Bioremediation of V^{5+} and Ni^{2+} by indigenous *Marinobacter Goseongensis* isolated from South African Vanadium Mine Waste

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Abstract Study assessed the bioremediation of V^{5+} and Ni^{2+} in wastewater by an indigenous bacterial species. Bacterial species was isolated from mine water and characterized by sequencing. The isolate appeared to tolerate V^{5+} and Ni^{2+} separately at concentrations ranging between 650 to 700 mg/L and 250 and 300 mg/L (24h-LC_5), respectively. Removal of V^{5+} and Ni^{2+} in the media was inversely proportional to the test metal concentrations. *Marinobacter goseongensis* could remove more than 99.99 % of both metals at 50 mg/L at below the maximum permissible limit of 0.1 mg/L. This indigenous bacterial isolate can be used in V^{5+} and Ni^{2+} bioremediation.

Keywords Mine water, microbial diversity, *Marinobacter*, vanadium, nickel, pollution, bioremediation

Introduction

Mine water remains one of the major problems of concern, not only in South Africa, but also worldwide. This is due to its environmental, socio-economic and public health impacts (Oelofse 2009). In South Africa in particular, mining activities have a long history and have played a major role in both economic development and environmental pollution countrywide (Adler *et al.* 2007). Although significant progress has been made to address mine water management, environmental pollution due to the disposal of untreated mine water still remains a challenge. Microorganisms, due to their ubiquitousness, have been viewed as one of the best ways to deal with this problem. Due to their ability to survive, grow and reproduce in such harsh environments, an interest in microorganisms was aroused among researchers worldwide (Zhou *et al.* 2010). Nevertheless, their presence in extreme environments such as mine water affects their species diversity (Wang *et al.* 2011). Wang *et al.* (2011) have pointed out that extreme conditions can be defined by levels of environmental factors, the effects of which pose difficulties for the survival of specific taxa or all taxa. In addition, both Johnson and Hallberg (2003) and Imarla *et al.* (2006) have also reported that a microbial community composition is largely bound to geochemical parameters such as pH and metal ion concentrations. As a result, microorganisms isolated from such environments are considered to constitute a valuable tool in the treatment of highly polluted mine water. Knowing that microorganisms, with bacteria in the lead, represent the largest reservoir of undescribed biodiversity (Tekere *et al.* 2011); their study has proved to be of great importance to science. This study aims at assessing the resistance and bioremediation ability of indigenous bacterial isolate to both V^{5+} and Ni^{2+} in a modified wastewater mixed liquor.

Methods

The *Marinobacter* species was isolated according to Huu *et al.* (1999) using Halomonas elongata (HMC) medium. Prior to be used, the isolate was identified by sequencing thereafter exposed to V^{5+} and Ni^{2+} ions at various concentration (from 50 to 800 mg/L) in the modified mixed liquor (Kamika and Momba 2011). Prior to assessing the metal tolerance ability of the isolates, the optimum growth temperature of MWI-1 was determined by incubating the isolates at various temperatures (25 °C, 30 °C and 35 °C) and in the HMC broth. Sample flasks as well as positive controls were inoculated with the isolates (approximately 100 cfu/mL) incubated at 30 °C ± 2 °C for 4 days. The median lethal concentration (LC_{50}) of the test metal for each of the test microbial isolates was determined as described by previous investigators (Kamika and Momba 2011). The minimum inhibitory concentration (MIC) of the test metal (referring to the smallest concentration of an antimicrobial agent necessary to inhibit growth of microorganisms) was determined according to Shirdam *et al.* (2006). MIC values were noted when the isolates failed to grow on the plates. After incubation, the microbial isolates were classified as being sensitive or tolerant to Ni^{2+} according to the inhibition of growth cells.

