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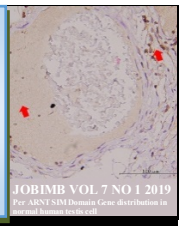

Pantoea sp. strain HMY-P4 Reduced Toxic Hexavalent Molybdenum to Insoluble Molybdenum Blue

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ABSTRACT

Bioremediation of pollutants such as heavy metals is an economic and environmentally friendly process. A novel molybdenum-reducing bacterium was isolated and characterized for its potential to reduce hexavalent molybdenum to molybdenum blue (Mo-blue). The bacterium reduces molybdate optimally at pH between 6 and 8, temperature between 35 and 40 ˚C. Glucose was the best electron donor source supporting molybdate reduction followed by sucrose, fructose, starch and glycerol in descending order. Other requirements include optimum phosphate concentration at 5.0 mM and molybdate concentration between 20 and 40 mM. 16S rRNA partial sequencing and phylogenetic analysis identified the bacterium as *Pantoea* sp. strain HMY-P4. The capacity of this bacterium to reduce toxic soluble molybdenum to a less toxic form is novel and makes the bacterium an important instrument for bioremediation of this pollutant.

INTRODUCTION

Pollution of the environment keeps on increasing at an alarming rate due to the activities of man such as urbanization, technological advancement, unsafe agricultural practices and rapid industrialization which degrades the environment. Heavy metals released into the environment are persistent due to their toxicity which poses a severe threat to organisms exposed to high levels of such pollutants. Metals are essential to the biological functions of plants and animals but at elevated levels, they interfere with metabolic reactions in systems of organisms. Toxic heavy metals e.g molybdenum which is useful to plants, are capable of reducing plant growth due to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of essential enzymes [1].

Toxic heavy metals like molybdenum could accumulate in the body when consumed in contaminated food through the food chain and become health risks to living organisms [2]. This causes oxidative stress, an unevenness involving the production of free radicals and the capacity of cells to eradicate them or repair the damage [3, 4]. This leads to base damage through formation of reactive oxygen species (ROS) which includes oxygen radicals like superoxide and hydroxyl and non-radical derivatives of molecular oxygen (O_2) such as hydrogen peroxide (H_2O_2), as well as breakage of the DNA molecule [3, 4]. Furthermore, heavy metal toxicity increases the production of ROS thereby decreasing the antioxidant systems (glutathione, superoxide dismutase, etc.) which protect cells. If this condition continues, the normal functioning of the organism is affected and may invariably lead to cell death.

Molybdate reduction to molybdenum blue is an old phenomenum that was reported over a century ago. According to Levin [5], the phenomenum was first reported in *E. coli* by capaldi and proskaue [6]. In 1985, microbial molybdate reduction resurfaced again in a report on its reduction by *E. coli* K12 [7]. Sugio *et al.* [8] reported the reduction of molybdate to molybdenum blue by *Thiobacillus ferreoxidants.* The effort of Sugio *et al.* [8] were followed by Ghani *et al.* [9], who reported on another heterotrophic bacterium *Enterobacter cloacae* strain 48 (EC48) was able to reduce molybdate to molybdenum blue.

A review on metal reduction by microbes has also confirmed this fact [10]. It was initially proposed that molybdate (Mo^{6+}) was first reduced to Mo^{5+} by molybdenum reductase prior to the joining of phosphate anions forming molybdenum blue [8, 9].

To date, about 25 molybdenum-reducing bacteria from 8 different genera (*Acinetobacter, Bacillus, Burkholderia, Enterobacter, Escherichia, Klebsiella, Pseudomonas and Serratia*) were reported to be isolated from different soils and polluted waters around the world. However, report on the isolation of molybdenum-reducing bacteria from Nigerian soil is scarce, making it necessary to conduct this research.
This work characterizes the potential of a locally isolated *Pantoea* sp. strain HMY-P4 to reduce toxic hexavalent molybdenum to insoluble molybdenum blue.

MATERIALS AND METHODS

Chemicals and equipment

All chemical reagents and media ingredients used in this research were of analytical grade. The glassware used for the experiment were washed with 10% (v/v) nitric acid and rinsed with double distilled water to avoid the effect of other metals contaminants. Following oven drying at 60 °C, glassware was sterilized by autoclaving at 121 °C, 115 kPa for 15 min.

Culture media preparation

All media preparations (solid and broth) were made according to the recipe of Ghani *et al.* [9] and Shukor *et al.* [11] except otherwise stated.

