Journal of Environmental Management 174 (2016) 79–86

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Short communication

Bioremediation of petroleum contaminated soil to combat toxicity on *Withania somnifera* through seed priming with biosurfactant producing plant growth promoting rhizobacteria

Amar Jyoti Das, Rajesh Kumar*

Rhizospheric Biology Laboratory, Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar (A Central) University, VidyaVihar, Raibareli Road, Lucknow 226 025, India

A R T I C L E I N F O

Article history:
Received 29 October 2015
Received in revised form
23 January 2016
Accepted 25 January 2016
Available online 25 March 2016

Keywords:
Petroleum toxicity
Bioremediation
Biosurfactant
PGPR
Seed priming
Withania sominfera

A B S T R A C T

Soil contaminated by Petroleum oil cannot be utilized for agricultural purposes due to hydrocarbon toxicity. Oil contaminated soil induces toxicity affecting germination, growth and productivity. Several technologies have been proposed for bioremediation of oil contaminated sites, but remediation through biosurfactant producing plant growth promontory rhizobacteria (PGPR) is considered to be most promising methods. In the present study the efficacy of seed priming on growth and pigment of *Withania somnifera* under petroleum toxicity is explored. Seeds of *W. somnifera* were primed with biosurfactant producing *Pseudomonas* sp. AJ15 with plant growth promoting traits having potentiality to utilized petroleum as carbon source. Results indicates that plant arose from priming seeds under various petroleum concentration expressed high values for all the parameters studied namely germination, shoot length, root length, fresh and dry weight and pigments (chlorophyll and carotenoid) as compared to non primed seed. Hence, the present study signifies that petroleum degrading biosurfactant producing PGPR could be further used for management and detoxification of petroleum contaminated soils for growing economically important crops.

© 2016 Published by Elsevier Ltd.

1. Introduction

Petroleum refinery is an industrial plant which refined the crude oil into useful product such as gasoline, petrol, diesel, kerosene, asphalt base, liquefied petroleum gas and heating oil (Gary and Handwerk, 1984). However in case of an unwanted leakage of petroleum oil and its product might act as a persistent soil and water pollutant (Graj et al., 2013). Soil pollution by petroleum oil induces major changes in the physical and chemical properties of soil ensuing in adverse effect on plant growth. Petroleum oil and its constituents can decrease the availability of oxygen, water and nutrients in soil, which as a result may decline the seed germination rate and effect the plant growth (Nogueira et al., 2011). Several technologies have been introduced for remediation of oil contaminated sites, but bioremediation through microorganisms with the appropriate metabolic capabilities is most promising. Although, optimal rates of hydrocarbon biodegradation by microorganism can be maintained by the adequate concentration of oxygen, nutrients and pH values, but high hydrophobic nature and low solubility of petroleum hydrocarbon compounds make them highly unavailable to microorganisms (Atlas, 1975; Amund and Nwokoye, 1993; Perry, 1984), Hencerelease of biosurfactants is one of the strategies used by microorganisms to influence the uptake of petroleum hydrocarbon and hydrophobic compounds (Marin et al., 1996; Johnsen et al., 2005; Obayori et al., 2009; Ron and Rosenberg, 2002). Biosurfactants increases the surface area of hydrophobic water insoluble substrates and increase their bioavailability, thereby enhancing the growth of bacteria and rate of bioremediation. Certain microbial species such as fluorescent pseudomonads are accounted to excrete a various form of partially or totally extracellular biosurfactant that facilitates the uptake of hydrocarbons by reducing the surface tension and enhance the removal of hydrocarbons from the oil contaminated soil (Bento et al., 2005; Franzetti et al., 2010). Various microorganisms have acquired a mechanism to thrive and grow in oil containing environment and play an immense role in treatment by degrading the pollutant (Pothuluri and Cerniglia, 1994; Jurelevicius et al., 2013; Pacwa-Płociniczak et al., 2016). Petroleum pollutants can be degraded by plants

* Corresponding author.
E-mail address: Rajesh4971@yahoo.com (R. Kumar).

http://dx.doi.org/10.1016/j.jenvman.2016.01.031
0301-4797/© 2016 Published by Elsevier Ltd.

through biochemical reactions taking place within the plants and in the rhizosphere (Hrynkiewicz and Baum, 2011). The remediation of soils containing petroleum is possible with the use of plants and their rhizosphere processes (Mirsal, 2004; Hrynkiewicz and Baum, 2011). In this interaction soil microorganism provides nutrients in the rhizosphere which leads to an increased microbial activity and degradation of toxic pollutants (Mirsal, 2004; Hrynkiewicz and Baum, 2011). Most of the soil bacteria with plant growth promoting traits can be well habituated to harsh soil conditions and enhance the remediation of disturbed soils directly and by plant growth promotion (Hrynkiewicz and Baum, 2011; Fomina et al., 2005; Wenzel, 2009). Fluorescent pseudomonad (versatile bacteria, gram-negative, motile, rod shaped and non-spore forming) have been reported to promote plant growth in rhizosphere directly by their plant growth promoting traits. These plant growth promoting traits can enhance various stages of plant growth development and combat abiotic stress (Kumar et al., 2013, 2015; Yang et al., 2008). *Withania somnifera* is a medicinal belonging to family Solanaceae, reportedly exhibit antipyretic, anti-inflammatory abortifacient, immunomodulatory and haematopoietic activity (Mishra et al., 2000). Leaves and roots of Indian *W. somnifera* content active components withaterin-A and withanolide-D, which serve as a source of drugs. But according to Red data book of threatened species *W. somnifera* is an endangered medicinal plant (Arumugam and Gopinath, 2013). So their cultivation is of urgent need, but due to scarcity of agricultural land no much attention has been given for their cultivation. Hence, managing the petroleum contaminated soil with biosurfactant producing bacterial strain for their cultivation could open a new vista for their conservation.

