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ORIGINAL PAPER

Tolerance and reduction of chromium by bacterial strains

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Abstract

Bacteria have potential to tolerate and reduce metals. This study evaluated the potential of selected bacterial strains in tolerating and reducing chromium (Cr). Six bacterial strains (*Rhizobium miluonense* LCC01, LCC04, LCC05, and LCC69; *Rhizobium pusense* LCC43; and *Agrobacterium deltaense* LCC50) showed tolerance to Cr(VI) (16 and 32 μg mL^{-1}), reduction potential of Cr(VI) (from 50 to 80%), and efficiency in producing exopolysaccharides. *Rhizobium pusense* LCC43 exhibited the highest tolerance (128 μg mL^{-1}), reduction potential of Cr(VI) (from 80 to 100%), and efficiency in producing exopolysaccharides. These results suggested that this strain may have the potential to be used in the bioremediation of soils contaminated with Cr(VI).

Keywords Cr contamination · Bioremediation · Pollution · Metals

Introduction

The production of solid wastes increases annually, and it is estimated that approximately 4.0 billion tons of solid waste would be released by 2050 (Kaza et al. 2018). Therefore, it is necessary to develop alternatives to recycle and reuse these wastes for decreasing their amounts in the environment, particularly in soils. Furthermore, these wastes can have high concentrations of metals that could promote soil contamination. For example, tannery wastes contain a high concentration of chromium (Cr) that can accumulate in the soil (Araujo et al. 2020) and promote negative effects on soil microbial biomass and diversity (Miranda et al. 2018; Araujo et al. 2020). However, this accumulation of Cr drives the selection of specific microbes that can tolerate its presence (Rocha et al. 2019).

The potential microbial tolerance to Cr is due to microbes having mechanisms that can overcome Cr toxicity, such as expression of genes involved in the reduction of Cr(VI), particularly the chromate reductase gene (*ChR*) (Cheung and Gu 2007), and production and release of exopolysaccharides. Exopolysaccharides (EPSs) are compounds that consist of proteins and polysaccharides and are released by microbes in response to abiotic stressors, such as metal toxicity (Sheng et al. 2010). The action of EPSs is mediated by sorption of metals, which decrease the availability and toxicity of metals; therefore, it is interesting for bioremediation strategies in metal-contaminated sites (Gupta and Diwan 2017). The *ChR* gene is efficient in reducing Cr(VI) to Cr(III), a less mobile and less toxic version of Cr, and thus reduces Cr toxicity (Cheung and Gu 2007). Some well-known bacteria carrying the *ChR* gene and having the ability to reduce Cr(VI) are *Bacillus* sp. (Wani et al. 2018) and *Rhizobium* sp. (Karthik et al. 2017).

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Recently, a preliminary study examining soils that were highly contaminated by Cr found bacterial strains with potential biochemical abilities (Rocha et al. 2019). However, it did not determine if those bacterial strains could tolerate and reduce Cr(VI). Thus, the present study aimed to identify and evaluate six potential bacterial strains, suggested by Rocha et al. 2019, for their potential in tolerating and reducing Cr(VI).

Materials and methods

Bacterial strains

Six bacterial strains (LCC01, LCC04, LCC05, LCC43, LCC50, and LCC69) with potential biochemical ability, measured by enzymes catalase, gelatinase, urease, lipase, phosphate solubilization, and cellulase, by Rocha et al. (2019) were used in our study. These strains were grown in tryptone Yeast (TY) broth (pH 7.2) under shaking incubation conditions (86×*g*) for 24 h at 28 °C. The bacterial cells were collected at log phase growth by centrifugation (Centrifuge Eppendorf™ 5418R with rotor FA-45-18-11) at 10,000 rpm for 10 min.

16S rRNA and *ChR* genes sequencing

The genomic DNA of each strain was extracted and the 16S rRNA and *ChR* gene were amplified using primers described by Weisburg et al. 1991 and Patra et al. 2010, respectively. All the sequences were deposited to NCBI. Phylogenetic analyses based on 16S rRNA and the *ChR* gene were performed through MEGA-X (Felsenstein, 1988) based on the maximum likelihood statistical method (Saitou and Nei 1987) and the branching support of 1000 bootstrap (Felsenstein 1988).

Minimum inhibitory concentration (MIC)

First, in each evaluation, Cr(VI) was used in the form of $K_2Cr_2O_7$. The method of plate dilution proposed by Alam and Malik (2008) was applied to verify both bacterial growth and Cr(VI) MIC. For this study, each strain was exposed to Cr(VI) concentrations varying from 16 to 1024 µg mL^{-1}. The lowest concentration of Cr(VI) without bacterial growth was considered the MIC (µg mL^{-1}).

