Characterisation of the simultaneous molybdenum reduction and glyphosate degradation by *Burkholderia vietnamiensis* AQ5-12 and *Burkholderia* sp. AQ5-13

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Received: 31 July 2017 / Accepted: 30 January 2018 / Published online: 7 February 2018
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Abstract

In this novel study, we report on the use of two molybdenum-reducing bacteria with the ability to utilise the herbicide glyphosate as the phosphorus source. The bacteria reduced sodium molybdate to molybdenum blue (Mo-blue), a colloidal and insoluble product, which is less toxic. The characterisation of the molybdenum-reducing bacteria was carried out using resting cells immersed in low-phosphate molybdenum media. Two glyphosate-degrading bacteria, namely *Burkholderia vietnamiensis* AQ5-12 and *Burkholderia* sp. AQ5-13, were able to use glyphosate as a phosphorous source to support molybdenum reduction to Mo-blue. The bacteria optimally reduced molybdenum between the pHs of 6.25 and 8. The optimum concentrations of molybdate for strain *Burkholderia vietnamiensis* strain AQ5-12 was observed to be between 40 and 60 mM, while for *Burkholderia* sp. AQ5-13, the optimum molybdate concentration occurred between 40 and 50 mM. Furthermore, 5 mM of phosphate was seen as the optimum concentration supporting molybdenum reduction for both bacteria. The optimum temperature aiding Mo-blue formation ranged from 30 to 40 °C for *Burkholderia vietnamiensis* strain AQ5-12, whereas for *Burkholderia* sp. AQ5-13, the range was from 35 to 40 °C. Glucose was the best electron donor for supporting molybdate reduction, followed by sucrose, fructose and galactose for both strains. Ammonium sulphate was the best nitrogen source in supporting molybdenum reduction. Interestingly, increasing the glyphosate concentrations beyond 100 and 300 ppm for *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. AQ5-13, respectively, significantly inhibited molybdenum reduction. The ability of these bacteria to reduce molybdenum while degrading glyphosate is a useful process for the bioremediation of both toxicants.

Keywords Molybdenum · Molybdenum blue · Glyphosate degrading · *Burkholderia vietnamiensis* · *Burkholderia* sp.

Introduction

The expansion of glyphosate-based herbicides is encouraged by the emergence of glyphosate-resistant transgenic varieties of significant agriculture species (soy, colza, maize, wheat, sugar beet, and cotton), which accommodate 90% of all transgenic plant grown worldwide (Padgett et al. 1996).

However, the introduction of glyphosate-resistant transgenic crops has resulted in farmers abusing the amount of glyphosate applied to their crops resulting in the pollution of glyphosate in the environment (Duke and Powles 2008). Glyphosate residues have been found in aquatic organisms, posing a significant health hazard for the current and future generations due to the uptake and accumulation of glyphosate and its metabolites from the food chain and drinking water.

In general, glyphosate is a unique inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the key enzyme in the shikimate pathway of aromatic compounds' biosynthesis in plants and microorganisms. EPSPS inhibition suppresses the synthesis of proteins and secondary metabolites as well as de-regulating energy metabolism (Duke and Powles 2008). Since this enzyme is not found in mammals

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or animals, it is often assumed to possess no adverse effect on them. Nevertheless, studies on various aquatic organisms including *Oreochromis aureus*, *Piaractus brachipomus*, *Prochilodus lineatus* and *Poecilia reticulata* showed that they exhibit significant toxicity to these organisms (Ayoola 2008; Langiano and Martinez 2008; Ramírez-Duarte et al. 2008; Rocha et al. 2015). On the other hand, residues of glyphosate have also been found in humans, cattle, hares and rabbits (Krüger et al. 2014). Additionally, surfactants found in agrochemical formulations are often associated with glyphosate toxicity as the surfactants reduce the surface tension on the surface of weeds improving glyphosate absorption (Song et al. 2012).

