CHAPTER II

RITERATURE REVIEWS

2.1 *Cannabis sativa*

2.1.1 Taxonomy and Classification

Cannabis belongs to the Cannabaceae family, a group of flowering plants comprising approximately 102 species. Despite exhibiting significant morphological, chemical, and utilitarian diversity, the prevailing view among botanists is that cannabis constitutes a single species, *Cannabis sativa* L. However, debate persists regarding whether cannabis should be classified as multiple species, particularly *Cannabis indica* and *Cannabis ruderalis*. Attempts to further categorize cannabis into subspecies or varieties, based on morphological traits, cannabinoid content, and geographic origin, have not gained widespread acceptance and are often inconsistently applied. This is particularly evident in the use of the terms "sativa" and "indica," which are frequently employed in the cannabis industry to describe distinct cannabis characteristics but do not accurately reflect true botanical classifications.

Kingdom: Streptophyta
Subkingdom: Viridiplantae
Phylum: Streptophyta
Superdivision: Spermatophyta (seed plants)
Division: Magnoliophyta (flowering plants)
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Rosales
Family: Cannabaceae
Genus: *Cannabis*
Species: *Cannabis sativa L*

2.1.2 Growth Habits

Cannabis is a versatile plant in the Cannabaceae family renowned for its wide range of growth and morphological characteristics. This variability reflects its long history of cultivation, breeding, and hybridization for various purposes, including fiber production, seed oil extraction, and medical and recreational use. Understanding the growth and morphology of cannabis is essential to improving cultivation, maximizing yield, and tailoring plant characteristics to specific applications.

Figure 1. Characteristics of *Cannabis sativa* L. (Retrieved April 29, 2024, from https://batsmg.m.wikipedia.org/wiki/Abruozdielis:Cannabis_sativa_001.JPG).

Cannabis is generally an herbaceous plant, although some varieties tend to be perennial under certain environmental conditions. Cannabis displays a wide range of growth characteristics, which are influenced by a complex interaction of genetic, environmental, al, and cultivation factors. Plant heights can vary greatly, from dwarf varieties less than 1 m tall to giant varieties exceeding 5 m tall (Clarke & Merlin, 2016). Much of this variability is due to genetic differences between strains and environmental factors, such as light intensity, nutrient availability, and water content. There are also significant differences in branching patterns, ranging from sparse to heavily branched, which affect the overall shape and yield of the plant. Cannabis flowering is largely influenced by photoperiod. The plant begins flowering as the days shorten in autumn (Small, 2015). However, some auto-flowering varieties have been developed that flower independently of photoperiod, depending on age or stage of development.

2.1.3 Morphology

Cannabis has a strong and adaptable root system, typically characterized by a prominent taproot with extensive lateral branching. This root structure allows the plant to efficiently absorb water and nutrients from different depths of soil and allows it to tolerate a wide range of environmental conditions (Clarke & Merlin, 2016). The stem is erect, ridged, and often hollow, providing structural support for the plant and facilitating nutrient transport (Small, 2015). The leaves are palmate, typically with 5–11 serrated leaflets radiating from a central point (Clarke & Merlin, 2016). The number of leaflets can vary depending on the variety, growth stage, and environmental conditions. The arrangement of the leaves on the stem, known as phyllotaxy, can vary, with opposite or alternating patterns occurring from one variety to another.

2.1.4 Flowers and inflorescences

Cannabis is generally an unisexual plant, meaning that male and female flowers are produced on separate plants. However, separate male and female flowers may also occur. Particularly in certain strains or under specific environmental conditions (Small, 2015), male flowers are small and grouped into inflorescences, which release pollen for fertilization. Female flowers are grouped into dense, conical inflorescences, often called “buds.” These inflorescences are covered with glandular trichomes that produce resins containing cannabinoids and terpenes, which are responsible for the plant’s diverse pharmacological effects.

2.1.5 Factors affecting growth and morphology

Genetic factors play a major role in determining the growth and morphology of cannabis (Small, 2015). Different strains and cultivated varieties display different growth patterns, branching habits, leaf patterns, and cannabinoid profiles due to their specific genetic makeup. Environmental conditions, including light intensity, temperature, water content, and nutrient levels, also significantly affect the growth and morphology of cannabis (Clarke & Merlin, 2016). For example, plants grown under high-intensity light tend to be shorter and bushier, while plants grown under low-intensity light tend to be taller and longer. In addition, cultivation practices, such as pruning, training, and spacing, can significantly affect the shape, size, and yield of a plant.

2.1.6 Adaptability and Varieties

Cannabis has demonstrated remarkable adaptability to a wide range of environments. From temperate to tropical regions, this adaptability is reflected in a wide range of morphological and growth characteristics. For example, some plant species adapt to arid environments by developing thick, leathery leaves to reduce water loss, while others adapt to humid environments by developing thin, delicate leaves to increase transpiration. Plant morphology can also be influenced by abiotic factors, such as herbivory and competition. For example, some plant species develop thorns or prickles to fend off herbivores, while others develop tall, slender stems to compete for light in dense vegetation.

2.1.7 Chemical Composition

Cannabis sativa L. is a species with a long history of use by humans and possesses a complex chemical profile that includes hundreds of different compounds. This phytochemical diversity is the foundation of the plant's multifaceted effects and therapeutic potential. A comprehensive understanding of the chemical makeup of cannabis is essential for researchers, growers, and consumers to make informed decisions regarding its cultivation, use, and safety.

Cannabinoids are a group of compounds unique to cannabis and are crucial for its pharmacological activity. These molecules interact with the endocannabinoid system (ECS) in humans and other mammals, a complex signaling network involved in regulating a wide range of physiological processes, including pain perception, appetite, mood regulation, and memory. The most widely studied cannabinoids are Δ9 - tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is responsible for the psychoactive effects of cannabis, which include euphoria, cognitive changes, and impaired judgment. In contrast, CBD is non-intoxicating and has garnered significant attention for its potential therapeutic benefits, including anti-inflammatory, anti-anxiety, and anti-seizure properties (Russo, 2011). In addition to these two prominent cannabinoids, other compounds such as cannabinol (CBN), cannabigerol (CBG), and tetrahydrocannabivarin (THCV) also contribute to the overall effects of cannabis and are being actively studied for their potential therapeutic applications (Hanuš et al., 2016).

