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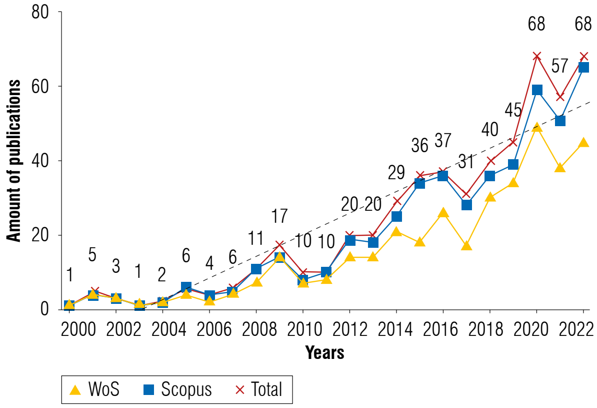
Application of molecular techniques in soil microbiology for the identification of bacteria with agricultural potential: a review and bibliometric analysis

Aplicación de técnicas moleculares en microbiología del suelo para la identificación de bacterias con potencial agrícola: una revisión y análisis bibliométrico



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Amount of scientific production in the years 2000-2022 in the subject of study by year.

Photo: C.A. Dodino

ABSTRACT

The excessive use of agrochemicals and poor agricultural practices have increased the negative effects on soil and crop biodiversity. In that sense, there is a need to identify potential bacteria by molecular techniques for sustainable agricultural production. The objective of this article was to develop a systematic and bibliometric mapping of the research carried out applying molecular techniques in soil microbiology for the identification of bacteria with agricultural potential. A search for research related to molecular techniques used for the identification of bacteria with agricultural potential was carried out in the Web of Science and Scopus databases, which were classified and analyzed by means of the R studio software. The origin, theoretical reference, bibliometric study and networks on the proposed topic were analyzed from the research obtained. A total of 527 researches related to molecular techniques used for the identification of bacteria with agricultural potential were reported, increasing by 52.75% in the last five years, with an annual growth rate of 17.4%, with India standing out as the country with the highest number of publications, contributing 25% of researches worldwide. Sequencing and PCR are the most common techniques to identify potential microorganisms,

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being *Bacillus*, *Pseudomonas*, *Enterobacter* and *Acinetobacter* the most frequent bacterial genera to be identified due to mechanisms used to favor sustainable agricultural production systems.

Additional key words: characterization; PCR; rhizobacteria; sustainable agriculture.

RESUMEN

El uso excesivo de agroquímicos y las malas prácticas agrícolas han aumentado los efectos negativos sobre el suelo y la diversidad biológica de los cultivos. En ese sentido, existe la necesidad de identificar bacterias potenciales por medio de técnicas moleculares para la producción agrícola sostenible. El objetivo de este artículo fue desarrollar un mapeo sistemático y bibliométrico acerca de las investigaciones realizadas en las que se aplican las técnicas moleculares en microbiología de suelos, para la identificación de bacterias con potencial agrícola. Se empleó la búsqueda de investigaciones relacionadas con las técnicas moleculares usadas para la identificación de bacterias con potencial agrícola en las bases de datos Web of Science y Scopus; los cuales fueron clasificados y analizados por medio del software R studio. A partir de las investigaciones obtenidas se analizó el origen, referente teórico, estudio bibliométrico y redes sobre la temática propuesta. Se reportaron un total de 527 investigaciones relacionadas con las técnicas moleculares empleadas para la identificación de bacterias con potencial agrícola, aumentado en los últimos cinco años en un 52,75%, con una tasa de crecimiento anual del 17,4%, destacándose India como el país con mayor número de publicaciones, aportando un 25% de investigaciones a nivel mundial. La secuenciación y la PCR son las técnicas más usuales para identificar a los microorganismos potenciales, siendo *Bacillus*, *Pseudomonas*, *Enterobacter* y *Acinetobacter* los géneros bacterianos más frecuentes en ser identificados debido a los mecanismos que utilizan para favorecer los sistemas de producción agrícola sostenible.

Palabras clave adicionales: caracterización; PCR; rizobacterias; agricultura sostenible.

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INTRODUCTION

Soil constitutes a very complex system where physical, chemical and biological processes take place in which microorganisms carry out important activities for the ecosystemic maintenance (Urgiles-Gómez *et al*., 2021). Currently, the implementation of intensive agriculture has led to the development of diseases, nutrient deficits, soil degradation, loss of biodiversity, erosion, among others (Pandey *et al*., 2019; Posada *et al*., 2021). One of the strategies to counteract this problem lies in the use of microorganisms with agricultural potential and among the most promising are plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) (Urgiles-Gómez *et al*., 2021); these microorganisms have the ability to minimize dependence on chemical fertilizers and pesticides, thus optimizing soil fertility and quality (Zuluaga *et al*., 2020; Issifu *et al*., 2022), are also attributed with the ability to offer resistance to pathogens and environmental stress (Tabacchioni *et al*., 2021) and facilitate the stimulation of plant growth through mechanisms such as biological nitrogen fixation (BNF), phytohormone production, synthesis of antibiotics and organic acids related to plant growth promotion, phosphate solubilization, siderophore production, cellulase and protease synthesis, among others (dos Santos *et al*., 2022; Mghazli *et al*., 2022; Sachman-Ruíz *et al*., 2022).

Thus, the soil contains microbial populations including bacteria, which play an important role in the cycling of organic matter and nutrient availability, and also participate in ecological processes that regulate plant growth (Tang *et al*., 2023), because soil microorganisms play a fundamental role in the maintenance and regulation of the biological cycles of soil elements and therefore in crop yields (Fan *et al*., 2021).

In addition, the microorganisms that inhabit the soil have an incidence in the formation and stabilization of aggregates, due to this, this type of organisms are related to the processes of soil construction (Cavael *et al*., 2020). Soil bacteria contribute to the decomposition of plant and animal residues to form compost, which improves soil structure, leading to the retention of nutrients and moisture, considered important factors in sustainable agriculture (Tanya and Leiva-Mora, 2019). The activity of enzymes produced by bacteria is linked to the physicochemical properties, diversity and microbial composition of the soil, which means that they act as important indicators of soil productivity and biological activity (Alkorta *et al*., 2003). It has even been claimed that indigenous soil bacteria can reduce environmental pollution by producing and utilizing enzymes that metabolize the pollutant, using it as a source of nutrients, whether organic or inorganic (Munawar *et al*., 2023).

It is important to make use of potential microbial populations, as they increase the restoration and development of soil components, maintaining the balance of biological interactions that favor agricultural production, applied as a natural and sustainable way with the environment (Gutiérrez-Calvo *et al*., 2022). In this sense, the consequences generated by the excessive use of agrochemicals are reduced, increasing the biological diversity of the soil (Aldonate *et al*., 2019; Diaz *et al*., 2019). In addition, the mechanisms used by PGPRs are useful for the productivity of different crop species in the face of abiotic stresses such as salinity and drought (Jatan *et al*., 2019; Vurukonda, 2020; Bécquer, 2022). Thus, the identification of soil microbial populations is of great value, since they can be used as biofertilizers and biocontrollers, depending on their metabolism and functionality within the strategy of sustainable agricultural production (Cruz *et al*., 2021). One way to identify microbial groups that are potentially beneficial in agriculture is by molecular techniques, which favor knowledge of the genes involved in obtaining productive systems focused on sustainable agriculture (Díaz-Rodríguez *et al*., 2021; Orozco-Mosqueda and Santoyo, 2021).

Molecular identification of microorganisms with agricultural potential is a topic of great significance, since it allows to make known to all those involved in agricultural production the different molecular tools that can be used for the identification of promising microorganisms with potential for agriculture. To this purpose, it is necessary to group these techniques and carry out a bibliographic and bibliometric mapping, which facilitates the development of the subject matter based on scientific documents, countries, authors, and journals that stand out in the area of study. It is important to mention that the Scopus and Web of Science databases were reviewed and no review articles were found that specifically compile the most used molecular biology techniques for the identification of PGPR and bacteria with agricultural potential, however in Scopus only one review article was reported, which was carried out by Urgiles-Gómez *et al*. (2021), who compiled research on the isolation and identification of PGPR where, in addition to using conventional methodologies, molecular analysis was performed, determining the presence of *Azospirillum, Pseudomonas, Bacillu*s and other microorganisms in the rhizosphere of coffee plants (*Coffea* spp.), which are considered potential biofertilizers.

Thus, the present study aimed to develop a systematic and bibliometric mapping of the research carried out, in which molecular techniques are applied in soil microbiology, for the identification of bacteria with agricultural potential (MTIBAP). For the development of the objective, a bibliographic search was carried out through the Web of Science (WoS) and Scopus databases. For this purpose, it was filtered in the years 2000-2022 and the information resulting from the search was processed by means of the R studio software. Subsequently, a bibliometric mapping was carried out by means of a network study, listing the most relevant documents; to achieve this, the metaphor of the tree of science was used, which is made up of three parts: root, trunk and leaves. In this way, the main lines of research of this study were determined. Finally, the most significant studies on each topic were investigated. This bibliometric document was developed in four important phases: 1) Studies were selected, analyzed and verified through the use of search criteria determined for the purpose of this research. 2) A beginning evaluation and analysis of the theory was developed. 3) The bibliometric study was carried out and the relationships. 4) Determining words, limitations and suggestions for further research related to the topic of MTIBAP were noted.

