## CAPÍTULO 4

# MICROBIAL CONSORTIA: A FEASIBLE ECOLOGICAL TECHNOLOGY FOR SUSTAINABLE AGRICULTURE

*Data de submissão: 17/12/2024* *Data de aceite: 02/01/2025*

**Rafael Luiz Frinhani Rocha**
Centro Universitário Multivix, Serra & Secretária Municipal de Meio Ambiente de Vargem Alta, Espírito Santo, Brasil.

**Fábio Lopes Olivares**
Laboratório de Biologia Celular e Tecidual & Núcleo de Desenvolvimento de Insumos Biológicos para a Agricultura (NUDIBA), Universidade Estadual do Norte Fluminense Darcy Ribeiro, UENF, Rio de Janeiro, Brasil.

**ABSTRACT**: The multispecies community drives the complex microbial process in the soil-plant system. Lessons from the environment suggest that microbial consortia have several advantages over single microbial strain-based products for sustainable agriculture. Due to physical and metabolic cooperation, proper synthetic microbial communities are potentially more efficient and stable in maintaining specific microbial processes. The mutualistic interaction that occurs in a stable microbial consortium has shown adaptative advantages compared to a single microbial application that results in better resilience under biotic and abiotic fluctuation and environmental disturbances, promoting benefits to the soil-plant system and plant growth by diverse mechanisms, such as bioavailability of nutrients or decomposition of organic waste. Representing a promising biotechnological advance, the use of microbial consortia is complex. It requires an integration of different areas, methodological approaches, and techniques for understanding the interaction elements and their development and efficient application in the field. Microbial consortia in agriculture correspond to a sustainable alternative to conventional practices, ensuring the productivity of crops and gains in terms of economy and ecology, fundamental for future agriculture. The present work aimed to present relevant concepts and information about biochemical mechanisms involved in microbial compatibility and synergy, methods of formulating microbial consortia, and proposals for use for environmentally sustainable and productive agriculture.

**KEYWORDS:** Microbial communities, microbial ecology, green agriculture, biological inputs.

## INTRODUCTION

Since the green revolution in the early 1970s, chemical fertilizers and pesticides in agriculture have increased considerably (Naik et al., 2019). In addition, the continuous population growth exerted intense pressure on agriculture to maintain such practices to meet market demands since the use of these agrochemicals resulted in considerable increases in agricultural productivity (Gupta, Bisaria, and Sharma 2015). However, sanitary, environmental, economic, and agricultural factors have demonstrated the infeasibility of using conventional methods, and the search for conciliatory agriculture between production and sustainability is increasing (Struik and Kuyper 2017; Valenzuela 2016; Weekley, Gabbard, and Nowak 2012). That is where biological inputs for agriculture arise.

The design and development of bio-based technologies convert natural processes in the soil-plant system into bio-input technologies (Sousa and Olivares 2016). However, when studied under laboratory conditions from tests with microorganisms in pure culture, some of these natural processes have unsatisfactory results, with methodological and efficiency limitations (Guo et al., 2011). In contrast, studies that address microbial communities characterized by the coexistence of different populations of microorganisms in each habitat and are imitated through synthetic microbial consortia are a way to overcome the limitations of individual microbes (Hays et al., 2017).

Despite advances in technologies and analysis methods, cultivation and laboratory approaches differ from microorganisms behavior in their natural habitats, grouped in complex communities (Stocker, 2012; Agapakis et al., 2012). The concept of manipulating microbial communities for consortia's artificial selection based on different microbial strains may increase tolerances to the overall conditions to which one intends to insert them (Lee, Show, and Wang, 2013). In this way, microbial consortia can reduce the metabolic load on a single species, optimizing processes and reducing cellular energy demand. From the presentation of the theme and its context, this review addresses microbial consortia and relevant aspects of technology and methodologies and their applications for more sustainable agriculture.

## Characterization of microbial consortiums

The use of microorganisms in agriculture is vast, and its benefits are diverse. Therefore, many approaches focus on maximizing the use of these microorganisms by optimizing beneficial characteristics. Studies with genetically modified microorganisms, for example, provide promising results. Still, in addition to not being an ecological approach, improving a desirable feature in a microorganism can result from the limitation of another element necessary for its development (Scott and Harsty, 2016). In this context, microbial consortia and their wide applications arise.

Microbial communities and their artificial representation, the microbial consortia, correspond to complex conglomerates that coexist in a habitat with strains with different physiological attributes (Lee, Show, and Wang, 2013). These microbial arrangements are widely distributed in nature, colonizing the most diverse environments, and correspond to a more promising and reliable environmental strategy than pure cultures (Jain et al., 2013; Gupta and Kumar, 2016). Furthermore, the spatial arrangement of these consortia corresponds to sites of constant competition and cooperation relationships, and these relationships are responsible for the specification and dynamics of the ecosystem (Kong et al., 2018).

Besides the relationship between microorganisms in agriculture, it is necessary to consider the plant and its rhizosphere as microbial habitats. In this habitat, several connections are established. The plant can directly reflect these relationships, modulating how microbial communities, mainly in the root and rhizosphere, are considered a hotspot of microbial interactions (Li et al., 2016). Some examples of existing relationships between microorganisms in the soil and the rhizosphere are shown in figure 1.

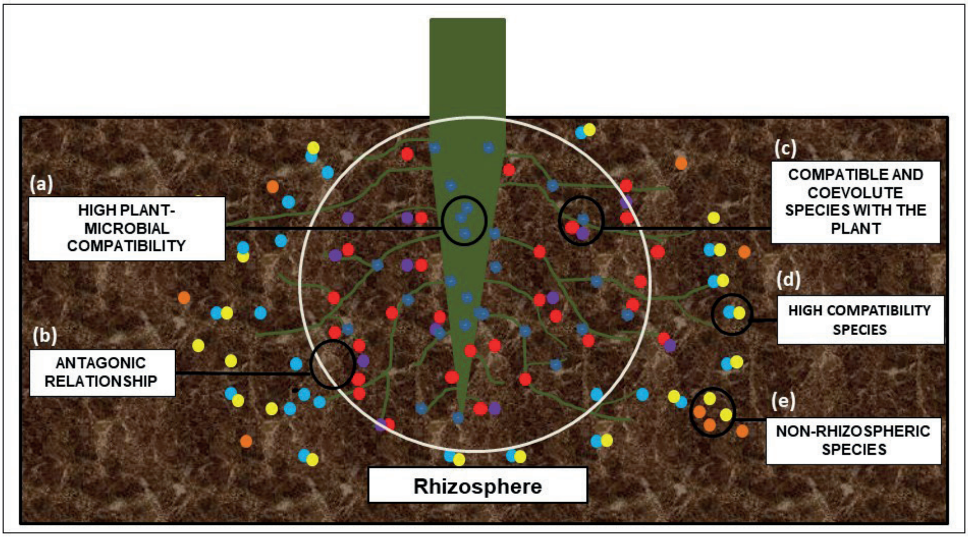

**Figure 1**. Different microbial relationships that occur in the rhizosphere: (a) microorganisms with high compatibility with the plant; (b) antagonistic relationships between varied species of microorganisms, not developing closely together; (c) species compatible and convoluted with the plant, existing only when associated with the plant; (d) microbial species that have high compatibility with each other; (e) non-rhizospheric microbial compatible species.

