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Biodegradation of Complex Hydrocarbon by a Novel *Bacillus cereus* Strain

Debajit Borah and R.N.S. Yadav
Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh, 786004, India

Corresponding Author: Debajit Borah, Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh, 786004, India

ABSTRACT

Contamination of soil and air due to hydrocarbon is a global issue and bioremediation provides probably the best way to remediate the contaminants. The current study shows the biodegradation of diesel, kerosene, crude oil and used engine oil by a newly isolated *Bacillus cereus* strain DRDU1 from an automobile engine. Hydrocarbon degrading strains were screened on BH (Bushnell and Haas broth) agar supplemented with 2% (v/v) of used engine oil as sole carbon source. Maximum degradation of hydrocarbon was observed at the addition of 2% (v/v) of hydrocarbon source in liquid BH (Bushnell and Haas broth) culture medium. The isolate was found to be degrading 67, 57, 72 and 42% of diesel, crude oil, kerosene and used engine oil respectively after 28 days in absence of nitrogen and phosphorus supplements. It was increased significantly up to 71, 64, 83 and 52% in presence of nitrate and phosphate supplements. It was further increased up to 77, 71, 88 and 61% upon addition of 0.01% (v/v) hydrogen peroxide, which acts as an additional oxygen source during oxygen stressed conditions. The strain proves itself to be a stress tolerating bacteria by withstanding 7% of salinity, 37% of glucose concentration and 52% of relative humidity. The thermal death point of the strain was found to be 86°C. The significance of the study is that the percentage degradation of the complex petroleum supplements used in the study was found to be far higher than some of the previously reported values.

Key words: *Bacillus cereus* strain DRDU1, complex hydrocarbon, degradation of complex petroleum oil

INTRODUCTION

Now a day's deliberate use of petroleum hydrocarbon products, such as diesel and engine oil increases the chance of soil pollution and gradually it is proving itself as a major environmental problem (NRC, 1985). Chemically crude oil contains paraffin (15-60%), naphthalene (30-60%), aromatics (3-30%) and asphaltic (6%), but the relative percentage of each varies from oil to oil (Mabro, 2006). Though the major constituents of crude oil may vary, but the percentage composition of the constituents may be given as, carbon (83-87%), nitrogen (0.1-2%), hydrogen (10-14%), oxygen (0.05-1.5%), sulphur (0.05-6%) and metals (<0.1%) (Hyne, 2001). Petroleum oil and PAH (poly aromatic hydrocarbon) has a wide spread effect on human body, as prolonged exposure to petroleum oil may induce liver and kidney diseases, bone marrow damage, or may lead to the development of cancer (Mandri and Lin, 2007).

Microbial bioremediation of hydrocarbon contaminated soil and water has emerged as a promising technology in recent years (Juwarkar, 2012). Most of the study has shown *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Acinetobacter* spp., *Flavobacterium* spp., *Yokenella* spp., *Alcaligenes* spp., *Roseomonas* spp., *Sphingobacterium* spp., *Capnocytophaga* spp., *Moraxella* spp., *Corynebacterium* spp., *Streptococcus* spp., *Providencia* spp., etc., as common hydrocarbon degraders (Mandri and Lin, 2007; Etkin, 1998; Juwarkar, 2012). Biodegradation of complex hydrocarbons, naphthalene and pyrene with the help of *Bacillus* spp., has been shown in many literatures and the degradation was found to be ranging from 20 to 60% (Ghazali *et al*., 2004; Das and Mukherjee, 2007; Bujang *et al*., 2013). Mukherjee and Bordoloi (2012) has evaluated the degradation patterns of benzene, toluene and xylene with the help of a consortium of *Bacillus subtilis* and *Pseudomonas aeruginosa*. The study was carried out both in presence and absence of external nitrate and phosphate supplements. Nwaogu *et al*. (2008) studied the biodegradation of diesel oil by *B. cereus* and it was found to be degrading upto 80% of diesel oil after 28 days of incubation at 30°C. But so far been no report has been found studying the possible role of H_2O_2 as an additional source of oxygen in the degradation of complex hydrocarbons by *Bacillus cereus*. Further, there is no other report found showing the growth of *Bacillus cereus* in hydrocarbon media in presence and absence of external N and P supplements in detail.

