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Ecological functions and environmental fate of exopolymers of Acidobacteria

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Chapter 1

General Introduction and Thesis Outline

Ohana Y. A. Costa & Eiko E. Kuramae

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1. Phylum *Acidobacteria*

Acidobacteria is a ubiquitous and abundant bacterial phylum in soil, but the factors underlying their ecological prevalence in the soil ecosystem remain unclear. This lack of fundamental knowledge is largely due to difficulties to isolate *Acidobacteria* and their slow growth *in vitro* (Kielak *et al.*, 2016). However, non-culturable approaches, mainly 16S rRNA-based sequence surveys, have revealed that *Acidobacteria* are metabolically diverse and widely distributed. Most *Acidobacteria* appear to be aerobes, but some can grow under reduced oxygen conditions (1%–2% O_2) (Eichorst *et al.*, 2018). The diversity and abundance of *Acidobacteria* have been reported in a variety of sites, such as diverse agricultural (Navarrete *et al.*, 2013) and contaminated soils (Wang *et al.*, 2016), sediments (Liao *et al.*, 2019), forest soils (Štursová *et al.*, 2012), peatland (Pankratov *et al.*, 2008), various water systems (Izumi *et al.*, 2012, López-López *et al.*, 2015), acid mine drainage (Wegner & Liesack, 2017) and surfaces of Paleolithic caves and catacombs (Schabereiter-Gurtner *et al.*, 2002, Zimmermann *et al.*, 2005). The few sequenced genomes of *Acidobacteria* indicate a broad substrate range of ABC transporters for nutrient uptake, suggesting an advantage of *Acidobacteria* in complex environments and adaptation to oligotrophic conditions, such as nutrient-limited soil conditions (Kielak *et al.*, 2016).

Acidobacteria form as much as 50% of the total soil bacterial community based on 16S rRNA gene phylogenetic sequence surveys (Pereira de Castro *et al.*, 2016) and compose on average 20% of the total microbial community in soils around the world (Janssen, 2006). Three subdivisions are particularly abundant in soils: class *Acidobacteriia* (former subdivision 1), class *Blastocatellia* (former subdivision 4) and subdivision 6. At present, members of class *Acidobacteriia* are the most readily culturable under laboratory conditions. Together with subdivision 3, *Acidobacteriia* are the most abundant groups in soils (Barns *et al.*, 1999, Janssen, 2006).

The existence of the phylum *Acidobacteria* was first recognized in 16S rRNA gene sequence-based studies, which revealed that *Acidobacteria* and *Proteobacteria* were the predominant phyla in diverse soil environments (Kielak *et al.*, 2016). It was predicted that *Acidobacteria* would be as diverse as the widely studied phylum *Proteobacteria* (Hugenholtz *et al.*, 1998). Only 4–5 subdivisions were initially described in 1997 (Kuske *et al.*, 1997, Ludwig *et al.*, 1997), a number that increased to 8 subdivisions in 1998 (Hugenholtz *et al.*, 1998) and 11 in 2005 (Zimmermann *et al.*, 2005). The diversity and phylogeny of *Acidobacteria* currently encompasses 26 known subdivisions (Figure 1) belonging to eleven described families: *Acidobacteriaceae*, *Bryobacteraceae* (within class *Acidobacteriia*), *Blastocatellaceae*, *Pyrinomonadaceae, Arenimicrobiaceae* (within class *Blastocatellia*), *Acanthopleuribacteraceae*, *Holophagaceae, Thermotomaculaceae* (within class *Holophagae*), *Vicinamibacteraceae* (within class *Vicinamibacteria*) and *Thermoanaerobaculales* (within class *Thermoanaerobaculia*).

Despite their high abundance in several environments, only 66 *Acidobacteria* species have been described so far. The first isolate belonging to this phylum was *Acidobacterium*

capsulatum, from which the name of the phylum was derived; the genus *Acidobacterium* was first proposed in 1991 for acidophilic, chemoorganotrophic bacteria isolated from an acidic mineral environment (Kishimoto *et al.*, 1991).

