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Influence of rhamnolipids, produced by *Pseudomonas aeruginosa* NCAIM(P), B001380 on their Cr(VI) removal capacity in liquid medium

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Abstract: *Pseudomonas aeruginosa* NCAIM(P), B001380, a propitious bacterial strain isolated from mineral cutting oil, was identified to be chromium tolerant and a producer of the biosurfactant rhamnolipid (RL) with potential application in heavy metal bioremediation. Culture growth, RL production and Cr(VI) removal capacity of the strain in the presence of 50 mg L^{-1} Cr(VI) (**I**) and 100 mg L^{-1} Cr(VI) (**II**) were studied. The maximums of RL production were found in the late-stationary phase at 72 h for both Cr(VI)-amended cultures: **I** (236 mg L^{-1}) and **II** (160 mg L^{-1}), as well as the maximums of the Cr(VI) removal capacity: 70 % (**I**) and 57 % (**II**). The amount of Cr in RL preparation **II** was 22 μg mg^{-1}, determined by flame atomic absorption spectroscopy (FAAS). Appearance of a new band at 914 cm^{-1} in infrared (IR) spectrum of RL (**II**) indicated significant proof for a possible coordination of CrO_4^{2-} with RL. The effect of Cr(VI) on monorhamnolipids (RL1) and dirhamnolipids (RL2) distribution and their ratio were studied by electrospray ionization mass spectrometry (ESI-MS). An increase was observed in the RL2/RL1 ratio for **II** compared to the control.

Keywords: rhamnolipids; chromium(VI); *Pseudomonas*; bioremediation.

INTRODUCTION

Their potential to metabolize chemical pollutants in the environment makes *Pseudomonas* species suitable for use as bioremediation agents capable of removing heavy metal pollutants and numerous toxic organic compounds.[1–4] *Pseu-*

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domonas produces and excretes small secondary metabolite rhamnolipids (RL), natural biosurfactants that could have unique metal binding capacities and selectivities to complex heavy metal ions.[1] Biosurfactants are a diverse group of surface-active substances divided based on molecular weight into low molecular-mass biosurfactants (glycolipids, phospholipids and lipopeptides) and high molecular-mass biosurfactants (polysaccharides, proteins, lipopolysaccharides and lipoproteins).[2] Rhamnolipids belong to the low-molecular mass molecules and, based on their chemical composition, the principal rhamnolipids are mono-rhamno-di-lipidic congeners (RL1) and di-rhamno-di-lipidic congeners (RL2).[2]

In comparison to their chemically synthesized equivalents, biosurfactants have many advantages. They are eco-friendly, biodegradable, less toxic and non-hazardous, with better foaming properties and higher selectivity.[5,6] Due to their potential advantages, special attention has been paid to the use of rhamnolipid biosurfactants in different aspects of environmental biotechnology.[2] Juwarkar *et al.* reported that di-rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* BS2 was used in the remediation of metals from multi-metal contaminated soil, confirming removal selectivity of metals in the order Cd = Cr > Pb = Cu > > Ni.[3] Removal of heavy metals from sediments could also be enhanced by the use of a solution of rhamnolipid.[4] On the other hand, application of rhamnolipid foam increases the efficiency and allows a higher percentage of removal of heavy metals in comparison to the use of rhamnolipid solution.[7,8]

While the complexation of RL with metal contaminants initiated numerous investigations on the potential use of RL as environmentally compatible soil washing agents, only a few publications were dedicated to the influence of heavy metal ions on the microbial production of RL.[9] Elevated concentrations of some divalent cations were shown to inhibit RL production, while iron limitations were correlated with the overexpression of the rhlI system.[10,11] A recent investigation on *P. aeruginosa* IGB83 evaluated a significant increase in the ratio of dirhamnolipids (RL2) to monorhamnolipids (RL1) congeners produced by cultures grown in the presence of Cd^{2+}.[12] In addition, it was demonstrated that environmental and growth conditions influence both RL production and quorum sensing (QS) or the cell-to-cell communication system.[11]

High solubility, rapid transport through the biological membranes and complexation with intracellular polymers, such as proteins and nucleic acids, imply high toxicity of Cr(VI) compounds for humans and microorganisms. Some microorganisms, however, can tolerate Cr(VI) using several distinct strategies: the plasmid mediated efflux system, absorbing heavy metals to their cell wall, by precipitation (including reductive precipitation) in the form of insoluble salts and binding metal ions to biological complexing agents, such as exopolymers and biosurfactants.[13–19] To date, a few chromium resistant *Pseudomonas* strains have been reported and their use in bioremediation of Cr(VI) suggested.[20–26] A re-

cently published proteomics study of chromium resistant *Pseudomonas aeruginosa*, indicated the existence of several resistance mechanisms as reasonable responses to Cr(VI) stress.[25]

