Nickel biosorption by alkalitolerant *Exiguobacterium* sp. 27 isolated from lake Mariout, Egypt

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Abstract: Water samples were collected from Lake Mariout and subjected to heavy metal analysis, the samples showed high metal content of Al and Ni (1.34, 4.62 mg/l, respectively). Forty one bacteria were isolated on LB, pH 9.0 and screened for their metal resistance pattern. Among the 41 tested isolates, H27 showed the highest tolerance, which able to grow in presence of 0.1 mM of Ni ion. The bacterium was identified as *Exiguobacterium* sp. 27 based on phyenotypic and genotypic characterization. It grew at pH range 8-10, temperature range 4 – 40°C and has the ability to grow in up to 10 % NaCl concentration. *Exiguobacterium* sp. 27 dry cells were able to biosorb Ni ion from solution recording 56 % Ni removal equivalent to 33.0 mg Ni^{+2}/g cells when cells were exposed to 0.1 M nickel solution for 1h.

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1. Introduction

Nickel is an important environmental inorganic pollutant with allowed level less than 0.04 mgl^{-1} in human consumption water. Higher concentrations affect normal flora in ecosystems and are toxic for human beings (Rodríguez *et al.*, 2006)**.** It is toxic to a variety of aquatic organisms, even at a very low concentration (Wang *et al.*, 2007)**.** Some authors described Nickel as one of the more resistant heavy metal to bioremediation (Malik, 2004, Moore *et al.*, 2008)**.** The Nickel (II) intake over the permissible levels results in different types of disease such as pulmonary fibrosis, renal edema, gastrointestinal distress (e.g. nausea, vomiting, diarrhea) (Cavani, 2005, Gupta *et al.*, 2010).

Biosorption has recently attracted growing interest. It has the advantage of achieving high purity of the treated wastewater involving inexpensive sorbents. Studies on various types of non-living biomasses such as algae, fungi, bacteria, yeast, nut hulls, and wood sawdust among some other lignocellulosic wastes, have shown that such biomaterials may be used for removal of toxic metal ions from wastewater (Allaboun and Abu Al-Rub, 2008, Aksu and Dönmez, 2006, Grimm *et al.*, 2008, Ghozlan *et al.*, 1999, El Helow *et al.*, 2000).

Several *Exiguobacterium* strains possess unique properties of interest for applications in biotechnology, bioremediation, industry and agriculture. *Exiguobacterium* strain Z8 was capable of neutralizing highly alkaline textile industry wastewater (Kumar *et al.*, 2006)**;** strain 2Sz showed high potential for pesticide removal (López-Cortés *et al.*, 2006)**;** strain WK6 was capable of reducing arsenate to arsenite (Anderson and Cook, 2004)**.** Other *Exiguobacterium* strains could rapidly reduce Cr^{6+}over a broad range of temperature, pH and salt concentrations (Alam and Malik, 2008)**.**

In Egypt the available limited water resources necessitates the reuse of agricultural drainage water in irrigation to meet the future agricultural expansion and development needs. However, there are some concerns about the quality of this water. Indeed, utilization of untreated or partially treated wastewater in irrigation can give rise to pollution problems in surface and ground waters, soils and plants (Abou-Zeid *et al.*, 2009). Therefore, the objective of this work was to isolate metal tolerant bacteria from a polluted area, and to select the most promising candidate for Ni biosorption.

2. Material and Methods

Sampling site and sample collection

Lake Mariut (Mariout, Maryut, Mareotis) brackish water lake located in the north of Egypt southeast of Alexandria city **Figure 1**, belong the Nile River Delta system, and is one of the most heavily populated urban areas in Egypt and in the world. Lake Mariout is highly polluted; it receives the drastic human impacts among the Egyptian lakes. It has been greatly deteriorated from a productive lake to a heavily polluted and highly eutrophicated basin (Arafa and Ali, 2008). The depth of the water in the lake is between is 3 – 5 meters.

Water samples were collected from three different sites of Lake Mariout close to Alexandria National Refining & Petrochemicals Company, and Alexandria-petroleum Company in El-Max region,

Alexandria, Egypt. Samples were transferred to lab and stored in a refrigerator for further analysis.

