 doi:10.20944/preprints202012.0736.v1

Can manipulation of soil microbiota enhance, stabilize and sustain cannabinoid production?

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Abstract

Cannabis is one of the oldest cultivated crops in the history for food, fiber and drugs for thousands of years. Extension of cannabis genetic variation developed in a wide-ranging choice of varieties with various complementary phenotypes and secondary metabolites. Cannabis grow practices is very diverse, especially indoor cultivation factors, such as different lighting conditions, pot size, humidity, fertilizers. These growth factors influence a lot on the production of cannabinoids. For medical or pharmaceutical purposes, ratio of CBD or THC is very important. Plants traits and metabolic compounds are related to various conditions produced by microbes. Investigating this crosstalk between plants and microbes can play a vital role not only for stimulating the biosynthetic and signaling pathways of the host plants for the production of agronomically or pharmaceutically essential metabolic compounds but also against pathogens. This study emphasis on decoding the crosstalk between cannabis and associated microbes in the belowground environmental niches that would unravel the complexity of stabilizing cannabinoid production.

Keywords

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***Cannabis*, cannabinoid, microbiota, inoculants**

Background

Domestication and cultivation of *Cannabis* plants for fiber, food and drug products dates back to approximately 6,000 years ago in Central and Southeast Asia. Over time, cultivation of the plant expanded to different parts of the world, arriving in North America in the 19th century. Cultivation and breeding of *Cannabis* plants led to the expansion of genetic variations, resulting in a range of cultivars with contrasting phenotypes, traits and secondary metabolite properties (Clarke and Merlin 2017; Li 1973). However, *Cannabis* plants are predominantly dioecious which makes breeding programs challenging. To avoid intercross, female flowers must be isolated from the males, which makes the development of true breed lines of *Cannabis* a costly and lengthy process (Clarke and Merlin 2017). In addition, like other cultivated plants, *Cannabis* can be susceptible to pest and pathogen attacks or abiotic stresses including nutrient deficiencies. Despite technological advances in *Cannabis* breeding, the proportion of cannabinoids – or more specifically, of cannabidiol (CBD) and tetrahydrocannabinol (THC) – will fluctuate greatly depending on various factors, including the sex of the parents (male or female), genotypes, cultivation practices, and biotic or abiotic stresses. Throughout history, farmers and breeders have preferred different qualitative and quantitative traits, which in turn has impacted plant selection and further diversified the genetics of new domesticated strains.

The *Cannabis* genus is comprised of two major groups of accessions: the *indica* gene pool and the *sativa* gene pool, which are closely-related subspecies of *Cannabis sativa* L. – although between these two subspecies, diverse cultivars have been domesticated throughout the hybridization process (Emboden 1981; Hillig 2005;

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Small and Cronquist 1976). Regardless of evolutionary relationships, *Cannabis* is largely cultivated for medicinal and recreational use, and this has led to further categorization with regard to its relative cannabinoid concentrations, which broadly vary between the male and female plants (Hillig and Mahlberg 2004; Schultes et al. 1974; Small 1979). Cannabinoids are unique secondary metabolites to *Cannabis* and are produced by trichomes of the plant. Although 61 true biosynthetic cannabinoids exist, *Cannabis* is generally cultivated for its CBD and THC, for both medicinal and recreational purposes. Previous studies have documented that the cannabinoid content in plants varies greatly depending on climatic conditions, plant genotypes, and cultivation practices that influence cannabinoid biosynthesis pathways (Beutler and Marderosian 1978; Small and Cronquist 1976). For example, high concentrations of THC in *Cannabis* plants were reported in cultivars originating from India, Nepal, Eastern Asia and Southern Africa, while high concentrations of CBD were found in Northeast Asian cultivars (Baker, Gough, and Taylor 1980; Fetterman and Turner 1972; Small and Beckstead 1973a; Small and Beckstead 1973b; Turner and Hadley 1973a; Turner and Hadley 1973b). In addition to the sex and maturity of the plant, biotic and abiotic stresses also affect cannabinoid biosynthesis (Doorenbos et al. 1971; Fetterman and Turner 1972). In addition, photoperiod (Valle et al. 1978), temperature (Bazzaz et al. 1975; Chandra et al. 2011), nutrients (Bócsa, Máthé, and Hangyel 1997; Coffman and Gentner 1977; Landi et al. 2019) and ultraviolet light (Lydon, Teramura, and Coffman 1987; Magagnini, Grassi, and Kotiranta 2018; Pate 1994), are among the factors that impact cannabinoid biosynthesis. However, as *Cannabis* is widely grown in indoor conditions with diverse growing substrates, artificial light and temperature control, these factors are not sufficient to stabilize ratios of THC and CBD in the plants, which brings another level of complexity to cannabinoid production and standardization. Many possibilities have been explored by *Cannabis* growers in the