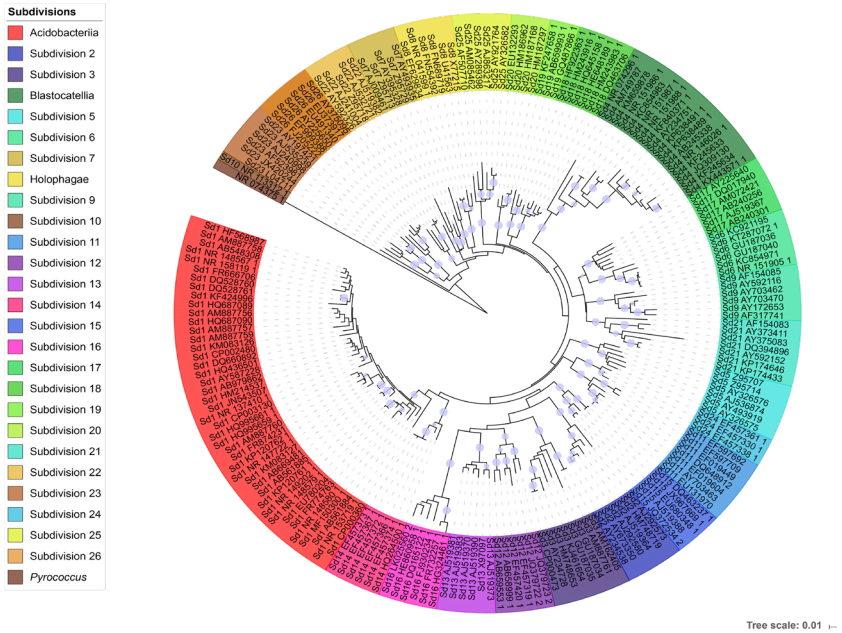

Figure 1: Dendogram of *Acidobacteria* subdivisions. The dendrogram was constructed using 16S rRNA gene sequences from 26 *Acidobacteria* subdivisions downloaded from the RDP 11 and NCBI databases. The sequences were aligned using the tool align.seqs in the software Mothur (Schloss et al., 2009) against Silva database version 132 (Quast et al., 2012). The software Mega 7 (Kumar et al., 2016) was used to build the dendrogram based on the neighbour-joining method with 1000 bootstraps. The circles represent bootstrap values above 0.75. Archaeon *Pyrococcus furiosus* was used as an outgroup.

Most *Acidobacteria* species belong to two classes: *Acidobacteriia* (46 species) and *Blastocatellia* (13 species), while four species belong to *Holophagae*, two to class *Vicinamibacteria*, and one species to class *Thermoanaerobaculia*. Two *Acidobacteria* isolates belong to *Candidatus* genera: '*Ca.* Koribacter', '*Ca.* Solibacter' (*Acidobacteriia*) and '*Ca.* Chloracidobacterium' (*Blastocatellia*). In 2018, three new *Candidatus* genera with features of dissimilatory sulfur metabolism were proposed based on metagenome-assembled genomes: '*Ca*. Sulfotelmatobacter', '*Ca*. Sulfotelmatomonas' and 'Ca. *Sulfopaludibacter*' (Class *Acidobacteriia*) (Hausmann *et al.*, 2018).

Recently, Dedysh and Yilmaz (2018) proposed a refinement to taxonomy of *Acidobacteria.* Based on 16S rRNA gene sequences, the authors distributed the 26 subdivisions to 15 class-level divisions, from which only 5 contain described members. Class *Acidobacteriia* contain subdivisions 1, 2, 3, 5, 11, 12, 13, 14, 15 and 24; class *Blastocatellia* contains subdivision 4; class *Vicinamibacteria* contains subdivisions 6, 9 and 17; class *Holophagae* consists of subdivisions

8 and 22, and class *Thermoanaerobaculia* contains subdivision 23.

The genus *Acidobacterium* (Kishimoto *et al.*, 1991) belongs to the family *Acidobacteriaceae* (subdivision 1), which also contains the genera *Edaphobacter* (Koch *et al.*, 2008), *Terriglobus* (Eichorst *et al.*, 2007), *Acidicapsa* (Kulichevskaya *et al.*, 2012), *Acidipila* (Okamura *et al.*, 2011), *Bryocella* (Dedysh *et al.*, 2012), *Granulicella* (Pankratov & Dedysh, 2010), *Occallatibacter* (Foesel *et al.*, 2016), *Telmatobacter* (Pankratov *et al.*, 2012), *Terracidiphilus* (García-Fraile *et al.*, 2016), *Silvibacterium* (Lladó *et al.*, 2016) and '*Candidatus* Koribacter' (Ward *et al.*, 2009). These bacteria are gram-negative chemoorganothrophs, with prevalent capsule formation and variable motility. They are aerobic or facultatively anaerobic and mostly mesophiles, although some are cold adapted. The members of this family use sugars as favorite source of carbon and energy and are able to degrade complex carbohydrates. Their genomic G+C content varies from 51.7 to 62.1% (Thrash & Coates, 2014, Foesel *et al.*, 2016).

The family *Bryobacteraceae* (subdivision 3) is formed by the genera *Bryobacter* (Kulichevskaya *et al.*, 2010), *Paludibaculum* (Kulichevskaya *et al.*, 2014) and '*Candidatus* Solibacter' (Ward *et al.*, 2009). These bacteria are chemoheterothrophic, gram negative, non-spore forming rods that are aerobes and facultative anaerobes and can use various sugars as growth substrates. In addition, members are mildly acidophilic, mesophilic and psychrotolerant. Their genomic G+C content varies from 55.5 to 61.9% (Dedysh *et al.*, 2017).

