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Hydrophobicity effect on oil degradation by two marine bacterial strains *Alcanivorax borkumensis* and *Thalassolituus oleivorans*

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Abstract

Variations on hydrophobicity were monitored in two marine obligate hydrocarbonoclastic bacteria: *Alcanivorax borkumensis* SK2T and *Thalassolituus oleivoras* MIL-1T. These strains were inoculated, separately in ONR7a mineral medium with different concentration of sodium acetate. During 10 days measurements of cellular abundance and cellular hydrophobicity (capacity to adhere at polystyrene) were carried out. Data obtained revealed that the natures of carbon source and growth phase are important factors in the regulation of adhesion of bacteria to the surfaces. Moreover *Alcanivorax* showed a major capability to colonize polystyrene respect on *Thalassolituus* in the experimented conditions. The understanding of capacity to adhesion of these bacteria for utilization of hydrophobic compounds is fundamental for their potential use in the mitigation of oil spills phenomenon.

Key words: Biodegradation, Bacteria, Crude oil, Marine Environment

Highlights

- The natures of carbon source and growth phase are important factors in the regulation of adhesion of bacteria to the surfaces.
- *Alcanivorax* have better hydrophobicity than *Thalassolituus*.
- Bacteria with better hydrophobicity have prevalent in oil biodegradation.

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Introduction

An important fraction of marine microbial community is characterized from "Obligate HydrocarbonoClastic Bacteria" (OHCB), (1). The group of OHCB is composed from indigenous bacteria of marine ecosystems able to utilize hydrocarbons as exclusive sources of carbon and energy (2).

Presence of these bacteria in marine environment is directly dependent on presence of hydrocarbons and/or crude oil that determine, in specific condition, a change of microbial community, with the selection of bacteria able to tolerate these substrates (2).

Several study showed that members of the genera *Alcanivorax* and *Thalassolituus* are of the most important microbes involved in removing the aliphatic fraction of crude oil from marine environment (3 and 4).

Alcanivorax sp. is a cosmopolitan marine bacterium (5) that exhibit a unique oligotrophic physiology with a specialized metabolism adapted to the degradation of aliphatic hydrocarbons (6) even if not effectively involved in degradation of aromatic compounds. However, members of the group of *Alcanivorax* are indeed able to use some organic substrates like n-paraffins, alkylic groups pertaining to n-alkylbenzene and n-alkylcycloalkane as only carbon source (6).

Thalassolituus sp. is a chemoorganoheterotrophic bacterium. Microbes related to this genus inhabite both marine and terrestrial environments subsurface caves and ground waters and are the dominant alkane degraders (7).

The importance of these bacteria resides mainly in biotechnological applications, especially in reference to bioremediation strategies. One of the barriers for the bioremediation processes in natural environments is the oil and water is immiscible liquids, and microbes grow only in an aqueous environment. Consequently, the interface between the oil and the water is of extreme importance for the success of degradation process. Microbial attachment to hydrophobic interfaces has been an area of interest for over 80 years, but there are relatively few studies about the controlled use of microbial adhesion as a means to promote reactions of oil. Attachment to hydrophobic surfaces is a common and natural strategy used by microorganisms to overcome limitations of solubility of hydrocarbons (7).

Several studies have demonstrated that environmental factor (temperature, pH) growth conditions (nutrients concentration) and features of bacteria (shape, dimension, and metabolic status) are the main factors influencing the process of adhesion which can determine variation in hydrophobicity of the bacterial cell surface (CSH) (8).

The aims of this work is to study the influence of carbon source concentration on the variation of cellular hydrophobicity (capacity to adhere at polystyrene) of two obligate hydrocarbonoclastic bacteria, *Alcanivorax borkumensis* SK_2^T and *Thalassolituus oleivorans* MIL-1^T.

The understanding of capacity to adhesion of these bacteria for utilization of hydrophobic compounds is fundamental for their potential use in the mitigation of oil spills phenomenon.

Materials and methods

Bacterial strains

A strain of *Alcanivorax borkumensis* SK2^T (2) and a strain of *Thalassolituus oleivorans* MIL-1^T (2) were used in all the experiments. *A. borkumensis* SK2^T and *T. oleivorans* MIL-1^T were isolated from sea water/sediment samples collected, respectively, around the Isle of Borkum (North Sea) and the harbour of Milazzo (Italy) using enrichment culture techniques with n-alkanes as sole carbon source.

