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BACTERIAL FLORA OF SOME EGYPTIAN ANTIQUITIES

By

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ABSTRACT

The bacterial flora of some Egyptian antiquities such as Tut Ankh Amen tomb, Nefertari tomb, and some royal kings mummies were investigated. A total of 148 bacterial isolates were obtained and identified down to the species levels as Alcaligenes denitrificans (16 strains), Acinetobacter calcoaceticus (15), Micrococcus rosus (18), Micrococcus halobius (20), Bacillus alcalophilus (22), Bacillus alkalophilus subsp halodurans (28), Bacillus firms (13), B. pasteuri (3), Brevibacterium casei (1) and Cytophaga aurantiaca (1). Some endospore forming Gram positive rod shaped isolates showed certain extremophilic characters, and did not match to the characteristics mentioned in the used identification keys, hence they were tentatively identified to the generic level as members of the genus Bacillus (11 strains).

INTRODUCTION

Bacterial growth and existence on different ancient Egyptian antiquities as well as certain soils has not been extensively studied. Few reports, which dealt with this subject, were concerned with some fermentation industries (Wilkinson, 1890; Erman, 1894; Arnold, 1911; Petrie, 1914; Lucas, 1928). Ruffer and Ferguson (1910) reported the presence of *Varicola* on the skin of an old Egyptain mummy of the twentieth Dynasty. Kowalik and Sadurska (1973) presented a study on the microflora of Papyrus samples obtained from the Egyptian Antiquities organization (EAO) in Cairo. Balout et al., (1985) studied the degradation of Ramses II mummy and reported the presence of some bacterial isolates such as *Micrococcus* and *Bacillus*. Recently a preliminary report by Ammar et al., (1987) gave a rapid presentation on the possibl microbial flora contaminating the wall paintings of Nefertari tomb.

The present investigation was therefore designed with collaboration with the EAO to study the extent and distribution of bacterial flora of some important antiquities.

MATERIALS AND METHODS

Isolation Sources and Sampling :

1- Nefertari tomb (at Luxor) : Swabs and air samples were collected as mentioned in the preliminary report of Ammar et al., (1987).

2- Tut Ankh Amen tomb (at Luxor), the tomb was closed and not allowed

for viewers to enter. Many greyish and brownish spots were scattered on the walls of the tomb. Swabs from the contaminated spots and some deciduous parts of the tomb as well as inside and out side air were sampled.

3- Three samples from the coffin of Auf Ankh mummy.

4- Three swabs from the scull of Ramses IV.

5- Thirty five samples and / or swabs from six Royal mummies presently exhibited in the Egyptian museum in Cairo were collected from different loci including hair, skull, eye cavities, nose cavities, teeth, neck, breast, arms, abdomen, abdominal cavity, legs, feet and nails. The six Royal mummies under investigation were, Nedjemet wife of Herihor, XXI Dynasty; Henttowi, wife of PaynedjemI, XXI Dynasty; Tuthmosis IV, XVIII Dynasty 1420 B. C.; Sety I XIX Dynasty 1313 B. C.; Meneptah XIX Dynasty 1225 B. C.; and Siptah XIX Dynasty 1215 B. C. All mummies were exhibited in a closed room, each inside a glass and wood show case.

Sampling were either collected as swabs, pieces of coffin, pieces of contaminating wall painting material. All samples were suspended in 25 ml saline solution. Air samples were considered after exposure of petri dishes containing the isolation media to the air for 30 min.

Isolation Media :

The following media were used for the purpose of isolation; **Medium I (g/1):** $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.5H_2O$,

0.01; yeast extract, 5.0; glucose, 2.0 and Agar - agar, 15.0. Different concentrations of NaCl were supplemented to aliquots of the medium to bring the following concentrations; 5; 10; 15; 20; 25 & 30%. The final pH was adjusted to 10.0 using 2N NaOH. **Medium II:** It was prepared according to Sato et al., (1983) using 10% Na_2CO_3 to adjust pH to 10.5. **Medium III:** medium I at pH 7.5. **Medium IV:** Nutrient agar base supplemented with 5% blood.

Isolation and Identification:

A total of 148 isolates were obtained, purified by streaking and kept on agar slopes. All isolates were preliminary characterized on the basis of Gram reaction, cell shape, aerobiosis, catalase, oxidase and spore staining (Malachite green). Most isolates showed alkalophilic characteristics were adapted to grow under neutrophilic conditions. Identification methods unless otherwise indicated were adopted from the Manual for Methods of General Bacteriology (1984). The following tests were performed: growth on different carbon and nitrogen sources, fermentation and oxidation of different carbohydrates, amino acids deamination and decarboxylation, nitrate reduction, aesculin hydrolysis, fat spiltting, coagulase, deoxyribonuclease, galactosidase, gelatin liquifaction, strach hydrolysis, levan formation, utilization of organic acids, temperature limits, pH limits, NaCl tolerance, Urease, phosphatase, H_2S, VP, MR, indole, lecithinase, growth on FTO agar and resistance to the vibriostatic agent 0 / 129, lysostaphin and the following antibiotics; Ampicillin, anisomycin, bacitracin, erythromycin, carbencillin chloramphenicol, streptomycin, and tetracyclin.

