Sludge oomph: harnessing the power of sediment microbiota

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In recent years, new methods of clean and environmentally friendly energy production have been the focus of intense research efforts. Microbial fuel cells are devices that utilize naturally occurring microorganisms that feed on organic matter while producing electrical energy. The natural habitats of bacteria thriving in microbial fuel cells are usually marine and freshwater sediments. In order to develop and improve applications of the vast metabolic repertoire of these microorganisms it is important to identify the relevant proteins and their functional mechanisms. Several sediment bacteria have been the focus of research efforts such as members of the genera Desulfuromonas, Geobacter, and Shewanella. The genomes of these organisms have the common characteristic of containing numerous genes for multiheme cytochromes. For Geobacter and Shewanella many of these are known to be essential for the extracellular respiration that is at the core of electricity production in microbial fuel cells. In this review, the current knowledge on these unusual redox chains and future perspectives on their understanding towards improved extracellular electrical contact are presented.

Keywords: Anaerobic respiration; Microbial Fuel Cells; Shewanella; Geobacter; Desulfuromonas; cytochromes

1. Introduction

Climate change is the pressing global problem of our time and energy generation to maintain our lifestyle is a major source of greenhouse gas emissions. In recent years, we have witnessed a surge in research efforts focusing on new forms of energy production that are more environmentally friendly. Most of the energy nowadays comes from processes that are inherently unsustainable due to the depletion of non-renewable resources. As a result, the development of new forms of sustainable and efficient energy generation capabilities is mandatory in order to allow industrial progress with a small environmental footprint. In this context, renewable energies will assume an increasing role, and biomass is a very promising source of energy, taking advantage of the metabolic versatility of microorganisms.

Microbial life is ubiquitous on planet Earth, having colonized successfully diverse ecological niches, including some that seem odd or extreme. Two recent examples illustrate the metabolic versatility that makes microbial life a crucial pillar of the geobiosphere: Sampling made in a gold mine in South Africa at a depth of 2.8 km has revealed an ecosystem composed of a single bacterial species capable of fixing nitrogen and carbon [1]; an alternative mode of independent lifestyle is maintained by a green sulfur bacterium isolated from a black smoker at a depth of 2.4 km in the Pacific Ocean which uses geothermal radiation to perform anoxygenic photosynthesis in the absence of sunlight [2]. As these examples illustrate, almost every ecological niche on Earth where a thermodynamically favorable reaction can be coupled to metabolism is known to be colonized [3]. Geological evidence indicates that unicellular life forms have inhabited Earth for at least 3.5 billion years [4]. Studies of present day microbes show that their ancient relatives had an impact on the chemistry of the planet’s lithosphere, hydrosphere and atmosphere [5, 6]. For example, different strains of the sediment bacterium Shewanella give rise to different mineral products when reducing hydrous ferric oxide [7]. Therefore, as the planet changed life on Earth also evolved, in a co-evolution process of biological and geological diversity [8].

Marine and freshwater sediments harbor microbial communities that are very rich in their metabolic variety. Vertical redox gradients in the sediment support respiratory processes relying on successively less oxidizing terminal electron acceptors [9]. Substrates for these anaerobic respiratory processes include a wide variety of organic and inorganic compounds [3, 9]. The list is extensive and includes also toxic elements and insoluble metallic compounds in ores. These are, nonetheless, redox active and therefore incorporated in the planetary biogeochemical cycles. The organisms capable of using metallic compounds, soluble or insoluble, to support their respiratory metabolism are called dissimilatory metal reducers.