Inoculums preparation and culture medium

Started cultures were prepared, separately, in ONR7a mineral salts medium (8) added with 0.1% of sodium acetate (CH_3COONa, Sigma-Aldrich, Italia). Mid-exponential-phase grown cells were harvested by centrifugation at 11.250×*g* for 10 min, washed twice with sterile medium, and inoculated at a final concentration of 0.1 of optical density (OD_{600nm}) in sterile ONR7a mineral medium.

Set-up and planning for experiments

The cells of strain SK2^T and MIL-1^T were inoculated in independent flasks with 500 ml of ONR7a medium and incubated at 20 °C with shaking (Certomat IS B. Braun Biothec International, 80 rpm) for 10 days. Four different series of experimentations have been performed. In the first three experiments bacterial cultures were carried out in ONR7a salts medium added with 0.6, 0.3 and 0.1% of sodium acetate, respectively. In negative control, bacteria were cultivated in the same medium without any source of carbon and energy.

Sampling strategy and assays

Every 24 hours during the whole experimental period sub-aliquots of cultures were collected to measure bacterial abundance and variation in hydrophobicity (measured as variation to adhesion at polystyrene). All the experiments were triplicate. Biomass variations were evaluated by optical density at 600 nm (Biophotomer Eppendorf), (OD).

Adhesion to polystyrene

For the assay of the adhesion to polystyrene an aliquot of bacterial culture has been directly withdrawn from the flask daily. The cells were washed twice in a phosphate-buffer saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) a final concentration of 0.3 (OD_{600nm}). The cells suspended in 10 ml were put in a polystyrene Petri plate (60 mm) and incubated for 30 min at 30 °C without shaking in order to study the initial adhesion process. The capacity of adhesion to polystyrene was analyzed, as well as the percentage of hydrophobicity (HP%). Cell surface hydrophobicity was determined by partitioning the cell suspensions into the polystyrene plate and aqueous phases after incubation time.

The percentage of hydrophobicity (HP%) of experimental bacterial suspension was calculated using the following equation, adapted to our purpose:

$$HP\% = (O.D_{init} - O.D_{exp}) \times 100 / O.D_{init}$$

Where $O.D_{init}$ stands for the optical density of the suspension before incubation in the polystyrene plate, and $O.D_{exp}$ was the optical density of the bacterial suspension after incubation (7).

Statistical analysis

Statistically significant differences between the experiments were detected by Analysis of variance (ANOVA).

Results

Effect of different concentration of carbon source on adhesion, as percentage of hydrophobicity (HP%) of *Alcanivorax borkumensis* SK_2^T

The quantitative estimation of the adhesion to polystyrene measured as percentage of hydrophobicity (HP%) to *A. borkumensis* $SK2^T$ on the various concentration of sodium acetate as only source of carbon and energy is shown in Fig. 1.

During cultivation in ONR7a salts medium amended with 0.6% of sodium acetate, *Alcanivorax* showed, in the first six days, an exponential growth, from a value of optical density of 0.1 to 0.85 (OD_{600nm}). After the first week, the cellular abundance remained constant up to the end of experiment (10 days). During the cultivation, the value of HP% showed an increment during the first 48 hrs, from value of HP% = 50 (time zero) to HP% = 68 and remaining constant up to the 5th day of experimentation. After the 6th day of cultivation a decrement of adhesion to polystyrene is observed, with values that are passing from a value of HP% = 62 (T_5) to HP% = 38 (T_8) and remaining constant for all periods of study (Fig. 1a).

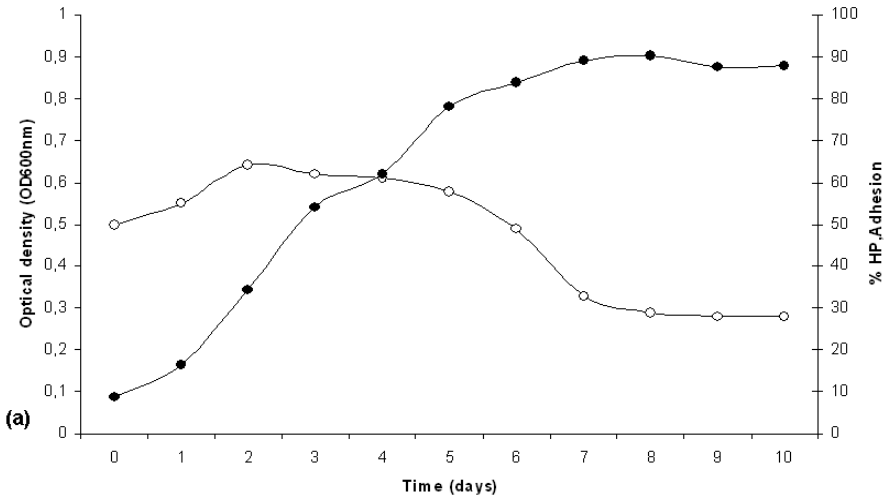

Fig. 1a- Dynamic of percentage of hydrophobicity (open squares) and bacterial abundance (filled squares) of *Alcanivorax borkumensis* SK_2^T during growth in 0.6% ONR7a medium

