Reduction of Mo(VI) by the bacterium *Serratia* sp. strain DRY5

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**Abstract:** The need to isolate efficient heavy metal reducers for cost effective bioremediation strategy have resulted in the isolation of a potent molybdenum-reducing bacterium. The isolate was tentatively identified as *Serratia* sp. strain DRY5 based on the Biolog GN carbon utilization profiles and partial 16S rDNA molecular phylogeny. Strain DRY5 produced 2.3 times the amount of Mo-blue than *S. marcescens* strain Dr.Y6, 23 times more than E. coli K12 and 7 times more than *E. cloacae* strain 48. Strain DRY5 required 37°C and pH 7.0 for optimum molybdenum reduction. Carbon sources such as sucrose, maltose, glucose and glycerol, supported cellular growth and molybdate reduction after 24 hr of static incubation. The most optimum carbon source that supported reduction was sucrose at 1.0% (w/v). Ammonium sulphate, ammonium chloride, glutamic acid, cysteine, and valine supported growth and molybdate reduction with ammonium sulphate as the optimum nitrogen source at 0.2% (w/v). Molybdate reduction was optimally supported by 30 mM molybdate. The optimum concentration of phosphate for molybdate reduction was 5 mM when molybdate concentration was fixed at 30 mM and molybdate reduction was totally inhibited at 100 mM phosphate. Mo-blue produced by this strain shows a unique characteristic absorption profile with a maximum peak at 965 nm and a shoulder at 700 nm. Dialysis tubing experiment showed that 95.42% of Mo-blue was found in the dialysis tubing suggesting that the molybdate reduction seen in this bacterium was catalyzed by enzyme(s). The characteristics of isolate DRY5 suggest that it would be useful in the bioremediation of molybdenum-containing waste.

**Key words:** *Serratia* sp., Molybdate-reduction, Molybdenum blue

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**Introduction**

Heavy metal pollution is a serious issue that warrants urgent attention. This pollution has been reported and studied all across the globe (Davis, 1991; Neunhäuserer et al., 2001; Ahtas et al., 2007; Sahu et al., 2007; Ozdilek et al., 2007; Ayas et al., 2007). A relatively recent technology to remediate heavy metal pollution is bioremediation. The technology utilizes microorganisms from a variety of genera. Metals that could be detoxified via biological reduction include mercury, chromium, molybdenum, arsenic, lead, copper, uranium, selenium, bismuth, and tungsten (Alexander, 1999). Studies on microbial molybdenum reduction as a potential molybdenum bioremediation are important because molybdenum pollution is an emerging global pollutant. Japan has recorded molybdenum pollution in Tokyo Bay where the level has reached hundreds of mg l⁻¹ (Davis, 1991). In Tyrol, Austria, molybdenum pollution is caused by industrial emissions and has contaminated large pasture areas, reaching as high as 200 ppm and causing scours in ruminants (Neunhäuserer et al., 2001). Molybdenum in the form of molybdenite is recovered as a by-product of copper mining in Malaysia (Kosaka and Wakita, 1978). This has led to reports of several cases of pollution caused by accidental leakage of the metal-carrying system of pipes and leaking of the metals from the mining site, resulting in contamination of 2000 acres of paddy fields and the Ranau River (Yong, 2000). Other cases of pollution by molybdenum and heavy metals come from scrap metal yards, scheduled heavy metal sludges and aquatic bodies near industrial complexes (DOE, 2007; Shukor et al., 2006; Yin et al., 2007; Shukor et al., 2008a). Bioremediation of molybdenum pollution in Tyrol, Austria has been shown to be successful (Neunhäuserer et al., 2001). This has prompted the isolation of molybdenum-reducing bacteria to be used for the bioremediation of polluted sites. Although the isolation of molybdate-reducing bacteria has been reported for more than one hundred years, only recently that their significance as potential molybdenum bioremediation agents were realized (Ghani et al., 1993). According to Levine (1925), the phenomenon is first reported in *E. coli* by Capaldi and Proskaeur (1896). Since then, reports on molybdate reduction by other bacteria have trickled in (Woolfolk and Whiteley, 1962; Bautista and Alexander, 1972; Campbell et al., 1985; Sugio et al., 1988; Ghani et al., 1993). *Enterobacter cloacae* strain 48 is the first locally isolated molybdenum-reducing bacterium and several works have been carried out on this bacterium. They include the development of a novel assay to quantify the molybdenum blue produced (Shukor et al., 2000), a method to determine whether molybdate reduction in this bacterium is mediated by enzymes (Shukor et al., 2002), attempts to purify the molybdenum-reducing enzyme (Ariff, 1997; Shukor et al., 2003), the development of a novel assay system for the enzyme (Shukor et al., 2008b) and a method to assay the effects of compounds on molybdate reduction to detect false negative and positive reactions.
(Shukor et al., 2008c). Very recently, we have isolated several new molybdate-reducing bacteria and our preliminary works showed that the reduction of molybdenum to molybdenum-blue in these bacteria is likely to proceed via a phosphomolybdate intermediate similar to that of C. bescens (Shukor et al., 2007). Recently, we have isolated and characterized another locally-isolated molybdenum-reducing bacterium, S. marcescens strain DR.Y6 (Shukor et al., 2008d). In this work, we report on the characterization and isolation of another locally-isolated molybdate-reducing bacterium from Malaysian soil. We discovered that this bacterium reduces molybdenum and producing Mo-blue at levels significantly greater than S. marcescens strain Dr.Y6, EC 48 and E. coli K12 and is therefore the best candidate so far for molybdenum bioremediation.

