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Cannabis Microbiome and the Role of Endophytes in Modulating the Production of Secondary Metabolites: An Overview

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Abstract

Plants, including Cannabis (*Cannabis sativa* subsp*. sativa)* host distinct beneficial microbial communities on and inside their tissues, designated the plant microbiota from the moment that they are planted into the soil as seed. They contribute to plant growth promotion, facilitating mineral nutrient uptake, inducing defense resistance against pathogens, higher yield and modulating plant secondary metabolites. Understanding the microbial partnerships with Cannabis has the potential to affect agricultural practices by improving plant fitness and the production yield of cannabinoids. Much less is known about this beneficial Cannabis-microbe partnership, and the complex relationship between the endogenous microbes associated with various tissues of the plant, particularly, the role that cannabis may play in supporting or enhancing them. This review will focus on Cannabis microbiota studies and the effect of endophytes on the elicitation of secondary metabolites production in Cannabis plants. The aim of this review is to shed light on the importance of Cannabis microbiome and how cannabinoid compounds concentration can be stimulated through symbiotic and or mutualistic relationships with endophytes.

Keywords: *cananbis sativa;* marijuana; hemp; microbiome; endophytes; secondary metabolites; Cannabinoids

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1. Introduction

Broadly, Cannabis (*Cannabis sativa L.*) refers to genetically different biotypes of both (non-intoxicant) industrial hemp and marijuana [1]. Differentiating strains of hemp from marijuana is based on an arbitrary threshold point of psychoactive compound, Δ9-tetrahydrocannabinol (THC) at 0.3%, a criterion established by Small and Cronquist [2].

Industrial hemp is cultivated for its fiber products, edible seeds products, and oilseed and non-psychoactive medicinal drugs [1]. Herbal marijuana, a term designated for the most frequently form of Cannabis, both for medical and recreational purposes produces some principal components of phytocannabinoids like Δ9-tetrahydrocannabinol (THC), the most intoxicating compound, cannabinol (CBN), cannabidiol (CBD), cannabidiol-carboxylic acid, cannabigerol, cannabichromene, and with therapeutic effect all of which are currently undergoing promising research [1]. These metabolites accumulate in all parts of the plant, however they are more concentrated in specialized secretory structures known as trichomes of the female flower buds [3,4]. In addition to phytocannabinoids, Cannabis produces a plethora of secondary metabolites which are produced as an adaptation for specific functions in plant mostly to improve plant growth or defense against biotic and abiotic stress that are important in agriculture [5]. These metabolites provide diverse biological activities for use in human medicine and the pharmaceutical industry [6]. Different commercial products include antibiotics, antifungal, and anticancer drugs [7].

Before the legalized use of *Cannabis sativa* in different countries, cultivation was restricted to hemp varieties of high-yielding fiber with significantly low levels of the psychoactive Δ9 -THC. The recent legalization of Cannabis in various countries, including Canada, Uruguay, and eleven states in the United States for the production of medical and/or recreational, have generated demand not only for high yielding varieties of Δ9 –THC and/or cannabinoids, but firm and reliable cannabinoid profiles. However, the legality of Cannabis for medical and recreational uses varies by country, in terms of its possession, distribution, and cultivation, and how it can be consumed and what medical conditions it can be used for [8,9]. The production of commercial and high-grade medical and/or recreational marijuana under indoor cultivation is influenced by production methods, and several environmental conditions [10], among them are light intensity, quality and photoperiod [11], storage temperatures and humidity [12], fertilization [13], abiotic elicitors

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including phytohormones [14,15], and the microbiome [16].

This Chapter aims to characterize the microbial diversity, associated with hemp and marijuana, show with recent examples the diversity of microbial communities (endophytes) that internalize their tissues and list the benefits that they confer to their hosts. We also highlight the values of the biologically active compounds produced by endophytes that contribute to increased plant fitness and tolerance against biotic and biotic stress. Moreover, we will provide some evidence that the microbial bioactive compounds produced by some endophytes are derivatives and/or analogs of their associated host plants.

2. The Plant Microbiome

Plants, including Cannabis host distinct beneficial microbial communities on and inside their tissues, designated the plant microbiota from the moment that they are planted into the soil as seed. The plant microbiome is composed of specific microbial communities associated with the roots and the soil surrounding the roots (i.e., the rhizosphere), the air-plant interface (i.e., the phyllosphere), and the internal tissues of the plant referred to as, the endosphere [17,18]. Seeds harbor diverse groups of microbiota that are a source of bio-inoculum for juvenile plants promoting protection against biotic and abiotic stress at seed germination and at later stages [19,20]. Each of these microhabitats provides suitable conditions for microbial life, which also has a respective function for the host. Plant microbiome is a contributing factor to plant health and productivity [21]. An increasing body of evidence highlights the importance of plant microbiome as a systemic booster of the plant immune system by priming accelerated activation of the defense system [22]. Many studies focused on the rhizosphere microbiome due to the soil-derived microbial diversity surrounding the root, and a potential source for selecting beneficial microbes that positively affects plant health [21,23,24]. Several reviews addressed the rhizosphere microbiome and the influence in conferring disease suppressiveness and improving drought resistance [21,25,26], while others studied contributing chemical components to selective enrichment of microorganisms in the rhizosphere [27,28]. Generally, above-ground plant microbiota mostly originated from the soil, seed and air adapt an endophytic lifestyle inhabiting tissues of the plant internally and play vital roles in plant development and fitness. These microbial

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communities internally inhabit plant tissues, are referred to as endophytes, and play a crucial role in plant development and growth [29]. In this study, we use the term endophyte based on the definition of Petrini to signify 'all organisms inhabiting plant organs that at some time in their life can colonize internal tissues without causing harm to their hosts' [30].

3. The Functions of Plant Microbiome are Essential for the Host

There is a considerable amount of information on the functional role of microbial communities associated with plants and their internal tissues. Plant growth promoting rhizobacteria (PGPR) and endophytes induce plant growth via the production of several phytohormones like auxins [22] gibberellins (GAs) abscisic acid (ABA) and ethylene (ET) induce plant growth and higher yield by producing these phytohormones or by eliciting the production of plant endogenous phytohormones including auxin [22], GAs, ABA and ET which modulate the endogenous phytohormone levels in the plant levels plant [31,32]. In general, Proteobacteria, and especially γ-Proteobacteria, such as *Bacillus, Staphylococcus, Pseudomonas,* and *Pantoea* were found to be the dominant endophytic bacteria isolated from a variety of plant species [33]. Moreover, gram–positive and gram-negative bacterial taxa including *Pseudomonas, Azospirillum, Azotobacter, Streptomyces, Enterobacter*, *Alcaligenes, Arthrobacter, Burkholderia* and *Bacillus* could enhance the plant growth and suppress phytopathogens [34]. Diverse strains of *Pseudomonas Bacillus*, *Arthrobacter* and *Pantoea* species associated with soybean and wheat roots exhibited growth-promotion properties like phytohormone production, mineral solubilization and the production of the enzyme 1-amino cyclopropnae-1-carbixylate (ACC) deaminase [35,36]. The role of ACC deaminase is to reduce the endogenous level of the stress hormone ET by limiting the amount of plant ACC deaminase , which in return prevents ET-induced root growth inhibition, and thus promotes plant growth and lowering stress susceptibility, in return, resulting more nitrogen supply for bacteria [37].