As observed in the image above, some microbial species co-evolved with plants and appear to be closely related to their respective host, growing in the region of influence of the root, the rhizosphere, or even directly associated with the rhizoplane, while in some cases, the plants produce metabolites that inhibit the growth of certain groups of microorganisms (Berg and Smalla, 2009). Also, in the soil, inhibition relationships between species with similar ecological niches (antagonism) and mutualistic relationships can occur, where species develop in the same habitat and benefit from this interaction (Stamford et al., (2005).

According to Jial et al. (2016), the main advantages of microbial consortia are the division of complex tasks, greater adaptability, and stability to the dynamism of environmental conditions. Furthermore, the multispecificity of microbial communities results in horizontal gene transfer, which in addition to altering the environment, influences the growth and development patterns of microbial populations (Hays et al., 2017). Therefore, the observance of the constancy of these relationships in their natural environments and agrosystems can make the consortia that may be developed more phenotypically complex and more adapted to environmental fluctuations compared to what is provided by microbial cultures while isolated (Alnahhas et al., 2020; Hays et al., 2017).

In addition to microbial interactions and their genetic variability, a spatial structure and availability of resources also exert selective pressure for efficient growth in their microbial assemblages. This selective pressure refers to the natural processes of changing the behavior and adaptability of microorganisms within the microbial system, spreading in more stable communities, whether in superficial microbial communities or even in dynamic soil-plant-microbe systems (Lee, Show, and Wang, 2013; Roller and Shmidt, 2015).

Despite the knowledge about the natural microbial consortia represented by microbial communities, more detailed studies on these interactions and interactions between microorganisms and their habitats, as well as their benefits, effective mechanism, and applications, are recent (Welbaum et al., 2004; Weekley et al. al., 2012). Thus, improving techniques for handling microbial consortia requires advances in methodological approaches and multidisciplinary integration of different areas.

## Study methods for design and formulation of microbial consortiums

The manipulation of microorganisms for the composition of a microbial consortium comprises several steps, and several techniques can be used. First, assuming a more ecological bias and without genetic alteration, the cultivation techniques associated with tools such as omics allow the development of a consortium with natural microorganisms without compatible and synergistic modifications it executing their desirable ecophysiological roles. Two prospects can formulate a microbial consortium (Figure 2).

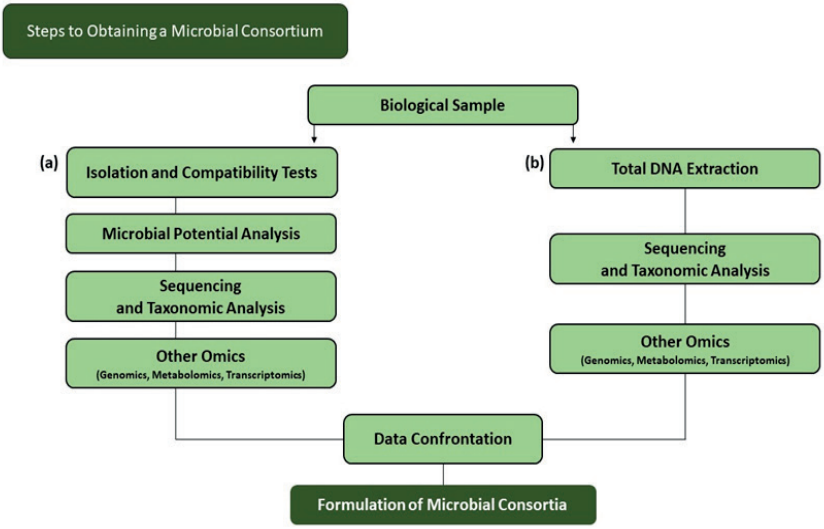

**Figure 2**. Steps for elaborating a microbial consortium consider two possibilities: (a) from the bottom up from isolation and cultivation; (b) and considering only refined techniques for understanding the consortia present in the biological sample.

Starting from good material related to the objective to be achieved, microorganisms can prospect, assessed in tests of biotechnological potential and their characteristics of compatibility or antagonism. Finally, they are identified and characterized, for example, concerning their metabolites and genes. Regarding direct use with omics, the total DNA is extracted, and the sequencing and metabolic and genomic characterization of microorganisms is carried out. Cultivable approaches are necessary to develop microbial consortia to manipulate and select microorganisms. Considering the need for a harmonious relationship between the strains involved, they must be confronted and present positive results; they are compatible and preferably synergistic concerning ecophysiological capacities. Therefore, techniques based on co-cultivation, or the "microbial antagonism and compatibility method," should be used, as shown in Figure 3 (Bell, Weels and Markham, 1982; Fuentes et al., 2016).

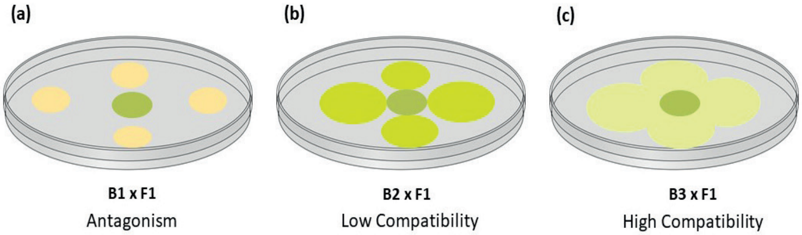

**Figure 2**. Scheme of antagonism and compatibility assays with different interactions. B1, B2, and B3: Generic bacteria; F1: Generic fungus. (a) Antagonism relationship; (b) Low compatibility; (c) High compatibility.

This method presents numerous possibilities for methodological adaptation and evaluation. Through this methodology, microorganisms of biotechnological interest are confronted and demonstrate characteristics of antagonism or interaction at diverse levels, facilitating their selection. Based on the interest of the experiment, it is possible to categorize microorganisms as antagonists (a) when there is no positive interaction between microorganisms involved or even inhibition, or as low (b) or high compatibility (c). The microbial compatibility and antagonism method allows confronting microorganisms to propose, for example, microbial consortia formulated based on a free-living $N_2$-fixing bacteria and a rhizospheric fungus (AMF). In this way, once the synergism of the microorganisms involved has been proven, the microbial consortium would be able to promote nutritional improvements to the soil and the associated culture through biological nitrogen fixation through bacterial action and greater efficiency in the absorption of nutrients, such as phosphorus, through fungal action (Paula et al., 1992; Miyauchi et al., 2008; Zhu et al., 2018).

