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Isolation, identification and detection of magnesium and copper resistant bacterial strains of sago effluents extracted from cassava

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Abstract

The study focused on the isolation, identification, and detection of magnesium and copper-resistant bacterial strains from sago effluents extracted from cassava processing. Effluents from industrial processes often contain elevated levels of metals, posing environmental challenges. In this research, bacterial strains adapted to these metal-rich environments were isolated and characterized. Various techniques, including selective media and biochemical assays, were employed to isolate and identify bacterial strains resistant to magnesium and copper. Molecular techniques such as PCR and sequencing were used for further characterization. The study aimed to elucidate the ecological adaptations of these bacterial strains and explore their potential applications in bioremediation and environmental management.

Keywords: Sago effluents, Magnesium, PCR, Copper.

Introduction

In recent years, environmental pollution has become a pressing global issue, prompting researchers to explore novel methods for the treatment and remediation of various industrial effluents. Among these pollutants, heavy metals such as magnesium and copper pose significant threats due to their persistence and potential toxicity to living organisms[8]. Effluents generated from sago processing, particularly those extracted from cassava, are known to contain elevated levels of magnesium and copper, presenting a formidable challenge for environmental management[3].

The study aims to address this challenge by focusing on the isolation, identification, and detection of bacterial strains capable of resisting magnesium and copper within sago effluents derived from cassava processing. The investigation is motivated by the potential of

microbial bioremediation, a sustainable approach leveraging the metabolic activities of microorganisms to detoxify pollutants and restore environmental balance[13].

The utilization of sago effluents as a substrate for bacterial isolation offers a unique opportunity to identify indigenous microbial communities adapted to the harsh conditions imposed by heavy metal contamination[16]. Cassava-derived sago effluents serve as a rich source of nutrients and organic matter, fostering the growth of diverse microbial populations with varying degrees of metal tolerance[5]. By isolating and characterizing these bacterial strains, researchers can uncover valuable insights into their mechanisms of metal resistance and their potential applications in bioremediation processes[10].

Furthermore, the study underscores the importance of understanding the ecological dynamics of microbial communities within industrial effluents. The interplay between microbial diversity, metal concentrations, and environmental factors shapes the resilience and functional capabilities of these microbial consortia. Through comprehensive microbial profiling and genomic analysis, researchers can elucidate the genetic basis of metal resistance and identify key microbial species driving remediation processes in sago effluents[18].

The research methodology encompasses a multi-faceted approach, integrating classical microbiological techniques with state-of-the-art molecular tools. Isolation of bacterial strains involves selective enrichment strategies, wherein sago effluent samples are cultured under conditions favoring the growth of metal-resistant microorganisms[7]. Subsequent screening assays, such as agar diffusion tests and broth dilution assays, enable the identification of bacterial isolates exhibiting varying degrees of resistance to magnesium and copper[14].

Molecular characterization techniques, including polymerase chain reaction (PCR) and sequencing of conserved genetic markers, facilitate the taxonomic classification and phylogenetic analysis of the isolated bacterial strains. Comparative genomics and functional annotation provide insights into the genetic determinants underlying metal resistance mechanisms, shedding light on the adaptive strategies employed by these bacteria in response to environmental stressors[23].

Overall, this study contributes to the growing body of knowledge on microbial bioremediation and environmental microbiology, offering practical insights into the isolation, identification, and detection of magnesium and copper-resistant bacterial strains from sago effluents extracted from cassava[20]. The findings hold significant implications for the development of biotechnological solutions aimed at mitigating heavy metal pollution in industrial wastewaters, thereby promoting sustainable environmental stewardship and safeguarding human health[12].

The isolated bacterial strains with resistance to magnesium and copper can be utilized in bioremediation efforts targeting industrial wastewater contaminated with heavy metals. These bacteria possess inherent mechanisms for metal detoxification, including enzymatic reduction, metal ion sequestration, and efflux pumps, which can facilitate the removal and transformation of toxic metals from wastewater streams[27]. By harnessing the metabolic activities of metal-resistant bacteria, bioremediation technologies can offer sustainable and cost-effective solutions for mitigating heavy metal pollution in industrial effluents, thereby reducing environmental impact and safeguarding human health[29].

