INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 3, Issue 2, April-June 2012: 87-92 www.ijcs.uaic.ro

THE EFFICACY OF SPECIFIC ESSENTIAL OILS ON YEASTS ISOLATED FROM THE ROYAL TOMB PAINTINGS AT TANIS, EGYPT

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Abstract
Yeast strains play an important role in the biodeterioration and biodegradation of paintings in ancient Egyptian tombs. Thirteen yeast were isolated from the royal tombs at Tanis (Oserkon II, Psunes and Shashanq), Sharkia Governorate, Egypt, dated back to 840 B.C., by using a sterile cotton swab. Those strains were identified as Saccharomyces cerevisiae, Candida albicans, C. lipolytica and Lodderomyces elongspous. The S. cerevisiae strains were halotolerant for sodium chloride, up to 10%. Moreover, they caused a fading for the azurite blue color in laboratory cultures and S. cerevisiae was the most potent agent in fading the color. Five essential oils (lemon, spearmint, fennel, marjonam and rosemary) were used to control their growth. Spearmint and lemon oils were the most effective oils in inhibiting the growth of those strains, whereas marjonam, fennel and rosemary had no effect on their growth.

Keywords: *Ancient Egyptian paintings; Royal tombs; Biodeterioration and biodegradation; Biocides;*

Introduction

Biodestruction and bioalteration processes of paintings caused by fungi, bacteria and lichens was investigated in several studies, but the effects of yeast in the biodeterioration and biodegradation of stone monuments and paintings were underestimated, especially in the case of Ancient Egyptian paintings. The tombs at Tanis (Oserkon II, Psunes and Shashanq), are considered the only royal tombs in Delta. Stone surfaces and paintings in royal tombs are exposed to continuous biodeterioration and biodegradation agents, such as microbial communities colonizining stone surface and paintings contain the greatest number and diversity of microorganisms, such as fungi, bacteria, actinobacteria and yeast [1].

Yeast-like fungi are regular inhabitants of rock and paintings and they are responsible for both the structural and the esthetic alteration and are considered among the most harmful microorganisms associated with monumental stone biodeterioration and biodegradation [2].

Saccharomyces cerevisiae was isolated from the lid of a sarcophagus with Clinea in Side Museum (Turkey). They are considered yeast-like fungi inhibitors of monuments and desert stones because of their special ability to form small nodose colonies on rock surfaces, and they could grow in adverse metrological conditions [3].

Yeast strains were isolated from various building materials: S*accharomyces petricola* on some marble monuments in the Mediterranean basine [4], *Saccharomyces sideticae* from marble monuments in Side, Turkey, and we attributed the colonization of yeast on limestone

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mounments due to its perference for alkalinity [5]. It was reported that yeast strains were isolated from paintings of façades from Brazil buildings and they observed an obvious discoloration [6]. Similarly, from granitic rocks in Germany, where it formed black, clump-like colonies consisting of isodiametrically dividing cells on the rock surface and inside the rock, although hyphal growth is exhibited in laboratory cultures.Yeast-like growth patterns turned out to be among the most successful inhabitants of marble, limestone, granite and other rock types especially in arid and semi-arid environments in Mediterranean area [7]. Shabereiter-Gurtner et al. [8] indicated that black yeast, such as *Capnobotryella spp., Coniosporum* (also called "meristematic black fungi") were resistant to desiccation and UV radiation and were associated with the deterioration and degradation of monuments and stone structures. Black yeast causes a black and stable staining, due to its melanin production.

An attempt to decrease the growth of yeast was done by the addition of synthetic preservatives, which caused harm for conservators and a color change in paintings and stone surfaces. In recent years, there has been an increasing interest in the application of essential oils as alternative antimicrobial agents. It is well known that essential oils of certain plants such as cinnamon, clove and cassia have preservative qualities and were used by Ancient Egyptians in mummification [9], due to their antimicrobial effect. Abdel-Mallek et al. [10] found that peppermint has inhibitory properties for yeast-like fungi, damaging their cell membrane by changing the lipid profile of the cell and also by impairing their normal metabolism.

