

## THE ROLE OF METHYLOBACTERIUM SYMBIOTICUM IN AGRICULTURE

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### Abstract

*The current scientific work presents a review of the role of Methylobacterium symbioticum not only as a tool for increasing agricultural yields but also as a strategic ally in transitioning to a greener and more sustainable agricultural model. Methylobacterium symbioticum is a symbiotic bacterium known for its ability to utilize single-carbon compounds, especially methanol, as a source of carbon and energy. In the context of modern agriculture, the use of beneficial microorganisms has become an essential component for enhancing sustainability and productivity. Among these, Methylobacterium symbioticum stands out as a symbiotic bacterium with significant potential for improving plant health and their adaptation to environmental stress. This bacterium has the capability to colonize plants and form symbiotic relationships, thereby contributing to increased nutrient use efficiency, stimulating growth and development, and enhancing plant tolerance to adverse conditions. It stimulates the physiological processes of the host plant by synthesizing phytohormones, such as auxins, and by producing enzymes that contribute to increased stress resistance.*

**Key words:** methylobacterium symbioticum, biofertilizer, phytohormones, biostimulant.

### INTRODUCTION

The use of beneficial bacteria for agriculture is a growing area of interest in sustainable crop production (Campobenedetto et al., 2020). With pressures rising for more production with less environmental impact, the agricultural sector is turning to biological solutions that can augment or even replace traditional chemical inputs (Kloepper & Schroth, 1978; Chițu et al., 2024; Sitnicki et al., 2024). Bacteria associated with plants and those found in soil are some of those that have demonstrated significant potential in plant growth promotion, nutrient enhancement, and soil structure and fertility improvement (Saharan & Nehra, 2011; Pandey et al., 2012; Hurek et al., 2002).

Moreover, the use of bacterial inoculants aligns with current efforts to reduce dependency on synthetic fertilizers and plant protection products, whose long-term use has been associated with soil degradation, biodiversity loss, and groundwater contamination. By enhancing natural soil microbial communities and promoting nutrient cycling, beneficial bacteria offer a promising alternative that supports both crop productivity and

environmental resilience. Recent advances in microbiome research, genomics, and bioformulation technologies have further expanded the possibilities for practical applications of bacteria in agriculture. However, successful implementation depends on factors such as strain selection, soil type, crop species, and environmental conditions. As such, continued interdisciplinary research is needed to optimize the use of microbial inoculants under field conditions and to integrate them effectively into modern farming systems (Amaresan et al., 2020; Butta et al., 2023). This article reviews the current understanding of the role of beneficial bacteria in agriculture, focusing on *Methylobacterium symbioticum*.

Bacteria from the *Methylobacterium* genus (Patt et al., 1976) are classified under the class *Alphaproteobacteria* and are Gram-negative bacteria characterized by a pink pigmentation due to carotenoid synthesis (Van et al., 2003), primarily xanthophylls. These bacteria have a bacillus shape, are strictly aerobic, and can grow by utilizing single-carbon (C1) compounds such as methanol or methylamine (Toyama et al., 1998; Kononova et al., 2007). *Methylobacterium* spp. can occupy various

habitats, including soil, water, leaf surfaces, root nodules, seeds, and air (Tani et al., 2012a; Wellner et al., 2012), having been detected in more than 70 plant species (Omer et al., 2004). In the context of modern agriculture, the use of beneficial microorganisms has become an essential component in increasing sustainability and productivity. Among these, *Methylobacterium symbioticum* is a symbiotic bacterial species with significant potential for improving plant health and enhancing adaptation to environmental stresses. This bacterium has the ability to colonize plants and form symbiotic relationships that contribute to increased nutrient use efficiency, stimulation of growth, and enhanced plant tolerance to adverse conditions (Szparaga et al., 2019).

While traditional fertilizers and plant protection products provide immediate effects, they often come with long-term ecological and economic risks. In contrast, *Methylobacterium symbioticum* and other symbiotic bacteria contribute to the development of a sustainable agricultural model by providing ecological solutions that support plants naturally, without negative effects on ecosystems. *Methylobacterium symbioticum* has been studied and reported to have the ability to produce phytohormones and other bioactive compounds that not only stimulate plant growth but also enhance resistance to pathogens and abiotic stresses such as drought or nutrient-poor soils (Green & Ardley, 2018; Zhang et al., 2024).