Usually, glyphosate in soils is removed by microbiological processes (Gimsing et al. 2004). Although chemical processes of degradation have been reported such as photodegradation (Chen et al. 2007), oxidation with chlorine, permanganate and ozone (Barrett and McBride 2005), the degradation rate is exceptionally slow due to the presence of the carbon–phosphate bond that is highly resistant to chemical breakdown (Gimsing et al. 2004). Nonetheless, there are studies conducted on glyphosate-degrading bacteria with capabilities of the biodegradation of glyphosate at contaminated areas. Some of these known bacteria include *Enterobacter cloacae* (Kryuchkova et al. 2014), *Bacillus cereus* (Fan et al. 2012), *Azotobacter* sp. (Moneke et al. 2010), and *Achromobacter* sp. (Ermakova et al. 2010). Microorganisms can degrade glyphosate by utilising glyphosate as the sole carbon or phosphorus source via the aminomethylphosphonate (AMPA) or glycine pathways (Dick and Quinn 1995).

Molybdenum (Mo) is a transition element with an atomic number of 48 as discovered by Carl Wilhelm Scheele in the early 1778 (Jonathan et al. 2007). It is an essential heavy metal mostly used in the steel industry for producing alloys. Application of Mo can also be found in the agrochemical industry where its powdered form is used for producing fertilizers (Bandyopadhyay et al. 2008). In addition, it is essential for the growth of leguminous plants due to its role in nitrogen fixation.

Although Mo is an essential element in maintaining the physiological balance of plants and animals, its toxic effects have been reported in *in vivo* studies on rats where the degeneration of testicular morphology due to the decrease in weight of the testes and other accessory organs when sodium molybdate is administered orally has been observed (Pandey and Singh 2002). Additionally, Mo is known to inhibit spermatogenesis, which leads to the arrest of embryogenesis in mice and rats (Yamaguchi et al. 2007).

Numerous uses of molybdenum in various fields have left an adverse effect to the environment. Poor mining, industrial and agricultural waste management practices have led to severe water and soil pollutions worldwide. Several cases of molybdenum contamination have been reported in western

Liaoning, northeast China (Yu et al. 2010), Newcastle, England (Okorie et al. 2010) and Colorado, the United States (Xie and MacKenzie 1991) as well as the waters of the Black Sea (King et al. 1992). The removal of Mo from wastewater can be done through chemical processes including electrocoagulation, which involves the ability of the electrical current to dissolve the anode of iron, thereby forming positive ions that bind with Mo and form very fine bubble (Ramirez 1976). However, this approach is costly. Another economically viable approach is through bioremediation (Ghazali and Johari 2015). One of the most successful cases of the bioremediation of molybdenum in agricultural soil occurred in Austria where molybdenum pollution has led to the deaths and scouring in cattle (Neunhäuserer et al. 2001). As agricultural soil is also polluted with the excessive usage of pesticides for instance glyphosate, there exists the need to isolate microorganisms with the ability to remediate both of these toxicants.

The current study is the first report on the use of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 isolated from glyphosate-contaminated sites from Malaysia to reduce molybdenum while using glyphosate as its phosphorus source. Previously, the strains AQ5-12 and AQ5-13 were discovered to be capable of utilising glyphosate as a sole phosphorus source of up to 600 ppm. (Manogaran et al. 2017).

Materials and methods

Chemicals

Glyphosate PESTANAL® 99.5% analytical standard was purchased from Sigma-Aldrich, USA. Sodium molybdate (VII) 99% analytical grade and other chemicals used in the media were obtained from Fisher (Fisher Scientific (M) Sdn Bhd.).

