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SHORT COMMUNICATION

Genomic repertoire of the *Woeseiaceae*/JTB255, cosmopolitan and abundant core members of microbial communities in marine sediments

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To date, very little is known about the bacterial core community of marine sediments. Here we study the environmental distribution, abundance and ecogenomics of the gammaproteobacterial *Woeseiaceae*/JTB255 marine benthic group. A meta-analysis of published work shows that the *Woeseiaceae*/JTB255 are ubiquitous and consistently rank among the most abundant 16S rRNA gene sequences in diverse marine sediments. They account for up to 22% of bacterial amplicons and 6% of total cell counts in European and Australian coastal sediments. The analysis of a single-cell genome, metagenomic bins and the genome of the next cultured relative *Woeseia oceani* indicated a broad physiological range, including heterotrophy and facultative autotrophy. All tested (meta)genomes encode a truncated denitrification pathway to nitrous oxide. The broad range of energy-yielding metabolisms possibly explains the ubiquity and high abundance of *Woeseiaceae*/JTB255 in marine sediments, where they carry out diverse, but yet unknown ecological functions.
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Introduction

Marine sediments are hot spots of microbially catalyzed element cycling and are Earth´s major carbon sink (Burdige, 2007). While there is consensus that some bacterial classes such as *Flavobacteria*, *Gamma-* and *Deltaproteobacteria* generally thrive in marine sediments (Amaral-Zettler *et al.*, 2010; Orcutt *et al.*, 2011; Zinger *et al.*, 2011), it is still not known whether bacterial lineages of lower taxonomic ranks belong to the 'core microbiome' of marine sediments. Recently, Bienhold *et al.* (2016) identified representatives of the gammaproteobacterial JTB255-marine benthic group (MBG) as ubiquitous core members of microbial communities in abyssal and bathyal surface sediments. Consistent with this, we identified the JTB255-MBG being among the dominant bacterial groups in 13 coastal sediments across Europe and Australia (Dyksma *et al.*, 2016). In some of these sediments, members of the JTB255-MBG assimilated inorganic carbon (Dyksma *et al.*, 2016), which would be in line with a hypothesized sulfur oxidation potential in this group (Bowman *et al.*, 2005). However, the genomic repertoire of this group is still unknown.

In this study, we surveyed published the next-generation-sequencing (NGS) datasets of 16S rRNA gene amplicons from marine sediments and determined relative JTB255-MBG cell abundances in five tidal marine sediments to underscore their environmental importance. By analyzing a single amplified genome (SAG), metagenomic bins from two distant sites and the genome of the recently isolated, cultured and closely related *Woeseia oceani* (Du *et al.*, 2016), we explored the genetic and metabolic potential of this group. Using 16S rRNA gene sequences and concatenated ribosomal proteins, we revisit the phylogenetic affiliation of the JTB255-MBG, indicating an affiliation with the *Woeseiaceae*.

Distribution and environmental importance in marine sediments

To assess the relative abundance of 16S rRNA gene sequences of JTB255-MBG in marine sediments, we surveyed recently published 16S rRNA gene amplicon studies that provided a sufficient taxonomic resolution along with relative sequence abundances (Figure 1). The JTB255-MBG occurs in high relative sequence abundances in all types of marine benthic habitats, including hydrothermal sites, coastal and deep-sea, and organic-rich and -poor sediments (Supplementary Figure S1, Supplementary Table S1).

