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Do Microbial Fuel Cells have Antipathogenic Properties?

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Abstract

During 2015-2017 we have conducted experiments in Japan to test the capacity of microbial fuel cells (MFC) to treat different types of wastewaters (swine farm, domestic, yeast fermentation, winery, etc.) and concomitantly collecting DNA samples from MFC anodic and planktonic bacterial communities. We analyzed these metagenomes in UK, using our new bioinformatics tool (ASAR) that allow integration of phylogenetic and functional data. Characteristic MFC communities and the associated functional signatures were shown to reflect effective waste water treatment. We also found that the fraction of opportunistic pathogenic bacteria DNA was reduced in metagenomes from MFC communities during swine waste treatment. The highest loss was recorded for Enterobacteriaceae family (such as *Yersinia, Vibrio, and Shigella*). The abundance of virulent genes responsible for adhesion, secretion systems, invasion and intracellular survival, as well as antibiotic resistance, associated with Firmicutes and Actinobacteria phyla of Gram-positive bacteria, also decreased in the MFC residential metagenomes. Key metabolic functions were redistributed among bacteria on the anode and archaea in plankton. We propose to use MFC, inoculated with electroactive bacterial communities, for waste disinfection, and potentially for development of novel antibacterial therapies. This approach promises to be effective and economically justified, especially in cases of epidemics of enterobacteria-associated diseases, and common residential hospital pathogens such as *Enterococcus.*

Keywords: Electrogenic; Metabolism; Virulence; Pathogenic; NA; Reduction; Microbial fuel cell

Introduction

The livestock industry around the world produces colossal amounts of wastes characterized by a high- loaded level of organic compounds and pathogenic microorganisms that are resistant to multiple antibiotics [1]. Pathogenic bacteria associated with fecal wastewaters and hospital wastes cause waterborne-disease outbreaks (typhoid and paratyphoid fever, salmonellosis, cholera, dysentery and other infections) [2]. Environmental issues attributed to remediation technologies and improvement of quality of treated wastes cause great public interest.

Among aerobic and anaerobic wastewater treatment systems microbial fuel cells (MFCs) representing novel electrochemical bioreactors efficiently convert organics directly into electricity has become one of the most promising technology for power generation and wastewater treatment [3-7]. The last decade the MFC design, anode and cathode materials, cation exchange membranes and oxygen reduction catalysts have been significantly advanced [5,7]. Different sources of inoculums (anaerobic digester sludge, food wastes, lignocellulose compost or brewery industrial sludge) were applied for the MFC aimed to electroactive microbial community adaptation and following wastewater treatment [3,8,9]. Among all, swine manure and wastewater are well studied as initial inoculum for electroactive community adaptation, treatment efficiency and power output [10].

Bacteria that are able to produce electricity are usually referred to as electroactive bacteria or 'electrogens' [3-10]. In addition to known electrogens (*Geobacter, Shewanella and Arcobacter genera)*, MFCs contain other genera, among which are well known probiotics [3-5,11,12]. From the other side, MFCs reportedly contain opportunistic pathogenic microbes, such as *Corynebacterium diphtheria*, *Shigella sp., Yersinia pestis,* and *Mycobacterium sp.* [3-5,13,14].

The composition of MFC populations can be manipulated by compounds involved in redox reactions or electron transfer. Ferric chelator compounds, for instance, enhance survival and growth of *Lactobacillus plantarum, Streptococcus lactis,* and *Erwinia dissolvens* [15-17]. In general, incubation in MFCs causes compositional shifts in bacterial communities [17-20]. The majority of pathogenic species are suggested to lose in competition to more MFC-adaptive electroactive bacteria or to bacteria that enter syntrophic interactions in the community [20-24]. Several microbial genera were shown to be dramatically reduced in MFC [18], including the phylum Actinobacteria (*Schlegelella*, P =0.045), and it was suggested, based on metagenome analysis, that MFCs could be directly used in more general waste disinfection [17,25].

Theoretically, MFCs can be used to improve bacterial community composition to favor probiotic and less pathogenic components on a large scale [15-17,19-21,24-26]. However, this concept still requires proof as there have been no systematic studies on composition and functional evolution of opportunistic pathogens in MFCs.

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Microbial fuel cells increasingly find application in technological spheres [26-29] and been suggested for wastes disinfection [25] to prevent bacterial resistance to the spectrum of chemical disinfectants in hospital settings [30-35]. We have previously reported changes in gene abundances in MFCs that were strongly characteristic of electroactive species [3,4]. Mechanisms underlying shifts in relevant gene abundances in bacterial communities are still not understood, however, we investigated potential traits of the dominating opportunistic pathogenic bacterial species, their virulent and selected antibiotic resistance genes [36-40] during MFC bacterial community incubation. Two different inocula have been used in a laboratory-scale MFCs to generate communities for short incubation periods and for further metagenomic analysis. This study demonstrates a potential benefit of applying MFC to waste disinfection.

Methods

MFC

The MFC configuration and operation was described in our previous work (Figure 1) [3,4]. Briefly, we inoculated two pairs of laboratory-scale MFCs in Okinawa Institute Science and Technology (Japan) with sludge granules from a beer wastewater treating anaerobic digester (IGBS) and from sludge taken from the bottom of a tank receiving swine wastewater (SS). The MFC chamber contained two anodes and two air-facing side cathodes. The anodes (approximately 6 x 8 cm), suspended 2-3 mm off the bottom of the chamber, composed of a layer of conductive carbon cloth to which 2 mm average size activated carbon granules were bound with conductive glue to provide more surface area. The granules had been prepared from birch precursor and were pre-treated with neutral red. We have been successfully using neutral red for many years and visible depletion or lack of performance have not been observed [41].

The cathodes were 3 mm thick graphite plates with 60% porosity. They were sprayed on the liquid -facing side of a plate with an aqueous 5% Fumion membrane polymer (Fumatech, Bietigheim- Bissingen, Germany), while activated carbon granules (treated with iron(II) phthalocyanine) were mechanically pressed to the air-facing side using netting frame. The cathode extended into a bath containing an electrolyte solution (maintained at pH 3 with regular additions of 0.1 N HCl. We are using wet cathodes since 2009 in different applications and never observed underperformance [42,43].

The MFC chambers were placed into a bath containing an electrolyte solution (1 M HCl, pH 3.0). The anode and cathode electrodes were connected with a multichannel logger (GraphtecMidi LOGGERGL820, Japan) for daily voltage measurements. The electric current was calculated using Ohm's law (V=IR). Power density was obtained according to $P=IV/A$, where I is the current, V is the voltage, and A is the projected surface area of the cathode. The external resistance was 1000 Ω. Anode potential and coulombic efficiency data are shown in Supplementary Table 1, please also refer to Khilyas et al. [4].

MFC inoculation procedures and operating conditions have been described previously [4]. Briefly, SS was collected from a local pig farm (Okinawa Livestock and Grassland Centre, Nago, Japan) and IGBS- from a wastewater-treating UASB reactor (Orion Brewery, Nago, Japan). Inocula were not chemically modified or diluted, though the SW inoculum was filtered through a 1 mm stainless steel mesh to remove large particles. Swine waste (SW) for use as MFC feed was stored at 4°C. Precautions were taken to keep the feed and inoculum anaerobic thus all the work was done in anaerobic cabinet.