Bacterial growth and maintenance of molybdenum-reducing isolates

Burkholderia vietnamiensis strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 were previously isolated from a glyphosate-contaminated site in the Kedah state, Malaysia, and were identified based on their 16S rRNA sequencing in a previous study (Manogaran et al. 2017). Both strains were cultured on low-phosphate media (LPM) at pH 7.0 with the ingredient composition (g/L) as follows: glucose (10), $(\text{NH}_4)_2\text{SO}_4$ (3), NaCl (5), yeast extract (0.5), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.05), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.242) and Na_2HPO_4 (0.71) (Yunus et al. 2009). Both bacteria were identified for their ability to reduce molybdate by streaking on LPM solid media. The presence of blue colonies indicates the presence of

molybdenum-reducing bacteria. Molybdenum reduction was carried out in liquid LPM. Both isolates were cultured in a 250-mL conical flask with 100 mL of LPM at room temperature (27 °C) for 72 h on an orbital shaker set at 150 rpm. Additionally, 1 mL of the 72-h culture was transferred into sterilised 250-mL conical flasks containing high-phosphate media for preparing resting cells using the same conditions.

Preparation of resting cells

Broth medium consisting of 10 g/L nutrient broth and 2 g/L yeast extract was prepared and autoclaved for 15 min at 121 °C. The medium was allowed to cool at room temperature, and then 2% (w/v) of 24-h pre-cultured *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 were added into separate 5-L conical flask. Aeration was provided via an aquarium air pump (BB-800, Chrometech Sdn. Bhd., Malaysia) fitted with a 0.45- μ m syringe filter (MCE, Chrometech Sdn. Bhd., Malaysia) followed by incubation at room temperature for 48 h. The bacterial cells were harvested at the early stationary phase by centrifuging at 10,000 $\times$ g at 4 °C for 10 min using a Beckman Coulter Avanti J-26 XPI centrifuge. The harvested cells were then washed twice using 10 mM Tris buffer and re-suspended in the same 10 mM Tris–base buffer (100 mL) and then stored at 8 °C until further use.

Effect of electron donors on Mo reduction

Twelve carbon sources, namely arabitol, glucose, glycerol, galactose, myo-inositol, lactose, maltitol, fructose, starch, sucrose, D-sorbitol and xylose were used as electron donors for molybdate reduction with the concentration fixed at 10 g/L while fixing the Mo and phosphate concentrations at 10 and 5 mM, respectively. The experiment was carried out by mixing 0.2 mL of resting cells preparation with 0.7 mL LPM (minus glucose) followed by the addition of 0.1 mL of carbon source from a stock solution for a total of 1 mL reaction mixture. The reaction mixture was incubated statistically for 24 h at 30 °C. Then the reaction mixture was centrifuged at 10,000 $\times$ g for 10 min and the supernatant was read at 865 nm.

Effect of phosphate and Mo concentrations on Mo reduction

Different phosphate concentrations ranging from 0 to 30 mM were used while fixing the molybdate concentration at 10 mM. Meanwhile, the effect of Mo concentration was studied using the concentrations ranging from 5 to 80 mM while fixing the phosphate concentration at 5 mM. The experiment was carried out by mixing 0.2 mL of resting cells preparation with 0.8 mL of LPM with the phosphate and

molybdate concentrations adjusted according to the above setting with glucose as the carbon source for a total of 1 mL reaction mixture. The reaction mixture was incubated statistically for 24 h at 30 °C. Then the reaction mixture was centrifuged at 10,000 $\times$ g for 10 min and the supernatant was read at 865 nm.

Effect of initial pH and temperature on Mo reduction

Different initial pHs ranging from 5.5 to 8.0 were set up by adjusting the media using either 1.0 M HCl or 1.0 M NaOH. A range of temperature from 10 to 60 °C was utilised in this work. The experiment was carried out by mixing 0.2 mL of resting cell preparation with 0.8 mL of LPM at various pHs (temperature set at 30 °C) and various temperatures (pH set at 7.0) with glucose as the carbon source for a total of 1 mL reaction mixture. The reaction mixture was incubated statistically for 24 h. Then the reaction mixture was centrifuged at 10,000 $\times$ g for 10 min and the supernatant was read at 865 nm.