As with bacterial endophytes, fungal endophytes can facilitate mineral nutrient uptake, promote plant growth and development, and induce defense resistance against pathogens [38,39]. Furthermore, they enhance abiotic stress tolerance, particularly, the dark septate endophytic fungus, *Curvularia* sp. provided thermal protection for host plant at high temperature [39]. Indeed,

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bacteria and non-mycorrhizal fungi have the advantage of axenic propagation that place them as an ideal model of the agri-horticulture application.

One of the tools to control plant pathogens with the least impact on the environment is biocontrol. There are numerous examples of biocontrol activities of bacterial and fungal endophytes against pathogen invasion and diseases [17,40,41]. Various mechanisms underlie the beneficial effects of bacterial endophytes on their hosts. These include antibiotic production, induction of host defenses and immunity via induced systemic resistance (ISR), parasitism, competition and quorum sensing [42]. Equally, endophytic fungi can protect plants against pathogens by triggering host resistance via systemic acquired resistance and ISR [43,44], or by antibiosis and mycoprasitisim [41].

4. The Microbiome of Hemp and Marijuana

Understanding microbial partnerships with industrial hemp and medical and recreational marijuana have the ability to influence agricultural practices by improving plant fitness and production yield. Furthermore, marijuana and hemp are attractive models to explore plant-microbiome interactions as they produce numerous secondary metabolic compounds [45]. Together, the plant genome and the microbial genome that the plant harbors inside its tissues (i.e., the endorhiza) forms the holobiont which is now considered as one unit of selection in plant breeding and also a contributor to ecological services of nutrient mineralization and delivery, protection from pests and diseases, and tolerance to abiotic stress [46]. Increasing evidence suggests that the host genotype influences the composition and function of certain microbial key groups in the endorhiza, which, in turn, affect how the plant reacts to environmental stresses [19] with plant traits essential for hosting and supporting beneficial microbes. Particularly, in disease suppressive soils, populations of rhizospheric bacteria are enriched and act as the first line of defence in the host plant against root pathogens, thereby activating secondary metabolite biosynthetic gene clusters that encode NRPSs and PKSs to enhance the level of defense metabolites [47].

A growing body of evidence signals that a two-step selection model where plant type and soil type are the main drivers of defining soil microbial community structure [48,49]. The soil type

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defines the composition of the rhizosphere and root inhabiting bacterial communities, and migration from the rhizosphere into the endorhiza tissue is dependent on plant genotype [50]. Accordingly, the influence of soil type and plant genotype on the microbial community structure of marijuana offered support of the two-tier system model whereby microbial communities in the rhizosphere is driven by soil type, and community structure in the endorhiza is determined by Cannabis cultivars [16]. This view that the rhizospheric microbiome has an influence in the selection of the next –generation Cannabis cultivars that are resilient to biotic and abiotic types of stress opens up a new approach of breeding. Of particular interest, the community structure of endorhiza was significantly correlated with cannabinoid concentration and composition [16]. Future studies should be directed at using microbial communities of Cannabis not only to increase fitness but augment derived metabolite production.

4.1. *Fungal Endophytes Associated with Different Organs of Hemp and Marijuana*

The diversity of fungal and bacterial endophytes associated with different tissues of hemp and marijuana sampled from various geographic and ecological regions is listed in **Figure 1**. Almost all of the non-symbiotic fungal endophytes reported by several studies belonged to the Ascomycetes, except for two studies reported the presence of strains belonging to the Basidiomycetes such as *Irpex, Cryptococcus* [51] and *Schizophyllum commune* [52]. Depending on the geographical region, the abundance of fungal endophytes associated with Cannabis tissues varied. For example, the abundance number of fungal strains belonging to *Aspergillus, Penicillium, Phoma, Rhizopus, Colletotrichum, Cladosporium, and Curvularia* in leaf samples from Himachal Pradesh, India [53] was higher as compared to those in stems and petioles [53], and so are fungal strains belonging to *Cochliobolus* and *Aureobasidium* of hemp samples of Canada [51]. Leaf, twig, and bud tissues of Bedrocan BV Medicinal marijuana from the Netherlands were associated with endophytic communities belonging to the *Penicillium* species (predominantly, *P. copticola*), *Eupenicillium rubidurum, Chaetomium globosum,* and *Paecilomyces lilacinus* [54]. Different species of *Aspergillus* (*A. niger, A. flavus,* and *A. nidulans*), *Penicillium* (*P. chrysogenum* and *P. citrinum*), and some pathogens as *Rhizopus stolonifera, Alternaria alternata*, and *Cladosporium* sp. were found in marijuana stem tissues [53]. Moreover, strains belonging to *Alternaria, Cryptococcus,*

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Aspergillus, Cladosporium Penicillium [51,53,55] were isolated from marijuana and hemp petioles, whereas, *Aureobasidium* and *Cladosporium* isolated from hemp seeds [51]. Recently, high levels of symbiotic fungi, the arbuscular mycorrhizal (AM) fungi belonging to *Diversispora* sp., *Funneliformis mosseae, F. geosporum*, *Glomus caledonium*, and *G. occultum* were associated with hemp plantation located on arable land contaminated with phosphogypsum and sewage sludge in Poland [56].

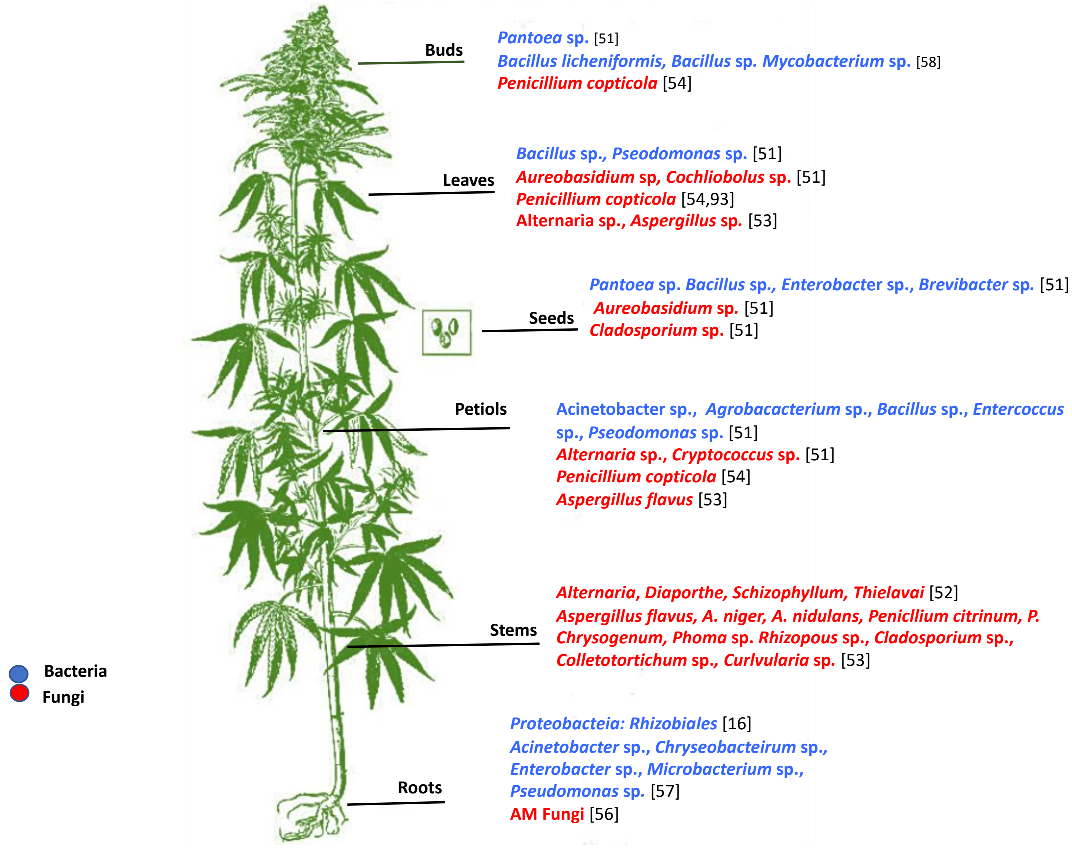

Fig.1. The most common endophytes harboured in different tissues of *Cannabis sativa* plants obtained from different geographical locations

