Comparative Gene Interaction Analysis of Electron Transfer Pathway for Efficient Power Generation in Microbial Fuel Cell

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Abstract. Exo-electrogenic bacteria possess the unique ability to transfer electrons to external electrodes, making them vital for microbial fuel cell (MFC) operations. This study investigated the genetic mechanisms underlying exo-electrogenic properties in various bacteria, which have significant potential for applications in MFCs. The study focused on targeting the genes responsible for these capabilities and examining the evolutionary relationships among these bacteria. Phylogenetic analysis assists in connecting Protein-Protein Interaction (PPI) results with the exo-electrogenic properties of the bacteria. This research delves into the genetic foundations and phylogenetic connections of various exo-electrogenic bacteria. It was found that some genes (*pilT, omcA, mtrA,* and *mtrB*) displayed high connectivity, emphasizing their importance in facilitating extracellular electron transport. These genes were primarily found in species such as *Shewanella* and *Geobacter*, renowned for their effective exo-electrogenic abilities. Gene Ontology analysis revealed that organisms with these highly interconnected genes produced higher electrical outputs. A functional and gene expression analysis of these genes was also performed using in-silico methods. This study highlights the genetic determinants of bioelectrochemical performance, offering insights for enhancing bacterial strains to boost energy production in microbial fuel cells.

Keywords: Exo-electrogenic bacteria, microbial fuel cells, phylogenetic relationships, gene interaction

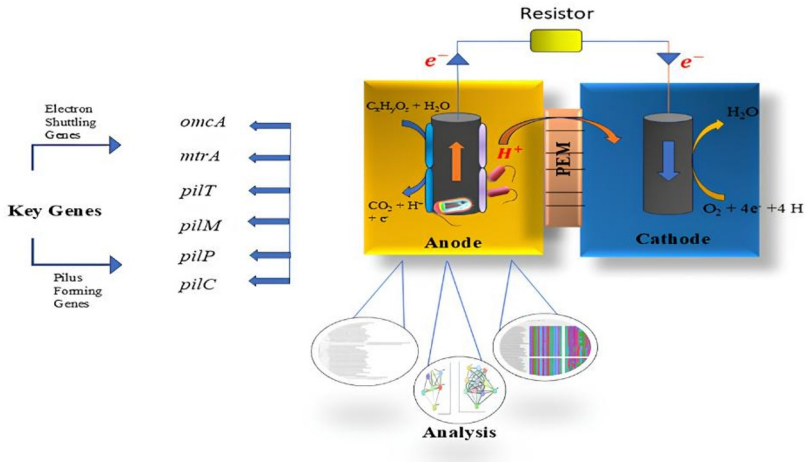

Fig 1: Schematic representation of extracellular electron transfer mechanisms and key genetic determinants in a microbial fuel cell (MFC).

1. Introduction

Microbial Fuel Cells (MFCs) constitute an eco-friendly and sustainable bio-electrochemical system that harnesses bacterial biofilms within a two-electrode configuration to generate electricity from organic waste [1]. MFCs facilitate the direct conversion of chemical energy into electrical energy, presenting a sustainable and environmentally friendly source of renewable energy. They have diverse applications, including wastewater treatment, power generation, desalination, and processing of agricultural, food, and industrial wastes such as those from the textile industry. This technology offers a cost-effective and eco-conscious solution to address various environmental challenges [2].

MFC consists of two chambers, anodic and cathodic, separated by a semi-permeable proton exchange membrane. The substrate, typically wastewater, is introduced into the anodic chamber, where exoelectrogenic bacteria such as *Bacillus subtilis*, *Rhodoferax ferrireducens*, and *Pseudomonas aeruginosa*, etc. are capable of facilitating electron transfer by metabolizing organic matter and transferring the released electrons to the anode surface [3]. These electrons then travel through an external circuit to the cathode, generating electricity, while the semi-permeable membrane facilitates proton

exchange to maintain electrochemical balance [4]. The electron transfer mechanisms employed by exo-electrogenic bacteria are classified into two types: indirect electron transfer via electron shuttling and direct transfer through nanowires and pilus formation [5].

Membrane proteins play a crucial role in enhancing the efficiency of electron transfer in microbial fuel cells. Among these, Cytochrome C family proteins, found in *Shewanella* and *Geobacter* species, are particularly significant. For instance, decaheme Cytochrome C is an outer membrane cytochrome that directly interacts with electrodes, facilitating extracellular electron transfer. Similarly, *cymA*, a periplasmic cytochrome, functions as a key connector between the electron transport chain and terminal electron acceptors, further enhancing the efficiency of microbial electron transport.