This paper examines the role of *Methylobacterium symbioticum* in agriculture, focusing on the mechanisms through which this bacterium supports plants and its potential agronomic applications. Additionally, the ecological and economic benefits of using this bacterium in sustainable agriculture will be explored, along with the challenges and perspectives for its integration into large-scale agricultural practices. Thus, *Methylobacterium symbioticum* stands out not only as a tool for increasing agricultural yields but also as a strategic ally in the transition toward a greener and more sustainable agricultural model.

## MATERIALS AND METHODS

The working methodology involved the selection of currently known information on the

fixed theme from academic databases such as Google Scholar, PubMed and Web of Science, aiming to integrate microbiological, physiological and agronomic approaches to comprehensively evaluate *Methylobacterium symbioticum* as a plant growth promoting bacterium (PGPB) on the one hand and as a support for the development of sustainable agricultural practices, which reduce dependence on synthetic fertilizers, while improving crop productivity and soil health, on the other hand.

## RESULTS AND DISCUSSIONS

The species *Methylobacterium symbioticum*, from the *Methylobacterium* genus, has garnered considerable attention in recent years due to its potential applications in promoting plant growth and increasing productivity. Studies have demonstrated that this bacterium forms symbiotic associations with numerous plant species, leading to improved physiological and biochemical performance, which is reflected in higher crop yields. The mechanisms through which *Methylobacterium symbioticum* exerts its beneficial effects include nitrogen fixation, phytohormone synthesis, and enhanced nutrient absorption processes that are essential for sustainable agriculture.

One of the major advantages of *Methylobacterium symbioticum* is its ability to fix atmospheric nitrogen, a vital nutrient for plant growth. Research has shown that species from the *Methylobacterium* genus, including *M. symbioticum*, can significantly increase nitrogen availability in the rhizosphere, thereby reducing the need for synthetic nitrogen-based fertilizers. For example, the role of leaf-resident *Methylobacterium* species in nitrogen fixation, a process that favors biomass accumulation and increased seed production in *Jatropha curcas*, has been highlighted (Madhaiyan et al., 2007). These findings are also supported by other studies that have observed that, under nitrogen stress conditions, plants can intensify their symbiotic relationship with *Methylobacterium symbioticum*, leading to improved photosynthetic activity and better overall plant health (Torres et al., 2024).

Beyond nitrogen fixation, *Methylobacterium symbioticum* is known for producing phytohormones such as auxins and cytokinins,

which play a crucial role in regulating plant growth and development. The production of these hormones has been correlated with enhanced root system development and increased nutrient uptake efficiency. Other studies have reported that foliar application of *Methylobacterium symbioticum* led to higher root length density (RLD) and an increase in root area density (RAD) in common wheat, indicating a positive impact on root architecture and function (Valente et al., 2024). Similarly, it has been observed that the beneficial effects of *Methylobacterium* spp. on plant growth are linked to their ability to produce growth regulators, supporting their role in promoting plant development through hormonal regulation (Senthilkumar et al., 2021).

The ability of *Methylobacterium symbioticum* to colonize plant tissues is another essential factor in manifesting its beneficial effects. Colonization efficiency significantly influences the extent of growth promotion observed in plants. Some studies suggest that efficient colonization of plant tissues by *Methylobacterium* species is crucial for establishing a symbiotic relationship that benefits both the plant and the bacterium (Yim et al., 2012). Additionally, the mutualistic symbiosis between *Methylobacterium* species and various plant species has been investigated, suggesting that this interaction is facilitated by methanol emission from plants, which serves as a carbon source for the bacteria (Tani et al., 2012b). This mutualistic relationship not only stimulates plant growth but also contributes to ecosystem health.

Moreover, the application of *Methylobacterium symbioticum* as a microbial inoculant has demonstrated its potential to reduce agricultural production costs. Studies have highlighted the potential for using lower amounts of *Methylobacterium*-based inoculants once substantial colonization has been achieved, thereby reducing expenses associated with traditional fertilizer use (Zhang et al., 2024). This characteristic is particularly relevant in the context of sustainable agriculture, where integrating biological inoculants can contribute to the development of more environmentally friendly farming practices.

