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Optimization of BH Medium for Efficient Biodegradation of Diesel, Crude Oil and Used Engine Oil by a Newly Isolated *Bacillus cereus* Strain DRDU1 from an Automobile Engine

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Abstract: Current study presents the optimization of growth conditions for efficient degradation of complex petroleum hydrocarbon oil, viz, diesel, crude oil and used engine oil by a newly isolated *Bacillus cereus* strain DRDU1 from an automobile engine. The growth pattern of the isolate was evaluated in BH (Bushnell and Haas) broth supplemented with the above mentioned hydrocarbon sources individually at different pH, temperature and salt concentrations. The optimum BH medium composition obtained by conventional experiments for the degradation of hydrocarbon oil by the strain at pH 7 and 37°C were found to be; magnesium sulfate (0.2), calcium chloride (0.02), potassium dihydrogen orthophosphate (1.0), dipotassium phosphate (1.0), ammonium nitrate (1.0) and ferric chloride (0.05) g L^{-1}. The novel strain was found to be degrading up to 99, 84 and 29% of diesel, crude oil and used engine oil, respectively under optimized condition. Hydrocarbon degradation was confirmed by monitoring the increase in colony forming units with the increase in duration of incubation time, followed by gas liquid chromatographic analysis.

Key words: *Bacillus cereus* strain DRDU1, media optimization, complex petroleum hydrocarbon degradation

INTRODUCTION

Because of the significant uses of oily hydrocarbons, toxicity due to oily sludge is an issue of highest importance (Gallego *et al*., 2007; Phillips *et al*., 2009; Su *et al*., 2011). Microbial bioremediation of hydrocarbon contaminated soil and water has proven itself as a promising technology in recent years (Juwarkar, 2012). Most of the literature showed various species of *Pseudomonas*, *Acinetobacter* and *Flavobacterium* as efficient hydrocarbon degraders (Juwarkar, 2012; Mandri and Lin, 2007). Some other showed *Yokenella*, *Alcaligenes*, *Roseomonas* and *Sphingobacterium*, *Capnocytophaga*, *Moraxella*, *Corynebacterium*, *Streptococcus*, *Providencia* and *Bacillus*, etc., as potential hydrocarbon degraders, both in pure form and as well as in consortia (Etkin, 1998; Borah and Yadav, 2014). For efficient degradation of hydrocarbon, optimization of culture condition and the media components is very important. BH (Bushnell and Haas) medium is widely as a selective media in studying hydrocarbon degradation studies (Mandri and Lin, 2007; Etkin, 1998; Borah and Yadav, 2014). The current study was carried out to optimize the components of BH media as well as the growth conditions (pH and temperature) for efficient complex hydrocarbon oil degradation by the isolate *Bacillus cereus* DRDU1, reported in previous study (Borah and Yadav, 2014).

MATERIALS AND METHODS

Crude oil, used engine oil and diesel oil used in the study were procured from Research and Development Laboratory of Oil India Limited, Duliajan, Assam. Mineral salts and other chemicals were purchased from Merck India Ltd. and all the media used in the study were purchased from Hi Media India Pvt. Ltd. Engine oil marketed by Honda India (P) Ltd. has been used during the study.

Preparation of starter culture: Pure culture of the bacterial isolate *Bacillus cereus* strain DRDU1 was inoculated in 100 mL BH broth (composition (g L^{-1}): $MgSO_4$ (0.2), $CaCl_2$ (0.02), KH_2PO_4 (1.0), K_2HPO_4 (1.0), NH_4NO_3 (1.0), $FeCl_3$ (0.05), agar-agar (20.0), pH (7.0) at 25°C) taken in 250 mL Erlenmeyer flask. It was then incubated for 72 h at 36°C and 135 rpm.

Optimization of pH, temperature and salt concentration for the bacterial isolate in BH broth: One milliliter of the bacterial broth (O.D.>0.1) was aseptically transferred into 100 mL freshly prepared BH broth supplemented with 2% v/v hydrocarbon supplement (used engine oil, crude oil and diesel oil) taken in four different air tight Erlenmeyer flasks. Appropriate blanks were maintained at the same

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condition for every case. The flasks were incubated maintaining the pH range from 1-14 in separate flasks and cfu count was carried out in every 7th days till 28th. Growth was monitored initially at different incubation temperatures (25, 30, 35, 40 and 45°C) after 7 days of incubation. The incubation temperature was increased gradually after obtaining the temperature range at which it gives maximum growth and the optimum temperature for growth was evaluated (Lakshmi *et al*., 2011).

The strain was inoculated in BH broth with various concentration of the salt components ($MgSO_4$, $CaCl_2$, KH_2PO_4, K_2HPO_4, NH_4NO_3 and $FeCl_3$) individually, keeping the rest of the components as it is described above for the determination of optimum formulation of BH medium (Lakshmi *et al*., 2011).