Wastewater feed was diluted with distilled water to adjust the chemical oxygen demand (COD) to 3.5-7.4 g O_2 L^{-1}. The MFCs microbial biofilm was formed for 3 days inoculation with SS and two with IGBS in open-circuit mode at room temperature. Wastewater was added to the MFCs semicontinuously using a peristaltic pump (HRT=24 h). After 67-day experiment swine wastewater, inoculum sludges, anodic biofilms (carbon felt and carbon granules) and planktonic samples of each MFC were used for DNA isolation (PowerMax soil DNA isolation kit (MO BIO laboratories, Inc.). A DNA library was constructed for shotgun sequencing and a 150 paired-end sequencing reaction was performed on MiSeq platform (Illumina, San-Diego, CA, USA). All experiments were set up in duplicate. The MFCs were disassembled after 67 days of the experiment and DNA was isolated in the same day. All experiments were set up in duplicate.

Data processing

The sequencing data were uploaded to the MG-RAST server as FASTAQ files for processing, primary analysis, and storage. *Sus scrofa* (pig) genome sequences were marked for exclusion during data submission. Primary submission data and results of the MG-RAST pipeline are available publicly (projectmgp19536). The MG-RAST representative hit organism abundances calculation was performed against the SEED database at the level of genera, based on a maximum *e*-value of 1×10^{-5}, minimum identity cut-off of 60%, and minimum sequence alignment of 15. Abundance data were downloaded as TSV files for further analysis. The representative hit data were downloaded from MG-RAST server via MGRASTer package [44] in R 3.1 environment. Abundance analysis was performed in metagenome Seq package [45] and ordination analysis was performed with phyloseq R packages [46]. Krona taxonomic community profiles were built by MG-RAST and stored as an image.

Data visualization

Functional, taxonomic, and KEGG Orthology [47] data were obtained from Illumina reads via MG- RAST pipeline. The functional and taxonomic annotations were merged based upon identical md5's corresponding to unique read sequences. Then read counts were aggregated for reads annotated with the same function and taxon. Functional and taxonomic read annotations to lowest level are matched to the lowest level annotations in their corresponding hierarchy trees to generate the whole phylogeny of each read. The result is the 3D dataset with axes of Functions, Taxonomy and Metagenome. Our post annotation analysis and visualization tool ASAR [48] uses data integration algorithm to merge taxonomic and functional data annotated at read level. The resulting 3D datasets with axes of Functions, Taxonomy and Metagenome samples were visualized via three heatmaps of each axis versus two others (F&T, F&M, T&M). Additionally, KEGG pathway enrichment sorting/heatmap and its map visualization were implemented. Advantages of the tool are: 1) Integrated functional and taxonomic analysis; 2) Comparative analysis of pathway enrichments; 3) KEGG pathway map visualization. The heatmaps show log abundance of reads annotated with selected functions in particular taxa within particular communities. On the KEGG map each functional box is split into sections corresponding

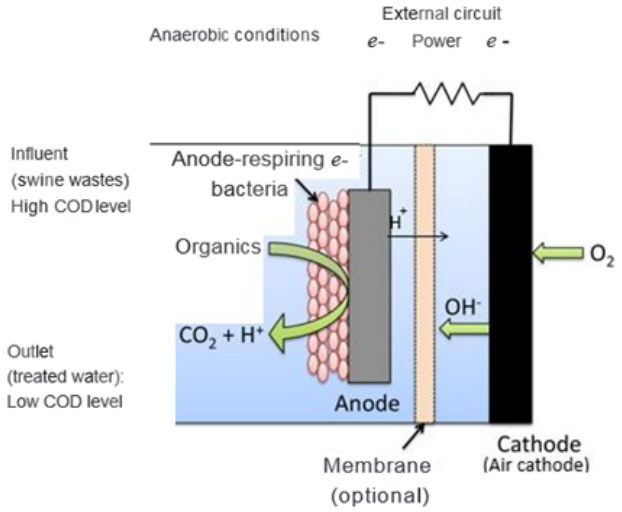

Figure 1: General schema of MFC.

to analyzed bacterial communities. The relative abundance of each function in each community is color coded from green (the lowest) to dark red (the highest proportion in the community). Taxonomical nomenclature is explained in Supplementary Table 2.

Results

Occurrence of pathogenic and opportunistic bacteria in MFC

We inoculated two pairs of laboratory-scale MFCs with sludge granules from a beer wastewater treatment anaerobic digester (IGBS) or with sludge (SS) taken from the bottom of a tank receiving swine wastewater (SW). The SS-inoculated MFC outperformed the IGBS-inoculated MFC with regard to volatile fatty acids removal, and electricity production [3,4]. Using a metagenomic approach we have previously described the microbial diversity of the MFC planktonic and anodic communities derived from two different inocula [3,4]. Along with electrogenic genus *Geobacter*, genera *Pelobacter, Pseudomonas, Arcobacter, Syntrophus, Syntrophobacter, Bacteroides*, and *Clostridium* and two acetoclastic methanogens (*Methanosarcina* and *Methanothermobacter*) were identified as the most highly abundant genera on both MFC anodes. Anodic communities of SS-inoculated MFCs had a higher proportion of *Clostridium* and *Bacterioides* genera relative to those of IGBS-inoculated MFCs, which were enriched with *Pelobacter*. Our results thus have shown a long-term influence of inoculum type on the performance and microbial community composition of swine wastewater treating MFCs [4].

This study mainly focuses on impact of the MFC treatment against pathogenic and opportunistic bacteria which are the major constituents in swine wastes. Particularly, bacteria related to Gammaproteobacteria (Enterobacteriaceae family, Pseudomonadales order) and Firmicutes phylum were analyzed in detail. With the current focus on an opportunistic pathogenic fraction of the MFC bacterial communities, we have found that multiple opportunistic pathogenic organisms, like *Shigella, Yersinia, Vibrio, Enterococcus, Haemophilus*, *Clostridium, Staphylococcus, Streptococcus* and *Bacillus* genera representatives are present in inoculum (SS) or waste influent (SW). Populations of these potentially pathogenic genera are strongly reduced after several days of incubation (Figure 2).

At the species level, most known pathogens is swine waste (SS) were suppressed in MFCs, including *Haemophilus influenza, Yersinia pestis, Vibrio cholerae, Clostridium difficile, Bacillus anthracis, Staphylococcus aureus, Bacillus cereus, Clostridium tetani, Shigella sonnei, Yersinia pseudotuberculosis, Streptococcus pneumonia, Streptococcus agalactiae, Streptococcus pyogenes, Enterococcus faecium, Yersinia enterocolitica, Staphylococcus epidermidis, Francisella tularensis, and Enterococcus faecaliis.* On contrary, *Campylobacter jejuni, Legionella sp., and Brucella* sp. Slightly increased their relative abundances (Figure 2).

The changes were the most dramatic for *Yersinia pestis, Yersinia pseudotuberculosis, Shigella sonnei,* and *Vibrio cholerae* for all inocula and in both anodic and planktonic communities (Figure 2). For other species, such as *Haemophilus influenzae, Yersinia enterocolitica*, *and Clostridium sp.*, the relative decline was strongly associated with SS inoculum and SW feedstock, with IGBS-inoculated MFC showing the poorest anti-pathogenic performance. Changes were not associated with a particular MFC compartment. The fact that species dynamics depend on inoculum source more than on MFC compartment suggests the role of community interactions/interspecies competition in the decline of certain bacterial groups. It also suggests that some species may be represented by differently adapted strains, depending upon the sample source. SW feedstock contains more vulnerable heterotrophs with limited metabolic capacity that cannot survive in the MFC environment. The ratio of *Pseudomonas spp.* increases in the MFC community is also reflected in an increase in the proportion of *Pseudomonas*-specific functions, such as biosynthesis of siderophores, for instance.