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effort to find a way to maintain the yield (Backer et al. 2019) and safety production against pathogen (Taghinasab and Jabaji 2020; Vujanovic et al. 2020), however scientific research for stabilization or balanced proportion of THC and CBD is not reported. Among these solutions, biostimulant substances, beneficial microbes belonging to Plant Growth Promoting Rhizobacteria (PGPR), and arbuscular mycorrhizal fungi are proposed.

Main text

Why should microbial crosstalk be considered for cannabinoid stabilization?

Advances made in high-throughput sequencing technologies offer possibilities for manipulating soil and plant microbiota to enhance crop yield and sustain agroecosystems (Ercolini 2013; Fadiji and Babalola 2020; Lucaciu et al. 2019). Microbiota manipulation refers here to human intervention to alter the taxonomic composition and abundance of microbial communities associated with *Cannabis* plants. Bacteria and fungi represent the most important groups of microbiota that closely or loosely interact with plants in a beneficial or adverse manner. Plants and their associated plethora of microbes nurture multifactorial interactive relationships where specific microorganisms including bacteria and fungi can stimulate the biosynthetic and signaling pathways of the host plants for the production of pharmaceutically or agronomically important metabolic compounds (Huang et al. 2014; Pascale et al. 2019; Ryffel et al. 2016; Scherling et al. 2009; van de Mortel et al. 2012). Recent literature has shown that root-associated microbes stimulate the systematically-induced root exudation of metabolites (SIREM) process and affect levels of root transcriptomes and metabolomes (Korenblum et al. 2020). Endophytic bacteria and fungi can influence the metabolic machinery for producing a specific

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medicinal compound. For example, the pharmaceutically-essential terpenoid indole type alkaloids vindoline, serpentine and ajmalicine showed a substantial increase when Madagascar periwinkle (*Catharanthus roseus* L.) plants were inoculated with the endophytic bacteria *Staphylococcus sciuri* and *Micrococcus* sp. (Etalo, Jeon, and Raaijmakers 2018). Recent review (Taghinasab and Jabaji 2020) discussed about the application of exogenous inducers, such as phytohormones abscisic acid (ABA), gibberellins (GA) and ethylene (ET) on probable recovery of secondary metabolites in cannabis, although the particular mechanism of these exogenous inducers on natural products in plants is unknown. Few bacterial (*Pseudonomas fulva* BTC8-1, *P. orientalis* BTG8-5 and *Panibacillus sp*. MOSEL-w13) and fungal endophytes (*Penicillium copticola* L3, *Paecilomyces lilacinus* A3 and *Alternaria niger* 2) in cannabis demonstrated their biocontrol potentiality effects against *Trichothecium roseum, Botrytis cineria, Fusarium solani, Curvularia lunata*, *Aspergillus niger* and *Fusarium oxysporum* (Scott et al. 2018; Kusari et al. 2012; Gautam, Kant, and Thakur 2013; Afzal, Shinwari, and Iqrar 2015; Qadri et al. 2013). It has been reviewed that PGPR from one crop to another plant species may stimulate yield and biocontrol effect (Smith et al. 2015; Backer et al. 2019), and the collective role of endophytes with the exogenous application of inducers in cannabis could stimulate improving THC and CBD contents, though its correlation and mechanism have not yet been fully revealed (Taghinasab and Jabaji 2020). Taghinasab and Jabaji (2020) did not discuss on how whole microbiome (not only endophytes) associated with cannabis root could be effectively used in stabilizing cannabinoid ratios, which is the key message of this opinion letter. Since the interplay between the microbes and plant stimulates various biochemical pathways, leading to the production of secondary metabolites, decoding the crosstalk between *Cannabis* and its rhizospheric microbiota would unravel the complexity of stabilizing cannabinoid production.

