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RESEARCH ARTICLE

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Potential of *Burkholderia cepacia* RQ1 in the biodegradation of heavy crude oil

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Abstract The potential of *Burkholderia cepacia* strain RQ1 in the biodegradation of heavy crude oil (Maya) was assessed to develop an active indigenous bacterial consortium for the bioremediation of crude oil-polluted systems in Nigeria. The heavy crude oil (Maya) was utilized as sole source of carbon, attaining maximum cell densities of 10^8 cfu ml^{-1} from an initial 10^5 cfu ml^{-1} in 15 days. Biomass also increased with oil concentrations up to 0.8% (w/v). Growth rates ranged from 0.028 h^{-1} to 0.036 h^{-1} and degradation rates decreased with increasing concentrations of oil from 0.009 day^{-1} to 0.004 day^{-1}. The quantity of oil metabolized increased significantly ($P < 0.05$) with increasing concentrations of oil. However, the growth of the bacterium was inhibited at crude oil concentrations beyond 6% (w/v). The pH of the culture media also dropped significantly ($P < 0.05$) during the 15-day test period, while the non-asphaltic fractions of the oil were significantly reduced (by about 89%) during the same period. The bacterium harbours a plasmid of about 10 kb that lacks restriction sites for the endonucleases *Asp*718, *Bam*HI and *Pst*I.

Keywords Heavy oil · Maya · *Burkholderia cepacia* · Biodegradation

Introduction

Burkholderia cepacia was known as *Pseudomonas cepacia* for a long time [27]. During that time, it was included in the rRNA homology group II of the *Pseudomonas* genus [20]. However, it was later identified as the type species of the new genus *Burkholderia*, on the basis of cellular lipid and fatty-acid composition, 16S rRNA sequences, DNA–DNA homology and phenotypic properties [27]. The bacterium is known to be a common rhizosphere isolate, phytopathogen and opportunistic pathogen of humans [25]. Hence its occurrence and distribution are not solely confined to environmental sources [2].

Microbial degradation of crude oil has been the subject of extensive studies and more recent studies have elucidated the kinetics of degradation of petroleum hydrocarbons and their constituents [18]. Recognized as one of the most metabolically versatile [24] of bacterial species, *B. cepacia* can degrade a wide range of compounds as carbon and energy sources [14], including a variety of aromatic compounds [10], pesticides and herbicides [3]. It has been suggested that the remarkable degradative potential of this bacterium may be due to the presence of large numbers of insertion elements in the genome of the organism [25] and extrachromosomal elements such as plasmids [9, 13, 16].

Nigeria has had her fair share of crude oil pollution since oil exploration started there in the mid-1950s. Nevertheless, there is no evidence in the literature to show that *B. cepacia* strains indigenous to the Nigerian environment have been characterized or assessed for their potential use in the bioremediation of crude oil-polluted systems in the country. We report here the heavy crude oil biodegradation potential of an axenic culture of a *B. cepacia* RQ1 strain isolated from a crude oil flow station saver pit in Nigeria.

Materials and methods

Bacterial isolation and identification

B. cepacia RQ1 used in this study was isolated in our previous study [19] from a crude oil flow station saver pit effluent in the Nigerian Niger delta region and was preserved in glycerol at –70 °C. The isolate was identified from its cultural, biochemical and enzymatic characteristics [6, 23] after resuscitation by growing it in Bushnell–Hass medium (BHM) supplemented with 0.1% (v/v) sterile Bonny light crude oil (BLCO). The composition of BHM

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includes (per litre): $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaCl_2$ (0.02 g), KH_2PO_4 (1.0 g), K_2HPO_4 (1.0 g), NH_4NO_3 (1.0 g) and $FeCl_3$ (two drops of 60% solution), pH 7.0–7.2. The biochemical and enzymatic assays were performed with the aid of a Becton Dickinson enteric/non-fermenter ID kit, BBL crystal identification system, as revised (September, 1997; US Patent 5,182,082).

Screening for biodegradation potential

The bacterial isolate was initially selected by enrichment, using 0.1% (v/v) sterile Nigerian BLCO in BHM. Turbidity and/or emulsification (after 15 days) were used as indices of biodegradation potential. The purity of the culture was confirmed by streaking on solidified 2% (w/v) glucose in minimal medium (GMM) followed by incubation at 35 °C for 48 h. A total of 10 ml of the pure enrichment culture was then centrifuged to pellet out the cells, washed three times with sterile physiological saline solution and the suspension was adjusted to an optical density at 546 nm (OD_{546}) of 0.1. From this, 1 ml was inoculated into 0.1% (w/v) sterile heavy crude oil (Maya) in 50 ml of BHM in 125-ml Erlenmeyer flasks. Incubation was at room temperature (25 ± 1 °C) on a horizontal shaker fixed at 150 rpm for 15 days. Turbidity and/or emulsification were used as indices of degradation activity. The crude oil (Maya) was kindly provided by the Instituto Mexicano del Petroleo, Mexico.