The general tendency of the observed community composition shift supports previous observations [16,19,27-29]. Though the MFC treatment is useful for Enterobacteriaceae (*Shigella, Yersinia, Vibrio*) disinfection, other groups of opportunistically pathogenic bacteria may be resistant to electroactive conditions, and for some of them (representatives of *Spirochaeta, Clostridium, Corynobacteria*, *Legionella, Streptococcus, Brucella,* and *Pseudomonas* genera) MFC may even constitute a stimulating environment. Propagation and evolution of virulent features of *Streptococcus, Staphylococus*, and *Bacillus* genera seem dependent on bacterial composition of the inoculum, as they had different tendencies in the two inocula. In general, disinfection/weakening of the virulence of Gram-positive organisms in the MFC does not seem very efficient, or at least, its outcome cannot be fully predicted yet. Further experiments are required. However, growth of few probiotic representatives of *Pseudomonades, Cyanobacteria* and *Streptococcus* genera may be supported in MFC if the initial inoculum and incubation conditions are optimized.

A decline of Enterobacteriaceae family in an operated MFC may certainly be attributed not to only a communal fitness factors but also a number of chemical and physical factors associated with operated MFC, such as increase in temperature which takes place in proximity to electrodes, global and local changes in pH and osmolality (cathodic space) [30]. In our experiments we see enrichment in a number of stress-resistance genomic attributes in bacteria which abundancies increases with time of cultivation in MFC (unpublished data), which suggests indeed an important role for this functional component

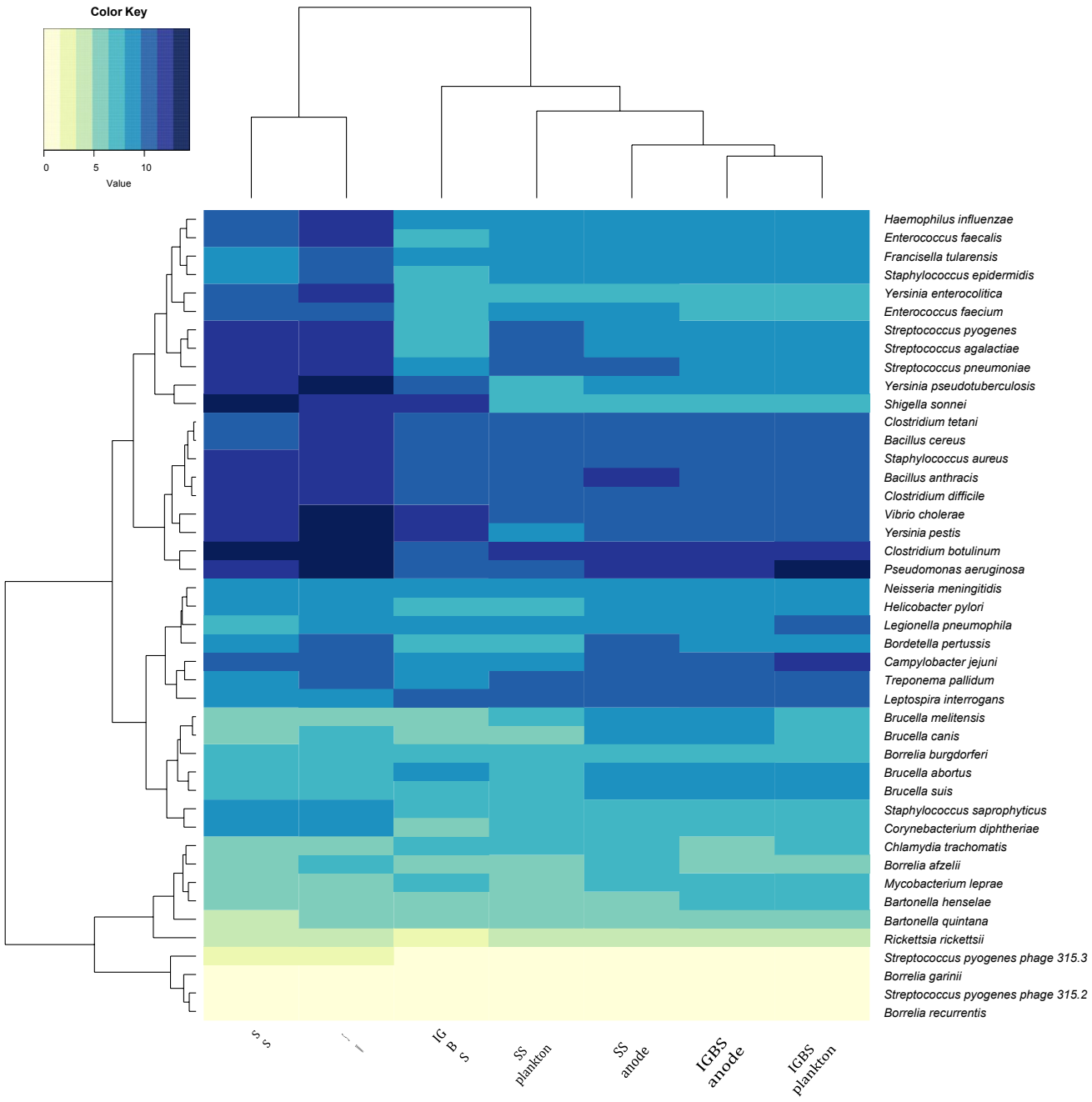

Figure 2: Relative abundancies of reads mapped to genomes of the pathogenic bacteria MFC residents. Generated via ASAR taxons to samples function mapper. The log abundance is shown for reads annotated with a selected function in a particular taxon within a particular community. SS- swine sludge inoculum, IGBS - industrial sludge inoculum, SW swine waste inflow, SS plankton and SS anode - planktonic and anodic community in the swine sludge inoculated MFC, IGBS plankton and IGBS anode - planktonic and anodic community in the industrial sludge inoculated MFC.

in developing composition of the bacterial communities. It may therefore be tempting to correlate the increased stress with the loss of genes that are not facilitating the electron transfer or other redox pathways. Similarly, such environmental pressure seems to positively influence those virulence genes that are involved in redox pathways (pyocyanin, siderophore in Pseudomonas spp.), or ion transfer (antibiotic and toxin resistance genes). We also observed differences between open circuit and active MFC which is in a support of the rather physical/chemical factors associated with electricity production having a key role in the observed suppression of the particular groups of bacteria. This theme is in focus of our current research and is a corner stone for many industrial applications of MFC technique.

Dynamics of pathogenicity-related genomic features in MFC's microbial community

The number of reads associated with the majority of functions

classified into bacterial 'pathogenicity'(merged virulence and antibiotics resistance) category diminished in MFC compared to initial inocula and are associated indiscriminately with all MFC compartments (Figure 3). However, sequences associated with several types of function: regulation of virulence (from IGBS inoculum), bacterial cytostatics, differentiation factors, and antibiotics (anodic communities from IGBS and SS inocula), siderophores (for all inocula, and especially for anodic communities), increased in abundance in the MFC. Genomic presentation of such functional categories as 'adhesion and secretion systems (III, IV, VI, ESAT)', 'invasion and intracellular resistance', 'cell wall of *Mycobacteria*' and 'NAD/NADP metabolism' increased in the IGBS-based MFC community compared to their levels in the initial IGBS inoculum.

Relative genomic presentation of pathogenicity islands decreases in the bacterial communities developed from SS inoculum. However, due to decreased representation of nearly all functions associated with opportunistic pathogenic bacteria in SS-based MFC, it was hard to conclude if the pathogenicity islands decline was a result of a targeted negative selection. Interestingly, functions from the 'toxins and superantigen' functional virulence category did not decline compared with the initial SS inoculum but increased instead. Only a slight decrease was observed for this group of functions in the anodic bacterial community developed from IGBS inoculum.

Analysis of categories responsible for bacterial virulence and virulence-like factors (Figure 4) suggests a potential role of MFC conditions in suppression of antibiotic resistance, invasion, intracellular resistance, and adhesion. However, it is not clear from the method whether this decline is proportional to a general decline of opportunistic pathogenic bacterial groups, especially from *Enterobacteria*, in the MFC community developed from SS inoculum.