P aeruginosa NCAIM(P), B001380 (earlier labeled as *P. aeruginosa* san ai) was isolated from mineral cutting oil used as a metal working fluid in the metal industry.[27] It was expected that *P. aeruginosa* NCAIM(P), B001380 isolated from such extreme conditions, could be a possible candidate for remediation of an environment polluted by the extremely toxic chromium. Therefore, the purpose of this study was to evaluate the chromium tolerance of the strain and to study the influence of their naturally produced rhamnolipids on their Cr(VI) removal capacity with aim of providing potential applications of the strain and its biosurfactants in bioremediation.

EXPERIMENTAL

Bacterial growth and chromium removal ability

The Cr(VI) tolerance of the bacterial strain was determined using 100 mL of nutrient Luria broth (LB) medium (0.5 % NaCl, 0.5 % yeast extract and 1 % tryptone) in 500 mL Erlenmeyer flasks, inoculated with 5 vol. % of a 24-h pre-culture and agitated at 250 cycles min^{-1} on a horizontal Kuhner shaker, Switzerland.[27] The inoculated medium was supplemented by two concentrations of Cr(VI): 50 (**I**), and 100 (**II**) mg L^{-1} at pH 7.2. A stock solution of chromium was prepared by dilution of $K_2Cr_2O_7$ to a final concentration of 10 g L^{-1} chromium. Samples were taken at regular intervals, centrifuged at 10000 rpm for 20 min (Sorvall, Rotor SS-1, New Town, Conn., USA) and analyzed for chromium removal. Controls were non-inoculated LB with 50 and 100 mg L^{-1} of Cr(VI). The chromium concentrations of samples **I** and **II** were calculated and compared to the controls. All determinations were performed in triplicate.

Growth of bacterial strains

Bacterial growth of the control (LB inoculated, without Cr(VI)) and Cr(VI)-amended cultures (**I** – 50 and **II** – 100 mg L^{-1}) were monitored as the change in optical density (*OD*) at 580 nm, using sterile LB medium as the control. The determination of the *OD* was performed in triplicate.

Evaluation of the chromium resistance

The minimal inhibitory concentration (*MIC*) was determined in LB medium supplemented with Cr(VI) concentrations ranging from 0.5–20 mM. Tubes containing the growth medium and various concentrations of Cr(VI) were inoculated with a 24-h culture to obtain an initial optical density of 0.06. Turbidity measurements were recorded after 24 h of growth. The minimum concentration of the metal inhibiting complete growth was taken as the *MIC*.[26]

Chromate reductase activity assay

Chromate reductase was assayed at 30 °C in 0.5 mL reaction mixture containing 0.05 mM K_2CrO_4 in 50 mM Tris–HCl pH 7.1, supplemented with 0.1 mM NADPH and 0.5 mL of enzyme preparation. The concentration of the residual Cr(VI) was determined spectrophotometrically at 540 nm using diphenylcarbazide.[28]

Determination of chromium(VI) in the culture broth

During the incubation period, a 3 mL sample of culture broths of **I** and **II** was taken from each flask. Samples were centrifuged at 10000 rpm for 5 min to precipitate the biomass. The concentration of chromium in the supernatant (c_{Cr}) was determined spectrophotometrically at 540 nm using diphenylcarbazide reagent in acidic solution as the complexing agent for Cr(VI).[24,29]

Total chromium determination

Total chromium was quantified by reducing air acetylene flame atomic absorption spectroscopy (FAAS) using a Perkin Elmer Model 2380 instrument at 359.3 nm after digestion of the samples with HNO_3 and H_2O_2 in a DS-6 digester, Tecator, Sweden.

Isolation and purification of rhamnolipids

A mixture of RL was isolated from the fermentation broth after separation of the bacterial cells by centrifugation. A crude preparation of RL was obtained by acidic precipitation using 1 M HCl (final pH 2). The precipitate was collected by centrifugation at 5000 rpm for 10 min (Sorvall, Rotor SS-1, UK) and the RL were dissolved in a mixture of chloroform and methanol (2:1). The clear supernatant obtained after centrifugation at 5000 rpm for 10 minutes (Janetzki T32c, Germany) was evaporated to dryness under vacuum and used for MS analysis and total chromium determination.[30] Further purification of crude preparation was performed by normal-phase column chromatography on silica gel (Kiesel gel 100, 70–230 mesh ASTM, Merck) as was demonstrated previously.[30] The purified samples were analyzed by FTIR and ^{1}H-NMR spectroscopy.

