Ecological functions and environmental fate of exopolymers of Acidobacteria

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Chapter 7

General Discussion

Acidobacteria is a phylum widely distributed in several soil types but their functional roles in ecosystem processes are still largely elusive. At present, cultured representatives of the 15 recently defined classes are available only for *Acidobacteriia*, *Blastocatellia*, *Holophagae*, *Vicinamibacteria* and *Thermoanaerobaculia* (Dedysh & Yilmaz, 2018). Therefore, most of the information and studies on the characterization of *Acidobacteria* are derived from subdivision 1 (class *Acidobacteriia*), which contains the majority of cultivated species to date. The overall goal of this thesis was to investigate and understand the metabolism of two strains belonging to the genus *Granulicella* (class *Acidobacteriia*), and the functions and environmental fate of extracellular polymeric substances (WH15EPS). The *Granulicella* genus is known for the copious production of EPS (Pankratov & Dedysh, 2010), and the EPS of our strains were chemically and physically characterized previously, showing interesting emulsification properties (Kielak *et al.*, 2017). The main objectives of my thesis were to i) study the responses *Granulicella* sp. strain WH15 to cellobiose as carbon source; ii) investigate the impact of manganese (Mn) and other trace elements, such as Fe, Zn, Cu, Co, Ni, B and Mo, on the growth and metabolism of *Granulicella* sp. strains WH15 and 5B5; iii) explore the assimilation of EPS of *Granulicella* sp. strainWH15 by other soil bacteria derived from the same environment where WH15 was isolated from; and iv) evaluate the potential of WH15EPS for enrichment of microbes, especially yet unclassified bacteria, capable of EPS degradation in culture medium. Here, the major findings of my thesis are discussed and future perspectives for studying the ecology of *Acidobacteria* are presented.

Culture media optimization and metabolism of *Granulicella*

One of the major hurdles of studying *Acidobacteria* is their isolation and propagation in culture media. Since they are slow growers, it can take weeks to months to develop colonies (Eichorst *et al.*, 2011, de Castro *et al.*, 2013). Recently, changes in traditional culture methods and the use of unconventional culture media composition have increased the number of *Acidobacteria* isolates that can be cultured in the lab. Currently, 62 species have been described compared to only 14 species in 2011 (de Castro, 2011). Media modifications include low concentration of nutrients (Janssen *et al.*, 2002, Stevenson *et al.*, 2004), unusual or complex polysaccharides as carbon sources (Pankratov *et al.*, 2008, Eichorst *et al.*, 2011), longer incubation periods (de Castro *et al.*, 2013), addition of humic acids and quorum-sensing molecules (Stevenson *et al.*, 2004), and the employment of soil solutions and inhibitors for unwanted microorganisms (de Castro *et al.*, 2013, Foesel *et al.*, 2013).

Once the isolates are obtained, the utilization of richer culture media, with higher concentrations of carbon and other nutrients can be applied for better cell proliferation (de Castro *et al.*, 2013, Kielak *et al.*, 2016). Campanharo *et al*. (2016) optimized a culture medium in order to improve growth of the *Acidobacteria* subdivision 1 strains used in my thesis, i.e. *Granulicella* strains WH15 and 5B5. In the solid culture medium PSYL 5 (**P**hosphate, **S**ucrose,

Yeast extract, **L**iquid medium, pH **5**), the strains produced visible colonies after around 3 days of incubation, a much shorter time frame as compared to earlier studies where *Acidobacteria* strains took 14-168 days to develop colonies (Eichorst *et al.*, 2011).

In order to study these strains and their EPS, further optimizations of the culture medium and growth conditions for extractable EPS yield were necessary. Our experiments showed that the agar brand was important for growth and EPS production in the laboratory. Initial experiments showed that, using Sigma Agar A1296-500, strain WH15 did not grow well and strain 5B5 was not able to grow at all. Our results demonstrated that both strains performed the best in BD agar (Figure 1). It is a common knowledge in laboratory procedures that the purity of the reagents is important for the reproducibility of results, since minor differences in medium composition can impact the growth of microorganisms (Atlas, 2010). However, few studies addressed the differences between agar brands, composition and preparation. For instance, Bosmans *et al.* (2016) showed that calcium content of the agar strongly affected antimicrobial activity of Firmicutes strain ST15.15/036 against *Agrobacterium*. Their study also addressed the metal ion composition of the different agar brands, showing that the products I used in this study had varied metal ion concentration, which could be affecting the performance of our strains in solid medium. Therefore, we tested if the addition of the trace element solution SL10 (described in chapter 4) to PSYL5 could enhance the growth of our *Granulicella* strains on media with the different agar brands. Indeed, the addition of SL10 (1X and 10X concentrations) enhanced bacterial growth, especially for Sigma (Figure1d) and Fluka (Figure 1e) brands, showing that the metal ion content of these agar brands was not fulfilling the requirements for the growth of our strains. In order to identify which component(s) was responsible for the growth enhancement, further experiments with the addition of metal ions separately were performed. Nevertheless, the best option was still BD agar (Figure 1c and 1f), since no extra requirements for bacterial development were necessary (Table 1).

