Metal-Tolerating ability of some selected *Rhizobia* strains

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Abstract
Heavy-metal pollution from many industrial processes is a major threat to human health and the environment resulting in loss of farming and grazing land. Many bacterial strains have been reported to tolerate metals but there is a dearth of research on the metal-tolerating ability of rhizobia strains, hence the need for this study to screen selected Rhizobial strains for their ability to tolerate varying concentrations of selected heavy metals. Ten Rhizobia strains including Bradhyrhizobium japonicum strains FA3, UDSA136, USDA 9032, USDA110, RANI 22, USDA 4675, RAUG and Bradhyrhizobium sp. strains B574, R25B and USDA 3541 obtained from the culture collection of the International Institute of Tropical Agriculture (IITA) were used for this study. They were screened for their ability to tolerate varying concentrations (10-150 µg/mL) of six selected metals (Copper, Cobalt, Cadmium, Lead, Zinc and Iron) on metal-incorporated Congo-red medium. FA3 (Bradhyrhizobium japonicum) showed the highest tolerance to iron (100 µg/mL), while FA3, USDA110 and USDA 4675 showed highest resistance to zinc with Minimum Inhibitory Concentration (MIC) of 150 µg/mL. Strains USDA 3451, USDA 4675, B547 were unable to grow on the cobalt-incorporated medium, while strains RAUG 1, USDA 4675, USDA136 and R25B had the highest MIC of 150 µg/mL for lead. Copper was the most toxic to the Rhizobial strains as the MIC recorded was between 10-20 µg/mL, while all the strains were able to tolerate 150 µg/mL concentration of Cadmium. Rhizobial strains could find a use in the bioremediation and recovery of soils contaminated with heavy metals as shown by their potentials to tolerate certain degree of metal concentration in this study.

Keywords: Heavy Metal tolerance, Minimum Inhibitory Concentration, Rhizobia strains, *Bradhyrhizobium* spp., International Institute of Tropical Agriculture (IITA).

1.0 INTRODUCTION

Our environment is daily being contaminated with metals especially in industrial areas and is becoming a major public health and environmental concern (Pazirandeh *et al*., 1998; Rajendra *et al*., 2003, Kulshresh *et al*., 2014). About 30% of land is estimated to be contaminated with several chemicals including metals, thereby posing a serious threat to agricultural practices and the environment at large (Alloway and Trevors, 2013; Valentín *et al*., 2013 and Teng *et al*., 2015). Heavy metal is a term used for describing a group of metals or metalloids (Table 1) that have density four or five times greater than that of water. They are usually toxic to plant, animals and humans even at very low concentrations resulting in several diseased conditions (Kulshresh *et al*., 2014). These high concentrations of metals can be cleaned up using several methods including chemical methods, but bioremediation which uses biological agents especially microbes to clean up contaminants seems to be a better alternative as it does not give toxic end-products like the other methods and is relatively cheap and affordable (Strong and Burgess, 2008).

Rhizobia are gram negative bacteria which are able to carry out biological nitrogen fixation in association with leguminous plants. Hydrogen (H_2) which is a by-product of the symbiotic nitrogen fixation has proven to enhance plant tolerance to abiotic factors including oxidative stress and heavy metal toxicity by its bioactive properties (Cui *et al*., 2013; Jin *et al*., 2013). Because nitrogen is of utmost importance for productivity in plants particularly legumes, rhizobia have attained a special position as a plant growth promoter in the field of agriculture but there is a dearth of information on the potentials of rhizobia to be used as agents in the bioremediation of metals, as most studies have focused on the metal-tolerating abilities and bioremediation potentials of bacteria and fungi in certain instances. This study therefore aimed at screening selected Rhizobia strains for their ability to tolerate varying concentrations of heavy metals, with a view of determining their metal-bioremediation potentials.

Table 1: Heavy metals, their common uses and associated clinical symptoms in humans

Metal	Industrial uses	Clinical symptoms	Reference
Pb	Batteries, lead sheets, pigments, alloys, aviation, weapons, construction, etc.	Neurotoxicity	Tiwari *et al*., 2013
Cd	Rechargeable batteries, bearing alloys, electronic items, semiconductors, cigarettes, pigments etc.	Liver cirrhosis, mental disturbance	Godt *et al*., 2006
Zn	Plastics, fungicides, concrete, fertilizers, pigments, anti-corrosives, fire retardants, etc.	Vomiting, renal damage and cramps	Chasapis *et al*., 2012
Fe	Machines, tools, automobiles, ship parts, aircrafts, stainless steel, construction, cooking utensils, etc.	Sarcoidosis Hemochromatosis	Gupta *et al*., 2014
Co	Radiography, gamma irradiation, paint, alloys for magnet, etc.	Diarrhoea, low blood pressure and paralysis	Czarnek *et al*., 2015
Cu	Generators, cars, electronics, transformers, heating cylinders, etc.	Kidney damage, Injury and mental Retardation	Manju, 2015

2.0 METHODOLOGY

2.1 Rhizobia strains used for the study

Ten already identified *Bradhyrhizobium* strains FA3, USDA 110, USDA 9032, USDA 4675, USDA 3451, USDA 136, RAUG 1, RANI 22, B574 and R25B were obtained from the culture collection of the International Institute of Tropical Agriculture (IITA), Idi-Ose, Oyo state, Nigeria.

2.2 Preparation of stock solution of metals

Stock solutions of the respective metals were prepared using the method of Narasimhulu and Setty (2012) . The metals used were: iron, zinc, cobalt, lead, copper and cadmium as shown in Table 2. The concentrations of each solution were calculated by determining the molar mass of the metal in the salt to determine the weight of the salt to be weighed to give the desired stock solutions of each metal.

