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Isolation and characterization of a molybdenum-reducing and SDS-degrading *Klebsiella oxytoca* strain Aft-7 and its bioremediation application in the environment

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Abstract. *Masdor N, Shukor MSA, Khan A, Halmi MIE, Abdullah SRS, Shamaan NA, Shukor MY. 2015. Taxonomy and distribution of species of the genus* Acanthus *(Acanthaceae) in mangroves of the Andaman and Nicobar Islands, India*. *Biodiversitas 16: 238-246.*Pollution as a result of anthropogenic activities is a severe global issue. These activities including inappropriate disposal, industrial and prospecting activities and unnecessary use of agricultural chemicals have triggered international initiatives to eliminate these contaminants. In this work we screen the ability of a molybdenum-reducing bacterium isolated from contaminated soil to grow and reduce molybdenum on various detergents. The bacterium was able to grow on SDS as a carbon source although the compound did not support molybdenum reduction. The bacterium reduces molybdate to Mo-blue optimally between pH 5.8 and 6.3 and between 25 and 34^{o}C. Glucose was the best electron donor for supporting molybdate reduction followed by sucrose, D-mannitol, D-sorbitol, lactose, salicin, trehalose, maltose and myo-Inositol in descending order. Other requirements include a phosphate concentration between 5.0 and 7.5 mM and a molybdate concentration between 5 and 20 mM. The absorption spectrum of the Mo-blue produced was similar to previous Mo-reducing bacterium, and closely resembles a reduced phosphomolybdate. Molybdenum reduction was inhibited by mercury (ii), silver (i) and copper (ii) at 2 ppm by 62.1, 33.9 and 33.6%, respectively. Biochemical analysis resulted in a tentative identification of the bacterium as *Klebsiella oxytoca* strain Aft-7. The ability of this bacterium to detoxify molybdenum and degrade detergent makes this bacterium an important tool for bioremediation.

Keywords: Bioremediation, isolation, *Klebsiella oxytoca*, molybdenum, SDS

INTRODUCTION

Pollution due to anthropogenic activities is a serious global problem. These activities such as improper disposal, industrial and mining activities and excessive use of agricultural chemicals have resulted in global efforts to remove these pollutants (Rieger et al. 2002). Molybdenum is one of the essential heavy metals that are required at trace amount and is toxic to a variety of organisms at elevated levels (Ahmad-Panahi et al. 2014). The estimated global molybdenum reserve base in 2008 was 19,000,000 metric tonnes. It has many uses in industries such as molybdenum grade stainless steels, high-temperature steel, tool and high speed steel, molybdenum grade cast irons, automobile engine anti-freeze component, component of corrosion resistant steel and as lubricant in the form of molybdenum disulphide. Widespread use of molybdenum in industry has resulted in several soil and water pollution cases all around the world such as in the Tokyo Bay, Tyrol in Austria, the Black Sea, where molybdenum level reaches in the hundreds of ppm (Davis 1991; Neunhäuserer et al.2001). In addition terrestrially, it has been recognized as a significant pollutant in sewage sludge (Lahann 1976). Molybdenum is very toxic to ruminants at levels of several parts per million being the most affected are cows (Underwood 1979; Kincaid 1980). Molybdenum toxicity in inhibiting spermatogenesis and arresting embryogenesis in organisms such as catfish and mice at levels as low as several parts per million have been reported (Meeker et al. 2008; Bi et al. 2013; Zhai et al. 2013; Zhang et al. 2013).

Aside from heavy metals, detergents are often present as co pollutants, especially in water bodies. Detergents have detrimental effects to aquatic life (Liwarska-Bizukojc et al. 2005). Anionic surfactants such as Sodium Dodecyl Sulfate (SDS) and Sodium Dodecyl Benzene Sulfonate (SDBS) (Figure 1) exhibited toxic effects at concentrations ranging from 0.0025 to 300 mg/L (Pettersson et al. 2000). Toxicity towards invertebrates and crustaceans has been documented in vivo and in vitro due to the massive amount of anionic surfactants continuously released into water bodies. For instance, a study on oyster digestive gland indicated that exposure to SDS has a detrimental effect.

The detrimental effects include perturbation of the metabolic and nutritional functions. All of these negative effects have a direct influence on oyster survival (Rosety et al. 2000). Detergents could also modify the behavior of the fish such as muscle spasms, erratic movement, and body torsion (Cserháti et al. 2002).

