THE RELATIONSHIP BETWEEN SALTS AND GROWTH OF *STREPTOMYCES* COLONIES ISOLATED FROM MURAL PAINTINGS IN ANCIENT EGYPTIAN TOMBS

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1. Introduction

Mural paintings in ancient Egyptian tombs are often deteriorated by microbial colonization and, particularly by *Streptomyces*. These bacterial species commonly colonize hypogea environments, such as caves and tombs, characterized by darkness, low temperature and nutrients [1], this may be attributed to the ability of *Streptomyces* to utilize a wide range of carbon sources and to produce a wide range of enzymes. Enzymatic activities that could decompose complex polymers of chopped straw used as filler, or organic matters present in limestone or dead fungal hyphae, could be used by microorganisms as a carbon source [2-3].

Streptomyces colonies on mural paintings and stone surfaces are considered as indicators of an advanced deterioration stage and are the most dominant in the microbiota colonizing these paintings [4]. Each *Streptomyces* species seems to play a specific role in the deterioration of mural paintings and stone supports [5], causing discoloration and enhancement of mechanical deterioration in relationship to salt cycles [6].

Identification of microorganisms colonizing mural paintings is an important step in putting forward sound conservation strategies, but due to problems imposed by morphological methods in identification, molecular methods based on 16S rDNA sequence analysis have been used to identify Actinomycetes isolated from deteriorated stones and mural paintings, confirming the morphological and biochemical identification keys [7-12].

Streptomyces are involved significantly in the deterioration of mural paintings in cooperation with other deterioration agents, salts in particular. Several salts were detected in deteriorated mural paintings in ancient Egyptian tombs from different sources. The sources of these salts are the composition of building stone and leakage of underground water containing salt ions from the soil to the walls of the tombs, since NaCl

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concentrations in the soil of investigated tombs reached up to 11574 ppm/g [13].

The most common salts detected in ancient Egyptian mural paintings were sodium chloride, potassium chloride, magnesium sulfate and sodium nitrate. Sodium chloride (NaCl) is a highly soluble and hygroscopic salt that absorbs humidity from the air and retains it in the wall surface, thus producing dark batches [14, 16].

Actinomycetes and halotolerant bacteria could thrive in hostile environments (solar energy, hypersalinity and radiation) where there is a little microbial competition. *Micrococcus*, *Arthrobacter* and *Streptomyces* were the most dominant in salted deteriorated mural paintings [4,6].

Streptomyces isolates were halotolerant NaCl, KCl and Mg SO_4. 7 H_2O, the most common salts occurring in deteriorated mural paintings, as well as *S. rishiriensis* colonizing mural paintings in the Altamira Cave, Spain and could grow up to 15 % NaCl [16]. Moreover, *Streptomyces diastaticus*, *S.cyaneus*, *S.antimycoticus, S.chromofuscus, S. exfoliatus, S.rochei, S. albidoflavus, S.anulatus, S. roseflavus, S.badius, S. violaceus, S. griseoflavus, S. macrosporus, S. phaeochromogenes, S. griseostramines* and *Streptomyces* sp. isolated from the tomb of Maat Tell Basta, were halotolerant to 7-13 and 10 % for NaCl and N_2SO_4. 10 H_2O respectivley [17].

Saiz-Jimenez and Laiz [16] reported that the optimum salt concentration for the growth of *Farnkia*, *S. griseus* and *Pseudonocardiaceae* isolated from mural paintings at Herberstein Castle, Austria, was 10 % of magnesium sulfate and 5 % sodium chloride, showing a rosy color, while higher salinity, 10-15 %, reduced the counts of microbial cells.

Furthermore, *Halococcus salifodinae* was isolated from white efflorescence of sodium nitrate and sodium chloride from the chapel of St. Virgil, Vienna, Austria, and their optimal growth occurred at 20-25% NaCl [18].

Streptomyces have a synergistic mechanical action with salts, where it was reported that salts enhance the mechanical deterioration of stone surfaces since the penetrating *Streptomyces* mycelium enlarge voids and cracks and resulted in the dissolving and recrystallization of salts. Moreover, sodium chloride is a hygroscopic salt and has high water retention, increasing the volume of *Streptomyces* mycelium that are mainly composed of extracellular-polymeric substances (EPS); whereas wetting and drying cycles cause the disintegration of stone surfaces [3,6,16,19]. On the other hand, salt action can roughen the stone surface that is the preferential site for colonization by various communities of microorganisms [20, 21].

It is reported that the presence of sulfate salts can lead to irreversible damage to historic constructions; in the presence of humidity it can dilute and withdraw the pigment layers of mural paintings and this high water content could dissolve the binding media that can also serve as nutrients for some micro-organisms [22].

In addition, there was an interrelation between the detected salts, and the growth and metabolic activity of isolated *Streptomyces* colonies, whereas salts obligate *Streptomyces* to choose different adaption mechanisms such as producing protective molecules for example melanin and carotenoids [23-27]. These pigments cause aesthical damage and irreversible staining of colonized paintings and plaster layers [28, 29].

In addition to pigment production *Streptomyces* have an osmotic balance mechanism of organic osmolytes responsible for osmotic balance (glycine betaine, ectoines, praline, N-acetylated diamino acids, N-derivatized glutamine amides and other amino acid derivatives), that have been found in all microbial colonies isolated from salt ef-

florescence [16, 25]. The main function of osmolytes could be to protect the protein conformations and so prevent their denaturation, or influence the conformational stability of nucleic acids induced by high salt concentrations [30].

