EFFECT OF ADAPTATION ON HEXANE BIODEGRADATION BY A *RHODOCOCCUS* STRAIN CAPABLE OF HYDROCARBON UTILIZATION

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Abstract

Although organic solvents such as hexane have harmful effects on human health and natural environments, they are commonly used chemicals in several industrial fields. Bioremediation is an environmentally friendly and cost effective waste management technique for rehabilitation of polluted environments. A hydrocarbon-utilizing bacterial strain identified as a *Rhodococcus sp.* PAE1 was previously isolated from mazut. This new isolate was adapted to hexane in order to assess the effects of adaptation. The adapted strain exhibited a wide range of hexane tolerance and faster hexane biodegradation rate compared to the non-adapted strains. Adaptation to hexane combined with changes in cell membrane fatty acids resulted in increased cell surface hydrophobicity and elongation of cells that can promote the bioavailability of a water insoluble substrate.

Introduction

Despite their harmful effects, organic solvents are widely used industrial chemicals posing a serious risk to human health and natural environments [1, 2]. Although hexane is a widespread solvent applied in the production of cleaning supplies [3] and glues [4], in footwear manufacturing [5], pharmaceutical [6] and food industry [7], it can do damage to the aquatic or terrestrial wildlife, if it is emitted into the environment [8]. Since hexane is highly volatile, it can easily evaporate into the atmosphere and contribute to the formation of photochemical smog by increasing the tropospheric ozone concentration [9, 10]. Taking these facts into consideration, adequate damage prevention and remediation technologies are needed. Out of the possible strategies, bioremediation involving the use of microorganisms or plants can be one of the most suitable techniques because it is an environmentally friendly and cost-effective method [11]. As a short chain alkane, hexane is extremely toxic for cells and even hydrocarbon degrader microorganisms can be sensitive for relatively low concentration of hexane. Therefore, we aimed to examine the tolerance of this strain to hexane, the effects of adaptation on hexane biodegradation and the phenotypical changes including cell length and cell surface hydrophobicity (adhesion% or ADH%) that might be involved in increasing hexane bioavailability.

Experimental

Rhodococcus sp. PAE1 isolated from mazut was adapted to hexane as sole carbon and energy source for 6 months. Cells were incubated with various concentrations of hexane, while their size and cell surface hydrophobicity were measured. Respiration activity and biodegradation efficacy were determined by following CO_2 release and remaining hexane content of each culture.

Results and discussion

Solvent tolerance was assessed by measuring respiration in liquid minimal medium (MM) supplemented with hexane in varying concentrations as sole carbon and energy source in closed vials. *Rhodococcus sp.* PAE1 was able to tolerate and even use hexane in a range of 0.1 - 1.5 V/V% (Fig. 1.).

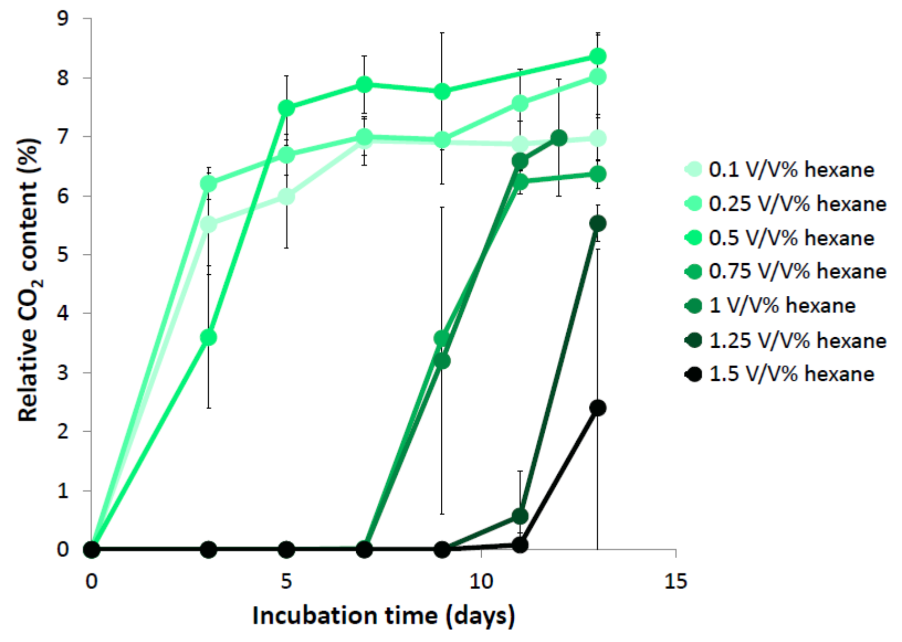

Figure 1. CO_2 production by *Rhodococcus sp.* PAE1 growing on hexane in varying amount as sole carbon and energy source

Respiration data were also used to examine the degradation of hexane by the adapted strains and the non-adapted ones (previously cultured in nutrient-rich LB medium or pregrown on hexane only once). Adapted culture was able to utilize hexane faster than the others but an increased rate of biodegradation was observed even in the cell line precultured on hexane only once compared to the non-adapted one indicating a quick adaption process to this solvent (Fig. 2.).

