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# Characterization of *Streptomyces* isolates causing colour changes of mural paintings in ancient Egyptian tombs

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ABSTRACT

Paintings in ancient Egyptian tombs often suffer colour changes due to microbial growth and colonization. *Streptomyces* strains were isolated from mural paintings of Tell Basta and Tanis tombs (East of Nile Delta, Egypt) and were identified using biochemical and molecular methods. The16S rDNA sequences data indicated that isolated strains were closely related to *S. coelicolor*, *S. albidofuscus*, *S. ambofaciens*, *S. canarius*, *S. parvullus*, *S. corchorusii*, *S. albidofuscus* and *S. nigrifaciens*. It could be shown that *Streptomyces* strains are involved on a large scale in the colour changes of paintings and stone support by producing a wide range of metabolites such as acids (oxalic, citric and sulphuric acids), biopigments of melanin, carotenoids, and hydrogen sulphide.



## 1. Introduction

Mural paintings in the ancient Egyptian tombs were carried out using a tempera technique either directly on stone surfaces or later in the Greek Roman period on plaster layers prepared from gypsum or from lime (Lucas 1948). Paintings in these tombs often suffer from biodeterioration due to growth and colonization of bacteria. *Streptomyces* have been described to cause different deterioration symptoms on mural paintings such as discoloration of stone surfaces, chemical alteration and physical alteration through penetrating mycelium into painting layers (Rölleke et al. 1996; Berdoulay and Salvado 2009). Deteriorability of *Streptomyces* for cultural heritage was attributed to ability of *Streptomyces* to produce a wide range of enzymes could decompose complex polymers into short chains polymers used as carbon source by *Streptomyces* (Goodfellow and Williams 1983; Ciferri 1999). Colour changes resulting in chemical alteration of pigments were used as indictors of the damage caused by growth and colonization of *Streptomyces*. *Streptomyces* are involved in colour change of paintings due to its ability to produce a wide range of metabolites such as organic and inorganic acids, enzymes, hydrogen sulphide and biopigments which react with various wall paintings constituents (Strzelczyk 1981; Groth and Saiz-Jimenez 1999; Gorbushina et al. 2004; Kubik 2010).

The spectrum of colour changes known to be caused by growth of Streptomyces species on ancient painitings and other artefacts is wide. It was reported that azurite blue on cartonnage and collars from Ancient Egypt was turned into black colour of covellite (Scott et al. 2004), due to the reaction of hydrogen sulphide produced by *Streptomyces* with azurite blue (Tresner and Danga 1958). It was also described that azurite blue was turned into green copper oxalate, the oxalate probably being a catabolic product of the microorganism (Liuveras et al. 2010). Furthermore, azurite blue may turn into malachite green due to increasing humidity caused by water retention of biofilm formed by *Streptomyces* strains. Azurite blue can be also turned into green color of brochantite by biogenic sulphuric acid. Other degradation products of azurite are the green pigment verdigris (basic copper acetate), and hydrated copper citrate, both, the acetic acid and the citric acid being produced by microbial activities (Grim and Allison 2003; Eremin et al. 2008) For this reason, azurite blue characterized with different shadows of green color in the deteriorated mural paintings. It was pointed out that acidification activity of *Streptomyces* strains by carboxylic and citric acids production might contribute into color change of paintings due to chemical alternation of pigments