These dissimilatory metal reducing organisms gained notoriety when it was realized that they represented an enormous untapped potential for bioremediation of contaminated sediments, soils and groundwater [3, 10]. Microorganisms are capable of changing the oxidation state of the elements, allowing for their easier removal, since most of them become insoluble when reduced [3]. This process can be an integral part of the bacterial metabolism. For example uranium, which is a soil and groundwater contaminant of great concern, can be reduced by sediment bacteria. It is converted from the soluble oxidized form, U(VI), to the insoluble reduced form, U(V), which precipitates and prevents its spread [11]. A dramatic counter example is the anaerobic respiration of arsenate. The arsenite released is water soluble and a cause of concern in multiple parts of the world because of its potential for poisoning drinking water sources. The capability of dissimilatory metal reducers to link their anaerobic respiratory metabolism to solid substrates such as the metallic ores, also lead to their application in devices where the solid electron acceptor is an inert electrode.
These bioelectrochemical devices called Microbial Fuel Cells (MFC) work as batteries when connected to an external circuit [12, 13]. The fuel to power these cells can be any source of organic matter, providing a novel environmentally friendly way to produce electricity.

2. Microbial Fuel Cells

Microbial fuel cells (MFCs) are devices that utilize microorganisms to produce electrical current while metabolizing nutrients in the medium. These devices consist of an anode that is kept under anoxic conditions and that receives the electrons from the bioenergetic metabolism of the microorganisms growing on its surface. MFCs also contain a cathode that transfers electrons to a terminal electron acceptor. The electrons flow from the anode to the cathode passing through an external circuit to perform electrical work. There is a wide variety of designs and the anode and cathode may be in a single compartment or separated by a physical barrier that is permeable to ions that close the circuit [14]. Research on microbial fuel cells leading to real world applications has surged in recent years [15, 16]. It is accepted that practical and economically viable applications of this technology require the use of atmospheric oxygen as the terminal acceptor in the cathode. Towards this end, original MFCs relied on expensive catalyst such as platinum for efficient reaction. However, the need to reduce the costs of the device spurred the development of new materials that considerably cut the use of precious metals or even eliminate their need. A laboratory model using stainless steel anodes lead to the report of a peak current of 2.4 A/m² [17]. Design evolution is also contributing to bringing this technology closer to real world applications. Tubular cell designs that facilitate the flow of substrate have improved the Coulombic efficiency of the devices up to 75% when using acetate as substrate and a solution of ferricyanide as cathode [18]. Miniaturization increases volumetric power output with values of up to 2.15 kW/m³ of internal volume reported in the literature [19]. On the microbiological side, isolation and characterization of new strains or microbial communities lead to power densities of up to 2.7 W/m² of cathode area [20]. The higher power obtained from MFCs operating with a mixed culture versus a pure culture was shown to be a consequence of lower internal resistance, and therefore dependent on the design of the MFC [21].

![Fig. 1 Schematic representation of a MFC. Gray ellipses represent cells growing on the anode.](image)

The capability of using organic waste, including wastewater, as substrate for MFCs has opened the possibility of producing electricity in a way that is close to carbon-neutral [22]. It also provides an alternative route for removal of organic matter in the treatment of municipal residues as well as residues derived from the food and beverage processing industries that is less power intensive and may even result in net power generation [23]. Electricity production is typically the objective when operating MFCs, but research has also lead to alternative designs and operation modes called Microbial Electrolysis Cells that can yield valuable commodities such as hydrogen or hydrogen peroxide [24, 25]. Different processes can be linked and tuned towards electricity production, hydrogen production, or chemical oxygen demand reduction. This allows for a versatile optimization of the most interesting outcome [26]. The knowledge that bacteria can produce electricity in laboratory is almost one century old, and organisms that produce electrical current have been named electricigens [12] or exoeletron [27]. Of all aspects of MFCs’ development, the
detailed knowledge of the molecular mechanisms that support extracellular electron transfer are the least advanced. Nonetheless a few general aspects are now well established.

3. Electron transfer to extracellular solid substrates

The key metabolic process for understanding how MFCs work is the mechanism of electron exchange with extracellular solids. Microorganisms that perform extracellular respiration are capable of transferring electrons to a terminal acceptor that is localized outside the cell. The molecular mechanisms of electron transfer to substrates in the extracellular space can be divided broadly in direct electron transfer and indirect electron transfer [28].

