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Bioleaching of Rare Earth Elements and Uranium From Sinai Soil, Egypt Using Actinomycetes

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Abstract

The use of microorganisms in the recovery of Rare Earth Elements (REEs) and Uranium (U) from low grade ores provides an appropriate eco-friendly alternative to chemical methods used in extraction and/or removal of metals from soils, with a higher efficiency, less destruction to the environment, and lower cost from a commercial view especially given that high-grade mineral resources that can be used in the chemical extraction of metals are being depleted. The feasibility of using actinomycetes to recover REEsand Ufrom a low-grade ore occurring in an Egyptian soil in Wadi Abu Thor, Sinai, Egypt, was investigated. The most effective isolate in REEs-bioleaching was *Streptomyces fungicidicus* YH04, while the most effective isolate in U-bioleaching was *Streptomyces aureofaciens* 3001(1). *Streptomyces fungicidicus* YH04 bioleached 37.13% of the REEs present in the sample, while *S. aureofaciens* 3001(1) bioleached of 12.56% of the U present in the sample. The isolated strains of actinomycetes can be used to extract REEs and U by better, more economic and less polluting bioleaching processes instead of the chemical processes which are in common use today.

Keywords: Actinomycetes, *Streptomyces*, Rare earth elements, Uranium, Bioleaching.

Introduction

Bioleaching is the extraction of metals from their ores using microorganisms. It offers an economic alternative to chemical extraction for the mining industry, at a time when high-grade mineral resources are being depleted (1,2,3). Among metal-solubilizing micro-organisms, actinomycetes are of special interest because these filamentous sporulating bacteria are adaptable to extremely different soils (4,5). The metals of interest in bioleaching are divided into 4 categories: (i) toxic heavy metals (6) (ii) strategic metals (7) (iii) precious metals (e.g. REEs) (8) and (iv) radionuclides (e.g. U) (9).

Rare earth elements and U are widely used in industry, agriculture, medicine, military industries and nuclear applications (10,11). However, they are more expensive to recover by conventional techniques (12,13). Bioleaching of REEs and U is of great importance as it represents a "clean techn-ology" with low cost and capital inputs required compared to conventional methods used in mining industries. Bioleaching allows the cycling of metals by a process close to natural biochemical cycles reducing the demand for resources such as ores, energy or landfill space (1,14). This study aims to evaluate the possibility of new actinomycetes isolates for their bioleaching capacities. The most active isolates in REE and U bioleaching were identified.

2 -Material and methods

2.1 Collection of soil samples

Soil samples were collected from Wadi Abu Thor, south western Sinai, Egypt. A sterile spatula was used to remove first 5 cm of the surface layer of the soil followed by the use of another sterile spatula in the layer subjacent (between 5 and 15 cm of depth). Soil samples (100 g) were collected from three different locations. They were further mixed, ground and sieved (< 2 mm) prior to storing in a sterile container at 4ºC (5).

2.2 Physical and chemical analysis

Physical and chemical properties of the soil samples were analyzed at Nuclear Materials Authority of Egypt at Qattamiya. The studied samples were subjected to X-Ray Diffraction (Model Phillips XL 30 with Energy Dispersive X-ray) to identify their mineral content. The major oxides were analyzed according to Shapiro and Brannock (15), while chemically extractable trace elements were analyzed using Flame Atomic Absorption (FAA Scan 1, Thermo Jarrel Ash USA). Rare earth elements (lanthanides) were analyzed using Prism High Dispersion Inductively

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Coupled Plasma Optical Emission Spectrometry ICP-OES, (Teledyne Leeman Labs) (16). The natural radionuclides, potassium 40K, uranium 238U and thorium 232Th, were estimated using Ortec HPGe radiation detector (Physics Department, College of Women for Arts, Science and Education, Ain Shams University).

2. 3 Microbial strains

Streptomyces chibaensis, previously isolated from Toshka's soil in the southern province of Aswan tourism, Egypt, was used as a reference strain in the bioleaching experiment.