Rhamnolipid determination

The orcinol assay for determination of amount of methyl pentose was used for quantification of RL in the culture supernatants of the controls, and samples **I** and **II**. A 150 μL aliquot of supernatant was diluted with water to attain a volume of 300 μL, which was extracted twice with 600 μL of diethyl ether. The pooled ether fractions were evaporated to dryness, the residue was dissolved in 100 μL of distilled water and mixed with 100 μL of 1.6 % orcinol and 800 μL of 60 % sulfuric acid. After heating at 80 °C for 30 min in a water bath, the absorbance at 421 nm was measured. The content of rhamnose in the samples was determined by comparing the absorption with those of rhamnose standards with defined concentrations (0.075–0.020 g L^{-1}). The RL concentration (c_{rl}) was calculated based on the assumption that 1 μg of rhamose corresponds to 2.5 μg of rhamnolipids.[31]

FTIR analysis of RL

Purified and dried RL samples of the control and sample **II** (1 mg) were dispersed in 100 mg of anhydrous KBr and pressed to a pellet. The FT-IR spectra were recorded at room temperature in the wave number range of 400–4000 cm^{-1} using a Perkin-Elmer 31725 X FTIR spectrophotometer.

NMR analysis of RL

The ^{1}H-NMR spectra of RL of the control and sample **II** were recorded in D_2O at room temperature using a Gemini 200 spectrometer at 200 MHz.

ESI-MS analysis of RL

Mass spectra of RL from the diethyl ether extracts of the culture filtrates of the control and sample **II** were recorded on MS system consisting of an HPLC (Agilent 1200 Series, Agilent Technologies) and a 6210 Time-of-Flight LC/MS (Agilent Technologies), using Zor-

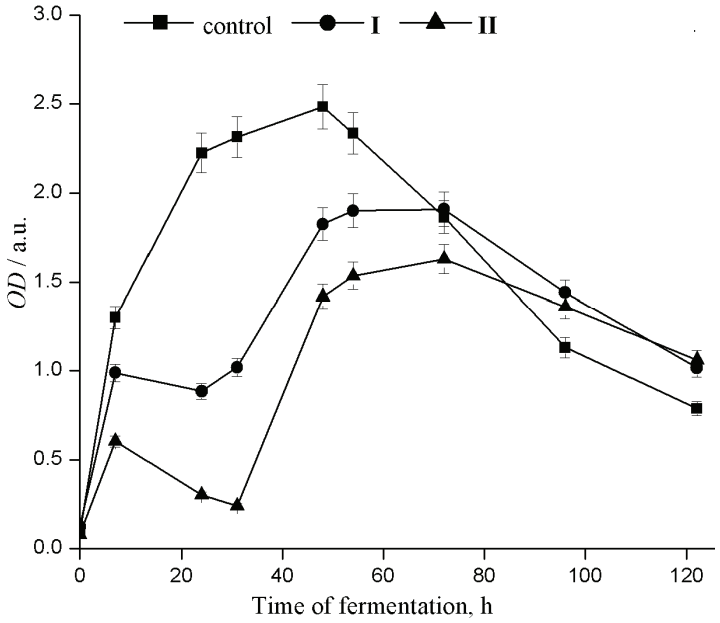
bax Eclipse Plus C18 column and a DAD detector. The mobile phase was a mixture of solvent A (0.2 % formic acid in water) and B (acetonitrile) in a gradient mode: 0–1.5 min 95 % A, 1.5–12 min 95–5% A, 12–15 min 5 % A, 15–16 min 5–95 % A. The data were processed by means of a Mass Hunter Workstation. The relative abundance of the RL congeners was calculated from the mass spectral abundance (counts) of the observed ions. The dirhamnolipids (RL2)/monorhamnolipids (RL1) ratio represents the sum of all RL2 congeners divided by the sum of all RL1 congeners present in a given sample.