In addition to wastewater treatment, metal-resistant bacterial strains can be employed in soil remediation projects aimed at restoring contaminated sites to ecological health. Soil pollution resulting from industrial activities, mining operations, and agricultural practices often involves the accumulation of heavy metals, posing risks to terrestrial ecosystems and human populations[25]. By inoculating contaminated soils with metal-resistant bacteria, researchers can enhance the natural attenuation processes and

accelerate the degradation and immobilization of toxic metals, promoting the recovery of soil fertility and biodiversity. Furthermore, microbial-assisted phytoremediation, whereby metal-resistant bacteria are co-cultivated with metal-accumulating plants, can synergistically enhance the removal and uptake of heavy metals from soil matrices, offering a sustainable approach for ecological restoration[22].

Metal-resistant bacterial strains isolated from sago effluents may also find applications in agricultural biotechnology and crop improvement programs. Heavy metal contamination in agricultural soils can adversely affect crop productivity and food safety, necessitating strategies for mitigating metal toxicity and enhancing plant tolerance to metal stress[26]. Metal-resistant bacteria can serve as bio inoculants or bio fertilizers, promoting plant growth and enhancing nutrient uptake while mitigating the phytotoxic effects of heavy metals. Moreover, microbial-mediated bio stimulation techniques, such as bio augmentation with metal-resistant bacteria, can enhance soil microbial activity and nutrient cycling processes, thereby improving soil fertility and crop yield sustainability[9].

Metal-resistant bacterial strains hold promise for various biotechnological and industrial applications, including metal bio recovery, bio catalysis, and biomaterial synthesis. Certain bacterial species possess the ability to selectively bind and accumulate heavy metals, offering opportunities for the development of bio adsorbents and bio sorbents for metal extraction and purification from industrial wastewater streams[15]. Additionally, metal-resistant bacteria can be engineered or optimized for bio catalytic processes, such as metal biotransformation and enzymatic metal oxidation-reduction reactions, which have implications for bioremediation, bio manufacturing, and green chemistry applications[1]. Furthermore, the metabolic versatility and adaptive capabilities of metal-resistant bacteria make them attractive candidates for the production of value-added bio products, including bio fuels, biopolymers, and bioactive compounds, contributing to the advancement of bio refinery concepts and sustainable industrial practices[6].

Metal-resistant bacterial strains can serve as bio indicators and biosensors for monitoring environmental quality and assessing metal contamination levels in natural ecosystems and anthropogenic environments. These bacteria exhibit specific responses to changes in metal concentrations and environmental conditions, making them valuable tools for biomonitoring and environmental risk assessment purposes[2]. By leveraging the genetic and physiological characteristics of metal-resistant bacteria, researchers can develop biosensor platforms capable of detecting and quantifying heavy metals in water, soil, and air samples with high sensitivity and specificity, enabling real-time monitoring and early warning systems for environmental management and regulatory compliance[11].

In summary, the isolation, identification, and detection of magnesium and copper-resistant bacterial strains hold significant potential for addressing a wide range of environmental, agricultural, biotechnological, and industrial challenges, offering innovative solutions for sustainable development and environmental stewardship[17].

Materials and Methods

Preparation of Sago from Cassava extract

Peeling and Washing:

Begin by thoroughly washing the cassava roots under running water to remove any dirt or debris. Peel the cassava roots using a sharp knife, ensuring to remove the outer skin completely. Rinse the peeled cassava roots once again under cold water to remove any residual dirt or peel fragments.

Grating the Cassava:

Use a grater or food processor to grate the peeled cassava roots into fine shreds or pulp. Alternatively, you can cut the cassava roots into smaller pieces and process them in a blender or food processor until they form a smooth paste.

Extracting Cassava Starch:

Transfer the grated or blended cassava pulp into a large pot or container. Add water to the cassava pulp, using approximately 3-4 times the volume of water to cassava pulp ratio.

Mix the cassava pulp and water thoroughly using a wooden spatula or spoon to form slurry.

