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Isolation and Characterization of a Molybdenum-reducing *Enterobacter aerogenes* strain Amr-18 in Soils from Egypt that Could Grow on Amides

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ABSTRACT

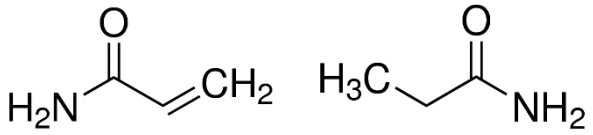
In the foreseeable future, bioremediation is by far the most cost-effective method for removing noxious chemical toxins and organic contaminants, especially at low concentrations when other methods like physical or chemical procedures wouldn't be successful. For the objective of bioremediation, we have isolated a molybdenum-reducing bacteria from agricultural soil. The ideal pH and temperature ranges for the bacterium to reduce molybdate to molybdenum blue (Mo-blue) are 6.3 and 6.8, respectively. The best electron donor for molybdate reduction was glucose, which was followed in descending order by sucrose, lactose, l-rhamnose, d-mannose, raffinose, d-adonitol, maltose, d-mannitol, melibiose, cellobiose, glycerol, and d-sorbitol. Phosphate concentrations of 7.5 mM and molybdate concentrations of 15 – 20 mM are also necessary**.** The Mo-blue that was formed had an absorption spectrum that was comparable to that of earlier Mo-reducing bacteria and closely resembled that of reduced phosphomolybdate. At 2 ppm, copper (II), mercury (I), and silver I hindered molybdenum reduction by 80.2, 74.8, and 30.4%, respectively. The bacterium was tentatively identified as *Enterobacter aerogenes* strain Amr-18 after phenotypic and biochemical identifications. The bacterium could thrive on the amides, acrylamide, acetamide, and propionamide and could use acrylamide as an electron donor for molybdenum reduction. This bacterium has a highly valued trait that makes it useful for bioremediation: the capacity to detoxify a variety of toxicants.

INTRODUCTION

Bioremediation clearly remains the most cost-effective method of removing contaminants when other methods like physical or chemical procedures are ineffective. One of the essential heavy metals, molybdenum is harmful to a number of organisms at high concentrations and is needed in trace amounts. It is used in a variety of industrial processes as an alloying agent, an antifreeze component for car engines, a corrosion-resistant steel component, and a lubricant in the form of molybdenum disulphide. The widespread use of molybdenum in industry has led to several incidents of water contamination worldwide, including in the Black Sea, Tyrol in Austria, and Tokyo Bay, where molybdenum levels reach hundreds of parts per million [1]. Molybdenum has also been identified as a significant pollutant in terrestrial sewage sludge contamination that poses a health risk [1]. It has been documented that molybdenum toxicity in organisms like catfish and mice can disrupt spermatogenesis and halt embryogenesis at concentrations as low as parts per

million [2,3]. Similarly, at a slightly high levels, molybdenum is extremely toxic to ruminants, with cows being the most impacted [4–6].

As a cost-effective technique for removing heavy metals and organic contaminants, bioremediation surpass especially at low concentrations where other techniques like physical or chemical procedures would fail [7]. Through reduction, several bacteria may detoxify a range of xenobiotics, including heavy metals [8,9]. Amides are harmful xenobiotics, including propionamide and acrylamide (**Fig**. 1). It serves as the foundation for the polymer; polyacrylamide. There are several applications for this polymer, including stabilizing tunnels and dams, sewage flocculating agents, and industrial adhesives [10]. In Sweden, environmental contamination with acrylamide has been linked to acute episodes of toxicity that have killed fish and cows [11]. Polyacrylamide, which is used in 20–30% of the formulation of the pesticide glyphosate as a dispersion agent [12], may be a significant source of acrylamide pollution in soil and runoff. There have been reports of acrylamide pollution at quantities as high as 1 g/L from the acrylonitrile-acrylamide industry [13].



(a) (b)

Fig. 1. The structure of acrylamide (a) and propionamide (b).

In the current study, a novel molybdenum-reducing bacterium that was isolated from agricultural soil for its capacity to either employ xenobiotics as independent electron donors for reduction or as carbon sources for growth was studied. Previously, the bacterium was isolated in order to biocontrol a plant fungal pathogen [14]. We intentionally use static growth or conditions because they are simple to achieve in a microplate environment where the oxygen concentration is lower than under aerobic conditions (20% environmental oxygen, EO), where most bioremediation conditions would have to be carried out in aquatic bodies or soils where the EO level is less than 20% EO and other electron acceptors such as nitrate would start to accumulate to be use [15]. This bacterium can thrive on the amides; acrylamide, acetamide and propionamide and can use acrylamide as a source of electron donor for molybdenum reduction. These properties make this bacterium suitable for bioremediation of both the organic pollutants (amides) and the heavy metal (molybdenum).