RESULTS AND DISCUSSION

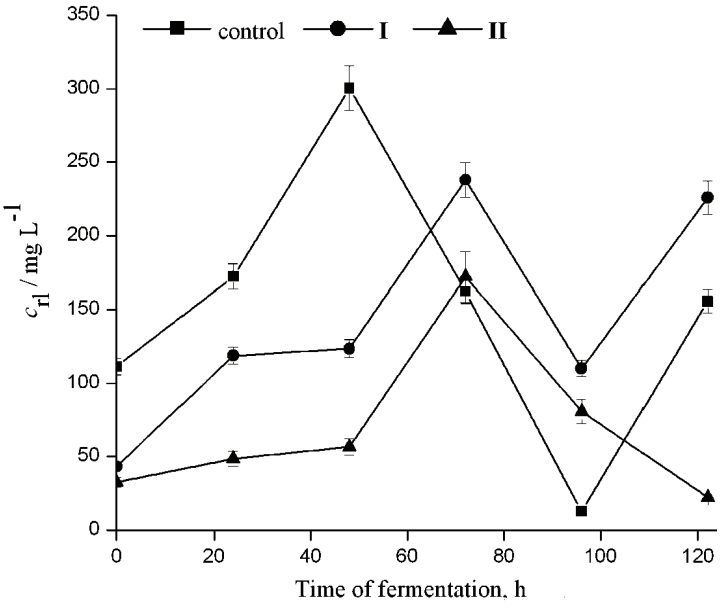
Cr(VI) removal capacity and RL production

The *MIC* of Cr(VI) for *P. aeruginosa* NCAIM(P), B001380 strain was 5 mM, similarly to *P. putida* as a model organism for Cr-bioremediation.[26] However, preliminary experiments suggested that sub-lethal concentrations of Cr(VI) of 50 (**I**) and 100 mg L^{-1} (**II**) had significant effects on RL production, so they were selected to monitor growth, *OD*, RL production, c_{rl}, and the concentration of Cr(VI), c_{Cr}, in the culture broth of *P. aeruginosa* NCAIM(P), B001380 (Fig. 1).

As Fig. 1a shows, Cr(VI)-amended cultures (**I** and **II**) had a slightly lower *OD* and a delay in growth phases compared to those of the control. The RL production c_{rl} in the control, **I** and **II** was observed in the stationary phase, when it was sustained on a high level until the late-stationary phase (Fig. 1b). A similar delay in c_{rl} induced by Cr(VI) was observed with the Cd-amended culture of *P. aeruginosa* IGB83.[12] The maximum RL concentrations, c_{rl}, were found in the late-stationary phase at 48 h in the control (310 mg L^{-1}), at 72 h in **I** (236 mg L^{-1}) and **II** (160 mg L^{-1}), Figs. 1a and 1b. Elevated concentrations of divalent cations, such as zinc, copper, and cadmium, were shown to inhibit c_{rl} but the correlation between Cr(VI) and RL production has not been evaluated yet.[12] In the current study, it was found that amount of Cr(VI) in the fermentation broth, c_{Cr}, (Fig. 1c) slowly decreased from the log to the late-stationary phase (Fig. 1a), along with enhancement of the RL production, c_{rl}, (Fig. 1b), reaching the maximum Cr(VI) removal capacity of 70 % (**I**) and 57 % (**II**) in the late-stationary phase at 72 h, which coincides with the maximum of c_{rl} for both Cr(VI)-amended cultures. This fact is in a good agreement with the observation that extra-cellular RL influences the bioavailability of heavy metals.[12] Namely, it was hypothesized that as the RL production increased, the content of Cd^{2+} was reduced due to complexation with the extra-cellular RL present in culture medium, similarly to the present observation that the amount of Cr(VI) slowly decreased with increasing RL production.[12] On the contrary, Kiliç *et al.* showed that a *P. aeruginosa* strain, isolated from tannery effluents, had the highest Cr(VI) removal (94.3 %) at 50 mg L^{-1} Cr(VI) at the end of a 96-h of incubation, which correlated with the maximum production of exo-polysaccharide, and that *Micrococcus* sp., from a media containing 100 mg L^{-1} Cr(VI), removed the heavy metal with a yield of 35.5 % after incubation for 72 h.[24]



(a)