4.2. *Bacterial Endophytes Composition in Different Organs of C. sativa*

The microbial community of bacterial endophytes associated with different cultivars of *C. sativa* belonged to ϒ-proteobacteria and α-proteobacteria including *Pseudomonadace*, *Oxalobacteraceae*, *Xanthomonadaceae*, *Actinomycetales*, and *Sphingobacteriales*, and all are well-known endophytic bacteria which substantiate observations from other plant systems (Fig.1)

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[57]. The most abundant strains isolated from leaves belong to *Pseudomonas* and *Bacillus*. Namely, *B. licheniformis*, *B. subtilis, B. pumilus* and *B. megaterium* formed the most abundant Gram-positive bacterial endophytes population in the leaf [51,58]. Strains of *Pantoea* and *Staphylococcus* were associated exclusively with Cannabis petioles [51], while strains of *Pantoa*, *Staphylococcus, Bacillus*, and *Enterobacter* were isolated from the seed [51]. The most prominent isolated genera from roots included *Acinetobacter, Chryseobacterium, Enterobacter, Microbacterium*, and *Pseudomonas* [57].

These findings prompted us to address the following questions whether Cannabis-associated bacterial and fungal communities could (i) increase hemp and marijuana yield, (ii) control plant pathogens infection of Cannabis plants and promote disease resistance, (iii) modulate the production of Cannabis secondary metabolites.

5. Endophytes, as Cannabis Microbial Biostimulants

Associated-bacterial endophytes with all plant species are able to promote plant growth in plants via several mechanisms: nitrogen fixation, siderophore production to chelate iron and make available to plant roots, mineral solubilization mainly phosphorus and calcium, and production of several phytohormones including auxins, ABA, cytokinins and GAs [17,45,59,60]. Production of such bioactive metabolites can enhance host plant growth and tolerate environmental stresses. There are limited studies on the use of growth-promoting bacterial endophytes and their effect on Cannabis growth and yield. Pagnani and co-workers [61] evaluated the suitability of multispecies consortium consisting of *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, and *Burkholderia ambifaria* isolated from roots or stems of corn, sorghum, sugarcane and Bermuda grass [62] to enhance hemp biomass. The bacterial consortium favoured plant growth development and the accumulation of secondary metabolites (i.e. CBD and THC). Conant et al. [63] reported on significant marijuana bud yield of 16.5% and plant height as a result of treatment with the microbial biostimulant Mammoth PTM, a multispecies consortium comprised of four bacterial taxa *Enterobacter cloacae*, *Citrobacter freundii*, *Pseudomonas putida* and *Comamonas testosteroni* [64]. In the case of fungal endophytes, root inoculation of hemp by AM fungi enhanced tolerance of hemp to accumulate Cd, Ni and Cr [65].

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Most of the above findings illustrate the use of endophytic bacteria isolated from plant species other than hemp or marijuana with the ability to trigger some physiological plant responses. Our laboratory along with other researchers have reported on the diversity of endogenous fungal and bacterial endophytes and the abundance of taxonomic groups in different tissues of hemp and marijuana with growth promotion capabilities (**Table 1**) and biological control potential (**Table 2**) [51-54,57,66]. Some of these isolates were able to trigger the production of IAA-like molecules in the plant, reinforcing the notion that beneficial endophytes modulate plant development and growth through the production of phytohormones, although the mechanism behind this has not been fully clarified. Performing experiments with endophytes as growth elicitors would facilitate the evaluation of secondary metabolites profiles, particularly for THC, cannabinoids compounds and terpenes of Cannabis plants inoculated with endophytes.

Table 1. Plant growth promoting bacteria and fungi associated with Cannabis and their mode of action

Organism	**Activity**	**References**
Bacillus sp.	P solubilizing	Joe et al. 2016 [91]
B. amyloliquefaciens	GAs	Shahzad et al. 2016 [92]
Pantoea vagans MOSEL-t13	IAA	Afzal et al. 2015 [57]
Pseudomonas fulva BTC6-3	P solubilizing & IAA	Scott et al. 2018 [51]
P. geniculata MOSEL-tnc1	IAA	Afzal et al. 2015 [57]
Serratia marcescens MOSEL-w2	IAA	Afzal et al. 2015 [57]
Bipolaris sp. CS-1	IAA and GAs	Lubna et al. 2019 [66]

IAA , indole acetic acid; GAs, gibberellins

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Due to past legal restrictions on the production of marijuana and hemp, growth promotion trials applying endogenous microbiome isolated from hemp and marijuana were constrained. It seems reasonable to hypothesize that endogenous endophytic bacteria and fungi possess the genetic information to trigger phenotypic drastic growth promotion, and positively increase Cannabis secondary metabolite in their respective hosts as compared to endophytes isolated from different plant species. With the legalization of marijuana in Canada and other countries, intensive investigations on how hormone-like molecules produced by endophytes influence plant adaptation and growth become possible.

Table 2. Cannabis endophytes with antagonistic effects against pathogens

Organism	Target pathogen	Activity	References
Penicillium copticola L3	*Trichothecium roseum*	Growth inhibition	Kusari et al. 2013 [54]
Paecilomyces lilacinus A3	*Botrytis cinerea*	Growth inhibition	Kusari et al. 2013 [54]
Pseudomonas fulva BTC8-1	*B. cinerea*	Cellulase,HCN, Siderophore	Scott et al. 2018 [51]
P. orientalis BTG8-5	*B. cinerea*	Cellulase, IAA, Siderophore	Scott et al. 2018 [51]
Alternaria alternata, CN1	*Fusarium solani*	Growth inhibition	Qadri et al. 2013 [52]
Aspergillus niger 2	*Curvularia lunata*	Growth inhibition	Gautam et al. 2013 [53]
Paenibacillus sp. MOSEL-w13	*Aspergillus niger* *Fusarium oxysporum*	Growth inhibition	Afzal et al. 2015 [57]

6. Cannabis endophytes with antagonistic effect against pathogens

There are limited bioprospecting studies on antagonistic activity of microbial endophytes associated with hemp and Marijuana against invading pathogens and contaminating mycotoxigenic fungi [51,54,57]. These studies used the bioprospecting rationale that hemp and

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Marijuana contain medicinal compounds that might also harbor competent microbial endophytes capable of providing health benefits to the host plant. The hemp-associated strains of *Pseudomonas fulva* (BTC6-3 and BTC8-1) and *P. orientalis* (BTG8-5 and BT14-4), exhibited antifungal activities against *Botrytis cinereal* in dual confrontation assays [51]. These strains were top producers of hydrogen cyanide (HCN), cellulose, siderophore, IAA and could solubilise P [51]. Additionally, *Pseudomonas* strains produce well-characterized secondary metabolites as diffusible antibiotics including phenazines like phenazine-1- carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyocyanine, pyoluteorin, pyrrolnitrin, phloroglucinols, lipopeptides, and the volatile metabolite as HCN [67]. All these attributes make *Pseudomonas* strains effective biocontrol agents. The endophytic bacterial strains, *Bacillus megaterium* B4, *Brevibacillus borstelensis* B8, and *Bacillus* sp. B11 and *Bacillus* sp. B3, employ quorum quenching as a strategy to disrupt cell- to cell quorum sensing signals in the target organism [58]. This strategy provides the Cannabis endophytes defense against plant pathogens and prevents the pathogen to develop resistance against the bioactive secondary compounds produced by the plant and or the endophytes.