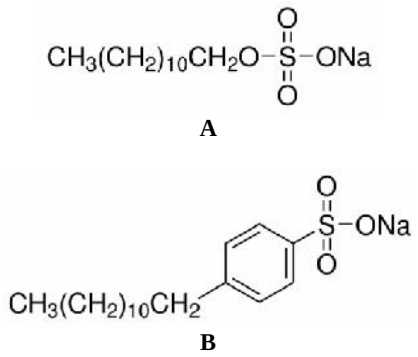

Figure 1. The structure of SDS (A) and SDBS (B).

Some microbes are able to degrade a variety of xenobiotics and detoxify heavy metals at the same time (Anu et al. 2010; Chaudhari et al. 2013; Bhattacharya et al. 2014) and the versatility of these microbes are in great demand in polluted sites where the presence of several contaminants are the norm (Ahmad et al. 2014). Heavy metals reduction coupled with azo dye decolorization have been reported (Chaudhari et al. 2013).

In the present work, we screen for the ability of a novel molybdenum reducing bacterium isolated from contaminated soil to grow on several detergents and hydrocarbons such as diesel and crude petroleum. Here we report on a novel molybdenum-reducing bacterium with the capacity to grow on the detergent SDS, isolated from a contaminated soil. The characteristics of this bacterium would make it suitable for future bioremediation works involving both the heavy metal molybdenum and dye as an organic contaminant.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and purchased from Sigma (St. Louis, MO, USA), Fisher (Malaysia) and Merck (Darmstadt, Germany).

Isolation of molybdenum-reducing bacterium

Soil samples were taken (5 cm deep from topsoil) from the grounds of a contaminated land in the province of Khyber Pakhtunkhwa, Pakistan, in 2013. One gram of soil sample was suspended in sterile tap water. 0.1 mL aliquot of the soil suspension was pipetted and spread onto agar of low phosphate media (pH 7.0), and incubated for 48 hours at room temperature. The composition of the low phosphate media (LPM) were as follows: glucose (1%), $(NH_4)_2.SO_4$ (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) (Yunus et al. 2009). The formations of blue colonies indicate molybdate reduction by molybdenum-reducing bacteria. Colony forming the strongest blue intensity was isolated and restreaked on low phosphate media (LPM) to obtain pure culture. Molybdenum reduction in liquid media (at pH 7.0) was carried out in 100 mL of the above media in a 250 mL shake flask culture at room temperature for 48 hours on an orbital shaker set at 120 rpm with the same media above but the phosphate concentration increased to 100 mM. Molybdenum blue (Mo-blue) absorption spectrum was studied by taking out 1.0 mL of the Mo-blue formed from the liquid culture above and then centrifuged at 10,000 xg for 10 minutes at room temperature. Scanning of the supernatant was carried out from 400 to 900 nm using a UV-spectrophotometer (Shimadzu 1201).The low phosphate media was utilized as the baseline correction.

Morphological, physiological and biochemical characterization of the isolated strain

Identification of the bacterium was carried out biochemically and phenotypically using standard methods such as colony shape, gram staining, size and color on nutrient agar plate, motility by the hanging drop method, 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 (Holt et al. 1994). Interpretation of the results was carried out via the ABIS online system (Costin and Ionut 2015).

Preparation of resting cells for molybdenum reduction characterization

Characterization works on molybdenum reduction to Mo-blue such as the effects of pH, temperature, phosphate and molybdate concentrations were carried out statically using resting cells in a microplate or microtiter format as previously developed (Shukor and Shukor 2014).Cells from a 1 L overnight culture grown in High Phosphate media (HPM) at room temperature on orbital shaker (150 rpm) with the only difference between the LPM and HPM was the phosphate concentration which was fixed at 100 mM for the HPM. Cells were harvested by centrifugation at 15,000 x g for 10 minutes and the pellet was washed several times to remove residual phosphate and resuspended in 20 mls of low phosphate media (LPM) minus glucose to an absorbance at 600 nm of approximately 1.00. In the low phosphate media, a concentration of 5 mM phosphate was optimal for all of the Mo-reducing bacteria isolated so far and hence this concentration was used in this work. Higher concentrations were found to be strongly inhibitory to molybdate

reduction (Campbell et al. 1985; Shukor et al. 2007, 2008, 2009a, b,2010a, b; Rahman et al. 2009; Yunus et al. 2009;Lim et al. 2012; Ghani et al. 1993; Abo-Shakeer et al. 2013; Ahmad et al. 2013; Halmi et al. 2013; Othman et al. 2013;Khan et al. 2014). Then 180 µL was sterically pipetted into each well of a sterile microplate. 20 µL of sterile glucose from a stock solution was then added to each well to initiate Mo-blue production. A sterile sealing tape that allows gas exchange (Corning® microplate) was used for sealing the tape. The microplate was incubated at room temperature. At defined times absorbance at 750 nm was read in a BioRad (Richmond, CA) Microtiter Plate reader (Model No. 680). 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 750 nm as the maximum filter wavelength available for themicroplate unit was 750 nm (Shukor et al. 2003).