Low phosphate-molybdate (LPM) agar

This medium was prepared by dissolving agar: 18 g, $(NH4)_2SO_4$: 3 g, $MgSO_4.7H_2O$: 0.5 g, NaCl: 5 g, $Na_2MoO_4.2H_2O$: 2.42g, Na_2HPO_4: 0.71g, yeast extract: 0.5 g and glucose: 10 g into a liter of deionized water and adjusting the pH to 7.5 prior to autoclaving at 121 °C, 115 kPa for 15 min. Glucose was separately autoclaved and added to the medium afterwards. The molten medium was allowed to cool to about 50 °C before pouring onto sterile disposable petri dishes. The plates were then kept at 30 °C in the incubator for overnight drying. In case of prolong storage, tape was used to seal and prevent the plates from drying out [12].

Low phosphate-molybdate medium (LPM)

This medium was prepared according to the method of Ghani *et al.* [9] with slight modification. Briefly, into a liter of deionized water, $(NH4)_2SO_4$: 3 g, $MgSO_4.7H_2O$: 0.5 g, NaCl: 5 g, $Na_2MoO_4.2H_2O$: 2.42 g, Na_2HPO_4: 0.71 g, yeast extract: 0.5 g and glucose: 10 g were dissolved and the medium was adjusted to pH 7.5 prior to autoclaving at 121 °C, 115 kPa for 15 min. Glucose was separately autoclaved and added to the medium afterwards.

Isolation of Mo-reducing bacteria

Soil sample was collected in March 2018 from agricultural land in Ungogo latitude 12.087077 and longitude 8.480645 Kano state, Nigeria. The sample was collected 5 cm beneath the top soil using a sterile spatula, transferred into suitable container and stored in refrigerator at 4 °C before use. After a careful serial dilution in sterile distilled water, an aliquot (0.1 ml) was spread-plated on LPM agar and incubated at 37 °C for possible Mo-blue production. According to Campbell *et al.* [7] bacterial molybdate reduction produces Mo-blue on a low phosphate molybdate medium. Therefore, colony that forms highest blue color intensity was isolated and re-streaked on low phosphate molybdate agar to obtain a pure culture. Following 24 h incubation on nutrient agar, a single isolated colony was inoculated into a fresh nutrient broth and incubated at 30 °C for 24 h. Molybdate reduction was determined by inoculating 2% (v/v) of the selected isolate (OD_{600} = 0.9-1.0) into 100 ml of freshly prepared low phosphate molybdate media and incubated at 25 °C for 24 h. The intensity of the molybdenum blue produced was measured at 865 nm. The isolate with the highest intensity of Mo-blue was selected for further study.

Identification of Mo-reducing bacteria

The morphological characteristics of the isolate were observed on LPM agar, nutrient agar and by Gram staining. The molecular phylogenetic analysis was used to identify the isolate at the species level [12].

Gram stain

This experiment classifies bacteria either as Gram positive or Gram negative. The former usually retains the purple primary stain color, while the later absorb the counter stain and become pink or red. A smear from fresh culture (18 h) was prepared and flooded with crystal violet and allowed for one min, then gently washed with distilled water. There follows the addition of Gram's iodine, ethyl alcohol (95%) and safranin respectively, each allowed for a min before washing. The slides were finally examined under a microscope.

16s rRNA Gene Sequencing

A single colony of the best molybdenum-reducing bacterium grown on nutrient agar was suspended in 1 ml of distilled water, and extracted the genomic DNA by alkaline lysis using Commercially prepared genomic DNA purification kit (Thermo Scientific). The forward (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse (5'-AAGGAGGTGATCCAGCCGCA-3') primers were used to amplify the 16S rRNA. PCR amplification was performed by initial denaturation at 94 °C for 3 min, 25 cycles at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, then a final extension at 72 °C for 10 min using Gradient touch thermo-cycler [16].

The resultant 1,050 bases were blast using NCBI server (http://www.ncbi.nlm.nih.gov/BLAST/) to compare with the GenBank database. Blast analysis revealed that the sequence is related to the family Enterobacteriaceae. The 16S ribosomal RNA gene sequence of this isolate will be deposited in the GenBank.

Phylogenetic analysis

The phylogenetic analysis was performed using clustal W by multiple alignments of twenty retrieved 16S rRNA gene sequences (from GenBank) that closely matched isolate D through PHYLIP output option. All possible missed-alignments were manually checked, and gaps were excluded from the computation. The phylogenetic tree was constructed using PHYLIP version 3.573, with *Serratia marcescens* as the out-group in the phylogram.