Table 2: Metal salts used in this study

Metal	Metal salts used
Iron	$FeSO_4.7H_2O$
Zinc	$ZnSO_4$
Cobalt	$Cl_2CoH_{12}O_6$
Copper	CuSO4
Cadmium	$CdCl_2$
Lead	$Pb(C_2H_3O_2)_2$

2.3 Screening on metal-incorporated medium

Young culture of each rhizobium strain was streaked on Congo red incorporated with increasing concentration (10-150 µg/mL) of filter-sterilized solution of each of the six metals selected for the study. The plates were incubated at 28 ^oC for 14 days after which the growth of the rhizobia strains was observed for visible growth. The rhizobium is then streaked on the next concentration of the metal, until it failed to show any visible growth on the medium. The concentration at which no visible growth was observed is taken as the Minimum Inhibitory Concentration (MIC) of the metal on the rhizobium strain.

3.0 RESULTS AND DISCUSSION

3.1 Results

A total of ten Rhizobia strains were used in this study to determine their metal-tolerance ability. Table 3 shows the MIC of the metals on each of the ten rhizobia strains. All the strains survived at the highest concentration of cadmium (150 µg/ml), while only USDA3451 and B574 grew at 50 µg/ml of lead, with the others growing at 150 µg/ml. Three of the strains; USDA 3451, USDA4657 and B574 did not grow at any concentration of cobalt. Only *Bradhyrhizobium japonicum* (FA3) grew at 100 µg/ml of iron, with the rest growing between 10-50 µg/ml of the metal. All the strains except USDA 9032, RAN122 and USDA136 grew at a range of 100-150 µg/ml of zinc, while copper appeared to be the least tolerated of the metals by the strains with MIC ranging from 10-20 µg/ml. FA3 grew on all the metals except for lead and copper on which it had an MIC of 10 µg/ml but showed no sign of growth at a higher concentration. USDA 9032 grew on all the metals but iron seemed to have an inhibitory action on it where its MIC was 20 µg/ml. USDA 3451 seems unaffected by all the metals expect for cobalt and iron where it had no growth after 10 µg/ml of the metal.

Table 3: Minimum Inhibitory Concentration (µg/mL) of metals on the selected Rhizobia strains used in this study

Strain	Iron	Zinc	Cobalt	Lead	Copper	Cadmium
***Bradhyrhizobium japonicum* FA3**	100	150	50	100	10	150
***Bradhyrhizobium japonicum* USDA 9032**	20	50	50	100	20	150
***Bradhyrhizobium sp.* USDA 3451**	10	100	NG	50	10	150
***Bradhyrhizobium japonicum* RAUG1**	50	150	50	150	20	150
***Bradhyrhizobium japonicum* USDA 4675**	50	150	NG	150	20	150
***Bradhyrhizobium sp.* B574**	20	150	NG	50	10	150
***Bradhyrhizobium japonicum* USDA 110**	20	150	50	100	20	150
***Bradhyrhizobium japonicum* RANI22**	10	20	10	150	10	150
***Bradhyrhizobium japonicum* USDA 136**	50	10	50	150	10	150
***Bradhyrhizobium sp.* R25B**	20	150	50	150	10	150

KEY: NG: No growth

3.2 Discussion

The rhizobia strains screened in this study survived at varying concentrations of the six selected metals on metal-incorporated Congo red medium. The isolation of *rhizobia* strains capable of tolerating high concentration of metals have been reported by several authors including El-Aziz *et al*. (1991).

3.2.1 Response to Iron: FA3 showed highest resistance to iron having growth at 100 µg/ml while RAUG 1, USDA 4675, USDA136 had an MIC of 50 µg/ml and all others were between 10 µg/ml - 20 µg/ml.

3.2.2 Response to Zinc: FA3, USDA110, USDA 4675 showed highest resistance to zinc with an MIC of 150 µg/ml, while RAN122, USDA 136 AND USDA 9302 had an MIC of 10 µg/ml, 20 µg/ml and 50 µg/ml, respectively (and were unable to tolerate zinc in high concentrations).

3.2.3 Response to Cobalt: USDA 3451, USDA 4675, B547 were not resistant to cobalt as they had no growth on cobalt incorporated medium, while RAN122 had an MIC of 10 µg/ml and the other an MIC of 50 µg/ml, showing that cobalt was also toxic to the *rhizobia* strains.

3.2.4 Response to Lead: RAUG 1, USDA 4675, USDA136 and R25B also had high resistance to lead having an MIC of 150 µg/ml.

3.2.5 Response to Copper: Copper showed most toxic effect on the rhizobia strains with an MIC that was between 10 µg/ml - 20 µg/ml which is similar to the findings of Arora *et al*., 2010, where copper had negative effects on the growth of tested bacteria species.

3.2.6 Response to Cadmium: Cadmium showed no negative effect on rhizobia strains as they were all able to tolerate and grow at 150 µg/ml concentration. This is however not in concordance with the work of Pereira *et al.* (2006) and Younis *et al*. (2007), where cadmium was reported to have some inhibitory effects on the growth of microsymbionts even at small concentrations thereby affecting their survival in the presence of the metal.

4.0 CONCLUSION

Legumes inoculated with *rhizobia* strains particularly *Bradhyrhizobium* strains can be useful for recovery of abandoned sites contaminated with heavy metals (at high concentration levels) by planting them on metal-contaminated sites which have been rendered useless. This study has highlighted that rhizobia could be potential candidates in the bioremediation of sites contaminated with metals as evident in their ability to tolerate different metals as shown in this study. Further study should be geared towards the evaluation of the initial and final concentrations of metals in soils after the application of rhizobia population to leguminous plants on soil.

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