Endophytic fungi obtained from Cannabis, such as *P. lilacinus* A3, *Penicillium* sp. T6, and *P. copticola* L3 were effective against two Cannabis pathogens, *B. cinerea* and *Trichothecium roseum* growth [54]. Using the endophytic strains of *Paenibacillus* sp. and *Pantoea vagans* in dual culture assays against *Fusarium oxysporum* were successful in antagonizing the pathogen [57]. Taken together, these studies, although limited in scope reveal the potency of endophytes in Cannabis plants, and their applications hold great promise not only as biocontrol agents against the known and emerging phytopathogens of Cannabis plants, but also as a sustainable resource of biologically active novel secondary metabolites. These bioactive metabolites are an ideal substitute for chemo-pesticide not only to support low pesticide residue levels in Cannabis flowers, but also for adopting zero-tolerance policy of pesticide residues in compliance with government regulatory bodies [68].

7. Endophytes of Medicinal Plants as Sources of Plants Secondary Metabolites

An exhaustive list of the natural products with antimicrobial activities and or therapeutic potential produced by endophytes that were isolated from plants is described in the recent review

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by Martinez-Klimova et al. [69]. To name a few, pharmaceutical molecules such as the antitumor drugs, vinblastine and vincristine [70], the anticancer drug camptothecin [71], the aneoplastic paclitaxel [72], and the insecticide azadirachtin [73].

There is compelling evidence that both the plant and their endophytes can produce a collection of secondary metabolites from similar precursors, possibly as an adaptation of the host environment [74]. Some examples include podophyllotoxin [75,76], camptothecin and structural analogs [71,77]. Some of these endophytes are able to biochemically produce compounds similar or identical to those produced by their host plants. It has been proposed that such molecular basis might be attributed to horizontal gene recombination or transfer during the evolutionary process. For example, the ability of the taxol producing fungus *Clasdosporium cladosporiodes* MD2 associated with the host plant *Taxus media* is attributed to the gene 10-deacetylbaccatin-III-10-*O*-acetyl transferase. This gene plays a role in the biosynthetic pathway of taxol and bears a 99% resemblance to the host plant gene [74]. The latter endophytic fungus being the original source of this important anti-cancer drug. The biosynthesis of the insecticide azadirachtin A and B by the fungal endophytes *Eupenicilliun purvium* isolated from the Indian neem plant lends another evidence on the ability of endophytes to produce similar host plant metabolites [73]. The recent progress in the molecular biology of secondary compounds and the cloning of genes of endophytic metabolites will offer insight on how the plant and endophyte genes of the metabolites are organized.

7.1. *Endophytes Modulate Secondary Metabolites of Medicinal Plants*

Accumulated evidence established that endophytes are capable of eliciting physiological plant responses which in turn influence the production of secondary metabolites in the host plant [78]. The production of bioactive secondary metabolites of *Rumex gmelini* seedlings was enhanced through co-culture with endophytic fungi [79]. An endophytic bacterium *Pseudonocardia* sp. induced artemisinin (antimalarial drugs) production in Artemisia plant [80]. Inoculation of the medicinal plant *Papaver somniferum* L. with a multispecies consortium increased the morphine yield by enhancing the expression of COR, a key gene for morphine biosynthesis [81]. The alkaloid drug Huperzine A (HupA) used to treat Alzheimer disease is not only derived from *Huperzia serrata*

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plant, but also is produced and biosynthesized by the fungal endophyte *Penicillium* sp. LDL4.4 isolated from *H. serratia* [82]. In Crotalaria (*Fabaceae*), biosynthesis of pyrrolizidine alkaloids (antiherbivore, nematicide) depends on the nodulation by *Bradyrhizobium* sp. [83]. In another example, the bacterial and fungal endophytes obtained from the Agarwood tree (*Aquilaria malaccensis*) enhanced the production of agarospirol, a highly sought after product in the pharmaceutical and perfumery industry, within 3 months of artificial infection [84]. Despite current research documents that endophytic microorganisms are capable of producing associated plant metabolites, the potential of production of plant metabolites by endophytes has not been fully explored and is far from exhausted. Exploiting this complex plant-microbe relationship can only enhance the sustained production of phytochemical by the associated microorganisms.

7.2 *Possible* ***Modulation of Cannabis Secondary Metabolite by Endophytes***

Endophytes are well known to produce biologically active secondary metabolites that mimic the effect of the host plant metabolites or produce precursors of host plant compounds to activate the signalling pathway aiming to modulate plant secondary metabolites [85]. They induce the production of phytohormones such as ABA, GA, and ET that may provide a significant potential for improving Cannabis secondary metabolites. Secondary metabolites including THC, CBN, and CBD, are the most prevalent of cannabinoid compounds and inherently are employed in Cannabis stress responses [86]. The precise role of cannabinoid in plant defense has not yet been elucidated. Evidently, the plant growth regulators including ABA, cycocel, ethephon, GAs, salicylic acid, γ-Aminobutyric acid (GABA) and mevinolin can manipulate cannabinoid biosynthesis and modulating Cannabis secondary metabolites [15,86-89].

The potential for secondary metabolite recovery can be improved by exogenous elicitation. For example, the application of plant hormone GA_3 at 100 µM level increased the amount of THC and CBD [88]. The exact mechanism of how the addition of exogenous hormones can affect the content of THC and CBD is not yet understood. One plausible hypothesis is that the exogenous application of GA_3 contributes to the regulation of 1-aminocyclopropane-1-carboxylic acid [62] content, which in turn elevates ET levels that lead to higher THC and CBD contents [88]. Ethephon, another plant growth regulator increased THC content of male flowers and CBD content of female

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flowers [86]. Such increase was attributed to ET levels that may function as switch between growth and secondary metabolites synthesis. Accordingly, exogenous application of two stress signalling molecules, salicylic acid (1 mM) and GABA (0.1 mM) improved THC content but deteriorated CBD content simultaneously, suggesting that these signalling molecules could affect the cannabinoid biosynthesis pathway through elicitation of expression of key genes leading to eventual changes in the amount of the final products [15].