This study was carried out to evaluate the role of yeast in the biodeterioration and biodegradation of paintings, the effect of different concentrations of NaCl on the growth of yeast strains and the of essential oils to inhibit the growth of yeast strains colonizing mural paintings in Ancient Egyptian tombs.

Materials and Methods

Sampling: Under investigation were tombs located at San El Haggar, Sharkia Governorate, 80 km east of Cairo. (Fig. 1).

Fig. 1. Isolation of yeast strains: Samples were taken from (a) azurite blue on the ceiling of the tomb of *Oserkon* II, (b) granite blocks in the tomb of Psunis with a high humidity and microorganisms, (c) the eastern wall of the tomb of Oserkon II, (d) the red pigment from the tomb of *Shashanq* III.

Samples from stone surfaces and paintings were taken with a cotton swab, cultured in a Sabouraud medium (40g glucose, 10g peptone and 20g agar in 1L distilled water), and incubated at 28°C for 3 days. The single colony of yeast was observed.

***Isolation and identification of yeast*:** Thirteen isolated types were identified biochemically and morphologically, according to reference identification keys [11].

The effect of yeast strains on azurite blue: Yeast strains were cultured on Sabouraud medium supplemented with the concentrations of 0.01, 0.02, 0.04, 0.08% of azurite blue (basic copper carbonate, $2CuCO_3 \cdot Cu(OH)_2$).

The effect of NaCl on yeast strains: Isolated yeast strains cultured on Sabouraud medium were supplemented with different concentrations of NaCl (1, 5, 10, and 13%).

The effect of essential oils on isolated yeast: Five essential oils (lemon, spearmint, fennel, marjonam, rosemary) were applied on isolated yeast strains, by using a disc-diffusion test [12]. The inhibition zone was estimated in mm.

Results

The tombs of Oserkon II, Pesunis, and Sashanq were characterized by high relative humidity and a high level of underground water. Limestone slabs contained a high percentage of halite (NaCl).

The isolated yeast strains were identified as *C. lipolytica*, *C. albicans*, L. *elongspous* and *S. cerevisiae* (Table 1).

Table 1. Sampling location

Isolates	Location	Observations
Candida albicans	Eastern wall of the tomb of Shashanq III.	Contains high relative humidity.
Candida lipolytica	Granite blocks from the tomb of Pesunis	Fragile and contain high percentage of water.
Lodderomyces elongspous	from faded red color, Northern wall of the burial chamber of Oserkon II	Fading of red pigment
Saccharomyces cerevisiae	In the salt from the ceiling of Oserkon II, blue pigment.	White efflorescence of sodium chloride.

In regard to the effect of azurite blue on the growth of yeast strains, results indicated that *S. cerevisiae* strains were the most effective in fading azurite blue in laboratory cultures, and could grow up to 0.08%, whereas C. *albicans*, C. *lipolytica* and L. *elongspous* were less tolerant to the Cu ions in azurite blue (Table 2).

Table 2. The effect of different concentrations of azurite blue on the growth of isolated yeast

Isolates	Azurite $2CuCO_3 \cdot Cu(OH)_2$ (%)			
	0.1	0.2	0.04	0.08
Candida albicans	++	++	+	+
Candida lipolytica	+	+	+	-
Lodderomyces elongspous	+	+	+	-
Saccharomyces cerevisiae	++	++	+++	+

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

Current results indicated that *S cerevisiae* could grow up to 10% in a NaCl medium, but the growth of *C. albicans*, *C. lipolytica* and *L. elongspous* was low (Table 3). From Table 4, it appears that spearmint oil showed a moderate inhibiting activity against the tested yeast strains and did not cause color change either in paintings or on stone surfaces, whereas fennel and lemon showed low efficiency. Marjonam and rosemary had no effect on isolated yeast strains.

The treatment with essential oils enhanced pigment production (Fig.2-4b) except whre *S. cerevisiae* was present (Fig 2a).

