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Short Communication

Dialysis Tubing Experiment Showed that Molybdenum Reduction in *S. marcescens* strain DrY6 is Mediated by Enzymatic Action

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ABSTRACT

Reduction of metal ions by microbes has long been afflicted by the contribution of abiotic and nonabiotic metal-reducing chemicals. The discrimination between enzymatic and chemical action upon metal must be solved to present a clear reduction mechanism. In this work, a previously developed method using dialysis tubing was employed to study the contribution of these agents upon molybdenum reduction to molybdenum blue in *S. marcescens* strain Dr.Y6. We discovered that approximately 95 % of molybdenum blue formed was found in the inside of the dialysis tubing. This suggests that the reduction of molybdate by this bacterium requires the presence of cells or mediated enzymatically.

Among the heavy metals, molybdenum is an emerging global metal pollution [4]. Microbial molybdenum reduction to molybdenum blue is a phenomenon that have been reported for more than one hundred years and is a potential bioremediation tool. Microbial molybdate reduction to molybdenum blue was first mentioned in 1896 by Capaldi and Proskauer [3]. Other reports on mirobial molybdate reduction were by Jan (1989) [9], Marchal and Gerard (1948) [10], Woolfolk and Whiteley (1962) [30] and Bautista and Alexander (1972) [1]. It was not until 1985 that Campbell revitalizes the phenomenon of molybdate reduction in *E. coli* K12 [2]. In 1988, Sugio and co-workers reported on the reduction of molybdate into molybdenum blue by *Thiobacillus ferrooxidans* strain AP19-3 [27]. In 1993, Ghani *et al*., 1993 reported that *Enterobacter cloacae* strain 48 (EC 48) could also reduce molybdate (molybdenum 6^+) to molybdenum blue (molybdenum 5^+) [6]. Recently, we have isolated and characterized a local molybdenum-reducing *Serratia marcescens* [17]. This is the second local strain isolated after EC 48. One of the biggest problems associated with microbial metal reduction is the problem with chemical reduction masking the role of enzyme

[8]. For instance, ferric reduction to ferrous iron in the environment has been suggested to be due to the lowering of the ambient redox potential as a result of microbial metabolism. According to equilibrium thermodynamics, this should shift the Fe(III)-Fe(II) equilibrium in favor of soluble Fe(II) [8]. This chemical reduction has been extrapolated to many metal ions negating the contribution of microbial enzymes. Fortunately, Munch and Ottow (1983) has designed a method using dialysis tubing to prove that ferric reduction in *Clostridium butyricum* is mediated by enzyme [11]. We modified the method by incorporating the molybdenum-reducing bacterium inside the dialysis tubing and prove that molybdenum-reduction in EC 48 is mediated by enzymatic action. In this method, using the same method we prove that the reduction of molybdenum to molybdenum blue in *S. marcescens strain* Dr.Y6 is mediated by enzymatic action.

S. marcescens was maintained in low phosphate molybdate media (LPM) media (pH 7.0) containing (w/v %) 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). Sucrose was autoclaved separately [17]. Growth in liquid media uses the same media as in the solid media above. Molybdenum blue is produced in this media but not at high phosphate media (100 mM phosphate). *S. marcescens* strain Dr.Y6 was grown and maintained on the above low phosphate liquid and solid media.

Bacterium was grown in 250 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) $(NH_4)_2SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), NaCl (0.5%), yeast extract (0.05%) and Na_2HPO_4 (5 mM). About 8 ml of this suspension was then placed in dialysis tubing (12,000 Dalton mwt cut-off, Sigma) previously boiled for ten minutes and immersed in sterile 100 ml of LPM 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 4 hours 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. Molybdenum blue was determined by means of a standard curve obtained using ascorbate-reduced 12-phoshomolybdate. The specific extinction coefficient for molybdenum blue is 16.7 $mM.^{-1}.cm^{-1}$ at 865 nm [15].

This method was developed to determine the possibility that molybdate reduction in this bacterium was chemically mediated as was discovered in *T. ferreoxidans* by Yong *et al.* (1997) [31]. In this method bacteria were enclosed in dialysis tubings and were allowed to reduce molybdate which was present in the outside and inside of the dialysis tubing. This method works because the reduced product; molybdenum blue, is colloidal and if placed in a dialysis tubing would diffuse very slowly [25]. If the reduction is mediated by chemical reductants produced abiotically by the bacterium, molybdenum blue would be observed in the inside and outside of the dialysis tubing in approximately equal concentration. If the reduction is mediated by enzyme(s) either extracellularly or intracellularly, reduction would only be observed in the inside of the tubings.

It is anticipated that even if the reduction of molybdenum occurs exclusively in the dialysis tubing, a certain percentage of the molybdenum blue would be found in the outside of the tubing due to diffusion as found in EC 48 [16].

Table 1. Amount of Mo-blue produced in the inside and in the outside of dialysis tubing after a static incubation period of 4 hours at room temperature. Data is mean± standard error (n=3).

Samples	Amount of Mo-blue produced (µmole)	Percent of total Mo-blue produced (%)
Inside of dialysis tubing	0.60±0.02	95.23
Outside of dialysis tubing	0.03±0.001	4.77

CONCLUSION

In this work, we found that approximately 95 % of Mo-blue was found in the inside of the dialysis tubing after the incubation period (Table 1). This suggests that the reduction of molybdate by this bacterium requires the presence of cells or mediated enzymatically. In EC 48 it was found that almost 90% of the Mo-blue produced was trapped in the dialysis tubing and the same conclusion was achieved [16].

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