Data presented on Figure 5 show functions from the 'antibiotic resistance' category in more detail. Only 'Multidrug resistance tripartile system' and 'Streptolysine biosynthesis and transport' pathways show an obvious decline in all MFC chambers' communities developed from IGBS inoculum. However, functions responsible for resistance to vancomycine, fostomycine, fluoroquinones and methicillin, as well as of beta-lactamase, cholera toxin, vibrio cytolysin, and the *Streptococcus pyogenes* virulome increased in IGBS MFC community. *PhnB, vanR, vanI* (genes for quorum sensing controlling biofilm formation and exopolysaccharide production in *Clostridium*) also declined after MFC incubation.

Given that antibiotic resistance mechanisms involve efflux pumps [36], as well as the role of cholera [37] and cytolysin [38] toxins in the ion flux, it is plausible that conditions within MFC favor those mechanisms.

In contrast to its dynamics in the IGBS-based MFC community, 'Multidrug resistance tripartile system' genomic presentation increased in the community developed from SS inoculum. Resistance to vancomycine and beta-lactamase, as well as genes for streptolysin biosynthesis and transport also had positive dynamics in the MFC community developed from SS inoculum. Sequences mapped to genes encoding functions associated with resistance to fluoroquinones and methicillin, as well as *Staphylococcus* adhesins, *Listeria* surface proteins, and *Streptococus pyogenes* virulence functions were less enriched in MFC compared with SS inoculum. However, these negative dynamics may have been caused by the total decline in opportunistic pathogenic bacteria in MFC chambers and may not allow conclusions to be drawn regarding the influence of the electroactive chamber on negative drift of virulent genes.

Metabolic functions of pathogens with differential presentation in bacterial communities before and after cultivation in MFC

Analyses of the reads mapped to KEGG pathways (Figure 6) confirm results of functional analyses based on SEED/RAST subsystems. Sequences relevant to biofilm metabolism were enriched in the MFC community produced from IGBS inoculum. Interestingly, we saw a clear enrichment for genomic presentation of functions relevant to biosynthesis of sulfur-containing cofactors, lipoate and thiamine, participating in microbial sulfur oxidation [39] and oxidation-reduction reactions of volatile acids [40] and pyruvate metabolism. The ratio of several thiamine biosynthetic genes increased in the MFC community developed from IGBS inoculum and the abundance of lipoate biosynthesis genes increased in MFC communities developed from IGBS, and for some bacteria (*Brucella, Bordetella, Clostridium*) also from SS inocula (Figures 6 and 7; Supplementary Figure 1). The results suggest engagement of redox-associated sulfur and/or pyruvate metabolic processes in MFC, particularly in the anodic community. At least for *Spirochaete*, *Fracisella, Legionella and Brucella,* these tendencies were strongly associated with increased genomic presentation of the TCA pathway (Supplementary Figure 2). Presentation of genes for biosynthesis of riboflavin, FMN, and glutathione metabolism are only slightly enriched in the MFC community compared to both inocula. Spirochaete (*Treponema, Leptospira*) and aerobic coccobacilli, *Franciella* and *Legionella*, became enriched in the MFC metagenomes of communities developed from both SW and IGBS inocula. Aerobic metabolism of bacteria that became abundant in MFCs, is reflected in a continuous enrichment of sequences corresponding to particular lipoate (LplA) [39,40] and thiamine salvage genes.

A link of lipoate salvage to hydrogen metabolism in a number of electroactive bacteria possessing non-methanogenic heterodisulfide reductase [39,40] can explain particular importance of the LplA genomic abundance in operating MFC. Electroactive properties of the bacteria are also based on pyruvate metabolic conversions that would strongly depend on presence of thiamine as an enzymatic cofactor.

As shown on examples of KEGG depiction of the corresponding pathways (Figure 8), relative abundance of reads mapped to genes encoding hydroxymethylpyrimidine kinase (EC2.7.1.49) and phosphomethylpyrimidine kinase (EC 2.7.4.7) strongly increased in *Spirochetes* population in MFC in compare to the initial inoculum. Interestingly, these three gene is were also the most differentially presented in MFC of all genes encoding bacterial metabolic functions. Hydroxymethylpyrimidine kinase ensures an assimilation of thiamine degradation product, 4-amino-2-methyl-5-pyrimidinemethanol (HMP), into the de-novo pathway where phosphomethylpyrimidine kinase performs the following reaction. Therefore, we can suggest that bacteria successfully adapted to MFC environment relay on mainly extracellular source of a precursor for thiamine biosynthesis. With a