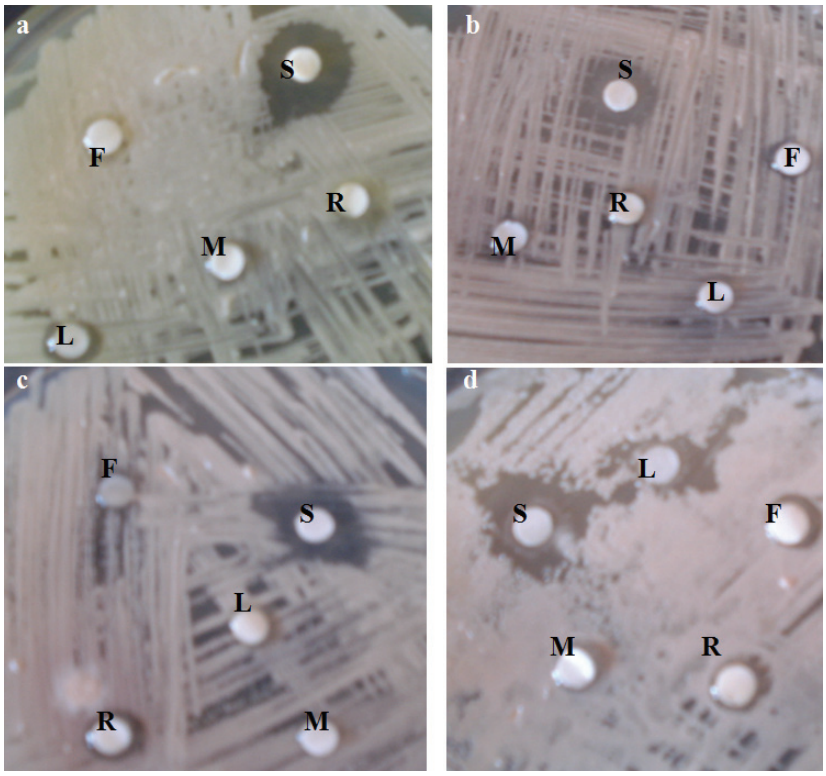

Fig. 2. The effect of essential oils on isolated yeast strains: (A) *Saccharomyces. cerevisiae*, (B) *Lodderomyces elongspous*, (C) *Candida albicans*, (D) *Candida lipolytica*. F. (Fennel oil); L. (Lemon oil); M. (Marjonam oil); R. (Rosemary oil); S. (Spearmint oil).

Table 3. The effect of different concentrations of NaCl on the growth of isolated yeast

Isolated	NaCl concentrations (%)			
	1	5	10	13
Candida albicans	++	++	+	-
Candida lipolytica	++	+	+	-
Lodderomyces elongspous	+	+	+	-
Saccharomyces cerevisiae	++	++++	++	+

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

Table 4. Average inhibition halo diameter (mm) at different concentrations of essential oils on different isolated yeasts

	Inhibition zone (mm)				
Isolated	Lemon	Spearmint	Fennel	Marjonam	Rosemary
Candida albicans	-	3.5	1.0	-	-
Candida lipolytica	2.5	4.0	-	-	-
Lodderomyces elongspous	-	6.0	-	-	-
Saccharomyces cerevisiae	-	5.0	-	-	-

Discussions

The royal tombs at Tanis were built of limestone and granite blocks characterized with high humidity. The biodeterioration of ancient buildings and monuments by yeast-like fungi depends on many factors, including environmental factors, such as light, moisture, weather, temperature and the type of microorganism [13].

Current results indicated that *S. cerevisiae* could tolerate NaCl, on average, up to 10%, as this strain was isolated from a blue paint layer on the ceiling of the tomb of Oserkon II, where pure crystals of sodium chloride were formed, the most common salts in Egyptian monuments [14]. Sterflinger [7] reported that *S. cerevisiae* strains were halotolerant to high concentrations of NaCl, due to an adaptation method, by producing glycerol as a response to increased salinity

Spearmint showed the most inhibiting effect on the isolated yeast strains, as Abdel-Mallek et al. [10] reported that peppermint containing 50-70% free methanol, 5-20% esters, L-limonen, cineol and phellandrene has inhibitory characteristics against yeast-like fungi, such as *Candida*, *Trichosporon*, *Cytococcus*.