MATERIALS AND METHODS

The strategy proposed in this study consisted of two stages: first, a bibliometric search was carried out in the Scopus and Web of Science (WoS) databases, which revealed scientific research focused on soil microbiology. Secondly, research related to molecular techniques used for the identification of microorganisms with agricultural potential was recognized. Thus, five bibliometric parameters were used to carry out these searches: citation analysis, co-occurrence of words, co-citations, co-authors and bibliographic coupling (Zupic and Čater, 2015). It is necessary to mention that Scopus and Web of Science are bibliographic databases that allow us to obtain the type of information necessary to carry out this review (Echchakoui, 2020), as well as providing information at the international level (Zhu and Liu, 2020; Pranckutė, 2021). The search criteria are listed in table 1.

After using the R studio software, the results obtained are 479 articles in Scopus and 373 in Web of Science, which after joining and discarding duplicate articles, gave a total of 527 records, which means between the two databases an overlap of 61.66%, showing the relevance of applying them together. As a result, it was found that 94% of the research published on this subject related to Web of Science and Scopus is in English and 3, 2, 1% and less than 1% are in Chinese, Korean, Portuguese and others, respectively (Fig. 1). The argument is because both journals and writers publish their studies in English to increase the H-index of these databases (Vera-Baceta *et al*., 2019). The software used for the bibliographic analysis is Bibliometrix (Aria and Cuccurullo, 2017), this is a free access application, which allows working with different databases (Scopus and Web of Science), its utilities are diverse, and it has been applied and evidenced by other studies (Tani *et al*., 2018; Landinez *et al*., 2019; Duque-Hurtado *et al*., 2020; Acevedo *et al*., 2021; Di Vaio *et al*., 2021; Duque *et al*., 2021c; Queiroz and Wamba, 2021; Secinaro *et al*., 2022; Rabelo-Flórez, 2023).

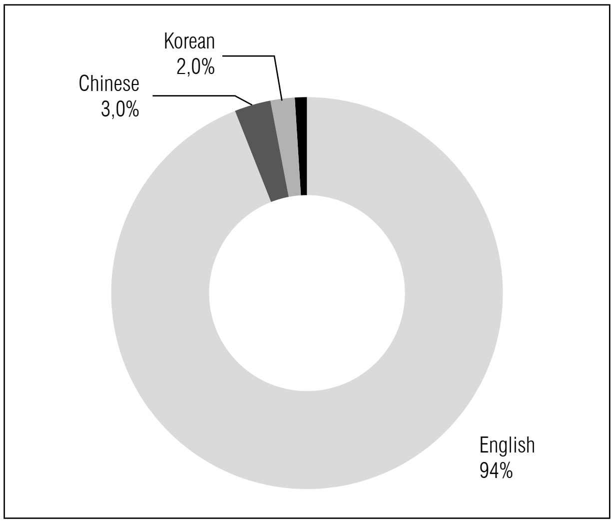

Figure 1. Languages of articles consulted in Web of Science and Scopus databases.

Network analysis

By means of the R study software, replicated documents from the Scopus and Web of Science databases

Table 1. Search criteria.

Databases	Web of Science (WoS)	Scopus
Consulting period	2000 - 2022	
Consulting date	17/01/2023	
Search criteria	Title	
Journal type	Alls	
Search terms	("Plant-Growth-Promoting Rhizobacteria" or "Plant growth-promoting bacterial endophytes" or "Plant growth promotion by phosphate solubilizing bacteria" or "Plant growth-promoting" or "plant growth-promoting bacterium" or "plant growth promoting fungus" or "plant growth promoting fungi" or "PGPF") and ("molecular identification" or "hybridization" or "sequencing" or "PCR" or "polymerase chain reaction" or "real-time PCR" or "qPCR" or "Reverse transcriptase polymerase chain reaction" or "RT- PCR" or "Digital PCR" or "Microarrays" or "Genome sequencing" or "Sanger sequencing" or "Massively parallel sequencing" or "SMP" or "Next-generation sequencing" or "NGS" or "DGGE" or "Pyrosequencing" or "DNA probe hybridization" or "Denaturing gradient gel electrophoresis" or "Maldi tof" or "RFLP" or "PCR-RFLP" or "RAPD" or "loop-mediated isothermal amplification" or "LAMP" or "restriction fragment length polymorphism fragment* length polymorphism" or "identification" or "molecular identification" or "isolation" or "characterization" or "selection")	
Results	373	479
Total results	527	

were excluded. Then, once the selected documents were obtained, the bibliographic sources were used in a grid of citations to build the graph theory, which consists of schematically representing the relationship between each of the appropriate documents that are part of this review (Wallis, 2007; Yang *et al*., 2017).

Three bibliometric indicators were taken into account, consisting of: the indegree, which refers to the greatest number of citations made by other authors about the article; the outdegree, which indicates the number of citations in which a given group uses ideas from other authors (Wallis, 2007). Therefore, they are not cited since they are recent articles; and finally, the betweenness that points out both the citation made by the authors of the article and the citation made in other documents about these same articles (Freeman, 1977; Zhang and Luo, 2017).

As a result, it was possible to acquire a mesh of data on the research on the subject of this study, acquired through Scopus and Web of Science. For this purpose, the Gephi software was used (Bastian *et al*., 2009), which facilitated to obtain in a representative way the map of cocitations, which confers to analyze the representation or the position that the researches on the MTIBAP topic acquire within the scientific studies, facilitating the visual obtaining of dynamic networks (Bastian *et al*., 2009; Gurzki and Woisetschläger, 2017; Zuschke, 2020).

The metrics of the bibliometric indicators were quantified for each of the corresponding network records, with the purpose of ordering the papers using the metaphor of the tree of science (Robledo *et al*., 2014; Valencia-Hernández *et al*., 2020). By means of this representation, three classifications can be obtained: the first one, the bases or roots (indegree), which integrates the most important studies or research within the subject of the study in question, which are highly referenced, but which do not cite others (Wallis, 2007); the second is the trunk (betweenness), where the articles or documents that are cited and also taken as a reference by others are located (Zhang and Luo, 2017). This section compiles the studies that relate the theoretical part of the bases or roots with the emerging research in the area of study and, thirdly, the out-of-degree studies, which correspond to current studies that cite the others but are not referenced (Wallis, 2007) and that also catalog current inclinations in the studied area. Thus, the methodological development of this study has been carried out and ratified by other authors in previous studies (Duque and Cervantes-Cervantes, 2019; Duque-Hurtado *et al*., 2020; Buitrago *et al*., 2020; Clavijo-Tapia *et al*., 2021; Duque, *et al*., 2021a; Duque *et al*., 2021b; Ramos-Enríquez *et al*., 2021; Torres *et al*., 2021; Trejos-Salazar *et al*., 2021; Barrera *et al*., 2022; Rabelo-Flórez, 2023).

RESULTS

Bibliometric analysis

The bibliometric production analysis and evaluation were carried out using the bibliometric procedures described by Zupic and Čater (2015), with modifications. The Web of Science (WoS) and Scopus databases were used simultaneously, due to the fact that they provide more extensive access to the bibliographic content (Echchakoui, 2020; Pranckutė, 2021), as well, that these are the main international databases (Bar-Ilan, 2010; Zhu and Liu, 2020), and the relevance of scientific production related to bacteria of agricultural potential that is published in therein.

Historical analysis of scientific production

Figure 2A shows the number of scientific papers that have been published in relation to MTIBAP in the years 2000-2022. The results showed a total of 527 scientific documents through the application of the Web of Science and Scopus databases; in addition, it is possible to show that the subject under study indicates an upward trend, consistent with the significant increase in the number of articles published, so it is feasible to determine that research in this area of study is booming, due to the fact that in the last five years 52.75% of the scientific production has been published and an annual growth rate of 17.4%. Thus, this background shows that the scientific community in general has shown interest in this field of study.

Country analysis

Figure 2B shows the scientific production according to countries and distinguishes the 10 most important at the international level. India is the country that publishes the most studies, with 137 studies (26%), followed by China, Korea and Pakistan with 70 (13%), 27 (5%) and 25 publications (5%), respectively. In fifth place is Brazil as the representative of Latin America with a total of 18 publications, equivalent to 3%. As for the top 10 countries, seven are from Asia with a total of 295 publications corresponding to 53% of the total scientific production worldwide, one country from the European continent with 11 publications (2%), one from South America with 18 publications (3%) and finally one country from North America with a total of 11 publications (2%). These 10 leading countries in the scientific production of the subject under study are of great relevance since they produce 62% of the publications generated worldwide, a situation in which the countries belonging to the Asian continent stand out as important pillars for research in the area of MTIBAP.

