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Probing the location of metal reduction in the bacterium *S. marcescens* strain DrY6 using respiratory inhibitors

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Abstract

This work was carried out to locate the site of molybdenum reduction in *S. marcescens* strain DrY6 using several respiratory inhibitors. Based on the non-inhibitory results of the respiratory inhibitors tested such as antimycin A, rotenone, azide and cyanide, the site of molybdenum reduction in this bacterium is suggested not to be components of the electron transport pathway.

Key words

Molybdenum reduction, Molybdenum blue, *Serratia marcescens*, Electron transport pathway

Introduction

Microbes have the ability to remediate heavy metals (AbdEl-Ghany and AbdEl-Mongy, 2009; Gupta *et al.,* 2012) through various mechanisms such as bioreduction, active efflux of heavy metals, biochelation and bioprecipitation (Nada *et al.*, 2009; Karelová *et al.,* 2011; Aggangan and Aggangan, 2012).

Molybdenum is one of the toxic significant heavy metal present in the environment. The ability of bacteria to detoxify molybdenum by converting molybdate ions into colloidal molybdenum blue is an important bioremediation tool (Ghani *et al.,* 1993). Recently, a local molybdenum-reducing bacteria, *Serratia marcescens* strain DrY6 (Shukor *et al.,* 2008a) has been isolated and characterized. The previous work of Ghani *et al.* (1993) demonstrated that the site of molybdenum reduction in *E. cloacae* strain 48-another molybdenum reducer, is downstream from cytochrome b in the electron transport pathway based on the inhibition of the enzyme activity by cyanide. In this work, the use of several respiratory inhibitors to determine whether the electron transport chain is the site of molybdenum reduction in this bacterium is reported.

Materials and Methods

The bacterium *S. marcescens* was maintained in low phosphate molybdate media (w/v) containing sucrose (1%), $(NH_4)_2SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), NaCl (0.5%), yeast extract (0.05%), $Na_2MoO_4.2H_2O$ (20 mM) and Na_2HPO_4 (5 mM) and pH 7.0 (Shukor *et al.,* 2008a).

Assay for molybdenum-reducing activity : Moly bdenum-reducing enzyme was assayed according to the method of Shukor *et al.,* (2008b). One unit of Mo-reducing activity is defined as that amount of enzyme that produce 1 nmole molybdenum blue per minute at room temperature. The specific extinction coefficient at 865 nm for the product; molybdenum blue, was determined by means of a standard curve obtained using ascorbate-reduced 12-phoshomolybdate. The specific extinction coefficient at 865 nm is 16.7 $mM^{-1}cm^{-1}$ (Shukor *et al.,* 2000).

Preparation of crude enzyme: Cells were harvested through centrifugation at 10,000 g for 10 min, washed once with distilled water, resuspended and recentrifuged. The pellet was reconstituted with 10 ml of 50 mM Tris buffer pH 7.5 containing 0.1

mM phenyl methan esulphonyl fluoride. Cells were sonicated for 1 min on an ice bath with 4 min cooling (total 20 min) and centrifuged for 20 minutes at 10 000 g. The supernatant containing crude enzyme fraction was taken.

Effects of respiratory inhibitors : Respiratory inhibitors such as antimycin A, sodium azide, potassium cyanide and rotenone were prepared as 20 mM, 50 mM, 60 mM and 50 mM stock solutions, respectively, in acetone. Inhibitors were added into the enzyme assay mixture to the final concentrations of 1.2, 10, 10 and 0.2 mM, respectively, in a volume not exceeding 20% of assay volume to prevent shifting in assay pH. The concentrations of inhibitors used in this assay were at least five times more than the suggested concentrations that would normally cause more than 50% inhibition of activity per mg of protein (Shukor et al., 2008a).