Table 1: Summary of *Granulicella* strains growth and EPS production in culture medium containing different agar sources at different concentrations of SL10 solution in two temperatures.

	20°C		**30°C**	
	WH15	**5B5**	**WH15**	**5B5**
Agar brand	**PSYL 5 (growth/ EPS)**	**PSYL5 (growth/EPS)**	**PSYL 5 (growth/ EPS)**	**PSYL5 (growth/EPS)**
Sigma	**+/+**	**-/-**	--	--
Sigma 1X	**+++/+**	**+/+**	**+++/+**	**+/+**
Sigma 10X	**+++/+++**	**++/+**	**+++/+++**	**+++/++**
Fluka	**++/++**	**+/+**	**++/++**	**+/+**
Fluka 1X	**+++/++**	**++/+**	**+++/+**	**+++/+**
Fluka 10X	**+++/++**	**++/+**	**+++/++**	**+++/+**
BD	**+++/++**	**+++/+**	**+++/+++**	**+++/+**
BD 1X	**+++/++**	**+++/+**	**+++/+++**	**+++/+**
BD 10X	**+++/++**	**+++/++**	**+++/+++**	**+++/++**

1X: trace element solution SL10 1ml/l; 10X: trace element solution SL10 10 ml/l.

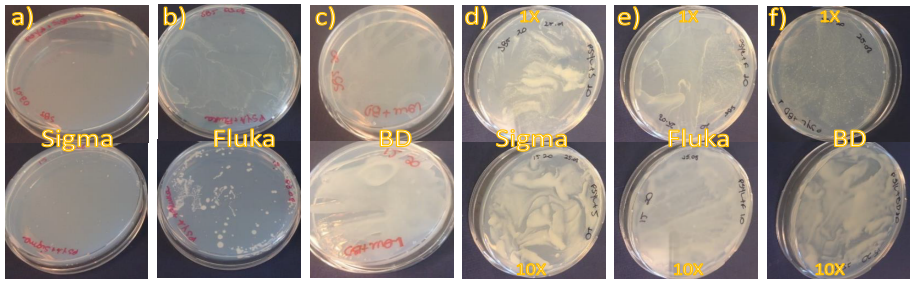

Figure 1: Growth of *Granulicella* strains 5B5 (top) and WH15 (bottom) in PSYL5 culture medium containing different agar brands after incubation for 15 days at 20 ^{0}C. a) Sigma agar, b) Fluka agar c) BD agar. Growth of both strains after the addition of trace element solution and incubation for 15 days at 20 ^{0}C. d) Sigma agar, e) Fluka agar, f) BD agar; 1X: trace element solution SL10 1ml/l; 10X: trace element solution SL10 10 ml/l.