Materials and Methods

Isolation of molybdate-reducing bacterium and phenotype identification: Soil samples, each measuring approximately 10 grams were taken randomly to a depth of 5 cm from the topsoil using sterile spatula and stored in sterile screw-capped polycarbonate tubes. The soil samples were taken from an abandoned metal recycling ground near the King Edward VII(2nd) Primary School in the city of Taiping, Perak, Malaysia. The samples were immediately placed on ice until returned to Universiti Putra Malaysia, Serdang, Selangor, Malaysia for further examinations. Five grams of a well-mixed soil sample were suspended in 45 ml of 0.9% saline solution. A suitable serial dilution aliquot (0.1 ml) of soil suspension was spread plated onto an agar of low phosphate (2.9 mM phosphate) media mixed soil sample were suspended in 45 ml of 0.9% saline solution. The soil samples were taken from an abandoned metal recycling ground near the King Edward VII(2nd) Primary School in the city of Taiping, Perak, Malaysia. The samples were immediately placed on ice until returned to Universiti Putra Malaysia, Serdang, Selangor, Malaysia for further examinations. Five grams of a well-mixed soil sample were suspended in 45 ml of 0.9% saline solution. A suitable serial dilution aliquot (0.1 ml) of soil suspension was spread plated onto an agar of low phosphate (2.9 mM phosphate) media (pH 7.0) containing glucose (1%), (NH4)2SO4 (0.3%), MgSO4.7H2O (0.05%), NaCl (0.5%), yeast extract (0.05%), NaMoO4.2H2O (0.242%) and Na2HPO4 (0.05%). Glucose was autoclaved separately. Growth in liquid media uses the same media as in the solid media above. Mo-blue is produced in this media but not at high phosphate (100 mM phosphate). The only difference between the high and low phosphate media is the phosphate concentrations. Several white and blue colonies appeared after overnight incubation at room temperature. One single colony exhibiting the strongest blue intensity observable by eye was inoculated into 50 ml of low phosphate media and incubated at 30°C for 24 hr. Enterobacter cloacae strain 48, originally isolated from Chengkau, Negeri Sembilan, Malaysia, was a kind gift from Prof. Dr. Mohd. Ismail Abd. Karim (Ghani et al., 1993). Enterobacter cloacae strain 48, S. marcescens strain Dr. Y6 and E. coli strain K12 (American type culture collection, rockville, USA) were grown and maintained on the above low phosphate liquid and solid media. For comparing molybdenum-reducing property, one single colony from each bacterium was inoculated into 50 ml of low phosphate media and incubated at 30°C for 24 hr. The production of Mo-blue from the media was measured at 865 nm. The molar extinction coefficient or molar absorbptivity at 865 nm for Mo-blue using 12-phosphomolybdate (Sigma) as a standard is 16.7 mM−1 cm−1 (Shukor et al., 2000). Identification at species level was performed by using Biolog GN MicroPlate (Biolog, Hayward, CA, USA) according to the manufacturer’s instructions and molecular phylogenetics studies.

