

Research Insight

Open Access

Role of Diazotrophic Bacteria in Promoting Sugarcane Growth and Yield

Ameng Li ✉

CRO Service Station, Sanya Tihitar SciTech Breeding Service Inc., Sanya, 572025, Hainan, China

✉ Corresponding email: ameng.hitar@hitar.orgField Crop, 2024, Vol.7, No.3 doi: [10.5376/fc.2024.07.0017](https://doi.org/10.5376/fc.2024.07.0017)

Received: 09 Apr., 2024

Accepted: 20 May, 2024

Published: 13 Jun., 2024

Copyright © 2024 Li, This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:Li A., 2024, Role of diazotrophic bacteria in promoting sugarcane growth and yield, Field Crop, 7(3): 171-181 (doi: [10.5376/fc.2024.07.0017](https://doi.org/10.5376/fc.2024.07.0017))

Abstract Sugarcane cultivation plays a crucial role in the global agricultural economy, especially in tropical and subtropical regions. Diazotrophic bacteria, capable of nitrogen fixation, are significant for enhancing the growth and yield of sugarcane. This study aims to explore the intricate interactions between diazotrophic bacteria and sugarcane, highlighting their impact on plant growth, yield, and agronomic practices. Various types of diazotrophic bacteria and their nitrogen fixation mechanisms are discussed, providing a comprehensive overview of their functions. The colonization and symbiosis processes, along with other plant growth-promoting activities, are examined to understand their contributions to sugarcane health. This study also delves into the enhancements in nutrient uptake, root development, biomass, and overall plant vigor resulting from these interactions. Statistical data on yield increases and improvements in sugarcane juice quality underscore the economic benefits of employing diazotrophic bacteria. Agronomic practices such as soil management, irrigation, crop rotation, and the use of biofertilizers are analyzed for their influence on diazotrophic activity. Advances in genetic engineering, inoculation techniques, and molecular approaches are highlighted as significant strides in research and technology. Despite these advancements, challenges to widespread adoption remain, necessitating further research and policy support. In summary, this study underscores the pivotal role of diazotrophic bacteria in sustainable sugarcane agriculture, advocating for continued research and policy initiatives to harness their full potential.

Keywords Diazotrophic bacteria; Sugarcane cultivation; Nitrogen fixation; Plant growth promotion; Sustainable agriculture

1 Introduction

Sugarcane (*Saccharum officinarum* L.) is a vital crop with significant economic, social, and environmental importance, particularly in countries like Brazil, which is the largest sugar producer and the second-largest bioethanol producer globally (Schultz et al., 2017). The cultivation of sugarcane is typically carried out in soils with varying levels of natural fertility, often requiring substantial nitrogen fertilization to achieve optimal growth and yield (Antunes et al., 2022). However, the high cost and environmental impact of chemical fertilizers have driven the search for sustainable agricultural practices, including the use of plant growth-promoting bacteria (PGPB) (Kruasuwan and Thamchaipenet, 2016).

Diazotrophic bacteria, which are capable of fixing atmospheric nitrogen, play a crucial role in promoting plant growth and enhancing crop yields. These bacteria, such as *Gluconacetobacter diazotrophicus* and *Herbaspirillum seropedicae*, can colonize sugarcane roots and tissues, providing a natural source of nitrogen through biological nitrogen fixation (BNF) (Sevilla et al., 2001; Lery et al., 2011). In addition to nitrogen fixation, diazotrophic bacteria produce plant growth-promoting substances like indole-3-acetic acid (IAA), which further stimulate plant growth. Studies have shown that inoculation with diazotrophic bacteria can significantly increase sugarcane yield, improve nutrient accumulation, and enhance the overall health of the plants (Renan et al., 2016; Schultz et al., 2017).

This study explores the role of diazotrophic bacteria in promoting sugarcane growth and yield. This includes evaluating the efficiency of different diazotrophic bacterial strains and their combinations in enhancing sugarcane productivity. By synthesizing findings from multiple studies, this study provides a comprehensive understanding of the potential of diazotrophic bacteria to improve sugarcane cultivation practices, thereby contributing to more sustainable and environmentally friendly agricultural systems. Investigating the mechanisms through which these bacteria promote plant growth, including nitrogen fixation and the production of growth-promoting substances, is

a key focus. Assessing the impact of environmental factors, such as soil type and nitrogen fertilization, on the effectiveness of bacterial inoculation, is also crucial. This study seeks to identify potential benefits and limitations of using diazotrophic bacteria as biofertilizers in sustainable sugarcane cultivation.

2 Overview of Diazotrophic Bacteria

Diazotrophic bacteria are a group of microorganisms capable of converting atmospheric nitrogen (N_2) into a form that is bioavailable to plants, a process known as biological nitrogen fixation (BNF). This ability is crucial for enhancing plant growth and yield, particularly in crops like sugarcane, which have high nitrogen demands. These bacteria can be found in various environments, including the rhizosphere, root tissues, and even within plant tissues as endophytes. Their role extends beyond nitrogen fixation to include promoting plant growth through the production of phytohormones, enhancing nutrient uptake, and providing resistance against pathogens.