The evolutionary distance matrices for the neighbor-joining/ UPGMA method were computed through DNADIST algorithm program, while nucleotide substitution was performed using Jukes and Cantor model. The confidence levels of individual tree branches were checked by repeating the PHYLIP analysis with 1,000 bootstraps. Majority rule (50%) consensus trees were constructed for the topologies found using a family of consensus tree methods using the CONSENSE program and the tree was viewed using Tree View [12].

Effect of electron donor sources on molybdate reduction
The effect of various electron donors such as glucose, fructose, sucrose, starch and glycerol on molybdenum reduction by this bacterium was determined by supplementing 1% (w/v) into LPM containing fixed concentrations of phosphate (5 mM) and sodium molybdate (10 mM) at pH 7.0. Into freshly prepared LPM, a 2% (v/v) of bacterial aliquot in nutrient broth (OD_{600}= 0.9-1.0) was inoculated, the culture media were then incubated for 24 h at 25 ºC. After the required incubation, an aliquot (3 ml) from the culture media was transferred into centrifuging tube and centrifuged at 4,000 rpm for 10 min. The resultant supernatant was spectrophotometrically measured at 865 nm for the amount of Mo-blue produced [12].

Effect of glucose concentrations
Following screening of the best electron donor source, glucose was found to be the best electron donor source that supports optimum Mo-blue production by this bacterium and thus analyzed for optimum concentration. In the present study, the effect of various concentrations of sucrose (0-50 g/L) supplemented into LPM were tested. After 24 h incubation, an aliquot (3 ml) from the culture media was centrifuged at 4,000 rpm for 10 min at room temperature. The resultant supernatant was spectrophotometrically measured at 865 nm for the amount of Mo-blue produced as before.

Screening of nitrogen source
Apart from a carbon source, the nitrogen source is also an absolute requirement for bacterial growth. Similarly, a balance in C:N ratio is important as this can influence the reduction process. Thus, it is necessary to screen for the suitable nitrogen sources that support molybdenum reduction in this bacterium. In this study, the effect of both organic and inorganic nitrogen sources like ammonium sulfate, phenyl alanine, urea, glutamate and glycine on molybdenum reduction was determined by supplementing 0.3% (w/v) each into LPM containing fixed concentrations of phosphate (5 mM) and sodium molybdate (10 mM) at pH 7.0. After 24 h incubation, an aliquot (3 ml) from the culture media was centrifuge at 4,000 rpm for 10 min at room temperature. The resultant supernatant was measured spectrophotometrically at 865 nm for the amount of Mo-blue produced.

Effect of initial pH of LPM
In this study, the effect of initial pH of the medium was used to determine the suitable pH that supports optimum Mo-blue production in this bacterium. The buffer system used was the disodium phosphate in LPM, which span the pH range between 5.5 and 8.0 (pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0). After 24 h incubation, an aliquot (3 ml) from the culture media centrifuge at 4,000 rpm for 10 min. The resultant supernatant was then measured spectrophotometrically at 865 nm for the amount of Mo-blue produced.

Effect of temperature
The effect of temperature was examined over a temperature range (25—50 ºC) was used to evaluate the effect of temperature. After 24 h incubation, an aliquot (3 ml) from the culture media was centrifuged at 4,000 rpm for 10 min at room temperature. The resultant supernatant was then read spectrophotometrically at 865 nm for the amount of Mo-blue produced.

Effect of phosphate and molybdate concentrations
Phosphate and molybdate concentrations are major parameters influencing microbial molybdate reduction to Mo-blue. In this study, the effect of phosphate concentration was evaluated by fixing molybdate concentration as 10 mM and varying the phosphate concentrations (1.5, 2.9, 3.5, 5.0, 7.5 mM). Similarly, the effect of molybdate concentration was evaluated by fixing phosphate concentration at 5 mM and varied the molybdate concentrations (10-100 mM). After the required incubation, an aliquot (3 ml) from the culture media was centrifuged at 4,000 rpm for 10 min at room temperature. The resultant supernatant was then read spectrophotometrically at 865 nm for the amount of Mo-blue produced.

Statistical Analysis
One-way analysis of variance was performed using a statistical software INSTAT GraphPad version 3.0.

