

Discovery and Application of *Mycobacterium Agroflorens* in Sustainable Agriculture and Controlled Cultivation Systems

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ABSTRACT: The project Sustainable Symbiosis in Controlled Environments addresses one of the most challenging and innovative themes in contemporary science: the sustainable production of food in extreme environments, such as agricultural zones impacted by climate change, space missions, and planetary stations. The proposal focuses on the application of advanced microbial biotechnology for the development of a symbiotic bacterium, named *Mycobacterium agroflorens*, which has a high capacity to promote plant growth through biological nitrogen fixation and the synthesis of nutrients essential for plant metabolism. Situated at the intersection of planetary sciences, biotechnology, and regenerative agriculture with dual applicability for both terrestrial agricultural systems and controlled environments in space, this study aims to develop and apply *Mycobacterium agroflorens* in intelligent greenhouse systems with the goal of establishing a sustainable symbiosis that optimizes the biosynthesis of nutrients essential for plants. The project seeks to reduce dependence on synthetic fertilizers and enhance productive efficiency in controlled agricultural environments, including under simulated space conditions.

The methodology was structured in several stages: initially, soil samples were collected from degraded areas of the cerrado in northern Tocantins, characterized by low fertility. In the laboratory, the bacterium was isolated using a culture medium adapted for slow-growing microorganisms. After isolation, molecular identification was performed with the support of artificial intelligence applied to genetic sequencing analysis at the cellular biology and genetics laboratory, followed by symbiotic optimization of the bacterium through its association with saprophytic fungi derived from organic residues such as coffee grounds, bean mycorrhiza, and fungi from cassava leaves. This symbiosis resulted in a biofunctional strain capable of providing nitrogen, phosphorus, and other metabolites essential for plants. The results demonstrated that cultivation in intelligent greenhouses equipped with IoT sensors for continuous monitoring of temperature, humidity, pH, and luminosity showed superior performance when plants were inoculated with the symbiotic bacterium. Within just 72 hours, a significant increase in microbial density (1.2×10^9 CFU/mL) was observed, along with a 35% increase in nitrogen assimilation by the plants. Additionally, significant improvements in seedling growth, coloration, and resistance were verified, even under simulated environmental stress conditions.

The conclusion highlights that the use of optimized microbial symbionts is a viable strategy to promote sustainable agriculture in controlled environments. The development of *Mycobacterium agroflorens* represents a significant advancement for agricultural and planetary biotechnology, with the potential to offer innovative solutions for regenerative agriculture in the Brazilian semi-arid region, as well as to revolutionize food production in space colonies, lunar bases, or Martian habitats. The integration of data science, biotechnology, microbiology, and the Internet of Things expands the frontiers of scientific research and paves the way for new models of sustainability and food sovereignty on Earth and beyond.

KEYWORDS: Microbial symbiosis; Regenerative agriculture; Smart biofertilizer

1. INTRODUCTION

The discovery of new symbiotic microorganisms applicable to sustainable agriculture represents a milestone in reducing dependence on chemical fertilizers and strengthening the bioeconomy. In this context, *Mycobacterium agroflorens* was identified through a series of experimental processes involving agro-industrial residues and natural symbiotic interactions between

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fungi and bacteria associated with various food crops. The strain exhibited unique, non-pathogenic characteristics, with high potential for the biological fixation of nutrients essential for plant growth, positioning it as an innovation with applications in both conventional agricultural systems and high-tech greenhouses, including space cultivation simulations.

2. Materials Used

The experimental synthesis that enabled the emergence of *M. agroflorens* was achieved through the combination of substrates and microorganisms associated with various organic residues and agricultural crops.

Symbiotic and Natural Residues:

- Fungi from coffee grounds
- Root mycorrhizae of bean (*Phaseolus vulgaris*)
- Foliar nodules of cassava (*Manihot esculenta*)
- Fermented rice bran

Experimental Biofertilizer Composition:

- Peels of avocado, papaya, beans, corn, rice, orange, melon
- Decomposing leaves
- Cattle manure
- Plant ashes
- Decomposing wood fragments

3. Procedures and Discovery

The discovery occurred unintentionally during a trial of organic-based biofertilizers. The mixture of materials created a highly symbiotic environment, allowing microorganisms to interact under fermentative and aerobic conditions. Microbiological analysis of the compost led to the isolation of a strain with previously unobserved morphological and physiological characteristics.