Kinetic studies

A 1-ml sample of the bacterial suspension prepared as described above was inoculated into separate 125-ml Erlenmeyer flasks containing 50 ml of BHM, to yield an initial bacteria count of approximately 10^5 cfu ml^{-1}. The final concentrations of crude oil (weathered by autoclaving) in the flasks were 0.1, 0.2, 0.4, 0.6 and 0.8% (w/v). Uninoculated controls were also set up to determine the extent of oil loss by evaporation. Incubation conditions were as explained earlier. Samples were taken at 0, 3, 9 and 15 days by sacrificing at least two flasks at a time, except for the final time when at least three flasks were sampled per isolate per oil concentration. The content of each flask was emptied into separate sterile separation funnels and allowed to stand for 1–2 h, during which almost all the oil fraction separated at the top. The aqueous fraction was then drained and analysed for pH and biomass and was returned into the separation funnels. The pH was determined with a glass electrode pH meter (model PHM210; MeterLab). Biomass was determined by estimating the viable count, as previously described [22]. Total residual crude oil was estimated by a gravimetric method, as described elsewhere [11, 17] with modifications, using dichloromethane as extraction solvent. Approximately 95% oil recuperation was achieved by this method. Degradation rate constants were estimated as described elsewhere [12].

Effect of high concentration of oil on biomass

The effects of high concentrations of heavy crude oil on bacterial growth were assessed. The method used was a modification of a previous method [26] and the concentrations of crude oil used were: 1.5, 4.0, 6.0, 8.0, 15.0 and 20% (w/v) respectively in BHM medium and prepared in triplicate. Incubation conditions were as described previously. The reaction was stopped after 5 days and the viable count was determined by a standard spread-plate technique [22], using sterile nutrient agar (Difco) as growth medium. Differences in viable count between the controls containing no crude oil and the experimental samples were estimated.

GC analysis

GC of residual crude oil was conducted in a Hewlett Packard HP5890 gas chromatograph, equipped with a flame ionization detector and a capillary column fused with methyl silicone (25.5 m length, 0.32 mm internal diameter). The operating temperature of the detector was 300 °C and that of the injector was 270 °C. The column temperature was set at 90 °C for the first 6 min, then increased to 150 °C at a rate of 4 °C min^{-1} and increased again to 280 °C at the rate of 10 °C min^{-1} until the final time (40 min). Helium was the carrier gas and the program used resolved all but asphaltic fractions.

Extraction of plasmid DNA

A colony of the bacterial isolate was subcultured from the GMM plate into sterile Luria broth (LB) and incubated overnight at 37 °C. Preliminary studies revealed that several rounds of subculturing on GMM did not affect isolation of plasmid DNA from this isolate. Plasmid DNA was then extracted from the LB culture by the alkaline lysis method [4], using *Escherichia coli* phage lambda DNA as the molecular weight marker. Plasmid DNA was separated by gel electrophoresis on 1% (w/v) horizontal agarose gels [15] and photographed under short-wave ultraviolet light after staining with ethidium bromide solution. Three restriction endonucleases that recognize six base pairs were used for digestion analyses of the plasmid DNA, including *Asp*718, *Bam*HI and *Pst*I (Boehringer).

Results

Bacterial identification

The bacterial identification assay revealed the bacterium was an indole-negative, oxidase-positive, Gram-negative rod and, through its enzymatic/biochemical characteristics, was identified (at 90% consistency index) to be *B. cepacia* RQ1. Stocks of the isolates are being maintained on agar slants and in glycerol at the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, and the Environmental Biotechnology laboratory, Centro de Investigación en Biotecnología, UAEM, Cuernavaca, Mexico.

Kinetic profiles

The preliminary screening for the ability of *B. cepacia* RQ1 suggested appreciable biodegradation potential, which was the basis for further investigation. Kinetic studies revealed a varying response of the bacterium to different concentrations of crude oil. Biomass increased with time in all cases, reaching maximal densities that ranged from 1.0×10^8 cfu ml^{-1} to 2.4×10^8 cfu ml^{-1} in 15 days for the various concentrations of crude oil (Table 1). Cell densities were appreciably higher for oil concentrations of 0.6% and 0.8% than for the lower concentrations. The specific growth rate was lowest (0.0028 h^{-1}) at 0.1% crude oil concentration (Table 2) and it peaked at 0.6% (0.036 h^{-1}). No correlation was observed between growth and degradation rates. The pH declined slightly but significantly in all cases, from an initial 6.83 to values of 6.47–6.76 in 15 days. Also, the amount of crude oil consumed by the bacterium during