Additionally, multiheme cytochromes are essential for long-range electron transfer through conductive biofilms, enabling efficient charge transport across bacterial communities. Beyond cytochromes, redox mediators such as flavins, secreted by *Shewanella*, further enhance electron transfer efficiency. Other mediators include phenazines, which serve as electron shuttles, and quinones, which facilitate electron transfer between intracellular components and external electron acceptors. Together, these proteins and mediators optimize extracellular electron transport, improving the overall performance of MFC [6].

The evolutionary basis of this mechanism remains incompletely understood; however, in certain bacteria, such as *Pseudomonas aeruginosa*, it appears to confer a selective advantage. This bacterium secretes phenazines, which exhibit antimicrobial properties, enabling it to suppress competing microbial populations. Additionally, research has shown that the phenazine pyocyanin induces the upregulation of genes and operons involved in electron transport pathways, further enhancing the organism's survival and adaptability through improved energy metabolism [7].

The genes associated with these electron transfer properties primarily belong to two major families, *omc* and *pil*. The *pil* genes are responsible for the construction of pilus or nanowires, which assist in extracellular electron conduction [4], and the *omcA* (outer membrane Cytochrome A protein) serves as an electron conduit to electron acceptors such as Fe(III) oxides and electrodes, working synergistically with *mtrC*. Additionally, *omcB* is essential for the reduction of Fe(III) and operates in conjunction with *omcZ*, which is critical for achieving high current density in biofilm-mediated electron transport. Similarly, *omcS*, a multi-heme c-type cytochrome, plays a pivotal role in long-range electron transfer, particularly in electrode reduction [8]. The *pil* family, consisting of proteins such as *pilA*, is fundamental for electron transfer along conductive pili; mutations in *pilA* can significantly affect current generation in MFC. The *pilB*, a pilin assembly ATPase, provides the necessary energy for pilus extension and collaborates with *pilC* to assemble pilus genes. The *pilT* is involved in the dynamic interaction with electron acceptors, while *pilO* and *pilQ* function as pilus secretion proteins [9]. These gene families function synergistically to optimize electron transfer pathways, thereby enhancing the efficiency of extracellular electron transport in microbial systems [6].

Previous studies have demonstrated that mixed cultures yield higher power outputs in MFCs compared to pure cultures [3]. Diverse bacterial species coexist in mixed cultures, leading to complex interspecies interactions that enhance electron transfer efficiency. Within these microbial communities, specific sets of genes work in synergy to optimize power generation by facilitating biofilm formation, extracellular electron transfer, and metabolic adaptability. However, the gene interaction and phylogenetic relationship between bacteria and their respective power output in mixed cultures is still not understood clearly in literature, which necessitates the study focused on gene network analysis.

In this regard, this study aims to identify and analyze the key genetic components present in mixed-culture bacteria that contribute to their higher electrochemical performance [10,11]. By understanding how these gene sets interact and regulate electron transfer pathways, valuable insights can be obtained into optimizing MFC efficiency. Such findings may aid in the development of engineered microbial consortia with enhanced power generation capabilities for sustainable bioenergy applications. By utilizing bioinformatics tools such as MEGA 11, Cytoscape, STRING, and InterPro, genomic analyses of exoelectrogenic genes were performed, protein interactions were examined, and this interaction was linked to ontological analysis. These bacteria are reported to produce higher power outputs [12] which can be attributed to the key genes involved, including *pilT, omcA, mtrA* (an inner membrane transporter), *mtrB* (a regulator of the Trp operon), and *pilC* & *pilP* (inner membrane components of the T4S system). For this study, 16S rRNA sequences from 38 distinct exo-electrogenic organisms were collected, encompassing both conserved and variable regions [13]. These sequences were aligned using MUSCLE to perform a detailed comparison of the exoelectrogenic species, highlighting their similarities and differences. Additionally, this alignment was used to explore the evolutionary relationships between the different bacterial species, providing valuable insights into their phylogenetic connections and how they have diverged over time.

Furthermore, it provided an opportunity to examine these bacteria at the functional level, prompting an investigation into the interconnections between them. This accounted for a detailed analysis of the *omc* and *pil* genes, constructing a network

to study their protein-protein interactions. The novelty of this study lies in elucidating the interplay between various genes in exo-electrogenic microorganisms and their influence on the extracellular electron transfer properties exhibited by the cells.