Culture media optimization demonstrated that both strains performed better when growing in culture media with higher carbon concentration. The two strains were originally isolated from wood-decay material using a 10X diluted culture medium TSB (3g of sugar) (Valášková *et al.*, 2009), while the optimized culture medium PSYL5 had a 10 times higher quantity of carbon (30g of sugar). In chapter 3, I investigated the impact of two concentrations of carbon source (0.025 g and 30g of cellobiose) on the metabolism of *Granulicella* sp. WH15 strain using a multi-omics approach. Firstly, we sequenced the complete genome of strain WH15, which has large number of genes dedicated to carbohydrate metabolism, involving the utilization of various carbon sources, hydrolysis of complex polysaccharides and biosynthesis of exopolymers (EPS) corroborating with other published *Granulicella* strains (Pankratov & Dedysh, 2010). The impact of higher concentrations in the metabolism of *Granulicella*, however, has not been studied previously. My proteomic and transcriptomic data analyses indicated that the higher cellobiose concentration, after 3 days of incubation, triggered a stress response in the strain studied. The transcriptomics profile of strain WH15 under high cellobiose concentration revealed the expression of several stress proteins, such as factor σW and toxin-antitoxin (TA) system HigAB, which in other bacterial genera are involved in responses to environmental stress stimuli, such as salt stress, presence of detergents (Zweers *et al.*, 2012), nutritional stress, heat shock and others (Tachdjian & Kelly, 2006, Jorgensen *et al.*, 2008). In addition, genes linked to osmoprotection were upregulated, such as *gfo4* (the glucose-fructose oxidoreductase), and glycerol transporter *glpF*, which could be important to balance the impact of the cellobiose quantity in the culture medium. The proteomic profile of strain WH15 at high cellobiose concentration was consistent with the transcriptomic response, revealing several membrane efflux pumps and transporter proteins, which could be involved in cell wall osmotic protection mechanisms. *Acidobacteria* in general possess a large variety of transporters involved in the acquisition and secretion of a wide range of substrates, which is suggested to be an adaptation advantage especially in low nutrient environments (Kielak *et al.*, 2016). Furthermore, I showed that higher cellobiose concentrations caused the

repression of pathways related to the metabolism of other carbon sources, especially the pentose phosphate (PP) pathway. Those results suggest that, *Granulicella* sp. strain WH15 reacts to higher carbon concentrations by upregulating genes associated with the transport of toxic compounds and by reallocating resources for cell maintenance of basic metabolism instead of growth. However, the mechanisms underlying adaptation towards enhanced growth still have to be addressed in further studies.

Another important question raised by bacterial growth optimization procedures was the impact of the trace elements on the metabolism of *Granulicella* sp. WH15 and 5B5 strains. Both strains were isolated from an environment (wood in late stage of decomposition) containing high amounts of manganese (chapter 5) ~17 times higher than the average observed in normal local soil without litter (5.8-8.0 mg Mn/Kg). Hence, in chapter 4, I examined the effects of trace element solution SL10 on the growth of both WH15 and 5B5 strains. The addition of the trace element solution had a clear impact in the growth yield of our strains, which responded similarly to 1X concentration of SL10 solution. However, the higher concentration of SL10 suppressed the growth of WH15 strain, while still enhancing the growth of 5B5 strain, highlighting differences in growth responses of the two strains. In order to identify the metal ion with the strongest effect on bacterial growth, I performed separate experiments for each of the 8 metal ions present in the SL10 solution. The analysis showed that only manganese (Mn) affected positively the growth of both strains, leading to a further investigation of their proteomic profile upon Mn exposure. Interestingly, the results did not show overexpression of any specific pathway, demonstrating that the presence of Mn did not have a clear impact in the regulation of protein expression. Nonetheless, higher number of upregulated proteins that can use Mn^{2+} as co-factor was detected, as well as several candidate transporters that could be involved in Mn homeostasis of *Granulicella*.

Both our strains showed tolerance to Mn at a level that is comparable to *Serratia* strains isolated from Mn water mines in Brazil (Barboza *et al.*, 2017). Even though *Acidobacteria* were already associated to heavy-metal contaminated sites in culture-independent surveys (Gremion *et al.*, 2003, Guo *et al.*, 2017), no study to date addressed heavy metal resistance of acidobacterial isolates. Nonetheless, the resistance to higher Mn concentrations, in comparison to other metal ions, presumably is linked to the survival of both *Granulicella* strains in decaying wood material. White rot fungi, for instance *Hypholoma fasciculare,* found in the same environment as our strains, can create deposits of Mn oxides in degraded wood (Blanchette, 1984).

Mn is an important co-factor of enzymes involved sugar, lipid and protein metabolism (Jensen & Jensen, 2014). The presence of Mn may have, for instance, enhanced *Granulicella* strains growth by improving metabolic activities linked to cell cycle and division. In addition, Mn can be a protection factor against reactive oxygen species (Jakubovics & Jenkinson, 2001, Jensen & Jensen, 2014) generated by fungal peroxidases and phenol oxidases during degradation of lignin and cellulose (Kielak *et al.*, 2016), that would further support our hypothesis that our

