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ORIGINAL ARTICLE

Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia

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Abstract Twenty three morphologically distinct microbial colonies were isolated from soil and sea water samples, which were collected from Jeddah region, Saudi Arabia for screening of the most potent biosurfactant strains. The isolated bacteria were selected by using different methods as drop collapse test, oil displacement test, blue agar test, blood hemolysis test, emulsification activity and surface tension. The results showed that the ability of *Virgibacillus salarius* to grow and reduce surface tension under a wide range of pH, salinities and temperatures gives bacteria isolate an advantage in many applications such as pharmaceutical, cosmetics, food industries and bioremediation in marine environment. The biosurfactant production by *V. salarius* decreased surface tension and emulsifying activity (30 mN/m and 80%, respectively). In addition to reducing the production cost of biosurfactants by tested several plant-derived oils such as jatropha oil, castor oils, jojoba oil, canola oil and cottonseed oil. In this respect the feasibility to reusing old frying oil of sunflower for production rhamnolipids and sophorolipids, their use that lead to solve many ecological and industrial problems.

1. Introduction

The surface-active compounds commonly used in many industries are chemically synthesized; they are widely used in almost every sector of recent industry (Samadi et al., 2007). The expansion in environmental carefulness has led to serious consideration of biological surfactants as the most promising alternative to existing product (Henkel et al., 2012).

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Biosurfactants are considered as one of the high values of microbial products, which have gained considerable interest in recent years that have become an important product of biotechnology for industrial and medical applications (Nitschke and costa, 2007; Makkar et al., 2011). The reasons for their publicity are lower toxicity, specificity of action, simplicity of preparation and extensive applicability. Moreover, they can be used as moistening agents, dispersing agents, emulsifiers, foaming agents, beneficial food elements and detergents in many industrial regions such as: organic chemicals, pharmaceuticals and cosmetics, beverages and foods, metallurgy, mining, petroleum, petrochemicals, biological control and management and many others (Banat et al., 2000; Perfumo et al., 2010; Vedaraman and Venkatesh, 2011). Over the above, biosurfactants have many advantages over synthetic ones, including bioavailability, structural diversity, specific activity at extreme salinity, temperatures and pH (Datta et al., 2011). In spite of these advantages, good attributions and a variety of potential uses of biosurfactants, efforts to commercial production have failed due to the low yield obtained and high production cost. The possibility of economical biosurfactant production to reduce pollution was caused by wastes. Increasing biosurfactant yields and decreasing production costs are essential factors affecting the efficiency of biosurfactant production process (Kosaric, 1992; Bognolo, 1999; Moussa et al., 2006). Syldatk and Hausmann (2010) found that the use of costly substrates, gave low yields and accumulation of undesirable product mixtures rather than refined biosurfactant compounds, such constraints explain why there is restricted production of biosurfactants in industry. Great varieties of agronomic, industrial by-products and material residues are recently available as nutrients for biosurfactant fermentation industry (Makkar and Cameotra, 2002; Savarino et al., 2007; Ferreira, 2008; Silva et al., 2009). Therefore hopefully tomorrow's microbial surfactants appear to depend particularly on the use of plentiful and cheap substrates for optimization of the operational cultivation conditions, which can markedly increment the yield (Mukherjee et al., 2007, 2008; Mutalik et al., 2008; Makkar et al., 2011). The world production of fats and oils is about 120 million tonnes, 81% of which are from plant sources (Brackmann and Deutschland, 2004). Most of the oils and fats are used in the food industry, which produces large amounts of waste frying oils. The disposal of frying oil waste is causing a great problem, which explains the increasing interest in the use of waste frying oils for microbial transformation (Vedaraman and Venkatesh, 2011). The beneficial effects of this field were paid attention to for isolation and characterization of biosurfactants produced by extremophiles such as halophilic and thermophilic bacteria (Mnif et al., 2009; Kumar et al., 2008; Joshi et al., 2008). This study aimed at isolation of some bacterial isolates from different sources (Oil contaminated soil, uncontaminated soil and from Red sea water at Jeddah region in kingdom of Saudi Arabia [KSA]), then screening assays for biosurfactant production from obtained isolates. The waste frying oil will be reused as a substrate for the production of cheaper biosurfactant.

2. Material and methods

2.1. Bacterial isolation

Bacterial isolates were obtained from different sources such as oil contaminated soil, uncontaminated soil and from Red sea water from Jeddah region at KSA. Ten grams of soil was collected from soil samples, in addition to 10 ml of water sample. Then all samples were transferred to the laboratory in sterilized polyethylene containers. The direct isolation of the microorganisms was carried out using serial dilution (up to 10^{-7}) of soil samples in 0.85% sterile saline (Bordoloi and Konwar, 2008). The different samples were agitated and serial dilutions, then 1 ml from each diluted samples was plated onto the surface of Nutrient Agar medium. The plates were incubated at 30 °C for 1–5 days. Pure cultures with different morphological distinct properties were obtained by picked repetitive streaking and stored in nutrient slants at 4 °C.

2.2. Screening assays for potential biosurfactant producing strains

The screening of the most potent surfactant strains was assayed qualitatively using different methods namely drop collapse test, oil displacement test, blue agar test, blood hemolysis test and quantitatively using emulsification activity and measurement of surface tension by the Du Nouy ring method. The strain which showed the lowest surface tension value was selected for a further study according to methods described by Bodour and Maier (1998), Youssef et al. (2004) and Sriram et al. (2011). All the experiments were done in triplicate.

2.3. Identification of biosurfactant producing bacteria

The bacterium identification was conducted by the molecular method depending on gene phylogenetic approximation. PCR amplifications of the 16S rRNA gene were performed with 16S rRNA Eu-bacterial primer (50-GAGTTTGATCCT GGCTCAG-30; 50-AGAAAGGAGGTGATCCAGCC-30) following the method described by Relman (1993). The phylogenetic tree was constructed by utilizing the neighbor-joining method and assessed with 1000 bootstrap replications. The minimum value of the nucleotide similitude percentage to define the species at the taxonomic level was 98% (Rossello-Mora and Amann, 2001).The 16S rRNA gene sequence obtained from the isolate Nrc-1 was compared with other bacterial sequences by using NCBI mega BLAST. The nucleotide sequence was aligned in CLUSTALX. The phylogenetic analyses were conducted using MEGA version 6 software (Tamura et al., 2007).

2.4. Production media and cultivation conditions

A minimal salt (MS) medium containing (g/l): KH_2PO_4, 1.4; Na_2HPO_4, 2.2; $(NH_4)_2SO_4$, 3; $MgSO_47H_2O$, 0.6; NaCl, 0.05; yeast extract, 1; $FeSO_4$ $7H_2O$, 0.01 and $CaCl_2$ $7H_2O$, 0.02; was used throughout the study. The basal minimal medium was supplemented with 2 ml of trace element solution and glucose 2% (v/v) was used as the sole carbon source. The composition of trace element solution involved (g/l): $ZnSO_4$, 0.29; $CaCl_2$, 0.24; $CuSO_4$, 0.25 and $MnSO_4$, 0.17. The trace element solution was added after the production media were autoclaved, prior to inoculation by filtering it through 0.2 μm membrane filters, sterilized by filtration (Millipore Corp., Bedford, MA, USA). After incubation the cultures at