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Current knowledge in microbiota research related to cannabinoids

The first draft genome sequencing of *Cannabis* solved one of the utmost ambiguities – that although they contain divergent pharmaceutical compounds, marijuana and hemp are derived from a single species: *Cannabis sativa L*. In addition, the genomic map provides clues to the scientific community for accelerating breeding programs to develop new cultivars with improved properties (van Bakel et al. 2011). The first report on the *Cannabis* plant microbiome highlighted cultivar-specificity and soil determinants of microbiome for five *Cannabis* cultivars – Bookoo Kush, Burmese, Maui Wowie, White Widow and Sour Diesel – and reported a core bacterial community composed of *Pseudomonas*, *Cellvibrio*, *Oxalobacteraceae*, *Xanthomonadaceae*, *Actinomycetales*, and *Sphingobacteriales* (Winston et al., 2014). This study included the biochemical correlations with the bacterial communities, outlining that the concentration and composition of CBD correlated to the structure of bacterial communities residing inside the root system, whereas THC concentrations correlated to the soil's edaphic factors. Another study on three *Cannabis sativa* L. (industrial hemp) cultivars grown in Quebec (Anka, CRS-1 and Yvonne) reported 18 bacterial and 13 fungal endophytic isolates where three bacterial genera of *Pseudomonas, Pantoea* and *Bacillus* and three fungal genera of *Aureobasidium, Alternaria* and *Cochliobolus* were found widely distributed in the above-ground tissues (Scott et al. 2018). Further experiments are needed to validate the effects of these isolates on *Cannabis* growth and secondary metabolite production. Among the microbial inoculants that have been engineered for *Cannabis* production, Mammoth P™ represents an example of microbial biostimulants designed to enhance bud growth, increase yield and elevate plant biotic stress (Conant et al. 2017). The authors claimed that application of Mammoth P™ in

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Cannabis sativa subsequently increased plant aerial biomass by 16.5%, however, its correlation to cannabinoid synthesis was not documented.

The first report on spatio-temporal and cultivar-dependent divergences in indoor commercial settings showed variations in the bacterial and fungal microbiome of *C. sativa*. The study included three cultivars – CBD Yummy, CBD Shark and Hash – and was carried out in strict indoor commercial settings (Comeau et al. 2020). The researchers did not apply microbial inoculants for the experiment. The study investigated spontaneous microbes that established themselves during the growth of *Cannabis* plants. Nevertheless, they found that the microbial communities changed during the growth cycle and were different between *Cannabis* strains. *Penicillium, Aspergillus*, *Zopfiella* and *Fusarium* genera of Ascomycota and Basidiomycota were recognized as the dominant fungi while Burkholderiaceae and Rhizobiaceae of the phylum Proteobacteria, and Streptomycetaceae and Norcardiodaceae of the phylum Actinobacteria were the dominant Bacteria. The authors did not quantify the metabolic profiling connecting microbes associated with the rhizosphere. They predicted the metabolic pathway based on bacterial abundance linked to glucose, pentose, lipid and amino acid metabolism, although this was not further validated (Comeau et al. 2020).

Determining core microbiota could enhance and sustain cannabinoid ratio

In this context, the core microbiota is defined as a microbial community common and essential to all healthy *Cannabis* plants. This core microbiota is anticipated to give indispensable indicators of crucial soil processes, of links between microbiota and their functional attributes (Delgado-Baquerizo et al. 2018), and of soil microbial communities (Lebeis 2014; Zamioudis and Pieterse 2011). However, plant genotype