Direct electron transfer occurs via contact with the extracellular solid. A recent vivid demonstration has been reported, by recording movies under a microscope of manganese oxide dissolution by the metal respiring bacterium *Shewanella oneidensis* MR-1. Individual cells are seen touching and swimming away from the solid that is slowly dissolved [29]. This contact can be mediated by redox proteins protruding from the cell surface. A great number of multiheme cytochromes are found in organisms capable of colonizing electrodes in MFCs, and several have been directly implicated in extracellular respiration [30]. Electrical contact was also proposed to occur via electrically conducting appendages called pili or nanowires [31, 32, 33].

Evidence for indirect electron transfer was reported as early as 2000 [34]. More recently, experiments showing that iron oxide entrapped within glass beads could be reduced by *Shewanella*, reveal that direct contact is not always required [35]. Indirect electron transfer has been proposed to take place via electron shuttles and siderophores. However, the relevance of siderophores in dissimilatory metal reducing processes has been questioned on the basis of the inadequate redox potential of the chelated metal [36]. More recently, deletion mutants on siderophore biosynthesis pathways as well as of their receptors and reductases in *Shewanella oneidensis* MR-1 showed that they do not play a role in dissimilatory metal reduction even though soluble forms of Fe(III) are detected [37]. Work on electron shuttles has focused mostly on flavins, which can be endogenously produced or added to the medium. Flavins allow a MFC inoculated with *Shewanella* to function even when the bacteria are prevented from contacting the electrodes [38]. Indirect electron transfer is also a necessity for cells in the bacterial biofilm that are not directly attached to the electrode surface [28]. In these conditions access to the solid electron acceptor is limited.

Electron acceptor limitation is among the conditions where *Shewanella oneidensis* [31] and *Geobacter sulfurreducens* [39] are capable of producing electrically conductive pili, called nanowires, which conduct electrons directly from the cell to the electron acceptor. Although they are not required for metal reduction, they show great affinity for metal oxides whenever they are produced [28]. There is evidence that *Geobacter*’s pili are not involved in motility [39], but in addition to their role in electron transfer, they are also very important in cell aggregation in biofilms. There, they will in turn, give a considerable contribution for the overall electricity production, since larger amounts of viable cells will produce more energy [40]. Biofilm morphology is an important aspect of fuel cell operation. *Shewanella* are capable of forming thick biofilms that rely on pili and redox shuttles to maintain viability of the cells not directly attached to the surface of the electron acceptor [28, 41]. *Geobacter* species were originally reported to form biofilms which are monolayers of cells [40] but later, the presence of thick biofilms on the surface of electrodes was deduced on the basis of high power production [42]. Also, transcriptional analysis across thick biofilms of *Geobacter sulfurreducens* showed different metabolic status depending on the distance of the sampling to the anode surface [43]. Biofilm structure appears to be a function of multiple parameters. Pure cultures of *Shewanella oneidensis* MR-1 display biofilms with different morphologies when the resistance of the circuit powered by the MFC is modified. The higher the resistance, the thicker the film [44]. When Gram-negative and Gram-positive bacteria are co-cultured, biofilms were shown to evolve towards segregation of the two species [45]. Of particular importance for novel designs of MFCs was the observation that once biofilms of *Geobacter sulfurreducens* had been established, it is the metabolic rate and not interfacial electron transfer the kinetically limiting step in current production [46].

Experimental MFC designs have been reported using bacteria, mostly Gram-negative, but also other organisms including Eukaryotes such as a yeast [47]. A few bacterial species, all Gram-negative, have gathered most of the attention, in particular concerns the molecular mechanisms of electron exchange with extracellular solids.