Also during cultivation in ONR7a medium with 0.3% of sodium acetate *A. borkumensis* $SK2^T$ showed, for the first five days, an exponential growth with values that are passing from a 0.1 to 0.7 (OD_{600nm}). Successively, at stationary phase the bacteria abundance remains constant with values around 0.75 (OD_{600nm}) up to the end of experimental period. After an initial increment (HP% = 50 to 62 in the first 2 days) the curve of percentage of hydrophobicity showed a progressive decrement with values starting from HP% = 62 (T_2) until reach, at the 6th day of analysis, HP% value of 26. This value remains constant for all periods of study (Fig.1b).

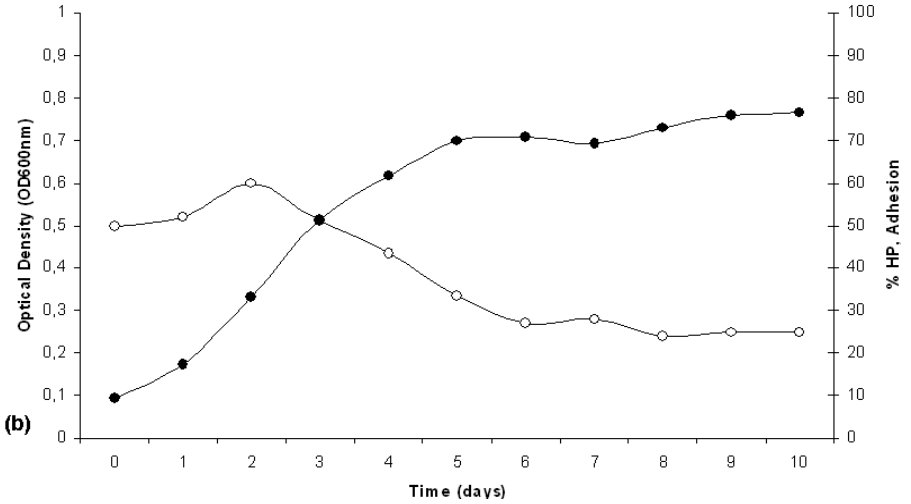

Fig. 1b- Dynamic of percentage of hydrophobicity (open squares) and bacterial abundance (filled squares) of *Alcanivorax borkumensis* SK_2^T during growth in 0.3 % ONR7a medium

In ONR7a medium with 0.1% of sodium acetate and without this carbon source (Fig. 1c and 1d), *Alcanivorax* growth seems to maintain values of optical density of 0.25 (0.1% of sodium acetate) and 0.1 (no carbon source). In both experiments HP% values start from 40 and decrease in very few hours remaining constant at 14 for ten days.

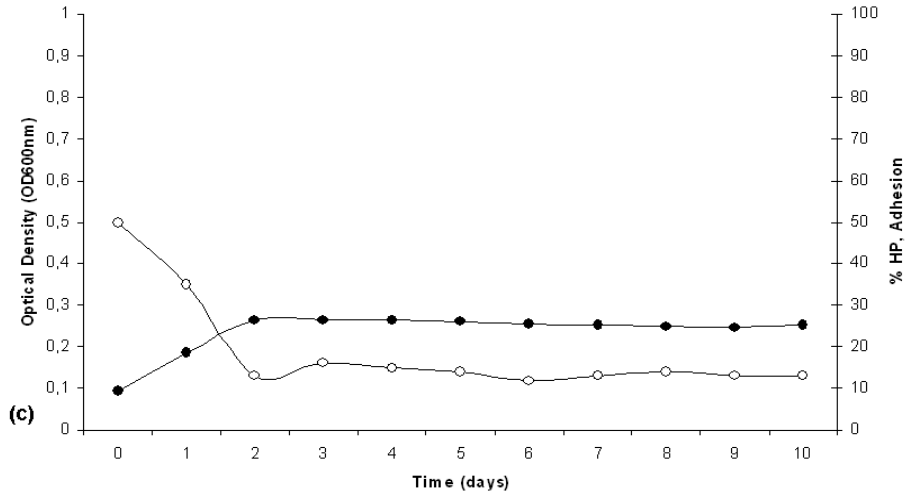

Fig. 1c- Dynamic of percentage of hydrophobicity (open squares) and bacterial abundance (filled squares) of *Alcanivorax borkumensis* SK_2^T during growth in 0.1 % ONR7a medium

