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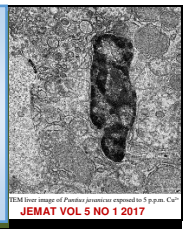

Isolation and characterization of Molybdenum-reducing and PEG-degrading *Enterobacter cloacae* strain KIK-14 in Agricultural soil from Nigeria

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ABSTRACT

Today, numerous researches have demonstrated the cost-effectiveness of bioremediation to waste removal from agricultural and industrial sectors particularly at lower levels of the toxicants, where other physicochemical techniques are ineffective. Multiple toxicant remediation by a single microorganism is important for remediation of sites contaminated with numerous toxicants. In this work, a molybdenum-reducing bacterium was screened for its ability to use the xenobiotic polyethylene glycol (PEG) as the sole source of carbon for growth and as electron donor source for molybdate reduction. Biochemical analysis results in the tentative identification of the isolate as *Enterobacter cloacae* strain KIK-14. The use of PEGs as an electron donor in this bacterium did not support molybdenum-blue production, even though the bacterium grew well on PEGs 200, 300, 600 and 1000 independent of molybdate reduction. Reduction of molybdate to Mo-blue was optimal at pH between 6.0 and 6.3, the temperature between 25 and 37 °C, molybdate and phosphate concentrations between 15 and 20 mM and between 5.0 and 7.5 mM respectively. The best electron donor source supporting the reduction process was glucose. The Mo-blue absorption spectrum resembles reduced phosphomolybdate and is similar to that of the previous Mo-reducing bacterium. At 2 ppm of silver, mercury and copper, molybdenum reduction was inhibited by 41.5, 57.1 and 40.5%, respectively. The ability of this bacterium to detoxify mixed toxicants makes it an important tool for bioremediation.

INTRODUCTION

Recent findings have demonstrated the cost-effectiveness of bioremediation to waste removal from agricultural and industrial sectors particularly at lower levels of the toxicants, where other physicochemical techniques are ineffective [1]. Polyethylene glycols (PEGs) are water-soluble polymers used in industrial production of cosmetics, pharmaceuticals, lubricants, antifreeze for automobile radiators and non-ionic surfactants. Polyethylene glycol is a nephrotoxic agent as evident in a wounded rabbit topically exposed to PEG-based antimicrobial cream, which produces symptoms of renal failure, and many tested animals died within one week of the therapy [2]. Effluents containing PEGs usually reached millions of tons globally, and are a significant pollutant in conventional sewage treatment systems [3]. During the last three decades, there was expressed concern about the fate of these polymers in the environment, which gave rise to several studies on their biodegradation potential, with the first documented work

reported in 1965 [4] and the subsequent isolations of PEG-degrading microorganisms were reported [3].

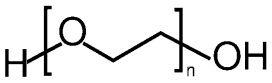

Fig. 1. The general structure of the PEG.

Molybdenum is an essential trace element and a heavy metal required by most organisms in a trace amount, but toxic to a variety of organisms at elevated levels. It has many industrial uses as an alloying agent, the lubricant in the form of molybdenum disulphide, automobile engine anti-freeze and a component of corrosion resistant steel. Reported molybdenum pollution in the Black Sea, Tokyo Bay and Tyrol in Austria are attributed to an increase in industrial activity [5]. Molybdenum at several p.p.m. is not toxic to human but it is known to be very toxic to ruminants leading to scouring and even death cases [6,7]. In addition, newer information has linked molybdenum toxicity to the arrest of spermatogenesis and embryogenesis in catfish and mice [8,9].

Previously, heavy metals reduction including both chromium [10–12] and molybdenum [13–20] coupled with xenobiotic degradation have been reported. As the use of foreign microbes in bioremediation is generally not recommended as such could cause ecological problems [21]. Thus, the need to screen for more local microbes for remediation works. In this work, an isolated molybdenum-reducing bacterium was screened for its ability to use polyethylene glycol (PEG) as the sole source of carbon for growth and as electron donor source for molybdate reduction. This novel molybdenum-reducer isolated from contaminated soil grow well on PEGs 200, 300, 600 and 1000. The characteristic tolerance of this bacterium would make it suitable for future bioremediation involving both heavy metal molybdenum and PEGs as contaminants.