Effect of heavy metals on molybdenum reduction

Seven heavy metals namely lead (ii), arsenic (v), copper (ii), mercury (ii), silver (i), chromium (vi) and cadmium (ii) were prepared from commercial salts or from Atomic Absorption Spectrometry standard solutions from MERCK. The bacterium was incubated with heavy metals in the microplate format at various concentrations. The plate was incubated for 24 hours at 30ºC. The amount of Mo-blue production was measured at 750 nm as before.

Screening of molybdenum reduction using detergents and hydrocarbon as source of electron donors and for growth

The ability of detergents such as Sodium Dodecyl Sulfate (SDS) and Sodium Dodecyl Benzene Sulfonate (SDBS) and the hydrocarbon diesel to support growth to support molybdenum reduction as electron donors was tested using the microplate format above by replacing glucose from the low phosphate medium with these xenobiotics at the final concentration of 200 mg/L for detergents. Diesel was initially added to the final concentration of 0.5 g/L in 10 mL media and sonicated for 5 minutes. Then 200 uL of the media was added into the microplate wells. The microplate was incubated at room temperature for three days and the amount of Mo-blue production was measured at 750 nm as before. The ability of these xenobiotics to support bacterial growth independent of molybdenum reduction was tested using the microplate format above using the following media at the final concentration of 200 mg/L. The ingredients of the growth media (LPM) were as follows: $(NH_4)_2.SO_4$ (0.3%), $NaNO_3$ (0.2%), $MgSO_4.7H_2O$ (0.05%), yeast extract (0.01%), NaCl (0.5%), Na_2HPO_4 (0.705% or 50 mM) and 1 mL of trace elements solution. The trace elements solution composition (mg/L) was as follows: $CaCl_2$ (40), $FeSO_4 \cdot 7H_2O$ (40), $MnSO_4 \cdot 4H_2O$ (40), $ZnSO_4 \cdot 7H_2O$ (20), $CuSO_4 \cdot 5H_2O$ (5), $CoCl_2 \cdot 6H_2O$ (5), $Na_2MoO_4 \cdot 2H_2O$ (5). The media was adjusted to pH 7.0. Then 200 uL of the media was added into the microplate wells and incubated at room temperature for 72 hours. The increase of bacterial growth after an incubation period of 3 days at room temperature was measured at 600 nm using the microplate reader (Bio-Rad 680).

Statistical analysis

Values are means ± SE. Data analyses were carried out using Graphpad Prism version 3.0 and Graphpad In Stat version 3.05 available from www.graphpad.com. A Student's t-test or a one-way analysis of variance with post hoc analysis by Tukey's test was employed for comparison between groups. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Identification of molybdenum reducing bacterium

strain AFt-7 was a rod-shaped, non-motile, gram-negative and facultative anaerobe bacterium. The bacterium was identified by comparing the results of cultural, morphological and various biochemical tests (Table 1) to the Bergey's Manual of Determinative Bacteriology (Holt et al. 1994) and using the ABIS online software (Costin and Ionut 2015). The software gave three suggestions for the bacterial identity with the highest similarity or homology (96%) and accuracy (100%) as *Klebsiella oxytoca*. However, more work in the future especially molecular identification technique through comparison of the 16srRNA gene are needed to identify this species further. However, at this juncture the bacterium is tentatively identified as *Klebsiella oxytoca* strain Aft-7. Previously, two molybdenum-reducing bacterium from this genus; *Klebsiella oxytoca* strain Dr.Y14 (Halmi et al. 2013) and *Klebsiella oxytoca* strain hkeem (Lim et al. 2012) have been isolated.

Table 1. Biochemical tests for *Klebsiella oxytoca* strain Aft-7

Motility	–	Acid production from:	
Pigment	–		
Catalase production (24 h)	+	α-methyl-D-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	–	Salicin	+
Urea hydrolysis	+	D-Sorbitol	+
Deoxyribonuclease	–	Sucrose (saccharose)	+
Lipase (corn oil)	–	Trehalose	+
Phenylalanine deaminase	–	D-Xylose	+

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

In this work using this bacterium, a rapid and simple high throughput method involving microplate format was used to speed up characterization works and obtaining more data than the normal shake-flask approach (Iyamu et al. 2008; Shukor and Shukor 2014). The use of resting cells under static conditions to characterize molybdenum reduction in bacterium was initiated by (Ghani et al. 1993). Resting cells have been used in studying heavy metals reduction such as in selenate (Losi and Frankenberger 1997), chromate (Llovera et al. 1993), vanadate (Carpentier et al. 2005) reductions and xenobiotics biodegradation such as diesel (Auffret et al. 2014), SDS (Chaturvedi and Kumar 2011), phenol (Sedighi and Vahabzadeh 2014), amides (Raj et al. 2010) and pentachlorophenol (Steiert et al. 1987).