2. Materials and Methods

2.1 Strains used in the Study

The bacterial 16S rRNA genomic sequence utilized in this study was obtained from GenBank. The selection of bacterial strains was guided by insights from existing literature [13], ensuring relevance to the study's objectives. In this study, 16S rRNA sequences were utilized (except for *Pseudomonas aeruginosa, Pseudomonas paraeruginosa,* and *Chlamydia trachomatis*, for which chromosomal genome was used), as the 16S rRNA gene encodes the small subunit of ribosomal RNA, which plays a crucial role in various cellular processes, including the translation of mRNA into functional proteins. The analysis of 16S rRNA sequences enables the identification of both conserved and variable regions, providing valuable insights into bacterial phylogeny and taxonomy [13]. Fig 2 describes a brief summary of the methodology used in the study.

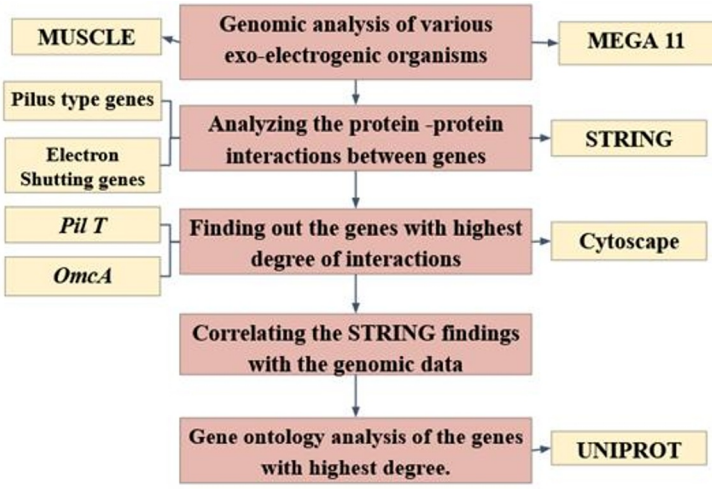

Fig 2: Workflow for genomic and protein–protein interaction analysis of exo-electrogenic organisms, highlighting sequence alignment (MUSCLE), phylogenetic analysis (MEGA 11), interaction network construction (STRING), hub gene identification (Cytoscape), and functional annotation (UniProt).

2.2 Sequence alignment of genes using MEGA 11

Genetic sequences, including 16S rRNA, were gathered for the selected organisms. To ensure consistency, the sequences were aligned using MEGA 11, and a MUSCLE alignment was performed (keeping gap penalties as: Gap Open: −400.00, Gap Extend: 0.00 and Minimum Diameter Length: 24) for a total of 38 different organisms. In this study, GAP (Gene Analysis and Protein-interaction) values were used to quantify the connectivity and functional significance of genes within protein–protein interaction networks. Genes exhibiting high GAP values, including *mtrA, mtrB*, and *omcA*, were identified as central nodes, indicating their critical role in maintaining the integrity of the extracellular electron transfer (EET) pathway. Elevated GAP values also supported the functional relevance of pilus-associated genes (*pilT, pilC, pilM*), highlighting the importance of structural connectivity in facilitating electron transfer alongside redox processes. Furthermore, the occurrence of high GAP values in conserved genes across *Shewanella oneidensis* and *Geobacter sulfurreducens* suggests the presence of a shared electroactive genetic framework. Overall, these high-connectivity genes were closely associated with key processes such as direct electron transfer and biofilm conductivity, thereby linking molecular-level interactions with enhanced power generation in MFC. Overall, these high-connectivity genes were closely associated with key processes such as direct electron transfer and biofilm conductivity, thereby linking molecular-level interactions with enhanced power generation in MFC. A phylogenetic tree was constructed using MEGA 11 to illustrate the evolutionary relationships among the organisms, employing appropriate models of sequence evolution. The phylogenetic analysis was performed using the Neighbor-Joining Method (NJM). This analysis enabled exploring the evolutionary similarities and connections between various exoelectrogenic species, shedding light on their genetic proximity and relationships.

2.3 Analyzing the protein-protein interaction between genes using STRING

Gene interaction data for each organism was retrieved from the STRING database. To construct the network, two main sets of genes were used: nanowire construction genes (*pilT, pilP, pilM, pilB, pilE, pilD, pilU, pilA, pilF, pilC*) and electron

shuttling genes (*omcA, mtrC, mtrB, mtrF, napB, petC, mtrA*). The data was then imported into Cytoscape, where a gene interaction network was developed. For degree analysis, Cytoscape's analysis tool CytoHubba was used to calculate the connectivity (degree) of genes within the network, helping identify genes with the highest connectivity, which could play a significant role in electron transfer mechanisms. The degree of a gene reflects how many other genes it interacts with in the system [14].

