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Production and characterization of microbial biosurfactants for potential use in oil-spill remediation

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ABSTRACT

Two biosurfactants, surfactin and fatty acyl-glutamate, were produced from genetically-modified strains of *Bacillus subtilis* on 2% glucose and mineral salts media in shake-flasks and bioreactors. Biosurfactant synthesis ceased when the main carbohydrate source was completely depleted. Surfactin titers were ~30-fold higher than fatty acyl-glutamate in the same medium. When bacteria were grown in large aerated bioreactors, biosurfactants mostly partitioned to the foam fraction, which was recovered. Dispersion effectiveness of surfactin and fatty acyl-glutamate was evaluated by measuring the critical micelle concentration (CMC) and dispersant-to-oil ratio (DOR). The CMC values for surfactin and fatty acyl-glutamate in double deionized distilled water were 0.015 and 0.10 g/L, respectively. However, CMC values were higher, 0.02 and 0.4 g/L for surfactin and fatty acyl-glutamate, respectively, in 12 parts per trillion (ppt) Instant Ocean® sea salt, which has been partly attributed to saline-induced conformational changes in the solvated ionic species of the biosurfactants. The DORs for surfactin and fatty acyl-glutamate were 1:96 and 1:12, respectively, in water. In Instant Ocean® solutions containing 12 ppt sea salt, these decreased to 1:30 and 1:4, respectively, suggesting reduction in oil dispersing efficiency of both surfactants in saline. Surfactant toxicities were assessed using the Gulf killifish, *Fundulus grandis*, which is common in estuarine habitats of the Gulf of Mexico. Surfactin was 10-fold more toxic than fatty acyl-glutamate. A commercial surfactant, sodium laurel sulfate, had intermediate toxicity. Raising the salinity from 5 to 25 ppt increased the toxicity of all three surfactants; however, the increase was the lowest for fatty acyl-glutamate.

1. Introduction

There is a growing demand for "green" and non-toxic dispersants for oil spill remediation. Due to the environmental and health risks from synthetic chemicals, surfactants from biological sources are favored for their biodegradability and potentially lower toxicity. Currently, surfactants are synthesized from petroleum-based hydrocarbons and are used in foods, medicines, industrial applications [1,2], and waste remediation [3,4]. It is estimated that current global surfactant production consumes about 7.4 billion kg of petrochemical intermediates and emits about 31.6 billion kg of CO_2 [5,6].

Biosurfactants include surface-active chemicals synthesized by a wide variety of microorganisms [7–9]. Examples include iturins, esperine, mycosubtilin and surfactin. These are cyclic lipopeptides produced by *Bacillus* sp., which have interesting physiological, biocidal, physicochemical, and surface-active properties. Identification and characterization of effective, environmentally friendly, and low-cost biodispersants is of critical importance. The selection of a dispersant for oil spills is influenced by its efficacy and environmental impact. Economic production of biodispersants from low-cost biological feedstocks with efficient green recovery methods will improve their acceptability, augment their use, and reduce or eliminate the need for synthetic dispersants.

Surfactin is a well-known lipopeptide with surface-active properties and was first co-produced with iturinic lipopeptides using

