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Research Article

Catabolism of sialic acids in an environmental microbial community

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One sentence summary: Catabolism of sialic acids in a microbial community with no host–microbe interaction resembles the one described for gut microbiota.
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Abstract

Sialic acids are a family of nine-carbon negatively charged carbohydrates. In animals, they are abundant on mucosa surfaces as terminal carbohydrates of mucin glycoproteins. Some commensal and pathogenic bacteria are able to release, take up and catabolize sialic acids. Recently, sialic acids have been discovered to be widespread among most microorganisms. Although the catabolism of sialic acids has been intensively investigated in the field of host–microbe interactions, very limited information is available on microbial degradation of sialic acids produced by environmental microorganisms. In this study, the catabolic pathways of sialic acids within a microbial community dominated by '*Candidatus* Accumulibacter' were evaluated. Protein alignment tools were used to detect the presence of the different proteins involved in the utilization of sialic acids in the flanking populations detected by 16S rRNA gene amplicon sequencing. The results showed the ability of *Clostridium* to release sialic acids from the glycan chains by the action of a sialidase. *Clostridium* and *Chryseobacterium* can take up free sialic acids and utilize them as nutrient. Interestingly, these results display similarities with the catabolism of sialic acids by the gut microbiota. This study points at the importance of sialic acids in environmental communities in the absence of eukaryotic hosts.

Keywords: biological phosphorus removal, environmental microbial community, extracellular polymeric substances, nonulosonic acids, '*Ca*. Accumulibacter'

Introduction

Sialic acids are part of a family of nine-carbon negatively charged carbohydrates called nonulosonic acids. These compounds are normally found as terminal residues on the glycan chain of the extracellular glycoconjugates of vertebrates, with *N*-acetylneuraminic acid (Neu5Ac) as the most studied representative (Chen and Varki 2010). In animals, sialic acids are extremely important for recognition, interaction processes and immune responses (Traving and Schauer 1998). In order to delay the immune response of the host, pathogenic bacteria utilize sialic acids for mimicking the host glycosylation pattern. They can either *de novo* synthesize these molecules or scavenge them after releasing them from the host glycoconjugates (Carlin *et al.* 2009). Additionally, some pathogenic bacteria are able to use host sialic acids as carbon, nitrogen and energy sources, which is crucial during bacterial colonization (Vimr 2013).

In animals, a major reservoir of sialic acids is found on mucosal surfaces, where sialic acids appear as the terminal carbohydrate of mucin glycoproteins. These mucins form a protective mucosal layer that acts as barrier for pathogens (McGuckin *et al.* 2011). In intestinal mucins, oligosaccharides make up to 80% of the mass (Capon *et al.* 1992), and over 65% of glycans contain residues of sialic acids as terminal carbohydrates (Robbe *et al.* 2004). These oligosaccharides can be used as nutrient sources by gastrointestinal commensal and pathogenic bacteria. The consumption of these complex oligosaccharides requires the synergic action of different bacteria. The presence of sialic acids as terminal carbohydrate makes them the first target of this degradation (Haines-menges *et al.* 2015).

The utilization of sialic acids as nutrients starts with their release from the host glycoconjugates under the action of sialidases (NanH), in the form of, e.g. Neu5Ac. Free sialic acids are then taken up by bacteria by the action of specific transporters, which can belong to four different families (i.e. ATP-binding cassette (ABC), tripartite ATP-independent periplasmic (TRAP), major facilitator superfamily (MFS) and sodium solute symporter (SSS)). Gram-negative bacteria first transport sialic acids across the outer membrane by a general porin (Thomas 2016). In the intracellular space, pyruvate is cleaved from Neu5Ac generating *N*-acetylmannosamine (ManNAc) by the action of *N*-acetylneuraminic acid lyase (NanA). ManNAc is then phosphorylated and converted to *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P) by the action of *N*-acetylmannosamine kinase (NanK) and *N*-acetylmannosamine-6-phosphate epimerase (NanE). GlcNAc-6-P is further deacetylated and deaminated by the action of *N*-acetylglucosamine-6-phopshate deacetylase (NagA) and glucosamine-6-phosphate deaminase (NagB), producing fructose-6-phosphate (Fru-6-P) that can be catabolized in the central carbon metabolism (Vimr 2013, Haines-menges *et al.* 2015). Figure 1 shows the canonical catabolic Neu5Ac pathway. In addition, other pathways have been described as well. For example, *Bacteroides fragilis* utilizes a novel

