Beyond the tip of the sulfite- and sulfate-reducer iceberg

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Abstract
Sulfite- and Sulfate-reducing microorganisms (SRM) play important roles in anoxic environments, linking the sulfur and carbon cycles. With climate warming, the distribution of anoxic habitats conductive to dissimilatory SRM is expanding. Consequently, we hypothesize that novel SRM are likely to emerge from the rare biosphere triggered by environmental changes. Using the dsrB gene as a molecular marker of sulfite- and sulfate reducers, we analyzed the diversity, community composition and abundance of SRM in 200 samples representing 14 different ecosystems including marine and freshwater environments, oil
reservoirs and engineered infrastructure. Up to 167,397 species-level OTUs affiliated with 47 different families were identified. Up to 96% of these can be considered as “rare biosphere SRM”. A third of the dsrB genes identified belonged to uncharacterized lineages. The dsrB sequences exhibited a strong pattern of selection in different ecosystems. These results expand our knowledge of the biodiversity and distribution of SRM, with implications for carbon and sulfur cycling in anoxic ecosystems.

Introduction

Sulfite- and Sulfate-reducing microorganisms (SRM) are widespread in anoxic environments such as marine sediments, hydrothermal vents, oil reservoirs, marine and freshwaters, where they play significant roles in the biogeochemical sulfur cycle (Holmer and Storkholm, 2001; Muyzer and Stams, 2008). In marine sediments, sulfate reduction activity can potentially oxidize up to 29% of the organic carbon pool (Bowles et al., 2014). Therefore, SRM are major players in the carbon cycle of anoxic environments, degrading, directly or indirectly through syntrophic associations, a broad range of complex substrates such as carbohydrates (Rabus et al., 2015) or aromatic compounds (Musat et al., 2009). SRM have an important impact on natural and engineered environments mainly through their production of hydrogen sulfide, which is both toxic and corrosive and can modify bioavailability of other chemical elements (Muyzer and Stams, 2008). With climate warming, enhancing rates of oxygen respiration and eutrophication leading to increased organic carbon deposition (Hoegh-Guldberg and Bruno, 2010), distribution of anoxic environments with various environmental settings (different carbon sources and interactions with other microorganisms, presence of trace elements) conducive to growth of SRM is projected to increase significantly (Harley et al., 2006). Consequently, we hypothesize that sulfate reducers are likely to emerge from the rare biosphere triggered by environmental changes and the spread of unusual anaerobic niches, with important consequences for ecosystem health (Kump et al., 2005). High throughput sequencing and quantitative PCR analysis of dissimilatory sulfite reductase dsrB genes from an unrivaled collection of 200 environmental samples, representing 14 different ecosystems, has allowed us to revise our knowledge of the global biodiversity of sulfite- and sulfate-reducing microorganisms and identify novel rare SRM lineages that may potentially become dominant organisms in new environments emerging with environmental changes.
Results and Discussion

In this study, the abundance of sulfite- and sulfate-reducers and the composition of the SRM community were investigated using DSR1728f/rDSR4R primer mixes (Supplementary Table 1), targeting the dissipatory sulfite reductase genes *dsrB*, involved in the last step of the energy producing dissimilatory sulfate reduction pathway and present in all known sulfate-reducing lineages (Loy *et al.*, 2008; Muller *et al.*, 2015; Wagner *et al.*, 2005). A total of $1.98 \times 10^7$ *dsrB* amplicon sequences were produced from 200 different environmental samples with an average of $1.2 \pm 0.9 \times 10^5$ *dsrB* sequences per sample. After quality filtering (Supplementary material), 167,397 different species-level OTUs (90% identity cut-off as recommended by Pelikan and coauthors (Pelikan *et al.*, 2016)) were identified, increasing substantially previous estimates of potential sulfate-reducing microbial diversity that proposed a minimum of 779 different species (OTU level at 90% similarity) (Muller *et al.*, 2015). Although this analysis includes a number of microorganisms that carry and express *dsrB* genes, but do not reduce sulfate such as *Pelotomaculum* species (Imachi *et al.*, 2006), *Desulfurivibrio alkaliphilus* (Thorup *et al.*, 2017); some members of the *Desulfobulbaceae* family (Trojan *et al.*, 2016), this clearly indicates that potential SRM diversity has been considerably underestimated by previous assessments (Colin *et al.*, 2013; Muller *et al.*, 2015). In addition, this analysis also includes sulfide oxidizers with oxidative-type DsrAB genes that operate in reverse direction (labeled as Ox. in Supplementary Material). However, from the total dataset these were represented by only 1885 OTUs (1.1% of the OTUs) with an average of 1% of such sequences per sample.

Considering 240 cultivated species of sulfate reducers, this result also indicates that <0.2% of the SRM have been cultivated. Analyses of the distribution of these OTUs in the dataset indicates that rare *dsrB* OTUs (<0.1% in all samples) represented 96.7% of the OTUs (Supplementary Table 3). This, coupled with the use of low coverage primers, might explain why sulfate reducer diversity has been underestimated previously, using low throughput analyses (e.g. Sanger sequencing of cloned dsrAB genes) (Hausmann *et al.*, 2016). However, these rare sulfite- and sulfate-reducers might represent an important ‘seed bank’ that can have a significant environmental role when triggered by environmental changes (Hausmann *et al.*, 2016; Pester *et al.*, 2010; Kalenitchenko *et al.*, 2018). The rare SRM biosphere might also include spore-forming sulfate reducers that were previously undetectable by Sanger sequencing without
modification of environmental conditions that would lead to germination of their dormant spores (de Rezende et al., 2013).