The purpose of this research is to isolate and identify *Streptomyces* colonies from deteriorated mural paintings in ancient Egyptian tombs and to determine the effect of salts detected in these mural paintings on the growth and metabolic activity of *Streptomyces* strains, in order to put forward the most suitable conservation strategies for these paintings.

2. Experimental

2.1. Location and Sampling

Eight samples of *Streptomyces* were collected from deteriorated mural paintings in the tombs of Ankh m b3st, Ist, Ankh h.f and Ihi at Tell Basta and the tomb of Oserkon II at Tanis, Lower Egypt (Figure 1), using a sterile cotton swab method. These samples were cultivated onto starch-nitrate-agar (SNA) medium (agar 20 g, starch 20 g; KH_2PO_4 1 g; $MgSO_4$ 0.5 g; NaCl 0.5 g; KNO_3 2 g; CaCO3 3 g in 1L distilled water), supplemented with an antifungal molecule (Dermatine 10-50 µg / l), to inhibit the growth of competitive fungi. Plates were incubated for 45 days at 30°C.

2.2. Identification of Streptomyces

Bacterial isolates were identified morphologically and biochemically according to the identification keys of Kämpfer, [31] and confirmed by analyzing the 16S rDNA sequences.

2.3. 16S rDNA sequencing

16S rDNA sequencing is one of the most promising techniques for identification of *Streptomyces*, whereas total DNA was extracted from eight isolated colonies of Actinobacteria [32]. The gene coding for 16S rRNA was amplified from each isolate by PCR with universal primers (forward primer, F27, 5'-AGAGTTTGATCCTGGCTCAG-3' [33] and reverse primer, R1492, 5'-GGTTACCTTGTTACGACTT-3') [34]. These primers bind to universally conserved regions and permit the amplification of a DNA fragment, approximately 1500 bp in length. The PCR amplification was carried out by a Gene-Amp PCR system 9600 thermocycler (Perkin Elmer). The amplification conditions were as follows: 94 °C for 10 min and 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 1 min, extension at 72 °C for 1 min; completed by a final extension step at 72 °C for 10 min. Presence and yield of specific PCR products (16S rRNA gene) were analyzed on 1% agarose gel. Before sequencing PCR product was cleaned up by using GeneJET™ PCR Purification Kit (Fermentas).

Amplified DNA fragments were partially sequenced at GATC Biotech AG (Konstanz, Germany) using an ABI 3730xl DNA sequencer, using forward primer (F27). The 16S rDNA sequences determined, were deposited into NCBI web server (www.ncbi.nlm.nih.gov). Sequence analysis and comparison were performed using the Basic Local Alignment Search Tool (BLAST) program (http://www.ncbi.nlm.nih.gov/blast) [35].

