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Research Article

Effect of Chromium and Organic Acids on Microbial Growth and Exopolymeric Substance Production by *Pseudomonas* Bacteria

Natural organic acids are capable of stimulating microbial chromium(VI) reduction, but little information is available about their behavior on microbial growth, exopolymeric substance (EPS) production, and subsequent microbial Cr(VI) reduction. Here, laboratory batch experiments were conducted to determine the effects of different natural organic acids (galacturonic, glucuronic, citric, and alginic acid) on microbial EPS production and the growth rates of four different naturally occurring soil bacteria (*Pseudomonas putida* P18, *P. aeruginosa* P16, *P. fluorescens* ATCC 55241, and *P. stutzeri* P40) as a function of pH and time in solutions containing toxic metal ions such as Cr(III) and Cr(VI). While the addition of Cr(VI) led to a negative impact on microbial growth in all strains studied, Cr(VI) significantly enhanced EPS release from cells due to extreme cell lysis. Organic acids diminished the toxic effects of Cr(VI) on cells, and thus significantly increased microbial cell growth and the EPS yield. The addition of Cr(III) with Cr(VI), on the other hand, led to a significant decrease in microbial cell growth rates relative to the systems containing only Cr(VI). This toxic effect decreased significantly in the presence of organic acids, and thus the EPS yield increased due to the formation of less toxic Cr(III)-EPS species. The overall results indicate that while the accumulation of free Cr(III) ion in aqueous phase during microbial Cr(VI) reduction may have an adverse influence on microbial cell growth, the EPS released by bacteria may bind with free Cr(III) ion in solution, and thus increase the cell growth rate due to the removal of toxic products of microbial reduction.

Keywords: Cell growth; Heavy metal toxicity; Organo–Cr(III) complex; Soil bacteria

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1 Introduction

Microbial exopolymeric substances (EPS), produced by bacteria to protect the cell walls from the toxic effects of environmental factors [e.g. pH, Cr(VI)] can affect the stability and transport behavior of chromium species in subsurface systems [1–6]. For instance, Dogan et al. [7] observed that microbial EPS significantly increased Cr(VI) reduction rates by *Pseudomonas* bacteria. Results from Harish et al. [6] and Sheng et al. [8] suggest that Cr(VI) species can stimulate the release of bacterial EPS in aqueous environment. Priester et al. [9] stated that free Cr^{3+} ion that formed as a result of microbial reduction of Cr(VI) with *P. putida* was partially bound by microbial EPS. Kantar et al. [10] found that microbial EPS significantly affected Cr(III) transport behavior in subsurface systems due to the formation of highly soluble and less sorbing Cr-EPS complexes.

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Abbreviations: **EPS**, exopolymeric substances; **TEM**, transmission electron microscopy

While data from Dogan et al. [7] suggest that the accumulation of free Cr(III) ion in aqueous phase during microbial Cr(VI) reduction led to the inhibition of the chromium reductases in *P. putida* and *P. aeruginosa*, this inhibitory effect decreased significantly with the addition of bacterial EPS and natural organic acids, thereby leading to increases in Cr(VI) removal rates relative to non-organic acid containing systems. In spite of the fact that there is significant information on the stimulatory effect of bacterial EPS on microbial Cr(VI) treatment [e.g. [6, 7]], knowledge on the role of low molecular weight natural organic acids on bacterial EPS production and subsequent bacterial Cr(VI) reduction is scarce in the literature. This present work was performed to evaluate the effects of natural organic acids on EPS yield and the cell growth rates of *P. putida* P18, *P. aeruginosa* P16, *P. fluorescens* ATCC 55241 and *P. stutzeri* P40 in aqueous environment containing toxic metal ions such as Cr(VI) and Cr(III). The organic substrates used in the study include glucuronic, galacturonic, citric, and alginic acids. Alginate, galacturonate, and glucuronate, recognized as primary components of microbial EPS that bind with metal ions, are released by some soil bacteria in the environment in response to environmental stress [11, 12]. Citric acid, a cellular organic metabolite, can strongly complex with Cr(III), and

form highly soluble Cr(III)–citrate complexes during microbial Cr(VI) reduction [13].

2 Materials and methods

2.1 Materials

All reagents used in this research were analytical grade or better. Water was obtained from a Millipore UV–water system. The stock solutions of Cr(VI) were made with potassium dichromate ($K_2Cr_2O_7$) (Merck) in UV–water. The stock solutions of Cr(III) were prepared from chromium(III)-nitrate-nonahydrate (Merck). Alginic acid sodium monohydrate was obtained from Aldrich. Stock solutions of galacturonate were made with D(+)-galacturonic acid ($C_6H_{10}O_7 \cdot H_2O$) (Fluka). Similarly, citric ($C_6H_8O_7 \cdot H_2O$) and glucuronic ($C_6H_9NaO_7 \cdot H_2O$) acids were supplied from Merck. The stock solutions were kept in a refrigerator at 4°C for further use, and allowed to equilibrate to room temperature prior to use in batch experiments.

2.2 Strains

While the strains of *P. fluorescens* ATCC 55241 were purchased from LGC Standards, the strains of *P. putida*, *P. aeruginosa* and, *P. stutzeri* were isolated from soils in various parts of Turkey (e.g. Aydin and Dalaman). Detailed information on isolation and purification of microbial EPS was provided in detail by Hung et al. [14].