Author analysis

Table 2 lists the 10 main authors who publish the largest number of articles related to the subject of this study; the classification is performed according to the total sum of publications in the WoS and Scopus databases, on the other hand, the number of citations and the H-index of the authors are useful to determine the impact of productivity and scientific quality; the H index is the measurement of the scientific production of a researcher, determined by the number of articles and the number of citations from them (Alonso *et al*., 2009; Cascón-Katchadourian *et al*., 2020). The authors with the highest publication (six published articles) are Kim Sang-dal linked to Yeungnam University - South Korea (903 citations and an H index of 15), Passaglia Luciane Maria Pereira from the Federal University of Rio Grande do Sul - Brazil (3,475 citations and an H index of 25) and Yao Tuo from the Agricultural University of Gansu, China (379 citations and an H index of 9). It is relevant to mention that Pandey, Ashok linked to the Indian Institute of Toxicology Research - India, is placed at number nine in the list, according to Scopus with a number of citations of 46,614 and with an H index of 99, warning that he has only four publications. It is also highlighted Maheshwari, Dinesh Kumar linked to Gurukul Kangri University - India, who is ranked number eight in the list and according to Scopus with a number of citations of 3,469 and an H index of 34 with a total of five publications. It is highlighted that some authors have fewer publications than others, but their H index is higher, indicating that they are researchers with greater impact within the scientific community.

Scientific journal analysis

Table 3 shows the 10 scientific journals with the highest number of publications on the subject of MTIBAP. The records are shown in relation to the Web of Science and Scopus databases, which have been used for this review, as well as the quartile, H-Index, impact factor, country and percentage of publications of the journals. The data show that of the ten journals with the highest impact, 40% are located in the Q2 category, the remaining 60% are distributed equally in 20% in each category Q1, Q3 and Q4 respectively;

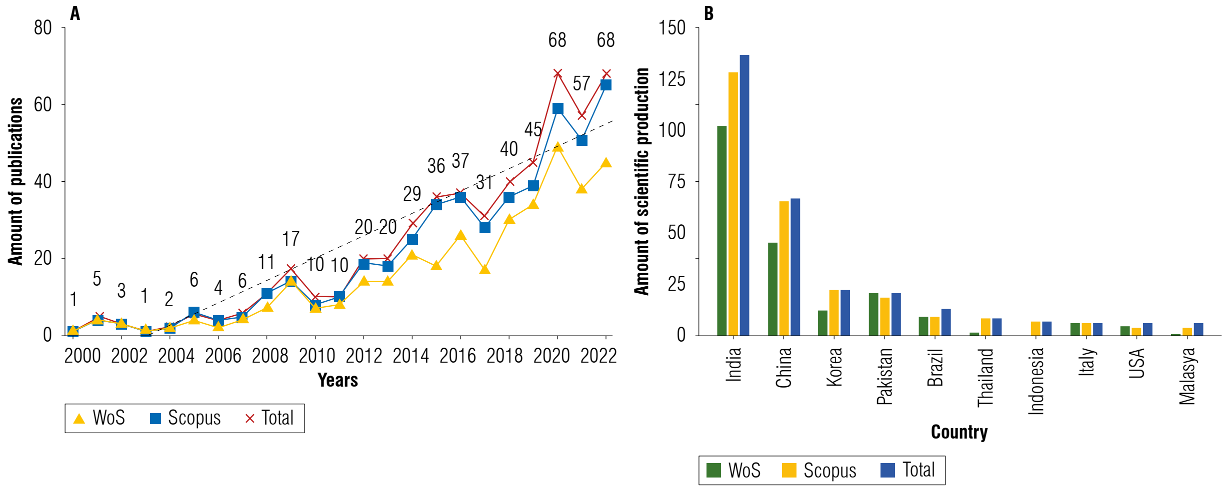

Figure 2. A. Amount of scientific production in the years 2000-2022 in the subject of study by year. The straight line represents the trend line. Figures represent total production. B. Amount of scientific production in the years 2000-2022 in the subject of study by country.

thus, the quartiles indicate the position of a journal in relation to all those in its area, ordered from highest to lowest impact using four quartiles. The journals with the highest impact will be the first quartile (Q1), the middle quartiles will be the second (Q2) and the third (Q3) and the lowest quartile will be the fourth (Q4). As for the journal with the most publications on the topic of study is Current microbiology from the United States, with a total of 15 publications, in second place is the journal microbiological research from Germany with 11 publications in total and in third position is the journal Frontiers in microbiology from Switzerland with a total of 10 publications; according to these data, the first three journals contribute 7% of the world's scientific production on MTIBAP. On the other hand, 50% corresponds to European countries (Germany, the Netherlands and Switzerland), 30% to North American countries (United States and Canada) and the remaining 20% to India. According to Scimago Journal and Country Rank (SJR), the journal with the highest H-index is the journal Frontiers in microbiology with an H-index of 166, the journal with the best SJR index is also Frontiers in microbiology with 1.31; in addition, it is observed that some journals have fewer publications than others, but their H-index and SJR index is higher, indicating that they are journals with a high impact on the scientific community.

Figure 3 shows the four relevant groups that make up the bibliographic study. In the first grouping, the network of co-citation of authors (A), this allows highlighting the most cited writers, in this case Glick Bernard, Schwyn Bernhard and Zhang Xiuhai

Table 2. Most relevant authors.

Authors	Total publications	Country	WoS			Scopus		
			Publications	Citations	H-Index	Publications	Citations	H-Index
Kim, Sang-dal	6	South Korea	-	-	-	6	903	15
Passaglia, Luciane María Pereira	6	Brazil	3	2,551	21	6	3,475	25
Yao, Tuo	6	China	-	-	-	6	379	9
Amaresan, Natarajan	5	India	4	487	15	5	592	15
Dastager, Syed Gulam	5	India	5	1,764	22	5	2,101	24
Hameed, Sohail	5	Pakistan	5	1,495	21	5	1,773	23
Khan, Mohammad Sayyar	5	China	5	347	11	5	662	14
Maheshwari, Dinesh Kumar	5	India	5	2,476	29	5	3,469	34
Pandey, Ashok	4	India	4	38,327	90	4	46,616	99
Castro, Paula M.L	4	Portugal	4	2,624	30	4	8,648	52

Table 3. Scientific journal analysis.

Journal	WoS	Scopus	Total	Percentage	Quartile	SJR (2021)	H- Index (SJR)	Country
Current microbiology	15	15	15	3 %	Q3	0.48	97	United States
Microbiological research	11	11	11	2 %	Q2	1.11	94	Germany
Frontiers in microbiology	11	10	10	2 %	Q1	1.31	166	Swiss
Journal of pure and applied microbiology	6	10	10	2 %	Q4	0.21	20	India
Biocatalysis and agricultural biotechnology	4	9	9	2 %	Q1	0.64	46	Netherlands
World journal of microbiology and biotechnology	NA	9	9	2 %	Q2	0.67	98	Netherlands
Canadian journal of microbiology	7	7	7	1 %	Q2	0.55	100	Canada
Journal of basic microbiology	7	7	7	1 %	Q3	0.46	60	Germany
Research journal of biotechnology	7	7	7	1 %	Q4	0.13	18	India
Biomed research international	6	6	6	1 %	Q2	0.65	147	United States

are the most referenced. The second grouping, the network of collaboration of authors (B), highlights the work in 10 groups, the first group is the most significant composed of six researchers, which stand out Zhang Xiuhai, Khan Mohammad Sayyar and Xue Jing, since they have better literary relationship; the second group is constituted by three researchers Pandey Ashok, Dastager Syed and Deepa C. K, the most collaboration is between Pandey, Dastager; the third group consists of three authors, Liu Ke, Kloepper Joseph and Hu C.H, which have equal literary relationship. The rest of the groups consist of two scientists each.

Now, the network of collaboration between countries (C) is the third constituent of the bibliometric analysis, showing that the country with the highest production of scientific articles about MTIBAP is India, with a marked collaboration with Korea, followed by China cooperating with Pakistan. Finally, the fourth group is shown, which consists of the network of cooccurrence of words (D), finding two broad groupings: the first in blue, the words microbiology, genetics, soil microbiology stand out; the second group in red, the words indoleacetic acid, plant growth and controlled study.

Network analysis

In this item, the most relevant studies on the subject were recognized. The documents with the most outstanding indicators were selected for analysis and organized using the metaphor of the tree of science. The 10 most significant (root), 10 pillars (trunk) and 40 recent documents (leaves). To establish the related research documents, the clustering algorithm was implemented according to Blondel *et al*. (2008), a simple method designed to extract community structures from large networks, allowing the visualization of distinct communities (clusters) in a network consisting of closely related nodes (Fig. 4).

