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Changes in respiration activities and bacterial communities in a bioaugmented oil-polluted soil in response to the addition of acyl homoserine lactones

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ABSTRACT

The effect of bacterial quorum sensing (QS) signals on the respiration activity of an oil-polluted soil with and without the addition of an alkane-degrading bacterial consortium was investigated. The addition of C_4–C_{12}-HSL N-acyl homoserine lactones (AHLs) to the contaminated soil with the bacterial consortium resulted in a significant increase in CO_2 evolution rates. Experiments with 1, 10 and 100 μM of C_{12}-HSL exhibited an increase in respiration activities with decreasing concentrations. This increase was concomitant with the degradation of hydrocarbons. 93% of the alkanes were degraded in the bioaugmented soil after C_{12}-HSL addition. Illumina MiSeq sequencing of soil communities at the end of the treatments showed that bacteria belonging to the classes *Deinococci* and *Alphaproteobacteria* dominated (i.e. 62–87% of total sequences) all bioaugmented and non-bioaugmented soils. Addition of C_{12}-HSL did not cause major changes in the detected genera of bacterial communities. Out of the five bacterial strains of the consortium, only *Alcanivorax* sp. and *Parvibaculum* sp. survived after 42 days. An additional experiment demonstrated that C_{12}-HSL increased biofilm formation in the consortium bacteria. In conclusion, the addition of AHLs has a simulative effect on bacterial respiration activities and degradation of hydrocarbons, hence can be useful in bioaugmentation treatments of oil-polluted soils.

1. Introduction

Bioaugmentation, which relies on the addition of oil-degrading microorganisms to speed up the degradation of hydrocarbons, is an environmental friendly approach that has been widely used in the bioremediation of contaminated ecosystems (Boopathy, 2000; Tyagi et al., 2011; Zouboulis and Moussas, 2011). The main pitfall of this method is the inability of the introduced microorganisms to cope with the harsh environmental conditions in the field and to compete with the indigenous bacteria. Thus, the selection of appropriate bacterial strains, with the ability to survive and degrade hydrocarbons under *in situ* conditions, is a key prerequisite for the success of bioaugmentation. Indeed, it was postulated that biofilm formation could potentially enhance biodegradation of pollutants, including hydrocarbons (Singh et al., 2006). Although several studies have tested bioaugmentation and reported on its success or failure (Ruberto et al., 2003; Abed et al., 2014; Colla et al., 2014; Fan et al., 2014; Hassanshahian et al., 2014; Qiao et al., 2014; Suja et al., 2014; Andreolli et al., 2015), very few studies have monitored the fate of introduced bacteria at the end of the treatment (Gertler et al., 2009a). Such studies are required in order to identify the most competent bacteria that are successful in the degradation of contaminants under field conditions.

In the environment, about 80–90% of soil microorganisms are sorbed to solid surfaces while enclosed in a matrix of hydrated extracellular polymeric substances (EPS) on soil particles (Maier et al., 2009; Flemming and Wingender, 2010). The formation of biofilms protects microorganisms from different environmental factors, such as desiccation, solar and UV radiation, heat, and allows cells to survive in hostile environments (Hall-Stoodley et al., 2004). The formation of biofilms is facilitated through the production, release and perception of QS signals, which allow cell-to-cell

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communication between bacteria (Waters and Bassler, 2005; Steinberg et al., 2011). Once QS signals accumulate in the environment to critical threshold concentrations they bind to the target receptors, which result in the activation of target genes and, thus, control "bacterial social behavior" (Crespi, 2001; Waters and Bassler, 2005; Hartmann and Schikora, 2012; Harder et al., 2014). The most common QS signals of Gram negative bacteria are AHLs (Decho et al., 2009). Different species of bacteria produce AHLs with the different side-chain length ranging from four to eighteen carbons (Harder et al., 2014). Due to the well-known role of AHLs in the formation of biofilms, we hypothesize that the addition of these compounds during bioaugmentation treatments will enhance the survival of introduced (exogenous) as well as native (endogenous) oil-degrading bacteria and consequently increase rates of oil degradation.

The main objectives of this study were to investigate the effect of five AHLs (i.e. N-butyryl-L-homoserine lactone (C_4-HSL), N-hexanoyl-L-homoserine lactone (C_6-HSL), N-octanoyl-L-homoserine lactone (C_8-HSL) and N-decanoyl-L-homoserine lactone (C_{10}-HSL), dodecanoyl-L-homoserine lacton (C_{12}-HSL) on respiration activities of a bacterial consortium and on oil-polluted soils with and without the consortium. Based on the results from this experiment and since C_{12}-HSL is more stable in the environment than shorter side chain AHLs, the effect of different concentrations of C_{12}-HSL on respiration activities of a bacterial consortium and oil-polluted soils and on biofilm formation of bacterial strains was further investigated. The fate of the bacterial consortium and changes in the bacterial community composition of the soils at the end of the experiment were followed using illumina MiSeq sequencing. The degradation of hydrocarbons was compared in the presence and absence of the bacteria consortium with and without C_{12}-HSL.