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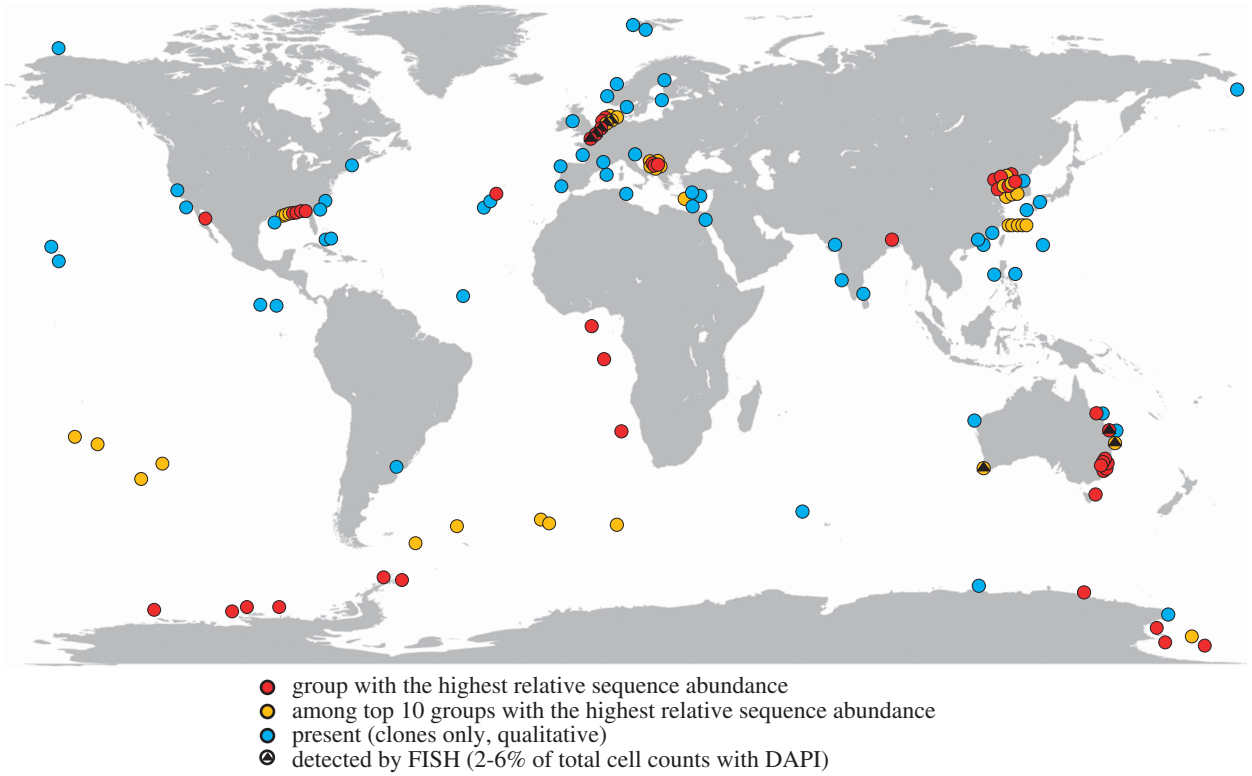

Figure 1 Global distribution and relative sequence abundances of 16S rRNA genes of the *Woeseiaceae*/JTB255 in marine benthic habitats. Color-coded circles refer to the relative sequence abundance of *Woeseiaceae*/JTB255 in NGS amplicon or clone studies according to SILVAngs (Quast *et al.*, 2013). Blue circles refer to the only qualitative detection of sequences of the *Woeseiaceae*/JTB255 in clone studies. Note that in previous publications, using outdated training sets for amplicon classification, the 16S rRNA gene sequences of the *Woeseiaceae*/JTB255 have often been classified as marine *Sinobacteraceae/Xanthomonadales*. An annotated map with references is given in the Supplementary Information (Supplementary Figure S1, Supplementary Table S1).

Intriguingly, among the lower taxonomic ranks (family to genus), the JTB255-MBG constituted the group with the highest relative sequence abundance at 32 sites (Figure 1, for example, Bowman *et al.*, 2005; Schauer *et al.*, 2010; Ruff *et al.*, 2014; Liu *et al.*, 2015). At 30 sites, the JTB255-MBG ranked among the top 10 sequence groups (for example, Tully and Heidelberg, 2013; Liu *et al.*, 2015). It was found in all tested NGS-amplicon datasets. In 13 selected sediment samples surveyed by Dyksma *et al.*, 2016, they accounted for an average of 9.8% of all bacterial sequences (Supplementary Table S2), which is a percentage close to their average relative cell abundance of 5.0% in some sediments as was determined by fluorescence *in situ* hybridization (Figure 1, Supplementary Table S2, Supplementary Figure S7).

To study the distribution patterns of JTB255-MBG populations in eight tidal sediments from Europe and Australia (Dyksma *et al.*, 2016), we performed a hierarchical cluster analyses of 16S rRNA genes. At a cutoff of 98% sequence identity, one-third of operational taxonomic units (OTUs) with a minimum relative sequence abundance of 0.5% of all bacterial pyrotags occurred at more than one site (Supplementary Figure S2). None of the OTUs occurred at all six tidal sediments sampled along the European Atlantic coastline. To identify potentially habitat-specific ecotypes among the JTB255-MBG, we phylogenetically analyzed nearly full-length 16S rRNA sequences of diverse origin (ARB-Silva SSU Ref release 123, Pruesse *et al.*, 2007). From this analysis, six stable sequence clusters emerged, which comprised mainly sequences of either coastal or deep-sea origin (Supplementary Figure S3). The majority of JTB255-MBG sequences did not show an apparent water depth-dependent phylogenetic pattern. In the future, the integration of full-length 16S rRNA sequences, meta- and single-cell genomes and physico-chemical parameters will be necessary to unravel potential ecotypes and factors shaping the JTB255-MBG communities in marine sediments.