MATERIALS AND METHODS

Isolation of a molybdenum-reducing bacterium

Soil samples (5 cm deep beneath the ground) were obtained from agricultural land in Kano, Nigeria, in 2014. Into 9 mL sterile water, 1 g of soil sample was suspended, and 0.1 mL aliquot of the soil suspension was spread onto agar of low phosphate media (LPM, pH 7.0) containing glucose (1%), $MgSO_4.7H_2O$ (0.05%), $(NH_4)_2.SO_4$ (0.3%), $Na_2MoO_4.2H_2O$ (0.242 % or 10 mM), NaCl (0.5%), Na_2HPO_4 (0.071% or 5 mM) and yeast extract (0.5%) and incubated at room temperature for 48 hours [22]. The formations of blue colonies after incubation indicates bacterial molybdate reduction. In order to obtain a pure culture, the colony with the strongest blue intensity was isolated and re-streaked on low phosphate media (LPM).

To characterize the molybdenum reduction in liquid media (pH 7.0), 100 mL of the above media in a 250 mL shake flask was used to culture the bacterium at room temperature on an orbital shaker (120 rpm) for 48 hours. Measuring the molybdenum blue (Mo-blue) absorption spectrum was done by taking out 1.0 mL aliquot form the culture medium and centrifuged at 10,000 × g for 10 minutes at room temperature. The supernatant was scanned from 400 to 900 nm using Shimadzu UV-spectrophotometer (Shimadzu 1201), with LPM used as baseline correction.

Morphological and biochemical characterization of a Mo-reducing bacterium

The bacterium was phenotypically and biochemically characterized according to the Bergey's manual of determinative bacteriology [23]. The parameters assessed include; colony shape, size and color on nutrient agar, Gram staining, motility, catalase and oxidase production (24 h), ONPG (beta-galactosidase), arginine dihydrolase (ADH), ornithine decarboxylase (ODC), lysine decarboxylase (LDC), nitrates reduction, Methyl red, indole production, Voges-Proskauer (VP), hydrogen sulfide (H_2S), acetate, citrate and malonate utilization (Simmons), esculin hydrolysis, gelatin hydrolysis, urea hydrolysis, deoxyribonuclease, lipase (corn oil), phenylalanine deaminase, acid production from various sugars and gas production from glucose, while the interpretation of the results was conducted via the ABIS online system [24].

Preparation of resting cells for molybdenum reduction characterization

The work on characterization of molybdate reduction to Mo-blue such as the effects of temperature, pH, molybdate and phosphate concentrations was conducted statically using resting cells in a microtiter plate as previously developed [25]. Cells grown overnight in 1 L of high phosphate media (HMP) at room temperature on orbital shaker (150 rpm) were harvested by centrifuging at 15,000 × g for 10 minutes and the pellet was rinsed thrice to remove residual phosphate, then re-suspended in 20 mL of low phosphate media with glucose excluded to an approximate absorbance 1.00 at 600 nm. A concentration of 5 mM phosphate in LMP was optimal for most of the Mo-reducing bacteria isolated so far.

Hence this concentration was used in this work. Higher phosphate concentrations were strongly inhibitory to Mo-blue production [22,26–40]. Into each well of a sterile microtiter plate, 180 µL LPM containing the suspended cells and 20 µL of sterile glucose from a stock solution (1%) were transferred to initiate Mo-blue production. A sterile sealing tape that enables gas exchange (Corning® microplate) was used to seal the tape and incubated at room temperature. Absorbance at defined time intervals was measured at 750 nm using a BioRad micro plate reader (Richmond, CA 680). The specific extinction coefficient of 11.69 $mM.^{-1}.cm^{-1}$ was used to determine the production of Mo-blue in a microtiter plate at 750 nm [41].