2.4 Microbiological media

For the bioleaching experiments, Nutrient broth medium (without agar) was used (17). Starch nitrate medium (18) was used for the count of actinomycetes. Yeast extract-malt extract agar medium (ISP2), oatmeal extract agar medium (ISP3), inorganic salts-starch agar medium (ISP4), glycerol-asparagine agar medium (ISP5), peptone yeast extract iron medium (ISP6), Tyrosine agar medium (ISP7), Bennet's agar medium and Carbon utilization agar medium (ISP9) were prepared according to Shirling and Gottlieb (19) for use in identifying actinomycetes.

2.5 Enumeration and isolation of actinomycetes

For the count of actinomycetes, soil samples were dried at 100ºC for 24 hrs (to reduce non-spore forming microorganisms) and 10ml of sterile saline solution (0.9% NaCl) was added to 1 g of each dried sample to obtain a 1:10 dilution. Serial dilutions were made from 10^{-1} to 10^{-10}. Approximately 100 µl of the diluted samples were spread on thesurface of starch nitrate agar (SNA) to which Cycloheximide (50 mg/L) and Nalidixic acid (20 mg/L) respectively were added (at 45°C) to prevent fungal and gram-negative bacterial growth (18,20). Triplicate plates, per dilution, were incubated at (28±2)ºC for 1 week. At the end of the incubation period, counts of the colonies were expressed as the mean number of colony forming units (CFU) per gram dry soil.

Colonies of actinomycetes were isolated and purified using the same cultivation medium and conditions. The isolates were selected by the morphological differences based on visible examination of the growth characteristics, aerial mycelium, substrate mycelium and diffusible pigments. Representatives of the resulting different colonies were checked for purity by microscopic examination of Gram-stained slides. Isolates were maintainned on plates for short-term storage and as suspensions in 20% (v/v) glycerol at -20ºC for long-term maintenance (20).

2.6 Screening of actinomycetes isolates for their REEs- and U-bioleaching efficiency

Nine actinomycetes that indigenous to the studied soil sample plus wild exogenous strain (*S. chibaensis*) were tested for their REEs and U bioleaching efficiencies.

1- Inoculum preparation

All isolates were inoculated in to 250 ml Erlenmyer flasks containing 100 ml nutrient broth. The flasks were incubated in a shaking incubator at 30ºC and 120 rpm for a week. Cells were collected by filtration through Whatman filter paper No.1, washed thoroughly with sterile deionized water (to be free of extraneous nutrients before exposure to soil) then dried to be used in REE bioleaching according to Tsuruta (21).

2- Bioleaching experiment

Bioleaching experiments were carried out in 250ml Erlenmyer flasks. Each flask contained 100 ml sterile distilled water and 1 gm of sterilized Abu Thor soil sample. The pH was adjusted to 7 ± 0.2. The experiment was initiated by the addition of 1mg of inoculum. All flasks were incubated in a shaking incubator at 30°C and 150 rpm for 48 hours. Cells and soil particles were discarded by filtration through Whatman filter paper No.1, supernatants were collected (21,22).

Supernatants were analyzed to estimate extractable REEs by the Nuclear Materials Authority of Egypt at Qattamiya, using a spectrophotometric method with Arsenazo-III as chromogenic reagent and a double beam Shimadzu 160-A UV-visible recording spectrophotometer. An intense pink violet complex of chromogenic reagent with REEs (REE^{+3}) could be read at λ_{max} 650 nm (23). Also, an intense pink violet complex of chromogenic reagent with uranyl ion (UO2+2) can be read at λ max 655 nm (24).

Identification of actinomycetes

The most effective isolates in REE and U bioleaching were identified according to morphological and physiological characteristics using the keys proposed by Bergey's Manual of Systematic Bacteriology (25) and

the description of Streptomyces species of the International Streptomyces Project (I.S.P) (19). In addition, they were tested for species identity using 16S rRNA sequencing according to Rochelle et al. (26) at Macrogen Inc., Seoul, South Korea.