2.1 Types of diazotrophic bacteria

Several types of diazotrophic bacteria have been identified as beneficial for sugarcane growth. Notable examples include *Pseudomonas* spp., *Pantoea* spp., *Enterobacter* spp., *GluconAcetobacter diazotrophicus*, *Acetobacter diazotrophicus*, *Herbaspirillum* spp., and *Burkholderia* spp. Strains such as *Pseudomonas koreensis* CY4 and *Pseudomonas entomophila* CN11 have been shown to significantly enhance nitrogen fixation and promote plant growth by increasing the activity of defense-related enzymes and *phytohormones* in sugarcane varieties GT11 and GXB9 (Singh et al., 2023). Similarly, strains like *Pantoea dispersa*-AA7 and *Enterobacter asburiae*-BY4 have demonstrated high nitrogenase activity and the ability to promote plant growth by inducing nitrogen uptake and defense-related gene expression (Figure 1) (Singh et al., 2021). *GluconAcetobacter diazotrophicus* is an endophytic bacterium well-known for its nitrogen-fixing capabilities and plant growth-promoting traits, including the synthesis of auxins and organic acids (Bertalan et al., 2009). *Acetobacter diazotrophicus* has been shown to enhance sugarcane growth under nitrogen-deficient conditions by transferring fixed nitrogen to the plant (Sevilla et al., 2001). *Herbaspirillum* spp. and *Burkholderia* spp. are also recognized for their role in promoting sugarcane growth and nutrient accumulation (Renan et al., 2016). These bacteria contribute to the overall health and productivity of sugarcane by facilitating nutrient uptake and enhancing plant vigor.

2.2 Nitrogen fixation mechanism

The nitrogen fixation mechanism in diazotrophic bacteria involves the enzyme nitrogenase, which catalyzes the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3). This process is energy-intensive and requires a low-oxygen environment to function effectively. The key steps include nitrogenase activity and gene regulation. The nitrogenase enzyme complex, which includes the *nifH* gene, is responsible for the reduction of N_2 to NH_3 . This enzyme is highly sensitive to oxygen and requires ATP and reducing power to function (Singh et al., 2021; Singh et al., 2022). The expression of nitrogenase and other related genes is tightly regulated by environmental factors such as oxygen levels and the availability of fixed nitrogen. For instance, the *nifH* gene is a common marker used to study nitrogen-fixing microbiomes (Singh et al., 2022). Diazotrophic bacteria can form symbiotic relationships with sugarcane, either in the rhizosphere or as endophytes within plant tissues. These symbiotic and endophytic associations facilitate the direct transfer of fixed nitrogen to the plant, enhancing its growth and reducing the need for chemical fertilizers (Kruasuwan and Thamchaipenet, 2016; Antunes et al., 2019). In addition to nitrogen fixation, diazotrophic bacteria produce phytohormones such as indole-3-acetic acid (IAA), gibberellic acid, and abscisic acid, which further promote plant growth and stress tolerance (Bertalan et al., 2009; Singh et al., 2021). In summary, diazotrophic bacteria play a multifaceted role in promoting sugarcane growth and yield through nitrogen fixation, phytohormone production, and enhancing plant defense mechanisms. Their application as biofertilizers offers a sustainable alternative to chemical fertilizers, contributing to improved crop productivity and environmental health.

3 Interaction Between Diazotrophic Bacteria and Sugarcane

3.1 Colonization and symbiosis

Diazotrophic bacteria, such as *GluconAcetobacter diazotrophicus* and *Acetobacter diazotrophicus*, establish a symbiotic relationship with sugarcane by colonizing its roots and stems. These bacteria are known to persist in

mature plants and can be found in various sugarcane-growing regions worldwide (Sevilla et al., 2001; Saravanan et al., 2007). The colonization process involves the bacteria entering the plant tissues and establishing themselves as endophytes, which allows them to interact closely with the plant cells and contribute to its growth and development (Saravanan et al., 2007; Bertalan et al., 2009). The genetic determinants that drive successful colonization include recognition and signaling pathways, which are crucial for the establishment of this symbiotic relationship (Pankievicz et al., 2021).

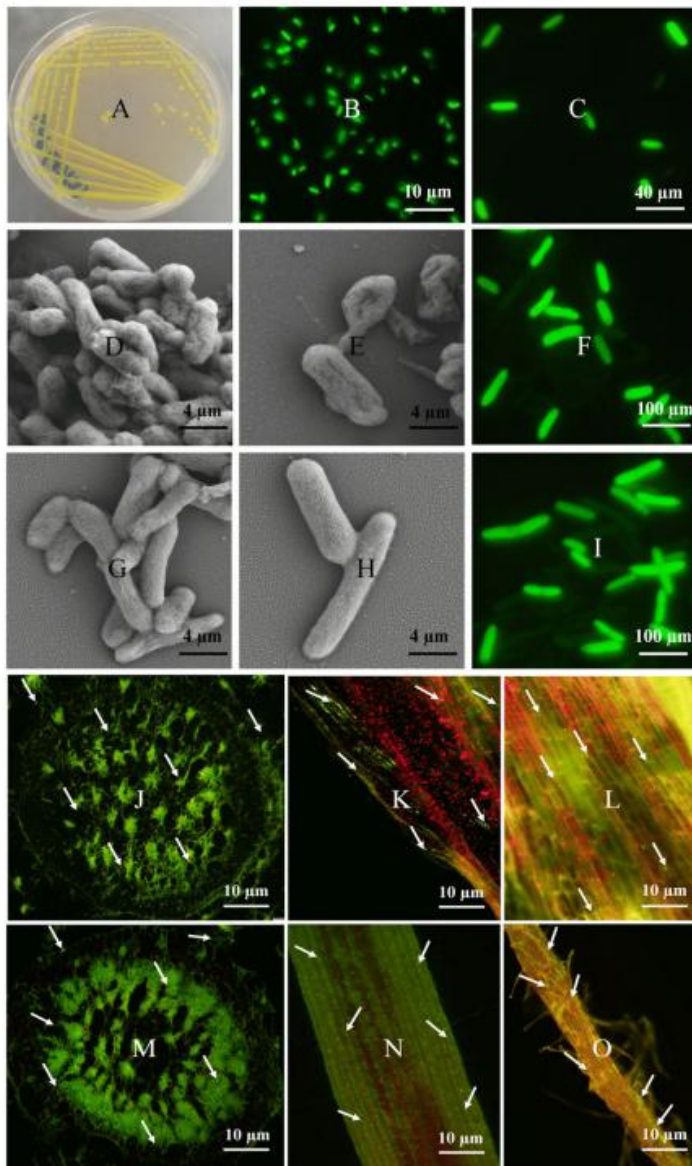


Figure 1 Fluorescence micrographs of GFP-tagged *P. dispersa* (AA7) and *E. asburiae* (BY4) rhizobacteria (Adopted from Singh et al., 2021)