PEGs as electron donor source and carbon source for growth

The potential use of polyethylene glycols like PEG 200, PEG 300, PEG 600, PEG 1000, PEG 2000, PEG 3000, PEG 4000, PEG 8000, PEG 10000 and PEG 20000 to be utilized as electron donor source for molybdate reduction using the microplate format was tested by replacing glucose in the LPM with these xenobiotics to a final concentration of 500 mg/L. Whereas, the ability of PEGs to support bacterial growth independent of molybdenum reduction was tested in a microplate using a growth medium with constituent as follows: $(NH_4)_2.SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), $NaNO_3$ (0.2%), Na_2HPO_4 (0.705% or 50 mM) NaCl (0.5%), yeast extract (0.01%) and 1 mL of trace elements solution consisting (mg/L) of $CaCl_2$ (40), $CoCl_2 \cdot 6H_2O$ (5), $CuSO_4 \cdot 5H_2O$ (5), $FeSO_4 \cdot 7H_2O$ (40), $MnSO_4 \cdot 4H_2O$ (40), $Na_2MoO_4 \cdot 2H_2O$ (5) and $ZnSO_4 \cdot 7H_2O$ (20) and the medium was adjusted to pH 7.0. The PEGs were initially added to a final concentration of 0.5 g/L in 10 mL medium and sonicated for 5 minutes, and then 200 µL of the medium was added to each well of the microplate and incubated at room temperature for 72 hours. The increased bacterial

growth following 3 days incubation period at room temperature was measured at 600 nm using the microplate reader (Bio-Rad 680).

Effect of heavy metals on molybdenum reduction

The bacterium was incubated (in a microplate) with various concentrations of the heavy metals such as arsenic (V), cadmium (II), chromium (VI) copper (II), lead (II), mercury (II) and silver (I) prepared from commercial salts or from Atomic Absorption Spectrometry standard solutions (MERCK). The amount of Mo-blue produced was measured at 750 nm.

Statistical analysis

InStat GraphPad (version 3.05) and GraphPad Prism version 3.0 available at www.graphpad.com were used to perform statistical analysis, and the result expressed as means ± SE. Student's t-test or a one-way ANOVA with Tukey's post hoc test was employed to compare between means, with $p<0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Identification of molybdenum reducing bacterium

Strain KIK-14 is a Gram-negative, short rod-shaped, motile and facultative anaerobe. Identification based on a comparison of the results from morphological and biochemical tests with the Bergey's manual of determinative bacteriology [23] through ABIS online software [24], three suggestions to the bacterial identity were given, with the highest similarity or homology greater 90% and 100% accuracy as *Enterobacter cloacae*. However, molecular identification techniques to compare the 16SrRNA gene may be required to further identify this species in future.

At this juncture, however, the bacterium is tentatively identified as *Enterobacter cloacae* strain KIK-14. Microbial reduction of molybdate to molybdenum blue was first described more than a century ago in 1896 by Capaldi, and Proskauer [42], though further reports followed by Jan in 1939 [43], Marchal and Gerard in 1948 [44], Woolfolk and Whiteley in 1962 [45], Bautista and Alexander in 1972 [46], Campbell et al. in 1985 [26], Sugio et al. (1988) and Ghani et al. in 1993 [27]. The work of Campbell et al. on *Escherichia coli* K12 was the earliest comprehensive studies on bacterial molybdate reduction to molybdenum blue. This work reported the isolation of new molybdenum reducing bacterium from the genus *Enterobacter*, though, two molybdenum-reducers, *Enterobacter cloacae* strain 48 [27], and *Enterobacter* sp. strain Dr.Y13 [31] have been previously isolated. The ability of the bacterium from this species to weakly degrade PEG-200 was similarly reported [47]. A simple and rapid throughput method involving microtiter plate format was utilized to speed up characterization work and obtain more data than the normal shake-flask approach [25,48]. Characterization of molybdenum reduction using resting cells at static was initiated by [27]. Resting cells have also been used to study heavy metals reduction like in selenate [49].

Table 1. Biochemical tests for *Enterobacter cloacae* strain KIK-14.

