Functional Genomics of Sulfate-Reducing Bacteria Degrading Hydrocarbons

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Abstract

Sulfate-reducing bacteria (SRB) are well known for their significance in the carbon and sulfur cycles of marine sediments. Despite their general energetic restriction, some SRB are capable of anaerobic degradation of aromatic hydrocarbons in the marine environments, such as deep sea sediments characterized by recent oil formation. Proteogenomics has allowed hitherto unknown insights into the physiology of SRB from single catabolic reactions to complex metabolic

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networks. Best studied aromatic compound-degrading SRB at present are toluene-degrading *Desulfobacula toluolica* Tol2 and the naphthalene-degrading enrichment culture N47.

1 Introduction

Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that thrive by coupling the oxidation of organic substrates to the respiratory reduction of sulfate (SO_4^{2-}) to sulfide (S^{2-}). Owing to the high concentrations (28 mM) of sulfate in sea water, SRB belong to the pivotal drivers of the biogeochemical cycles of carbon and sulfur in the marine seafloor (Jørgensen 1982). Most recently, the global rates of sulfate-reduction in marine sediments were predicted to account for 12–29% of the organic carbon mineralization in these environments (Bowles et al. 2014). Bacterial sulfate reduction has previously been reported to occur at elevated temperatures (80 to above 100 °C) in the production fluids of oil reservoirs in the North Sea (Stetter et al. 1993) and deep-sea hydrothermal vent sediments of the Guaymas Basin in the Gulf of California (Jørgensen et al. 1992). In fact SRB enriched/isolated from these two environments were shown to anaerobically grow with crude oil as sole source of organic carbon and energy by depleting *n*-alkanes or alkylbenzenes directly from the crude oil (Rueter et al. 1994; Harms et al. 1999; Wilkes et al. 2000). This capacity of SRB is of great interest for reservoir geochemistry, albeit an undesired process in oil industry as it deteriorates the quality of the oil by compositional alterations and by souring due to H_2S generation (Head et al. 2014). Formed H_2S can also contribute to the corrosion of iron in technical installations, e.g., oil rigs and pipelines, and some SRB can even use metallic iron as electron donor for sulfate reduction (Enning and Garrelfs 2014). Then again, anaerobic degradation of hydrocarbons represents a beneficial process for bioremediation efforts at hydrocarbon contaminated sites, such as aquifers (e.g., Townsend et al. 2003; Griebler et al. 2004) or marine/estuarine systems (e.g., Miralles et al. 2007; Kimes et al. 2014). For a more general overview on SRB and their role in global biogeochemical cycles, technical processes, biomedicine, and fundamental microbiology, refer to other reviews (e.g., Muyzer and Stams 2008; Rabus et al. 2015). In the following, an overview on aromatic compound-degrading, sulfate-reducing bacteria that have been studied by functional genomics is provided.

2 *Desulfobacula toluolica* Tol2

Desulfobacula toluolica Tol2 represents the first pure culture of a sulfate-reducing bacterium that was demonstrated to completely oxidize toluene to CO_2, with this capacity found to be inducible in whole cell cultures (Rabus et al. 1993). Subsequent experiments indicated initial activation of toluene not to proceed via benzyl alcohol, but via benzylsuccinate (Rabus and Widdel 1995; Rabus and Heider 1998). The occurrence of *Desulfobacula* phylotypes has been reported for a tidal sand flat in the German Wadden Sea (Gittel et al. 2008), for a reduced organic-rich surface sediment in the Baltic

Sea (Sinkko et al. 2013), and for a sediment core from the Caspian Sea subjected to simulated petroleum seepage in laboratory experiments (Stagars et al. 2017).