Root

The researches that make up the root of this bibliographic search are categorized as the studies with the highest indegree (group of most cited documents),

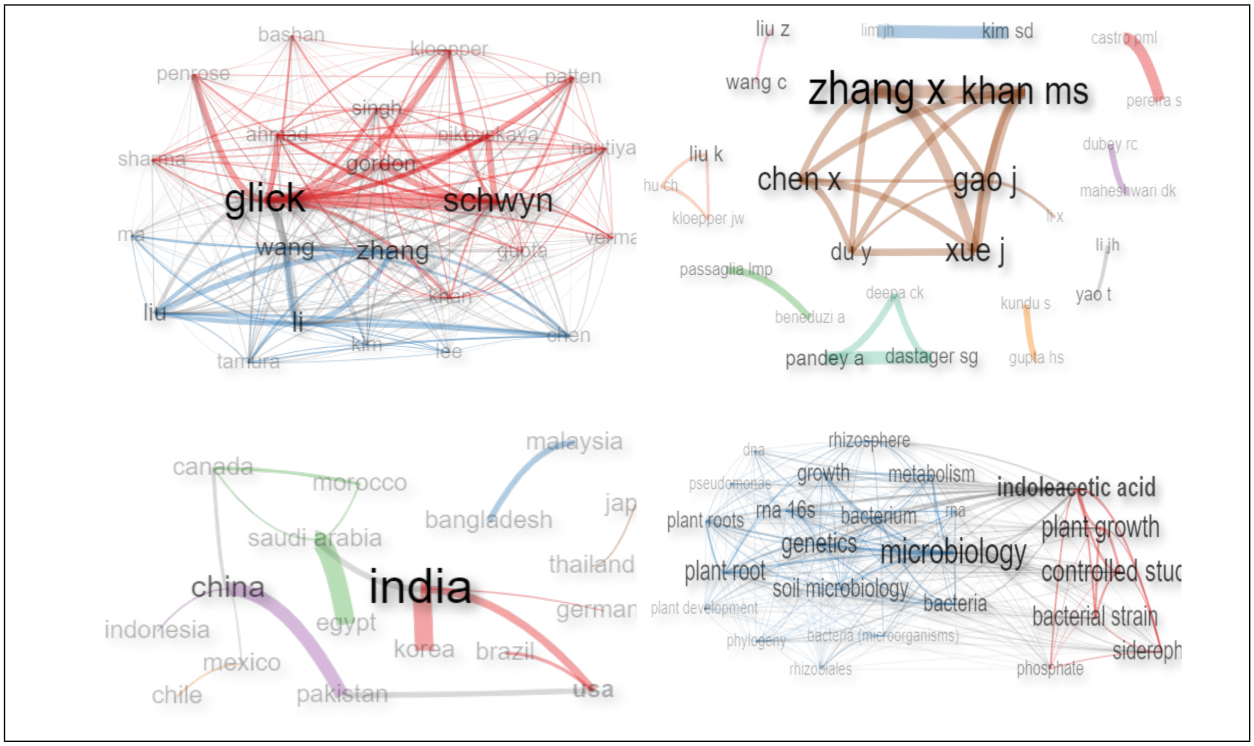

Figure 3. Elements of bibliometric analysis. (A) author co-citation network, (B) author collaboration network, (C) cross-country collaboration network and (D) keyword co-occurrence network.

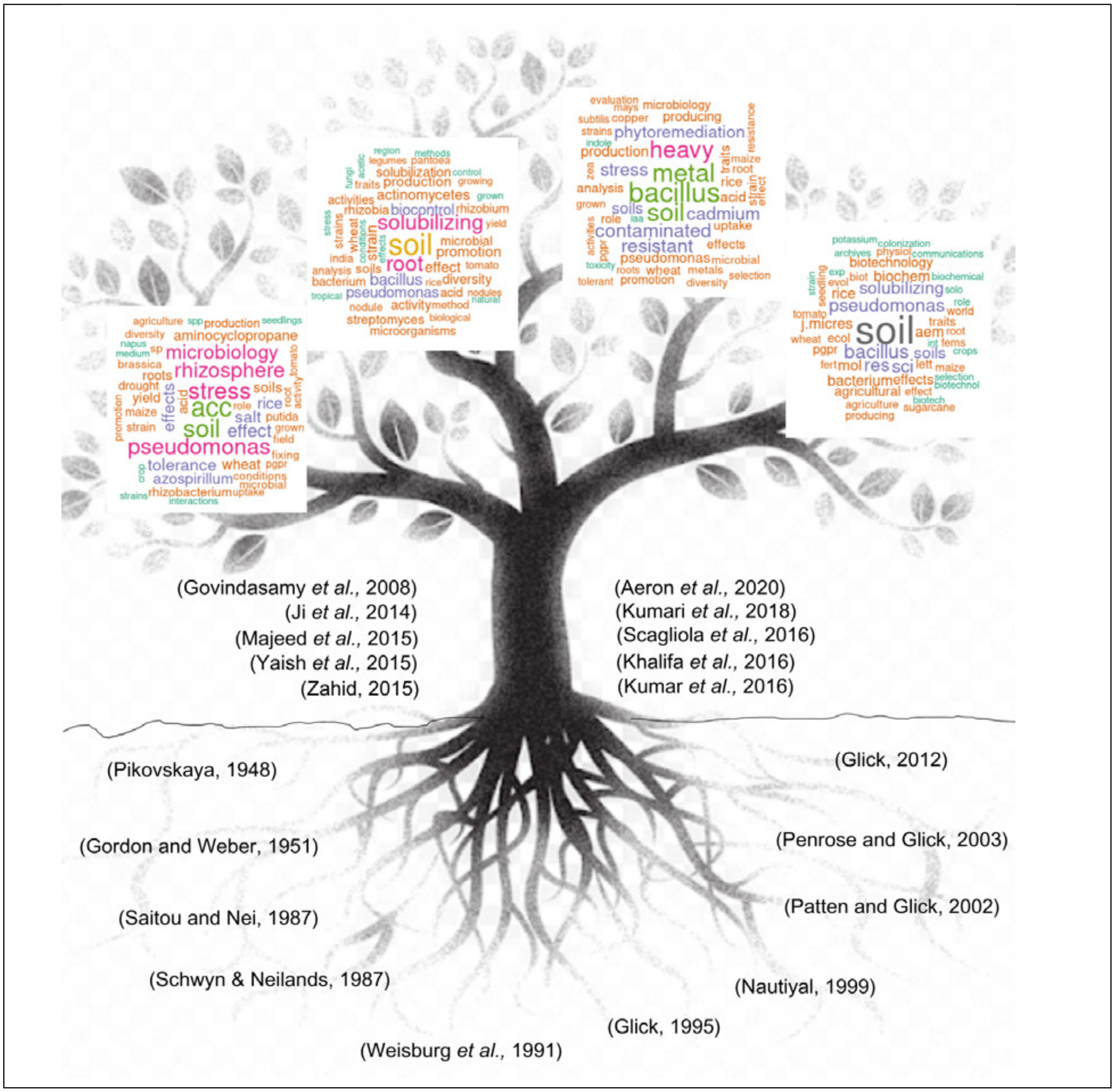

Figure 4. **Diagram of the metaphor of the tree of science. The graphic description of the hegemonic authors (root), those who have consolidated the predominant research (trunk) and the trends with respect to the subject matter (leaves) is presented.**

establishing their superior and predominant condition. Thus, 10 publications were studied, cited as predominant according to the aforementioned. In this section, reference is made to methods for detecting certain compounds such as siderophores and indoleacetic acid (IAA); also procedures for isolating PGRP, accompanied by two literature reviews on microorganisms that facilitate plant growth. In addition, they refer to techniques for the identification of phosphate solubilizing microorganisms. They include phylogenetic studies from the taxonomic point of view applied to these microorganisms and studies related to the improvement of root development in plants. These are specified below:

Before the implementation of molecular techniques such as polymerase chain reaction (PCR), the study of plant growth-promoting microorganisms was initiated using conventional methods as described below. In the middle of the 20th century, Pikovskaya (1948) was the first to develop a reliable and useful approach for the detection of phosphate solubilizing bacteria (PSB), which is currently applied and is based on visual detection of clarified zones in the culture medium, containing insoluble mineral phosphates. The performed approach is used by having 0.1 mL or 1 mL of suspension of a rhizosphere-associated soil sample serially diluted in sterile Pikovskaya (PVK) medium, supplemented with insoluble tricalcium phosphate as the sole source of phosphorus, dextrose and numerous inorganic components, for optical detection of clear zones in the medium. Also, the solubilization index (SI) was described by Pikovskaya (1948) as: SI=colony diameter (cm)+halozone diameter (cm)/colony diameter (cm). Years later, Gordon and Weber (1951) modified the ferric chloride-sulphuric acid method proposed by Tang and Bonner (1948) or the colorimetric estimation of indoleacetic acid (IAA), these changes produced greater stability, color, specificity and modification of the density more rapidly with respect to the variation in the concentration of IAA; they were able to conclude that by substituting sulfuric acid for perchloric acid in the Tang and Bonner reagent, the color intensity was improved and therefore it had the advantage of being a relatively sensitive method with greater specificity than the methods that had been used up to that time. On the other hand, at the end of the 80's Schwyn and Neilands (1987) developed as a research objective to describe a highly sensitive chemical method for the detection of siderophores based on their affinity for iron(III). Thus, they developed a global assay using chromium azurol S (CAS) and hexadecyltrimethylammonium bromide (HDTMA) as indicators, based on the fact that CAS/HTDMA forms complexes with iron (III) to produce a blue color and when a strong chelator, such as a siderophore, suppresses the iron in the coloring complex, its coloration varies from blue to orange.

