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Comparative genomics of the protocatechuate branch of the β-ketoadipate pathway in the Roseobacter lineage

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ABSTRACT

The protocatechuate branch of the β-ketoadipate pathway is the most common pathway for degradation of monoaromatic compounds in the Roseobacter lineage. We analyzed 43 Roseobacter genomes in order to determine if they possessed all genetic elements for this pathway and if there were common patterns in gene organization. The eight genes of the pathway (*pcaG, -H, -B, -C, -D, -I, -J, and -F*), possible regulators, and genes encoding for proteins with related function (i.e. catabolism of 4-hydroxybenzoate, catechol, and meta-cleavage of protocatechuate) were predicted by sequence homology analysis. Most of the Roseobacters studied had putatively a complete protocatechuate branch of the β-ketoadipate pathway while 11 of them would probably have an incomplete pathway. Thirty-one Roseobacters would be potentially able of transforming 4-hydroxybenzoate to protocatechuate, and 13 of them might transform catechol via ortho-cleavage, the starting reaction of the catechol branch of the β-ketoadipate pathway. We observed variability in gene organization, with no clear relationship between gene order and Roseobacter taxonomy. Genes were usually organized in several gene clusters. One of the clusters (*pcaRIJF*) was not reported previously in Roseobacters. The presence of the putative regulator *pcaR* in these bacteria was also a novel finding. The conserved ORF (*chp*), encoding for a protein of family DUF849 whose functional role has been proven recently, was detected in 34 genomes. Sequence homology confirmed that proteins encoded by *chp* corresponded to putative BKACE G4 proteins, which are able to transform β-ketoadipate. Therefore, most Roseobacters seemed to possess two different enzymes for transforming β-ketoadipate. We also report two possible regulation mechanisms of gene *pobA* (encoding for the enzyme transforming 4-hydroxybenzoate to protocatechuate): via PcaQ, the regulator commonly found with *pca* genes, and via an independent regulator (PobR). The results of this study evidence the relevance of 4-hydroxybenzoate, protocatechuate and β-ketoadipate degradation pathways in Roseobacters and provide a more complex view of possible regulation mechanisms.

1. Introduction

The Roseobacter lineage is a diverse, phylogenetically-coherent, group within the *Alphaproteobacteria*, formed mainly by marine bacteria (Buchan et al., 2005; Newton et al., 2010). Roseobacters are key components of marine bacterioplankton in surface waters, particularly in coastal areas where they can represent more than 20% of total prokaryotes (Buchan et al., 2005). They have a generalist lifestyle and are metabolically versatile, with a suite of mechanisms for energy production and carbon acquisition that include aerobic anoxygenic phototrophy, litotrophy (carbon monoxide and sulfide oxidation), and chemoorganotrophy (Moran et al., 2007; Newton et al., 2010; Christie-Oleza et al., 2012).

One of the characteristics of marine Roseobacters is the ability of using aromatic compounds for growth. The first evidence came from the isolation of a lignin-transforming bacterium, *Sagittula stellata* E-37, from an enrichment culture prepared with seawater of the coast of Georgia, USA, and effluent of a pulp mill (Gonzalez et al., 1997). Lignin is a complex, chemically stable, aromatic heteropolymer present in cell walls of vascular plants. It is one of the most abundant natural polymers on the planet and constitutes an important component of the pool of dissolved organic matter in marine environments, such as coastal salt marshes and estuaries (Moran and Hodson, 1994; Sleighter and Hatcher, 2008). Degradation of lignin by fungi and bacteria proceeds through different pathways and leads to the release of several aromatic acids, such as benzoic acid derivatives and other phenolic compounds like gallate, ferulate, 4-hydroxybenzoate, vanillate, coumarate, protocatechuate, etc. (Bugg et al., 2011). Six Roseobacter isolates, including strain E-37, were tested for the ability to degrade lignin-related aromatic compounds, leading to the first report of the presence of a pathway for aromatic compound degradation in marine Roseobacters, the protocatechuate branch of the β-ketoadipate pathway (Buchan et al., 2000). More recently, genome analysis has revealed that marine Roseobacters possess up to six catabolic routes for

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monoaromatic compound degradation, namely pathways for benzoate, gentisate, homoprotocatechuate, phenylacetate, homogentisate, and finally protocatechuate, the one most frequently found (Moran et al., 2007; Buchan and González, 2010; Newton et al., 2010). Based on these and other evidences coming from experiments with hydrocarbons (see Buchan and González, 2010 for a review), Roseobacters have been proposed to participate in aromatic hydrocarbon degradation in marine environments.

