Studii şi Cercetări Ştiinţifice 2015, **16** (4), pp. 353 – 369
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER / REVIEW

ISOLATION AND CHARACTERIZATION OF A MOLYBDENUM-REDUCING, PHENOL- AND CATECHOL-DEGRADING *PSEUDOMONAS PUTIDA* STRAIN AMR-12 IN SOILS FROM EGYPT

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Received: November, 11, 2015
Accepted: December, 16, 2015

Abstract: Sites contaminated with both heavy metals and organic xenobiotic pollutants warrants the effective use of either a multitude of bacterial degraders or bacteria having the capacity to detoxify numerous toxicants simultaneously. A molybdenum-reducing bacterium with the capacity to degrade phenolics is reported. Molybdenum (sodium molybdate) reduction was optimum between pH 6.0 and 7.0 and between 20 and 30 °C. The most suitable electron donor was glucose. A narrow range of phosphate concentrations between 5.0 and 7.5 mM was required for optimal reduction, while molybdate between 20 and 30 mM were needed for optimal reduction. The scanning absorption spectrum of the molybdenum blue produced indicated that Mo-blue is a reduced phosphomolybdate. Molybdenum reduction was inhibited by the heavy metals mercury, silver and chromium. Biochemical analysis identified the bacterium as *Pseudomonas putida* strain Amr-12. Phenol and phenolics cannot support molybdenum reduction. However, the bacterium was able to grow on the phenolic compounds (phenol and catechol) with observable lag periods. Maximum growth on phenol and catechol occurred around the concentrations of 600 $mg \cdot L^{-1}$. The ability of this bacterium to detoxify molybdenum and grown on toxic phenolic makes this bacterium an important tool for bioremediation.

Keywords: *catechol, molybdenum blue, molybdenum-reducing bacteria, phenol, Pseudomonas putida*

INTRODUCTION

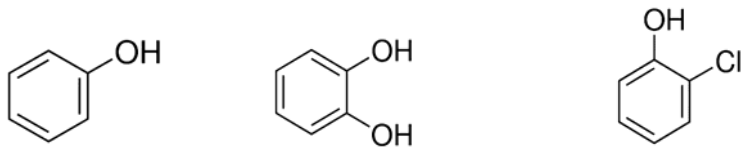
Polluted sites often contain both heavy metals and organic xenobiotic contaminants. This warrants the usage of either a great number of bacterial degraders or bacteria having the ability to detoxify several toxicants simultaneously. Molybdenum, like many metal ions such as chromium and copper, is required at very low concentration but toxic at elevated levels [1]. Its uses in industries include as steel-hardening agent, anti-freeze component of automobile engine and as lubricant [2]. Molybdenum pollution from mining and industrial activities is the two major routes of water bodies and soil pollutions due to molybdenum. At one time, waters of the Black Sea and Japan Bay are polluted with this element at levels above the standards regulatory limits [2]. Soils of a cattle-grazing area in Austria polluted with molybdenum over a long period of time led to several deaths and scouring cases in cattle [3]. In southern Colorado, U.S.A., dissolved molybdenum as high as 900 $mg \cdot L^{-1}$ have been reported in the tailing waters from an abandoned uranium mine. While level as high as 6,500 $mg \cdot kg^{-1}$ was discovered in soils in the same site [4]. In Malaysia, molybdenum is produced as a by-product of a copper mine in Ranau, Sabah. It was discovered that there were episodic leakage of metal waste-carrying pipes during the mine operation days. This have polluted huge agricultural areas especially paddy fields, resulting in damage ecosystems, and affecting the livelihood of people near the region [5].

Literature search has shown that molybdenum posed a serious threat to metabolic process including spermatogenesis. This was observed in drosophila [6] and other organisms. Male rats exposed to molybdenum between 50 and 150 $mg \cdot kg^{-1}$, result in a significant decrease in the relative weight of the testes coupled with aberrations of histomorphy and histology, indicating testicular abnormality [7]. Hypocuprosis is another toxicity effect of molybdenum. Ruminants such as sheep and cattle are the most susceptible, with dietary intake of between 5 to 10 ppm produces scouring and in certain cases deaths [8].

Bioremediation of molybdenum has been pursued through two routes. Both routes convert the soluble molybdenum (molybdate ions) into insoluble products, which are molybdenum disulphide or MoS_2 (Mo^{4+}) and molybdenum blue (Mo-blue). Reduction of molybdenum to MoS_2 by sulfate-reducing bacteria required completely anaerobic conditions. The bacteria from the genera *Desulfovibrio* and *Desulfotomaculum* are candidates that have been proposed. However, this process generates toxic hydrogen sulphide [9]. The second route is through the reduction of molybdenum into molybdenum blue under low oxygen tension. Mo-blue is a colloidal and can be confined in dialysis tubing easily [10], suggesting a method for its removal from aqueous solution [11]. The phenomenon of bacterial reduction of molybdenum to molybdenum blue was reported in *E. coli* for more than one century ago [12]. Further studies in *E. coli* K12 was carried out in a greater detail [13]. Since then, numerous Mo-reducing bacteria as candidates for bioremediation [14 – 26]. The successful purification of the Mo-reducing enzyme has only been recently carried out [27].