The concentration of cannabinoid compounds can be conceivably stimulated through biotic elicitation by symbiotic and or mutualistic relationships with endophytes. This raises the question of whether the production of identical molecules to plant hormones by endophytes in the plant would be effective as with the exogenous application of elicitors. A mixture of four bacterial endophytes significantly improved CBD and THC contents [61]. Endophyte could manipulate ACC deaminase level in the plant [31,37,90] which is the precursor of THC biosynthesis. Despite these advances, the mechanisms underlying the regulation of THC synthesis have not been completely elucidated.

It might be useful to draw an analogy between the medicinal plant-endophyte association and the engagement of the endophytes to produce structurally similar secondary metabolites of medicinal Cannabis. However, the exact role of the natural products produced by endophytes inside Cannabis in the perspective of helping in plant fitness is not precisely known. Unfortunately, this potential has not yet achieved.

8. Challenges and Future Directions

To date, basic information on Cannabis endophytes diversity and composition is published. Most publications are restricted to isolation and identification of Cannabis endophytes but their biological effects on Cannabis growth promotion and modulating of secondary compounds are unrevealed. Thus, it is imperative to understand the microbial partnerships with Cannabis as it has the potential to affect agricultural practices by improving plant fitness and the production yield of cannabinoids. Interestingly, the active metabolites of microbial endophytes possess excellent biological activities that not only have the potential to wage war on plant biotic and abiotic stress but also useful for human health to prevent or cure fatal illness. The above observations highlight

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the wealth of untapped, and as of yet unknown functional traits of endophytes harbouring Cannabis that need to be discovered and characterize their role in the enrichment of Cannabis secondary metabolites. The importance of endophytic microorganisms producing compounds similar to their plants has gained momentum. Synthesized plant compounds by microbial endophytes are being studied to produce secondary metabolites that are originally identified in their host plants. They could turn out some important medicinal compounds independently, which will enable the pharmacological industry to large-scale fermentation of cannabinoids, independent of Cannabis cultivation. This review puts emphasis on the great importance of more studies on Cannabis endophytes and their biological properties. The examples presented in this review indicate that there is an urgent need to understand the molecular and biochemical mechanisms that might elicit similar responses in both plants and their associated endophytes that leads to the production of similar secondary metabolites.

Publication bibliography

1. Small, E. *Cannabis : a complete guide*; 2017. CRC Press
2. Small, E.; Cronquist, A. A practical and natural taxonomy for Cannabis. *Taxon* **1976**, *25*, 405–435, doi:10.2307/1220524.
3. Andre, C.M.; Hausman, J.-F.; Guerriero, G. *Cannabis sativa*: The plant of the thousand and one molecules. *Front Plant Sci* **2016**, *7*, 19, doi:10.3389/fpls.2016.00019.
4. Rodziewicz, P.; Loroch, S.; Marczak, Ł.; Sickmann, A.; Kayser, O. Cannabinoid synthases and osmoprotective metabolites accumulate in the exudates of *Cannabis sativa* L. glandular trichomes. *Plant Science* **2019**, *284*, 108–116, doi:10.1016/j.plantsci.2019.04.008.
5. Gonçalves, J.; Rosado, T.; Soares, S.; Simão, A.Y.; Caramelo, D.; Luís, Â.; Fernández, N.; Barroso, M.; Gallardo, E.; Duarte, A.P. Cannabis and its secondary metabolites: Their use as therapeutic drugs, Toxicological Aspects, and Analytical Determination. *Medicines (Basel, Switzerland)* **2019**, *6*, 31, doi:10.3390/medicines6010031.
6. Deshmukh, S.K.; Gupta, M.K.; Prakash, V.; Reddy, M.S. Mangrove-associated fungi: A novel source of potential anticancer compounds. *Journal of fungi (Basel, Switzerland)* **2018**, *4*, 101, doi:10.3390/jof4030101.
7. Verpoorte, R.; Contin, A.; Memelink, J. Biotechnology for the production of plant secondary metabolites. *Phytochemistry Reviews* **2002**, *1*, 13–25, doi:10.1023/A:1015871916833.
8. Schuermeyer, J.; Salomonsen-Sautel, S.; Price, R.K.; Balan, S.; Thurstone, C.; Min, S.-J.; Sakai, J.T. Temporal trends in marijuana attitudes, availability and use in Colorado compared to non-medical marijuana states: 2003-11. *Drug and alcohol dependence* **2014**, *140*, 145–155, doi:10.1016/j.drugalcdep.2014.04.016.
9. Hall, W. The future of the international drug control system and national drug prohibitions. *Addiction* **2018**, *113*, 1210–1223, doi:10.1111/add.13941.

Peer-reviewed version available at *Microorganisms* **2020**, *8*, 355; doi:10.3390/microorganisms8030355

10. Potter, D.J. A review of the cultivation and processing of cannabis (*Cannabis sativa* L.) for production of prescription medicines in the UK. *Drug Testing and Analysis* **2014**, *6*, 31–38, doi:10.1002/dta.1531.
11. Eichhorn Bilodeau, S.; Wu, B.-S.; Rufyikiri, A.-S.; MacPherson, S.; Lefsrud, M. An update on plant photobiology and implications for Cannabis production. *Front Plant Sci* **2019**, *10*, 296, doi:10.3389/fpls.2019.00296.
12. Taschwer, M.; Schmid, M.G. Determination of the relative percentage distribution of THCA and Δ9-THC in herbal cannabis seized in Austria – Impact of different storage temperatures on stability. *Forensic Science International* **2015**, *254*, 167–171, doi:10.1016/j.forsciint.2015.07.019.
13. Caplan, D.; Dixon, M.; Zheng, Y. Optimal rate of organic fertilizer during the vegetative-stage for Cannabis grown in two coir-based substrates. *HortScience horts* **2017**, *52*, 1307–1312, doi:10.21273/HORTSCI11903-17.
14. Mansouri, H.; Asrar, Z.; Szopa, J. Effects of ABA on primary terpenoids and Δ9-tetrahydrocannabinol in *Cannabis sativa* L. at flowering stage. *Plant Growth Regulation* **2009**, *58*, 269–277, doi:10.1007/s10725-009-9375-y.
15. Jalali, S.; Salami, S.A.; Sharifi, M.; Sohrabi, S. Signaling compounds elicit expression of key genes in cannabinoid pathway and related metabolites in cannabis. *Industrial Crops and Products* **2019**, *133*, 105-110, doi:https://doi.org/10.1016/j.indcrop.2019.03.004.
16. Winston, M.E.; Hampton-Marcell, J.; Zarraonaindia, I.; Owens, S.M.; Moreau, C.S.; Gilbert, J.A.; Hartsel, J.; Kennedy, S.J.; Gibbons, S.M. Understanding cultivar-specificity and soil determinants of the Cannabis microbiome. *PLoS One* **2014**, *9*, e99641, doi:10.1371/journal.pone.0099641.
17. Berg, G.; Grube, M.; Schloter, M.; Smalla, K. Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology* **2014**, *5*, 148, doi:10.3389/fmicb.2014.00148.
18. Turner, T.R.; James, E.K.; Poole, P.S. The plant microbiome. *Genome Biology* **2013**, *14*, 209, doi:10.1186/gb-2013-14-6-209.
19. Gopal, M.; Gupta, A. Microbiome selection could spur next-generation plant breeding strategies. *Frontiers in Microbiology* **2016**, *7*, 1971, doi:10.3389/fmicb.2016.01971.
20. Khalaf, E.M.; Raizada, M.N. Bacterial seed endophytes of domesticated cucurbits antagonize fungal and Oomycete pathogens including powdery mildew. *Frontiers in Microbiology* **2018**, *9*, 42, doi:10.3389/fmicb.2018.00042.
21. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends in Plant Science* **2012**, *17*, 478–486, doi:10.1016/j.tplants.2012.04.001.
22. van der Ent, S.; van Hulten, M.; Pozo, M.J.; Czechowski, T.; Udvardi, M.K.; Pieterse, C.M.J.; Ton, J. Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: differences and similarities in regulation. *New Phytologist* **2009**, *183*, 419–431, doi:10.1111/j.1469-8137.2009.02851.x.
23. Weller, D.M.; Raaijmakers, J.M.; Gardener, B.B.M.; Thomashow, L.S. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual review of phytopathology* **2002**, *40*, 309–348, doi:10.1146/annurev.phyto.40.030402.110010.
24. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; van der Putten, W.H. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **2013**, *11*, 789–799, doi:10.1038/nrmicro3109.