Using consortia, it is necessary to understand the ecological, metabolic, and genetic aspects eliminated for further manipulation. Regarding microbial ecology, incompatibility between species can result in physiological or metabolic commitment. In this scenario, the integration of omics provides information on the mechanisms involved in the interaction and establishment of the consortia so that the natural attributes of the proposed consortia become stable and viable and their applications (Hays et al., 2017; Woo and Pepe, 2018) Greater detailing of microbial consortia considering sophisticated techniques can offer a different strategic view to increasing the persistence and activity of microorganisms (Li et al., 2017). We highlight cross-feed and Quorum sensing among the possibilities of integrating omics, metabolomics, and genomics (Figure 4).

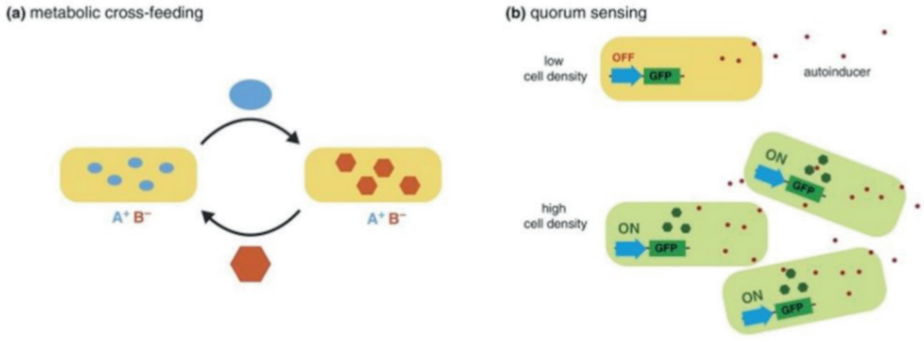

**Figure 4**. (a) Cross-feed: One cell produces metabolite A but does not create metabolite B. The second strain cannot produce metabolite A but makes B, benefiting each other and allowing survival. (b) Quorum sensing: Cells have an auto inductive molecule that increases at high cell concentrations, causing an increase in a responsive promoter, resulting in changes in gene expression. (Hays et al., 2017- Adapted).

In Cross-feed, a microorganism supplies another with a metabolite that it produces in excess while feeding on a metabolite that does not produce but is released by another organism present in the same microbial community (Hays et al., 2017). For example, in a co-cultivation study, Deveau et al. (2010) demonstrated that a Pseudomonas fluorescence bacterial strain produces thiamine. This water-soluble vitamin belongs to the B complex, enabling the development of the mycorrhizal fungus *Laccaria*, which in turn supplies the bacterium with trehalose disaccharide, produced and accumulated in its hyphae.

As for quorum sensing (QS), it is a mechanism for the secretion of molecular (self-inducing) signals, common in bacteria; through it, the microorganisms of a given habitat can detect the population density of the microbial community so that it is possible to coordinate the gene expression and affect its differentiation (Scott and Harsty, 2016; Stephens and Bentley, 2020). About quorum sensing and microbial consortia, Liu et al. (2021) investigated the hydrogen recovery from the action of microbial consortia on residues via electro-fermentation and pretreatment with free nitrous acid together with quorum sensing. According to the author, the distinct species showed synergistic relationships with biofilm formation and quorum sensing, resulting in higher yields in hydrogen recovery.

Still, conventional techniques with isolation of individuals allow for microbial prospecting, but omics provide different insights into the persistence and activity of microorganisms involved in consortia (Li et al., 2017). The integration of the two approaches makes it possible to understand the influence of environmental variations on the modulation and production of metabolites by microorganisms and how they react to these disturbances, expressing or repressing related genes, resulting in improved formulation and establishment of an efficient microbial consortium.

## Applications in agriculture

The so-called plant growth promotion microorganisms (PGPM) are naturally endowed with many traits with extensive agricultural uses (Naik et a., 2019). The benefits of microbial action to soil and plants are widely described in the literature (Olivares et al., 2017; Igiehon and Babalola, 2017; Khan, Pimentel et al., 20120; Bano and Curá, 2020). Among these, the decomposition of crop residues, biological nitrogen fixation, production of phytohormones, phosphorus solubilization, and optimization of the root system, among others, stand out. Some of the applications proposed in studies with microbial consortia in the last two decades are shown in Table 1.

| Microorganisms Involved | Major Functions | Reference |
|---|---|---|
| *Pichia guilermondii* and *Bacillus mycoides* | Biocontrol of pests and diseases | Guetsky et al., 2001 |
| *Ceriporiopsis subvermispora, Cellulomonas* sp. and *Azospirillum brasilense* | Lignocellulosic biomass deconstruction | Beary, Boopathy and Templet, 2002 |
| *Chryseobacterium* sp., *Comamonas* sp. and others | Bioremediation of contaminated soils | Radianingtyas, Robinson and Bull, 2003 |
| *Trichoderma reesei, Aspergillus niger* and *Saccharomyces cerevisiae* | Lignocellulosic biomass deconstruction | Yang et al., 2004 |
| Multispecific consortium formulated with many bacteria and fungi | Bioremediation of contaminated soils | Viñas et al., 2005 |
| *Bacillus subtilis, Streptomyces* sp. and *Cellulomonas* sp. | Lignocellulosic biomass deconstruction | Guevara and Zambrano, 2006 |
| *Pseudomonas* spp., *Burkholderia* sp., *Flavobacterium* sp. and *Vibrio* sp. | Bioremediation of contaminated soils | Murthy and Manonmani, 2007 |
| *Escherichia coli* and *Ochrobactrum* sp. | Bioremediation of contaminated soils | Zhang et al., 2008 |
| *Bacillus subtilis* and *Bacillus licheniformis* | Plant growth promotion | Lim et al., 2009 |
| *Bacillus subtilis* and *Bacillus licheniformis* | Biocontrol of pests and diseases | Chung, Lim and Kim, 2010 |
| *Azospirillum* sp., *Azotobacter* sp., *Pseudomonas* sp. and *Bacillus sp.* | Plant growth promotion | Rajasekar and Elango, 2011 |
| *Pseudomonas aeruginosa, Trichoderma harzianum* and *Bacillus subtilis* | Biocontrol of pests and diseases | Jain et al., 2012 |
| Undefined microbial consortium | Lignocellulosic biomass deconstruction | Hui et al., 2013 |
| *Pseudomonas aeruginosa, Bacillus cereus* and *Bacillus amyloliquefaciens* | Biocontrol of pests and diseases | Thakkar and Saraf, 2014 |
| *Bacillus megaterium, Pseudomonas fluorescens* and *Trichoderma harzianum* | Plant growth promotion | Gupta et al., 2015 |
| *Bacillus* sp., *Providencia* sp. and *Ochrobactrum* sp. | Lignocellulosic biomass deconstruction | Poszytek et al., 2016 |
| *Rhizobium* sp. and *Trichoderma* sp. | Bioremediation of contaminated soils | Madariaga-Navarrete et al., 2017 |
| Multispecific complex (Proteobacteria and other phyla) | Lignocellulosic biomass deconstruction | Liang et al., 2018 |
| *Azotobacter* sp., *Rhizobium japonicum, Trichoderma harzianum* and others | Stress relief and growth promotion | Bradacová et al., 2019 |
| *Planomicrobium chinense* and *Bacillus cereus* | Stress relief and growth promotion | Khan et al., 2020 |
| *Rhizoglomus irregulare* and *Pseudomonas putida* | Plant growth promotion | Pacheco et al., 2021 |
| *Halomonas aquamarina and Pseudomonas extremorientalis* | Plant growth promotion | Devi et al., 2022 |
| *Azotobacter vinelandii, Bacillus amyloliquefaciens, Burkholderia ambifaria* and others | Plant growth promotion | Hett et al., 2023 |

| | | |
|---|---|---|
| *Cellulomonas iranensis, Pseudomonas* sp. | Plant growth promotion | Che et al., 2024 |

**Table 1**. Microorganisms and their applications in microbial consortia have been proposed in biotechnological studies for agriculture in the last two decades.