In addition to heavy metals, hydrocarbons in the forms of oil, grease and phenolics are ranked as the number one scheduled wastes generated from industries [28]. Phenol and phenolic compounds (Figure 1) are not only toxic to humans but to many other organisms as well [29]. Their vapours are corrosive to the mucous membranes, skin, eyes, and the respiratory tract. Prolonged skin contact causes dermatitis with a

possibility of a third-degree burn. Chronic exposure has harmful effects on the liver and kidneys. Toxicity is due to its hydrophobicity and to a certain extent the formation of phenoxyl radicals [30].



a) Phenol b) Catechol c) 2-Chlorophenol

Figure 1. *The structure of some toxic phenolic compounds*

It is well known that pollutes sites often contain a mixture of heavy metals and organic contaminants. Hence, the isolation of microorganisms with the ability to degrade a variety of xenobiotics and detoxify heavy metals simultaneously is in great need. An example of such microorganisms are the reduction of chromate coupled with phenol biodegradation in several bacteria [31, 32].

With this in mind, the aim of the present work is to isolate novel molybdenum reducing bacterium from contaminated soil having the ability to support molybdenum reduction to Mo-blue or grow on various toxic organic compounds specifically phenol and phenolic compounds. A Mo-reducing bacterium having the capacity to utilize phenol and catechol as carbon sources have been isolated from Egyptian soil. The multiple detoxification characteristics of the bacterium can be utilized in the future bioremediation of the heavy metal molybdenum and phenolics as the organic contaminant.

MATERIALS AND METHODS

Isolation of Mo-reducing bacterium

Soils from the ground of a contaminated land in Sadat City, Egypt were taken about 5 cm deep from the topsoil in 2014. About one gram of the soil sample was then suspended in sterile tap water. An aliquot of 0.1 mL of the soil suspension was then spread onto an agar of low phosphate media (*p*H 7.0). The Low Phosphate Media (LPM) was composed as follows: glucose (1 %), NaCl (0.5 %), $MgSO_4 \cdot 7H_2O$ (0.05 %), yeast extract (0.5 %), $Na_2MoO_4 \cdot 2H_2O$ (0.242 % or 10 mM), $(NH_4)_2 \cdot SO_4$ (0.3 %) and Na_2HPO_4 (0.071 % or 5 mM) [18]. The bacteria were incubated for 48 hours at room temperature. The presence of blue colonies suggests the presence of bacteria capable of molybdenum reduction. Colony showing the strongest blue intensity indicated that it is the best reducer, and was restreaked on LPM until a pure culture was obtained. The scanning of the absorption spectrum from 400 to 900 nm (UV-spectrophotometer, Shimadzu 1201) of molybdenum blue was carried out by taking out 1.0 mL of the culture media followed by centrifugation at 10,000 x g for 10 minutes at room temperature. The LPM was the baseline correction. Identification of the bacterium was carried out biochemically and phenotypically according to the Bergey's Manual of

Determinative Bacteriology [33]. Interpretation of the results was carried out via the ABIS online system [34].

Resting cells preparation

Optimal conditions for Mo-blue production from this bacterium was carried out utilizing resting cells in a microplate (microtiter) format as before [35]. Cells were grown in a volume of 1 L in several shake flasks (250 mL) aerobically trough shaking at 120 rpm on an orbital shaker in High Phosphate Media (HPM) at room temperature. The growth media was HPM. The difference between LPM and the HPM is the phosphate concentration, where the latter had a phosphate concentration of 100 mM. Cells were centrifuged at 15,000 x g for 10 minutes, and the pellets were washed several times using deionized water, and resuspended in 20 mL of low phosphate media (LPM) with glucose omitted. The characterization of Mo-blue production needs appropriate modifications to the LPM in order to accommodate variations in phosphate, molybdate and pH conditions. About 180 µL was sterically transferred into the wells of a sterile microplate. This was followed by the addition of 20 µL of sterile glucose from a stock solution to the final concentration of 0.1 % (w/v). This started Mo-blue production. A sterile sealing tape was utilized to seal the plate. The seal allowed gas exchange (Corning® microplate). Incubation of the microplate was carried out at room temperature. Readings at 750 nm was periodically taken using a BioRad (Richmond, CA) Microtiter Plate reader (Model No. 680). The specific extinction coefficient of 11.69 $mM^{-1} \cdot cm^{-1}$ at 750 nm was utilized to quantify Mo-blue production. This wavelength is the maximum filter available for the microplate unit [35]. The effect of several heavy metals was studied utilizing Atomic Absorption Spectrometry calibration standard solutions from MERCK.

