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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 $1 \times 10^5$ 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-

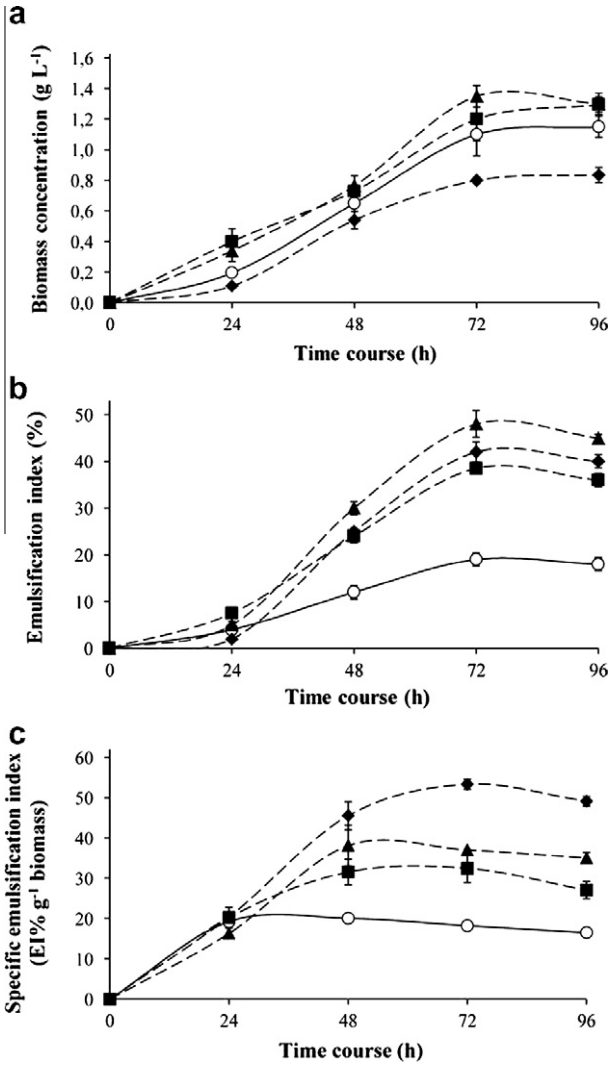


**Fig. 1.** *Streptomyces* sp. MC1 growth kinetics and bioemulsifier production in MM at 30 °C: (–○–) control, (- -▲- -) at initial pH 8, (- -■- -) in the presence of 2 g $L^{-1}$ $PO_4^{-3}$, (- -◆- -) in the presence of 1 g $L^{-1}$ $Ca^{+2}$. (a) Emulsification index (EI). (b) Biomass concentration.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