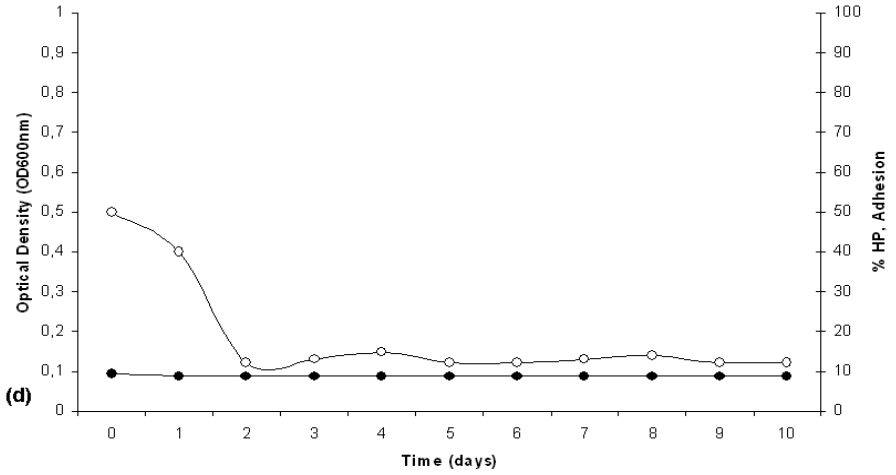

Fig. 1d- Dynamic of percentage of hydrophobicity (open squares) and bacterial abundance (filled squares) of *Alcanivorax borkumensis* SK_2^T during growth in ONR7a medium without carbon and energy source

Effect of different concentration of carbon source on adhesion, as percentage of hydrophobicity (HP%) of *Thalassolituus oleivorans* MIL-1^T

During growth with 0.6% of sodium acetate *T. oleivorans* MIL-1^T showed a long lag phase with values of optical density around 0.1 (OD_{600nm}) up to the 5th day of experimentation. After the 5th day, a rapid exponential growth was observed with an increment of bacterial abundance that was passing from a value of 0.1 (T_5) to 0.6 (OD_{600nm}) at the end of experiment. During the cultivation the value of HP% showed a decrement during the first 3 days, from value of HP% = 50 (time zero) to HP% = 12 (T_3) and remains constant up to the 5th day of experimentation. After the sixth day of cultivation an increment of adhesion to polystyrene is observed, with values that are passing from a value of HP% = 12 (T_5) to HP% = 60 (T_7) and remaining constant for all period of study (Fig. 2a).

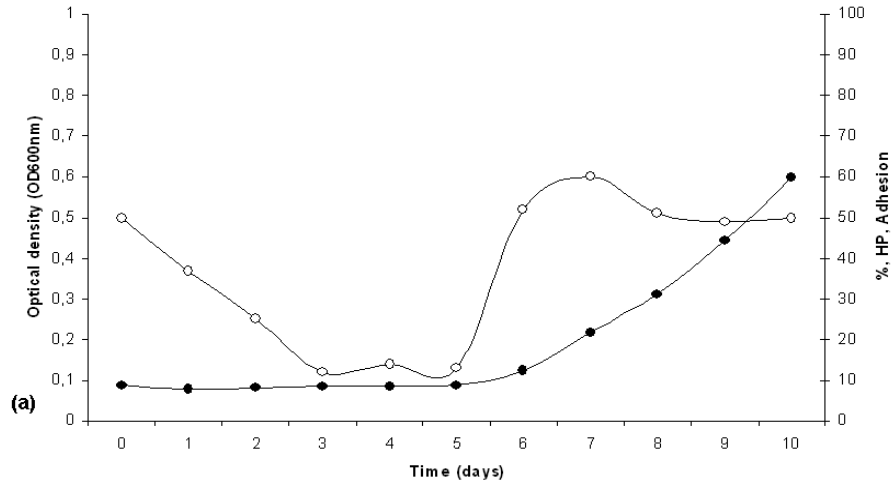

Fig. 2a- Effect of different concentration of carbon source on adhesion, as percentage of hydrophobicity (HP%) of *Thalassolituus oleivorans* MIL-1^T. Optical abundance (filled squares) and percentage of hydrophobicity (open squared) during growth in 0.6 % ONR7a medium

