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Production and partial characterization of bioemulsifier from a chromium-resistant actinobacteria

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HIGHLIGHTS

- The first study on emulsifiers production by *Streptomyces* sp. MC1 is presented.
- The cultivation factors have a significant influence on emulsifier production.
- *Streptomyces* sp. MC1 is able to produce emulsifier in presence of Cr(VI).
- Emulsifier presented high thermo-stability and partial water solubility.
- Emulsifiers possess promising prospects for remediation of metal-contaminated sites.

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ABSTRACT

Surface-active compounds such as synthetic emulsifiers have been used for several decades, both for the degradation of hydrocarbons and increasing desorption of soil-bound metals. However, due to their high toxicity, low degradability, and production costs unaffordable for use in larger ecosystems, synthetic emulsifiers have been gradually replaced by those derived from natural sources such as plants or microbes. In previous studies, the bacterium *Streptomyces* sp. MC1 has shown the ability to reduce and/or accumulate Cr(VI), a highly promising advance in the development of methods for environmental clean-up of sites contaminated with chromium. Here, new studies on the production of emulsifier from this strain are presented. The cultivation factors that have a significant influence on emulsifier biosynthesis, as well as the interactions among them, were studied by factorial design. Based upon optimization studies, maximum bioemulsifier production was detected in the culture medium having an initial pH of 8 with phosphate 2.0 g L^{-1} and Ca^{+2} 1.0 g L^{-1} added, with an emulsification index about 3.5 times greater compared to the basal value. Interestingly, in the presence of 5.0 g L^{-1} Cr(VI), *Streptomyces* sp. MC1 retained about 65% of its emulsifier production ability. Partially purified emulsifier presented high thermo-stability and partial water solubility. These findings could have promising future prospects for the remediation of organic- and metal-contaminated sites.

1. Introduction

The production of microbial emulsifiers, also called bioemulsifiers, has increased in recent years because of their higher biodegradability and reduced toxicity compared to their synthetically produced alternatives (Colin et al., 2010). Bioemulsifiers are amphiphatic molecules secreted by (micro)organisms, which facilitate the uptake of water-insoluble substrates (Shete et al., 2006). They are therefore commonly produced in response to microbial growth on hydrocarbons (Luna-Velasco et al., 2007; Martínez-Checa et al., 2007). However, there are also a few examples of bioemulsifier production during microbial growth on carbohydrates (Sarubbo et al., 2001; Colin et al., 2010).

Bioemulsifiers have a wide range of applications in multiple areas of biotechnology (Kiran et al., 2010), including in primary mechanisms for removal of petroleum and other hydrocarbon pollutants from the environment (Calvo et al., 2009; Edward et al.,

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2011). There are also reports in the literature regarding the removal of heavy metals from wastewater and soils using biological agents as bioemulsifiers (Juwarkar et al., 2007; Gutierrez et al., 2008; Aniszewski et al., 2010). Although a wide diversity of bioemulsifiers has been produced up until now using a large variety of microorganisms, only a few reports have appeared in relation to actinobacteria emulsifier producers such as *Streptomyces* sp., isolated from marine environments (Kokare et al., 2007), or *Streptomyces* sp. S22, isolated from garden soil (Maniyar et al., 2011).

In previous studies, *Streptomyces* sp. MC1, an actinobacteria isolated from sugar cane, has shown the ability to reduce hexavalent chromium [Cr(VI)] to less toxics species (Polti et al., 2007) with this biological reduction seeming to occur largely on the cell surface (Pereira, 2010). In fact, chromate reductase activity has been effectively detected in all cellular fractions of this strain (Polti et al., 2010). Considering that Cr(VI) is highly toxic to living organisms, its reduction to less toxic species using whole cells of *Streptomyces* sp. MC1 appears to be a highly promising approach in the development of suitable methods for cleaning up environments contaminated with Cr(VI). Many studies have been done for microbial reduction of Cr(VI) to Cr(III). However, the metabolic versatility that makes the actinobacteria useful as cellular factories for the production of metabolites of biotechnological interest (Nakashima et al., 2005; Maniyar et al., 2011) has not yet been deeply exploited in this case. Here, a first study related to *Streptomyces* sp. MC1's ability to produce a bioemulsifier is reported. Also, optimization of production and partial purification/characterization of the emulsifier produced are presented.