The advantages of *Methylobacterium symbioticum* are not limited to a specific plant

species but can be extended to various agricultural crops. Research has shown that *Methylobacterium* can enhance the growth and yield of economically valuable crops such as strawberries and maize. The effectiveness of *Methylobacterium symbioticum* as a biological inoculant for these crops has been particularly evident under nitrogen stress conditions, where the symbiotic relationship proved highly beneficial (Torres et al., 2024). Furthermore, the diversity of *Methylobacterium* species in the phylloplane of different crops has been documented, indicating a broad potential for promoting plant growth across various agricultural systems (Cheng et al., 2022).

Additionally, the role of *Methylobacterium symbioticum* in phytoremediation has become a promising research direction. Studies have highlighted the potential of *Methylobacterium*-enriched *Crotalaria pumila* seeds in improving phytoremediation efforts for soils contaminated with heavy metals (Sánchez-López et al., 2018). This application not only supports plant growth but also contributes to soil health and environmental sustainability by facilitating the restoration of contaminated lands.

Research indicates that *Methylobacterium symbioticum* can effectively reduce the need for synthetic nitrogen-based fertilizers without compromising crop productivity. Studies have demonstrated that its application in maize and strawberry crops has led to comparable or even superior results in terms of growth and yield compared to conventional fertilization methods (Torres et al., 2024). This aspect is particularly relevant in the context of sustainable agriculture, where the ecological impact of chemical fertilizers is an increasing concern. As a biofertilizer, *Methylobacterium symbioticum* not only stimulates plant growth but also contributes to soil health by enhancing microbial diversity and activity (Valente et al., 2024; Torres et al., 2024; Rodrigues et al., 2024).

**Mechanisms of Action of *Methylobacterium symbioticum***

The mechanisms through which *Methylobacterium symbioticum* exerts its beneficial effects are complex and multidimensional. One of the fundamental mechanisms is the production of phytohormones such as auxins and cytokinins, which are essential for plant growth and development.

These hormones regulate processes such as root elongation, nutrient uptake, and stress tolerance, thereby improving overall plant health and productivity (Senthilkumar et al., 2021; Bogas et al., 2016; Grossi et al., 2024). Additionally, *Methylobacterium* species are known for their ability to produce indole-3-acetic acid (IAA), a type of auxin essential for root architecture and function, which significantly enhances plants' ability to absorb water and nutrients from the soil (Grossi et al., 2024; Kwak et al., 2014). Moreover, the ability of *Methylobacterium symbioticum* to fix atmospheric nitrogen represents a significant advantage, especially in nitrogen-deficient soils. This characteristic reduces dependence on chemical nitrogen-based fertilizers, which are often associated with negative environmental effects such as water eutrophication and soil degradation (Torres et al., 2024; Rodrigues et al., 2024). Integrating *Methylobacterium symbioticum* into agricultural practices can thus contribute to the development of more sustainable farming systems that maintain high productivity levels while minimizing ecological impact. In addition to its role in nitrogen fixation and phytohormone production, *Methylobacterium symbioticum* improves plant resistance to abiotic stresses such as drought and salinity. By modulating metabolic pathways associated with stress responses and stimulating root growth, this bacterium helps plants withstand unfavorable environmental conditions, ultimately leading to stabilized yields under fluctuating climatic conditions (Palberg et al., 2022; Choudhury et al., 2023). Its ability to mitigate stress effects is particularly important in the context of global climate change, which is placing increasing pressure on agricultural systems. The application of *Methylobacterium symbioticum* is not limited to a single type of crop; it has been successfully tested on various species, including rice, olive, and potato. For example, studies have demonstrated that inoculating olive trees with *Methylobacterium symbioticum* resulted in improved growth and increased nitrogen fixation efficiency, highlighting its versatility as a biofertilizer (Rodrigues et al., 2024; Grossi et al., 2020). Similarly, in potato cultivation, *Methylobacterium* has been reported to enhance phosphorus and nitrogen absorption, further

reinforcing its role as a valuable agricultural agent (Grossi et al., 2020).