Results and Discussion

None of the respiratory inhibitors tested showed any significant inhibition of more than 10% to the Mo-reducing activity in this bacterium (Table 1). Rotenone is an inhibitor to NADH dehydrogenase while sodium azide and cyanide are inhibitors to the terminal cytochrome oxidase. Antimycin A inhibits cytochrome b. In contrast to the results obtained from EC 48, the results suggested that the electron transport pathway of this bacterium is not the site of molybdenum-reduction. Initially, it was thought that the different assay composition employed might affect the outcome of the results. Thus, the original assay was employed using molybdate as the electron acceptor substrate. Again, it was observed that there was no inhibition of more than 10% to the molybdenum-reducing activity (Data not shown). The use of respiratory inhibitors to probe location of metal-reducing or oxidizing enzyme showed mixed results. Respiratory inhibitors such as rotenone, azide and cyanide failed to inhibit chromate reduction in E. coli (Shen and Wang, 1993) and in Pseudomonas mendocina (Rajwade *et al.,* 1999). Metal-reducing enzyme like mercury reductase is known to be not associated with the electron transport system (Lovley *et al.,* 1993) of bacteria and it is suggested that Mo-reducing activity in *S. marcescens* strain Dr.Y6 is also similar. In conclusion, the use of respiratory inhibitors has shown that the site of the molybdenum-reducing activity in this bacterium is not the respiratory inhibitors. Future works are needed to identify the molybdenum-reducing activity so that the site of molybdenum reduction can be determined.

Table 1 : Effect of respiratory inhibitors on molybdate reduction in *S. marcescens* strain DrY6.

Respiratory inhibitors	Concentration which normally gives 50% inhibition per mg protein	% Activity
Rotenone	10-8 M	89.21 ± 2.88
Azide	1 mM	95.68 ± 5.54
Cyanide	~ 0.5 mM	100.48 ± 5.74
Antimycin A	0.5 uM	108.41 ± 3.75

Values are mean of 3 replicates ± SD

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References

Abd El-Ghany, T.M. and M. Abd El-Mongy: Bioremoval of heavy metals in presence of oxalic and citric acid using *Aspergillus tamarii*. *Egypt. J. Exp. Biol. (Bot.)*, **5**, 53–58 (2009).

Aggangan, N.S. and B.J.S. Aggangan: Selection of ectomycorrhizal fungi and tree species for rehabilitation of Cu mine tailings in the Philippines. *J. Environ. Sci. Manag.*, **15**, 59–71 (2012).

Ghani, B, M. Takai, N.Z. Hisham, N. Kishimito, M.I.A. Ismail, T. Tano and T. Sugio: Isolation and characterization of a Mo^{6+}-reducing bacterium. *Appl. Environ. Microbiol.*, **59**, 1176–1180 (1993).

Gupta, K., C. Chatterjee and B. Gupta: Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India. *Biologia,* **67**, 827–836 (2012).

Karelová, E., J. Harichová, T. Stojnev, D. Pangallo and P. Ferianc: The isolation of heavy-metal resistant culturable bacteria and resistance determinants from a heavy-metal-contaminated site. *Biologia,* **66**, 18–26 (2011).

Lovley, D.R.: Dissimilatory metal reduction. *Annu. Rev. Microbiol.*, **47**, 263–290 (1993).

Nada, A.M., S. Abd El-Mongy and E.S. Abd El-Sayed: Effect of different treatments on cellulose toward carboxylation and its application for metal ion absorption. *Bio Res.*, **4**, 80–93 (2009).

Rajwade, J.M., P.B. Salunke and K.M. Pknikar: Biochemical basis of chromate reduction by Pseudomonas mendocina. Proc. Inter. Biohydrometall, Symp. Elsevier, New York, pp. 105–114 (1999).

Shen, H. and Y. Wang: Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. *Appl. Environ. Microbiol.*, **59**, 3171–3777 (1993).

Shukor, M.Y., N.A. Shamaan, M.A. Syed, C.H. Lee and M.I.A. Karim: Characterization and quantification of Mo-blue production in *Enterobacter cloacae* strain 48 using 12-phosphomolybdate as the reference compound. *Asia Pac. J. Mol. Biol. Biotechnol.*, **8**, 167–172 (2000).

Shukor, M.Y., S.H.M. Habib, M.F.A. Rahman, H. Jirangon, M.P.A. Abdullah, N.A. Shamaan and M.A. Syed: Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain DrY6. *Appl. Biochem. Biotechnol.*, **149**, 33–43 (2008a).

Shukor, M.Y., M.F.A. Rahman, N.A. Shamaan, C.H. Lee, M.I.A Karim and M.A. Syed: An improved enzyme assay for molybdenum-reducing activity in bacteria. *Appl. Biochem. Biotechnol.*, **144**, 293–300 (2008b).