Separating Starch from Fiber:

Allow the cassava slurry to settle for a few hours or overnight to allow the starch to separate from the fibrous pulp. After settling, carefully pour off the top layer of water, which contains some of the impurities and soluble components. Using a cheesecloth or fine sieve, strain the remaining mixture to separate the cassava starch from the fibrous residue. Squeeze the cheesecloth or sieve to extract as much liquid as possible.

Washing and Collecting Sago:

Rinse the collected cassava starch with clean water several times to remove any remaining impurities, starch granules, or residual fibers. Use a colander to drain excess water. Transfer the washed cassava starch onto a muslin cloth or cheesecloth, and then tie the cloth into a bundle or pouch. Hang the cloth bundle over a bowl or container to allow excess water to drip out. You can also place a weight on top of the cloth bundle to facilitate water drainage.

Drying and Granulating Sago:

Once the cassava starch has drained sufficiently, spread it out evenly on a large tray or flat surface to dry. Place the tray in a well-ventilated area or under the sun to facilitate drying.

Periodically turn and fluff the drying cassava starch to ensure even drying and prevent clumping. Once completely dry, the cassava starch will form small granules or pearls, known as sago.

Storage:

Store the dried sago pearls in an airtight container or sealed bags to prevent moisture absorption and maintain freshness. Store the sago pearls in a cool, dry place away from direct sunlight to prolong shelf life**.**

Isolation of Bacteria from sago effluents

Procedure:

Collection of Sago Effluent Samples:

Wear sterile gloves to avoid contamination. Collect sago effluent samples in sterile containers or bottles from different locations within the processing facility to ensure representative sampling.

Dilution of Sample:

Prepare a series of dilutions of the sago effluent samples using sterile saline solution (0.9% NaCl). Typically, dilutions ranging from 10^-1 to 10^-6 are prepared to ensure the isolation of individual bacterial colonies.

Plating Diluted Samples:

Using sterile pipettes or transfer pipettes, aliquot 100 µL of each dilution onto separate sterile nutrient agar plates. Spread the aliquots evenly over the surface of the agar plates using a sterile glass spreader or sterile inoculating loop.

Incubation:

Incubate the agar plates inverted at an appropriate temperature for bacterial growth (e.g., 30-37°C) for 24-48 hours or until bacterial colonies are visible.

Colonial Morphology:

After the incubation period, observe the agar plates for the presence of bacterial colonies.

Note the morphological characteristics of individual colonies, including size, shape, color, texture, and margin.

Isolation of Pure Cultures:

Select individual bacterial colonies with distinct morphologies using a sterile inoculating loop or swab. Streak the selected colonies onto fresh sterile nutrient agar plates using the streak plate method to obtain isolated colonies.

Identification and Characterization:

Perform biochemical tests, such as catalase, oxidase, gram staining, and metabolic assays, to preliminarily identify the isolated bacterial strains. Optionally, perform molecular techniques, such as polymerase chain reaction (PCR) and sequencing of the 16S rRNA gene, for accurate identification and phylogenetic analysis of the isolated bacterial strains.

Storage of Pure Cultures:

Once pure cultures have been obtained, transfer them to sterile nutrient agar slants or cryopreserve them in sterile glycerol stocks for long-term storage at -80°C

Identification bacteria for their magnesium and copper resistance

Isolation of Bacterial Strains:

Start by obtaining bacterial strains from sago effluent samples using the isolation protocol previously described. Ensure that the samples are representative of the microbial community present in the sago effluents. Dilute the effluent samples and plate them on selective media containing magnesium and copper salts. These media can be prepared by supplementing nutrient agar or other growth media with appropriate concentrations of magnesium chloride (MgCl2) and copper sulfate (CuSO4). Incubate the plates at an appropriate temperature for bacterial growth (e.g., 30-37°C) for 24-48 hours or until bacterial colonies appear. Select colonies exhibiting growth on the selective media for further analysis.