According to the study of Saitou and Nei (1987) standardized a new method known as Neighbor-joining (NJ), which aimed to find pairs of operational taxonomic units (OTU) that decreased the total branch length at different stages of OTU clustering, from a star-shaped phylogenetic tree of any organism, which showed that the NJ method provided reliability and efficiency for the correct production of the tree topology. For their part, Weisburg *et al*. (1991) were among the pioneers in the use of molecular techniques, since they described a set of primers capable of initiating enzymatic amplification, that is to say PCR, in a wide phylogenetic and taxonomic variety of bacteria, demonstrating the method for its use and a respective example; arguing that by means of oligonucleotides designed for genetic fractions, bacterial species considered difficult to cultivate or with the potential to be pathogenic can be studied.

In addition, Glick (1995) in his literature review noted the importance of considering and discussing the ways in which PGPR facilitate plant growth; the author described in his study the direct mechanisms of plant growth promotion of PGPR and highlighted the relevance of the extensive biochemical and molecular studies carried out in symbiotic diazotrophs such as rhizobia, which have served as a starting point for understanding these mechanisms, due to the fact that rhizobia offer plants fixed nitrogen in exchange for carbon in photosynthesis; he also emphasized the processes of iron-siderophore bacterial complexes as a mechanism for obtaining iron from the soil, highlighting that without the presence of this process, plant growth would be severely limited; he also mentioned the effects of the synthesis of phytohormones, such as indoleacetic acid (IAA), an auxin phytohormone well known for stimulating rapid responses such as cell elongation, and participating in the processes of cell division and differentiation. On the other hand, Nautiyal (1999) formulated a defined medium for the detection of phosphate solubilizing microorganisms; these authors used the Pikovskaya medium (PVK) mentioned above, to perform the comparison, demonstrating that phosphate solubilization was three times more effective in the growth broth of the National Botanical Research Institute (NBRIP) compared to the PVK medium. For their part Patten and Glick (2002), demonstrated that the *ipd*c gene is essential to increase the phytohormone indole acetic acid (IAA) of *Pseudomonas putida* from tryptophan, which has a significant impact on stimulating the growth of primary roots of seedlings, taking into account that they used PCR to isolate the gene of interest and carry out the research.

In the study of Penrose and Glick (2003), described a method to isolate and characterize plant growth-promoting rhizobacteria containing the enzyme 1-Aminocyclopropane-1-carboxylic acid (ACC deaminase) as they reduce ethylene levels, based on research by Glick (1995); this type of procedure allows other researchers to easily isolate new strains of PGPR containing ACC deaminase as an agricultural potential. According to the study of Glick (2012) continued his own research cited above; he mentioned that soil bacteria in general and plant growth promoting bacteria (PGPB), produce cytokinins and gibberellins or both; he described that cytokinins (molecules that have the ability to promote cytokinesis or cell division in plants), have been detected in cells of *Azotobacter* spp., *Bacillus subtilis, Pseudomonas fluorescens, Rhizobium* spp., *Pantoea agglomerans,* among others; he also concluded that the use of PGPB is recognized as an integral component of agricultural practices whose time has come and that the usefulness of these bacteria has been successfully demonstrated in growing countries around the world.

Trunk

In this section, the R studio program yielded 10 researches classified as the most representative in relation to the research topic. Thus, the concomitant documents in this item are classified as those whose betweenness or intermediation are the highest (authors who cite those of the root section and are cited in turn by the most recent scientific production, that is to say the clusters); in this way, research related to isolation and molecular identification techniques that allow characterizing plant growth promoting microorganisms in cereals, vegetables, fruits, legumes and other crops such as palm are included.

In the study of Govindasamy *et al*. (2008), by PCR, cloned and sequenced the ACC deaminase (*acd*S) gene, managing to identify two bacterial isolates from wheat rhizosphere; thus, by phylogenetic analysis, the presence of *Achromobacter* sp. and *Pseudomonas stutzeri* was observed. Now, Ji *et al*. (2014) identified through specific primers of the nifH gene, two species of *Paenibacillus*, three species of *Microbacterium*, three species of *Bacillus* and four species of *Klebsiella*, which were isolated from 10 rice fields, in addition, they demonstrated that the characterized diazotrophic endophytic bacteria could be used as plant growth promoters and induce fungal resistance in plants. Subsequently, Majeed *et al*. (2015) managed to obtain nine bacterial isolates, of which seven produced indole-3-acetic acid; seven were nitrogen fixers and four were able to solubilize inorganic phosphate *in vitro*; they also evidenced genotypically four different morphotypes, based on IGS-RFLP (Intergenic Spacer - Restriction Fragment Length Polymorphism) fingerprinting and by 16S rRNA sequencing, identified *Stenotrophomonas* spp. and another bacterial isolate with genetic traits equal to *Acetobacter pasteurianus* and *Stenotrophomonas*; affirming the potential of PGPR in the early growth of wheat (*Triticum aestivum* L.). In addition, Scagliola *et al*. (2016) isolated, characterized and molecularly identified bacterial strains from the rhizosphere of barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicum* L.), previously cultivated under hydroponic conditions; isolation was performed using the standard plate dilution technique with nutrient agar supplemented with 0.1% (w/v) cycloheximide as fungal inhibitor; for PCR amplification of the 16s rDNA gene, the template of the genetic material was extracted by heat shock and using the universal primers 357f and R1401, amplicons of 1,060 bp were evidenced; the results showed that of the 800 isolated strains only 80 excreted siderophores, of which belonged to 12 different genera, the most representative being *Pseudomonas* (approximately 80%), but also genera such as: *Enterobacter, Azotobacter, Stenotrophomonas, Chryseobacterium* and *Rhizobium.*

In another study, Yaish *et al*. (2015) isolated and characterized endemic endophytic bacteria from date palm (*Phoenix dactylifera* L.) and identified some of the mechanisms that these bacteria can use, to facilitate the growth of this plant in saline environments; for the isolation of bacterial endophytes they used two techniques; in the first, root tissues were extracted using Ringer's solution and then seeded directly on agar media rich in different components and in the second, aliquots of Ringer's solution were taken, extracted as inocula from the selective media in sequential procedures, for the isolation of ACC deaminase-producing strains; as for molecular identification, they amplified the 16S rRNA gene using primers 27F and 1492R for bacteria; 16S rRNA gene sequence analysis led to the isolation of bacterial strains using the first method related to the genera *Bacillus, Chryseobacterium, Paenibacillus, Rhodococcus* and *Staphylococcus*, where *Bacillus* was dominant, and the second method led to the isolation of endophytes related to the genera *Achromobacter, Acinetobacter, Escherichia, Enterobacter* and *Klebsiella*, where *Enterobacter* was dominant. On the other hand, Zahid *et al*. (2015) isolated and identified PGPR associated with maize (*Zea mays* L.) crops in Pakistan; for molecular identification and authentication, eubacterial primers fD1 and rD1 corresponding to the 16S rRNA gene of *Escherichia coli* were applied; the results of the application of 16s rRNA gene sequencing and phylogenetic analysis evidenced that the isolates belonged to bacteria of the genus *Pseudomonas* and *Bacillus*.

So, the study of Kumar *et al*. (2016) designed an investigation to isolate and characterize PGPB

(*Pseudomonas* spp.) from the rhizosphere of common bean (*Phaseolus vulgaris* L.), from which they selected 8 isolates of *Pseudomonas* (PPR1-PPR8) and carried out the amplification of the PPR8 16s rRNA gene by PCR, using the universal eubacterial primers fD1 and rD1; the sequence of the gene used was aligned for similarity using the BLAST program of the GenBank database (NCBI) and thus, the authors obtained as a result of the sequencing of the 16s rRNA gene of *Pseudomonas* sp. PPR8, a sequence of 904 bp showing 100% similarity with *Pseudomonas* sp. BP-1 and 99% similarity with *Pseudomonas* sp. MBEA06 and *Pseudomonas* sp. HNS021. For their part, Khalifa *et al*. (2016) also performed 16s rRNA gene amplification using the universal primers 27F and 1492R, from a DNA template of an endophytic *Enterobacter cloacae* (MSR1) strain, isolated from inside roots of alfalfa (*Medicago sativa*) plants; the 16s rRNA gene sequencing results exhibited 99% homology with *E. cloacae* subsp. *cloacae* strain DSM 30054T, *E. cloacae* strain 5621A, *E. cloacae* strain AB6, and *E. cloacae* strain E717. In the study of Kumari *et al*. (2018) isolated and identified *Pseudomonas* spp., *Bacillus* spp., and *Acinetobacter* spp., by biochemical analysis and 16S rDNA gene sequencing, which were isolated from the rhizosphere of mung bean plants; they even demonstrated that all bacterial strains turned out to be PGPR, capable of producing indole-3-acetic acid and solubilizing phosphate. On the other hand, Aeron *et al*. (2020) identified *Stenotrophomonas maltophilia* by 16S rRNA sequence, which was recognized as a non-rhizobial endophytic bacterial strain, isolated from the root nodules of the medicinal legume *Mucuna utilis* var. *capitata* L.; demonstrating that it promotes plant growth directly and indirectly.