The JTB255-MBG are affiliated with the *Woeseiaceae*

Given the high sequence diversity and abundance of JTB255-MBG in marine sediments, we aimed at resolving the phylogenetic affiliation of the JTB255-MBG in more detail. To this end, we analyzed high quality, nearly full-length 16S rRNA gene sequences ($n = 825$) using four different treeing methods. In a consensus tree, 96% of all sequences previously classified as JTB255-MBG formed a monophylethic cluster supported by all four treeing methods (Supplementary Figure S3). In contrast to their

earlier classification as members of the order *Xanthomonadales*, our phylogenetic reconstruction indicates that the JTB255-MBG rather forms a stable deep-branching monophyletic cluster within the *Gammaproteobacteria*. Intriguingly, the recently isolated type strain *Woeseia oceani* XK5, the only cultured member of the family *Woeseiaceae* (Du *et al*., 2016) forms a monophyletic cluster with the JTB255-MBG that is supported by different treeing methods (Supplementary Figure S3). Moreover, the minimum sequence identity within this cluster is 87%, which is above the accepted 86.5% cut-off to discriminate families (Yarza *et al*., 2014). On basis of these findings, we propose that the JTB255-MBG belongs to the family *Woeseiaceae*.

To confirm the phylogenetic affiliation of JTB255-MBG with *W. oceani,* we analyzed a single amplified genome (SAG 1868_B) from a tidal surface sediment in the German Wadden Sea, site Janssand. It encodes 2.27 Mbp and is 48% complete (Table 1). The 16S rRNA displays 93% sequence identity to *W. oceani* and 92.4% to the gammaproteobacterial metagenomic bin WOR_SG8_31 from White Oak River estuary sediments (Table 1, Baker *et al*., 2015). From these, we extracted and concatenated 16 ribosomal protein (Rpo) sequences, which are considered to be barely affected by lateral gene transfer (Sorek *et al*., 2007). Consistent with the 16S rRNA gene phylogeny and supported by four treeing methods, the Rpo-phylogeny confirmed an affiliation of the JTB255-MBG with *W. oceani* (Supplementary Figure S4). Together, 16S rRNA and Rpo phylogenies provide strong support that the JTB255-MBG are actually members of the family *Woeseiaceae*. Thus, we henceforth refer to the JTB255-MBG as *Woeseiaceae*/JTB255.

Genomic repertoire of the *Woeseiaceae*/JTB255

To recover additional genomic data from this family, we analyzed two incomplete metagenomic bins that we extracted from a bulk metagenome of the Janssand tidal sediment (Table 1, Supplementary Figure S5). The metagenomic bin 20_j1 (2.4 Mbp) was generated by recruitment and assembly of metagenomic reads using SAG 1868_B as reference. Consequently, the bin 20_j1 displays a high average nucleotide identity (ANI) of 99.7% to SAG 1868_B and shows a highly similar genome content. In addition, using GC-content, tetranucleotide frequencies and read coverage, we extracted the metagenomic bin JSS_woes1 (8.1 Mbp). The single-copy genes in this metagenomic bin occur as non-identical dupli- or triplicates, which indicates two to three bacterial genomes in this bin. Two homologous operons encode ribosomal proteins (Rpo) that are most closely affiliated with *W. oceani* (Supplementary Figure S4, 82 and 84% SI). Consistent with this, the recovered fragments of 5S, 16S, 23S rRNA gene sequences were all highly similar to those of W. *oceani* (97, 100, 96% SI, respectively). The fact that the metagenomic bin JSS_woes1 attracted in total 6% of all metagenomic reads further supports the high *in situ* abundance of *Woeseiaceae*/JTB255 in this tidal sediment.

Potential for chemolithoautotrophy

The recent description of type strain *W. oceani* XK5 (Du *et al*., 2016) and the fully sequenced genome (NZ_CP016268) provide first insights into physiology and genetic potential of the *Woeseiaceae*/JTB255. Physiological tests and the genome content indicate that *W. oceani* is an obligate chemoorganoheterotroph. This finding, however, contrasts with the recently proposed chemolithoautotrophic potential of the *Woeseiaceae*/JTB255-MGB (Dyksma *et al*., 2016). Therefore, we screened SAG 1868_B and all metagenomic bins for pathways that could power growth with inorganic energy and carbon sources.