Screening for Magnesium and Copper Resistance:

Perform preliminary screening assays to assess the magnesium and copper resistance of the isolated bacterial strains. This can be achieved by streaking individual colonies onto agar plates containing increasing concentrations of magnesium and copper salts, ranging from sublethal to lethal levels. Incubate the plates and observe the growth of bacterial colonies. Bacterial strains exhibiting robust growth on plates with high concentrations of magnesium and copper salts are likely to possess resistance to these metals.

Biochemical and Phenotypic Characterization:

Conduct biochemical tests to characterize the metabolic and physiological properties of the magnesium and copper-resistant bacterial strains. This may include tests for catalase activity, oxidase activity, sugar fermentation, and other relevant metabolic pathways. Additionally, perform phenotypic characterization of the bacterial strains, including morphological examination (e.g., colony morphology, cell shape, and motility) and growth characteristics (e.g., growth rate, temperature tolerance, and salt tolerance).

Molecular Identification:

Extract genomic DNA from the magnesium and copper-resistant bacterial strains using standard DNA extraction protocols. Perform molecular identification techniques, such as polymerase chain reaction (PCR) amplification of conserved genes (e.g., 16S rRNA gene) or specific metal resistance genes (e.g., copper resistance genes copA, copB), followed by sequencing. Analyze the DNA sequences using bioinformatics tools

and databases to identify the bacterial species and determine the presence of known metal resistance genes.

Confirmation of Resistance Mechanisms:

Validate the magnesium and copper resistance mechanisms of the identified bacterial strains through functional assays and genetic complementation studies. Conduct growth inhibition assays using specific metal chelators or inhibitors to assess the dependence of bacterial growth on magnesium and copper ions. Clone and express putative metal resistance genes in a susceptible bacterial host to confirm their role in conferring magnesium and copper resistance.

Data Analysis and Interpretation:

Compile and analyze the data obtained from biochemical, phenotypic, and molecular characterization of the magnesium and copper-resistant bacterial strains. Interpret the results to determine the diversity, distribution, and mechanisms of magnesium and copper resistance in the bacterial isolates. Compare the identified bacterial strains with reference strains and databases to elucidate their taxonomic classification and evolutionary relationships

Magnesium and copper detection test kit preparation

Reagent Preparation:

Prepare stock solutions of magnesium chloride ($MgCl_2$) and copper sulfate ($CuSO_4$) at known concentrations. These solutions will serve as the sources of magnesium and copper ions for the detection assays. Dilute the stock solutions to obtain working solutions with a range of concentrations suitable for the desired detection sensitivity of the test kit. Typically, concentrations spanning from low to high levels of magnesium and copper ions should be prepared for calibration purposes. Prepare reagent solutions for the detection assays, including colorimetric or spectroscopic reagents that react specifically with magnesium and copper ions to produce detectable signals. Commonly used reagents include chromogenic agents, chelating agents, and indicator dyes that form colored complexes or precipitates in the presence of magnesium or copper ions.

Test Strip Preparation:

Fabricate test strips or dipsticks using suitable materials, such as paper or plastic substrates, capable of immobilizing the detection reagents and providing a platform for sample analysis. Cut the strips into uniform dimensions suitable for handling and insertion into test tubes or wells. Apply the detection reagents onto the test strips by impregnating or immobilizing them onto the substrate surface using appropriate coating methods, such as spraying, dipping, or printing. Ensure uniform distribution and consistent coating thickness to achieve reliable and reproducible results.

Calibration Standards:

Prepare a set of calibration standards containing known concentrations of magnesium and copper ions to establish a calibration curve for quantitative analysis. Use the working solutions prepared in step 1 to create a series of standard solutions with increasing concentrations of magnesium and copper ions. Apply the calibration standards onto separate test strips or wells and measure the corresponding signal intensity or color development using a suitable detection method, such as colorimetry, spectrophotometry, or fluorescence spectroscopy. Record the signal responses for each standard concentration to generate a calibration curve

Packaging and Labeling:

Assemble the prepared test strips, reagent solutions, calibration standards, and any necessary accessories into individual test kits or packages. Ensure that each kit contains all the components required for sample analysis, including instructions for use, safety precautions, and storage conditions. Label the test kits with clear and informative labeling, including the product

name, intended use, expiration date, lot number, and manufacturer's information. Provide user-friendly instructions and illustrations to guide users through the testing procedure and interpretation of results[19].