Figure 5. **Cluster 1, most mentioned words: Solubilization, *Bacillus*, *Pseudomonas*, Root.**

Leaves

From the bibliographic review, four perspectives or clusters were characterized, in this thematic under study, the most relevant lines of research in the area addressed are manifested. These are specified below:

In this first cluster, there are the investigations that mention PGP bacterial genera such as *Bacillus*, *Pseudomonas*, and also the importance of the identification of microorganisms with mineral solubilizing potential, because often the availability of these nutrients in the soil is limited; based on this Chakdar *et al*. (2018) carried out the isolation, identification and molecular characterization of mineral phosphate solubilizing bacteria from thermitorial soils of Sanjivani Island, India; the results of molecular amplification of 16s rRNA gene with primers (16S F27 and 16S R1525) demonstrated highly efficient isolates, such *Pantoea* sp. A3, *Kosakonia* sp. A37, who produced and increase of 37 and 53% of tomato root length; In addition, *Bacillus* sp. AH9 was identified, this isolate showed the highest phosphate index and the highest solubilization efficiency in Pikovskaya Agar. In the study of Mushtaq *et al*. (2019) developed the screening of bacterial endophytic isolates through citrus leaves for plant growth promoting activities using molecular characterization by using bacterial primers (27F and 1492R); the results evidenced, that out of 16 isolates characterized, *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Brevibacterium borstelensis*, *Enterobacter hermachei*, *Klebsiella pneumoniae*, *Staphylococcus haemolyticus*, *Bacillus subtilis*, *Psychrobacterium pulmonis*, *Bacillus megaterium*, *Proteus mirabilis* and *Bacillus cereus*, showed phosphate solubilization activity. For their part, Jana and Yaish (2020), isolated a rhizobacterium from date palm grown in salinity and by 16s rRNA gene amplification, using genomic DNA and universal primers 27F and 1492R, identified *Enterobacter cloacae* SQU-2, which in addition to the production of siderophores and indoleacetic acid (IAA), showed zinc, phosphorus and potassium solubilization characteristics. For their part, Lelapalli *et al*. (2021) isolated and performed molecular characterization using the universal primers described by Weisburg *et al*. (1991), finding that *Lysinibacillus pakistanensis* PCPSMR15, is a potential bacterium of the rice rhizosphere, capable of solubilizing inorganic phosphorus from insoluble compounds and promoting plant growth in terms of root, shoot and leaf length.

Liu *et al*. (2020) isolated and identified a plant growth-promoting rhizobacterium from root soil samples of rice plants; the researchers used 16s rRNA gene sequencing and confirmed the close relationship with 99.93% similarity to *Bacillus methylotrophicus* (DD-1), an isolate that exhibited potassium solubilizing activity, production of indoleacetic acid, gibberellic acid, siderophores and increased growth parameters in dry weight, number of tillers, root and shoot length compared to plants not treated with the DD-1 isolate. In turn Zhu *et al*. (2020) also isolated and identified PGPR in rhizosphere soil samples from rice plants; they performed molecular identification for amplification and sequencing using the primers 27F and 1492R, but also primers for *gyr*A and *gyr*B, specific genes of the auxin-producing rhizobacterium *Bacillus megaterium*; the authors concluded that, of the thirteen isolates from the rice rhizosphere, three strains, *B. megaterium*, *B. subtilis* subsp. *subtilis* and *B. subtilis* were auxin-producing and salt-tolerant strains. In addition, Fasusi *et al*. (2021) performed genomic sequencing and PCR amplification with the universal primer for bacteria 341F and 907R of the 16s rRNA gene; finding isolates with potential plant growth-promoting activities in rhizospheric soil samples of maize (*Zea mays* L.) and soybean (*Glycine max*), identifying *Bacillus* spp. (80.77%), *Rhodocyclaceae* (3.85%), *Enterococcus* spp. (3.85%), *Massilia* spp. (3.85%) and *Pseudomonas* (7.69%); the bacterial isolates possessed antifungal activity against *Fusarium graminearum* and promoted the development of corn and soybean seeds under laboratory conditions.

In another study, Gu *et al*. (2020) isolated and characterized at the molecular level, employing sequencing and amplification of 16s rDNA using 27F and 1494R oligonucleotides in rhizospheric soil samples of tobacco plants; strain S58 identified as *Pseudomonas mediterranea*, showed significant antagonism against plant pathogenic fungi and bacteria and efficiently controlled acute eyespot of wheat. For their part, Aldayel and Khalifa (2021) identified *Pseudomonas monteilii* in rhizospheric soil samples of tomato (*Solanum lycopersicum* L.) with 99.86% homologous regions by sequencing the 16s rRNA gene and applying the universal primers 27F and 1494R; the results evidenced that this strain significantly promoted primary root length, stem and leaf number in *Eruca sativa* (arugula) and *Raphanus sativus* (radish), growing under 1,000 mg kg^{-1} salt stress conditions. Finally, Aloo *et al*., (2020), investigated the diversity of cultivable rhizobacteria associated with potato (*Solanum tuberosum* L.) in Tanzania; of the 152 isolates obtained, 52 of them were selected for molecular identification based on preliminary screening for PGP activities; thus for PCR amplification, primers 27F and 1492R were used; The results showed that the isolates were all Gammaproteobacteria, belonging to four families (*Enterobacteriaceae, Yersiniaceae, Pseudomonadaceae* and *Morganellaceae*) and nine genera (*Enterobacter, Klebsiella, Serratia, Pseudomonas, Morganella, Buttiauxella, Pantoea* and *Cedecea*).

In this second cluster, it refers that currently agricultural practices have been revolutionized by microbiology, in search of sustainable agriculture by means of microorganisms with the potential to favor the factors or conditions that plants require to grow in a better way. In the study of Saengsanga (2018) molecularly identified *Enterobacter* sp. and *Bacillus* sp. strains isolated from the rhizosphere of early Thai jasmine rice plants with the ability to produce high concentrations of indoleacetic acid (IAA). On the other hand, Khezrinejad *et al*. (2019) characterized plant growth promoting rhizobacteria (PGPR) in sunflowers by phenotypic analysis and rep-PCR method; obtaining strains of *Pseudomonas fluorescens, P. aeruginosa, P. geniculata, Bacillus subtilis, B. pumilus, Stenotrophomonas maltophilia* and *Brevibacterium frigoritolerans*; in addition, by means of experimentation, they showed that the variability of microbial species obtained as PGPR improved the morphophysiological

Figure 6. Group 2, most mentioned words: *Bacillus*, Soil, Metal, Resistant, Heavy.

characteristics of sunflower shoots and roots. According to the study of Helal *et al*. (2022) the authors proposed a hypothesis on the effect of rhizocompetence in the rhizosphere of tomato, based on bacterial isolates from different microhabitats of this plant in different soils, identifying the genera *Pseudomonas, Bacillus* and six other bacteria by BOX-PCR; concluding that soil type and plant sphere can influence both genotypic diversity and rhizocompetence capacity of the same bacterial species.

In another research, Hyder *et al*. (2020) isolated and identified *Pseudomonas putida, P. libanensis*, *P. aeruginosa*, *Bacillus subtilis*, *B. megaterium and B. cereus* from chili rhizosphere by 16S rRNA sequence analysis, where it demonstrated the fundamental function of these microorganisms obtained for the antagonism of a fungus, in addition, their ability to improve the growth characters of the chili plant. For their part, Alotaibi *et al*. (2022) demonstrated that the combination of PGPR variety and petroleum hydrocarbon degradation (PHC) potential of bacteria contribute in the positive effect on canola shoot length growth and phytoremediation capacity to PHCs, where they were able to prove the mechanisms of *Pseudomonas plecoglossicida, Bacillus* and other microbial species to perform this type of beneficial processes, which could lead to the development of innovative bacterial inoculants for plants to remediate contaminated soils. Taking into account Andleeb *et al*. (2022), they conducted a research based on the evaluation and identification of plant growth promoting bacteria from the digestive tract of *Eisenia fetida* (Striped Red Worm) achieving the characterization of *Bacillus mycoides*, *B. aryabhattai*, *B. megaterium*, *Staphylococcus hominis*, *B. subtilis*, *B. spizizenii*, *B. licheniformis*, *B. mojavensis*, *B. toyonensis*, *B. anthracis*, *B. cereus*, *B. thuringiensis* and *B. paranthracis* as producers of siderophores, indoleacetic acid, and other compounds, in addition to the ability to solubilize phosphate, demonstrating the ability of most strains of the genus *Bacillus* isolated to be used as potential microbial biofertilizers to increase crop production.

