RESEARCH DESCRIPTION

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The main theme of my research is microbial ecology. The research covers topics at the community, cellular, molecular and genomic levels, and focuses on three areas: microbiology of flooded rice paddies, ecology and molecular biology of methanotrophic bacteria, and microbial ecology of *Sphagnum*-dominated peatlands. The following text provides an overview of my research activities at the MPI for Terrestrial Microbiology (Marburg, Germany). Methods that are not mentioned below were developed for the detection and analysis of betaproteobacterial ammonia oxidizers (*Nitrosomonas*/*Nitrosospira* group) and aerobic methanotrophs (PCR and T-RFLP fingerprinting of *amoA* and *pmoA* [1, 2, 3]).

Microbiology of flooded rice paddies

The studies were carried out using soil from rice fields of the Italian Rice Research Institute in Vercelli, Italy. Our initial research focused on the analysis of the diversity and structure of microbial communities in anoxic paddy soil. We used a combination of molecular and cultivation techniques as outlined in [4]. Members of the *Verrucomicrobia*, CFB group (*Bacteroidetes*), clostridial cluster XIVa, and *Bacillaceae* were identified as major populations, as derived from close similarities between 16S rRNA gene sequences directly retrieved from paddy soil and those of bacterial isolates obtained by most-probable-number-techniques [5, 6, 7; reviewed in 8]. The same dual approach of direct 16S rRNA gene sequence retrieval and cultivation was applied to analyze the archaeal community composition. The results indicated *Methanosaeta* spp. and *Methanobacterium* spp. to be the dominant methanogenic populations in the anoxic rice soil, whereas *Methanosarcina* spp. appeared to be less abundant [9]. T-RFLP fingerprinting of 16S rRNA genes and 16S rRNA, combined with clone libraries, was used to assess spatial and temporal changes in bacterial community composition along the oxygen gradient that develops in the surface layer of paddy soil after flooding. The analyses revealed major changes in community composition along a depth profile of 2 mm, in response to oxygen decline [10, 11]. Temporal dynamics were most pronounced from 2 to 21 days after flooding, for both oxic and anoxic zones. No effect of time or oxygen zone on the community dynamics was observed from 21 to 168 days after flooding. Members of the *Betaproteobacteria* (oxic zone) and clostridial cluster I (anoxic zone) were identified as the dominant populations in early succession. Dominant late-successional populations belonged to the *Acidobacteria*, *Verrucomicrobia*, *Nitrospira*, and *Myxococcales*. The overall findings suggested that the principles of *r*- and *K*-selection could be applied to explain the successional changes in community composition in the oxygen gradient system studied [11].

The analysis of root-associated bacterial communities revealed a composition that mirrored the spatial and temporal heterogeneity of the rice rhizosphere. *Alphaproteobacteria*, *Betaproteobacteria* (in particular *Comamonadaceae*), *Deltaproteobacteria* (*Geobacteraceae*, *Myxococcales*), *Firmicutes* (*Clostridium*, *Sporomusa*), *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes* were identified as major groups. Members of these groups were detected with varying frequencies in 16S rRNA gene clone libraries obtained from roots of rice plants grown for either 45 days or 90 days [12]. A phylogenetic survey of root-associated archaea revealed several new euryarchaeal lineages. One of these was initially named Rice Cluster I [13]. RC-I formed a distinct clade within the phylogenetic radiation of the *Methanosarcinales* and *Methanomicrobiales*, suggesting a methanogenic phenotype of this archaeon. First

attempts to isolate members of RC-I in pure culture failed, but resulted in the enrichment culture MRE50 [14, 15]. The high enrichment level of a single RC-I genotype in MRE50 (approx. 50% of total cells) let us attempt to reconstruct the complete genome of this RC-I_{MRE50} genotype by a metagenomic approach. The complete RC-I genome sequence (3.18 Mb) suggested that RC-I_{MRE50} has an aerotolerant H_2/CO_2-dependent lifestyle and enzymatic capacities for assimilatory sulfate reduction and oxygen detoxification, hitherto unknown among methanogens [16]. More recently, the RC-I_{MRE50} genotype was obtained in pure culture and taxonomically described as *Methanocella avoryzae* sp. nov. [17]. Phenotypic characterization of the isolate confirmed its hydrogenotrophic lifestyle, utilizing H_2/CO_2 and formate for growth and methane production.