Molybdenum absorbance spectrum

The absorption spectrum of Mo-blue produced by *Klebsiella oxytoca* strain Aft-7 exhibited a shoulder at approximately 700 nm and a maximum peak near the infra-red region of between 860 and 870 nm with a median at 865 nm (Figure 2). The identity of the Mo-blue is not easily ascertained as it is complex in structure and has many species (Shukor et al.2007). Briefly It has been suggested by Campbell et al. (Campbell et al. 1985) that the Mo-blue observed in the reduction of molybdenum by *Escherichia coli* K12 is a reduced form of phosphomolybdate but did not provide a plausible mechanism. The Mo-blue spectrum from the phosphate determination method normally showed a maximum absorption around 880 to 890 nm and a shoulder around 700 to 720 nm (Hori et al. 1988). This spectrum is different from other heteropolymolybdates such as molybdosilicate and molybdosulphate (Figure 3).

We hypothesize that microbial molybdate reduction in media containing molybdate and phosphate must proceed via the phosphomolybdate intermediate and the conversion from molybdate to this structure occurs due to the reduction of pH during bacterial growth, in other words the reduction of molybdenum to Mo-blue requires both chemical and biological processes. The absorption spectrum of the Mo-blue from this bacterium if it goes through this mechanism should show a spectrum closely resembling the phosphate determination method. To be exact, the spectrum observed showed a maximum absorption in between 860 and 870 nm and a shoulder at approximately 700 nm. We have shown previously that the entire Mo-blue spectra from other bacteria obey this requirement (Shukor et al. 2007). In this work the result from the absorption spectrum clearly implies a similar spectrum and thus provides evidence for the hypothesis. Exact identification of the phosphomolybdate species must be carried out using n.m.r and e.s.r. due to the complex structure of the compound (Chae et al. 1993). However, spectrophotometric characterization of heteropoly-molybdate species via analyzing the scanning spectroscopic profile is a less cumbersome and accepted method (Glenn and Crane 1956; Sims 1961; Kazansky and Fedotov 1980; Yoshimura et al. 1986). Although the maximum absorption wavelength for Mo-blue was 865 nm, measurement at 750 nm, although was approximately 30% lower, was enough for routine monitoring of Mo-blue production as the intensity obtained was much higher than cellular absorption at 600-620 nm (Shukor and Shukor 2014). Previous monitoring of Mo-blue production uses several wavelengths such as 710 nm (Ghani et al. 1993) and 820 nm (Campbell et al. 1985).

Effect of pH and temperature on molybdate reduction

Klebsiella oxytoca strain Aft-7 was incubated at different pH ranging from 5.5 to 8.0 using Bis-Tris and Tris.Cl buffers (20 mM). Analysis by ANOVA showed that the optimum pH for reduction was between 5.8 and 6.3. Inhibition of reduction was dramatic at pH lower than 5 (Figure 4). The effect of temperature (Figure 5) was observed over a wide range of temperature (20 to 60°C) with an optimum temperature ranging from 25 to 34°C with no significant different ($p>0.05$) among the values measured as analyzed using ANOVA. Temperatures lower than 30°C and higher than 37^{o}C were strongly inhibitory to Mo-blue production from *Klebsiella oxytoca* strain Aft-7.

Temperature and pH play important roles in molybdenum reduction, since this process is enzyme mediated, both parameters affect protein folding and enzyme activity causing the inhibition of molybdenum reduction. The optimum conditions would be an advantage for bioremediation in a tropical country like Malaysia which have average yearly temperature ranging from 25 to 35^{o}C (Shukor et al. 2008). Therefore, *Klebsiella oxytoca* strain Aft-7 could be a candidate for soil bioremediation of molybdenum locally and in other tropical countries. The majority of the reducers shows an optimal temperature of between 25 and 37ºC (Shukor et al. 2008, 2009a,2010a, b, 2014; Rahman et al. 2009; Yunus et al. 2009; Lim et al. 2012; Abo-Shakeer et al. 2013; Othman et al. 2013; Halmi et al. 2013; Khan et al. 2014)as they are isolated from tropical soils with the only psychrotolerant reducer isolated from Antartica showing an optimal temperature supporting reduction of between 15 and 20ºC (Ahmad et al. 2013).