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

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

Molybdenum absorbance spectrum

Previous studies revealed that Mo-blue absorption spectrum from many bacteria exhibited a maximum absorption at 865 nm and a shoulder at approximately 710 nm. Scanning absorption spectra in this work revealed a similar result with a maximum peak between 860 and 870 nm, and a shoulder at approximately 710 nm for all sampling periods (**Fig. 2**). The reduction product of molybdenum (Mo-blue) has a complex structure, with many species and a mixed valency (between 5+ and 6+) occur in the oxidation state in many of these species [28,50], making exact identification of the species difficult.

It was earlier suggested by [26] that the Mo-blue produced during molybdenum reduction by *E. coli* K12 is a reduced form of phosphomolybdate and resembles the Mo-blue formed in the phosphate determination method. In the phosphate determination method, Mo-blue absorption spectrum normally gives a maximum absorption around 880 to 890 nm and a shoulder around 700 to 720 nm [33].

The fact that the Mo-blue absorption spectra from most other previous bacteria exhibited the same characteristic implies that the same Mo-blue species is possibly involved [28]. The precise identification of the phosphomolybdate species should be done using NMR and ESR [50], though, scanning spectroscopic profile of the heteropolymolybdate species is simple and well accepted spectrophotometric method for characterization [51]. The intensity of the wavelength selected for this work (750 nm) was approximately 30% lower than at 865 nm, but it is enough for routine monitoring of Mo-blue production, especially that the intensity obtained is high enough not to be masked by cellular absorption at 600-620 nm [25]. Previously, monitoring Mo-blue production uses other wavelengths such as 820 nm [26] and 710 nm [27], both of which are claimed to have less interference by bacterial cellular absorption.

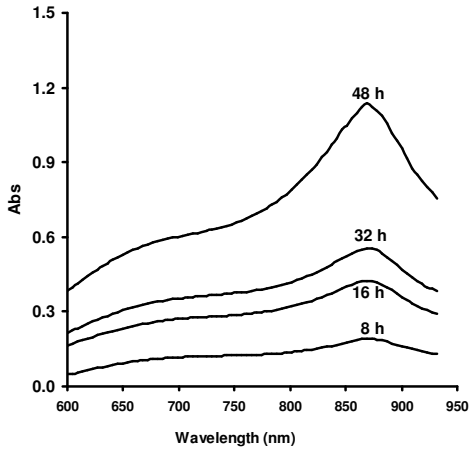

Fig. 2. Absorption spectrum scanning of Mo-blue from *Enterobacter cloacae* strain KIK-14 at different time intervals.

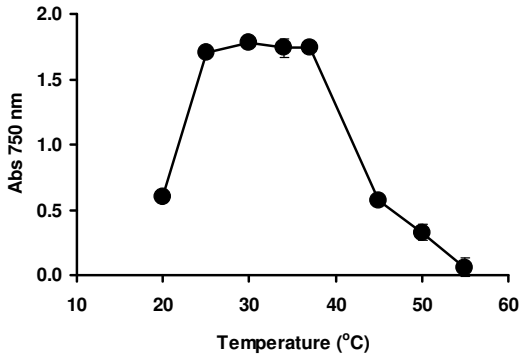

Fig. 4. Effect of temperature on molybdenum blue production by *Enterobacter cloacae* strains KIK-14, after 48 hours incubation of resting cells under an optimized condition in a microplate. Error bars represent mean ± SE (n=3).

Effect of temperature and pH on molybdate reduction

The effect of pH and temperature was determined by incubating strain KIK-14 at different pH ranging from 5.5 to 8.0 using 20 mM Tris.Cl and Bis-Tris buffers and temperature ranging from 20 to 60 °C. Analysis of variance showed that the optimum pH and temperature supporting Mo-blue production was between 6.0 and 6.3 (**Fig. 3**) and between 25 to 37°C (**Fig. 4**), respectively, with no significant difference ($p>0.05$) between the means. Temperature higher than 37 °C was found to strongly inhibit Mo-blue production by *Enterobacter cloacae* strain KIK-14.