2. Materials and methods

2.1. The microorganism, its maintenance and culture conditions

The microorganism used in this work was *Streptomyces* sp. MC1 (PROIMI Collection, NCBI accession number: AY741287), which has shown resistance to Cr(VI) (Polti et al., 2007). This strain was maintained on Starch–Casein agar slants (SC agar) containing (g L^{-1}): starch, 10.0; casein, 1.0; K_2HPO_4, 0.5; and agar, 12.0.

Streptomyces sp. MC1 spore suspensions harvested from SC agar were inoculated in liquid minimal medium (MM) as formulated by Amoroso et al. (1998) (final concentration of 1×10^5 CFU mL^{-1}), with the following composition (g L^{-1}): glucose as carbon source (C), 10.0; L-asparagine as nitrogen source (N), 0.5 (C/N = 20); K_2HPO_4, 1.0; $MgCl_2 \cdot 7H_2O$, 0.20; and $FeSO_4 \cdot 7H_2O$, 0.01. The basal production of the bioemulsifier detected in this medium (initial pH 7, 30 °C) was used as control or reference.

To identify the factors that most significantly affect bioemulsifier production, an univariate analysis using dose–response experiments was performed for the effects of initial pH of the MM (5–8), growth temperature (25–37 °C), concentration of oxanions (as SO_4^{-2} and PO_4^{-3}, 0.5–2.5 g L^{-1}), and concentration of cations (as Mg^{+2}, Ca^{+2}, and Fe^{+3}, 0.5–2.5 g L^{-1}) (data not shown). Based upon these experiments, initial pH of the MM and the concentration of PO_4^{-3} or Ca^{+2} were chosen to perform the optimization studies. Emulsifier biosynthesis was also evaluated in the presence of a range of Cr(VI) concentrations (5–20 mg L^{-1}). The SO_4^{-2}, PO_4^{-3}, and Cr(VI) were added as Na_2SO_4, K_2HPO_4, and $K_2Cr_2O_7$, respectively, from stock solutions, while the Mg^{+2}, Ca^{+2}, and Fe^{+3} were added as chlorides. Cultures were conducted in Erlenmeyer flasks, incubated on an orbital shaker (170 rpm) at 30 °C for 96 h.

2.2. Emulsification index and emulsion stability

The emulsification index (EI) of the cultures was determined by mixing equal volumes of a hydrocarbon (kerosene) and the culture supernatant, with the mixture then vortexed for 2 min and left to settle. The EI was calculated as the percentage produced by dividing the height of the emulsified layer (mm) by the total height of the liquid column (mm) (Cooper and Goldenberg, 1987). Specific emulsification index (q_{EI}) was also calculated and expressed as EI% per g of biomass. Finally, emulsion stability (ES) was determined, with an emulsion being defined as stable if the EI was 50% or higher after being left to settle for 24 h (Bosch et al., 1988).

Table 1
Factors and their levels for a 2^3 full factorial design.

Factors	Process parameters	Level (−)	Level (+)
A	pH	7	8
B	PO_4^{-3} (g L^{-1})	1	2
C	Ca^{+2} (g L^{-1})	0	1

2.3. Determination of biomass

To estimate the microbial biomass, the samples were centrifuged at 10000*g* for 15 min at 4 °C, and cells were washed twice with bi-distilled water. Dry weight was determined using alumi-

