







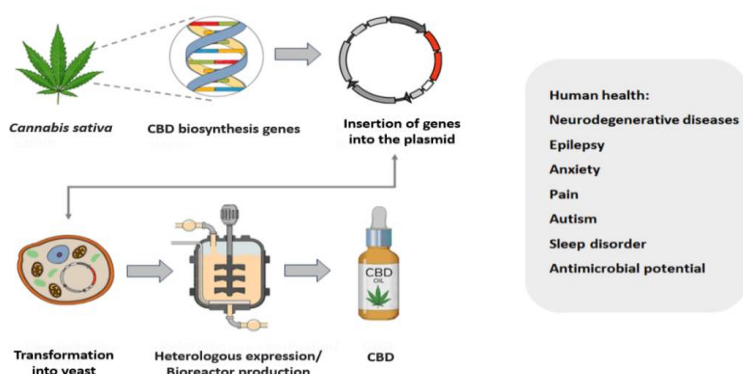
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# Insights of Cannabinoid CBD: The Pharmacological Potential and Heterologous Expression

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In this review, a thorough approach was taken to the therapeutic potential of CBD and the possibilities of heterologous expression for its synthesis. Synthesizes the most recent discoveries on the medicinal benefits of CBD, its mode of action, and different methodologies that aim to maximize CBD production using heterologous expression systems. This review contributes to increasing knowledge about the wide range of uses of CBD and its potential in industries, including biotechnology and medicine.

## Graphical abstract



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## 1. Introduction

Cannabis, also known as marijuana, is a Cannabaceae plant that originated in Southeast and Central Asia. It consists of four species: *Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis* and *Cannabis afghanica* [1]. The qualitative qualities of this plant make it suitable for the production of textile fibers, biofuel, plastic products, seed oil, a substitute for wood and a possible medicinal agent [2].

The cannabis plant includes a large number of chemical components, including approximately 100 types of cannabinoids known as terpenophenolics. These chemicals are synthesized from fatty acids and isoprenoid precursors

during the secondary metabolism of Cannabis [3, 4]. The primary chemicals produced are  $\Delta^9$ -tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) [5]. Thermal decarboxylation converts THCA and CBDA into  $\Delta^9$ -tetrahydrocannabinol (THC) and cannabidiol (CBD), respectively [6].

CBD and THC (Figure 1) are mainly sourced from the *Cannabis sativa* plant or produced using chemical techniques [7, 8]. This plant suffers from a number of obstacles during cultivation, including vulnerability to environmental conditions and infections.<sup>3</sup> Furthermore, they have extensive growth

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cycles that influence changes in profile and low CBD concentration as the plant grows, matures, and ages [7, 9]. However, constraints in chemical synthesis include the creation of CBD isomers and the use of organic solvents that can be difficult to remove. All of these linked parameters are costly and complicate the isolation of very pure CBD [7].

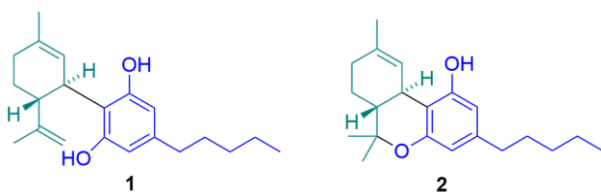


Fig. 1. Chemical structures of CBD (1) and THC (2).

The CBD/THC production ratio of cannabis has resulted in three distinct categories: industrial hemp with THC less than 0.3%, marijuana with THC equal to or greater than 0.3%, and medicinal cannabis with a high concentration of CBD and THC less than 0.3%. Due to the increasing use of these molecules as an alternative in the treatment of many pathologies, particularly neurological illnesses, the global legal market is expected to reach an average of more than \$100 million by 2030 [10].