The family *Blastocatellaceae* (subdivision 4) contains the genera *Blastocatella* (Foesel *et al.*, 2013), *Aridibacter* (Huber *et al.*, 2014), *Tellurimicrobium* and *Stenotrophobacter* (Pascual *et al.*, 2015). The members of this family are gram-negative, non-spore forming, non-capsule forming bacteria. In addition, these aerobic bacteria are unable to reduce nitrate or ferment glucose and are slightly acidophilic to neutrophilic mesophiles, with a preference for complex proteinaceous growth substrates, although a few complex carbohydrates can be used. Their genomic G+C content ranges from 46.5% to 59.4%. (Pascual *et al.*, 2015).

The family *Pyrinomonadaceae* (subdivision 4) is composed of the genus *Pyrinomonas* (Crowe *et al.*, 2013). Members of this genus are gram-negative non-spore-forming, non-capsule-forming aerobic chemoheterothrophs that are unable to grow phototrophically, reduce nitrate or ferment glucose. They are thermophiles and mildly acidophiles. Furthermore, they prefer complex proteinaceous growth substrates and have a variable capability to hydrolyze polymers. The genomic G+C of the type strain *Pyrinomonas methylaliphatogenes* is 59.6 %(Crowe *et al.*, 2013).

The family *Arenimicrobiaceae* (subdivision 4) is formed by the genera *Brevitalea* and *Arenimicrobium* (Wüst *et al.*, 2016). These bacteria are gram-negative non-spore-forming and non-capsule forming rods. They are aerobic chemoorganoheterotrophs that prefer proteinaceous growth substrates and are not capable of nitrate reduction and glucose fermentation. Additionally, they are mesophiles with a wide pH tolerance range (Dedysh & Yilmaz, 2018). Their genomic G+C content varies from 54.7% to 66.9% (Wüst *et al.*, 2016).

Family *Acanthopleuribacteraceae* (subdivision 8) contains only the genus *Acanthopleuribacter*.

Cells belonging to this genus are gram-negative, motile, strictly aerobic rods that are able to use α-D-glucose, L-alanine, hydroxy-L-proline, L-serine, L-threonine, inosine, uridine and thymidine for growth. The genomic G+C content of the type species, *Acanthopleuribacter pedis* is 56.7% (Fukunaga *et al.*, 2008).

Family *Vicinamibacteraceae* (subdivision 6) contains the genera *Vicinamibacter* (Huber *et al.*, 2016) and *Luteitalea* (Vieira *et al.*, 2017). These bacteria are gram-negative, non-spore forming, aerobic chemoorganoheterotrophs that are capable of growth on organic/nucleic acids and simple sugars but prefer complex proteinaceous compounds. They are neutrophils that tolerate a wide range of pH and can be from psychrotolerant to mesophiles. Genomic G+C content varies from 64.7 to 65.9% (Huber & Overmann, 2018).

Family *Holophagaceae* (subdivision 8) is formed by genera *Holophaga* (Liesack *et al.*, 1994) and *Geothrix* (Coates *et al.*, 1999). Both are strict anaerobes chemoorganothrophs, non-spore-forming, gram negative, mesophile, neutrophilic, and non-motile. The genomic DNA G+C content of the type species *Holophaga foetida* is 62.5 % (Liesack *et al.*, 1994).

Family *Thermotomaculaceae* (subdivision 10) contains only the genus *Thermotomaculum,* a gram-negative, non-spore forming anaerobic heterotrophic thermophile that was isolated from a deep-sea hydrothermal vent. The genomic DNA G+C content of the type species *Thermotomaculum hydrothermale* is 51.6% (Izumi *et al.*, 2012).

The family *Thermoanaerobaculales* (subdivision 23) accommodates the genus *Thermoanaerobaculum,* a strictly anaerobic, thermophilic and chemo-organotrophic genus that was isolated from a freshwater hot spring. The genomic DNA G+C content of the type species *Thermoanaerobaculum aquaticum* is 62.7% (Losey *et al.*, 2013).

Last, *Chloracidobacterium* genus is not assigned to any currently described *Acidobacteria* family. These bacteria are thermophilic, anoxygenic, chlorophototrophic members of class *Blastocatellia* isolated from a hot spring (Tank & Bryant, 2015). The genus potentially represents a novel family and novel order, but studies addressing the taxonomy status of *Chloracidobacterium* are still ongoing (Dedysh & Yilmaz, 2018). In addition, genomes assigned to candidate phyla '*Candidatus* Aminicenantes' and '*Candidatus* Fischerbacteria' might belong to *Acidobacteria* (Dedysh & Yilmaz, 2018).