The genera *Marinobacter* were isolated (MWI-1) from the mine water samples and assessed for their possible ability to resist V^{5+} and Ni^{2+}. The phylogenetic analysis using the neighbour-joining method with a bootstrap value of 100 replicates indicated that an MWI-1 [AB793286] isolate belonged to the genus *Marinobacter* and was most closely related to *Marinobacter goseongensis* strain En6 [EF660754.1 and NR044340.1] at a similarity of approximately 97 % (fig. 1).

During the study the growth curves of the MWI-1 in a metal-free medium (HMC broth) revealed a lag-phase between time 0 to 2 followed by an exponential phase from time 2 h to 8 h when inoculated at 25 °C, 30 °C and 35 °C with bacterial counts of 7 log CFU/mL, 8 log CFU/mL and 7 log CFU/mL, respectively. At 30 °C, MWI-1 indicated a second exponential growth (10 logCFU/mL) from time 16 h to the end of the experiment, whereas a death phase was observed at 25 °C and 35 °C, repectively.

As MWI-1 grew very well at 30 °C, its tolerance to V^{5+} and Ni^{2+} in the modified mixed liquor was tested at the said temperature and at pH 7.2 ± 0.2. The growth performance of this isolate in the modified mixed liquor containing either V^{5+} or Ni^{2+} or both at two different concentrations in order to highlight the difference of the toxic effect between the two metals (fig. 2).

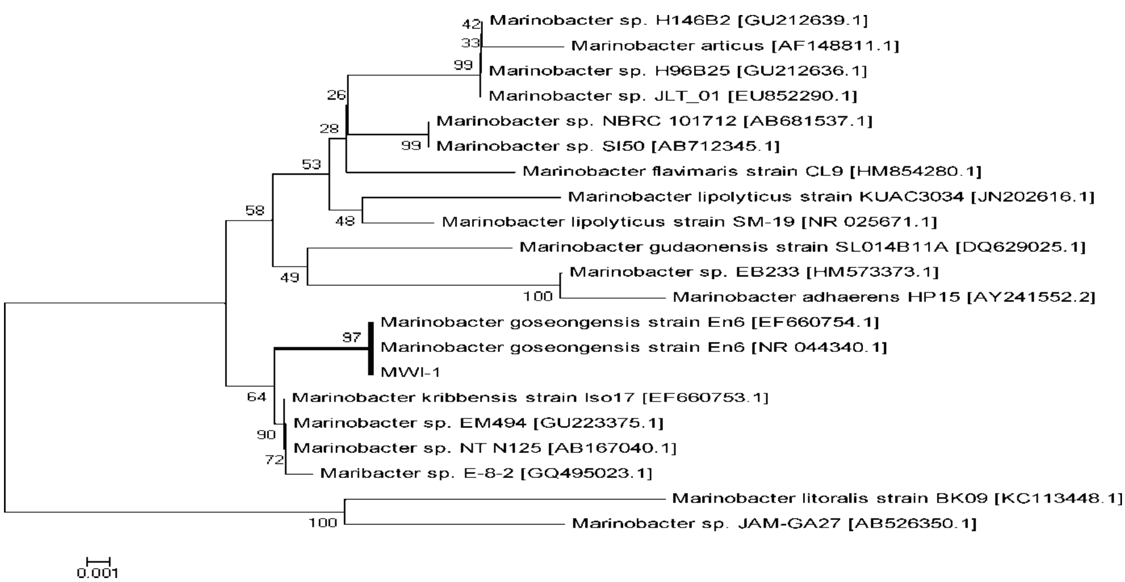

Fig. 1 *Phylogenetic tree using the neighbour-joining method, constructed and based on the bacterial 16S rRNA gene sequence detected in the present study along with similar sequences detected from the NCBI and RDP databases.*