16S rDNA gene sequencing: Genomic DNA was extracted from bacterial colonies by alkaline lysis. PCR amplification was performed using Biometra T Gradient PCR (Montreal Biotech Inc., Kirkland, QC). The PCR mixture contained 0.5 μl of each primer, 200 mM of each deoxynucleotide triphosphate, 1x reaction buffer, 2.5 U of Taq DNA polymerase (Promega) to achieve a final volume of 50 μl. The 16S rDNA gene from the genomic DNA was amplified by PCR using the following primers; 5’-AGAGTTTGTATCTGCTGCTAG-3’ and 5’-AAGAGGTTGATCCAGCAGCA-3’ (Devereux and Wilkinson, 2004). PCR was performed under the following conditions: initial denaturation at 94°C for 3 min; 25 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 10 min. Cycle sequencing was subsequently performed with the Big Dye terminator kit (Perkin-Elmer Applied Biosystems) as recommended by the manufacturer. The resultant 1282 bases were compared with the GenBank database using the Blast server at NCBI (Altschul et al., 1990). This analysis showed this sequence to be closely related to rrs from Gammaproteobacteria. The 16S rRNA ribosomal gene sequence for this isolate have been deposited in GenBank under the accession number DQ226206.

Phylogenetic analysis: A multiple alignment of 20 16S rRNA gene sequences closely matches Isolate DRY5 were retrieved from GenBank and were aligned using ClustalW (Thompson et al., 1994). A phylogenetic tree was constructed by using PHYLIP, version 3.573 (Retief, 2000), with Bacillus subtilis as the outgroup in the cladogram. Evolutionary distance matrices for the neighbour-joining / UPGMA method were computed using the DNADIST algorithm program. The model of nucleotide substitution is those of Jukes and Cantor (1969). Phylogenetic tree (Fig. 1) was inferred by using the neighbour-joining method of Saitou and Nei (1987). With each algorithm, confidence levels for individual branches within the tree were checked by repeating the PHYLIP analysis with 1000 bootstraps (Felsenstein, 1985) by the SEQBOOT program in the PHYLIP package. Majority rule (50%) consensus trees were constructed the Ml methods (Margush and McMorris, 1981) and the tree was viewed using TREEVIEW (Page, 1996).

Dialysis tubing experiment: Isolate DRY5 was grown in 50 ml high phosphate media overnight with shaking at 150 rpm at room temperature. Cells were harvested by centrifugation at 15,000 g for 10 minutes and the pellet resuspended in low phosphate solution (pH 7.0) containing (w/v) (NH4)2SO4 (0.3%), MgSO4.7H2O (0.05%), NaCl (0.5%), yeast extract (0.05%) and Na2HPO4 (0.05%). About 8 ml of this suspension was then placed in dialysis tubing (12,000 Dalton cut-off, Sigma) previously boiled for ten minutes and immersed in sterile 100 ml of low phosphate media (pH 7.0) as described previously. Aliquots (1 ml) of the media were taken at the beginning of the experiment and after a static incubation period of 6 hr at room temperature and then read at 865 nm. At the same time, 1 ml was taken out from the content of the dialysis tubing and centrifuged at 15,000 g for 10 minutes. The supernatant was then read at 865 nm. Experiments were carried out in triplicate.
Statistical analysis: Values are means ± SE of at least three replicates. All data were analyzed using Graphpad Prism version 3.0 and Graphpad InStat version 3.05. Comparison between groups was performed using a Student's t-test or a one-way analysis of variance with post hoc analysis by Tukey's test (Miller and Miller, 2000). p < 0.05 was considered statistically significant.

