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MINI-REVIEW

# Production of natural value-added compounds: an insight into the eugenol biotransformation pathway

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**Abstract** During the past few years, the production of natural value-added compounds from microbial sources has gained tremendous importance. Due to an increase in consumer demand for natural products, various food and pharmaceutical industries are continuously in search of novel metabolites obtained from microbial biotransformation. The exploitation of microbial biosynthetic pathways is both feasible and cost effective in the production of natural compounds. The environmentally compatible nature of these products is one major reason for their increasing demand. Novel approaches for natural product biogeneration will take advantage of the current studies on biotechnology, biochemical pathways and microbiology. The interest of the scientific community has shifted toward the use of microbial bioconversion for the production of valuable compounds from natural substrates. The present review focuses on eugenol biotransformation by microorganisms resulting in the formation of various value-added products such as ferulic acid, coniferyl alcohol, vanillin and vanillic acid.

**Keywords** Biotransformation · Metabolites · Eugenol · Ferulic acid · Vanillin

## Introduction

Although the market is currently dominated by chemically synthesized compounds, the recent trend of investigation has shifted toward the exploitation of metabolic and biocatalytic potential of microorganisms to transform conventional as well as nonconventional substrates into value-added products. The shift in this trend has been observed due to the environmental incompatibility of the production process, reduced substrate selectivity and high downstream cost [26]. The estimated worldwide market demand for natural flavor compounds was approximately $21.8 billion during the year 2011 [24]. These compounds are mainly aromatic in nature and produced through biotransformation of substrates isolated from plants by physical (extraction from natural resource), enzymatic or microbial processes [44].

Although several different natural substrates such as eugenol (4-allyl-2-methoxyphenol), isoeugenol (4-hydroxy-3-methoxy-1-propenylbenzene), and ferulic acid (4-hydroxy-3-methoxycinnamic acid) can be biotransformed into value-added products, eugenol seems to be one of the more ideal potential substrates because it is economically and commercially available [36]. Eugenol belongs to the phenylpropanoid class, is aromatic in nature, and is isolated from the buds and leaves of *Syzygium aromaticum* (clove tree) by steam distillation [11]. Natural value-added compounds such as ferulic acid, coniferyl alcohol (4-hydroxy-3-methoxy cinnamyl alcohol), coniferyl aldehyde, vanillin, vanillic acid (4-hydroxy-3-methoxybenzoate) and protocatechuatuic acid (3,4-dihydroxybenzoate) have been produced by different microorganisms through the biotransformation process using eugenol as a substrate [38]. These intermediate compounds are produced through various biotechnological routes which include microbial, plant tissue culture techniques and enzymatic routes for natural flavor production.

Among the different intermediates in the eugenol biotransformation pathway, coniferyl alcohol is a precursor responsible for lignification in many plants [42]. Coniferyl

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aldehyde is an oxidation product of coniferyl alcohol, catalyzed by the enzyme coniferyl alcohol dehydrogenase. Another product of eugenol biotransformation is vanillin, obtained from vanilla pods or prepared from the phenolic volatile oil constituent eugenol and from the glycoside coniferin lignin. Large-scale commercial production of vanillin has been achieved through hydrolysis of lignin, producing coniferyl alcohol as an intermediate which is subsequently used as a substrate for large-scale production of commercial vanillin. The oxidized form of vanillin is vanillic acid, also an intermediate in the production of vanillin from ferulic acid [36]. After the demethylation of vanillic acid, protocatechuic acid is produced [36].

Although a substantial amount of research has been done on the biotransformation of various phenylpropanoids during recent years, the biotechnology of the biotransformed products obtained from eugenol is still in its nascent stage. In the present study, we reviewed the various metabolic pathways followed during eugenol biotransformation by microorganisms, to give an insight of how they may be exploited to obtain natural value-added compounds.

## Biotechnological methods leading to the production of value-added compounds

The production of natural aromatic compounds can be catalyzed by the following three possible methods: plant cell culture, enzymes and microorganisms. A wide range of natural aroma compounds can be produced through the plant cell culture method from their plant source [26]. The ability of plant tissue cultures to yield value-added compounds is based upon their distinctive biochemical capacities, genetic capabilities and the totipotency of their cells [14, 40, 43]. Thus every cell of plant tissue contains the genetic information necessary to construct the several chemical components (or their precursors) that comprise natural value-added compounds [39].