3.1 *Shewanella*

*Shewanella oneidensis* MR-1 (originally *putrefaciens*) was isolated from brackish water in Lake Oneida [48]. This bacterium gained prominence by being the first organism shown to be capable of powering a MFC without the need of mediators [49]. This facultative anaerobic bacterium has the great experimental advantage of being easily cultivated in laboratory under a relatively wide range of conditions [13]. Furthermore, its genome is fully sequenced since 2002 facilitating the study of the role of the different genes and proteins [50].

Direct electron transfer between *Shewanella* cells and the extracellular substrates, such as metal oxides and anodes of the MFCs, involves c-type multi-heme cytochromes [30, 51]. The genome sequence revealed that there are forty-two possible cytochrome c genes, many of which are predicted to be multiheme [52]. Several of these cytochromes are known to be essential for these respiratory pathways. The inner-membrane tetraheme cytochrome c, known as CymA,
proteins inserted in the outer membrane, called MtrB and MtrE, respectively [58, 59]. These β-barrel proteins are essential for the correct localization of the outer-membrane cytochromes [60] and are proposed to promote the electron transfer between the periplasmic cytochromes MtrA and MtrD and the outer-membrane-associated decaheme cytochromes, MtrC and OmcA [61], and MtrF [59], respectively.

All of the three outer-membrane cytochromes MtrC, OmcA and MtrF have been shown to be exposed to the exterior [62, 63, 64, 65], which has made them of extreme interest as plausible terminal reductases for metal oxides and MFCs anodes. The majority of the present day studies focus on the extracellular electron transfer pathway involving the decaheme cytochromes MtrC and OmcA. These cytochromes are terminal reductases crucial for the reduction of insoluble substrates and electron transfer to MFC anodes [64, 66, 67, 68, 69]. Nonetheless, a series of knock-out mutations of all the outer-membrane cytochromes and subsequent expression of each one individually, showed that MtrC is pivotal for extracellular electron transfer and that mutants containing only the OmcA cytochrome were not capable of transferring electrons to iron [65]. This fact suggests that while OmcA is an iron terminal reductase [61, 70], its contact with the periplasmic redox chain is mediated by MtrC [65]. Recent studies have additionally shown that MtrC is responsible for most of the electron transfer to carbon electrodes, while OmcA is involved in cellular attachment to solid surfaces and plays a smaller role in electron transfer [69]. This is coherent with data obtained by antibody functionalized Atomic Force Microscopy (AFM) tips that showed OmcA in the interface between the cell and insoluble substrate, while MtrC displays a more uniform distribution across the cell surface [64]. Furthermore, it has also been shown that the OmcA has a higher binding affinity to insoluble iron substrates than MtrC [72, 73]. MtrF also has the ability to reduce metals and MFC anodes but its physiological function was recently proposed to be reduction-based detoxification of radionuclides [44, 65].

Electron transfer via direct contact in Shewanella can also be mediated by electrically conductive pili, also known as nanowires, which are hypothesized to assist in the electron transfer from the bacterial cells to extracellular electron acceptors [31]. Scanning tunneling microscopy (STM) showed various thin filaments with about 8 nm in diameter and tens of microns in length. Shewanella nanowires display non-linear electrical transport behaviour, where the voltage dependence of the conductance reveals peaks that indicate the presence of discrete energy levels with higher electronic density of states [33].

Although in terms of morphology Shewanella and Geobacter pili are relatively similar [31, 39, 74], in terms of composition, Shewanella nanowires are thought to be partially composed of c-type cytochromes. This was demonstrated by deleting the genes coding for the outer-membrane cytochromes, MtrC and OmcA, resulting in non-conductive pili-structures [31]. Also, deleting the gspG gene which is involved in the type II secretion pathway, that is required for the proper export of the outer membrane cytochromes MtrC and OmcA to the cell exterior [75, 76], resulted in non-conductive pili-structures. Recently, it was concluded that the pili structures were not essential for extracellular electron transfer from Shewanella to metals or anode surfaces. Deletion of the genes involved in the synthesis of both types of pili (Msh and type IV) showed that in the case of type IV pili the mutant generated more current than the wild-type and that the loss of the Msh pili only resulted in a decrease of the output from the MFCs over time [76].

