International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 9, 2019, pp.-1705-1707.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

BIOSURFACTANT PRODUCTION FROM CRUDE OIL SOIL BACTERIUM

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Received: August 15, 2019; Revised: September 24, 2019; Accepted: September 26, 2019; Published: September 30, 2019

Abstract- The main criteria of this research were to produce Biosurfactant by bacteria isolated from the soil sample contaminated with crude oil. To carry out this work we have isolated organism from the garage soil (crude oil spill). This strain was subjected to screening test (Bath Assay) for biosurfactant production. The isolated strain was characterised by 16s RNA sequencing (NCIM-CSIR-NCL) found to be resembling *Lysinibacillus tabacifolii* strain K3514. Crude oil used was waste engine oil as a source of carbon for Biosurfactant production. Biosurfactant are extracellular surface-active compounds produced by bacteria, fungi and yeast. Most microbial surfactant are complex molecules, comprising different structures that include lipopolypeptide, glycolipid, polysaccharide protein complexes, fatty acids and phospholipids. Fermenter production of biosurfactant was carried out in MSM and in Whey broth with waste crude oil from garage as a source of carbon. Biosurfactant was extracted and purified in solvent system Ethyl acetate: methanol (4:1). The biosurfactant produced was dissolved in chloroform and separated on TLC (thin layer chromatography). The separated spots were characterised by GC- MS (Gas chromatography-mass spectrophotometer).

Keywords- *Biosurfactant, waste engine oil, Lysinibacillus*

Citation: Yadav D.J., *et al.*, (2019) Biosurfactant Production from Crude Oil Soil Bacterium. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 9, pp.-1705-1707.

Introduction

Biosurfactants are surface-active molecules/chemical compounds synthesized by microorganisms. These amphiphilic compounds are produced mostly on microbial cell surfaces, or excreted extracellularly. These are amphipathic molecules have the ability to accumulate between fluid phases, thus reducing surface and interfacial tensions at the surface and interface respectively. Most biosurfactants are can be a carbohydrate, an amino acid, a phosphate group, or some other compounds. The hydrophobic moiety is mostly long carbon chain fatty acid either anionic or neutral and the hydrophilic moiety. These molecules reduce surface and interfacial tensions. Because of surface active property of biosurfactants, microemulsions are built in which micelle formations occur where hydrocarbons can solubilize in water or water in hydrocarbons. Biosurfactants are biodegradable in nature. Biodegradability is a very important in controlling environmental pollution. and it is safe in the environment. Properties and Applications of biosurfactants. All surfactants are nowadays chemically synthesized, much attention has been directed towards biosurfactants due to their property of oil recovery and various other applications. Applications-Biosurfactants enhance the emulsification of hydrocarbons, solubilize hydrocarbon contaminants and microbial degradation. The use of chemicals for the treatment of a hydrocarbon polluted site may pollute the environment with their by-products, whereas biological treatment may efficiently destroy pollutants, while being biodegradable themselves

Material and Methods

Isolation on Bushnell Hass-Agar

Take 0.1gm of soil sample and dissolved in sterile saline.
Make a dilution upto 10-6 from this saline sample.
Take a loopful of soil from dilutions $10^{-4,-5,-6}$.
Take a 0.1ml of crude oil and put it on agar medium used.
Isolate it on Bushnell Hass-Agar.
Incubate it at room temperature for 24hrs.
Colonies appear slimy. 5.2.2. Heamolytic Assay.
Take a loopful of culture from Bushnell Hass-Agar plate.
Isolate it on Blood agar.
Incubate it at room temperature for 24hrs.
Observe for clear zone.

Bath Assay (Bacterial adhesion to hydrocarbon assay).

Bacterial cells were wash twice and suspended in buffer salt solution (gm/l 16.9K_2HPO_4, 7.3 KH_2PO_4) to give an optical density (OD) of~0.5.
The cell suspension (2ml) with 100microliter crude oil added was vortex-shaken for 3min.
After shaking, crude oil and aqueous phase were allowed to separate for 1hr.
O.D of aqueous phase was measured at 600nm spectrophotometer
Hydrophobicity is expressed as the percentage of cell adherence to crude oil calculate as follow
100(1- O.D of aqueous phase) (O.D of initial cell)*

Cell growth and biosurfactant production.

