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# Isolation and characterization of actinomycetes from Mural paintings of Snu- Sert-Ankh tomb, their antimicrobial activity, and their biodeterioration



Abeer F. Elhagrassy

Lecturer of Mural paintings and microbiology, Conservation Department, Faculty of Archaeology, Fayoum University, Egypt

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## ABSTRACT

A total of 35 actinomycetes were isolated from the surface of mural paintings of snu- sort- ankh in El Leasht, Egypt during four seasons all over 2016-2017. In 2009 this tomb was deteriorated by "*Aspergillus niger*, *A. flavus*, *Fusarium moniliforme*, *Alternaria alternate*, *Rhizopus stolonifera*, *Bacillus subtilis*, *Bacillus cereus*, and *micrococcus iuteus*." In 2017 the isolation of swabs presents only *Aspergillus niger* and about 35 actinomycetes classified to three different groups "*Streptomyces*, *Nocardia*, and *Micromonospora*" only five species belong to *Streptomyces* group showed antimicrobial activity against the previous microorganisms. These actinomycetes were identified according to their sequences in the GenBank to "*Streptomyces spectabilis*, *S. alborgriseolus*, *S. globus*, *S. corchorstt*, *S. ambofactens*." In the other hand, the pigments of the wall paintings of the tomb (Egyptian blue, Egyptian green, Goethite) that analyzed by SEM-EDX, FTIR were measured by the spectrophotometer in both 2009 and 2017. The results showed that the actinomycetes could produce extracellular pigments causing a color change of archaeological pigments otherwise it helps in inhibition the growth of the previous microorganisms found in 2009. The optimization factors for increasing the antibiotic production of the *Streptomyces* were 3% NaCl and temperature between 30:35°C in Alkin pH (pH = 7.5).

## 1. Introduction

Actinomycetes are prokaryotes "order of Actinomycetales"; they interpose in function and shapes between bacteria and fungi. Actinomycetes are Gram-positive mycelial bacteria that have a high metabolic diversity (Waksman, 1940). *Streptomyces* are mainly known to produce a wide variety of industrially and medically relevant compounds (antibiotics, chemotherapeutics, fungicides, herbicides and immune suppressants). (Subramani and Aalbersberg, 2012).

Actinomycetes as microorganisms recognized as a deterioration factor that played an essential role in decayed of archaeological stone and mural paintings, (Gorbushina et al., 2004; Berdoulay and Salvado, 2009; Kubik, 2010). However, little consideration has been carried on the bioactive of the actinomycetes in the cultural heritage field which called bioremediation of cultural heritage.

*Streptomyces* species are considered as the most group of Actinomycetes that deteriorated stones and mural paintings (Abdulla et al., 2008; Pepe et al., 2010; Abdel-Haliema et al., 2013). Otherwise, they are rich sources of thousands of bioactive natural product that has commercial important as antibiotics for several diseases, antifungal and antibacterial agent for most species that deteriorated the mural paintings. (Augustine et al., 2005; Abdel Fattah, 2009; Arifuzzaman et al., 2010; Holkar et al., 2013).

Egyptian pharaonic tombs have taken the value from the mural paintings in them that always holding messages from past, either tempera technique or fresco technique carried these mural paintings. (Lucas, 1948).

Egyptian tombs are always suffering from the biodeterioration that caused by the growth of microorganisms especially the mural paintings (Milanesi et al., 2006a,b; Berdoulay and Salvado, 2009; Rosado et al., 2015; Unković et al., 2015; Unković et al., 2016). The effect of the biodeterioration on these paintings appeared in the discoloration of pigments, the weakness of the mural paintings structure, and the weakness of the pigment layer as a result of the chemical reaction of the biofilm of the microorganisms or the physical damage during the penetration of the hypha. (Rölleke et al., 1996; Saarela et al., 2004; Veneranda et al., 2017)

The tomb of Snu sert ankh is an Egyptian tomb its mural paintings executed by tempera technique. In 2009, swab analysis was taken from all over the burial chamber; the analysis showed that the tomb was bio deteriorated by fungi and bacteria. In 2017, this tomb was suffering from crystallization of salt damage, but when another swab was taken from the same burial chamber, the analysis showed that the mural paintings were deteriorated by actinomycetes and only one species of fungi.

E-mail address: [afa01@fayoum.edu.eg](mailto:afa01@fayoum.edu.eg).

