Plant Growth Promoting and Heavy Metal-Tolerant Rhizobia from Algeria

Mouloud Ghadbane, Laid Benderradji, Samir Medjekal, Hani Belhadj, and Harzallah Daoud

Abstract Heavy metal pollution of the soil is a relevant environmental problem. Plant growth promoting and tolerance heavy metal bacteria are a promising technique for phytoremediation. The aim of this study was to isolate heavy metal-tolerant rhizobia from *Calobota saharae.* Tolerance to a range of heavy metal ions was determined by minimal inhibitory concentration (MIC) for Rhizobia which was isolated from *Calobota saharae.* The *Rhizobium leguminosarum* GM01 strain exhibited strong tolerance to a range of heavy metals including lead (Pb), Cobalt (Co), and zinc (Zn). *R. leguminosarum* GM01 have great potentials for plant growth promotion and phytoremediation of contaminated soils.

Keywords Rhizobia · Phytoremediation · *Rhizobium leguminosarum* · Pollution · Heavy metal

1 Introduction

Fabaceae were proposed as relevant species for phytoremediation, largely due to their ability to colonize marginal lands and nutrient-poor soils aided by legume-rhizobia symbiosis [1]. Heavy metal removal from soils and water can be achieved by a microorganism [2], microalgae [3], or enzymes [4]. Rhizobia are a paraphyletic group of nitrogen-fixing bacteria belonging to the Alpha- and Betaproteobacteria classes.

M. Ghadbane (✉) · L. Benderradji
Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, M'sila, Algeria
e-mail: mouloud.ghadbane@univ-msila.dz

S. Medjekal
Department of Microbiology and Biochemistry, Faculty of Science, University Mohamed Boudiaf of M'sila, M'sila, Algeria

M. Ghadbane · H. Belhadj · H. Daoud
Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University Ferhat Abbas Setif 1, 19000 Setif, Algeria

M. Ksibi et al. (eds.), *Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions (2nd Edition)*, Environmental Science and Engineering, https://doi.org/10.1007/978-3-030-51210-1_110

The symbiosome is the basic nitrogen-fixing unit of the nodule, and the nitrogen fixed by bacteroids is exported as ammonium to the host plant cytoplasm [5]. Previous studies demonstrated that inoculation of legume by heavy metal-resistant Rhizobia could be useful in phytoremediation process. Therefore, this study focused on the heavy metal-resistant and plant growth-promoting rhizobia isolated from *Calobota saharae* (Coss. & Durieu), in order to provide information for phytoremediation.

2 Materials and Methods

2.1 Isolation of Rhizobia

Ten strains were isolated from root nodules of *Calobota saharae* (Coss. & Durieu) using a standard method described by Somasegaran and Hoben [6]. The 16S rRNA gene of GM01 was amplified by polymerase chain reaction (PCR) using two universal primers: forward primer, 5′-AGAGTTTGATCCTGGCTCAG-3′, and reverse primer, 5′-AAGGAGGTGATCCAAGCC-3′. DNA electrophoresis, DNA purification, restriction, ligation, and transformation were all performed according to the method previously described by Sambrook et al. [7]. The nucleotide sequence of the 16S rRNA gene was determined on both strands using a BigDye® Terminator v3.1 Cycle Sequencing Kit and an automated DNA sequencer ABI Prism® 3100-Avant Genetic Analyser (Applied Biosystems). 16S rDNA sequence analysis was performed by means of the BLAST program (www.ncbi.nlm.nih.gov/blast). Phylogenetic and molecular evolutionary analyses were conducted by means of molecular evolutionary genetics analysis (MEGA) software version 10.0.5. Distances and clustering were calculated by the neighbor-joining method.

2.2 Nucleotide Accession Number

The 16S rRNA nucleotide sequence data of the newly isolated *Rhizobium leguminosarum* GM01 has been submitted to NCBI GenBank database and assigned accession number FJ663017.