Effect of nitrogen source on Mo reduction

Different nitrogen sources such as acetamide, ammonium sulphate, acrylamide, nicotinamide, sodium nitrate and urea were studied for their effect on Mo reduction at 3 g/L while fixing the Mo and phosphate concentrations at 10 and 5 mM, respectively. The experiment was carried out by mixing 0.2 mL of resting cell preparation with 0.8 mL of LPM containing various nitrogen sources with glucose as the carbon source for a total of 1 mL reaction mixture. The reaction mixture was incubated statistically for 24 h at 30 °C. Then the reaction mixture was centrifuged at 10,000 $\times$ g for 10 min and the supernatant was read at 865 nm.

Glyphosate as the phosphate source

The ability of glyphosate in aiding Mo reduction has been previously studied (Sabullah et al. 2016). Glyphosate was substituted as the phosphate source in LPM, whereas different concentrations of glyphosate ranging from 100 to 600 ppm were studied using both strains while fixing the Mo concentration at 10 mM. The experiment was carried out by mixing 0.2 mL of resting cells preparation with 0.8 mL of LPM with glyphosate at various concentrations replacing phosphate and glucose as the carbon source for a total of 1 mL reaction mixture. The reaction mixture was incubated statistically for 24 h at 30 °C. Then the reaction mixture was centrifuged at 10,000 $\times$ g for 10 min and the supernatant was read at 865 nm.

Statistical analysis

All the experiments were conducted in triplicate. Experimental errors are shown as error bars (standard deviation of three determinations). All the data were statistically analysed using GraphPad v3.5. One-way ANOVA analysis of variance using the Tukey's test was carried out and $p < 0.05$ was considered statistically significant.

Results

Twelve different types of carbon source were tested on both strains for their ability as electron donors for Mo reduction. These include arabinose, fructose, galactose, glucose, glycerol, lactose, maltitol, myo-inositol, sorbitol, starch, sucrose and xylitol (Fig. 1). *Burkholderia vietnamiensis* strain AQ5-12 preferred glucose as the best electron donor, while *Burkholderia* sp. strain AQ5-13 showed the highest Mo reduction when sucrose was supplemented as the electron source. Both strains only showed positive results for Mo reduction when fructose, galactose, glucose and sucrose were utilised as carbon sources. None of the strains were able to utilise the remaining carbon sources as electron donors for Mo reduction even though they were able to grow on them. No bacterial growth was observed on glycerol (data not shown).

Effect of different Mo concentrations ranging from 5 to 80 mM was examined while maintaining the phosphate concentration at 5 mM (Fig. 2). The optimum concentration of Mo for *Burkholderia vietnamiensis* strain AQ5-12 was observed between 40 and 60 mM where ANOVA analysis showed no significant difference in values within this range. For *Burkholderia* sp. strain AQ5-13, the optimum concentration of Mo occurred between 40 and 50 mM. Significant difference in Mo-reducing ability was observed in both strains

Fig. 1 Effect of different carbon sources on Mo reduction by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13. Data represent mean $\pm$ SD ($n = 3$)

Fig. 2 Effect of different Mo concentrations on reduction ability by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13. Data represent mean $\pm$ SD ($n = 3$)

between 35 and 40 mM based on ANOVA analysis. Higher concentrations were seen to strongly inhibit the reduction for both strains.

The optimum phosphate concentration occurred at 5 mM for both AQ5-12 and AQ5-13 strains (Fig. 3). *Burkholderia vietnamiensis* strain AQ5-12 showed better reduction compared to *Burkholderia* sp. strain AQ5-13 at 5 mM with an absorbance of 1.481 and 1.210, respectively. Higher phosphate concentrations beyond 5 mM were observed to strongly inhibit reduction.