MATERIALS AND METHODS

Bacteria isolation of molybdate-reducing bacterium

In 2014, soil samples were gathered from the grounds of a polluted site (5 cm below the topsoil) in Sadat City, Egypt. Into a sterile tap water, 1 g of the soil sample was suspended. Agar with low phosphate media (pH 7.0) was pipetted and spread with an aliquot of the soil suspension equal to 100 µL. It was then incubated at room temperature for 48 hours. Glucose (1%), $(NH4)_2SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), yeast extract (0.5%), NaCl (0.5%), $Na_2MoO_4.2H_2O$ (0.242% or 10 mM) and Na_2HPO_4 (0.071% or 5 mM) made up the low phosphate media (LPM). To avoid the formation of molybdenum blue, which hampers analytical tasks, the HPM uses a greater phosphate content. In HPM, the bacterium still exhibits molecular activity [16].

The development of blue colonies is a sign that molybdenum-reducing bacteria are reducing molybdate. To establish pure culture, the colony with the greatest blue intensity was selected and re-streaked on low phosphate media (LPM). Molybdenum reduction in liquid media (at pH 7.0) was performed in 100 mL of the aforementioned media in a 250 mL shake flask culture at room temperature for 48 hours on an orbital shaker set at 120 rpm with the aforementioned media but with a higher phosphate content of 100 mM. By removing 1.0 mL of the Mo-blue produced by the liquid culture above and centrifuging it at 10,000 × g for 10 minutes at room temperature, it was possible to analyze the Mo-blue absorption spectra. Using a UV-spectrophotometer, the supernatant was scanned from 400 to 900 nm (Shimadzu 1201). The baseline adjustment used the low phosphate medium.

Identification of Mo-reducing bacterium

The bacterium was phenotypically and biochemically characterized using conventional methods such as colony shape, gram staining, size and colour on nutrient agar plate, motility, oxidase (24 h), ONPG (beta-galactosidase), catalase production (24 h), ornithine decarboxylase (ODC), arginine dihydrolase (ADH), lysine decarboxylase (LDC), nitrates reduction, Methyl red, indole production, Voges-Proskauer (VP), hydrogen sulfide (H_2S), acetate utilization, malonate utilization, citrate utilization (Simmons), esculin hydrolysis, tartrate (Jordans), gelatin hydrolysis, urea hydrolysis, deoxyribonuclease, lipase (corn oil), phenylalanine deaminase, gas production from glucose and production of acids from various sugars were carried out according to the Bergey's Manual of Determinative Bacteriology [17]. The ABIS online system was used to interpret the findings [18].

Resting cells preparation to characterize molybdate reduction

The effects of pH, temperature, phosphate, and molybdate concentrations on molybdenum reduction to Mo-blue were studied statically using resting cells in a microplate or microtiter format, as previously developed [19]. The only difference between the High Phosphate Media (HPM) and Low Phosphate Media (LPM) was the phosphate concentration, which was fixed at 100 mM for the HPM. Cells from a 1 L overnight culture were grown in the HPM at room temperature on an orbital shaker (150 rpm). Centrifugation at 15,000 × g for 10 minutes was used to remove any remaining phosphate from the cells' pellet before it was resuspended in 20 mL of low phosphate media (LPM) without glucose, resulting in an absorbance of roughly 1.00 at 600 nm. All of the Mo-reducing bacteria that have been isolated to date preferred a phosphate concentration of 5 mM in the low phosphate media, so this concentration was used in this study.

Molybdate reduction was found to be strongly inhibited by higher concentrations [16,20–34]. A sterile microplate was then filled with 180 L using a sterile pipette. Then, to start the production of Mo-blue, 20 µL of sterile glucose from a stock solution was added to each well. The tape was sealed using a sterile, gas-exchange-permitting Corning® microplate. A room-temperature incubator was used for the microplate. A BioRad (Richmond, CA) Microtiter Plate reader was used to measure absorbance at 750 nm at predetermined intervals (Model No. 680). The maximum filter wavelength that could be used with the microplate unit was 750 nm, so the production of Mo-blue from the media in a microplate format was measured using the specific extinction coefficient of 11.69 $mM.^{-1}.cm^{-1}$ at that wavelength. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours.