Biotransformation in plant tissues is expensive and yields only small amounts of value-added compounds. Another disadvantage associated with plant cells is their much longer duplication period and susceptibility to damage due to their size or composition of the cell wall. The price of the medium for plant tissue culture is approximately ten-fold of that required for microorganisms [16]. The yield is also affected by climate conditions and plant diseases [26]. Plant cell cultures flourish best in only the systems in which the biochemical pathways of target compounds are well-known, and therefore the use of this method is currently limited.

Apart from the previously mentioned method, many enzymes have been used to produce natural value-added compounds. Enzymes such as lipolytic enzymes (lipases), proteases, and glucosidases may directly produce value-added products by hydrolysis of precursors. The major advantages associated with both enzymatic and microbial methods are high-substrate selectivity and fewer by-products, which makes product isolation procedures effortless [54]. But the drawback of enzyme-catalyzed synthesis is the biocatalyst's high price and the difficulty of maintaining its activity and stability. Therefore, immobilized enzyme approaches have been considered that help in recovery, reuse and, in few cases, an increase of enzyme thermostability [26]. But this method is expensive and economically not feasible, so the research trend has inclined more toward microbial catalysis.

Microorganisms play a vital role in the production of natural aromatic compounds. It has been reported that they catalyze oxidation of the side-chain of eugenol, which is the first step in the biocatalysis of important value-added products such as coniferyl alcohol, coniferyl aldehydes, ferulic acid and vanillin [54]. The major value-added compounds produced by microbial catalysis of eugenol are summarized in Table 1.

## Metabolic pathways of eugenol biotransformation

Studies revealed that microorganisms follow different catabolic routes for eugenol degradation. In Fig. 1, we present all the possible (six) degradation pathways, which indicates the varied nature of microorganisms and their role played in eugenol biotransformation. They are labeled from *a* to *f* and are discussed below.

Starting with pathway *a*, a report suggested that *Corynebacterium* sp. transformed eugenol through ferulic acid, vanillin, vanillic acid and protocatechuic acid, which was further biotransformed to keto-adipic acid by "*ortho*" fission [49]. The formation of eugenol epoxide as the initial reaction of the eugenol degradation in *Pseudomonas* sp. was proposed in pathway *b*. Moreover, few metabolites like eugenol oxide, eugenol-diol, coniferyl alcohol and coniferyl aldehyde exist as intermediates before the formation of ferulic acid [48]. In pathway *c*, *Pseudomonas* sp. HR199 produced coniferyl alcohol, coniferyl aldehyde, ferulic acid and vanillic acid as intermediates through the biotransformation of eugenol. In this catabolic pathway, eugenol was oxidized to coniferyl alcohol with a 43.5 % molar yield via coniferyl aldehyde and then further oxidized to ferulic acid with a 52 % molar yield [37]. This pathway corresponds to the pathways present in *Corynebacterium* sp. [49] and *Pseudomonas* sp. [48], except that vanillin was not formed as an intermediate. *Pseudomonas nitroreducens* Jin1 [52] follows the same pathway of eugenol degradation as found in *Pseudomonas* sp. HR199 [37], except that coniferyl aldehyde was not formed as an intermediate. Another

**Table 1** Microorganisms involved in eugenol biotransformation

| Microorganisms | Products | References |
|---|---|---|
| *Corynebacterium* sp. | Ferulic acid, vanillin, vanillic acid, protocatechuic acid and keto-adipic acid | [49] |
| *Pseudomonas* sp. | Eugenol oxide, eugenol-diol, coniferyl alcohol, coniferyl aldehyde and ferulic acid | [48] |
| *Enterobacter* sp. | Vanillin | [38] |
| *Pseudomonas* sp. | Coniferyl alcohol, coniferyl aldehydes, ferulic acid and vanillic acid | [37] |
| *Pseudomonas* sp. HR199 | Ferulic acid, coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and vanillic acid | [35] |
| *R. eutropha* H16 (genetically modified) | Coniferyl alcohol, coniferyl aldehyde and ferulic acid | [30] |
| *Escherichia coli* (genetically modified) | Coniferyl alcohol, coniferyl aldehyde, ferulic acid and vanillin | [31] |
| *P. fluorescens* E118 | Coniferyl alcohol | [11] |
| *Amycolatopsis* sp. HR167 (genetically modified) | Coniferyl alcohol, coniferyl aldehyde, ferulic acid, guaiacol and vanillic acid | [32] |
| *P. nitroreducens* Jin1 | Coniferyl alcohol, ferulic acid, vanillin and vanillic acid | [52] |
| *Bacillus cereus* PN24 | 4-vinyl guaiacol, vanillin, vanillic acid, protocatechuic acid and keto-adipic acid | [19] |
| *Streptomyces* sp. | Coniferyl alcohol, ferulic acid and vanillin | [22] |
| *P. resinovorans* SPR1 | Ferulic acid, coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillin and vanillic acid | [2] |
| *Pseudomonas* sp. OPS1 | Coniferyl alcohol, coniferyl aldehyde, ferulic acid, vanillic acid and protocatechuic acid | [4] |