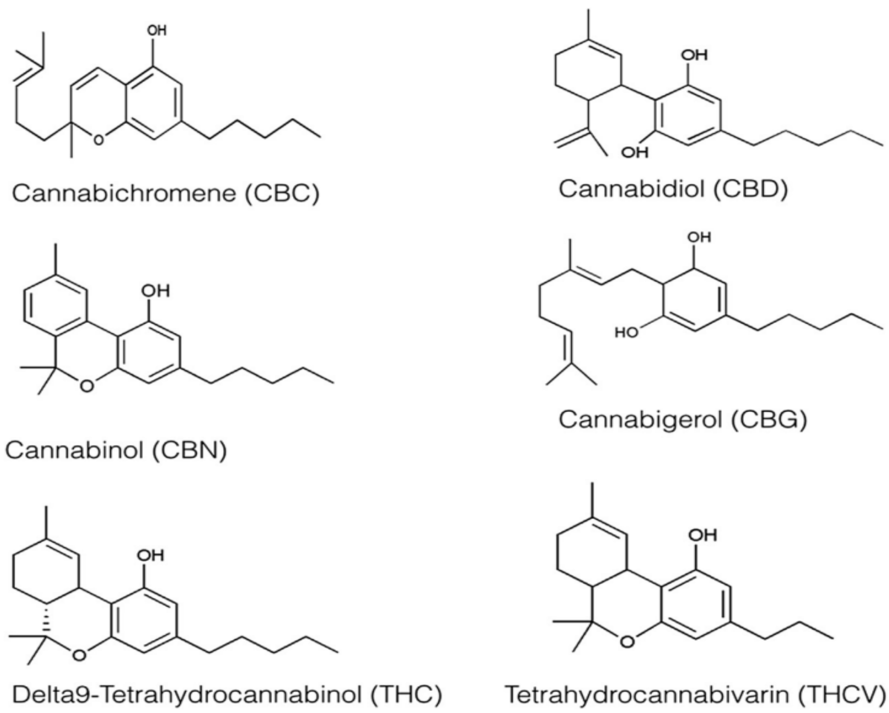

Figure 2. The structure of cannabinoids present in Cannabis (Marcu, 2016).

Terpenes are aromatic compounds commonly found throughout the plant kingdom and are responsible for the distinct aromas and flavors of different cannabis strains. However, the role of terpenes extends beyond sensory perception. They can interact with other cannabinoids and compounds in the plant, potentially modifying their pharmacological effects. This synergistic interaction, often referred to as the "entourage effect," suggests that the combined effects of these compounds exceed the sum of their individual effects (Russo, 2011). Common terpenes in cannabis include myrcene, limonene, pinene, and linalool, each with its unique aroma and potential therapeutic properties (Booth & Bohlmann, 2019). For example, myrcene is associated with sedative effects, while limonene is believed to enhance mood. The diverse terpene profiles of cannabis strains contribute not only to their sensory characteristics but also to their therapeutic potential.

Flavonoids, a group of polyphenol compounds found in many plant species, are another important component of the chemical makeup of cannabis. These compounds contribute to the plant's pigmentation and have been linked to various health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties (Andre et al., 2016) Cannabis contains a variety of flavonoids, including cannaflavin A, cannaflavin B, and apigenin. Cannaflavins A and B, which are unique to cannabis, have potent anti-inflammatory properties comparable to traditional non-steroidal anti-inflammatory drugs (NSAIDs) (Barrett & Bradley, 2016). These flavonoids may play a role in cannabis's effectiveness in treating inflammatory conditions such as arthritis and inflammatory bowel disease.

The chemical composition of cannabis is constantly changing and is influenced by several factors, including genetics, environment, and cultivation practices. Different strains or chemotypes exhibit unique chemical profiles due to their genetic makeup, resulting in variations in cannabinoid and terpene content. Environmental factors, such as light intensity, temperature, and nutrient availability, can affect the production of these compounds (Wanas et al., 2020). Additionally, cultivation practices, including harvest timing and drying methods, can influence the final chemical composition of the plant material. Understanding these factors is crucial for cultivating cannabis with specific chemical profiles suited to various therapeutic or recreational uses.

2.1.8 Uses and Applications

Cannabis, which includes a variety of chemovars with different concentrations of cannabinoids such as Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), has seen a renewed interest in its therapeutic potential. Beyond its historical use for recreational and spiritual purposes, modern research is uncovering the diverse applications of cannabis in managing various health conditions, leading to a reevaluation of its role in contemporary medicine. One of the most promising areas for cannabis-based therapies is the treatment of neurological disorders. CBD, in particular, has gained significant attention for its anticonvulsant properties. Clinical trials have demonstrated its effectiveness in reducing the frequency of seizures in individuals with refractory epilepsy, especially in cases such as Dravet syndrome and Lennox-Gastaut syndrome. This often leads to a reduced need for multiple medications and improves the quality of life (Devinsky et al., 2017; Thiele et al., 2018) Additionally, preclinical and observational studies suggest that cannabinoids may have neuroprotective effects in conditions like multiple sclerosis and Parkinson's disease, potentially helping to slow disease progression and alleviate symptoms (Giacoppo et al., 2014).

In addition to its neurological applications, cannabis shows promise in the realm of mental health. Although the evidence is still developing and more rigorous research is needed, preliminary findings indicate that CBD may have anxiety-reducing (anxiolytic) and antipsychotic properties. Research has investigated its potential to alleviate anxiety symptoms in individuals with social anxiety disorder and post-traumatic stress disorder (PTSD) (Blessing et al., 2015). Furthermore, some studies suggest that CBD may help reduce psychotic symptoms in schizophrenia, potentially serving as an alternative or complement to traditional antipsychotic medications (McGuire et al., 2018). Cannabis also holds promise in palliative care, as its ability to relieve a variety of symptoms can significantly improve the well-being of patients with life-limiting illnesses. It is effective in managing chronic pain, reducing nausea and vomiting caused by chemotherapy, and stimulating appetite, thus enhancing the quality of life for patients with cancer and other debilitating conditions (Portenoy et al., 2012). Additionally, emerging evidence suggests that cannabinoids may have antitumor properties, inhibiting the growth and spread of cancer cells in preclinical models (Velasco et al., 2012). While further research is required to translate these findings into clinical practice, the potential benefits of cannabis in palliative care cannot be overlooked.

Despite the increasing evidence supporting the therapeutic uses of cannabis, concerns about its application persist. Significant issues include the potential for abuse, dependence, and negative effects on cognitive function, especially among adolescents and young adults (Volkow et al., 2014). Additionally, the complex relationship between cannabinoids and the endocannabinoid system requires further investigation to fully understand the mechanisms of action, optimal dosages, and long-term effects of cannabis use. As our scientific understanding of cannabis evolves and the legal landscape changes, it is crucial to adopt a balanced approach that weighs the potential benefits against the associated risks, ensuring the safe and effective integration of cannabis into medical practice.

2.2 Residues in cannabis and other crops cultivation

Continuous sources of both leadership and observation pose significant challenges for agriculture today, particularly in the health of organisms and the quality of cannabis seeds and other crop production. Orange residues can result from the use of pesticides, heavy fertilizers, and microbial additives, which can persist and accumulate as compounds. This accumulation influences both growers and regulatory agents, especially in medical applications, where longer residue limits are often required due to potential inhalation or deficiencies (Small, 2017). Unlike traditional crops, certain products can be developed without needing to meet initial food control regulations, allowing access to a broader range of compounds. Regulatory frameworks are evolving primarily to address these issues (Eichler et al., 2023), but a crucial area for progress lies in harmonizing global standards (Upton, 2018).