2.4 Finding the key genes using Cytoscape

In the Cytoscape analysis, two gene sets were used: Set 1 included *pilA, pilB, pilC, pilD, pilE, pilF, pilM, pilP, pilT,* and *pilU*, while Set 2 comprised *omcA, mtrA, mtrB, napB, mtrC, mtrF,* and *petC*. The interaction data for these genes was uploaded to STRING to build a network based on their interactions. The data was then exported to Excel and converted into the appropriate format for Cytoscape. Using Cytoscape, a degree analysis was performed to rank the top 10 genes with the highest degree of interaction, reflecting their significance in the network. The genes with the highest degree in the network were considered as "key/Hub genes" of the network, and parameters were set to determine the shortest path between genes.

2.5 Gene ontological analysis

Gene ontology analysis was conducted by first obtaining the UniProt IDs from the UniProt database. These gene IDs were then used as input in UniProt to retrieve detailed information integrated by InterPro. They work together to give more accurate and richer information regarding the molecular functions and biological processes linked to each gene. This data was crucial for comparing and analyzing the roles of these genes, helping to understand how they contribute to the overall mechanism of electron transfer. By examining their specific functions and processes, their collective involvement in facilitating efficient electron transfer within the system can be assessed effectively.

3. Results and Discussion

3.1 Sequence alignment of genes using MEGA 11

The sequence alignment facilitated the construction of a phylogenetic tree, providing crucial insights into the evolutionary relationships among the exoelectrogenic bacteria analyzed in this study. This phylogenetic framework enhances understanding of their genetic similarities and divergence, shedding light on the potential mechanisms underlying their extracellular electron transfer capabilities. The phylogenetic analysis demonstrated that bacteria exhibiting high power output, such as *Paracoccus denitrificans* and *Rhodopseudomonas palustris*, are closely related. According to existing literature [12], these two species are well-documented for their ability to generate significant power outputs, highlighting a possible evolutionary link between their extracellular electron transfer mechanisms. Fig 3 shows the phylogenetic tree constructed using NJM. Similarly, *Geobacter sulfurreducens* is closely related to *Desulfuromonas*, showing almost similar power outputs [15,3]. The study revealed a close evolutionary relationship between *Shewanella oneidensis* and *Pseudomonas aeruginosa*; these bacteria have been found abundant in mixed culture [16,17] MFCs and show a higher power output [3].