Cannabis is currently allowed for therapeutic use in the Netherlands, Canada, the Czech Republic, Israel and several regions of the United States [3]. On the contrary, Brazil's anti-drug law 11,343/2006 forbids the planting, growth, harvesting and exploitation of cannabis due to its recreational use, particularly the euphoric effects generated by THC. As a result, due to the positive benefits attributed to CBD derived from cannabis and the legal restrictions on the production of this plant, the primary objective of this study is to conduct a bibliographic survey of studies and methodologies on heterologous CBD expression.

## 2. Mechanism of Action of Cannabinoids

The endocannabinoid system consists of two types of cannabinoid receptors: type 1 (CB1), which is found in the central nervous system, and type 2 (CB2), which is found largely in immune cells and the peripheral nervous system. It includes the endogenous ligands anandamide and 2-arachidonoylglycerol, as well as metabolic and synthetic enzymes. The system inhibits neurotransmitters such as dopamine, acetylcholine, glutamate, histamine, serotonin and GABA [1, 11, 12].

THC is the main ingredient in cannabis that interacts with the endocannabinoid system by binding to the CB1 and CB2 receptors. Its medicinal properties include analgesic, anti-inflammatory, anticonvulsant, and muscle relaxant actions. However, significant affinity for these receptors can result in adverse/toxic consequences such as psychoactive effects, anxiety, paranoia, euphoria, sleeplessness, and withdrawal syndrome [1, 5, 10, 13].

Cannabidiol (CBD) is a potential chemical that has been extensively explored due to its low affinity for the CB1 and CB2 receptors, which does not result in adverse effects.<sup>1</sup> CBD interacts complexly with receptors, enzymes, and neurotransmitters in the human body; however, the mechanism of these interactions has not yet been fully understood [1, 13, 14].

The anticonvulsant effect of CBD is believed to be due to

its ability to block voltage-dependent calcium channels (VGCC) and to function as an antagonist against the G protein-coupled receptor 55 (GPR55) [4, 6]. These channels regulate calcium influx into cells and play an important role in neuronal transmission and excitability control [12]. Furthermore, CBD acts as an agonist on transient receptor potential vanilloid type 1 (TRPV1) receptors, which can modulate pain response and inflammatory processes, providing analgesic and anti-inflammatory properties [13-15]. However, due to its multifunctional properties, CBD can be used as a medication against a variety of illnesses, particularly those affecting the central nervous system.

## 3. Cannabidiol and its Applications as Potential Pharmaceuticals

### 3.1 Effects on human health

During the past decade, researchers around the world have led to studies on the usefulness and safety of CBD for the treatment of a variety of diseases. As a result, the FDA and the European Medicines Agency (EMA) have approved CBD Epidiolex (GW Pharmaceuticals) as an adjunctive treatment for Dravet syndrome (DS), Lennox-Gastaut syndrome (LGS) and tuberous sclerosis complex (TSC) [12].

Epilepsy is a complex condition that includes genetic causes, acquired brain traumas, and metabolic abnormalities. Due to the variety of causes, defining treatment requires a personalized strategy. Furthermore, currently available medications may have low efficacy and unacceptable side effects, reducing patient quality of life [15].

Devinsky et al. [16] conducted a double-blind, randomized study to assess the efficacy of CBD in treating Dravet syndrome (DS) in 120 children. This syndrome is a drug-resistant epileptic condition that causes high rates of newborn mortality. Initially, 100 mg.mL<sup>-1</sup> doses were administered to patients for 18 weeks, with a maximum concentration of 20 mg/kg/day. Patients who received CBD (38.9%) had a significantly lower frequency of seizures than the placebo group (13.3%).

After a year, the same author investigated the effect of CBD on another rare disease known as Lennox-Gastaut (LGS), which is characterized by seizures resistant to traditional treatments. The 225 subjects were divided into three groups: placebo (76), CBD 20 mg/kg/day (76), and CBD 10 mg/kg/day (73). The results show a reduction of 41.9% and 37.2% reduction in epileptic seizures in the CBD 20 mg/kg/day groups, respectively, compared to the placebo group (17.2%) [17].

