Abstract

Background and Objectives: Microbial derived biosurfactants are amphipathic molecules consisting of both the hydrophobic and hydrophilic domains which allow them to be aggregated at interfaces of immiscible liquids such as water and oil. The study focused on isolation and identification of biosurfactant-producing bacterial strain from oil contaminated soil samples, characterization of produced biosurfactant and evaluation of factors affecting on biosurfactant production and also antioxidant potential and wound healing activity of produced biosurfactant.

Methods: An enrichment program for hydrocarbon-utilizing bacteria capable of biosurfactant production isolated from soil samples was carried out on mineral salt medium supplemented with ILCO, 1%. The organic solvent extraction method was performed in order to extract the biosurfactant. Produced biosurfactant was characterized by physicochemical properties and analytical characterization such as FTIR, ^{1}HNMR, HPTLC, CD and UV spectroscopy. The aggregation behavior of the biosurfactant was evaluated in a PBS solution (pH 7.4) by using DLS technique, turbidity measurement, and TEM inspection. DPPH radical scavenging activities and FRAP assays were used to measure the antioxidant properties. To evaluate the wound healing activity, 36 rats (previously wounded in the depilated thoracic region) were randomly distributed into six groups and chromatic, wound contraction, and histopathological feature were examined. Assessments the level of ROS after biosurfactant exposure were determined using MDA, H_2O_2, and glutathione GSH assay kits. In addition, the acute toxicity of the obtained biosurfactant was also determined.

Results: The most promising isolate was identified as *Acinetobacter junii* B6 using 16S rDNA sequencing and biochemical characterization .Application of two-level fractional factorial design showed that surface tension of culture broth was maximally reduced to 38 mN/m in the optimize condition (NaNO3 2 g/L, ILCO 5%, temperature of 25 °C, aeration rate of 300 rpm, and inoculum size of 2%). GC-MS analysis of the culture broth showed the ability of *A. junii* B6 to degrade most of the alkanes' components of ILCO when used as sole carbon source. According to the analytical characterization, the biosurfactant was lipopeptide compound. The produced biosurfactant decreased the surface tension of water to 36 mN m^{-1} with the CMC of approximately 300 mg/l. The biosurfactant showed the spherical-shaped vesicles at a concentration higher than its CMC and the circular dichroism (CD) spectra showed that the secondary structure of the biosurfactant vesicles is dominated by the β sheet. The biosurfactant showed an HLB value of 10 that reflects suitable O/W emulsifying property. DPPH assay showed notable scavenging activities at the corresponding concentrations with the IC50 value of 0.7 mg/ml. The best histopathological remission was achieved following treatment by 5 mg/ml of the LBS. Scar wounds at day 13 showed the lowest lesion sizes, increased re-epithelialization, hair follicle detection, and decreased amounts of neutrophilic inflammation, the immaturity of the wound bed, erythema, edema, capillary, and retention of necrotic tissue. Results from MDA, H_2O_2, and GSH levels of the treated sample confirmed the scavenging property of the bacterial - derived LBS through ROS

Conclusion: It can be concluded that both strain and product has potential applications in various industries such as oil and pharmaceutical.

Keywords: Lipopeptide biosurfactant; *Acinetobacter junni*; Aggregation behaviors; physicochemical properties; Wound healing; Oxidative stress.