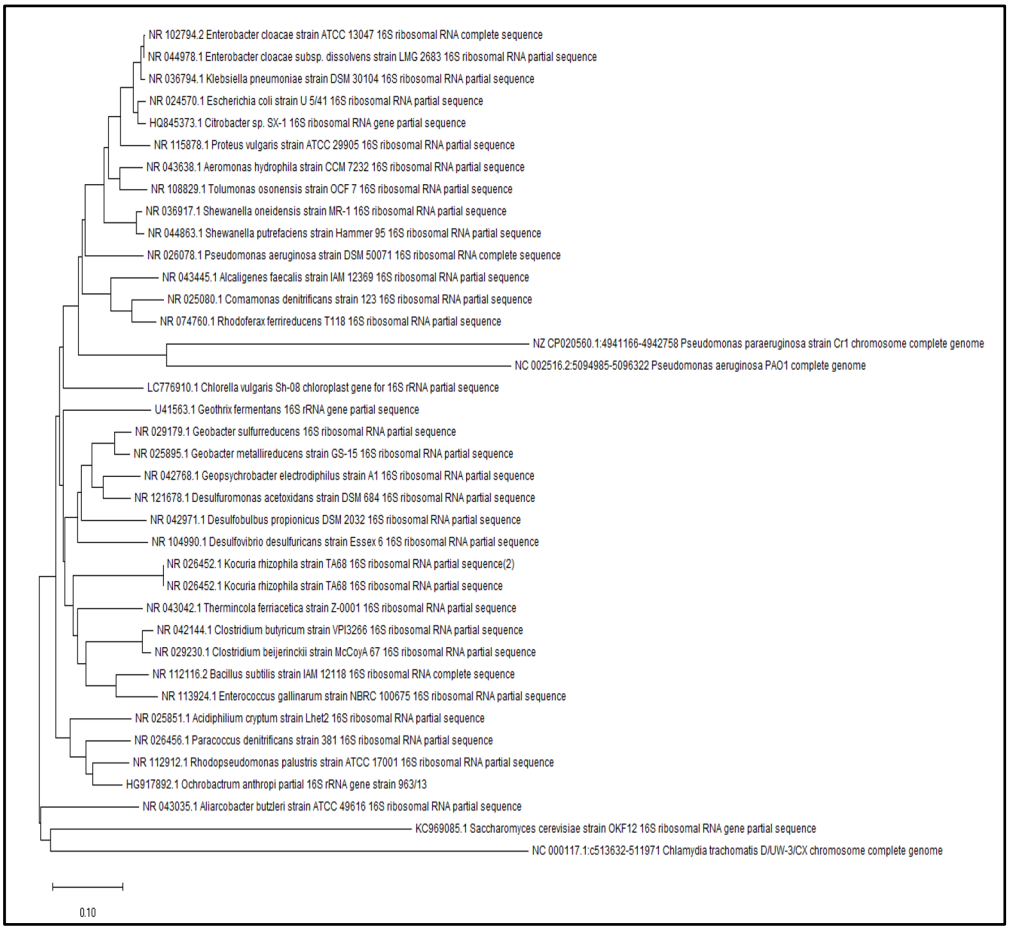

Fig 3: Phylogenetic tree constructed in MEGA11 using NJM

3.2 Analyzing the protein-protein interaction between genes using STRING

The Protein-Protein Interaction (PPI) analysis performed using STRING unveiled the structural organization of the interaction network encompassing both gene families, pilus-forming genes, and electron-shuttling genes. This analysis provides valuable insights into the functional connectivity and potential regulatory mechanisms governing extracellular electron transfer in the studied bacteria. Fig 4 (a) & (b) show PPI networks for electron shuttling genes and pilus forming genes, respectively. This analysis reveals the homologous relationship between *mtrF* and *mtrC*, both involved in electron transfer processes, implying their origin from a common ancestral gene. As a result, these proteins preserve conserved structural features that are essential for their role in electron transport. This conservation is evident in their similar domain organization and overall structural framework, which supports their involvement in extracellular electron transfer pathways. In contrast, *omcA* in characterized as a paralog of *mtrC*, suggesting that although it retains comparable domains and structural attributes, it has diverged functionally over the course of evolution. Such divergence is reflected in differences in their specific contributions within the electron transfer system, potentially influencing their interaction dynamics, efficiency, or functional context despite maintaining a similar structural basis. Conversely, *omcA* is a paralog of *mtrC*, sharing similar domains but exhibiting functional divergence [18]. Despite these differences, these proteins collaborate seamlessly to ensure efficient electron transfer. The key interactions identified in this study include *mtrF, omcA, mtrA, mtrB,* and *mtrC*, each playing an interconnected role in the electron transfer mechanism.

Similarly, in the pilus family, *pilT* interacts with *pilC, pilM,* and *pilN* to support pilus retraction and motility. *pilC*, in conjunction with *pilM* and *pilN*, is critical for pilus assembly. The *pilP* links *pilO* to *pilQ*, forming the *pilM/pilN/pilO/pilP* complex. The *pilM* and *pilN* coordinate to assemble the pilus, enabling the binding of *pilB, pilT,* and *pilC*, thus facilitating the overall function of the type IV pilus and secretion systems [19].