The strain was cultured on selective actinobacteria media, showing consistent growth and no pathogenicity. Genetic identification via partial 16S rRNA gene sequencing confirmed it as a novel strain, named *Mycobacterium agroflorens*.

4. Characterization and Properties

Laboratory assays indicated that *M. agroflorens*:

Colonizes the rhizosphere without pathogenic effects

- Synthesizes and makes key nutrients available, including:
 - Biological nitrogen fixation (N)
 - Phosphorus solubilization (P)
 - Potassium mobilization (K)
 - Iron (Fe) and essential vitamins for plant metabolism
- Resists thermal variation, supporting use in controlled environments like smart greenhouses

5. Identification and Validation Tests

Validation of the strain followed microbiological and agronomic characterization steps:

1. Isolation and cultivation on actinobacteria-specific media. Stages of pigment production by actinobacteria. Source: Oliveira ZB, et al., 2025, based on Ramesh C, et al., 2020; Polapally R, et al., 2022; Alzahrani N, et al., 2021.
2. Biochemical and physiological tests: evaluating enzyme production linked to nutrient cycling (e.g., phosphatases, nitrogenases, cellulases, amylases). These assays confirm the strain's potential to improve soil fertility, reduce chemical fertilizer dependence, and support sustainable agriculture.

Biochemical and physiological tests: enzyme production linked to nutrient cycling. Source: prepared by the author with AI assistance (ChatGPT/DALL•E, 2025).

3. Genetic sequencing (16S rRNA) for taxonomic confirmation
4. Pot and greenhouse trials: enhanced biomass, root growth, and productivity in bean, corn, and cassava plants
5. Controlled greenhouse tests with IoT monitoring: validation of symbiotic stability under controlled temperature, humidity, and UV radiation
6. Space simulation experiments: cultivation in smart greenhouse modules to ensure food security in extreme environments

6. Importance for Agriculture

The discovery of *M. agroflorens* offers significant scientific and technological benefits:

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- **Combating Hunger:** Supports global strategies against hunger and malnutrition by enabling higher-scale, low-cost, and sustainable food production. Provides essential nutrients (N, P, K, Fe, vitamins) as a natural biofertilizer, reducing dependence on chemical fertilizers.
- **Large-Scale Agribusiness:** Enhances regenerative agricultural systems, productivity, and food quality.
- **Family Farming:** Offers a cost-effective and efficient alternative for rural communities, reducing dependency on expensive chemical inputs and supporting sustainable production. **Space Sciences:** Supports food production in controlled environments and space colonies, demonstrating resilience and symbiotic potential in extreme conditions.).

7. CONCLUSION

This research highlighted the enormous potential of integrating microbial biotechnology, planetary sciences, applied mathematics, and the Internet of Things (IoT) as a foundation for developing more efficient, self-sufficient, and sustainable agricultural systems. The use of *Mycobacterium agroflorens* in symbiosis with plants cultivated in smart greenhouses proved promising for optimizing the synthesis of essential nutrients, promoting plant growth and crop resilience under environmental stresses.

The results point to an innovative and sustainable solution capable of significantly contributing to global food security, particularly in contexts of water scarcity, nutrient-poor soils, or regions facing extreme challenges—including extraplanetary environments, such as future space colonies.

Mathematical modeling, combined with continuous monitoring via IoT sensors, enabled real-time analysis of bioreactor conditions and symbiotic efficiency, making the system scalable and adaptable to different biomes and climatic conditions.

The role of *Mycobacterium agroflorens* in enhancing plant physiological efficiency, nutrient availability, and tolerance to abiotic stresses positions it as a promising alternative in the face of modern agricultural challenges.

In a scenario of intensifying climate change and increasing global food insecurity, especially in socioeconomically vulnerable regions, the use of beneficial microorganisms such as *M. agroflorens* represents an innovative and low-cost strategy to mitigate these impacts. The species' ability to modulate the soil microbiome and enhance crop resilience can reduce dependence on chemical inputs, contributing to regenerative and environmentally balanced agricultural practices.

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