In addition to reducing the frequency of epileptic seizures by 71.7%, another study used functional magnetic resonance imaging (fMRI) to assess the effect of CBD on functional connectivity of the resting state (rs-FC). This method enables the study of how different regions of the brain communicate and coordinate their activities using images, even when the person is not doing a specific job. The findings revealed that after using CBD, patients improved in areas such as seizure severity, adverse events, and emotional states, similar to the results reported in healthy individuals [18].

It is vital to note that changes in rs-FC can suggest neuronal network breakdown and resistance to antiepileptic medications. The evaluation of rs-FC when studying CBD use altered and perhaps normalized these brain connections in patients with treatment-resistant epilepsy. Furthermore, fMRI revealed alterations in how different parts of the brain

communicate with each other, indicating that CBD may be positively impacting these connections.

Kühne et al. [19] recently reviewed retrospective data from medical records from epileptic centers in Germany from 311 patients with Dravet Syndrome (SD), Lennox-Gastaut Syndrome (LGS), and Tuberous Sclerosis Complex (TSC) treated with CBD. They observed that, while seizures improved, CBD's antiepileptic effects were similar to those of conventional drugs.

These findings suggest that CBD could be used as an effective treatment for treatment-resistant epilepsy, thereby improving the quality of life of these patients.

In 2020, the United Nations (UN) withdrew cannabis from Schedule IV of the 1961 Single Convention on Narcotic Drugs, allowing greater flexibility in the use of therapeutic cannabis. This action has prompted more research into compounds derived from this plant for other illnesses that have not yet been licensed by the FDA and EMA [20].

Accordingly, a systematic study examined the effects and safety of CBD in patients with primary psychotic illnesses, such as schizophrenia. Eight studies were chosen, including four randomized clinical trials (RCTs) and four observational studies. Although data analysis revealed good benefits, specifically reduction in general psychopathological symptoms, both positive and negative, in certain studies, there are contradictions in the lack of improvement in cognitive or functional impairment in patients. Furthermore, CBD was deemed safe, with moderate side effects such as drowsiness and gastrointestinal disturbances, even at large doses, compared to drugs used to treat this disorder [21].

The efficacy of CBD was examined for social anxiety disorder, psychotic disorder, substance use disorders [22] and sleep disorders using the same criteria [23]. Bilbao and Spanagel reported persistent discomfort, spasticity, nausea/vomiting, and increased hunger [20]. CBD is a promising alternative for all medical diseases studied due to its potential efficacy and manageable side effects. However, evidence on the clinical use of CBD in these disorders is still inconclusive, due to limitations such as study heterogeneity, low number of participants, varying dosages and drug interactions, and a high risk of bias. However, these limitations can have an impact on overall research conclusions, reducing the robustness of findings and the certainty about the therapeutic efficacy of CBD, emphasizing the need for additional studies to determine a more comprehensive and consistent perspective for the safe and effective use of this molecule in various clinical conditions [20-23].

### 3.2 Antimicrobial activity

Antimicrobial resistance (AMR) is a growing threat to human and animal health. In 2022, the global average of antibiotic resistance reached alarming levels, with strains of *Klebsiella pneumoniae* and *Acinetobacter* spp. growing by 50%, while *Escherichia coli* and *Staphylococcus aureus* increased by 42% and 35%, respectively [24].

However, because of the multifunctional qualities of CBD, research has been done to assess its antibacterial activity. Table 1 summarizes the antimicrobial assays performed against Gram positive and Gram-negative bacteria that are sensitive or resistant to antibiotics used in clinical practice.

Appendino et al. found that CBD is highly effective (MICs ranging from 0.5 to 1  $\mu\text{g}\cdot\text{mL}^{-1}$ ) against all bacteria tested, despite their various resistance mechanisms [25]. *S. aureus* EMRSA-15 is resistant to methicillin, while RN4220 is resistant

to macrolides. Two strains carry genes encoding overexpression of the efflux pump, SA-1199B and XU212, which give resistance to certain fluoroquinolones and tetracycline antibiotics, respectively.

