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Biochemical Profiling and Characterization of *Mesorhizobium ciceri* Isolates from Chickpea Root Nodules: A Comprehensive Review

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ABSTRACT

Mesorhizobium ciceri is a pivotal bacterium in the nitrogen fixation process of chickpea (Cicer arietinum), playing a crucial role in enhancing soil fertility and crop productivity. This comprehensive review delves into the biochemical profiling and characterization of M. ciceri isolates from chickpea root nodules. It details the methodologies employed for isolating and identifying these bacterial isolates, encompassing morphological, biochemical, and molecular techniques. The review highlights various biochemical assays, including carbon source utilization, enzyme activity, and antibiotic resistance profiles, alongside genetic and phenotypic characterizations through 16S rRNA sequencing, multilocus sequence typing, and genomic fingerprinting and explores the implications of these findings for sustainable agriculture, focusing on biofertilizer development, soil health, and improved crop management. By providing an in-depth understanding of M. ciceri's role in chickpea symbiosis, this review aims to enhance strategies for sustainable agricultural practices and legume crop productivity.

Keywords: *Biochemical, Chickpea, Root Nodules, rRNA sequencing*

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most significant legume crops globally, providing essential nutrients and contributing to food security, particularly in semi-arid regions. As a legume, chickpea has the unique ability to form a symbiotic relationship with nitrogen-fixing bacteria, particularly *Mesorhizobium ciceri*, which colonize the root nodules and convert atmospheric nitrogen into a form that the plant can utilize [1]. This biological nitrogen fixation is a critical process that reduces the need for synthetic nitrogen fertilizers, thereby promoting sustainable agriculture and enhancing soil fertility. *Mesorhizobium ciceri* plays a crucial role in this symbiosis, yet the full extent of its biochemical and genetic diversity remains underexplored. Understanding the biochemical profiling and characterization of M. ciceri isolates from chickpea root nodules is essential for optimizing their application in agriculture [2]. Such knowledge can lead to the development of more effective biofertilizers, tailored to specific environmental conditions and chickpea varieties, ultimately improving crop yields and soil health.

This review aims to provide a comprehensive overview of the current state of research on the biochemical and genetic characterization of *M. ciceri* isolates [3] will discuss the various methodologies employed for isolating and identifying M. ciceri from chickpea root nodules, the biochemical assays used for profiling these isolates, and the molecular techniques for genetic characterization. Additionally, we will explore the implications of these findings for sustainable agriculture and the development of biofertilizers [4]. By synthesizing existing research and identifying gaps in knowledge, this review seeks to offer valuable insights for researchers and agricultural practitioners. The ultimate goal is to enhance our understanding of M. ciceri and its symbiotic relationship with chickpea, thereby contributing to more sustainable and productive agricultural systems.

Isolation of *Mesorhizobium ciceri* from Chickpea Root Nodules

The isolation of *Mesorhizobium ciceri* from chickpea root nodules is a meticulous process that begins with the collection of healthy chickpea plants [5]. Plants are carefully uprooted from either field conditions or greenhouse environments to ensure the integrity of the root system and the nodules attached to it. The root nodules, which are small, swollen structures on the roots, are then detached using sterilized tools to prevent contamination. This initial step is crucial as it sets the stage for obtaining pure isolates of *M. ciceri*.

Surface sterilization is a critical procedure to ensure that the nodules are free from external contaminants. This typically involves a sequential treatment starting with immersion in 70% ethanol for about 30 seconds to eliminate surface microbes [6]. This is followed by a treatment with a sodium hypochlorite solution (1-3%) for 2-5 minutes, effectively killing any remaining external microorganisms. The nodules are then thoroughly rinsed multiple times with sterile distilled water to remove any residual sterilizing agents. This ensures that only the bacteria present inside the nodules are subsequently cultured. Once surface sterilization is complete, the nodules are crushed using a sterile mortar and pestle to release the bacteria contained within [7]. The nodule extract is then streaked onto yeast extract mannitol agar (YEMA) plates. YEMA is a selective medium that supports the growth of rhizobia while inhibiting

other bacteria, often supplemented with Congo red or bromothymol blue to aid in differentiating rhizobial colonies based on their absorption of these dyes. The plates are incubated at 28-30°C for 3-7 days to allow the development of bacterial colonies, which are then subjected to further identification processes.

Identification of *Mesorhizobium ciceri*

Identification of *M. ciceri* isolates involves a combination of morphological, biochemical, and molecular techniques to ensure accurate and comprehensive characterization. Morphological observation is the first step, where the colonies are examined for characteristics such as shape, size, color, and texture on the YEMA plates. Gram staining is performed to confirm that the isolates are Gram-negative, which is a hallmark of rhizobia. Motility tests are also conducted to determine whether the isolates are motile, which can provide further phenotypic information [7].