Temperature and pH are factors that play important roles in enzymatic molybdenum reduction to Mo-blue, as both parameters affect the ionization of the constituent amino acids and protein folding, thus inhibiting molybdenum reduction. The optimum conditions obtained in this work could be of advantage to bioremediation in a tropical country like Nigeria, with average annual temperature ranging from 25 to 30 °C. Thus, strain KIK-14 is a suitable candidate for local soil molybdenum bioremediation and in other tropical countries. The vast majority of molybdenum-reducers show the optimal temperature of between 25 and 37 °C [22,29,30,32–36,38–40,52] as they are isolated from tropical soils with only psychrotolerant-reducer isolated from Antarctica showing optimum temperature supporting molybdenum reduction to be between 15 and 20 °C [37].

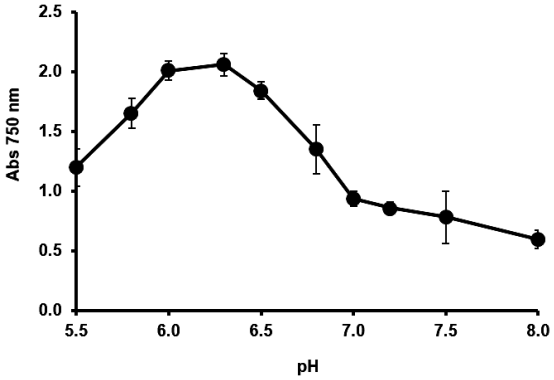

Fig. 3. Effect of pH on molybdenum blue production by *Enterobacter cloacae* strains KIK-14, after 48 hours incubation of resting cells under an optimized condition in a microplate. Error bars represent mean ± SE (n=3).

The optimal pH range supporting Mo-blue production by this bacterium reflects strain KIK-14 as a neutrophile. A characteristic neutrophile grows best at pH between 5.5 and 8.0. Perhaps, it was generally observed that Mo-blue production in bacteria is optimal at a slightly acidic pH ranging from 5.0 to 7.0 [26,27,29–40,52]. It was earlier suggested that acidic pH plays a vital role in the formation and stability of phosphomolybdate intermediate before being reduced to Mo-blue. Thus, optimal molybdate reduction occurs by balancing between substrate stability and enzyme activity [28].

Effect of electron donor on molybdate reduction

Bacterial molybdenum reduction to Mo-blue requires simple assimilable carbon sources such as glucose and sucrose. Mo-blue production by strain KIK-14 was highest when glucose is used as electron donor source, followed by sucrose, d-mannose, maltose, d-sorbitol, trehalose, d-mannitol, glycerol, salicin and myo-inositol in descending order (**Fig. 5**), while other carbon sources did not support molybdenum reduction. Previous works revealed that some Mo-reducing bacteria like *Enterobacter cloacae* strain 48 [27], *Serratia* sp. strain Dr.Y5 [30], *Serratia marcescens* strain DRY6 [29] and *S. marcescens* strain Dr.Y9 [22] prefer sucrose as the best carbon source. Other molybdenum reducers such as E*scherichia coli* K12 [26], *Serratia* sp. strain Dr.Y5 [30], *Enterobacter* sp. strain Dr.Y13 [31], *Pseudomonas* sp. strain DRY2 [33], *Acinetobacter calcoaceticus* strain Dr.Y12 [34], *Pseudomonas* sp. strain DRY1 [37], *Bacillus pumilus* strain lbna [36] and *Bacillus* sp. strain A.rzi [39] showed glucose as the carbon source, whereas, *Klebsiella oxyto*ca strain hkeem prefers fructose [35].

Both glucose and sucrose are enzymatically metabolized via glycolytic, tricarboxylic acid cycle, hexose monophosphate shunt and electron transport chain to generate reducing equivalent NADH and NADPH that serve as electron donors for metal reductase such as in molybdenum [41,52] and chromate [53–55]. Perhaps, to be realistic sucrose is much more preferred because it is a cheaper alternative being present in large quantity in industrial waste, for example, molasses and cane sugar. The concentration of sucrose in molasses could reach as much as 45% (w/v). The use of affordable and simple electron donor source is to promote growth and energy options that improve the performance of a system and lowering the overall costs.

The use of molasses and pure sucrose has been effective in decreasing chromate concentration from 5 mg/L to 0.1 mg/L [56]. Thus, sucrose as molasses would be as electron donor source for future studies.