RESULTS

Identification of Molybdenum-Reducing Bacterium

The bacterial isolate was screened for its capacity to reduce molybdate to Mo-blue. The colony morphology on nutrient agar showed a smooth, circular, while Gram's stain microscopic observation revealed that the bacterium is Gram-negative, rod-shaped. Phylogenetic analysis of the 16S rRNA gene sequence (**Fig. 1**) using the neighbor-joining method revealed a bootstrap value of 75% similarity to *Pantoea agglomerans* strain LMG 1286 indicating that the phylogenetic relationship of this isolate to a particular species will be difficult. Thus, the isolate was tentatively assigned as *Pantoea* sp. strain HMY-P4.

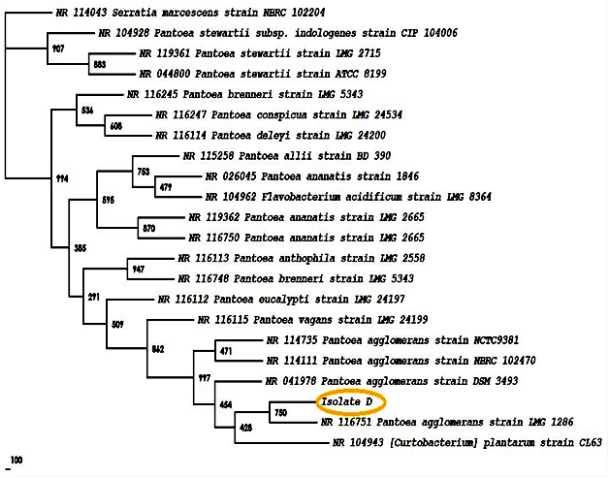

Fig. 1. Phylogram (neighbor-joining method) indicating the genetic relationship between isolate A and referenced related microorganisms based on 16S rRNA gene sequence analysis. Accession numbers are accompanied by the species names of their 16S rRNA sequences. The numbers at branching points or nodes refer to bootstrap values, based on 1000 re-samplings. Scale bar represents 100 nucleotide substitutions. *Serratia marcescens* is the out-group.

Effect of electron donor sources on molybdate reduction
Different electron donor sources such as glucose, sucrose, fructose, starch and lactose were used at an initial concentration of 1% (w/v) to study their effects on the molybdate reduction efficiency of the bacterium. The bacterium utilizes all the carbon sources. However, glucose was the best carbon source (**Fig. 2**). There was no significant difference between sucrose and fructose. The least utilized carbon was starch. The best glucose concentration was at 5 mM (**Fig. 3**).

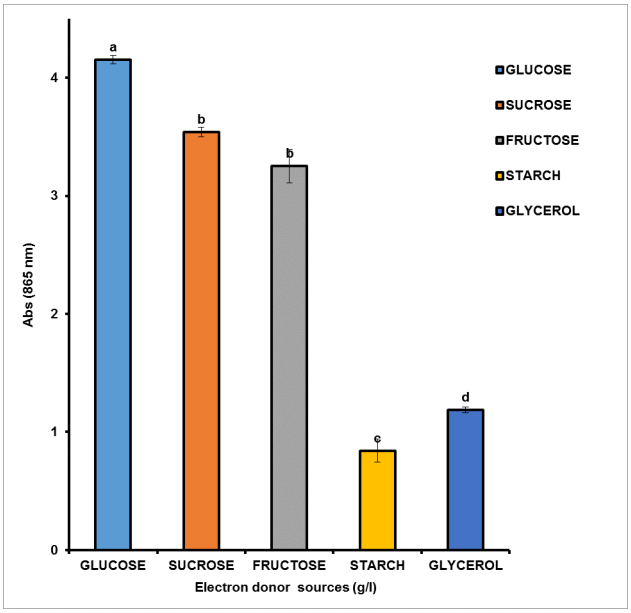

Fig. 2. Effect of various electron donor sources on molybdenum reduction by *Pantoea* sp. after 24 h incubation at the final concentration of 1% (w/v). Data represent mean ± standard deviation of triplicate. Values with different letters over the bars are significantly different ($p<0.05$)

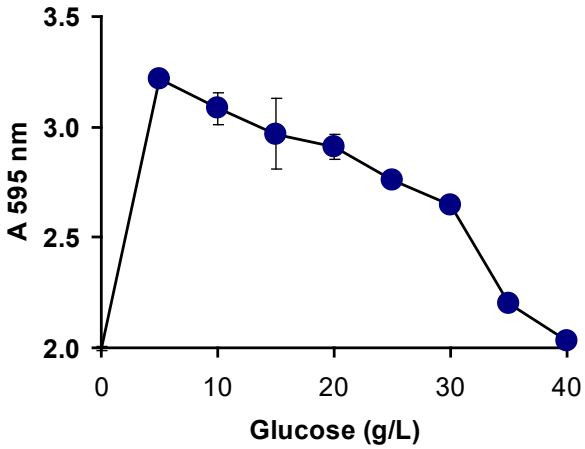

Fig. 3. Effect of different glucose concentrations on molybdenum reduction by *Pantoea* sp. after 24 h incubation. Data represent mean ± standard deviation of triplicate.