Biochemical tests are essential for understanding the metabolic capabilities of the *M. ciceri* isolates. One of the primary biochemical assays involves testing the ability of the isolates to utilize various carbon sources [8]. This can be assessed using systems like the Biolog Microbial Identification System, which provides a comprehensive profile of carbon utilization. Enzyme activity assays are also crucial, particularly for enzymes involved in nitrogen fixation and plant growth promotion. For instance, nitrogenase activity can be assessed using the acetylene reduction assay (ARA), phosphatase activity can be measured using p-nitrophenyl phosphate as a substrate, and siderophore production can be evaluated using chrome azurol S (CAS) agar. Additionally, antibiotic resistance profiles are determined by exposing the isolates to a range of antibiotics, which helps in understanding their robustness and potential adaptability to different soil environments [9].

Molecular techniques provide a high level of precision in identifying and characterizing M. ciceri isolates. Polymerase Chain Reaction (PCR) is widely used to amplify specific DNA regions, such as the 16S rRNA gene, which serves as a reliable marker for bacterial identification. Sequencing of the 16S rRNA gene allows for detailed phylogenetic analysis and confirmation of the isolates' identity at the species level. Moreover, genomic fingerprinting techniques such as Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) are employed to assess genetic diversity and establish genetic relationships among different isolates. These molecular methods are indispensable for providing a comprehensive genetic profile, which is essential for understanding the diversity and evolutionary relationships of *M. ciceri* isolates.

By employing a robust combination of morphological, biochemical, and molecular methodologies, researchers can accurately isolate and identify *M. ciceri* from chickpea root nodules. This thorough approach ensures the reliability and purity of the isolates, paving the way for subsequent studies on their biochemical and genetic characteristics, which are crucial for applications in biofertilizer development and sustainable agricultural practices.

Biochemical Profiling of *Mesorhizobium ciceri* Isolates

Carbon Source Utilization

Carbon source utilization profiles of *Mesorhizobium ciceri* isolates are pivotal for understanding their metabolic flexibility and ecological adaptation.

By determining which carbon sources the isolates can metabolize, researchers can infer the potential of these bacteria to thrive in various soil environments and symbiotic conditions. The Biolog Microbial Identification System is frequently employed for this purpose, offering a comprehensive array of carbon substrates to test. Isolates are grown in microtiter plates containing different carbon sources, and their growth is monitored through colorimetric changes. This assay provides detailed metabolic fingerprints, revealing insights into the nutritional preferences and ecological niches that M. ciceri can occupy [10].

Enzyme Activity

Enzyme activity assays are critical for assessing the functional capabilities of *M. ciceri* isolates, particularly those enzymes involved in nitrogen fixation and plant growth promotion. Several key enzymes are typically examined:

Nitrogenase Activity

Nitrogenase is the enzyme complex responsible for the reduction of atmospheric nitrogen to ammonia, a form that plants can utilize. The acetylene reduction assay (ARA) is a standard method for measuring nitrogenase activity. In this assay, isolates are incubated with acetylene gas, which nitrogenase reduces to ethylene. The production of ethylene is then quantified using gas chromatography, providing a direct measure of nitrogenase activity and, consequently, the nitrogen-fixing potential of the isolates.

Phosphatase Activity

Phosphatases play a vital role in phosphorus metabolism, which is crucial for plant growth. The activity of phosphatases can be assessed using substrates such as p-nitrophenyl phosphate, which, upon hydrolysis by the enzyme, releases p-nitrophenol. The amount of p-nitrophenol produced is measured spectrophotometrically, offering a quantitative assessment of phosphatase activity [11]. This information helps in understanding how *M. ciceri* isolates contribute to phosphorus cycling and availability in the soil.

Siderophore Production

Siderophores are compounds secreted by bacteria to scavenge iron from the environment, which is essential for many biological processes. The production of siderophores by M. ciceri isolates is assessed using the chrome azurol S (CAS) assay [12]. In this assay, the presence of siderophores results in a color change in the CAS agar, which can be quantified to measure the amount of siderophores produced. Siderophore production indicates the potential of M. ciceri to enhance iron uptake in chickpea plants, thereby promoting plant health and growth.

Antibiotic Resistance

Assessing antibiotic resistance profiles of *M. ciceri* isolates is crucial for understanding their resilience and adaptability to various soil environments. This involves exposing the isolates to a range of antibiotics and observing their growth responses. Antibiotic resistance tests are typically performed using disk diffusion or broth microdilution methods. In disk diffusion, antibiotic-impregnated disks are placed on agar plates inoculated with the isolates, and the zones of inhibition are measured after incubation [13]. In broth microdilution, isolates are grown in liquid media containing different concentrations of antibiotics, and their growth is monitored.