Screening for phenolics as source of electron for molybdenum reduction and source or carbon for growth

The ability of various phenolics as electron donors was tested by replacing the best carbon source with these xenobiotics to the final concentration of 800 $mg \cdot L^{-1}$. The solubility of these xenobiotics was taken into account during the preparation of the xenobiotics [36]. The reduction media contained 40 µL of resting cells, 170 µL of LPM and 40 µL of xenobiotics. The phenolic compounds tested were phenol, pentachlorophenol, 2-chlorophenol, 2,4-dinitrophenol, 4-chlorophenol, catechol, salicylic acid, 4-nonylphenol, p-hydroxybenzoic acid, benzoate and 2-napthol. The microplate was sealed and incubated at room temperature for three days. Mo-blue production was monitored as before. When studying the ability of the phenolics above to support growth of this bacterium independent of molybdenum-reduction, molybdenum and glucose were omitted. The reduction media contained 40 µL of resting cells resuspended in HPM, 170 µL of HPM and 40 µL of xenobiotics. The increase of bacterial growth was measured at 600 nm after three days of incubation at room temperature for the initial screening.

RESULTS AND DISCUSSION

The reduction of molybdate to molybdenum blue by bacteria was first described more than one hundred years ago in 1896 by Capaldi, and Proskauer [12]. Further isolation of Mo-reducing bacteria in the last century were reported in 1939 [37], in 1948 [38], in 1962 [39], in 1972 [40], in 1985 [13], and in 1993 [41]. Ghani et al. [41] quickly recognize the potential application of this phenomenon for the bioremediation of molybdenum. Since then, numerous Mo-reducing bacteria have been isolated (Table 1). The ability of the newly isolated Mo-reducing bacterium to grow on other xenobiotics is indeed a highly sought after property.

Table 1. *Numerous molydenum-reducing bacteria that have been isolated by researchers*

Bacteria	Optimal C source	Optimal Temperature	Optimal *p*H	Optimal Molybdate [mM]	Optimal Phosphate [mM]	Heavy Metals that inhibit reduction	Author
Klebsiella oxytoca strain Aft-7	Glucose	25 and 34 °C	5.8-6.3	5-20	5-7.5	Cu^{2+}, Ag^{+}, Hg^{2+}	[26]
Bacillus pumilus strain lbna	Glucose	37 °C	7.0-8.0	40	2.5-5	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}	[22]
Bacillus sp. strain A.rzi	Glucose	28-30 °C	7.3	50	4	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}, Co^{2+},Zn^{2+}	[24]
Serratia sp. strain Dr.Y8	Sucrose	37 °C	6.0	50	5	Cr, Cu, Ag, Hg	[17]
S. marcescens strain Dr.Y9	Sucrose	37 °C	7.0	20	5	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[18]
Serratia sp. strain Dr.Y5	Glucose	37 °C	7.0	30	5	n.a.	[15]
Pseudomonas sp. strain DRY2	Glucose	40 °C	6.0	15-20	5	Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[19]
Pseudomonas sp. strain DRY1	Glucose	15-20 °C	6.5-7.5	30-50	5	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}	[23]
Enterobacter sp. strain Dr.Y13	Glucose	37 °C	6.5	25-50	5	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[16]
Acinetobacter calcoaceticus strain Dr.Y12	Glucose	37 °C	6.5	20	5	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[20]
Serratia marcescens strain DRY6	Sucrose	35 °C	7.0	15-25	5	Cr^{6+}, Cu^{2+}, Hg^{2+}*	[14]
Enterobacter cloacae strain 48	Sucrose	30 °C	7.0	20	2.9	Cr^{6+}, Cu^{2+}	[41]
Escherichia coli K12	Glucose	30-36 °C	7.0	80	5	Cr^{6+}	[13]
Klebsiella oxytoca strain hkeem	Fructose	30 °C	7.3	80	4.5	Cu^{2+}, Ag^{+}, Hg^{2+}	[21]

Identification of molybdenum reducing bacterium

The bacterium was a short rod-shaped, motile and Gram-negative. Results from the various tests conducted (Table 2) were computed into the ABIS online software [34]. The software gave three suggestions with the highest homology (81 %) and accuracy at 85 % given as *Pseudomonas putida*. Currently, molecular identification technique *via* 16srRNA gene sequencing and analysis through comparison with similar strains from gene database is being carried out to identify the species further. At this juncture, the bacterium is tentatively identified as *Pseudomonas putida* strain Amr-12. This is not the first Mo-reducing bacterium from the genus as there are two Mo-reducing bacteria from this genu that have been isolated, which include *Pseudomona*s sp. strain DRY2 [19] and the Antarctic bacterium *Pseudomona*s sp. strain DRY1 [23]. Bacterial species from this genus are known for their phenol- and phenolics-degrading capacity [42 – 46]. Analysis by ANOVA indicated that the optimum *p*H supporting molybdate reduction range between 6.0 and 7.0 (Data not shown) and the optimum temperatures ranged from 20 to 30 °C (Data not shown).