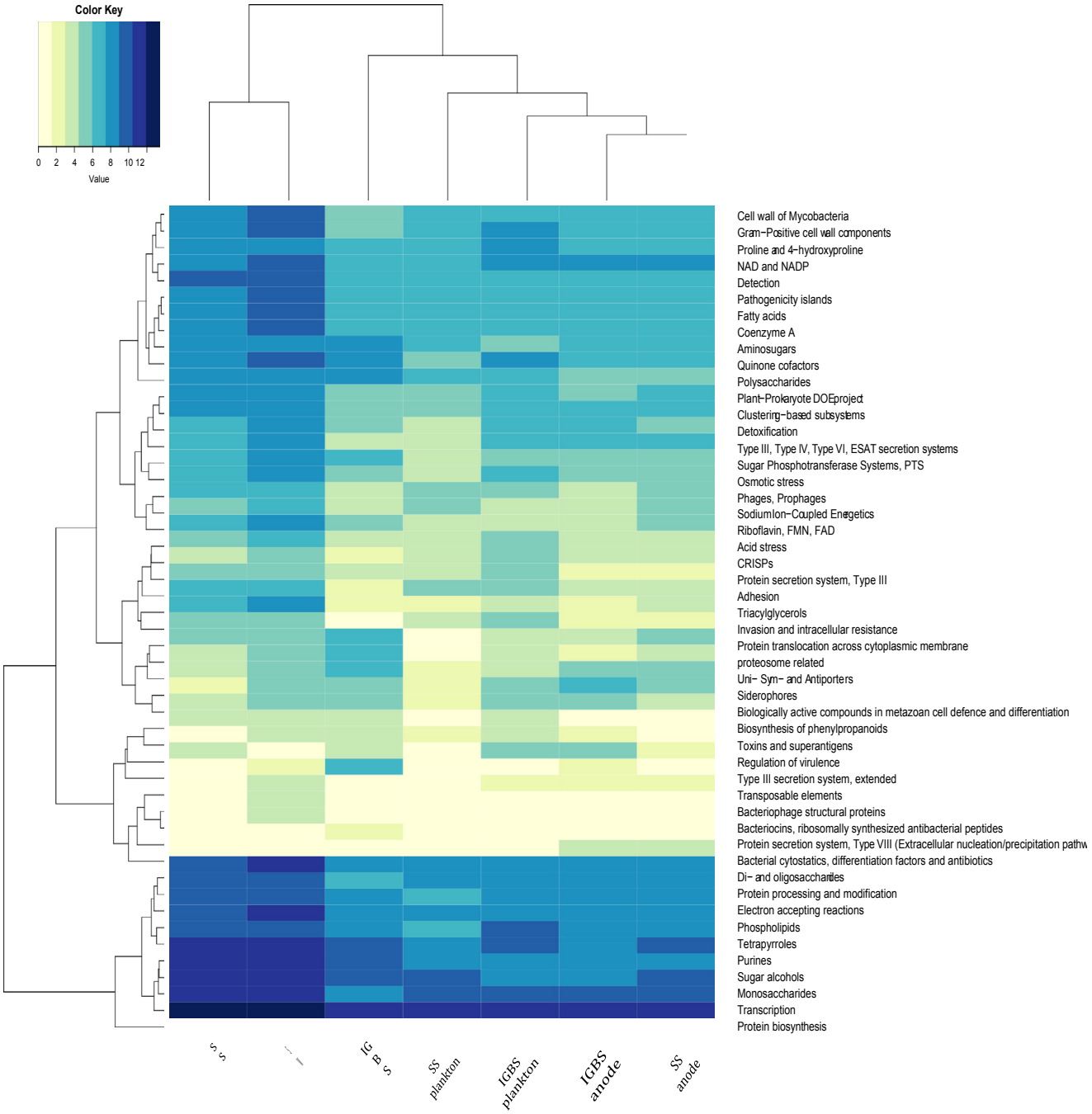

Figure 3: Relative abundancies of reads mapped to genomes (species level) of the pathogenic bacteria MFC residents. Generated via ASAR taxons to samples function mapper. The log abundance is shown for reads annotated with a selected function in a particular taxon within a particular community. SS- swine sludge inoculum, IGBS - industrial sludge inoculum, SW swine waste inflow, SS plankton and SS anode - planktonic and anodic community in the swine sludge inoculated MFC, IGBS plankton and IGBS anode - planktonic and anodic community in the industrial sludge inoculated MFC.

diminished survival of a fraction of groups of bacteria, and a potential accumulation of thiamine products in the media, adaptation of the *Spirochetes* may be of a competitive nature.

In contrast, a strong decline of the hydroxymethylpyrimidine kinase and phosphomethylpyrimidine kinase matching reads was observed for declining Enterobacteriaceae family and *Haemophilus* and *Bordetella* genera, with an example depicted in the KEGG maps (Figure 9). Another thiamine–biosynthetic gene, encoding for the function of thiamine-phosphate pyrophosphorylase (EC 2.5.1.3), KEGG maps (Figure 8A and 9A) also showed specific pattern of enrichment, depending on bacterial genera and MFC chambers. For instance, increased number of the corresponding *Neisseria* and *Vibrio* reads was detected in the

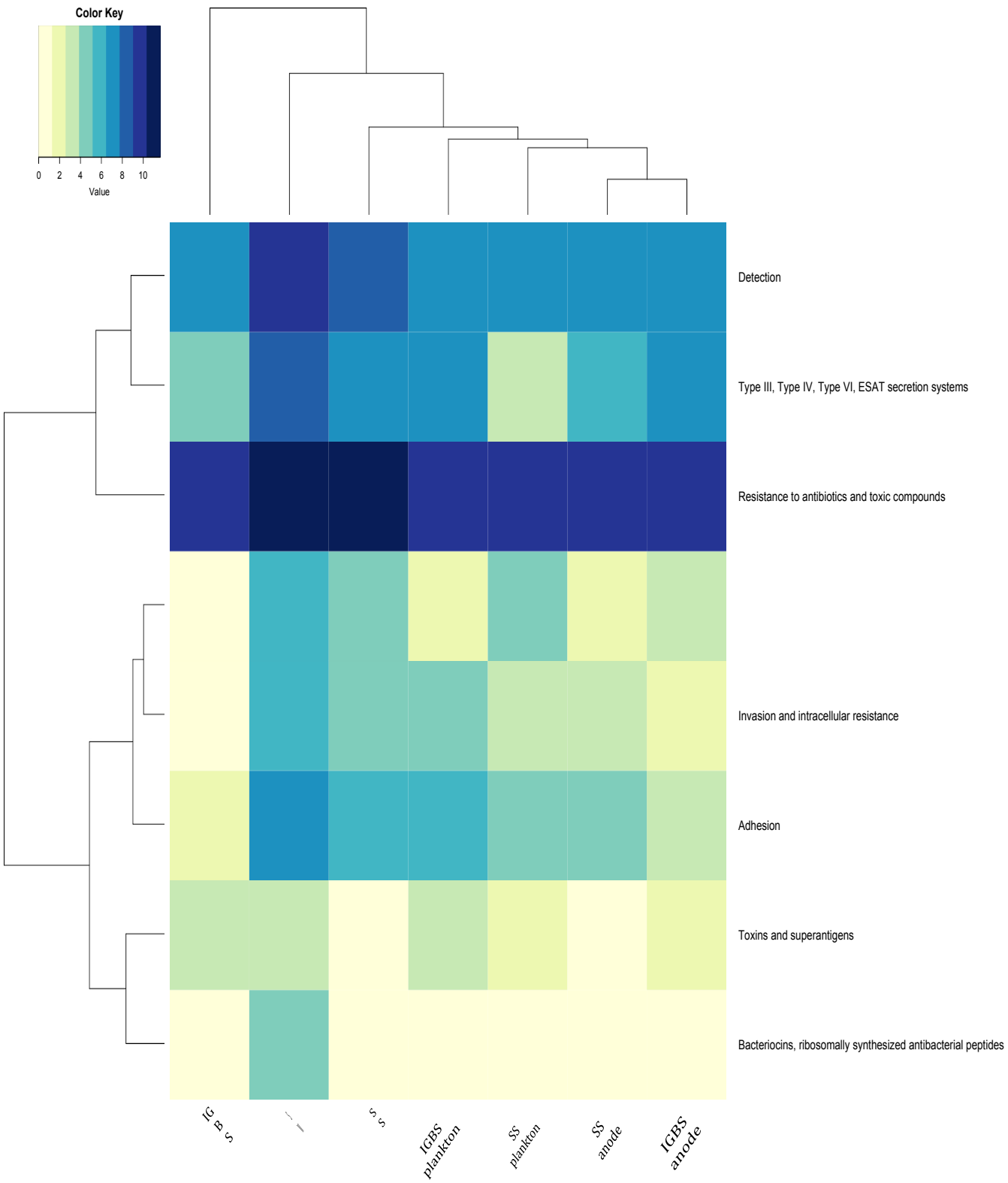

Figure 4: Relative abundancies of reads mapped to virulent components of the genomes of the pathogenic bacteria MFC residents. Generated via ASAR taxons to samples function mapper. The log abundance is shown for reads annotated with a selected function in a particular taxon within a particular community. SS- swine sludge inoculum, IGBS - industrial sludge inoculum, SW swine waste inflow, SS plankton and SS anode - planktonic and anodic community in the swine sludge inoculated MFC, IGBS plankton and IGBS anode - planktonic and anodic community in the industrial sludge inoculated MFC.

