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Surfactin restores and enhances swarming motility under heavy metal stress

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ABSTRACT

The present work reports the importance of lipopeptide biosurfactant on swarming motility of multi-metal resistant (MMR) bacterium under heavy metal stress. The MMR bacteria strain CM100B, identified as *Bacillus cereus*, was isolated from the coal mine sample. The strain was able to grow and reduce several metals namely Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+} and Pb^{2+} ions which are common environmental pollutants. Presence of toxic heavy metal ions in the swarming medium significantly altered the motility of CM100B. Presence of Cd^{2+} and Pb^{2+} ions inhibited development of peritrichous flagella, thus inhibiting swarming motility. However, the addition of anionic biosurfactant surfactin restored (in case of Cd^{2+} and Pb^{2+} ions) or enhanced (in case of Co^{2+}, Cu^{2+}, Ni^{2+} and Mn^{2+}) the swarming ability of CM100B. Zeta potential studies for determining bacterial cell surface charge indicated that surfactin provided a suitable swarming environment to bacteria even under metal stress by chelating to cationic metal ions. Non-ionic surfactant Triton X-100 was unable to restore swarming under Cd^{2+} and Pb^{2+} ion stress. Thus, suggesting that surfactin can aid in motility not only by reducing the surface tension of swarming medium but also by binding to metal ions in the presence of metal ions stress.

1. Introduction

Microbial encounter with metals and metalloids is inevitable in natural environment. Anthropogenic activities and geographic makeup of certain regions has bestowed soil and water present in such regions with alarming levels of metal and metalloid contamination. Microorganisms residing in such regions or encountering such conditions alter their physiological behavior to minimize or nullify the toxicity of metals.

Motility is undoubtedly the most remarkable feature of a microbe's physiology and liable to alteration under metal stress [1–3]. Motile prokaryotic microorganisms move in aqueous environments by using distinct modes of surface translocation like swimming, swarming, twitching, gliding, sliding and darting [4]. Microbial motility plays an important role not only for microorganisms but also for human in diverse fields ranging from soil microbiology, water purification and microbial pathogenesis. Importance of motility and chemotaxis in biodegradation of various pollutants has been highlighted in several reports [5–7]. Response of a bacterium toward pollutant largely depends on its physiological capability to utilize or resist the pollutant, along with the nature and concentration of pollutants.

Microorganisms to support their motile behavior produce array of compounds like flagellar proteins, lipopolysaccharide (LPS) and surfactants [4,8]. Biosurfactants produced by microbes assist in the bacterial motility by reducing the interfacial tension between the migrating cells and the surface beneath them [9,10]. Apart from helping in motility, biosurfactant are also known to protect microorganism from metal ion stress by acting as metal ion chelators [11–13].

The aim of the present investigation was to study the effect of common metal pollutants on the swarming behavior of multi-metal resistant soil bacterium. Also, to assess the hypothesis that microbial anionic surfactant can aid in the motility under metal ion stress not only by reducing the surface tension but also by acting as metal ion chelators.

2. Materials and methods

2.1. Microorganism

The microorganism used in the present study was isolated from Asansol coal mines of West Bengal, India for selenite (SeO_3^{2-}) transformation studies. The bacterial isolate was characterized by morphological, biochemical and 16S rRNA gene sequencing [14].

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2.2. Metal ions and surfactant solutions

Stock solutions of heavy metals were prepared by dissolving $CdCl_2{\cdot}2.5H_2O$, $CoCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$ and $(CH_3COO)_2$ $Pb{\cdot}3H_2O$ salts in deionized water. Solutions were filter sterilized using 0.22 μm filter (Millipore). Concentration of the metal ions in the growth medium ranged from 0.1 mM to 1 mM. Data given in the paper is for 1 mM concentration of metal ions. Stock solution of surfactin (≤98% pure; Sigma) and Triton X-100 was prepared in methanol and deionized water, respectively.

2.3. Surface tension and CMC determination of surfactants

The surface tension was measured at 25 °C using a duNouy tensiometer (CSC Scientific Company Inc., USA) based on the ring detachment method. The critical micelle concentration (CMC) was determined by plotting the surface tension as a function of the surfactant concentration and the point where a sudden change in the surface tension was observed was designated as CMC.

2.4. Growth profile of bacterial isolate in the presence of metals and surfactants

Growth of the strain CM100B was determined in the presence of various metals and surfactants. Briefly, the growth medium Tryptone soy broth (TSB) was amended with different metals ions or surfactant and inoculated with 1% inoculum of overnight grown seed culture. Flasks were incubated at 37 °C with an agitation rate of 200 rpm. Growth of the isolate was determined by estimating the protein content of bacterial biomass at different time interval as described by Dhanjal and Cameotra [14].

