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MICROBIOLOGY

Cannabis-Bacteria Interaction: Trends in Secondary Metabolites, Biocontrol, and By-products

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Abstract: In the last decade, the interest in cannabis cultivation has grown significantly, driven by its various applications and the presence of key cannabinoids such as cannabidiol (CBD) and cannabigerol (CBG). However, this increase in production has heightened susceptibility to contaminants, especially microorganisms, such as pathogenic bacteria, which pose risks to both human health and the plant itself. Nonetheless, a promising outlook emerges with the biological utilization of certain bacterial species. This study focused on a bibliometric evaluation of the interaction between Cannabis and bacteria, analyzing research trends related to metabolites, biocontrol, and by-products. To achieve this, scientific literature was reviewed in databases such as Scopus and Web of Science (WOS) from 2000 to 2023. The data was processed and analyzed using methodologies such as "Tree of Science" (ToS) and Prisma, using tools like R-Studio and Bibliometrix. The results highlight two predominant areas in this interaction: the application of biotechnology to enhance cultivation and utilize metabolites for medical purposes and the assessment of antibacterial properties in Cannabis extracts and essential oils, along with the use of beneficial bacteria for biocontrol in cannabis plants. Several avenues for future research are proposed, considering identified knowledge gaps.

Key words: Bibliometric analysis, Biological control, Beneficial microorganisms, *Cannabis sativa* L., Microbial contamination, Phytopathology.

INTRODUCTION

In the last decade, there has been a noticeable increase in interest in cannabis cultivation due to its numerous multifunctional applications and the presence of various cannabinoids (ElSohly et al. 2017, Rodriguez-Yzquierdo et al. 2021). This plant is widely used to produce textiles, food, cosmetics, pharmaceuticals, and biofuels (Chandra et al. 2020). Furthermore, approximately 120 cannabinoids are known today, with the most studied ones being tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) (Perez et al. 2022). However, this increase in production has led to greater susceptibility to various contaminants (Rodriguez-Yzquierdo et al. 2021), such as pesticides, mycotoxins, heavy metals, and microorganisms (Fitzcharles et al. 2023).

On a global scale, various bacterial genera have been identified as part of microbial contamination. Among the strains found, *Acinetobacter baumannii, Acinetobacter pitti, Corynebacterium diphtheriae, Coxiella burnetii, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Ralstonia pickettii, Salmonella entérica, Listeria* spp., *Clostridium* spp., and *Klebsiella* spp. Some of these bacteria possess pathogenic characteristics and produce toxins that pose a health risk to cannabis consumers. (McKernan et al. 2016, Unger et al. 2014, Vujanovic et al. 2020).

There are specific microorganisms that can directly affect the cultivation of Cannabis. Among these are *Xanthomonas campestris* pv. *cannabis*, which is the cause of bacterial leaf spot (Netsu et al. 2014), compared with the species complex *Agrobacterium tumefaciens*, which can cause galls in the crown (Holmes et al. 2023). Additionally, *Pseudomonas syringae* pv. *cannabina*, which triggers bacterial blight (McPartland et al. 2000), and *Pseudomonas syringae* pv. *mori*, which induces ulcerative streak (McPartland & Hillig 2004).

Research has been conducted on the relationship between bacteria and Cannabis cultivation. Firstly, the enhancement of phytocannabinoids and plant production using such microorganisms (Akhtar et al. 2016, Balthazar et al. 2021, Favero et al. 2022). Simultaneously, the presence of pathogenic bacteria that pose a risk to human health or have the capacity to cause diseases in the crop directly has been investigated (Montoya et al. 2020, Punja 2021). Finally, various studies related to the antimicrobial capacity of Cannabis against certain pathogenic bacteria in humans (Alfei et al. 2023, Hernández-Cervantes et al. 2017, Klahn 2020, Saleemi et al. 2022, Schofs et al. 2021, Singh et al. 2022, Sionov & Steinberg 2022). However, no research studies currently relate literature reviews on the interaction between Cannabis and bacteria using methodologies like the Tree of Science (ToS) in globally recognized databases such as Scopus and Web of Science (WoS).

A review was conducted on Cannabis cultivation and the presence of bacteria. A systematic literature review was performed using bibliographic tools and methods, with the Scopus database as the primary reference source from 2000 to 2023. The organization, observation, and systematization of information followed standard management practices, with some tools such as Scopus and Web of Science used for data collection. In total, 379 publications were reviewed as part of the bibliometric analysis on Cannabis cultivation and bacterial colonization. The results obtained were analyzed using the R-studio software, where a network analysis based on the reviewed studies was implemented, utilizing the ToS and Prisma methodologies for document identification and selection. Finally, emerging trends in the study topic were defined, and scientific documents in each category were highlighted.

MATERIALS AND METHODS

This article was developed in two phases. The first stage involved scientific mapping of the knowledge field using bibliometric analysis of scientific production identified in Scopus and Web of Science (WoS). The second phase involved network analysis using the Tree of Science (ToS) methodology to identify and classify key research contributions. This approach enabled the identification of the most prominent studies related to production management and the classification of research according to its relevance and impact within the field.

Five bibliometric procedures recommended by Zupic and Čater (2015) were applied to carry out scientific mapping and productivity analysis. These procedures included citation studies, term co-occurrence analysis, citation analysis of other documents, co-citation analysis, and a study of citation clusters related to multiple authors. The Scopus and Web of Science databases were chosen for this research due to the abundance of registered articles in these databases and their global recognition (Bar-Ilan 2008, Zhu & Liu 2020).

The search process used the equation combining "cannabis" and "bacteria," initially yielding 474 results. After applying filters for the publication period (2000-2023), document type—focusing on articles, books, book chapters, and reference documents—15 records were excluded. Additionally, removing duplicates between the WoS and Scopus databases resulted in a final selection of 379 documents considered in this study. The search and inclusion criteria are detailed using the PRISMA methodology in Figure 1.

In total, the search indices yielded a total of 379 results in Scopus and Web of Science. To carry out the bibliometric analysis, the Bibliometrix tool (Aria & Cuccurullo 2017) was employed, selected for its ease of use and the proven effectiveness of its multiple functions, as demonstrated in previous studies (Acevedo-Meneses et al. 2020, Aria et al. 2020, Bond et al. 2019, Demiroz & Haase 2019, Duque et al. 2021, Duque-Hurtado et al. 2020, Tani et al. 2018).