strains have a competitive advantage in this environment, not only tolerating Mn but also using the metal for protection against reactive oxygen species.
Furthermore, the gene for the most common transcriptional Mn homeostasis regulator, *mntR*, could not be detected in the genome, suggesting that manganese homeostasis in these two *Granulicella* strains is under the control of a different regulator. In these analyses, I identified a wide range of transporters that could be involved in metal ion homeostasis for both *Granulicella* strains. It was already observed that strains of *Acidobacteria* have a large proportion of their genomes dedicated to transport (Kielak et al., 2016). Most of the genes are related to drug/metabolite transport; however, a broad range of substrate categories can be observed, suggesting that *Acidobacteria* may have advantages in nutrient uptake especially in oligotrophic, nutrient-limited conditions (Kielak *et al.*, 2016). Efflux is one of the key mechanisms that bacteria have developed to tolerate the presence of metal ions (Porcheron *et al.*, 2013). Along with potential transporters involved in manganese homeostasis, such as metal translocating P-type ATPase copA and metal ABC transporter ATP-binding protein troB, I detected 78 genes in strain WH15 and 23 genes in strain 5B5 that are candidates for the transportation of a diverse range of metal ions, such as Cu, Ag, As, Zn. Even though the strains did not show a high resistance to most of the metals in culture medium, further tests in liquid culture media with absorbance measurements should be performed in order to examine the range of metal resistance of our *Granulicella* strains and determine their minimum inhibitory concentration (MIC). Combining the results of chapter 3 and chapter 4, I showed that our strains are equipped with several types of transporters, which could be involved not only in nutrient uptake but also in detoxification and resistance important for their survival in soil and wood decay environments.
The growth optimization tests that I have carried out in chapters 3 and 4 allowed for improvements in obtaining higher bacterial growth yields, as well as enhanced EPS production, steps that were crucial for further examination of the ecological functions and environmental fate of *Granulicella* EPS. Furthermore, the investigations I performed in chapters 3 and 4 demonstrated that *Granulicella* strains are equipped with several features, namely tolerance to higher concentrations of carbon sources and Mn, presence of several types of glycosyde hydrolases and a wide range of transporters, which might be fundamental characteristics for their development and prevalence in soil environments.

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Granulicella EPS – ecological role and application

An additional bacterial trait associated with protection against harsh environments is the production of extracellular polymeric substances (EPS). *Granulicella* species are known to produce large amounts of EPS (Pankratov & Dedysh, 2010), biopolymers with protective capabilities against antimicrobial compounds and environmental stresses such as drought (Souli & Giamarellou, 1998, Krembs *et al.*, 2011, Upadhyay *et al.*, 2011). Microbial EPS typically

consist of mucoid, viscous substances that mediate contact and exchange of nutrients among cells and the environment, as well as providing ideal moisture conditions for enzymatic reactions (Wingender *et al.*, 1999). EPS protective capacity was reported for several types of environmental stresses, such as drought, temperature (Bhatnagar *et al.*, 2014), pH, salinity, metal ions (Upadhyay *et al.*, 2011, Upadhyay *et al.*, 2017), as well as antimicrobial compounds (Davenport *et al.*, 2014). The main components of EPS are carbohydrates (Flemming & Wingender, 2010), yet few studies addressed the importance of EPS as carbon source. To this end, I applied Stable Isotope probing (SIP) coupled to rRNA gene sequencing to identify microbes capable of using EPS as a carbon source (Chapter 5). I used WH15EPS, since strain WH15 produces higher amounts of EPS in the laboratory than strain 5B5. The characterization of WH15EPS showed that the biopolymer is composed of xylose, mannose, glucose, galactose, rhamnose, glucuronic and galacturonic acids (Kielak *et al.*, 2017). Hence, metabolization of WH15EPS would demand a variety of glycoside hydrolases. I showed that WH15EPS enriched for a specific microbial community from the topsoil litter and I identified the main EPS metabolizers within 3 timepoints. After 35 days of incubation, the addition of WH15EPS to soil enriched mostly bacteria belonging to the phylum *Planctomycetes*, especially *Singulisphaera*. Interestingly, *Planctomycetes*, similar to *Acidobacteria*, possess few isolated representatives due to difficulties in cultivation. However, genomic studies of the available isolates, as well as cultivation-independent studies identified a wide range of glycosyl hydrolases in *Planctomycetes*, enzymes involved in degrading complex polysaccharides (Ivanova *et al.*, 2017, Ivanova *et al.*, 2017). Unclassified *Planctomycetes* were also enriched in a similar study by Wang *et al.* (2015) in which the authors applied a microbial biopolymer with a different composition to soil. In addition, we also observed the enrichment of *Rhodanobacter,* a bacterial genus associated with the degradation of aromatic compounds (Uhlik *et al.*, 2012), and other poorly characterized groups, such as *Pedospheraceae*, which belongs to the phylum *Verrucomicrobia* (Spring *et al.*, 2016). Among the fungal genera, I detected *Trichoderma, Scleroderma* and *Mortierella* and also several ectomycorrhizal fungi, known producers of extracellular enzymes for organic material decomposition (Valášková *et al.*, 2009, Urbanová *et al.*, 2015).