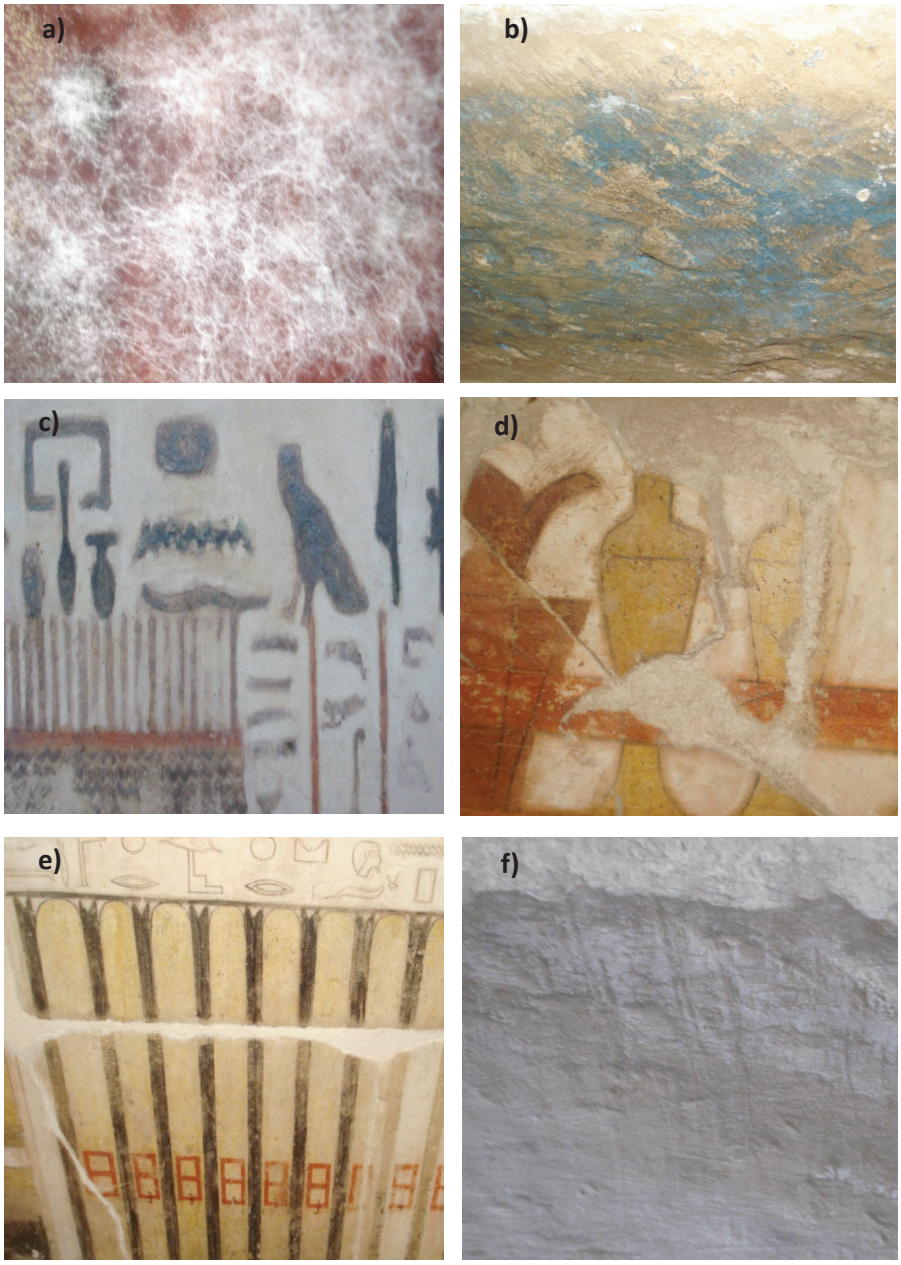


Figure 1. Location of sampling: (a) Azurite blue in tomb of Oserkon II with white crust, Tanis. (b): Red ochre, tomb Ankh m b3st. (c) Azurite blue, tomb of Ihi, Tell Basta. (d) Red and yellow paintings, tomb Ankh h3 f, Tell Basta (e) Black color from tomb of Ankh h3 f, Tell Basta. (f) White crust of soduim chloride, tomb of Ist, Tell Basta.

2.4. Salts analysis

White crust, sampled from the blue color in the ceiling room of the King Oserkon II at Tanis, was analyzed by SEM-EDS (Energy Dispersive Spectrometry) microscopy, using a Philips XL 30 ESEM scanning electron microscope; National Research Centre, Dokky, Cairo, Egypt.

2.5. Effect of different concentrations of salts NaCl, KCl and $MgSO_4$. 7 H_2O (2.5, 5 - 10 %) on metabolic activity

To investigate the mechanical destruction caused by *Streptomyces*, limestone discs were inoculated with *S. coelicolor* and *S. canarius*, incubated for 3 weeks at 30°C.

2.6. Scanning Electron Microscope (SEM) investigations

Limestone discs (diameter 1.5 cm), initially treated with different concentrations of NaCl, KCl and $MgSO_4$. 7 H_2O, were inoculated under aseptic conditions with *Streptomyces* isolates and incubated for 6 months at 30°C, in beakers containing 50 ml distilled water to establish a humid environment. Samples of inoculated limestone discs were prepared for SEM investigations according to Milanesi *et al*., [21], whereas these samples were fixed in 4% glutaraldehyde for 24 h and then dehydrated by ethyl alcohol series 25, 50, 75 and 100 %, (one hour per step). Samples were then coated with gold microparticles and investigated by using the Scanning Electron Microscopy, National Research Centre, Dokky, Cairo, Egypt.

2.7. Extraction and purification of red pigment

Extracellular red pigment produced by *S. canaries* under stress conditions (different NaCl concentrations) was extracted and purified according to Sterflinger, [36] and Baskar *et al*. [37]. The *S. canaries* strain was cultured on broth starch-nitrate medium, Erlenmeyer flasks (250 ml), containing 50 ml of medium supplemented with 10 % NaCl and incubated for 7 days at 30 °C.

5 grams of biomass were added to a mixture of *n*-hexane and acetone (92/8 v/v), then centrifuged for 10 min. at 5000 rpm. The volume of the organic mixture where the red pigment was dissolved, was reduced by up to 0.1 ml. The resulting volume of pigment was purified on TLC plates and the fractions of pigment were analyzed by JASCo. FT / IR 61000, National Research Centre, Dokky, Cairo, Egypt.

2.8. Determination of amino acids

Amino acids produced by *Streptomyces* strains under stress of salinity were valued in broth medium, by using a HPLC amino acid analyzer, LC300 Eppendorf-Germany, connected to a flourescence detector, National Research Centre, Dokky, Egypt, according to Callejon et al. [38]. *Streptomyces* strains were cultured on broth starch-nitrate-agar medium in Erlenmeyer flasks 250 ml, and incubated at 30°C for 7 days. 20µL of filtrate was injected in the column (Luna C18,5 µm, 250 × 4.6 mm and guard column 4.0 × 3.0 mm). Detection was performed by fluorescence excitation at 250 nm and emission at 395 nm. Fractions were obtained at a flow rate of 1 mL min-1 at 34 °C. Results were normalized against standard a solution of 22 amino acid.

3. Results

3.1. Identification of Streptomyces

Our finding indicated that all identified isolates were of *Streptomyces* genus and were attributed to *S. albidofuscus, S. ambofaciens*, *S. canaries*, *S. chibaensis*, *S. coelicolor*, *S. corchorusii*, *S. nigrifaciens* and *S. parvullus*. The authors' accession numbers in the International Gene Bank were illustrated in Table 1.