Image caption: (A~C) is a plasmid pPROBE-pTetr-OT containing the green fluorescent protein (GFP), (D, E) and (G, H) is the colony morphology of *P. dispersa* (AA7) and *E. asburiae* (BY4) observed by SEM, (F, I) are the GFP/pPROBEpTetr -TT tagged *P. dispersa* and *E. asburiae*. (J~L) and (M~O) CLSM of GFP/pPROBEpTetr -TT-tagged *P. dispersa* and *E. asburiae* colonizing root, leaf, and stem tissues of micropropagated sugarcane plantlets. The images represent bacterial cells in green dots (white arrow), indicating the colonization of rhizobacteria with autofluorescence in every part of sugarcane tissues, respectively (Adopted from Singh et al., 2021)

3.2 Nitrogen fixation process in sugarcane

One of the primary benefits of diazotrophic bacteria to sugarcane is their ability to fix atmospheric nitrogen, converting it into a form that the plant can utilize. This biological nitrogen fixation (BNF) significantly contributes

to the nitrogen requirements of sugarcane, with some varieties obtaining up to 70% of their nitrogen from this process (Oliveira et al., 2006). Studies have shown that inoculation with diazotrophic bacteria can lead to increased nitrogen content in sugarcane plants, especially under nitrogen-deficient conditions (Sevilla et al., 2001; Renan et al., 2016). The efficiency of nitrogen fixation can vary depending on the bacterial strain, plant genotype, and environmental conditions (Oliveira et al., 2006; Antunes et al., 2019). For instance, *G. diazotrophicus* has been shown to actively fix nitrogen within sugarcane plants, enhancing their growth and nitrogen content (Sevilla et al., 2001; Bertalan et al., 2009).

3.3 Other plant growth-promoting activities

In addition to nitrogen fixation, diazotrophic bacteria promote sugarcane growth through various other mechanisms. These include the production of phytohormones such as indole-3-acetic acid (IAA), which stimulates root growth and development, and the solubilization of phosphate and zinc, making these nutrients more available to the plant (Saravanan et al., 2007; Bertalan et al., 2009; Kruasuwan and Thamchaipenet, 2016). Furthermore, these bacteria can enhance the plant's resistance to pathogens and abiotic stresses, such as drought. For example, *G. diazotrophicus* has been shown to confer drought tolerance to sugarcane by modulating hormone pathways and activating stress-responsive genes (Vargas et al., 2014). Co-inoculation with diazotrophs and actinomycetes has also been found to significantly enhance sugarcane growth compared to individual inoculations, indicating the potential for synergistic effects among different plant growth-promoting bacteria (Kruasuwan and Thamchaipenet, 2016). In summary, diazotrophic bacteria play a crucial role in promoting sugarcane growth and yield through their ability to fix atmospheric nitrogen, produce growth-promoting substances, and enhance the plant's resistance to various stresses. The interaction between these bacteria and sugarcane is complex and influenced by multiple factors, including bacterial strain, plant genotype, and environmental conditions.

4 Impact on Sugarcane Growth

Diazotrophic bacteria, known for their ability to fix atmospheric nitrogen, play a significant role in enhancing the growth and yield of sugarcane. These bacteria not only provide essential nutrients but also improve various physiological and morphological aspects of the plant.

4.1 Enhanced nutrient uptake

Inoculation with diazotrophic bacteria has been shown to significantly enhance the uptake of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) in sugarcane. For instance, a study demonstrated that the accumulation rates of N, P, and K were highest around 180 days after planting (DAP) for N and P, and around 160 DAP for K, in treatments involving bacterial inoculation, compared to the control (Renan et al., 2016). This enhanced nutrient uptake is crucial for the overall growth and development of the plant, leading to improved biomass and crop growth rates.

4.2 Improved root development

The application of diazotrophic bacteria also positively impacts root development in sugarcane. Research has shown that inoculation with a mixture of diazotrophic strains can lead to a significant increase in root dry mass, root initiation, volume, and area, especially of the fine roots (Santos et al., 2019). This improved root architecture enhances the plant's ability to absorb water and nutrients from the soil, thereby supporting better growth and resilience against environmental stresses.

4.3 Increased biomass and plant vigor

The overall biomass and vigor of sugarcane plants are markedly improved with the inoculation of diazotrophic bacteria. Studies have reported that inoculated plants exhibit higher total dry matter accumulation and stem yield compared to non-inoculated controls. For example, inoculation with a consortium of five diazotrophic bacterial strains resulted in significant increases in stem yield and total dry matter in different sugarcane varieties (Schultz et al., 2017). Additionally, the presence of these beneficial bacteria can enhance the plant's tolerance to abiotic stresses, such as drought, further contributing to increased biomass and plant vigor (Figure 2) (Vargas et al., 2014). In conclusion, the role of diazotrophic bacteria in promoting sugarcane growth and yield is multifaceted,

involving enhanced nutrient uptake, improved root development, and increased biomass and plant vigor. These benefits underscore the potential of using diazotrophic bacteria as a sustainable agricultural practice to boost sugarcane productivity.