Table 2. *Biochemical tests for Pseudomonas putida strain Amr-12*

Biochemical tests	**Result**	**Biochemical tests**	**Result**
Motility	+	Utilization of:	
Hemolysis	+	L-Arabinose	+
Growth at 4 °C	–	Citrate	+
Growth at 41 °C	+	Fructose	+
Growth on MacConkey agar	–	Glucose	+
Arginine dihydrolase (ADH)	+	*meso*-Inositol	–
Alkaline phosphatase (PAL)	+	2-Ketogluconate	+
Indole production	–	Mannose	+
Nitrates reduction	–	Mannitol	–
Lecithinase	–	Sorbitol	–
Lysine decarboxylase (LDC)	–	Sucrose	+
Ornithine decarboxylase (ODC)	–	Trehalose	–
ONPG (beta-galactosidase)	–	Xylose	–
Esculin hydrolysis	–		
Gelatin hydrolysis	–		
Starch hydrolysis	–		
Urea hydrolysis	+		
Oxidase reaction	+		

Note: "+" - positive result, "–" - negative result / indeterminate result

As molybdenum reduction to Mo-blue require low oxygen tension environment [41], the use of resting cells under static conditions is an advantage for this study. The reductions of selenate [47], chromate [48] and vanadate [49], as well as studies on the biodegradation of several xenobiotics such as diesel [50], SDS [51] and phenol [52] also take advantage of resting cells for characterizations of these processes.

Molybdenum absorbance spectrum

The Mo-blue produced by *Pseudomonas putida* strain Amr-12 exhibited a shoulder at 700 nm and a maximum peak at 865 nm (Figure 2). The spectrum obtained is very similar to previously characterized Mo-reducing bacteria, and all of them are similar to the Mo-blue from the method to determine phosphate. It has been discussed previously that this similar spectrum strongly suggests that Mo-blue from bacterial reduction of molybdate is a reduced phosphomolybdate (Figure 2). However, it is well known that phosphomolybdate has numerous lacunary species, which include Keggin and Dawson's structures. The exact identification of the species is normally conducted using NMR and ESR [53]. Phosphomolybdate falls under the family of heteropolymolybdates, with other main species include silicomolybdate and sulphomolybdate. Spectrophotometric characterization of heteropolymolybdate species can be easily carried out by scrutinizing the absorption spectrum of the reduced products [54 – 57], which was adopted in this work. Monitoring of Mo-blue production at 750 nm, although was approximately 30 % lower than 865 nm, was not interfered with bacterial absorption at 600-620 nm [35]. Previous monitoring of Mo-blue production in bacteria uses other wavelengths, which include 710 nm for monitoring Mo-blue production from *Enterobacter cloacae* strain 48 [41] and 820 nm for monitoring Mo-blue production from *E. coli* K12 [13].

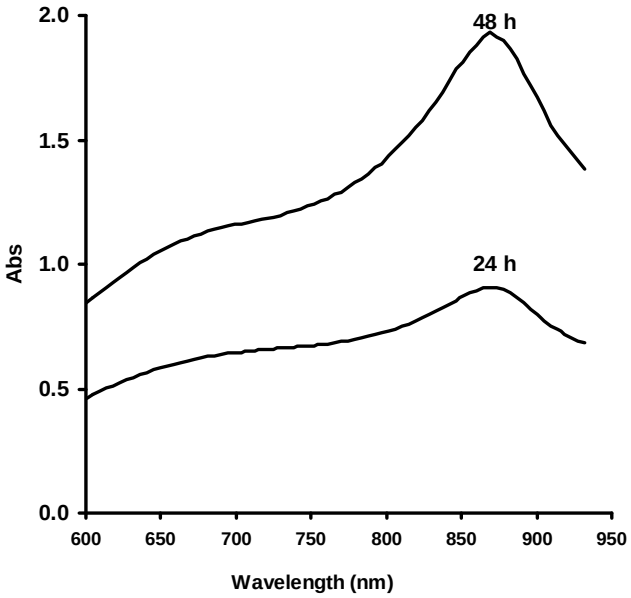


***Figure** 2. Scanning absorption spectrum of Mo-blue from Pseudomonas putida strain Amr-12*

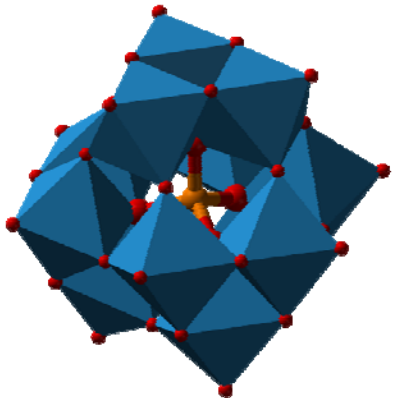


***Figure** 3. Structure of phosphomolybdate, a heteropolymolybdate [58]*

Effect of electron donor on molybdate reduction

Nearly all of the Mo-reducing bacteria isolated to date prefer either glucose or sucrose as source of electron donor for Mo-blue production, with *Klebsiella oxytoca* strain hkeem being the only bacterium that prefers fructose (Table 1). The results showed that