Table 1. Phylogenetic affiliation of inoculated strains (Homology of 16S r DNA and similarity in comparison with NCBI Data)

Location	Homology Approximately	Similarity enter genes 16S r DNA	Authors' accession number	G+C content
Azurite blue, tomb of Ihi, Tell Basta	*S. albidofuscus*	99 %	Later name is *S. pyridomyceticus* BankIt1507621 JQ625331	58 %
Yellow color of Southern wall of Ankh h3 f tomb	*S. ambofaciens*	99 %	BankIt1507642 JQ625332	60.6 %
Blue color, ceiling burial tomb of Oserkon II, Tanis	*S. canaries*	99 %	BankIt1507650 JQ625337	58.9 %
Black color, tomb Ankh h.f, Tell Basta	*S. chibaensis*	100%	BankIt1507649 JQ625336	58.9 %
Red color, tomb Ankh m b3st, Tell Basta	*S. coelicolor*	99 %	BankIt1507648 JQ625335	59.2 %
Limestone, tomb of Oserkon II, Tanis	*S. corchorusii*	98 %	BankIt1507647 JQ625334	58.9 %
North wall, tomb of Ist, Tell Basta	*S. nigrifaciens*	98 %	Later name is *S. flavovirens* BankIt1507149 JQ625330	~56 %
Yellow color of Southern wall of Ankh h3 f tomb	*S. parvullus*	99 %	BankIt1507645 JQ625333	56.3 %

3.2. SEM-EDX spectra of white crust

SEM-EDX spectra of white crusts covering the blue pigment in the tomb of *Oserkon* II, revealed that sodium chloride (NaCl) was in approximately pure needle form as illustrated in Figure 2 a. Moreover, SEM micrograph in Figure 2 b pointed out that halite in needle shape was formed, before reaching the cubic crystals, the typical form of halite

and veins of halite within the limestone blocks in the burial chamber of King Oserkon II, Tanis (Figure 2 c). Figure 2 d showed dissolving and precipitation of calcite by underground water in the form of stalactites. The EDX pattern of white crusts on the surface of the tomb of Ihy in Figure 2 e showed that crusts were of nearly pure halite.

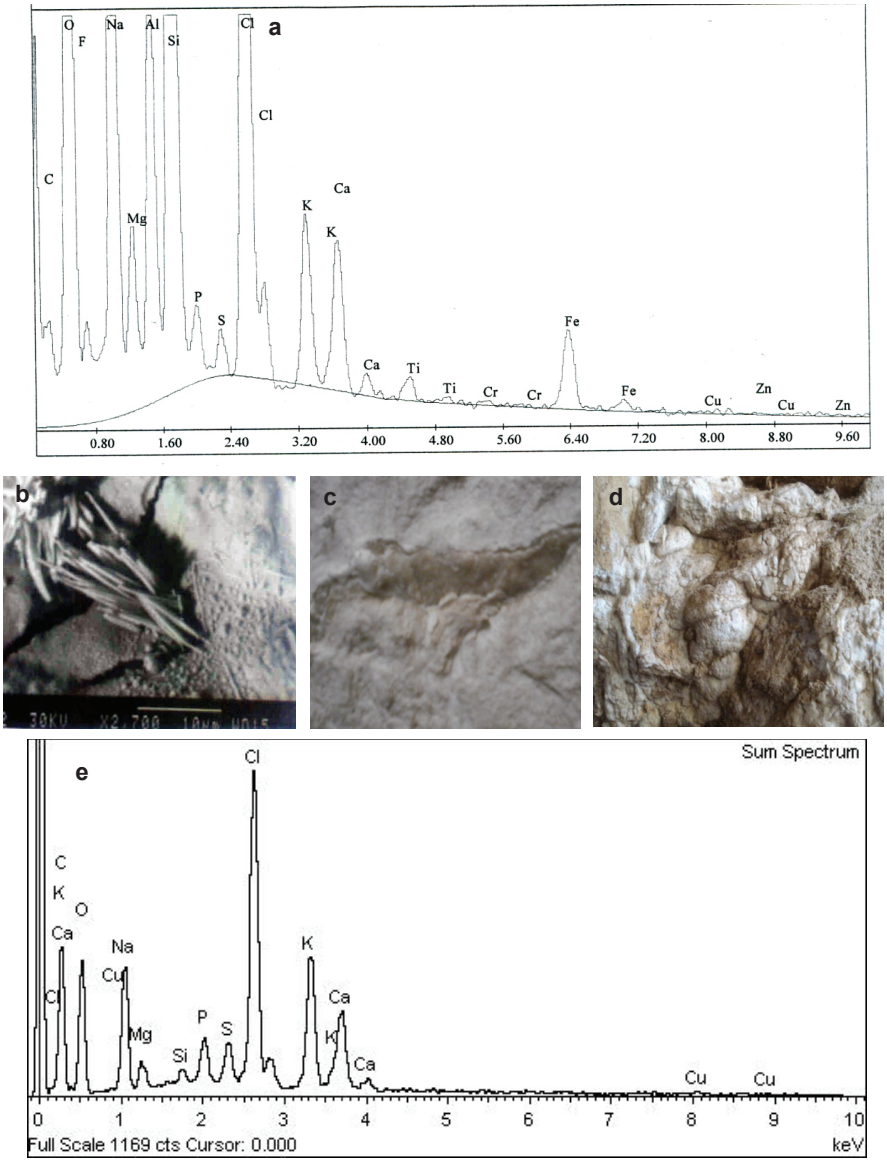


Figure 2. (a) SEM-EDX spectra of white efflorescence from ceiling room, tomb of Oserkon II, Tanis. (b) needle form of halite from the ceiling room tomb of Oserkon II, Tanis, (c) veins of halite within the limestone blocks, burial chamber of King Oserkon II. (d) dissolving and precipitation of calcite, tomb of King Oserkon II (e) EDX pattern of white crust from limestone surface, tomb of Ihy, Tell Basta.

3.3. Effect of sodium chloride

With regard to the effect of Nacl on the growth of *Streptomyces*, Figure 3 and Table 2 showed that *S. coelicolor*, *S. ambofaciens*, *S. canarius* grew very well at 10% NaCl. *S. parvullus* had good growth, *S. chibaensis*, *S. corchorusii*, *S. nigrifaciens* had moderate growth, while *S. albidofuscus* had reduced growth at the same NaCl concentration. It was found that the growth rate decreased with the increase of NaCl concetntration up to 10 %, and *S. canaries* showed a high tolerance to NaCl 10% and produced red pigment, not produced under normal culture conditions (Figure 4).