To increase the inhibitory potential of CBD, two studies created analogs by acetylating and methylating phenolic hydroxyls, and concluded that these were the primary modifications that significantly interfered with the antimicrobial action of these molecules [25, 26].

Martinenghi et al. found MIC values of 1  $\mu\text{g}\cdot\text{mL}^{-1}$  for susceptible strains of *S. aureus* (ATCC 25923) and methicillin-resistant (MRSA USA300). Strains of *Staphylococcus epidermidis* MRSA and clinical isolate (CA#71) showed susceptibility at 4  $\mu\text{g}\cdot\text{mL}^{-1}$ , but did not show MICs higher than the antibiotics tested, except clindamycin. However, CBD proved ineffective against Gram-negative bacteria [26].

In contrast to previous investigations by Blaskovich et al., where the antimicrobial action of CBD was successful against Gram-positive bacteria, resistance was shown in various Gram-negative species, including *E. coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Surprisingly, it demonstrated antibacterial activity against *Neisseria gonorrhoeae* and *Neisseria meningitidis*, which cause gonorrhea and meningitis, respectively. This activity could be attributed to structural changes in this bacterial genus that allow for the permeabilization of the lipopolysaccharide membrane and hence the action of CBD [27].

Given the significance of this molecule and the limitations in its acquisition, such as the difficulty in obtaining high levels of purity, drug laws, and environmental impacts, the prospect of production via heterologous recombination indicating biosynthetic pathways as well as compatible vectors has become very appealing.

## 4. Cannabinoid Biosynthesis Pathway

In this biosynthetic route, some critical phases can be highlighted. The pathway involves the biosynthesis of olivetolic acid (OA) and geranyl diphosphate (GPP), the prenylation of OA with GPP to form cannabigerolic acid (CBGA), and the action of the enzymes  $\Delta^9$ -tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS) and cannabichromenic acid synthase (CBCAS) for the synthesis of the major cannabinoids tetrahydrocannabinol (THC), cannabidiol (CBCAS).

Cannabinoids originate from multiple biosynthetic pathways, including fatty acids and isoprenoid precursors [28]. To begin the biosynthesis of OA, an acyl-activating enzyme converts hexanoic acid to hexanoyl-CoA. According to Stout et al. [28] the olivetol synthase enzyme (OLS) condenses hexanoyl-CoA with three molecules of malonyl-CoA to create olivetol, which is then cyclized by the olivetolic acid cyclase enzyme [29].