Effect of different pH values ranging from 5.5 to 8.0 on the Mo reduction ability by both strains were studied (Fig. 4). The optimum pH range exhibited by *Burkholderia vietnamiensis* strain AQ5-12 was between pH 6.25 and

Fig. 3 Effect of different phosphate concentrations on Mo reduction by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13. Data represent mean $\pm$ SD ($n = 3$)

Fig. 4 Effect of different pH level on molybdenum reduction by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 ($n = 3$)

Fig. 5 Effect of temperature on Mo reduction by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13. Data represent mean $\pm$ SD ($n = 3$)

8.0, where there was no significant difference in that range ($p > 0.05$). Meanwhile, while *Burkholderia* sp. strain AQ5-13 showed optimum pH range of between 6.5 and 8.0 with no significant difference based on ANOVA analysis although the highest Mo reduction was observed at pH 8.0. *Burkholderia vietnamiensis* strain AQ5-12 showed better Mo reduction compared to strain *Burkholderia* sp. AQ5-13 at the same pH values.

The optimum temperature range for Mo reduction by strain AQ5-12 was between 30 and 40 °C where ANOVA analysis showed no significant difference between that range, while the optimum temperature for *Burkholderia* sp. strain AQ5-13 was between 35 and 40 °C, with no significant differences ($p > 0.05$) (Fig. 5). Lower temperatures

Fig. 6 Effect of nitrogen sources on Mo reduction by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13. Data represent mean $\pm$ SD ($n = 3$)

Fig. 7 Effect of different glyphosate concentrations on Mo reduction by resting cells of *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13. Data represent mean $\pm$ SD ($n = 3$)

beyond 30 °C and higher temperatures above 40 °C showed a decrease in Mo-blue formation.

The effect of nitrogen sources on Mo reduction was studied using acetamide, ammonium sulphate, acrylamide, nicotinamide, sodium nitrate and urea (Fig. 6). Ammonium sulphate was found to be the most effective supplement for supporting Mo reduction in both strains. *Burkholderia vietnamiensis* strain AQ5-12 showed the lowest Mo reduction when acrylamide was used, whereas sodium nitrate was found to be the least to aid in the formation of Mo-blue for *Burkholderia* sp. strain AQ5-13.

The effect of different glyphosate concentrations as the phosphate (PO_4^{3-}) substitute was studied on Mo reduction by both *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 (Fig. 7). Increasing glyphosate

concentrations from 100 to 500 ppm showed a decrease in Mo reduction rate by *Burkholderia* sp. strain AQ5-13, where the highest reduction rate was observed at 100 ppm. However, *Burkholderia* sp. strain AQ5-13 showed an increase in Mo reduction rate from 100 to 300 ppm. Nevertheless, the intensity of Mo-blue formation was significantly decreased when 400 and 500 ppm of glyphosate were introduced into the media. Both strains showed bacterial growth at 600 ppm of glyphosate (data not shown); however, no Mo-blue formation was observed.

Discussion

It is well known that Mo reduction in bacteria is best supported by easily assimilable sugars such as sucrose and glucose. Several previously isolated bacteria such as *Bacillus* sp. strain Neni-10 (Mansur et al. 2017), *Klebsiella oxytoca* strain SAW-5 (Sabullah et al. 2016), *Klebsiella oxytoca* strain Aft-7 (Masdor et al. 2015), *Pseudomonas* sp. strain DRY1 (Ahmad et al. 2013), *Bacillus* sp. strain A.rzi (Othman et al. 2013), *Pseudomonas* sp. strain DRY2 (Shukor et al. 2010a), *Acinetobacter calcoaceticus* strain Dr.Y12 (Shukor et al. 2010b) prefer glucose as their electron donor. Meanwhile, *Serratia marcescens* strain Dr.Y9 (Yunus et al. 2009) and *Serratia* sp. strain DRY5 (Rahman et al. 2009) require sucrose as the ideal carbon source to aid reduction. The ability of fructose in aiding Mo reduction is in agreement with a previous finding (Lim et al. 2012). Even though *Klebsiella* sp. strain hkeem (Lim et al. 2012) was able to utilise both glucose and sucrose, higher Mo reduction rate was observed when fructose is introduced as an electron donor. In any Mo reduction by bacterial strains, the presence of reducing equivalents, namely NADH (or reduced nicotinamide adenine dinucleotide) and NADPH (or reduced nicotinamide adenine dinucleotide phosphate), which act as substrates for Mo-reducing enzymes, is able to effect reduction rate. Both glucose and sucrose show high molybdenum reduction due to their affinity to produce more electron-donating substrates using generic metabolic pathways including glycolysis, Krebs' cycle and the electron transport chain compared to other carbon sources.