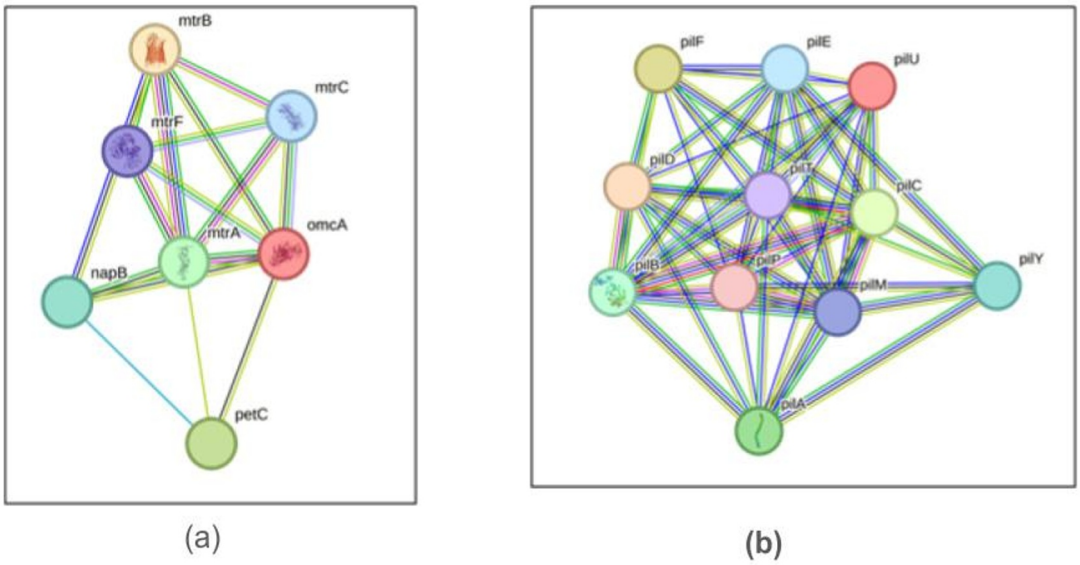

Fig 4 (a): PPI network of electron shuttling genes (b) PPI network of pilus forming genes

3.3 Finding the key genes using Cytoscape

The data obtained from the PPI analysis using STRING was subsequently analyzed in Cytoscape to identify "Hub Genes" with the help of the cytoHubba extension. This comprehensive analysis revealed that among the pilus-forming gene family, the genes *pilT, pilP, pilC,* and *pilM* exhibited the highest degree scores, each with a score of 20. This indicates that these genes are highly interconnected with other genes within the network, suggesting their pivotal role in the formation and function of pili, which are crucial for various bacterial processes such as adhesion, motility, and biofilm formation. Additionally, in the electron transport gene family, *omcA* and *mtrA* stood out with the highest degree scores of 12, highlighting their central role in the process of extracellular electron transfer.

These findings underscore the central importance of these hub genes in both pilus formation and electron transport processes. The high degree scores observed for these genes reflect their greater connectivity to a wide range of other genes, which points to their significant functional roles in coordinating complex biological pathways. This increased connectivity further emphasizes the crucial contribution of these genes in facilitating key cellular processes such as electron transfer, and underscores their elevated functional significance in the overall cellular machinery. Fig 5 (a) & (b) show the degree analysis of electron shuttling genes and pilus forming genes respectively. Fig 6 (a) & (b) shows the gene interaction network reflecting degree of each gene for pilus forming gene and electron shuttling genes respectively.

Top 7 in network string_interactions.tsv ranked by Degree method

Rank	Name	Score
1	omcA	12
1	mtrA	12
3	mtrB	10
4	napB	8
4	mtrC	8
4	mtrF	8
7	petC	6

(a)

Top 10 in network string_interactions.tsv_1 ranked by Degree method

Rank	Name	Score
1	pilT	20
1	pilP	20
1	pilC	20
1	pilM	20
5	pilB	18
5	pilE	18
5	pilD	18
5	pilU	18
9	pilA	16
9	pilF	16

(b)

Fig 5 (a): Degree analysis of electron shuttling genes (b) Degree analysis of pilus

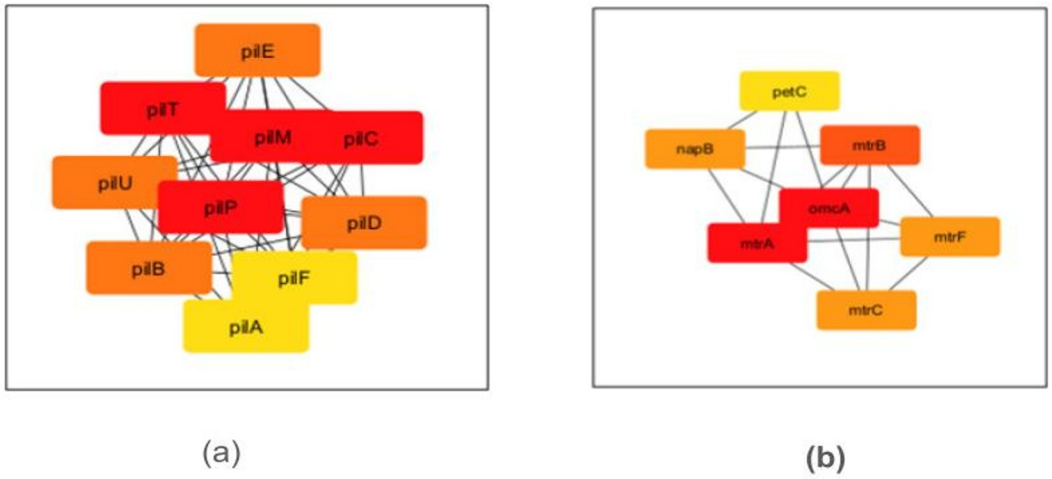

Fig 6: Gene interaction network reflecting degree of each gene for (a) pilus forming gene (b) for electron shuttling genes

3.4 Gene ontological analysis

The Gene Ontology (GO) study helps to understand the functions of genes and their associated gene products. It classifies biological information into three main domains: biological processes, which describe the pathways and functions a gene is involved in; molecular functions, which define the biochemical activity of gene products; and cellular components, which indicate the specific locations within the cell where gene products are active [17].