Furthermore, integrating *Methylobacterium symbioticum* into agricultural practices aligns with agroecological principles, which emphasize the importance of biodiversity and ecological balance in farming systems. By promoting beneficial microbial communities in the soil and rhizosphere, *Methylobacterium symbioticum* contributes to the overall health of agroecosystems, fostering sustainable agricultural practices capable of addressing the challenges of modern farming (Valente et al., 2024; Christian et al., 2021).

*Methylobacterium symbioticum* offers multiple benefits for plant growth and productivity through mechanisms such as nitrogen fixation, phytohormone production, and optimized nutrient absorption. Its ability to establish symbiotic relationships with various crops positions it as a valuable tool for sustainable agriculture, potentially reducing dependence on chemical fertilizers while enhancing crop yields. Ongoing research into the diverse applications of *Methylobacterium symbioticum* underscores its importance in modern agricultural practices and environmental management.

Recent studies and practical applications have confirmed that *Methylobacterium symbioticum* can bring significant benefits to essential agricultural crops, contributing to increased yield, improved quality, and enhanced plant tolerance to stress. As research progresses, *Methylobacterium symbioticum* is on its way to becoming a fundamental tool in sustainable agricultural practices, providing farmers with a method to boost productivity in an environmentally friendly and economically viable manner.

In recent years, the application of foliar biostimulant products has gained universal acceptance as a promising practice due to their ease of application and cost-effectiveness, particularly when combined with other agronomic practices such as herbicide, fungicide, and fertilizer application. Numerous studies have investigated the efficacy of these products, which often contain growth-promoting substances such as amino acids, humic acids, or beneficial microorganisms, including plant growth-promoting bacteria (PGPB) (Glick et al., 1998; Dal et al., 2017).

These studies have explored the possibility of reducing chemical fertilization while maintaining production and quality standards. However, the *Methylobacterium symbioticum* strain, isolated by Pascual et al. in 2020, has not yet been extensively studied, and the effects of an inoculant based on this bacterium on common wheat remain untested.

Consistent with previously reported results for crops such as maize and strawberry (Torres et al., 2024; Pascual et al., 2020), this study confirmed the beneficial effects on vegetative parameters (SPAD and NDVI) following inoculation with *Methylobacterium symbioticum*. The inoculant extended the crop's stay-green period, prolonging photosynthetic activity throughout the growth cycle. In biomass crops such as maize, this aspect represents an agronomic advantage, as it allows the plant to continue the photosynthesis process for a longer period, thereby increasing CO<sub>2</sub> assimilation and total biomass accumulation at harvest.

In the case of common wheat, as expected, SPAD values before harvest were significantly lower in plots fertilized with a reduced nitrogen dose (75%N) compared to control plots. However, the "75%N + bact" treatment mitigated this difference, demonstrating the bacterium's ability to delay chlorophyll degradation in the final stages of the growth cycle. Additionally, although not statistically significant, a trend was observed in which plots treated with the microbial biostimulant exhibited higher SPAD values than untreated ones, particularly at the reduced nitrogen dose. This suggests that the bacteria express their effects more effectively under nitrogen deficiency conditions. It is presumed that plants in a nitrogen-deficient nutritional state are more favorable for establishing and maintaining an endophytic relationship with these beneficial bacteria. Similar *Biofertilizer* results were obtained in an *in vitro* study on maize (Torres et al., 2024), where bacterial application at a reduced nitrogen dose (50%N) resulted in higher SPAD values compared to the fully fertilized control, further confirming a more efficient association between plants and bacteria under nutritional stress conditions.

NDVI analysis demonstrated that, at the end of the growth cycle, values were similar across all

treatments but were higher in the "75%N + bact" treatment compared to "75%N" alone.

Previous studies have shown that various strains from the *Methylobacterium* genus possess ACC deaminase activity (Yim et al., 2014). Based on this hypothesis, preliminary *in vitro* tests confirmed that the tested strain exhibits this enzymatic activity, allowing it to delay senescence by breaking down the ethylene precursor ACC, thereby prolonging the stay-green period of leaves. This enzyme degrades ACC, reducing ethylene synthesis and enhancing plant stress tolerance (Madhaiyan et al., 2007).