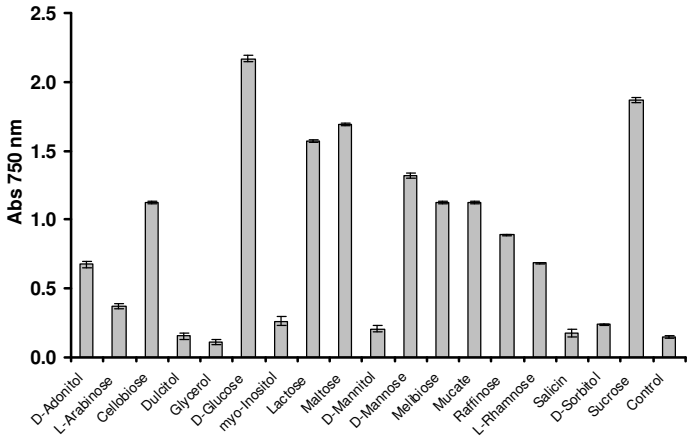

Fig. 5. Effect of various electron donor sources (1% w/v) on molybdenum blue production by *Enterobacter cloacae* strain KIK-14. Resting cells were grown in LMP containing 10 mM molybdate and various electron donors for 48 hours in a microplate under optimized conditions. Error bars represent mean ± SE (n = 3).

Effect of phosphate and molybdate concentrations to molybdate reduction

The phosphate concentration required for optimum Mo-blue production in this bacterium was between 5.0 and 7.5 mM, with concentrations higher than this being inhibitory to the reduction process (**Fig. 6**). All isolated molybdenum-reducers so far require phosphate concentration not more than 5 mM for the optimal reduction. High phosphate concentration has been suggested to inhibit phosphomolybdate formation and stability since the complex requires acidic condition, which is hindered due to the strong buffering power of the phosphate buffer [57–59]. Determining the optimal phosphate and molybdate concentrations supporting molybdenum reduction is necessary as the ratio of these anions has been shown to affect Mo-blue production in bacteria [26,27,29–40,52].

The effect of molybdate concentration on Mo-blue production was found to be optimal between 15 and 20 mM, but the newly isolated bacterium was able to reduce up to 60 mM molybdenum but with reduced Mo-blue production intensity (**Fig. 7**). Reduction as high as this molybdate concentration to an insoluble form (Mo-blue) would allow the strain to remediate high concentration of molybdenum pollution. The least optimal concentration of molybdenum reported was 15 mM in *Pseudomonas* sp. strain Dr.Y2 [33], while the highest molybdenum required for optimal reduction was 80 mM in *E. coli* K12 [26] and *Klebsiella oxytoca* strain hkeem [35]. Other molybdenum-reducers like EC 48 [27], *S. marcescens* strain Dr.Y6 [29], *Enterobacter* sp. strain Dr.Y13 [31], *S. marcescens*. Dr.Y9 [22], *Serratia* sp. strain Dr.Y5 [30], *Pseudomonas* sp. strain Dr.Y2 [33] and *Acinetobacter calcoaceticus* [34] produce optimal Mo-blue at optimal molybdate concentrations at 50, 25, 55, 30, 30, 50 and 20 mM, respectively. Perhaps, the highest concentration of molybdenum reported as pollutant in the environment was around 2000 ppm (20 mM) [60].

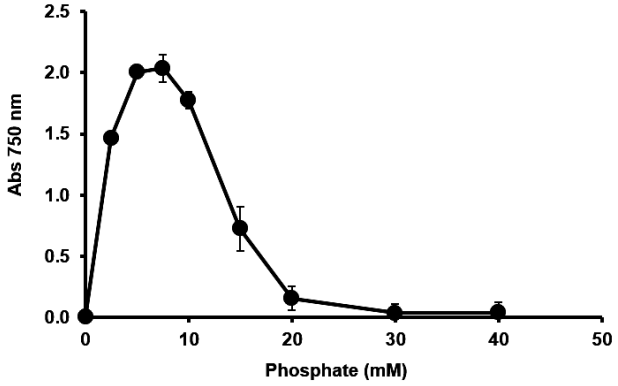

Fig. 6. Effect of phosphate concentration on Mo-blue production by *Enterobacter cloacae* strain KIK-14, after 48 hours incubation of the resting cells in a microplate under optimized conditions. Error bars represent mean ± SE (n = 3).