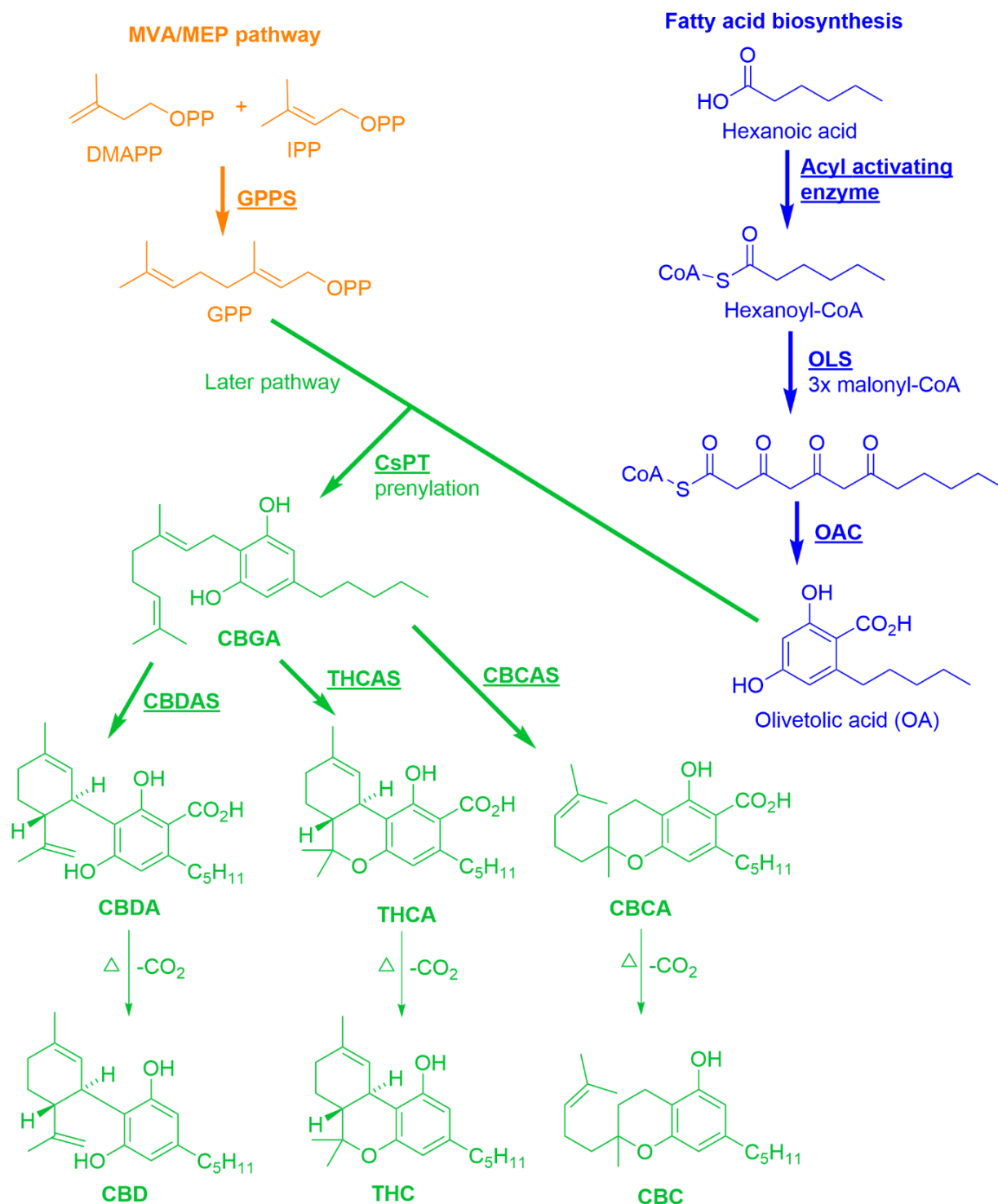
The terpenoid portion of cannabinoids is derived from two biosynthetic pathways: the mevalonate pathway (MVA) and the 2-C-methyl-D-erythritol 4-phosphate or 1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway), which are located in the cytosol and plastid, respectively, and are responsible for the production of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) [3]. The enzyme geranyl diphosphate synthase (GPPS) converts IPP and DMAPP to GPP [30].