Heavy metals effect on molybdate reduction

Lead (II), arsenic (V), copper (II), mercury (ii), silver chromium (VI), and cadmium (II) were among the highly toxic heavy metals that were prepared from either commercial salts or MERCK standard solutions for atomic absorption spectrometry (AAS). In a microplate format, the bacterium was incubated with heavy metals at various concentrations. The same wavelength of 750 nm was used to gauge the production of Mo-blue.

Screening for growth and molybdate reduction on amides and nitriles

Using the aforementioned microplate format, it was determined whether amides and nitriles like acrylamide, nicotinamide, acetamide, iodoacetamide, propionamide, 2-chloroacetamide, acetamide, acetonitrile, acrylonitrile, and benzonitrile could support molybdenum reduction as electron donors. This was done while taking into account the general toxicity and solubility of [35]. After that, 200 uL of the media and 50 L of a suspension of resting cells were applied to the microplate wells. The microplate was incubated for three days at room temperature, and as before, the quantity of Mo-blue generation was detected at 750 nm. Using the microplate format described above, the media below were used with the exception of molybdate, and glucose was substituted with the xenobiotics at a final concentration of 200 mg/L in a volume of 50 L to test whether the aforementioned compounds could support the growth of this bacterium independently of molybdenum-reduction. The growth media (LPM) contained the following components: yeast extract (0.01%), (NH4)2SO4 (0.3%), NaNO3 (0.2%), MgSO4.7H2O (0.05%), NaCl (0.5%), and Na2HPO4 (0.705% or 50 mM). The media were changed to a pH of 7.0. After three days of incubation at room temperature, the rise in bacterial growth was observed and measured at 600 nm.

Statistical analysis

The data represent means ±SD. Graphpad Prism 3.0 and Graphpad InStat 3.05, both accessible from www.graphpad.com, were used to analyze the data. For group comparisons, either a student t-test or one-way analysis of variance with *post hoc* analysis by Tukey's test was used. Statistics were deemed significant at $P<0.05$.

RESULTS AND DISCUSSION

Identification of molybdenum-reducing bacterium

The bacterium was a short, motile, facultative anaerobe and Gram-negative. The colonies were round, smooth, cream-colored, glossy, and between 1 and 3 mm in diameter. Bergey's Manual of Determinative Bacteriology [17] and the ABIS online software [18] were used to compare the results of the cultural, morphological, and numerous biochemical tests (**Table** 1) to the results of the bacteria. The software provided three choices for the bacterial identity, with *Enterobacter aerogenes* having the highest homology (87%) and accuracy (100%). To further identify this species, more research will be required in the future, particularly using molecular identification methods based on comparison of the 16srRNA gene. The preliminary name for the bacterium at this time is *Enterobacter aerogenes* strain Amr-18.

Table 1. Biochemical tests for *Enterobacter aerogenes* strain Amr-18.

Motility	+	Acid production from:	
Pigment	–		
		Alpha-Methyl-D-	
Catalase production (24 h)	+	Glucoside	+
Oxidase (24 h)	–	D-Adonitol	+
ONPG (beta-galactosidase)	+	L-Arabinose	+
Arginine dihydrolase (ADH)	+	Cellobiose	+
Lysine decarboxylase (LDC)	–	Dulcitol	+
Ornithine decarboxylase (ODC)	+	Glycerol	+
Nitrates reduction	+	D-Glucose	+
Methyl red	–	myo-Inositol	+
Voges-Proskauer (VP)	+	Lactose	+
Indole production	–	Maltose	+
Hydrogen sulfide (H2S)	–	D-Mannitol	+
Acetate utilization	+	D-Mannose	+
Malonate utilization	+	Melibiose	+
Citrate utilization (Simmons)	+	Mucate	+
Tartrate (Jordans)	+	Raffinose	+
Esculin hydrolysis	+	L-Rhamnose	+
Gelatin hydrolysis	d	Salicin	+
Urea hydrolysis	+	D-Sorbitol	+
Deoxyribonuclease	–	Sucrose (Saccharose)	+
Lipase (corn oil)	–	Trehalose	+
Phenylalanine deaminase	–	D-Xylose	+

Note: + positive result, − negative result, d indeterminate result

A quick and easy high throughput method utilizing a microplate format was utilized in this study to speed up characterization works and collect more data than the typical shake-flask methodology [19,36]. Ghani et al. [21] pioneered the use of stationary, resting cells to characterize molybdenum reduction in bacteria. Resting cells have been utilized to research the biodegradation of xenobiotics such dyes [37,38], diesel [39], SDS [40,41], phenol [42], amides [43], and pentachlorophenol [44] as well as the reduction of heavy metals like selenate [45], chromate [46], and vanadate [47].