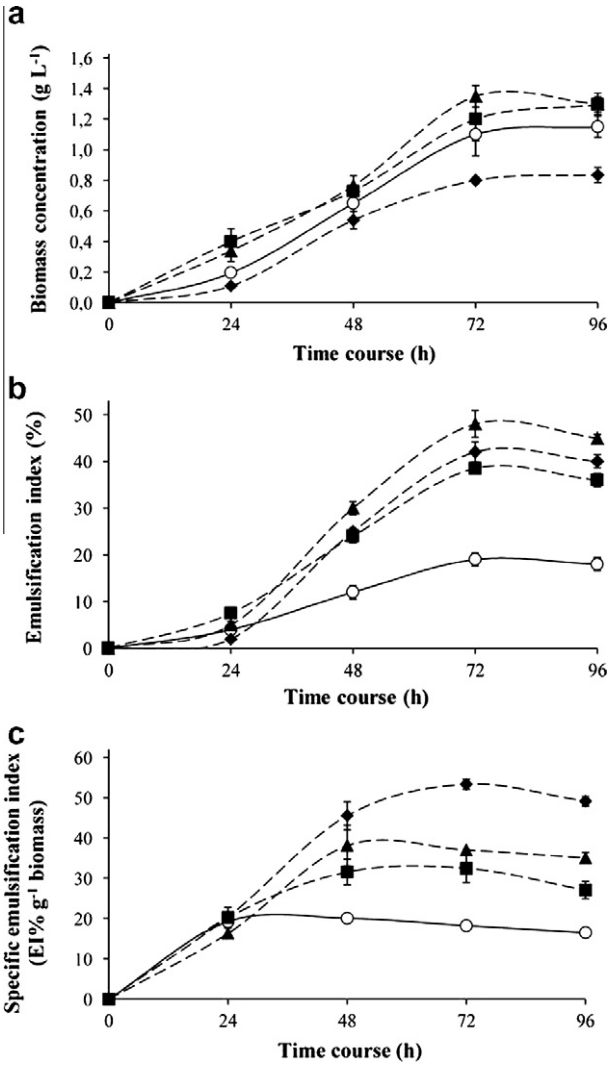

Fig. 1. *Streptomyces* sp. MC1 growth kinetics and bioemulsifier production in MM at 30 °C: (–○–) control, (- -▲- -) at initial pH 8, (- -■- -) in the presence of 2 g L^{-1} PO_4^{-3}, (- -◆- -) in the presence of 1 g L^{-1} Ca^{+2}. (a) Emulsification index (EI). (b) Biomass concentration. (c) Specific emulsification index (q_{EI}). Error bars represent the standard deviation calculated from three independent experiments.

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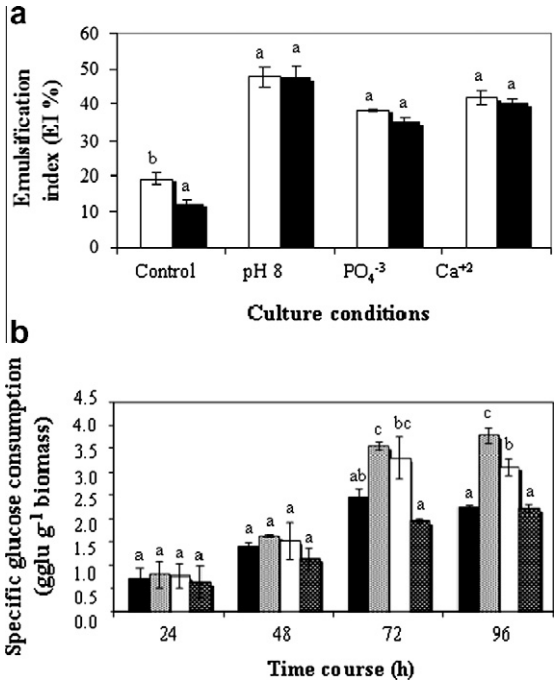

Fig. 2. (a) Stability of emulsions formed between the kerosene and the culture supernatants after 72 h of growth: initial EI (*open bars*) and EI after settling for 24 h (*solid bars*), (b) Time course of the specific glucose consumption: (■) control, (▨) at initial pH 8, (□) with 2 g L^{-1} PO_4^{-3}, (▩) with 1 g L^{-1} Ca^{+2}. Error bars represent the standard deviation calculated from three independent experiments. The values with different letters are significantly different ($P < 0.05$).

num foil cups dried at 80 °C to constant weight. Supernatants were stored at −20 °C for subsequent analysis.

2.4. Determination of residual glucose

Residual glucose was determined using the dinitrosalicylic acid method as described by Miller (1959). Readings were interpolated from a standard curve prepared using a series of glucose dilutions (0–1 g L^{-1}). Specific glucose consumption (q_{glu}) was also calculated and expressed as *g* of glucose consumption per *g* of biomass.