Reduction potential of Cr(VI)

The reduction potential of Cr(VI) was evaluated according to the method proposed by Baldiris et al. (2018). Here, each strain was exposed to varying concentrations of Cr(VI) (0, 25, 50, and 100 µg mL^{-1}). The control was TY broth without bacterial inoculation. The remaining Cr(VI) concentration was determined by the 1,5-diphenylcarbazide method (Pattanapipitpaisal et al. 2001), and the results were read using a spectrophotometer (OD 540 nm). The reduction of Cr(VI) was calculated as follows: Remaining Cr (VI) = $(Cr_s/Cr_c) \times 100\%$, where Cr_s and Cr_c are the Cr(VI) found in supernatants from each strain and control, respectively.

Production of biofilm and exopolysaccharides (EPSs)

Each strain was subjected to varying Cr(VI) concentrations (0, 25, 50, and 100 µg mL^{-1}). Crystal violet staining was used to evaluate biofilm formation (Baldiris et al. 2018). The production of biofilm was determined by spectrophotometer at OD 570 nm.

The production of EPSs was determined according to the method proposed by Castellane et al. (2014). Each strain was subjected to Cr(VI) concentrations of 0 and 25 µg mL^{-1}. After biofilm growth, EPSs produced were separated, washed, and determined gravimetrically.

Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean values. The values of cell biomass, total EPSs, and efficiency were subjected to analysis of variance, and the means were compared by Tukey's test at 5% probability.

Results and discussion

16S rRNA and *ChR* genes sequencing

In this study, six potential bacterial strains (Rocha et al. 2019) were evaluated to verify if they could grow efficiently in the presence of Cr(VI), produce EPSs, and reduce Cr(VI). The phylogenetic classification (16S rRNA) evidenced three bacterial species, *Rhizobium miluonense* (LCC01, LCC04, LCC05, and LCC69), *Rhizobium pusense* (LCC43), and *Agrobacterium deltaense* (LCC50) (Table S1; Figure S1). Notably, *ChR* amplification demonstrated the presence of the gene only in *R. pusense* (LCC43) (Figs. S2, S3).

The sequencing of the 16S rRNA gene identified *Rhizobium* (*R. miluonense* and *R. pusense*) and *Agrobacterium* (*A. deltaense*) as bacterial species in this study. The identification of *Rhizobium* and *Agrobacterium* suggested that both the bacterial species have the potential to tolerate Cr(VI) and validates previous studies that reported *Rhizobium* and *Agrobacterium* as the main genera found in soils contaminated with Cr(VI) (Raaman et al. 2012; Chaudhary et al. 2021; Gutiérrez et al. 2010).

MIC

All strains grew under Cr(VI) concentrations of 0 and 25 μg mL^{-1} (Fig. 1). However, *R. miluonense* (LCC01, LCC04, LCC05, and LCC69) and *A. deltaense* (LCC50) did not grow under Cr(VI) concentrations of 50, 100, and 200 μg mL^{-1}, while *R. pusense* LCC43 grew under a Cr(VI) concentration of 100 μg mL^{-1}. *R. miluonense* (LCC69) and *A. deltaense* (LCC50) showed tolerance to Cr(VI) concentrations of 16 and 32 μg mL^{-1}, respectively, while *R. miluonense* (LCC01, LCC04, and LCC05) showed tolerance to a Cr(VI) concentration of 64 μg mL^{-1} (Table 1). *R. pusense* (LCC43) showed the highest tolerance to Cr(VI), showing growth while exposed to a Cr(VI) concentration of 128 μg mL^{-1}.

All bacterial strains showed tolerance to low concentrations of Cr(VI) (25 μg mL^{-1}). However, *R. pusense* LCC43 tolerated a higher concentration of Cr(VI) (100 μg mL^{-1}).