glucose was the best electron donor. This was followed by galactose, fructose and citrate, while other carbon sources cannot support reduction (Figure 4). One of the reasons why simple carbohydrates such as sucrose and glucose are the preferred electron donor is these sources can produce the reducing equivalents NADH and NADPH thorough bacterial pathways, which include glycolysis, Kreb's cycle and electron transport chain. Both of these reducing equivalents are substrates for the molybdenum reducing-enzyme [27, 59]. Nevertheless, when it comes to bioremediation, affordability is the most important factors, and a cheaper carbon source may be needed for an economic process. Molasses is preferred over simple carbohydrates as it is less expensive, and can be obtained in bulk from the sugarcane manufacturing industries as waste materials in Malaysia [60]. The use of molasses in supporting metal reduction has been utilized in the bacterial reductions of chromate [61, 62] and selenate [63]. Thus, the potential of molasses as an alternative to sucrose or glucose will be carried out in future research.

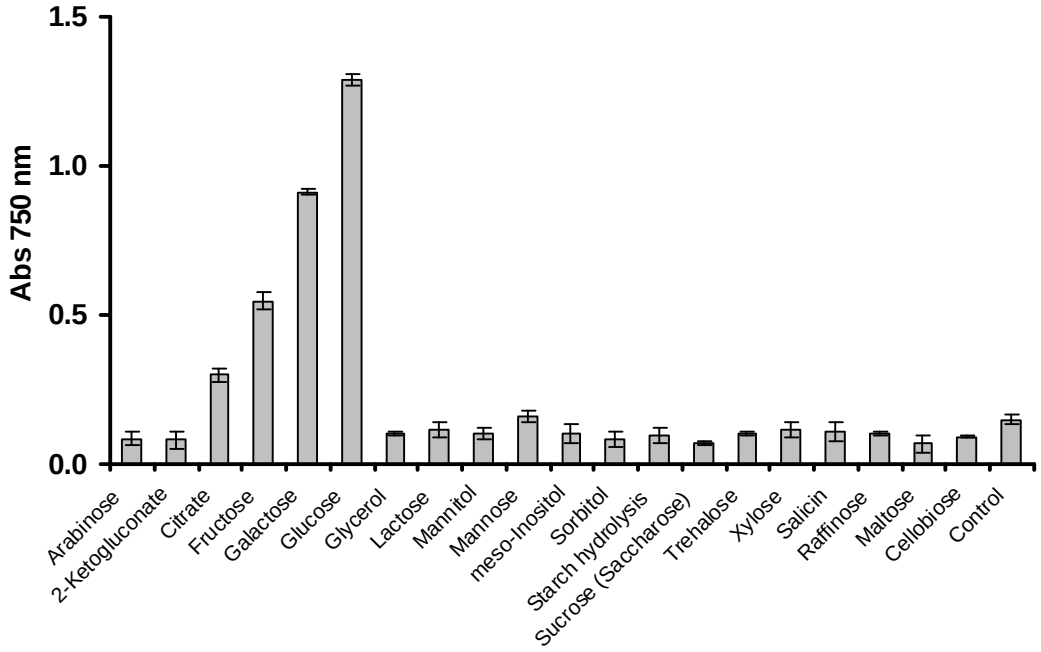


Figure 4. *Effect of various carbohydrates as electron donor sources on molybdenum reduction (error bars indicate mean ± standard deviation of triplicates)*

Mo-blue production under various phosphate and molybdate concentrations

Mo-blue production is optimally supported by a wide range of sodium molybdate concentrations, with a reported range of between 5 and 80 mM (Table 1). On the other hand, the concentrations of phosphate required for optimal production is very narrow and ranged between 5 and 7.5 mM (Table 1). Similar to previous works, a phosphate concentration of 5 mM was required for optimal reduction in this bacterium. Higher concentrations strongly inhibited reduction. A phosphate concentration of 100 mM ceased Mo-blue production completely (Figure 5). As the phosphomolybdate complex itself is unstable in the presence of high phosphate [54, 55, 64], this could explain the