Pesticide residues are the most common problem for crops, especially without synthetic lead, such as pyrethroids, organophosphates, and neonicotinoids. Control and disease systems, but most residues may persist on the processing core. Chemicals that can be monitored for quantification and filtration of the endonuclease Carcinogenic and morbidity to pain (Van Maele-Fabry et al., 2017). In the long-term control of signaling, substances can be monitored normally due to specific reasons and quality control and accumulate lipophilic substances (Citti et al., 2018). Analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and later tandem chromatography-mass spectrometry (LC-MS/MS) have been recorded for a long time for the number of residues, providing both a control system and a memory of the meat (Strashnov et al., 2024).

Fertilizer waste to fertilizer waste with important components that cause more problems. Inside most fertilizers, nitrates dissolve into water causing eutrophication and directions in drinking water sources (López-Bellido et al., 2010). In the cultivation of cannabis, Hydronic systems and the use of concentrated nutrients have clearly shown increased accumulation of manure residues. Increased nitrate and phosphate residues in cannabis products (Caplan et al., 2017) have been observed to be a good balance of toxic aromas such as nitrosamines. Scientists must strike a balance between delivering good nutrients and environmentally absorbable spices, including vegetarian,s and slow release fertilizers (Chien et al., 2009).

Residues of heavy metals such as lead, cadmium, and arsenic continue to be a concern in important plant species. Heavy metals are particularly toxic to plants, and heavy metals are particularly toxic to soils or water sources (Ahmed et al., 2022). The highest levels can be accumulated in leaves, which is why consumers may use cannabis for medical purposes (Potter, 2014). Strategies to monitor heavy metal residues to soil remediation methods such as Soil remediation with plants and the use of biochar in the re-examination of more time-consuming raw materials (Komárek et al., 2013). Developing methods to reduce heavy metal concentrations to a minimum is important for both health and agricultural purposes. Microbial residues and pathogens such as Escherichia coli and Aspergillus species pose a risk to both hardware and public health. Cannabis, which often takes time to view without processing or visible components, is the source of the investigation found to be cruise and checkpoint (McPartland & Guy, 2017). For other crops, investigations are often based on food loss at frequent or adherence to practices. Organizational microbial management strategies, the use of control agents, and post-storage inspections are most important in controlling safety standards. In standard microbial residue testing, safety aspects (Ganguly et al., 2017),quality studies using advanced techniques provide assurance and reliability (Shah et al., 2020). This highlights the long-term persistence of cannabis and other crops. It emphasizes the need to manage pesticides, fertilizers, heavy metals, and microbial residues that can be harmful to consumers and the environment. Effective management is essential in farming practices to ensure that these harmful substances are controlled. Research should focus on developing strategies that establish and adapt safety standards compatible with both consumer health and environmental sustainability.

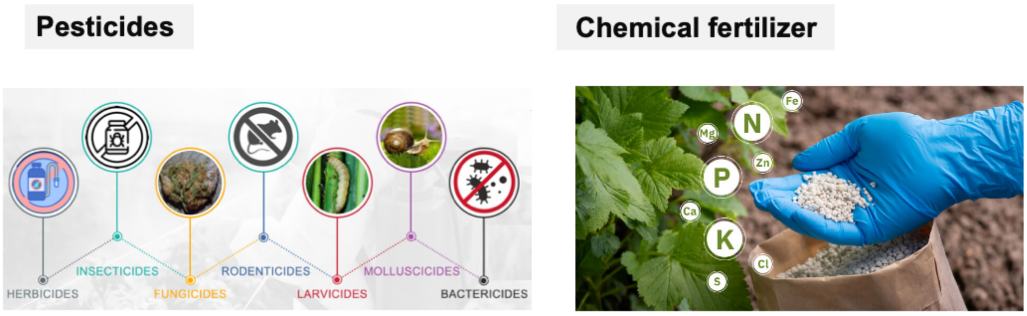

Figure 3. Residues associated with the agricultural practices of cannabis and various crops (Moscariello et al., 2021).

2.3 Plant Growth-Promoting Microorganisms (PGPM)

Plant growth-promoting microorganisms (PGPM) represent a diverse group of microbes that foster plant growth and enhance agricultural productivity by improving nutrient availability, promoting pathogen resistance, and aiding in stress tolerance. PGPM includes a wide range of organisms such as bacteria, fungi, and actinomycetes, all of which interact with plants in unique ways to improve plant health and yield. The use of PGPM in sustainable agriculture has gained significant attention due to its potential to reduce the reliance on chemical fertilizers, pesticides, and herbicides. The beneficial effects of these microorganisms on plant growth are driven by a range of mechanisms, including nitrogen fixation, phosphorus solubilization, hormone production, and disease suppression.

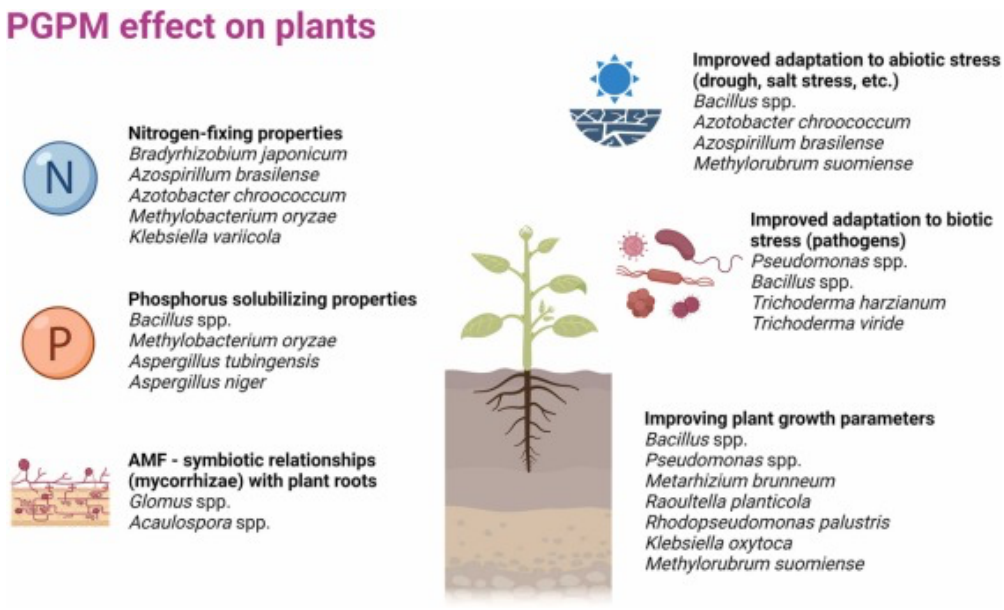

Figure 4. The impact of Plant Growth-Promoting Microorganisms (PGPM) on plant growth. Types of Plant Growth-Promoting Microorganisms (PGPM) (Szopa et al., 2022).

PGPMs can be broadly categorized based on their biological classification and mechanisms of action. The most commonly studied PGPM include bacteria, fungi, and actinomycetes.

2.3.1. Bacterial PGPM

Bacteria are the most studied and diverse group of PGPM. They inhabit various niches within the rhizosphere, endosphere, and soil, where they interact with plants. Some common genera of plant growth-promoting bacteria include Pseudomonas, *Bacillus*, *Azospirillum*, *Rhizobium,* and *Enterobacter*. These bacteria enhance plant growth through several mechanisms, such as nitrogen fixation, nutrient solubilization, production of plant growth regulators, and pathogen suppression.