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plays a key role in shaping the microbial communities of the rhizosphere (Marques et al. 2014; Sapkota et al. 2015). Plant root bound microbes are so crucial for plant health that they are often referred to as the second genome of the plant(Berendsen, Pieterse, and Bakker 2012). It is important to select microbes that improve nutrient security, plant health and biosynthesis of chemical compounds for a specific genotype. Genotype plays a vivacious ecological role in maintaining complex connections between microbial taxa in spite of being allied with below-ground nutrient cycling. However, introduction of unspecified microbes could be ineffective or have an antagonistic effect due to competition with native soil microbial communities or reduced colonization efficiency (Qin et al. 2016). The core microbiota could be a baseline for selecting beneficial microbes, whose communities could then be manipulated to enhance desired functions and services of plant hosts such as biochemical compound production. In order to achieve this, we must prioritize study of the microbial community structure of cannabis and the influencing factors in its different stages of growth. Microbial communities are not static, and their dynamic is impacted by growth stages and cultivation environments. Many studies review multiple investigations of root-associated microbial communities – for example, mycorrhizal fungi and their associated microbes triggering plant growth promotion in an agricultural context (Hijri 2016; Ismail and Hijri 2012; Zarik et al. 2016) or microbial community in contaminated environments (Bell et al. 2014; Bourdel et al. 2016; Dagher, Pitre, and Hijri 2020; Hassan, Hijri, and St-Arnaud 2013; Iffis, St-Arnaud, and Hijri 2017; Dagher et al. 2019). Such studies could be adapted and applied to marijuana and industrial hemp so as to decipher the underlying core microbiota.

Here we propose a technical workflow for how the microbial community structure of *Cannabis* could be decoded and used to determine core microbiota, tested and

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validated for stable cannabinoid production. Members of the core microbiota should be tested for compatibility, synergy, plant growth promoting activities and positive correlation secondary metabolite production. We propose the following hypothesis: *Cannabis* plants recruit their specific core microbiota when they are inoculated with a microbial suspension made from naturally microbial-rich environments such as forest soils. Amplicon sequencing targeting the bacterial 16S rRNA gene, the fungal ITS gene and fungal 18S rRNA gene, coupled with whole metagenome and/or metatranscriptome sequencing using high-throughput sequencing (e.g, Illumina platforms), will allow us to decipher the microbial communities and their spatio-temporal changes in different *Cannabis* cultivars. Bioinformatics and network analyses within highly-connected microbial species will assist us in finding hub microbial taxa across cultivars (Floc'h et al. 2020). These hub taxa will be the candidates for identifying core microbes influencing plant growth and cannabinoid biosynthesis (Fig. 1), while evidence of the hub microbial species will serve as a first step towards screening microbes exclusively for cannabinoid stabilization.

Conclusions

Cannabis has been banned by various countries around the globe over many centuries, limiting research and development of its cultivation. *Cannabis* cultivation is highly variable on a global scale, but cultivation practices can greatly influence yield and cannabinoid quality even in the same cultivar. Mounting concerns regarding the topic of stable and sustained cannabinoid production have recently drawn the meticulous attention of scientists. Our main purpose here is to speculate on the most sustainable way to minimize the imbalance of cannabinoid production in *Cannabis*. Therefore, contingent upon related and high-throughput research, we propose that studies to decode microbial communities and their interactions with

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Cannabis plants would be a promising way to formulate bioinoculants for improvement of cannabis quality in sustainable agricultural practices. Although beneficial microbes for biomass improvement are available for other plant species, the practice of microbe-based organic farming for cannabis cultivation is still in its infancy. We propose that the identification of core microbiota and their correspondence with secondary metabolites production through metagenomics in combination with metabolomics will offer new leads for exploring the underlying mechanisms of *Cannabis* cultivation for improved, sustainable and stable production of cannabinoids. Conclusively, deeper understanding of microbiota-biochemical talk may rationalize our current gaps in knowledge correlating microbial mechanisms for stabilizing cannabinoid biosynthesis.

List of abbreviations

CBD: Cannabidiol
THC: Tetrahydrocannabinol
PGPR: Plant growth promoting rhizobacteria
SIREM: Systematically induced root exudation of metabolites
ABA: Abscisic acid
GA: Gibberellins
ET: Ethylene

Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

 doi:10.20944/preprints202012.0736.v1

This study does not involve any animals for experimental studies.

Consent for publication

Not applicable.

Competing interests

Both authors, BA and MH work for Institut de Recherche en Biologie Végétale, Université de Montréal

Funding

This study was supported by The Fonds de recherche du Québec - Nature et technologies (FRQNT) B3X fellowship to BA and by the Natural Sciences and Engineering Research Council (NSERC) Discovery grant to MH. Health Canada issued a licence number LIC-QB7LHCB3BL-2020 to MH in accordance with the Cannabis Act and Cannabis Regulations.

