



# Engineering microbes to overproduce natural products as agrochemicals

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## ABSTRACT

Current agricultural practices heavily rely on the excessive application of synthetic pesticides and fertilizers to meet the food demands of the increasing global population. This practice has several drawbacks including its negative impact on the environment and human health. Recently, the use of natural products has gained interest as alternatives to these synthetic agrochemicals due to their selective working mechanisms and biodegradability. In order to efficiently produce these natural agrochemicals, engineering microorganisms is emerging as an increasingly viable approach, and it is anticipated that it will have a significant market share in the near future. This approach manipulates the metabolism of microbes to manufacture the desired natural compounds from low-cost starting materials. This review discusses recent examples of this approach. The produced natural products can serve as biopesticides or plant growth regulators for the sustainable improvement of plant growth and disease control. The challenges in further developing these strategies are also discussed.

## 1. Introduction

The rapid growth of the human population and the resulting rise in food demands have imposed a large burden on agriculture [1]. In the past, plant productivity and crop yields have been considerably enhanced through the extensive use of synthetic fertilizers and pesticides. However, significant concerns were raised regarding their undesired impacts on the environment and ecosystems [2]. Many of these non-natural chemicals remain in the soil for an extended amount of time [3], affecting soil fertility and long-term agricultural productivity. In addition, chemical pesticides frequently result in the death of non-targeted beneficial organisms and the development of pesticide resistance [4]. Many pesticides are also lost to the atmosphere or water resources causing environmental pollution [5,6]. Some pesticide residues could also contaminate the produce and pose health threats to the farmers who apply them due to their prolonged exposure [7–9].

Because of the above concerns, the use of natural products to control plant diseases has recently received increasing attention. These products are non-toxic, selective towards target pests, and biodegradable [10,11]. An example is azadirachtin, a natural insecticide derived from neem oil, which is widely used in organic farming [12,13]. Many of these molecules are extracted from plants. Unfortunately, they often incur high extraction and purification cost due to their low content in plant materials [14].

With the recent advances in metabolic engineering, some of these plant natural products can be sustainably produced from affordable building blocks by using engineered microorganisms [15]. In this review, we summarize recent examples of this approach (Table 1), primarily concerning production of biopesticides and plant hormones (Fig. 1). We also share our personal views on the opportunities and challenges that may be faced in the future when pursuing this research direction.

## 2. Biopesticides

Engineering microbes to produce naturally occurring biopesticides has been investigated as a new way of supplying the natural agrochemicals. Three recent examples are reviewed below.

### 2.1. Insecticide

The first study used genetically manipulated *Escherichia coli* to produce cinnamaldehyde (a natural nematocide) from glucose [16]. The essential precursor for cinnamaldehyde synthesis is L-phenylalanine. Extensive research has been conducted on the biosynthesis of L-phenylalanine from glucose [17]. In this study, the strain was engineered to increase the intracellular pool of L-phenylalanine in accordance with published methods. The L-phenylalanine-producing strain

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was further engineered by overexpressing the enzymes that convert L-phenylalanine into cinnamaldehyde: phenylalanine-ammonia lyase (PAL), 4-coumarate CoA ligase (4CL), and cinnamoyl-CoA reductase (CCR) (Fig. 2a). PAL catalyzes the deamination of L-phenylalanine into cinnamic acid; 4CL activates cinnamic acid into cinnamoyl-CoA which was subsequently reduced into cinnamaldehyde by CCR. The authors examined the key genes from different organisms and found that the combination of 4CL from *Streptomyces coelicolor* (ScCCL), CCR from *Arabidopsis thaliana* (AtCCR) and PAL from *Streptomyces maritimus* (SmPAL) resulted in higher production of cinnamaldehyde. To evaluate its nematocidal activity, the authors treated pine wood nematode (*Bursaphelenchus xylophilus*) directly with the culture supernatant containing 60 mg/L cinnamaldehyde. Comparable nematocidal activity was observed between commercially available cinnamaldehyde at equivalent concentration and the one produced in this study. This approach is promising in cost reduction because the product can be provided to farmers without purification. However, the use of *E. coli* as a host raises concerns. *E. coli* has not been approved as GRAS (generally recognized as safe). Vegetables can be contaminated with *E. coli* which may fail to meet the food safety standards. Future research may focus on transferring the cinnamaldehyde biosynthetic pathway into GRAS organisms such as *Saccharomyces cerevisiae* or *Corynebacterium glutamicum*.