In the last two decades, the proposed use of consortia has grown with several applications for agriculture and the environment and industry. This advance is due to the need to implement ecological alternatives for crops and modern technologies to formulate inoculants. Microbial consortia formulations can be based on microorganisms from the same or different habitats. These combinations can improve their capabilities, enabling biotechnologies to benefit the soil and increase crop productivity (Weekley et al., 2012). In this way, attention is drawn to microbial communities as drivers for developing these microbial consortia.

Soil microbial communities, mainly represented by bacteria and fungi, live in an intimate and close relationship with the rhizosphere, providing several direct and indirect benefits to associated plants (Mondal et al., 2020). The approaches in the development of microbial consortia aim to obtain and insert the help of these microorganisms in a potentialized way through modified microbial consortia for bioprocesses, studies of projected micro-ecosystems, or in agrosystems (Alnahhas et., 2020). Some more specific examples of the performance of microbial consortia with effects on the growth promotion of plants and crops will be presented below.

## Minerals solubilization

The mineral solubilization process takes place through the conversion of insoluble forms of these minerals into biologically assimilable soluble forms (Liu et al., 2016; Kalayu, 2019). In this process, several recognized microorganisms are being declared as solubilizers, the so-called phosphate solubilizing microorganisms (PSM's) and potassium solubilizing microorganisms (KSM's) (Meena, Maurya and Verma, 2014; Kalayu, 2019). Although bacteria and fungi have this ability, most works on solubilizing microorganisms are based on the use of pure cultures, even though, in nature, the action of these microorganisms occurs during constant interaction processes. According to Xiao et al. (2020), in reports from their research group, they proposed microbial consortia, if possible, to solubilize phosphate sources more efficiently.

Although there are several ways to solubilize phosphate minerals, such as lowering the pH at the site of action and chelation of K-linked cations, for example (Meena et al., 2014; Alori, Glick and Babalola, 2017; Soumare et al., 2019), several works describe the production of liquids as an efficient strategy in the bioavailability of minerals (Benbrik et al., 2020). The production of organic acid, mainly malic acid, acetic acid, oxalic acid, citric acid, and gluconic acid, results in acidification of the microbial cell as well as its surroundings, promoting mineral solubilization (Meena et al., 2014, Mukhtar et al., 2017; Mendoza-Arroyo et al., 2020). Considering the production of these acids, it is essential to consider that not all microorganisms can produce them, indicating that the combined use of different microorganisms becomes an exciting approach. According to a survey conducted by Meena et al. (2014), other acids are found in varied bacteria and fungi. Thus, greater enzymatic complexation would be provided by a multispecific microbial mix.

In an experiment comparing solubilization activity, Gupta and Kumar (2015) report greater efficiency in assimilation by microbial consortia than microorganisms evaluated alone, through the greater synergy provided by the microbial multispecificity involved. Several reports with comparable results are presented in a survey conducted by Behera et al. (2021), showing the application of consortia as a promising strategy. Common to the experiments mentioned here, the results are attributed to the physiological complementation provided by the released metabolites or even by establishing new synergistic microbial associations (Dipak and Sankar, 2015; Woo and Pepe, 2018).

## Decomposition of cultural residues

The performance of microbial consortia even in the natural environment can be evidenced in the breakdown of lignocellulose compounds. The decomposition of these highly recalcitrant residues requires the joint action of several enzymes that, in nature, are divided into distinct microbial groups, forming complex enzymatic associations (Gomes et al., 2017). In this process, fungi, essentially saprophytic organisms, act in the initial stage of putrefaction of the essential components of plants, such as lignocelluloses. Then the bacteria end the process by eating the digestible materials (Mondal et a., 2020).

Lignocellulose polymers have a complex and organized structural arrangement and are primarily composed of cellulose, a crystalline matrix of linear β-(1,4)-d-glucans which requires different enzymatic groups for its breakdown (Moreira et al., 2011; Vyas et al., 2018; Houfani et al., 2020) as showed in Figure 5.

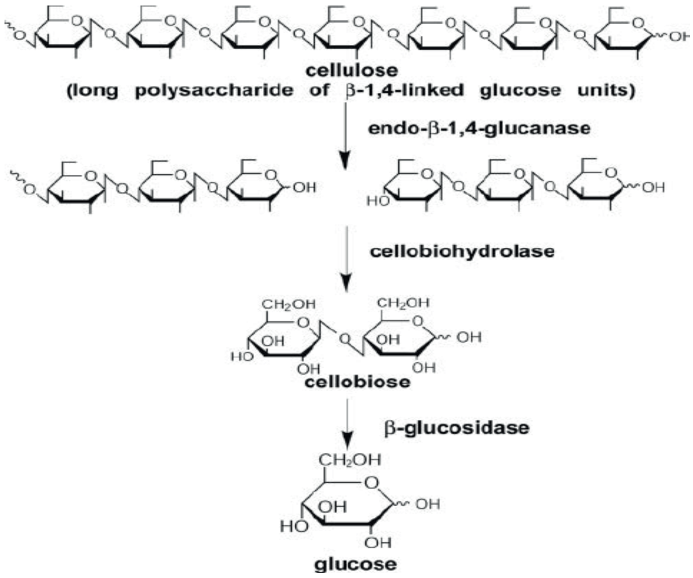

**Figure 5**. Enzymes are involved in breaking down the complex structure of the cellulose molecule. (Xie et al., 2007).

Cultural residues such as corn stalk and sugarcane straw are rich in lignocellulosic compounds and correspond to potential raw materials for the generation of energy or other value-added products (Yamaguchi et al., 2017; Reinehr et al., 2021). Although the microbial decomposition of these residues has been extensively researched, most studies have been based on inoculums with pure cultures, resulting in unsatisfactory lignocellulolytic activities with a low capacity to decompose the complex structure of natural lignocellulose (Guo et al., 2011).