The present study was initiated to asses and to compare the degradation of used engine oil, crude oil, diesel and kerosene in liquid media by *Bacillus cereus* strain DRDU1 (isolated from automobile engine) in presence and absence of N, P and H_2O_2 supplements. Further, bacterial growth was evaluated in various stressed conditions (relative humidity, salinity, glucose concentration) in addition to its Thermal Death Point (TDP).

MATERIALS AND METHODS

Chemicals and materials: Crude oil, used engine oil, kerosene 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 HiMedia India Pvt. Ltd. Engine oil marketed by Honda India (P) Ltd. has been used during the study.

Isolation and screening of hydrocarbon degrading microorganisms from various parts of automobile engine: Hydrocarbon residues from various parts of automobile engine were collected and inoculated on BH agar plates (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) and incubated (Bacteriological incubator, Sciegenics Biotech India (P) Ltd) at 37°C for 36 h. Bacterial isolates obtained were subsequently sub cultured twice on Bushnell and Haas (BH) agar plates spread with 200 μL (±2.0 μL) used engine oil to obtain pure colonies of hydrocarbon degrading microorganisms. Pure strains obtained were inoculated in 100 mL BH broth supplemented with 2% (v/v) (2000±2.0 μL) used engine oil as sole carbon source. These were kept in 250 mL air tight Erlenmeyer flask to minimize the evaporation of hydrocarbon supplements. The flasks were allowed to incubate at 37°C for 5 days at 135 rpm on rotary shaker (CERTOMAT®BS-1 shaker incubator, Sartorious Germany Ltd.).

Identification of the potential isolates: The bast isolates was identified on the basis of various staining techniques and biochemical characteristics prescribed by Bergey's Manual of Systematic Bacteriology and finally by 16S rDNA sequencing.

Isolation of genomic DNA and 16S rDNA sequencing: DNA was isolated from the best potential bacterial isolate. Its quality was evaluated on 1.2% Agarose Gel, "a single band of high-molecular weight DNA has been observed". Fragment of 16S rDNA was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel. The PCR amplicon was purified to remove contaminants. "Fragment of 16S rDNA was amplified". Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1262 bp 16S rDNA was generated from forward and reverse sequence data using aligner software. The 16S rDNA sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W™. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4. The 16S rDNA sequence obtained was submitted to GenBank.

Growth in liquid medium in presence and absence of nitrate, phosphate and H_2O_2: The potential isolate was inoculated in 100 mL BH broth (N and P supplements were added or eliminated as per the requirements) in 250 mL air tight Erlenmeyer flask, supplemented with 2, 4, 6 and 8% (v/v) used engine oil, crude oil, kerosene and diesel separately. The growth of the isolate was evaluated both in presence and absence of nitrate (1 g NH_4NO_3 l^{-1}, v/v) and phosphate (1 g KH_2PO_4 l^{-1}, v/v). Two hundred and fifty millileter air tight Erlenmeyer flasks devoid of inoculums maintained at same condition were used as control for each case. Each experiment was performed in triplicate. The Colony Forming Unit (CFU) and the protein content were monitored in every 7 days till 28 days of incubation at 37°C and 135 rpm. The cell pellet obtained after centrifuging the broth at 8000×g for 10 min ("Sigma Germany" was re suspended in 1 mL of distilled water and sonicated (Sartorius Stedim Labsonic, Germany Ltd.) for 10 sec at 100% amplitude for one cycle to lyse the cells. Protein content was determined by the method described by (Lowry *et al.*, 1951).

Role of H_2O_2 as an additional oxygen source and electron acceptor on hydrocarbon biodegradation was studied by aseptically supplementing the final concentration of 0.01% (v/v) H_2O_2. Each hydrocarbon supplements were then aseptically added separately to give a final concentration of 2% (v/v). Un-inoculated flasks and flasks without H_2O_2 served as controls (Mukherjee and Bordoloi, 2012).