The (meta)genomes encode three pathways to gain energy from inorganic compounds. SAG 1868_B and bin 20_j1 encode the Sox-pathway (SoxABCDHXYZ) for thiosulfate oxidation (Table 1). For sulfite oxidation, some *Woeseiaceae*/JTB255 may employ a dissimilatory adenosine-5'-phosphosulfate reductase (AprABM) that is encoded in metagenomic bin JSS_woes1. Moreover, we identified genes encoding an oxygen-tolerant [NiFe] uptake hydrogenase (Hup) plus accessory proteins in SAG 1868_B and in metagenomic bin JSS_woes1 (Table 1).

Furthermore, in metagenomic bin JSS_woes1, we detected genes encoding a Rubisco form II for carbon fixation via the Calvin–Benson-Bassham (CBB) cycle and a phosphoribulokinase (Table 1). These co-localize with three non-CBB-related genes that are most similar to homologs in *W. oceani*, further supporting an autotrophic potential within the *Woeseiaceae* (Supplementary Figure S6). To find additional support of a chemolithoautotrophic potential in the *Woeseiaceae*/JTB255, we re-analyzed the metagenomic bin WOR_SG8_31 (Baker *et al*., 2015). As the (meta)genomes from the tidal flat, it encodes an aerotolerant [NiFe] uptake hydrogenase, the Sox-pathway for thiosulfate oxidation and a Rubisco for carbon fixation (Table 1).

Collectively, our analyses show that some members of the *Woeseiaceae*/JTB255 have the genetic potential for chemolithoautotrophy powered by sulfur or hydrogen oxidation. It confirms the previously measured carbon fixation activity of *Woeseiaceae*/JTB255 cells at our sampling site Janssand (Dyksma *et al*., 2016).

Potential for heterotrophy

Since the next cultured relative strain *W. coeani* is an obligate chemoorganoheterotroph, we tested whether the uncultured *Woeseiaceae*/JTB255 from Janssand tidal sediment also have the potential to use organic

Table 1 Genome statistics and selected metabolic features encoded in available (meta)genomes of *Woeseiaceae*/JTB255

(Meta)genome name	*SAG 1868_B*	*bin 20_j1*	*bin JSS_woes1*	*bin WOR_SG8_31*[a]	*Woeseia oceani*[b]
Accession	IMG genome ID 2651869504	IMG genome ID 2651869885	IMG genome ID 2695420981	Genbank LJTI00000000	Genbank (NZ_CP016268)
Sample origin	Tidal sediment Janssand/GER	Tidal sediment Janssand/GER	Tidal sediment Janssand/GER	Estuarine sediment WOR/USA	Coastal sediment Xiaoshi Island/CN
Genome statistics					
Assembly size (bp)	2 277 554	2 404 210	8 121 065	5 968 366	4 059 891
GC (%)	59.9	56.5	57.8	62.6	57.8
	2333	2424	9718[c]	5821	3646
Predicted genes (IMG gene calling)					
Scaffolds	358	298	607	266	1
N50	22 940	20 713	17 507	37 817	—
Marker gene completeness (%)	48.3	48.4	(92.5[d])	100[a]	100
tRNAs	26	25	54	68	45
rRNAs	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S	5S, 16S, 23S
Metabolism					
Autotrophic carbon fixation	–	–	Rubisco II, PRK	Rubisco I, PRK	–
Dissimilatory sulfur oxidation	SoxABCDHXYZ	SoxABCDXYZ	AprAB	SoxABCDHWXYZ	—
Group 1d [NiFe] uptake hydrogenase	+	–	+	+	–
Cytochrome c oxidase (O_2 respiration)	+	+	+	+	+
Denitrification	NirS, NorB	NorB	NirS, NorB	NirS, NorB	NirS, NorB
Glycosyl hydrolases (families)	22 (6)	20 (7)	54 (17)	13 (6)	19 (9)[e]
Glycosyl hydrolases per Mbp	10	8	7	2	5
Peptidases (S = serine;	114	119	486	262	222
M = metallo)	(S:40, M: 44)	(S:43, M: 40)	(S:170, M: 205)	(S:97, M: 94)	(S: 82, M: 85)
Peptidases per Mbp	50	50	60	44	55
Peptidase/glycosyl hydrolase per Mbp	5	6.3	8.6	22	11