The number of isolates and described genera of *Acidobacteria* has gradually increased due insights into the metabolism of these bacteria provided by genomic and metagenomics studies, as well as through improvement of cultivation methods (Pascual *et al.*, 2015). Currently, in 2020, there are 16,286 *Acidobacteria* 16S rRNA gene sequences in RDP 11 Database (Cole *et al.*, 2014) and 51 complete genomes (42 *Acidobacteriia*, 3 *Blastocatellia*, 3 *Holophagae*, 1 *Thermoanaerobaculia*, 1 *Vicinamibacteria* and 1 unclassified *Acidobacteria*) in NCBI database (Table 1) (NCBI Resource Coordinators, 2016). Further efforts to unravel the metabolism of uncultured microorganisms through modern technologies, such as high-throughput sequencing and Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS), may provide new insights into how to cultivate novel genera, thereby increasing the present knowledge on the

characteristics and potential functions of members of the phylum *Acidobacteria*.

Table 1: *Acidobacteria* complete genomes listed in NCBI*.

Genome	Accession	Size (Mb)
Acidobacteriia		
Acidipila dinghuensis str. DHOF10	NZ_SDMK00000000	5.1
Acidipila rosea str. DSM 103428	NZ_SMGK00000000	4.2
Acidipila sp. str. 4G-K13	NZ_QVQT00000000	5.0
Acidipila sp. str. EB88	NZ_QWEV00000000	4.5
Acidisarcina polymorpha str. SBC82	-	7.6
Acidobacteria bacterium str. KBS 146	NZ_JHVA00000000	5.0
Acidobacteriaceae bacterium str. KBS 83	-	12.5
Acidobacteriaceae bacterium str. KBS 89	-	12.0
Acidobacteriaceae bacterium str. KBS 96	-	13.4
Acidobacteriaceae bacterium str. TAA166	-	12.3
Acidobacteriaceae bacterium str. URHE0068	-	2.2
Acidobacteriia bacterium str. SbA2	NZ_OKRG00000000	2.7
Acidobacterium ailaaui str: PMMR2	NZ_JIAL00000000	3.7
Acidobacterium capsulatum str. ATCC 51196	-	8.3
Bryobacter aggregatus str. MPL3	NZ_JNIF00000000	5.7
Bryocella elongata str. DSM 22489	NZ_FNVA00000000	5.7
Candidatus Koribacter versatilis str. Ellin345	-	11.3
Candidatus Solibacter usitatus str. Ellin6076	-	19.9
Candidatus Sulfopaludibacter sp. str. SbA3	NZ_OKRF00000000	8.5
Candidatus Sulfopaludibacter sp. str. SbA4	NZ_OMOG00000000	10.0
Candidatus Sulfopaludibacter sp. str. SbA6	NZ_OKRH00000000	3.5
Candidatus Sulfotelmatobacter kueseliae str. SbA1	NZ_OMOD00000000	5.4
Candidatus Sulfotelmatobacter sp. str. SbA7	NZ_OKRE00000000	2.8
Candidatus Sulfotelmatomonas gaucii str. SbA5	NZ_OKRB00000000	5.3
Edaphobacter aggregans str. EB153	NZ_RSDW00000000	0.6
Edaphobacter aggregans str. DSM 19364	NZ_JQKI00000000	0.9
Edaphobacter dinghuensis str. EB95	NZ_RBIF00000000	4.5
Edaphobacter modestus str.DSM 18101	NZ_SHKW00000000	7.4
Granulicella mallensis str. MP5ACTX8	-	12.5
Granulicella pectinivorans str. DSM 21001	NZ_FOZL00000000	5.3
Granulicella rosea str. DSM 18704	NZ_FZOU00000000	5.3
Granulicella sibirica str.AF10	NZ_RDSM00000000	6.1
Granulicella sp. GAS466 str. GAS466	NZ_RJKT00000000	6.2
Granulicella tundricola str. MP5ACTX9	-	11.0
Occallatibacter savannae str. AB23	NZ_QFFY00000000	6.3
Silvibacterium bohemicum str. S15	NZ_LBHJ00000000	6.5
Terracidiphilus gabretensis str. S55	NZ_LAIJ00000000	5.3
Terriglobus albidus str. ORNL	NZ_CP042806	6.4
Terriglobus roseus str. GAS232	-	9.7
Terriglobus roseus str. DSM 18391	-	10.5
Terriglobus saanensis str. SP1PR4	-	10.2
Terriglobus sp. str. TAA 43	NZ_JUGR00000000	5.0
Blastocatellia		
Chloracidobacterium thermophilum str. OC1	NZ_LMXM00000000	3.6
Chloracidobacterium thermophilum B str. B	-	7.4
Pyrinomonas methylaliphatogenes str. K22	NZ_CBXV000000000	3.8
Holophagae		
Geothrix fermentans str. DSM 14018	-	2.0
Holophaga foetida str. DSM 6591	NZ_AGSB00000000	4.2
Holophagae bacterium str. FeB_10	NZ_PQAJ00000000	4.2
Thermoanaerobaculia		
Thermoanaerobaculum aquaticum str. MP-01	-	5.3
Vicinamibacteria		
Luteitalea pratensis str. DSM 100886	NZ_CP015136	7.5
Unclassified Acidobacteria		
Acidobacteria bacterium AB60	NZ_VANK00000000	6.7