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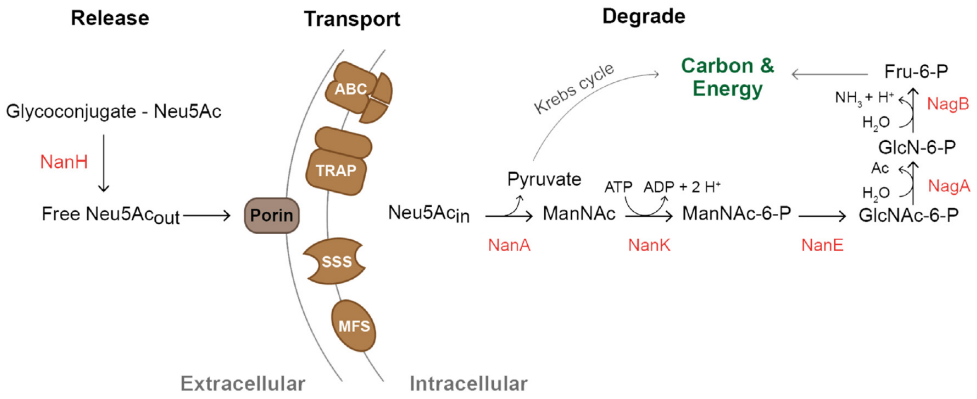

Figure 1. Schematic representation of the catabolic pathway of sialic acids (i.e. Neu5Ac) in bacteria. NanH: sialidase; MFS: major facilitator superfamily; TRAP: tripartite ATP-independent periplasmic; ABC: ATP-binding cassette; SSS: sodium solute symporter; NanA: *N*-acetylneuraminic acid lyase; NanK: *N*-acetylmannosamine acid kinase; NanE: *N*-acetylmannosamine-6-P epimerase; NagA: *N*-acetylglucosamine-6-P deacetylase; NagB: glucosamine-6-P deaminase.

N-acetylmannosamine-6 epimerase converting ManNAc into GlcNAc, which is further phosphorylated to GlcNAc-6P, and the rest of the pathway is the same as the canonical catabolic Neu5Ac pathway (Brigham *et al.* 2009). On the other hand, *Ruminococcus gnavus* releases sialic acids in the form of 2,7-anhydro-Neu5Ac by the action of a *trans*-sialidase. This chemical form is transported into the cell and converted back to Neu5Ac, which is processed as described in Fig. 1 (Bell *et al.* 2020).

Various interactions have been described between gastrointestinal bacteria and sialic acids present in the host glycans. Some bacteria, such as the pathogen *Vibrio cholerae*, can secrete extracellular sialidases that release sialic acids from the host glycans, which are then transported into the cells and used as nutrients (Sharma *et al.* 2011). On the other hand, other microorganisms can excrete sialidases to release sialic acids, but are not able to consume them as nutrients (i.e. *Bacteroides thetaiotaomicron*) (Brigham *et al.* 2009). This action exposes the underlying sugars, making them available for consumption. As a result, the released sialic acids can be used by other bacteria, such as some commensal strains of *Escherichia coli*. These strains of *E. coli* lack sialidases to liberate sialic acids from the host glycans but have the enzymes necessary for the uptake and catabolism of free sialic acids (Huang *et al.* 2015).

Sialic acids have been mainly studied in animal cells and pathogenic bacteria, due to the assumption that presence of sialic acids is confined to pathogenic and commensal species. Only very recently, a mass spectrometry based survey has revealed an unexpectedly wide distribution of sialic acids among non-pathogenic environmental bacteria (Kleikamp *et al.* 2020). This suggests that sialic acids might be an important component of the extracellular polymeric substances (EPS) of environmental bacteria, where they have been largely overlooked. In addition, although catabolism of sialic acids has been intensively investigated under host-microbe circumstances, little information is available on the microbial degradation of sialic acids produced by other environmental microorganisms. Therefore, understanding the catabolism of sialic acids among environmental bacteria is important to reveal the role of sialic acids within the microbial world other than pathogens and commensals.

