ROLE OF SIDEROPHORES PRODUCED BY *ALCANIVORAX BORKUMENSIS* DURING BIOREMEDIATION PROCESSES

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Abstract
Hydrocarbonoclastic bacteria exhibit genetic and phisiological features to use hydrocarbons as sole source of carbon and to compete for the uptake of nutrients. As an example the iron uptake system includes the production of siderophores, small high-affinity iron chelating compounds. We identified the siderophore produced by *Alcanivorax borkumensis*, which behaves as a shuttle transferring iron from marine environment within the cell. Such siderophore belongs to the hydroxamates group and it is one of the few isoxazolidine found in nature which mimics the antibiotic action of the β-lactams. The alternance of dominant members of microbial communities in oil polluted marine samples and in pure cultures, revealed that the siderophores produced by Alcanivorax sp act as inhibitor factors during the competition for substrates.

Keywords: Pollution, Petroleum, Biotechnologies, Messina Strait

Bioremediation in marine environment is a promising cheap biotechnology which proposes the recovery of hydrocarbons-polluted sites exploiting the self-cleaning capacity of the sea. A special group of hydrocarbons-degrading marine bacteria (HCB), namely *Alcanivorax* sp., *Cycloclasticus* sp., *Thalassolituus* sp., *Oleispira* sp., *Oleiphilus* sp., exhibit both genetic and phisiological features to use hydrocarbons as sole source of carbon and energy and to compete for the uptake of nutrients. Among these, iron is one of the essential nutrients for the success of in situ biodegradation by microrganisms. In fact, for in situ biocatalysis, the iron is one of the limiting factors because of its very low availability in marine environment. To bypass this matter, bacteria have developed the capability to produce siderophores, small high-affinity iron chelating compounds which behave as a shuttle taking iron from marine environment and transferring it within the cell. Bacterial siderophores have been associated to many biotechnological application: drug delivery, bacterial communication, antibiotic function, iron chelator in iron metabolism diseases, inducers of bacterial growth, quorum sensing, but up to day there are very few study on the role of siderophore within natural occurring biodegradation processes.

The aim of the present study was to study the role of siderophores produced by *Alcanivorax borkumensis* during the processes of bioremediation.

We have performed a first screening on the production of siderophores both in solid and liquid CAS-assay using HCB as samples. *Alcanivorax*, *Thalassolituus* and *Oleispira* were positive to the test, on the contrary, *Cycloclasticus* didn't show siderophore activity, data confirmed by the the lack of genes involved in siderophore production in *Cycloclasticus* genome.

After the first screening we decided to proceed with the isolation and characterization of the siderophore produced by the most known hydrocarbonoclastic strain, *Alcanivorax borkumensis*. *Alcanivorax* produces a siderophore which belongs to the hydroxamates group and is one of the few isoxazolidine found in nature, very similar to pesudomonine produced by *Pseudomonas fluorescens*, it seems to mimic the antibiotic action of the β-lactams and show a high affinity for iron. Experiments carried out in pure culture of *Alcanivorax borkumensis* showed that when the growth was in the medium containing optimal concentration of iron we were not able to detect siderophores production. On the contrary, in iron limiting conditions we have registered the highest siderophore activity during the esponential phase of growth. Moreover, at the same phase we didn't registered significant differences in the rate of tetradecane degradation between optimal and iron limiting conditions, suggesting that siderophores play a pivotal role in natural environment during biodegradation processes.

One of the interesting feature of microbial siderophores is that some of the microbial community members (helpers) are usually the most active in the production of siderophores, such siderophore–iron complexes can be used by the no-siderophore-producer (cheaters). In the present study we observed whether such crossfeeding occurs among the HCB strains during bioremediation processes. We monitored the growth of *Cycloclasticus* (no siderophores-producers) in a medium amended with siderophore produced by *Alcanivorax*. We observed that the addition of siderophore didn't facilitate *Cycloclasticus* growth in iron-limiting conditions. On the contrary, siderophores seemed to have an inhibitor effect on the growth of *Cycloclasticus* during the first 10 days.

At the moment we are not able to explain this behaviour. On the basis of our previous studies, we know that during treatment of bioremediation of oil-polluted marine sediments, an alternance of *Alcanivorax* and *Cycloclasticus* as active dominant members occurred. Further studies are suggested to verify if *Alcanivorax* is able to modulate, by the use of siderophores, interspecific interaction during the processes of bioremediation for the competition of substrates.

References
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