*Desulfobacteraceae* and *Desulfuvirionaceae* were the most frequently detected families followed by *Desulfobulbaceae, Desulfobulbiaeae, Syntrophobacteraceae, Archaeoglobaceae*, the uncultured cluster 9 of the Environmental supercluster 1 and the uncultured cluster 5 of the *Firmicutes* supercluster (Figure 1b, Supplementary Figure 2; Müller et al., 2015). Additionally, with the exception of oil reservoirs and corrosive biofilms growing on engineered infrastructure, our results also indicated that 28±12 % of the detected SRM were affiliated with uncharacterized groups without cultured representatives. Despite the extensive diversity uncovered by deep sequencing, no species-level OTU nor sulfate reducer family was detected as ubiquitous in all environments. Furthermore, community composition among the different environments was significantly different (NPMANOVA, p<0.04) (Figure 1a), suggesting that environmental conditions apply considerable selection pressure on SRM and result in communities that are specialized for particular environments. This is also reflected in the sulfate-reducing community richness estimated for different environments (average: 24 dsrB families, min:1, max:44) and abundances observed amongst the various ecosystems analyzed (average: 9.56x10^5 dsrB genes ng^-1 of gDNA; min:4.32x10^3, max:2.33x10^7) (Figure 2b).

Marine sediments presented the highest richness of SRM (>25 dsrB families), suggesting a lower selective pressure and/or environmental heterogeneity and confirming that the marine environment, by virtue of high sulfate concentration and the variety of degradable carbon substrates is the main biotope of sulfate reducers (Figure 2b) (Rabus et al., 2015). Consistently, community composition in all marine environments was strongly predominated by members of the *Desulfobacteraceae* (46.1±6% of the sequences), (Figure 1b) which is considered to be catabolically versatile SRB family.

Although the diversity of SRM was similar across marine environments, relative abundances varied considerably. The highest abundance of sulfate reducers in marine environments was quantified in organic carbon-rich, salt marsh sediments, whereas the lowest abundances were estimated in subsurface sediments with refractory organic carbon (Figure 2a). Therefore, the relative abundance of sulfate reducers in marine sediments potentially could be influenced by the availability of utilizable organic carbon (Rabus
et al., 2015) and decreases in abundance as labile carbon pools decline. Since climate warming might be associated with increased organic matter deposition, abundance of sulfate-reducing microbes is likely to increase accordingly. The proportion of uncharacterized SRM lineages as well as members of the *Syntrophobacteraceae* increased in subseafloor organic poor sediments, suggesting that quality and/or quantity of the labile organic matter might also play a role in shaping the SRM community composition.

Q-PCR results indicated that the abundance of SRM in niches that will likely expand in the future due to environmental change (e.g.: urban freshwater ecosystems, anoxic aquifers, flooded soils and wetlands) was comparable to their counterparts in marine environments (Figure 2a). However, a lower richness was observed (<25 dsrB families) with members of the *Desulfobulbaceae* and *Desulfovibrionaceae*, as well as organisms belonging to the Uncultured clusters 9 and 10, potentially related to peatland sulfate-reducing *Acidobacteria* (Hausmann et al., 2017). Although these bacteria might have alternative metabolic capabilities (syntrophic or fermentative lifestyles, nitrate reduction, microaerophilia or sulfide oxidation for some members of the *Desulfobulbaceae* (Trojan et al., 2016)), these results indicate that, if sulfate concentrations can support their metabolism, unknown lineages of SRM could become important components of microbial communities in these expanding environments, potentially leading to substantial release of toxic and corrosive hydrogen sulfide gas.

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References


Legend of the figures

Figure 1: a) Non-Metric multidimensional scaling (NMDS) of the dsrB community composition. b) Distribution of the 19 most abundant dsrB-bearing families in the different ecosystems. Brown, infrastructures; Dark red, mine drainage ponds; purple, wetlands; dark green, freshwaters; orange, groundwater; grey, oil reservoirs; yellow, hydrothermal vents; dark blue, subseafloor; blue, mud volcanoes; light green, cold seeps; green, deep sea fans; salmon, salt marshes; dark blue, estuary.

Figure 2: a) DsrB gene abundance (copy per ng of gDNA) in the different sampled environments. Boxes were drawn using 25% and 75% quartiles, x represents the mean, horizontal line the median, whiskers the variability outside quartiles and points outside whiskers are outliers b) relationship between dsrB gene abundance and estimated richness at the family level. Each dot represents a sample. Color of the samples corresponds to the caption in a): Brown, infrastructures; Dark red, mine drainage ponds; purple, wetlands; dark green, freshwaters; orange, groundwater; grey, oil reservoirs; yellow, hydrothermal vents; dark blue, subseafloor; blue, mud volcanoes; light green, cold seeps; green, deep sea fans; salmon, salt marshes. High gene abundance coupled to low richness, as detected in oil reservoirs (grey dots), suggests a strong selective pressure and specialized microorganisms.
a) dsrAB relative abundance

b) dsrAB relative abundance vs richness diversity index