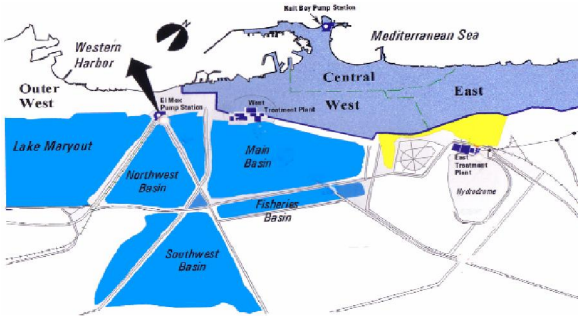

Figure1. The Basins of Lake Mariout. Source: Comprehensive Strategic Development Plan for Lake Mariout Zone

Chemicals and reagents

All bacteriological media components, chemicals, solvents and reagents, were of analytical grade and obtained from commercial suppliers. Metal salts used were nickel chloride hexa hydrate ($NiCl_2.6H_2O$), potassium dichromate ($K_2Cr_2O_7$), lead acetate trihydrate ($PbCH_3COO(H_2O)_3$), cadmium chloride ($CdCl_2$), mercuric chloride ($HgCl_2$) and cobalt chloride hexahydrate ($CoCl_2.6H_2O$).

Analysis of water samples for heavy metal content

Samples were analyzed for heavy metal content according to the Standard Methods for the Examination of Water and Wastewater (Eaton and Franson, 2005). The digestion was performed using conc. HNO3 and H2O2 as oxidizing agent. Concentration of each of the following metal ions Cu, Cd, Cr, Zn, Pb, Fe, Co, Mn, Al, and Ni was measured using Perkin-Elmer model 5000, an Atomic Adsorption Spectrophotometer (AAS) at City of Scientific Research and Technological Applications.

Enrichment of alkali-tolerant bacteria

Water samples (50 ml) were filtered through 0.45 µm sterile bacterial filters, then used to inoculate flasks containing 50 ml LB-broth medium, pH 9.0, and incubated at 25°C under shaked condition for 48 h. Flasks showing turbidity were used to inoculate LB agar plates, pH 9.0 and incubated at 25°C for 48 h. All developed colonies were purified and preserved on LB agar slants pH 9.0 at 4°C.

Screening for metal resistant pattern

In order to investigate the metal resistant pattern of bacterial isolates, the method of Kirby-Bauer disc diffusion was used (Sabdono *et al.*, 2011)**.** Under aseptic condition, LB plates of pH 9.0 were inoculated by each bacterial isolates. Filter paper disks, 8 mm in diameter **(Toyobo, Japan)** were saturated with either 1 mM Cu, Pb or Co, 0.5 mM Ni, 0.1 mM Hg, 2 mM Cr, or 0.2 mM Cd and placed over inoculated plates. Plates were then incubated at 25 °C and clear zone formed around each disc was measured.

Growth measurement

The ability of bacteria to grow in presence of 1 mM Ni was evaluated in liquid cultures. Flasks containing 50 ml of LB broth, pH 9.0 were amended with 1 mM Ni. Flasks were inoculated and incubated for 3 days at 25 °C with shaking at 300 rpm. Growth was determined by measuring the optical density **(OD_{600nm})** of the culture using JENWAY 6305 Sectrophotometer at Faculty of Science, Alexandria University.

Nickel biosorption experiment

Biosorbents were prepared by growing each isolate in 250 ml flask, containing 50 ml LB medium and incubated shaken at 300 rpm at 25°C for 24 h. Cells were then removed by centrifugation at 14000 rpm for 3 min, washed twice with distilled water and dried until a constant weight was achieved.

Biosorption was performed by suspending 0.1g dry weight of each strain in 100 ml of 0.1 M of nickel salt ($NiCl_2.6H_2O$) solution dispensed in 250 ml flask and shaked for one h at 100 rpm. After centrifugation at 14000 rpm for 3 min, residual Ni concentration was estimated in culture supernatants by colorimetric titration according to (Chojnacka *et al.*, 2005, Naja *et al.*, 2005). In a total volume of 100 ml distilled water, one ml of cell free extract was mixed with 2 ml buffer then few crystals of Murexide indicator were added, a yellow color developed after titration against 0.01M EDTA. End point was detected when color turned from yellow to wine red.

Effect of culture age on Ni^{2+} uptake was examined by using cells collected at different growth periods 6, 9, 12, 18, 20, 22 and 24 h. Ni^{2+} uptake experiments proceeded previously as described.