*(https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=57723, January 2020).

2. Carbohydrate metabolism

Carbon usage is one of the physiological requirements in *Acidobacteria* that has been widely studied. Genomic analyses demonstrated the presence of 131 glycoside hydrolase (GH) families across 24 *Acidobacteria* genomes, including important enzymes for plant cell wall breakdown (Eichorst *et al.*, 2018). Overall, *Acidobacteria* are able to use D-glucose, D-xylose, lactose, maltose, cellobiose, glucose and xylose as carbon sources and can degrade simple and polymeric carbohydrates. The ability to use glucose and xylose is evident, since those are the main carbon sources employed for acidobacterial isolation (Kielak *et al.*, 2016). However, most of subdivision 1 *Acidobacteria* are not able to use fucose or sorbose, sugars rarely observed in plant cell wall and soil (Li *et al.*, 2013, Kielak *et al.*, 2016). Genes related to the biosynthesis, transfer, breakdown and/or modification of carbohydrates typically represent 5%–9% of acidobacterial genomes. The genomes of the non-soil isolates *G. fermentans*, *H. foetida* and *C. thermophilum* B have the lowest percentages of genes related to carbohydrate-active enzymes, which indicates that soil *Acidobacteria* might have a higher proportion of their genomes involved in carbohydrate metabolism (Eichorst *et al.*, 2018). Moreover, members of subdivision 1 (*Acidobacteriia*) have a broader glycolytic capability than other subdivisions (Kielak *et al.*, 2016).

The genomes of *Acidobacteria* include genes encoding pathways for the degradation of various polysaccharides (starch, cellulose, hemicellulose, laminarin, xylan, xyloglucan, and gellan gum), but experimental evidence for hydrolytic capabilities not always support genomic predictions, which could be due to errors in gene annotation, variations in gene regulation or culture conditions (Kielak *et al.*, 2016, Belova *et al.*, 2018).

The GH families related to polymeric carbohydrate degradation with the highest percentages across sequenced acidobacterial genomes are GH109 and GH74 (Eichorst *et al.*, 2018). GH109 contais α-N-acetylgalactosaminidases that act on O-linked oligosaccharides, which are typically found in chitin, bacterial peptidoglycan and lipopolysaccharides (Liu *et al.*, 2007), while GH74 contains endoglucanases that act on β-1,4-linked glucans (Lombard *et al.*, 2014). Families involved in cellulose degradation, such as GH5 were observed in subdivisions 1, 3, 4 and 6, while families GH8, GH9, GH44 and GH12 were found in a few subdivision 1 and 3 genomes (*Terriglobus*. sp., '*Ca*. K. versatilis', *T*. *gabretensis*, and *G*. *mallensis* and 'Ca. S. usitatus'). Family GH3 β-glucosidases were detected in all sequenced genomes, while GH18 and GH19 putative chitinases were present in genomes belonging to subdivision 1, 3, 4, 6, and 8 (Eichorst *et al.*, 2018). Until recently, chitin usage had not been experimentally demonstrated for any member of *Acidobacteria* subdivision 1 (Kielak *et al.*, 2016). Nevertheless, Belova *et al.* (2018) isolated two strains of a novel genus and species, *Acidisarcina polymorpha*, bacteria with a wide repertoire of enzymes for the degradation of chitin, cellulose and xylan. The strains secreted chitinases linked to family GH18 and were capable of using chitin as carbon and nitrogen sources. In earlier studies, Ivanova (2016) identified *Acidobacteria* increased SSU rRNA transcript abundance in response to chitin availability in an acidic peatland

investigation.