2.3 Resistance to Heavy Metals of Rhizobia

Rhizobia metal tolerance levels were determined by broth dilution method [8]. After incubation at 28 °C for 5 days [9], the minimum inhibitory concentration (MIC) was defined as the lowest concentration of metal salt inhibiting bacterial growth.

Table 1 Minimal inhibitory concentration of various heavy metals (μg mL^{-1}) and plant growth promoting (PGP) traits (+ present; − absent) for used Rhizobia isolates

Isolates	MIC (μg mL^{-1})					PGP traits		
	Pb	Co	Cd	Zn	Ni	IAA production	Siderophore production	Phosphate solubilization
GM01	3	2	0.2	2	0.1	+	+	+
GM24	0.8	0.5	0.2	0.4	0.1	+	−	−
GM30	0.8	0.2	0.1	0.8	0.2	+	+	−
GM34	1.5	0.4	0.2	0.8	0.1	+	−	−
GM36	2	0.4	0.2	0.4	0.2	+	+	+

2.4 Characterization of Rhizobia for Plant Growth Promoting (PGP) Traits

Rhizobia isolates were tested for the production of plant growth-promoting substances such as indole acetic acid (IAA) [10], phosphatase [11], and Siderophore [12].

3 Results

3.1 Heavy Metal Tolerance

Heavy metal tolerance ability of rhizobia was tested by minimum inhibitory concentration (MIC). The five isolated Rhizobia also showed a high tolerance to lead (Pb), Cobalt (Co), zinc (Zn) (Table 1). All isolates were characterized by low tolerance to Cd and Ni. Isolate GM01 was found to have the highest MIC value for Pb, Co, and Zn.

3.2 PGP Traits

The results of PGP traits (Table 1) showed that all five strains of rhizobia (GM01, GM24, GM30, GM34, and GM36) and identified with phenotypic characteristic were able to produce IAA in the presence of L-tryptophan. Three strains (GM01, GM30, and GM36) were able to produce siderophore and the phosphate solubilization activity was shown by two strains namely GM01 and GM36.

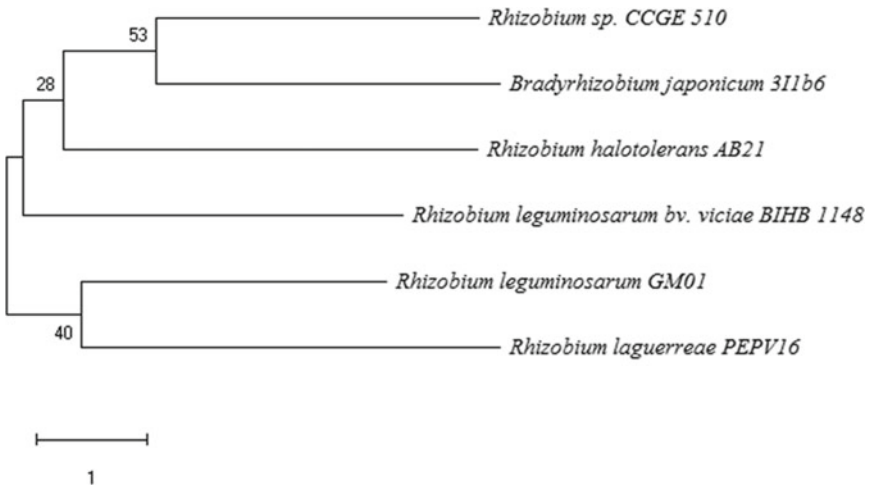

Fig. 1 Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain GM01 within the radiation of the genera *Rhizobium* and *Bradyrhizobium*

3.3 Identification of Rhizobia

The selected GM01 strain based on PGP characteristics and resistance to high heavy metal concentration (Table 1) was identified by 16S rRNA gene sequence analysis (Fig. 1). The comparison of the 16S rRNA gene sequences revealed that GM01 strain was phylogenetically related to Rhizobia, belonging to genus *Rhizobium* (Fig. 1). On the basis of the phonotypical characteristics (data not shown), and 16S rRNA gene sequence analysis, strain GM01 was recognized as *R. leguminosarum* GM01 (Fig. 1).