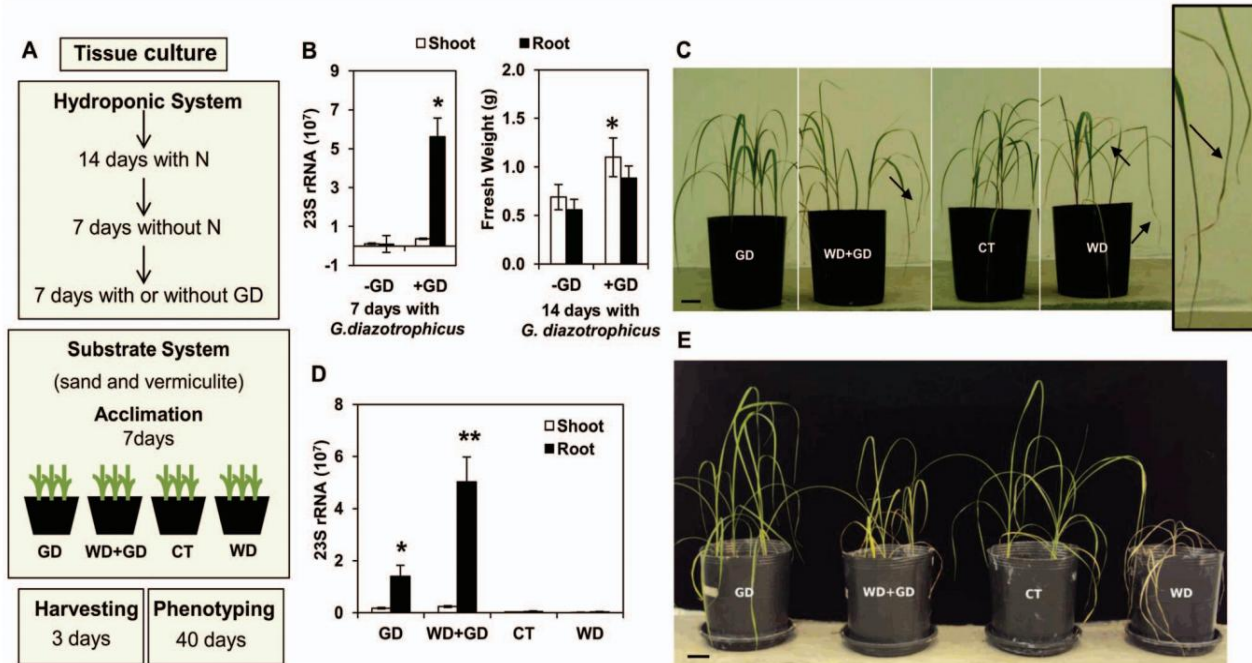


Figure 2 Water deficit assay of sugarcane cv. SP70-1143 colonized with the beneficial endophytic diazotrophic bacteria *G. diazotrophicus* strain PAL5 (Adopted from Vargas et al., 2014)

Image caption: (a) Simplified pipeline for water deficit assay. (b) left panel shows qRT-PCR quantification of sugarcane colonization by *G. diazotrophicus* 7 days after inoculation in hydroponic solution. Bacterial 23S rRNA levels are presented relative to rice 28 S rRNA levels. Right panel shows root and shoot fresh weight measurements 14 days after inoculation and 1 day before treatments. (c) Phenotype of sugarcane cv. SP70-1143 inoculated or not with *G. diazotrophicus*, 3 days after withholding water. Senescent edges are indicated by arrows and are shown in the amplified image from a WD+GD plant. (d) qRT-PCR quantification of sugarcane colonization by *G. diazotrophicus* after 3 days under water deficit assay. Bacterial 23S rRNA levels are presented relative to rice 28 S rRNA levels. (e) Phenotype of sugarcane cv. SP70-1143 inoculated or not with *G. diazotrophicus*, after 40 days withholding water. Bar 5 cm. Error bars indicate standard error of the mean. Asterisk mark statistical significance between GD-R vs CT-R (* $p < 0.05$) and WD+GD-R vs GD-R (** $p < 0.01$), performed by statistical t-test (unpaired) (Adopted from Vargas et al., 2014)

5 Impact on Sugarcane Yield

5.1 Yield increase statistics

The inoculation of sugarcane with diazotrophic bacteria has shown significant improvements in yield across various studies. For instance, inoculation with a consortium of five diazotrophic strains resulted in an increase of stem yield by 22.3 to 38.0 Mg/ha in different sugarcane varieties compared to control treatments (Schultz et al., 2017). Another study demonstrated that inoculation with *Gluconacetobacter diazotrophicus* and other diazotrophs led to higher biomass accumulation and nutrient uptake, which in turn enhanced the growth rates of sugarcane (Renan et al., 2016). Additionally, the use of mixed inocula in micropropagated sugarcane plantlets showed a synergistic effect, significantly increasing colonization and growth (Oliveira et al., 2009).

5.2 Quality of sugarcane juice

The quality of sugarcane juice, an important parameter for both sugar and bioethanol production, is also positively influenced by diazotrophic bacteria. Inoculation with diazotrophic bacteria not only increased the stem yield but also improved the total recoverable sugar yield (Schultz et al., 2017). Furthermore, the industrial characteristics of sugarcane, such as juice quality, were enhanced by the presence of plant growth-promoting bacteria (PGPB) (Antunes et al., 2019). These improvements are attributed to the better nutrient assimilation and stress tolerance conferred by the bacteria.

5.3 Economic benefits

The economic benefits of using diazotrophic bacteria in sugarcane cultivation are multifaceted. The increase in yield directly translates to higher revenue for farmers. For example, the yield increases observed in various studies suggest a substantial boost in productivity, which can significantly enhance profitability (Renan et al., 2016; Schultz et al., 2017). The improved quality of sugarcane juice can lead to better market prices for both sugar and bioethanol. The use of diazotrophic bacteria can reduce the dependency on chemical fertilizers, thereby lowering input costs and promoting sustainable agricultural practices (Kruasuwan and Thamchaipenet, 2016; Matoso et al., 2021). This reduction in chemical usage not only cuts costs but also benefits the environment, aligning with the goals of sustainable agriculture.