Determination of hydrocarbon degradation by the isolate: The remaining oils from each isolate were extracted using n-hexane (HPLC grade), then the dry weight was determined and the oil degradability was calculated based on the weight loss (Shirai *et al.*, 1995) as follows:

$$\text{Oil degradation (\%)} = \frac{\text{Weight of the oil in negative control} - \text{Weight of the oil in sample}}{\text{Weight of the oil in negative control}} \times 100$$

Growth of the isolate in different relative humidity (RH), salinity, osmoticum and temperature: The effect of relative humidity on the growth of the isolates was analyzed by preparing relative humidity chambers using saturated solutions of chemicals. Where $CaSO_4.5H_2O$ gives 98% R.H., KH_2PO_4 gives 96.6% R.H, $Na_2HPO_4.12H_2O$ gives 95% R.H., $NH_4H_2PO_4$ gives 93% R.H., $ZnSO_4$ gives 88.5% R.H., KCl gives 85% R.H., NH_4Cl/KBr gives 79% R.H., NaCl gives 76%

R.H. and $CaNO_3.4H_2O$ gives 52% (Aneja, 2010). The isolates were incubated at 37°C for 3-5 days on separate nutrient agar plates containing 0.5 to 50% glucose for the determination of the effect of osmotic pressure on the bacterial isolates. The effect of saline on the isolates was determined by growing the isolate in nutrient agar plates containing NaCl (concentration ranging from 1 to 20%). The Thermal Death Point (TDP) of the isolate was also determined.

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

RESULTS AND DISCUSSION

The work presented was conducted for the isolation of hydrocarbon degrading bacteria from various parts of automobile engine, as it was expected that an automobile engine would provide comparatively unfavourable conditions for the growth of microbes. A total of 71 hydrocarbon degrading bacterial isolates were screened. These were isolated from hydrocarbon residues from various parts of a total of 25 automobile engines. Each isolates were provided an identification code (IDs), viz, AA, AB,...YD (Fig. 1a-b), prior to the identification of the best isolate. No fungal strain was obtained during the study. Survival of microorganisms in a medium supplemented with petroleum hydrocarbon after their inoculation is a key deciding factor in the rate of biodegradation of hydrocarbon (Ramos *et al*., 1991). The bacterial sample XI was found to be shown most promising isolate for further studies on the basis of protein content 20 μg mL^{-1} just after 5 days of incubation (Fig. 1a-b) in BH broth supplemented with used engine oil. An increase in protein content signifies the increase in cell number and utilization of hydrocarbon supplement as a sole carbon source by the isolates (Mandri and Lin, 2007).

The potential isolate XI was identified as *Bacillus cereus* strain DRDU1 (GenBank Accession Number: KF273330) (Fig. 2) based on biochemical characterizations and 16S rDNA sequencing. The strain was found to be fermenting sucrose, trehalose, Arabinose, glucose and mannitol. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). Sequence producing significant alignments and the optimal tree is shown (Fig. 2). The percentage of imitate trees in the bootstrap test (500 replicates) was shown next to the twigs (Saitou and Nei, 1987). The evolutionary distances were computed using the Kimura 2-parameter method (Felsenstein, 1985). Phylogenetic analyses were conducted by using MEGA4 software (Kimura, 1980; Tamura *et al*., 2007).

The bacterial isolate *Bacillus cereus* strain DRDU1 utilized crude oil, kerosene, diesel and used engine oil as a sole source of carbon and energy. This was evident from the simultaneous increase in CFU and protein content in the medium after each 7 days of incubation till 28 days. The increase in the CFU count and the respective protein content with the increase in incubation time clearly indicates the hydrocarbon degradation by the potential isolate (Mandri and Lin 2007; Das and Mukherjee, 2007). The maximum CFU count was found to be 7.5×10^9 at 2% (v/v) of kerosene supplement in absence of N and P supplements after 28 days of incubation. It was found to be increasing upto 4×10^{10} in presence of N and P supplements (Table 1). The gradual increase in CFU count and the respective protein content has been observed in all concentrations of hydrocarbon used. The detailed growth profile of the isolate in various concentrations of diesel, crude oil, kerosene and used engine oil has been shown in Table 1-4. The lowest CFU was found to be 2.4×10^6 and 4.8×10^8, respectively in absence and presence of N and P supplements along with 8% (v/v) used engine oil as sole carbon source (Table 4).