Ni^{2+} biosorption **(%)** was determined as follow:

Removal (%) = [(C_i – C_f) / C_i] * 100

Where;

R, Ni^{2+} biosorption **(%)**

C_i, Initial Ni^{2+} concentration **(mg/l)**

C_f, Final Ni^{2+} concentration **(mg/l)**

While specific biosorption **q (mg/g)** is calculated as follow:

q = [(C_i – C_f) / Wt] * V

Where

q, specific biosorption **(mg/g)**

C_i, Initial Ni^{2+} concentration **(mg/l)**

C_f, Final Ni^{2+} concentration **(mg/l)**

Wt, weight ofsorbents **(g)**

V, volume of solution **(L)**

Characterization of isolate H27

Colony morphology characters as appearance, configuration and elevation were examined on LB agar plates after 24 h incubation. The

tests included Gram reaction, KOH test and some biochemical reactions such as bio-oxidation, hydrolysis and oxidation reactions in addition to some miscellaneous tests according to Podschun and Ullmann (1998). Antibiotic susceptibility test was performed on Mullar-Hinton agar plates (Hudzicki, 2010).

The effect of medium pH was tested by adjusting portions of LB-broth to different pHs (8, 9 and10). Growth at different temperatures was determined by allowing the bacterium to grow at 4, 15, 25, 30 and 37^{o}C. Requirement for sodium chloride was determined by allowing cells to grow in portions of LB-broth containing different concentrations (1, 5, 10, 15, 20, 25 and 30 %) of NaCl. The tests were carried in conical flasks each containing 20 ml LB-broth medium. Results were taken by measuring OD_{600} nm.

Scanning electron microscopy (SEM)

Cell morphology was examined microscopically in presence and absence of Ni. Cell morphology of fresh cultures was examined by scanning electron microscope (JEOL JEM-5300, Japan). A part from fresh cultures was dried; specimen was sputter-coated with gold and examined with the scanning electron microscope at 10 kV.

Phylogenetic analysis

The genomic DNA was extracted from the purified isolates according to Sambrook *et al.* (2001). The purified DNA was subjected to polymerase chain amplification reaction (PCR) using primers designed to amplify 1500 bp fragment of the 16S rDNA region. The forward primer sequence was 5'AGAGTTTGATCMTGGCTCAG3' and the reverse primer sequence was 5'TACGGYTACCTTGTTACGACTT3' The PCR mixture was composed of 100 ng of genomic DNA, 30 pmol of each primer, 200 µM of dNTPs, 1U of Taq polymerase and 10 µl of 10X PCR reaction buffer, the reaction volume was adjusted to 50 µl in 0.5 ml Eppendorf tube. PCR amplification was carried out using Progene Techn. Thermocycler, and a touchdown PCR program, that was achieved as follow: a) denaturation at 94 °C for 1 min., b) annealing at 55 °C for 1 min. then temperature was decreased with a decrement of 1° C every cycle till it reached 52 °C (30 cycles), and c) extension at 72° C for 2 min, in addition to a final incubation step at 72 °C for 10 min. The PCR products were purified using PCR purification kit (GeneJET PCR Purification Kit, Thermo Scientific). To check the purity and size of the amplified DNA, PCR amplification was confirmed by gel electrophoresis then the two strands of each amplicon were sequenced using chain terminator method (Bioneer Company, Korea).

Sequences were then subjected to similarity BLAST searches (www.ncbi.nlm.nih.gov/blast) and the closely related sequences from GenBank were detected. The phylogenetic relationships of the experimental isolates and closely related species were analyzed using the multisequence alignment program (MEGA 5.1)

The selected sequences were aligned using CLUSTALW integrated into MEGA5.1 package (Tamura *et al.*, 2011). Sequence comparison and phylogenetic analyses of the partial sequencing of 16S rDNA were performed using the software MEGA5.1 (Tamura *et al.*, 2011). Neighbor-joining (Saitou and Nei, 1987) with Kimura 2-paramter (Kimura, 1980) method were used for computing the evolutionary distance, thousand bootstraps replicates were used to build the corresponding phylogenetic trees. The 16S rDNA gene sequence of strain H27 has been deposited in the GenBank database under Accession No. JF909557.

3. Results

Metal content of Lake Mariout water samples

Data in Table 1 reveal that Ni was recorded in relatively high level ranging from 2.949 to 6.997 mg/l by average of 4.62 mg/l compared to other tested metals. Average concentrations of Al, Mn, Pb and Fe were detected in low values (1.34, 0.42, 0.27 and 0.3 mg/l, respectively). Other elements such as Cu, Cd, Cr, Zn and Co were below the limit of detection.