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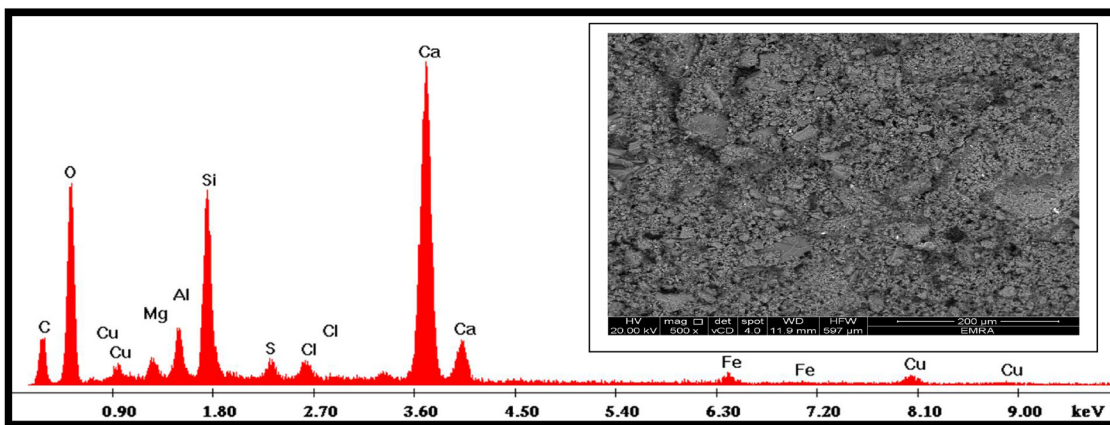


Fig. 1. Shows the ground layer of the mural paintings 500 × and the EDX spectrum of the components.

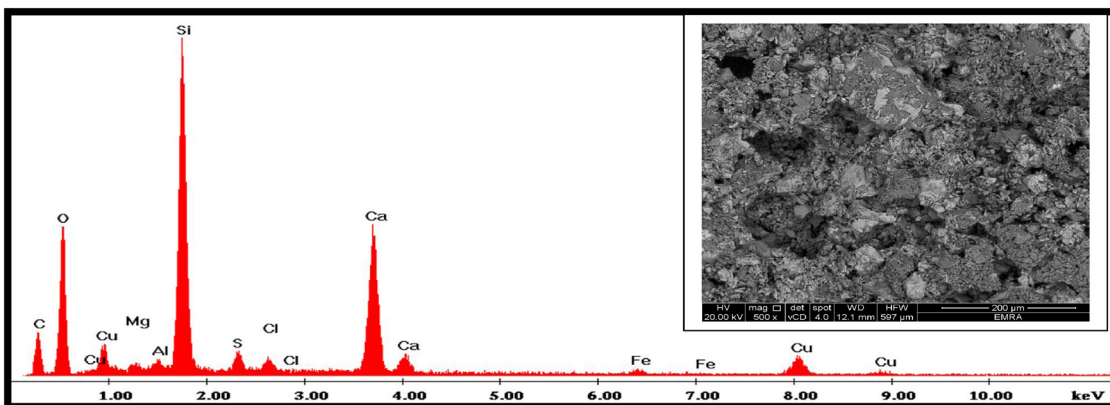


Fig. 2. The EDX Spectrum analysis of the Egyptian blue and the SEM Morphology of the pigment (500x).

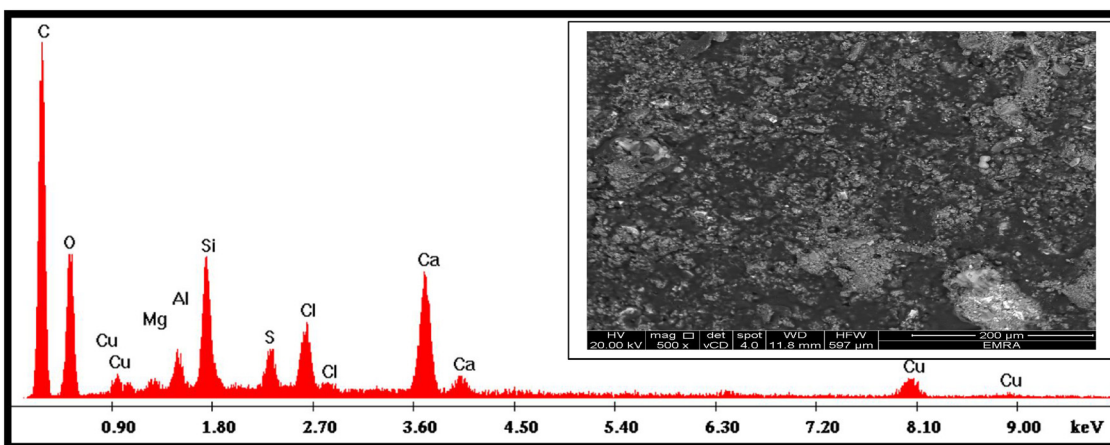


Fig. 3. The EDX Spectrum analysis of the Egyptian Green and the SEM Morphology of the pigment (500x).

2. Material and methods

2.1. (SEM) Scanning Electron microscope

The Examination and Analysis of Mural paintings carried by SEM microscope "the micrographs obtained by Jeol JSM (5600 L V), Philips XL 30; the EDX Unit attached to the SEM, 30 K.V., magnification started from (10x to 400.000x), the resolution for W. is (3.5 nm)".

2.2. FTIR spectroscopy

The Fourier transform infrared (FTIR) analyses executed by "Nicolet Nexus spectrophotometer (Washington, USA), which coupled with (Nicolet Continuum)," Fourier transforms infrared spectroscopy microscope equipped with a "HgCdTe detector cooled with liquid N2. Spectra recorded by a Graseby-Specac diamond cell accessory in transmission mode between 4000 and 700 cm<sup>-1</sup>".

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