**Table 1.** Minimum inhibitory concentration of CBD against Gram positive and Gram-negative bacteria.

Minimum Inhibitory Concentration [MIC $\mu$ g.mL <sup>-1</sup> ]						
Bacterial strain	CBD	Norfloxacin	Erythromycin	Tetracycline	Oxacillin	Reference
<i>S. aureus</i> -1199B	1	32	0.25	0.25	0.25	[25]
<i>S. aureus</i> RN-4220	1	1	64	0.25	0.25	
<i>S. aureus</i> XU212	1	4	> 128	128	128	
<i>S. aureus</i> (ATCC 25923)	0.5	1	0.25	0.25	0.125	
<i>S. aureus</i> EMRSA-15	1	0.5	> 128	0.125	32	
<i>S. aureus</i> EMRSA-16	1	128	> 128	0.125	> 128	
Bacterial strain	CBD	Clindamycin	Tobramycin	Meropenem	Ofloxacin	Reference
<i>S. aureus</i> (ATCC 25923)	1	> 128	0.25	0.06	0.5	[26]
<i>S. aureus</i> MRSA USA300	1	128	1	16	64	
<i>S. epidermidis</i> (ATCC 51625)	4	0.06	1	2	1	
<i>S. epidermidis</i> (CA#71)	4	> 128	0.06	0.12	0.25	
<i>P. aeruginosa</i> (PA01)	> 64	> 128	0.12	0.5	1	
<i>E. coli</i> (ATCC 25922)	> 64	8	2	0.06	0.06	
Bacterial strain	CBD	Clindamycin	Vancomycin	Daptomycin	Trimethoprim	Reference
<i>S. aureus</i> (ATCC 25923)	1-2	0.06–0.25	1–2	1–2	2–4	[27]
<i>S. aureus</i> MRSA (ATCC 43300)	1-2	>64	0.5–1	0.5–1	2	
<i>S. epidermidis</i> (ATCC 12228)	1-2	0.06–0.25	1–2	1–8	1–4	
<i>S. epidermidis</i> (NRS-60 VISE)	4-8	0.06–0.125	2–4	1–4	>64	
<i>E. faecalis</i> (clinical isolate)	2-4	>64	>64	8	>64	
Bacterial strain	CBD	Gentamicin	Vancomycin	Meropenem	Levofloxacin	
<i>E. faecalis</i> (MMX486 VRE)	1	>32	>16	4	>32	[27]
<i>E. faecium</i> (MMX485 VRE)	1	>32	>32	>32	1	
Bacterial strain	CBD	Clindamycin	Colistin	Mupirocin	Trimethoprim	
<i>P. aeruginosa</i> (ATCC 27853)	>64	>64	0.25–1	>64	>64	[27]
<i>E. coli</i> (ATCC 25922)	> 64	>64	0.06–0.125	>64	0.5–2	
Bacterial strain	CBD	Gentamicin	Vancomycin	Meropenem	Levofloxacin	
<i>A. baumannii</i> (ATCC 19606)	>64	8	>32	0.5	0.25	[27]
<i>N. gonorrhoeae</i> (ATCC 19424)	1	4	8	≤0.03	0.002	
<i>N. meningitidis</i> (MMX 7515)	1	1	0.12	0.25	0.5	

Cannabigerolic acid (CBGA) is a frequent precursor of cannabinoids. CBGA is produced by the cannabis sativa prenyltransferase enzyme (CsPT) through the prenylation of OA and GPP.<sup>31</sup> The enzymes  $\Delta^9$ -tetrahydrocannabinolic acid

synthase (THCAS), cannabidiolic acid synthase (CBDAS) and cannabichromenic acid synthase (CBCAS) convert CBGA into THCA, CBDA, and CBCA [32]. As shown in Figure 2, CBDA, THCA and CBCA are thermally decarboxylated to provide the neutral forms CBD, THC and CBC, respectively [33].



**Fig. 2.** Cannabinoid biosynthesis pathway: Orange provides GPP from the MVA/MEP pathway, blue provides fatty acid biosynthesis resulting in OA, and green begins with prenylation of GPP with OA to produce the common precursor CBGA and ends with the formation of the cannabinoids CBD, THC, and CBC via a thermal process. The enzymes of each step have been underlined.

#### 4.1 Enzymes of the Cannabinoid Biosynthesis pathway

The enzymes involved in the biosynthesis of cannabinoids belong to various families, and each has an intrinsic three-dimensional structure with a specific active site for the

reaction it will catalyze, distinguishing it as a unique enzyme. Previously, it was assumed that OA synthesis was completely dependent on the action of OLS, a type III polyketide synthase (PKS). Taura et al. [34] observed that a type III PKS cloned



from cannabis leaves produced olivetol and  $\alpha$ -pyrones (PDAL and HTAL), but not OA. OAC, a dimeric  $\alpha$ + $\beta$  barrel protein (DABB) that catalyzes C2-C7 aldol condensation with carboxylate retention, is necessary for OLS to cycle OA [29].

CsPT1 and CsPT4 are two membrane proteins of *C. sativa* that can pre-nylate OA and GPP to generate CBGA; they have identical 62% amino acid sequence [35]. In vitro studies indicate that CsPT4 has stronger enzyme activity than CsPT1, at 6.72  $\mu$ M [36] and 60 mM [33].