6 Agronomic Practices Influencing Diazotrophic Activity

6.1 Soil management and fertility

Soil management and fertility play a crucial role in the effectiveness of diazotrophic bacteria in promoting sugarcane growth. Studies have shown that the interaction between sugarcane varieties and diazotrophic bacteria is highly influenced by soil type and fertility levels. For instance, sugarcane grown in Alfisol (low fertility soil) showed significant increases in stem yield and biological nitrogen fixation (BNF) when inoculated with diazotrophic bacteria, compared to Oxisol (medium fertility) and Ultisol (high fertility) soils (Oliveira et al., 2006). Additionally, the use of biofertilizers produced from rocks and organic matter enriched with diazotrophic bacteria has been found to improve nutrient absorption and plant growth, leading to increased sugarcane yield (Oliveira et al., 2015).

6.2 Irrigation practices

Irrigation practices can significantly impact the activity and effectiveness of diazotrophic bacteria in sugarcane cultivation. Proper irrigation ensures that the soil remains conducive for bacterial activity, promoting better colonization and interaction with the plant roots. Although specific studies on irrigation practices were not detailed in the provided content, it is generally understood that maintaining optimal soil moisture levels is essential for the survival and activity of diazotrophic bacteria.

6.3 Crop rotation and intercropping

Crop rotation and intercropping can influence the microbial diversity and activity in the soil, including diazotrophic bacteria. While it did not specifically address crop rotation and intercropping, it is known that these practices can enhance soil health and microbial diversity, potentially benefiting diazotrophic bacteria. For example, the diversity of culturable plant growth-promoting bacterial endophytes, including diazotrophs, was found to be beneficial for sugarcane growth when co-inoculated with actinomycetes (Kruasuwan and Thamchaipenet, 2016).

6.4 Use of biofertilizers

The use of biofertilizers containing diazotrophic bacteria has been extensively studied and shown to promote sugarcane growth and yield. Inoculation with diazotrophic bacteria such as *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, and *Azospirillum amazonense* has been demonstrated to enhance biomass accumulation, nutrient uptake, and crop growth rates (Schultz et al., 2014; Renan et al., 2016). Additionally, biofertilizers produced from rocks and organic matter enriched with diazotrophic bacteria have been found to be effective in increasing sugarcane productivity and improving plant characteristics (Oliveira et al., 2015). In summary, agronomic practices such as soil management and fertility, irrigation, crop rotation, and the use of biofertilizers significantly influence the activity and effectiveness of diazotrophic bacteria in promoting sugarcane growth and yield. These practices help create a favorable environment for bacterial colonization and interaction with the plant, leading to enhanced growth and productivity.

7 Advances in Research and Technology

7.1 Genetic engineering of diazotrophic bacteria

Genetic engineering of diazotrophic bacteria has emerged as a promising approach to enhance their plant growth-promoting capabilities. By manipulating the genetic makeup of these bacteria, researchers aim to improve

their efficiency in nitrogen fixation and other plant growth-promoting traits. For instance, studies have shown that genetically modified strains of diazotrophic bacteria can significantly enhance sugarcane growth and yield. The introduction of specific genes responsible for nitrogen fixation and other beneficial traits can lead to more robust and efficient bacterial strains (Kruasuwan and Thamchaipenet, 2016; Renan et al., 2016; Antunes et al., 2019). Additionally, the use of high-throughput sequencing and other molecular techniques has allowed for a deeper understanding of the genetic diversity and functional potential of these bacteria, paving the way for more targeted genetic modifications (Figure 3) (Malviya et al., 2022).

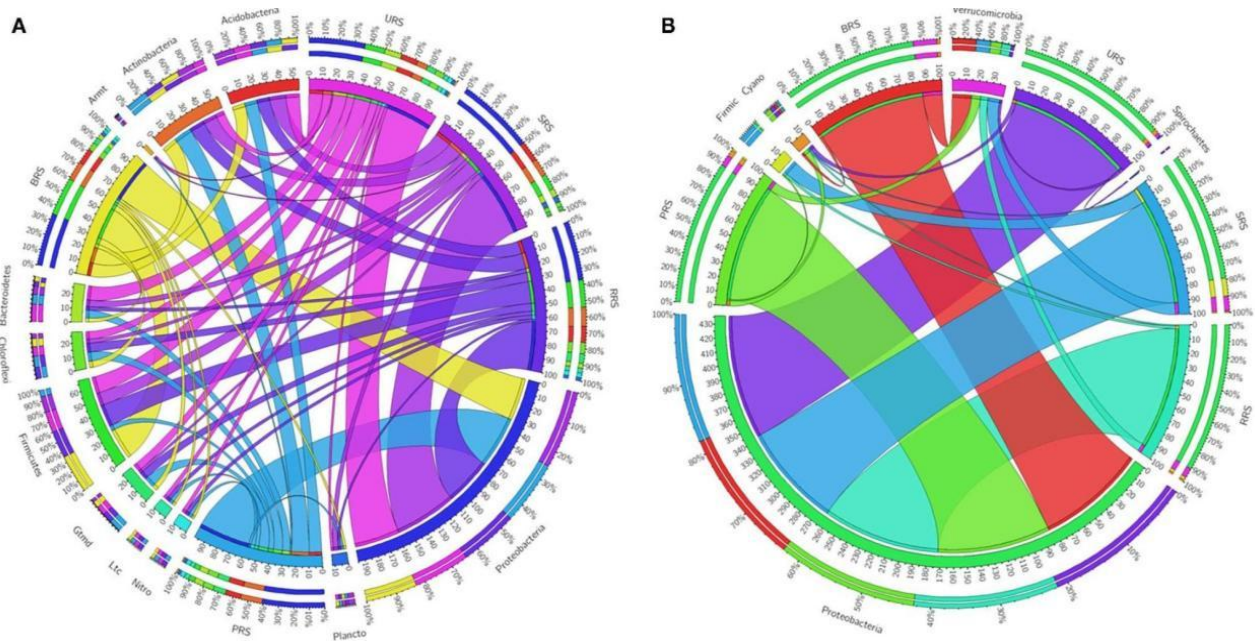


Figure 3 Circular representation of the proportional structure of bacterial communities at the phylum level based on 16s rRNA (A) and *nifH* gene (B) associated with the sugarcane rhizosphere of different species (Adopted from Malviya et al., 2022)
 Image caption: Values within the inner circle indicate the number of reads of a phylum within the normalized dataset. *S. officinarum* L. cv Badila (BRS), *S. barberi* Jesw. cv Pansahi (PRS), *S. robustum* (RRS), *S. spontaneum* (SRS), and *S. sinense* Roxb. cv Uba (URS) (Adopted from Malviya et al., 2022)