Besides reduction through direct contact, Shewanella oneidensis can also reduce extracellular substrates through indirect electron transfer, namely by production of electron shuttles that mediate the electron transfer between the cell surface and the exogenous acceptors [36]. In the case of electron shuttles, small organic molecules serve as the terminal electron acceptor and transfer electrons to the iron oxides or to the MFC anode, becoming reoxidized and capable to be re-used. It was initially proposed by Newman and Kolter that Shewanella excreted some unidentified quinones to mediate extracellular electron transfer [34]. Recent studies showed that flavins are the main endogenous electron shuttle in the Shewanella genus. Shewanella oneidensis MR-1 accumulates flavins to high concentrations in solution (250-500 nM) to be used as electron shuttles for extracellular electron transfer to the electrodes [41]. As further confirmation, FMN and riboflavin were shown to be the major electron shuttles in Shewanella sp. for mediating the electron transfer [77].

Recent, kinetic results showed that direct contact between the outer membrane cytochromes (OmcA and MtrC) and insoluble iron substrates or MFC anodes could not account for the rates of electron transfer observed when using whole cells assays [61, 78]. This gap in electron transfer rates was resolved with the addition of flavins. This demonstrated that multiheme outer membrane cytochromes are not the only elements responsible for the electron transfer to insoluble iron at relevant kinetic rates and that direct and indirect electron transfer occur in tandem in Shewanella oneidensis MR-1 [61]. Moreover, recently it has been shown that the outer membrane cytochromes account for at least 95 % of the reduction of extracellular flavins at physiological relevant rates [69].

Shewanella oneidensis MR-1 possess a highly versatile respiratory system, able to use nearly any electron acceptor more electronegative than sulphate [79]. However, in terms of the usable carbon sources, it is relatively limited, catabalising mainly fermentation end products such lactate, some amino acids, formate, and hydrogen [79]. The
oxidation of these carbon sources is incomplete, producing acetate. For applications on MFCs where wastewater is the substrate, an incomplete oxidizer is less interesting because the removal of dissolved organic matter will be less complete. This is in contrast with *Geobacter* and *Desulfuromonas* species which are able to perform the complete oxidation of their carbon sources [80]. Species from the genera *Geobacter* and *Desulfuromonas* represent more than half of the population on the energy harvesting electrodes. In freshwater environments the predominant species is *Geobacter sulfurreducens*, and *Desulfuromonas acetoxidans* in marine studies [81].

### 3.2 Geobacter

The genus *Geobacter* groups species of Gram-negative δ-proteobacteria capable of completely oxidizing organic electron donors to carbon dioxide [82]. The environments inhabited by *Geobacter* are typically anoxic where the available electron acceptors for respiration can be insoluble. Thus, these bacteria are amongst the predominant species present in anodes that harvest energy from diverse sediment environments [32]. *Geobacter sulfurreducens* was originally isolated from sediments contaminated with petroleum, and is routinely studied as a model for the Geobacteraceae family because its genome was the first to be sequenced and there is an available genetic system [83]. This species has 111 putative c-type cytochromes. Many display higher levels of expression during growth with insoluble electron acceptors, and 91 (82% of the total predicted) were detected under diverse growth conditions [82].

OmcE (GSU0618) and OmcS (GSU2504) are two predicted outer membrane proteins with four and six c-type cytochrome motifs, respectively, which are highly expressed when *Geobacter sulfurreducens* grows with Mn(IV) and Fe(III) oxides as terminal electron acceptors [30]. The gene omcT (GSU2503) is located immediately downstream of omcS and encodes a hexaheam c-type cytochrome which is only transcribed with omcS [30]. Deletion of either gene diminishes the ability of the cells to reduce Mn(IV) and Fe(III) oxides, but it does not affect the reduction of soluble Fe(III)-citrate. This indicates that the three proteins are involved in the reduction of insoluble electron acceptors [84]. Another membrane bound cytochrome that appears to be involved in metal reduction is OmcB (GSU2737), which receives electrons from a variety of periplasmic electron carriers [85,86]. When *Geobacter sulfurreducens* is grown with an energy harvesting electrode as electron acceptor, besides the cytochromes mentioned for Fe(III) and Mn(IV) oxides, the c-type cytochrome with seven hemes, OmcZ (GSU2076), is highly expressed [86, 87]. Furthermore, three other uncharacterized cytochromes are highly expressed only when there’s an insoluble terminal electron acceptor, GSU0105, GSU0701 and GSU2515 [82].