2.5. Nature of the emulsifier

The emulsifier agent was extracted from the optimized culture medium by precipitation using cold acetone (1:1, v/v), at 4 °C for 16 h. Emulsifier precipitate was recovered by filtration through a 0.45 μm membrane, then dissolved in distilled water and subjected to the treatments next: To investigate the role of peptides and lipids on emulsification ability, the extract was treated with proteinase *K* (30 U mg^{-1}, at 37 °C for 4 h) and Lipolase 100 L from *Thermomyces lanuginosus* (10 U mg^{-1}, at 37 °C for 2 h), respectively. The role of sugars was estimated by acid hydrolysis of the extract at 100 °C. The presence of reducing sugar was determined according to Miller (1959). Stability of the emulsifier was done by incubating crude extract at 37 °C for 1 h in buffers of different pH values: citric acid–Na_2HPO_3 (pH 3, 4 and 5), Na_2HPO_3–NaH_2PO_3 (pH 6, 7 and 8), and Na_2CO_3–$NaHCO_3$ (pH 9 and 10). Finally, thermal resistance of the emulsifier was tested by measuring the EI (%) after incubation at 100 °C for 10 min.

2.6. Statistical analysis

Statistical analysis was performed using Infostat (version 2004) and Minitab (version 14; Minitab) software for Windows. Results are presented as the mean ± standard deviation. Statistical significance values for the means were evaluated using one-way analysis of variance. Subsequent comparisons were performed using Tukey's post hoc test. Differences were accepted as significant when $P < 0.05$. Associations between variables were assessed by using Pearson's correlation coefficient. In order to identify the main effects and interactions of the selected variables, a 2^3 full factorial design was used. The independent variables and their levels are presented in Table 1. The "+ and −" notation is used to represent the highest and lowest levels for each factor.

3. Results

3.1. Significant factors for bioemulsifier production

Based on univariate analysis, the most influential factors in terms of bioemulsifier production were the initial pH of the MM and the PO_4^{-3} and Ca^{+2} concentration (Fig. 1). It is important to mention that either above or below 30 °C, growth of *Streptomyces* sp. MC1 was very low (data not shown), and therefore the production assays were carried out at 30 °C. Under all cultivation conditions tested, the maximum EI was observed after 72 h of incubation (Fig. 1a). Compared to the results at the control condition of pH 7, adjustment of the initial pH to 8 increased the EI of the supernatants by 150%, while the addition of 2 g L^{-1} PO_4^{-3} or 1 g L^{-1} Ca^{+2} increased the emulsification ability of the supernatants by 94% and 128%, respectively.

The growth kinetic of this strain was also significantly affected by the changes in the environmental culture conditions (Fig. 1b). After 72 h of incubation, the biomass concentration at initial pH 8 was increased by 18%. In contrast, the addition of 1 g L^{-1} Ca^{+2} to the MM decreased bacterial growth by 19%, while no significant

Table 2
Matrix for a 2^3 full factorial design and experimental results measured after 72 h of cultivation.

Run	Factors			EI (%)	Biomass (g L^{-1})	q_{EI} (EI% g^{-1} biomass)	ES (%)	q_{glu} (gglu g^{-1} biomass)
	pH	PO_4^{-3}	Ca^{+2}					
1	+	+	−	54 ± 2	1.36 ± 0.06	40 ± 3	98 ± 2	5.3 ± 0.2
2	+	−	−	50 ± 3	1.35 ± 0.07	37 ± 0	99 ± 0	3.6 ± 0.1
3	−	+	+	52 ± 1	1.04 ± 0.16	50 ± 3	99 ± 0	2.0 ± 0.2
4	+	−	+	58 ± 2	1.23 ± 0.04	48 ± 0	95 ± 1	3.8 ± 0.1
5	−	−	−	20 ± 1	1.10 ± 0.14	18 ± 1	66 ± 2	2.4 ± 0.2
6	−	−	+	44 ± 2	0.82 ± 0.02	53 ± 1	95 ± 0	2.0 ± 0.1
7	+	+	+	70 ± 1[a]	1.30 ± 0.01	54 ± 1[a]	100 ± 1	3.6 ± 0.2
8	−	+	−	39 ± 0	1.20 ± 0.14	32 ± 4	91 ± 1	3.3 ± 0.4

EI: emulsification index.
q_{EI}: specific emulsification index.
ES: emulsion stability.
q_{glu}: specific glucose consumption.
[a] The maximum values of the emulsification indices.