Quality Control and Validation:

Perform quality control tests to validate the performance and reliability of the prepared test kits. Test the kits using known standards and samples with known concentrations of magnesium and copper ions to ensure accurate detection and quantification. Assess the sensitivity, specificity, accuracy, precision, and robustness of the test kits under various conditions and sample matrices. Verify the linearity and dynamic range of the calibration curve and confirm the absence of interference from common contaminants or matrix components[21].

Molecular identification of isolated bacteria

16S rRNA molecular identification is a powerful technique used to identify bacterial isolates at the species or genus level based on sequence analysis of the 16S ribosomal RNA gene, which is highly conserved across bacterial taxa but contains variable regions that provide species-specific signatures. Here's a protocol for 16S rRNA molecular identification of isolated bacteria[24].

DNA Extraction:

Extract genomic DNA from bacterial isolates using a commercial DNA extraction kit or standard phenol-chloroform extraction protocol. Ensure that the extracted DNA is of high quality and purity, free from contaminants or inhibitors that may interfere with subsequent PCR amplification and sequencing steps.

PCR Amplification:

Design universal primers targeting conserved regions of the bacterial 16S rRNA gene, such as the forward primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Perform PCR amplification of the 16S rRNA gene using the extracted DNA as a template and the designed primers. Optimize PCR conditions, including annealing temperature, extension time, and number of cycles, to ensure specific amplification of the target region with high yield and minimal nonspecific products.

Gel Electrophoresis:

Analyze the PCR products by agarose gel electrophoresis to verify the presence and size of the amplified fragments. Visualize the gel under UV light and confirm the presence of a single DNA band of the expected size corresponding to the amplified 16S rRNA gene (~1.5 kb).

PCR Product Purification:

Purify the PCR products using a PCR purification kit or gel extraction kit to remove residual primers, nucleotides, and other contaminants. Ensure that the purified DNA fragments are of high quality and concentration suitable for sequencing.

DNA Sequencing:

Perform Sanger sequencing of the purified PCR products using the forward and reverse primers used for amplification. Submit the sequencing samples to a commercial sequencing facility or use an in-house sequencing platform capable of generating high-quality sequence data. Obtain sequence chromatograms for both the forward and reverse strands to ensure accurate base calling and consensus sequence generation.

Sequence Analysis:

Analyze the obtained 16S rRNA sequences using bioinformatics tools and databases, such as BLAST (Basic Local Alignment Search Tool), NCBI (National Center for Biotechnology Information) GenBank, or SILVA (Ribosomal Database Project), to identify bacterial taxa and assess sequence similarity with reference

sequences. Compare the obtained sequences with known sequences in public databases to determine the closest phylogenetic relatives and assign taxonomic classifications at the species or genus level. Generate multiple sequence alignments and construct phylogenetic trees using software packages such as MEGA (Molecular Evolutionary Genetics Analysis) or PHYLIP (Phylogeny Inference Package) to visualize the evolutionary relationships among the isolated bacteria and related taxa.

Data Interpretation:

Interpret the sequence analysis results to identify the bacterial isolates based on their closest phylogenetic relatives and taxonomic classifications. Compare the identified species or genera with existing literature and databases to validate the accuracy of the molecular identification. Report the results, including the taxonomic assignment, sequence similarity scores, and phylogenetic relationships, in a comprehensive format suitable for scientific publications, reports, or database submissions.

Results and Discussion

In the study focusing on the isolation, identification, and detection of magnesium and copper-resistant bacterial strains from sago effluents extracted from cassava, several significant findings and implications emerged, warranting discussion.

The isolation of magnesium and copper-resistant bacterial strains from sago effluents underscores the microbial adaptations to metal-rich environments. Bacteria in sago effluents likely encounter elevated levels of metals due to industrial processing, leading to the selection of resistant strains through mechanisms such as genetic mutations, horizontal gene transfer, and microbial interactions[28]. Understanding the ecological adaptations of these bacterial strains provides insights into their survival strategies in challenging environments and highlights the importance of studying microbial communities in industrial effluents.