3. Extracellular polymeric substances (EPS) production

EPS production has been reported for the *Acidobacteria* species *Granulicella paludicola*, *G. pectinivorans*, *G. aggregans*, *G. rosea* (Pankratov & Dedysh, 2010), *Acidicapsa borealis*, *A. ligni* (Kulichevskaya *et al.*, 2012) and *Terriglobus tenax* (Whang *et al.*, 2014). In addition, most acidobacterial genomes belonging to subdivision 1 (with the exception of '*Ca*. K. versatilis strain Ellin345) contain genes involved specifically in cellulose biosynthesis (Kielak *et al.*, 2016), which might be related to EPS production (Flemming *et al.*, 2007). EPS production is possibly contributing to acidobacterial cell protection and long-term survival in soil. The production of large amounts of EPS is related to abiotic stress, likely supporting dominance of *Acidobacteria* in acidic environments, resistance to heavy metals and pollutants like uranium, antimonium (Wang *et al.*, 2016), cadmium, lead, zinc, mercury (Guo *et al.*, 2017), petroleum compounds, linear alkylbenzene sulfonate (Sanchez-Peinado *et al.*, 2010) and p-nitrophenol (Paul *et al.*, 2006). To date, the only two acidobacterial EPSs that have been isolated and chemically characterized are produced by two strains of *Acidobacteria* subdivision 1, *Granulicella* sp. strain WH15 and strain 5B5. Both WH15EPS and 5B5EPS are able to emulsify oils and hydrocarbons, producing emulsions that are more thermostable over time than those of commercial EPS (Kielak *et al.*, 2017). In addition, EPS production allows these strains to colonize *Arabidopsis* roots, promoting plant growth (Kielak *et al.*, 2016). Interestingly, most of the characterized and industrially relevant EPS are composed of a maximum of four different monosaccharides (Rehm, 2010), while WH15EPS and 5B5EPS are heteropolysaccharides composed of 7 different monosaccharides, with xylose, mannose, glucose and galactose as main components (Kielak *et al.*, 2017). The composition of those EPS might be responsible for additional biological properties not present in EPS composed of more common sugar monomers (Roca *et al.*, 2015).

4. Genus *Granulicella*

The genus *Granulicella* currently contains 11 species: *G. paludicola* (T), *G. pectinivorans*, *G. aggregans*, *G. rosea,* isolated from *Sphagnum* peat bogs in Russia (Pankratov & Dedysh, 2010); *G. arctica*, *G. mallensis*, *G. tundricola*, *G. sapmiensis*, isolated from tundra soil in Finland (Mannisto *et al.*, 2012); *G. cerasi*, isolated from cherry bark in Japan (Yamada *et al.*, 2014); *G. acidiphila*, isolated from abandoned metal mines in Spain (Falagán *et al.*, 2017); and *G. sibirica*, isolated from organic tundra soil layer in Siberia (Oshkin *et al.*, 2019).

Granulicellas are gram-negative, non-spore forming, non-motile rods, occurring singly, in pair of short chains. These bacteria produce copious amount of EPS in culture media, and their colony colours vary from pale-pink to red, due to the production of carotenoid pigments. Strictly aerobic chemo-organotrophs, their preferred growth substrates are mono and polysaccharides. They grow in acidophilic and mesophilic conditions and are capable

to hydrolyze several polysaccharides, but cellulose and chitin breakdown has not yet been demonstrated (Pankratov & Dedysh, 2010).

The two strains used in my thesis, *Granulicella* sp. 5B5 and WH15, were isolated from decaying wood in the Netherlands (Valášková *et al.*, 2009). Comparisons among 16S rRNA gene sequences demonstrated that strain 5B5 is phylogenetically more related to *G. cerasi* and *G. paludicola*, while strain WH15 is closer to *G. tundricola* and *G. rosea* (Figure 2). The Average Nucleotide Identity (ANI) values (Table 2) showed that both strains do not belong to any of the species for which the genomes have been sequenced.

Table 2: Average Nucleotide Identity percentage values between *Granulicella* strains 5B5 and WH15 and other *Granulicella* strains' whole genomes available.

	G5B5	GHW15	GTUN	GPEC	GMAL	GSIB
G5B5	100	72.75	72.42	72.81	73.58	71.81
GHW15	72.75	100	74.12	73.32	73.03	73.19
GTUN	72.42	74.12	100	73.31	71.57	72.58
GPEC	72.81	73.32	73.31	100	71.57	74.88
GMAL	73.58	73.03	71.57	71.57	100	71.01
GSIB	71.81	73.19	72.58	74.88	71.01	100

GTUN –*G. tundricola*; GPEC-*G. pectinivorans*; GMAL-*G. mallensis*; GSIB-*G. sibirica*