Determination of hydrocarbon degradation by the isolate: Percentage hydrocarbon degradation was confirmed by both gravimetrically (Borah and Yadav, 2014) and also by GLC analysis.

Nucleotide sequences: NCBI GeneBank accession number for 16S rDNA partial sequence of *B. cereus* strain DRDU1 was KF273330.

Statistical analysis: Student's t-test was performed for analysis. Each experiment was performed in triplicate and results were presented in Mean±SD.

RESULTS AND DISCUSSION

The study was carried out for the optimization of growth conditions for a novel *Bacillus cereus* strain DRDU1 isolated from an automobile engine. The strain was identified on the basis of biochemical characteristics and 16S rDNA sequencing. A total of 1262 bp sequence has been submitted to GenBank under accession number KF273330. The strain was found to be proven itself as an efficient hydrocarbon degrader in our previous study (Borah and Yadav, 2014). The phylogenetic relationship of the strain was shown with nine closely related bacterial strains shown in Fig. 1. Colonies of the novel isolate

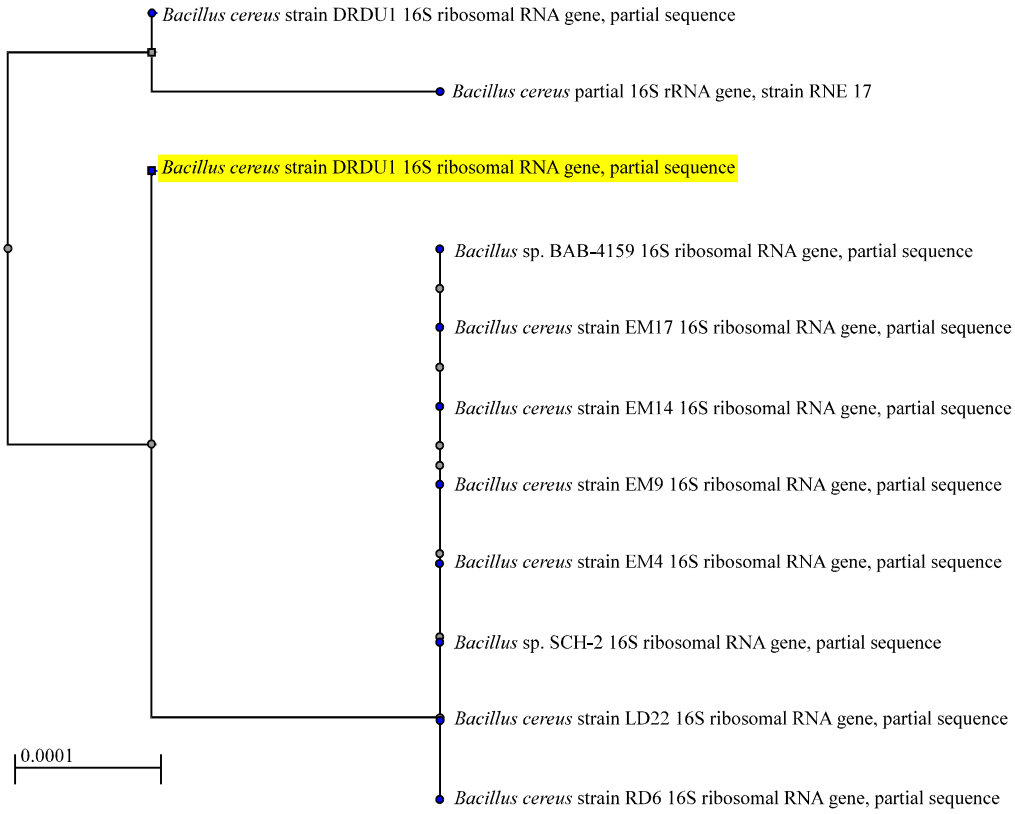

Fig. 1: Phylogenetic relationship of the novel *Bacillus cereus* strain DRDU1 with nine closely related bacterial strains

Bacillus cereus strain DRDU1 showing chemotaxis towards hydrocarbon supplement (used engine oil) on BH agar plates has been shown in Fig. 2 and it also confirms the strain a potential hydrocarbon degrader (John and Okpokwasili, 2012). BH medium has been found to be extensively used for carrying out hydrocarbon degradation studies (Mandri and Lin, 2007). Therefore, optimization was carried out in BH broth supplemented with diesel, crude oil and used engine oil individually for optimum growth of the isolate for optimum hydrocarbon degradation. The optimum BH medium composition obtained by conventional experiments for the degradation of hydrocarbon oil by *Bacillus cereus* strain DRDU1 were found to be, $MgSO_4$ (0.2) (Fig. 3a), $CaCl_2$ (0.02) (Fig. 3b), KH_2PO_4 (1.0) (Fig. 3c), K_2HPO_4 (1.0) (Fig. 3d), NH_4NO_3 (1.0) (Fig. 3e), $FeCl_3$ (0.05 g L^{-1}) (Fig. 3f), for all the four hydrocarbon supplements used in the study. Hydrocarbon degradation was confirmed by monitoring the increase in colony forming unit counts with the increase in duration of incubation time (Borah and Yadav, 2014). The optimum temperature for the maximum growth of the strain for hydrocarbon oil degradation was found to be 37°C and at pH 7, respectively. The strain was found to be degrading upto 99, 84 and 29% of diesel, crude oil and used engine oil under optimized condition (Fig. 4).