Spectrum for Mo-blue absorption

Mo-blue generated by *Enterobacter aerogenes* strain Amr-18 had an absorption spectrum with a shoulder at around 700 nm and a maximum peak close to infrared between 860 and 870 nm with a median at 865 nm (**Fig**. 2). Due of its complicated structure and wide variety of species, it is difficult to determine the identity of the Mo-blue formed [22]. In a nutshell, isopolymolybdate and heteropolymolybdate are two types of molybdenum complexes that are reduced to form Mo-blue. According to Campbell et al. [20], the Mo-blue that was seen when molybdenum was reduced by *E. coli* K12 might actually be a reduced form of phosphomolybdate. When using the phosphate determination method, the Mo-blue spectra typically displayed a maximum absorption between 880 and 890 nm and a shoulder between 700 and 720 nm [27]. We have previously demonstrated that the entire spectrum of other bacteria's molybdenum blue displayed similarities to the spectrum of molybdenum blue obtained using the phosphate determination method, and we propose the theory that phosphomolybdate is a crucial intermediate between molybdenum and Mo-blue [22]. In this study, the absorption spectrum result strongly suggests a comparable spectrum, supporting the phosphomolybdate theory. Due to the compound's intricate structure, precise identification of the phosphomolybdate species must be done by NMR or ESR.

A less time-consuming and well-accepted technique is to characterize heteropolymolybdate species spectrophotometrically by looking at the scanning spectroscopic profile [48]. Optimal Mo-blue absorption wavelength was 865 nm, but measurements at 750 nm, is around 30% lower, but sufficient for routinely monitoring the production of Mo-blue because the intensity was substantially higher than cellular absorption at 600–620 nm [19]. Several wavelengths have been used in the past to monitor the synthesis of Mo-blue, including 710 nm [21] and 820 nm [20].

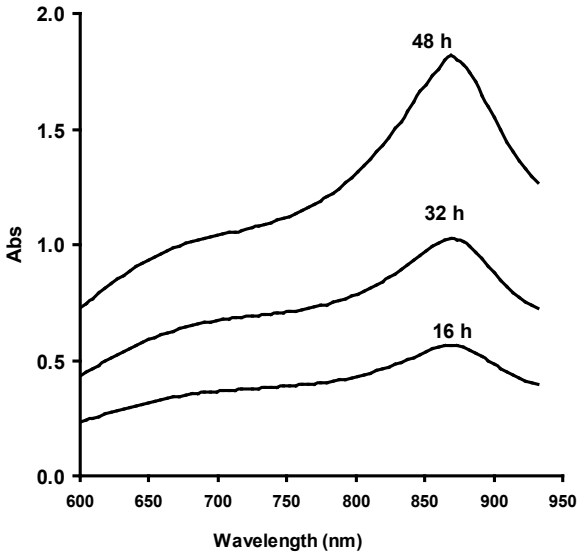


Fig. 2. Scanning absorption spectrum of Mo-blue from *Enterobacter aerogenes* strain Amr-18 at different time intervals.

Effects of pH and temperature on molybdate reduction

Using Bis-Tris and Tris.Cl buffers, *Enterobacter aerogenes* strain Amr-18 was cultured at pH values ranging from 5.5 - 8.0. (20 mM). The best pH range for reduction, according to ANOVA analysis, was between 6.0 and 6.8. At pH levels below 5, there was a significant inhibition of reduction (**Fig**. 3). With an optimal temperature range between 30 °C and 37 °C, the influence of temperature (**Fig**. 4) was found over a broad temperature range (20 to 60 °C), with no significant differences ($p>0.05$) among the measured values as analyzed using ANOVA. The growth of *Enterobacter aerogenes* strain Amr-18 was severely inhibited by temperatures lower than 30 °C and higher than 37 °C [49,50].