## 2.2. Herbicide

The second study used *Streptomyces albus*J1074 to produce thaxtomins, a group of natural products with herbicidal activities [18]. The native host of thaxtomins is a pathogenic *Streptomyces* species (*Streptomyces scabiei*) that causes common scab potato disease. Thaxtomins produced from the native *Streptomyces* strain also has a very low yield. In the study, the thaxtomin biosynthetic gene cluster was transferred into the non-pathogenic *S. albus* J1074, resulting in a 10-fold increase in yield compared to the native producer. Thaxtomins are cyclic dipeptides (2,5-dike-topiperazines) composed of phenylalanine and a nitrated tryptophan (Fig. 2b). The gene cluster TxtDEABCRH contains seven genes. Nitric oxide synthase (TxtD) converted L-arginine into nitric oxide. TxtE (P450) used this nitric oxide and O<sub>2</sub> to catalyze the nitration of L-tryptophan, resulting in L-4-nitrotryptophan. TxtA and TxtB (two nonribosomal peptide synthetases (NRPSs)) cyclized L-4-nitrotryptophan with L-phenylalanine to make N, N'-dimethyldiketopiperazine thaxtomin D, which is subsequently hydroxylated twice to produce thaxtomin A, by TxtC (P450). TxtR, which is induced by cellobiose, controls the expression of the thaxtomin gene cluster. The authors integrated this gene cluster into the chromosome of *S. albus*J1074. The resulting strain correctly recognized the native promoters and produced thaxtomin A and its analogs after a 6-day fermentation. One nonnatural fluorinated analog with comparable herbicidal activity was produced when the unnatural precursor

5-fluoro-L-tryptophan was supplemented in the culture medium. With further process engineering, the yield of thaxtomins analogs reached 222 mg/L. The thaxtomins pathway was not transferred to other hosts including *E. coli*, because those hosts may not recognise the native promoters of *Streptomyces* and/or have the machineries for expressing the proteins and supplying the needed precursors. Using the same or similar hosts is preferable for many complex molecules like thaxtomins.

## 2.3. Antimicrobial agents

Many biopesticide molecules remain less exploited due to the potential pathogenicity of their hosts. For example, numerous species of the genus *Burkholderia* are potential biocontrol organisms as they can produce antimicrobial agents. However, *Burkholderia* species are pathogenic to humans. A recent study characterized the *Burkholderia ambifaria* biosynthetic gene cluster (BGC) of cepacin A (an anti-oomycete natural product) using phylogeny-based genome mining approach [19]. Cepacin is an acetylenic antibiotic belonging to the polyynes class of compounds, which are characterized by the alternation of single and triple carbon-carbon bonds (Fig. 2c). The BGC is composed of 13 biosynthetic genes arranged in a single operon with regulatory genes placed upstream. The BGCs in the genome of 64 *B. ambifaria* strains were analysed and in vitro antimicrobial activity of the strains was evaluated to define the biocontrol efficacy. The virulence component encoded on *B. ambifaria*'s third replicon was deleted. The resultant mutants significantly reduced respiratory infection levels in mouse models while keeping its biopesticidal activity against *Pythium*, the pathogen responsible for crop damping-off disease. The approach used by this study will be useful to future works that aim to identify BGCs and turn pathogenic strains into safe ones.

The production of biopesticides was approached differently in each of the aforementioned studies. Before selecting strategies for developing the microbial cell factories, the biopesticides to be produced should be carefully analysed in terms of their structural complexity, the synthesis chemistry, and the application potential. For the biosynthesis of simple compounds such as cinnamaldehyde, metabolic engineering approaches can be used to establish total biosynthesis in model host species using cheap carbon source as substrate. This entails the overexpression of the essential genes involved in its biosynthesis. For compounds with complex chemical structures such as thaxtomins, transferring the pathway to common model organisms such as *E. coli* or other non-native producers may be difficult as each microorganism has a different metabolism, prefers different carbon sources, and recognises different promoters. In such cases, selecting a microorganism that is similar to the native species can be explored. For novel compounds such as cepacin A, which is even more complex and whose biosynthetic pathway is unknown, using genome mining is needed to predict the gene clusters. Avoiding pathogenic hosts should be an important consideration in this instance. The