On the other hand, in agriculture, efforts have been made to select plant growth-promoting bacteria resistant to heavy metals to improve the efficiency of phytoremediation and in turn increase crop production. Accordingly, Fan *et al*. (2018) isolated endophytic bacterial strains from root nodules of *Robinia pseudoacacia*, grown in a Pb-Zn mining area; demonstrating that some of these microorganisms had the ability to resist Cd, Zn, Pb and Cu, also the biological activity to produce indole-3-acetic acid, siderophores and carry out 1-aminocyclopropane-1-carboxylate deaminase activity, where *Mesorhizobium loti* HZ76 was the best strain to promote plant growth and perform phytoremediation processes. Likewise, Wu *et al*. (2019) also characterized heavy metal resistant plant growth promoting bacteria from acid mine drainage contaminated crop soil, which evaluated plant growth, nutrient uptake, antioxidant enzyme activities, and plant growth promoting characteristics were determined; thus, they identified strains S6-1 of *Burkholderia* sp., and S2-3 of *Pseudomonas* sp. demonstrating higher resistance and activity as PGPs. On the other hand, Idaszkin *et al*. (2021) isolated bacteria from the rhizosphere of the halophyte plants *Spartina densiflora* and *Sarcocornia perennis* in a polluted marsh, obtaining 60 strains, of which 25% of them produced siderophores, 16% were able to solubilize phosphate, 11% were able to produce auxins and 7% chitinase; evidencing that bacteria isolated from the rhizosphere of *S. densiflora* demonstrated higher PGP properties related to heavy metal phytostabilization. Finally, Bennis *et al*. (2022), aimed to characterize plant growth-promoting rhizobacteria from the rhizosphere of naturally grown *R. pseudoacacia* plants grown naturally in mining tailings contaminated with Pb and Zn, obtaining as a result the alteration of the properties of the three isolated PGPs when different concentrations of Pb or Zn were added to the culture medium; as for the analysis of

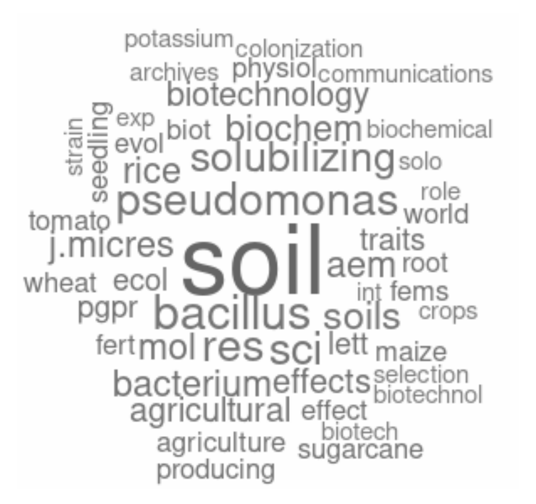

Figure 7. Cluster 3, most mentioned words: Soil, *Bacillus*, *Pseudomonas*, Solubilization.

the 16S rRNA gene of each strain, it was evidenced that strains 7MBT, 17MBT and 84MBT had 99.34, 100 and 99.72% of similarity with con *Priestia endophytica*, *B. pumilus* NBRC 12092 T and *B. halotolerans* NBRC 15718 T, respectively.

In the third cluster, according to its great relevance and repeated application, additional studies are mentioned, related to the isolation and molecular identification of the bacterial genera *Pseudomonas* and *Bacillus* that present plant growth promoting mechanisms; also, the capacity of PGPB to solubilize insoluble minerals such as phosphates and potassium, attributed as vital microbial functions for plant development, is mentioned. Accordingly, Ong *et al*. (2018) characterized diazotrophic bacteria present in saline soils, plant rhizosphere and plant roots, amplifying by PCR employing primers (polF, polR) and (27F, 1492R) targeting *nif*H genes and 16S rRNA gene respectively; the results indicated that, out of 147 isolates, 10 demonstrated the best characteristics related to plant growth mechanisms, identified as strains AS6. 3ES, SDSO11.2 and AS6.3ES, with 99, 100 and 99% sequence similarity to *Pseudomonas* sp., *Pseudomonas plecoglossicida* strain LJ3 and several strains of *Pseudomonas putida*, respectively, which were positive for ACC deaminase activity, IAA production, siderophore synthesis and phosphate solubilization. Years later, Singh *et al*. (2020) sequenced and performed molecular identification of three PGPRs based on 16s rDNA gene sequence and PCR amplification by primers 27F and 1492R; the three isolates were identified as *Burkholderia arboris*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and showed multiple PGP activities *in vitro*, for example, *P. aeruginosa* showed maximum IAA production and *B. arboris* showed maximum mineral solubilization and production of siderophores and ammonia. According to the study of Leontidou *et al*. (2020), after molecular identification through sanger sequencing and amplification of the 16s rRNA gene with Bakt_341F and Bakt_805R primers in samples of wild plants and a tomato cultivar, the presence of *Pseudomonas* (48%) and *Bacillus* (20%), as well as *Acinetobacter*, *Pedobacter*, *Pantoea*, *Enterobacter*, *Luteibacter*, *Lysobacter* and *Chryseobacterium* was determined; In addition, the isolates of *Pseudomonas* sp. showed genes related to possible PGP and included the *gdh* and *ppq* genes encoding glucose dehydrogenase and pyrroquinoline quinone synthase respectively, sequences that are associated with the production of gluconic acid, a substance involved in the solubilization of inorganic phosphate.

On the other hand, Singha *et al*. (2017) revealed the presence of nitrogen fixation genes (*nif*H) and nodulation genes (*nod*C) in isolates selected using fresh root nodules from pigeon pea (*Cajanus cajan*) and *Lablab purpureus* plants; for the molecular identification of the *nif*H genes they used the primers zehrf and zehr, while for the identification of the *nod*C genes they used the primers *nod*C1 and *nod*C2, likewise, they performed PCR-RFLP of the 16s rRNA gene applying the primers 27F and 1492R, thus obtaining that all isolates belonging to the genera *Rhizobium*, *Mesorhizobium* and *Burkholderia* were able to solubilize phosphates confirmed by a zone of clarification in the Pikovskaya agar medium. For their part, Malisorn *et al*. (2020), also amplified a DNA fragment by PCR, using the 16s rRNA gene using primers 27F and 1492R, a technique from which they identified 20 isolates obtained from legume nodules and rhizospheric soil samples, corresponding to the genera *Rhizobium*, *Ochrobactrum*, *Pseudomonas* and *Acinetobacter*. The results of the plant promotion mechanisms showed that the isolate SN-5 *Pseudomonas geniculata* showed the highest production of IAA and the highest rate of phosphate and zinc solubilization. In the study of Mghazli *et al*. (2022), conducted an investigation with the objective of isolating, characterizing and identifying autochthonous bacterial strains *in vitro*, for the best performing strains in plants, they performed sequencing and PCR amplification of the 16S rRNA gene using the primers 27f/1492r and fD1/rD1; the results of molecular identification reflected that the 41 isolates, corresponded to the genera *Microbacterium*, *Bacillus*, *Acinetobacter*, *Agrococcus*, *Brevibacterium*, *Neobacillus*, *Paenibacillus*, *Peribacillus*, *Pseudarthrobacter*, *Stenotrophomonas* and *Raoultella*; all isolates were able to tolerate up to 5% NaCl; *Bacillus paramycoides* showed the best catalase activity, positive reaction to cellulase synthesis and the highest hydrogen cyanide (HCN) production activity, a compound known for its ability to control and counteract plant pathogens.

Javoreková *et al*. (2021) identified PGPRs of the genera *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Pseudomonas* and *Serratia* by molecular analysis of the 16S rRNA gene using primers 27F and 1492R; isolates of which, the bacterial strains *B. altitudinis*, *B. aryabhattai* and *B. megaterium*, showed the best PGPR properties, thus representing an alternative for plant growth promotion in maize (*Zea mays* L.). Similarly Umapathi *et al*. (2022), conducted an investigation to identify drought-tolerant endophytic bacteria and to know their PGP effect on sorghum plants (*Sorghum bicolor* L.); to meet this objective, they isolated and identified bacteria of the genus *Acinetobacter pittii*, *Bacillus licheniformis*, *Bacillus* sp., *Pseudacidovorax intermedius* and *Acinetobacter baumannii* by 16S rRNA sequencing; they selected the strains *A. pittii*, *Bacillus* sp. and *P. intermedius*, to evaluate growth on sorghum seedlings, the results concluded that *Bacillus* sp. and *P. intermedius* improved growth characteristics: root length, shoot length, plant vigor index and total dry matter production, and protected the seedling against water stress.

For their part, Bhutani *et al*. (2022) recognized that the bacterial endophyte *Bacillus licheniformis* isolated from nodules and roots of *Vigna radiata* (mung bean), this strain was the only one of the 46 isolates that managed to grow at a salt concentration of 15%, so that after determining its molecular identity mediated by 16S rDNA sequencing, it was declared positive for ACC deaminase activity, IAA production, siderophores, ammonia, hydrogen cyanide (HCN), which means a potential isolate to promote plant growth in soils where there is a seasonal variation of salinity as a consequence of climate change. Also, Gohil *et al*. (2022) isolated and identified *Bacillus* sp. PG-8 from fermented Panchagavya, (mixture among five cow products including manure, urine, milk, rennet and butter), by applying 16s rRNA gene sequence using universal primers 27F and 1492R; due to its PGB characteristics such as IAA production, gibberellic acid, exopolysaccharide production, ammonia synthesis and phosphate solubilization, strain PG-8 was tested on *Arachis hypogea* (peanut), showing a germination rate of 70% in seeds treated with this inoculum, optimizing growth compared to the control and considerably improving root length, number of root hairs, number of leaves and leaf area.