The network analysis was conducted using the results obtained from the Scopus and WoS databases by processing information in R-studio software. This tool was used to present the bibliography and structure the reference chain, following the principles of graph theory as a guide. Graph theory was employed as a practical resource to streamline the collection

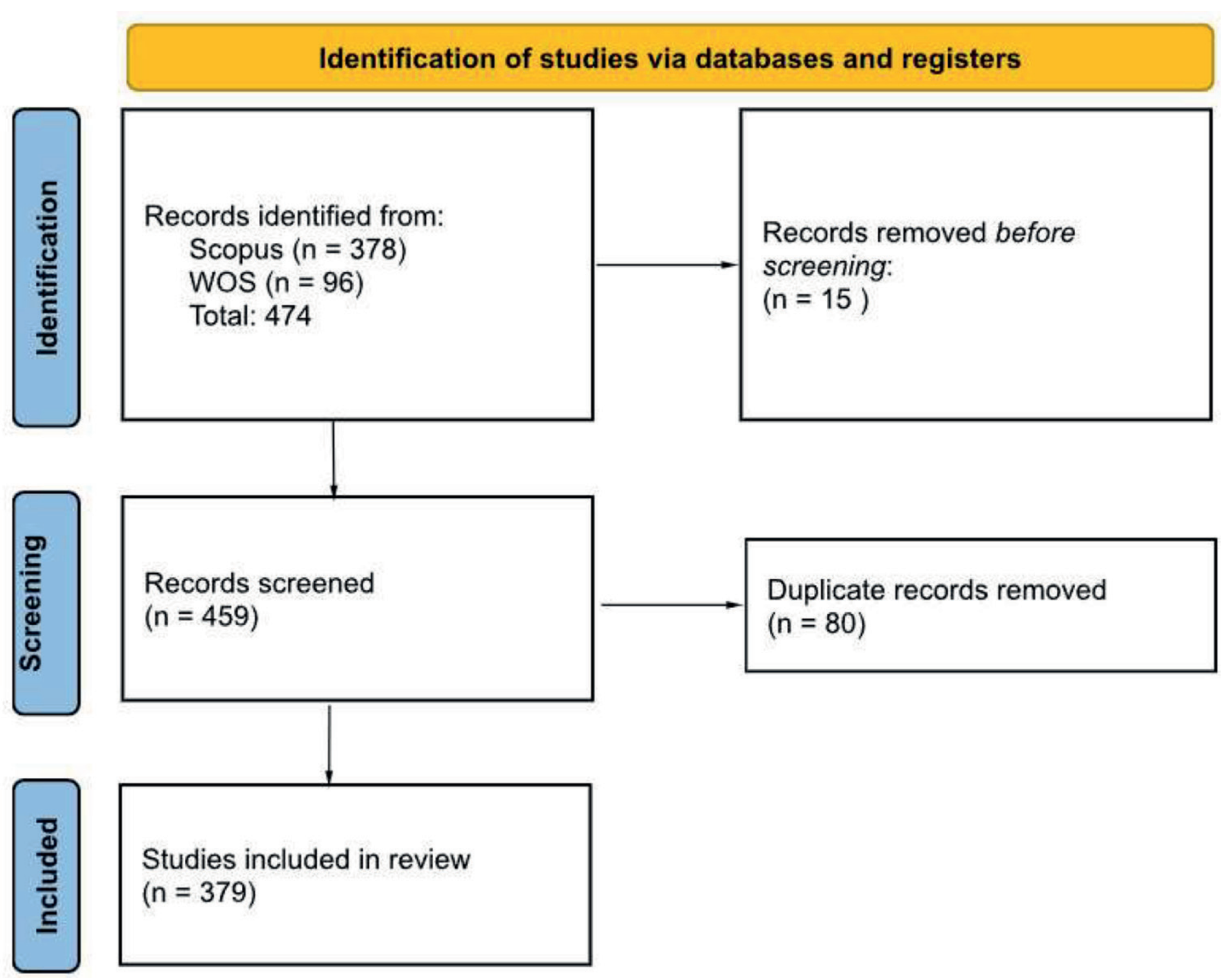

Figure 1. Search Criteria using the PRISMA Methodology (Page et al. 2021).

of data related to the typologies and distinctive characteristics of the network, as well as to analyze each of the articles comprising it (Wallis 2007, Yang et al. 2016).

Subsequently, three bibliometric indicators recommended by Wallis (2007) were applied: Indegree, which quantifies how frequently other authors cite a text; Outdegree, which determines the number of times a specific node references other nodes or the number of connections it establishes with other writings; and betweenness, which identifies the degree of mediation and the position of each component within the network (Freeman 1977), considering the use of references in the work and citations from other authors (Zhang & Luo 2017).

The indicators of indegree, outdegree, and betweenness were implemented in the network to facilitate its organization following the ToS (Robledo et al. 2014, Valencia-Hernandez et al. 2020). Based on this strategy, three different categorizations were carried out:

Indegree: These roots focused on analyzing traditional and theoretically influential articles within the subject. Specifically, they concentrated on reference works that do not mention other authors (Wallis 2007).

Betweenness: In this category, articles that, in addition to referencing, were cited by other authors were included (Zhang & Luo 2017). These works were key connections between classical theory and contemporary studies, bridging the gap.

Outdegree: The leaves focused on current articles referencing other authors (Wallis 2007). These documents represent contemporary trends in the field and reflect emerging proposals that underpin studies on the subject and the criteria mentioned by other authors.

It is important to note that this methodological approach has been applied and validated in previous research (Buitrago et al. 2019, Duque et al. 2021, Duque & Cervantes-Cervantes 2019, Duque-Hurtado et al. 2020, Hernández-Betancur et al. 2020, Ramos-Enríquez et al. 2021, Toro-Cardona et al. 2020, Trejos-Salazar et al. 2021).