**Table 1**  
Recent examples of natural agrochemicals produced by engineered microorganisms.

Agrochemicals	Type/Action	Microbial hosts	Titer/Yield	Ref.
Cinnamaldehyde	Nematicide	<i>Escherichia coli</i>	75 mg/L from 20 g/L glucose (Shake flask)	(Bang, Hyun Bae et al., 2016)
Thaxtomin A	Herbicide	<i>Streptomyces albus</i> J1074	222 mg/L from 10 g/L cellobiose (Shake flask)	(Jiang, Guangde, et al., 2018)
Cepacin A	Anti-oomycete	<i>Burkholderia ambifaria</i>	NA	(Mullins, Alex J. et al., 2019)
Indole-3-acetic acid (IAA)	Plant hormone	<i>E. coli</i>	744 mg/L from 20 g/L of glucose (Shake flask)	(Guo, Daoyi, et al., 2019)
Indole-3-acetic acid (IAA)	Plant hormone	<i>Cupriavidus pinatubonensis</i> JMP134	NA	(Zuniga, Ana, et al., 2018)
Gibberellins (GAs)	Plant hormone	<i>Yarrowia lipolytica</i>	13 mg/L GA <sub>3</sub> and 18 mg/L GA <sub>4</sub> (24-well plate)	(Kildegaard, Kanchana R. et al., 2021)
Abscisic acid (ABA)	Plant hormone	<i>Y. lipolytica</i>	264 mg/L (24-well plate)	(Arnesen, Jonathan Asmund et al., 2022)
Strigolactones (SLs)	Plant hormone	<i>E. coli</i> – <i>Saccharomyces cerevisiae</i> consortium	48 µg/L 5-deoxystrigol (5DS) from 40 g/L Xylose (Shake flask)	(Wu, Sheng, et al., 2021)

NA: Not available.

pathogenic hosts can either be engineered to remove their virulence factor or the metabolic pathway can be transferred to a non-pathogenic host after understanding its metabolism.

### 3. Plant hormones

Plant hormones are small molecules that regulate plant growth and development [20]. They are naturally produced by plants and some plant associated microbes [21]. The exogenous application of plant hormones has increased productivity of plants and improved their resilience to environmental stresses such as drought, cold, flood, heavy metals and salt [22]. The industrial scale production of these chemicals mainly relied on plant extraction and chemical synthesis. However, the hormone concentration in plants is extremely low, which makes the extraction method inefficient. The complex chemical structures have also prevented using chemical synthesis as the manufacturing method for acquiring many hormone molecules. Microbial biosynthesis is being investigated as an alternative route for producing these chemicals [21].

#### 3.1. Indole-3-acetic acid (IAA)

In a recent study, *E. coli* was engineered to synthesize IAA [23], the most common plant auxin and a major regulator of plant development. The most common precursor of IAA production is L-tryptophan. There are at least five different biosynthetic IAA pathways [24]. The most prevalent and well-studied one is the indole-3-pyruvate (IPyA) pathway [25]. An aminotransferase first converts L-tryptophan into indole-3-pyruvic acid which is subsequently decarboxylated and oxidized into IAA [25]. This study overexpressed *S. cerevisiae* aminotransferase (ARO8), *S. cerevisiae* decarboxylase (KDC) and an *E. coli* aldehyde dehydrogenase (AldH) to convert L-tryptophan into IAA via the three steps as described above (Fig. 3a). The L-tryptophan biosynthetic pathway from glucose was introduced into this engineered *E. coli*. The resultant strain produced up to 744 mg/L IAA from 20 g/L glucose in 24 h. Despite the relatively high product titer, future research may consider transferring the auxin biosynthetic pathway into a safer host.

*Cupriavidus pinatubonensis* JMP134 is a neutral non-plant growth promoting rhizobacteria (non-PGPR), plant-associating rhizobacterium. It was engineered in another study to produce IAA from L-tryptophan based on quorum sensing (QS) signal [26]. Microbes in the soil communicate with each other through QS signals to maximize their survival in their complex fast-changing habitats. In the process, microbes secrete signaling molecules called autoinducers which diffuse across the cell membrane and accumulate in the extracellular environment. The authors designed a cell density dependent QS genetic circuit to regulate the IAA synthesis genes. The well-known luxI/luxR-type QS system from marine bacterium *Vibrio fischeri* was selected. LuxI produces homoserine lactones (HSL), which serves as inducers for the expression of the target genes in this system. As the cell density increases, concentration of HSL increases. Once the HSL reaches a threshold, it activates the lux promoter ( $P_{lux}$ ), which transcribes the target genes. For IAA

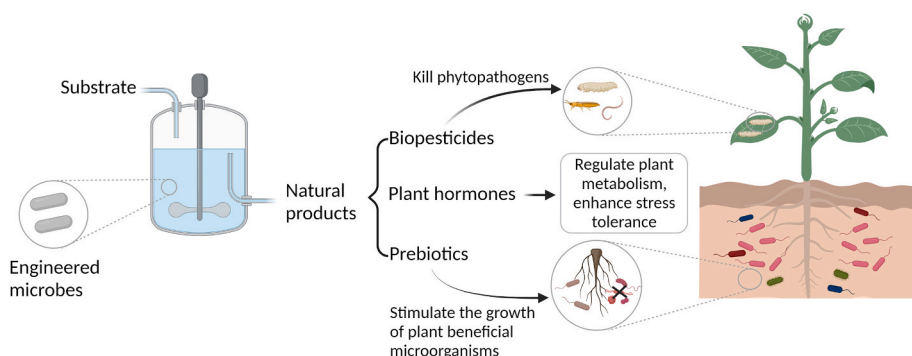
synthesis, the two key genes 2-tryptophan monooxygenase (*iaaM*) and indole-3-acetamide hydrolase (*iaaH*) were overexpressed downstream of  $P_{lux}$  (Fig. 3b). The cells harboring this plasmid were able to produce sufficient inducer to activate the IAA-gene expression and synchronize the entire population to produce IAA. Inoculating *A. thaliana* with the *C. pinatubonensis* strain significantly enhanced root development.