7.2 Innovations in inoculation techniques

Innovative inoculation techniques have been developed to maximize the benefits of diazotrophic bacteria in promoting sugarcane growth. Traditional methods of inoculation often face challenges such as uneven distribution and low survival rates of the bacteria. Recent advancements include the development of more effective carrier materials and formulations that enhance the viability and colonization efficiency of the bacteria. For example, co-inoculation with multiple strains of diazotrophic bacteria and other beneficial microbes has shown synergistic effects, leading to improved plant growth and nutrient uptake (Sevilla et al., 2001; Oliveira et al., 2006; Kruasuwan and Thamchaipenet, 2016). Additionally, the use of micropropagated sugarcane plantlets has been optimized to ensure successful colonization by diazotrophic bacteria, further enhancing their growth-promoting effects (Oliveira et al., 2009).

7.3 Molecular approaches to study bacteria-plant interactions

Molecular approaches have significantly advanced our understanding of the interactions between diazotrophic bacteria and sugarcane plants. Techniques such as fluorescence in situ hybridization (FISH), quantitative PCR, and high-throughput sequencing have been employed to study the colonization patterns, population dynamics, and functional roles of these bacteria within the plant rhizosphere and tissues (Oliveira et al., 2006; Oliveira et al., 2009). These methods have revealed the complex and dynamic nature of bacteria-plant interactions, highlighting the importance of specific bacterial strains and their interactions with plant genotypes and environmental conditions. For instance, studies have shown that different sugarcane varieties respond differently to bacterial

inoculation, with some varieties showing greater benefits in terms of growth and nutrient uptake (Oliveira et al., 2006; Santos et al., 2019). Understanding these interactions at the molecular level can inform the development of more effective inoculants and management practices to optimize the benefits of diazotrophic bacteria in sugarcane cultivation.

8 Challenges and Future Directions

8.1 Barriers to widespread adoption

Despite the promising results of diazotrophic bacteria in promoting sugarcane growth and yield, several barriers hinder their widespread adoption. One significant challenge is the variability in response among different sugarcane varieties and environmental conditions. For instance, the effectiveness of bacterial inoculation can vary significantly depending on the sugarcane variety and the substrate used, as observed in studies where different varieties showed varied responses to inoculation (Matoso et al., 2021). Additionally, the inconsistency in the colonization efficiency of different bacterial strains poses a challenge. Some strains may exhibit antagonistic interactions when used in mixed inoculations, which can affect their overall efficacy (Oliveira et al., 2009). Furthermore, the lack of standardized protocols for the application of these bacteria in different agricultural settings complicates their adoption. The need for specific conditions for optimal bacterial performance, such as particular soil types or nutrient levels, adds another layer of complexity (Renan et al., 2016; Schultz et al., 2017).

8.2 Areas for further research

To overcome these barriers, further research is needed in several key areas. First, there is a need for comprehensive studies to understand the interactions between different sugarcane varieties and diazotrophic bacteria. This includes identifying the genetic and physiological traits that make certain varieties more responsive to bacterial inoculation (Matoso et al., 2021). Additionally, research should focus on optimizing the formulation and application methods of bacterial inoculants to enhance their colonization efficiency and consistency across different environmental conditions (Oliveira et al., 2009). Another critical area is the investigation of the long-term effects of bacterial inoculation on soil health and crop productivity. Understanding the sustainability and potential cumulative benefits of using diazotrophic bacteria over multiple crop cycles is essential (Antunes et al., 2019). Moreover, exploring the synergistic effects of co-inoculating diazotrophic bacteria with other plant growth-promoting microorganisms, such as actinomycetes, could provide new insights into enhancing sugarcane growth (Kruasuwan and Thamchaipenet, 2016).

8.3 Policy and institutional support

For the successful integration of diazotrophic bacteria into mainstream agricultural practices, robust policy and institutional support are crucial. Governments and agricultural institutions should promote research and development in this field by providing funding and resources for large-scale field trials and the development of standardized application protocols (Schultz et al., 2014). Additionally, policies that incentivize the adoption of sustainable agricultural practices, including the use of microbial inoculants, can encourage farmers to transition from conventional chemical fertilizers to biofertilizers (Schultz et al., 2017). Extension services and training programs should be established to educate farmers about the benefits and application methods of diazotrophic bacteria. Collaborations between research institutions, agricultural extension services, and farmers can facilitate the dissemination of knowledge and best practices (Bertalan et al., 2009). Furthermore, regulatory frameworks should be developed to ensure the quality and efficacy of commercial bacterial inoculants, protecting farmers from substandard products and fostering trust in this technology (Sevilla et al., 2001). By addressing these challenges through targeted research and supportive policies, the potential of diazotrophic bacteria in enhancing sugarcane growth and yield can be fully realized, contributing to more sustainable and productive agricultural systems.

9 Concluding Remarks

The role of diazotrophic bacteria in promoting sugarcane growth and yield has been extensively studied, revealing several key findings. Inoculation with diazotrophic bacteria has been shown to significantly increase stem yield, dry matter accumulation, and nitrogen content in various sugarcane varieties. The use of bacterial inoculants,

either alone or in combination with nitrogen fertilization, has demonstrated enhanced growth rates and nutrient accumulation, particularly nitrogen, phosphorus, and potassium. Additionally, co-inoculation with diazotrophs and actinomycetes has been found to further improve growth parameters compared to individual inoculations. The benefits of inoculation are influenced by factors such as sugarcane variety, soil type, and substrate used, highlighting the importance of understanding plant-bacteria-environment interactions.