Peer-reviewed version available at *Microorganisms* **2020**, *8*, 355; doi:10.3390/microorganisms8030355

25. Raaijmakers, J.M.; Paulitz, T.C.; Steinberg, C.; Alabouvette, C.; Moënne-Loccoz, Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and soil* **2009**, *321*, 341–361, doi:10.1007/s11104-008-9568-6.
26. Marasco, R.; Rolli, E.; Ettoumi, B.; Vigani, G.; Mapelli, F.; Borin, S.; Abou-Hadid, A.F.; El-Behairy, U.A.; Sorlini, C.; Cherif, A., et al. A drought resistance-promoting microbiome is selected by root system under desert farming. *PLoS One* **2012**, *7*, e48479, doi:10.1371/journal.pone.0048479.
27. Bais, H.P.; Weir, T.L.; Perry, L.G.; Gilroy, S.; Vivanco, J.M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* **2006**, *57*, 233–266, doi:10.1146/annurev.arplant.57.032905.105159.
28. Doornbos, R.F.; van Loon, L.C.; Bakker, P.A.H.M. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development* **2012**, *32*, 227–243, doi:10.1007/s13593-011-0028-y.
29. Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and molecular biology reviews : MMBR* **2015**, *79*, 293–320, doi:10.1128/MMBR.00050-14.
30. Petrini, O. Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves*, Andrews, J.H., Hirano, S.S., Eds. Springer New York: 1991; pp. 179–197.
31. Khan, A.L.; Halo, B.A.; Elyassi, A.; Ali, S.; Al-Hosni, K.; Hussain, J.; Al-Harrasi, A.; Lee, I.-J. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. *Electronic Journal of Biotechnology* **2016**, *21*, 58–64, doi:10.1016/j.ejbt.2016.02.001.
32. Gagné-Bourque, F.; Bertrand, A.; Claessens, A.; Aliferis, K.A.; Jabaji, S. Alleviation of drought stress and metabolic changes in Timothy (*Phleum pratense* L.) colonized with *Bacillus subtilis* B26. *Front Plant Sci* **2016**, *7*, 584-584, doi:10.3389/fpls.2016.00584.
33. Truyens, S.; Weyens, N.; Cuypers, A.; Vangronsveld, J. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* **2015**, *7*, 40–50, doi:10.1111/1758-2229.12181.
34. Lugtenberg, B.; Kamilova, F. Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology* **2009**, *63*, 541–556, doi:10.1146/annurev.micro.62.081307.162918.
35. Win, K.T.; Tanaka, F.; Okazaki, K.; Ohwaki, Y. The ACC deaminase expressing endophyte *Pseudomonas* spp. enhances NaCl stress tolerance by reducing stress-related ethylene production, resulting in improved growth, photosynthetic performance, and ionic balance in tomato plants. *Plant Physiology and Biochemistry* **2018**, *127*, 599–607, doi:10.1016/j.plaphy.2018.04.038.
36. Rascovan, N.; Carbonetto, B.; Perrig, D.; Díaz, M.; Canciani, W.; Abalo, M.; Alloati, J.; González-Anta, G.; Vazquez, M.P. Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. *Scientific Reports* **2016**, *6*, 28084, doi:10.1038/srep28084.
37. van de Poel, B.; van der Straeten, D. 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene! *Front Plant Sci* **2014**, *5*, 640, doi:10.3389/fpls.2014.00640.
38. Taghinasab, M. The root endophytes *Trametes versicolor* and *Piriformospora indica* increase grain yield and P content in wheat. *Plant and soil* **2018**, *v. 426*, pp. 10-348-2018 v.2426 no.2011-2012, doi:10.1007/s11104-018-3624-7.

39. Redman, R.S.; Sheehan, K.B.; Stout, R.G.; Rodriguez, R.J.; Henson, J.M. Thermotolerance generated by plant/fungal symbiosis. *Science* **2002**, *298*, 1581, doi:10.1126/science.1078055.
40. Arnold, A.E.; Mejía, L.C.; Kyllo, D.; Rojas, E.I.; Maynard, Z.; Robbins, N.; Herre, E.A. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **2003**, *100*, 15649, doi:10.1073/pnas.2533483100.
41. Silva, N.I.; Brooks, S.; Lumyong, S.; Hyde, K.D. Use of endophytes as biocontrol agents. *Fungal Biology Reviews* **2019**, *33*, 133–148, doi:10.1016/j.fbr.2018.10.001.
42. Eljounaidi, K.; Lee, S.K.; Bae, H. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases – Review and future prospects. *Biological Control* **2016**, *103*, 62–68, doi:10.1016/j.biocontrol.2016.07.013.
43. Busby, P.E.; Ridout, M.; Newcombe, G. Fungal endophytes: modifiers of plant disease. *Plant Molecular Biology* **2016**, *90*, 645–655, doi:10.1007/s11103-015-0412-0.
44. Burketova, L.; Trda, L.; Ott, P.G.; Valentova, O. Bio-based resistance inducers for sustainable plant protection against pathogens. *Biotechnology Advances* **2015**, *33*, 994–1004, doi:10.1016/j.biotechadv.2015.01.004.
45. Compant, S.; Samad, A.; Faist, H.; Sessitsch, A. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research* **2019**, *19*, 29–37, doi:10.1016/j.jare.2019.03.004.
46. Alegria Terrazas, R.; Giles, C.; Paterson, E.; Robertson-Albertyn, S.; Cesco, S.; Mimmo, T.; Pii, Y.; Bulgarelli, D. Chapter One - plant–microbiota interactions as a driver of the mineral turnover in the rhizosphere. In *Advances in Applied Microbiology*, Sariaslani, S., Michael Gadd, G., Eds. Academic Press: 2016; Vol. 95, pp. 1–67.
47. Carrión, V.J.; Perez-Jaramillo, J.; Cordovez, V.; Tracanna, V.; Hollander, M.; Ruiz-Buck, D.; Mendes, L.W.; van Ijcken, W.F.J.; Gomez-Exposito, R.; Elsayed, S.S., et al. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* **2019**, *366*, 606, doi:10.1126/science.aaw9285.
48. Garbeva, P.; van Veen, J.A.; van Elsas, J.D. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual review of phytopathology* **2004**, *42*, 243–270, doi:10.1146/annurev.phyto.42.012604.135455.
49. Berg, G.; Smalla, K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* **2009**, *68*, 1–13, doi:10.1111/j.1574-6941.2009.00654.x.
50. Bulgarelli, D.; Schlaeppi, K.; Spaepen, S.; van Themaat, E.V.L.; Schulze-Lefert, P. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **2013**, *64*, 807–838, doi:10.1146/annurev-arplant-050312-120106.
51. Scott, M.; Rani, M.; Samsatly, J.; Charron, J.-B.; Jabaji, S. Endophytes of industrial hemp (*Cannabis sativa* L.) cultivars: identification of culturable bacteria and fungi in leaves, petioles, and seeds. *Canadian Journal of Microbiology* **2018**, *64*, 664–680, doi:10.1139/cjm-2018-0108.
52. Qadri, M.; Johri, S.; Shah, B.A.; Khajuria, A.; Sidiq, T.; Lattoo, S.K.; Abdin, M.Z.; Riyaz-Ul-Hassan, S. Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. *SpringerPlus* **2013**, *2*, 8, doi:10.1186/2193-1801-2-8.
53. Gautam, A.K.; Kant, M.; Thakur, Y. Isolation of endophytic fungi from *Cannabis sativa* and study their antifungal potential. *Archives of Phytopathology and Plant Protection* **2013**, *46*, 627–635, doi:10.1080/03235408.2012.749696.