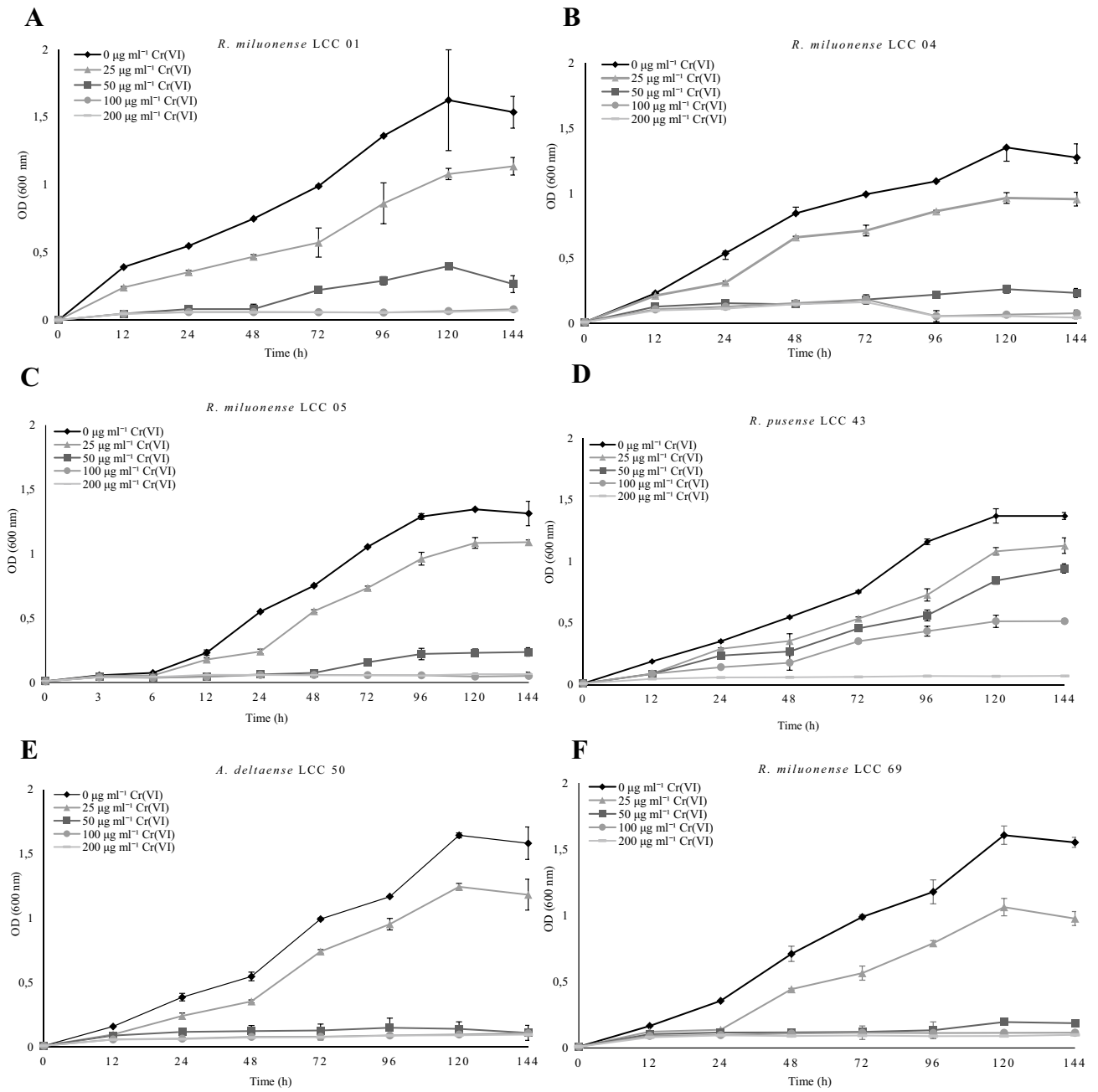

Fig. 1 Growth of bacterial strains *R. miluonense* LCC01 **A**, *R. miluonense* LCC04 **B**, *R. miluonense* LCC05 **C**, *R. pusense* LCC43 **D**, *A. deltaense* LCC50 **E** e *R. miluonense* LCC69 **F** in the presence of concentration of Cr(VI) at various time intervals. The bars represent the standard deviation

Table 1 Minimum inhibitory concentration of bacterial strains

Strain	Cr(VI) (μg mL^{-1})								
	16	32	64	128	200	400	512	800	1024
R. miluonense LCC01	+	+	+	–	–	–	–	–	–
R. miluonense LCC04	+	+	+	–	–	–	–	–	–
R. miluonense LCC05	+	+	+	–	–	–	–	–	–
R. pusense LCC43	+	+	+	+	–	–	–	–	–
A. deltaense LCC50	+	+	–	–	–	–	–	–	–
R. miluonense LCC69	+	–	–	–	–	–	–	–	–

+indicates growth; – indicates no growth

These results suggested that these bacterial strains have mechanisms to tolerate Cr, such as the removal of the metal from the solution (Karthik et al. 2017) and extracellular reduction of Cr(VI) (Chovanec et al. 2012). In addition, the higher tolerance to Cr(VI) found in *R. pusense* LCC43 suggested that this strain could possess important microbial traits to tolerate Cr(VI), such as distinct proteins, functional genes involved in the cell wall formation, and metabolism of aromatic compounds (Chaudhary et al. 2021).