'*Candidatus* Accumulibacter phosphatis', a well-studied polyphosphate-accumulating organism dominant in wastewater treatment systems designed for enhanced biological phosphorus removal (EBPR), has been recently described to produce different types of sialic acids as part of their EPS (Tomás-Martínez *et al.* 2021). In these engineered ecosystems alternating anaerobic feast and aerobic famine regimes, the carbon sources are rapidly scavenged by '*Ca*. Accumulibacter' under anaerobic conditions making them unavailable for other organisms in the community. Under substrate limitation, it is assumed that, the extracellular polymers, such as polysaccharides and glycoproteins, produced by '*Ca*. Accumulibacter' might be utilized as substrate by other organisms, especially during the aerobic phase of the EBPR process. As sialic acids mostly appear as the terminal carbohydrate of the glycan chain in glycoproteins present in the EPS (de Graaff *et al.* 2019), they are likely the first target for cleavage and degradation. In this study, the pathways involved in the catabolism of sialic acids within the EBPR microbial community were evaluated by using protein alignment tools. This gave insight on the catabolism of sialic acids in environmental bacterial aggregates, where no interaction with a vertebrate host is present.

Materials and methods

EBPR community characterization

Biomass enriched with '*Ca*. Accumulibacter' from a laboratory-scale sequencing batch reactor performing EBPR was used for this study (Tomás-Martínez *et al.* 2021). In order to characterize the microbial community and identify flanking populations present in the biomass, 16S rRNA gene amplicon sequencing was applied. DNA was extracted from the granules using the DNeasy UltraClean Microbial kit (Qiagen, Venlo, The Netherlands), using the manufacturer's protocol. The extracted DNA was quantified using a Qubit 4 (Thermo Fisher Scientific, Waltham, MA). Samples were sent to Novogene Ltd (Hong Kong, China) for amplicon sequencing of the V3–4 hypervariable region of the 16S rRNA gene (position 341–806) on a MiSeq desktop sequencing platform (Illumina, San Diego, CA) operated under paired-end mode. The raw sequencing reads were processed by Novogene Ltd (Hong Kong, China) and quality filtered using the QIIME software (Caporaso *et al.* 2010). Chimeric sequences were removed using UCHIME (Edgar *et al.* 2011) and sequences with ≥97% identity were assigned to the same operational taxonomic units (OTUs) using UPARSE (Edgar 2013). Each OTU was taxonomically annotated using the Mothur software against the SSU rRNA database of the SILVA Database (Quast *et al.* 2013).

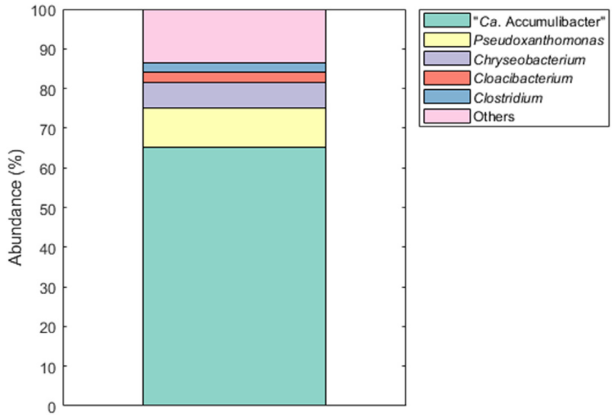

Figure 2. Relative genus-level microbial community distribution based on 16S rRNA gene copy numbers. All OTUs contributing <1% are grouped as 'Others'.

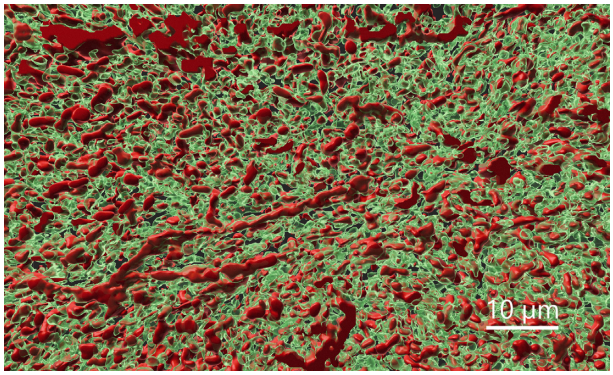

Figure 3. Confocal laser scanning microscopy of a bioaggregate enriched with '*Ca*. Accumulibacter' presented as a 3D isosurface view. The green channel is projected semi-transparent in order to show the foam-like protein matrix. The red channel shows the glycoconjugates indicating individual bacterial cell surfaces and microcolonies as well as groups of bacteria linked via a glycoconjugate matrix. Color allocation: green—Sypro Orange protein stain, red—HMA-Alexa633 lectin. A volume view of the same area is shown in Fig. S1 (Supporting Information).