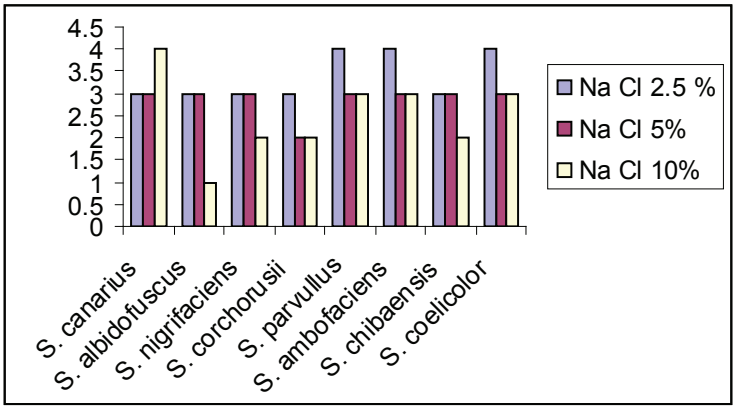


Figure 3. Effect of different NaCl concentrations on the growth of isolated Streptomyces

Streptomyces	Salts concentrations (%)								
	NaCl			KCl			$MgSO_{4\cdot7}H_2O$		
	2.5	5	10	2.5	5	10	2.5	5	10
S. albidofuscus	+++	+++	+	++	++	+++	+++	+++	+++
S. ambofaciens	++++	+++	+++	++++	+++	+++	+++	+++	+++
S. albidofuscus	+++	+++	+	++	++	+++	+++	+++	+++
S. ambofaciens	++++	+++	+++	++++	+++	+++	+++	+++	+++
S. canarius	+++	+++	++++	++	++	+++	++	++	+++
S. chibaensis	+++	+++	++	++++	+++	+++	++++	+++	+++
S. coelicolor	++++	+++	+++	++++	+++	+++	++	++	+
S. corchorusii	+++	++	++	+++	+++	+++	+++	+++	+++
S. nigrifaciens	+++	+++	++	++	++	++	++++	+++	+++
S. parvullus	++++	+++	+++	++++	+++	+++	++++	+++	+++

(++++) very good growth (+++) good growth (++) moderate growth (+) low growth.

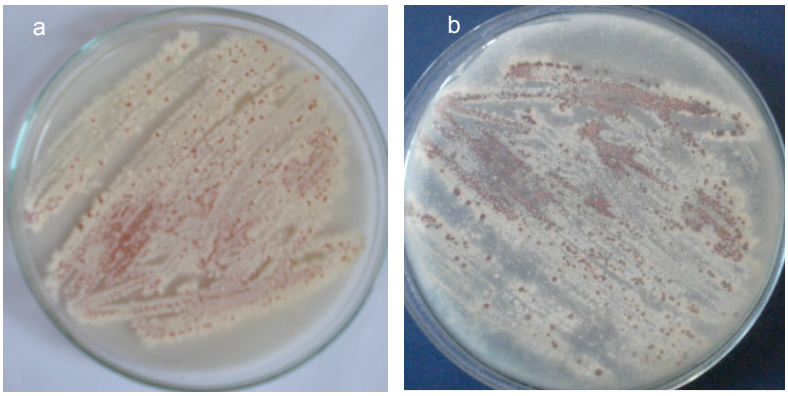


Figure 4. Effect of NaCl on S. canaries: (a) 5 % (b) 10 % NaCl

3.4. Effect of potassium chloride

The second most common salt identified in deteriorated mural paintings was KCl (Sylvite). Figure 5 and Table 1 showed that *S. coelicolor, S. chibaensis, S. ambofaciens, S. parvullus, S. corchorusii* have very good growth in the presence of 10% KCl; good growth for *S. canarius, S. albidofuscus*; reduced growth for *S. nigrifaciens*, at the same KCl concentration. *Streptomyces* could grow up to 10%, whereas growth was poor at 15% and impaired at 20% KCl. Our finding indicated that KCl up to 10% enhanced *S. ambofaciens* to produce a rosy pigment (Figure 7 c) and enhanced sporulation of *S. coelicolor* after 14 days of incubation.

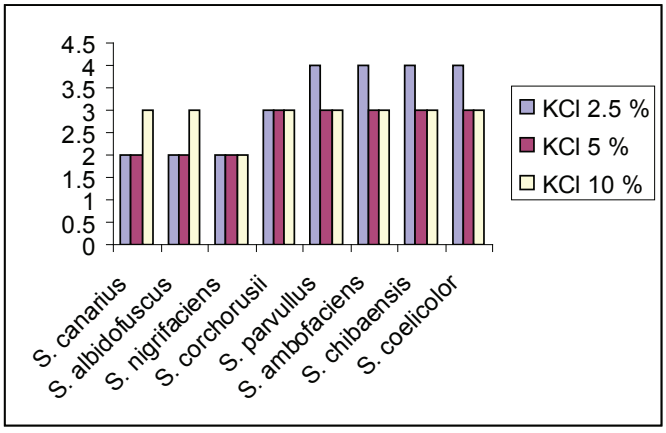


Figure 5. Effect of different KCl concentrations on the growth of isolated Streptomyces

3.5. Effect of magnesium sulfate

Figure 6 shows that *S. nigrifaciens, S. corchorusii, S. parvullus, S. ambofaciens* and *S. chibaensis* have very good growth with 10% magnesium sulfate ($MgSO_4.7\ H_2O$), but

S. canarius, S. albidofuscus have good growth, and *S. coelicolor* had reduced growth with the same salt concentration. Current results indicated that different concentrations 2.5, 5 and 10% of NaCl, KCl and $MgSO_4$ -7 H_2O, enhanced sporulation of *S. coelicolor,* increasing the pigmentation of *S. ambofaciens* (Figure 7).