The determination of Mo and phosphate concentration in aiding optimal molybdenum reduction is crucial since both anions have been shown to inhibit Mo-blue production by bacteria (Shukor et al. 2010a; Ahmad et al. 2013). The inhibitory effect of high phosphate concentrations obtained in this study shows similarities to previous studies. Most of the previously isolated molybdenum-reducing bacteria such as *Bacillus* sp. strain Neni-10 (Mansur et al. 2017), *Klebsiella oxytoca* strain SAW-5 (Sabullah et al. 2016), *Pseudomonas* sp. strain DRY1 (Ahmad et al. 2013) and *Pseudomonas* sp. strain DRY2 (Shukor et al. 2010a) showed an optimum

reduction at 5 mM of phosphate, whereby increasing the concentration results in a lower reduction. This is due to the inhibitory effect of high phosphate concentration on the stability of the phosphomolybdate species, a key intermediate from molybdate to molybdenum blue (Glenn and Crane 1956).

Mo reduction steadily increased with Mo concentration up to 50 mM for both strains when phosphate concentration was fixed at 5 mM. This finding is in agreement with previously isolated bacteria such as *Serratia marcescens* strain Dr.Y6 (Shukor et al. 2008), *Pseudomonas* sp. strain DRY1 (Ahmad et al. 2013) and *Bacillus* sp. strain A.rzi (Othman et al. 2013), which showed the highest reduction rate at 50 mM. The toxicity of Mo beyond 50 mM results in a significant reduction of bacterial growth, and also the production of Mo-blue. Mo reduction increased steadily as the temperature increases culminating with a sharp optimum at 30 °C for *Burkholderia vietnamiensis* strain AQ5-12, while the highest reduction was observed at 40 °C for *Burkholderia* sp. strain AQ5-13. Beyond 40 °C, the production of Mo-blue was significantly decreased for both strains. Increase in temperature caused the enzyme to be more kinetically active, therefore, resulting in a higher yield of NADH⁺ and NADPH⁺, which are crucial substrates for Mo-blue formation. Similar pattern on the effect of temperature was observed in mesophilic molybdenum-reducing bacteria such as *Klebsiella oxytoca* strain SAW-5 (Sabullah et al. 2016) and *Serratia* sp. strain Dr.Y (Shukor et al. 2009).

Both strains showed optimum pH ranging from 6.25 to 8.0 for molybdenum reduction, which reflects a classic property of neutrophilic bacteria. The pH plays an important role in the formation and stability of phosphomolybdate complex in the reducing process (Shukor et al. 2008). The optimum pH for other isolated bacteria was pH 6.5 for *Klebsiella Oxytoca* strain Saw-5 (Sabullah et al. 2016), 6.5–7.5 for *Pseudomonas* sp. strain DRY1 (Ahmad et al. 2013), pH 7.0 for *Klebsiella oxytoca* strain DRY14 (Halmi et al. 2013) and pH 7.3 for *Klebsiella* sp. strain hkeem (Lim et al. 2012). Thus far, the only known *Burkholderia* genus able to reduce Mo is *Burkholderia* sp. strain Neni-11 (Mansur et al. 2016) with an optimum pH of between 6.0 and 6.3. Having a wide range of optimum pH range for *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 allows for better efficiency in application in contaminated sites, as the pH might differ in values.