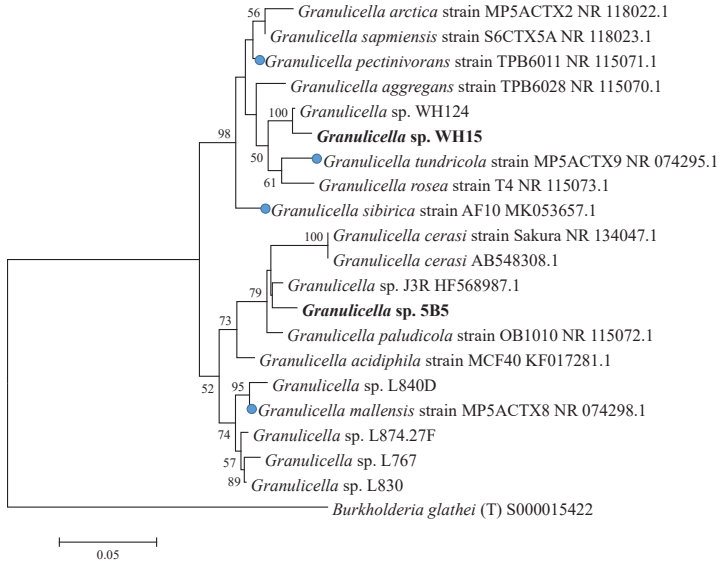

Figure 2: Dendogram showing the phylogenegtic relationships between *Granulicella* species based on comparisons between 16S rRNA gene sequences. The dendogram was constructed by using Maximum Likelihood (Tamura-Nei model) method (Tamura & Nei, 1993). Bootstrap values (expressed as percentages of 1,000 replications) are shown at branch points. *Burkholderia glathei* was used as outgroup. The strains used in this thesis are highlighted in bold letters. Blue circles indicate genomes compared to those of strains WH15 and 5B5 in ANI analysis

Thesis Outline

The high abundance and ubiquity of *Acidobacteria* in different environments, especially soils, raises intriguing questions about the physiological traits underlying their marked abundance. Genome sequences have provided relevant information, especially about *Acidobacteria* subdivision 1, the group for which various pure cultures are available. The increase in shotgun metagenomic studies and postgenomic analyses have enabled *de novo* assembly of acidobacterial genomes from environmental datasets and new insights into genome traits. Increased knowledge of the genomic features of different *Acidobacteria* subdivisions is critical for understanding their persistence in soil as well as their interactions with other soil microorganisms. Efforts to culture different acidobacterial genera and strains remain a top priority to decipher their genomic potential and to study their physiology and ecological functions.

The research presented in my thesis aimed at i) optimizing the growth of two strains of Granulicella, and ii) investigating the assimilation of the extracellular polymeric substances (EPS) of Granulicella strain WH15 by litter-topsoil microbial communities. To this end, I integrated different 'omic' approaches, including genomics, metagenomics, transcriptomics and proteomics, to expand the fundamental knowledge of their metabolism and interactions with other soil microbes, including the environmental fate of extracellular polymeric substances (EPS) produced by *Granulicella* (Figure 3).

EPS consists of highly hydrated polymers comprising polysaccharides, proteins and DNA (Wingender *et al.*, 1999) (Figure 4, Costa., unpublished). In **chapter 2**, we provide an overview

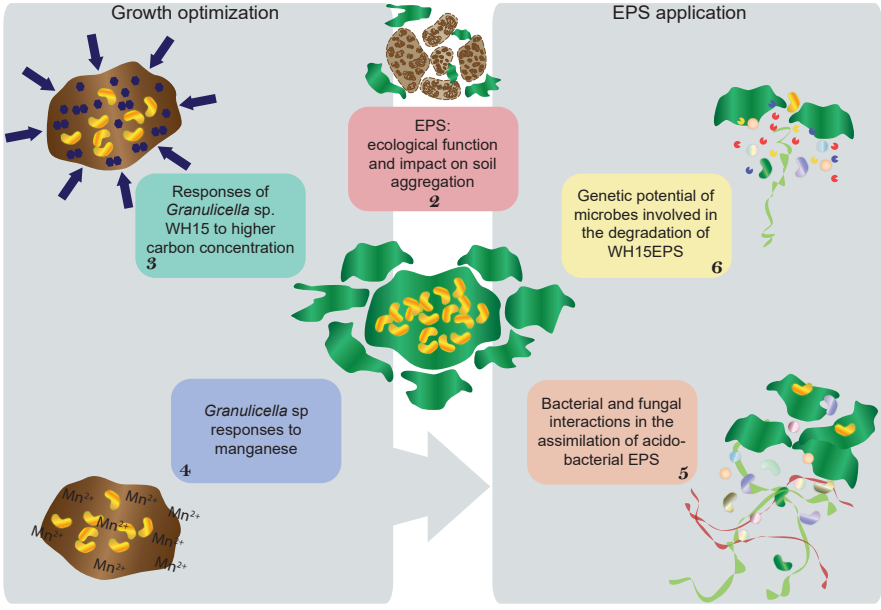

Figure 3: Schematic overview of the chapters presented in this thesis.

of the current knowledge on EPS biosynthesis, its chemical composition, factors influencing EPS production, the ecological functions of EPS and its application to improve soil particle aggregation. To study acidobacterial EPS, we first optimized growth conditions for higher bacterial biomass, necessary for EPS production and extraction. Simultaneously, we studied the impact of such growth optimizations on the metabolism of the *Granulicella* strains.