#### 3.2. Gibberellins (GAs) and abscisic acid (ABA)

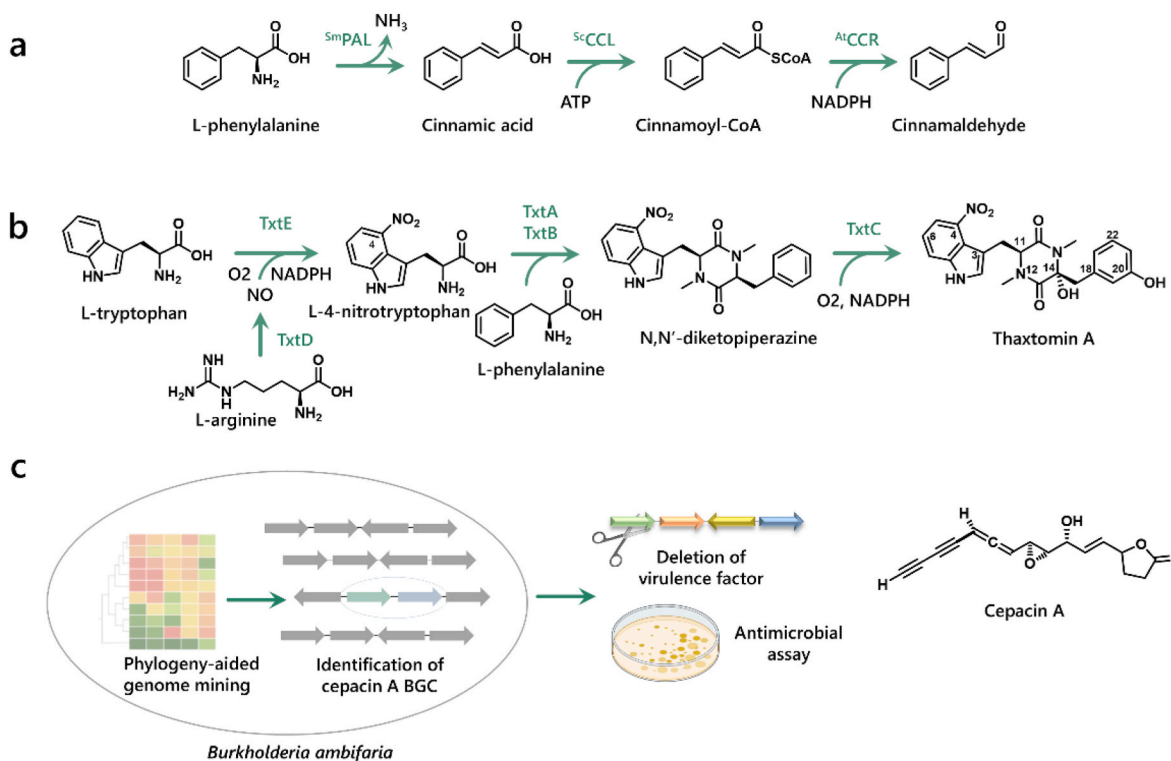
GAs and ABA are important isoprenoid plant hormones that are of great interest in the agricultural sector. In plants, their building blocks are mainly produced through the plastidial MEP pathway. Currently, the industrial production of GAs and ABA relies on native fungi *Fusarium fujikuroi* and *Botrytis cinerea* respectively, which support the production of these chemicals through the mevalonate (MVA) pathway. These fermentation processes incur high cost due to extensive separation steps and lengthy cultivation time. There are limited genetic engineering tools available for the two species. As a result, establishing the biosynthesis in model host organisms has been extensively explored.