Co-occurrence analysis demonstrated that *Singulisphaera* had potential positive interactions with other bacterial phyla, suggesting cooperation with other bacteria for the metabolization of WH15EPS, and some negative connections, possibly due to competition with fungal genera. Even though there is no experimental evidence that strain WH15 can use its own EPS as a carbon source, *Granulicella* was detected in the labeled treatment. It should be emphasized that our co-occurrence network analysis should be evaluated cautiously. I propose that more targeted metabolic studies can be more insightful for the generation of ecological inferences, while only pure evaluation of total DNA content of samples can yield misleading interpretations. Furthermore, studies show that the total DNA pool of an environment can contain non-active and dormant microorganisms, masking the more active components of

microbial communities (Kuramae *et al.*, 2013, Harkes *et al.*, 2019, Lupatini *et al.*, 2019).
In addition to the microbial taxonomic classification, SIP allows targeting functions associated with the incorporation of the labeled material. With this in mind, I analyzed metagenomics sequences originated from the SIP experiment of chapter 5 in order to find interesting genes that encoded enzymes involved in the hydrolysis of WH15EPS (Chapter 6). I also employed WH15EPS as an enrichment factor and carbon source in culture medium, to increase the number of unidentified microorganisms. Taxonomic analyses of the SIP metagenomics dataset were consistent with those from chapter 5, showing an increase in the abundance of *Planctomycetes*. In addition, I also observed a high abundance of sequences affiliated to the phyla *Proteobacteria*, *Actinobacteria* and *Acidobacteria*, which are widely known to be involved in carbon degradation processes, such as degradation and assimilation of cellulose, xylan, pectin and chitin (Haichar *et al.*, 2007, Kielak *et al.*, 2016). On the other hand, the culture-based metagenomics showed the predominance of *Proteobacteria*, which are usually easier to isolate from environmental samples in culture medium, but also a high abundance of unclassified bacteria, demonstrating the potential of EPS for the enrichment and isolation of previously uncultivated microbes. Futhermore, the culture-based metagenomics dataset allowed the assembly of 4 metagenome-assembled genomes (MAGs) of unclassified *Proteobacteria*. Besides unknown microbes, I was able to isolate 201 strains of bacteria and fungi, among them 143 *Dyella*, 27 *Burkholderia*, 11 *Pseudomonas*, 1 *Mucilaginibacter, 2 Buttiauxella*, 4 *Rahnella*, 3 *Enterobacter,* 2 *Penicillium,* 1 *Trichoderma* and 1 *Pseudogymnoascus,* which will be tested in further studies for enzymatic activity.
Collectively, these results confirm and extend the study of Verastegui *et al.* (2014), in which the presence of a complex polysaccharide promoted the enrichment of microorganisms capable of breaking down several plant-derived carbohydrates. In further functional analyses, I showed that the microbial community enriched in both cultivation dependent and independent approaches was abundant with carbohydrate-associated enzymes (CAZymes) with potential applications in diverse sectors of industry. Overall, the most abundant CAZyme families were glycosyl transferases, which can be explained by the high proportion (~1.5% of total genes) of GTs in the genomes of most microorganisms in general (Lairson *et al.*, 2008). GTs are not widely explored in industries since their application requires activated donor precursors (Yuan *et al.*, 2018). However, they can be employed for manufacturing of modified natural products with medical applications, such as anticancer compounds (Schmid *et al.*, 2016).
Overall, I showed the presence of 310 CAZyme families, from which 38.4% (119) were GH families. Even though the potential enzymes might belong to slow growing microorganisms in laboratory conditions, such as *Acidobacteria*, *Planctomyctes* and *Verrucomicrobia*, sequences can still be targeted for further heterologous expression and characterization. Within the most abundant GHs, I found families of amylases, acetylgalactosaminidases, glucuronidases, agarases, invertases and endoglucanases. For instance, amylases are widely employed in food industry, where they can be used for production of glucose and maltose