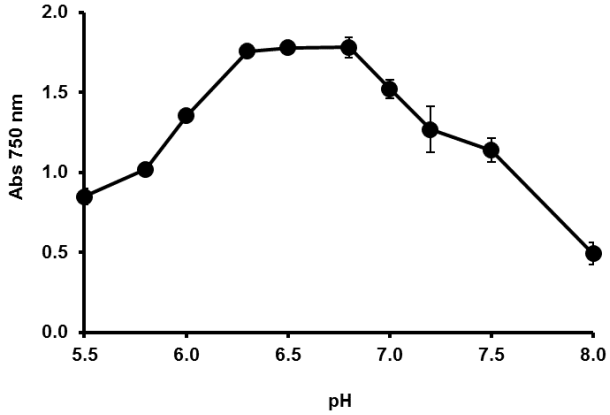


Fig. 3. pH effect on molybdenum reduction by *Enterobacter aerogenes* strain Amr-18. Data represent mean ± standard deviation (n=3).

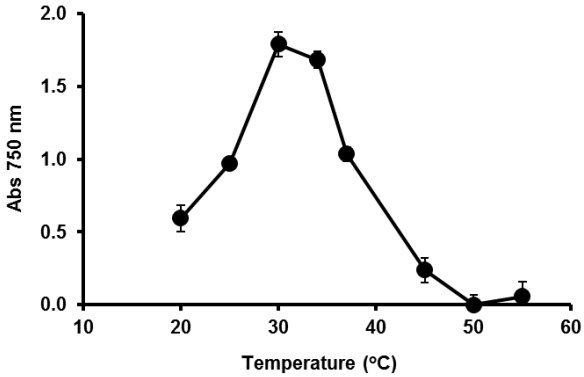


Fig. 4. Temperature effect on molybdenum reduction by *Enterobacter aerogenes* strain Amr-18. Data represent mean ± standard deviation (n=3).

Since molybdenum reduction is an enzyme-mediated, temperature and pH are significant factors in this process. These factors have an impact on protein folding and enzyme activity, which inhibits molybdenum reduction. An advantage for bioremediation would be the ideal environmental conditions in a tropical nation like Malaysia, where the average annual temperature ranges from 25 to 35 ºC [23]. As a result, both locally and in other tropical nations, *Enterobacter aerogenes* strain Amr-18 may be a contender for molybdenum soil bioremediation. Due to their isolation from tropical soils, the majority of the reducers exhibit an optimal temperature range of 25 – 37 ºC [16,23,24,26–30,32–34,51], with the sole psychrotolerant reducer isolated from Antarctica exhibiting an optimal temperature range of 15 – 20 ºC [31].

The *Enterobacter aerogenes* strain Amr-18 preferred pH range for molybdenum reduction is a reflection of the bacterium's neutrophilic nature. The ability of neutrophile to thrive between pH values of 5.5 and 8.0 is one of their properties. The best pH for molybdenum reduction in bacteria is somewhat acidic, with optimal pHs ranging from pH 5.0 – 7.0 [20,21,23–34,51,52]. This is an important finding. It has been proposed in the past that phosphomolybdate's production and stability before it is converted to Mo-blue depend significantly on the acidity. In order to get the best reduction, substrate stability and enzyme activity must be balanced [53].

Effects of electron donors on molybdate reduction

The best electron donor enabling molybdate reduction to Mo-blue among the sources of carbon studied was glucose, which was followed in descending order by sucrose, lactose, l-rhamnose, d-mannose, raffinose, d-adonitol, maltose, d-mannitol, melibiose, cellobiose, glycerol, and d-sorbitol (**Fig**. 5), however, production of Mo-blue was not supported by other carbon sources. Previous research by Shukor et al. indicated that sucrose as the most suitable source of carbon for a number of Mo-reducing bacteria, including *Enterobacter cloacae* strain 48 [21], *Serratia* sp. strain Dr.Y5 [24], *S. marcescens* strain Dr.Y9 [16], and *Serratia marcescens* strain DRY6 [23]. In other molybdenum reducers like *Escherichia coli* K12 [20], *Pseudomonas* sp. strain DRY2 [27], *Pseudomonas* sp. strain DRY1 [31], *Enterobacter* sp. strain Dr.Y13 [25], *Acinetobacter calcoaceticus* strain Dr.Y12 [28], *Bacillus pumilus* strain lbna [30], *Bacillus* sp. strain A.rzi [33] glucose is most preferred, whereas, fructose is best for *Klebsiella oxytoca* strain hkeem [29].