Table 1. Plant growth-promoting bacterial strains are utilized as biocontrol agents to combat pathogenic microbes.

Host	Pathogen	Disease	strains	References
Apple	*Mucor piriformis*	*Mucor* rot	*Pseudomonas fluorescens*	(Wallace et al., 2018)
Banana	*Fusarium* spp.	Postharvest diseases	*Trichoderma* spp.	(Snehalatharani et al., 2021)
Wheat	*Stagonospora nodorum*	*Stagonospora nodorum*	*Bacillus subtilis* 26DCryChS	(Maksimov et al., 2020)
Soybean	*Fusarium solani, Macrophomina phaseolina*	Root rot	*Bradyrhizobium* sp.	(Parveen et al., 2019)
	Sclerotinia sclerotiorum	White mold	*Butia archeri*	(Vitorino et al., 2020)
Strawberry	*Macrophomina phaseolina*	Charcoal rot disease	*Azospirillum brasilense*	(Viejobueno et al., 2021)
	Botrytis cinerea	Gray mold	*Bacillus amyloliquefaciens* Y1	(Maung et al., 2021)
Maize	*Fusarium graminearum*	Stalk rot	*Bacillus methylotrophicus*	(Cheng et al., 2019)
Mungbean	*Cercospora canescens*	*Cercospora* Leaf Spot	*Bacillus velezensis* S141	(Songwattana et al., 2023)
Tomato	*Fusarium oxysporum* f. sp. *lycopersici*	*Fusarium* wilt	*Brevibacillus brevis*	(Chandel et al., 2010)
Tea	*Colletotrichum* sp,	Shoot necrosis	*Trichoderma camelliae*	(Chakruno et al., 2022)

2.3.2 Fungal PGPM

Fungi, especially mycorrhizal fungi, play an important role in promoting plant growth through symbiotic relationships with plant roots. These fungi enhance nutrient uptake, particularly for nutrients such as phosphorus, and also provide protection against soil-borne pathogens.

Table 2. Plant growth-promoting fungal strains are utilized as biocontrol agents to combat pathogenic microbes.

Host	Pathogen	Disease	strains	References
Rice	*Helminthosporium oryzae, Bipolaris oryzae*	*Leaf brown spot*	*Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum*	(Khalili et al., 2012; Mau et al., 2022)
Tomato	*Sclerotium rolfsii*	Southern blight	*Stenotrophomonas maltophilia PPB3*	(Sultana & Hossain, 2022)
	Fusarium oxysporum f. sp. lycopersici	Wilt	*Penicillium oxalicum*	(Murugan et al., 2020)
	Rhizophagus intraradices	*Verticillium* wilt	*Penicillium pinophilum*	(Ibiang et al., 2021)
Sweet potato	*Ceratocystis fimbriata*	Black rot disease	*Pseudomonas chlororaphis subsp. aureofaciens SPS-41*	(Zhang et al., 2021)

2.3.3 Mechanisms of Plant Growth Promotion by PGPM

PGPM promotes plant growth through a variety of mechanisms, which can be broadly categorized into direct and indirect mechanisms.

2.3.3.1 Direct Mechanisms

- Nutrient Acquisition: PGPM enhances the availability of essential nutrients, particularly nitrogen and phosphorus, through nitrogen fixation and phosphate solubilization. These processes increase the nutrient supply to plants, leading to enhanced growth (Malgioglio et al., 2022).

- Hormonal Regulation: Many PGPMsM produce plant hormones such as auxins, cytokinin's, and gibberellins. These hormones promote root elongation, stimulate shoot growth, and enhance flowering. By regulating these processes, PGP contributes to overall plant development (Glick, 2014)

- Improvement of Root System: The application of PGPM can stimulate root growth by producing auxins and other growth regulators. Enhanced root growth improves the plant's ability to absorb water and nutrients, leading to better overall plant health (Bashan et al., 2013).

2.3.3.2 Indirect Mechanisms

- Pathogen Suppression: PGPM can suppress plant diseases by outcompeting pathogens for space and nutrients, producing antimicrobial compounds, or inducing systemic resistance in plants. This reduces the need for chemical pesticides and supports healthy crop growth (Vinale et al., 2014)

- Environmental Stress Resistance: PGPM enhances plant resistance to abiotic stresses such as drought, salinity, and temperature extremes. These microorganisms produce Osmo protectants, modulate ion uptake, and activate plant defense mechanisms to protect plants from environmental stress (Sharma et al., 2013).

2.3.4 Applications of PGPM in Agriculture

PGPMsM are utilized in agriculture to improve soil health, enhance plant growth, and protect crops from diseases. The following are some common applications of PGPM.

2.3.4.1 Biofertilizers

PGPM such as nitrogen-fixing bacteria (*Rhizobium*, *Azospirillum*) and phosphate-solubilizing bacteria (*Bacillus*, *Pseudomonas*) are used as biofertilizers to supplement or replace chemical fertilizers. These microorganisms promote nutrient availability, enhance soil fertility, and reduce the environmental impact of synthetic fertilizers (Bashan et al., 2013)

2.3.4.2 Biocontrol

PGPMsPM are used as biocontrol agents to manage plant diseases caused by fungi, bacteria, and viruses. Trichoderma species, Pseudomonas species, and Bacillus species are examples of microorganisms that can suppress plant pathogens and reduce the reliance on chemical pesticides (Vinale et al., 2014)

2.3.4.3 Stress Management

PGPM can also be employed to improve crop resilience under stressful conditions such as drought, salinity, and extreme temperatures. By enhancing nutrient uptake and promoting plant growth, PG helps plants tolerate environmental stress, leading to higher yields and improved crop productivity (Malgioglio et al., 2022).

2.4 Plant growth-promoting bacteria (PGPBs)

Plant growth-promoting bacteria (PGPB) are indispensable in enhancing agricultural productivity by alleviating biotic and abiotic stressors. These microorganisms inhabit the rhizosphere, facilitating plant growth through diverse mechanisms, including nitrogen fixation, production of phytohormones, phosphate solubilization, and the synthesis of siderophores. For instance, *Azospirillum* and *Pseudomonas spp*. are renowned for promoting root architecture modification and enhancing nutrient uptake under stress conditions like salinity (Egamberdieva et al., 2019; Poria et al., 2022). Moreover, PGPB can mitigate oxidative stress in plants by enhancing antioxidant enzyme activities, contributing to improved drought and salt tolerance (Ramakrishna et al., 2020; Saikia et al., 2018).

One of the pivotal attributes of PGPB is their ability to confer resilience against saline soils, a challenge affecting approximately 20% of the world's arable land (Ondrasek et al., 2021). Salinity induces osmotic stress in plants, limiting water uptake and nutrient assimilation. Specific strains of *Bacillus* and *Halomonas* have demonstrated the ability to modulate the expression of salt-stress-responsive genes, thereby enhancing plant survival in saline environments (Qurashi & Sabri, 2012). Additionally, the production of exopolysaccharides by these bacteria helps in soil aggregation, thus improving root adhesion and water retention in the rhizosphere (Chen et al., 2016).