The color of aerial mycelium, substrate mycelium, and soluble pigments, other than melanin, were observed by the naked eye (I.S.P. standard measure is not available) after 7, 14 and 21 days of incubation on 4 standard medium: ISP2, ISP3, ISP4 and ISP5 media (19). Spore chain morphology and spore surface ornamentation were examined by light microscope (27) on ISP4 medium after 14 days of incubation at 28±2°C. The form of the spore chains of the selected actinomycetes was described in terms of the morphological groups of Pridhamet et al. (28) modified by Shirling and Gottlieb (19). The production of melanoid pigment was tested on ISP6 and ISP7 media (19). The A1 and A8 selected isolates were tested for their ability to grow on Czapek's agar medium (29) and for sodium chloride tolerance. Sodium chloride was used at concentrations of 2.5%, 5%, 7.5% and 10% in starch nitrate agar medium (19). Sensitivity of the selected isolates to Streptomycin sulphate (50 µg ml-1) was tested on Bennet's agar medium (30) by using a filter paper disc method according to the method described by Qadrieet et al. (31). Determination of antimicrobial activities of the selected isolates was performed by using thespektra-plak method (32). Gram positive bacteria (*Bacillus subtilis*and *Staphylococcus aureus*), gram negative bacteria (*Escherechia coli* and *Pseudomonas aeruginosa*), yeast (*Candida albicans*) and fungi (*Aspergillus niger*, *A. flavus* and *Fusarium oxysporum*) were used as indicator microbes. Finally, the selected isolates were studied for their ability to utilize different carbon sources (monosaccharides: D-glucose (as positive control), D-fructose, D-galactose, D-mannitol, inositol, L-arabinose, D-mannose, salicin, D-xylose; disaccharides: lactose, D-sucrose, maltose; trisaccharides: D-raffinose and polysaccharides: starch) in ISP9 medium.

For the 16S rRNA sequencing, genomic DNA was isolated by using 2 consensus primers: forward primer 518F (5'-CCAGCAGCCGCGGTAATACG-3') and reverse 800R (5'- TACCAGGGTATCTAAT-CC-3') then amplified using AmpliTaq® DNA polymerase. The amplified product was sequenced using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied Bio-Systems, USA) and also analyzed in the National Centre for Biotechnology Information (NCBI), and application of USA database using Basic Local Alignment Search Tool for Nucleotides (BLASTN) (33).

Statistical analysis

Data obtained were analyzed by using Origin software, version 8 (Origin Lab). They were fitted and smoothed to approximate and reduce statistical fluctuations. Mean values were compared by Least Significant Difference (LSD) at 5%. Means and standard deviations are illustrated in the figures made by using Origin software.

Results

The studied soil sample was dark grey to black in color. It was composed mainly of sand and silt grains embedded in clay matrix with high amount of organic matter and many coarse fragments; hence, it is characterized as gravelly sand according to United States Department of Agriculture-Natural Resources Conservation Service classification (34). Tables 1 and 2 summarize the chemical and physical properties and elemental analysis of the tested sample. The count of actinomycetes in Abu Thor sample was considerably low (267 CFU/ml).

Nine isolates of actinomycetes were tested for their ability to make bioleach of REEs and U under the same conditions. The A1 and A8 actinomycetes were isolated from the study samples and *Streptomyces chibaensis* isolated from Toshka's soil. The aim of using microorganisms isolated from Abu Thor local soil samples and a "foreign" actinomyces isolated from Toshka's soil was to compare the bioleaching capabilities of two indigenous soil actinomycetes. Those which grow in an environment with high concentration of heavy metals and one which is a reference as *S.chibaensis*.

All the tested actinomycetes had different REEs and U bioleaching activity (Figure 1). The highest recovery of REEs in leach liquor from bioleaching were with isolates A1 (37.13%) and A4 (30.8%). The

highest concentrations of U in leach liquor were 57 and 50 ppm by isolates A8 and A2, respectively i.e. 12.56% and 10.99% of the original concentration of U in the study sample, respectively.