Table 1 Maximum cell densities attained during 15 days of incubation of *Burkholderia cepacia* RQ1 in various concentrations of the heavy crude oil (Maya)

Crude oil concentration (%, w/v)	Starting cell density ($\times 10^7$ cfu ml^{-1})	Maximum cell density ($\times 10^7$ cfu ml^{-1})
0.1	0.02 ± 0.005	10 ± 0.8
0.2	0.01 ± 0.008	12 ± 0.8
0.4	0.02 ± 0.005	11 ± 0.8
0.6	0.01 ± 0.008	23 ± 2.0
0.8	0.01 ± 0.008	24 ± 0.6

Table 2 Effect of crude oil concentration on the specific growth rates of RQ1, degradation rates of the crude oil and pH decline of the cultures (after 15 days of degradation reaction). Specific growth rates and degradation rates were calculated from mean values of cell densities and residual total crude oil respectively

Crude oil concentration (%, w/v)	Specific growth rates (h^{-1})	Degradation rates (day^{-1})	Decline in pH
0.1	0.028	0.009	6.76 ± 0.04
0.2	0.032	0.005	6.74 ± 0.06
0.4	0.030	0.004	6.67 ± 0.02
0.6	0.036	0.008	6.64 ± 0.02
0.8	0.033	0.005	6.47 ± 0.05

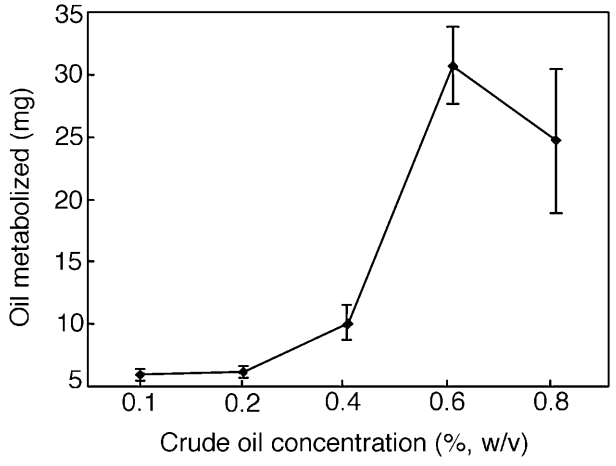

Fig. 1 Effect of crude oil concentration on amount of oil metabolized by *Burkholderia cepacia* RQ1 after 15 days of degradation reaction

the 15-day incubation increased significantly ($P < 0.05$) with increasing concentrations of crude oil, ranging from 5.9 mg to 30.7 mg (Fig. 1). GC analysis revealed that the bacterium significantly degraded the non-asphaltic fractions of the crude oil (Fig. 2).

Effect of high concentration of crude oil and plasmid extraction

To determine the effect of high concentrations of the heavy crude oil on *B. cepacia* RQ1, we observed biomass increases up to 6.0% crude oil concentration, beyond which a decline set in (Fig. 3). Also, the bacterium harbours a plasmid of about 10 kb in size, that lacked sites for the restriction endonucleases *Asp*718, *Bam*HI and *Pst*I (Fig. 4).

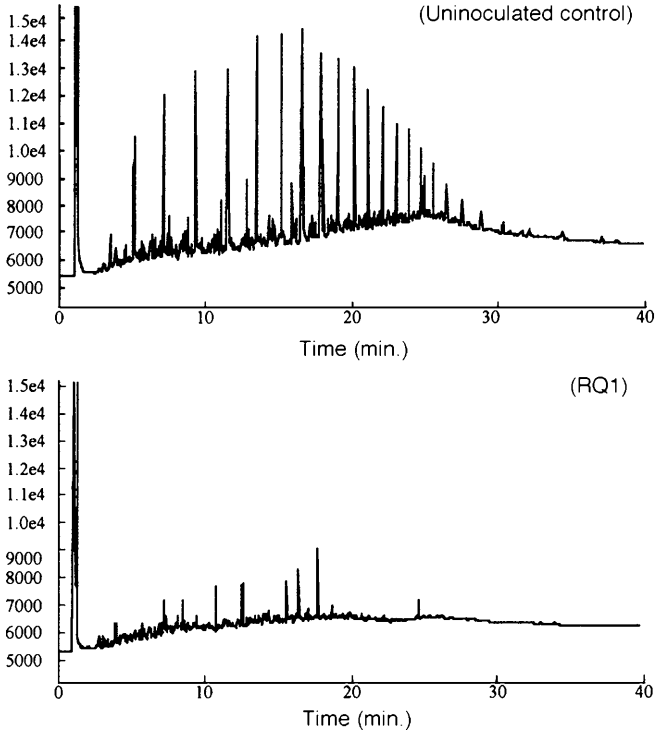

Fig. 2 Chromatogram of residual crude oil after 15 days of reaction for the inoculated control (*above*) and RQ1 (*below*)

Discussion

This study assessed the abilities and limitations of *B. cepacia* RQ1 in the biodegradation of heavy crude oil (Maya) in shake flasks. No attempt was made at ascertaining the degradation kinetics of the various fractions, nor were we interested in elucidating the profile of the intermediate metabolites.