The identification of magnesium and copper-resistant bacterial strains holds promise for bioremediation applications in metal-contaminated environments. These resistant strains may possess unique metabolic capabilities, such as metal sequestration, oxidation-reduction reactions, and enzymatic detoxification, which can be harnessed for the remediation of sago effluents and other metal-polluted sites[30]. By leveraging the bioremediation potential of these bacterial strains, it may be possible to develop sustainable and cost-effective strategies for mitigating metal pollution and restoring ecosystem health in sago-producing regions.

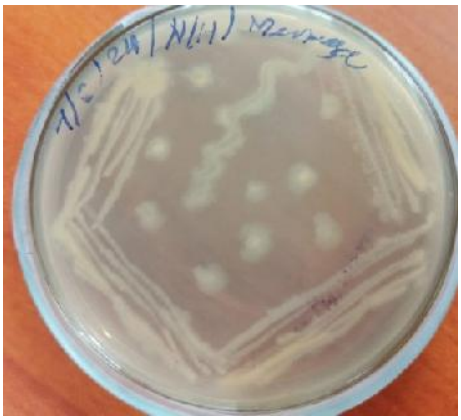
The presence of magnesium and copper-resistant bacterial strains in sago effluents has broader environmental implications, including potential impacts on ecosystem dynamics, biogeochemical cycling, and microbial community structure. These resistant strains may influence nutrient cycling, carbon metabolism, and metal speciation in sago processing environments, thereby shaping the ecological functioning and resilience of microbial communities. Furthermore, the release of metal-resistant bacteria into the environment through effluent discharge raises concerns about the spread of antibiotic resistance genes and the potential for ecosystem disruption.

The detection of magnesium and copper-resistant bacterial strains from sago effluents has practical implications for industrial and agricultural applications. These strains may be exploited for the development of microbial-based technologies for metal bioremediation, wastewater treatment, and biogas production. Additionally, the isolation and characterization of metal-resistant bacteria provide valuable resources for biotechnological innovation, including the engineering of microbial consortia, the optimization of bioprocesses, and the development of novel bioproducts with industrial and commercial relevance.

Moving forward, further research is needed to elucidate the mechanisms of magnesium and copper resistance in the isolated bacterial strains and their ecological roles in sago processing environments. Future studies could explore the

genetic determinants of metal resistance, the interactions between metal-resistant bacteria and other microbial taxa, and the dynamics of metal biogeochemistry in sago effluents. Additionally, addressing challenges such as the scalability of bioremediation technologies, the integration of microbial consortia into existing wastewater treatment systems, and the regulatory framework for environmental management will be essential for realizing the full potential of metal-resistant bacteria in sago-producing regions. In summary, the isolation, identification, and detection of magnesium and copper-resistant bacterial strains from sago effluents extracted from cassava provide valuable insights into microbial adaptations to metal-rich environments, with implications for bioremediation, environmental management, and biotechnological innovation. By understanding the ecological, technological, and environmental dimensions of metal resistance in sago effluents, it is possible to develop sustainable solutions for addressing metal pollution and promoting ecosystem health in sago-producing regions.