In general, strains of *Acidobacteria* exhibit slow growth under laboratory conditions, requiring low nutrient concentrations (Kielak *et al.*, 2016). However, growth at higher carbon concentrations was demonstrated for some isolates (de Castro *et al.*, 2013), including strains WH15 and 5B5 for which culture medium PSYL5 was developed (Campanharo *et al.*, 2016). In **chapter 3,** we evaluated transcriptional and proteomic responses of *Granulicella* strain WH15 grown at different concentrations of cellobiose. Our results demonstrated that higher cellobiose concentrations resulted in the higher expression of excretory functions and the reallocation of resources to maintenance of basic cell metabolism instead of production of new cell material.

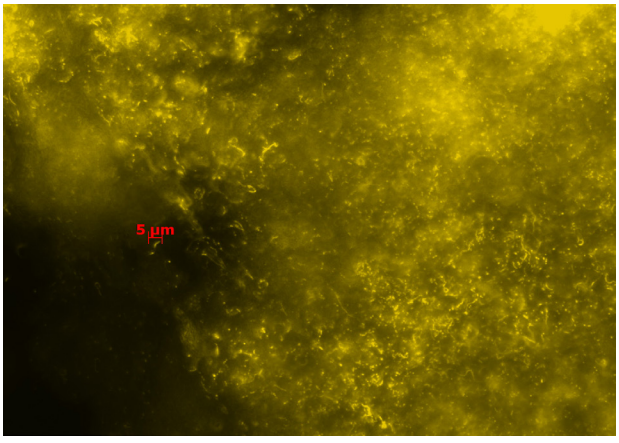

Figure 4: Extracellular DNA present in the EPS of liquid cultures of *Granulicella* sp.WH15 detected by Sytox™ Orange fluorescent nucleic acid stain (Invitrogen ™) (Unpublished data).

It is widely known that trace elements are important for microbial metabolism (Puri *et al.*, 2010). In **chapter 4,** we evaluated the impact of different trace elements on the growth of *Granulicella* strains WH15 and 5B5. The addition of trace element solution SL10 improved significantly the growth of both strains. When we further evaluated the effect of each of the trace elements separately, the results showed that primarily manganese (Mn) had a positive effect on the growth of both strains. To understand the effect of Mn on the metabolism of the two *Granulicella* strains, we adopted proteomics and genomics. Our results showed that the strains had different proteomic profiles and several uncharacterized metal ion transporters that could be involved in metal ion homeostasis. We postulate that these transporters could contribute to survival under high manganese concentrations present in the wood decomposition environment from where the strains were originally isolated.

Optimization of carbon concentration and manganese in culture medium allowed our strains, especially WH15, to grow faster in laboratory conditions, producing extractable amounts of EPS. The EPS of strain WH15 (WH15EPS) is mainly composed of polysaccharides (Kielak *et al.*, 2017) with a unique sugar composition that can be used as a nutrient source for other microorganisms. In **chapter 5**, we labeled WH15EPS with ^{13}C and investigated its effect on the assembly and co-occurrence of the active bacterial and fungal communities in topsoil by the stable isotope probing (SIP) approach. Our results demonstrated that WH15EPS was mainly assimilated by *Planctomycetes*, *Verrucomicrobia, Ascomycota* and *Basidiomycota* and co-inertia analysis suggested overall relationships between these kingdoms. Furthermore, comparisons among co-occurrence networks from labeled and unlabeled treatments demonstrated that hidden potential interactions can be unraveled by more specific and targeted metabolism studies. For instance, we observed the incorporation of WH15EPS by *Singulisphaera* and its connections to other *Planctomycetes* and *Acidobacteria*, which were not reported before.

The metabolization of WH15EPS and other biopolymers requires the production of a wide range of enzymes, such as glycoside hydrolases (GHs). GHs have applications in several industrial sectors, including biofilm removal, food processing and biofuel production. In **chapter 6,** we applied WH15EPS as an enrichment factor to target microorganisms and functions involved in EPS degradation through culture-independent and culture-dependent techniques. For this, we used topsoil samples obtained from the environment where *Granulicella* sp. WH15 was originally isolated. Our results showed a large diversity of glycoside hydrolase families with biotechnological potential and a high number of unclassified microorganisms that could be targeted for further studies.

In **chapter 7,** I integrate the overall findings of my thesis and discuss the most important observations concerning the impact of carbon sources and trace elements on the physiology of *Granulicella* and, more general, the ecological functions and environmental fate of EPS of *Acidobacteria*.