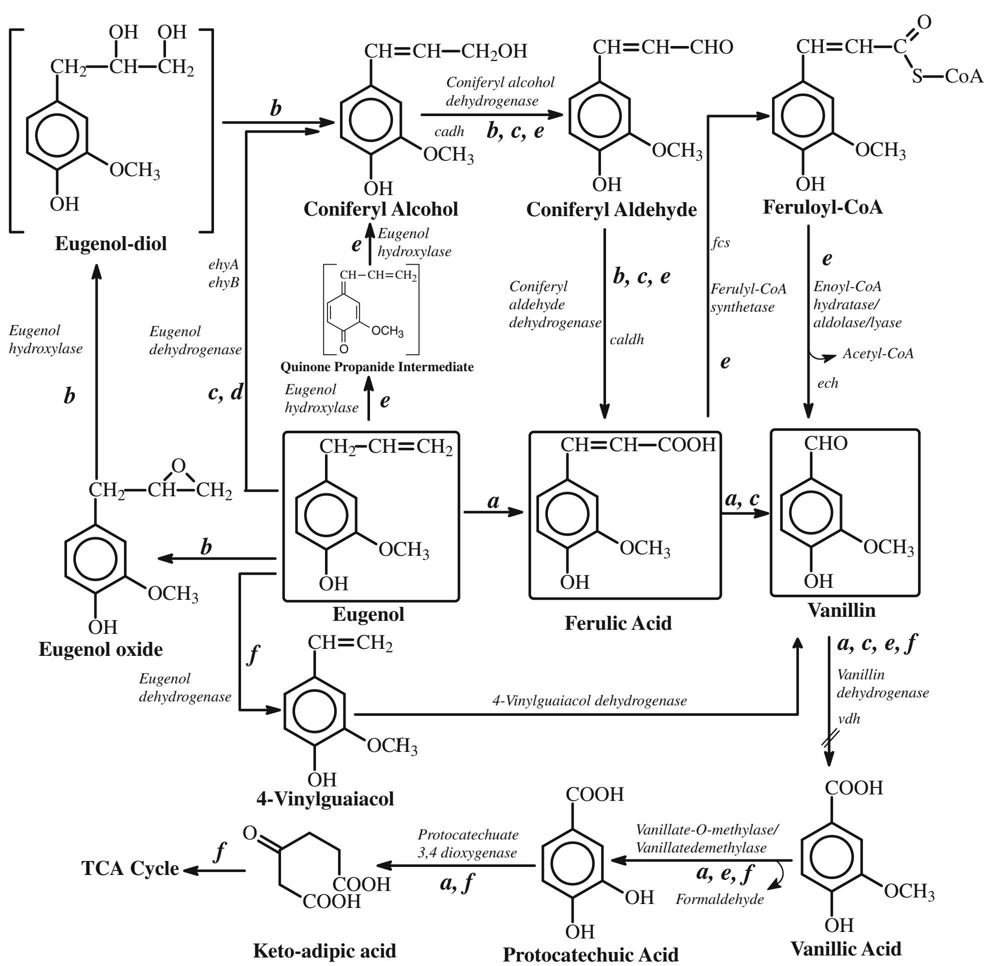


**Fig. 1** Outline of various pathways followed by microorganisms in eugenol degradation

pathway for the production of coniferyl alcohol, ferulic acid and vanillin was proposed through the bioconversion of eugenol by *Streptomyces* sp [22]. In order to enhance the yield of value-added compounds obtained from eugenol biotransformation, various recombinant strains have been constructed. The genes *ehyAB*, *calA* and *calB* of *Pseudomonas* sp. HR199 encoded for the enzyme eugenol hydroxylase, coniferyl alcohol dehydrogenase and coniferyl aldehyde dehydrogenase, respectively, were cloned in the vector pBBR1-JO2 (pBBR1-JO2ehyABcalAcalB) and transferred to *Ralstonia eutropha* H16. This recombinant strain catalyzed eugenol into ferulic acid with an increase of 41.8 % molar yield [30] in contrast to *Pseudomonas* sp. HR199. The vanillyl alcohol oxidase (*vaoA*) gene from *P. simplicissimum* CBS 170.90 and genes of *Pseudomonas* sp. strain HR199 (*calA* and *calB*) were expressed in *Escherichia coli* XL1-Blue. This recombinant strain of *E. coli* XL1-Blue is capable of transforming eugenol into coniferyl alcohol, coniferyl aldehyde, ferulic acid and vanillin as intermediates with a 93.3 % molar yield of ferulic acid [31]. *Pseudomonas fluorescens* E118 follows a similar pathway of eugenol degradation as that found in *Pseudomonas* sp. HR199 [37], producing ferulic acid by intermittent addition of eugenol into the basal medium with a molar conversion yield of 54 % [11]. The *vaoA* gene from *Penicillium simplicissimum* CBS 170.90 was expressed in *Amycolatopsis* sp. HR167. This recombinant strain degraded eugenol into coniferyl alcohol, coniferyl aldehyde, ferulic acid, guaiacol and vanillic acid [32]. In pathway *d*, a novel enzyme eugenol dehydrogenase was produced by the *P. fluorescens* E118 strain, which catalyzed the conversion of eugenol into coniferyl alcohol [10]. In pathway *e*, structural genes *ehyA* and *ehyB* were identified in *Pseudomonas* sp. strain HR199 for an enzyme eugenol hydroxylase, which was responsible for the biotransformation of eugenol to coniferyl alcohol. In this case, quinine propanide was found as an intermediate during the conversion of eugenol to coniferyl alcohol with coniferyl aldehyde, ferulic acid, feruloyl-CoA, vanillin, vanillic acid and protocatechuic acid [35]. Conversely, in pathway *f*, the *Bacillus cereus* strain PN24 degraded eugenol into different metabolic intermediates such as 4-vinylguaiacol (4-ethenyl-2-methoxyphenol), vanillin, vanillic acid and protocatechuic acid, which was further metabolized by the keto-adipic acid pathway [19]. In this pathway, ferulic acid was not produced.