syrups, reduction of viscosity of syrups, production of clarified fruit juices, solubilization of starch for brewing processes and manufacture of baked products (Liu & Kokare, 2017). Other GHs can be applied for the production of oligosaccharides with antioxidant activities for food, pharmaceutical and cosmetic industries (Fu & Kim, 2010), as well as be used in biological control, bioremediation processes (Dahiya *et al.*, 2005), production of biofuels and paper (McKee *et al.*, 2016). Another important application of GHs is the removal or inhibition of biofilm. Amylase and cellulose solutions have already been used to inhibit biofilm formation of *S. aureus* and *P. aeruginosa* decreasing significantly bacterial biomass and increasing the effectiveness of antibiotics treatments (Craigen, 2011, Flemming *et al.*, 2016).

In chapters 5 and 6, I showed that WH15EPS can be a potential carbon source for a diverse range of microorganisms, suggesting direct and indirect associations between *Granulicella* and other soil bacteria, especially *Singulisphaera*. I demonstrated that WH15EPS can be used as an enrichment factor for the isolation of known and unknown microbes with biotechnological potential, as well as for targeting sequences of a wide range of CAZYmes. In conclusion, I showed that targeted metabolic studies can be effective to unravel microbial interactions and reinforce characterization of these groups to better understand their ecological functions in the environment.

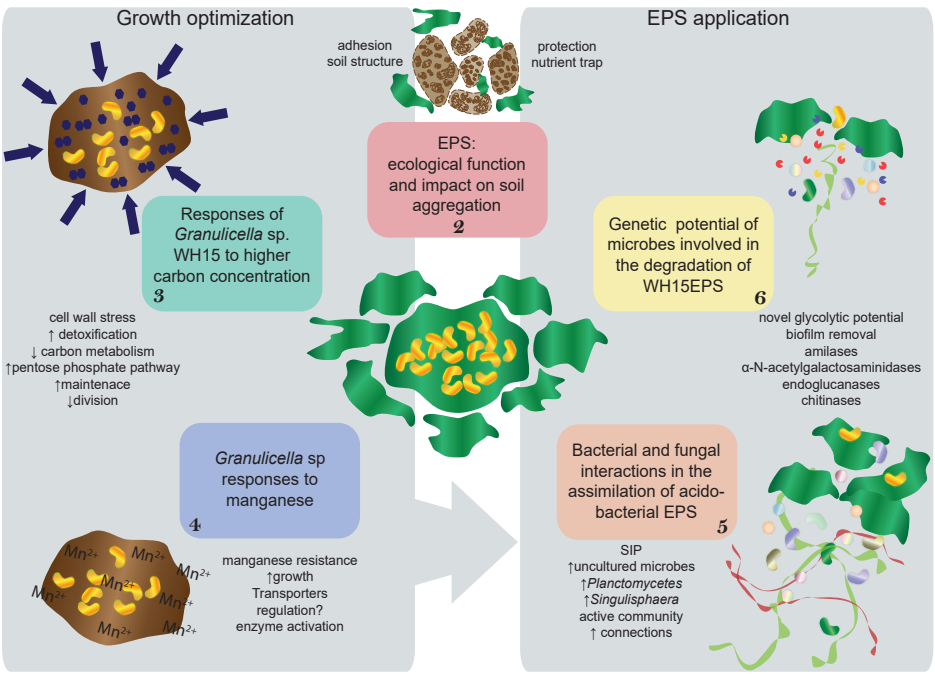

Figure 2:: Schematic overview of the main topics and most important findings described in this thesis.

Concluding remarks and future prospects

Our understanding of *Acidobacteria* has gradually increased throughout the years, but there is still a huge knowledge gap, especially concerning subdivisions with no cultivated representatives. To bridge this gap, I studied two strains of *Granulicella*, *Acidobacteria* belonging to the subgroup 1, class *Acidobacteriia*. This is the first time that the impact of trace elements and carbon source concentration on the metabolism of *Acidobacteria* strains was tested with different 'omics approaches. With my research, I showed that trace elements, especially Mn, are important for the growth of *Granulicellla* strains and can be used to enhance their growth in culture medium. Together with a higher carbon concentration, the addition of trace elements is necessary for improving *Acidobacteria* growth and can be used in future research for isolation and propagation of other *Acidobacteria* strains.