![Fig. 2 Cartoon representation of the respiratory chains leading to extracellular electron transfer in *Geobacter sulfurreducens*. OM – outer membrane, IM – inner membrane, PilC - inner membrane insertion, protein, PilQ - outer membrane insertion protein; all other proteins are described in the main text. Circles with lines represent the hemes in the cytochromes.](image)

Periplasmic cytochromes known to be involved in Fe(III) reduction are MacA (GSU0466), a diheme protein possibly associated with the inner membrane [88], and PpcA (GSO0612) a small soluble protein containing three hemes that belongs to the cytochrome c₃ superfamily [89]. In addition to electron transfer by direct contact using the outer membrane cytochromes, these proteins are also essential for the reduction of humic acids and soluble quinines [87].
Geobacter cells also display pili that favor biofilm growth and electron transfer, processes that are crucial for electricity generation [90]. Pili are not required for electricity production, but they are necessary for maximum power generation [32, 39, 86, 91]. Two genes have been identified that are responsible for pili formation, oxpG (GSU1776) which encodes for type II secretion system or pseudopilins and is involved in protein secretion to the outer membrane [39], and pilA (GSU1496) which encodes a protein homologous with pilus subunits of other Geobacteraceae [86]. These unique pili are called geopili, because there are some differences between Geobacter sulfurreducens pili and other bacterial pili. For example, they are shorter (less than 20 µm) and only the N-terminal domain of type IV pili is highly conserved [39]. The gene pilA seems to be essential for pili development. When this gene is deleted, no pili are detected and there is no reduction of insoluble electron acceptors, but soluble substrates, like fumarate, are reduced.

One of the roles of type IV pili in most bacteria is to establish contact with surfaces. However, pilA mutants are able to attach to the electron acceptor, but there is no increase in biomass. This might mean that the pili apparatus is not only involved in electron transfer from one cell to the extracellular insoluble acceptor, but it might also be involved in cell to cell transfer [32]. These studies also demonstrated that geopili are highly conductive and since the apparatus is anchored in the periplasm and outer membrane of the cells, they can accept electrons from the periplasm or from redox proteins localized in the outer membrane [30].

It is clear from the above description that although several of the proteins essential for extracellular electron transfer in Geobacter have been identified, their characterization remains to be done. Geobacter sulfurreducens is a freshwater organism, and in the context of MFCs applications, a marine, or salt tolerant organism is more adequate. Recently, a phylogenetic tree of the Geobactereaceae family showed that it is divided into two clades, with Desulfuromonas acetoxidans, a marine organism, belonging to a different clade from Geobacter sulfurreducens [92].

3.3 Desulfuromonas

Desulfuromonas acetoxidans is a Gram negative, anaerobic δ-proteobacterium containing a single flagellum isolated from sediments in the Antarctic Ocean. This microorganism reduces elemental sulfur to sulfide in order to obtain energy, although it can also use fumarate, ferric iron (Fe$^{3+}$) or manganese (Mn$^{4+}$) as terminal electron acceptors [93]. Desulfuromonas acetoxidans obtains energy from the complete oxidation of organic compounds to carbon dioxide while reducing extracellular electron acceptors [92,94]. In its natural habitat, this bacterium participates in a symbiotic consortium with the green sulfur bacterium Chloropseudomonas ethylica strain 2K. The elemental sulfur produced is reduced back to sulfide by Desulfuromonas acetoxidans, establishing a closed sulfur cycle [93].