Peer-reviewed version available at *Microorganisms* **2020**, *8*, 355; doi:10.3390/microorganisms8030355

54. Kusari, P.; Kusari, S.; Spiteller, M.; Kayser, O. Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens. *Fungal Diversity* **2013**, *60*, 137–151, doi:10.1007/s13225-012-0216-3.
55. McKernan, K.; Spangler, J.; Zhang, L.; Tadigotla, V.; Helbert, Y.; Foss, T.; Smith, D. Cannabis microbiome sequencing reveals several mycotoxic fungi native to dispensary grade Cannabis flowers. *F1000Research* **2015**, *4*, 1422, doi:10.12688/f1000research.7507.2.
56. Zielonka, D.; Sas-Paszt, L.; Derkowska, E.; Lisek, A.; Russel, S. Occurrence of arbuscular mycorrhizal fungi in Hemp (*Cannabis sativa*) plants and soil fertilized with sewage sludge and phosphogypsum. *Journal of Natural Fibers* **2019**, 10.1080/15440478.2019.1618779, 1–11, doi:10.1080/15440478.2019.1618779.
57. Afzal I, S.Z., Iqrar I. Selective isolation and characterization of agriculturally beneficial endophytic bacteria from wild hemp using Canola. *Pak J Bot.* **2015**, *5*, 1999–2008.
58. Kusari, P.; Kusari, S.; Lamshöft, M.; Sezgin, S.; Spiteller, M.; Kayser, O. Quorum quenching is an antivirulence strategy employed by endophytic bacteria. *Applied Microbiology and Biotechnology* **2014**, *98*, 7173–7183, doi:10.1007/s00253-014-5807-3.
59. Köberl, M.; Schmidt, R.; Ramadan, E.M.; Bauer, R.; Berg, G. The microbiome of medicinal plants: diversity and importance for plant growth, quality and health. *Frontiers in Microbiology* **2013**, *4*, 400, doi:10.3389/fmicb.2013.00400.
60. Egamberdieva, D.; Wirth, S.; Behrendt, U.; Ahmad, P.; Berg, G. Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. *Frontiers in Microbiology* **2017**, *8*, 199, doi:10.3389/fmicb.2017.00199.
61. Pagnani, G.; Pellegrini, M.; Galieni, A.; D'Egidio, S.; Matteucci, F.; Ricci, A.; Stagnari, F.; Sergi, M.; Lo Sterzo, C.; Pisante, M., et al. Plant growth-promoting rhizobacteria (PGPR) in *Cannabis sativa* 'Finola' cultivation: An alternative fertilization strategy to improve plant growth and quality characteristics. *Industrial Crops and Products* **2018**, *123*, 75–83, doi:10.1016/j.indcrop.2018.06.033.
62. Botta, A.L.; Santacecilia, A.; Ercole, C.; Cacchio, P.; Del Gallo, M. In vitro and in vivo inoculation of four endophytic bacteria on *Lycopersicon esculentum*. *New Biotechnology* **2013**, *30*, 666–674, doi:10.1016/j.nbt.2013.01.001.
63. Conant, R.T.; Walsh, R.P.; Walsh, M.; Bell, C.W.; Wallenstein, M.D. Effects of a microbial biostimulant, Mammoth PTM, on *Cannabis sativa* bud yield. *Journal of Horticulture* **2017**, *04*, 5, doi:10.4172/2376-0354.1000191.
64. Baas, P.; Bell, C.; Mancini, L.M.; Lee, M.N.; Conant, R.T.; Wallenstein, M.D. Phosphorus mobilizing consortium Mammoth P(™) enhances plant growth. *PeerJ* **2016**, *4*, e2121-e2121, doi:10.7717/peerj.2121.
65. Citterio, S.; Prato, N.; Fumagalli, P.; Aina, R.; Massa, N.; Santagostino, A.; Sgorbati, S.; Berta, G. The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L. *Chemosphere* **2005**, *59*, 21–29, doi:10.1016/j.chemosphere.2004.10.009.
66. Lubna; Asaf, S.; Khan, A.L.; Waqas, M.; Kang, S.-M.; Hamayun, M.; Lee, I.-J.; Hussain, A. Growth-promoting bioactivities of *Bipolaris* sp. CSL-1 isolated from *Cannabis sativa* suggest a distinctive role in modifying host plant phenotypic plasticity and functions. *Acta Physiologiae Plantarum* **2019**, *41*, 65, doi:10.1007/s11738-019-2852-7.

Peer-reviewed version available at *Microorganisms* **2020**, *8*, 355; doi:10.3390/microorganisms8030355