Metal resistant profile of bacterial isolates

A total of 41 bacterial isolates were isolated and purified. They were screened for metal resistant profile using seven different metal ions (Cu, Pb, Ni, Co, Hg, Cr, and Cd) by disc diffusion method and the clear zones appeared around the disks were measured in mm.

Table 1 Heavy metal concentration (mg/l) in samples collected from Lake Mariout

Element	Lake water samples			
	(1)	(2)	(3)	Average
Pb	0.221	0.294	0.296	0.27
Fe	< LOD*	< LOD	0.295	0.30
Mn	0.503	0.323	0.441	0.42
Al	3.852	0.115	0.06	1.34
Ni	6.997	3.9	2.949	4.62

*LOD (limit of detection) for Cu 0.4 ppb (µg/l); Cd ppb: 1 ppb; Cr ppb: 2 ppb; Zn ppb: 1 ppb; Fe ppb: 2 ppb; Co ppb: 1 ppb

For the purpose of defining metal resistance, those strains which were not inhibited by 2 mM Cr, Cu, Co, Pb, 0.5 mM Ni, 0.2 mM Cd and 0.1 mM Hg were regarded as being resistant. It is well known that there are no currently acceptable concentrations of metal ions, which can be used to distinguish metal-

resistant from metal-sensitive bacteria. However, the concentrations used in this study have been employed in a similar study (Sabry *et al.*, 1997, Pandit *et al.*, 2013)**.** The concentrations of added heavy metals used in this study do exceed the values of the total metal concentrations in the contaminated sites. However, understanding the toxicity of free metal ion in relation to the complexes that it forms and the relative concentrations of these species in natural systems is crucial (Teitzel and Parsek, 2003)**.** The response of the microbial community to environmental stress is the result of a combination of factors, thus showing the selection for microbial populations with genetic resistance mechanism (Thacker and Madamwar, 2005).

Percentage of isolates resistant to each metal in the defined concentration is given in **Figure 2.** As clearly observed, almost 53.4 % of the isolates were resistant to Cr, whereas 32.0% were resistant to Ni and Cu. Lower percentages (19.5, 14.6 and 12.2%) were resistant to Co, Pb and Hg, respectively. The lowest value (7.3 %) was observed for Cd. As shown in **Figure 3** multiple resistance was common among examined isolates as only 5 % showed multiple resistance to 6 metals.Triple, tetra, and penta resistance were recorded in 7.3 % of the isolates, whereas 12.0 % showed double resistance. Resistance to only one metal was found in 29.0 % of the total. On the other hand, 32.0 % were sensitive to all metals**.**

Bacterial growth in presence of Ni^{2+}

A data in **Figure 4** depict that almost 32.0% of the isolates were capable to grow in presence of 1Mm Ni2. Isolate H27 was the most potent recording a relatively higher growth value (OD600nm 0.72) compared to other tested strains followed by isolates H25, H23, and H32.These strains showing the highest resistance to nickel were thus selected to test their efficacy in Nickel ion biosorption, whereas the two sensitive isolates H1 and H2 were taken for comparison.

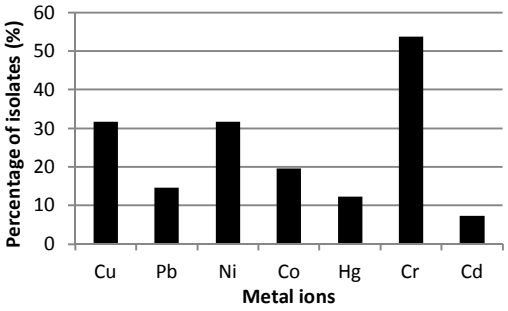

Figure 2. Percentage of isolates resistant to each tested metal ion. (metal concentrations were 2 mM Cr, Cu, Co, Pb, 0.5 mM Ni, 0.2 mM Cd and 0.1 mM Hg)

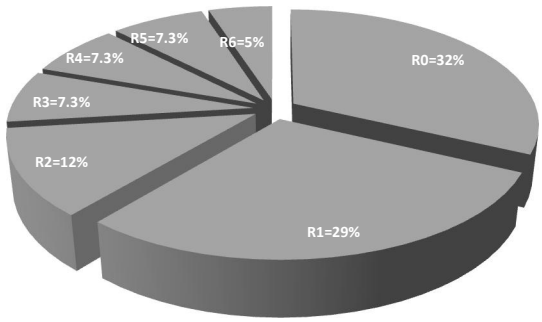

Figure 3. Percentages of metal multiple resistance among tested isolates (R1, resistance to one metal; R2, resistant to two metals; R3, resistant to three metals; R4, resistant to four metals; R5, resistant to five metals; R6, resistant to six metals and R0, sensitive to all metals).

