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MICROBIAL DETERIORATION OF A MESOAMERICAN AND HISPANIC MUMMIES

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Bibliographical review. Most of the biological ancient remains degradation depends on the microbes biodeteriorant potential (Sterflinger & Piñar, 2013). The degradation effect on mummified remains caused by the microorganisms, such as bacteria present in mummified tissues, actually does not correspond to the microorganisms presented at the death of the individuals. But they are commonly acquired from the museum or the environment surrounded where the remains have been stored, as happens with filamentous fungi (Aufderheide, 2003). Thus, the study of microbiota presented on the mummified remains is a good indicative of its conservation. On the other hand, metagenomic study of ancient microbial DNA, may offers an accurate analysis of specific historical trends. One of them is represented by tuberculosis. Tuberculosis is an acute or chronic infection of soft tissues or skeleton produced by *Mycobacterium tuberculosis* or *Mycobacterium bovis*. (Sotomayor *et al*., 2004) This infection had been spreaded throughout the world to become pandemic between XVIII-XX centuries what makes its study of special interest.

Abstract. This study is focused on the potential biodegradative microbiota presented in samples taken from two Spanish mummies located in Alpandeire (Málaga, Spain), from the 18th century and from another pre-Hispanic mummy known as "La Mexicana" originally from Perú and dated in 13th century. It were obtained 15 isolates among filamentous

fungi and bacteria. Microbial potential biodegradation capacity on mummies were evaluated studying cellulase, amylase and protease activity, because of the fundamental role its substrates play in biological and ornamental composition of mummified remains. Isolation strains were identified by PCR and Sanger sequenciation. A possible presence of *Mycobacterium tuberculosis* was also studied by PCR technique amplification of *M. tuberculosis*-specific CYP-141 gene. Tuberculosis reached its epidemic state in XVIII-XIX century and there are already evidence of its presence in pre-Columbian Americas.

Keywords. Mummification. Biodeterioration. *Mycobacterium tuberculosis.*

1. INTRODUCTION

Mummies represent a valuable and delicate cultural legacy (Valentin, 2015). Interest of microbiological approaches on mummified remains research, mean an important application and a few very historical considerations plenty of importance. On one hand, microbiological study of the mummified remains infers an analysis of the biodeterioration and conservational state of the own remains. The correct conservation of ancient remains depends on several factors, among them the chemical, physical, biological and environmental origin are critical (Valentin, 2015). Mummies studied of Alpandeire, were subject to a natural mummification process and they were exposure to a high longer environmental conditions until their final emplacement, currently located in the crypt of Padúa church. Its lack of further protection could have favored the presence of microbes that could be developed a biodeteriorant activity.

On the other hand, metagenomics studies of ancient DNA from mummified remains, that is the study of DNA sequences recovered directly from uncultivated samples (Jacqueline *et al.*, 2013), allow us to obtain information about the different historical conditions, environmental, biological and cultural moments of the mummified "organism", such as the diagnosis of diseases and the study of their evolution. One of the diseases that has had the deepest repercussion throughout our history is tuberculosis. This disease has been presented in a large part of human history, including "Pott's disease", which were well demonstrated in Peruvian mummies (Allison *et al.*, 1973). Because of the bone signals observed on the remains, thoracic tissues and bones samples were studied to diagnose the possible presence of Tuberculosis.

2. MATERIAL AND METHODS

Sampling was done by Institute for the Scientific Study in Mummies Bioethics protocol, keeping sterile conditions through all the analysis. It were used 30 mg of sample which were incubated in 50 ml of TSB culture medium (Tripticasein Soy Broth) for bacteria culture and in PDB medium (Potato Dextrose Broth) with Chloramphenicol (50 ppm) for fungal growing. Both prepared with an additional 1ml enrichment medium (2.0 g / 100 ml yeast extract and 7.4 g / 100 ml heart brain extract). After very seedings in TSA (Tripticasein Soy Agar) culture medium, at 37°C (48-96 hours) pure cultures were obtained. Filamentous fungi isolation were performed incubating spherical mycelium from broth cultures in PDA media at 28°C from 4 to 7 days.