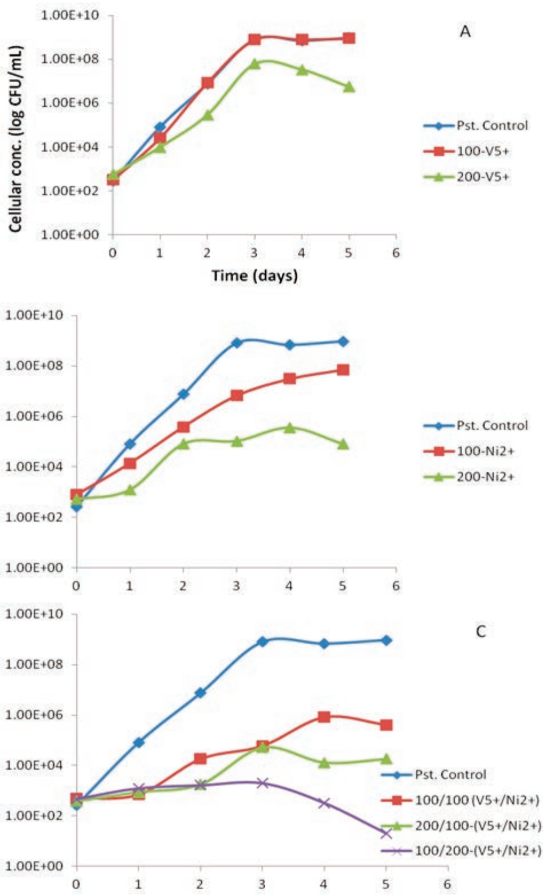

Fig. 2 *Growth performance of MWI-1 in a medium containing either V^{5+} (A) or Ni^{2+} (B) or both (B) at 100 mg/L and 200 mg/L, 30 °C, pH 7.2±0.2.*

In general, the growth of the isolates decreased with the increases of the metal concentrations. The MWI-1 was able to significantly grow in the presence of V^{5+} at 100 mg/L (9 log CFU/mL) and 200 mg/L (8 log CFU/mL), while in the presence of Ni^{2+} MWI-1 could only grow at 100 mg/L. Concomitantly, Ni^{2+} toxicity was able to inhibit the growth in all the volume ratios. Statistical evidence revealed a significant difference ($p<0.05$) in terms of growth performance between the positive controls and those samples treated with Ni^{2+} while no significant difference ($p>0.05$) was indicated for MWI-1 treated with V^{5+}. Another significant difference was also noted for positive controls when compared with those samples treated with both metals concomitantly.

A general observation indicated that the MWI-1 isolate was more tolerant to V^{5+} than to Ni^{2+} in the modified mixed liquor (Table 1). The MWI-1 isolate could resist V^{5+} up to 650–700 mg/L (MIC) and only reached 200–250 mg/L in the presence of Ni^{2+}. The tolerance or sensitivity of the MWI-1 isolate was also revealed by its ability to remove V^{5+} and Ni^{2+} (Table 1 and 2). In the presence of V^{5+} or Ni^{2+} separately, the MWI-1 was able to remove up to 99.95 % of 100 mg/L V^{5+} and 86.42 % of 100 mg/L Ni^{2+}. When in concomitance with each other (Table 2), the Ni^{2+} toxicity disturbed the removal of V^{5+} in the modified mixed liquor. In consortium, none of the metals (V^{5+}/Ni^{2+}) was removed at a percentage of over 30 %, with the exception of V^{5+} (30.15 %) that was removed at a ratio of 1:1 (100 mg/L/100 mg/L).

Due to their abundance, the *Marinobacter* genus of the *Proteobacteria phylum* was isolated in the present study and assessed for their tolerance to and removal of V^{5+} and Ni^{2+} in the modified mixed liquor. The isolate (MWI-1) was firstly sequenced and found to be closely related to *Marinobacter goseongensis*.