Effect of nitrogen source on molybdate reduction

Different nitrogen sources such as ammonium sulphate, glycine, phenylalanine, glutamic acid and urea were used to study the effect of nitrogen source on the bacterium. **Fig. 4**, shows that ammonium sulphate was the best followed by glycine and then phenylalanine, glutamate and the least was urea.

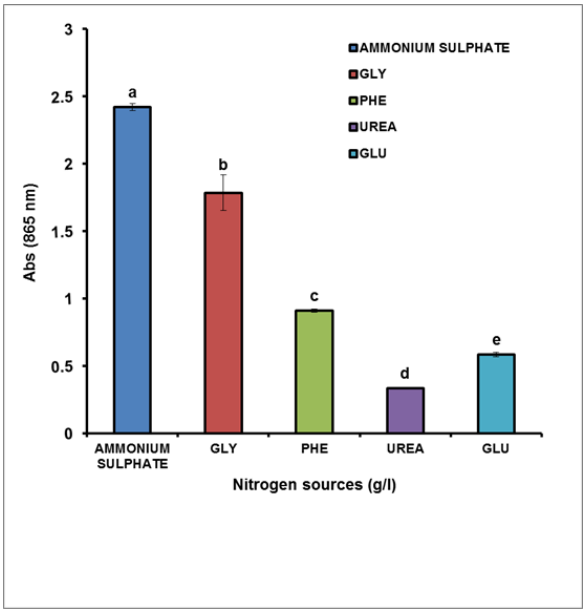

Fig. 4. Effect of various nitrogen sources on molybdenum reduction by *Pantoea* sp. after 24 h incubation at the final concentration of 0.3% (w/v). Data represent mean ± standard deviation of triplicate. Values with different letters over the bars are significantly different ($p<0.05$).

Effect of pH and temperature on molybdate reduction

The effect of temperature on molybdate reduction of bacterium was studied at temperature ranging from 25, 30, 35, 40, and 50. The result shows that the optimum temperature is between 35 and 40 °C. After 45 °C, the bacteria growth dropped rapidly and almost no growth occurred at temperature 50 °C and above (**Fig. 5**). High level of Mo-blue complex production was between pH 6 and 8. The pH of the medium affected the reduction of molybdenum. Most of the best pH for best reduction of the bacteria is ranging between pH 6 and 8 (**Fig. 6**)

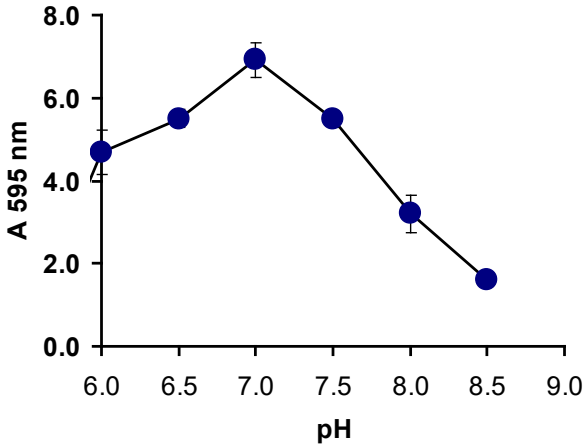

Fig. 5. Effect of different pH on molybdenum reduction by *Pantoea* sp. after 24 h incubation. Data represent mean ± standard deviation of triplicate.

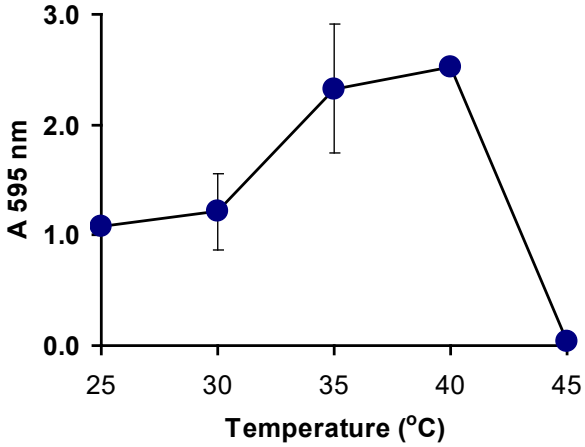

Fig. 6. Effect of various temperature on molybdenum reduction by *Pantoea* sp. after 24 h incubation. Data represent mean ± standard deviation of triplicate.