Results and Discussion

Identification of the isolate: A low bootstrap value (37.6%) was seen when strain DRY5 was associated to Serratia sp. strain DS001 and S. marcescens strain CPO1[4]CU, indicating that the difference in phylogenetic relationships between the species was not strong (Fig. 1). The identifications performed by Biolog GN also gave no conclusive identification to the species level. For now, strain DRY5 is assigned tentatively as Serratia sp. strain DRY5. In an earlier work of Jan (1939), another genus of Serratia sp. was reported to be able to reduce ammonium molybdate to Mo-blue making strain DRY5 as the third bacterium from the genus Serratia reported for molybdate reduction aside from strain Dr.Y6 (Shukor et al., 2008b). Table 1 shows the amount of Mo-blue produced by a 24 hr culture of Serratia sp. strain DRY5, E. cloacae strain 48 and E. coli K12. An analysis of the variance showed that isolate DRY5 produced a significantly higher quantity of Mo blue (p<0.05) with 2.3 times more Mo-blue than S. marcescens strain Dr.Y6, 7 times more than E. coli K12 and 10 mM molybdate. Molybdate reduction in isolate DRY5 had an optimum growth temperature of 37 °C and an optimum initial pH for growth of 7.0. The high optimum temperature suggested that this bacterium should be useful in a tropical climate like Malaysia where normal soil temperatures can reach as high as 35°C (Sinnakkannu et al., 2004). The optimum temperature for the reduction of molybdate by S. marcescens strain Dr.Y6 slightly differs at 35°C (Shukor et al., 2008d). Ghani et al. (1993) grew EC 48 at 30°C while the optimum temperature range for molybdate reduction in E. coli K12 was from 30 to 36°C (Campbell et al., 1985). With EC 48 the optimum pH supporting molybdate reduction was at pH 7.0 (Ghani et al., 1993). With E. coli K12, it was between 5.0 and 6.0 (Campbell et al., 1985) whilst optimum pH studied was not carried out in S. marcescens strain Dr. Y6 (Shukor et al., 2008d).

Nitrogen sources for molybdate reduction: Since molybdate reduction in the heterotrophic bacterium EC 48 has been reported to be growth-associated (Ghani et al., 1993; Ariff et al., 1997), factors that increase growth would also increase molybdate reduction. It was found that ammonium sulphate, ammonia chloride, glutamic acid, cysteine, and valine supported growth and molybdate reduction while alpha-ketoglutarate, alanine, asparagine, aspartic acid, glycine, histidine, hydroxyproline and leucine supported cellular growth, but not molybdate reduction (Fig. 2). An analysis of variance showed that ammonium sulphate gave significantly higher Mo-blue production at 7.20±0.13 micromoles of Mo blue, in comparison to ammonium chloride (p<0.01) at 4.50±0.05 micromoles of Mo-blue. The latter showed no significant difference in terms of Mo-blue production with glutamic acid (3.66±0.25 micromoles of Mo-blue) and cysteine (3.54±0.15 micromoles of Mo blue) (p>0.05), but was significantly greater than valine (2.22±0.15 micromoles of Mo blue) (p<0.05). The results for cellular growth with amino acids were consistent with those with Biolog’s amino acids. The optimum concentration of ammonium sulphate supporting molybdate reduction was 0.2% (w/v) after 24 hr of static incubation, whilst the concentration of ammonium sulphate giving optimum molybdate reduction in EC 48 was at 0.3% (w/v). A further increase in ammonium sulphate concentration reduced molybdate reduction considerably. In S. marcescens strain Dr.Y6, ammonium sulphate, glycine, glutamic acid, OH-proline (in

![Table - 1: Amount of Mo-blue produced from a 24-hr culture of bacteria.](image)

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>μ mole Mo blue produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia sp. strain DRY5</td>
<td>7.61 ± 0.05*</td>
</tr>
<tr>
<td>Serratia marcescens strain Dr.Y6</td>
<td>3.24±0.14d</td>
</tr>
<tr>
<td>E. cloacae strain 48 (EC 48)</td>
<td>1.14 ± 0.31c</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>0.335 ± 0.22d</td>
</tr>
</tbody>
</table>

![Fig. 1: A phylogram (neighbour-joining method) showing genetic relationship between strain DRY5 and other related reference microorganisms based on the 16S rRNA gene sequence analysis.](image)
the order of decreasing efficiency) support molybdate reduction while nitrate, nitrite, alanine, asparagine, aspartic acid, valine, cysteine, histidine, leucine, oxaloacetate, alpha-ketoglutarate and urea do not support reduction (Shukor et al., 2008d). The amino acids cysteine and valine were found to support molybdate reduction in this work but not in strain Dr.Y6 and the reverse was true for the amino acids glycine and OH-proline probably as a result of a different metabolic route involved in nitrogen metabolism due to species differences. Similarly, ammonium sulphate at 0.3% (w/v) was the optimum nitrogen source for supporting molybdate reduction after 24 hr of static incubation. Similar to the works of Campbell et al. (1985), both nitrate and nitrite inhibited molybdate reduction. Our results showed that the
inhibitory effect was at the cellular level and not just molybdate reduction per se, since the cellular growth was also inhibited (Fig. 2). It would be interesting in the future to study why certain amino acids support molybdate reduction and others support only cellular growth, but not molybdate reduction, in these bacteria.