2.3 Preparation of media and growth conditions

While the growth media (Tryptic Soy Broth: TSB) for *P. fluorescens* ATCC 55241 and *P. putida* P18 contained dipotassium hydrogen phosphate (2.5 g/L), NaCl (5 g/L), glucose (2.5 g/L), peptone from soy meal (3 g/L) and peptone from casein (17 g/L), the growth media (Lysogeny Broth (LB)-Miller) for *P. aeruginosa* P16 and *P. stutzeri* P40 consisted of NaCl (10 g/L), yeast extract (5 g/L) and tryptone (10 g/L). The bacterial strains were grown under aerobic conditions in 250 mL flasks containing 100 mL of growth media at 30°C (for *P. putida* P18 and *P. fluorescens* ATCC 55241) and 37°C (for *P. stutzeri* P40 and *P. aeruginosa* P16). The cell suspensions were continuously stirred at 150 rpm, and the cell growth was observed by checking optical density (OD) at 600 nm wavelength. The McFarland 0.5 standards provided turbidity comparable to a bacterial suspension containing 1.5×10^8 cfu/mL.

2.4 Role of pH and organic acids on EPS production

Kinetic experiments were conducted to determine the influence of solution pH and organic acids on EPS production by *P. putida* P18, *P. stutzeri* P40, *P. fluorescens* ATCC 55241, and *P. aeruginosa* P16. The flasks with 100 mL of growth media at a desired solution pH and organic acid concentration (1 g/L) were injected with 2 mL cultures at log phase. Prior to use in experiments, all media were autoclaved at ~121°C for 15 min. The pH adjustments were done using 0.1 M NaOH and 0.1 M HCl. The strains were then incubated under aerobic conditions at 37°C for *P. stutzeri* P40 and *P. aeruginosa* P16, and 30°C for *P. fluorescens* ATCC 55241 and *P. putida* P18 with constant shaking at 150 rpm. Immediately after inoculation with bacterial strains, samples were withdrawn at selected time intervals, and analyzed for their total EPS contents using ethanol precipitation procedure described by Frengova et al. [15]. The growth of microbial cells was continuously observed by checking OD at 600 nm wavelength.

2.5 Role of Cr(VI), Cr(III) and organic acids on EPS production

The influence of Cr(III) and Cr(VI) on EPS production in the absence or presence of an organic acid (1 g/L) was investigated in 250 mL flasks

Table 1. Experimental conditions for EPS production and microbial growth experiments

Experiment	Strain	pH[a)]	Growth media	Organic substrate[b)] (1 g/L)	Cr(VI) (mg/L)	Cr(III) (mg/L)
1	*P. putida*	7.0	TSB	-	–	–
2	*P. putida*	7.0	TSB	+	–	–
3	*P. putida*	7.0	TSB	-	17.5	–
4	*P. putida*	7.0	TSB	+	17.5	
5	*P. putida*	7.0	TSB	-	12.5	7.5
6	*P. putida*	7.0	TSB	+	12.5	7.5
7	*P. fluorescens*	7.0	TSB	-	–	–
8	*P. fluorescens*	7.0	TSB	+	–	–
9	*P. fluorescens*	7.0	TSB	-	40	–
10	*P. fluorescens*	7.0	TSB	+	40	
11	*P. fluorescens*	7.0	TSB	-	28	12
12	*P. fluorescens*	7.0	TSB	+	28	12
13	*P. stutzeri*	7.5	LB-Miller	-	–	–
14	*P. stutzeri*	7.5	LB-Miller	+	-	–
15	*P. stutzeri*	7.5	LB-Miller	-	30	–
16	*P. stutzeri*	7.5	LB-Miller	+	30	
17	*P. stutzeri*	7.5	LB-Miller	-	21	9
18	*P. stutzeri*	7.5	LB-Miller	+	21	9
19	*P. aeruginosa*	7.5	LB-Miller	-	-	–
20	*P. aeruginosa*	7.5	LB-Miller	+	-	–
21	*P. aeruginosa*	7.5	LB-Miller	-	130	–
22	*P. aeruginosa*	7.5	LB-Miller	+	130	
23	*P. aeruginosa*	7.5	LB-Miller	-	100	30
24	*P. aeruginosa*	7.5	LB-Miller	+	100	30

[a)] All solutions were buffered to pH 7 or 7.5 with an appropriate amount of $NaHCO_3$.
[b)] Galacturonic, glucuronic, alginic, citric acid, or microbial EPS.

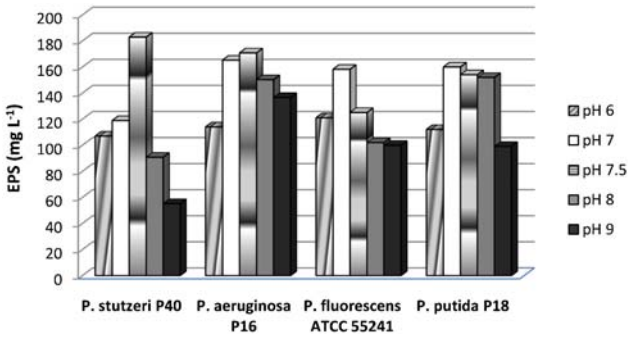

Figure 1. Effects of pH on EPS production by *P. putida* P18, *P. aeruginosa* P16, *P. stutzeri* P40, and *P.fluorescens* ATCC 55241. All solutions were prepared in either LB-Miller or TSB nutrient medium.

with a desired Cr(VI) and/or Cr(III) concentration. The total solution volume was 100 mL in all experiments. The experiments were performed in a similar fashion as explained above. The Cr(VI) concentrations used in the batch experiments were selected using data obtained from minimum inhibitory concentration tests as outlined by Dogan et al. [7]. In all experiments, samples were withdrawn at selected time intervals for EPS analysis. The experimental conditions are summarized in Tab. 1.