Also during cultivation on ONR7a added with the 0.3% of sodium acetate *Thalassolituus oleivorans* MIL-1^T showed a long lag phase with values around 0.1 (OD_{600nm}) just the 3rd day of experimentation (Fig. 2b); after the 3rd day of experimentation an exponential growth is showed with values of cellular abundance that passing from 0.1 (T_3) to 0.7 (T_{10}). Curve of percentage of hydrophobicity present a trend with value of HP% = 50 (time zero), 12 (T_2 and T_3), 60 (T_5) and 30 (T_{10}).

Similarly to *Alcanivorax*, the addition of 0.1% of sodium acetate induces a stationary phase with means values of optical density (OD_{600nm}), respectively, of 0.17 and 0.1. of *Thalassolituus* MIL-1, while the values of HP% decrease during the first 48h, passing from 50 at time zero to HP% = 14 and remaining constant for all periods of study (Fig. 2c and 2d).

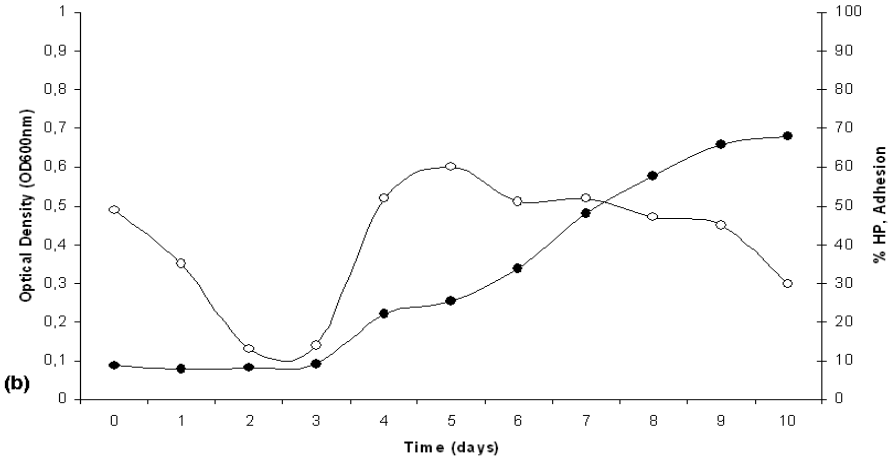

Fig. 2b- Effect of different concentration of carbon source on adhesion, as percentage of hydrophobicity (HP%) of *Thalassolituus oleivorans* MIL-1^T. Optical abundance (filled squares) and percentage of hydrophobicity (open squared) during growth in 0.3 % ONR7a medium

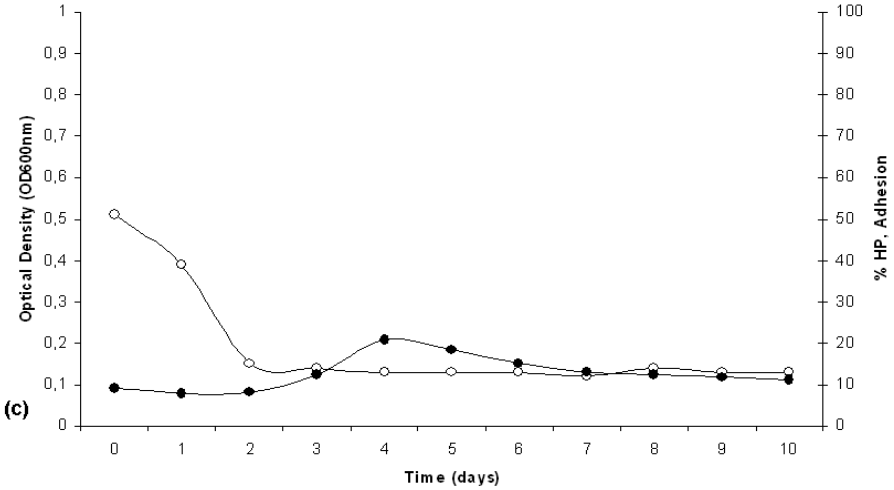

Fig. 2c- Effect of different concentration of carbon source on adhesion, as percentage of hydrophobicity (HP%) of *Thalassolituus oleivorans* MIL-1^T. Optical abundance (filled squares) and percentage of hydrophobicity (open squared) during growth in 0.1% ONR7a medium