Diazotrophic bacteria play a crucial role in sustainable agriculture by reducing the need for chemical fertilizers and enhancing plant growth through biological nitrogen fixation (BNF) and other plant growth-promoting mechanisms. These bacteria can fix atmospheric nitrogen, making it available to plants, thus reducing the dependency on synthetic nitrogen fertilizers. The use of diazotrophic bacteria as biofertilizers aligns with the goals of sustainable agriculture by promoting environmentally friendly practices and improving soil health. Moreover, the ability of these bacteria to enhance nutrient uptake and plant growth can lead to increased crop yields and better resource use efficiency, contributing to the overall sustainability of agricultural systems.

The integration of diazotrophic bacteria into sugarcane cultivation practices offers a promising avenue for enhancing crop productivity and sustainability. Future research should focus on optimizing inoculation strategies, including the selection of effective bacterial strains and understanding the interactions between different sugarcane varieties, soil types, and environmental conditions. Additionally, long-term field studies are needed to evaluate the consistency and scalability of the benefits observed in controlled experiments. It is recommended that farmers and agricultural practitioners consider incorporating diazotrophic bacteria into their crop management practices to reduce reliance on chemical fertilizers and improve crop yields sustainably. Collaborative efforts between researchers, industry stakeholders, and policymakers will be essential to promote the adoption of this technology and realize its full potential in sustainable agriculture.

Acknowledgments

The author thanks the two anonymous peer reviewers for their thorough review of this study and for their valuable suggestions for improvement.

Conflict of Interest Disclosure

Author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Antunes J., Freitas A., Bonifácio A., Oliveira L., Santos C., Lyra M., Oliveira J., Ollero F., Araújo A., and Figueiredo M., 2022, Isolation and characterization of plant growth-promotion diazotrophic endophytic bacteria associated to sugarcane (*Saccharum officinarum* L.) grown in Paraíba, Brazil, Brazilian Archives of Biology and Technology, 65: e22200439.
<https://doi.org/10.1590/1678-4324-2022200439>
- Antunes J., Freitas A., Oliveira L., Lyra M., Fonseca M., Santos C., Oliveira J., Araújo, A., and Figueiredo M., 2019, Sugarcane inoculated with endophytic diazotrophic bacteria: effects on yield, biological nitrogen fixation and industrial characteristics, Anais da Academia Brasileira de Ciências, 91(4): e20180990.
<https://doi.org/10.1590/0001-3765201920180990>
PMid:31778453
- Bertalan M., Albano R., Pádua V., Rouws L., Rojas C., Hemerly A., Teixeira K., Schwab S., Araújo J., Oliveira A., França L., Magalhães V., Alquéres S., Cardoso A., Almeida W., Loureiro M., Nogueira E., Cidade D., Oliveira D., Simão T., Macedo J., Valadão A., Dreschsel M., Freitas F., Vidal M., Guedes H., Rodrigues E., Meneses C., Brioso P., Pozzer L., Figueiredo D., Montano H., Junior J., Filho G., Flores V., Ferreira B., Branco A., Gonzalez P., Guillobel H., Lemos M., Seibel L., Macedo J., Alves-Ferreira M., Sachetto-Martins G., Coelho A., Santos E., Amaral, G., Neves A., Pacheco A., Carvalho D., Lery L., Bisch P., Rössle S., Ürményi T., Pereira A., Silva R., Rondinelli E., Krüger W., Martins O., Baldani J., and Ferreira P., 2009, Complete genome sequence of the sugarcane nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* Pal5, BMC Genomics, 10: 1-17.
<https://doi.org/10.1186/1471-2164-10-450>
PMid:19775431 PMCID:PMC2765452
- de Oliveira A., de Canuto E., Urquiaga S., Reis V., and Baldani J., 2006, Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria, Plant and Soil, 284: 23-32.
<https://doi.org/10.1007/s11104-006-0025-0>