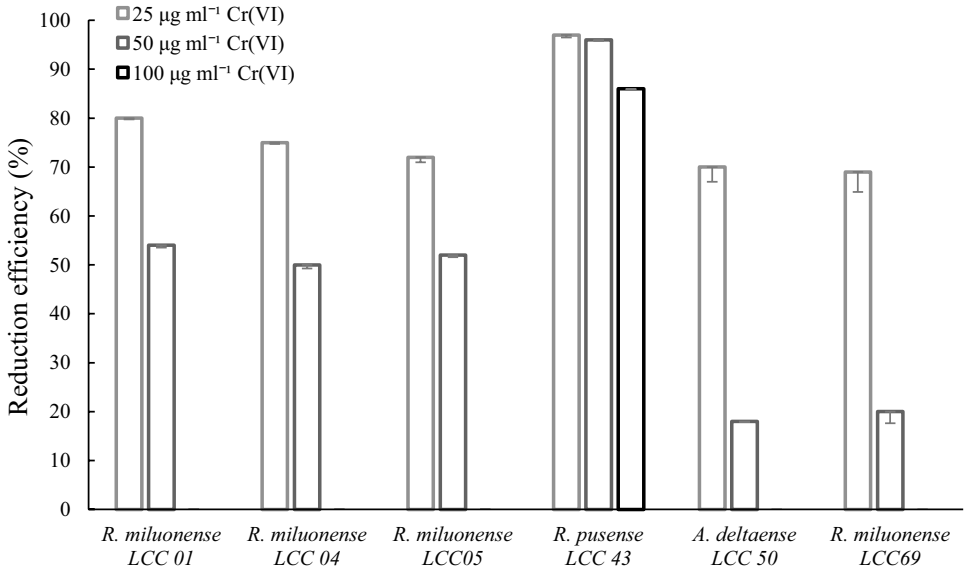

Fig. 2 Reduction efficiency of Cr(VI) by *R. miluonense* LCC01, *R. miluonense* LCC04, *R. miluonense* LCC05, *R. pusense* LCC43, *A. deltaense* LCC50 and *R. miluonense* LCC69 in the presence of concentration of Cr(VI). The bars represent the standard deviation

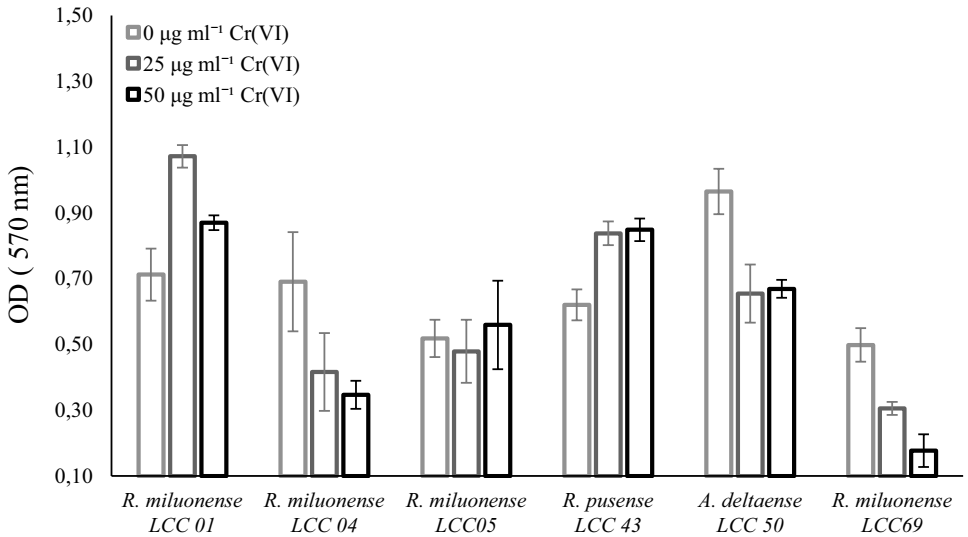

Fig. 3 Biofilm production by *R. miluonense* LCC01, *R. miluonense* LCC04, *R. miluonense* LCC05, *R. pusense* LCC43, *A. deltaense* LCC50 and *R. miluonense* LCC69 in the presence of concentrations of Cr(VI). The bars represent the standard deviation