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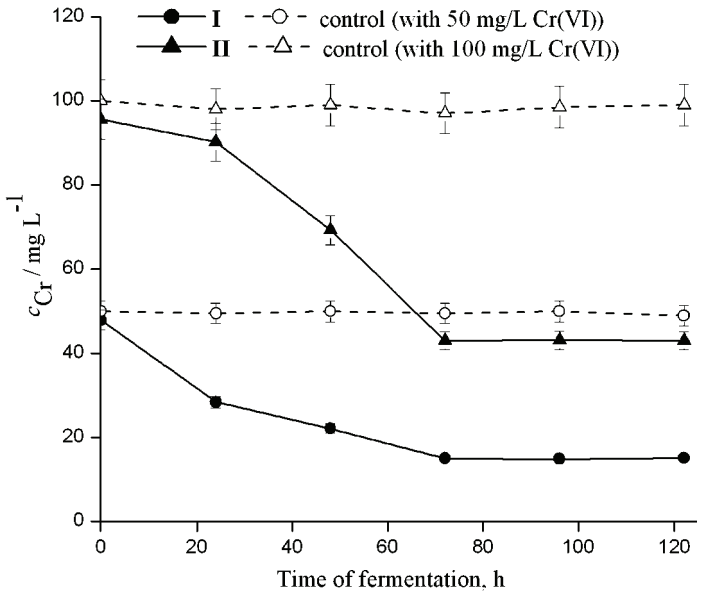

(c)

Fig. 1. a) Dynamics of the *OD* growth and RL production by *P. aeruginosa* NCAIM(P) as c_{rl} (b); B001380 grown in LB (control) and Cr(VI) supplemented LB medium with 50 (**I**) and 100 mg L^{-1} of Cr(VI) (**II**); c) concentration of Cr(VI) c_{Cr} in the culture broth of *P. aeruginosa* NCAIM(P), B001380 in the controls (non-inoculated LB mediums with 50 and 100 mg L^{-1} of Cr(VI)) and Cr(VI) supplemented LB mediums **I** and **II**. The symbols represent the average values of triplicate data and the error bars are standard errors.

With regard to the hypothesis that complexation with extracellular RL occurs, total chromium was determined in the RL samples by flame absorption atomic spectroscopy (FAAS) and it was found that amount of Cr in the RL preparation isolated from the culture filtrate of *P. aeruginosa* NCAIM(P), B001380 grown in LB with 100 mg of Cr(VI) (II), was 22 μg of Cr per mg of RL. Interestingly, the same amount of 22 μg of heavy metal Cd^{2+} per mg of RL was found to be complexed.[1] Actually, from the culture filtrate of **II**, it was confirmed that 57 % of total added Cr(VI) was absorbed. The amount of Cr measured in the RL preparation suggested that less than 5 % of total added Cr(VI), that is about 10 % of overall absorbed Cr(VI), was bound to RL, while all remaining chromium was found in the biomass, indicating absorption of Cr on exopolymers (EPS) on the surface of cells, as previously reported.[24] Aside from absorption, the strain *P. aeruginosa* NCAIM(P), B001380 can reduce Cr(VI) by chromate reductase, which was found to be an intracellular, soluble, non-inducible enzyme, with a specific activity of 40 μg Cr(VI) h^{-1} mg^{-1} proteins, as was expected.[28] Based on the given data, at least two mechanisms are proposed by

which *P. aeruginosa* NCAIM(P), B001380 controls the amount of toxic Cr(VI): reduction to Cr(III) and its efficient efflux, accompanied with biological complexing of Cr(VI) to RL and EPS.

Structural elucidation of RL–Cr(VI) adduct/complex

Heavy metals efficiently form complexes with RL obtained by *P. aeruginosa*, but structural details of complexes are lacking.[12,32,33] The coordination chemistry of Cr(VI) as a ligand is mainly the chemistry of anions as chromates, dichromates and polychromates with T_d symmetry.[34] RL–Cr(VI) interaction could be based on binding of CrO_4^{2-} as a ligand for protonated carboxylate groups of fatty acids residues of RL in complexes $(RL–COOH_2^+)_2CrO_4^{2-}$. RL preparations obtained by *P. aeruginosa* NCAIM(P), B001380 without (control) and with Cr(VI) (**II**) were analyzed by FT-IR, EI-MS and NMR spectroscopy to find and elucidate the structural relationship between RL and Cr(VI).