Ultimately, the knowledge network of the research field is composed entirely of articles retrieved from the database and their respective references. This implies that texts from various bibliographic sources are included, not limited solely to those identified in Scopus and Web of Science but also to other information sources and relevant scientific works. This network exploration approach, or co-citation map, provides valuable insights into the structure and organization of a subject while also facilitating the identification of subfields and study methodologies (Gurzki & Woisetschläger 2017, Zuschke 2020). To effectively visualize and analyze the structure of this scientific knowledge network in the field of research on social and solidarity economics (SSE), the Gephi tool is employed (Bastian et al., 2009).

RESULTS AND DISCUSSION

Scientific mapping

Bibliometric analysis

To assess trends in productions related to the presence or use of bacteria in medicinal Cannabis, an analysis of the number of publications identified in the Scopus and WoS databases was conducted between 2000 and 2023. The results are displayed in Figure 2, indicating a progressive increase in publications over time. Notably, the first publication on the subject of interest occurred in 2000. In the last five years, more than 70 % of the publications were recorded, validating the recent interest in the subject. This is also correlated with the legalization of this product in various countries

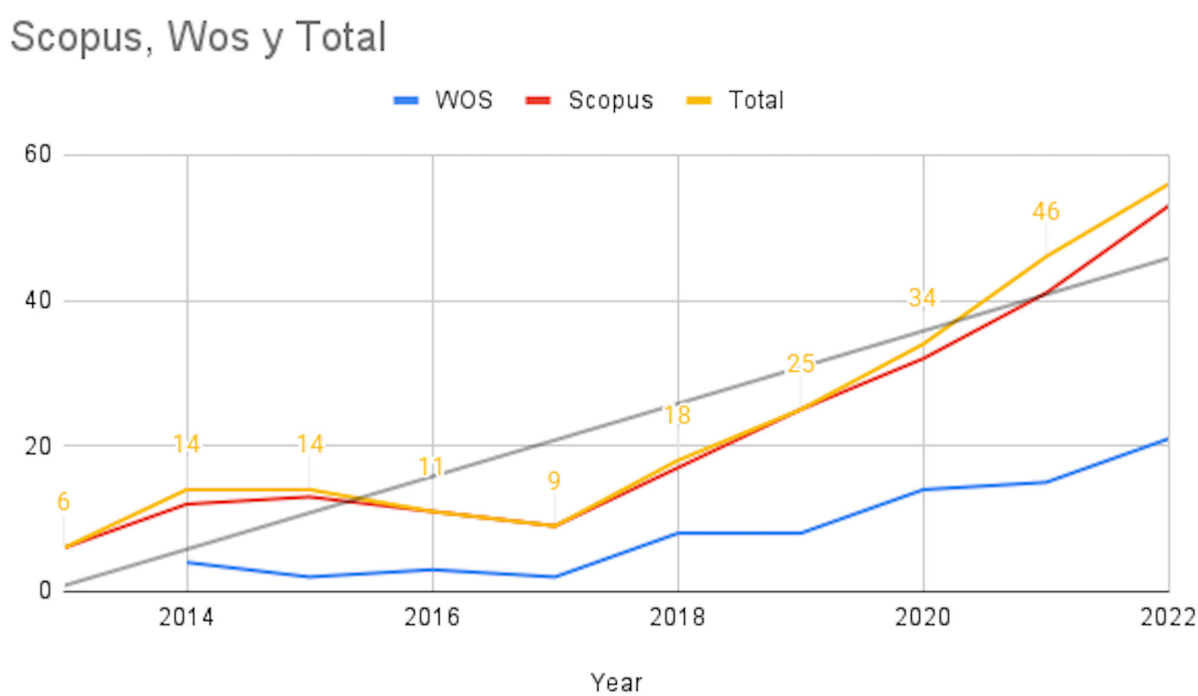

Figure 2. History of publications in Scopus and WoS.

worldwide, reflecting an annual growth rate exceeding 25 % in these scientific research studies (Mollner 2022, United Nations 2022). Additionally, it is observed that the number of articles published in Scopus surpasses the quantity published in WoS.

Table I provides a detailed overview of the key contributors in research about bacteria and medicinal Cannabis, demonstrating that the top ten leading countries in this domain account for 57.52 % of the global academic output. Topping the list is the United States, accounting for 17.15 %, followed by Italy at 10.03 %, and Canada in third place with 6.60 %. Upon examining the distribution by continent, North America, comprising the United States and Canada, makes a substantial contribution of 41 %, Asia at 32 %, and Europe at 27 %. The significance of the United States in medicinal cannabis research can be attributed to its early legalization in the 1990s and, notably, the removal of research barriers by the federal government in 2015. This policy shift markedly propelled studies in this field (ProCon 2023). According to Table I, the countries listed have some degree of cannabis legalization (except for Pakistan), whether for recreational, medicinal, or exclusively industrial purposes, as seen in China and India, evidencing a higher contribution to research on the topic. These differences in academic output reflect how public policies regarding Cannabis in each country influence its prevalence and shape research approaches in various regions (Ransing et al. 2022).

Figure 3 illustrates the collaboration network among countries, highlighting significant interaction among pertinent nations within each continent. A robust partnership is evident between the United States and Canada and between the United States and Europe. In Europe, countries tend to engage with one another, as evidenced by a cooperative network involving Italy, Germany, the United Kingdom, and Sweden. A comparable pattern emerges

Table I. List of countries.

Country/Region	Wos	Scopus	Number of publications	
			Total	% of total
USA	17	63	**65**	**17.15 %**
ITALY	14	35	**38**	**10.03 %**
CANADA	9	25	**25**	**6.60 %**
INDIA	7	21	**23**	**6.07 %**
CHINA	2	15	**15**	**3.96 %**
PAKISTAN	6	13	**13**	**3.43 %**
UNITED KINGDOM	1	12	**12**	**3.17 %**
ISRAEL	5	8	**9**	**2.37 %**
GERMANY	3	9	**9**	**2.37 %**
AUSTRALIA	2	8	**9**	**2.37 %**
			379	57.52 %

among Asian nations, with collaboration networks between Israel, China, Australia, and Pakistan.

Author Analysis

Table II highlights the top 10 authors with a notable number of publications in the databases under study and significant relevance in the field of research. These ten authors have produced 53 publications on the subject of interest. According to their institutional affiliation, five authors are based in the United States, two in Finland, and one each in Italy, Iceland, and Pakistan. The institutions from which the authors originate include five universities, one from the United States government and one from an international private company.