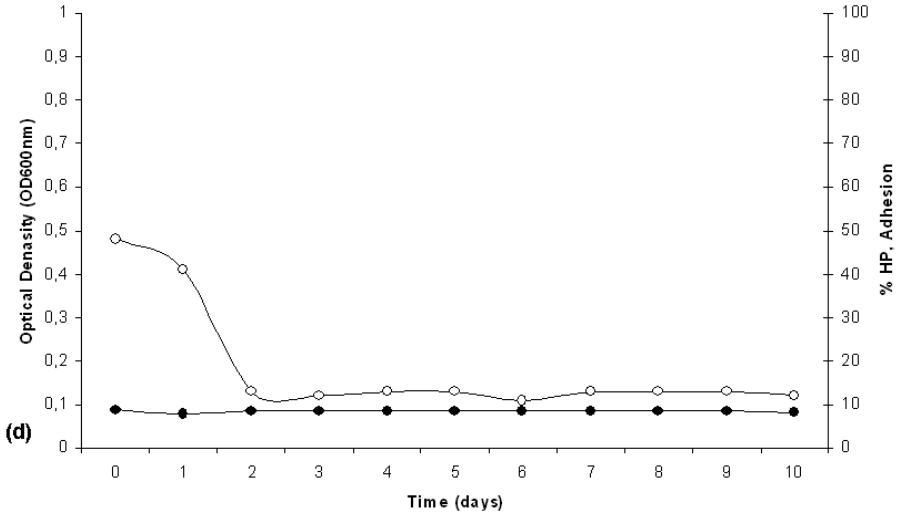

Fig. 2 d- Effect of different concentration of carbon source on adhesion, as percentage of hydrophobicity (% HP) of *Thalassolituus oleivorans* MIL-1^T. Optical abundance (filled squares) and percentage of hydrophobicity (open squared) during growth in ONR7a medium without carbon and energy source

Discussion and conclusion

In this preliminary study, for the first time, we have analyzed the effect of different concentrations of substrate (sodium acetate) and the phase of growth on hydrophobicity in two marine obligate hydrocarbonoclastic bacteria, *Alcanivorax borkumensis* SK2^T and *Thalassolituus oleivorans* MIL-1^T. Among the hydrocarbon-utilizing bacteria, *Alcanivorax borkumensis* and *Thalassolituus oleivorans*, are two of the most frequently isolated from hydrocarbon-contaminated environments.

Marine OHCB occupy a special tropic niche among marine heterotrophic bacteria participating in the global carbon cycle, as they mediate degradation of chemically stable saturated and aromatic hydrocarbon species that are not substrates for most bacteria (9). However, the eco-physiology of these bacteria has not been studied extensively for different motivations as discovery relatively recent, it is difficult to isolate these organisms in pure culture, etc.

The analysis of the variation of bacterial adhesion during optimal growth conditions is a fundamental data in order to understand the adhesion and the potential use, of hydrocarbonoclastic bacteria (HCB), for the mitigation of oil spills. Bacteria and other microorganisms have a natural tendency to adhere to surfaces as a survival mechanism and bacterial colonization of solid surfaces have been described as a basic and natural bacterial stratagem in a wide variety of environment (9 and 10).

As showed, during cultivation in ONR7a medium with 0.6 and 0.3% of sodium acetate, *Alcanivorax* present, in the first days, an exponential growth during which is possible to observe an increment of cellular hydrophobicity that decrease when bacteria enter the stationary phase. Moreover it is very important to remind that this growth-phase transition is controlled by a genetic regulatory network

integrating various environmental signals (11). This genetic network determines a reduction of proteic synthesis (12) characterized from specific stress proteins synthesis (13) to disadvantage of the synthesis of proteins and structures naturally implied in the processes of bacterial adhesion.

The reduction of cellular hydrophobicity is more visible using the 0.1% of sodium acetate and without sources of carbon and energy. The concentration of 0.1% of sodium acetate is not sufficient to support bacterial growth and after complete utilisation of this substrate the population enter in a stationary phase presumably associate at a carbon starvation. In this case, the drastic decrement of cellular hydrophobicity, can be justified both from reduction of protein synthesis and from characteristic of carbon starvation. Both were through the dimensional reduction that the bacterial cells endure in that condition (14).