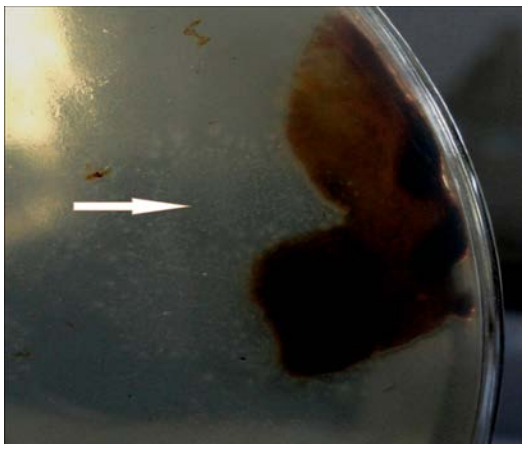

Fig. 2: Colonies of the newly isolated *Bacillus cereus* strain DRDU1 showing chemotaxis towards hydrocarbon supplement (used engine oil) on BH agar plates

The current study showed detailed growth profile of *Bacillus cereus* strain DRDU1 in hydrocarbon containing media with various concentrations of macro and micro nutrients. The strain has proven itself a potential tool for hydrocarbon degradation (Borah and Yadav, 2014). Therefore, the study has been carried out to determining the optimum conditions for better growth of the strain for enhanced hydrocarbon degradation. $KH_2PO_4.2H_2O$ and $K_2HPO_4.2H_2O$ favoured the growth due to their buffering capacity. Moreover, phosphorus on other hand, helps in the synthesis of ATP and DNA. $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and $FeCl_3.2H_2O$ favoured the growth as Ca^{2+}, Mg^{2+} and Fe^{2+} ions has well established role in enhancing the enzymatic action of hydrocarbon degrading enzymes. The enhancement in the degradation in the case of $NH_4NO_3.2H_2O$ may be explained by the fact that hydrocarbons exist in a reduced state and they are oxidized by microbes using electron acceptors. Nitrate possesses high oxidation potential for the removal of hydrocarbon contamination (Mukherjee and Bordoloi, 2012). Moreover, additional N supplements acts