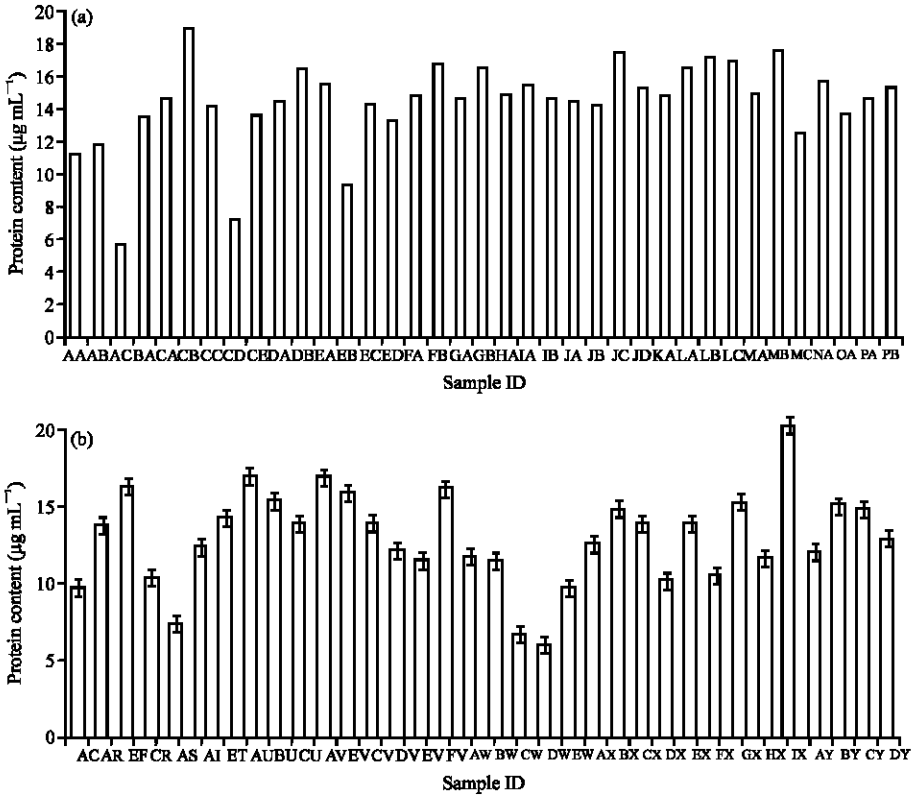

Fig. 1(a-b): (a-b) Protein contents (µg mL^{-1}) of 71 bacterial isolates after 5 days of incubation in BH broth supplemented with 2% v/v used engine oil as sole carbon source, Sample IDs provided to the isolates and the protein content after incubation in the media has been shown on X and Y-axis, respectively, Presence of protein after incubation in the media, where only used engine oil acts as a carbon source clearly indicates the cell division, proliferation and hydrocarbon degradation in the media, Isolate XI shows maximum protein content (Fig. 1b), followed by the isolate CC (Fig. 1a)

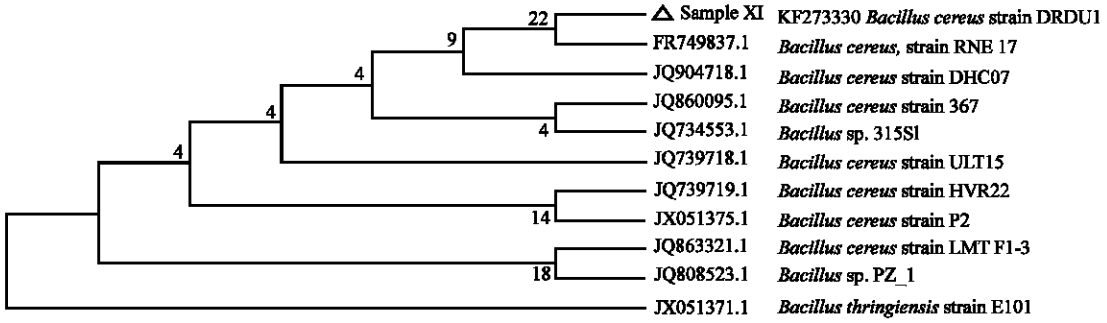

Fig. 2: Phylogenetic relationships of *Bacillus cereus* strain DRDU1 (sample XI, GenBank accession no. KF273330) and other closely related *Bacillus* species based on 16S rDNA sequencing, The tree was generated using the neighbour-joining method, The data set was resampled 1,000 times by using the bootstrap option and percentage values are given at the nodes