The optimal pH range exhibited by *Klebsiella oxytoca* strain Aft-7 for supporting molybdenum reduction reflects the property of the bacterium as a neutrophile. The characteristics neutrophiles are their ability to grow between pH 5.5 and 8.0. An important observation regarding molybdenum reduction in bacteria is the optimal pH reduction is slightly acidic with optimal pHs ranging from pH 5.0 to 7.0 (Campbell et al. 1985; Ghani et al. 1993; Shukor et al. 2008,2009a,2010a, b,2014; Rahman et al. 2009; Lim et al. 2012; Abo-Shakeer et al. 2013; Ahmad et al. 2013; Halmi et al. 2013; Othman et al. 2013; Khan et al. 2014). It has been suggested previously that acidic pH plays an important role in the formation and stability of phosphomolybdate before it is being reduced to Mo-blue. Thus, the optimal reduction occurs by balancing between enzyme activity and substrate stability (Shukor et al. 2007).

Effect of electron donor on molybdate reduction

Among the electron donor tested, glucose was the best electron donor for supporting molybdate reduction followed by sucrose, D-mannitol, D-sorbitol, lactose, salicin, trehalose, maltose and myo-Inositol in descending

order (Figure 6). Other carbon sources did not support molybdenum reduction. Previous works by Shukor et al. demonstrated that several of Mo-reducing bacteria such as *Enterobacter cloacae* strain 48 (Ghani et al. 1993), *Serratia* sp. strain Dr.Y5 (Rahman et al. 2009), *S. marcescens* strain Dr.Y9 (Yunus et al. 2009) and *Serratia marcescens* strain DRY6 (Shukor et al. 2008) showed sucrose as the best carbon source. Other molybdenum reducers such as *Escherichia coli* K12 (Campbell et al. 1985), *Serratia* sp. strain Dr.Y5 (Rahman et al. 2009), *Pseudomonas* sp. strain DRY2 (Shukor et al. 2010a), *Pseudomonas* sp. strain DRY1 (Ahmad et al. 2013), *Enterobacter* sp. strain Dr.Y13 (Shukor et al. 2009a), *Acinetobacter calcoaceticus* strain Dr.Y12 (Shukor et al. 2010b), *Bacillus pumilus* strain lbna (Abo-Shakeer et al. 2013) and *Bacillus* sp. strain A.rzi (Othman et al. 2013) prefer glucose as the carbon source while *Klebsiella oxytoca* strain hkeem prefers fructose (Lim et al. 2012). In the presence of carbon sources in the media, the bacteria could produce electron donating substrates, NADH and NADPH thorough metabolic pathways such as glycolysis, Kreb's cycle and electron transport chain. Both NADH and NADPH are responsible as the electron donating substrates for molybdenum reducing-enzyme (Shukor et al. 2008,2014).

Effect of phosphate and molybdate concentrations to molybdate reduction

The determination of phosphate and molybdate concentrations supporting optimal molybdenum reduction is important because both anions have been shown to inhibit Mo-blue production in bacteria (Shukor et al. 2008, 2009b, 2010a, 2014; Yunus et al. 2009; Lim et al. 2012; Ahmad et al. 2013; Othman et al. 2013). The optimum concentration of phosphate occurred between 5.0 and 7.5 mM with higher concentrations were strongly inhibitory to reduction (Figure 7). High phosphate was suggested to inhibit phosphomolybdate stability as the complex requires acidic conditions of which the higher the phosphate concentration the stronger buffering power of the phosphate buffer used. In addition, the phosphomolybdate complex itself is unstable in the presence of high phosphate through an unknown mechanism (Glenn and Crane 1956; Sims 1961; Shukor et al. 2000). All of the molybdenum-reducing bacterium isolated so far requires phosphate concentration not higher than 5 mM for optimal reduction (Campbell et al. 1985; Ghani et al. 1993; Shukor et al. 2008, 2009b, 2010a, b, 2014; Rahman et al. 2009; Lim et al. 2012; Abo-Shakeer et al. 2013; Ahmad et al. 2013; Halmi et al. 2013; Othman et al. 2013; Khan et al. 2014). Studies on the effect of molybdenum concentration on molybdenum reduction showed that the newly isolated bacterium was able to reduce molybdenum as high as 60 mM but with reduced Mo-blue production. The optimal reduction range was between 5 and 20 mM (Figure 8). Reduction at this high concentration into an insoluble form would allow the strain to reduce high concentration of molybdenum pollution. The lowest optimal concentration of molybdenum reported is 15 mM in *Pseudomonas* sp strain Dr.Y2 (Shukor et al. 2010a), whilst the highest molybdenum required for optimal reduction was 80 mM in *Escherichia coli* K12 (Campbell et al. 1985) and *Klebsiella oxytoca* strain hkeem (Lim et al. 2012). Other Mo-reducing bacteria such as EC48 (Ghani et al. 1993), *S. marcescens* strain Dr.Y6 (Shukor et al. 2008), *S. marcescens*. Dr.Y9 (Yunus et al. 2009), *Pseudomonas* sp. strain Dr.Y2 (Shukor et al. 2010a), *Serratia* sp. strain Dr.Y5 (Rahman et al. 2009), *Enterobacter* sp. strain Dr.Y13 (Shukor et al. 2009a) and *Acinetobacter calcoaceticus* (Shukor et al. 2010b) could produce optimal Mo-blue using the optimal molybdate concentrations at 50, 25, 55, 30, 30, 50 and 20 mM, respectively. In fact the highest concentration of molybdenum as a pollutant in the environment is around 2000 ppm or about 20 mM (Runnells et al. 1976).