The bacteria could generate NADH and NADPH by metabolic processes such glycolysis, Kreb's cycle, and electron transport chain when carbon sources were present in the media. The most efficient electron donor appears to be either glucose or sucrose in most situations. This may be due to the fact that glucose and sucrose produce reducing equivalents like NADH and NADPH through normal metabolic pathways much more readily than other electron donors, and both reducing equivalents serve as an electron donor for molybdate-reducing enzyme activity [51,54]. The same situation is seen in chromate reduction, numerous bacteria's chromate reductase enzyme uses both NADH and NADPH as electron donor substrates, and glucose and sucrose are both effective electron donors for chromate reduction [55–57].

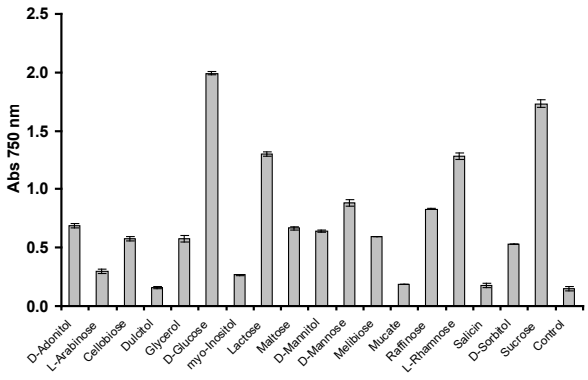


Fig. 5. Effect of different electron donor sources (1% w/v) on molybdenum reduction. *Enterobacter aerogenes* strain Amr-18 was grown in low phosphate media containing 10 mM molybdate. Data represent mean ± standard deviation (n = 3).

Effects of phosphate and molybdate concentrations to molybdate reduction

Determining the phosphate and molybdate concentrations supporting optimal molybdenum reduction is important because both anions have been reported to impede Mo-blue synthesis in bacteria [16,23,25–29,31,33,51], it is crucial to determine the phosphate and molybdate concentrations that enable optimal Mo-blue production. Phosphate concentrations above 7.5 mM greatly inhibited reduction, while lower amounts were optimal (**Fig**. 6). Since the complex needs acidic conditions and the higher the phosphate content, the stronger the buffering power of the phosphate buffer utilized, it was proposed that high phosphate levels hinder phosphomolybdate stability. Additionally, a reason why the phosphomolybdate complex itself is unstable in the presence of high phosphate is still unknown [58–60]. For optimum reduction, phosphate concentrations not greater than 5 mM is needed by all the molybdenum-reducing bacteria that has been discovered to date [20,21,23–34,51].

Findings on the impact of molybdenum concentration on the reduction process, reveals that the optimum concentration for reduction ranged 15 – 20 mM (**Fig**. 7). However, a newly identified bacterium was able to reduce molybdenum up to 60 mM, despite lower Mo-blue synthesis. This strain would therefore be able to combat high molybdenum pollution due to its ability for reducing high molybdenum concentration into an insoluble form.

E. coli K12 [20] and *Klebsiella oxytoca* strain hkeem [29] needed the highest molybdenum concentration (80 mM) for optimum reduction, the lowest optimal concentration of molybdenum was found in *Pseudomonas* sp. strain Dr.Y2 at 15 mM [27]. Other Mo-reducing bacteria, including EC48 [21], *S. marcescens* strain Dr.Y6 [23], *S. marcescens* strain Dr.Y9 [16], *Pseudomonas* sp. strain Dr.Y2 [27], *Serratia* sp. strain Dr.Y5 [24], *Enterobacter* sp. strain Dr.Y13 [25] and *Acinetobacter calcoaceticus* [28], could also produce excellent Mo-blue. In reality, the maximum amount of molybdenum that may be found in the atmosphere is roughly 2000 ppm or 20 mM [61].

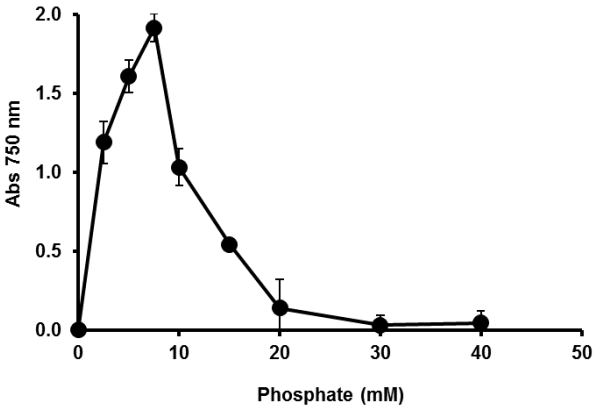


Fig. 6. Effect of phosphate concentrations on molybdenum reduction by *Enterobacter aerogenes* strain Amr-18. Data represent mean ± standard deviation (n = 3).