FT-IR spectroscopy of RL

The FT-IR spectra of purified RL of the control and Cr(VI)-amended sample **II** were recorded at room temperature in the wavenumber range of 400–4000 cm^{-1} and compared in Fig. 2. Both IR spectra showed characteristic bands for mono- and di-rhamnolipids, as reported by Leitermann *et al.*[35]

Broader and red shifted O–H band at 3288 cm^{-1} of **II** compared to control at 3450 cm^{-1} (Fig. 2), might be the first indication for protonation of the COOH group of RL in complex with CrO_4^{2-}. The red shift of 59 cm^{-1} for the C=O stretching band of **II** (1650 cm^{-1}) compared to the control (1709 cm^{-1}) is a further indication for a possible coordination of CrO_4^{2-} and hence weaker C=O bond. The red shift of the deformation C–OH band at 1399 cm^{-1} and the blue shift of O–C–O symmetric band at 1072 cm^{-1} for **II** compared to the control (1459 and 1043 cm^{-1}) could also be explained by coordination of the CrO_4^{2-}, as an anion ligand, with the protonated $COOH_2^+$ group of the fatty acid residue of RL.

In addition, another significant difference in spectrum of **II** is appearance of a new, weak and sharp band at 914 cm^{-1} (Fig. 2). This observation might be crucial proof for the coordination of CrO_4^{2-} to RL in **II** because it is known that when the chromate ion is coordinated, it shows a new, weak band of the Cr=O vibration at about 900 cm^{-1}.[36,37]

^{1}H-NMR spectra of RL

The ^{1}H-NMR spectra of RL of the control and **II** gave characteristic proton signals of rhamnose 1 and rhamnose 2 from monorhamnolipids (RL1) and dirhamnolipids (RL2) and signals of lipid components of RL.[30] The NMR results indicate that the products of the RLs of the control and **II** were a mixture of RL1 and RL2, but without any significant difference in their spectra. Many of their proton signals are overlapped, especially chemical shift of $–CH_2–COO^–$ protons

at 2.48–2.57 ppm, where a difference was expected between the control and **II**, due to the interaction of CrO_4^{2-} and RL of **II**, as it was evidenced by the IR spectra.

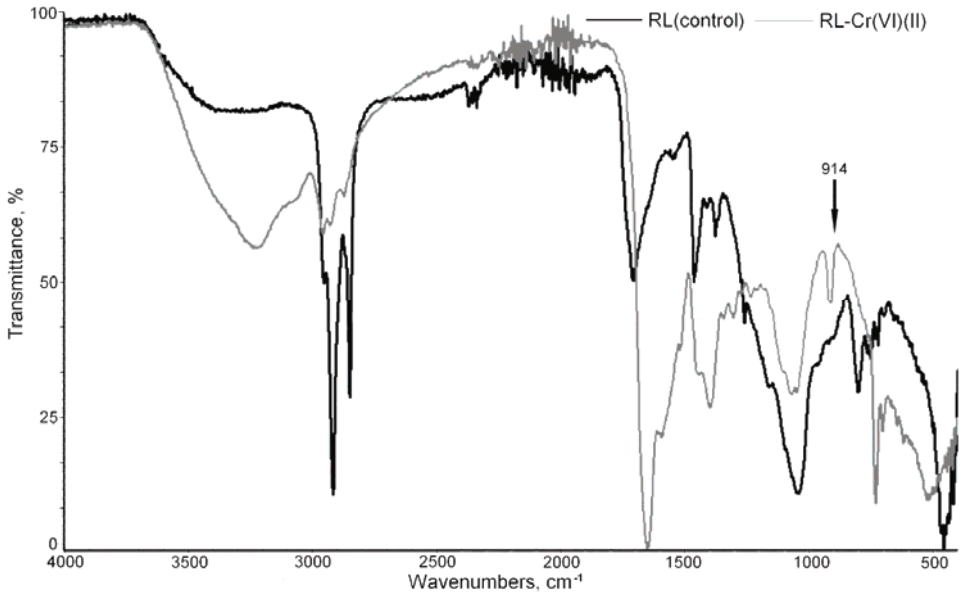

Fig. 2. Comparison of rhamnolipids IR spectra of the control and Cr(VI)-amended sample **II** (100 mg L^{-1}) obtained by *P. aeruginosa* NCAIM(P), B001380 grown in LB medium.

Influence of Cr(VI) on the distribution of RL congeners

RLs were isolated from the culture broths of the control and sample **II** at the time of its maximum yield (48 and 72 h, respectively), and they were analyzed by electrospray ionization mass spectrometry ESI/MS.[38,39] In the negative ESI mode, only negatively charged molecules can be observed. Under such conditions, the RLs, as molecules with an acidic function, show an intense $[M–H]^-$ peak without the occurrence of fragmentation. The effect of Cr(VI) on the distribution of the RL congeners is presented in Table I.