Effect of heavy metals

Molybdenum reduction was inhibited by mercury (ii), silver (i) and copper (ii) at 2 ppm by 62.1, 33.9 and 33.6%, respectively (Figure 9). The inhibition effects by others metal ions and heavy metals present a major problem for bioremediation. Therefore it is important to screen and isolate bacteria with as many metal resistance capability. As described previously (Shukor et al. 2002), mercury is a physiological inhibitor to molybdate reduction. A summary

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

Bacteria	Heavy metals that inhibit reduction	Author
Acinetobacter calcoaceticus strain Dr.Y12	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	Shukor et al. (2010b)
Bacillus pumilus strain lbna	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}	Abo-Shakeer et al. (2013)
Bacillus sp. strain A.rzi	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, $Hg^{2+,}$ Co^{2+},Zn^{2+}	Othman et al. (2013)
Enterobacter cloacae strain 48	Cr^{6+}, Cu^{2+}	Ghani et al. (1993)
Enterobacter sp. strain Dr.Y13	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}	Shukor et al. (2009a)
Escherichia coli K12	Cr^{6+}	Campbell et al. (1985)
Klebsiella oxytoca train hkeem	Cu^{2+}, Ag^{+}, Hg^{2+}	Lim et al. (2012)
Pseudomonas sp. strain DRY1	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}	Ahmad et al. (2013)
Pseudomonas sp. strainDRY2	Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	Shukor et al. (2010a)
Serratia marcescens strain Dr.Y9	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	Yunus et al. (2009)
Serratia marcescens strain DRY6	Cr^{6+}, Cu^{2+}, Hg^{2+}*	Shukor et al. (2008)
Serratia sp. strain Dr.Y5	n.a.	Rahman et al. (2009)
Serratia sp. strain Dr.Y8	Cr, Cu, Ag, Hg	Shukor et al. (2009b)

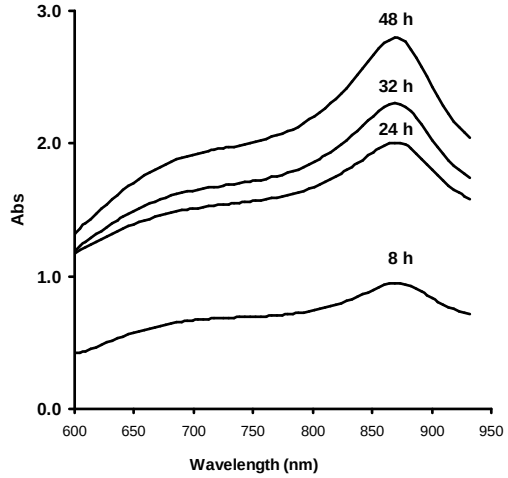

Figure 2. Scanning absorption spectrum of Mo-blue from *Klebsiella oxytoca* strain Aft-7 at different time intervals.

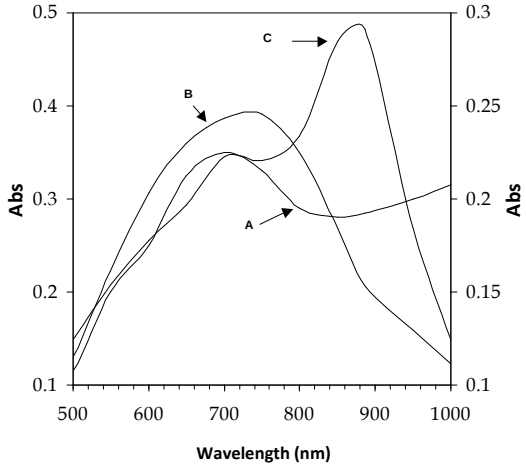

Figure 3. Scanning spectra of Mo-blue from molybdosilicate (A), molybdosulphate (B) and molybdophosphate (C) (from Shukor et al. 2000).