Effect of phosphate and molybdate concentrations on Mo-blue production

The study of the effect of molybdate concentrations on molybdate reduction was carried out at 10, 20, 40, 60, 80, and 100 mM respectively. **Fig. 7** shows that molybdate reduction was found to increase linearly as molybdate concentration was increased from 10 mM attaining optimum at 40 mM, while molybdate reduction was inhibited at higher concentrations. Phosphate concentration for molybdate reduction was set at 1.5, 2.9, 3.5, 5.0, and 7.5 mM, and 5 mM was found to be optimum in this bacterium (**Fig. 8**).

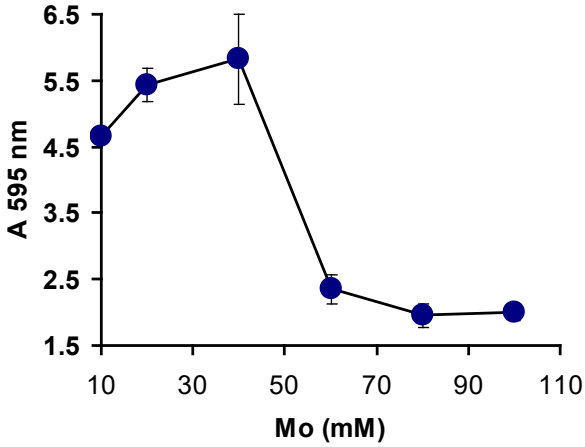

Fig. 7. Effect of molybdate concentration on Mo-blue production by *Pantoea* sp. after 24 h incubation at fixed phosphate concentration. Data represent mean ± standard deviation of triplicate.

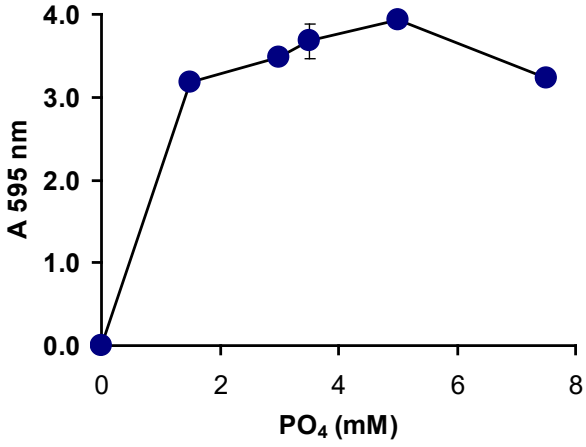

Fig. 8. Effect of phosphate concentrations on molybdenum reduction by *Pantoea* sp. after 24 h incubation at fixed molybdate concentration. Data represent mean ± standard deviation of triplicate.

DSICUSSION

The vast majority of the molybdenum-reducing bacteria reported to date are heterotrophs belonging to the Enterobacteriaceae family [9, 13-15] with the exception of *Acidothiobacillus thiooxidans* [8] and several *Bacillus* spp. [16-19] The heterotrophic nature of this family of bacteria enables them to ferment simple sugars resulting in lowering the pH of the growth medium and thus inducing the formation of phosphomolybdate, a vital intermediate in the reduction of molybdate to Mo-blue [13].

Study on the effect of various parameters such as source of electron donors, temperature, molybdate, and phosphate on molybdate reduction is important. This knowledge not only is important for contributing to the fundamental understanding of the mechanism of reduction but also will be beneficial in the area of bioremediation of molybdenum especially with pH and source of electron donors as these parameters can be controlled by addition of suitable compounds into the soil. Glucose appears to be the main carbon source supporting Mo-blue production in this bacterium. In contrast, sucrose was the best source of electron donor for *E. coli* 48, *S. marcescens* strain Dr.Y6, and *Serratia* sp. strain Dr.Y5 [9,13,20]. Fructose was the best carbon source in strain *hkeem* [21].

It was previously demonstrated in *E coli* 48 that molybdate reduction is growth-associated and this is also true for several other Mo-reducing bacteria [13]. Hence, it is not surprising that glucose supported the highest reduction as it is the best substrate for growth and source of carbon as well as the best substrate for producing reducing equivalents in the form of NADH or NADP. However, in terms of cost effectiveness, sucrose in the form of cane and sugar molasses is preferred to pure and simple carbohydrates as it is a cheaper alternative found in abundance in industrial wastes [21]. Thus, sucrose in the form of molasses would be employed for future molybdenum bioremediation using this bacterium.