instability of the complex. Previously isolated molybdenum-reducing bacterium exhibited requirement for phosphate concentration around 5 mM for optimal reduction (Table 1). Mo-blue production was optimal between 20 and 40 mM, and higher concentrations were inhibitory, with concentrations of molybdate higher than 70 mM nearly ceased Mo-blue production (Figure 6). A time course Mo-blue production results show a lag period of about 10 hours before Mo-blue production commenced. Maximum production of Mo-blue occurred around 40 hours of incubation (Figure 7). Other Mo-reducing bacteria showed optimal reduction at concentrations in between 5 to 80 mM (450 to 7,600 $mg \cdot L^{-1}$) (Table 1). One of the highest concentrations of molybdenum found in the environment is from an abandoned uranium mine in Colorado. In one of the waste tailing sites, a molybdenum concentration of 6,550 $mg \cdot Kg^{-1}$ has been reported [4]. Several of the Mo-reducing bacteria isolated can be utilised for the bioremediation of molybdenum pollution in this site due to their high tolerance towards molybdenum (Table 1).

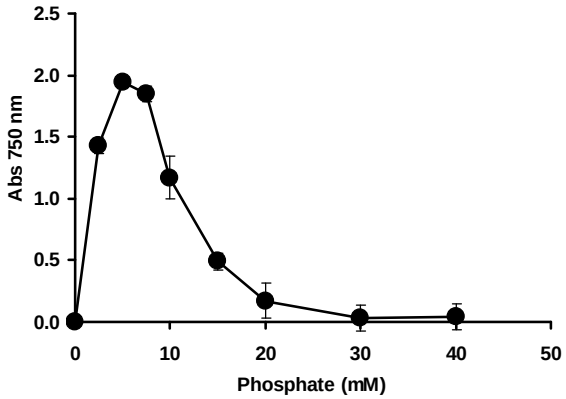


Figure 5. *Mo-blue production under various concentrations of phosphate (error bars indicate mean ± standard deviation of triplicates)*

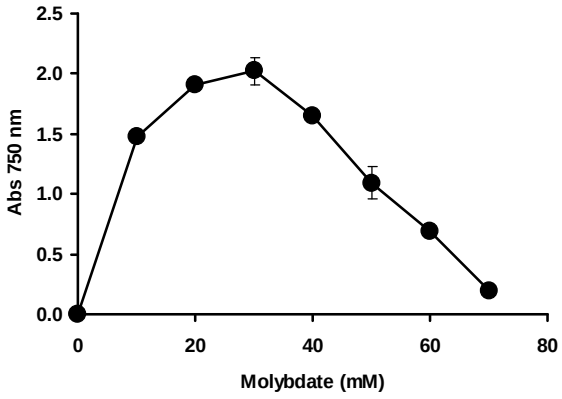


Figure 6. *Mo-blue production under various concentrations of sodium molybdate (error bars indicate mean ± standard deviation of triplicates)*

Effect of heavy metals on molybdenum reduction

Mo-blue production was inhibited by the heavy metals mercury, silver and chromium at 2 ppm by 87.1, 70.6 and 35.0 %, respectively (Figure 8). Similar toxic heavy metals have been found to inhibit many of the Mo-reducing bacteria (Table 1). The heavy metals mercury and copper are also strong inhibitors to bacterial reduction of another similar heavy metal, which is chromate. The inhibition of these toxic metal ions is seen in the bacteria *Bacillus* sp. [65] and *Enterobacter cloacae* strain H01 [66]. Since these metal ions interact with critical protein and enzyme active sites, which include carboxyl, amide, sulfhydryl, phosphoryl and amine groups, this interaction is the reason for the generic toxicity of these metal ions. Mercury and copper interact with the sulfhydryl group of chromate reductase in both of the bacteria above, which lead to inhibition of enzyme reduction activity [65, 66]. A potential way to reduce the toxicity effect of these toxic metal ions is by adding metal-sequestering or chelating substances, which preferably bind to these cations. This include calcium carbonate, manganese oxide,

phosphate, and magnesium hydroxide [67, 68]. As an alternative, an immobilization of the Mo-reducing bacterium membrane or other matrices can offer some resistance against the toxicity of these heavy metals [10].

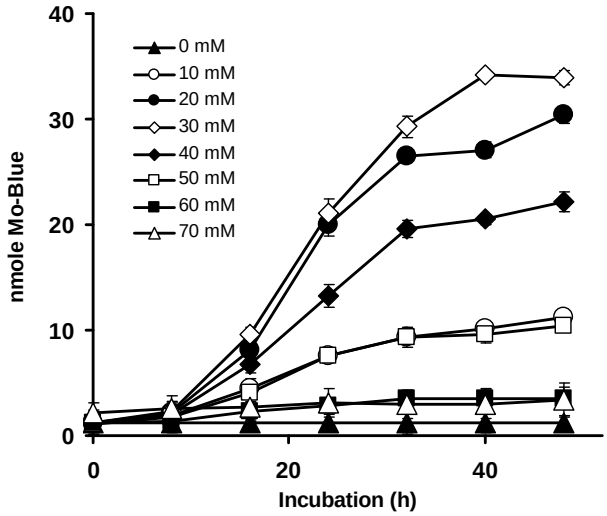


Figure 7. *The effect of various concentrations of sodium molybdate on molybdenum reduction by Pseudomonas putida strain Amr-12 monitored over time (error bars indicate mean ± standard deviation of triplicate)*