A recent study engineered *Yarrowia lipolytica* to produce gibberellins (GAs) GA3, GA4 and GA7 [27], which are involved in the plant development and salt tolerance in many higher plants such as soybeans, maize and sugarcane. GA is a tetracyclic dihydroxy lactonic acid (Fig. 3c). Ent-kaurenoic acid (ent-KA) is the precursor for GA synthesis. The authors evaluated both plant (*A. thaliana*) and fungal (*F. fujikuroi*) biosynthetic pathways for ent-KA production and found that expressing plant enzymes performed better than the fungal enzymes. ent-KA is derived from the phosphorylated C<sub>20</sub>-terpene precursor geranylgeranyl diphosphate (GGPP) which is formed by the sequential condensation of DMAPP (C<sub>5</sub>) with three IPPs (C<sub>5</sub>). These C<sub>5</sub> building blocks were supplied using the MVA pathway. They overexpressed 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (tHMG), the rate-limiting enzyme in the MVA pathway) and the native geranylgeranyl diphosphate synthase (GGPPS) to enhance the supply of GGPP. Copalyl diphosphate synthase (AtCPS), ent-kaurene synthase (AtKS) and ent-kaurene oxidase (AtKO) were next overexpressed to transform GGPP into ent-KA. AtCPS and AtKS catalyze the cyclization of GGPP into ent-copalyl diphosphate and then into ent-kaurene (Fig. 3c). AtKO catalyzes a three-step hydroxylation/oxidation cascade to transform ent-kaurene into ent-kaurenol, ent-kaurenal and then into ent-KA. Furthermore, ent-kaurenoic acid oxidase (AtKAO), YlCyb5 (the native *Y. lipolytica* cytochrome p450) and NADPH cytochrome p450 reductase (AtATR2) were overexpressed to catalyze the conversion of ent-KA into GA12. The pathway from GA12 to the active gibberellin GA4 was extended by the overexpression of *A. thaliana* GA C20-oxidase (C20ox) and GA C3-oxidase (C3ox). The pathway was further extended from GA4 to GA7 and GA3 by over expressing a cytochrome P450 monooxygenase (P450-3p) and GA<sub>4</sub> desaturase (DESp) from *F. fujikuroi*. Through protein engineering, GA3 production was increased to 12.8 mg/L.

Similarly, Arnesen et al. engineered *Y. lipolytica* to produce abscisic acid (ABA) [28]. The genes involved in ABA biosynthesis in *Botrytis*



**Fig. 1.** Engineering microbes to produce natural products for improving plant growth. These microbes are cultivated on renewable and cost-effective substrates to produce natural compounds with the potential to function as biocontrol agents. Biopesticides protect crop health by killing phytopathogens. Plant hormones play versatile roles in regulating plant metabolism and improving resilience to abiotic and biotic stresses. Application of prebiotics to stimulate the growth of plant-beneficial microorganisms could be a future research direction.



**Fig. 2.** Engineering microbes to produce metabolites that can serve as biopesticides. (a) *E. coli* was genetically engineered to produce cinnamaldehyde, which demonstrated comparable nematocidal activity as commercial biopesticide against pine wood nematode (*Bursaphelenchus xylophilus*). PAL: phenylalanine-ammonia lyase. CCL: coumarate:CoA ligase. CCR: cinnamoyl-CoA reductase. (b) *Streptomyces albus*J1074 was engineered to produce thaxtomins, an effective bioherbicide, by integrating a biosynthetic gene cluster for thaxtomin biosynthesis into its genome. TxtD encodes for Nitric oxide synthase, TxtE and TxtC are thaxtomin cytochrome P450s, TxtA and TxtB are nonribosomal peptide synthetases (NRPSs), TxtR is the regulator induced by cellobiose. (c) Cepacin A biosynthetic gene cluster (BGC) was identified using phylogeny-led genome mining approach in *B. ambifaria*, a proven plant beneficial microorganism with potential pathogenic risk. The removal of virulence component from the genome made this microorganism a safe biocontrol agent.

*cinerea* (BcABA1, BcABA2, BcABA3, BcABA4 and BcCPR1) were over-expressed in this study with an engineered mevalonate pathway (Fig. 3d). ABA is synthesized in fungi from farnesyl diphosphate (FPP) which is formed by the sequential condensation of DMAPP with two IPPs. FPP is cyclized by  $\alpha$ -ionylideneethane synthase (BcABA3p) into  $\alpha$ -ionylideneethane, which is subsequently oxidized into  $\alpha$ -ionylideneacetic acid by a cytochrome P450 (BcABA1p). Another cytochrome P450 (BcABA2p) then oxygenated  $\alpha$ -ionylideneacetic acid into 1',4'-trans-dihydroxy- $\alpha$ -ionylideneacetic acid, which was lastly oxidized into ABA by a dehydrogenase (BcABA4p). A previously engineered strain with improved FPP production was used in the study [29]. Using two copies of BcABA3 and upregulating ERG20 (FPP synthase) substantially improved ABA titer to 252 mg/L. The above studies have demonstrated *Y. lipolytica* as a promising host which requires less cultivation time compared with naturally isolated fungi that have filamentous growth. The phytohormones produced by these engineered strains could be exogenously added to crops to improve their growth and development.