Campbell *et al*. [7] were the first to note the inhibitory effects of elevated concentrations of both phosphate and molybdate ions on bacterial Mo-blue production. Hence, it is very important to ascertain the effects of phosphate and

molybdate ions on molybdate reduction in this bacterium. A similar ratio of phosphate to molybdate was seen in *E. coli* K12 and *Klebsiella* sp. strain Hkeem [21], where, at 5 mM phosphate, 40 mM molybdate was the optimum concentration for supporting Mo-blue production [7]. In Ec 48, the optimum ratio is 5 mM phosphate to 20 mM molybdate.

In all of the Mo-reducing bacteria studied so far, phosphate concentrations higher than 5 mM inhibited molybdate reduction. Phosphate disrupts the phosphomolybdate complex preventing reduction to Mo-blue [22]. The highest reported concentration of molybdenum as a pollutant is at 2000 ppm (20.8 mM molybdate) from a molybdenum mine runoff [23]. At this concentration, all of the bacteria studied so far can reduce molybdate provided that the soil phosphate concentrations do not exceed 20 mM for appreciable reduction to take place. Fortunately, phosphate soil concentrations rarely exceeded this value [9].

The study of temperature optimal for the growth of microbes would be very useful for bioremediation and maximizing enzyme yield for purification purposes. *Pantoea* sp. isolated shows optimal temperature between 35 and 40 ºC. This correlate with the bacterium *Pseudomonas* sp. strain DRY2 [15], which shows maximum temperature at 40 ºC. However, in contrast to *E. coli* K12 which reduces molybdenum optimally between 30 and 36 ºC [7]. Ghani *et al.* [9] reported 30 ºC as the optimum. Other Mo-reducing bacteria isolated so far have optimal temperature between 30 and 37 ºC.

Although, it is not generally possible to change temperature when performing onsite bioremediation, screening for indigenous microbes for local bioremediation works is the norm since these microbes would have an optimum temperature close to the temperature of the site chosen for bioremediation. The optimal initial pH supporting reduction is also shared by majority of the Mo-reducing bacteria [7, 9, 13, 20, 21, 24]. The obligation for neutral pH and a moderate temperature range ensures that bioremediation treatments will be economical, and these observable facts are also shared by many bacteria. However, most soils with active metabolic activity usually exhibited lowering in pH due to several factors such as carbohydrate fermentation and carbon dioxide production leading to the lowering of pH [25].

CONCLUSION

A locally isolated bacterium with potential to reduce toxic hexavalent molybdenum to precipitable Mo-blue was isolated and characterize. The bacterium was tentatively identified as *Pantoea* sp. strain HMY-P4 based on the 16S rRNA partial sequencing and molecular phylogenetic analysis. Glucose, an easy assimilable carbon source was the best electron donor at 5 g/L. Also, ammonium sulphate was the best nitrogen source. Other requirements include a strict phosphate concentration at 5.0 mM, molybdate concentration between 20 and 40 mM, pH between 6 and 8 and temperature at 35 and 40 ºC. This bacterium could be suitable for bioremediation in temperate regions like Nigeria.