Isolates A1 and A8 belonged to the genus *Streptomyces*. They formed well developed branching, non-septate, non-fragmented aerial mycelium bearing long spore chains and non-motile spores that were not borne in verticillate sporophores (35).The morphological and physiological analysis of isolates A1 and A8 are summarized in Tables3 and 4. For isolate A1, 16S rRNA sequencing showed 100% identity with *Streptomyces fungicidicus* YH04. Thus, it is most probably *S. fungicidicus*. While the 16S rRNA sequencing of isolate A8 showed 100% identity with *S. aureofaciens* 3001(1). Thus, it is most probably *S. aureofaciens*.

Discussion

Actinomycetes grow extensively in soils rich in organic matter and the number of actinomycetes in soil is positively correlated to the level of organic matter and moisture content (36,37). In the current study, the low actinomycetes count in Abu Thor sample may be due to soil properties, as a combination of other environmental and biological factors would control the distribution of actinomycetes in the studied sample. This decrease may be due to the high heavy metal content of the sample as the presence of heavy metals at elevated concentrations in the soil environment has a range of effects on microbial species. These include a decrease in microbial count due to disruption of nucleic acid and enzyme function (38). In a pot experiment, La had a slightly stimulative effect on soil bacteria and actinomycetes when applied at low concentrations while it had inhibitory effect on soil bacteria, actinomycetes and fungi at high concentrations (39).

Ten actinomycetes were tested for their ability to bioleach REEs and U under the same conditions. They included nine actinomycetes isolated from the study sample (from A1 to A9) and *S. chibaensis* (isolated from Toshka's soil). The results indicated that all the tested isolates had REEs- and U-bioleaching activites. All bioleaching studies done by other authors utilized bacteria (especially *Thiobacillus ferrooxidans*) or fungi (especially *Aspergillus niger*) but none have reported utilizing actinomycetes (22,40,41). During the bioleaching process, the micro-organisms do not attack metal ore directly but create the chemical conditions necessary for its dissolution. However, Zajic and Ng (42) suggest that the bacteria might attack uranium oxides directly because oxidation is more rapid in the presence of thiobacilli than of ferric ions alone. A clear picture of the microbial diversity in a bioleaching habitat is required. This type of information is essential to advance our understanding of microbial metal solubilization in biomining applications (1).

The results of this investigation are expected to lead to enhancing the extraction of REEs using actinomycetes that could be less polluting, more economical and more effective than traditional chemical extraction especially from low grade ores.

Conclusion

The nine isolated strains of actinomycetes from the Egyptian desert, in Sinai peninsula, which contains many yet to be mined unexploited sites that are rich with precious and /or toxic metals such as REEs and U are mentioned. We proved that actinomycetes extraction of REEs and U is better, more economic and less polluting bioleaching processes. We strongly recommend its use instead of the chemical processes which are in common use today.

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Table 1: Physical and chemical characteristics of Abu Thor soil.

Property	Abu Thor
Electrical conductivity EC (dSm^{-1})	4.1
PH	7.3
Organic matter O.M (%)	1.19
Cation exchange capacity CEC (meq/100g)	48.2
Sand (%)	35.5
Silt (%)	7
Clay (%)	5
Gravel (%)	46
Granules (%)	6.5
Texture	Gravelly sand (GrS)

Table 2: Elemental analysis of Abu Thor samples.

Major element	Concentration (%)	Trace element	Concentration (mg/kg)
Na	0.77	Zr	235
Mg	1.78	Nb	3
Al	17.5	Pb	914
Si	35.4	Ba	737
P	0.39	Ra	95
S	20	LREEs	Concentration (mg/kg)
Cl	4.4	Y	170.7
K	6.82	La	200
Ca	5.96	Ce	238
Ti	0.49	Pr	76
Mn	0.6	Nd	270.5
Fe	18.9	Sm	200
Trace element	Concentration (mg/kg)	HREEs	Concentration (mg/kg)
V	258	Gd	122.7
Co	0.26	Dy	20.6
Ni	1414	Er	886.8
Cu	461	Total REEs	Concentration (mg/kg)
Zn	5089	ΣREEs	2165.3
Cd	0.04	Actinides	Concentration (mg/kg)
Rb	13	Th	236.78
Sr	490	U	318.57

LREEs :light rare earth elements
HREEs : Heavy rare earth elements

Table 3: Cultural and physiological characteristics of the tested actinomycetes.