In heavy metal contaminated soils, PGPB enhances phytoremediation efficacy by transforming metals into less bioavailable forms or by sequestering them within plant tissues. For example, *Bacillus* and *Proteus* strains have shown significant potential in promoting plant growth while remediating lead and cadmium from polluted sites (Sorour et al., 2022).This dual role of PGPB in bioremediation and plant growth promotion underscores their utility in reclaiming degraded lands for agriculture (Kaya et al., 2024).

PGPB also demonstrates the potential to improve the productivity of marginal lands through integrated phytotechnologies. Marginal soils often suffer from nutrient deficiencies, salinity, or contamination. The use of microbial consortia tailored to specific soil conditions has proven to stabilize plant growth and optimize soil fertility. For instance, microbial formulations with strains such as Pseudomonas thivervalensis have significantly increased biomass and nutrient uptake in Brassica napus grown in copper-contaminated soils (Ren et al., 2019). Such approaches exemplify sustainable agricultural practices to rehabilitate degraded ecosystems.

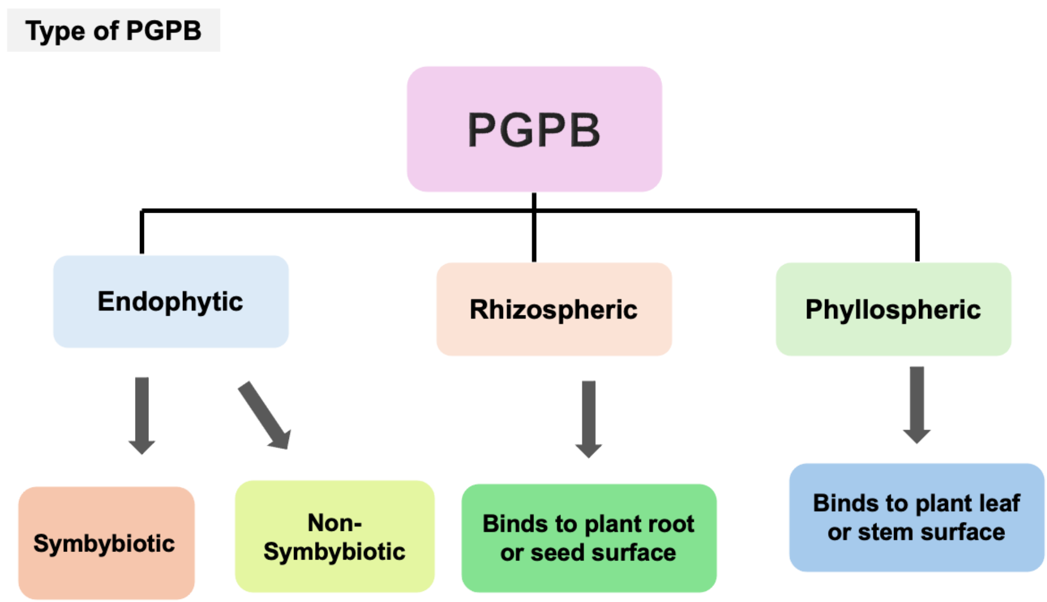

Figure 5. Plant growth-promoting bacteria (PGPB) (Stegelmeier et al., 2022).

2.4.1 Benefits of Plant Growth-Promoting Bacteria (PGPBs) in Cannabis and Other Crops

Plant growth-promoting bacteria (PGPBs) are vital for enhancing the growth, productivity, and resilience of cannabis and other economically significant crops. In cannabis, PGPBs like Bacillus and Pseudomonas species facilitate nutrient acquisition by solubilizing phosphate and fixing atmospheric nitrogen, which are critical for the plant's rapid vegetative and flowering phases (Backer et al., 2018). These microorganisms also produce phytohormones such as indole-3-acetic acid (IAA), which stimulates root elongation and branching, leading to improved nutrient uptake (Numan et al., 2018). Moreover, the inoculation of cannabis with PGPBs has been linked to higher cannabinoid yields, emphasizing their role in secondary metabolite production under controlled cultivation conditions (Lyu et al., 2020). In addition to nutrient acquisition, PGPBs protect plants from abiotic stresses like salinity and drought, challenges commonly faced in cannabis cultivation. *Bacillus subtilis* and *Azospirillum brasilense* produce exopolysaccharides that improve soil structure, enhancing water retention and alleviating salt stress in the rhizosphere (Egamberdieva et al., 2019). Furthermore, these bacteria increase the expression of stress-responsive genes, enabling crops like rice and wheat to withstand adverse conditions, a mechanism equally applicable to cannabis (Poria et al., 2022) Cannabis grown in saline soils showed enhanced growth and biomass production when treated with PGPBs capable of modulating ionic balance through sodium exclusion.

PGPBs are also instrumental in mitigating biotic stresses caused by pathogens and pests. They produce antifungal metabolites, including lipopeptides and siderophores, which inhibit the growth of phytopathogenic fungi such as Fusarium and Pythium species (Palaniyandi et al., 2014). This is particularly significant for cannabis, a crop prone to fungal infections in its humid cultivation environment. Studies on tomatoes and cucumbers have shown that Pseudomonas fluorescens reduces the incidence of fungal diseases by up to 70% (Numan et al., 2018). Similar results have been observed in cannabis cultivation systems, where PGPB application leads to reduced disease prevalence and enhanced plant vigor (Lyu et al., 2020). PGPBs also contribute to sustainable agriculture by reducing the reliance on chemical fertilizers and pesticides. In maize and wheat, PGPBs have been reported to increase nitrogen use efficiency by up to 40%, leading to substantial reductions in synthetic fertilizer applications (Egamberdieva et al., 2019). Similarly, in cannabis cultivation, the use of microbial consortia has been shown to lower chemical inputs while maintaining high yields and quality (Poria et al., 2022). This aligns with the increasing demand for environmentally sustainable practices in commercial cannabis and other crop production. Lastly, PGPBs facilitate the phytoremediation of contaminated soils, enabling cannabis and other crops to thrive in marginal lands. By producing organic acids and chelating agents, PGPBs enhance the bioavailability of heavy metals, which plants can then accumulate or stabilize (Ledin, 2000). For instance, cannabis treated with Pseudomonas spp. has shown enhanced growth on cadmium-contaminated soils (Sorour et al., 2022). These findings underscore the potential of PGPBs not only in agricultural productivity but also in environmental remediation.