2.6 Statistical analysis

All experiments were carried out in duplicate. Differences between treatments were identified by using a Student *t*-test ($p < 0.05$) via Microsoft Excel.

3 Results

3.1 Effect of pH on EPS production

Figure 1 shows the effects of solution pH on EPS production by bacteria in growth media with a solution pH ranging from 6 to 9. In these experiments, the EPS released by bacteria were determined at the end of 58 h incubation. In all strains analyzed, the EPS production reached a maximum at near-neutral pH (~7 to ~7.5), and decreased sharply towards more acidic or more alkaline conditions. While the maximum EPS production by *P. aeruginosa* P16 (170.8 mg/L) and *P. stutzeri* P40 (182.9 mg/L) was observed at pH 7.5, *P. fluorescens* ATCC 55241 (158 mg/L) and *P. putida* P18 (160 mg/L) exhibited maximum EPS yield at pH 7 (Fig. 1). This pH dependence of EPS production has also been observed by other strains [16, 17]. For example, Bonet et al. [18] reported that the maximum amount of EPS production by *Aeromonas salmoicida* was observed at pH valuess between 7 and 7.5. Similarly, Kilic and Donmez [17] found that a solution pH of 7 was required to obtain a maximum EPS yield by *P. aeruginosa*.

3.2 Effect of organic acids on EPS production

Kinetic experiments were conducted to evaluate the influence of organic acids on microbial EPS production (Fig. 2). The experimental conditions for these experiments (Exp. 2, 8, 14, 20) are tabulated in Tab. 1. Note that the EPS yield initially increased, and approached a maximum value at incubation times between 48 and 58 h, and decreased sharply at much higher incubation times. It is clear that all organic acids used in the study led to certain degrees of increases in EPS yield relative to systems containing only the growth media ($p < 0.05$), and that the amount of EPS released by bacteria was highly

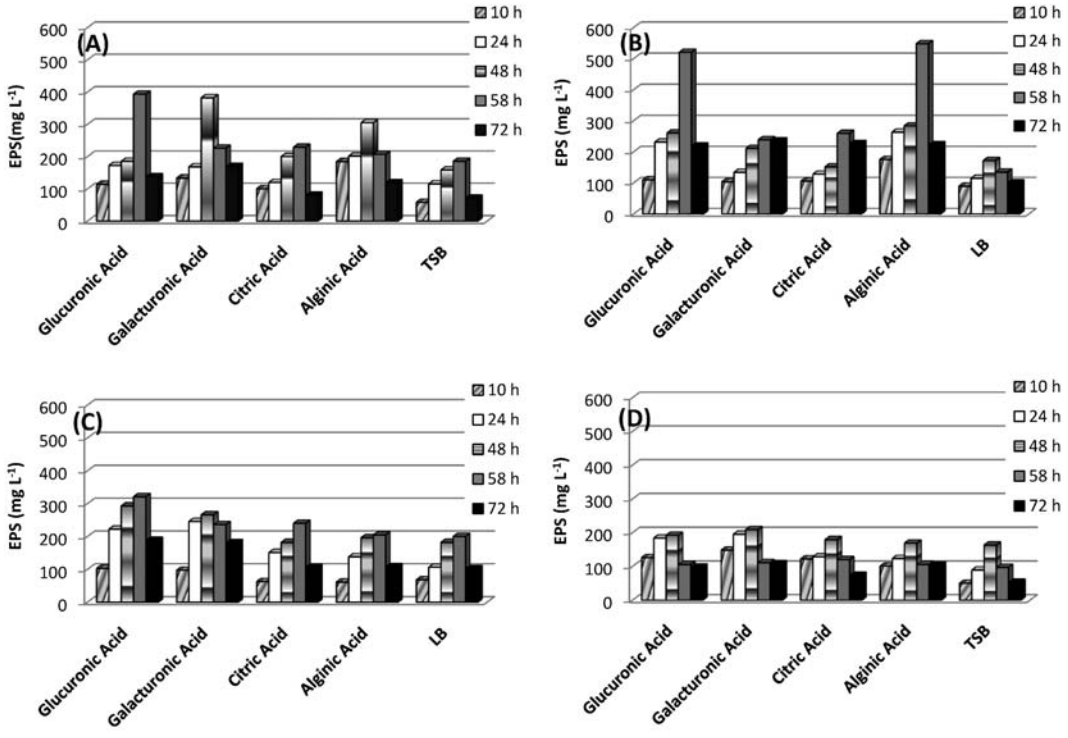

Figure 2. Effects of organic acids (1 g/L) on EPS production by (A) *P. putida* P18, (B) *P. aeruginosa* P16, (C) *P. stutzeri* P40, and (D) *P. fluorescens* ATCC 55241. Experimental conditions are summarized in Tab. 1.

dependent on the type of organic acid used. For instance, among all organic acids studied, glucuronic acid was very effective in stimulating EPS production by all bacterial strains (Fig. 2). *P. putida* P18, *P. stutzeri*, and *P. fluorescens* produced maximum EPS in growth media with 1 g/L glucuronate or galacturonate. For *P. aeruginosa*, on the other hand, the maximum EPS formation occurred in growth media with 1 g/L alginate or glucuronate.