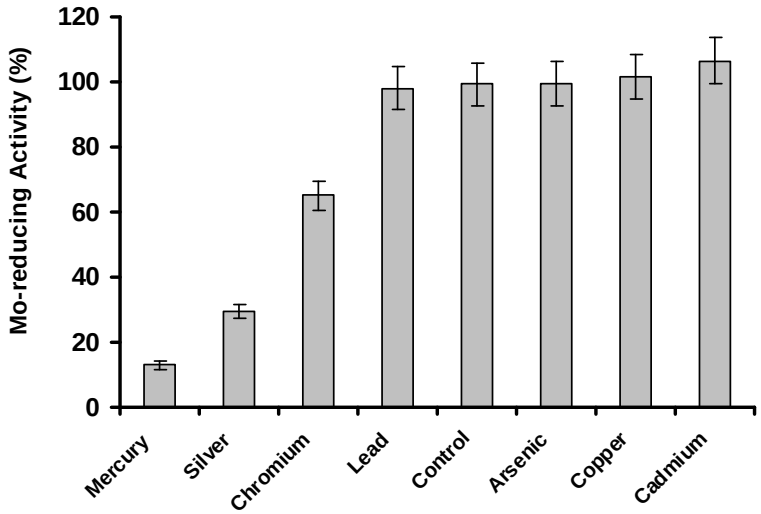


Figure 8. *Mo-blue production under various heavy metals (error bars indicate mean ± standard deviation of triplicate)*

Phenolics as electron donor sources for molybdenum reduction and independent growth

Screening of phenolics as electron donors supporting molybdenum reduction failed to give positive results. However, the bacterium was able to grow on the phenolic compounds phenol and catechol (Figure 9). A time course data on growth of the bacterium on phenol showed a lag period of approximately one day for phenol

concentrations lower than 600 mg·L^{-1} and approximately two days at higher concentrations before growth commenced. A maximum growth on phenol occurred around six days of incubation. Maximal growth occurred between phenol concentrations of between 600 and 750 mg·L^{-1}, while higher concentrations were inhibitory to growth (Figure 10). Similarly, growth on catechol showed a lag period of about one day for catechol concentrations lower than 600 mg·L^{-1} and approximately two days at higher concentrations before growth commenced. Maximum growth on catechol occurred around day six (Figure 11).

Phenol- and phenolics-degrading bacteria are ideal for phenol remediation due to economic factors. Biodegradation of phenol and phenolic compounds by microorganisms has long been an object of intense research globally. Bacteria that could degrade phenol and phenolic compounds include *Pseudomonas* species [43 – 46], *Bacillus brev*is [69], *Alcaligenes* sp. [70], *Ochrobactrum* sp. [71], *Acinetobacter* sp. [72, 73] and *Rhodococcus* species [36]. Each of these degraders have its own unique properties such as the ability to tolerate high concentration of phenol, salt tolerant, heavy metals tolerant and the ability to grow at either extreme *p*Hs or temperature. The presence of plethora of bacteria with catechol and phenol-degrading capacity can make bioremediation of these phenolic compounds a reality. Nonetheless, hardly any bacteria are actually stated to be competent to biodegrade phenolics and to detoxify heavy metals simultaneously. The potential of this bacterium to perform the two functions indicates that the bacterium can be really beneficial as a bioremediation instrument.

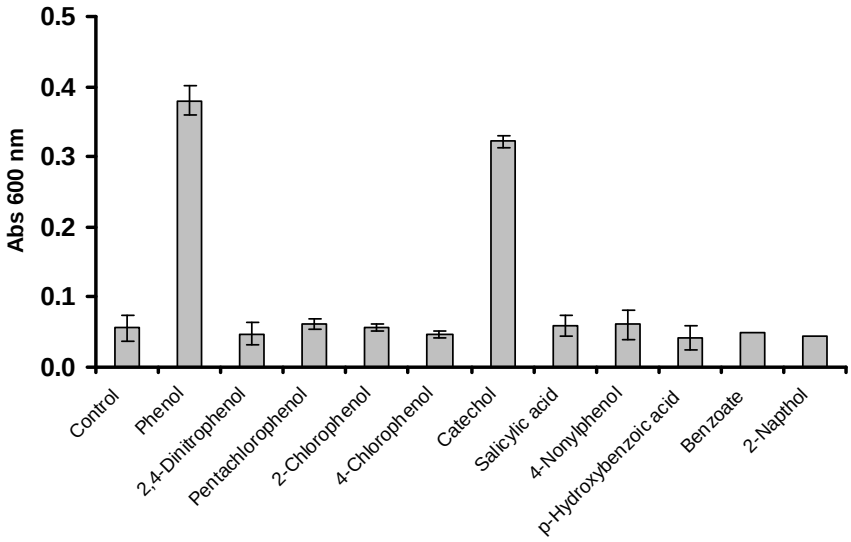


Figure 9. *Growth of Pseudomonas putida strain Amr-12 on phenolic compounds (error bars indicate mean ± standard deviation of triplicate)*