The action of FAD-dependent cyclase enzymes (CBDAS, THCAS, and CBCAS) occurs in the final step to the production of the main cannabinoids. These enzymes facilitate the oxidative cyclization of CBGA [35], and share a high protein identity >80%, including the conserved berberine bridge type (BBE) domain involved in binding a FAD moiety [37].

## 5. Heterologous Expression of CBD

When selecting a host organism for the heterologous expression of a metabolite, consider the availability of genetic information, tools for the expression of heterologous proteins, genetic approaches, and the need for specific molecular capabilities such as precursor or cofactor production [3].

Genetic engineering and heterologous expression provide a fresh and beneficial approach to producing cannabinoids, particularly CBD, in some microorganisms. This critical technique is a cornerstone in the realm of cannabis research, giving higher yields and better procedures. A recent study has shown that sugar can be converted into cannabis by introducing particular genes and increasing enzyme levels. This technique ensures the integrity of final products while increasing production yield, implying a substantial advancement in CBD research [38].

Given the features of late cannabinoid pathway enzymes, it is clear that a prokaryotic host is not particularly viable. Because CBGAS is an integral membrane protein, it is unlikely that bacteria will express a considerable amount of functional protein. The FAD-dependent oxygenases THCAS and CBDAS feature a disulfide bond and several N-glycosylation sites, making them incompatible with prokaryotic hosts [39]. Furthermore, the expression of certain enzymes involved in cannabis biosynthesis, such as cannabidiolic acid synthase (CBDAS), in bacteria can be difficult due to their properties. CBDAS, for example, has a covalently attached flavin adenine dinucleotide (FAD) linker, which may limit expression in bacterial hosts because the FAD cofactor requires molecular oxygen to replenish [3].

Despite restrictions, *Lactobacillus paracasei* has been genetically engineered to express cannabidiol (CBD) and  $\Delta^9$ -tetrahydrocannabinolic acid (THCA), allowing the synthesis of these cannabinoids in the digestive tract. The host could be a human, a non-human primate, or another organism that benefits from the modified probiotic [40].

Yeasts are the most widely employed species for cannabinoid synthesis. Studies have shown that cannabinoids and their artificial equivalents can be completely produced by genetically modifying yeast to manufacture CBGA, THCA, CBDA, THCVA, and CBDVA from galactose. Furthermore, the structural diversity of cannabinoids was achieved by including several fatty acids in the biosynthetic process, broadening the range of chemicals generated [36]. Yeasts such as *Saccharomyces cerevisiae* and *Komagataella phaffii* have been used in cannabinoid synthesis, and these cells provide benefits such as cofactor generation, post-translational

modifications, and eukaryotic protein folding, ensuring enzyme performance [37]. *K. phaffii* was effectively used to produce THCA, with a yield of 1 mM (0.36 g of THC per liter) [39].

In addition to the benefits listed above, *S. cerevisiae* is one of the most well-known host species with well-established molecular tools, which makes it attractive for heterologous biosynthesis of cannabinoids [3]. In this context, and knowing that the precursors GPP, malonyl-CoA and hexanoyl-CoA are required for biosynthesis and are all obtained from acetyl-CoA [41], rewired core carbon metabolism in *S. cerevisiae* with four genes. They obtained an increase in cytosolic acetyl-CoA production while reducing CO<sub>2</sub> loss and ATP expenditure, as well as improving the redox balance.

The cannabis biosynthetic process contains steps that are difficult to execute in the context of heterologous expression, and efficient yeast platforms have been designed to avoid these limiting steps and ensure cannabinoid expression. One of these issues is the generation of the primary molecule (CBGA), which has low titers due to the slow conversion of hexanoate to AO and the limited activity and stability of the enzyme CsPT4. Zhang et al. [31] used modified *S. cerevisiae* to produce a 78.64-fold increase in CBGA production, reaching 510.32  $\pm$  10.70 mg.L<sup>-1</sup> from glucose and hexanoate. They stopped the consumption of hexanoate through the beta-oxidation route, reduced its incorporation into fatty acids, increased the endoplasmic reticulum, and fused an auxiliary protein to CsPT4.