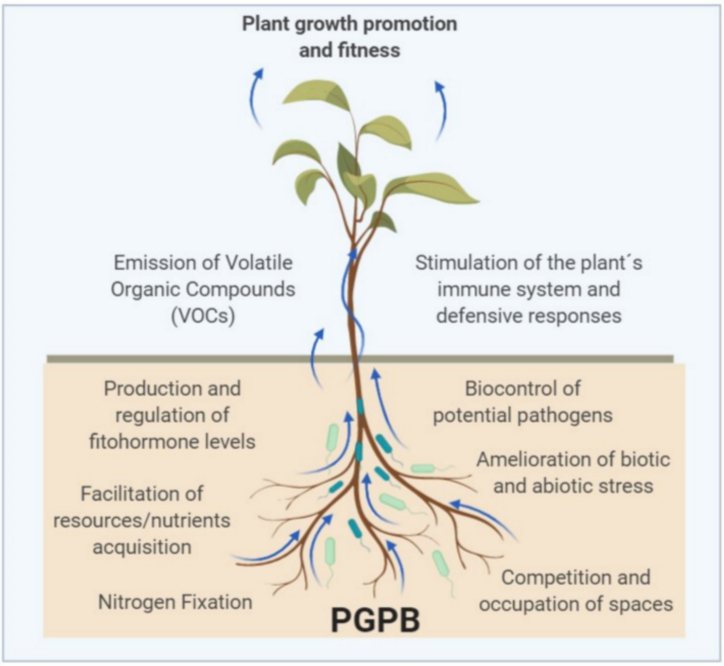

Figure 6. Beneficial activities performed by plant growth-promoting bacteria (PGPB) to promote optimal plant growth and fitness (Orozco-Mosqueda et al., 2021).

2.4.2 Mechanisms of Action of Plant Growth-Promoting Bacteria (PGPBs)

PGPBs utilize multifaceted mechanisms to enhance plant growth and resilience, acting directly or indirectly on plant physiology and soil chemistry. A primary mechanism involves nutrient acquisition facilitation, such as nitrogen fixation and phosphate solubilization. Nitrogen-fixing bacteria, including *Rhizobium* and *Azospirillum* species, convert atmospheric nitrogen into ammonia, making it accessible to plants (Kumar et al., 2018). Phosphate-solubilizing PGPBs like *Bacillus* and *Pseudomonas* secrete organic acids that release phosphate bound in soil minerals, thereby increasing its bioavailability for uptake by plant roots (Egamberdieva et al., 2019). Additionally, potassium- and zinc-solubilizing bacteria enhance the availability of these essential nutrients, contributing to optimal plant development. Another critical mechanism is phytohormone production, which directly influences plant growth and stress response. PGPBs produce hormones like indole-3-acetic acid (IAA), gibberellins, and cytokinins that modulate root architecture, leading to improved nutrient and water uptake (Patten & Glick, 2002). IAA promotes root elongation and lateral root formation, while gibberellins stimulate shoot elongation and seed germination. Furthermore, some PGPBs regulate ethylene levels in plants under stress by synthesizing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which lowers ethylene concentrations that would otherwise inhibit plant growth under adverse conditions (Glick, 2014). PGPBs also enhance plant defense mechanisms by inducing systemic resistance and producing antimicrobial compounds. Induced systemic resistance (ISR) involves priming the plant's immune system to better respond to pathogen attacks, mediated by signaling molecules such as jasmonic acid and salicylic acid (Van Wees et al., 2008). PGPBs like *Bacillus subtilis* and *Pseudomonas* fluorescens produce lipopeptides, siderophores, and enzymes that inhibit the growth of phytopathogens, providing an effective biological control mechanism (Choudhary & Johri, 2009). These interactions reduce the dependence on chemical pesticides, promoting sustainable agricultural practices.

PGPBs influence soil structure and function through their interactions with the rhizosphere microbiome. They secrete exopolysaccharides that enhance soil aggregation, improving water retention and aeration (Qurashi & Sabri, 2012). These bacteria also alter rhizosphere microbial communities by fostering beneficial interactions and suppressing harmful microorganisms. For example, some PGPBs promote the growth of arbuscular mycorrhizal fungi, which synergistically improve nutrient uptake and plant health (Smith & Read, 2010). This microbial synergy underscores the holistic impact of PGPBs on plant-microbe-soil interactions. Finally, PGPBs mitigate the effects of abiotic stresses such as salinity, drought, and heavy metal contamination. They achieve this by producing osmolytes, enhancing antioxidant enzyme activities, and altering ionic balances in plants under stress (Egamberdieva et al., 2019). Moreover, PGPBs facilitate phytoremediation by transforming toxic heavy metals into less bioavailable forms or by promoting their accumulation in plant tissues (Poria et al., 2022). These mechanisms not only improve plant survival under challenging conditions but also contribute to environmental sustainability by reclaiming contaminated soils.

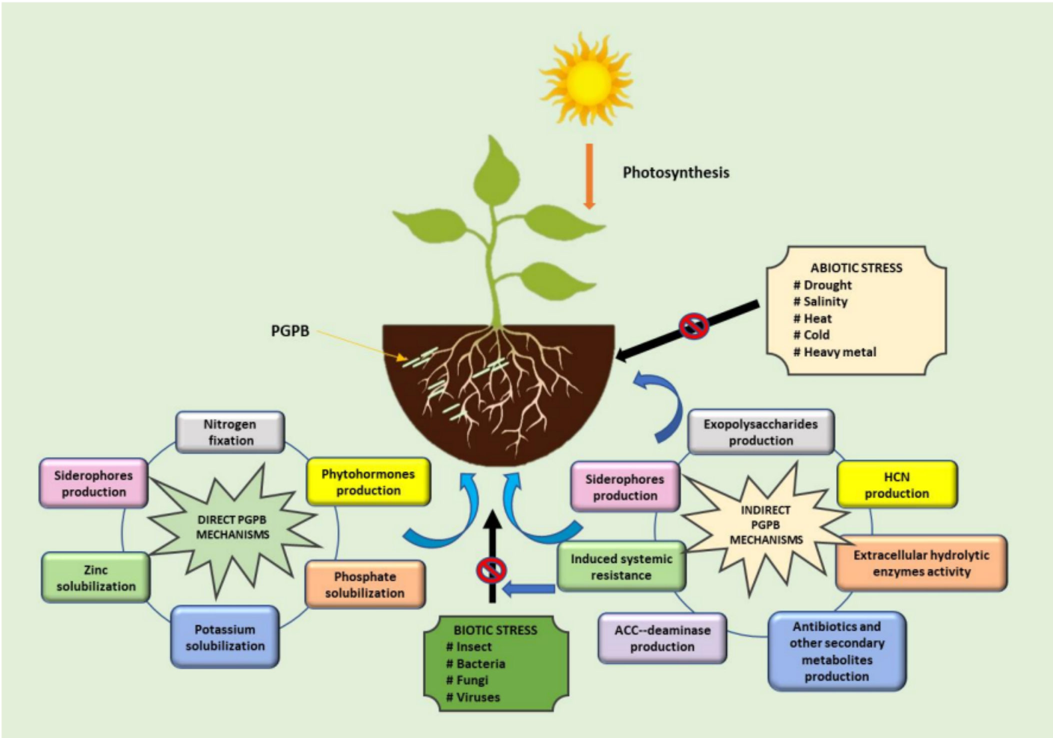

Figure 7. Mechanisms of PGPB, both direct and indirect, that contribute to plant development (Ajijah et al., 2023).