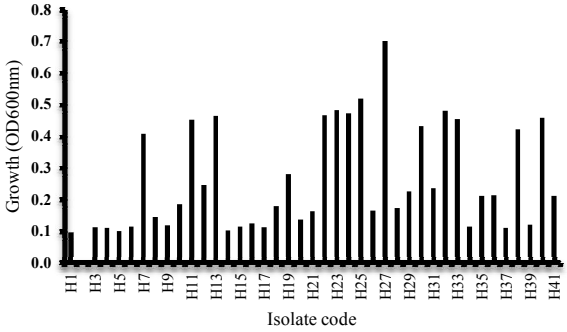

Figure.4. Growth of bacterial isolates in presence of 1mM Ni^{2+}. Growth in absence of Ni^{2+} (control) = 1.0

Biosorption of nickel by bacterial biomass

Based on previous experimental data, five isolates (H13, H22, H25, H27 and H40) which exhibited better growth in presence of 1mM Ni^{2+} were selected. Two sensitive strains H1 and H2 were also taken as negative control. The capability of the selected isolates to biosorb 0.1 M Nickel from solution was investigated. Bacterial cells were prepared as previously described, standard weight (0.1g) of dry cell mass was suspended in 0.1 M nickel solution and residual metal was measured after 60 min of exposure using the titration method.

Data obtained revealed that the two sensitive strains H1 and H2 gave a much lower biosorption values compared to resistant strains. The five resistant isolates exhibited different degrees of biosorption values (**Figure 5**). Isolate H27 was the most potent recording a biosorption value of 48.5 %, 28.5 mg Ni/g dry cells.

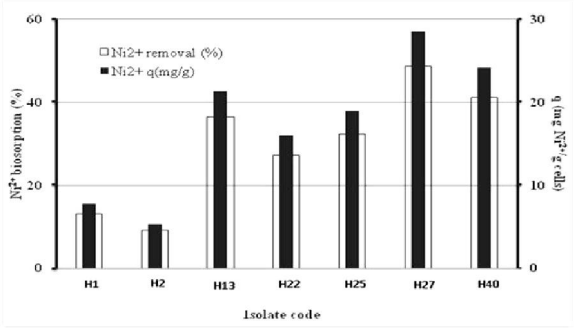

Figure5. Ni^{2+} biosoption (%) and specific biosorption (q) by some chosen isolates

Characterization of isolate H27

Phenotypic characterization

The morphological characterization on LB agar plates showed orange circular colonies (1-5 mm in diameter), with entire margins. They were Gram-positive able to grow at pH 8 – 10 with NaCl concentration varied from 1– 10%. H27 was able to grow at temperature range 4 - 37°C. It was positive for methyl red hydrolysis, but negative for Voges-Proskaure test, indole test, urea hydrolysis and citrate utilization **(Table 2)**. Resistance to only 4 antibiotics Tobramycin, Ampicillin, Nitrofurantoin and Azithromycin.

Phylogenetic analysis

The extracted and purified DNA was used to amplify the 16S rDNA gene, the PCR product were purified, sequenced, and aligned against other 16S rDNA sequences of the ribosomal database project http://www.cme.msu.edu/RDP/html/index/html (Maidak *et al.*, 1994, Rainey *et al.*, 1996) sequences deposited in GenBank. The sequence analysis of isolate H27 showed 99 % similarity to *Exiguobacterium* sp. EdyKolEs20 16S ribosomal RNA gene with accession no. JX625999. Therefore, H27 was affiliated to genus *Exiguobacterium*, Order *Bacillales*, Phyllum *Firmicutes*. The sequence of isolate H27 was aligned with other sequences of related bacteria on the database to determine its phylogenetic relationship to other bacteria. The sequence was deposited in GenBank with accession number JF909557, and the phylogenetic tree was constructed as shown in **Figure 6**

The experimental bacterium *Exiguobacterium* sp. 27 was compared to other species *Exiguobacterium* spp. in the database with regard to some physiological tests. Data depict that our strain shares some properties with some strains and is different in some other characters, which indicate the great possibility of being a new species **(Table 3).**