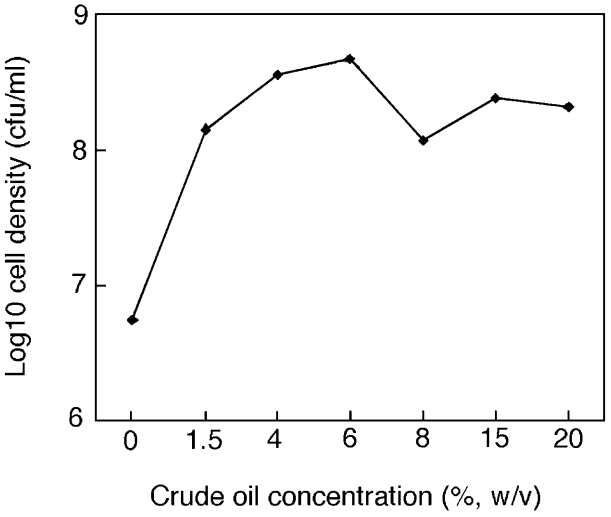

Fig. 3 Effect of high concentrations of crude oil (Maya) on cell density of *B. cepacia* RQ1 during a 5-day reaction period

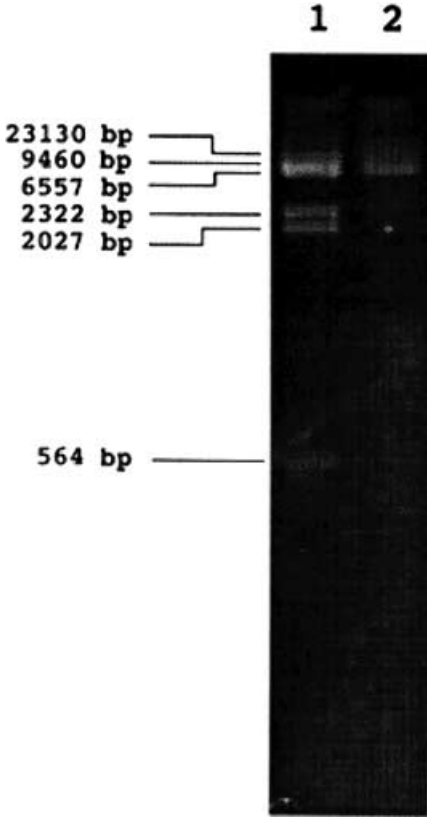

Fig. 4 Gel electrophoresis of *B. cepacia* RQ1 plasmid DNA. *Lane 1* contains the molecular weight marker (lambda-phage DNA), while *lane 2* contains the RQ1 plasmid DNA

The results of this study suggest a high crude oil degradation potential of the *B. cepacia* strain used. Degradation rates obtained for this bacterium (ranging from 0.004 day^{-1} to 0.009 day^{-1}) are far higher than some previously reported values, even for mixed cultures [7]. The maximum biomass profile correlated positively ($r = 0.92$) with increasing concentrations of the heavy oil, within the limits of the concentrations used (0.1–0.8%), thus corroborating earlier reports on the versatility of this bacterium [2, 21]. The irregular profile of cell growth resulted in a lack of correlation between growth and degradation rates. This phenomenon can be associated with the observation that various interactions of the components within the oil matrix may affect microbial activities in different ways [1, 5, 8]. Fig. 3 shows that *B. cepacia* RQ1 can tolerate high concentrations of heavy crude oil, up to 6% (w/v), beyond which growth became static, up to 20% (w/v) oil concentration. The GC resolved non-asphaltic fractions indicated an appreciable degradation of these fractions, by about 89%. Harayama and co-workers [11] reported a lower efficiency of degradation of Maya by some strains of *Acinetobacter*.

There have been reports describing plasmids from *B. cepacia* [9, 13]. The implication of plasmids in the degradation of petroleum hydrocarbons has also been a subject of investigation. The RQ1 plasmid with unusual resistance to digestion by the restriction enzymes *Asp*718, *Bam*HI and *Rst*I , as observed in this study, could contribute to the bacterium's versatility. Lennon and Decicco [13] also reported similar plasmids that were resistant to digestion by *Eco*RI, *Pst*I and *Pvu*II. Resistance to digestion by restriction endonucleases has been suggested to be an important survival mechanism of bacteria against certain stress situations (G. Nadathur, pers. comm.). The findings in this study shows that *B. cepacia* RQ1 could be useful in bioremediation of petroleum hydrocarbon contaminated systems.

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