2.4.3 Application of PGPBs

The application of Plant Growth-Promoting Bacteria (PGPBs) spans diverse agricultural and environmental domains, leveraging their multifaceted benefits for sustainable development. One of their primary roles is enhancing crop productivity in nutrient poor soils. PGPBs such as *Rhizobium*, *Azospirillum*, and *Bacillus* species are used as biofertilizers to augment nitrogen fixation, phosphorus solubilization, and micronutrient availability (Kumar & Verma, 2018). Their use has proven effective in crops like legumes, cereals, and vegetables, where they increase yields and reduce dependence on chemical fertilizers. For instance, inoculation with *Azospirillum* in maize enhances nitrogen uptake, leading to higher grain production (Egamberdieva et al., 2019).

In the context of abiotic stress management, PGPBs play a pivotal role in mitigating the adverse effects of salinity, drought, and heavy metal toxicity. For instance, *Bacillus* and *Pseudomonas* strains enhance the tolerance of crops like rice and wheat to salinity by modulating ionic balances and producing exopolysaccharides (Ramakrishna et al., 2020). Drought resilience is improved through the production of osmolytes and phytohormones, which regulate water use efficiency in crops such as tomatoes and soybeans (Glick, 2014). Additionally, in phytoremediation, PGPBs like *Pseudomonas* and *Bacillus facilitate* the reclamation of contaminated soils by aiding in the uptake or transformation of heavy metals into less bioavailable forms, enabling plants like mustard and sunflower to thrive in polluted environments (Poria et al., 2022).

The use of PGPBs in disease management highlights their potential as eco-friendly alternatives to synthetic pesticides. These bacteria produce antimicrobial compounds, including siderophores, lipopeptides, and enzymes, which inhibit the growth of plant pathogens (Palaniyandi et al., 2014). For example, *Pseudomonas* fluorescens has been shown to suppress Fusarium wilt in tomatoes, significantly reducing disease incidence (Van Wees et al., 2008). Furthermore, PGPBs induce systemic resistance in plants, priming their immune systems against biotic stressors without the need for direct microbial contact (Choudhary & Johri, 2009).

In horticulture and controlled environment agriculture, PGPBs are gaining attention for enhancing the quality and yield of high value crops like cannabis and strawberries. By stimulating root development and nutrient uptake, they improve biomass production and the concentration of secondary metabolites, such as cannabinoids in cannabis and anthocyanins in strawberries (Lyu et al., 2020). Similarly, the integration of PGPBs into hydroponic systems has demonstrated increased efficiency in nutrient cycling and disease suppression, contributing to higher productivity (Backer et al., 2018).

Beyond agriculture, PGPBs contribute to ecological restoration and land reclamation efforts. They are applied to revegetate degraded lands and enhance soil fertility in marginal environments, including saline or eroded soils (Qurashi & Sabri, 2012). The combined use of PGPBs with native plant species has been particularly effective in stabilizing soil structure and promoting biodiversity in degraded ecosystems (Smith & Read, 2010). Their broad applicability underscores their role as a cornerstone of sustainable agricultural practices and environmental stewardship.

2.4.4 Strain Selection for Plant Growth-Promoting Bacteria (PGPBs)

Strain selection is a pivotal step in harnessing Plant Growth-Promoting Bacteria (PGPBs) for agricultural and environmental applications, as the effectiveness of PGPBs depends significantly on the strain's intrinsic properties and compatibility with target crops. Ideal strains should exhibit robust plant growth-promoting traits, including nitrogen fixation, phosphate solubilization, and the production of phytohormones like indole-3-acetic acid (IAA) (Kumar & Verma, 2018). For instance, *Azospirillum brasilense* and *Pseudomonas* fluorescens have demonstrated exceptional efficacy in enhancing root development and nutrient uptake across multiple crop species (Egamberdieva et al., 2019). The ability of a strain to colonize plant roots effectively and establish a stable rhizosphere population is another critical criterion for selection (Compant et al., 2010).

Abiotic stress tolerance is a key consideration in strain selection, especially for applications in challenging environments such as saline, drought-prone, or heavy metal-contaminated soils. Strains like *Bacillus subtilis* and *Halomonas spp.* are often prioritized for their resilience under high salinity and their production of exopolysaccharides, which improve soil structure and plant water retention (Ramakrishna et al., 2020). Similarly, *Pseudomonas putida* and *Arthrobacter globiformis* are noted for their abilities to degrade organic pollutants and detoxify heavy metals, enabling plant growth in contaminated sites (Poria et al., 2022). Advanced screening techniques, including stress-specific assays and genomic analyses, are increasingly employed to identify strains with superior stress tolerance and environmental adaptability.

Compatibility with the host plant is a critical determinant of PGPB efficacy. Host-specific interactions between plants and bacterial strains often dictate the success of inoculation. For example, rhizobial strains must be compatible with the legume species they colonize to establish functional nitrogen-fixing nodules (Smith & Read, 2010). Similarly, certain strains of *Bacillus* and *Trichoderma* exhibit a preference for specific crops, enhancing growth through unique biochemical pathways (Lugtenberg & Kamilova, 2009). Researchers often perform greenhouse and field trials to evaluate the symbiotic compatibility and agronomic benefits of candidate strains.

Molecular and omics-based approaches have revolutionized strain selection by providing insights into the genetic determinants of PGP traits. Genome sequencing and comparative genomics allow for the identification of genes responsible for nitrogen fixation, ACC deaminase production, and secondary metabolite biosynthesis (Glick, 2014). Metagenomic studies of rhizosphere microbiomes further aid in discovering novel strains with untapped potential for plant growth promotion (Compant et al., 2010). These tools facilitate the development of custom-tailored PGPB consortia optimized for specific agroecological conditions.

To maximize the benefits of strain selection, regulatory considerations must also be addressed. Strains intended for commercial use must undergo rigorous safety evaluations to ensure they are non-pathogenic and environmentally benign (Authority et al., 2018). Additionally, they should exhibit genetic stability to prevent horizontal gene transfer that could compromise biosafety (Pal & Gardener, 2006). By integrating biological, molecular, and regulatory criteria, strain selection can advance the effective and sustainable use of PGPBs in agriculture and environmental management.

2.4.5 Regulations for the Use of Plant Growth-Promoting Bacteria (PGPBs)

The use of Plant Growth-Promoting Bacteria (PGPBs) in agriculture and environmental management is subject to rigorous regulatory frameworks to ensure safety, efficacy, and environmental sustainability. In the United States, the Environmental Protection Agency (EPA) regulates PGPBs under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as microbial pesticides or biostimulants (Insecticide). Manufacturers must provide comprehensive data on the identity, mode of action, and potential environmental and human health impacts of the microbial product. This process includes risk assessments for pathogenicity, allergenicity, and genetic stability, ensuring that only strains with minimal risk profiles are approved for commercial use (Pal & Gardener, 2006).