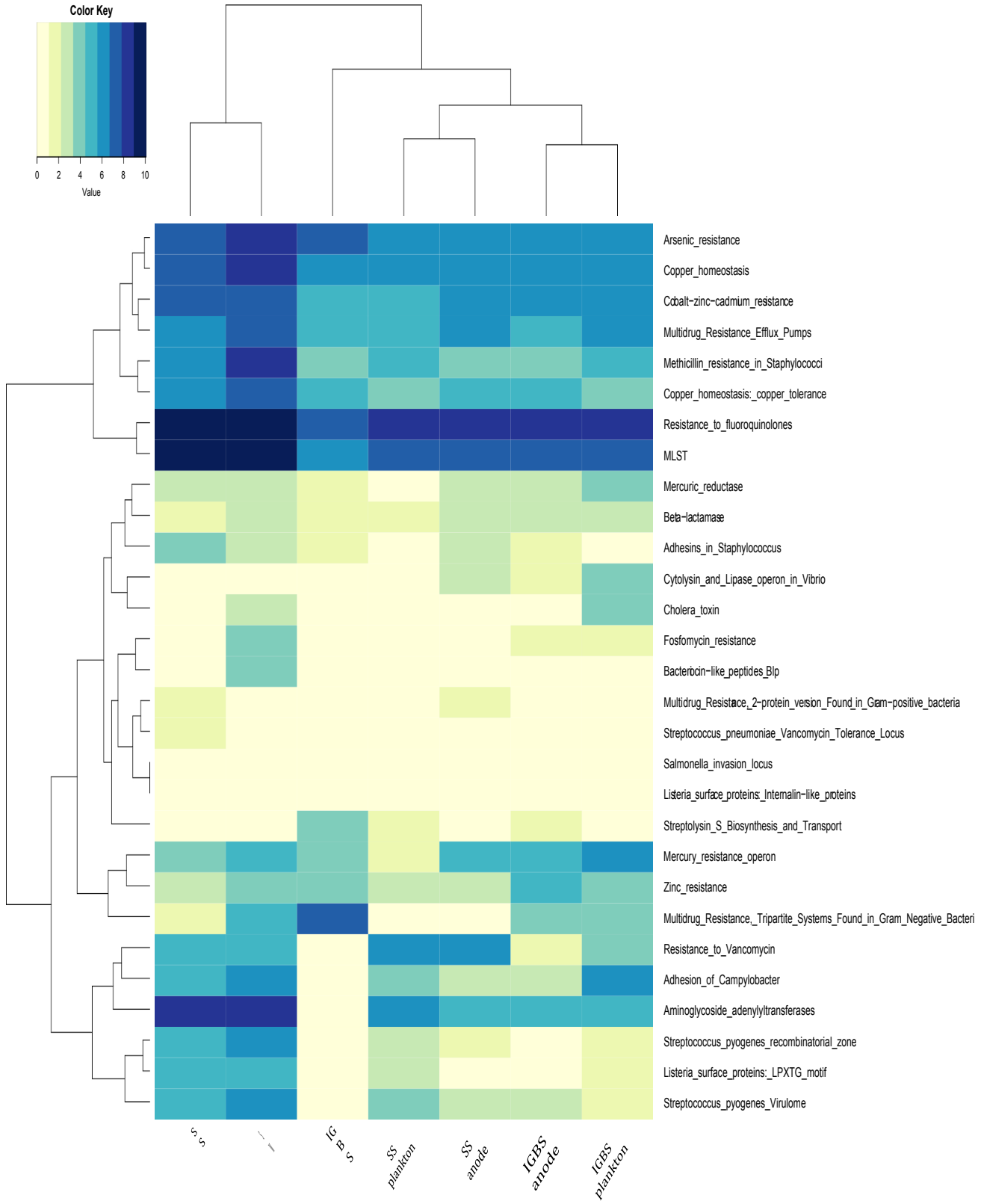

Figure 5: Relative abundancies of reads mapped to genomes of the pathogenic bacteria (family level) MFC residents. Generated via ASAR taxons to samples function mapper. The log abundance is shown for reads annotated with a selected function in a particular taxon within a particular community. SS- swine sludge inoculum, IGBS - industrial sludge inoculum, SW swine waste inflow, SS plankton and SS anode - planktonic and anodic community in the swine sludge inoculated MFC, IGBS plankton and IGBS anode - planktonic and anodic community in the industrial sludge inoculated MFC.

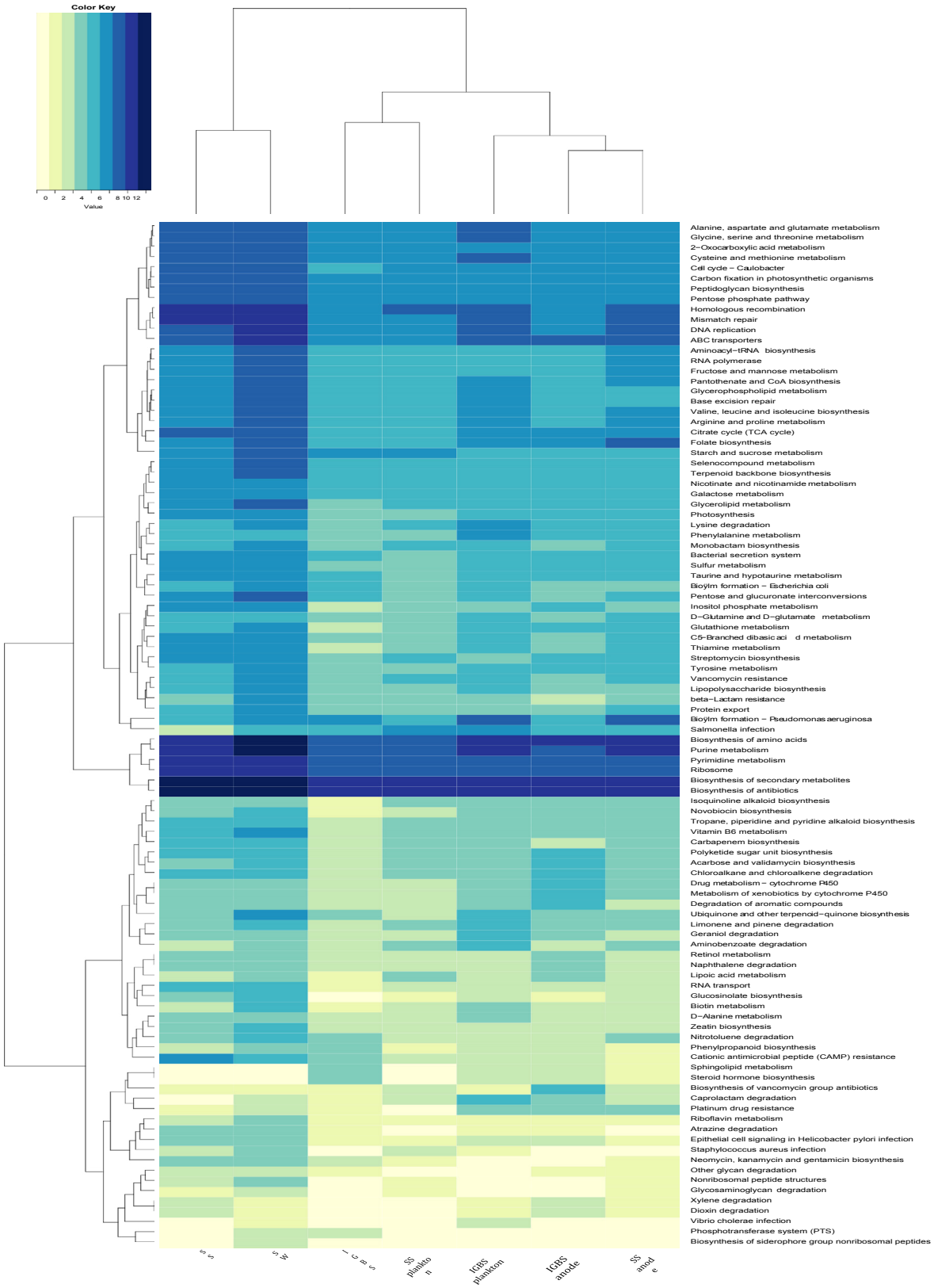

Figure 6: Relative abundancies of reads mapped to KEGG functions associated with genomes of the pathogenic bacteria MFC residents. Generated via ASAR taxons to samples function mapper. The log abundance is shown for reads annotated with a selected function in a particular taxon within a particular community. SS- swine sludge inoculum, IGBS - industrial sludge inoculum, SW swine waste inflow, SS plankton and SS anode - planktonic and anodic community in the swine sludge inoculated MFC, IGBS plankton and IGBS anode - planktonic and anodic community in the industrial sludge inoculated MFC.