BLASTp analysis of enzymes involved in the catabolism of sialic acids

Amino acid sequences of enzymes involved in the catabolic pathway of sialic acids (Fig. 1) from different well-described sialic acid consumers were retrieved from the online database NCBI RefSeq (O'Leary *et al.* 2016). The accession number of the sequences and the reference microorganisms are listed in Table S1 (Supporting Information). These sequences were used to predict the degradation capability of the different bacterial populations of the EBPR community. A protein sequence alignment versus a protein database (BLASTp) from the NCBI website (blast.ncbi.nlm.nih.gov/Blast.cgi) with default parameters was performed for each of the most abundant members detected in the EBPR community by 16S rRNA gene amplicon sequencing (Fig. 2). The presence of the enzymes involved in the catabolism of sialic acids in the flanking populations was confirmed with an *e*-value lower than 5e-25 (Petit *et al.* 2018).

Fluorescence lectin-binding analysis

Lectin staining of bioaggregates of the EBPR biomass was conducted according to earlier works (Weissbrodt *et al.* 2013, Boleij *et al.* 2018, de Graaff *et al.* 2019, Tomás-Martínez *et al.* 2021). Bioaggregates were stained and mounted in CoverWell imaging chambers with various spacers in order to avoid squeezing of the samples. The basis for the final staining protocol was fluorescent lectin bar-coding (i.e. screening with all commercially available lectins). In a second step, the examination was detailed with a small screening using lectins specific to sialic acids such as CCA, HMA, LFA, LPA, MAA, SNA and WGA. Finally, the lectin approach was combined with a number of other fluorochromes specific for nucleic acids or proteins. For the image data set shown (Fig. 3), protein staining with Sypro Orange was applied according to the suppliers instruction (Thermo Fisher Scientific, Waltham, MA). After incubating for 1 h, samples were washed three times with tap water and stained with the sialic acid-specific lectin HMA-Alexa633 (Turonova *et al.* 2016). After incubation with the lectin solution, the sample was washed again with tap water for three times in order to remove unbound lectins. Samples were examined with a TCS SP5X confocal laser scanning microscope (CLSM; Leica, Wetzlar, Germany) equipped with an upright microscope and a super continuum light source (white laser). The microscope hardware was controlled by the software LAS AF 2.4.1. Confocal datasets were recorded by using 25× NA 0.95 and 63× NA 1.2 water immersion lenses. Excitation was performed at 475 and 630 nm, and fluorescence emission signals were detected simultaneously with two photomultipliers from 520 to 600 nm (Sypro Orange) and 645 to 720 nm (HMA-Alexa633). Image data sets were deconvolved with Huygens version 21.04 using blind deconvolution (SVI, Hilversum, The Netherlands) and projected with Imaris version 9.8 (Oxford Instruments, Abingdon, UK).

Results and discussion

Predominance of '*Ca*. Accumulibacter' and flanking populations in the EBPR biomass

Although '*Ca*. Accumulibacter' has been widely studied for its importance in the EBPR process (Mino *et al.* 1998, Barr *et al.* 2016, Rubio-Rincón *et al.* 2019, Silva *et al.* 2020, Tomás-Martínez *et al.* 2021), this organism has not been isolated as pure culture up to date. In this study, one of our previously described enrichment culture of '*Ca*. Accumulibacter' was used (Guedes da Silva 2020, Tomás-Martínez *et al.* 2021). Although the relative abundance of '*Ca*. Accumulibacter' is underrepresented by 16S rRNA gene sequencing, this method was used to determine the flanking populations present in the bioreactor. Methods such as fluorescence *in situ* hybridization or metaproteomics allow a better representation of the contribution of the different microorganisms to the population mass (Kleiner *et al.* 2017, Stokholm-Bjerregaard *et al.* 2017, Kleikamp *et al.* 2021). '*Ca*. Accumulibacter' was shown to account for 95% of the mass of the enriched microbial community (Kleikamp *et al.* 2021). Genomics based techniques are more suitable to identify the low abundance flanking population. The genomics based microbial community composition of the enriched EBPR biomass can be observed in Fig. 2. '*Ca*. Accumulibacter' appeared as dominant organism (65% of 16S rRNA gene amplicon sequencing read counts). '*Ca*. Accumulibacter' was mainly accompanied by the genera *Pseudoxanthomonas*, *Chryseobacterium*, *Cloacibacterium* and *Clostridium*, which all showed an abundance of >1% of the total 16S rRNA gene counts. Since these genera are not capable to sequester acetate under anaerobic conditions it is inferred that they grow on products produced by the dominant population of '*Ca*. Accumulibacter'.