The morphologic variation can determine a change in the contact angle that the bacteria have with the surface of adhesion with consequent variation in the cellular adhesion (15, 16 and 17). *Thalassolituus* shows a different behaviour when is cultivated with higher concentrations of substrate. In fact, HP% shows two phases of adhesion process: the first one, in the first hours of growth and the latter during the exponential phase of the growth. This capability is maintained at high values during the 10 days (18, 19 and 20).

In conclusion, although *Alcanivorax* reaches absolute values of HP% a little higher than *Thalassolituus*, the efficiency of hydrophobicity is maintained for a longer period (21, 22 and 23). The preliminary data obtained showed a correlation between the physiological status (identified from curve of bacterial abundance) and cellular hydrophobicity (24). The maximum and minimum values of cellular hydrophobicity were obtained during cultivation of *Alcanivorax borkumensis* SK2 and *Thalassolituus oleivorans* MIL-1 depend to the bacterial status. The importance of these preliminary data resides mainly in biotechnological applications, especially in reference to application of these bacteria in bioremediation strategies.

References

(1) Yakimov, M. M.; Golyshin, P. N.; Timmis, K. N. Obligate oil-degrading marine bacteria. *Curr. Opin. Biothecnol.* **2007**, *18(3), pp* 257-266.

(2) Yakimov, M. M.; Golyshin, P. N.; Lang, S; Moor E. R. B.; Abraham, H.; Luensdorf, W. R.; Timmis, K. N. *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int. J. Syst. Bacteriol.***1998,** *48*, pp 339-348.

(3) Kasai, Y.; Kishira, H.; Sasaki, T.; Syutsubo, K.; Watanabe, K.; Harayama, S. Predominant growth of *Alcanivorax* strains in oil-contaminated and nutrient-supplemented seawater. *Environ. Microbiol*. **2002**, *4*, pp 141-147.

(4) Harayama, S.; Kishira, H.; Kasai, Y.; Shutsubo, K. Petroleum biodegradation in marine environments. *J. Mol. Microbiol. Biotechnol.* **1999**, *1*: pp 63-70.

(5) Head, I. M.; Jones, D. M.; Roling, W. F. Marine microorganisms make a meal of oil. *Nat. Rev. Microbiol.* **2006**, *4*: pp 173-182.

(6) Sabirova, J. S.; Ferrer, M; Regenhardt, D.; Timmis, K. N; Golyshin, P. N. Proteomic Insights into Metabolic Adaptations in *Alcanivorax borkumensis* Induced by Alkane Utilization. *J. Bacteriol*. **2006,** *188(11)*, pp 3763-3773.

(7) Mckew, B. A.; Coulon, F.; Yakimov, M. M.; Denaro, R. Genovese, M, Smith, C. J. Efficacy of intervention strategies for bioremediation of crude oil in marine systems and effects on indigenous hydrocarbonoclastic bacteria. *Environ. Microbiol.* **2007**, *9***,** pp 1562-1571.

(8) Kjellfeberg, S.; Hermansson, M. Starvation-Induced Effects on Bacterial Surface Characteristics. *Appl. Environ. Microbiol*. *1984*, *48(3)*, pp 497-503.

(9) Hunt, S. M.; Werner, E. M.; Hamilton, M. Hypothesis for the role of nutrient starvation in biofilm detachment. *Appl. Environ. Microbiol.* **2004,** *70(12)*, pp 7418-7425.

(10) Jana, T. K.; Sarivastava, A. K.; Csery, K.; Arora, D. K. Influence of growth and environmental conditions on cell surface hydrophobicity of *Pseudomonas fluorescens* in non-specific adhesion. *Can. J. Microbiol.* **2000**, *46(1)*, pp 28-37.

(11) Ropers, D.; Hidde, S.; Geiselmann, J. Qualitative simulation of the carbon starvation response in *Escherichia coli*. *Biosystems*. **2006,** *84(2)*, pp 124-152.

(12) Givskov, M.; Eberl, L.; Moller, S.; Molin, S. Response to nutrient starvation in *Pseudomonas. putida* KT2442: Analysis of general cross-protection, cell shape and macromolecular content. *J. Bacteriol*. **1994**, *176*, pp 7-14.

(13) Baikun, L.; Logan, B. F. Bacterial adhesion to glass and metal-oxide surfaces. Colloids and Surfaces B. *Biointerfaces.* **2004**, *36*, pp 81-90.

(14) Bariandet, R.; Meylheuc, T.; Maher, C. Scott A. *Listeria monocytogenes* Cell Surface Charge, Hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions. *Appl. Environ. Microbiol*. **1999**, *65*, pp 5328-5333.