The author with the highest number of publications in Cannabis and bacterial interaction is Zhang L. from Neogen Corporation, with eight publications accounting for 15.09 % of the subject of interest. This author's lines of research focus on validating rapid microbial detection methods, mainly in food and cosmetic products, using automated systems such as Soleris® and ANSR®. His studies include the detection of various bacteria (such as *Burkholderia cepacia* and *Listeria* spp.) in specific products, including dried Cannabis and environmental surfaces. These investigations validate AOAC-approved methods, providing reliable quality and safety control techniques in regulated industries. Followed by Boyette C. and Pellegrini M. with six publications each. Boyette's lines of research focus on the use of bioherbicidal fungi and the interaction of natural compounds with synthetic herbicides for weed control, especially in crops. His work includes studies on the fungus *Albifimbria verrucaria* to control glyphosate-resistant weeds, analysis of chemical interactions that affect seedling growth, and the expansion of the host range of certain fungi. He also evaluates formulation methods to improve the efficacy of fungi as biological control agents in different agricultural environments. Pellegrini M. specializes in soil microbiology, biological control, and plant growth promotion. Her work focuses on using

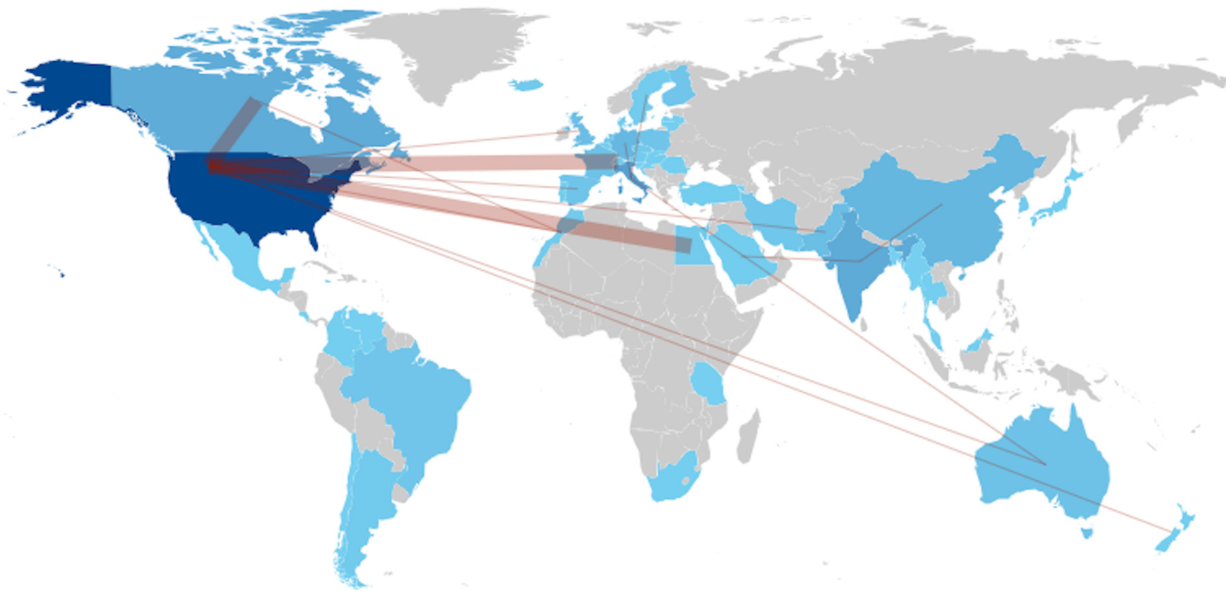

Figure 3. **Intercountry collaboration network.**

microorganisms and mycorrhizal fungi to improve plant health and yield, as well as the application of biopesticides and biofertilizers. Meanwhile, Adnan M. has the highest H-index (29). It is essential to clarify that the number of citations for the authors corresponds to their entire research experience and not specifically to citations within the subject of interest.

Three main networks were identified in the analysis of the collaboration network among authors. The first network consists of Newton C., Klein TW., and Friedman H. from Morsani College of Medicine in Tampa, United States. They focus on various research areas in biomedical sciences, mainly related to immune response, infections, and the use of cannabinoids in therapy. These authors contribute to the therapeutic effects of Cannabis and its application in treating diseases. Their experience in biomedical research can help to understand better the mechanisms of action of the active compounds in Cannabis, such as THC and CBD, and their potential in medicine.

The second network is formed by Sjöberg A.M. and Kymäläinen H.R. from Finland, who focus mainly on host-pathogen interactions, microbiota, and the application of molecular biology, which can contribute to the study of diseases affecting cannabis plants. Research on the interactions between soil microorganisms and crops can be crucial to develop effective integrated disease management strategies to ensure healthier and more productive crops.

The third network includes Boyette C.D. and Hoagland R.E. from the Biological Pest Control Research Unit in Stoneville, USA, who focus on using natural enemies, such as predators and biological controllers, to reduce reliance on chemical pesticides. They also analyze the interaction between pests and plant pathogens, allowing them to create integrated crop management solutions. This confirms that the United States is leading in publications in the study area. Their research on natural enemies and biopesticides can influence the development of agricultural practices that minimize the use of chemical pesticides in cannabis cultivation, thus improving the quality and safety of products.

Table II. Main authors.

Number	Author	# of publications	# of citations	H-index	Institution	Country
1	ZHANG L.	8	24	3	Neogen Corporation	United States
2	BOYETTE C.	6	1.247	22	Biological Control of Pests Research Unit	United States
3	PELLEGRINI M.	6	749	15	Università degli Studi dell'Aquila	Italy
4	HOAGLAND.R.	5	435	13	USDA Agricultural Research Service	United States
5	KYMÄLÄINEN H.	5	1.159	18	Helsingin Yliopisto	Finland
6	ORLYGSSON J.	5	823	18	Háskólinn á Akureyri	Iceland
7	SJÖBERG A.	5	2.233	24	Helsingin Yliopisto	Finland
8	WANG K.	5	1.665	22	University of Hawai'i at Mānoa	United States
9	ADNAN M.	4	2.449	29	University of Sargodha	Pakistan
10	BISWAS P.	4	36	4	Neogen Corporation	United States

Journal Analysis

To identify the top ten journals with the highest number of publications on the topic of Cannabis and its interaction with bacteria, a specific search was conducted (Table III), the impact of the journals and the scientific effect of an article within a journal were analyzed using the SCImago Journal Ranking (SJR), the H-index obtained from SJR, and the nationality to which each journal belongs.