The β-ketoadipate pathway is present in many bacterial groups, including gram-positive and gram-negative bacteria (Harwood and Parales, 1996). This is a convergent central pathway for the degradation of aromatic compounds that is particularly important in microorganisms from soil (El Azhari et al., 2008). Two different branches of the pathway (named the protocatechuate or catechol branches, respectively) convert either protocatechuate or catechol to β-ketoadipate (Fig. 1). Both protocatechuate and catechol are central metabolites derived from the

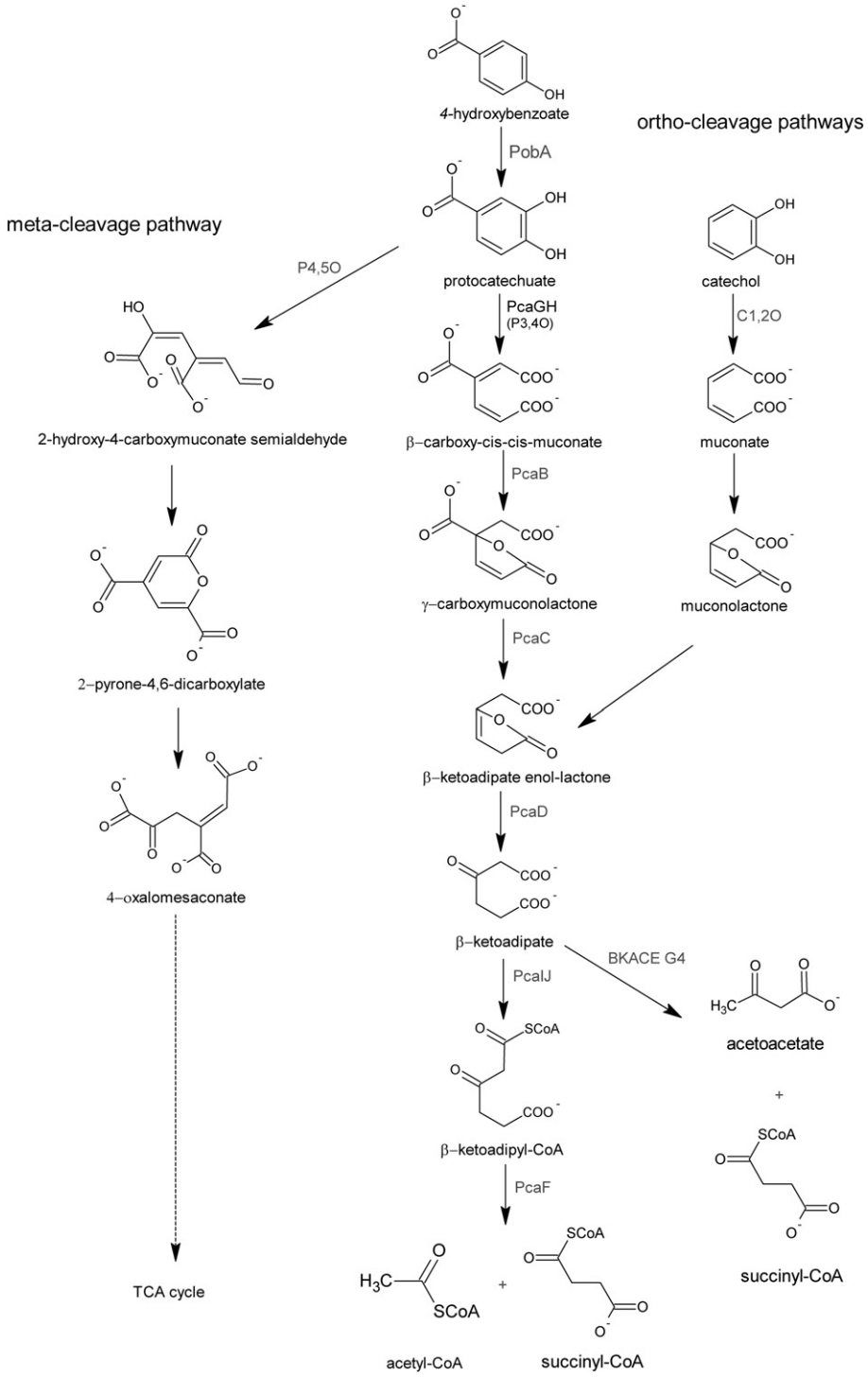

Fig. 1. The bacterial β-ketoadipate pathway (protocatechuate and catechol branches) and additional reactions for degradation of 4-hydroxybenzoate and meta-cleavage of protocatechuate. Only relevant enzymes for the contents of the manuscript are shown. Enzyme abbreviations: PobA, 4-hydroxybenzoate-3-monooxygenase; PcaGH (P3,4O), protocatechuate 3,4-dioxygenase; PcaB, β-carboxy-cis,cis-muconate cycloisomerase; PcaC, γ-carboxymuconolactone decarboxylase; PcaD, enol-lactone hydrolase; PcaIJ, β-ketoadipate succinyl-CoA transferase; PcaF, β-ketoadipyl-CoA thiolase; C1,2O, catechol 1,2-dioxygenase; P4,5O, protocatechuate 4,5-dioxygenase; BKACE G4, β-ketoacid cleaving enzyme group G4.