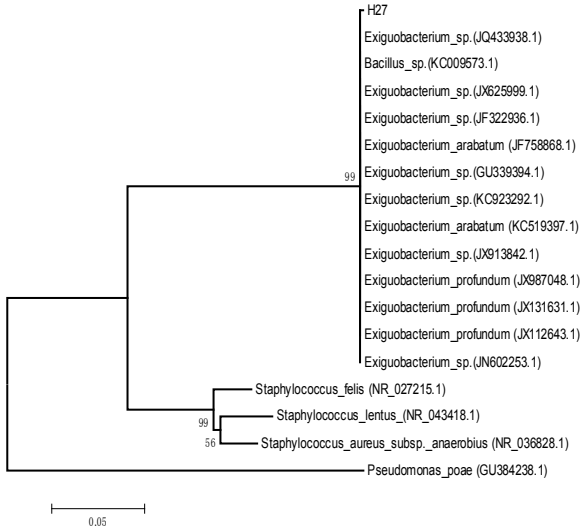

Figure 6. Neighbor-joining tree represents phylogenetic relationship isolate H27 based on 16S rDNA partial sequences isolated in this study. Evolutionary distances were calculated using the Kimura 2 model using MEGA5 software. The numerals show the results of the bootstrap analysis values from 1000 replicates (only bootstrap values above 50% were shown).

Scanning Electron Microscopy

The morphological characteristics of strain *Exiguobacterium* sp. 27 visualized by microscope was shown in **Figure 7**. Cells were rod-shaped (0.8-1.3 x 0.4-0.6 µm), non-spore former. **Figure 7a** shows cells of H27 in absence of nickel and **Figure 7b** cells exposed to nickel ions solution. The cells without nickel were smooth and had certain dimensions but after their exposure to nickel solution, they become distorted and swollen, their surface has meanders. This may be due to nickel ions precipitate around the cell surface and linked with their functional groups.

Table 2. Some phenotypic characters of *Exiguobacterium* sp. 27

Growth at	**Result**
Temperature (°C)	4 – 37
NaCl tolerance (%)	0 – 10
pH	8 – 10
Catalase	+
Oxidase	+
Acid produced from:	
Glucose	-
Lactose	-
Maltose	-
Methyl red test	+
Voges Prauskauer test	
Indole test	-
Urea hydrolysis	-
Gelatin hydrolysis	+
Citrate utilization	-
Antibiotic sensitivity	
Tobramycin (TOB 10)	R
Ampicillin (sulbactam) (SAM20)	R
Streptomycin (S10)	S
Amikacin (AK25)	I
Ciprofloxacin (CIP5)	I
Levofloxacin (LEV5)	S
Tetracyclin (TE30)	S
Cefoperazone (CEP75)	S
Erythromycin (E15)	I
Imipenem (IPM10)	S
Ceftazimide (CAZ30)	I
Nitrofurantoin (F300)	R
Norfloxacin (NOR10)	S
Gentamycin (CN10)	S
Piperacillin (Tazobactam) (TZP110)	S
Oxicillin (OX1)	S
Azithromycin (AZM15)	R
Ciprofloxacin (CPR5)	S
Gentamycin (GM10)	S
Amikacin (AK30)	I

R, resistance S, sensitive I, intermediate

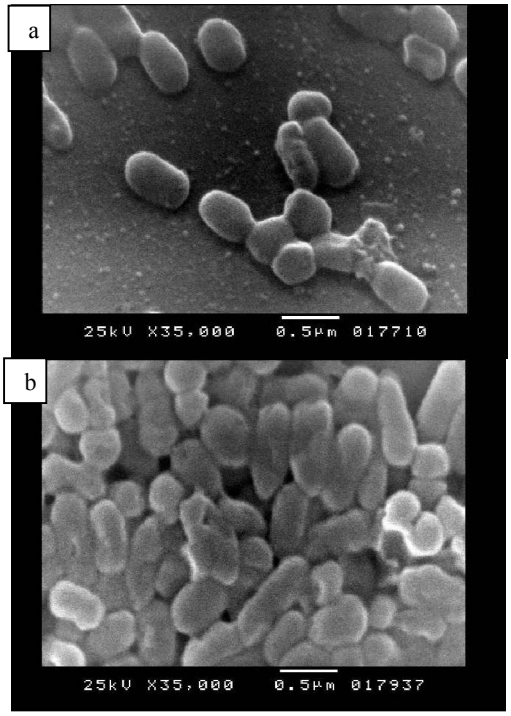

Figure.7. Scanning electron micrograph of H27 (a) in absence of Ni^{2+}; (b) in presence of Ni^{2+}.

