

COMPARATIVE GENOMICS OF AROMATIC COMPOUNDS DEGRADATION PATHWAYS IN THE MARINE ROSEOBACTER CLADE

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Abstract

Members of the Roseobacter clade predominate in marine environments chronically polluted with hydrocarbons. Genomic analyses shows that these bacteria might possess up to six pathways for aromatic hydrocarbon degradation. Our hypothesis is that bacteria of the Roseobacter clade have genetic structures and strategies for degrading and tolerating hydrocarbons that are different from those of the well-known marine hydrocarbon degraders, and that these differences might underlay their predominance in chronically-polluted marine environments. We have analysed gene organization for aromatic hydrocarbon degradation pathways in all available genomes from the Roseobacter clade. We present here the results for the beta-ketoadipate pathway, the one most frequently found in Roseobacter genomes

Keywords: Bacteria, Petroleum, Balearic Islands

The Roseobacter clade is a key component of marine bacterioplankton (Buchan *et al.*, 2005), and plays key roles in biogeochemical cycles. They are generalist and metabolically versatile, with a suite of mechanisms for energy production and carbon acquisition that include aerobic anoxygenic phototrophy, litotrophy (carbon monoxide and sulfide oxidation), anaerobic carbon dioxide fixation and chemoorganotrophy. Marine roseobacters are readily cultured in the laboratory and consequently there are more than sixty genera described. As a result, and due to their ecological interest, they have been the subject of many genome sequencing projects (there are about forty-three genome sequences, complete or as draft available so far). Comparative genomic analyses of members of the clade have been published (Moran *et al.*, 2007; Newton *et al.*, 2010).

Degradation of aromatic compounds by marine roseobacters was reported in year 2000, with the isolation of strains able to degrade aromatic compounds resulting from lignin degradation, and the confirmation of the presence of the protocatechuate branch of the beta-ketoadipate pathway in these bacteria (Buchan *et al.*, 2000, 2004). More recently, genome analysis has revealed that marine roseobacters possess up to six catabolic routes for monoaromatic compound degradation, namely pathways for: benzoate, gentisate, homoprotocatechuate, phenylacetate, homogentisate, and protocatechuate (Moran *et al.*, 2007; Newton *et al.*, 2010). This has led to proposing a potential role for these bacteria in aromatic hydrocarbon degradation in marine environments. In addition, there are accumulating evidences showing a positive response of roseobacters after treatment with hydrocarbons in the laboratory (McKew *et al.*, 2007; Lanfranconi *et al.*, 2010), and their predominance of chronically-polluted environments (Nogales *et al.*, 2007).

We are analyzing the catabolic potential for hydrocarbon degradation by isolates from the roseobacter clade obtained in Mallorca Island. As part of our research we are interested in analyzing genetic structures of aromatic compound degradation pathways in marine roseobacters, and therefore we are performing comparative genome analysis. We followed two different approaches. Firstly, we searched the available genomes for determining the presence of all genes involved in the six routes for aromatic hydrocarbon degradation proposed in marine roseobacters. We started with the protocatechuate branch of the beta-ketoadipate pathway, which is the pathway most commonly found in these genomes (Newton *et al.*, 2010). The key genes *pcaH* and *pcaG*, encoding the two subunits of protocatechuate 3,4 dioxygenase, were found in 70% of the genomes analyzed. In all but one of these genomes, the *pobA* gene for degradation of p-hydroxybenzoate, the precursor of protocatechuate, was also found. In most cases, *pobA* gene was located next to *pca* gene cluster and apparently under a common regulatory control, although this should be confirmed experimentally. Many differences in *pca* gene content and order were observed, but a basic structure of genes *pca(DC)HG* was observed. The presence of the LysR-like regulatory protein (PcaQ), and a hypothetical chaperone protein (Chp) was predicted in sixty percent of the roseobacter genomes analysed, confirming the proposed relevance of this regulatory mechanism of the protocatechuate pathway, as well as the importance of this putative uncharacterized chaperone in marine roseobacters (Buchan *et al.*, 2004). Our second approach has been the generation of novel genomic

data from a collection of seventeen roseobacters obtained from chronically-polluted environments in Mallorca Islands. As first approach, and with the aim of maximizing the exploration of the diversity of catabolic genes in these genomes, we grouped the isolates in four phylogenetically-coherent groups before pyrosequencing (Roche 454 technology). We analyzed the presence of genes for each one of the six predicted catabolic pathways in marine roseobacters. The genomic groups differed in the number of catabolic pathways that we could predict: one in group 3, three in group 4, 5 in group 2 and 6 in group 1. Genes for the protocatechuate pathway were present in all genomic groups. Except for group 2, these results agreed with the results of cultivation experiments, showing that the majority of the isolates were able to grow on protocatechuate as sole carbon and energy source.

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