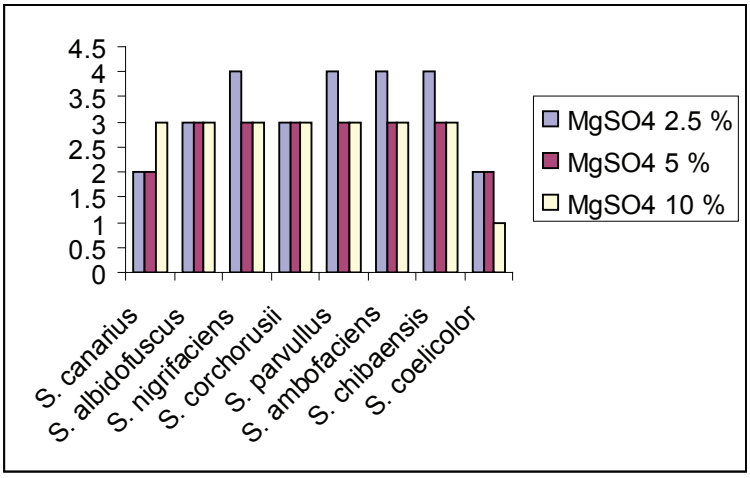


Figure 6. Effect of different $MgSO_4$ 7 H_2O concentrations on the growth of isolated Streptomyces

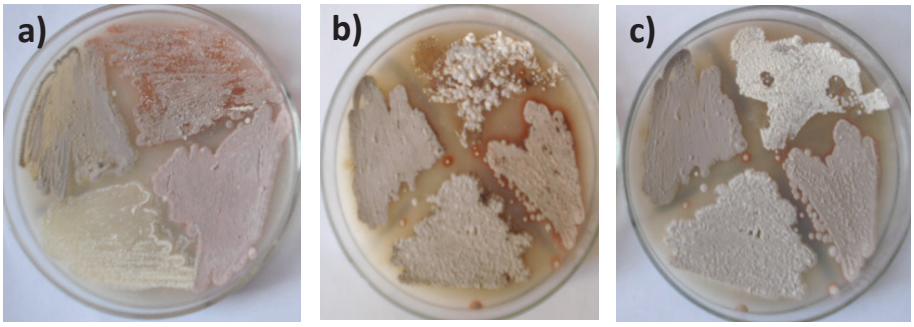
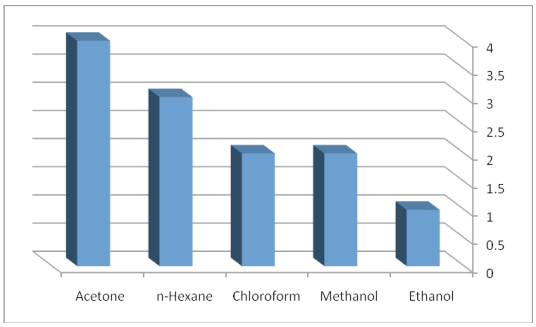


Figure 7. Effect of KCl on growth of Streptomyces strains: (a) control, (b) effect of KCl 2.5% on the growth of Streptomyces coelicolor. (c): pigmentation of S. ambofaciens

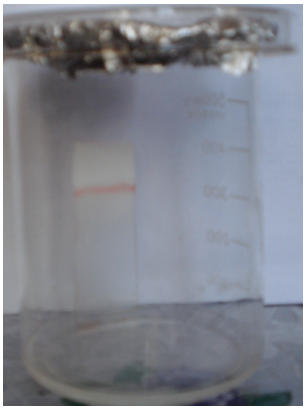
3.6. IR pattern of red pigment

Red pigment was extracted by a mixture of *n*-hexane and acetone (92/8 v/v) on TLC plates (Figure 8 a). On the one hand, Figure 8 b showed that organic solvents have different dissolvability for red pigment, since acetone has high dissolvability, *n*-Hexan, chloroform and methanol have moderate dissolvability, but ethanol has low dissolvability. On the other hand, the IR pattern of extracted red pigment indicated the presence of quinon (ON-O-R) group at 3457 cm^{-1}, carbonyl compounds (R_2C = O) at 1638 cm^{-1} and alkanes $(CH_3)_2$ CH at 957 cm^{-1} (Figure 8 c). The red pigment is

non pH sensitive, its red color did not change with the changing of pH values. Finally, HPLC data indicate that all *Streptomyces* strains produced glutamic acid at NaCl 10% (unpublished data).



a)



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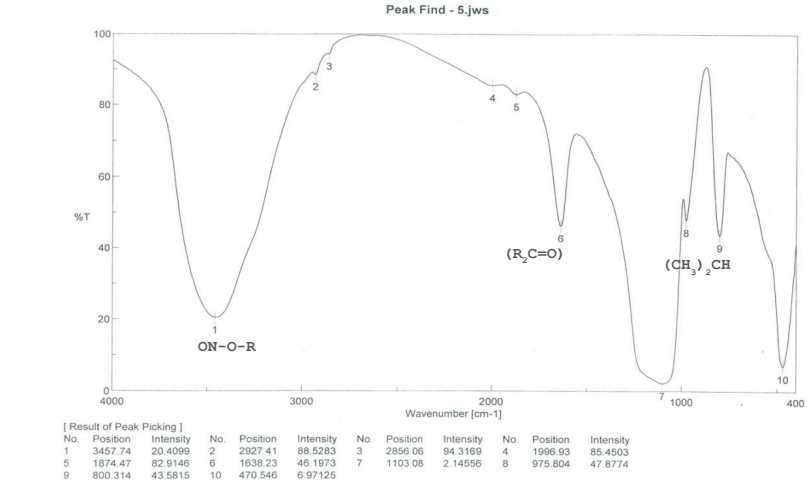