3.3 Combined effects of organic acids and Cr(VI) on EPS production

Figure 3 shows the effects of Cr(VI) on microbial EPS yield in the absence or presence of an organic acid at an incubation time of 58 h (log phase). The experimental conditions for these experiments (Exp. 3 and 4 for *P. putida*; Exp. 9 and 10 for *P. fluorescens*; Exp. 15 and 16 for *P. stutzeri*; Exp. 21 and 22 for *P. aeruginosa*) are given in Tab. 1. Except for *P. stutzeri*, all bacterial strains spiked with Cr(VI) produced more EPS relative to the growth media with no organic acid. While the co-addition of organic acids with Cr(VI) led to a significant increase in EPS yield for *P. stutzeri* and *P. aeruginosa* ($p < 0.05$), all organic acids, except for alginic acid, had little or no effect on EPS yield by *P. fluorescens* and *P. putida*. Especially, of the four bacterial strains tested, *P. aeruginosa* P16 was able to produce the highest amount of EPS in growth media with glucuronic, galacturonic, or alginic acid with Cr(VI).

The influence of Cr(VI) and organic acids on EPS yield at an incubation time of 10 h (lag phase) is presented in Fig. 4. Note that during lag phase ($t = 10$ h), the EPS yield for all strains was very low relative to the log phase ($t = 58$ h) under all experimental conditions studied. Except for *P. stutzeri*, all bacterial strains inoculated with Cr(VI) in growth media produced slightly higher EPS relative to the growth media with no ligand. In growth media with Cr(VI), the highest EPS yield was observed in *P. fluorescens* relative to the growth media with no Cr(VI). The addition of a ligand with Cr(VI) in growth media resulted in slightly higher EPS yield relative to the growth media without any ligand. The highest EPS yield was obtained with *P. putida* in growth media with 17.5 mg/L Cr(VI) and 1 g/L alginate (Fig. 4C).

3.4 Combined effects of organic acids, Cr(VI), and Cr(III) on EPS production

Free Cr(III) ion is commonly produced as a result of microbial Cr(VI) reduction [7, 9, 13]. For instance, Puzon et al. [13] determined that Cr(VI) reduction with cellular organic metabolites led to the formation of both soluble and insoluble organo–Cr(III) complexes in aqueous phase. Although Cr(III) is an end-product of microbial reduction, there is limited information on the effects of Cr(III) species on EPS production and overall microbial Cr(VI) reduction rates in the literature. Dogan et al. [7] observed that the accumulation of Cr(III) species in solution during microbial Cr(VI) reduction adversely