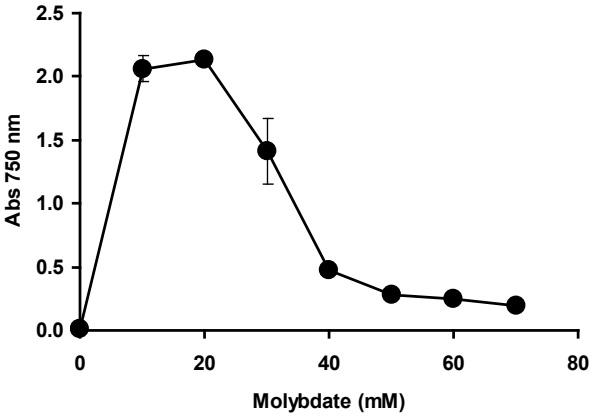


Fig. 7. The effect of molybdate concentration on molybdenum reduction by *Enterobacter aerogenes* strain Amr-18. Data represent mean ± standard deviation (n = 3).

Heavy metals effect on molybdate reduction

At 2 ppm, copper (II), mercury (II) and silver hindered molybdenum reduction by 80.2, 74.8, and 30.4%, respectively (**Fig**. 8). In bioremediation, the inhibitory effects of other metal ions offer a significant challenge. Therefore, it's critical to identify and isolate bacteria that have a wide range of metal resistance capabilities. Mercury is a physiological inhibitor of molybdate reduction, as previously stated by Shukor et al. [62]. Mo-reducing bacteria were shown to be inhibited by a variety of heavy metals, and it was shown that hazardous heavy metals block nearly all of the reducers (**Table** 2). Silver, copper, cadmium and other heavy metals frequently target the sulfhydryl group of enzymes [63]. It is well known that chromium inhibits enzymes like glucose oxidase [64].

The ability of the enzyme(s) responsible for the reduction to reduce metals was deactivated by the binding of these metals.

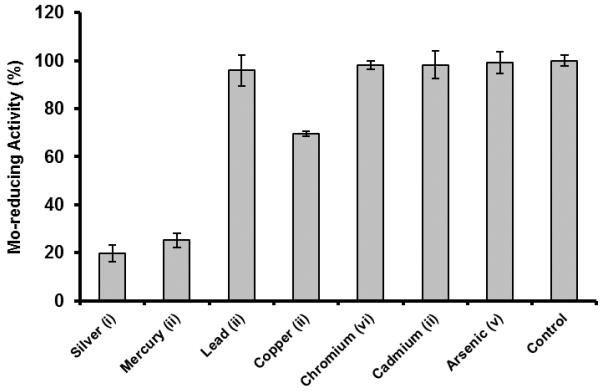


Fig. 8. The effect of metals on Mo-blue production by *Enterobacter aerogenes* strain Amr-18. Data represent mean ± standard deviation (n = 3).

Table 2. Heavy metals inhibition of Mo-reducing bacteria.

Bacteria	Heavy Metals that inhibit reduction	Author
Bacillus pumilus strain lbna	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}	[30]
Bacillus sp. strain A.rzi	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}, Co^{2+},Zn^{2+}	[33]
Serratia sp. strain Dr.Y8	Cr, Cu, Ag, Hg	[26]
S. marcescens strain Dr.Y9	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[16]
Serratia sp. strain Dr.Y5	n.a.	[24]
Pseudomonas sp. strain DRY2	Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[27]
Pseudomonas sp. strain DRY1	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}	[31]
Enterobacter sp. strain Dr.Y13	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[25]
Acinetobacter calcoaceticus strain Dr.Y12	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[28]
Serratia marcescens strain DRY6	Cr^{6+}, Cu^{2+}, Hg^{2+}*	[23]
Enterobacter cloacae strain 48	Cr^{6+}, Cu^{2+}	[21]
Escherichia coli K12	Cr^{6+}	[20]
Klebsiella oxytoca strain hkeem	Cu^{2+}, Ag^{+}, Hg^{2+}	[29]

Utilization of amides and nitriles as electron donors for growth and molybdate reduction