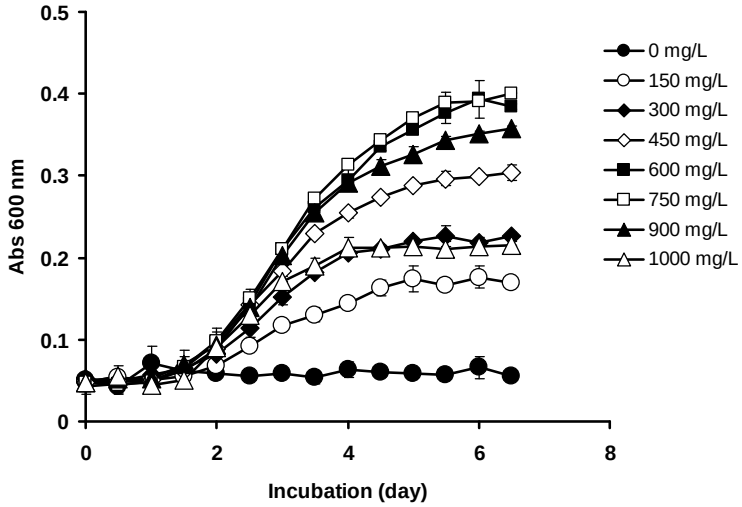


***Figure 10**. Growth of Pseudomonas putida strain Amr-12 on various concentrations of phenol; resting cells of Pseudomonas putida strain Amr-12 were grown in phenol in a microtiter plate format (error bars indicate mean ± standard deviation of triplicate)*

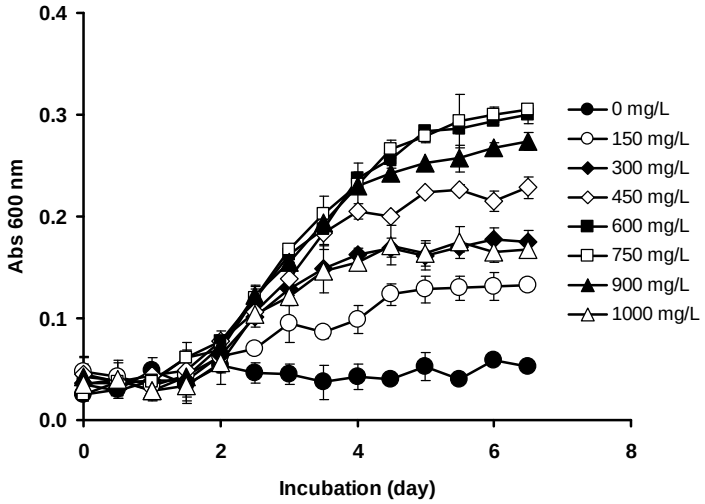


***Figure 11**. Growth of Pseudomonas putida strain Amr-12 on various concentrations of catechol; resting cells of Pseudomonas putida strain Amr-12 were grown in catechol in a microtiter plate format (error bars indicate mean ± standard deviation of triplicate)*

CONCLUSION

A Mo-reducing bacterium has been isolated from Egyptian soil. The bacterium was able to biodegrade the phenolic compounds phenol and catechol. Glucose, an easily assimilable sugar was the best electron donor for molybdenum reduction. Phosphate concentrations were critically needed between 5.0 and 7.5 mM. The scanning absorption spectrum of the molybdenum blue produced indicated that Mo-blue is a reduced

phosphomolybdate. Molybdenum reduction was inhibited by the heavy metals mercury, silver and chromium. Biochemical analysis identified the bacterium as *Pseudomonas putida* strain Amr-12. Phenol and phenolics cannot support molybdenum reduction. However, the bacterium was able to grow on the phenolic compounds phenol and catechol with observable lag periods. Maximum growth on phenol and catechol occurred around the concentrations of 600 $mg \cdot L^{-1}$ indicating good tolerance to these toxic compounds. The multiple detoxification capacity of this bacterium indicates that the bacterium can be an important tool for bioremediation. Currently, the 16s rRNA gene from this bacterium is being sequenced for molecular phylogenetic analysis, a process important for bacterial identification. In addition, the molybdenum-reducing enzyme from this bacterium is being purified and the characterization of the bacterial degradation of phenolics is being carried out.

ACKNOWLEDGEMENTS

This project was supported by funds from Snoc International Sdn Bhd, Malaysia.

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