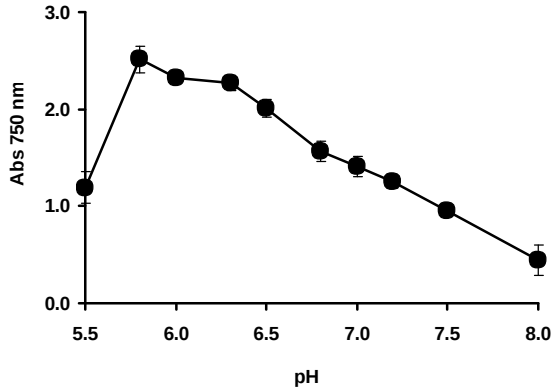

Figure 4. Effect of pH on molybdenum reduction by *Klebsiella oxytoca* strain Aft-7. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n=3).

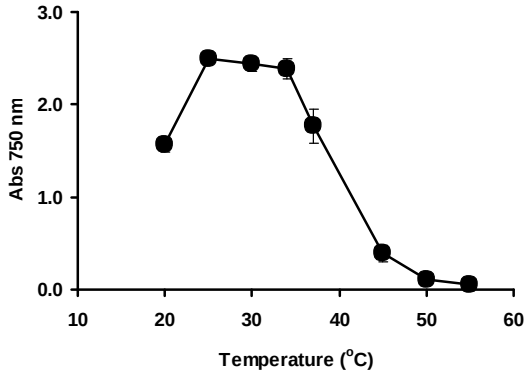

Figure 5. Effect of temperature on molybdenum reduction by *Klebsiella oxytoca* strain Aft-7. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n=3).

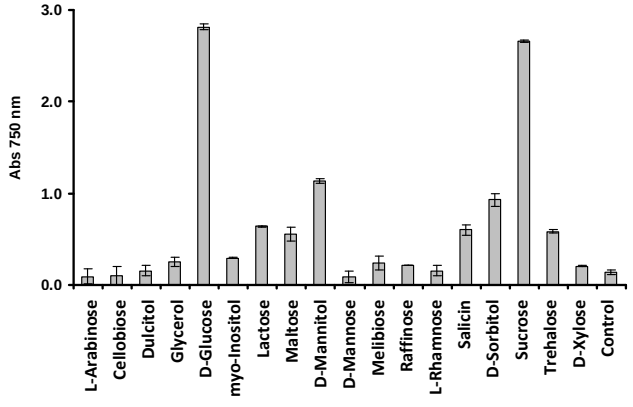

Figure 6. Effect of different electron donor sources (1% w/v) on molybdenum reduction. *Klebsiella oxytoca* strain Aft-7 was grown in low phosphate media containing 10 mM molybdate and various electron donors. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n = 3).

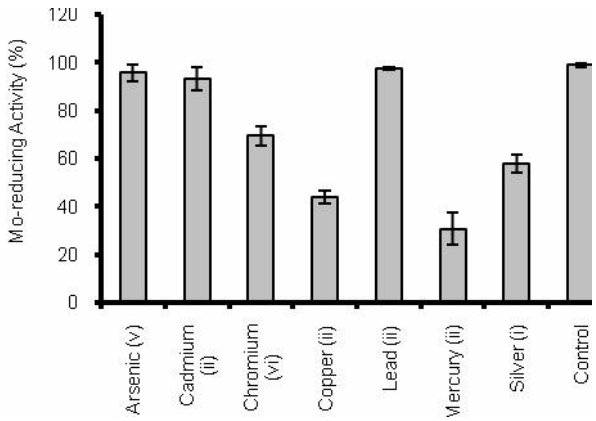

Figure 9. Effect of metals on Mo-blue production by *Klebsiella oxytoca* strain Aft-7. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n = 3).

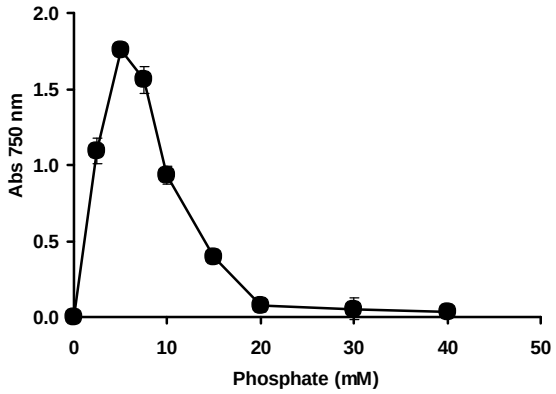

Figure7. Effect of phosphate concentration on molybdenum reduction by *Klebsiella oxytoca* strain Aft-7. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n = 3).