The mono-rhamnolipid (RL1) congeners **1**–**7** were observed in both samples, except **3**, which was detected only in the control (Table I). All of the di-rhamnolipid (RL2) congeners **8**–**12** were observed in both samples, except **12**, which was present only in sample **II** (Table I). Obviously, the slight difference between the control and the Cr(VI)-amended culture (**II**) comes from the minor congeners.

While the major RL congeners were the same in both cultures, the ratio of RL2 to RL1 was changed. The Cr(VI) treatment (**II**) resulted in a RL2/RL1 ratio

of 1.11, while in control, the RL2/RL1 ratio was 0.56. Similarly, it was reported that the ratio was significantly higher for a Cd-amended culture.[12]

TABLE I. List of rhamnolipid RL1 (**1**–**7**) and RL2 (**8**–**12**) congeners obtained in the control and Cr(VI)-amended sample **II** (100 mg L^{-1}), by *P. aeruginosa* NCAIM(P), B001380 grown in LB medium, with molecular formula, molecular weight, elution time and ratio of relative ion abundance control/Cr(VI)-amended culture (**II**), observed by ESI/MS

No	RL congener	Molecular formula	Molecular weight, g mol^{-1}	Elution time, min	Ratio of the relative ion abundance[a] control/**II**
1	Rha–C8	$C_{14}H_{26}O_7$	306.35	9.48	0.9/0.5
2	Rha–C10	$C_{16}H_{30}O_7$	334.41	8.95	0.4/0.5
3	Rha–C12	$C_{18}H_{34}O_7$	362.23	11.12	0.2/–[b]
4	Rha–C10–C10 / Rha–C8–C12 / Rha–C12–C8	$C_{26}H_{48}O_9$	504.65	11.43	28.8/37.6
5	Rha–C10–C12 / Rha–C12–C10	$C_{28}H_{52}O_9$	532.36	12.49	22.9/4.5
6	Rha–C10–C12:1 / Rha–C12:1–C10	$C_{28}H_{50}O_9$	530.69	12.06	9.8/3.3
7	Rha–C10–C10–CH3	$C_{27}H_{50}O_9$	518.68	11.97	1.0/0.9
8	Rha–Rha–C10–C10	$C_{32}H_{58}O_{13}$	650.79	10.74	20.1/30.9
9	Rha–Rha–C10–C12 / Rha–Rha–C12–C10	$C_{34}H_{62}O_{13}$	678.84	11.80	8.8/11.1
10	Rha–Rha–C10–C12:1 / Rha–Rha–C12:1–C10	$C_{34}H_{60}O_{13}$	676.40	11.36	5.5/3.5
11	Rha–Rha–C10–C10–CH_3	$C_{33}H_{60}O_{13}$	664.40	11.26	1.6/5.8
12	Rha–Rha–C10–C14:1/ Rha–Rha–C12–C12:1/ Rha–Rha–C12:1–C12	$C_{36}H_{64}O_{13}$	704.43	12.38	–[b]/1.4

[a]Abundance taken as the count number of the observed molecular ions. The relative ion abundance was calculated as the abundance of a given RL congener divided by the sum of abundances of all observed RLs; [b]Congener was not detected

The relative ion abundance (%) of the most abundant RL1 (**4**–**7**) and RL2 (**8**–**11**) congeners in the control and sample **II** are shown in Fig. 3. Monorhamnolipids **4**–**7** showed a higher relative abundance in the control, except **4** and even 5- and 3-fold increases for **5** and **6** in the expression ratio compared to those in **II** (Fig. 3, Table I). The dirhamnolipids congeners **8**–**11** were slightly more present in **II**, except for **10**. This resulted in the increase in the RL2/RL1 ratio of 1.11 for **II** compared to that of the control (0.56). This observation clearly shows that the presence of Cr(VI) influenced the rhamnolipid profile produced by *Pseudomonas*. This in turn would impact the bioavailability of the metal.