There is a known diversity in the metabolic types of soil microbiota, which is capable of extracting nitrogen sources from organic and soil substances and then releasing them in the form of microbial nitrogen into the soil pool (Parimoolam and Dahalan 2013; Zulkharnain et al. 2013). Although soil nitrogen pool is naturally maintained by microbial activity, the availability of organic nitrogen is obtained from herbicides, fertilizers and decaying organic

matter (Kusnin et al. 2015). Since *Burkholderia vietnamiensis* strain AQ5-12 and *Burkholderia* sp. strain AQ5-13 were isolated from agricultural soil with a history of herbicide and fertilizer usage, it is crucial to screen the effect of various nitrogen sources on Mo reduction. Both strains showed maximum Mo reduction when ammonium sulphate was utilised as a nitrogen source. Most fertilizers contain ammonium sulphate or urea as the nitrogen-based ingredient, and these simple forms of nitrogen are easily assimilable by these bacteria (Gülser 2005). Frequent exposure to these types of fertilizer allows the isolated strains to efficiently utilise ammonium sulphate compared to other available nitrogen sources. Moreover, both strains were able to utilise urea and acrylamide, which can be found in fertilizers. Fertilizer can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil (Khonje et al. 1989). Therefore, increasing microbial biomass could result in better Mo reduction.

Glyphosate is an organophosphate made of three functional groups known as carboxyl, amino and phosphate where the molecules are held together by hydrogen bonds (Nandula 2010). The presence of phosphorus group in glyphosate makes it a suitable candidate for replacing Na_2HPO_4 in the LPM. Both strains showed positive results in Mo reduction when glyphosate is used as a sole phosphorus source. Although both strains were able to tolerate up to 600 ppm of glyphosate (Manogaran et al. 2017), increasing glyphosate concentration has resulted in poor Mo reduction rate. *Burkholderia* sp. strain AQ5-13 was able to reduce Mo better at 300 ppm compared to *Burkholderia vietnamiensis* strain AQ5-12. Even though the bacterial growth was observed at 600 ppm (data not shown), no Mo-blue formation was seen, therefore, significantly affecting reduction ability. This can be explained by the increasing glyphosate concentration resulting in the presence of more phosphate, of which high concentrations of the latter have been proven to reduce molybdenum blue production through the reduction of the stability of the phosphomolybdate complex (Glenn and Crane 1956; Shukor et al. 2002).

Conclusion

Two Mo-reducing bacteria, *Burkholderia* sp. strain AQ5-12 and *Burkholderia vietnamiensis* strain AQ5-13, with the novel ability to use glyphosate as the phosphorus source are reported. The highest Mo-reducing activity was observed when glucose was utilised as the carbon source. Strain AQ5-12 and strain AQ5-13 demonstrated optimum reduction when phosphate concentration was fixed at 5 mM. The highest Mo-blue formation for both strains occurred when Mo concentration was in the range from 40 to 50 mM while

the phosphate concentration was fixed at 5 mM. In addition, their ability to use glyphosate as a phosphate substitute has displayed positive results in aiding molybdenum reduction. Therefore, this is the first report of a molybdenum-reducing bacterium with the capacity to grow on glyphosate as the sole phosphorus source. Thus, the potential of this bacterium to accomplish the two functions shows that both bacteria can be beneficial as bioremediation agents in molybdenum-contaminated sites co-contaminated with pesticides.

Acknowledgements This project was supported by Putra-IPS fund received from Universiti Putra Malaysia under the Grant number 9486100 and Science fund received from the Ministry of Science, Technology and Innovation (MOSTI) Malaysia under the Grant number 02-01-04-sf1473. We also thank Universiti Putra Malaysia for providing a GRF scholarship to Mr. Motharasan Manogaran.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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