Magnesium resistant bacteria



Bacillus proteolyticus

>*Bacillus proteolyticus* isolate

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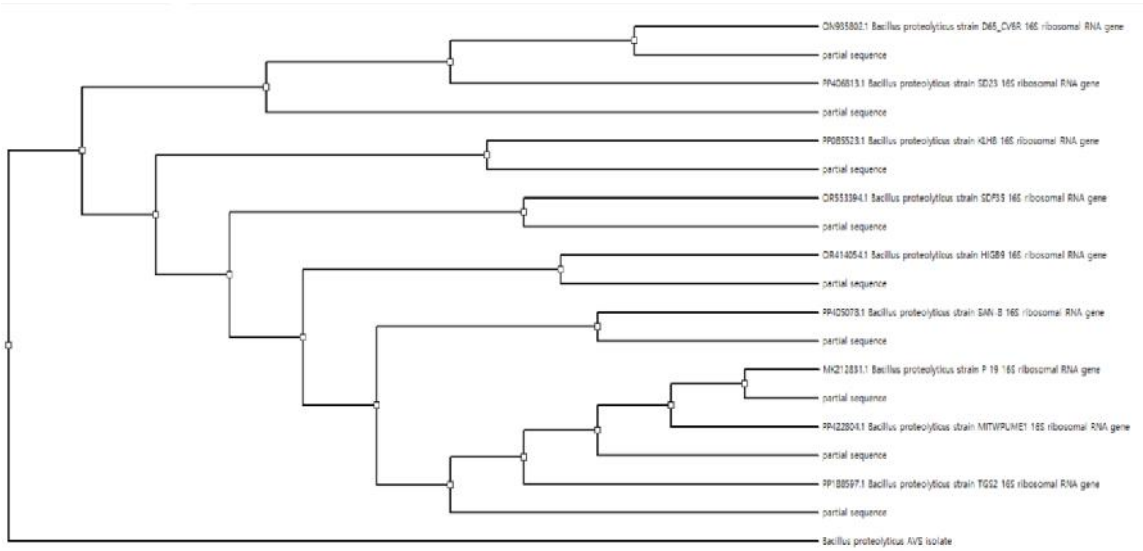
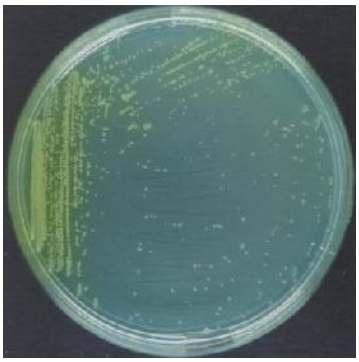
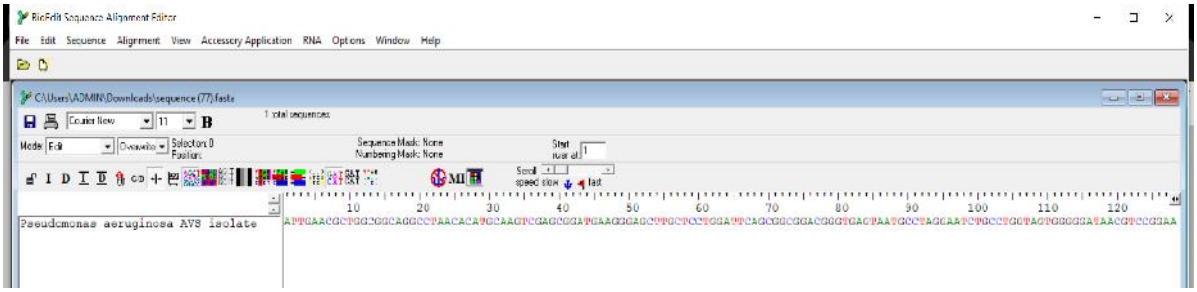