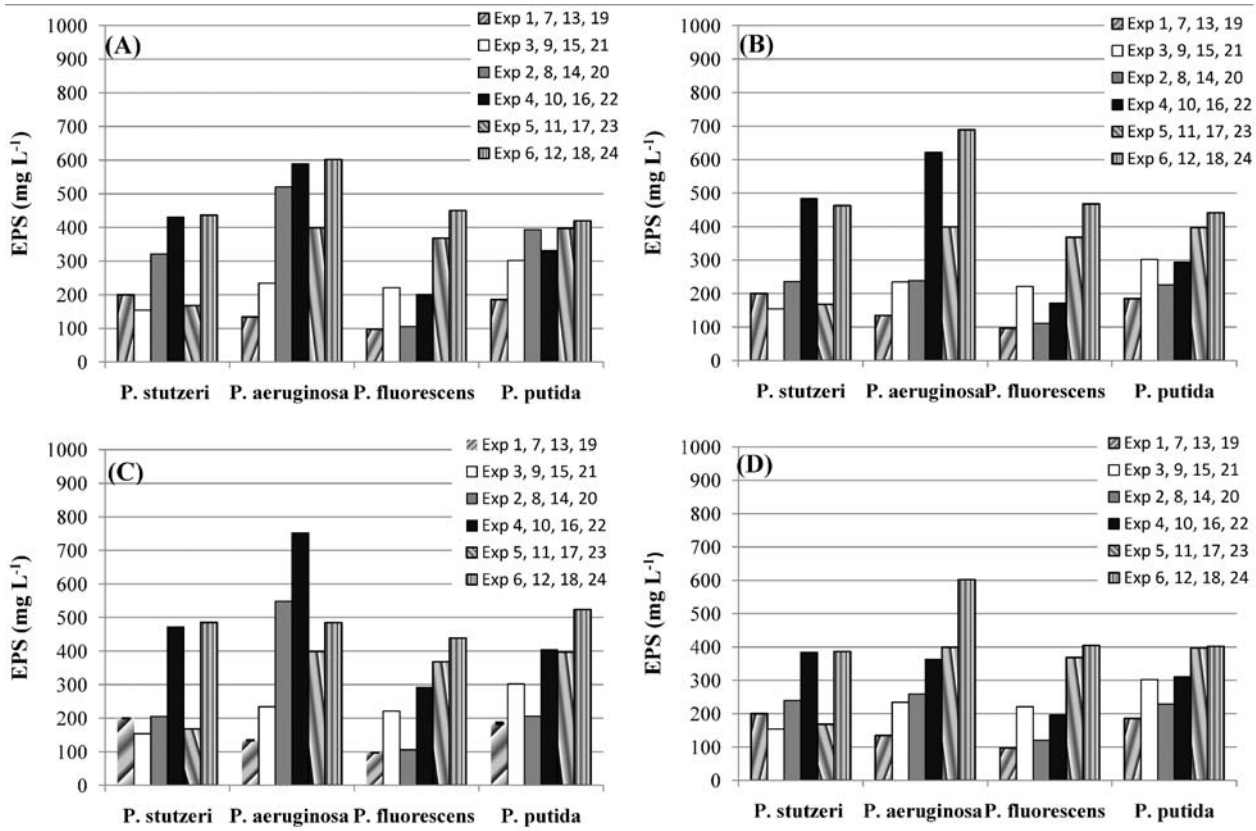

Figure 3. Effects of Cr(III) and/or Cr(VI) on EPS production by bacteria incubated in growth media for 58 h (log phase) in the absence or presence of (A) 1 g/L glucuronic acid, (B) 1 g/L galacturonic acid, (C) 1 g/L alginic acid, and (D) 1 g/L citric acid. Experimental conditions are summarized in Tab. 1. Exp. 1, 7, 13, and 19 [growth media]; Exp. 3, 9, 15, and 21 [growth media + Cr(VI)]; Exp. 2, 8, 14, and 20 [growth media + ligand]; Exp. 4, 10, 16, and 22 [growth media + ligand + Cr(VI)]; Exp. 5, 11, 17, and 23 [growth media + Cr(III) + Cr(VI)]; Exp. 6, 12, 18, and 24 [growth media + ligand + Cr(III) + Cr(VI)].