In breaking down these highly recalcitrant compounds, microbial consortia composed of bacteria, actinomycetes, and fungi are more promising compared to inoculums consisting of pure cultures, as they can provide a multi enzymatic action, being even more resistant to non-sterile conditions (Hui et al., 2013; Liang et al., 2018). Three main classes of cellulases are described concerning these enzymes: the endoglucanases, which act randomly on the soluble and insoluble cellulose chains. In addition, the expeinclude cellobiohydrolases work to release cellobiose and sometimes glucose, and glycosidases release glucose from cellobiose and exoglycosidases (Himmel et al., 2010; Moreira et al., 2011; Fülöp and Ecker, 2020).

Thus, the application of microbial consortia with high multi enzymatic complementation in the decomposition of organic compounds rich in lignocellulose has agroecological and economic potential, as it can reduce the impacts of these residues on the soil, such as the proliferation of pests and diseases and compromise the development of shoots (Campos et a., 2010). Furthermore, its benefits may further extend to optimizing the supply of organic matter and nutrient release to the soil (Rasche and Diego, 2019; Cherubin et al., 2019).

## Biocontrol and biosynthesis of phytohormone

Most approaches to biological control of plant diseases are based on techniques such as increasing the disease suppression mechanism in the biological control agents (BCAs), inhibiting competitive communities and developing microbial consortia with high biological control activity (Thakkar and Saraf, 2014). However, using multiple microorganisms as biocontrol agents is more robust as it encompasses different antagonistic and stress tolerance characteristics. Therefore, it can be assumed that at least one biological control mechanism will be functional in the circumstances faced by released BCAs (Jain et al., 2012).

The role in biocontrol may result from the direct relationship between healthy microorganisms and the host plant or indirectly through the antagonistic relationship with phytopathogenic microbes (Mondal et al., 2020). In both cases, the microbial action can be stimulated via exudates released into the rhizosphere, with the microbial species co-evolved with the host plant tending to prevail. In addition to the antagonistic relationship potentiated by the synergism of the consortium, their use provides more effective disease management, as they occupy different rhizospheric microbial niches, thus restricting competition between them and increasing disease suppression (Sarma et al., 2015).

Assessing the effect of bacteria in the control of *Fusarium spp.*, Palmieri et al. (2017) observed better results when they used a consortium composed of four bacteria to the detriment of other less diverse consortia and mainly to the applications of isolated species. However, it is crucial to consider that the test used bacteria with growth-promoting characteristics, such as phosphate solubilization and phytohormones production. According to Sarma et al. (2015), multispecific biocontrol agents containing microbial species with distinct characteristics can promote benefits beyond the suppression of pathogens, thus optimizing plant growth promotion through at least two forms of action.

Regarding the production of phytohormones, studies with microbial consortia are still lacking. However, some works highlight this ability. Assessing the effect of mixed inoculation of *Pseudomonas fluorescence* and *Trichoderma asperellum*, Singh et al. (2018) observed, among other advantages, an increase in hormone synthesis and more significant growth in chickpea seedlings. In a co-inoculation assay, Bilal et al. (2018) observed strict hormonal regulation in soybean plants, reducing metallic stress by inhibiting metal absorption and translocation. Also, according to the authors, higher absorption of essential nutrients was observed and, consequently, better plant growth.

## Biological nitrogen fixation

Atmospheric nitrogen is not available to most living beings, except for a selected group of archaea and bacteria called diazotrophs, which convert it into assimilable forms through biological nitrogen fixation (FBN) (Batista and Dixon, 2019). The FBN process is widely studied. Several bacterial genera are described as diazotrophic, directly associated with plants (endophytic) or free-living, establishing close plant-microbe relationships (Conalghi et al., 1997; Serrato, 2014; Olivares et al., 2017).

Although this characteristic is restricted to specific microbial groups, some legumes have greater nitrogen assimilation and develop better when co-inoculated with diazotrophic bacteria and arbuscular mycorrhizal fungi (AMF). Furthermore, in the presence of compatible fungal strains, the nodulation and fixation processes are more efficient, indicating the mutualistic relationship of the intercropping application of these microorganisms when compared to plants with isolated inoculum, even under stress conditions (Linderman, 1991; Saia et al., 2014).

Moreover, Pereira et al. (2013) observed synergistic effects in mixed inoculations with arbuscular mycorrhizal fungi, actinomycetes, and rhizobia evaluated, with gains in area biomass and nodule biomass. However, despite reports indicating the effects of intercropping inoculations, the mechanisms of this cooperation and the effects on the rhizosphere's resident microbiota are not fully understood (Woo and Pepe, 2018). Thus, there is a commitment to propose these applications at the commercial level, especially concerning FBN, one of the principal areas of application of microbial-based inputs due to the relevance of nitrogen in agriculture (Chianu et al., 2010; Olivares et al., 2017; Soumare et al., 2020).

## Application vehicles for microbial consortia

The microbial consortia to be used need an application vehicle. These vehicles make up most of the inoculant volume and help deliver a physiologically viable number of microbial cells and favor the inoculant dispersion through the favorable conditions provided. Despite this knowledge, one of the biggest challenges in using microbial consortia is the difficulty of maintaining the viability of microorganisms throughout the storage and logistics period (Reis, 2007). In this sense, several studies have been carried out to improve the survival of microbial cells, thus ensuring the efficiency of the microbial consortium.

Various materials and substances have been analyzed as support for inoculation, including peat, coal, clay, rock phosphate and natural gums (Rocha, 2018). Among these, peat stands out, as it has better quality and availability than other potential vehicles and offers physical protection against soil adversities (Zilli et al., 2010; Santos et al., 2022). Other alternatives have also been valuable, such as the inoculation of stalks in sugarcane and seeds, such as corn. In these cases, inoculation is carried out under controlled conditions, and when the stems or seeds go to the field, the consortia are already in the colonization phase. In general, there is still no consensus or vehicle that helps apply consortia in different cultures. Thus, it is necessary to know the microorganisms involved so that the consortium, the vehicle and the plant are compatible and the expected results are obtained.

## FINAL CONSIDERATIONS AND PROSPECTS

The successful use of consortia depends on the existing compatibility between the microorganisms involved since two antagonistic organisms, even if beneficial, can compromise stability and reach the expected results. Thus, understanding the molecular and physiological bases involved can be a way to improve manipulation techniques in microbial consortia, suppressing or expressing genes of interest. Therefore, using multidisciplinary approaches can facilitate the development and improvement of stable consortia.

Finally, understanding microbial ecology in the most diverse environments and the requirements of organisms for their joint action is necessary for the development of studies and techniques that enable the application of microbial consortia. By being an engaging area that still requires deep investigations, and insertion of modern technologies and methods, including to be developed, microbial consortia still fit as promising natural alternatives. However, they will collaborate to create and establish productive, healthy, and sustainable future agriculture.

## REFERENCES

Alnahhas, Razan N. et al. (2020). Majority Sensing in Synthetic Microbial Consortia. *Nature Communications* 11(1).

Alori, E. T., Glick, B. R., e Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in microbiology, 8, 971.

Batista, Marcelo Bueno, and Ray Dixon. 2019. “Manipulating Nitrogen Regulation in Diazotrophic Bacteria for Agronomic Benefit.” *Biochemical Society Transactions* 47(2): 603–14.