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effect on bacterial growth was observed in the presence of 2 g L^{-1} PO_4^{-3}.

In order to express emulsifier production in relation to the growth of *Streptomyces* sp. MC1, q_{EI} was also calculated (Fig. 1c). The maximum values were detected between 48 and 72 h of cultivation, with q_{EI} increasing by 90% at initial pH 8, and by 57 and 127% in the presence of 2 g L^{-1} PO_4^{-3} or 1 g L^{-1} Ca^{+2}, respectively, compared to the control. However, it is important to point out that the biomass concentration was lower than 1 g L^{-1} when the strain was cultivated in the medium with added Ca^{+2} (Fig. 1b).

Under control condition the EI decreased by 40% after settling for 24 h (Fig. 2a). In contrast, the emulsions formed between the kerosene and the culture supernatants obtained at initial pH 8, as well as those formed in the presence of 2 g L^{-1} PO_4^{-3} or 1 g L^{-1} Ca^{+2}, were highly stable, maintaining almost 100% of their initial height. Finally, q_{glu} during the growth of *Streptomyces* sp. MC1 and the strain's bioemulsifier production was estimated (Fig. 2b). As expected, a high correlation between this parameter and microbial growth was revealed ($r = 0.775$; $P = 0.024$). However, no correlation was seen between q_{glu} and EI ($r = 0.371$; $P = 0.365$) or q_{EI} ($r = 0.304$; $P = 0.401$).

3.2. Multi-factorial design for improving bioemulsifier production

In order to identify the main effects of the selected factors and the interactions among them, a 2^3 full factorial design was applied. Based upon the results of the univariate study presented in Fig. 1, the effects of initial pH and concentrations of PO_4^{-3} and Ca^{+2} on the emulsification indices (EI, q_{EI}) and growth parameters of *Streptomyces* sp. MC1 were analyzed at 72 h of cultivation. After this time there were no significant further increases in the responses studied (data not shown). The levels at which the variables show their maximum and minimum values are presented in Table 1. Maximum values for both EI and q_{EI} were obtained in MM at initial pH 8 and when PO_4^{-3} and Ca^{+2} were added (Table 2). Table 3, it was estimated that both parameters, EI and q_{EI}, increase as the initial pH value and PO_4^{-3} and Ca^{+2} concentrations increase. In terms of the interaction effects, a significant negative effect was detected between the initial pH and the presence of Ca^{+2} for both indices (EI, q_{EI}). However, interaction among all three factors (initial pH, PO_4^{-3} and Ca^{+2} concentration), had positive effects on bioemulsifier production. In terms of ES, the main effect as well as the interaction among the three factors increased the stability of the emulsions formed. Finally, it can be seen that the variables most relevant for growth are the initial pH and Ca^{+2} concentration. While increase in pH had significantly positive effect on both biomass and q_{glu}, a decrease in both parameters was detected in the presence of Ca^{+2}. Based upon the R-$Sq_{(adj)}$ (Table 3), it is concluded that multi-factorial model applied explains largely the variability of the data.

3.3. Bioemulsifier production in the presence of hexavalent chromium

Since *Streptomyces* sp. MC1 is known to be a chromium-resistant strain, the effects of Cr(VI) on its ability to synthesize bioemulsifier was also evaluated. After 72 h of cultivation, a linear decrease in all of the measured parameters (EI, q_{EI}, ES, biomass concentration, and q_{glu}) was observed with increasing Cr(VI) concentration (Table 4). In the medium with 20.0 mg L^{-1} Cr(VI) added, no bioemulsifier production could be detected. According to the concept of the stability of emulsions (Bosch et al., 1988), the emulsion formed under the assay conditions was stable over time only in the presence of 5.0 mg L^{-1} Cr(VI). In contrast, when *Streptomyces* sp. MC1 was cultivated in the presence of between 10 and 15 mg L^{-1} of Cr(VI), the EI remaining after 24 h was less than 50%. In terms of microbial growth, biomass concentration and q_{glu}

Table 3
Estimated effects analysis for EI, biomass, q_{EI}, ES, and q_{glu}, determined after 72 h of cultivation.