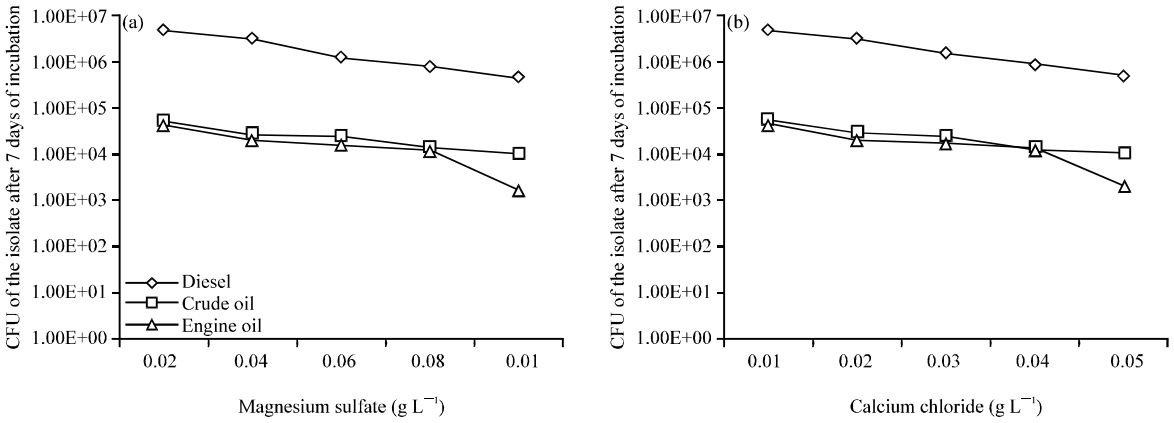

Fig. 3(a-f): Countinue

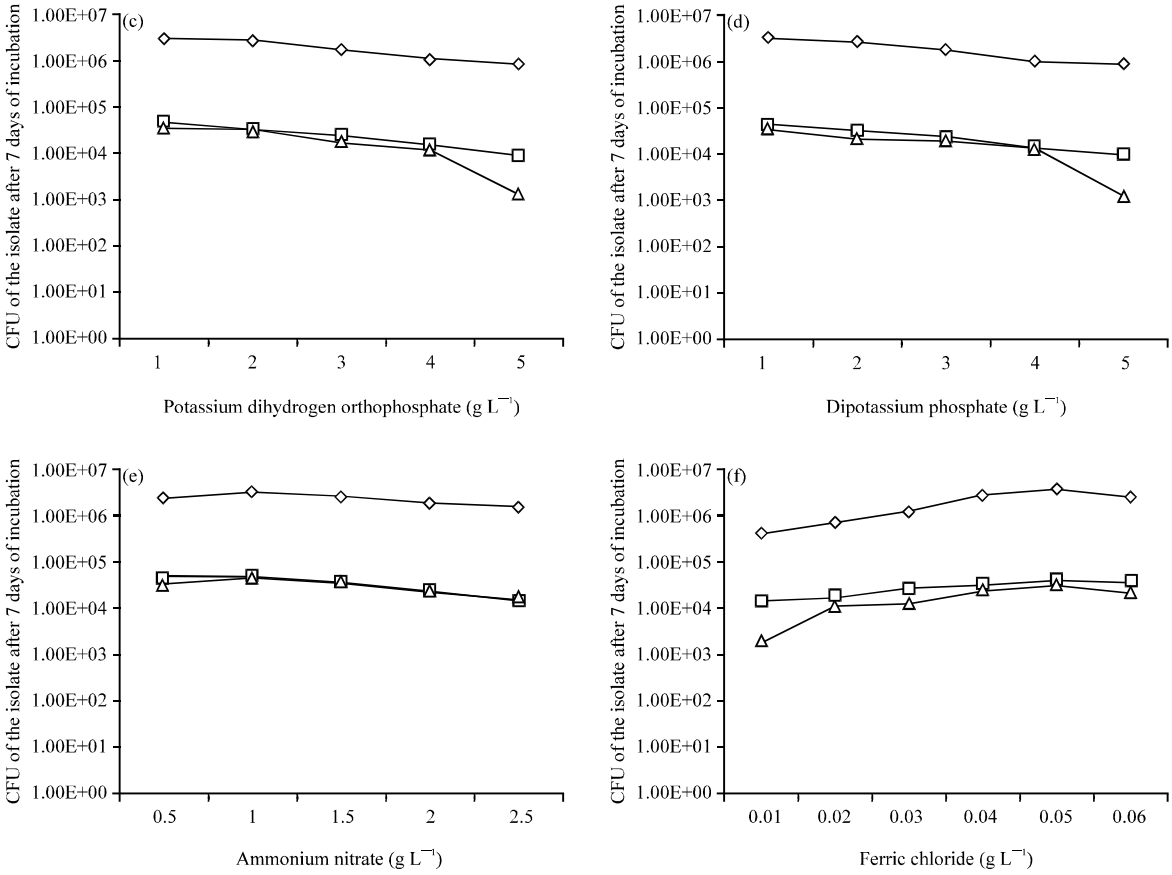

Fig. 3(a-f): Effect of minimal salt concentrations, (a) Magnesium sulfate, (b) Calcium chloride, (c) Potassium dihydrogen orthophosphate, (d) Dipotassium phosphate, (e) Ammonium nitrate and (f) Ferric chloride on hydrocarbon (diesel, crude oil and engine oil) degradation in terms of CFU count after 7 days of incubation at 37°C and 135 rpm

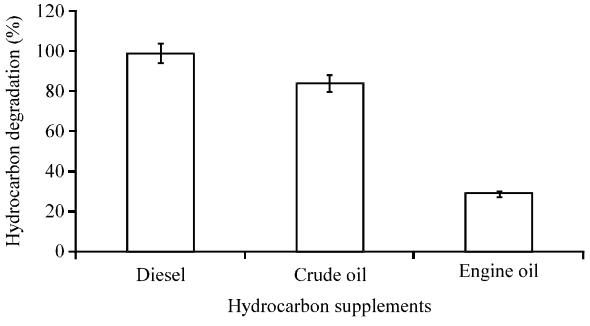

Fig. 4: Percentage hydrocarbon degradation after 28 days of incubation under optimized condition

as macronutrient for the synthesis of amino acids and nucleic acids for the rapid cell growth in the medium. The hydrocarbon degradation potential of the novel isolate was found to be far better than previously reported values (Shirai *et al*., 1995; Nwaogu *et al*., 2008).

CONCLUSION

The current study showed successful hydrocarbon degradation by a novel strain of *B. cereus* isolated from an unconventional source. Optimization of the factors led towards efficient hydrocarbon degradation which was found far higher than some of the previously reported values. With all these features, the strain proves itself a promising tool for petroleum hydrocarbon degradation.

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