Environmental transcriptomics

We explored global gene expression of microbial communities in oxic versus anoxic paddy soil. Using conventional Sanger sequencing, the random analysis of 800 cDNA sequences allowed us to identify gene expression related to cellular processes, metabolism and transport mechanisms, and to detect changes in the active components of the microbial communities [18]. Expressed sequence tags assigned to *Alpha*- and *Betaproteobacteria* were predominantly found in the oxic zone, while those affiliated with *Deltaproteobacteria* were more frequently detected in the anoxic zone. At the genus level, multiple assignments to *Bradyrhizobium* and *Geobacter* were unique to the oxic and anoxic zone, respectively. In general, there was a good correspondence between phylogenetic rRNA-based analysis and the taxonomic assignment of mRNA transcripts. Plotting the cDNA lengths against the *E*-values in BLASTX showed that sequence read lengths of ≥ 400 base pairs, as provided by Roche 454 GS FLX Titanium pyrosequencing, are sufficient to functionally annotate the majority of cDNAs with high significance.

More recently, we developed an efficient method for extracting high quality mRNA from soil [19]. Key steps in the isolation of total RNA are low-pH extraction (pH 5.0) and Q-Sepharose chromatography. The removal efficiency of humic acids was 94 to 98% for all soils tested. To enrich mRNA, subtractive hybridization of rRNA is most efficient. The mRNA is of sufficient purity and integrity (size range of 0.3 to 4 kb) for cDNA library construction and next-generation sequencing (NGS).

Using flooded paddy soil as a model system, our current metatranscriptomic approaches involve (i) extraction of total RNA, (ii) enrichment of mRNA, (iii) cDNA synthesis and NGS (454 pyrosequencing, Illumina HiSeq/MiSeq), (iv) preprocessing of raw reads, and (v) data analysis using either online tools (rRNA: QIIME, Mothur, Silva; mRNA: Camera, MG-Rast) or a local bioinformatic pipeline [20].

Methane-oxidizing bacteria

Methane-oxidizing bacteria, or methanotrophs, are crucial players in the global cycle of the greenhouse gas methane. They are strict aerobes that use methane as their major source of carbon and energy. Their activity attenuates methane emission from various environments, such as wetlands, rice paddies, and landfills, and constitutes the only biological sink for atmospheric methane in upland soils. In the methane oxidation pathway, the first step is mediated by particulate methane monooxygenase (pMMO). This key enzyme of methanotrophy converts methane to methanol and is encoded by *pmoCAB*. All known methanotrophs belong to the *Proteobacteria*, except for members of the NC10 phylum and a particular subgroup of the *Verrucomicrobia* [21].

***Methylocystis* sp. strain SC2.** It has long been believed that type I and type II methanotrophs possess a single type of pMMO. However, we showed that the type II methanotroph *Methylocystis* sp. strain SC2 contains two pMMO isozymes (pMMO1, pMMO2) with different methane oxidation kinetics. The conventional pMMO1 is expressed and oxidizes methane only at elevated and high concentrations (>600 ppmv). By contrast, pMMO2 is expressed and oxidizes methane at low mixing ratios, even at the trace level of atmospheric methane [22, 23]. Most type II methanotrophs possess *pmoCAB2*, encoding pMMO2 [24]. The existence of two pMMO isozymes with different thresholds for methane oxidation in type II methanotrophs, but not in type I methanotrophs, has important implications for our understanding of niche competition between members of these two major subgroups. The pMMO2 provides type II methanotrophs with a selective advantage, enabling them to survive under methane conditions at which most type I methanotrophs do not thrive, presumably <450-600 ppmv.