Experiment	Tolerance limit (mg/L)				Percentage removal (%)			
	24h-LC50 (V^{5+})	MIC (V^{5+})	24h-LC50 (Ni^{2+})	MIC (Ni^{2+})	100 mg/L V^{5+}	200 mg/L V^{5+}	100 mg/L Ni^{2+}	200 mg/L Ni^{2+}
1st	550	700	250	350	99.95	96.38	81.36	47.28
2nd	500	650	200	300	99.98	91.28	86.25	43.95
3rd	500	700	200	350	98.97	94.65	79.64	39.78
	500-550	650-700	200-250	300-350	99.63	94.10	82.42	43.67

Table 1 *Tolerance limit and removal ability of MWI-1 in the modified mixed liquor containing V^{5+} and Ni^{2+} separately.*

	100/100 (mg/L) [V^{5+}/Ni^{2+}, v/v]		200/100 (mg/L) [V^{5+}/Ni^{2+}, v/v]		100/200 (mg/L) [V^{5+}/Ni^{2+}, v/v]	
	V^{5+} (%)	Ni^{2+} (%)	V^{5+} (%)	Ni^{2+} (%)	V^{5+} (%)	Ni^{2+} (%)
1st	27.05	26.15	16.31	11.07	9.87	3.68
2nd	25.13	21.08	18.05	13.12	7.38	5.28
3rd	38.27	24.65	17.15	10.24	11.28	9.17
	30.15	23.96	17.17	11.48	9.51	6.04

Table 2 *Removal ability of MWI-1 in the modified mixed liquor containing V^{5+} and Ni^{2+} in consortium.*

This MWI-1 showed very good growth at 30 °C in the HMC broth, while at 25 °C and 35 °C a prompt die-off was noted when inoculated in a metal-free media. Findings of this study corroborated those of Roh *et al.* (2008) who reported that the optimum temperature for *Marinobacter goseongensis* sp. nov. should be between 25 °C and 30 °C. When inoculated in modified mixed liquor, culture media containing V^{5+} and Ni^{2+}, separately or combined and incubated at 30 °C, reveals a significant growth ($p<0.05$) of the MWI-1 in the media with V^{5+} when compared to the media with Ni^{2+}. The MWI-1 bacterial isolate was more tolerant towards V^{5+} than towards Ni^{2+} (Table 1). The toxicity of the test metals in MWI-1 appeared to have a relatively negative effect on the metal-removal ability of the test isolate in the modified mixed liquor with V^{5+} indicating the highest level of removal (Table 1). It has been reported that bacterial strains can be characterised as being tolerant towards metal such as Ni^{2+}, if it is capable of expressing growth at concentrations higher than 100 mg/L of the metal (Gikas 2008).

Zucconi *et al.* (2003) stated that isolating microorganisms from extreme environments represent an appropriate practice to select metal-resistant strains that could be used for metal removal and bioremediation purposes. Since it has been reported that most of the species of the genus *Marinobacter* are halophilic, heterotrophic neutrophiles and living under extreme environmental conditions such as pH and high salinity, they have been isolated from several habitats such as seawater, petroleum refineries, oil-refineies, and so forth (Guo *et al.* 2007). According to Brito *et al.* (2006), the *Marinobacter* species are the best degraders of hydrocarbons and have been associated with the removal of hydrocarbons in seawater. Owing to their ability to grow under extreme habitats, strains found in *Marinobacter* could have the ability to remove metal in the environment. Researchers have reported that several strains of *Marinobacter spp.* such as *Marinobacter aquaeolei* possess iron transport capabilities and are also capable of oxidising iron (Amin *et al.* 2012).

Conclusion

This study revealed that highly contaminated and toxic mine water effluents are a reservoir of novel microbial species which can adequately be used for the removal of metals in highly polluted effluents. The MWI-1 isolate, closely related to *Marinobacter goseongensis*, demonstrates high tolerance to both V^{5+} and Ni^{2+}. Further studies on geochemistry and microbial diversity need to be conducted in order to unveil how the chemistry of the effluent from the vanadium mine in South Africa can affect the microbial diversity of the environment. Furthermore, studies carried out on microbial diversity in extreme environments such as mine water are needed in order to isolate novel hyper-tolerant microbial species for the removal of metals.

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