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3457.74	20.4099	2	2927.41	88.5283	3	2856.06	94.3169	4	1996.93	85.4503
5	1874.47	82.9146	6	1638.23	46.1973	7	1103.08	2.14556	8	975.804	47.8774
9	800.314	43.5815	10	470.546	6.97125						

c)

Figure 8. (a) Extraction of red pigment, (b) Extractability of red pigment with different organic solvents, (c) IR spectra of red pigment produced by S. canaries under hyper-salinity induced by NaCl.

3.7. Mechanical deterioration

Figure 9, showed *Streptomyces* mycelia, enlarging the fissures and cracks caused by salt, penetrating within inter-granular spaces of calcite crystals, in deteriorated limestone discs.

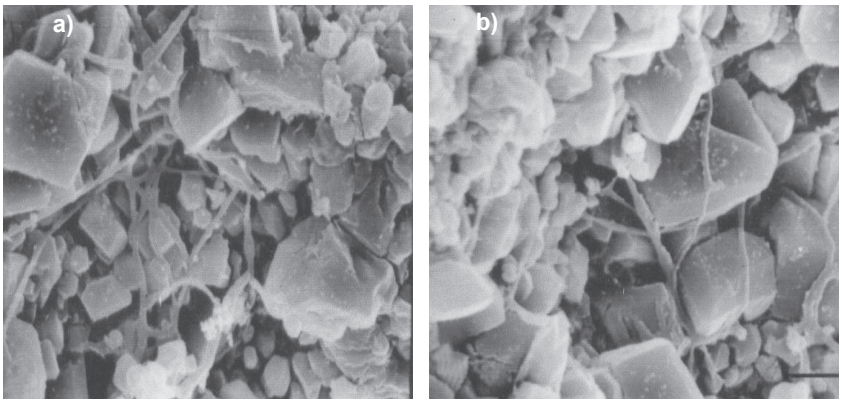

Figura 9. SEM micrographs illustrate the synergistic action of Streptomyces and salts, in the deterioration of limestone discs, Tomb of Maat, Tell Basta (Magnitude X 1500).

Figure 10 showed that *Streptomyces* isolates varied in their productivity of amino acids under stress of hypersalinity of sodium chloride, since *S. albidofuscus* was the main producer, *S. canarius*, *S. ambofaciens*, *S. nigrifaciens* and *S. chibanensis* showed moderate productivity, but *S. coelicolor, S. corchorusii* and *S. parvullus* showed low productivity of amino acids.

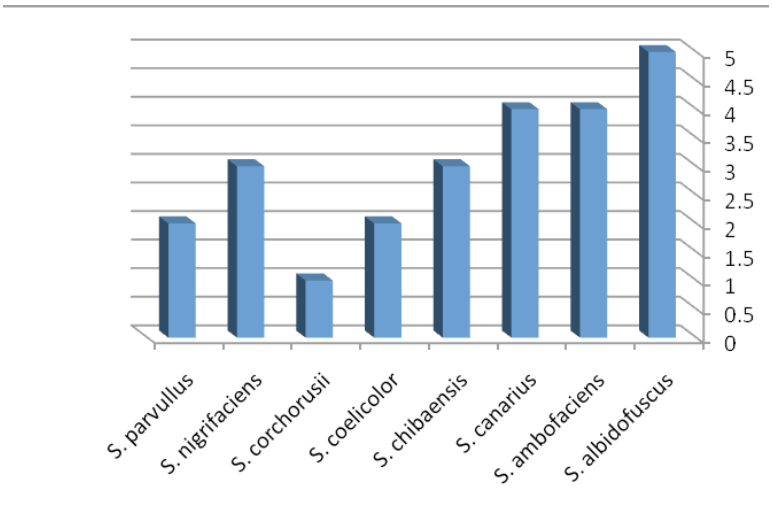

Figura 10. Productivity of amino acids by Streptomyces isolates

4. Discussion

16S rDNA data showed that all isolates had a similarity of more than 98% with *Streptomyces* genus, the most dominant in the microbiota colonizing deteriorated mural paintings [4,36]. On the other hand, it was reported that *Streptomyces* are the first colonizers of deteriorated mural paintings and an indicator of an advanced phase of deterioration [28,39]. From the survey of the salts detected in the investigated tombs, it was found that the most common salts in the deteriorated mural painting were sodium chloride, potassium chloride and magnesium sulfate, this is in agreement with the results of Arnold [41]. Sodium chloride commonly occurs as impurities in gypsum precipitations used as plaster layers or in limestone supports, as veins within the limestone blocks or infiltrated from underground water to a stone support and paintings since this salt is not produced by microorganisms [13]. Sodium chloride is a hygroscopic salt with high solubility, so it is usually observed on the higher zones of paintings in tombs, in the form of a white margin [41].