Abundancy of sialic acids on the surface of EBPR bioaggregates

EPS consist of different biopolymers, such as polysaccharides or (glyco)proteins (Flemming and Wingender 2010). Recently, it was shown that '*Ca*. Accumulibacter' can produce different types of sialic acids (i.e. neuraminic, pseudaminic and legionaminic acids) as part of their extracellular matrix (Tomás-Martínez *et al.* 2021). In the current research, aggregates were first stained with Sypro Orange (specific for proteins), washed and counterstained with the fluorescent labelled lectin HMA (specific for sialic acids) to visualize the distribution of extracellular proteins and glycoconjugates containing sialic acids (Fig. 3). The protein specific stain indicates a foam-like and tubular frequently covering bacterial cells, showing that proteins (and/or glycoproteins that do not contain sialic acids) are widely distributed throughout the EPS matrix as a fibrous network. The sialic acid specific signal appears directly on the individual bacterial cell surfaces or around microcolonies as a more extended signal linking groups of bacteria, which are embedded in the fibrous network. Those heavily sialylated 'covers' could be part of cell surface polymers, such as capsular polysaccharides, lipopolysaccharides, flagella or S-layer glycoproteins as reported in literature (Haines-menges *et al.* 2015). The corresponding volume view of the same area as Fig. 3 is shown in Fig. S1 (Supporting Information). Furthermore, for clarity, Fig. S2 (Supporting Information) shows a single slice of the data stack after deconvolution revealing a dotted protein signal around the bacterial cell surface (Saarimaa *et al.* 2006, Peltola *et al.* 2008).

The extracellular polymers could potentially be used as carbon, nitrogen and energy sources by the organisms present in the community when no other carbon source is available (Weissbrodt *et al.* 2013). In this case, both the proteinaceous fibrous network and the sialylated glycoconjugates could be used. The complexity of those polymers might require the synergetic action of different microorganisms to fully hydrolyze and metabolize them; this is common in other microbial communities such as the gut microbiota (Flint *et al.* 2012). Sialic acids appear as terminal carbohydrate residue in glycoconjugates in the EPS and have been described to protect the underlying carbohydrates and proteins from hydrolysis (Nishiyama *et al.* 2000, de Graaff *et al.* 2019, Boleij *et al.* 2020). Their protective role and terminal position at the surface makes sialic acid the first target for the utilization of glycoproteins as substrate.

Enzymes involved in sialic acid utilization in the genomes of predominant populations

To check the potential of different organisms in the EBPR system to utilize sialic acids as nutrient source, the amino acid reference sequences of the enzymes involved in the release (NanH), transport (MFS, TRAP, ABC, SSS) and degradation (NanA, NanK, NanE, NagA, NagB) of sialic acids (Fig. 1) were blasted against the sequences of the different genera present in the EBPR community (Fig. 2). The presence or absence of the gene encoding for each protein for each of the organisms are summarized in Table 1. More details can be found in Table S2 (Supporting Information).

These results reflect different possible interactions between the microorganisms present in the EBPR biomass and the sialic acids. Organisms affiliating with the genera *Pseudoxanthomonas* and *Cloacibacterium* do not harbor the necessary enzymatic machinery for the catabolism of this type of carbohydrates. Although *Pseudoxanthomonas* showed a positive match for different transporters, this can be a false positive due to the absence of most of the catabolic proteins and the similarity with other transporters from the same families (Thomas 2016).

In comparison, microorganisms from the genus *Clostridium* can encode all the proteins involved in the degradation of sialic acids. This starts with the cleavage from the glycoconjugates by the action of a sialidase (NanH). After sialic acids are liberated, they can be taken up into the intracellular space with the help of a transporter. Once sialic acids are in the cytoplasm, they can be degraded by the action of the different enzymes and enter the general carbon catabolism. Bacteria belonging to the genus *Chryseobacterium* lack the genes encoding for sialidases, but possess the transporters and the rest of enzymes for the use of sialic acids as nutrients. This means that after the cleavage by *Clostridium* sialidases, *Clostridium* and *Chryseobacterium* might compete for the uptake of free sialic acids.