We obtained the complete genome sequence of strain SC2 [25, 26]. It possesses a circular chromosome of 3.77 Mb and two plasmids of 229.6 kb (pBSC2-1) and 143.5 kb (pBSC2-2) with an average GC content of 63, 61 and 60 %, respectively. All genes required for a methanotrophic lifestyle were identified. Presence of two identical copies of *pmoCAB1* and one copy of *pmoCAB2* was confirmed. In addition, we could detect three singleton *pmoC* paralogs, with one present in the plasmid pBSC2-2. Absence of genes encoding the soluble methane monooxygenase was validated. A large repertoire of genes involved in nitrogen metabolism was detected. This includes genes whose products are involved in transport and assimilation of ammonia, hydroxylamine detoxification, nitrogen fixation, and denitrification. The genome sequence of *Methylocystis* sp. strain SC2 provides a blueprint for its ability to thrive in environments with varying methane or nitrogen availability.

Strain SC2 possesses a complete denitrification pathway. Denitrification-mediated accumulation of 0.7 nmol $^{30}N_2$/hr/mg dry weight of cells under anoxic conditions was detected by tracer analysis. N_2 production was related to the activities of plasmid-borne nitric oxide and nitrous oxide reductases. Presence of a complete denitrification pathway in strain SC2, including the plasmid-encoded *nosRZDFYX* operon, is unique among known methanotrophs [27]. Most recently, we showed that *pmoCAB2* and genes involved hydroxylamine detoxification (*haoAB* encoding hydroxylamine oxidoreductase) are differentially expressed in response to increasing ammonium concentrations [see PDF file “Recent Research (Liesack 2012-2014)” for details].

Upland Soil Cluster alpha (USCα). Members of USCα are assumed to be methanotrophic bacteria specifically adapted to the trace level of atmospheric methane. These bacteria have escaped all cultivation attempts. While the 16S rRNA phylogeny of USCα is still not known, phylogenies constructed for *pmoA* placed USCα next to *M. acidiphila* B2. In order to assess whether the *pmoA* tree reflects the evolutionary identity of USCα, we obtained a 42-kb genomic contig of a USCα representative from acidic forest soil using a metagenomic approach. For comparison, a 101-kb genomic contig from *M. acidiphila* was analyzed, including the *pmoCAB* gene cluster. Several lines of evidence confirmed a close phylogenetic relationship between USCα and *M. acidiphila* [28]. The study also confirmed that atmospheric methane oxidation by members of USCα is most likely based on the well-known type of pMMO, encoded by *pmoCAB*.

More recently, we assessed (i) the activity and diversity of methane-oxidizing bacteria in glacier forefields of the Swiss Alps [29] and (ii) seasonal changes in atmospheric methane

oxidation and the underlying methanotrophic communities in grassland near Giessen (Germany). In the latter study, soil samples were taken from the surface layer (0–10 cm) of three sites in 2007 and 2008, along a soil moisture gradient. The sites showed seasonal differences in hydrological parameters. Net uptake rates varied seasonally between 0 and 70 µg CH_4 m^{-2} h^{-1}. Greatest uptake rates coincided with lowest soil moisture in spring and summer. Over all sites and seasons, the methanotrophic communities were dominated by USCα-like populations related to the RA14, MHP and JR1 clades of uncultured methanotrophs. Temperature was positively correlated with CH_4 uptake rates ($P<0.001$), but had no effect on methanotrophic population dynamics. Soil moisture was negatively correlated with CH_4 uptake rates ($P<0.001$), but showed a positive correlation with changes in USCα-like diversity ($P<0.001$) and *pmoA* gene abundance ($P<0.05$). These changes were greatest at low net CH_4 uptake rates during winter times and coincided with an overall increase in bacterial 16S rRNA gene abundances ($P<0.05$). Overall, soil moisture had a significant but opposed effect on CH_4 uptake rates and methanotrophic population dynamics. The latter was increasingly stimulated by soil moisture contents >50 vol% and primarily related to members of the MHP clade [30].

Microbial ecology of northern *Sphagnum*-dominated peatlands

This research is carried out in close collaboration with Dr. Svetlana N. Dedysh (Laboratory of Wetland Microbiology, Winogradsky Institute of Microbiology RAS, Moscow, Russia) and her group. The research has been funded by the Deutsche Forschungsgemeinschaft and the Russian Fund for Basic Research.