2.5. Determining bio-reduction potential of various metal ions by strain CM100B

The metal ion reducing abilities of CM100B were determined by quantifying the concentration of different metal ions in the TSB amended with respective ions after 48 h of bacterial growth. Metal content was quantified after acid digestion of sample by atomic absorption spectrophotometer (AA-6800, Shimadzu) with appropriate standards and conditions applicable to the respective metals. Cd, Cu, Co, Pb, Mn and Ni were estimated in Flame mode at 228.8, 324.8, 240.7, 357.9, 283.3 and 352.5 nm respectively. The reduction ability was given in the terms of percentage reduction of metal ions in the growth medium.

2.6. Biosurfactant producing ability of the strain CM100B

The strain was grown in TSB for 72 h at 37 °C with 200 rpm and reduction in surface tension was taken as the criteria for assessing the biosurfactant production ability. The surface tension of the cell free broth after different time intervals was measured using DuNouy Tensiometer. Un-inoculated TSB and water (72 mN/m) were taken as controls.

2.7. Swarm plate assay for motility

In this assay, an appropriate heavy metal ion (final concentration 1 mM) was added to TSB medium containing 0.7% bacto agar. Culture was grown in TSB till log phase, centrifuged at 1800 × *g* for 6 mins and the cell pellet washed and re-suspended in phosphate saline buffer (PBS; pH 7.2). 5.0 μl of washed cell suspension (2.0×10^5 cells/ml) was gently poured in the center of the plate. Plates were incubated at 37 °C and observed for ring formation. Plates without heavy metal ions, only Triton X-100 and only surfactin in the swarming medium were taken as controls. All the experiments were done in triplicate. Growth of bacterium on swarm plates was observed following the method described by Salvetti et al. [15].

2.8. Cell surface charge measurements of bacterial cell suspension

Bacterial suspensions were prepared from the 24 h bacterial cultures growing on the TSB either in the presence or absence of above mentioned metal ions or surfactants. The bacterial cultures were centrifuged (1800 × *g*, 6 min) and washed with sterile water to remove any residue. Bacteria was re-suspended in water to obtain OD of 0.20–0.30 (λ = 600 nm). For the Zeta potential measurements an appropriate volume of bacterial suspension was transferred to polystyrene U-shaped cell. Zetasizer nano ZS (Malvern Instrument, Malvern, UK) was used for measuring the zeta potential (ζ), which is determined from electrophoretic mobility (μ) based on Smoluchowski's formula.

2.9. Electron microscopy

Bacteria from the leading edge of the moving colony were analyzed for the presence or absence of flagella using electron microscopy. The bacterial cells were gently scraped off with a toothpick and subsequently suspended in a drop of water mounted on the top of a carbon coated copper grids (mesh: 200). The grids were washed once with distilled water and stained with 1% uranyl acetate. The negatively stained cells were visualized by transmission electron microscope (JEOL, JEM-2100).

3. Results

3.1. Growth and bio-reduction potential of CM100B in the presence of metal ions

The strain CM100B exhibited luxuriant growth in the presence of 0.1 mM and 0.5 mM amount of metal ions namely Cu^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+} and Mn^{2+}. However, exposure to 1 mM concentration of above mentioned ions partially hindered the growth of CM100B as compared to controls lacking these ions (Fig. 1a and b). The strain CM100B also demonstrated excellent bio-reduction ability by reducing aforementioned metal ions. It was observed that after 48 h of growth, CM100B was able to reduce nearly 50% of Cu^{2+}, Ni^{2+} and Mn^{2+} ions (Fig. 1c).

3.2. Effect of heavy metal ions on the motility of Bacillus cereus (strain CM100B)

The toxic effect of heavy metals on the motility of strain CM100B was studied using soft agar swarm plate assay having a low percentage of agar in the growth medium. Within 4–5 h of incubation the bacterium formed a colony of 2–3 mm diameter in standard swarm plates. However, after 5 h of incubation there was a continuous increase in the colony diameter that reached almost edge of the 90 mm plates within 14 h.

However, the presence of 1 mM concentration of metal ions significantly altered the motility of bacterial cell as compared to control in which no metal ions were present. The presence of metal ions namely Cu^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} moderately reduced the motility of bacterium. However, exposure to Pb^{2+} and Cd^{2+} ions inhibited swarming ability of bacterial cells (Fig. 2a). When CM100B was exposed to 0.1 mM concentration of Pb^{2+} and Cd^{2+} ions swarming was observed thus suggesting that metal induced swarming motility inhibition is primarily concentration dependent.