Behera, Biswaranjan et al. (2021). Microbial Consortia for Sustaining Productivity of Non-Legume Crops: Prospects and Challenges. *Agricultural Research* 10(1).

Bell, D. K. (1982). *In Vitro* Antagonism of Trichoderma Species Against Six Fungal Plant Pathogens. *Phytopathology* 72(4): 379.

Berg, Gabriele, and Kornelia Smalla. (2009). Plant Species and Soil Type Cooperatively Shape the Structure and Function of Microbial Communities in the Rhizosphere. *FEMS Microbiology Ecology* 68(1): 1–13.

Bilal, Saqib et al. (2018). Endophytic Microbial Consortia of Phytohormones-Producing Fungus Paecilomyces Formosus Lhl10 and Bacteria Sphingomonas Sp. Lk11 to Glycine Max l. Regulates Physio-Hormonal Changes to Attenuate Aluminum and Zinc Stresses. *Frontiers in Plant Science* 9(September): 1–18.

Bradáčová, Klára et al. (2019). Microbial Consortia versus Single-Strain Inoculants: An Advantage in PGPM-Assisted Tomato Production?” *Agronomy* 9(2).

de Campos, Luiz Henrique Franco et al. 2010. “Sistemas de Manejo Da Palhada Influenciam Acúmulo de Biomassa e Produtividade Da Cana-de-Açúcar (Var. RB855453). *Acta Scientiarum - Agronomy* 32(2): 345–50.

Chantre, N. C. S. (2018). Veículo de inoculação à base de exopolissacarídeo em formulações bacterianas para cana-de-açúcar.

Che, S., Xu, Y., Qin, X., Tian, S., Wang, J., Zhou, X., ... & Yang, X. (2024). Building microbial consortia to enhance straw degradation, phosphorus solubilization, and soil fertility for rice growth. *Microbial Cell Factories*, *23*(1), 232.

Cherubin, Maurício R. et al. (2019). Sugarcane Straw Removal: Implications to Soil Fertility and Fertilizer Demand in Brazil. *Bioenergy Research* 12(4): 888–900.

Chung, Soohee, Jong Hui Lim, and Sang Dal Kim. (2010). Powder Formulation Using Heat Resistant Endospores of Two Multi-Functional Plant Growth Promoting Rhizobacteria Bacillus Strains Having Phytophthora Blight Suppression and Growth Promoting Functions. *Journal of Applied Biological Chemistry* 53(4): 485–92.

Colnaghi, Rita et al. (1997). Strategies for Increased Ammonium Production in Free-Living or Plant Associated Nitrogen Fixing Bacteria. *Plant and Soil* 194(1–2): 145–54.

De Souza Moreira, Fatima Maria, Krisle Da Silva, Rafaela Simão Abrahão Nóbrega, and Fernanda De Carvalho. (2010). Bactérias Diazotróficas Associativas: Diversidade, Ecologia e Potencial de Aplicações. *Comunicata Scientiae* 1(2): 74–99.

Devi, R., Kaur, T., Kour, D., Yadav, A. N., & Suman, A. (2022). Potential applications of mineral solubilizing rhizospheric and nitrogen fixing endophytic bacteria as microbial consortium for the growth promotion of chilli (Capsicum annum L.). *Biologia*, *77*(10), 2933-2943.

Dos Santos, L. G., Baldani, V. L. D., Ferreira, J. S., Bahia, B. L., Santana, M. S., & Peixouto, L. S. (2022). Sobrevivência de bactérias diazotróficas em suporte inoculante alternativo de casca de algodão. Conjecturas, 22(2), 1386-1397.

Fuentes, María S., Verónica L. Colin, María J. Amoroso, and Claudia S. Benimeli. (2016). Selection of an Actinobacteria Mixed Culture for Chlordane Remediation. Pesticide Effects on Microbial Morphology and Bioemulsifier Production. *Journal of Basic Microbiology* 56(2): 127–37.

Fülöp, László, and János Ecker. (2020). An Overview of Biomass Conversion: Exploring New Opportunities. *PeerJ* 8.

Gomes, Helder Andrey Rocha et al. (2017). Identification of Multienzymatic Complexes in the Clonostachys Byssicola Secretomes Produced in Response to Different Lignocellulosic Carbon Sources. *Journal of Biotechnology* 254(January): 51–58.

Guetsky, R., D. Shtienberg, Y. Elad, and A. Dinoor. (2001). Combining Biocontrol Agents to Reduce the Variability of Biological Control. *Phytopathology* 91(7): 621–27.

Guevara, Claudia e María Mercedes Zambrano. (2006). Sugarcane Cellulose Utilization by a Defined Microbial Consortium. *FEMS Microbiology Letters* 255(1): 52–58.

Gupta, Rashi; Bisaria, V. S., Sharma, Shilpi. (2015). Effect of agricultural amendments on *Cajanus cajan* (pigeon pea) and its rhizospheric microbial communities - A comparison between chemical fertilizers and bioinoculants. PLoS One, v. 10, n. 7, p. 1–17.

Gupta, Rashi, V. S. Bisaria, and Shilpi Sharma. (2015). Effect of Agricultural Amendments on *Cajanus Cajan* (Pigeon Pea) and Its Rhizospheric Microbial Communities - A Comparison between Chemical Fertilizers and Bioinoculants. *PLoS ONE* 10(7): 1–17.

Hays, Stephanie G., Leo L.W. Yan, Pamela A. Silver, and Daniel C. Ducat. (2017). Synthetic Photosynthetic Consortia Define Interactions Leading to Robustness and Photoproduction. *Journal of Biological Engineering* 11(1): 1–14.

Hett, J., Döring, T. F., Bevivino, A., & Neuhoff, D. (2023). Impact of microbial consortia on organic maize in a temperate climate varies with environment but not with fertilization. European Journal of Agronomy, 144, 126743.

Himmel, Michael E. et al. (2010). Microbial Enzyme Systems for Biomass Conversion: Emerging Paradigms. *Biofuels* 1(2): 323–41.

Houfani, Aicha Asma et al. (2020). Insights from Enzymatic Degradation of Cellulose and Hemicellulose to Fermentable Sugars – a Review. *Biomass and Bioenergy* 134(February): 105481.

Hui, Wang et al. (2013). Bioconversion of Un-Pretreated Lignocellulosic Materials by a Microbial Consortium XDC-2. *Bioresource Technology* 136: 481–87.

Igiehon, Nicholas O., Olubukola O. Babalola. 2017. “Biofertilizers and Sustainable Agriculture: Exploring Arbuscular Mycorrhizal Fungi.” *Applied Microbiology and Biotechnology* 101(12): 4871–81.

Jain, A., S. Singh, B. Kumar Sarma Ee H. Bahadur Singh. (2012). Microbial Consortium-Mediated Reprogramming of Defence Network in Pea to Enhance Tolerance against *Sclerotinia sclerotiorum*. *Journal of Applied Microbiology* 112(3): 537–50.

Kalayu, Girmay. (2019). Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers. *International Journal of Agronomy*.