Reduction potential of Cr(VI)

All strains had potential to reduce Cr(VI). Specifically, *R. miluonense* (LCC01, LCC04, LCC05, and LCC69) and *A. deltaense* (LCC50) reduced 80% of Cr(VI) under a Cr(VI) concentration of 25 µg mL^{-1}, and reduced 50% of Cr(VI) under a Cr(VI) concentration of 50 µg mL^{-1} (Fig. 2). These strains did not reduce Cr(VI) under a Cr(VI) concentration of 100 µg mL^{-1}. However, considering absolute values per ug of reduced Cr(VI), the reducing potential of the strains LCC01, LCC04, and LCC05 remained unchanged or narrowly increased in the presence of 25 and 50 µg Cr(VI), since 80% of 25 µg nearly corresponds to 20 µg and 50% of 50 µg correspond to 25 µg Cr(VI). In addition, the strain LCC50 showed an increased ability to reduce Cr(VI), while LCC69 showed a decreased ability. However, *R. pusense* (LCC43) showed high potential in reducing Cr under Cr(VI) concentrations of 25, 50, and 100 µg mL^{-1}, reaching a reduction rate higher than 90% under Cr(VI) concentrations of 25 and 50 µg mL^{-1}.

The results showed that each strain has a mechanism to tolerate and reduce Cr(VI); thus, its ability to reduce Cr could be the basis for bacterial strains selection for bioremediation purposes in soils contaminated with Cr(VI) (Karim