Desulfuromonas acetoxidans is the least explored of the three organisms mentioned in this review. However, the draft genome shows that several key bioenergetic pathways are conserved and codes for various cytochromes with significant homology to those assigned to metal respiration or anode reduction in Geobacter sulfurreducens [95].

<table>
<thead>
<tr>
<th>G. sulfurreducens</th>
<th>Predicted hemes</th>
<th>D. acetoxidans</th>
<th>Predicted hemes</th>
<th>Identity</th>
</tr>
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<tbody>
<tr>
<td>OmcB (GSU2737)</td>
<td>12</td>
<td>Q1JWB8</td>
<td>11</td>
<td>30%</td>
</tr>
<tr>
<td>OmcT (GSU2503)</td>
<td>6</td>
<td>Q1K3G8</td>
<td>7</td>
<td>26%</td>
</tr>
<tr>
<td>OmcS (GSU2504)</td>
<td>6</td>
<td>Q1K3G8</td>
<td>7</td>
<td>26%</td>
</tr>
<tr>
<td>MacA</td>
<td>2</td>
<td>Q1JXK4</td>
<td>2</td>
<td>45%</td>
</tr>
<tr>
<td>ppcA (cyt $c_3$)</td>
<td>3</td>
<td>$c_7$ (cyt $c_3$)</td>
<td>3</td>
<td>44%</td>
</tr>
<tr>
<td>GSU0701</td>
<td>6</td>
<td>Q1K1I4</td>
<td>5</td>
<td>23%</td>
</tr>
<tr>
<td>GSU0105</td>
<td>3</td>
<td>Q1K1I4</td>
<td>10</td>
<td>25%</td>
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The membrane associated diheme MacA from Geobacter sulfurreducens shows 45% identity with Q1JXK4 which is annotated as Cyt-c peroxidase precursor in Desulfuromonas acetoxidans. Cell suspensions of Desulfuromonas acetoxidans have an absorption spectrum typical of reduced c-type cytochromes, and several cytochromes have been identified and purified from this bacterium [96]. The most abundant and best characterized of these heme-proteins is the triheme cytochrome $c_7$, which belongs to the tetraheme cytochrome $c_3$ superfamily [97]. This cytochrome has 44% identity with ppcA from Geobacter sulfurreducens (table 1). The cytochromes belonging to the periplasmic cytochrome pool of Geobacter sulfurreducens that were expressed solely when an extracellular electron acceptor was present, GSU0701 and GSU0105, also have homologous in Desulfuromonas acetoxidans with identities of 23% with Q1K1I4.
and 25% with Q1KJ4, respectively. Finally, the draft genome of *Desulfuromonas acetoxidans* also codes for cytochromes Q1JWB8, Q1K3G8 and Q1K3G8 which have over 25% identity with the outer membrane cytochromes from *Geobacter sulfurreducens*, OmcB, OmcT and OmcS, respectively.

Therefore, it appears that the molecular components of the trans-periplasmic electron transfer chain in *Desulfuromonas acetoxidans* and *Geobacter sulfurreducens* are very similar.

### 4. Conclusions and outlook

As can be appreciated from the current literature, although the mechanism of extracellular respiration differs within species, Gram-negative bacteria appear to rely on multiheme cytochromes for key roles in the bioenergetic respiratory chains linked to respiration of extracellular minerals or anodes in MFCs. These cytochromes form a highly complex network that extends from the periplasm to the outer membrane of the cell, so that electrons may flow to the outside, where solid acceptors are reduced [98, 99, 100]. At this moment only a few of these cytochromes have been characterized structurally, none of them the putative terminal reductases [101]. The structures of the terminal reductases will undoubtedly provide essential information for developing a molecular description of the electron transfer taking place at the bio-mineral interface. We look forward to these exciting results.

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