spain). Geomi-crobiol J; this issue.

Caumartin V. 1963. Review of the microbiology of underground environments. Natl Speleol Soc Bull 25:1±14.

Cigna AA. 1993. Environmental management of tourist caves. Environ Geol 21:173±180. Crawford DL, Pometto AL, Crawford RL. 1983.

Lignin degradation by *Streptomyces viridosporus*:

isolationand characterizationof a new polymeric lignin degradationintermediate. Appl Environ Microbiol 45:898±904.

Cunningham KI, Northup DE, Pollastro RM, Wright WG, LaRock EJ. 1995. Bacteria, fungi and biokarst in Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico. Environ Geol 25:2±8.

Dari K, Bechet M, Blondeau R. 1995. Isolation of soil *Streptomyces* strains capable of degrading humic acids and analysis of their peroxidase activity. FEMS Microbiol Ecol 16:115±122.

Fliermans CB, Schmidt EL. 1977. *Nitrobacter* in Mammoth Cave. Int J Speleol 9:1±20. Ghiorse WC. 1997. Subterranean life.
Science 275:789±790.

Giacobini C, De Cicco MA, Tiglie I, Accardo G. 1987. Actinomycetes and biodeterioration in the field of fine art. Biodeterioration7:418±423.

Gonzalez de los Reyes-Gavilán C, Barbes C, Hardisson C. 1984. Estudio de la ¯ ora microbiana de la cueva de Altamira. Rev Biol Univ Oviedo 2:41±50.

Goodfellow M, Williams ST. 1983. Ecology of actinomycetes. Ann Rev Microbiol 37:189±216. Groth I, Vettermann R, Schuetze B, Schumann P, Saiz-Jimenez C. 1999. Actinomycetes in karstic

caves of Northern Spain (Altamira and Tito Bustillo). J Microbiol Methods; in press. GuggenbergerG, Zech W. 1994.Dissolved organic carbon in forest oorleachates:simple degradation

products or humic substances. Sci Total Environ 152:37±47.

Hardisson C, Barbes C, Gonzalez CR. 1982. Contaminacion microbiana en las cuevas de Altamira. Unpublished report.

Hayakawa M, NonomuraH. 1987. Humic acid±vitamin agar, a new medium for the selectiveisolation of soil actinomycetes. J Ferment Technol 65:501±509.

Hernandez-MarineM, Canals T. 1994. *Herpyzonema pulverulentum* (Mastigocladaceae),a new cavernicolous atmophytic and lime-incrusted cyanophyte. Algol Stud 75:123±136.

Hoyos M, Soler V. 1993. La cueva de Nerja (Malaga):Â ejemplo de degradacionÂ microambiental. In: Fortea FJ, editor. La ProteccionÂ y ConservacionÂ del Arte Rupestre Paleolítico. Oviedo: Servicio de Publicaciones del Principado de Asturias; p 95±107.

Hoyos M, Soler V, Fortea J. 1993. Principales resultados sobre las características microambientales de la Cueva de San Román de Candamo (Asturias). In: Fortea FJ, editor. La Protección y Conservación del Arte Rupestre Paleolítico. Oviedo: Servicio de Publicaciones del Principado de Asturias; p 77±85.

Iliopoulou-Georgoudaki J, Pantazidou A, Theoulakis P. 1993. An assessment of cleaning photoau-totrophic micro¯ ora: the case of Perama cave, Ioannina, Greece. Mem Biospeol 20:117±120.

Komagata K, Nakase T. 1997. *Cryobacterium psichrophilum*gen. nov., sp. nov., nom. rev., comb. nov., an obligately psychrophilic actinomycete to accommodate ª *Curtobacterium psychrophilum*º Inoue and Komagata 1976. Int J System Bacteriol 47:474±478.