Biosorption of Ni^{+2} by *Exiguobacterium* sp. 27 as influenced by cell age

In this experiment cells were grown in LB broth, incubated at 37ºC shaked at 150 rpm. At time intervals, growth was measured by determining the optical density at wavelength 600 nm. Cells were then collected, dried and used for biosorption examination. **Fig. 8** illustrates that data cells grew exponentially from the beginning of the inoculation and reached maximal growth (OD_{600nm} 5.5) after 12 h, thereafter the stationary phase began and growth remained constant till the end of incubation period. Concerning biosorption, it was observed that cells of late exponential growth (12 h) showed the highest biosorption values (56 % and 33.0 mg /g cells) when exposed to Ni^{+2} in solutions for 1 h. In general, older cells (of stationary phase) possessed better biosorption capacity compared to younger cells (of mid-exponential).

Table 3. Differential characteristics between strain H27 and the type strains of members of *Exiguobacterium*

Characteristic	*E. profundum*	*E. aestuarii*	*E. marinum*	*E. mexicanum*	*E. aurantiacum*	*E.* sp. 27
Temperature range (°C)	12–49	10–47	10–43	20–41	7–43	4 - 40
NaCl tolerance %	0–11	0–19	0–17	ND	ND	0 – 10
Oxidase test	-	-	-	+	-	+
Nitrate to nitrite	+	+	+	+	ND	ND

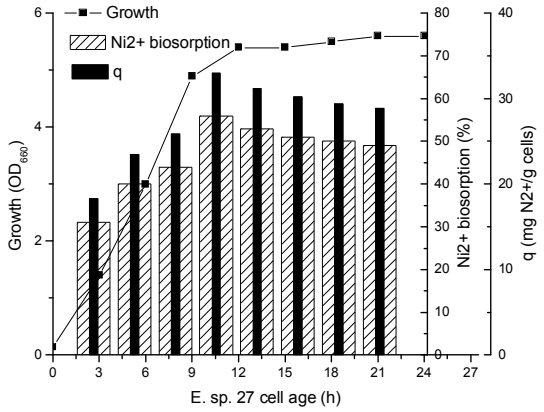

Figure.8. Growth curve, Ni^{2+}biosorption (%) and specific biosorption (q) of Exiguobacterium sp. 27

4. Discussions

The results show how Lake Mariout has been abused and misused severely, this abuse made the lake in a highly deteriorated, highly eutrophied and polluted status. This is totally in agreement with reported published (El-Bestawy, 2000, Arafa and Ali, 2008**).** Because of heavy pollution (industrial, domestic and agricultural), the economic value of the lake, as a commercial fishing area had declined `tremendously, and its productivity decreased by about 75% during the last twenty years (Mateo, 2009). It was reported before that lake Mariout is highly polluted with different heavy metals such as iron, copper and zinc (El-Bestawy, 2000**).** Water samples of Lake Mariout contained high concentrations of Hg and Cd that were associated with differences in electrophoretic patterns of proteins prepared from Mariout and other locations (El-Shehawi *et al.*, 2007).

All metals exceeded the legal limit, which is 1 ppm according to Egyptian law (48/1982-article 69) of water quality parameters that regulates the limits of wastewater discharge into water bodies. In addition, their values exceeded the limits recommended by U.S. Environmental Protection Agency (USEPA) (EPA, 2009) for heavy metals concentration in drinking water Pb, Cu, and Ni must not exceed 0.1, 0.05 and 0.03 ppm, respectively.

Data obtained in this study show that in spite of the low nickel ion concentration used (0.5 mM, 29.5 mg/l), only 31.7 % of the isolates were resistance to such concentration. Resistance to such metals has been previously reported (Sabry *et al.*, 1997, Knotek-Smith *et al.*, 2003**).** Higher resistance was reported by bacterial strains isolated from Serpentine soil (Pal *et al.*, 2004). The data also show that the degree of growth in response to metal ions varied with the metal ion species and bacterial strain. Bacterial growth in the presence of each metal was consistently lower than that of the control. Similar observation has been reported earlier (Pal *et al.*, 2004, Raja *et al.*, 2006). Reduction in growth is mainly because of the interaction between the cell surface and of metal cations along with phosphates carboxyl, hydroxyl and amino-groups (Sag and Kutsal, 2001**).** These differences are due to the chemical properties of each metal such as valence and atomic weight and due to the properties of the biomass such structure, functional groups and surface area (Sari *et al.*, 2007**).** The growth in presence of metals can be due to two conditions, metabolism dependent metal uptake inside the cell or because of energy independent process of biosorption to the membrane.