Syntiva Therapeutics Inc. filed a patent that permitted the manufacture of hexanoate in yeast during the stationary phase using particular genes and overexpression of the fadD gene, resulting in costs of less than \$1,000 per kilogram of pure cannabis. The higher number of patents filed demonstrates that these genetic alterations considerably boosted process yield. This innovative technology suggests more enhancements, such as altering peroxisomal  $\beta$ -oxidation in yeast to broaden sources of fatty acids and including alternate mechanisms for turning glucose into cannabinoids [38].

Currently, yeast is believed to be the most feasible species for the expression of CBD. For example, yeast *Kluyveromyces marxianus* obtained the highest documented expression of hexanoic acid to date, reaching up to 142 mg.L<sup>-1</sup> using a method that can be applied to other yeasts [42]. Researchers demonstrated that the yeast *K. marxianus* can express and produce various cannabinoids, such as cannabigerolic acid (CBGA), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabigerol (CBG) and tetrahydrocannabinol (THC), which resulted in a patent [43]. Luo et al. [36] used *S. cerevisiae* to synthesize CBD, including cannabigerolic acid,  $\Delta^9$ -tetrahydrocannabinolic acid,  $\Delta^9$ -tetrahydrocannabivarinic acid, and cannabidivarinic acid, from galactose. This research group engineered the native mevalonate pathway to obtain IPP and GPP, established a heterologous biosynthetic pathway for the production of hexanoyl-CoA, and introduced cannabis genes encoding the enzymes involved in the synthesis of AO, a geranyltransferase and the specific synthases for each cannabinoid. They achieved a CBDA titer of 4.2  $\mu$ g.L<sup>-1</sup>.

The primary cannabinoids (THC, CBD, and CBC) share most of the same biochemical process, with the exception of the final stage, where each is generated from its particular synthase. These synthase enzymes have a high protein identity with each other, reaching 80%. Wiles et al. [37] and do not demonstrate perfect selectivity, creating secondary by-

products and other small cannabinoids [32].

Careful analysis of the structure, activity and subtle variations of these enzymes is critical for more targeted heterologous expression with higher titers, whether for THC, CBD, or CBC. Mutagenic changes were made to the THCAS and CBDAS enzymes to explore the glycosylation pattern, the BBE domain, the active site, and the selectivity of the product to increase the enzymatic performance. Two versions of CBDAS were developed, with catalytic activity increased 2.8 and 3.3 times for CBDA production [32]. Other creatures have already been employed for this purpose. A study proved the capability to produce heterologous cannabinoids in tobacco plants. Cannabinoids such as CBDA can be produced and released into the secretory cavity of the trichome using trichome-specific promoters, according to research. Furthermore, the soluble bacterial prenyltransferase NphB simplifies the cannabis manufacturing process by replacing a difficult step that requires an integral membrane protein. These findings point to tobacco as a promising organism for cannabinoid synthesis [44].

Advances in genetic engineering, as well as methodological tools that allow the regulation of biosynthetic pathways, have resulted in significant breakthroughs in the production of cannabinoids from various living organisms, particularly yeast. This area remains a fertile ground for the development of techniques that allow for greater output and purity of CBD, resulting in the expansion of heterologous biosynthesis

## 6. Conclusions

Based on all bibliographic research conducted, we were able to verify the exploration of the great pharmacological potential of CBD and the crucial role of heterologous expression in overcoming production challenges. Through continuous research and innovation, CBD promises to be a versatile therapeutic agent with diverse applications in medicine and biotechnology, paving the way for advances in healthcare and drug development.

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## Author Contributions

#IBP and CM contributed equally. AB conceived the idea. IBP and CM designed the manuscript. IBP, CM, DRSB, GBS and DPL, investigated current literature. All the authors contributed for the writing of the manuscript.

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