2. Materials and methods

2.1. Soil collection

Oil contaminated soils were collected from an area close to an oil production facility in Mina Al-Fahal, a coastal area in Muscat, Oman (23° 37′ 49.92″N; 58° 31′ 23.84″E). Soils from this area have been previously investigated for their physical and chemical properties as well as for their response to different bioremediation treatments including the addition of inorganic nutrients and exogenous microorganisms (Abed et al., 2014). In brief, the concentration of total petroleum hydrocarbons (TPH) in this soil was 22 ± 0.7 mg g^{-1} soil and the pH was 7.46. The soils contained 62% silt, 30% sand and 8% clay and thus had a silt loam texture. The concentration of phosphate and nitrate reached 0.5 and 21 mg l^{-1}, respectively. Soils in sterile plastic boxes were brought back to the laboratory, where all experiments were performed.

2.2. Bioaugmentation experiments

2.2.1. Preparation of consortium

Prior to the experiment, a consortium composed of five alkane-degrading bacterial strains (i.e. *Alcanivorax* sp. MH3, *Parvibaculum* MH21, *Azospirillum* sp. AH2, *Marinobacter* sp. AH3 and *Marinobacter* sp. AH6) was prepared by cultivation in an artificial seawater medium supplemented with acetate at 30 °C. Detailed biochemical, physiological and phylogenetic characterization of the strains has been published elsewhere (Abed et al., 2014). The strains were selected based on their ability to degrade different alkanes and to represent different bacterial genera. The optical density of each strain was monitored at 600 nm until it reached 0.2. The cells were then collected at the exponential phase by centrifugation, washed three times with sterile seawater to remove traces of acetate and re-suspended in carbon- and nutrient-free (i.e. without KH_2PO_4 and NH_4Cl) minimal salt medium. Equal volumes of each bacterial suspension were mixed together in artificial seawater medium and 1 ml of this mixture was added to 19 ml medium for each treatment (see below 2.2.2.). The artificial seawater medium contained $MgCl_2 \cdot 6H_2O$ (5.6 gl^{-1}), $MgSO_4 \cdot 7H_2O$ (6.8 gl^{-1}), $CaCl_2 \cdot 2H_2O$ (1.47 gl^{-1}), KCl (0.66 gl^{-1}), KBr (0.09 gl^{-1}) and was supplemented with trace elements mixture (Widdel and Rabus, 2001) and vitamins (Heijthuijsen and Hansen, 1986).

2.2.2. Experimental setup

The experiment was performed in glass bottles (volume 165 ml) containing 10 g of oil polluted soil mixed with 20 ml of carbon- and nutrient-free minimal salt medium. These soils were incubated, each in triplicate: 1) with five different acyl homoserine lactones (i.e. C_4-HSL, C_6-HSL, C_8-HSL, C_{10}-HSL and C_{12}-HSL), each added separately to the oil-polluted soils in the presence of the bacterial consortium at the concentration of 1 μM and 2) with different concentrations of C_{12}-HSL (i.e. 1 μM, 10 μM and 100 μM) added to the soil with and without consortium as well as to the consortium alone. A fresh dose of crude Arabian light oil 0.5% (v/v) was added to all soils, in order to follow the degradation of alkanes. The same incubations in the absence of AHLs and in the absence of extra oil served as controls. All used AHLs were ordered as pure compounds from Sigma–Aldrich (USA). The bottles were sealed with thick, black rubber stoppers to ensure no gas leakage and incubated for 42 days at 30 °C. CO_2 evolution was followed at different time intervals using gas chromatography (GC) (see below 2.2.3.).

2.2.3. Chemical analyses

CO_2 evolution was measured in the headspace of the gas tight serum bottles by withdrawing 250 μl of gas and injecting it manually into the GC (GC, Agilent model 6890N). The GC used helium gas as a carrier gas at a flow rate of 4 ml min^{-1}. The GC was equipped with a thermal conductivity detector and a 30 m × 250 μm capillary column (HP-PLOT Q). The thermal conductivity detector was maintained at 200 °C while the injector temperature was 210 °C. The oven temperature was programmed from 50 °C to 80 °C with a final hold time 3 min at a rate of 20 °C min^{-1}. Oil mineralization rates were calculated by comparing the experimentally quantified CO_2 with the theoretical amount of CO_2 that would be produced by complete oxidation of the present oil (Abed et al., 2014, 2015a, b). CO_2 evolution data over time were statistically analyzed using two-way ANOVA using the SPSS software (10th edition, Chicago, USA). The assumption of normality of data was verified with the Shapiro–Wilk's test (Shapiro and Wilk, 1965). Turkey post-hoc test was used in order to compare individual means. In all cases, the threshold for significance was 5%.

Oil degradation was assessed by extracting samples 3 times with 10 ml dichloromethane (DCM) and the pooled extract was filtered with non-absorbent cotton to remove solid particles. Anhydrous sodium sulfate was used to remove traces of water from the filtrate. The filtrate was then evaporated using a rotary evaporator. The dry extract was re-dissolved into DCM and passed through silica gel prior to injection. Gas chromatography-mass spectrometry (GC–MS) was equipped with a 30 m × 250 μm capillary column (Rtx®-5MS) and individual hydrocarbons were quantified by comparing them to an external standard (C_7–C_{30}). Helium gas was used as a carrier at a flow rate of 1 ml min^{-1} and the injector and detector were maintained at 290 °C. The oven temperature was programmed from 80 °C (initial hold time 2 min) to 290 °C (final hold time 30 min) at a rate of 10 °C min^{-1}.

2.2.4. Illumina MiSeq 16S rRNA amplicon sequencing

At the end of the experiments, the fate of the bacterial