External Enzymatic Activities :

The following enzyme activities were detected: Lipolytic activities using Nile blue sulfate (Skerman, 1967); amylolytic activities using iodine as indicator; gelatin hydrolysis using the charcoal - gelatin discs (Difco laboratories); Cellulolytic activities (Smibert and Krieg, 1984); and Chitinolytic activities (Reichenbach and Dworkin, 1981).

RESULTS AND DISCUSSION

Total Viable bacterial counts were representeed as CFU/mL or CFU/30 min. Table (1) describes the total counts after isolation. A total of 148 colonies were selected on the basis of morphological differences and subjected to identification. All isolates were preliminary grouped on the basis of Gram reaction, cell shape, endospore formation, acid fastness, arobiosis, catalse and cytochrome oxidase production into five groups. The major characteristics of these groups are presented in Table (2).

Characterization of isolates of group I:

Thirty one bacterial isolates were obtained following the proposed group I. They were differentiated into two genera according to oxidase test where 15 isolates were negative and rod in shape, therefore they were tentatively identified as *Acinetobacter* spp. The rest of isolates were oxidase positive and form rod and coccal rod cells, they tolerate higher pHs, therefore indentified as *Alcaligenes* spp. According to the taxonomy of *Acinetobacter* only one species exists (Doudoroff and Stanier, 1968). Hence

they were identified as *A. calcoaceticus*. Isolates identified as *Alcaligenes* spp. were tested for certain taxonomic characteristics including assimilation of various nitrogen and carbon sources, acid production from carbohydrates and nitrate reduction. They were accordingly identified as the species *A. denitrificans* according to Ruger and Tan (1983). Results are represented in Table (3).

Identification of isolates of group II:

One isolate was obtained. It is gram positive, forming coccus - rod cycle. It is identified as a genus of the irregular non sporing rods. It was separated from other genera in being non - acid fast and strict aerobic growth. Therefore it was identified as *Brevibacterium* sp. Due to its survival after heating at 60 C for 30 min it was identified as *Brevibacterium casei* according to Collins et al., (1989).

Identification of isolates of group III:

One isolate only was obtained. The isolates is orange in color, cells appeared with tapered ends, glucose is the sole carbon and energy source, cells are long and thin (3.8 - 4.5 X 0.3 um), color is not changed with KOH, colonies on filter paper spread slowly and become very large and bright orange. It attacks cellulose, carboxy methyl cellulose. Therefore it was identified as *Cytophaga aurantiaca*.

Identification of isolates of group IV:

Results are given in Table (4). A total of 38 isolates were obtained. They were identified as members of the family *Micrococcaceae* due to their

shape, gram reaction and positive oxidase and catales tests. They were separeated from other genera of the family *Micorcoccaceae as Micrococcus* due to the following characters: being non - motile, grow on FTO, resistant to lysostaphin and non glucose fermenting. They were separated into two groups according to the color of colonies, red isolates (18) and non - pigmented isolates (20). The red pigmented isolates were identified as *M. roseus* due to the following characters: non - fermentative, nitrate reduced, and esculin not hydrolysed. Other isolates (20) were identified as *M. halobius* due to unpigmentation. (Schleifer, 1984).

Identification of isolates of group V:

All isolates of this group (77) were spore forming. They resemble the majority of isolates (52%). They are identified as *Bacillus* spp Table 5). They were identified as :

1 - *B. alcalophilus* (22 isolates) due to VP negative; strict aerob; grow at pH 10 - 0 and no growth at pH 6.8; citrate not utilized; casein, starch and gelatin were hydrolysed; maximum temperature, 40 C; and NaCl not required for growth.

2- *B. alcalophilus* subsp. *halodurans* (28 isolates). They were idenfied to this species and sub species due to their close similarity to *B. alcalophilus* isolates with the exception of the rquirements for NaCl for growth.

3- *B. firms* (13 isolates). The presence of parasporal bodies in spore stained smears support their identification.

4- *B. pasteuri* (3 isolates). The main distinctive criteria of these isolates were

their rapid conversion of urea to ammonium carbonate, and the absolute requirement for alkaline media.

5- The rest of endospore forming isolates (11) showed some distinctive characteristics such as growth at pH 10.0 and above, hlophilic nature (15-25% NaCl), and thermophilic nature. The inabilty of these isolates to grow under neutral pH conditions made their identification by the ordinary methods not reliable. They require new standard methods for identification hence we suggest their identification as *Bacillus* spp. only due to the presence of endospores. Their proper taxonomic position will be reported elsewhere together with other Bacilli that grow preferentially or obligatorily in alkaline madia.

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