Term	EI		Biomass		q_{EI}		ES		q_{glu}	
	Effects	*t*-Values (*P*-values)	Effects	*t*-Values (*P*-values)	Effects	*t*-Values (*P*-values)	Effects	*t*-Values (*P*-values)	Effects	*t*-Values (*P*-values)
pH (A)	19.438	21.83 ($P < 0.001$)	0.26875	6.36 ($P < 0.001$)	5.975	5.67 ($P < 0.001$)	11.125	17.62 ($P < 0.001$)	1.6375	14.93 ($P < 0.001$)
PO_4^{-3} (B)	10.688	12.00 ($P < 0.001$)	0.10125	2.40 ($P = 0.043$)	5.050	4.79 ($P = 0.001$)	9.125	14.46 ($P < 0.001$)	0.6375	5.81 ($P < 0.001$)
Ca^{+2} (C)	15.437	17.34 ($P < 0.001$)	−0.15875	−3.76 ($P = 0.006$)	19.550	18.56 ($P < 0.001$)	9.500	15.05 ($P < 0.001$)	−0.8125	−7.41 ($P < 0.001$)
A B	−3.062	−3.44 ($P = 0.009$)	−0.06125	−1.45 ($P = 0.185$)	−0.600	−0.57 ($P = 0.585$)	−7.000	11.09 ($P < 0.001$)	0.1875	1.71 ($P = 0.126$)
A C	−3.063	−3.44 ($P = 0.009$)	0.06375	1.51 ($P = 0.170$)	−6.950	−6.60 ($P < 0.001$)	−10.875	17.23 ($P < 0.001$)	0.0875	0.80 ($P = 0.448$)
B C	−0.562	−0.63 ($P = 0.545$)	0.04625	1.09 ($P = 0.306$)	−3.375	−3.20 ($P = 0.013$)	−4.875	7.72 ($P < 0.001$)	−0.6625	−6.04 ($P < 0.001$)
A B C	4.437	4.98 ($P = 0.001$)	−0.01625	−0.38 ($P = 0.711$)	5.225	4.96 ($P = 0.001$)	7.250	11.49 ($P < 0.001$)	−0.2625	−2.39 ($P = 0.044$)
R-$Sq_{(adj)}$	0.985		0.797		0.969		0.989		0.959	

EI: emulsification index.
q_{EI}: specific emulsification index.
ES: emulsion stability.
q_{glu}: specific glucose consumption.

Table 4
Streptomyces sp. MC1 growth and its bioemulsifier production, determined after 72 h of cultivation in the presence of Cr(VI).

Cr(VI) concentration (mg L^{-1})	EI (%)	Biomass (g L^{-1})	q_{EI} (EI% g^{-1} biomass)	ES (%)	q_{glu} (gglu g^{-1} biomass)
0	70 ± 1[a]	1.30 ± 0.01	54 ± 1[a]	100 ± 1	3.6 ± 0.2
5	46 ± 2	1.25 ± 0.03	36 ± 1	53 ± 5	3.5 ± 0.2
10	17 ± 3	0.79 ± 0.04	22 ± 5	38 ± 3	2.8 ± 0.2
15	4 ± 1	0.73 ± 0.04	5 ± 2	–	2.2 ± 0.3
20	–	0.63 ± 0.06	–	–	1.9 ± 0.4

[a] Bioemulsifier production in the optimized culture medium.