About half of the total annual flux of CH_4 to the atmosphere is contributed by wetlands. The massive northern wetlands account for 50% of the global wetland area and their most extensive type, as found in northern Europe, West Siberia, the United States, and Canada, is the acidic *Sphagnum* peat bogs, which have pH values from 3.5 to 5. Methanotrophic bacteria reduce the potential flux of CH_4 from these environments to the atmosphere.

Our initial research focused on the taxonomic characterization of two novel acidophilic methanotrophs, *Methylocella palustris* [31, 32] and *Methylocapsa acidiphila* [33]. *M. acidiphila* possesses pMMO like all other methanotrophs [34], while *M. palustris* was the first known methanotroph which possesses soluble methane monooxygenase but no pMMO. Later, additional members of *Methylocella* and *Methylocapsa* were reported by us and others (e.g., *Methylocella tundrae* [35]).

We assessed the abundance of *Methylocella*, *Methylocapsa*, and other methanotrophic populations in native *Sphagnum* peat by fluorescence *in situ* hybridization (FISH). Besides *Methylocella* and *Methylocapsa*, a distinct subgroup of *Methylocystis* was identified to be dominant and widely distributed in *Sphagnum*-dominated wetlands [36, 37]. A moderately acidophilic isolate of this numerically abundant and ecologically important *Methylocystis* population was recently shown to be able to grow slowly on acetate in the absence of methane (preferred sustrate), which may be a survival strategy of *Methylocystis* spp. in environments with varying methane availability such as peat bogs [38].

We studied the overall bacterial community composition in *Sphagnum*-dominared peatlands by combining 16S rRNA gene clone library analysis, rRNA-targeted FISH, and cultivation. *Alphaproteobacteria*, *Acidobacteria*, and *Planctomycetes* were the dominant groups. *Firmicutes* and *Bacteroidetes* were detected only with minor population sizes. Representatives

of the *Acidobacteria* and *Planctomycetes* were obtained in pure culture by a novel biofilm-mediated enrichment strategy [39]. More recently, we examined the culturability of members of the poorly characterized phylum *Acidobacteria*. Using low-nutrient media and FISH-mediated monitoring of the isolation procedure, we obtained nine acidobacterial strains in pure culture. Their phenotypes, including acidophily and low-temperature growth, are consistent with the occurrence of acidobacteria in cold and acidic northern wetlands [40, 41].

In order to assess the potential impact of global warming on microbial processes, we investigated temperature-induced changes in the activity and composition of the cellulolytic bacterial community in *Sphagnum* peat. Acidic *Sphagnum*-dominated ombrotrophic bogs are characterized by extremely low rates of plant debris decomposition. The degradation of cellulose, the major component of *Sphagnum*-derived litter, was almost undetectable at 10°C and occurred at low rates at 20°C, while it was significantly accelerated at both temperature regimes by the addition of available nitrogen. The indigenous bacterial community in peat was dominated by *Alphaproteobacteria* and *Acidobacteria*. The addition of cellulose induced a clear shift in the community structure towards an increase in the relative abundance of the *Bacteroidetes*. Increasing temperature and nitrogen availability resulted in a selective development of bacteria phylogenetically related to *Cytophaga hutchinsonii*. Among isolates obtained from the native community only some subdivision 1 *Acidobacteria* were capable of degrading cellulose, albeit at a very slow rate. These acidobacteria represent indigenous cellulolytic members of the microbial community in acidic peat and are easily out-competed by *Cytophaga*-like bacteria under conditions of increased nitrogen availability. Members of the phylum *Firmicutes*, known to be key players in cellulose degradation in neutral habitats, were not detected in the cellulolytic community enriched at low pH [42].

Most recently, we compared the bacterial community composition in the surface (0–5 cm depth) and subsurface (45–50 cm) peat layers of an acidic (pH 4.0) *Sphagnum*-dominated wetland, using 454 pyrosequencing of 16S rRNA genes [see PDF file “Recent Research (Liesack 2012-2014)” for details].

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