The complete 5.2 Mbp genome of *D. toluolica* Tol2 was the first to be determined for an aromatic compound-degrading, marine SRB, and functional predictions were refined by comprehensive differential proteomics and targeted metabolite analyses uses of substrate-adapted cells (Wöhlbrand et al. 2013). The high genome plasticity is reminiscent of the genome from the denitrifying degradation specialist "*Aromatoleum aromaticum*" EbN1 (Rabus et al. 2005). Anaerobic degradation of toluene and *p*-cresol was found to be initiated by addition to fumarate followed by modified β-oxidation of the formed succinate derivative to benzoyl-CoA and 4-hydroxybenzoyl-CoA, respectively. Notably, separated gene clusters could be assigned to these two substrates, with the *p*-cresol-specific initial enzyme ((4-hydroxybenzyl)succinate synthase, HbsA catalytic subunit) forming an own branch in the phylogenetic tree of alkyl-/arylalkylsuccinate synthases. Interestingly, the dehydrogenation reactions in both β-oxidation branches are apparently linked via the activity of dedicated electron transfer proteins directly to the membrane redox pool. Anaerobic degradation of phenylalanine involves nonoxidative deamination to cinnamate. Dearomatization of the central intermediate benzoyl-CoA expectedly involves an ATP-independent class II benzoyl-CoA reductase (BCR) as known from *Geobacter metallireducens* GS-15 (Kung et al. 2009). Complete oxidation of acetyl-CoA to CO_2 proceeds via the Wood-Ljungdahl pathway as widespread in the *Desulfobacteraceae* family which strain Tol2 is affiliating with (Rabus et al. 2015). Detailed analysis by 1D blue native-PAGE complexome profiling and 2D blue native-/SDS-PAGE demonstrated an elaborated system of membrane protein complexes for electron transport, including, e.g., dimer and quadruple formation of the central DsrMKJOP complex and the potential capacity for Na^+-based bioenergetics via the RnfABCDEG complex (Wöhlbrand et al. 2016).

3 *Desulfobacula toluolica* TS

A microcosm established from oil-contaminated tidal flat sediment was demonstrated to degrade toluene coupled to sulfate reduction by determining the formation of $^{13}CO_2$ from ^{13}C-labeled toluene. A metagenomics approach was applied to this microcosm yielding a draft genome of 125 scaffolds that showed highest similarity to the genome of *D. toluolica* Tol2. Accordingly, the degradation pathways for several aromatic compounds could be reconstructed, mirroring the network established before for strain Tol2 and underpinning its model character (Kim et al. 2014).

4 Strains NaphS2, NaphS3, and NaphS6

Strain NaphS2 originates from sulfidic marine sediment, represents the first pure culture of an anaerobic bacterium demonstrated to degrade naphthalene completely to CO_2 (Galushko et al. 1999), and is closely related to sulfate-reducing strain EbS7 anaerobically degrading ethylbenzene via addition to fumarate (Kniemeyer et al.

2003). Differential proteogenomics revealed that strain NaphS2 and two further naphthalene-degrading SRBs (strains NaphS3 and NaphS6) activated 2-methylnaphthalene via addition to fumarate and that the presumptive (2-naphthylmethyl)succinate synthase was absent in naphthalene-adapted cells of all three strains. This finding indicated that the three studied marine strains did not employ a methylation as initial activation of anaerobic naphthalene degradation (Musat et al. 2009), as previously suggested for the sulfate-reducing enrichment culture N47 (Safinowski and Meckenstock 2006). Instead, carboxylation of naphthalene is discussed as a possible activation reaction (DiDonato et al. 2010; Musat et al. 2009).

5 Enrichment Culture N47

The sulfate-reducing enrichment culture N47 was obtained from soil material of a contaminated aquifer and by labeling studies (with [^{13}C]bicarbonate) suggested to anaerobically degrade naphthalene via a possible carboxylation to 2-naphthoic acid (Meckenstock et al. 2000). Anaerobic degradation of 2-methylnaphthalene was proposed to proceed in analogy to the anaerobic degradation of toluene, i.e., via addition to fumarate followed by β-oxidation of the succinate moiety (Annweiler et al. 2000). The interim hypothesis of direct methylation of naphthalene to connect with the 2-methylnaphthalene pathway (Safinowski and Meckenstock 2006) was not sustained, as proteomic and enzymatic evidence for the possible involvement of a putative naphthalene carboxylase showing sequence similarity to phenylphosphate carboxylase emerged (Bergmann et al. 2011a; Mouttaki et al. 2012). Subsequently, a draft 4.7 Mbp genome comprised of 17 contigs was obtained for the enrichment culture N47 (Bergmann et al. 2011b), which formed the basis for a proteomics investigation. The latter allowed identification of all genes required for anaerobic degradation of 2-methylnaphthalene as well as a class I benzoyl-CoA reductase (Selesi et al. 2010). Initial biochemical studies suggested that the four-electron reduction of naphthoyl-CoA to 5,6,7,8-tetrahydro-2-naphthoyl-CoA is catalyzed by a novel type of dearomatizing reductase containing FAD, FMN, and an Fe-S cluster as cofactors (Eberlein et al. 2013). Meanwhile, it could be shown that actually two distinct enzymes are involved in the anaerobic reduction of the naphthyl ring, each catalyzing a two-electron reduction (Estelmann et al. 2015).