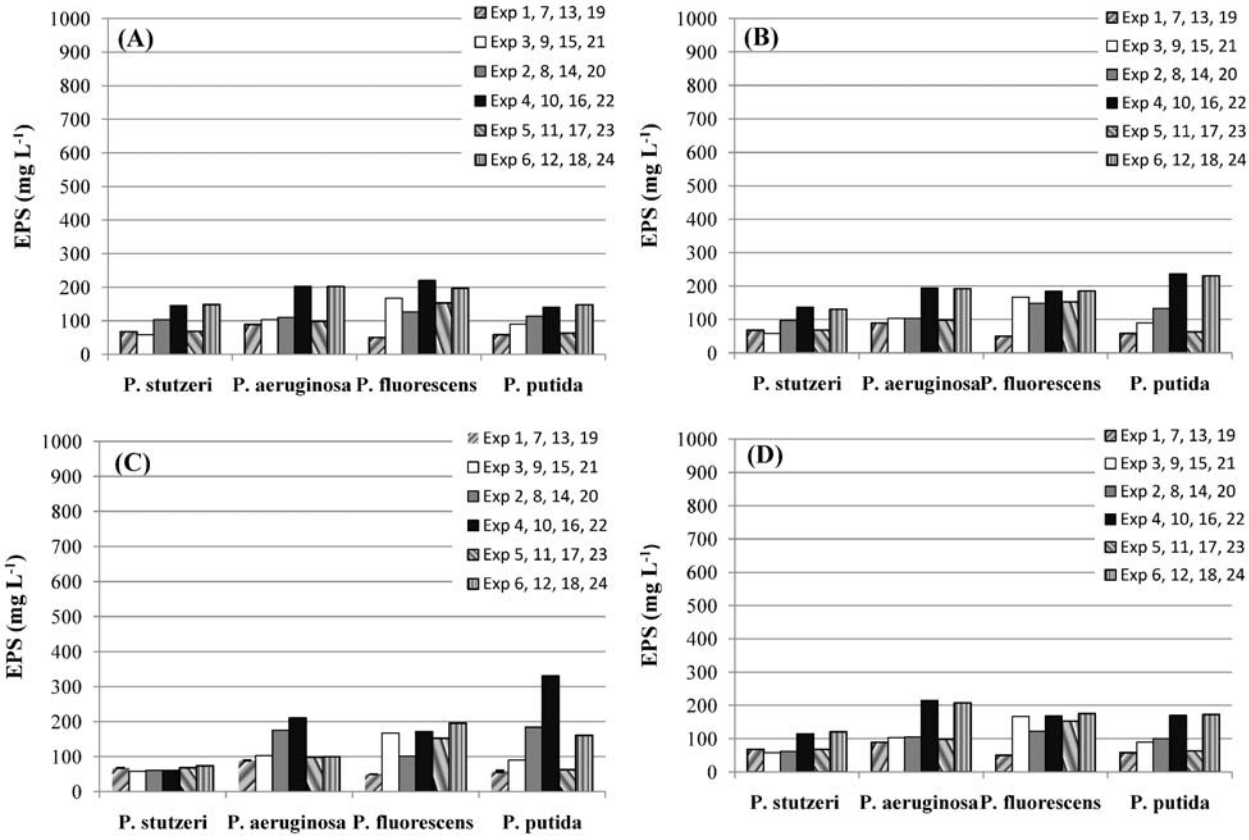

Figure 4. Effects of Cr(III) and/or Cr(VI) on EPS production by bacteria incubated in growth media for 10 h (lag phase) in the absence or presence of (A) 1 g/L glucuronic acid, (B) 1 g/L galacturonic acid, (C) 1 g/L alginic acid, and (D) 1 g/L citric acid. Experimental conditions are summarized in Tab. 1. Exp. 1, 7, 13, and 19 [growth media]; Exp. 3, 9, 15, and 21 [growth media + Cr(VI)]; Exp. 2, 8, 14, and 20 [growth media + ligand]; Exp. 4, 10, 16, and 22 [growth media + ligand + Cr(VI)]; Exp. 5, 11, 17, and 23 [growth media + Cr(III) + Cr(VI)]; Exp. 6, 12, 18, and 24 [growth media + ligand + Cr(III) + Cr(VI)].

affected microbial Cr(VI) reduction rates. Figure 3 shows the influence of organic acids on microbial EPS production in growth media with both Cr(III) and Cr(VI) (e.g. Exp. 5 and 6 for *P. putida*; Exp. 11 and 12 for *P. fluorescens*; Exp. 17 and 18 for *P. stutzeri*; Exp. 23 and 24 for *P. aeruginosa*). The results indicate that all bacterial strains inoculated in growth media with both Cr(III) and Cr(VI) produced more EPS relative to the growth media with only Cr(VI) ($p < 0.05$). Of all the bacterial strains studied, *P. aeruginosa* and *P. putida* were the most effective bacterial strains in EPS production in growth media with both Cr(VI) and Cr(III) in the absence of organic ligands. While the addition of organic acids with both Cr(III) and Cr(VI) elevated EPS yield to a certain degree by all strains relative to non-organic acid systems ($p < 0.05$), the highest impact of organic acids on EPS production was observed in *P. stutzeri*. These results indicate that the toxic effect of Cr(III) on microbial cells decreased in the presence of organic acids. Of all the strains analyzed, *P. aeruginosa* produced the highest amount of EPS in systems containing 1 g/L galacturonate with Cr(III) and Cr(VI). Except for alginic acid, all organic acids used had negligible effect on EPS production with *P. putida* in growth media with both Cr(III) and Cr(VI).

For lag phase ($t < 10$ h) (Fig. 4), the co-injection of Cr(III) with Cr(VI) in growth media had very little or no effect on EPS yield relative to the growth media with Cr(VI) alone. However, the addition of a ligand with Cr(III) and Cr(VI) resulted in slightly higher EPS yield in all strains relative to the growth media with only Cr(III) and Cr(VI).

4 Discussion

A large number of studies have been performed over the last two decades to determine the potential role of microbial EPS on metal ion transport as an important factor to be evaluated for the improvement of risk assessments and remedial actions [6, 9, 10, 19]. Data by Dogan et al. [7] suggest that while the formation of Cr(III) species during microbial Cr(VI) reduction led to the inhibition of Cr(VI) reductases associated with *P. aeruginosa* and *P. putida*, the inhibitory effect caused by Cr(III) species decreased significantly with the addition of bacterial EPS and natural organic acids (e.g. glucuronate). Similarly, Harish et al. [6] found that the EPS extracted from *Enterobacter cloacae* SUKCr1D was able to chemically reduce Cr(VI) to Cr(III) at low Cr(VI) concentrations (e.g. 10 mg/L). This current work provides further data on the role of bacterial EPS on Cr(VI) reduction and defense mechanisms that *Pseudomonas* bacteria develop to cope with the toxic effect of Cr(III) and Cr(VI) on microbial cells. In our previous work, we found that Cr(III) strongly complexes with microbial EPS released by bacteria during microbial Cr(VI) reduction [19]:

$$\text{Organic acids} + \text{Cr(VI)} \xrightarrow{\text{Cells/Enzymes}} \text{Cr(III)} + \text{EPS} \rightarrow \text{Cr(III)} - \text{EPS} \quad (1)$$

Microbial EPS may strongly complex with Cr(III) through a variety of functional groups (e.g. carboxylic) [19]. The Cr(III)–EPS complexes not only dominate Cr(III) speciation, but also lead to enhanced Cr(III) solubility even under alkaline pH environment [7]. In addition, data by

Dogan et al. [7] suggested that the formation of such less toxic organo-Cr(III) complexes during microbial Cr(VI) reduction protected the chromate reductases from inactivation, thereby leading to an increase in Cr(VI) reduction rates. Mabbett et al. [20] determined that microbial Cr(VI) reduction rates were closely related to the strength of Cr(III)–ligand complexes in systems containing low molecular weight organic ligands. Similarly, Desai et al. [21] found that organic ligands such as citrate may stimulate Cr(VI) reduction in *Pseudomonas* sp. G1DM21.