4 Discussion

Heavy metal tolerance ability of rhizobia was tested. The five Rhizobia isolated also showed a high tolerance to Pb, Co, and Zn. All isolates characterized low tolerance to Cd and Ni. *R. leguminosarum* GM01 was found to have the highest MIC value for Pb, Co, and Zn. Rhizobia which were isolated from heavy metal-contaminated soils or plants often exhibit much higher heavy metal-resistant ability to adapt to such environments [13]. Rhizobia have been recognized as plant growth-promoting bacteria and some of them could produce auxin, siderophore, and solubilized of phosphate. The PGP traits may also enhance metal extraction, or stabilization in soil.

5 Conclusion

The isolate GM01 was identified as *R. leguminosarum* by phenotypic and genotypic characteristics. The high heavy metal resistance of GM01 indicates its promising adaptation abilities for practical applications. This result clearly proves the high potential for plant growth promotion and for use in phytoremediation of soils contaminated with heavy metals.

References

1. Fagorzi, C., Checcucci, A., diCenzo, G.C., Debiec-Andrzejewska, K., Dziewit, L., Pini, F., Mengoni, A.: Harnessing rhizobia to improve heavy-metal phytoremediation by legumes. Genes **9**, 542 (2018). https://doi.org/10.3390/genes9110542
2. Dutta, P., Karmakar, A., Majumdar, S., Roy, S.: *Klebsiella pneumoniae* (HR1) assisted alleviation of Cd(II) toxicity in *Vigna mungo*: a case study of biosorption of heavy metal by an endophytic bacterium coupled with plant growth promotion. Euro-Mediterranean J. Environ. Integr. **3**, 27 (2018)
3. Das, D., Chakraborty, S., Bhattacharjee, C., Chowdhury, R.: Biosorption of lead ions (Pb^{2+}) from simulated wastewater using residual biomass of microalgae. Desalin. Water Treat. **57**, 4576–4586 (2016)
4. Nath, A., Chakraborty, S., Bhattacharjee, C.: Bio-reactorand enzymatic reactions in bioremediation. In: Das, S. (ed.) Microbial Biodegradation and Bioremediation, pp. 455–495. Elsevier, Amsterdam (2014)
5. Coba de la Peña, T., Fedorova, E., Pueyo, J.J., Lucas, M.M.: The symbiosome: legume and rhizobia co-evolution toward a nitrogen-fixing organelle? Front. Plant Sci. **8**, 2229 (2018)
6. Somasegaran, P., Hoben, H.J.: The Handbook for Rhizobia. Methods in Legume-Rhizobium Technology. Springer, New York (1994)
7. Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989)
8. Ma, Y., Rajkumar, M., Zhang, C., Freitas, H.: Inoculation of *Brassica oxyrrhina* with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. J. Hazard. Mater. **320**, 36–44 (2016)
9. Yu, X., Li, Y., Zhang, C., Liu, H., Liu, J., Zheng, W., Kang, X., Leng, X., Zhao, K., Gu, Y., Zhang, X., Xiang, Q., Chen, Q.: Culturable heavy metal-resistant and plant growth promoting bacteria in V-Ti magnetite mine tailing soil from Panzhihua, China. PLoS ONE **9**(9), e106618 (2014)
10. Khamna, S., Yokota, A., Lumyong, S.: Actinobacteria isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World J. Microbiol. Biotechnol. **25**(4), 649–655 (2009)
11. Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodríguez, M.X., Barea, J.-M.: Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl. Soil Ecol. **45**, 209–217 (2010)
12. Schwyn, B., Neilands, J.B.: Universal chemical assay for the detection and determination of siderophores. Ann. Biochem. **160**, 47–56 (1987)
13. Guo, J., Chi, J.: Effect of Cd-tolerant plant growth-promoting rhizobium on plant growth and Cd uptake by *Lolium multiflorum* Lam. and *Glycine max* (L.) Merr. in Cd-contaminated soil. Plant Soil **375**, 205–214 (2014)