67. Haas, D.; Keel, C. Regulation of antibiotic production in root-colonizing *Peudomonas* spp. and relevance for biological control of plant disease. *Annual review of phytopathology* **2003**, *41*, 117–153, doi:10.1146/annurev.phyto.41.052002.095656.
68. Nate, S. Into the Weeds: Regulating pesticides in Cannabis. *Environmental Health Perspectives* **2019**, *127*, 042001, doi:10.1289/EHP5265.
69. Martinez-Klimova, E.; Rodríguez-Peña, K.; Sánchez, S. Endophytes as sources of antibiotics. *Biochemical Pharmacology* **2017**, *134*, 1–17, doi:10.1016/j.bcp.2016.10.010.
70. Kumar, A.; Patil, D.; Rajamohanan, P.R.; Ahmad, A. Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus*. *PLoS One* **2013**, *8*, e71805-e71805, doi:10.1371/journal.pone.0071805.
71. Puri, S.C.; Verma, V.; Amna, T.; Qazi, G.N.; Spiteller, M. An endophytic fungus from *Nothapodytes foetida* that produces Camptothecin. *Journal of Natural Products* **2005**, *68*, 1717–1719, doi:10.1021/np0502802.
72. Stierle, A.; Strobel, G.; Stierle, D. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science* **1993**, *260*, 214, doi:10.1126/science.8097061.
73. Kusari, S.; Hertweck, C.; Spiteller, M. Chemical ecology of endophytic fungi: Origins of secondary metabolites. *Chemistry & Biology* **2012**, *19*, 792–798, doi:10.1016/j.chembiol.2012.06.004.
74. Zhang, P.; Zhou, P.-P.; Yu, L.-J. An Endophytic taxol-producing fungus from *Taxus media*, *Cladosporium cladosporioides* MD2. *Current Microbiology* **2009**, *59*, 227, doi:10.1007/s00284-008-9270-1.
75. Puri, S.C.; Nazir, A.; Chawla, R.; Arora, R.; Riyaz-ul-Hasan, S.; Amna, T.; Ahmed, B.; Verma, V.; Singh, S.; Sagar, R., et al. The endophytic fungus *Trametes hirsuta* as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. *Journal of Biotechnology* **2006**, *122*, 494-510, doi:https://doi.org/10.1016/j.jbiotec.2005.10.015.
76. Eyberger, A.L.; Dondapati, R.; Porter, J.R. Endophyte fungal isolates from *Podophyllum peltatum* produce Podophyllotoxin. *Journal of Natural Products* **2006**, *69*, 1121-1124, doi:10.1021/np060174f.
77. Shweta, S.; Zuehlke, S.; Ramesha, B.T.; Priti, V.; Mohana Kumar, P.; Ravikanth, G.; Spiteller, M.; Vasudeva, R.; Uma Shaanker, R. Endophytic fungal strains of *Fusarium solani*, from Apodytes dimidiata E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. *Phytochemistry* **2010**, *71*, 117-122, doi:https://doi.org/10.1016/j.phytochem.2009.09.030.
78. Pandey, S.S.; Singh, S.; Babu, C.S.V.; Shanker, K.; Srivastava, N.K.; Shukla, A.K.; Kalra, A. Fungal endophytes of *Catharanthus roseus* enhance vindoline content by modulating structural and regulatory genes related to terpenoid indole alkaloid biosynthesis. *Scientific Reports* **2016**, *6*, 26583, doi:10.1038/srep26583.
79. Ding, C.-h.; Wang, Q.-B.; Guo, S.; Wang, Z.-y. The improvement of bioactive secondary metabolites accumulation in *Rumex gmelini* Turcz through co-culture with endophytic fungi. *Brazilian Journal of Microbiology* **2018**, *49*, 362–369, doi:10.1016/j.bjm.2017.04.013.
80. Li, J.; Zhao, G.-Z.; Varma, A.; Qin, S.; Xiong, Z.; Huang, H.-Y.; Zhu, W.-Y.; Zhao, L.-X.; Xu, L.-H.; Zhang, S., et al. An endophytic pseudonocardia species induces the production of Artemisinin in *Artemisia annua*. *PLoS One* **2012**, *7*, e51410, doi:10.1371/journal.pone.0051410.

Peer-reviewed version available at *Microorganisms* **2020**, *8*, 355; doi:10.3390/microorganisms8030355

81. Ray, T.; Pandey, S.S.; Pandey, A.; Srivastava, M.; Shanker, K.; Kalra, A. Endophytic consortium with diverse gene-regulating capabilities of Benzylisoquinoline Alkaloids biosynthetic pathway can enhance endogenous Morphine biosynthesis in *Papaver somniferum*. *Frontiers in Microbiology* **2019**, *10*, 925, doi:10.3389/fmicb.2019.00925.
82. Thi Minh Le, T.; Thi Hong Hoang, A.; Thi Bich Le, T.; Thi Bich Vo, T.; van Quyen, D.; Hoang Chu, H. Isolation of endophytic fungi and screening of Huperzine A–producing fungus from *Huperzia serrata* in Vietnam. *Scientific Reports* **2019**, *9*, 16152, doi:10.1038/s41598-019-52481-2.
83. Irmer, S.; Podzun, N.; Langel, D.; Heidemann, F.; Kaltenegger, E.; Schemmerling, B.; Geilfus, C.-M.; Zörb, C.; Ober, D. New aspect of plant–rhizobia interaction: Alkaloid biosynthesis in Crotalaria depends on nodulation *Proceedings of the National Academy of Sciences* **2015**, *112*, 4164, doi:10.1073/pnas.1423457112.
84. Chhipa, H.; Kaushik, N. Fungal and bacterial diversity isolated from *Aquilaria malaccensis* tree and soil, induces Agarospirol formation within 3 months after artificial infection. *Frontiers in Microbiology* **2017**, *8*, 1286, doi:10.3389/fmicb.2017.01286.
85. Kusari, P.; Kusari, S.; Spiteller, M.; Kayser, O. Cannabis endophytes and their application in breeding and physiological fitness. In *Cannabis sativa L. - Botany and Biotechnology*, Chandra, S., Lata, H., ElSohly, M.A., Eds. Springer International Publishing: Cham, **2017**; 10.1007/978-3-319-54564-6_20pp. 419–437.
86. Mansouri, H.; Salari, F.; Asrar, Z. Ethephon application stimulats cannabinoids and plastidic terpenoids production in *Cannabis sativa* at flowering stage. *Industrial Crops and Products* **2013**, *46*, 269–273, doi:10.1016/j.indcrop.2013.01.025.
87. Mansouri, H.; Salari, F. Influence of mevinolin on chloroplast terpenoids in *Cannabis sativa*. *Physiology and molecular biology of plants : an international journal of functional plant biology* **2014**, *20*, 273–277, doi:10.1007/s12298-014-0222-x.
88. Mansouri, H.; Asrar, Z.; Amarowicz, R. The response of terpenoids to exogenous gibberellic acid in *Cannabis sativa* L. at vegetative stage. *Acta Physiologiae Plantarum* **2011**, *33*, 1085–1091, doi:10.1007/s11738-010-0636-1.
89. Mansouri, H.; Asrar, Z. Effects of abscisic acid on content and biosynthesis of terpenoids in *Cannabis sativa* at vegetative stage. *Biologia Plantarum* **2012**, *56*, 153–156, doi:10.1007/s10535-012-0033-2.
90. Etesami, H.; Mirseyed Hosseini, H.; Alikhani, H.A. Bacterial biosynthesis of 1-aminocyclopropane-1-caboxylate (ACC) deaminase, a useful trait to elongation and endophytic colonization of the roots of rice under constant flooded conditions. *Physiology and molecular biology of plants : an international journal of functional plant biology* **2014**, *20*, 425–434, doi:10.1007/s12298-014-0251-5.
91. Joe, M.M.; Devaraj, S.; Benson, A.; Sa, T. Isolation of phosphate solubilizing endophytic bacteria from Phyllanthus amarus Schum & Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress. *Journal of Applied Research on Medicinal and Aromatic Plants* **2016**, *3*, 71-77, doi:https://doi.org/10.1016/j.jarmap.2016.02.003.
92. Shahzad, R.; Waqas, M.; Khan, A.L.; Asaf, S.; Khan, M.A.; Kang, S.-M.; Yun, B.-W.; Lee, I.-J. Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. *Plant Physiology and Biochemistry* **2016**, *106*, 236–243, doi:10.1016/j.plaphy.2016.05.006.

Peer-reviewed version available at *Microorganisms* **2020**, *8*, 355; doi:10.3390/microorganisms8030355

93. Punja, Z.K. Flower and foliage-infecting pathogens of marijuana (*Cannabis sativa* L.) plants. *Canadian Journal of Plant Pathology* **2018**, *40*, 514-527, doi:10.1080/07060661.2018.1535467