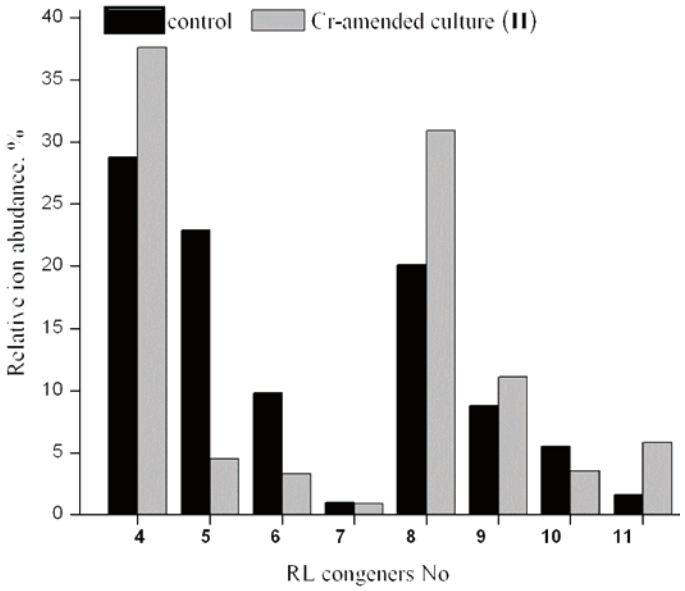

Fig. 3. Relative ion abundances (%) of the most abundant RL1 (**4**–**7**) and RL2 (**8**–**11**) congeners obtained in the control and Cr(VI)-amended sample **II** (100 mg L^{-1}), by *P. aeruginosa* NCAIM(P), B001380 grown in LB mediums.

CONCLUSIONS

This work confirmed the potential of *Pseudomonas aeruginosa* NCAIM(P), B001380 for application in bioremediation. The effect of different sub-lethal concentrations of Cr(VI) ion (**I** – 50 mg L^{-1} and **II** – 100 mg L^{-1}) on the growth and production of RL by strain was investigated. Cr(VI) was shown to inhibit RL production slightly, but the maximum of RL production was found in the late-stationary phase at 72 h for both Cr(VI)-amended cultures: **I** (236 mg L^{-1}) and **II** (160 mg L^{-1}). The relationship between RL production and Cr(VI) removal capacity was closely observed, showing that the maximum of the Cr(VI) removal capacity: 70 % (**I**) and 57 % (**II**) was reached with the maximum RL production. Although the amount of Cr measured in the RL preparation (22 μg mg^{-1}) indicated that less than 5 % of the total added Cr(VI) was bound to RL, in a further investigation, the yield could be enhanced in a two-phases experiment with first the production of rhamnolipids and then treatment with Cr(VI). Structural proof for RL–Cr(VI) interaction, which might have a pronounced impact on chromium bioremediation, was established by FTIR spectroscopy. An increase in a RL2/RL1 ratio to 1.11 for **II** compared to the control (0.56) was observed by ESI-MS, which also supports the finding that the presence of Cr(VI) may influence the rhamnolipid congeners produced by *Pseudomonas*, which in turn would impact the bioavailability of the metal.

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ИЗВОД

УТИЦАЈ РАМНОЛИПИДА ДОБИЈЕНИХ ПОМОЋУ *Pseudomonas aeruginosa* NCAIM(P), B001380 НА КАПАЦИТЕТ ЗА УКЛАЊАЊЕ Cr(VI) ПРИ РАСТУ У ТЕЧНОЈ ПОДЛОЗИ

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Утврђено је да бактерија *Pseudomonas aeruginosa* NCAIM(P), B001380, изолована из минералног уља за сечење метала, има високу толеранцију на хром и да производи биосурфактант рамнолипид који има потенцијал за примену у биоремедијацији тешких метала. Испитан је раст културе, продукција рамнолипида и капацитет за уклањање Cr(VI) у присуству 50 (**I**) и 100 mg L^{-1} Cr(VI) (**II**) у течној подлози. Утврђен је максимум продукције рамнолипида у касној стационарној фази при расту бактерије у присуству Cr(VI): **I** (236 mg L^{-1}) и **II** (160 mg L^{-1}), при чему је уклоњен маскимум Cr(VI): 70 % (**I**) и 57 % (**II**). Атомском апсорпционом спектроскопијом је утврђено присуство Cr у препарату екстрацелуларног рамнолипида **II** у концентрацији од 22 μg mg^{-1}. Појављивање нове траке на 914 cm^{-1} у инфрацрвеном спектру рамнолипида **II** се може узети као значајна индикација могуће координације јона CrO_4^{2-} са рамнолипидом. Ефекат Cr(VI) на расподелу и однос ди- и моно- рамнолипида је испитан помоћу електроспреј-јонизационе масене спектрометрије (ESI–MS), при чему је, у поређењу са контролом, уочено повећање односа ди-/моно-рамнолипида у узорку **II**.

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