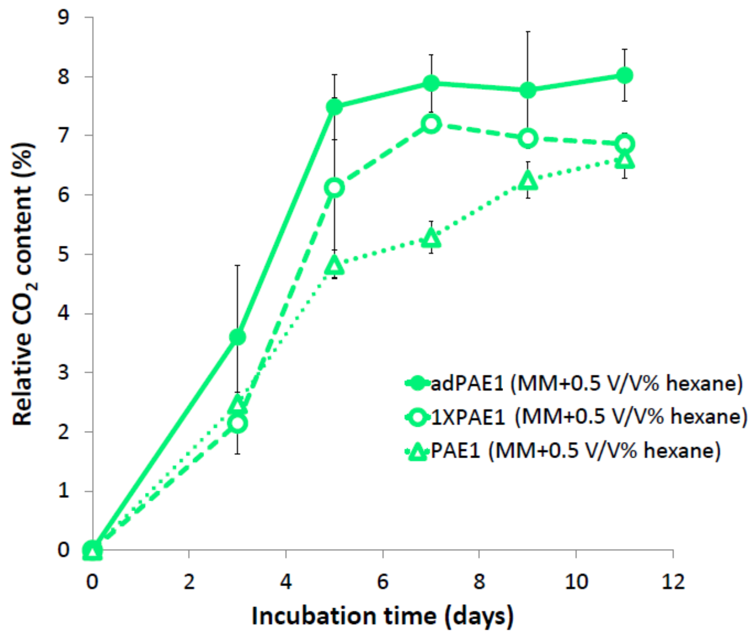

Figure 2. Effect of adaptation on CO_2 production by *Rhodococcus sp.* PAE1 growing on 0.5 V/V% hexane as sole carbon and energy source

Remaining hexane content from these samples were determined after solvent-solvent extraction and GC-MS analysis. Our results revealed that available oxygen concentration in the headspace of closed vials limited hexane biodegradation and it was only sufficient for oxydizing about 25% of the initial hexane concentration (Fig. 3.).

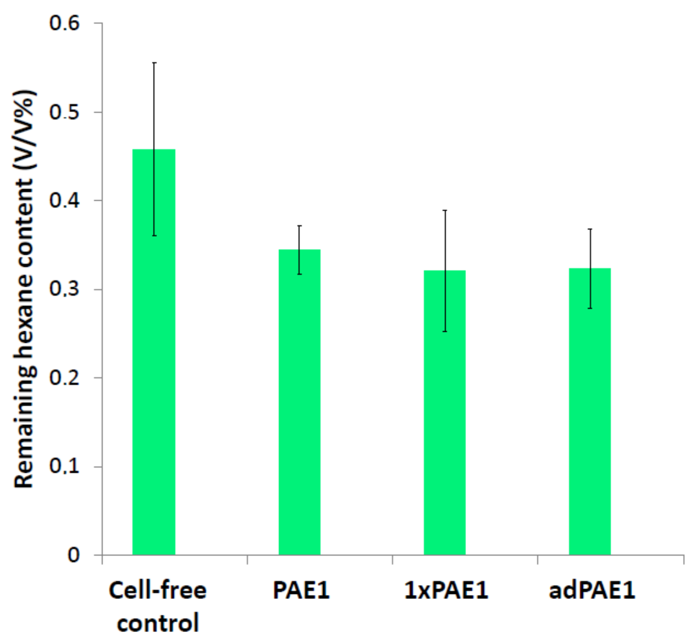

Figure 3. Remaining hexane content after 11 days of incubation

Cell surface hydrophobicity is a widespread phenomenon in *Rhodococci* due to chains of mycolic acid located in their cell walls. Using the method of Qiao et al. [13] and analysing bright field microscopy pictures, cell surface hydrophobicity (ADH%) and cell length of *Rhodococcus sp*. PAE1 from the previous samples were determined (Table 1.).

Table 1. Remaining hexane content after 11 days of incubation

	non-adapted PAE1 (LB)	**non-adapted 1xPAE1**	**long term adapted PAE1**
ADH%	41.67 ± 19.97	55.39 ± 12.02	61.89 ± 8.76
Cell length (µm)	2.02 ± 0.14	2.21 ± 0.16	2.38 ± 0.10

Based on our data, we suppose that properties of culturing medium and the bioavailability of carbon source essentially influence the surface parameters and length of bacterial cells grown in that specific medium. Increased hydrophobicity of the cell surface and elongation can be beneficial for utilization of a not water-soluble substrate. In accordance with our previous results (Fig. 2.), adapted *Rhodococcus* sp. PAE1 strain showed to be different from non-adapted ones (precultured in LB or on hexane only once) and exhibited higher cell surface hydrophobicity and cell length while the non-adapted strain, which was precultured on hexane only one time, seemed to have intermediate properties between the adapted *Rhodococcus sp*. PAE1 and the non-adapted PAE1 strain precultured in nutrient-rich LB medium.

Conclusion

Hexane utilization by adapted *Rhodococcus sp.* PAE1 was increased compared to the non-adapted cells presumably caused by adaptation-induced changes, such as elongation and increased cell surface hydrophobicity facilitating hexane bioavailability. Cells were tended to be aggregated in hexane containing media. We suppose that adaptation might result in increased level of unsaturated bonds in membrane fatty acids playing a crucial role in making cells more resistant to organic solvents. These observations could be useful in future application of bacteria capable of hexane biodegradation.

Acknowledgements

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