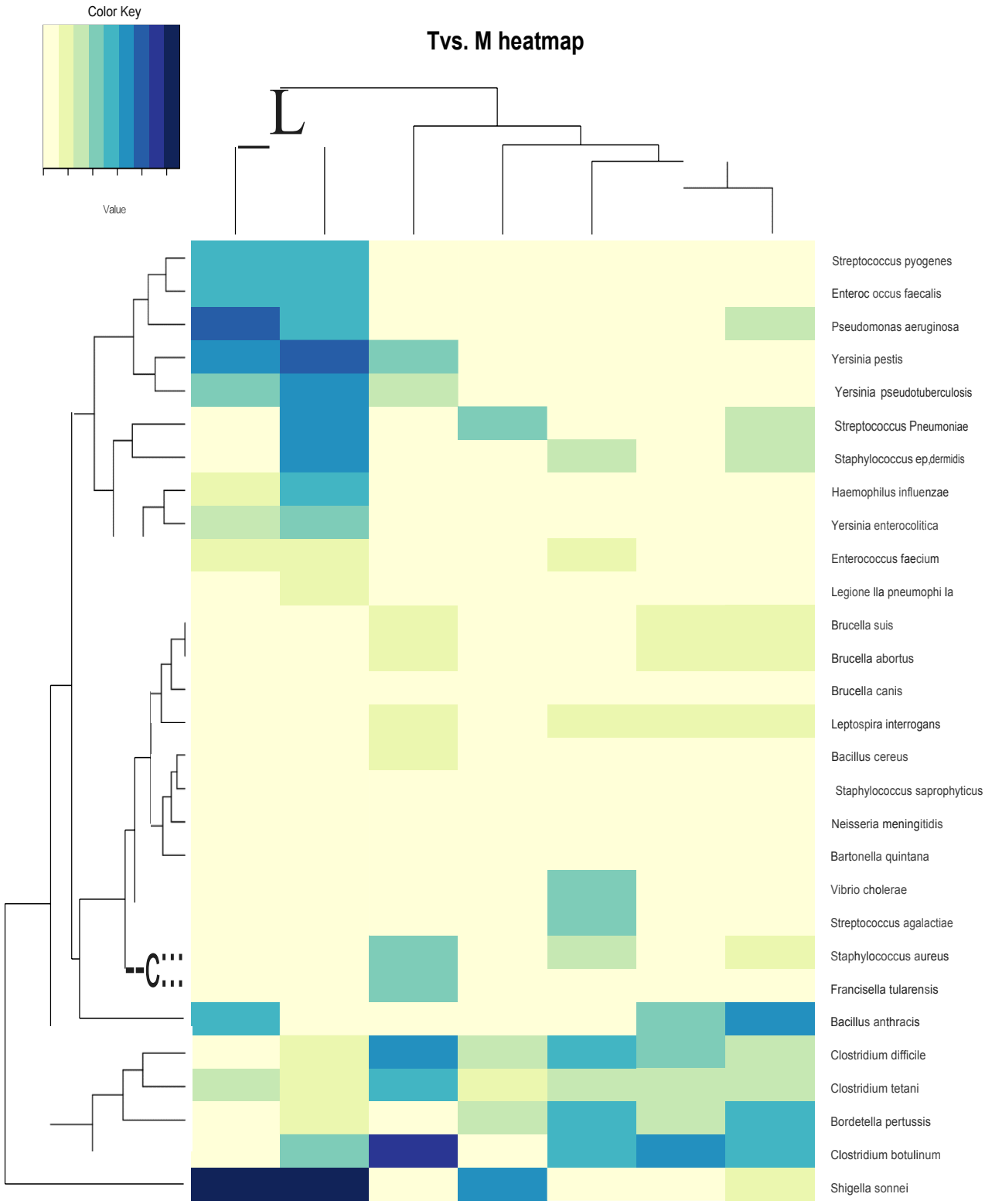

Figure 7: Heatmap for normalized number of reads mapped to Lipoate biosynthesis genes across the pathogen's bacteria species. Generated via ASAR taxons to samples function mapper. The log abundance is shown for reads annotated with a selected function in a particular taxon within a particular community. SS- swine sludge inoculum, IGBS - industrial sludge inoculum, SW swine waste inflow, SS plankton and SS anode - planktonic and anodic community in the swine sludge inoculated MFC, IGBS plankton and IGBS anode - planktonic and anodic community in the industrial sludge inoculated MFC.

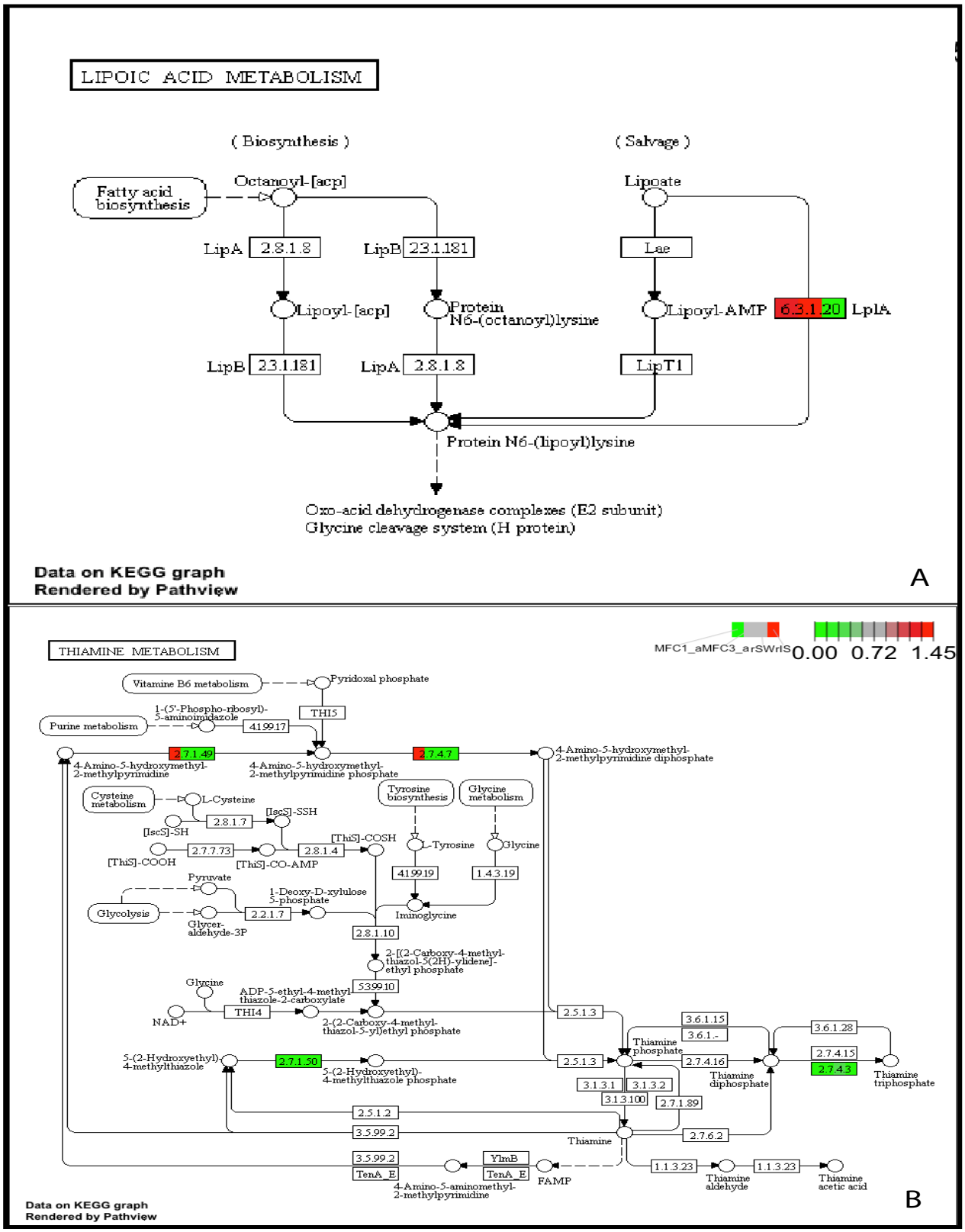

Figure 8: KEGG maps [47] presenting examples of differential relevant abundancies of reads from MFC anodic chamber (left section of a block) and inflow SW (right section of a block) metagenomes mapped to Spirochetes' Lipoate (A) and Thiamine salvage (B) pathways. The Function boxes are split into sections corresponding to the analyzed metagenomes; color of each section encodes the proportion of the function provided by a selected taxon.