### 3.3. Strigolactones (SLs)

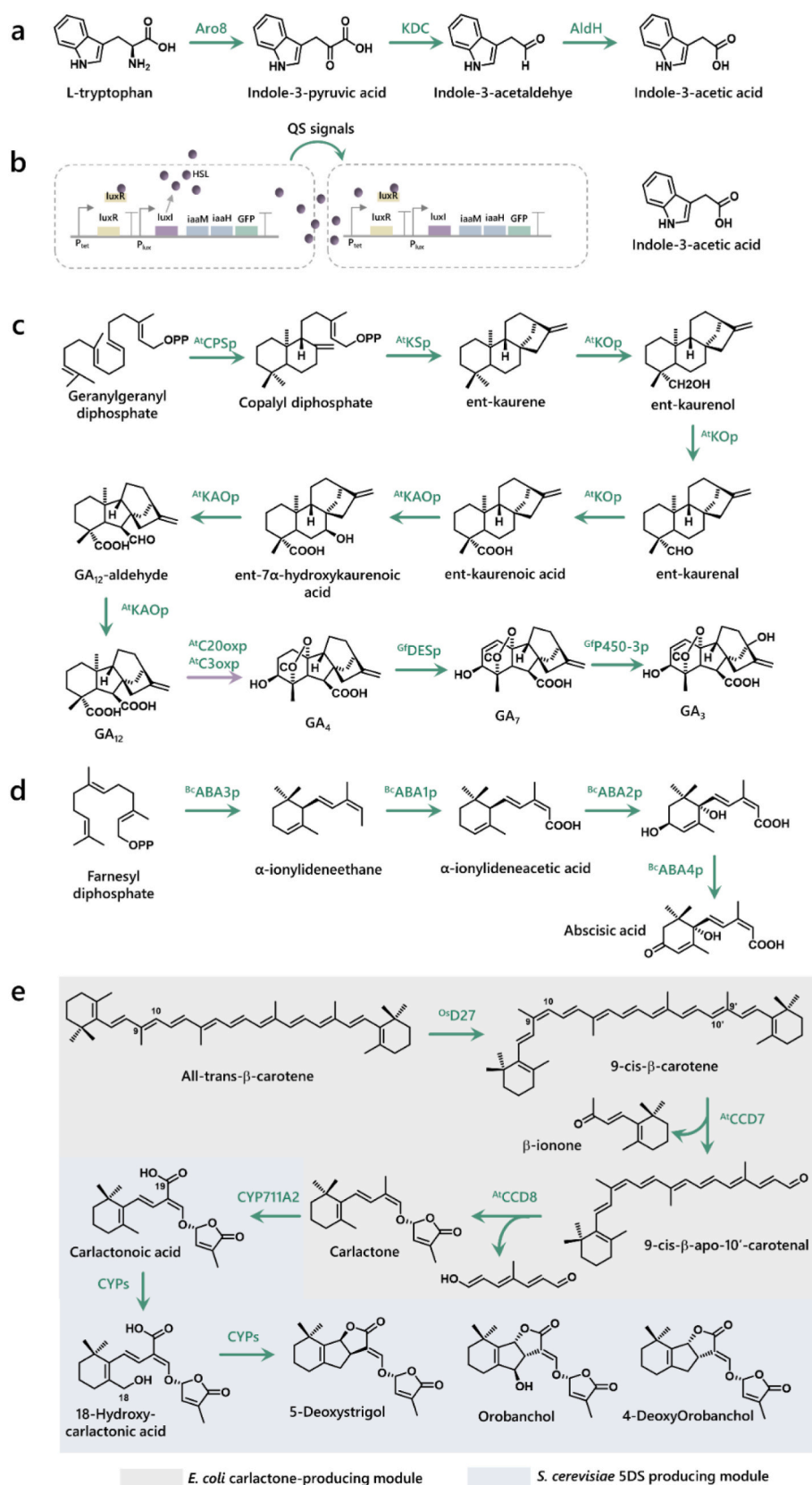
SLs are a new class of plant hormones derived from  $\beta$ -carotene. They were first recognized to promote the germination of root parasitic weeds and were later discovered to regulate several aspects of plant growth, including root architecture, shoot branching, leaf senescence, and nodulation [30–32]. The major branching point for SLs is carlactone (CL), which is produced by  $\beta$ -carotene isomerase DWARF27 (D27) and two carotenoid cleavage dioxygenases (CCD7 and CCD8) (Fig. 3e). D27 catalyzes the reversible isomerization of all-trans- $\beta$ -carotene (AT $\beta$ C) into 9-cis- $\beta$ -carotene (9C $\beta$ C). CCD7 cleaves the 9C $\beta$ C into 9-cis- $\beta$ -apo-10-carotenol, which is cleaved by CCD8 to produce the CL,

