Characterization of Molybdenum-reduction by an Acrylamide-degrading Antarctic bacterium

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Abstract

A molybdenum reducing bacterium previously isolated as an acrylamide-degrading bacterium from Antarctica is reported. The bacterium reduces sodium molybdate to molybdenum blue. The bacterium is able to use acrylamide as an electron donor source for molybdenum reduction. Reduction of molybdenum at various concentrations of acrylamide (100 to 700 mg/L) showed sigmoidal profiles with concentrations of acrylamide higher than 300 mg/L were inhibitory to growth. The modified Gompertz model was successfully used to fit the growth data with good fitting. The results of the reduction parameters from the modified Gompertz model will be useful to model the effect of the substrate to reduction rate in the future.

Introduction

Molybdenum has many uses in industries such as alloying agent, automobile engine anti-freeze component, component of corrosion resistant steel and as a lubricant in the form of molybdenum disulphide. Molybdenum toxicity in inhibiting spermatogenesis and arresting embryogenesis in organisms such as catfish and mice at levels as low as several parts per million have been reported (Yamaguchi et al., 2007). Furthermore, molybdenum is very toxic to ruminants at levels of several parts per million is the most affected are cows (Kincaid, 1980). Molybdenum deposits have been identified in Antarctica, and although these deposits have been banned from mining by the Madrid protocol, the ban is up for review in 2041 (Heim, 1990). Even without the presence of mining activities, molybdenum has been found as a pollutant in Antarctica due to anthropogenic sources. There is a very limited study on the potential bioremediation of microorganism isolated from Antarctica.

Here we report on a previously isolated acrylamide-degrading Antarctic bacterium with the novel capacity to reduce molybdenum but with much less efficiency than glucose. The bacterium was able to use acrylamide as a source of electron donor for reduction. The characteristics of this bacterium would make it suitable for future bioremediation works in Antarctica and temperate regions involving both the heavy metal molybdenum and amides as organic contaminants.

Preliminary screening of our inhouse culture collection resulted in the discovery that a previously isolated acrylamide-degrading Antarctic bacterium (Shukor et al., 2009) can reduce molybdenum into molybdenum blue (Mo-blue). The composition (w/v) of the low phosphate media (LPM) for supporting and maintenance of molybdenum reduction is as follows: glucose (1%), $(NH_4)_2.SO_4$ (0.3%), $MgSO_4.7H_2O$ (0.05%), yeast extract (0.5%), NaCl (0.5%), $Na_2MoO_4.2H_2O$ (0.242 % or 10 mM) and Na_2HPO_4 (0.071% or 5 mM). Molybdenum reduction in liquid media (at pH 7.0) was carried out in 100 mL of the above media in a 250 mL shake flask culture at 15 °C for 72 hours on an orbital shaker set at 120 rpm with the same media above, but the phosphate concentration increased to 100 mM. To study the effect of acrylamide as a carbon source for molybdenum reduction, the glucose in the media was replaced with various concentrations of acrylamide, and the absence of both carbon source acts as a control. Molybdenum reduction profile for each acrylamide concentrations was then modelled using the modified Gompertz as follows;

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\}$$

Where A is a Mo-blue lower asymptote, μ_m= maximum specific Mo-blue production rate, λ is a lag time, y_{max} is Mo-blue upper asymptote, e is an exponent (2.718281828) and t is sampling time.

The course of molybdenum reduction, measured as the production of molybdenum blue measured at 865 nm at various concentrations of acrylamide indicated good reduction activity with sigmoidal profiles at 100, and 200 mg/L of acrylamide, whilst higher concentrations were inhibitory to growth. In addition, significant lag periods were observed for all concentrations (**Fig. 1a**). The modified Gompertz model was then utilized to fit the growth

data on various concentrations of acrylamide. The result was good fitting (**Fig 1b**) with correlation of coefficient values from 0.97 to 0.99. Curves for acrylamide concentrations of 500, 600 and 700 mg/L were not fitted to the model as the software reported a failure of convergence, probably due to the very low growth absorbances obtained.

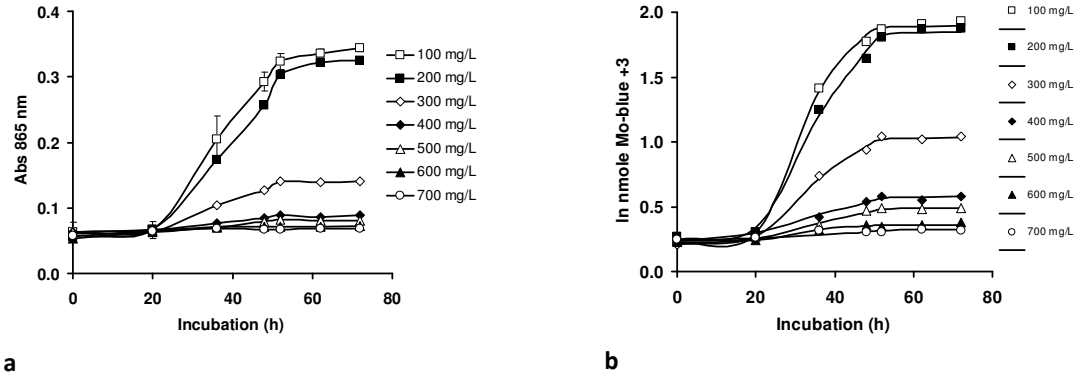

Fig. 1. The effect of various concentrations of acrylamide as an electron donor source for molybdenum reduction—(**a**) and its subsequent fitting using the modified Gompertz model—(**b**). Data from (**a**) was log natural (ln) transformed prior to modelling.

The reduction parameters (**Table 1**) indicate a trend of an increase in the lag period as the concentrations of acrylamide was increased. In addition, the maximum absorption at 600 nm (Y_{max}) and the specific reduction rate (μ_{max}) decreased to zero as the concentrations of acrylamide was increased. Acrylamide is toxic to bacterial growth as it binds to thiol groups of proteins at high concentrations forming adducts that denatures the activity of enzymes and proteins (Cavins and Friedman, 1968). This is the second bacteria from Antarctica that has shown molybdenum reduction capacity. The first bacterium was a *Pseudomonas* species (Ahmad et al., 2013).

Table 1. Modified Gompertz parameters at various concentrations of acrylamide (±standard error).

	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L	600 mg/L	700 mg/L
Lag (h)	23.54±1.73	25.15±2.122	27.303±1.935	30.12±1.52	n.a.	n.a.	n.a.
Y_{max}	0.81±0.01	0.341±0.11	0.325±0.005	0.009±0.001	n.a.	n.a.	n.a.
μ_{max}	0.026±0.003	0.01±0.001	0.011±0.002	0.005±0.002	0	0	0

Note:
n.a. not available because software failed to fit the data

In conclusion, this is the first report of a bacterium with dual biotransformation capacity from Antarctica. The bacterium was able to use acrylamide as an electron donor for molybdenum biotransformation to molybdenum blue. The modified Gompertz model was successfully used to fit the growth curve at various concentrations of acrylamide.

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