Table 1: Detailed growth profile of the isolate *Bacillus cereus* strain DRDU1 in liquid medium containing 2% (v/v) hydrocarbon source and in presence and absence of N and P supplements

Name of the hydrocarbon added (2% v/v)	CFU and protein content of the isolate in different hydrocarbon without P and N (weeks) 1st	2nd	3rd	4th	CFU and protein content of the isolate in different hydrocarbon with P and N (weeks) 1st	2nd	3rd	4th
Diesel								
CFU	6×10^{6}	3×10^{7}	7×10^{8}	9×10^{8}	8×10^{6}	5×10^{8}	14×10^{8}	5×10^{9}
Protein content (μg mL^{-1})	84.8±0.04	97.733±0.02	152.667±0.06	193.867±0.09	86.544±0.02	126.782±0.08	184.562±0.09	236.46±0.08
Crude oil								
CFU	5×10^{4}	3.5×10^{6}	4×10^{8}	4×10^{5}	4×10^{5}	6×10^{5}	7×10^{8}	14×10^{8}
Protein content (μg mL^{-1})	19.733±0.02	62.267±0.02	114.067±0.08	71.467±0.08	32.946±0.06	83.644±0.04	146.248±0.09	192.468±0.09
Kerosene								
CFU	3×10^{5}	4×10^{5}	4×10^{8}	7.5×10^{9}	3×10^{5}	2×10^{6}	13×10^{8}	4×10^{10}
Protein content (μg mL^{-1})	30.933±0.09	40.8±0.04	119.933±0.04	268.4±0.08	29.424±0.09	68.946±0.03	184.624±0.06	326.342±0.04
Used engine oil								
CFU	5×10^{4}	3×10^{5}	5×10^{8}	5×10^{6}	3×10^{5}	1.7×10^{6}	6×10^{8}	11×10^{8}
Protein content (μg mL^{-1})	20.667±0.04	31.6±0.09	129.2±0.08	97.56±0.05	28.762±0.04	56.832±0.02	142.642±0.04	164.348±0.09

Table 2: Detailed growth profile of the isolate *Bacillus cereus* strain DRDU1 in liquid medium containing 4% (v/v) hydrocarbon source and in presence and absence of N and P supplements

Name of the hydrocarbon added (4% v/v)	CFU and protein content of the isolate in different hydrocarbon without P and N (weeks) 1st	2nd	3rd	4th	CFU and protein content of the isolate in different hydrocarbon with P and N (weeks) 1st	2nd	3rd	4th
Diesel								
CFU	5.6×10^{6}	1.9×10^{7}	5.2×10^{8}	6×10^{8}	6.4×10^{6}	3.7×10^{8}	11×10^{8}	4.3×10^{9}
Protein content (μg mL^{-1})	79.42±0.06	94.622±0.04	145.424±0.08	184.324±0.08	82.442±0.04	119.414±0.08	176.216±0.08	227.212±0.08
Crude oil								
CFU	4.2×10^{4}	3×10^{6}	3.2×10^{8}	3.4×10^{5}	3×10^{5}	5.4×10^{5}	5×10^{8}	11×10^{8}
Protein content (μg mL^{-1})	16.412±0.04	57.116±0.04	107.102±0.08	62.226±0.08	29.846±0.06	77.614±0.08	137.216±0.08	183.242±0.08
Kerosene								
CFU	2.8×10^{5}	3.4×10^{5}	3.3×10^{8}	6.9×10^{9}	2.6×10^{5}	1.7×10^{6}	11×10^{8}	3.3×10^{10}
Protein content (μg mL^{-1})	25.213±0.08	37.04±0.08	110.164±0.06	261.2±0.08	26.114±0.08	66.242±0.04	177.412±0.08	318.214±0.08
Used engine oil								
CFU	3.4×10^{4}	2.3×10^{5}	3.8×10^{8}	4.2×10^{6}	2×10^{5}	1.2×10^{6}	4.9×10^{8}	8×10^{8}
Protein content (μg mL^{-1})	16.316±0.06	24.412±0.08	121.42±0.06	93.219±0.06	26.612±0.06	50.114±0.04	132.112±0.06	149.946±0.08