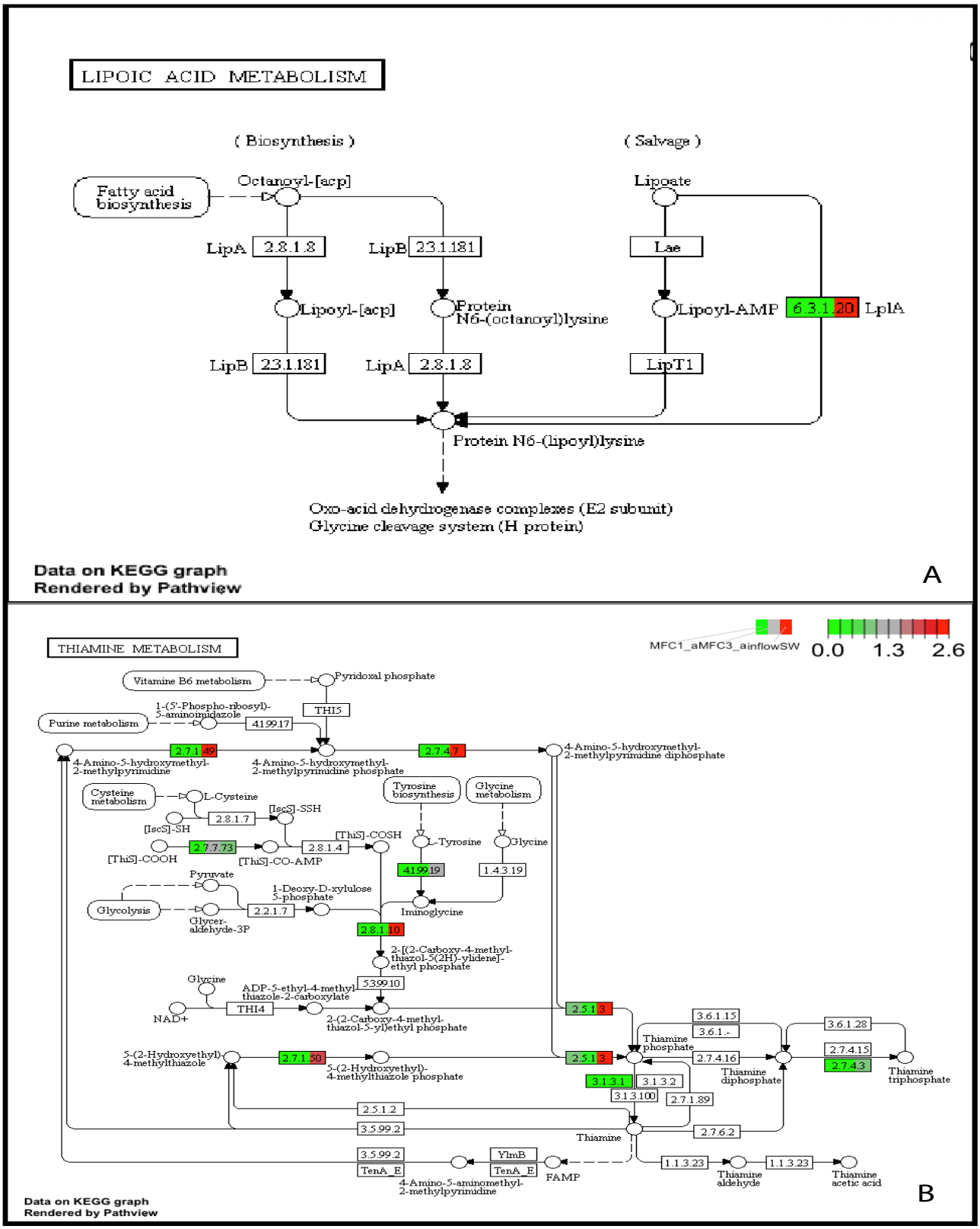

Figure 9: KEGG maps [47] presenting examples of differential relevant abundancies of reads from MFC anodic chamber (left section of a block) and inflow SW (right section of a block) metagenomes mapped to Enterobacterial Lipoate (A) and Thiamine salvage (B) pathways. Function boxes are split into sections corresponding to the analyzed metagenomes; color of each section encodes the proportion of the function provided by a selected taxon.

planktonic community metagenome, but strongly decreased number of the thiamine-phosphate pyrophosphorylase matching Vibrio reads was detected in the metagenome of the anodic community).

The data suggest that thiamine-based oxidative pyruvate metabolism is likely suppressed in bacteria selected against in the MFC chamber, especially in opportunistic pathogens: *Shigella, Yersinia, Vibrio*, and *Haemophilus*. However, those pathways seem to be of increasing metabolic significance for *Spirochaete,* and aerobic cocobacilli *from Francisella and Legionella* genera. Relative abundance of reads mapped to diverse *Staphylococcus and Clostridium species, Streptococus agalactae and Enterococcus faecium* lipoate pathway was also increased in the MFC community developed from IGBS inocula (Figure 6).

Conclusions

Our results support the suggestion to use of MFC to disinfect wastes enriched in Enterobacteriaceae *(Shigella, Yersinia, Vibrio)* species which would help to prevent bacterial resistance to the spectrum of chemical disinfectants. As our data show, it may be also effective against *Haemophilus* and a number of Gram-positive bacterial genera, such as *Enterococcus,* that recently comprise the leading causes of hospital-associated infections as possessing resistance to multiple antibiotics.

However, for some groups of opportunistic pathogenic bacteria (representatives of *Spirochaete, Clostridium, Corynobacteria*, *Legionella, Streptococcus, Brucella*, and *Pseudomonas genera*) MFC may even constitute a stimulating environment. Propagation and evolution of virulent features of *Streptococcus, Staphylococus*, and *Bacillus* genera and a disinfection effectiveness of MFC in general, depend on the source or a bacterial composition of the original inoculum that suggests the role of community interactions/interspecies competition in the decline of certain bacterial groups. It also suggests that some species may be represented by differently adapted strains, depending upon the sample source.

A negative drift of genes for lipoate and thiamine salvage occurred to be characteristic for the declining enterobacterial groups, though strong enrichment of these genes was demonstrated for *Spirochaeta*, *Clostridium, Fraciella and Legionella* which abundancy increases during MFC incubation and also may be treated as a result of intracommunity competition. Those questions are subjected for more detailed interdisciplinary research with mathematical modeling of metabolic communal interactions and comparative genomics efforts involved.

A decline of Enterobacteriacae family in an operated MFC may certainly be attributed to not only a communal fitness factors but also a number of chemical and physical factors associated with operated MFC, which comprises a focus of our current investigation. With more data available, optimized MFC technology may become an efficient approach to be applied to disinfection of hospital wastewaters and medical wastes.

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Author Contributions

IG and OV drafted the manuscript. AS developed ASAR software. OV, IG and LS analysed the results. All authors reviewed and edited the manuscript.

Competing Interests

None.

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