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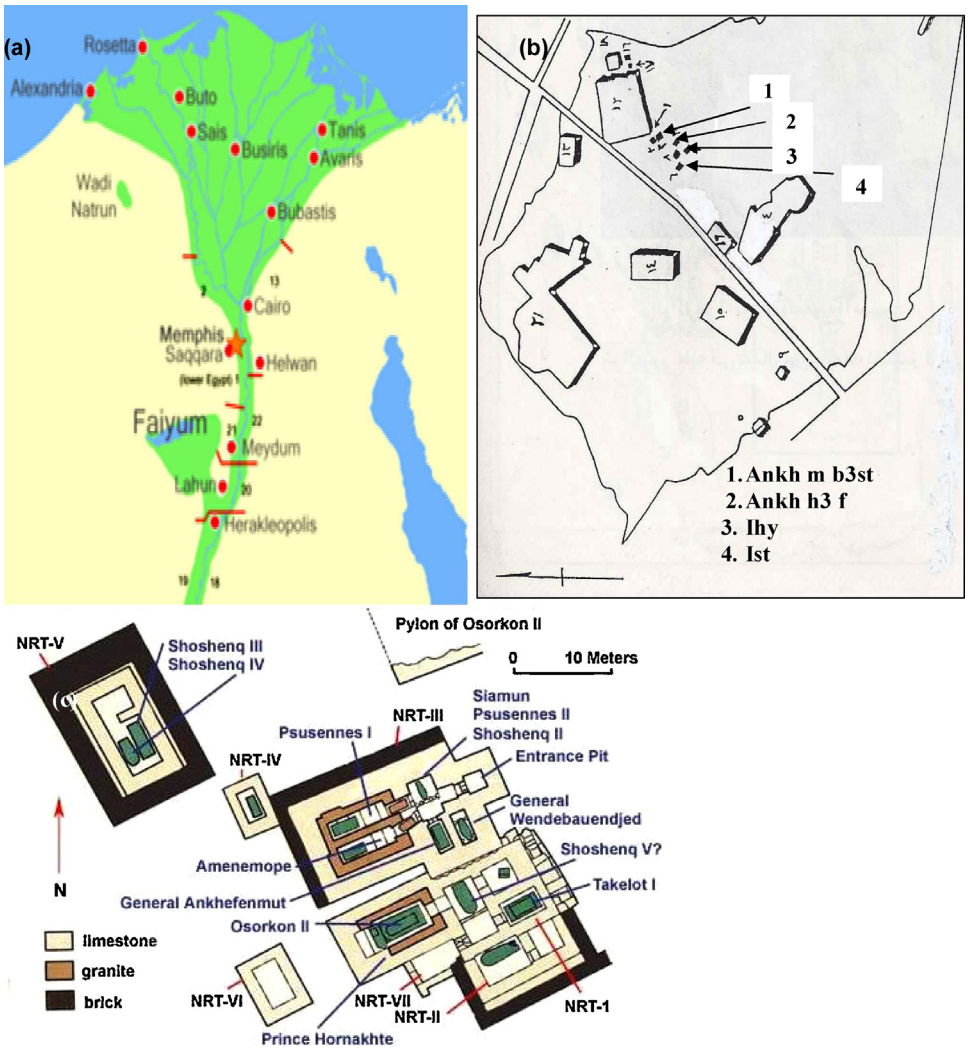


**Fig. 1.** (a) Location of Tanis and Tell Basta (Bubastsis in the Greek-period), (b) Bubastis tombs, (c) Tomb of the King Oserkon II among Tanis tombs.

(Milanesi et al. 2006a). This microbial activity was responsible for color change of the manuscript parts in the library of Vercelli, Italy, dated back to IXth century (Aceto et al. 2006).

Moreover, alteration of paintings should depend on the chemical composition of pigments, with copper-based pigments being more sensitive to acids than iron based pigments. Strzelczyk (1981) delineated that *Streptomyces* grow and colonize paintings containing heavy metals with high toxicity such as Hg, As, Cu and Pb better than other microorganisms.

Another enzymatic activity of *Streptomyces* strains involved in color change of paintings by producing peroxidase enzyme acted as strong oxidizing agent of organic pigments such as indigo and lake (Mercer et al. 1996) that were used as blue and red color respectively in tempera paintings dated back to the Greek–Roman period. Peroxidase activity has been described to cause the decolorization red azo dyes (Pasti-Grigspy et al. 1992).

The growth and colonization of *Streptomyces* cause irreversible stainings and chromatic alteration of stone and mural paintings by producing carotenoids with red, orange, yellow, pink and violet color and melanins with black and brown olivey colors (Estellés et al. 2006).

Finally, mycelium of *Streptomyces* colonizing mural paintings penetrates into these paintings. The mechanical pressure due to alternating wetting and drying of the mycelium causes binding media in these paintings to lose their cohesion (Strzelczyk 1981).

Although *Streptomyces* have been described to cause the deterioration of ancient paintings, the role of *Streptomyces* in color change of mural paintings in ancient Egyptian tombs has never been investigated in detail. Here we describe the isolation and identification of *Streptomyces* strains colonizing mural paintings in some ancient Egyptian tombs. In order to put forward the most appropriate conservation strategy we wanted to accurately identify the deteriorating *Streptomyces* species. We show how the isolated *Streptomyces* strains cause color changes and other symptoms of deterioration in these paintings such as chromatic alteration of stone surfaces, physical alteration through penetrating mycelium and chemical alteration of pigments.

## 2. Materials and methods

### 2.1. Sampling

Forty six samples were taken from tombs of Ankh h3 f, Ankh m b3st, Ihy, Ist (Tell Basta, 2420 B.C.) and the tomb of Oserkon II (Tanis, 860–848 B.C.) using sterile cotton swabs (Fig. 2). The maps illustrate the locations of Tanis and Tell Basta in Egypt and show the location of tombs within every site (Fig. 1). Samples were taken from representative sites showing deterioration symptoms of staining and scaling. Samples were cultivated onto starch-nitrate-agar (SNA) plates (agar 20; starch 20; $KH_2PO_4$ 1; $MgSO_4$ 0.5; NaCl 0.5; $KNO_3$