Gene Ontology analysis of the highest-degree genes revealed that, despite belonging to distinct families, these genes function collaboratively to facilitate the electron transfer process. The *omcA* gene, a multi-heme cytochrome located in the outer membrane, interacts with *mtrC* to mediate electron transfer between Mn/Fe ions. Additionally, *mtrA* and *mtrB* contribute to nutrient transport and the regulation of biosynthetic pathways [18].

Within the pilus-forming gene family, *pilT*, an ATPase, plays a crucial role in pilus retraction and bacterial virulence. *pilC, pilP,* and *pilM* are essential for the assembly and function of type IV pili and secretion systems, which are pivotal for bacterial adhesion, motility, and biofilm formation. Collectively, these findings underscore the interdependence of genes across different families, illustrating their cooperative roles in supporting efficient electron transfer mechanisms, bacterial survival, and pathogenesis [19]. Table 1 describes a brief summary of ontological features of some of the "key/hub genes".

Table 1: A brief summary of ontological features of some of the "key genes"

Genes	Location	Functions	Key Features
omcA	Outer membrane	*OmcA* forms a decaheme complex with *MtrC,* facilitating electron transfer to Mn/Fe ions. This interaction plays a crucial role in extracellular electron transport, enabling efficient redox reactions essential for microbial respiration and bioelectrogenesis.	Multiheme cytochrome (200–743 bp) with heme groups covalently attached to cysteine residues via thioether bonds, playing a crucial role in electron transport and redox reactions in exoelectrogenic bacteria. [17]
mtrA	Inner membrane	Facilitates amino acid transport in coordination with amino acid permease, playing a crucial role in nutrient uptake and metabolic regulation within the bacterial cell.	Essential for amino acid uptake and transport efficiency, ensuring proper nutrient acquisition and metabolic regulation in bacterial cells. [17]
mtrB	Inner membrane	Regulates the trp operon by controlling transcription through attenuation, a mechanism that modulates	Binds RNA exclusively in the presence of L-tryptophan, playing a key role in transcriptional regulation and

		gene expression based on intracellular tryptophan levels.	attenuation of the trp operon. [18]
pilT	Cytoplasm	Facilitates pilus retraction and twitching motility, essential for bacterial adhesion, surface exploration, and biofilm development.	Crucial for bacterial adhesion, biofilm maturation, and virulence, facilitating host colonization and persistence in various environments. [19]
pilC	Inner membrane	Component of T4P; enables pilus assembly/disassembly, adhesion, and colonization.	Essential for twitching motility, enabling surface translocation, biofilm formation, and host colonization in bacteria. [19]
pilP	Inner membrane	Facilitates pilus retraction and twitching motility, playing a critical role in bacterial adhesion, surface colonization, biofilm formation, and host-pathogen interactions.	Anchors secretion components, stabilizing the pilus structure, and ensuring proper assembly and function of the type IV pilus. [19]
pilM	Inner membrane	Works in conjunction with *PilN* to assemble a functional type IV (T4) pilus, which is essential for bacterial motility, adhesion, biofilm formation, and horizontal gene transfer.	Anchors secretion components, stabilizing the pilus structure and supporting its function in adhesion, motility, and host interactions. [19]

3.5 Discussion

The study employed bioinformatics platforms, including STRING and Cytoscape, to identify pivotal genes exhibiting the highest interaction degrees, such as *pilT* and *omcA*, highlighting their essential roles in the electron transfer mechanism. The constructed interaction network between these gene families further illustrates that even genes with fewer interactions play a supportive role in facilitating extracellular electron transfer, emphasizing the complexity and cooperative nature of the system. Through a detailed literature review and gene ontology analysis using InterPro, investigation on the roles of key genes in the electron transfer mechanisms of exoelectrogenic bacteria was conducted. These genes, despite belonging to different families, collaborate to facilitate the electron transfer process. For instance, *omcA*, a multi-heme cytochrome in the outer membrane, interacts with *mtrC* to transfer electrons between Mn/Fe ions. The *mtrA* and *mtrB* are involved in nutrient transport and regulation of biosynthesis, while *pilT*, an ATPase, plays a vital role in pilus retraction and bacterial virulence [18]. The *pilC, pilP,* and *pilM* contribute to the assembly and function of type IV pili and secretion systems, which are crucial for bacterial adhesion, motility, and biofilm formation [20]. Overall, these findings highlight the interdependence of genes from various families, demonstrating how they work together to support efficient electron transfer mechanisms, bacterial survival, and pathogenesis.