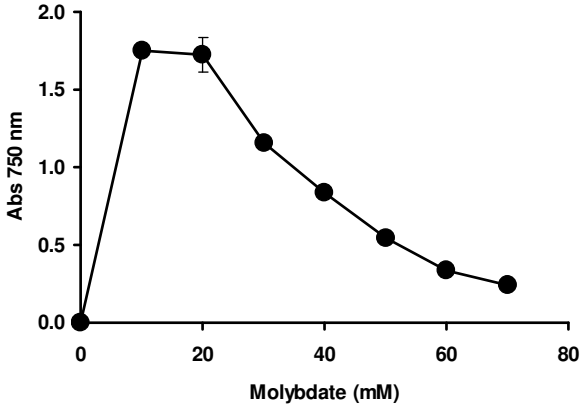

Fig. 7. Effect of molybdate concentration on Mo-blue production by *Enterobacter cloacae* strain KIK-14, after 48 hours incubation of the resting cells in a microtiter plate under optimized conditions. Error bars represent mean ± SE (n = 3).

Effect of heavy metals

The inhibitory effect of other interacting metal ions and heavy metals present a major challenge to the success of bioremediation. It is therefore of importance to isolate and screen bacteria with as many metal resistance capability as possible. The reduction process was inhibited by 40.5, 57.1 and 41.5% at 2 ppm copper (II), mercury (II) and silver (I) respectively (**Fig. 8**). A previous result has shown that mercury is a physiological inhibitor to molybdate reduction whilst metals including stannous and ferrous ions are chemical reducers of molybdenum to molybdenum blue and hence are not physiological inhibitors [61]. A brief summary of the heavy metals inhibiting molybdenum-reducers revealed that almost all the reducers are affected in a similar fashion by toxic heavy metals (**Table 2**).

Heavy metals such as cadmium, copper mercury and silver usually target the sulfhydryl group of enzymes [62], thereby, inactivating the metal-reducing capacity of the enzyme(s). Chromate for example was known to inhibit enzymes such as glucose oxidase [63]. Binding of heavy metals responsible for the reduction.

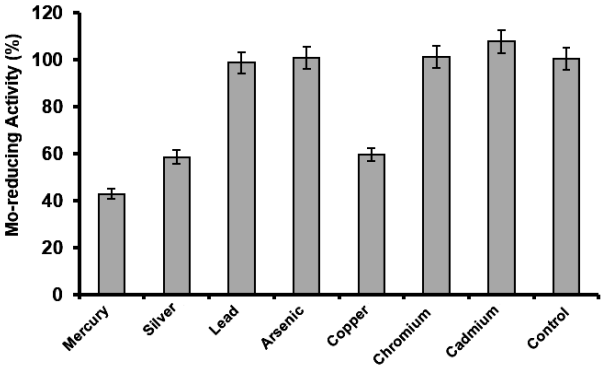

Fig. 8. Effect of metal ions on Mo-blue production by *Enterobacter cloacae* strain KIK-14, after 48 hours incubation of resting cells in a microplate under optimized condition. Error bars represent mean ± SE (n = 3).

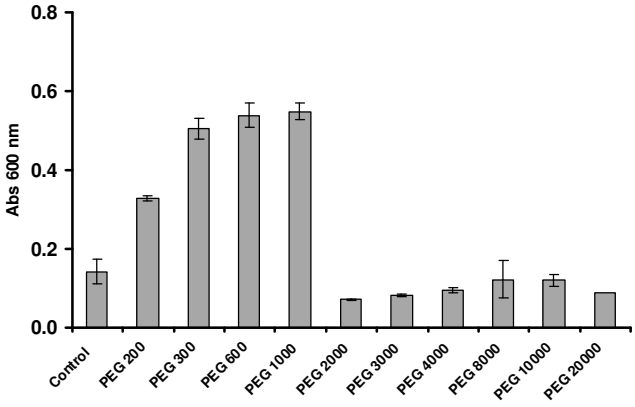

Fig. 9. The growth of *Enterobacter cloacae* strain KIK-14 on various PEGs after 48 hours incubation of resting cells in a microplate under optimized condition. Error bars represent mean ± SE (n = 3).