which can be functionalized in two major pathways. One produces strigol- and orobanchol-type compounds such as 5-deoxystrigol (5DS), 4-deoxyorobanchol and orobanchol (OB), while the other leads to non-canonical SLs that lack the canonical ABC tricyclic structure and whose biosynthesis is not fully understood. Engineering microbes to produce SLs has not been widely explored yet. In a recent study, *E. coli*-*S. cerevisiae* were co-cultured to synthesize various SLs [33]. The authors initially attempted to construct the SL-biosynthetic pathway in *S. cerevisiae* but were unsuccessful because the host was unable to reconstitute the function of D27 and CCD7. Engineered *E. coli* was able to produce CL by the overexpression of D27, CCD7 and CCD8, but could not functionalize it further. Using a mixed culture of *E. coli* and *S. cerevisiae* completed the task. A beta-carotene-producing *E. coli* strain was engineered to transform xylose into CL by the overexpression of D27, CCD7 and CCD8, while *S. cerevisiae* was engineered to convert CL into different SLs by the overexpression of the corresponding CYPs (Fig. 3e). 5DS was chosen to be the model SL in this study and further pathway engineering and process optimization improved the 5DS titer. Although the final titer was low (50  $\mu$ g/L), the system allowed the authors to quickly characterize CYPs from various plants. Future efforts could focus on improving the product titer towards manufacturing SLs as agrochemicals.

## 4. Prebiotics for the plant probiotic microorganisms

The application of plant probiotic microorganisms (PPMs) in the soil has been an efficient alternative to chemical fertilizers and pesticides. PPMs are naturally occurring microorganisms associated with plants that promote the growth of the host plant. They may suppress growth of phytopathogens when applied in sufficient quantities [34]. Reported





**Fig. 3.** Engineering microbes to produce important plant hormones. (a) *E. coli* was engineered to produce indole-3-acetic acid (IAA), the main plant auxin by overexpression of ARO8, KDC and AldH. ARO8: aminotransferase. KDC: decarboxylase. AldH: aldehyde dehydrogenase. (b) *Cupriavidus pinatubonensis* JMP134, a neutral non-PGPR, plant-associating rhizobacterium was engineered to synthesize IAA in an autoregulated manner under the regulation of a quorum sensing (QS) signal. IaaM encodes tryptophan monooxygenase, which oxidatively decarboxylated tryptophan to indoleacetamide. IaaH encodes indole-3-acetamide hydrolase which catalyzed the hydrolysis of indole-3-acetamide into IAA. HSL: Homoserine lactone, inducer for expression of the target genes. (c) *Y. lipolytica* was engineered to produce gibberellins GA<sub>3</sub>, GA<sub>4</sub> and GA<sub>7</sub>. CPS: Copalyl diphosphate synthase. KS: ent-kaurene synthase. KO: ent-kaurene oxidase. C20ox: GA C20-oxidase. C3ox: GA C3-oxidase. P450-3p: Cytochrome P450 monooxygenase. DESp: GA<sub>4</sub> desaturase. (d) *Y. lipolytica* was engineered to produce abscisic acid (ABA) by overexpression of B<sup>c</sup>ABA1, B<sup>c</sup>ABA2, B<sup>c</sup>ABA3, B<sup>c</sup>ABA4 and B<sup>c</sup>CPR1 from *Botrytis cinerea*. ABA3p encodes  $\alpha$ -ionylideneethane synthase. ABA1p and ABA2p encodes cytochrome P450s. ABA4p encodes a dehydrogenase. CPR1: cytochrome P450 reductase. (e) *E. coli* and *S. cerevisiae* was engineered and co-cultured to produce strigolactones, a novel family of plant hormones. *E. coli* was engineered to produce CL by the overexpression of D27, CCD7 and CCD8. *S. cerevisiae* was engineered to convert CL into different SLs by the overexpression of different cytochrome P450s (CYPs). D27 encodes  $\beta$ -carotene isomerase. CCD7 and CCD8 are carotenoid cleavage dioxygenases.

major plant-growth promoting probiotic microorganisms include species of *Bacillus*, *Pseudomonas*, *Paraburkholderia*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, and *Serratia* [35,36]. They give favorable benefits to host plants by producing various regulating compounds, such as plant hormones, antibiotics, siderophores, and lytic enzymes. They may enhance nutrient acquisition by fixing atmospheric nitrogen and solubilizing soil mineral nutrients (such as P, K, Zn, Fe). It has also been reported that PPMs could bioremediate contaminated soils by absorbing harmful heavy metals, and help the host plants to develop systemic resistance against abiotic and biotic stresses [37]. However, the inoculation of these microorganisms in the actual fields often shows a different behavior than the laboratory results due to the limited survival of these bacteria in the soil under the field conditions [38], casting doubts on the broad applications in fields. In this scenario, selectively stimulating the growth and/or activity of those may be a useful approach. Future research may focus on the use of prebiotics, which are carbon sources that exert a selective pressure to promote growth of these specific plant beneficial bacteria. To the best of our knowledge, no study involving prebiotics for PPMs has been reported so far.