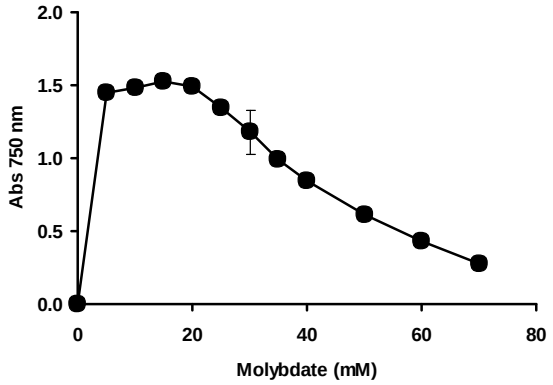

Figure 8. Effect of molybdate concentration on molybdenum reduction by *Klebsiella oxytoca* strain Aft-7. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n = 3).

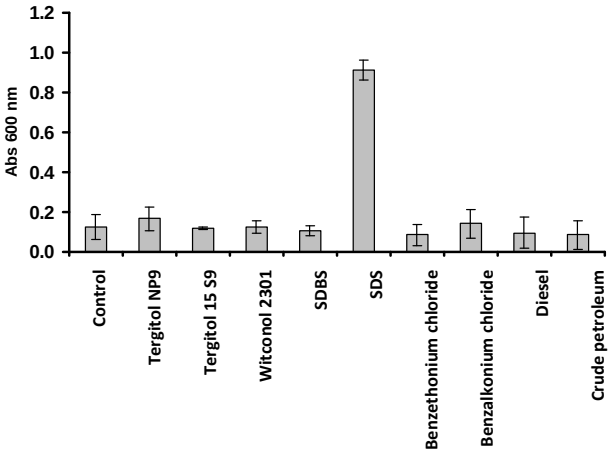

Figure 10. Growth of *Klebsiella oxytoca* strain Aft-7 on xenobiotics independent of molybdenum reduction. Resting cells of the bacterium were incubated in a microtiter plate under optimized conditions for 48 hours. Error bars represent mean ± standard deviation (n = 3).

of the type of heavy metals that inhibited Mo-reducing bacteria showed that almost all of the reducers are inhibited by toxic heavy metals (Table 2). Heavy metals such as mercury, cadmium, silver and copper usually target sulfhydryl group of enzymes (Sugiura and Hirayama 1976). Chromate is known to inhibit certain enzymes such as glucose oxidase (Zeng et al. 2004) and enzymes of nitrogen metabolism in plants (Sangwan et al. 2014). Binding of heavy metals inactivated metal-reducing capability of the enzyme(s) responsible for the reduction.

Detergents and hydrocarbons as electron donor sources for molybdenum reduction and independent growth

Screening of detergents and hydrocarbons as electron donors supporting molybdenum reduction failed to give positive results (data not shown). However, the bacterium was able to grow on the detergent SDS (Figure 10). SDS-degrading bacteria are ideal for SDS remediation due to economic factors. Microbes are known for their ability to degrade organics including SDS and their use as bioremediation agents is important for economical removal of xenobiotic pollutants (Syed et al. 2010). Biodegradation of anionic surfactant under aerobic conditions by the bacterium *Pseudomonas* sp. strain C12B was among the first to be studied (Payne and Feisal 1963), and to date quite a number of SDS-degrading bacteria have been isolated and characterized (Thomas and White 1990; Lee et al. 1995; Kostal et al. 1998; Roig et al. 1998; Dhouib et al. 2003; Ke et al. 2003; Yin et al. 2005; Wu 2006; Khleifat et al. 2010; Asok and Jisha 2013; Chaturvedi and Kumar 2013,2014; Cortés-Lorenzo et al. 2013; Halmi et al. 2013; Venkatesh 2013; Yilmaz and Icgen 2014). Works on cold-adapted microbes with ability to degrade SDS are rare, and were first reported by Margesin and Schinner (1998).The existence of multitude of bacteria with detergent-degrading ability makes bioremediation the more ideal method for detergent degradation. However, very few bacteria have been reported to be able to degrade xenobiotics and detoxify heavy metals, and the ability of this bacterium to do both suggest that this bacterium will be very useful as a bioremediation agent in polluted sites co-contaminated with xenobiotics and heavy metals.

In conclusion, a local isolate of Mo-reducing bacterium with the novel ability to degrade SDS has been isolated. This is the first report of a molybdenum reducing bacterium with the ability to degrade SDS. The ability of this bacterium to detoxify multiple toxicants is a sought after property, and this makes the bacterium an important tool for bioremediation. Currently, work is underway to purify the molybdenum-reducing enzyme from this bacterium and to characterize the detergent degradation studies in more detail.

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