The findings highlight how these genes, despite belonging to different gene families, are interdependent and work together to improve and coordinate electron transfer by the bacteria more efficiently. In addition, this study highlights the selected gram-negative bacteria such as *Geobacter sulfurreducens* and *Shewanella oneidensis*, which are capable of reducing metal ions like Fe(III), Mn(III), and Mn(IV) [18]. Bacteria such as *Shewanella* and *Geobacter*, which are frequently studied for their electroactive capabilities, harbor these genes to facilitate efficient power generation through electron transfer mechanisms. Similarly, other bacteria, including *Rhodopseudomonas*, also possess these traits, demonstrating that such mechanisms are common across various bacterial species involved in electron transfer and energy production [21].

In this study, the identification of the key genes involved in the electron transfer mechanisms of exoelectrogenic bacteria was performed and their functional interactions within a network were analyzed successfully. Such an analysis enhances understanding of the molecular framework governing extracellular electron transport. Ultimately, these insights contribute to a deeper comprehension of exoelectrogenic bacteria behavior, which can be leveraged to optimize and enhance the efficiency of MFCs.

The ability to optimize and harness the power of bacterial systems can potentially improve the efficiency of MFCs, particularly in scale-up studies. The high cost of electrodes and membranes currently results in lower power output and

limits the widespread adoption of this technology [22]. This study, however, could provide a basis for developing systems with bacterial species that enhance power output, which, when applied in large-scale environments, could efficiently coordinate to produce higher energy, offering significant promise as a sustainable bioenergy solution.

Moreover, these insights could be applied to environmental bioremediation strategies, where exoelectrogenic bacteria play a key role in detoxifying pollutants through electron transfer processes. Ultimately, by elucidating the molecular foundations of bacterial electron transfer, this study contributes to the development of innovative applications in bioenergy and environmental sustainability.

4. Conclusions

This study underscores the crucial role of specific genes in mediating electron transfer mechanisms in exoelectrogenic bacteria. Through the analysis of key genes in species such as *Shewanella oneidensis* and *Geobacter sulfurreducens*, it is demonstrated how genes from different families interact to facilitate efficient power generation, biofilm formation, motility, and virulence. The interplay among these genetic elements highlights the intricate regulatory networks that govern bacterial electron transport, emphasizing the complexity of microbial systems involved in bioelectrogenesis.

The efficiency of electron transfer in these bacteria is largely governed by the presence of specific cytochromes, pilus-associated proteins, and redox mediators, which collectively optimize extracellular electron transport. For example, genes encoding Cytochrome C family proteins, such as *omcA* and *mtrC*, are essential for direct electron transfer to electrodes, while pilus-associated genes, including *pilT, pilC*, and *pilM*, facilitate biofilm formation and improve conductivity. Furthermore, metabolic pathways regulated by genes like *mtrA* and *cymA* enhance the bacteria's ability for long-range electron transport. These findings suggest that *Shewanella oneidensis* and *Geobacter sulfurreducens* exhibit a high abundance of periplasmic/outer-membrane multiheme cytochromes (*PmC)* and pilus genes, which are critical for boosting power output. Additionally, there is interconnected relationship between these bacterial species. Ultimately, these insights underscore the potential of using a mixed bacterial culture to achieve more efficient power generation in MFC. The identification of coordinated interactions and functional complementarity among key electron transfer proteins provides a mechanistic basis for optimizing electron transport processes. This understanding enables the strategic selection or engineering of microbial systems with enhanced and synergistic electron transfer capabilities. Consequently, these insights support the development of engineered microbial consortia with improved efficiency and stability, ultimately contributing to enhanced MFC performance.

These findings provide valuable insights into the fundamental mechanisms of microbial electroactivity, shedding light on the evolutionary significance of extracellular electron transfer. Understanding these genetic interdependencies not only advances our knowledge of microbial physiology but also has significant biotechnological implications.

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