6 *Desulfococcus multivorans*

Desulfococcus multivorans strain 1be1 is a nutritionally versatile sulfate-reducing bacterium originally isolated from a sewage digester (Widdel 1980). *D. multivorans* is a representative of the *Desulfosarcina-Desulfococcus* clade within *Desulfobacteraceae* that has been repeatedly reported to dominate the SRB community in diverse marine sediments (e.g., Ravenschlag et al. 2000; Mußmann et al. 2005) and represent the bacterial partner in anaerobically methane-oxidizing

consortia (Boetius et al. 2000). The complete 4.46 Mbp genome of *D. multivorans* contains a single chromosome and represents the first to be reported for a member of the *Desulfosarcina-Desulfococcus* clade (Dörries et al. 2016). The metabolic reconstruction was fostered by detailed differential proteome profiling across 17 different substrate conditions, revealing a catabolic network comprising 170 proteins (154 detected; ~91% coverage). Peripheral degradation of aromatic compounds feeds via the benzoyl-CoA pathway (class II benzoyl-CoA reductase) into the Wood-Ljungdahl (WL) pathway for terminal oxidation to CO_2. While peripheral degradation pathways (including benzoyl-CoA pathway) displayed high substrate-specific formation, the central catabolic modules (WL and methylmalonyl-CoA pathways) as well as the membrane protein complexes involved in electron transfer were constitutively formed. This pattern appears to be a common property of to date proteogenomically studied members of the *Desulfobacteraceae*.

7 *Desulfotomaculum gibsoniae* GrollT

The mesophilic sulfate-reducing bacterium strain Groll was isolated from a black mud sample from a small freshwater ditch and shown to anaerobically grow with several aromatic compounds, including phenol, cresols, and catechol (Kuever et al. 1993). The bacterium was subsequently classified as *Desulfotomaculum gibsoniae* Groll^T (Kuever et al. 1999). The ~4.9 Mbp genome allowed for a metabolic reconstruction (Kuever et al. 2014). The anaerobic degradation of cresols is suggested to follow the route previously suggested for *D. toluolica* Tol2 (see above Sect. 2), i.e., initial activation via addition to fumarate is followed by modified β-oxidation to feed via class II BCR into the central benzoyl-CoA pathway and from there to the WL-pathway for terminal oxidation to CO_2. Catechol degradation is proposed to involve initial activation by phosphorylation followed by carboxylation as previously proposed for the denitrifying bacterium *Thauera aromatica* K172 (Ding et al. 2008).

8 Research Needs

While the biogeographical significance and biogeochemical imprint of SRB on a global scale are well established, the understanding of the catabolic properties that form the basis of an organism's functional role in the habitat is far less advanced. Thus, more complete expert-annotated genomes of model SRBs being relevant for an intriguing degradation ability and/or abundance in the habitat are required. The integration of genomics, proteomics, and metabolite analysis has proven particularly rewarding for elucidating novel reactions and reconstructing complete catabolic networks and needs to be further developed. A broader functional genomics understanding of aromatic compound-degrading SRB promises new catabolic discoveries, insights into how these bacteria operate when degrading recalcitrant compounds at the thermodynamic limit, and to what extent the catabolic networks revealed from

pure cultures of SRB can be actually found in the natural habitat (e.g., Michas et al. 2017). In this respect, metaOMICS investigations of sulfidic sites rich in aromatic compounds will be highly interesting.

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