Take 250ml MSM broth and WHEY broth in500ml of conical flask.
Add 1ml of crude oil in the MSM and WHEY broth.
Take loopful of culture and inoculate it in the broths.
Incubate it for 7 days at room temperature.
Observe for crude oil degradation and biosurfactant production.
Purification of biosurfactant.
Add 5ml of conc. HCL and store in fridge for 24hrs.
Add 50ml of mixture of Ethyl Acetate: Methanol (4:1).
Separate organic and inorganic layer by separating funnel (allow to stand for 15min).
Organic layer was separated and dried in the petri plate for 5 days at room temperature.
Solid extract was obtained.

Characterization of biosurfactant.
Solid extract was dissolved in chloroform.
The dissolved samples were spotted on TLC plate.
TLC plate was run in solvent system
{(Petroleum Ether:Ethyle Acetate)(7%)}
Separated spots were visualised under 254 & 366nm under TLC visualiser.

Spots were eluted and dissolved in chloroform and further sent for GC-MS Bacteria was characterised and identified by 16s rRNA sequencing method.

Sequencing result
Strain SEQ171Culture3_NC150319B showed closest homology with *Lysinibacillus* sp. (Closer totabacifolii) as identities: 655/656(99%)

Raw data
Yellow region indicates good quality sequence used for analysis
>530F_Seq 171_Culture
3AAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTATCTGGTC
TGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGC
CCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTC
GCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCC
GTTGACCACTGTAGAGATATGGTTTTCCCTTCGGGGACAACG
GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGC
ACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA
AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGATA
CAAACGGTTGCCAACTCGCGAGAGGGAGCTAATCCGATAAAGTCGTTCTCAG
TTCGGATTGTAGGGCTGCAACTCGCCTACA TGAAGCCGGAATCGCTA.

NCBI-BLASTn
BLASTn shows 100% similarity with *Lysininbacillus Tabacofolii* Strain K3514 16srRNA, Partial sequence [NR_132691.1] having E-value 0.0.

Fig-1 The biosurfactant was produced in WHEY and MSM broth by using these bacteria.
The biosurfactant was extracted by using solvent system Ethyle Actate : Methanol (4:1).

Fig-2 The biosurfactant was been separated using TLC plate and separated spots were eluted and were further send for the GCMS for structural characterisation.

Conclusion
The main purpose of carrying out this research was to isolate the biosurfactant producing bacteria from soil sample collected at near the garage area. The growth of bacteria was carried out on Bushnell Hass-Agar and hydrophobicity was confirmed by BATH Assay. The bacteria were found to be citrate, urease, catalase, oxidase positive and nitrate, indole, methyl red negative tests. The bacteria were identified by 16s rRNA sequencing and the bacteria was concluded as *Lysinibacillus tabacofolii*. Biodegradable to the environment Helps in degrading oil spills and safe to the environment

Future Prospective
It is an economical method of producing biosurfactant by using cheap carbon source. Since chemically synthesize surfactant are not biodegradable and pollute environment, biological biosurfactant are stable and biodegradable. Since the production of biosurfactant do not produce any waste material and cost effective.

Application of research: The bacteria is inoculated on MSM and WHEY with engine oil and biosurfactant production was carried out the biosurfactant produced was extracted and separated on TLC and for structural characterisation biosurfactant was send for GCMS

Research Category: Biodegradation

Acknowledgement / Funding: Authors are thankful to Department of Biotechnology, Ismail Yusuf College, Mumbai, 400060, University of Mumbai, Mumbai, Maharashtra, India. Authors are also thankful to National Collection of Industrial Microorganisms (NCIM), Pune for Sequencing

***Research Guide or Chairperson of research: Dr H A Pathan**
University: University of Mumbai, Mumbai
Research project name or number: MSc Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Garage soil collected from Malad, Mumbai

Strain name: *Lysinibacillus tabacifolii* strain K3514

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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