Kociã-Tanackov et al. [12] investigated the effect of essential oils on yeast and reported that oils limit the growth, due to the phenolic compounds that have an antagonistic activity against yeast. They also stated that these oils also cause changes in the morphology of colonies as well as changes on a cellular level (destruction of fungal cells, decreasing the oxygen intake, reducing the cellular growth, inhibiting the synthesis of lipids, proteins and nucleic acids. Sacchetti et al. [15] reported that lemongrass oil has an antimicrobial effect against phytopathogens and dermatophytes. On the other hand, marjonam and rosemary had no effect on the isolated yeast strains. Results indicated that treatment of yeast strains with essential oils enhance pigment production as a defense mechanism [16].

Conclusions

We could conclude that yeast strains were halotolerant up to 10% NaCl and caused fading of paintings. Spearmint oil had a good antimicrobial activity against the isolated yeast strains.

References

[1] M. Thornbush, H. Viles, *Changing patterns of soiling and microbial growth on building stone in Oxford, England after implementation of a major traffic scheme*, **Science of the Total Environment, 367**, 2006, pp. 203-211.

[2] M. Marvasi, F. Donnarumma, A. Frandi, G. Mastromei, K. Sterflinger, P. Tiano, B. Perito, *Black micro colonial fungi as deteriogens of two famous marble statues in Florence, Italy*, **International Biodeterioration and Biodegradation, 68**, 2012, pp. 36-44.

[3] H. Sert, K. Sterflinger, *A new Coniosporium species from historical marble monuments*, **Mycology Progress, 9**, 2010, pp. 353–359.

[4] M. Thornbush, H. Viles, *Changing patterns of soiling and microbial growth on building stone in Oxford, England after implementation of a major traffic scheme*, **Science of the Total Environment, 367**, 2006, pp. 203-211.

[5] H. Sert, H. Sümbül, K. Sterflinger, *Microcolonial fungi from antique marbles in Perge, Side and Termessos (Antalya, Turkey),* **Antonie van Leeuwenhoek, 91**, 2007, pp. 217–227.

[6] M.A. Shirakawa, V.M. John, C.C. Gaylarde, W. Gambale. *Mould and phototrophy growth on masonery façade repainting*, **Materials and Structures**, **37,** 2004, pp. 472-479.

[7] K. Sterflinger. *Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi,* **Antonie van Leeuwenhoek, 74**, 1998, pp. 271–281.

[8] C. Schabereiter-Gunter, G. Pinăr, W. Lubitz, S. Rölleke, *Analysis of fungal communities on historical church window glass by denatu ring gradient gel electrophoresis and*

phylogenetic 18S rDNA sequence analysis, **Journal of Microbiological Methods, 47**, 2001, pp. 345-354.
[9] A. Lucas, **Ancient Egyptian Materials and Industries**, Edward Arnold & Co., London, 1948.
[10] AY. Abdel-Mallek, M.M. Bagy, H.A. Hasan, *The in vitro anti-yeast activity of some essential oils*, **Journal of Islamic Academy of Science, 7**, 1994, pp. 10-12.
[11] J.A. Barnett, R.W. Payne, D. Yarrow, **Yeast Characteristics and Identification** (3rd edition), Cambridge, 2000.
[12] S.D. Kociã - Tanackov, G.R. Dimic, A.N. Tepic, B.L. Vujiaic, *Influence of Allium Ampeloprasum L., and Allium Cerpa L., Essential oils on the growth of some yeast and moulds*, **Proc. Nat. Sci, Matica Srpska Novi Sad, 116**, 2009, pp. 121-130.
[13] K. Sharma, S. Lanjewar, *Biodeterioration of ancient monuments (DEVARBIJA) of Chhattisgarh by fungi,* **Journal of Phytology, 2**, 2010, pp. 47-49.
[14] A. Arnold. *Determination of mineral salts from monuments*, **Studies in Conservation, 29**, 1984, pp. 129-138.
[15] G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, R. Bruni, *Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods*, **Food Chemistry, 91**, 2005, pp. 621–632.
[16] D. Conner, L. Beuchat, *Senstivity of heat stressed yeast to essential oils of plants*, **Applied and Environmental Microbiology, 47**, 1984, pp. 229-233.

Received: March, 04, 2012
Accepted: April, 27, 2012