The relation between the salts and *Streptomyces* colonization is a close one, since the obtained results indicated that *Streptomyces* isolates varied in their tolerance profile to NaCl, so different *Streptomyces* isolates could grow and colonize specific zones of deteriorated mural paintings according to the occurring salts [42]. Our findings indicated that most *Streptomyces* isolates were halotolerant to sodium chloride up to 10%, and it was reported that the optimum of NaCl, for most *Streptomyces* strains, was 5-10 % [43,44]. Moreover, *Streptomyces* ssp., *S. amctimycoticus* and *S. etfoliatus* isolated from the tomb of Maat at Tell Basta could grow in cultures supplemented with NaCl up to 13% [17]. On the other hand, Milanesi *et al*. [20] showed that *Streptomyces* isolated from deteriorated fresco paintings had a high tolerance to NaCl concentrations in reality and under laboratory conditions. Moreover, Saiz-limenez and Samson [46] studied the efflorescence of the frescoes of the monastery of Santa Maria de la Rabida (Spain), and identified gypsum, calcium chloride, sodium chloride, potassium chloride, quartz and silicates, where *Micrococcus luteus*, *Bacillus*, *Pseudomonas* and *Streptomyces* were identified.

The current results indicated that *S. canarius* was halotolerant and could grow up to 10 % of NaCl; at this concentration, this isolate produced reddish pigment of carotenoid that was not produced under normal cultures. *S. canarius* was isolated from white efflorescence, nearly pure sodium chloride, covering the ceiling of the burial room of King Oserkon II's tomb, at Tanis and the tomb of Ihy at Tell Basta. Pigment production corresponds to a defense mechanism *of Streptomyces,* against adverse environmental conditions such as hyper salinity, where this pigment acts as a shield between the microbial cells and surrounding adverse environmental conditions, so these pigments were called protective pigments [25,35].

The second common salt detected in the investigated tombs was potassium chloride, identified on the paintings of Ankh h3 f tomb, at Tell Basta, probably due to the surrounding cultivated lands, where a consistent amount of nitrogen fertilizers were utilized [47]. Our results indicated that potassium chloride (KCl Sylvite) enhanced the production of carotenoid pigment and sporulation, corresponding to a *Streptomyces* mechanism against hyper-salinity.

Our results indicated, in the tombs' mural painting surfaces, the presence of magnesium sulfate, frequently found in deteriorated mural paintings [48], indicating that isolated *Streptomyces* strains could grow up to 10% $MgSO_4 \cdot 7H_2O$, detected in Ibsmite

present on the paintings of Ankh h3f at Tell Basta [47]; Magnesium sulfate crystalized in different forms such as Schonite ($MgSO_4.6H_2O$); Keiserite ($MgSO_4.H_2O$); Hexahydrate ($MgSO_4.6H_2O$), and Ibsmite [40]. The action of crystallization and hydration of different salts was linked with the biodeterioration of paintings colonized by *Streptomyces* in the investigated tombs [19,40].

SEM micrographs showed the penetration of *Streptomyces* mycelium into the intergranular structure of calcite crystals, at a considerable depth and forming a microbial network, mainly composed of polymeric substances. These polymers are able to expand and shrink in relationship to wetting and drying cycles, and to the presence of sodium chloride (high water retention). This in agreement with the results of Suihko *et al*., [3] pointed out that the growth of *Streptomyces* colonizing the stone surfaces enhanced mechanical deterioration, caused by the penetration of biofilm within the pores, related to salt cycles, penetration that increases the width and depth of the pores [40]. *Streptomyces* sp. colonizing limestone discs from the Maat tomb at Tell Basta, increased the porosity of these discs up to 7%, after salt treatment (50 cycles) with 10% NaCl [16].

IR spectra of the extracted red pigment, related to the presence of the quinon group (ON-O-R) that appeared at 3457 cm^{-1}, allow us to attribute the red pigment produced by S. *canarius* under stress of 10% NaCl to carotenoid. Carotenoids ($C_{40}H_{50}$) are lipophilic pigments with a yellow-red color, including three molecules: *ß*-carotene, γ-carotene and rhodoxanthin. These pigments act as membrane stabilizers, protecting the microbial cells against adverse environmental conditions such as hypersalinity, irradiation and nutrient depletion [36], so this pigment is termed a protective pigment [26,27]. The problem imposed by carotenoid production by *Streptomyces* colonizing mural paintings causes chromatic alteration to these paintings, stone surfaces and plaster layers with irreversible stains, in particular if this pigment is exolithic in nature [48,50]. This phenomenon was well documented in the fresco paintings from the Chapel of St. Virgil, Vienna (Austria) since *Rubrobacter* sp. isolates showed a moderate halophilic behaviour to NaCl up to 6% and caused discoloration of the fresco paintings with rosy stains [18].

Furthermore, HPLC data indicated that all identified *Streptomyces* isolates were able to produce glutamic acid at 5-10% NaCl and KCl concentrations, this in agreement with Killham and Firestone [51]. Amino acids production by *Streptomyces* is considered a defense mechanism against hypersalinity. This was attributed to the preference of *Streptomyces* for amino acid groups including glutamic, proline and arginine acids for supporting their growth; the productivity of amino acids varied even if between closely related strains [52].

5. Conclusions

In conclusion *Streptomyces* were found to be the main bacterial species on deteriorated mural paintings in the tombs of Tanis and Tell Basta. These bacteria were halotolerant up to 10% NaCl, KCl and $MgSO_4$. 7 H_2O. Moreover, 10 % NaCl enhanced the production of red carotenoid pigment in *S. canarius*.

Glutamic acid and other amino acid derivatives, produced by *Streptomyces* isolates as defense mechanisms against hypersalinity, were also found.

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