Khan, Naeem, Asghari Bano, and José Alfredo Curá. (2020). Role of Beneficial Microorganisms and Salicylic Acid in Improving Rainfed Agriculture and Future Food Safety. *Microorganisms* 8(7): 1–22.

Kong, Wentao, David R. Meldgin, James J. Collins, and Ting Lu. (2018). Designing Microbial Consortia with Defined Social Interactions. *Nature Chemical Biology* 14(8): 821–29.

Lee, Duu Jong, Kuan Yeow Show, and Aijie Wang. (2013). Unconventional Approaches to Isolation and Enrichment of Functional Microbial Consortium - A Review. *Bioresource Technology* 136: 697–706.

Lee, Myung Hwan, and Seon-Woo Lee. (2013). Bioprospecting Potential of the Soil Metagenome: Novel Enzymes and Bioactivities. *Genomics e Informatics* 11(3): 114.

Li, Renyi, Ulrike Dörfler, Jean Charles Munch, and Reiner Schroll. (2017). Enhanced Degradation of Isoproturon in an Agricultural Soil by a *Sphingomonas* sp. Strain and a Microbial Consortium. *Chemosphere* 168: 1169–76.

Liang, Jiajin, Xiuxiu Fang, Yunqin Lin, and Dehan Wang. (2018). A New Screened Microbial Consortium OEM2 for Lignocellulosic Biomass Deconstruction and Chlorophenols Detoxification. *Journal of Hazardous Materials* 347(Jan.): 341–48.

Lima, Rissele Paraguai et al. (2015). Aporte e Decomposição da Serapilheira na Caatinga no Sul do Piauí. Litter Contribution and Decomposition in the Caatinga In.” 22(November 2011): 42–49.

Linderman, R. G. (1991). Mycorrhizal Interactions in the Rhizosphere. *The Rhizosphere and Plant Growth*: 343–48.

Liu, Chenggang et al. (2019). Drivers of Soil Bacterial Community Structure and Diversity in Tropical Agroforestry Systems. *Agriculture, Ecosystems and Environment* 278(March): 24–34.

Liu, Min et al. (2016). Selection and Evaluation of Phosphate-Solubilizing Bacteria from Grapevine Rhizospheres for Use as Biofertilizers. *Spanish Journal of Agricultural Research* 14(4).

Liu, Zhihong et al. (2021). Quorum Sensing Shaped Microbial Consortia and Enhanced Hydrogen Recovery from Waste Activated Sludge Electro-Fermentation on Basis of Free Nitrous Acid Treatment. *Science of the Total Environment* 766: 144348.

Meena, Vijay Singh, B. R. Maurya, and Jay Prakash Verma. (2014). Does a Rhizospheric Microorganism Enhance $K^+$ Availability in Agricultural Soils? *Microbiological Research* 169(5–6): 337–47.

Mendoza-Arroyo, Gustavo Enrique et al. (2020). Inorganic Phosphate Solubilization by a Novel Isolated Bacterial Strain *Enterobacter* sp. Itcb-09 and Its Application Potential as Biofertilizer. *Agriculture (Switzerland)* 10(9): 1–15.

Miyauchi, Marina Yumi Horta et al. (2008). Interactions between Diazotrophic Bacteria and Mycorrhizal Fungus in Maize Genotypes. *Scientia Agricola* 65(5): 525–31.

Mondal, Subhadeep, Suman Kumar Halder, Ajar Nath Yadav, and Keshab Chandra Mondal. 2020. *Microbial Consortium with Multifunctional Plant Growth-Promoting Attributes. Future Perspective in Agriculture*.

Mukhtar, Salma, Samina Mehnaz, and Kauser Abdulla Malik. (2019). Microbial Diversity in the Rhizosphere of Plants Growing under Extreme Environments and Its Impact on Crop Improvement. *Environmental Sustainability* 2(3): 329–38.

Murthy, H. M.Rajashekara, and H. K. Manonmani. 2007. “Aerobic Degradation of Technical Hexachlorocyclohexane by a Defined Microbial Consortium.” *Journal of Hazardous Materials* 149(1): 18–25.

Naik, Kalyani; Mishra, Snehasish; Srichandan, Haragobinda; Singh, Puneet Kumar; Sarangi, Prakash Kumar. (2019). Plant growth-promoting microbes: Potential link to sustainable agriculture and environment. Biocatalysis and Agricultural Biotechnology, [S. l.], v. 21, n. July, p. 101326.

Olivares, Fábio Lopes et al. (2017). Plant Growth Promoting Bacteria and Humic Substances: Crop Promotion and Mechanisms of Action. *Chemical and Biological Technologies in Agriculture* 4(1): 1–13.

Pacheco, Inês et al. (2021). Microbial Consortium Increases Maize Productivity and Reduces Grain Phosphorus Concentration under Field Conditions. *Saudi Journal of Biological Sciences* 28(1): 232–37.

Palmieri, D., D. Vitullo, F. De Curtis, and Giuseppe Lima. (2017). A Microbial Consortium in the Rhizosphere as a New Biocontrol Approach against Fusarium Decline of Chickpea. *Plant and Soil* 412(1–2): 425–39.

Paul, Dipak, and Sankar Narayan Sinha. (2015). Biological Removal of Phosphate Using Phosphate Solubilizing Bacterial Consortium from Synthetic Wastewater: A Laboratory Scale. *EnvironmentAsia* 8(1): 1–8.

Paula, M A, S Urquiaga, and J O Siqueira. (1992). And Diazotrophic Bacteria on Nutrition and Growth of Sweet Potato. 61–66.

Pereira, Guilherme Henrique Almeida et al. (2013). Decomposição Da Serrapilheira, Diversidade e Funcionalidade de Invertebrados Do Solo Em Um Fragmento de Floresta Atlântica. *Bioscience Journal* 29(5): 1316–26.

Poszytek, Krzysztof, Martyna Ciezkowska, Aleksandra Sklodowska, and Lukasz Drewniak. (2016). Microbial Consortium with High Cellulolytic Activity (MCHCA) for Enhanced Biogas Production.” *Frontiers in Microbiology* 7(MAR): 1–11.

Radianingtyas, Helia, Gary K. Robinson, and Alan T. Bull. (2003). Characterization of a Soil-Derived Bacterial Consortium Degrading 4-Chloroaniline. *Microbiology* 149(11): 3279–87.

Rajasekar, S, and R Elango. (2011). “Effect of Microbial Consortium on Plant Growth and Improvement of Alkaloid Content in Withania Somnifera (Ashwagandha).” *Current Botany* 2(8): 27–30.

Rasche, Livia, and Ruth Sos Del Diego. (2020). Pros and Cons of Sugarcane Straw Recovery in São Paulo. *Bioenergy Research* 13(1): 147–56.

Razafimbelo, Tantely et al. (2006). Effect of Sugarcane Residue Management (Mulching versus Burning) on Organic Matter in a Clayey Oxisol from Southern Brazil. *Agriculture, Ecosystems and Environment* 115(1–4): 285–89.