(15) Rahman, K. S. M.; Banat, I. M. Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresou. Technol.* **2004**, *85*, pp 257-261.

(16) Hasanshahian, M.; Emtiazi, G. Investigation of alkane biodegradation using the microtiter plate method and correlation between biofilm formation, biosurfactant production and crude oil biodegradation. *Int. Biodeterior.Biodegrad*. **2008**, *62*, pp170-178.

(17) Hassanshahian, M.; Emtiazi, G.; Kermanshahi, R.; Cappello, S. Comparison of oil degrading microbial communities in sediments from the Persian Gulf and Caspian Sea. *Soil Sediment Contaminat*. **2010,** *19 (3)*, pp 277-291.

(18) Hassanshahian, M.; Tebyanian, H.; Cappello, S. Isolation and characterization of two crude-oil degrading yeast strains, Yarrowia lipolytica PG-20 and PG-32 from Persian Gulf. *Mar. Pollut. Bull.* **2012,** *64*, pp 1386-1391.

(19) Hassanshahian, M.; Emtiazi, G.; Cappello, S. Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. *Mar. Pollut. Bull.* **2012,** *64*, pp 7-12.

(20) Hassanshahian, M.; Ahmadinejad, M.; Tebyanian, H.; Kariminik, A. Isolation and characterization of alkane degrading bacteria from petroleum reservoir waste water in Iran (Kerman and Tehran provenances). *Mar. Pollut. Bull.* **2013,** *73*, pp 300-305.

(21) Hassanshahian, M.; Emtiazi, G.; Caruso, G.; Cappello, S. Bioremediation (bioaugmentation/biostimulation) trials of oil polluted seawater: a mesocosm simulation study. *Mar. Environ. Res.* **2014**, *95*, pp 28-38.

(22) Hassanshahian, M.; Zeynalipour, M.S.; Hosseinzadeh Musa, F. Isolation and characterization of crude oil degrading bacteria from the Persian Gulf (Khorramshahr provenance). *Mar. Pollut. Bull*. **2014**, *82*, pp 39-44.

(23) Hassanshahian, M.; Yakimov, M.M.; Denaro, R.; Genovese, M.; Cappello, S. Using Real-Time PCR to assess changes in the crude oil degrading microbial community in contaminated seawater mesocosms. *Inte. Biodeterior. Biodegrad.* **2014,** *93*, pp 241-248.

(24) Tebyanian, H.; Hassanshahian, M.; Kariminik, A. 2013. Hexadecane-degradation by *Teskumurella* and *Stenotrophomonas* strains iso lated from hydrocarbon contaminated soils. *Jundishapur. J. Microbiol*. **2014,** *26 (7)*, pp e9182.

بررسی اثر هیدروفوبیسیته بر روی تجزیه زیستی نفت خام توسط دو باکتری دریایی *Alcanivorax borkumensis* و *Thalassolituus oleivorans*

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چکیده

تفاوت‌ها در هیدروفوبیسیتی به وسیله دو باکتری دریایی هیدروکربنوکلاستیکوس تجزیه کننده اجباری نفت بررسی شد. این سویه‌های باکتریایی شامل: *Alcanivorax borkumensis* SK2T و *Thalassolituus oleivoras* MIL-1T بودند. هر دوی این سویه‌ها به شکل جداگانه در محیط کشت ONR با غلظت‌های متفاوتی از استات سدیم انکوبه شدند. در طی ۱۰ روز، فراوانی باکتری‌ها و همچنین، هیدروفوبیسیته سلولی (قابلیت اتصال به پلی استرن) مطالعه و سنجش شد. نتایج به دست آمده از این پژوهش نشان داد که نوع منبع کربن و مرحله رشد، فاکتورهای مهمی در تنظیم چسبندگی باکتری‌ها به سطوح هستند. علاوه بر این، باکتری *Alcanivorax* قابلیت بالاتری برای کلونیزه شدن به پلی استرن در مقایسه با *Thalassolituus* در شرایط آزمایشگاه نشان داد. فهم قابلیت اتصال این باکتری‌ها برای استفاده از ترکیبات هیدروکربنی پایه‌ای برای کاربرد آن‌ها در عملیات‌های میدانی نشت نفت و آلودگی نفتی است.

واژه‌های کلیدی: تجزیه زیستی، باکتری، نفت خام، محیط دریایی

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* نویسنده مسؤول مکاتبات

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