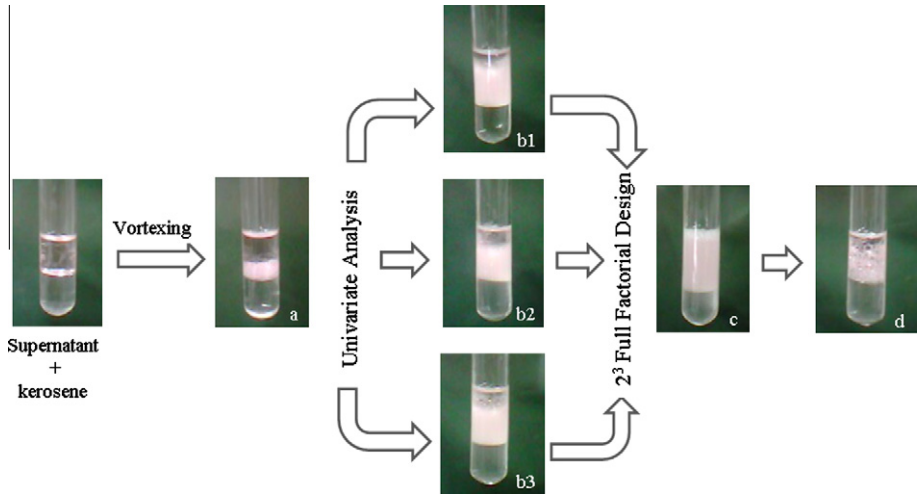

Fig. 3. Flow chart summarizing the experimental procedure used to optimize bioemulsifier production by *Streptomyces* sp. MC1. The figures show the emulsification index (EI) determined after 72 h of cultivation: (a) under the control conditions, (b1) at initial pH 8, (b2) with 2 g L^{-1} PO_4^{-3}, (b3) with 1 g L^{-1} Ca^{+2}, (c) in the optimized medium, and (d) in the optimized medium with 5 mg L^{-1} Cr(VI) added.

decreased by about 50% in the presence of 20 mg L^{-1} Cr(VI), while in the medium with 5 mg L^{-1} Cr(VI) added, these parameters were not significantly affected ($P > 0.05$). Fig. 3 summarizes the experimental procedure used to optimize emulsifier production by *Streptomyces* sp. MC1.

3.4. The nature of the emulsifier

Finally, the nature of the emulsifier agent obtained from the optimized culture medium was determined. The yields of partially purified compound from the cell-free supernatant were found to be 6.8 g L^{-1} and 0.05 g g^{-1} biomass. In order to investigate the nature of the emulsifier, acetone extracts were exposed to a variety of treatments. The emulsifier crude extract was active within the pH range tested. The EI was only slightly reduced (about 5–7%) after incubation in the pH range of 6–10; while in the pH range of 3–5, the emulsification ability of the extract decreased by about 14%. The emulsification ability of the extract remained virtually constant after heating at 100 °C for 10 min. However, acid hydrolysis at 100 °C completely destroyed this ability, suggesting the presence of sugars in the emulsifier agent's structure. Subsequently, a concentration of 1.7 g L^{-1} of reducing sugar was detected in the extract. Concerning the role of peptides, proteinase *K* treatment decreased the EI by 45%. However, after Lipolase 100 L treatment, the extract retained 100% of its emulsification ability. The results of these experiments suggest that the emulsifier compound could be a glycoprotein.

4. Discussion

Surface-active compounds produced by microorganisms are of two types: those that reduce surface tension at the air–water interface (biosurfactants), and those that reduce the interfacial tension either between immiscible liquids or at the solid–liquid interface (bioemulsifiers). The factors affecting the biosynthesis of surface-active compounds have been extensively studied in the recent years (Cunha et al., 2004; Albuquerque et al., 2006; Franzetti et al., 2009). Researchers found that the inclusion of hydrocarbons in the growth medium markedly increased biosynthesis of these compounds (Martínez-Checa et al., 2007; Zheng et al., 2011). However, this also creates a challenge in terms of subsequent separation of the compound produced. Under the assay conditions used here, *Streptomyces* sp. MC1 was able to produce bioemulsifier in the absence of any water-immiscible substrate. Among the factors studied, the initial pH of the medium, and PO_4^{-3} or Ca^{+2} concentrations were found to be culture-related factors that significantly influenced this strain's emulsifier biosynthesis (Tables 2 and 3). Like us, Franzetti et al. (2009) have reported on the positive effect of PO_4^{-3} in surfactant biosynthesis by *Gordonia* sp. BS29. Also, the synthetic pathway of surface-active compounds has been elucidated in *Rhodococcus*, where one of the key reactions for the synthesis of the final resulting sugar residue, trehalose-6-phosphate, is catalyzed by the trehalose-6-phosphate synthesis enzyme (Lang and Philp, 1998). Among the actinobacteria, a high phosphate concentration in liquid medium has been reported to lead to an accumulation of glucose-6-phosphate in *Streptomyces* cells grown in glucose, which is an immediate precursor of trehalose-6-phosphate (Madry et al., 1979). Based upon this background, Pagilla et al. (2002) have hypothesised that the presence of PO_4^{-3} could promote the synthesis of certain sugar residues, thereby improving the production of glycosidic compounds by certain microbes. With regard to the effects of initial pH and Ca^{+2} concentration, Colin et al. (2010) found that emulsifier production by *Aspergillus niger* MYA 135 decreased as pH or Ca^{+2} concentration in the culture medium increased. In contrast, under our assay conditions,