Table 3: Detailed growth profile of the isolate *Bacillus cereus* strain DRDU1 in liquid medium containing 6% (v/v) hydrocarbon source and in presence and absence of N and P supplements presence and absence of N and P supplements

Name of the hydrocarbon added (6% v/v)	CFU and protein content of the isolate in different hydrocarbon without P and N (weeks) 1st	2nd	3rd	4th	CFU and protein content of the isolate in different hydrocarbon with P and N (weeks) 1st	2nd	3rd	4th
Diesel								
CFU	4×10^{6}	1.4×10^{7}	4×10^{8}	5.1×10^{8}	5.9×10^{6}	3×10^{8}	8×10^{8}	3.6×10^{9}
Protein content (μg mL^{-1})	70.114±0.08	92.146±0.06	138.411±0.08	178.224±0.08	73.062±0.04	107.112±0.08	162.662±0.08	218.114±0.08
Crude oil								
CFU	3.4×10^{4}	2.2×10^{6}	2.1×10^{8}	2.9×10^{5}	2.3×10^{5}	4.9×10^{5}	4.3×10^{8}	7×10^{8}
Protein content (μg mL^{-1})	11.225±0.04	54.112±0.06	101.214±0.06	49.114±0.08	21.136±0.06	64.402±0.06	122.102±0.06	164.229±0.08
Kerosene								
CFU	1.8×10^{5}	2.7×10^{5}	2.7×10^{8}	5×10^{9}	1.9×10^{5}	2.9×10^{5}	7×10^{8}	2.9×10^{10}
Protein content (μg mL^{-1})	19.104±0.08	29.026±0.08	102.146±0.04	249.462±0.08	18.108±0.08	37.022±0.08	173.104±0.06	309.334±0.04
Used engine oil								
CFU	2.6×10^{4}	1.6×10^{5}	3.2×10^{8}	3.3×10^{6}	1.9×10^{5}	78×10^{5}	3.2×10^{8}	6.2×10^{8}
Protein content (μg mL^{-1})	11.114±0.06	18.496±0.08	109.12±0.08	82.107±0.08	24.149±0.08	39.178±0.06	119.14±0.06	139.119±0.07

Table 4: Detailed growth profile of the isolate *Bacillus cereus* strain DRDU1 in liquid medium containing 8% (v/v) hydrocarbon source and in presence and absence of N and P supplements

Name of the hydrocarbon added (8% v/v)	CFU and protein content of the isolate in different hydrocarbon without P and N (weeks)				CFU and protein content of the isolate in different hydrocarbon with P and N (weeks)			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Diesel								
CFU	1.9×10^{6}	84×10^{5}	3.3×10^{8}	4.2×10^{8}	4.8×10^{6}	2.1×10^{8}	6.2×10^{8}	2.8×10^{9}
Protein content (μg mL^{-1})	52.076±0.08	70.119±0.08	121.127±0.08	143.122±0.06	66.124±0.04	95.064±0.07	147.194±0.08	199.194±0.04
Crude oil								
CFU	2.3×10^{4}	1.8×10^{6}	1.6×10^{8}	1.9×10^{5}	1.8×10^{5}	4.1×10^{5}	3.6×10^{8}	5.4×10^{8}
Protein content (μg mL^{-1})	7.494±0.02	52.214±0.04	95.107±0.08	43.324±0.06	16.108±0.08	63.227±0.04	115.117±0.07	152.129±0.04
Kerosene								
CFU	1.1×10^{5}	1.8×10^{5}	1.8×10^{8}	3×10^{9}	1.2×10^{5}	2×10^{5}	4×10^{8}	2.2×10^{10}
Protein content (μg mL^{-1})	18.224±0.07	21.249±0.08	92.136±0.07	232.042±0.08	14.229±0.08	33.114±0.07	158.172±0.06	303.624±0.08
Used engine oil								
CFU	1.7×10^{4}	1.1×10^{5}	2.6×10^{8}	2.4×10^{6}	1.2×10^{5}	54×10^{5}	2.6×10^{8}	4.8×10^{8}
Protein content (μg mL^{-1})	8.274±0.04	13.478±0.06	92.142±0.04	75.149±0.07	16.772±0.06	29.946±0.07	104.226±0.08	122.108±0.08