**Carbon sources for molybdate reduction:** Carbon sources, such as sucrose, maltose, glucose and glycerol, supported cellular growth and molybdate reduction after 24 hr of static incubation (Fig. 3). Acetate, formate, starch, tartarate, citric acid and lactose did not support cellular growth and molybdate reduction in the presence of 10 mM molybdate. There is a possibility that growth is retarded in some of the carbon sources studied in the presence of molybdate. For instance, Biolog results showed that the isolate was able to grow using formate and citric acid as a carbon source, but that both carbon sources were unable to support growth in the presence of 10 mM molybdate. An analysis of the variance showed that sucrose gave a significantly higher (p<0.01) amount of Mo-blue at 6.99±0.88 micromole Mo blue than glucose, maltose or glycerol. There was no significant difference (p>0.05) in the amounts of Mo blue produced by glucose (3.05±0.47 micromoles of Mo blue), maltose (4.19±0.13 micromoles of Mo blue) and glycerol (2.39±1.17 micromoles of Mo blue). A sucrose concentration at 1.0% (w/v) was optimum after 24 hr of static incubation, similar to the results obtained by Ghani et al. (1993) in EC 48 and by Shukor et al. (2008b) in S. marcescens strain Dr.Y6. The same results in terms of type of carbon sources supporting molybdate reduction were seen in S. marcescens strain Dr.Y6 with sucrose, maltose, glucose, glycerol (in decreasing order) supported molybdate reduction after 24 hr of static incubation while acetate, formate, citric acid, lactose, fructose, mannitol, tartarate, and starch did not support reduction. This phenomenon is probably due to the same metabolic route involved in sugar metabolism in producing reducing equivalent (NADH) for molybdate reduction. Campbell et al. (1985) reports that glucose at 1% (w/v) is the best substrate. In EC 48, carbon sources, such as glucose, fructose, galactose, mannose, maltose, lactose, raffinose and sorbitol, support molybdate reduction. In contrast, 2-ketoglutarate, citrate, pyruvate, xylene, acetic acid and ribose do not support molybdate reduction in EC 48 (Ghani et al., 1993). It would be interesting to study in the future why certain carbon sources supported molybdate reduction and others supported only cellular growth, but not molybdate reduction in these bacteria.

**The effect of phosphate and molybdate concentrations:** Both phosphate and molybdate ions have been previously reported to inhibit molybdate reduction at certain concentrations (Campbell et al., 1985; Ghani et al., 1993; Shukor et al., 2000). Thus, it is very important to ascertain the effects of phosphate and molybdate ions to molybdate reduction in this bacterium. Molybdate reduction was found to increase as molybdate concentrations were increased from 0 to 30 mM and reached an optimum point at 30 mM. At higher concentrations, molybdate reduction was inhibited (Fig. 4). We found that the optimum concentration of phosphate for molybdate reduction was 5 mM when molybdate concentration was fixed at 30 mM and molybdate reduction decreased rapidly at much high phosphate concentrations and was totally inhibited at 100 mM phosphate (Fig. 5). The results are similar to the results obtained from S. marcescens strain Dr.Y6. In the latter, molybdate reduction is supported optimally by 15 to 25 mM molybdate and 5 mM phosphate. Similarly, very high phosphate concentrations (>50 mM) inhibited molybdate reduction. Campbell et al. (1985) reported that, at 5 mM phosphate, 80 mM molybdate is the optimum concentration for molybdate reduction. Ghani et al. (1993) reported that, at 5 mM phosphate, 20 mM molybdate is the optimum concentration. In both works, phosphate concentrations higher than 50 mM strongly inhibit molybdate reduction with complete cessation of molybdate reduction occurring at 100 mM phosphate. It is speculated that, at 5 mM phosphate, other bacteria would exhibit more variation in the optimum molybdate concentration that would give the optimum result. High phosphate inhibits molybdate reduction probably by maintaining the pH at neutral; a pH that is undesirable for the formation, and stability of phosphomolybdate (Lee, 1977; Sidgwick, 1984).