Author contribution

Bulbul Ahmed: Conceptualisation, Writing of the manuscript.

Mohamed Hijri: Conceptualisation, Funding acquisition, Supervision, Revision of the manuscript.

Acknowledgments

Not applicable

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Figure 1

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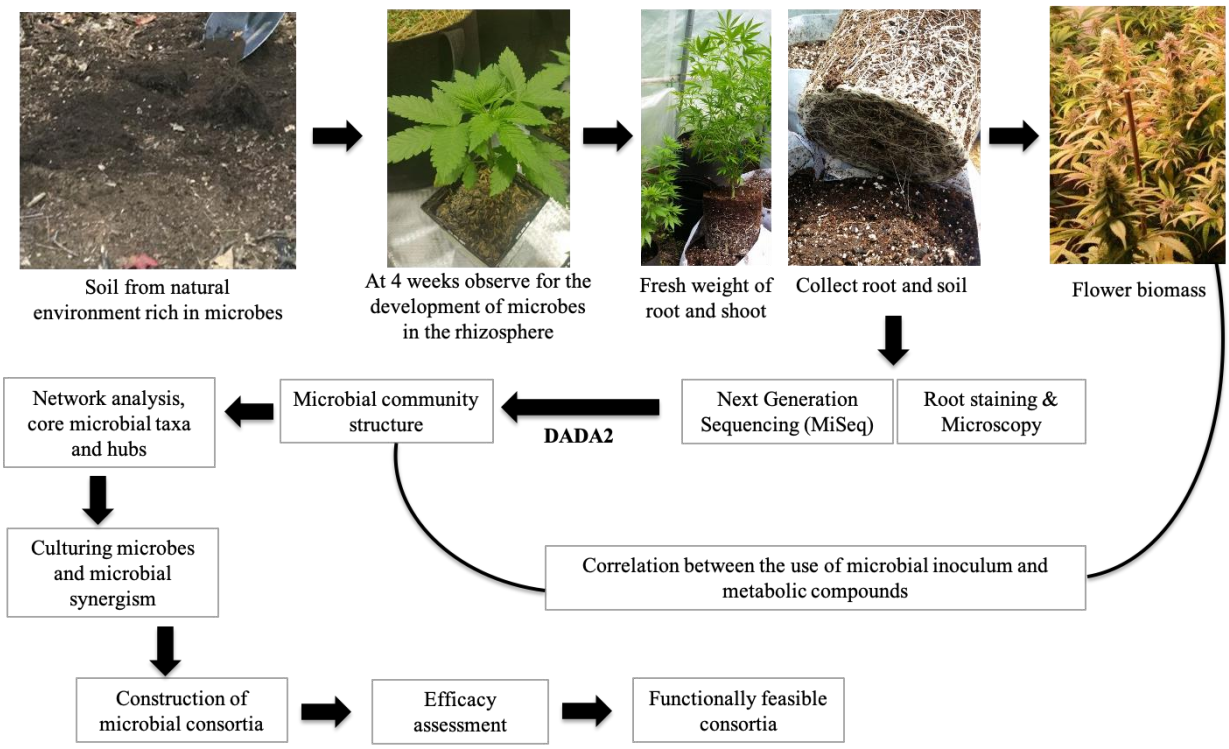

Figure 1. This illustration shows the technical workflow for the development of microbial consortia for *Cannabis* (hemp and marijuana) to enhance the yield and quality of metabolic compounds. The source is a microbial suspension from a natural environment rich in microbes. A root colonization study at 4 weeks allows assessment of whether or not microbes have been established. Next-generation sequencing and the DADA2 pipeline in R can determine community composition, and core microbes can be isolated from cannabis roots and rhizospheric soils. Network analysis will provide insight into the core microbial taxa and hub microbes. Later the core microbes can be cultured in different microbial growth mediums and microbial synergism can be evaluated. Then, different microbial consortia can be considered for efficacy assessment, and functionally feasible consortia considered for utilization in the cultivation of marijuana and hemp.