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REFERENCES

1. Nematian MA & Kazemeini, F. Accumulation of Pb, Zn, C and Fe in plants and hyperaccumulator choice in Galali iron mine area, Iran. Int. J. Agric. Crop Sci. 2013: **5**, 426.
2. Tak HI, Ahmad F. & Babalola OO. Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. In Reviews of Environmental Contamination and Toxicology, USA. 2013: **223**: 33–52.
3. Chandra K, Salman AS, Mohd A, Sweety R. & Ali KN. Protection against fca induced oxidative stress induced DNA damage as a model of arthritis and in vitro anti-arthriti potential of costus speciosus rhizome extract. Int. J. Pharm. Phytopharmacol Res. 2015: **7**(2) 383–389.
4. Mani S. Production of Reactive Oxygen Species and its Implication in Human Diseases. In Free Radicals in Human Health and Disease; Vibha, R., Umesh, C.S.Y., Eds.; Springer: New Delhi, India. 2015; pp. 3–15.
5. Levin VE. The reducing properties of microorganisms with special reference to selenium compounds. J Bacteriol. 1925: **10**:217-263.
6. Capaldi A & Proskauer B. Beitr&ge Zur Kenntniss Der S&urebildung Bei Typhus-Bacillen Und Bacterium Coli - Eine Differential-Diagnostische Studie, Zeitschrift f&r Hygiene und Infectionskrankheiten. 1896: **23**: 452-474.
7. Campbell MA, Campbell AD & Villaret DB. Molybdate reduction by *Escherichia coli* K-12 and its chl mutants. Proc Natl Acad Sci. USA.1985: **82**(1): 227-31.
8. Sugio T, Tsujita Y, Katagiri T, Inagaki K. & Tano T. Reduction of Mo^{6+} with elemental sulphur by *Thiobacillus ferrooxidants*. J Bacteriol. 1988: **170**(12):5956-5959.
9. Ghani B, Takai M, Hisham NZ, Kishimoto N, Ismail AKM, Tano T. & Sugio T. Isolation and characterization of a Mo^{6+}-reducing bacterium. Appl. Environ. Microbiol. 1993: **59**(4): 1176–1180.
10. Lloyd JR. Microbial reduction of metals and radionuclides. FEMS Microbial Rev. 2003: **27**(2-3):11-25.
11. Shukor MY, Habib SH, Rahman MF, Jirangon H, Abdullah MP, Shamaan NA. & Syed MA. Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain Dr. Y6. Appl. Biochem. Biotechnol. 2003: **149**(1):33– 43.
12. Yakasai HM. Microbial molybdate reduction to Mo-blue by a cyanide-degrading bacterium. Thesis submitted to the school of Graduate studies, Universiti Putra Malaysia, in fulfilment of the requirements for the degree of Doctor of Philosophy. 2017.
13. Shukor MY, Habib SH, Rahman MF, Jirangon H, Abdullah MP, Shamaan NA. & Syed MA. Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain Dr. Y6. Appl. Biochem. Biotechnol. 2008: **149**(1):33– 43.
14. Shukor MY, Rahman MF, Shamaan N. & Syed MA. Reduction of molybdate to molybdenum blue by *Enterobacter sp.* strain Dr.Y13. J. Basic Microbiol. 2009: **49**:43–54.
15. Shukor MY, Rahman MF, Suhaili Z, Shamaan NA. & Syed MA. Hexavalent molybdenum reduction to Mo-blue by *Acinetobacter calcoaceticus*. Folia Microbiol. 2010: **55**(2):137–143.
16. Abo-shakeer LKA, Ahmed SA, Shukor MY, Shamaan NA. & Syed NA. Isolation and characterization of a molybdenum-reducing *Bacillus pumilus* strain lbna. J Environ Microbiol Toxicol. 2013: **1**(1):9-14.
17. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmed SA. & Jirangon H. Kinectics of molybdenum reduction to molybdenum-blue by *Bacillus* sp. strain A. rzi. BioMed Res Int. 2013. 371058.
18. Halmi MIE, Wasoh H, Sukor S, Ahmad SA, Yusof MT & Shukor MY. Bioremoval of molybdenum from aqueous solution. Int. J. Agric Biol. 2014: 16: 848–850.
19. Khayat ME, Rahman MFA. & Shukor MS. Characterization of a molybdenum reducing *Bacillus* sp. strain Khayat with the ability to grow on SDS and diesel. Rend Acc Lincei. 2016: 29:547-556.
20. Rahman MFA, Shukor MY, Suhaili Z, Mustafa S, Shamaan NA. & Syed MA. Reduction of Mo (VI) by the bacterium *Serratia* sp. strain DRY5. J. Environ. Biol. 2009: 30(1): 65–72.
21. Lim HK, Syed MA. & Shukor MY. Reduction of molybdate to molybdenum blue by *Klebsiella* sp. strain hkeem. J. Basic Microbiol. 2012: 52(3):296–305.

22. Glenn JL. & Crane FL. Studies on metalloflavoproteins: The action of silicomolybdate in the reduction of cytochrome c by aldehyde oxidase. Biochim. Biophy. Acta. 1956: **22**(1): 111–115.
23. Runnells DD, Kaback DS. & Thurman EM. Geochemistry and sampling of molybdenum in sediments, soils, and plants in Colorado. Molybdenum in the environment. New York. 1976.
24. Shukor MY, Rahman MF, Suhaili Z, Shamaan NA. & Syed MA. Bacterial reduction of hexavalent molybdenum to molybdenum blue," World J Microbiol. & Biotechnol. 2009: **25**, (7): 1225–1234.
25. Gupta VK, Nayak A. & Ajarwal S. Biosorbents for remediation of heavy metals, current status and their future prospect. Environ. Eng. Res. 2012: **20**, 1-18.