In the analysis of journals, it can be observed that, in the top 10, four of the journals are from Switzerland, followed by the United States (2) and the United Kingdom (2), and finally, Germany (1), showing a trend in countries where both medicinal and recreational consumption is legal. Legalization in countries such as the United States, Canada, and several European countries, such as Switzerland and the United Kingdom, has allowed greater access to research funds, which has facilitated more in-depth studies on the therapeutic benefits of Cannabis, as well as its interaction with microorganisms. Thus, for example, in the United States, a notable growth in cannabis-related research has been observed in universities and research centers, especially in those states that have adopted more liberal policies. In contrast, in countries where Cannabis remains illegal, such as many countries in Asia and Africa, scientific production is scarce. This phenomenon highlights how public policies can directly influence research opportunities and the amount of scientific literature available on a specific topic.

The journal with the highest number of publications on the analyzed topic was Frontiers in Microbiology, from Switzerland, a journal in Microbiology that seeks to understand the role microorganisms play in addressing global challenges such as healthcare, food safety, and climate change (Table III). This journal focuses on microorganisms and their impact on various

Table III. Representative Journals.

No	Journal	Number of records	% of the total	SJR 2020	Quartile SJR	H-index (SJR)	Country	Thematic Area
1	Frontiers in Microbiology	7	0.02	1.19	Q1	201	Switzerland	Microbiology
2	Industrial Crops and Products	7	0.02	0.9	Q1	158	Netherlands	Agricultural and Biological Science
3	Molecules	6	0.02	0.7	Q1	199	Switzerland	Biochemistry, Genetics and Molecular Biology
4	Applied Microbiology and Biotechnology	6	0.02	0.97	Q1	253	Germany	Immunology and Microbiology
5	Frontiers in Pharmacology	5	0.01	01.06	Q1	129	Switzerland	Pharmacology, Toxicology and Pharmaceutics
6	Journal of Applied Microbiology	5	0.01	0.77	Q2	176	United Kingdom	Immunology and Microbiology Biochemistry, Genetics and Molecular Biology
7	Journal of AOAC International	4	0.01	0.36	Q2	94	United States	Agricultural and Biological Sciences
8	Scientific Reports	4	0.01	0.97	Q1	289	United Kingdom	Multidisciplinary
9	Plos One	4	0.01	0.89	Q1	404	United States	Multidisciplinary
10	Frontiers in Cellular and Infection Microbiology	3	0.01	1.31	Q1	105	Switzerland	Immunology and Microbiology

fields, including agriculture and health, making it ideal for publishing research on interactions between Cannabis and bacteria.

The journal Plos One, from the United States, has a multidisciplinary scope and the highest H-index, with a score of 404 and a share of 0.01% of the published works, representing a smaller proportion than the journal in the first place. However, both journals have the same quartile (Q1), concluding that Plos One has a higher h-index because it is a multidisciplinary journal. Likewise, it can be evident that journals publishing on the topic of interest have a high impact factor in science; as of the top ten, eight are Q1, and two are Q2 (Table III). Having a more general and less specialized approach to certain aspects of Cannabis allows it to attract a more significant number of citations due to its accessibility and thematic diversity.

Knowledge network

Following the methodology proposed by Robledo et al. (2022) and Valencia-Hernandez et al. (2020), which employ a metaphor based on the anatomy of a tree to structure information into three distinct components - the root, the trunk, and the leaves - the identification of research trends and the determination of essential texts corresponding to each of these categories have been undertaken.

Figure 4 displays the analysis conducted on documents about Cannabis and bacteria, revealing the absence of roots and trunks and emphasizing exclusively the presence of "leaves." In this context, "leaves" tend to reflect contemporary trends that prevail in studies within this field, including frequently cited fundamentals, thus categorizing them as emerging works in the subject (Wallis 2007).

The sole presence of "leaves" in this analysis also underscores the novelty of the research focus on the topic of Cannabis and Bacteria from various thematic perspectives, corroborating the trend observed previously in Figure 2, which indicated a significant increase starting from the year 2015. Furthermore, the analysis identified two clearly defined trend areas or clusters: the first one focuses on biotechnological applications and the relationship of bacteria with Cannabis cultivation, covering over 300 documents, while the second one centers on the antibacterial properties of extracts and essential oils derived from *Cannabis sativa* L., comprising a set of fewer than 50 documents.

Research Trend 1: Biotechnological Applications to Enhance Cannabis Cultivation and Utilize Secondary Metabolites in Medical Applications.

In general, this research trend was analyzed from two perspectives. One of them is the ability to enhance Cannabis cultivation using microorganisms, specifically bacteria, and the second refers to the use of phytocannabinoids in various medical applications.

Several studies have been conducted in biotechnological applications involving bacteria to enhance cultivation or as a pathogen