The present investigation was carried out for the management and detoxification of petroleum contaminated soil to reduce the toxic effect of petroleum on the growth and pigments of *W. somnifera* (economically important crop) through seed priming with biosurfactant producing plant growth promoting bacteria having the potentiality utilize petroleum as carbon source.

2. Materials and methods

2.1. Microorganism

In this present study, a biosurfactant producing microorganism, identified as *Pseudomonas* sp. AJ15, was selected to perform the experiments on the basis of its plant growth promoting traits and ability to degrade petroleum oil. This strain was isolated from petroleum oil contaminated soil and stored in our laboratory. The inoculum was prepared by transferring a loopful of bacterial culture into 25 ml nutrient medium (composition g/l beef extract-3 g, peptone-5 g, NaCl-5 g) in 250 ml Erlenmeyer flask and incubated at 30 °C for 24 h. This inoculum contained 10^8 cells/ml.

2.2. Screening of the bacterial strain for biosurfactant production

Bacterial strain was tested for biosurfactant production on 500 ml minimal salt medium, MSM (composition g/l MgSO4 (anhydrous) −0.5, $NaNO_3$ −2.5, KH_2PO_4 −1.0, $FeSO_4$ −0.01, KCl −0.1, Na_2HPO_4 −5.67, $CaCl_2$ −0.1,NH_4NO_3 −0.39, $MnSO_4$ −0.002, dextrose −15). Further the biosurfactant production was confirmed by various test. Drop collapse test was performed by following the method of Bodour et al., 2003. Haemolytic assay was performed by modifying the method of Plaza et al., 2006 and Mulligan et al., 1984. Emulsification index E_{24} (%) was determined by following the method of Cooper and Goldenberg (1987). Surface tension reducing ability was measured by following the method of Viramontes-Ramos et al. (2010). Oil displacement test of the of isolated crude biosurfactant was done by Ohno et al., 1993.

2.3. Screening the potentiality of the bacterial strain to utilized petroleum as carbon source

2% (v/v) petroleum oil was mixed with 100 ml minimal salt medium (MSM) to check the potentiality of the bacterial strain to utilized petroleum as carbon source for production of biosurfactant. The incubation was carried out on shaker for 72 h at 30 °C. Culture medium samples were drawn once at every 24 h for estimation of bacterial biomass, biosurfactant production. A minimal salt medium with dextrose as carbon source was used for comparative study. All the experiments were performed in triplicate.

2.4. Plant growth promoting traits

Phosphate solubilization activity was screened on NBRIP medium as per the method described by Nautiyal (1999) and Quantitative analysis of phosphate solubilization (tricalcium phosphate) in liquid medium was estimated by following the method of Jackson 1973. Siderophores production was assayed on the Chrome azurol S agar medium according to the method of Schwyn and Neilands (1987). Quantitative analysis of IAA was performed by following the method of Loper and Scroth (1986) at concentrations of 100 and 500 μg/ml of tryptophan.

2.5. Seed priming

Five certified seeds of *W. somnifera* were primed with 1 ml of 48 h grown bacterial cell suspensions of *Pseudomonas* sp. AJ15 by incubating at 22 °C for 4 h. During incubation, seeds were agitated at 150 rpm on a rotary shaker and were air dried overnight at 28 °C. After 2 days storage of the seeds at room temperature, to assure that each seed had between $\log 10^7$ CFU $seed^{-1}$, the number of bacteria per seed were assessed by grinding samples of ten seeds for 1 min in1 ml 0.85% NaCl using a sterilised mortar and pestle. Suspensions were serially diluted and plated on TSA (TSB supplemented with 1.6% agar and 100 mg/mL rifampicin). Plates were then incubated at 28° C in the dark for 48 h before CFUs were counted (Abuamsha et al. 2011).

2.6. Growth profiling to assess the stress effect

The effect of petroleum oil on the growth was conducted according to Sagar et al., 2012. The primed and non primed seeds (10 each) were sown in pots with 1 kg sterilized sandy clay soil with chemical properties [C organic (%) 1.4 ± 0.05, Ca (g Kg^{-1}) 3.5 ± 0.4, N (g Kg^{-1}) 1.19 ± 0.8, Na (g Kg^{-1}) 3.8 ± 0.1, P (g Kg^{-1}) 0.75 ± 0.05, K (g Kg^{-1}) 2.94 ± 0.4, Fe (g Kg^{-1}) 0.155 ± 0.03, Zn (g Kg^{-1}) 0.0038 ± 0.02] amended with 0.88% (10 ml), 2.2% (25 ml) and 4.4% (50 ml) of petroleum oil concentration. Each experimental set consisted of 3 pots. Pots were placed in a growth chamber at 30 °C with a 12 h dark–light cycle. Percentage germination, root/shoot length and fresh/dry weight were recorded after 30 days. Rhizospheric colonization for 5 representative plants from each set was determined by dilution plating of 1 g of rhizospheric soil at 20 day on TSA amended with rifampicin.

2.7. Effect on the pigments

Method of Arnon (1949) was followed to study the effect petroleum oil contamination on carotenoid and chlorophyll content. Briefly, 500 mg of fresh leaf was cut into small piece and homogenized with 10 ml of 80% acetone and centrifuged at 2500 rpm for 10 min at 4 °C.The extract obtained was mixed with 80% acetone

Download English Version:

https://daneshyari.com/en/article/1055395

Download Persian Version:

https://daneshyari.com/article/1055395

Daneshyari.com