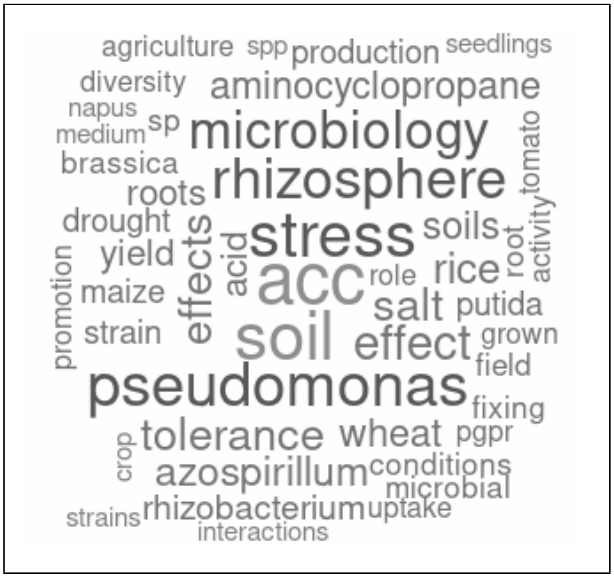

Figure 8. **Cluster 4, most mentioned words: *Pseudomonas*, Rhizosphere, ACC (1 - Aminocyclopropane - 1 - carboxylate (ACC) deaminase), Stress.**

In this last cluster, the influence of biotic or abiotic factors is analyzed; however, some microorganisms have the capacity to diminish the negative effects of stress, increasing plant tolerance to these factors. South *et al*. (2021) conducted a study based on the search for PGPR, which could improve the performance of ornamental plants at greenhouse level, with a lower amount of fertilizer, considered as an abiotic stress factor; so they genetically identified *Caballeronia zhejiangensis* C7B12, considering it as a potential PGPR under the given conditions of stress. On the other hand, Panigrahi *et al*. (2020) performed morphological, biochemical, and 16S rRNA gene sequencing characterization of an endophytic bacterium, identified as *Enterobacter cloacae*, where they determined that this isolated strain, was able to tolerate 9% NaCl and grow at 54 °C under osmotic stress in the medium, in addition to significantly improve the growth of four crop plants. However, Saravanakumar (2019) states that there are other types of mechanisms to benefit plants in the face of a variety of stress factors, where microorganisms may contain genes that favor the production of enzymes as a defense against adverse compounds that harm plant health, in this case 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD), produced by microorganisms, to promote the growth of plants that are influenced by environmental stress due to high ethylene accumulation.

There are other abiotic stress factors such as temperature; thus, Vega-Celedón *et al*. (2021) affirm that cold is one of the factors that minimizes plant growth, therefore, these authors aimed to isolate and characterize PGPB psychrotolerant of wild flora, which have the ability to protect and promote plant growth at low temperatures; thus, they identified the 16 rRNA gene of the isolated strains, and formulated PGPB consortia; obtaining that the bacterial consortium composed of *Pseudomonas* sp. TmR5a and *Curtobacterium* sp. BmP22c were the best, since it showed the presence of ACC deaminase and ice recrystallization inhibition activities. According to Goyal *et al*. (2022), they argue that PGPBs must have dual functional capacity to be implemented in arid crops; firstly the expression of ACC deaminase and secondly, the ability to tolerate higher temperature and salt concentration; so they isolated PGPB from the rhizosphere of *Cyamopsis tetragonoloba*, and sequenced the 16S rRNA gene of the microorganisms, identifying *Pseudomonas*, *Enterobacter* and *Stenotrophomonas*, they were ACC deaminase positive, thermohalotolerant and drought tolerant successfully. Govindasamy *et al*. (2022) presented the first report of isolation and characterization of cactus endophytic actinobacteria, where they identified strains of *Streptomyces* sp. by 16S rRNA gene sequencing and phylogenetic analysis, which were noted for their plant growth promotion such as ACC deaminase activity; even, the potential to improve crop plant growth under abiotic stress conditions such as drought. In the same year, Gao *et al*. (2022) aimed to isolate and identify endophytic antagonistic bacteria from lily roots, evaluating their antifungal activity and plant growth promotion characteristics; they recognized *Bacillus halotolerans* by 16S rRNA sequence, demonstrating ACC deaminase activity, salt and drought stress tolerance in an *in vitro* experiment, in addition to other mechanisms that contributed to plant growth promotion in this research.

On the other hand, Jhuma *et al*. (2021) confirm that the application of PGPR is a strategy of great viability to counteract biotic and abiotic stresses; these authors isolated 65 endophytic bacteria from the roots of healthy *Oryza sativa*, naturally grown in a saline environment; thus, by analyzing ribosomal DNA, they identified four genomically diverse groups, which were *Enterobacter*, *Achromobacter*, *Bacillus* and *Stenotrophomonas*, evidencing the tolerance of these microorganisms to NaCl ranging from 1.37 to 2.57 mol L^{-1} in nutrient agar medium; furthermore, under a salt stress of 200 mmol L^{-1} *in vitro*, the isolated strains of 108 CFU/mL demonstrated competitive production of exopolysaccharides (EPS). However Djebaili *et al*. (2021), analyzed fourteen isolates for phosphate solubilization, indoleacetic acid, hydrocyanic acid and ammonia production under different salt concentrations, showing that all isolates had halotolerant ability, improved morpho-biochemical parameters of durum wheat plants, and also 86% had 1-aminocyclopropane-1-carboxylate deaminase activity. According to Li *et al*. (2022), one of the main factors that threaten tall fescue (grasses) growth and turf quality is soil salinity; however, 10 genera were obtained from isolation, demonstrating the importance of *Bacillus zanthoxyli* and *Bacillus altitudinis* as promoters of tall fescue growth, based on salt tolerance.

CONCLUSION

Since 2012, there has been a significant increase in scientific production on the subject of MTIBAP, due to the need to identify and apply plant growth promoting bacteria on different agricultural crops, as an alternative to the use of agrochemicals. Regarding the molecular biology techniques used by different authors, sequencing and PCR amplification are the most frequent to identify and characterize the microorganisms that have shown a better response in *in vitro* and *ex vitro* research, using the universal eubacterial primers 27F and 1492R or 1494R.

Since the creation of PCR and sequencing techniques, greater speed, precision, sensitivity and specificity have been obtained for the identification of microbial genera isolated from agricultural soils, with potential for application as biofertilizers. The diversity of microorganisms isolated from the soil have been studied for their capacity to favor plant growth, mainly by solubilization mechanisms, production of siderophores and phytohormones and expression of ACC deaminase; the genera that have been most identified molecularly by using these mechanisms are *Bacillus, Pseudomonas, Enterobacter* and *Acinetobacter.*

In the purpose of continuing to address the subject and apply future research, it is suggested to continue developing the perspectives alluded to by the authors (Tab. 4); some of these are specified below:

Table 4. Perspectives.

Perspective	Topic	Reference
Solubilization, *Bacillus, Pseudomonas*, root	Bacterial endophytes could be more useful in controlling diseases of field crops	Mushtaq *et al*. (2019)
	B. subtilis subsp. *subtilis* and *B. subtilis* show potential for inducing salt tolerance in alfalfa plants	Zhu *et al*. (2020)
	Apply the use of *L. pakistanensis* as a biofertilizer and conduct further research due to its enzymatic characteristics and eventual commercial applications	Lelapalli *et al*. (2021)
Bacillus, soil, metal, resistance, heavy	Heavy metal contaminated areas can be biologically remediated by the interaction between heavy metal resistant rhizobia and *Robinia pseudoacacia*.	Fan *et al*. (2018)
	Importance of native rhizobacteria for the control of soil oomycetes and the potential use of *Bacillus* and *Pseudomonas* spp. in the development of biofertilizers and biofungicides	Hyder *et al*. (2020)
	Several strains obtained by these authors have the potential to be biofertilizers, demonstrated by the *in vitro* activities performed, as well as their high capacity of rhizocompetence, which is influenced by the type of soil and the plant	Helal *et al*. (2022)
Soil, *Bacillus*, *Pseudomonas*, solubilization	Conduct future research from a more holistic approach of PGP characteristics and isolation of microbial consortia as an agricultural application in unfavorable environments	Leontidou *et al*. (2020)
	Continue to test *in vivo* bacterial strains of *B. megaterium*, *B. altitudinis* and *B. aryabhattai* to promote maize growth without or with minimized use of industrial fertilizers and pesticides	Javoreková *et al*. (2021)
	Molecular mechanisms for adaptation to salinity due to climate change can be understood using the halotolerant bacterium *Bacillus licheniformis*	Bhutani *et al*. (2022)
Pseudomonas, rhizosphere, ACC (1-Aminocyclopropane-1-carboxylate (ACC) deaminase), stress	*E. cloacae* strain MG001451 could be an invaluable resource for applied agriculture, reducing the use of synthetic fertilizers and contributing to sustainable agriculture with further scientific research	Panigrahi *et al*. (2020)
	Further studies should be conducted to evaluate the possibility of producing biofertilizers with strains *S. iakyrus* G10, *S. ambofaciens* J27. *S. xantholyticus* K12, making consortia to evaluate possible synergistic effects, and to investigate the effectiveness of the formulations in open field experiments and on different crops for the development of biofertilizers useful to overcome high salinity in soils	Djebaili *et al*. (2021)
	Bacillus halotolerans LBG-1-13 can be considered a potential biocontroller and biofertilizer in lily, due to its antifungal capabilities, drought and salt tolerance, and production of ACC deaminase	Gao *et al*. (2022)

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