These tests provide valuable information on the robustness of the isolates and their potential to survive and function in diverse and possibly antibiotic-contaminated environments. By conducting comprehensive biochemical profiling, including carbon source utilization, enzyme activity assays, and antibiotic resistance tests, researchers can gain a detailed understanding of the metabolic capabilities and ecological adaptability of *Mesorhizobium ciceri* isolates. This knowledge is instrumental in selecting and optimizing strains for use as biofertilizers, ultimately contributing to enhanced chickpea cultivation and sustainable agricultural practices.

Genetic and Phenotypic Characterization

In addition to biochemical profiling, genetic and phenotypic characterization of *Mesorhizobium ciceri* isolates provides deeper insights into their diversity, functionality, and potential for application in sustainable agriculture. Genetic characterization involves the use of molecular techniques to analyze the DNA of the isolates, offering a precise understanding of their genetic makeup and evolutionary relationships [14].

Genetic Characterization

16S rRNA sequencing: One of the most common methods for genetic characterization is 16S rRNA gene sequencing. This technique involves extracting the DNA from the isolates and amplifying the 16S rRNA gene using PCR. The resulting sequences are compared with known sequences in databases to confirm the identity of the isolates and to explore their phylogenetic relationships [15]. This method provides high-resolution data that can differentiate M. ciceri from other closely related species.

Multilocus Sequence Typing (MLST): MLST is another powerful technique used to assess the genetic diversity of M. ciceri isolates. It involves sequencing several housekeeping genes and comparing the sequences to categorize isolates into distinct sequence types. This method offers a more comprehensive view of genetic variation and can reveal patterns of evolutionary divergence and adaptation among different isolates.

Genomic Fingerprinting: Techniques such as Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) are employed to create genetic fingerprints of the isolates. These methods involve the digestion of DNA with specific restriction enzymes or the amplification of random DNA segments, respectively. The resulting patterns of DNA fragments or bands are analyzed to assess genetic diversity and to identify unique genetic markers that distinguish different isolates.

Phenotypic Characterization

Nodulation Efficiency: Phenotypic characterization involves evaluating the functional traits of *M. ciceri* isolates, particularly their ability to form effective symbiosis with chickpea plants [16]. Nodulation efficiency is assessed by inoculating chickpea plants with the isolates and measuring the number, size, and health of the nodules formed. This assessment provides insights into the compatibility of the isolates with the host plant and their effectiveness in establishing a symbiotic relationship.

Symbiotic Effectiveness: The symbiotic effectiveness of *M. ciceri* isolates is determined by measuring the growth and nitrogen content of inoculated chickpea plants. Parameters such as plant height, biomass, chlorophyll content, and nitrogen concentration in plant tissues are recorded. These measurements indicate the extent to which the isolates contribute to plant growth and nitrogen fixation, highlighting their potential utility as biofertilizers [17].

Implications for Sustainable Agriculture

The comprehensive biochemical, genetic, and phenotypic profiling of *Mesorhizobium ciceri* isolates has significant implications for sustainable agriculture. By selecting isolates with superior metabolic capabilities, genetic robustness, and symbiotic effectiveness, researchers can develop high-quality biofertilizers that enhance chickpea production while minimizing environmental impact [18].

Development of Biofertilizers: Detailed characterization of M. ciceri isolates allows for the identification of strains with optimal traits for biofertilizer production. These biofertilizers can improve soil fertility by enhancing nitrogen fixation, phosphorus availability, and iron uptake in chickpea plants. The use of well-characterized biofertilizers can reduce the dependence on synthetic fertilizers, promoting more sustainable agricultural practices.

Soil Health and Crop Management: The introduction of effective *M. ciceri* isolates into agricultural systems can improve soil health by increasing microbial diversity and nutrient cycling. These beneficial bacteria enhance soil structure, water retention, and overall soil fertility. Additionally, the use of *M. ciceri*-based biofertilizers can improve crop management by reducing the need for chemical inputs, thereby lowering production costs and environmental risks.

Enhancing Chickpea Production: By leveraging the symbiotic relationship between *M. ciceri* and chickpea, farmers can achieve higher crop yields and better quality produce. Well-characterized isolates ensure consistent and reliable performance under various environmental conditions, making chickpea cultivation more resilient to stress factors such as drought and soil nutrient deficiencies [18-21]. This not only boosts food security but also contributes to the economic well-being of farming communities.

Conclusion

The comprehensive review of biochemical profiling and characterization of *Mesorhizobium ciceri* isolates from chickpea root nodules underscores the importance of these bacteria in sustainable agriculture. Through meticulous isolation, identification, and characterization processes, researchers can unlock the full potential of *M. ciceri* as a biofertilizer, enhancing chickpea production and promoting environmentally friendly farming practices. The insights gained from this review pave the way for future research and application, contributing to the development of more sustainable and productive agricultural systems.

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