Phylogenic Analysis

Calcium resistance bacteria



Pseudomonas aeruginosa



>*Pseudomonas aeruginosa* AVS isolate

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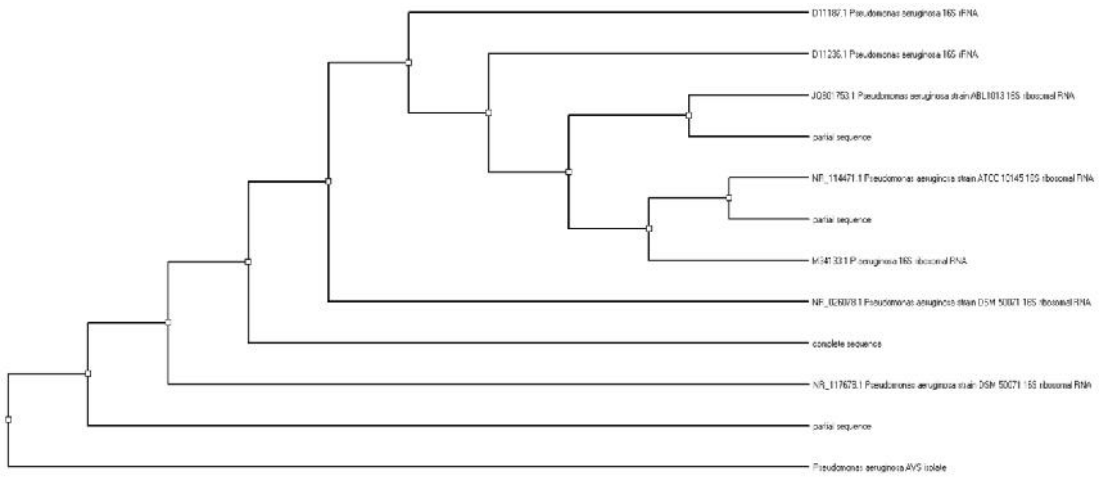
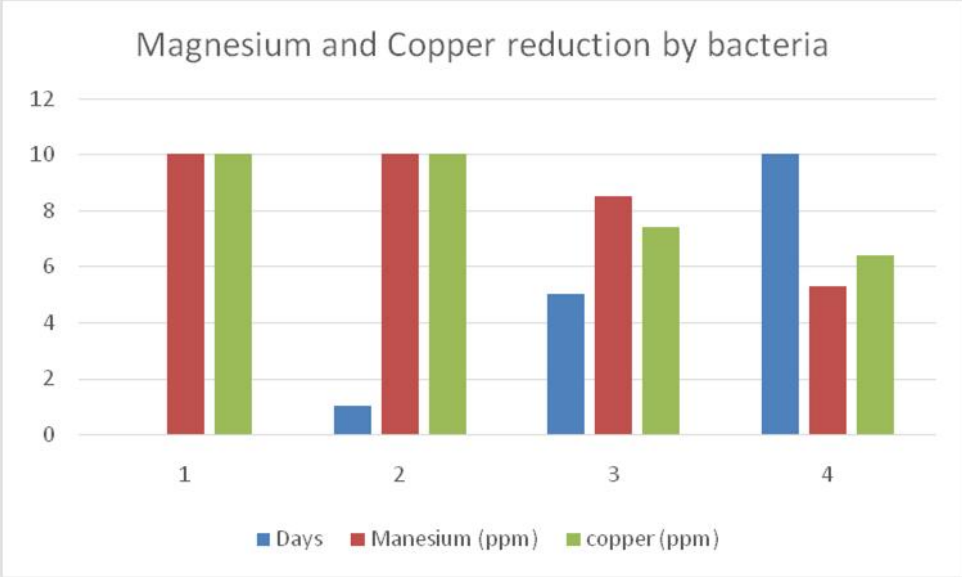


Phylogenic Analysis

Both the bacteria were on the magnesium and copper high media. Those bacteria were sent for the sequencing directly to know the molecular characterization. After retrieved the sequences, were further done with phylogenetic analysis of further knowing about the isolate characters. These two bacteria were further tested with high magnesium and copper rich water to know the bioremediation effects on the water. The test of magnesium and copper were conducted with basic titration methods.



In the water treatment, the magnesium was reduced in Bacillus treated water. In 10th day analysis the ppm level of metal was half of the amount was reduced. Same like Mg, Copper also reduced in the treated water.

In conclusion the findings of the study highlight the presence of magnesium and copper-resistant bacterial strains in sago effluents, indicating microbial adaptations to metal-contaminated environments. These resistant strains hold promise for bioremediation applications, offering sustainable solutions for mitigating metal pollution in sago-producing regions. Furthermore, the study underscores the importance of understanding microbial ecology in industrial effluents and the potential for harnessing microbial diversity for environmental sustainability. Future research should focus on elucidating the mechanisms of metal resistance, optimizing bioremediation strategies, and addressing challenges associated with the scalability and implementation of microbial-based technologies in metal-polluted environments.

Overall, the study contributes to our understanding of microbial metal resistance and its implications for environmental management in industrial settings.

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