Table 2. Inhibition of Mo-reducing bacteria by heavy metals.

Bacteria	Heavy Metals that inhibit reduction	Author
Klebsiella oxytoca strain	Cu^{2+}, Ag^{+}, Hg^{2+}	[35]
Bacillus sp. strain A.rzi	Cd^{2+}, Cr^{6+}, Cu^{2+}, Ag^{+}, Pb^{2+}, Hg^{2+}, Co^{2+}, Zn^{2+}	[39]
Bacillus pumilus strain lbna	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}	[36]
Escherichia coli K12	Cr^{6+}	[26]
Serratia sp. strain DRY5	n.a.	[30]
S. marcescens strain DRY9	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[22]
Pseudomonas sp. strain DRY2	Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[33]
Enterobacter cloacae strain 48	Cr^{6+}, Cu^{2+}	[27]
Enterobacter sp. strain DRY13	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[31]
Pseudomonas sp. strain DRY1	Cd^{2+}, Cr^{6+}, Cu^{2+}, Ag^{+}, Pb^{2+}, Hg^{2+}	[37]
Acinetobacter calcoaceticus strain DRY12	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[34]
Serratia marcescens strain DRY6	Cr^{6+}, Cu^{2+}, Hg^{2+}	[29]

PEGs as electron donors for molybdenum reduction and independent growth

The capability of strain KIK-14 to degrade PEGs was explored, and it was found that this bacterium was able to grow on PEGs 200, 300, 600 and 1000 independent of molybdenum reduction, but none of the PEGs supports Mo-blue production (**Fig. 9**). Other bacteria reported to degrade PEG include *Acetobacterium* sp. [64], *Comamonus acidovorans* [65], *Alcaligenes denitrificans and Xanthomonas maltophilia* [66], *Alcaligenes xylosoxidans, Enterobacter diversus, Pseudomonas vesicularis* and *P. solanecearum* [67], *Pseudomonas* spp., Rhodococcus spp., *Williamsia* spp., *Mycobacterium* spp. and *Bacillus* spp. [68], *Pseudomonas* sp., *Sphingomonas* sp., *S. macrogoltabida* and *Stenotrophomonas maltophilia,* [69], *Myroides pelagicus* and *Pseudomonas stutzeri* [70], *Flavobacterium* sp. [71] and Bacteroides strain PG1 [72]. Bacteria that detoxify heavy metals as well degrade xenobiotics are rarely reported, for instance, in chromate reduction phenol could be used as an electron donor source for reduction [65].

CONCLUSION

A local molybdenum-reducer with novel ability to use PEGs 200, 300, 600 and 1000 as a carbon source for growth has been isolated. Based on morphology and biochemical tests, the bacterium was tentatively identified as *Enterobacter cloacae* strain KIK-14. This is the first report of molybdenum-reducing bacterium with PEG-degrading ability. The bacterium optimally reduces molybdate to Mo-blue at pH between pH 6.0 and 6.3, temperature between 25 and 37 ^{o}C, phosphate concentration between 5.0 and 7.5 mM and a molybdate concentration between 15 and 20 mM. Glucose was the best electron donor source supporting maximum Mo-blue production, followed by sucrose, maltose, lactose, d-mannose, cellobiose, melibiose, mucate, raffinose, l-rhamnose, d-adonitol and l-arabinose in descending order. The Mo-blue absorption spectrum was similar to that of previous Mo-reducing bacterium, and resembles a reduced phosphomolybdate. The reduction process was inhibited by 40.5, 57.1 and 41.5% at 2 ppm copper (II), mercury (II) and silver (I) respectively. The capacity of strain KIK-14 to detoxify several toxicants makes it an important bioremediation tool. Research is still ongoing to further characterize the PEG-degrading ability and purify the molybdenum-reducing enzyme from this bacterium.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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