Table 5: Percentage biodegradation of diesel, crude oil, kerosene and used engine oil in liquid medium in presence and absence of N, P and H_2O_2 after 28 days of incubation

Name of the hydrocarbon added	Quantity added (% v/v)	Degradation after 28 days of incubation without external P and N supplements (%)	Degradation after 28 days of incubation in presence of additional P and N supplements (%)	Degradation after 28 days of incubation in presence of additional P, N and H_2O_2 supplements (%)
Diesel	2	67	71	77
	4	63	68	73
	6	58	63	69
	8	51	56	61
Crude oil	2	57	64	71
	4	55	62	65
	6	49	54	58
	8	43	46	52
Kerosene	2	72	83	88
	4	68	78	85
	6	62	73	77
	8	51	67	81
Engine oil	2	42	52	61
	4	39	48	57
	6	32	44	51
	8	26	35	40

The strain was found to be degrading maximum 67, 57, 72 and 42% of diesel, crude oil, kerosene and used engine oil, respectively in absence of additional N and P sources in the medium containing 2% (v/v) hydrocarbon supplements. The degradation was increased upto 71, 64, 83 and 52% in addition of N and P supplements and further upto 77, 71, 88 and 61% upon addition of 0.01% (v/v) H_2O_2. The degradation was found to be increasing in every concentration of hydrocarbon during the study after addition of N, P and H_2O_2 supplements (Table 5). The enhancement in the degradation may be explained by the fact that hydrocarbons exist in a reduced state and they are oxidized by microbes using electron acceptor. Nitrate possesses high oxidation potential for the removal of hydrocarbon contamination (Mukherjee and Bordoloi, 2012). Moreover additional N supplements acts as macronutrient for the synthesis of amino acids and nucleic acids

for the rapid cell growth in the medium. Phosphorus on other hand helps in the synthesis of ATP and DNA. H_2O_2 stimulated hydrocarbon degradation by providing additional oxygen to the isolate in oxygen stressed conditions.

The stress tolerant potential of the isolate was determined by growing the isolate at 37°C for 3-5 days on separate nutrient agar plates containing 0.5 to 50% glucose, 2 to 20% NaCl. It was done for the determination of the effect of osmotic pressure and salinity on the bacterial isolate *Bacillus cereus* strain DRDU1. The isolate was found to be tolerating 7.5% saline (moderately halophilic) and 37% sugar content, 52% RH. The strain was inoculated in nutrient broth maintained at temperature 40, 50, 60, 70, 80, 90 and 100°C for a duration of 10 min for the determination of its Thermal Death Point (TDP). When no growth was observed at 90°C and above, the above procedure was repeated again from 80 to 90°C with an increase in 1°C for the determination of TDP of the isolate. The TDP of the isolate was found to be at 86°C.

The degradation potential and the cell growth were found to be decreasing with the increase in hydrocarbon supplements. This may be due to the increase in cytotoxicity in the medium with the increase in hydrocarbon supplements in the medium (Borah and Yadav, 2012).

The current study showed detailed growth profile of *Bacillus cereus* (isolate XI) in hydrocarbon containing media, both in presence and absence of external N and P supplements and also successful enhancement in hydrocarbon degradation in presence of H_2O_2 in the media. Also the isolate was found to be showing its potential to grow under stressed conditions such as less relative humidity (52%), higher osmotic pressure (37%) and moderate salinity (7%), with a TDP 86°C. These features may play a key role for proving its survival in wide range of climatic conditions. Moreover, till date, no report is published on the detailed study of the growth of *Bacillus cereus* in liquid media supplemented with used engine oil, crude oil, kerosene and diesel oil in presence and absence of N, P and H_2O_2. Therefore, the current study had shown the newly isolated *Bacillus cereus* strain DRDU1 as a potential tool for bioremediation.

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