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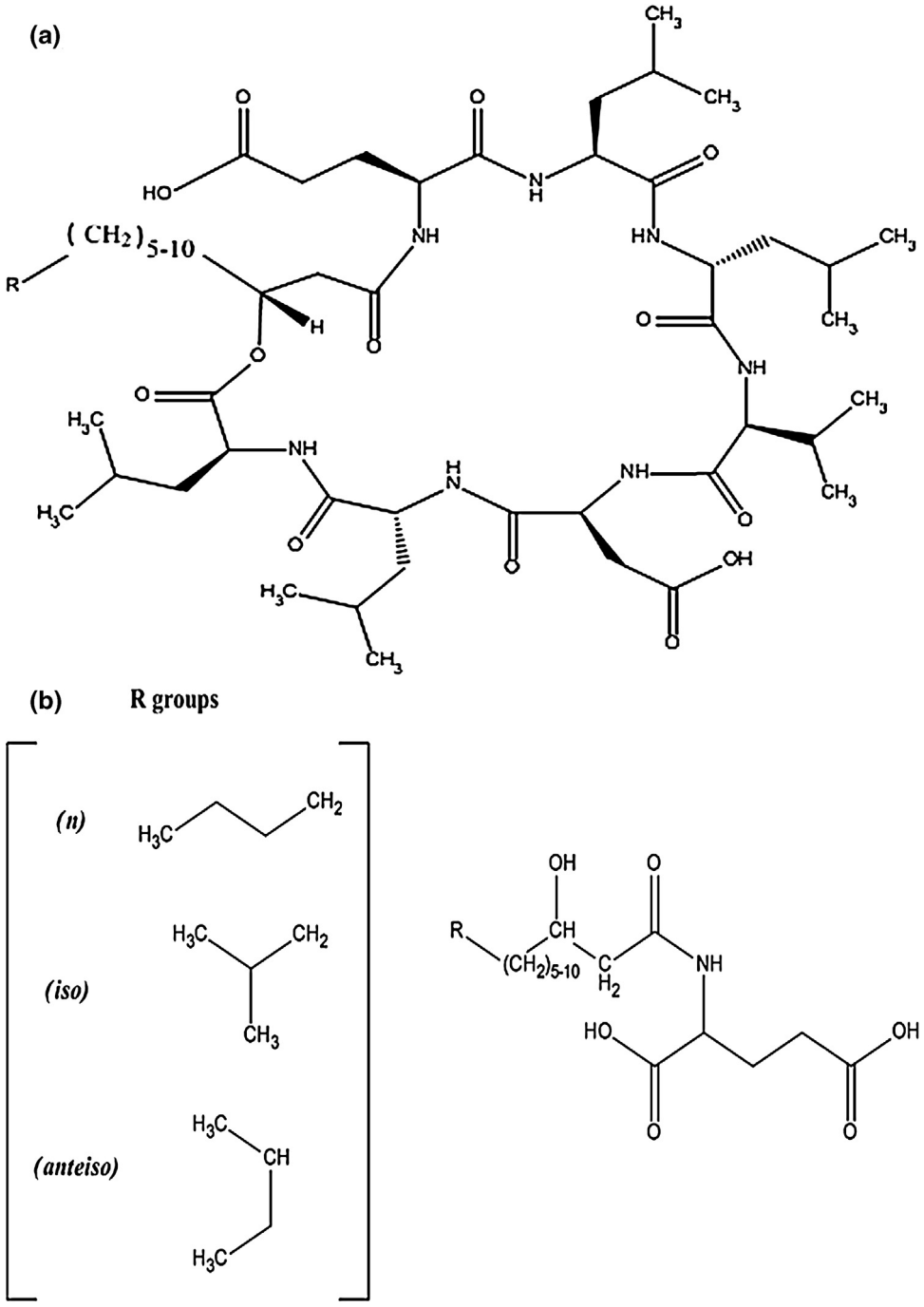

Fig. 1. Structures of lipopeptide biosurfactants: (a) surfactin and (b) FA-Glu. The variability in the fatty acid chain length of both surfactants is responsible for the presence of several isoforms of different molecular weight. The molecular weights of surfactin's isoforms are 978.59, 992.59, 1006.62, 1020.64 and 1034.64 and 1048.69; those of FA-Glu are 344.07, 358.07, 372.08, 386.08, 400.09 and 414.09.

Bacillus subtilis [10,11]. It consists of a long-chain β-hydroxy fatty acid joined by an amide bond to the glutamic acid residue of a heptapeptide having the chiral sequence: L-Glu/L-Leu/D-Leu/L-Val/L-Asp/D-Leu/L-Leu (Fig. 1a). As its fatty acid chain has from 12 to 17 carbons, surfactin exists as a population of surfactant isoforms that range in molecular weight from 978.59 to 1048.69 and differ by 14 amu, the molecular weight of a methylene (—CH_2—) residue. The production of surfactin and similar lipopeptides from *Bacillus* species is reported in the literature. Liu et al. showed with *B. subtilis TD7* that the type of amino acid present in the growth medium resulted in surfactants with certain characteristics: for example, inclusion of Arg, Gln or Val in medium resulted in predominance of surfactin molecules with even-numbered carbon chains, whereas when Cys, His, Ile, Leu, Met, Ser or Thr were included, surfactin molecules with odd-numbered carbon chains predominated [12]. de Faria and co-workers (2011) used *B. subtilis LSFM-05* to produce a surfactin variant with an amino acid sequence of GluOMe-Leu-Leu-Asp-Val-Leu-Leu and a C14 fatty acid moiety from glycerin derived from biodiesel production.

The CMC and emulsification index (E_{24}) of the surfactant are also reported and compared with those of sodium dodecyl sulfate (SDS) and Triton X [13]. Liu and co-workers (2010) determined CMCs and surface tensions in phosphate buffered saline (PBS) of nC14- and anteiso C15-surfactins produced by *Bacillus velezensis*