Some of the potential prebiotics can be the cheap substrates such as ethylene glycol (EG), which can be obtained from plastic and cellulosic wastes [39]. Only a few *Pseudomonas* species such as *P. putida* JM37 can catabolize EG [39,40]. The genus *Pseudomonas* is one of the most significant genera of bacteria that promote plant development. It produces 1-aminocyclopropane-1-carboxylate (ACC) deaminase [34], an enzyme that PGPM uses to combat biotic stress [38]. A variety of biotic stressors may trigger the synthesis of ethylene in plants. ACC deaminase cleaves ACC, which is a direct precursor of ethylene, into ammonia and  $\alpha$ -keto butyrate. This decreases the quantity of ethylene in the roots of growing plants, hence minimizing the associated negative consequences, such as the obstruction of normal plant growth, leaf abscission, leaf senescence, chlorosis, and flower wilting [41]. Various strains of ACC-producing *Pseudomonas* have been shown to benefit plant growth such as growth of wheat crops [42,43], maize plants under salt and aluminum stress [44]. Moreover, *Pseudomonas* strains promote the growth of asparagus seedlings in water-scarce environments, safeguard *Medicago sativa* seeds in soils contaminated with copper and serve as PGPR for a number of crops growing in the salty soils of coastal biomes [34]. Hence, selectively increasing the population of beneficial microbes such as *Pseudomonas* species can have a significant impact on plant growth and development. Application of the proposed potential prebiotics such as EG may selectively increase the population of *Pseudomonas* species in the soil. Although PPMs may naturally utilize these potential prebiotics, genetic engineering could further improve the uptake rate of the prebiotics. In addition, PPMs could also be engineered to use the prebiotic molecules, helping them to gain competitive growth advantages in the field.

## 5. Conclusions and perspectives

Natural agrochemicals that are produced from engineered microbes are an attractive alternative to the synthetic agrochemicals. However, there are only a small number of published reports of these agrochemicals being applied in the fields despite their successful and effective laboratory trials. Farmers have developed a strong trust in the effectiveness and quick results of chemical fertilizers and pesticides, making it difficult to adopt and/or replace them with alternative techniques. Furthermore, the titers of the majority of natural agrochemicals remain low, affecting their large-scale production. New fermentation strategies, gene editing tools, and novel computational tools would be useful to improve the product titer.

Advanced sequencing techniques can be used to better understand the microbial community of soil and discover novel plant-beneficial microbes which can be further engineered. Protein engineering approaches could be useful to enhance the activity of the enzymes or to eliminate feedback regulation on enzymes. Protein-ligand docking for

virtual screening of new plant hormones may be another potential direction for future research. Based on the crop of interest, the properties of the plant hormones can be improved by designing and introducing modifications using computational simulations.

It would be interesting to engineer microbes that can harvest sunlight and improve the net photosynthetic rate when deployed in the field. Another interesting idea is to engineer microbes that might improve water absorption and retention in arid lands. The plant-associated microbes from desert plants might be isolated and subjected to adaptive evolution in dry conditions followed by genome sequencing. The identified mutations might be introduced into model microbes, which might then be applied in water-scarcity lands. The development of biosensor strains that can sense plant's needs and then release agrochemicals in a controlled manner would be another attractive strategy.

For successful deployment of the above-mentioned engineered microbes in the field, the selection strategy must include evaluation of the microbes' ability to survive in the environment of interest, including tolerance to the associated stressors such as aridity, heat, and low light. Another major risk is the possibility of gene transfer into native microbes [45], which must be carefully evaluated during the selection process. Other considerations are their biocompatibility, non-pathogenicity, ability to colonize the plant rhizosphere, effectiveness in competing with the existing microbes, enhanced shelf-life, and eco-friendliness.

We envision that a large number of natural agrochemicals can be produced by using engineered microbes as discussed in this review. These strategies could play an important role in the sustainable agriculture over the coming years.

## CRedit authorship contribution statement

**Smaranika Panda:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Kang Zhou:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare no conflict of interest.

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