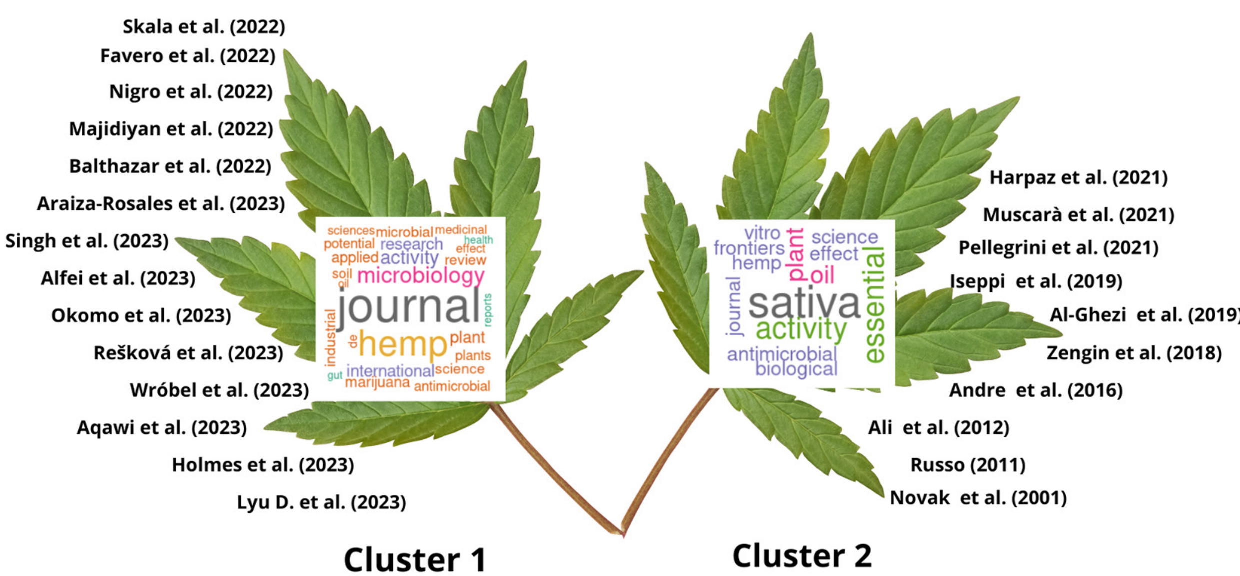

Figure 4. Analysis tree of Cannabis + Bacteria documents.

controller. Lyu et al. (2023) explored the potential of plant growth-promoting rhizobacteria (PGPR) to improve the growth and chemical composition of Cannabis. Cultures of three PGPR strains in King's B medium were used to inoculate Cannabis plants at two different stages of growth. The results revealed that Mucilaginibacter sp. increased the dry weight of flowers and the content of CBD and THC during the vegetative stage. In contrast, *Pseudomonas* sp. increased stem dry matter and cannabinoid content. *Bacillus* sp. demonstrated that it significantly contributed to the accumulation of neutral THC and CBD. Overall, the inoculation of PGPR during the vegetative stage improved the yield and chemical profiles of Cannabis, suggesting its potential for sustainable cultivation.

In other similar research, rhizospheric bacterial communities associated with *Cannabis sativa* L. growing in distillery sludge were examined. Four bacterial species were characterized, displaying attributes related to plant growth promotion and lignin degradation. Analysis using GC-MS revealed bacterial activity in the conversion and reduction of organic contaminants present in the sludge, and the accumulation of metals in Cannabis plants was observed, suggesting their potential for phytoremediation of contaminated sites (Singh et al.2023).

Another study addressed the biocontrol potential of *Pseudomonas protegens* Pf-5 against the pathogenic fungus *Botrytis cinerea* in *Cannabis sativa* L. crops. During this investigation, mutants lacking key antibiotic-producing genes were created. It was discovered that the absence of pyoluteorin (PLT) reduced the effectiveness of biocontrol, while the lack of 2,4-diacetylphloroglucinol (DAPG) resulted in greater pathogen inhibition. Plant assays demonstrated a significant reduction in disease in mutants lacking these antibiotics, highlighting their importance in biocontrol (Balthazar et al. 2022).

Furthermore, it is important to mention various research studies, such as the one conducted by Araiza-Rosales et al. (2023), in which they analyzed the concentration of multiple components in agroindustrial residues of *Cannabis sativa* L. after subjecting them to two different processes of cannabinoid extraction. These processes reduced the content of lipids and cannabinoids. However, they increased the fiber content, neutral sugars of the cell wall (NSC), and in vitro dry matter digestibility, demonstrating potential as livestock feed. In contrast to this study, conducted by Wróbel et al. (2023), it was determined that industrial hemp (*Cannabis sativa* L.) is a valuable source for fiber extraction, while its biomass finds applications in food and energy production. Additionally, the impact of inoculating hemp shoots (HS) and hemp flowers (HF) with a starter culture of lactic acid bacteria during the ensiling process was explored. Overall, ensiling hemp with biological additives proved effective for biofuel production and biomass preservation, ensuring the availability of raw materials throughout the year.

However, it is relevant to highlight studies that have addressed fermentation in Cannabis cultivation, such as the one conducted by Okomo Aloo et al. (2023). This research explored the effects of fermentation and germination on the metabolite profile and bioactive properties of 'Cheongsam' hemp seeds. Various extracts were analyzed for antinutrients, metabolites, and bioactivity, including raw, fermented, germinated, and fermented sprouts. The results revealed significant changes in antinutrient levels, an increase in polyphenols, flavonoids, individual polyphenols, and cannabinoids, as well as improved functional properties in the fermented sprouts. In comparison, the study conducted by Favero et al. (2022), which focused on cannabinoid fermentation as an alternative to traditional cannabis cultivation for the production of Δ9-THC, CBD, and Δ9-THCV, highlights the advantages of this method, such as reduced resource usage and faster production. However, it also addresses the limitations of replicating the diverse phytochemical profile of Cannabis.

Regarding the medicinal applications of phytocannabinoids, the study conducted by Alfei et al. (2023) emphasized the urgent need for new antimicrobial agents due to the increase in pathogens resistant to multiple drugs. The potential of phytocannabinoids present in Cannabis sativa L., such as CBD, Δ8-THC, Δ9-THC, CBC, CBG, and CBN, was highlighted for combating gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA), and inhibiting biofilm formation. These findings align with those described by Nigro et al. (2022), whose research underscored the need for efficient purification methods and investigated the potential nutraceutical and cosmeceutical properties of cannabidiolic acid (CBDA), modulating the release of proinflammatory cytokines and chemokines, suggesting anti-inflammatory properties as well as antimicrobial and anti-biofilm activities.