Additionally, '*Ca*. Accumulibacter' only presented the genes encoding for the transporters, which could act as a reuptake mechanism to compete with the organisms consuming sialic acids, as proposed previously (Tomás-Martínez *et al.* 2021). In this way, '*Ca*. Accumulibacter' can reutilize this specific type of carbohydrate, saving cellular resources by not synthesizing them *de novo*. A proposed illustration of the interaction between the bacteria in the EBPR system and the sialic acids is shown in Fig. 4.

Similarities in the catabolism of sialic acids between EBPR biomass and gut microbiota

Interestingly, the possible catabolism of sialic acids in EBPR bioaggregates resembles what has been described for gut microbiota. In the fermentative environment of guts, there is a fierce competition for scarce resources among densely populated gut inhabitants. One way many bacterial species compete is specialization on alternative carbon sources instead of the highly utilized glucose (Hooper *et al.* 2002). One of the nutrient sources are host mucins, which are the main structural component of the mucus layer covering the epithelium surface, while sialic acids are present as terminal carbohydrates in mucin glycoproteins (McGuckin *et al.* 2011).

Mucosal sialic acids serve as nutrients for some organisms like the commensal bacterium *Akkermansia muciniphila* or the pathogen *V. cholerae*. These bacteria can excrete sialidases that cleave and release sialic acids present in mucins. Sialic acids can be transported into the bacteria, where they can be fully catabolized (Sharma *et al.* 2011, van Passel *et al.* 2011). *Clostridium* present in the EBPR community might occupy a similar niche in the EBPR community as *A. muciniphila* or *V. cholerae* in the gut microbiome.

Some commensal strains of *E. coli* lack sialidases genes, but are still able to take up and utilize sialic acids as nutrients. The growth of these *E. coli* strains relies on the release of sialic acids by the sialidase activity of other microorganisms (Huang *et al.* 2015). This behavior correspond to the strategy of *Chryseobacterium* in the EBPR system.

In addition, other organisms (e.g. *Bacteroides thetaiotaomicron*) that can secrete sialidases but cannot take up free sialic acids have been described in the gut microbiota (Brigham *et al.* 2009). However, there is no comparable behavior observed by the genomic search in the current research.

Tracking the catabolism of sialic acids in environmental microbiology

Although this study focused on one specific type of sialic acids (i.e. *N*-acetylneuraminic acid, Neu5Ac), other sialic acid structures have been described in '*Ca*. Accumulibacter', such as pseu-

Table 1. Presence (blue) or absence (white) of genes coding for the main proteins involved in the utilization of sialic acids in the genomes of the five most abundant populations present in the examined EBPR biomass. Detailed information can be found in Table S2 (Supporting Information). NanH: sialidase; NanA: *N*-acetylneuraminic acid lyase; NanK: *N*-acetylmannosamine acid kinase; NanE: *N*-acetylmannosamine-6-P epimerase; NagA: *N*-acetylglucosamine-6-P deacetylase; NagB: glucosamine-6-P deaminase; MFS: major facilitator superfamily; TRAP: tripartite ATP-independent periplasmic; ABC: ATP-binding cassette; SSS: sodium solute symporter.

		Catabolic proteins						Transporters			
Organism	**TaxID**	**NanH**	**NanA**	**NanK**	**NanE**	**NagA**	**NagB**	**MFS**	**TRAP**	**ABC**	**SSS**
"*Ca.* Accumulibacter"	327159								■		
Pseudoxanthomonas	83618		■			■		■	■	■	■
Chryseobacterium	59732		■	■	■	■	■	■	■	■	■
Cloacibacterium	501783						■				
Clostridium	1485	■	■	■	■	■	■		■	■	■