Although the bacterial treatment did not have significant effects on morphological traits (plant height, upper internode length, LAI, and aerial biomass), notable effects were recorded on photosynthetic parameters. The "75%N + bact" treatment increased stomatal conductance and transpiration rate compared to the "100%N" and "75%N" plots without inoculation. Additionally, improved CO<sub>2</sub> assimilation rates and enhanced PSII efficiency were observed, confirming that plants establish associations with microorganisms to compensate for nutritional deficits.

Previous studies on other plant growth-promoting bacteria (PGPB) have highlighted similar benefits. For example, *Azospirillum brasilense* improves the growth of various crops by fixing atmospheric nitrogen and synthesizing growth-promoting substances (Bhattacharyya & Jha, 2012). Similarly, *Pseudomonas fluorescens* has demonstrated its ability to increase plant tolerance to saline stress by producing phytohormones and ACC deaminase, thereby reducing ethylene levels and improving plant development (Egamberdieva & Lugtenberg, 2014).

Regarding yield, the bacterial treatment did not significantly impact wheat productivity. However, other studies have reported yield increases in crops such as maize, grapevine, rice, and strawberry, particularly under reduced nitrogen doses (Torres et al., 2024; Pascual et al., 2020). These results suggest that improved crop performance is associated with the microorganisms' ability to assimilate ammonium through nitrogen fixation, reducing the plant's energy requirements for mineral nitrogen conversion.



Regarding wheat quality, the microbial biostimulant did not significantly increase protein content but influenced protein quality by increasing the glutenin-to-gliadin ratio and the proportion of high-molecular-weight (HMW) subunits relative to low-molecular-weight (LMW) subunits. This modification led to an increase in dough tenacity and stability.

From an economic perspective, applying the inoculant does not incur additional costs for agricultural equipment, as it can be integrated with post-emergence weed control treatments or fungicides, thereby saving time and fuel. Although potential interactions between the bacterium and applied plant protection products need further investigation, economic viability is largely determined by fertilizer prices, which have risen due to raw material shortages. Beyond economic considerations, the ecological benefits of reducing chemical fertilizer applications such as decreased nitrogen leaching after heavy rainfall represent another agronomic advantage.

## CONCLUSIONS

A rapid transition to a sustainable and productive agricultural system is necessary to maintain soil fertility and reduce soil biodiversity loss.

Microbial biostimulants represent an innovative solution for achieving safe and long-term agricultural production with high nutritional value (Castiglione et al., 2021). Given the continuous growth of the global population, the reduction of arable land, and the depletion of crops' genetic potential, the implementation of innovative agricultural technologies is essential. Agronomic solutions with low environmental impact, aimed at improving plant resilience to challenging soil conditions, are becoming indispensable for meeting the high demand for nutrient-rich food (Szparaga et al., 2019; Mannino et al., 2021).

Plant biostimulants (PBs) are a new generation of products available on the market that can contribute to achieving sustainability goals in agriculture. According to EU Regulation 2019/1009, they are defined as "products that stimulate plant nutrition processes, independently of their nutrient content, with the sole purpose of improving one or more of the

following characteristics of the plant or its rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality characteristics, and the availability of limited nutrients in the soil or rhizosphere" (Campobenedetto et al., 2020; Campobenedetto et al., 2021).

PBs can consist of substances, mixtures, or microorganisms and are thus classified into microbial and non-microbial biostimulants (European Commission). A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms included in CMC-7 (Component Material Categories, number 7), which comprises four distinct genera: *Azotobacter* spp., mycorrhizal fungi, *Rhizobium* spp., and *Azospirillum* spp.

However, although the existence of regulations and limitations can be beneficial for ensuring food safety and product quality, the strictness and exclusivity of the positive list may significantly affect the potential benefits of these innovative products. Economically, the application of the inoculant does not entail additional spending on farm machinery, as it can be mixed with post-emergence weed control treatments or fungicides, thereby conserving time and fuel. Although the possible interaction of the bacteria with sprayed plant protection products must be the subject of further research, economic viability is decided primarily by the cost of fertilizers, which has increased because of the shortage of raw materials. Therefore, it would be appropriate to consider reducing the negative list and expanding the positive list with new microorganisms, provided that scientific evidence demonstrates and supports their safety for both the environment and consumers.

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