It was investigated whether these amides and nitriles could aid molybdenum reduction. Only acrylamide, out of all the xenobiotics examined, was demonstrated to facilitate molybdenum reduction at a lesser efficiency than glucose (**Fig**. 9). Independent of molybdenum reduction, the bacterium could thrive on the amides; acrylamide, acetamide and propionamide (**Fig**. 10). This is the first report on carbon sources besides carbs that might enable bacterial Mo-reduction. Xenobiotics like phenol could be used as electron donors in the chromate reduction process [65]. Millions of tonnes of amides are manufactured annually, including acetamide, propionamide and acrylamide. Numerous bacteria that could exploit these amides and nitriles as sources of carbon or nitrogen for growth and development have been discovered [10,66,67,69–77]. The ability to tolerate large concentrations of xenobiotics, salt tolerance, heavy metal tolerance, and the capacity to thrive at either extreme pHs or temperatures are just a few of the special qualities that each of these degraders have. Bioremediation is the preferred method for amide degradation due to the abundance of bacteria that can degrade amides. The fact that this bacterium can both breakdown amide and detoxify heavy metals, which nearly no other bacteria have been found to be able to, suggests that it will be a particularly helpful bioremediation agent in polluted areas having xenobiotics and heavy metals as contaminants.

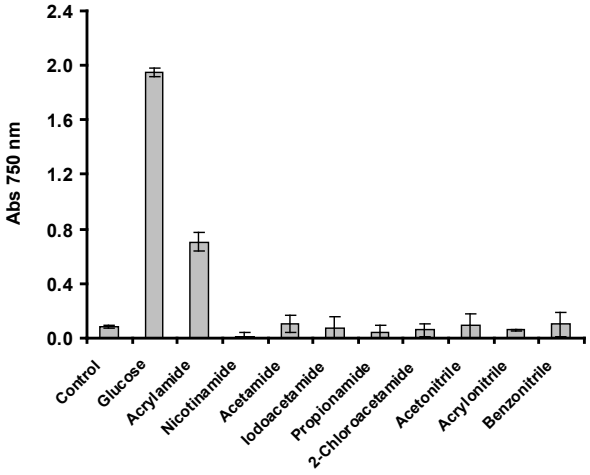


Fig. 9. Mo-blue reduction by xenobiotics at 10 mM in low phosphate media. Glucose was the positive control. Data represent mean ± standard deviation (n = 3).

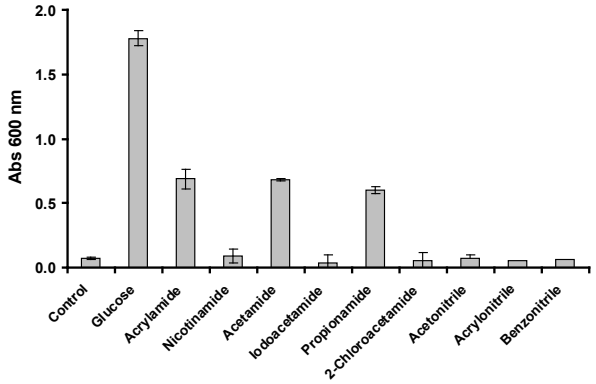


Fig. 10. Growth of *Enterobacter aerogenes* strain Amr-18 on xenobiotics independent of molybdenum reduction. Glucose was the positive control. Data represent mean ± standard deviation (n = 3).

CONCLUSION

An indigenous bacterium capable of utilizing acrylamide as electron donor for molybdenum reduction has been isolated locally. The ideal pH and temperature ranges for the bacterium to reduce molybdate to Mo-blue are 6.3 – 6.8 and 30 – 35 °C, respectively. The best electron donor for molybdate reduction was glucose, which was followed by sucrose, lactose, L-rhamnose, D-mannose, raffinose, D-adonitol, maltose, D-mannitol, melibiose, cellobiose, glycerol and D-sorbitol in descending order. Phosphate concentrations of 7.5 mM and molybdate concentrations between 15 and 20 mM. The Mo-blue that was formed had an absorption spectrum that was comparable to that of earlier Mo-reducing-bacteria and closely resembled that of reduced phosphomolybdate. At 2 ppm, copper (II), mercury (II) and silver hindered molybdenum reduction by 80.2, 74.8, and 30.4%, respectively. This bacterium has a highly valued trait that makes it useful for bioremediation: the capacity to detoxify

several toxicants. Currently, efforts are being made to characterize the amide degradation studies and to purify the molybdenum-reducing enzyme from this bacterium.

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