On the other hand, there is the study by Skala et al. (2022), which analyzed the increasing popularity of Cannabis preparations for the treatment of skin diseases, emphasizing the use of high-potency Cannabis extracts, primarily the non-psychoactive tetrahydrocannabinolic acid (THCA), in topical treatments. This research examined the antimicrobial and antifungal properties of two potent cannabis strains extracted with different solvents, focusing on their chemical profiles and effectiveness against microorganisms. Additionally, in the study conducted by Aqawi et al. (2023), it was determined that cannabigerol (CBG) exhibits antibacterial and anti-biofilm properties against Streptococcus mutans. The study explored how the Quorum Sensing (QS) systems, ComCDE and LuxS, affect the susceptibility of S. mutans to CBG and the anti-QS effects of CBG. Finally, in the research by Majidiyan et al. (2022), the use of industrial hemp essential oil, whey protein nanofibrils, and mung bean protein nanoparticles as a novel nano-carrier was explored. The study successfully demonstrated the incorporation of essential oil and nanoparticles, resulting in effective antimicrobial coatings for fish products and extending their shelf life.

Research Trend 2: Antibacterial Properties of Extracts and Essential Oils from Cannabis sativa L. and the Use of Beneficial Bacteria for Biocontrol in Cannabis Plants.

This research trend was addressed through two approaches, one related to applying phytocannabinoids in various areas of medicine and the other involving using bacteria for biocontrol.

In the field of utilizing chemical compounds from medicinal Cannabis, various research studies have been conducted. Among these investigations, the review conducted by Andre et al. (2016) stands out, which addresses the versatile applications of *Cannabis sativa* L., analyzing cannabinoids, terpenes, and phenolic compounds, emphasizing their pharmaceutical and industrial potential. Similarly, the study carried out by Novak et al. (2001) examined the essential oils of five different varieties of *Cannabis sativa* L., identifying key compounds such as α-pinene, myrcene, trans-β-ocimene, α-terpinolene, trans-caryophyllene, and α-humulene. Additionally, the research conducted by Iseppi et al. (2019) focused on characterizing volatile terpene compounds in different varieties of essential oils from *Cannabis* *sativa* L. using GC-MS and GC-FID techniques. Seventeen essential oils were analyzed, revealing that α- and β-pinene, β-myrcene, and β-caryophyllene were the major components. Furthermore, the antibacterial properties of hemp essential oils against various pathogenic and spoilage microorganisms were evaluated, suggesting their potential for reducing bacterial contamination in food processing.

Similarly, Russo (2011) delved into the importance of cannabis terpenoids such as limonene, myrcene, and α-pinene, among others, along with cannabinoids like THC and CBD. Their therapeutic potential was highlighted, including pain relief, anti-inflammatory properties, and their role in counteracting the intoxicating effects of THC. In contrast, the study conducted by Harpaz et al. (2021) investigated the physiological effects of nine selected cannabinoids using a bioluminescent bacterial panel. The panel consisted of *Escherichia coli* modified with stress promoters to identify bacterial defense mechanisms in response to cannabinoids, revealing that THC and THCA had genotoxic effects. In contrast, others caused cytotoxicity or oxidative damage. Cannabis extracts were mainly genotoxic, and cannabinoid mixtures showed unique response patterns, shedding light on potential synergistic or antagonistic interactions. Finally, the study conducted by Zengin et al. (2018) provided information on cannabinoid content, volatile compounds, phenolic patterns, flavonoids, and color characteristics. Additionally, it explored the plant's potential as a source of antioxidants, its effects on various enzymes and microbial strains, and its cytotoxicity against normal and malignant cells.

Concerning various medical conditions, evaluations of the medicinal components of *Cannabis sativa* L. have been conducted. Among them, there is a study conducted by Al-Ghezi et al. (2019), which assessed the impact of a combination of marijuana cannabinoids, THC, and CBD on experimental autoimmune encephalomyelitis (EAE). The research explored how cannabinoids influence neuroinflammation by modulating the gut microbiota. Treatment with THC and CBD led to a reduction in EAE symptoms, as well as a decrease in inflammatory cytokines and an alteration in the gut microbiome. Additionally, there is the research by Ali et al. (2012), who investigated the antimicrobial properties of various extracts of *Cannabis sativa* L. against different bacteria and fungi; seed oil demonstrated significant antibacterial effects against *Bacillus subtilis* and *Staphylococcus aureus*. Similarly, a study by Muscarà et al. (2021) determined that *Cannabis sativa* L. oils have antioxidant and antimicrobial properties, particularly against *Staphylococcus aureus*.

Regarding the use of bacteria as biocontrol agents, the study conducted by Pellegrini et al. (2021) stands out, in which a consortium of microorganisms composed of *Azospirillum brasilense, Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae,* and *Burkholderia ambifaria* was evaluated for effectively addressing *Fusarium* sp. infection.

Future research agenda

Table IV presents the identified research lines in the two generated clusters. These lines primarily focus on improving cultivation during the productive stage, the utilization of agro-industrial residues, and the primary uses of phytocannabinoids.

According to the identified trends and their research lines, potential research horizons include studying the specific mechanisms underlying the anti-inflammatory and antimicrobial actions of CBDA, as well as modifications of CBC, CBG, and CBD to enhance

Table IV. Future lines of research.

Research trend	Research areas	References
Research Trend 1: Biotechnological Applications to Enhance Cannabis Cultivation and Exploit its Secondary Metabolites for Medical Applications.	Use of Plant Growth-Promoting Bacteria for Physiological Enhancement of Cannabis Cultivation.	(Lyu et al. 2023, Singh et al. 2023)
	Use of Lactic Acid Bacteria for Physiological Enhancement of Cannabis Cultivation.	(Okomo Aloo et al. 2023, Wróbel et al. 2023)
	Antimicrobial Effect of Phytocannabinoids.	(Alfei et al. 2023, Aqawi et al. 2023, Skala et al. 2022)
	Phytocannabinoid production via fermentation	(Favero et al. 2022)
	Use of Cannabis-Derived Essential Oils to Extend Shelf Life in Food.	(Majidiyan et al. 2022)
	Utilization of Agro-Industrial Residues from Cannabis Plants as a Potential Source of Forage in Ruminant Feeding.	(Araiza-Rosales et al. 2023)
Research Trend 2: Antibacterial Properties of Extracts and Essential Oils from Cannabis sativa L. and the Use of Beneficial Bacteria for Biocontrol in Cannabis Plants.	Antimicrobial Potential of Compounds Derived from Cannabis Plants.	(Ali et al. 2012, Iseppi et al. 2019, Novak et al. 2001, Pellegrini et al. 2021, Russo 2011, Zengin et al. 2018)
	Industrial and Pharmaceutical Applications of Phytocannabinoids.	(Al-Ghezi et al. 2019, Andre et al. 2016)

their antibacterial activity against Gram-negative bacteria. Similarly, delving into the specific mechanisms by which cannabinoids from different cannabis varieties modulate the gut microbiota and their potential therapeutic applications for multiple sclerosis and other neuroinflammatory conditions is suggested. Additionally, applications in nutraceutical products and skincare may yield valuable insights. Likewise, it is proposed to optimize the formulation and application of nanoparticles fortified with cannabis-derived essential oils for various food products, along with exploring their potential as natural preservatives in the food industry.