Reinehr, Thiago Olinek et al. (2021). Study of Pyrolysis Kinetic of Green Corn Husk. *Journal of Thermal Analysis and Calorimetry* 143(4): 3181–92.

Reis, V. M. Uso de Bactérias Fixadoras de Nitrogênio como Inoculante para Aplicação em Gramíneas. Seropédica: Embrapa Agrobiologia, 2007. 22 p. (Documentos, Embrapa Agrobiologia. ISSN 1517-8498: 232).

Rocha, J. F. D. (2018). Estudo da mistura carboximetilcelulose/Lithothamnium como veículo para inoculantes rizobianos.

Rocha, R. L. F. Atributos químicos e microbiológicos associados ao sistema solo-palha de cana-de-açúcar (2018). Dissertação (Mestrado). Programa de Pós Graduação em produção Vegetal - Universidade Estadual do Norte Fluminense Darcy Ribeiro. Campos dos Goytacazes, RJ.

Roller, Benjamin R.K., and Thomas M. Schmidt. (2015). The Physiology and Ecological Implications of Efficient Growth. *ISME Journal* 9(7): 1481–87.

Roscoe, R.; Machado, P.L.O.A. (2002). Fracionamento físico do solo em estudos da matéria orgânica Dourados: Embrapa Agropecuária Oeste. 86p.

Saia, Sergio et al. (2014). Influence of Arbuscular Mycorrhizae on Biomass Production and Nitrogen Fixation of Berseem Clover Plants Subjected to Water Stress. *PLoS ONE* 9(3).

Sarma, Birinchi Kumar, Sudheer Kumar Yadav, Surendra Singh and Harikesh Bahadur Singh. (2015). Microbial Consortium-Mediated Plant Defense against Phytopathogens: Readdressing for Enhancing Efficacy. *Soil Biology and Biochemistry* 87: 25–33.

Scott, Spencer R., and Jeff Hasty. (2016). Quorum Sensing Communication Modules for Microbial Consortia. *ACS Synthetic Biology* 5(9): 969–77.

Singh, Radha, and Ashok K. Dubey. (2018). Diversity and Applications of Endophytic Actinobacteria of Plants in Special and Other Ecological Niches. *Frontiers in Microbiology* 9(AUG).

Soumare, Abdoulaye, Kenza Boubekri, et al. (2020). From Isolation of Phosphate Solubilizing Microbes to Their Formulation and Use as Biofertilizers: Status and Needs. *Frontiers in Bioengineering and Biotechnology* 7(January): 1–14.

Soumare, Abdoulaye, Abdala G Diedhiou, Moses Thuita, and Mohamed Hafidi. (2020). Exploiting Biological Nitrogen Fixation : A Route. *Plants*: 1–22.

Stephens, Kristina, and William E. Bentley. (2020). Synthetic Biology for Manipulating Quorum Sensing in Microbial Consortia. *Trends in Microbiology* 28(8): 633–43.

Stocker, Roman. (2012). Marine Microbes See a Sea of Gradients. *Science* 338(6107): 628–33.

Sun, Hai et al. (2017). Effects of Different Leaf Litters on the Physicochemical Properties and Bacterial Communities in Panax Ginseng-Growing Soil. *Applied Soil Ecology* 111: 17–24.

Sun, X., Tao, R., Xu, D., Qu, M., Zheng, M., Zhang, M., & Mei, Y. (2023). Role of polyamide microplastic in altering microbial consortium and carbon and nitrogen cycles in a simulated agricultural soil microcosm. *Chemosphere*, *312*, 137155.

Sousa, Jucimara Anunciação De Jesus; Olivares, Fabio Lopes. (2016). Plant growth promotion by streptomycetes: Ecophysiology, mechanisms and applications. Chemical and Biological Technologies in Agriculture, v. 3, n. 1, p. 1–12.

Struik, Paul C.; Kuyper, Thomas W. (2017). Sustainable intensification in agriculture: the richer shade of green. A review. Agronomy for Sustainable Development, [S. l.], v. 37, n. 5.

Thakkar, A., and M. Saraf. (2015). Development of Microbial Consortia as a Biocontrol Agent for Effective Management of Fungal Diseases in Glycine Max L. *Archives of Phytopathology and Plant Protection* 48(6): 459–74.

Valenzuela, Hector. Agroecology: A global paradigm to challenge mainstream industrial agriculture. Horticulturae, [S. l.], v. 2, n. 1, 2016.

Viñas, Marc et al. (2005). Culture-Dependent and -Independent Approaches Establish the Complexity of a PAH-Degrading Microbial Consortium. *Canadian Journal of Microbiology* 51(11): 897–909.

Vyas, Preeti, Ashwani Kumar, and Suren Singh. (2018). Biomass Breakdown: A Review on Pretreatment, Instrumentations and Methods. *Frontiers in Bioscience - Elite* 10(1): 155–74.

Welbaum, Gregory E., Antony V. Sturz, Zhongmin Dong, and Jerzy Nowak. (2004). Managing Soil Microorganisms to Improve Productivity of Agro-Ecosystems. *Critical Reviews in Plant Sciences* 23(2): 175–93.

Weekley, Jonathan; Gabbard, Joseph; Nowak, Jerzy. (2012). Micro-Level Management of Agricultural Inputs: Emerging Approaches. Agronomy, v. 2, n.4, p.321–357.

Woo, Sheridan L., and Olimpia Pepe. (2018). Microbial Consortia: Promising Probiotics as Plant Biostimulants for Sustainable Agriculture. *Frontiers in Plant Science* 9(2003): 7–12.

Xiao, Chunqiao et al. (2020). Biosolubilization of Low-Grade Rock Phosphate by Native Microbial Consortia from Phosphate Mines: Effect of Sampling Sources and Culture Media. *Geomicrobiology Journal* 37(9): 859–66.

Xie, Gary et al. (2007). Genome Sequence of the Cellulolytic Gliding Bacterium *Cytophaga hutchinsonii*." *Applied and Environmental Microbiology* 73(11): 3536–46.

Yamaguchi, Carina Sayuri et al. (2017). Sugarcane Straw Decomposition and Carbon Balance as a Function of Initial Biomass and Vinasse Addition to Soil Surface. *Bragantia* 76(1): 135–44.

Yang, Y. H. et al. (2004). Research on Solid-State Fermentation on Rice Chaff with a Microbial Consortium. *Colloids and Surfaces B: Biointerfaces* 34(1): 1–6.

Zhang, Heng et al. (2008). Functional Assembly of a Microbial Consortium with Autofluorescent and Mineralizing Activity for the Biodegradation of Organophosphates. *Journal of Agricultural and Food Chemistry* 56(17): 7897–7902.

Zhu, Chen et al. (2018). N-Fertilizer-Driven Association between the Arbuscular Mycorrhizal Fungal Community and Diazotrophic Community Impacts Wheat Yield. *Agriculture, Ecosystems and Environment* 254(June 2017): 191–201.