**Mo-blue absorption spectra:** The result shows that Mo-blue produced by this strain revealed the same characteristic absorption...
profile as the Mo-blue from *S. marcescens* strain Dr.Y6 (Shukor et al., 2008d), EC 48 (Ghani et al., 1993) and the phosphate determination method (Shukor et al., 2000; Yoshimura et al., 1986) with a maximum peak at 865 nm and a shoulder at 700 nm (Fig. 6). This characteristic profile was preserved as the incubation period increases, especially with the peak maximum at 865 nm. The concurrent increase in Mo-blue production suggests that the Mo-blue produced from this isolate is likely to be a reduced phosphomolybdate.

The mechanism of molybdate reduction to Mo-blue by the heterotrophic microbes has previously been a subject of uncertainty. Campbell et al. (1985) suggest that the Mo-blue observed in *E. coli* K12 reduction of molybdate is a reduced form of phosphomolybdate due to the spectrum obtained, but claim that the mechanism of reduction is unknown. Ghani et al. (1993) suggest that molybdenum(vi) (Mo\(^{6+}\)) is first enzymatically reduced to molybdenum(v) (Mo\(^{5+}\)) before the addition of phosphate leads to the formation of the Mo-blue. The involvement of phosphomolybdate as the principal substrate for the molybdenum-reducing enzyme in bacteria is probably likely than molybdate as a substrate since enzymes such as aldehyde- and xanthine oxidase are known to reduce phosphomolybdate but not molybdate to Mo-blue (Glenn and Crane, 1956). There are many phosphomolybdate species aside from 12-phosphomolybdate. Although identification of the exact phosphomolybdate species must be carried out using n.m.r and e.s.r. (Shukor et al., 2000), identification of phosphomolybdate species by analysing the scanning spectroscopic profile has been accepted as a simple and quick method to distinguish between phosphomolybdate and other heteropoly molybdates such as silicomolybdate and sulphomolybdate (Glenn and Crane, 1956; Sims, 1961; Kazansky and Fedotov, 1980; Yoshimura et al., 1986; Hori et al., 1988; Shukor et al., 2000).

**Dialysis tubing experiment:** Many reducing agents, biotic and non-abiotic can reduce phosphomolybdate to Mo-blue (Sidgwick, 1984). Hence, works on molybdate reduction by microbes for the purpose of remediation must provide evidence of biological reduction. Boiling the bacteria denatures enzymes that produce bioreductants and hence can falsely indicate enzymatic reduction. Such is the problem that early studies of ferric reduction have been suggested to be mediated by bio- or chemical reductants and is not of enzymatic origin (Hem, 1972). Munch and Ottow (1983) were the first to prove that ferric reduction is an enzymatic process by enclosing insoluble ferric materials in dialysis tubing and immersing them in media containing ferric-reducing bacteria. We modified this experiment to suit molybdate reduction in EC 48 and found a similar result, concluding that molybate reduction in EC 48 is enzymatic in origin (Shukor et al., 2002). In this experiment, it was discovered that 95.42± 1.31% of the Mo-blue was found in the dialysis tubing suggesting that the molybdate reduction seen in this bacterium was catalyzed by enzyme. For EC 48, almost 90% of

![Fig. 6: Scanning spectrum of Mo-blue from Isolate DRY5 after 12, 14, 16, 18 and 20 hr of static incubation labeled A, B, C, D and E, respectively](image-url)
the Mo-blue produced is found in the dialysis tubing (Shukor et al., 2002). The dialysis tubing method was not tested to S. marcescens strain Dr.Y6 and we are currently applying this method to strain Dr.Y6 to glean on the involvement of enzyme on the reduction of molybdate by this strain.

In conclusion, we have isolated and characterized a molybdate-reducing bacterium from soil. Due to the limited literature on the variety of molybdate-reducing bacterium, our work sought to increase the repertoire of bacterial species able to reduce molybdate to Mo-blue. We have studied the effect of various parameters, such as nitrogen and carbon sources, temperature, molybdate, yeast and phosphate, on molybdate reduction from this bacterium. We found that phosphate inhibited molybdate reduction from this bacterium similar to previous reports. This knowledge is not only an important contribution to the fundamental understanding of the mechanism of reduction, but will also be beneficial in the area of bioremediation of molybdenum. We also provide some evidence on the involvement of phosphomolybdate as an intermediate compound that must form before reduction to molybdate can occur. Currently, work is underway to purify the molybdenum-reducing enzyme from this bacterium.

References