Figure 5 shows the role of chromium and microbial EPS on cell growth. In all cases studied, the cell growth rate significantly increased with an increase in incubation times, and approached a maximum value at approximately $t = 58$ h. However, at much higher incubation times ($t > 58$ h), the cell growth rate decreased sharply. The decreases in cell growth rate at $t > 58$ h may be explained through endogenous respiration, which usually occurs under nutrient-limited conditions. In growth media with no chromium (e.g. Exp. 2, 8, 14, and 20), the bacterial EPS significantly enhanced the cell growth relative to non-EPS systems, indicating that bacterial EPS can be used as a C source under nutrient-limited conditions.

In growth media with Cr(VI) (e.g. Exp. 3, 9, 15, 21), Cr(VI) had a negative impact on microbial growth ($p < 0.05$) in all strains studied due to the adverse effects of Cr(VI) on bacterial cells (Fig. 5). Note that despite the negative impact of Cr(VI) on cell growth, the addition of Cr(VI) led to enhanced EPS yield in some bacterial strains such as *P. aeruginosa*, *P. fluorescens* and *P. putida* (Fig. 3). The enhanced EPS yield observed in the presence of Cr(VI) may be explained through cell lysis. Figure 6 shows transmission electron microscopy (TEM) images of *P. aeruginosa* P16 cells incubated in growth media for about 58 h with or without Cr(VI) exposure. Note that the TEM images show significant cell wall destruction when exposed to Cr(VI) relative to the cells incubated without Cr(VI). The images also indicate that Cr(VI) not only led to alterations in cell shapes, but also initiated serious damages on the cell walls. Note that the cell walls of bacteria without Cr(VI) exposure were thicker than those of cells exposed to Cr(VI), indicating that the cell lysis may play a major impact on microbial Cr(VI) reduction, especially in growth media without organic ligands. Priester et al. [9] found that cell lysis led to the production of Cr(VI) reductase enzymes that can catalyze extracellular Cr(VI) reduction with *P. putida*. McLean and Beveridge [22] reported that the chromate reductase enzymes, originally found in the cytoplasm, were released into solution due to extreme cell destruction, thereby reducing Cr(VI) extracellulary. On the contrary, Puzon et al. [13] reported that chromium(VI), initially reduced in the cell cytoplasm, left cells due to extreme cell lysis.

Figure 5 displays the effects of microbial EPS on cell growth in growth media with Cr(VI) and/or Cr(III). The microbial growth in growth media with microbial EPS and Cr(VI) (e.g. Exp. 4, 10, 16, 22) was significantly enhanced relative to the growth media with Cr(VI) ($p < 0.05$). These results indicate that the adverse effects of Cr(VI) on bacterial cells decreased in the presence of organic acids and bacterial EPS which, in turn, led to an increase in microbial growth. The increase in cell growth in the presence of organics acids strongly correlated with the enhanced EPS yield for some bacterial strains, e.g. *P. aeruginosa* and *P. stutzeri* (Fig. 3). Note that compared to the EPS yield at $t = 58$ h (Fig. 3), the EPS yield was much lower at $t = 10$ h