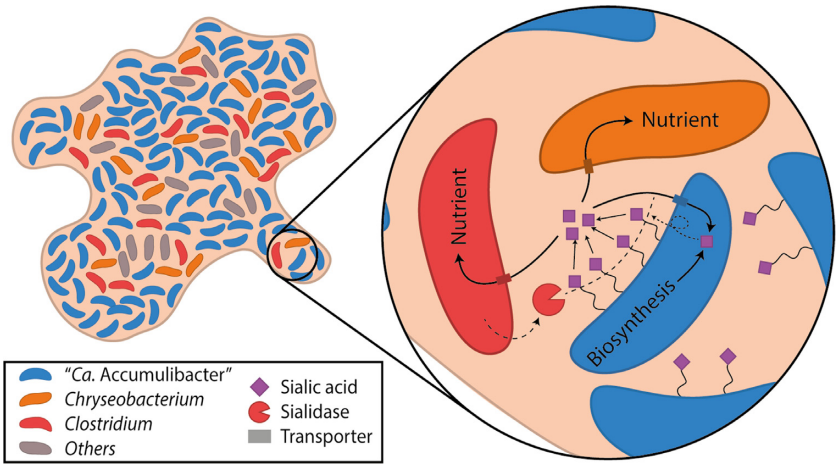

Figure 4. Illustration of the interaction between bacteria and sialic acids in bioaggregates of the EBPR biomass, proposed from the protein elucidations out of the genomes retrieved for the predominant populations. Sialic acids are synthesized by '*Ca.* Accumulibacter' and placed in their extracellular molecules. Once in the extracellular matrix, sialic acids are exposed to sialidases produced by *Clostridium*, releasing them as free sialic acids. *Clostridium* and *Chryseobacterium* compete for these free sialic acids and take them up for utilization as carbon, nitrogen and energy sources. Additionally, '*Ca.* Accumulibacter' can reuptake the free sialic acids and reutilize them as building blocks.

daminic or legionaminic acids (Tomás-Martínez *et al.* 2021). Currently, most of the studies reported in literature have focused on the catabolism of Neu5Ac or other related structures and no biochemical data are available for the degradation of other bacterial sialic acids (i.e. pseudaminic or legionaminic acids). Because of their chemical differences, different types of sialidases are needed to cleave the sialic acids structures present in the matrices (Juge *et al.* 2016). It could be speculated that '*Ca.* Accumulibacter' (and most environmental bacteria) take advantage of the need for specific catabolisms for each sialic acid as protection mechanism: different enzymes would be needed to degrade their variety of sialic acids. However, no matter what type of sialic acid it is, in microbial communities, due to its crucial terminal position at the glycan chain present in the EPS, it is likely that the consumption of any type of sialic acid will follow a similar pattern as Neu5Ac. Further investigations should be carried out to validate the catabolic conversion of the various sialic acids present in EBPR and other microbial communities.

The catabolism of sialic acids might be connected with the degradation of the relevant glycan chain as well. Once sialic acids are released, the underlying carbohydrates become available as substrate for the organisms in the community. Interestingly, it is reported that members of the here-detected genus *Chryseobacterium* can grow on complex polymeric compounds by degrading them into simpler substrates, which can be further used by other microorganisms (McBride 2014). Apparently, the degradation of these complex polymers requires the synergic action of different organisms, similar to cross-feeding phenomena in the gut microbiota (Flint *et al.* 2012). Such synergistic action can possibly apply to EPS macromolecules as well.

Up-to-date studies on the sialic acid metabolism (sialometabolism) have mainly focused on commensal or pathogenic bacteria in contact with animal cells (Almagro-Moreno and Boyd 2009). Based on our investigations, we suggest that the complex microbial interactions related to sialic acid metabolism in the gut microbiota can occur in an environmental community without the presence of a host. The catabolism of the host sialic acids plays an important role in microbial colonization. The same situation can arise in a microbial competitive environment, where sialic acids can be heavily expressed by the dominant species in the EPS for their protective roles. As a survival strategy, other bacteria may develop mechanisms to utilize the sialic acids in many different ways. This implies that the study of sialometabolism needs to take microbe–microbe interaction into account in environmental microbiomes besides the current main focus of host–microbe interactions. Using this

study as a starting point, future research on this emerging field with sialic acids as important monosaccharides in EPS and source of nutrients, electrons and energy for microbial growth will help elucidate the complex food networks in microbial communities.

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Supplementary data

Supplementary data are available at *FEMSEC* online.

Authors' contributions

ST-M, LMC and YL planned the research based on intensive discussions among all the authors, especially with MCML and DGW. ST-M performed the analysis with the support of LMC. TN performed the fluorescence lectin-binding analysis. ST-M and YL interpreted the data and played major roles in drafting, writing and revising the manuscript. All authors read and approved the manuscript.

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Conflict of interest statement. None declared.

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