2.1 Study of the biodeteriorant potential of isolated microbial strains

The biodeteriorative potential of microbial strains were analyzed by studying amylase, cellulase and protease enzymatic activities. Potential amylolitic activity of

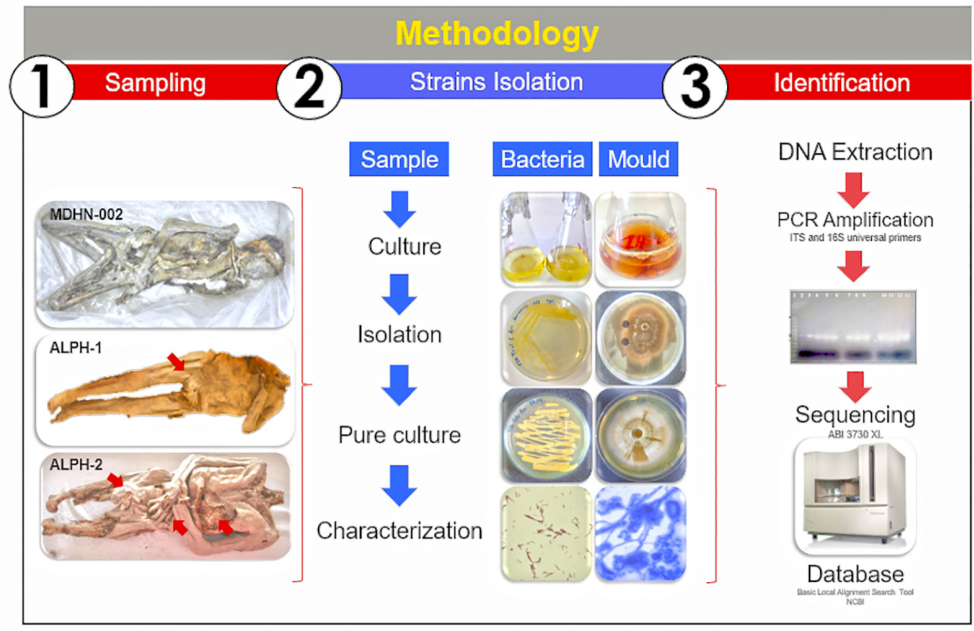

Fig. 1. Working scheme for the detection of microbial present degradation in mummified remains.

the strains were determined incubating bacteria strains in TSA (40.0 g/l) for bacterial or PDA (40.0 g/l) for fungal strains with starch (10.0 g/l), at 37 ° C, 48 to 96 h and at 28 °C, 4-7 days, for filamentous fungi strains. The assay was revealed with Lugol. Determination of strains cellulase activity, was performed by growing them on a Whatman® no. 1 cellulose surface disc placed on the top of a base-medium composed of ammonium nitrate (1.0 g/l), bipotassium phosphate (1.0 g/l), magnesium sulfate heptahydrate (0.5 g/l), casein peptone (2.5 g/l) and agar (15.0 g/l) final pH of 5.5, and incubated at 28°C and 37°C for bacterial and fungal growth respectively. To determine the protein degradation of isolated strains, gelatin agar medium was employed: TSA (40.0 g/l) or PDA (40.0 g/l) and gelatin (10.0 g/l). After the same period of time of seeding and incubating used in amylolitic activity, this assay was reveal with Frazier reagent.

2.2 Molecular identification of the isolated strains

DNA extraction and purification from isolated microorganisms was performed by the "Rapid extraction of fungal DNA for PCR amplification protoco" (Cenis, 1992). Molecular identification of strains was done by using specific primers of the hypervariable genomic region encoding for rRNA 16S (Young *et al*., 1991) for bacteria identification. Fungal identification was done using specific primers for the hypervariable genomic region of the ribosomal ITS 26/28S. The amplification of the regions mentioned was performed by PCR. Sanger sequencing of amplicons was performed in Illumina© MiSeq equipment. Alignment of sequences obtained were done in the "Basic Local Alignment Search Tool" of the National Center for Biotechnology Information obtaining a threshold of identity of 95%.

2.3 *Mycobacterium tuberculosis* diagnosis

The possible presence of *Mycobacterium tuberculosis* was determined using ancient DNA silica extraction techniques (Rohland and Hofreiter, 2007; Gomes *et al*., 2017) by PCR amplification of the CYP-141 gene, using specifically primers and PCR program (Rabiee-Faradonbeh *et al*., 2014). And *M. tuberculosis* genomic DNA was used as positive control at a concentration of 10 ng/µl in PCR reaction.