Generally, passive uptake of heavy metals using dead cells (heat dried or lyophilized cells) is more efficient than active uptake using untreated cells (living cells) (Srinath *et al.*, 2002, Choi and Yun, 2004, Tunali *et al.*, 2006). This may be attributed to the passive uptake being energy independent (Gabr *et al.*, 2008). Therefore, in the present study dead cells were examined as bio-sorbent materials. H27 isolate was the most potent isolate capable of biosorb 48.5 % Ni^{+2}. Same observation was found by Ansari and Malik (2007) who reported that the biosorption of nickel increased from 6.96 to 55.31 mg/g of cells, at a concentration ranging from 50 to 400 µg/ml after 2 h of incubation in a single metal solution. Similarly, maximum accumulation of nickel (0.59 mg/g dry weight) was observed when the bacterium was grown in media containing 2 mmol/l of nickel (Patel *et al.*, 2006**).** Parameswari *et al.* (2009) reported maximum metal removal 86.16 %, 84.32 % and 90.98 % of Ni^{2+} by *A. chroococcum, Bacillus* sp. and *P. fluorescens,* respectively) was found to be 72 h at an initial metal concentration of 25 ppm.

The phenotypic and phylogenetic analysis depicted that isolate H27 which is able to biosorb nickel efficiently was affiliated to genus *Exiguobacterium* and designated as *Exiguobacterium* sp. 27. As with other members of the genus *Exiguobacterium,* strain H27 exhibited growth under alkaline conditions (up to pH 10) and was halotolerant, growing in the presence of NaCl concentration up to 10 g /l. *Exiguobacterium* have been reported to be alkali-tolerant or alkaliphilic (Suga and Koyama, 2000, Yumoto *et al.*, 2004, Lee *et al.*, 2009).

The *Exiguobacterium* genus comprises psychrotrophic, mesophilic, and moderate thermophilic species (Vishnivetskaya and Kathariou, 2005**),** with pronounced morphological diversity

(ovoid, rods, double rods, and chains) depending on species, strain, and environmental conditions (Vishnivetskaya *et al.*, 2007)**.** Currently, the NCBI database contains 439 *Exiguobacterium* entries, including 158 uncultured *Exiguobacterium* spp.

The fact that members of this genus seem to be successful in cold environments, have very distinct physiologies (Ponder *et al.*, 2005, Rodrigues *et al.*, 2008) make it a good candidate. Results suggest that these cold-adapted microorganisms are not restricted to cold environments but are also found intemperate and tropical soils (Rodrigues *et al.*, 2009). *Exiguobacterium* spp. have been isolated from or molecularly detected in an impressive diversity of habitats, including Arcticperma frost, mats of Lake Fryxell in the Antarctic, surface water, several types of food processing plants, and a range of saline or alkaline environments (Vishnivetskaya and Kathariou, 2005)**.**

Based on phylogenetic and phenotypic characteristics and comparison with other genera of *Exiguobacterium*, it is suggested that strain 27 may be considered as a novel species of the genus *Exiguobacterium*. More genotypic analyses are still needed to confirm our suggestion. It is worth mentioning that up to literature available, this is the first time to isolate a member of this genus in Egypt. Moreover, metal biosorbtion by cells of this genus was not previously reported, although, a novel strain with high capability for azo dye decolorization was previously isolated from a contaminated soil system and identified as *Exiguobacterium* sp. (Tan *et al.*, 2009). Also, Alam and Malik (2008) isolated *Exiguobacterium* sp. ZM-2 resistant to high level of chromate and other heavy metal ions, and reduce significantly the hexavalent chromium.

As a conclusion, this work provides information on a newly isolated alkaliphilic *Exiguobacterium* sp. 27 which grows well at pH range 8 – 10, and salinity up to 10 %. It is able grows over a wide range of temperature (4 – 40°C) and in presence of 0.1M of Ni^{2+}. It showed the highest biosorption capacity (48.5 %, 28.5 mg /g dry cells) when 0.1g cells were exposed to 0.1M Ni solution for one hour. The data obtained revealed that *Exiguobacterium* sp. 27 can be considered a novel alkalophilic metal tolerant bacterium. The dried biomass proved to be a good biosorbent for nickel removal. It is worth mentioning that up to literature available, this is the first time to isolate a member of this genus in Egypt. More genotypic analyses are still needed to confirm our suggestion.

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