- Kruasuwan W., and Thamchaipenet A., 2016, Diversity of culturable plant growth-promoting bacterial endophytes associated with sugarcane roots and their effect of growth by co-inoculation of diazotrophs and actinomycetes, *Journal of Plant Growth Regulation*, 35: 1074-1087.
<https://doi.org/10.1007/s00344-016-9604-3>
- Lery L., Hemerly A., Nogueira E., Krüger W., and Bisch P., 2011, Quantitative proteomic analysis of the interaction between the endophytic plant-growth-promoting bacterium *GluconAcetobacter diazotrophicus* and sugarcane, *Molecular Plant-Microbe Interactions*, 24(5): 562-576.
<https://doi.org/10.1094/MPMI-08-10-0178>
PMid:21190439
- Malviya M., Li C., Lakshmanan P., Solanki M., Wang Z., Solanki A., Nong Q., Verma K., Singh R., Singh P., Sharma A., Guo D., Dessoky E., Song X., and Li Y., 2022, High-throughput sequencing-based analysis of rhizosphere and diazotrophic bacterial diversity among wild progenitor and closely related species of sugarcane (*Saccharum* spp. inter-specific hybrids), *Frontiers in Plant Science*, 13: 829337.
<https://doi.org/10.3389/fpls.2022.829337>
PMid:35283913 PMCid:PMC8908384
- Matoso E., Reis V., Giacomini S., Silva M., Avancini A., and Silva S., 2021, Diazotrophic bacteria and substrates in the growth and nitrogen accumulation of sugarcane seedlings, *Scientia Agricola*, 78(1): e20190035.
<https://doi.org/10.1590/1678-992x-2019-0035>
- Oliveira A., Stoffels M., Schmid M., Reis V., Baldani J., and Hartmann A., 2009, Colonization of sugarcane plantlets by mixed inoculations with diazotrophic bacteria, *European Journal of Soil Biology*, 45(1): 106-113.
<https://doi.org/10.1016/j.ejsobi.2008.09.004>
- Oliveira F., Stamford N., Neto D., Oliveira E., Oliveira W., Rosália C., and Santos S., 2015, Effects of biofertilizers produced from rocks and organic matter, enriched by diazotrophic bacteria inoculation on growth and yield of sugarcane, *Australian Journal of Crop Science*, 9(6): 504-508.
- Pankievicz V., Amaral F., Ane J., and Stacey G., 2021, Diazotrophic bacteria and their mechanisms to interact and benefit cereals, *Molecular Plant-Microbe Interactions*, 34(5): 491-498.
<https://doi.org/10.1094/MPMI-11-20-0316-FI>
PMid:33543986
- Renan O., Nivaldo S., Rafael C., Willian P., Adelson P., Segundo U., and Veronica M., 2016, Growth analysis of sugarcane inoculated with diazotrophic bacteria and nitrogen fertilization, *African Journal of Agricultural Research*, 11(30): 2786-2795.
<https://doi.org/10.5897/AJAR2016.11141>
- Santos S., Chaves V., Ribeiro F., Alves G., and Reis V., 2019, Rooting and growth of pre-germinated sugarcane seedlings inoculated with diazotrophic bacteria, *Applied Soil Ecology*, 133: 12-23.
<https://doi.org/10.1016/j.apsoil.2018.08.015>
- Santos S., Ribeiro F., Alves G., Santos L., and Reis V., 2019, Inoculation with five diazotrophs alters nitrogen metabolism during the initial growth of sugarcane varieties with contrasting responses to added nitrogen, *Plant and Soil*, 451: 25-44.
<https://doi.org/10.1007/s11104-019-04101-1>
- Saravanan V., Saravanan V., Madhaiyan M., Osborne J., Thangaraju M., and Sa T., 2007, Ecological occurrence of *GluconAcetobacter diazotrophicus* and nitrogen-fixing *Acetobacteraceae* members: their possible role in plant growth promotion, *Microbial Ecology*, 55: 130-140.
<https://doi.org/10.1007/s00248-007-9258-6>
PMid:17574542
- Schultz N., Pereira S., Silva P., Baldani J., Boddey R., Alves B., Urquiaga S., and Reis V., 2017, Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium, *Plant Production Science*, 20(4): 366-374.
<https://doi.org/10.1080/1343943X.2017.1374869>
- Schultz N., Silva J., Sousa J., Monteiro R., Oliveira R., Chaves V., Pereira W., Silva M., Baldani J., Boddey R., Reis V., and Urquiaga S., 2014, Inoculation of sugarcane with diazotrophic bacteria, *Revista Brasileira de Ciência do Solo*, 38: 407-414.
<https://doi.org/10.1590/S0100-06832014000200005>
- Sevilla M., Burris R., Gunapala N., and Kennedy C., 2001, Comparison of benefit to sugarcane plant growth and ¹⁵N₂ incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild-type and Nif- mutants strains, *Molecular Plant-Microbe Interactions*, 14(3): 358-366.
<https://doi.org/10.1094/MPMI.2001.14.3.358>
PMid:11277433
- Singh P., Singh R., Li H., Guo D., Sharma A., Lakshmanan P., Malviya M., Song X., Solanki M., Verma K., Yang L., and Li Y., 2021, Diazotrophic bacteria *Pantoea dispersa* and *Enterobacter asburiae* promote sugarcane growth by inducing nitrogen uptake and defense-related gene expression, *Frontiers in Microbiology*, 11: 600417.
<https://doi.org/10.3389/fmicb.2020.600417>
PMid:33510724 PMCid:PMC7835727
- Singh P., Singh R., Li H., Guo D., Sharma A., Verma K., Solanki M., Upadhyay S., Lakshmanan P., Yang L., and Li Y., 2023, Nitrogen fixation and phytohormone stimulation of sugarcane plant through plant growth promoting diazotrophic *Pseudomonas*, *Biotechnology and Genetic Engineering Reviews*, pp.1-21.
<https://doi.org/10.1080/02648725.2023.2177814>

- Singh R., Singh P., Guo, D., Sharma A., Li D., Li X., Verma K., Malviya M., Song X., Lakshmanan P., Yang L., and Li Y., 2021, Root-derived endophytic diazotrophic bacteria *Pantoea cyripedii* AF1 and *Kosakonia arachidis* EF1 promote nitrogen assimilation and growth in sugarcane, *Frontiers in Microbiology*, 12: 774707.
<https://doi.org/10.3389/fmicb.2021.774707>
PMid:34975800 PMCID:PMC8714890
- Singh R., Singh P., Sharma A., Guo D., Upadhyay S., Song Q., Verma K., Li D., Malviya M., Song X., Yang L., and Li Y., 2022, Unraveling nitrogen fixing potential of endophytic diazotrophs of different *Saccharum* species for sustainable sugarcane growth, *International Journal of Molecular Sciences*, 23(11): 6242.
<https://doi.org/10.3390/ijms23116242>
PMid:35682919 PMCID:PMC9181200
- Vargas L., Brígida A., Filho J., Carvalho T., Rojas C., Vanechoutte D., Bel M., Farrinelli L., Ferreira P., Vandepoele K., and Hemerly A., 2014, Drought tolerance conferred to sugarcane by association with *Gluconacetobacter diazotrophicus*: a transcriptomic view of hormone pathways, *PLoS ONE*, 9(12): e114744.
<https://doi.org/10.1371/journal.pone.0114744>
PMid:25489849 PMCID:PMC4260876



Disclaimer/Publisher's Note

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and do not represent the views of the publishing house and/or its editors. The publisher and/or its editors disclaim all responsibility for any harm or damage to persons or property that may result from the application of ideas, methods, instructions, or products discussed in the content. Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