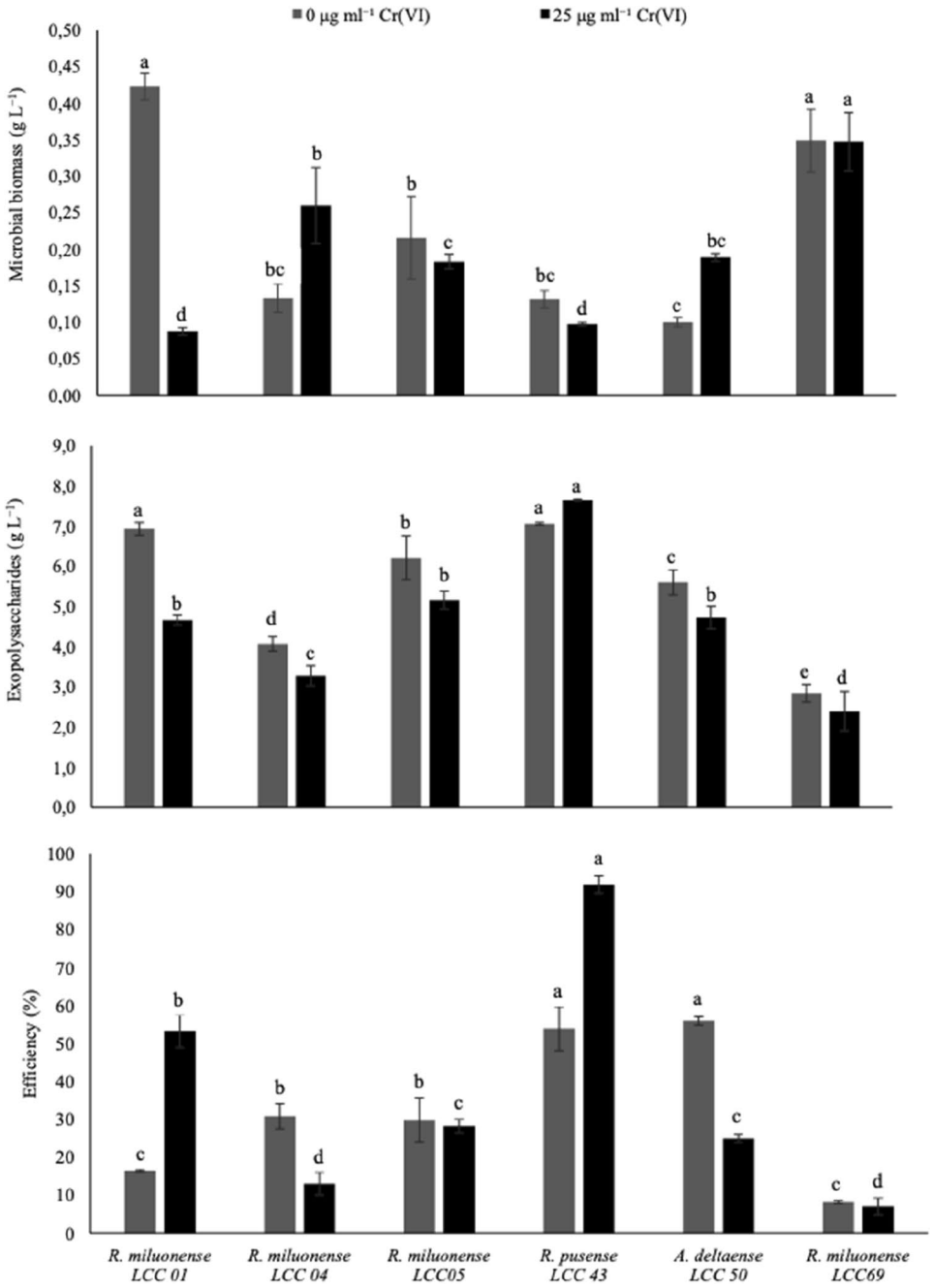

Fig. 4 Production of microbial biomass, exopolysaccharides and efficiency by *R. miluonense* LCC01, *R. miluonense* LCC04, *R. miluonense* LCC05, *R. pusense* LCC43, *A. deltaense* LCC50 and *R. miluonense* LCC69 with and without Cr(VI). Different letters indicate a significant difference ($p < 0.05$) between treatments. The bars represent the standard deviation

et al. 2020). We highlighted *R. pusense* LCC43 for its ability to promote higher reduction of Cr(VI) compared to the other bacterial strains. The genus *Rhizobium* seems to have high potential in reducing Cr(VI) as reported by Karthik et al. (2017) who reported *Rhizobium* sp. reducing Cr(VI) by approximately 80 and 100% under concentrations of 50 and 150 µg mL^{-1} Cr, respectively. Recently, a strain of *R. pusense* isolated from water containing Cr showed the ability to reduce Cr(VI), thereby showing promising Cr(VI) detoxification applications (Sahoo et al. 2022).

Production of biofilms and EPSs

We observed variations in the production of biofilms (Fig. 3), where both *R. miluonense* (LCC01) and *R. pusense* (LCC43) produced more biofilms than *R. miluonense* (LCC04, LCC05, and LCC69) and *A. deltaense* (LCC69) under Cr(VI) concentrations of 25 and 50 µg mL^{-1}. The highest values of biofilm biomass and EPSs were from *R. miluonense (*LCC69) (Fig. 4A). However, *R. pusense* (LCC43) showed the highest production and efficiency in producing EPSs in the presence of Cr(VI) (Fig. 4B, C).

All strains showed efficiency in producing biofilms and EPSs. The ability to produce biofilms is important since they protect bacteria against metals (Haque et al. 2021) by assisting bacteria in metal sorption (Pan et al. 2014). Similarly, EPSs are efficient in protecting bacterial cells and removing metals from solutions by flocculation (Aljuboori et al. 2013). Ayangbenro et al. (2019) reported the presence of EPSs as an important factor to reduce Cr. In our study, *R. pusense* LCC43 showed a higher ability to produce EPSs in the presence of Cr(VI) compared to the other strains, which was in accordance with previous studies reporting *Rhizobium tropici* and *Ochrobactrum intermedium* producing EPSs in response to Cr(VI) (Leonel et al. 2019). Thus, *R. pusense* LCC43 is highly efficient in producing EPSs. Consequently, this is an important finding for the development of approaches targeting bioremediation of soils contaminated with Cr. Finally, *R. pusense* LCC43 was found to be the unique strain amplifying *ChR* gene fragment that can confer higher tolerance and has mechanisms to reduce Cr(VI) (Soni et al. 2013; Zhu et al. 2019).

Conclusions

This study demonstrated that bacterial strains isolated from soil contaminated with Cr presented different abilities to tolerate and reduce Cr(VI). *Rhizobium pusense* LCC43 had higher potential in tolerating and reducing Cr(VI), efficiently producing EPSs, and carrying the *ChR* gene. These findings suggested that *Rhizobium pusense* LCC43 has potential to be used in the bioremediation of soils contaminated with Cr(VI).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03329-3.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by SMBR, MRdaA, MKLC, TCdaSS, RMC, JELA, and LMdeSO, The first draft of the manuscript was written by FdeAN, EVdeM, APdeAP, and ASFA: and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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