3. RESULTS AND CONCLUSIONS

Fifteen isolations were obtained among mould of genus *Aspergillus* and *Chaetomium*, and bacteria of the genus *Paenibacillus*, *Staphylococcus Microbacterium*

and *Micrococcus*. Microorganism isolated seems to share an environmental origin noticing how bacterial diversity is greater in comparison with fungal one. Strains isolated manifested high biodeteriorant potential activity.

Table 1. Molecular identification of bacterial strains and evaluation of its biodegradation potential.

Sampling	Identification	Collagen degradation	Starch degradation	Cellulose degradation
Alpandeire female mummy				
Thoracic cavity	*Staphylococcus* sp.	1+	0	0
Abdominal-rectal cavity	*Paenibacillus dendritiformis*	3+	3+	0
Left arm bend	*Paenibacillus* sp.	0	3+	1+
Alpandeire male mummy				
Epigastric	*Paenibacillus* sp.	3+	3+	0
Epigastric	*Paenibacillus* sp.	1+	3+	1+
Thoracic rub	*Staphylococcus* sp.	2+	0	0
Thoracic rub	*Staphylococcus epidermidis*	2+	0	0
Andean mummy				
Hair fragment	*Micrococcus luteus*	3+	0	1+
Hair fragment	*Micrococcus* sp.	2+	0	1+
Textile	*Microbacterium foliorum*	2+	1+	0
Textile	*Microbacterium* sp.	2+	0	0

3+: Very positive activity (radius of growth halo in cellulose substrate / radius halo amylolitic revealed test / proteolytic test > 2.0 cm). **2+:** Positive result (1.0 cm ≤ halo ≤ 2.0 cm). **1+:** Result slightly positive (halo < 1.0 cm). **0:** Negative result (no halo).

The andean mummy biodeterioration, seems to be caused by enzymatic bacterial synergical action of *Micrococcus sp*. and *Microbacterium sp.* strains. The absence of mould in the andean mummy and the presence of filamentous fungi in both hispanic mummies (*Table 2*), may be indicative of their greater exposure to environmental agents. The two more critical factors which may inhibit growth of strains are humidity and aeration. Under this conditions they tend to growth in protected areas like cavities (Aufderheide, 2003) conditions very different in hair and textile locations. Moreover, biodeterioration depends on the type and frequency of cares and cleaning of surfaces in museums (Sterflinger and Piñar, 2013), like the museum where this remains are currently exposed. Likewise, the possible biodeterioration

observed in hispanic mummies may be consequence of bacteria enzymatic activities, *Paenibacillus sp*. and *Staphylococcus sp*., like *Staphylococcus epidermidis* as well as filamentous fungi of the genera *Aspergillus sp*. and *Chaetomium sp.*

Table 2. Molecular identification of fungal strains and evaluation of its biodegradation potential.

Sampling	Identification	Collagen degradation	Starch degradation	Cellulose degradation
Alpandeire female mummy				
Abdominal-rectal cavity	*Aspergillus* sp.	0	1+	0
Left arm bend	*Aspergillus* sp.	0	0	3+
Left arm bend	*Chaetomium longicolleum*	0	0	3+
Alpandeire male mummy				
Epigastric	*Aspergillus* sp.	0	1+	0

3+: Very positive activity (radius of growth halo in cellulose substrate / radius halo amylolitic revealed test / proteolytic test > 2.0 cm). **1+:** Result slightly positive (halo < 1.0 cm). **0:** Negative result (no halo).

3.1. Molecular *Mycobacterium tuberculosis* diagnosis

No gene amplification of *M. tuberculosis* were obtained from any sample. Thus, we can infer that analyzed remains are exempted of the presence of *M. tuberculosis*. However, while PCR technique has been effective to obtain amplification of short segments of DNA extracted from archaeological samples, it is still complicated to identify nuclear aDNA with segments greater than 200 base pairs which also means a low recovery success (Aufderheide, 2003). Although other researchers had obtained amplification of similar sizes of aDNA in relative studies of tuberculosis diagnosis (Sotomayor *et al*., 2004), that makes the study difficult especially when our target region of primers used here (amplifying Cyp-141 gene), consists on 1203 base pairs size. We conclude that molecularly there is no evidence of tuberculosis infection in these valuable mummified remains.

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