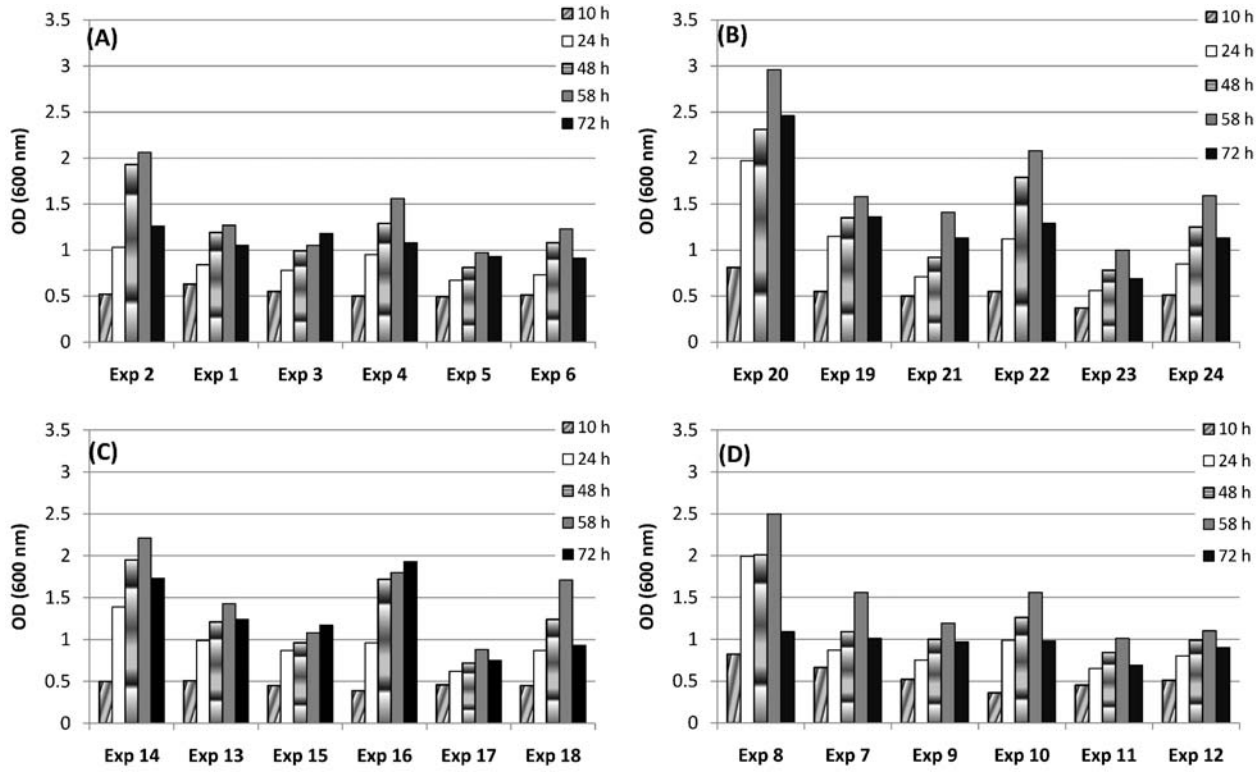

Figure 5. Effects of Cr(VI) and Cr(III) on cell growth in absence or presence of 1 g/L microbial EPS by (A) *P. putida* P18, (B) *P. aeruginosa* P16, (C) *P. stutzeri* P40, and (D) *P. fluorescens* ATCC 55241. Experimental conditions are summarized in Tab. 1. Here, the microbial EPS directly isolated and purified from bacteria was used as the organic ligand.

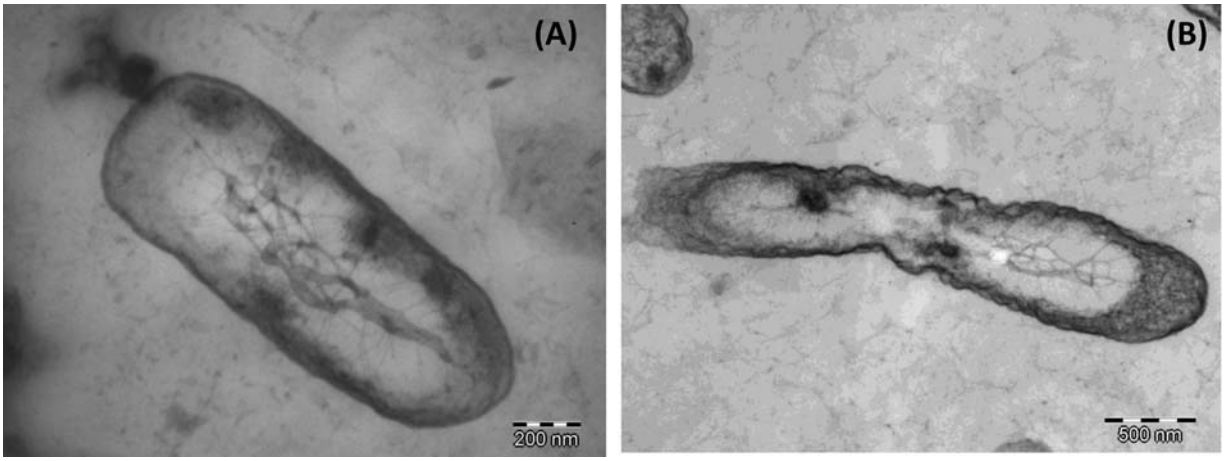

Figure 6. TEM images of *P. aeruginosa* P16 incubated for about 58 h in LB-Miller nutrient medium containing 1 g/L glucuronic acid with (A) no Cr(VI), and (B) 130 mg/L Cr(VI).

(Fig. 4) due to less cell growth (Fig. 5) as well as less cell lysis during lag phase.

The co-addition of Cr(III) with Cr(VI) (e.g. Exp. 5, 11, 17, 23), on the other hand, led to a significant decrease in microbial growth relative to the growth media with Cr(VI) (Fig. 5). This indicates that Cr(III) exhibited more toxic effects on cells than Cr(VI). Despite the toxicity of Cr(III) on microbial cells, the EPS yield for some bacterial strains e.g. *P. aeruginosa* increased significantly relative to the growth media with only Cr(VI), which is indicative of extreme cell lysis (Fig. 3). Fang et al. [23] found that trivalent chromium resulted in a nearly 82% increase in extracellular carbohydrate in sulfate-reducing bacterial biofilms. The addition of an organic acid with Cr(III) significantly improved the EPS yield for some bacterial strains such as *P. aeruginosa* and *P. stutzeri* due to the formation of non- or less toxic Cr(III)–ligand complexes (e.g. Cr(III)–EPS complex). In addition to protection of Cr(VI) reductase enzymes, it is clear that the formation of such soluble and less toxic organo–Cr(III) complexes may also protect the bacterial strains from the adverse effects of Cr(III) species. In our previous paper [7], we found that organic ligands significantly decreased Cr(III) binding by cell surfaces relative to non-organic systems. Similarly, Mabbett et al. [20] reported that free Cr(III) ion bound with an organic ligand does not interact with bacterial surface sites responsible for Cr(VI) reduction.

In brief, chromium exposure to microbial cells resulted in lower cell yield, but enhanced EPS release partly due to cell lysis. The toxic effect caused by chromium species decreased significantly in the presence of organic acids, leading to enhanced cell growth and EPS yield due to the formation of less toxic organo–Cr(III) complexes. The enhanced EPS yield in the presence of organic acids also provided a source of carbon for bacteria, which, in turn, may affect bacterial survival under nutrient-limited conditions.

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The authors have declared no conflict of interest.

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