I further demonstrated that our strains harbour a wide range of transporters involved in nutrient transport and detoxification, which can play a role in surviving stressful and harsh conditions. In addition, the transporters can be involved in homeostasis and resistance to the high Mn concentrations released by fungi in wood decomposition environments, the natural habitat from where they were isolated. The variety of transporters found in the genomes and proteomes of our *Granulicella* strains suggest they contribute to stress resistance of several *Acidobacteria*. Further studies, however, are needed to validate these hypotheses.

The research presented in this thesis highlights the importance of targeted metabolic studies to disentangle potential microbial interactions and unknown functions. In this context, the application of modern techniques, such as nanoscale secondary ion mass spectrometry (nanoSIMS) and DNA-SIP coupled to high-throughput sequencing can be useful for unraveling further metabolic mechanisms and interactions among microbes *in situ*. Additional studies can be performed involving isolated strains between which we found connections, such as *Granulicella* and *Singulisphaera*, evaluating the interactions of these genera during the metabolization of labeled carbon and nitrogen sources, such as glucose, cellulose or chitin, for instance. Furthermore DNA-SIP can be applied for the study of other microbial EPS in an ecological context. In general, the cultivation of novel microbes is still necessary, since it allows their functional characterization and application in studies that include pairwise or group interactions, either in culture media or in environmental samples. Furthermore, the isolation of new species belonging to *Acidobacteria* classes that do not have cultured representatives is fundamental for the exploration of their functions, since the variation within the phylum is broad, not allowing generalizations (Kielak *et al.*, 2016).

In this thesis I showed that the use of a complex polymer (WH15EPS) enriched microbial communities in unclassified microbes using culture-dependent and independent approaches; therefore, WH15EPS and other microbial biopolymers with different compositions may be an interesting source of variation for the cultivation of new species. Furthermore, the analysis of new metagenome-assembled genomes may provide novel information that could be applied for their isolation. The isolation of unclassified microbes may rely in the development of new

cultivation techniques, but also in the use of unconventional compounds in the preparation of culture media, as well as different growth conditions or the combination of both.

Using WH15EPS as an enrichment factor, I showed that *Acidobacteria* and other slow-growing phyla have an unexplored genetic and biotechnological potential that can be investigated through heterologous studies, not needing their cultivation in laboratory. My results indicated that microorganisms that incorporated and used WH15EPS as a carbon source have a wide glycolytic potential, possessing genes involved in the degradation of several complex polymers. I found several enzymes with potential applications in industry and the extent of their efficiency can be evaluated in further studies. Gene sequences belonging to enzymes of interest in metagenome data can be recovered from assembled metagenome data and used for expression. In addition, labeled DNA can be used for library construction and functional screening (Verastegui *et al.*, 2014), which also can be performed for the microorganisms I isolated. Overall, my findings in this thesis demonstrate that *Granulicella* are resourceful bacteria, which produce EPS with diverse ecological functions and a wide range of applications. Furthermore, the use of EPS allowed the enrichment and cultivation of unclassified *Proteobacteria*, demonstrating that even the biotechnological potential of well studied phyla is far from being fully explored.

In conclusion, the data presented in this thesis establish a solid fundamental basis for more mechanistic studies of *Acidobacteria* and other uncultivated microbes. Here I list questions to be answered in future studies on this high abundant and ubiquitous unexplored group of bacteria, *Acidobacteria* inhibiting soil ecosystems. More studies are needed to address the role of Mn in protection against oxidative stress and gene regulation of Mn homeostasis in *Granulicella* and other *Acidobacteria* strains. In this thesis we observed that *Planctomycetes* are capable to incorporate WH15EPS. Thus, we raise questions related to the potential interactions of *Acidobacteria* with other soil microbes. Does *Granulicella* produce EPS in presence of *Singulisphaera*, in laboratory and field conditions? How is the interaction between *Granulicella* and *Singulisphaera* during the degradation of cellulose or hemicellulose? Do they compete or collaborate? Is the dynamics of interaction dependent on nutrient source or concentration? Those are interesting questions that, once addressed will expand further our knowledge on *Acidobacteria*, providing more insights into their ecological roles and the survival strategies of this yet underexplored bacterial phylum, the interactions of these bacteria with other soil dwelling microbes, and possible biotechnological applications of their genes and their biopolymers.