In the European Union, PGPBs are categorized under the Plant Protection Products Regulation (Regulation (EC) No 1107/2009) (Villaverde et al., 2014). The European Food Safety Authority (EFSA) evaluates microbial products for their safety and efficacy, requiring detailed dossiers on production processes, strain identification, and ecological risks (Insecticide). Specific attention is given to the potential for horizontal gene transfer and the impact of released PGPBs on non-target organisms, including beneficial soil microbes (Compant et al., 2010). Furthermore, the EU enforces stringent traceability and labeling requirements to ensure transparency and consumer confidence in microbial products.

Countries in Asia, such as India and China, have also established frameworks for PGPB regulation, albeit with varying levels of stringency. In India, the Fertilizer (Control) Order 1985 governs biofertilizers, including PGPBs, and mandates quality testing for microbial density, purity, and performance (Yadav et al., 1985). China, through its Ministry of Agriculture and Rural Affairs, requires that microbial inoculants undergo multi-year field trials to verify their agronomic benefits and absence of adverse environmental effects (Meng et al., 2022). Both nations emphasize the importance of aligning microbial product development with sustainable agricultural goals, reflecting global trends toward eco-friendly farming practices.

Globally, the regulatory landscape for PGPBs is evolving to accommodate advances in biotechnology and microbial genomics. The Cartagena Protocol on Biosafety provides an international framework for regulating living-modified organisms, including genetically engineered PGPBs (Diversity, 2000). It mandates risk assessments and promotes information exchange among nations, facilitating the safe cross-border movement of microbial products. This is particularly relevant for multinational corporations developing microbial solutions for diverse agro-ecological zones.

While regulations aim to safeguard human and environmental health, they also present challenges such as high costs and lengthy approval processes, potentially stifling innovation. Calls for harmonized international guidelines have emerged to streamline regulatory pathways while maintaining rigorous safety standards

(Ravensberg, 2011). Policymakers and stakeholders are increasingly advocating for risk-based, tiered approaches that balance safety with the need for rapid deployment of PGPBs to address global challenges such as food security and climate resilience.

2.5 *Bacillus velezensis*

Bacillus velezensis, a Gram-positive, spore-forming bacterium within the Bacillus subtilis species complex, has garnered attention due to its dual role as both a biocontrol agent and a plant growth-promoting rhizobacterium (PGPR). Commonly inhabiting the rhizosphere, *B. velezensis* establishes beneficial interactions with a wide variety of plant species. Its ability to adapt to different environmental conditions, coupled with the synthesis of a diverse array of bioactive compounds, makes it a highly effective and sustainable alternative to synthetic agricultural inputs, such as chemical fertilizers and pesticides. Consequently, *B. velezensis* is considered an essential tool in the development of environmentally sustainable farming practices (Fan et al., 2018; Rabbee et al., 2019).

One of the key attributes of *B. velezensis* is its production of secondary metabolites that exhibit potent antimicrobial activity. These bioactive compounds include lipopeptides such as surfactin, fencing, and iturin, as well as polyketides, including prolactin and difficidin. These metabolites play a crucial role in controlling plant pathogens by disrupting cell membranes and inhibiting essential metabolic processes. For instance, *B. velezensis* FZB42 has demonstrated substantial efficacy against soil-borne pathogens, including Fusarium spp. and Pythium spp., highlighting its utility in integrated pest management systems (Rabbee et al., 2019). Moreover, B. velezensis can form biofilms, which enhances its survival in the soil, ensuring long-term disease control and crop protection (Chowdhury et al., 2015).

In addition to pathogen suppression, *B. velezensis* significantly contributes to plant growth promotion by enhancing nutrient availability and producing phytohormones. The bacterium can solubilize phosphate, making it more accessible to plants, and synthesizing siderophores to sequester iron, alleviating micronutrient deficiencies in the rhizosphere (Singh, 2019). Furthermore, *B. velezensis* produces indole-3-acetic acid (IAA), a crucial plant hormone that stimulates root development, thereby improving the plant's ability to access water and nutrients, especially under suboptimal conditions (Hamid et al., 2021). This multifaceted functionality of *B. velezensis* underscores its potential to improve crop productivity, particularly in challenging environmental contexts.

The genomic analysis of *B. velezensis* has revealed a wealth of biosynthetic gene clusters responsible for the production of antimicrobial peptides and growth-promoting compounds. Notably, strains such as *B. velezensis* S141 contain unique genes associated with auxin production and nitrogen fixation. These genetic traits are particularly beneficial in symbiotic relationships with legumes, enhancing nodule formation and nitrogen fixation efficiency when co-inoculated with *Bradyrhizobium diazoefficiens*. Such genetic insights facilitate the development of precision microbial formulations tailored to specific crops and environmental conditions, thereby enhancing the bacterium's efficacy in agricultural applications (Sibponkrung et al., 2020).

Field studies conducted in Thailand have demonstrated the practical effectiveness of *B. velezensis* in controlling fungal diseases and promoting plant growth. For example, *B. velezensis* S141 has been successfully used to manage fungal pathogens in mung bean crops, while also improving nodulation and nitrogen fixation in soybean plants (Prakamhang et al., 2015; Songwattana et al., 2023). Beyond its pathogen-suppressing abilities, *B. velezensis* promotes soil health by enhancing soil aggregation and increasing water retention, thus supporting sustainable farming practices (Kenfaoui et al., 2024). These ecological benefits contribute to improved crop yields and reduce environmental degradation, aligning with global efforts to reduce the ecological footprint of agriculture and promote eco-friendly farming practices. Despite the promising applications of *B. velezensis*, several challenges remain that limit its widespread adoption. Environmental factors such as soil pH, texture, and temperature can influence the bacterium's performance, and the stability of microbial formulations during storage and transportation continues to be a significant hurdle (Rabbee et al., 2019). Ongoing research is focused on developing more robust microbial consortia and advanced delivery systems to address these challenges. By overcoming these limitations, *B. velezensis* has the potential to become a cornerstone of sustainable agriculture, contributing to food security and reducing the environmental impact of conventional farming practices (Chowdhury et al., 2015).

2.6 Sustainable agriculture

Sustainable agriculture embodies the integration of environmental stewardship, economic viability, and social equity to meet the needs of current and future generations. It focuses on preserving natural resources, enhancing soil fertility, and reducing ecological degradation while ensuring stable food production (Tilman et al., 2002). Central to this approach is the adoption of practices that maintain the balance of agroecosystems, including crop diversification, conservation tillage, and the judicious use of organic and inorganic inputs (Pretty, 2008). Such practices aim to mitigate climate change impacts and promote biodiversity, thus securing long-term agricultural productivity.

The integration of biological approaches, such as the use of Plant Growth-Promoting Bacteria (PGPBs) and natural pest control agents, exemplifies sustainable agricultural practices. PGPBs enhance nutrient acquisition, stress tolerance, and soil fertility while reducing the dependency on chemical fertilizers (Backer et al., 2018). Similarly, biological pest control using predators, parasitoids, and entomopathogenic fungi offers an environmentally friendly alternative to synthetic pesticides, mitigating the risks of pest resistance and environmental contamination (Koller et al., 2023). These approaches align with the principles of ecological intensification, which prioritize ecosystem services over external inputs.