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increasing the levels of both of these factors improved emulsifier biosynthesis by *Streptomyces* sp. MC1. It is also worth mentioning that below pH 7, no significant production could be detected.

The ability of *Streptomyces* sp. MC1 to produce emulsifiers in the presence of Cr(VI) has also been evaluated. Although overall the addition of Cr(VI) to the culture medium had a negative effect on emulsifier biosynthesis, this strain retained about 65% of its production ability at a final concentration of 5.0 g L^{-1} of Cr(VI) (Table 4). In contrast to these results, Paraszkiewicz et al. (2007) observed that the addition of certain heavy metals including cadmium and lead to the culture medium enhanced emulsifier production in *Curvularia lunata*. However, direct application of bioemulsifiers in the bioremediation area, could be unquestionable advantages compared to the use of whole-cell microbes because microorganisms able to produce surface active compounds do not need to have survival ability in heavy metal-contaminated soil.

It is known that the chemical structures and functional properties of bioemulsifiers are strongly influenced by the producing microorganism as well as by factors related to the culture environment. While the emulsifier agents isolated from *Streptomyces* sp. S1 and *Streptomyces* sp. S22 were protein polysaccharide and peptidoglycolipidic in nature, respectively (Kokare et al., 2007; Maniyar et al., 2011), the partial characterization studies presented here suggest that the emulsifier produced by *Streptomyces* sp. MC1 could be a glycoprotein. Interestingly, this compound presents high thermo-stability and partial water solubility, which could have promising future prospects for biotechnological applications. For example, bioemulsifier technology can be an effective and non-destructive method for remediation of organic and inorganic pollutants. Certain remediation techniques for metal-contaminated soils involve washing strategies that use surface-active compounds (Franzetti et al., 2009). These can increase desorption of the soil-bound metals and facilitate their transport through the soil matrix. In relation to these processes, the wide variety of chemical structures found in microbial emulsifiers may lead to different metal selectivities and thus varying efficiencies in terms of removal. Also along these lines, Gutierrez et al. (2008) reported on a glycoprotein with emulsifying activity produced by the strain *Pseudoalteromonas* sp. TG12, which demonstrated a differential capacity to desorb diverse mono-, di- and tri-valent metal species from marine sediments. Juwarkar et al. (2007) have also reported on the effectiveness of a dirhamnolipid biosurfactant produced by the *Pseudomonas aeruginosa* BS2 strain for the removal of metals including cadmium and lead from soil matrix. Aniszewski et al. (2010), on the other hand, reported on the differential removal of cadmium and zinc from industrial residues, by using four bioemulsifier-producing *Microbacterium* strains named Mc1, Mc6b, Mc24, and Mc60. Based upon this previous research, studies on the ability of bioemulsifiers produced by *Streptomyces* sp. MC1 to bind metals could lay the groundwork for future applications in environmental bioremediation techniques.

5. Conclusions

In summary, we have documented here the production of a possible glycoprotein with emulsifying activity by a chromium-resistant actinobacterium from our culture collection. Biosynthesis of this compound was found to be strongly influenced by environmental factors, with the EI increasing by about 3.5 times in our optimized medium compared to the EI detected in the control condition. It was also demonstrated that this strain is able to produce emulsifier when it is grown in the presence of Cr(VI). These studies could mark a turning point in conventional approaches for the development of environmental clean-up strategies, where the use of microbial whole-cell metabolism is exploited to reduce metal contaminations. Based upon our results, evaluation of the emulsifier agent's ability to bind and remove metals from liquid systems and soils will be analyzed in future.

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