Another future application involves using *Cannabis sativa* L. plants to optimize phytoremediation techniques and their associated rhizospheric bacteria for efficient detoxification of industrial waste, aiding in the ecological restoration of contaminated sites for sustainable development. This will lead to developing specific cleaning and sanitation strategies to mitigate different types of pollution.

Furthermore, to expand our understanding of the cannabis-bacteria interaction, it is proposed to delve deeper into the characteristics of phytopathogenic bacteria with potential economic impact on cultivation. Additionally, exploring the use of phytocannabinoids in controlling microorganisms in the same crop or others would enable the broader application of these metabolites in agriculture. Another field of study suggested by Araiza-Rosales et al.

(2023) is the use of agro-industrial residues from cannabis plants. Expanding this perspective to consider the long-term effects of incorporating these residues into ruminant diets and evaluating their impact on animal performance and health would support the emerging trends in the circular economy.

It is also proposed that further research on the synergistic effects of different biocontrol strategies be conducted to integrate them into an integrated disease management approach. Additionally, exploring the level of colonization of plant growth-promoting rhizobacteria (PGPR) in cannabis plants and the interactions between PGPR and the host for deeper insights is recommended. Furthermore, elucidating the underlying molecular mechanisms of co-regulation between the pioluteorin (PLT) and 2,4-diacetylphloroglucinol (DAPG) biosynthesis pathways and exploring the potential synergistic effects of other compounds in biological control is crucial. Finally, advancing studies on the genetic resistance of Cannabis plants to diseases of bacterial and fungal origin could bring significant advancements to cultivation and production (Sirangelo et al. 2023).

In all future research lines, it is proposed to explore the application of omics technologies, such as metabolomics, transcriptomics, volatolomics, and other next-generation sequencing tools, to understand this interaction and its potential applications comprehensively.

CONCLUSIONS

In summary, this bibliometric analysis reveals a steady growth in publications related to bacteria and medicinal Cannabis from 2000 to 2023. The increasing attention in the last five years, accounting for over 70% of the total publications, aligns with the legalization of Cannabis in various countries. The United States leads the research, followed by Italy and Canada. International collaboration is evident, especially between the United States, Canada, and Europe. Removing research barriers in the United States in 2015 was a turning point. This analysis provides a comprehensive insight into the evolution and collaboration in this constantly growing scientific field.

The top 10 authors in the field of study of Cannabis and bacteria have a total of 53 joint publications. The United States is the leading country in author contributions, followed by Finland, Italy, Iceland, and Pakistan. Most authors come from universities, the U.S. government, and international private companies. Zhang L from Neogen Corporation leads the list with eight publications, followed by Boyette C and Pellegrini M, each with six publications. Adnan M stands out with a high H-index of 29.

The collaboration network analysis reveals three main networks, with authors from Morsani College of Medicine, Tampa, Unit°ed States, leading the first network. This confirms the prominence of the United States in publications related to Cannabis and bacteria in this field of study. It is important to note that the citation figures for authors encompass their entire research experience and are not limited to the specific topic of interest.

The top ten journals with the highest number of publications on Cannabis and its interaction with bacteria reveal an interesting geographical distribution, with four journals from Switzerland, followed by the United States and the United Kingdom. This reflects the legalization of both medicinal and recreational consumption in these countries. Frontiers in Microbiology, based in Switzerland, leads the list of publications and focuses on microbiology and its role in global challenges. Plos One, from the United States, stands out with the highest H-index, although it represents a smaller proportion than the leading journal. Furthermore, most of these journals have a high impact factor in science, with eight in quartile Q1 and two in quartile Q2. This suggests strong interest and recognition in the scientific community regarding this topic.

Research is primarily concentrated in the "leaves" category, indicating a trend toward contemporary and emerging approaches in the field. This observation reinforces the growth trend in research since 2015, highlighting two key areas: biotechnology and the bacterial relationship in cannabis cultivation, with over 300 documents, and the antibacterial properties of extracts from *Cannabis sativa* L., with less than 50 documents. These findings underscore the novelty and diversity of approaches in this constantly evolving area.

Research trend one focuses on Cannabis and bacteria and has been explored from two perspectives: the potential of cultivation and the medical applications of phytocannabinoids. In the biotechnological realm, plant growth-promoting rhizobacteria have been studied to enhance the production and chemical composition of Cannabis, suggesting their potential for sustainable cultivation. Additionally, research has addressed using bacteria to degrade contaminants and phytoremediation in contaminated soils. The study also addressed the fermentation of hemp seeds and biofuel production. Regarding medical applications, phytocannabinoids have been highlighted as potential antimicrobial and anti-inflammatory agents, their use in topical treatments, and the exploration of new nano-carriers. These studies demonstrate the diversity and evolving research potential in this interdisciplinary area.

Research trend two focuses on the medical application of phytocannabinoids and using bacteria for biocontrol. Studies on non-psychoactive compounds of Cannabis in medical applications show promise in a variety of areas, from pain relief to modulation of the gut microbiota. On the other hand, using bacteria as biocontrol agents in agriculture offers innovative solutions to address pathogens and improve crop yields. Both approaches reflect the versatility and growing importance of research around Cannabis and bacteria in medicine and agriculture.

Finally, it is proposed as future research lines to delve deeper into the host (Cannabis) regarding the uses of its by-products and residues and its resistance to bacterial diseases. Similarly, continuing to explore alternative methods of controlling phytopathogenic bacteria and fungi to be more competitive in the market and provide safe products for consumers, considering that most are immunocompromised patients.

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