Abbreviation: PRK, phosphoribulokinase.
[a]For details see Baker *et al*., 2015.
[b]Du *et al*., 2016.
[c]RAST gene calling (see Supplementary Information).
[d]Includes a 2.2× duplication of single copy genes.
[e]Available at www.cazy.org.

molecules as energy (and carbon) sources. The SAG 1868_B and both metagenomic bins encode several organic molecule transporters, alpha-, beta-glucosidases and beta-glucanases, and carbohydrate-active enzymes of glycosyl hydrolase families (Table 1, Supplementary Figure S8). Some also occur in metagenomic bin WOR_SG8_31 and were linked to cellulolysis and amylolysis (Baker *et al*., 2015). These data indicate a potential to utilize oligo- or polysaccharides, however, we did not detect polysaccharide utilization loci (PUL), typically found in polymer-degrading marine microorganisms (Fernández-Gómez *et al*., 2013). The type strain *W. oceani* consumes sugars but does not hydrolyze cellulose, alginate or starch and also seems to lack the typical carbohydrate-hydrolyzing enzymes (Du *et al*., 2016).

All (meta)genomes encode alcohol dehydrogenases, which is consistent with the observed consumption of glycerol by *W. oceani* (Du *et al*., 2016). Moreover, the SAG 1868_B, metagenomic bins and *W. oceani* encode diverse proteases and peptidases (Supplementary Figure S8). For example, dipeptidyl-peptidases M48 and S46 occur in SAG 1868_B but not in any autotrophic gammaproteobacterial sulfur-oxidizer cultured to date. Dioxygenases, such as tryptophan-dioxygenase are found in all tested (meta)genomes and are employed to aerobically cleave aromatic ring structures (Supplementary Figure S8). Consistent with this, *W. oceani* was isolated on peptone agar and displays proteolytic enzyme activities (Du *et al*., 2016). In summary, the ability to consume organic compounds such as carbohydrates and peptides appears to be common among the studied (meta)genomes of *Woeseiaceae*/JTB255. The varying peptidase/GH ratios (Table 1), however, also suggest distinct substrate preferences among the studied members of *Woeseiaceae*/JTB255.

Aerobic and anaerobic respiration

Our (meta)genomic analyses and the physiological tests with *W. oceani* (Du *et al*., 2016) indicated the capability for aerobic and anaerobic growth. Oxygen is respired using aa_3-type and cbb_3-type cytochrome c oxidases. For oxygen-independent respiration SAG 1868_B, metagenomic bins JSS_woes1 and WOR_SG8_31, and *W. oceani* may employ consistently a truncated

denitrification pathway, including the periplasmic dissimilatory nitrite NirS and the membrane-bound nitric oxide reductase NorB (Supplementary Figure S8). While *W. oceani* does not respire nitrate (Du *et al.*, 2016) and only possesses *nirS* and *norB* genes, we cannot exclude that we missed additional denitrification genes in the non-sequenced parts of the (meta) genomes. However, truncated denitrification pathways and modularity are common among denitrifying microorganisms (Graf *et al.*, 2014) and also appear to drive denitrification at our sampling site (Marchant *et al.*, under review). In fact, coastal sandy sediments have a more important role in N-loss than assumed and may account for a significant fraction of the global oceanic emission of the potent greenhouse gas nitrous oxide (Marchant *et al.*, under review). If a truncated denitrification is common among the *Woeseiaceae*/JTB255, this ubiquitous and abundant group might substantially contribute to nitrous oxide emissions from coastal sediments.

Conclusion

Our study highlights the role of the *Woeseiaceae*/JTB255 in marine surface sediments. The genomic repertoire presented in this study and the physiology of the first isolated strain *W. oceani* suggest that this family is heterogeneous and covers a broad physiological spectrum ranging from facultative sulfur- and hydrogen-based chemolithoautotrophy to obligate chemorganoheterotrophy. This could provide adaptations to various biogeochemical settings and possibly explains their success in marine sediments worldwide. Given their substantial sequence and cell frequencies in sediments across the oceans, we propose that the *Woeseiaceae*/JTB255 are important members of microbial benthic communities and are likely among the most abundant microorganisms in both deep-sea and coastal sediments. Thus, it is imperative to unravel their *in situ* activity and function in carbon, sulfur and nitrogen cycling in diverse types of marine sediment.

Conflict of Interest

The authors declare no conflict of interest.

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