Characteristic		A1	A8
1- Cultural characteristics			
	Color of aerial mycelium	**White or buff**	**Grey**
	Color of substrate mycelium	**-**	**-**
	Diffusible pigments	**-**	**-**
2- Morphological characteristics			
	Spore chain morphology	**Flexuous**	**Flexuous**
	Spore surface ornamentation	**Smooth**	**Smooth**
3- Physiological characteristics			
	Melanoid pigment produced	**-**	**-**
	Growth on Czapeck's medium	+	**W**
	Sodium chloride tolerance (< 4)		
Concentration:	**2.5%**	**W**	+
	5%	**-**	+
	7.5%	**-**	**W**
	10%	**-**	**W**
	Sensitivity to streptomycin (50 µg ml^{-1})	**Resistant**	**Sensitive**
	Antimicrobial activity		
Tested organism:			
	B. subtilis	+	+
	S. aureus	+	+
	E. coli	-	-
	P. aeruginosa	-	-
	C. albicans	+	+
	A. niger	+	+
	A. flavus	+	+
	F. oxysporum	+	+

W : weak growth

Table 4: Cultural and physiological characteristics of two actinomycetes isolates from sites in Sinai desert.

Characteristic		A1	A8
	Utilization of carbon sources		
Source:	**No carbon**	-	-
	D-Glucose	+	+
	D-Fructose	-	+
	D-Galactose	+	+
	D-Mannitol	+	+
	Inositol	+	-
	L-Arabinose	-	+
	D-Mannose	+	+
	Salicin	-	-
	D-Xylose	+	+
	Lactose	-	+
	D-Sucrose	-	-
	Maltose	+	+
	D-Raffinose	+	+
	Starch	+	+

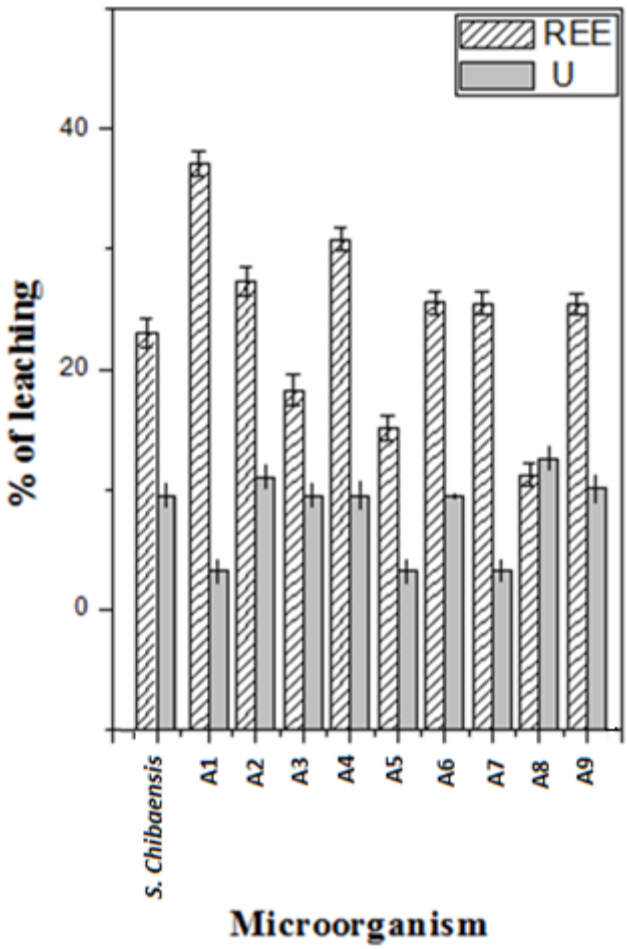

Figure 1: Bioleaching capacity of REEs and U using the tested actinomycetes.