## Applications of the value-added products obtained upon biotransformation of eugenol

Ferulic acid is used as an effective component of Chinese medicinal herbs such as *Angelica sinensis, Cimicifuga heracleifolia* and *Lignsticum chuangxiong*. It has been reported to have many physiological benefits including antioxidant, antimicrobial, anti-inflammatory, antithrombosis, and anticancer activities [12]. It also protects against coronary disease, lowers cholesterol and increases sperm viability [29]. It stimulates the production of human white blood cells and increases the secretion of interferon-gamma (IFN-$\gamma$), an immune system stimulatory protein. This suggests a possible value of ferulic acid as an immune stimulant and provides some support for traditional usages of ferulic acid-containing plants as treatments for cancer and infectious diseases [5]. Due to its natural antioxidant potential, ferulic acid exhibits strong anti-inflammatory properties. It is especially good at neutralizing the free radicals known as superoxide, hydroxyl radical, and nitric oxide [28]. In addition, ferulic acid constitutes the active ingredient in many skin lotions and sunscreens designed for photo protection. It is used in hair creams to prevent alopecia, seborrhea, and pruritis. It is even used in the manufacture of golf wear because of its ultra-violet absorption properties [51]. Ferulic acid also helps in the treatment of diabetes [1, 3], different types of cancers [29, 46], neuroprotection [20, 25, 45], bone degeneration [41, 50] and menopause [17, 27, 34]. It is used to enhance athletic performance, both in humans and in race horses, as it decreases fatigue by counteracting free radicals which spoil the power generating arrangement in cells [9]. Ferulic acid can also be used to preserve foods because of its antioxidant and antimicrobial action. In food-related systems such as lecithin-liposomes, ferulic acid was capable of inhibiting lipid and protein oxidation [15]. Ferulic acid is an active element of some plant extracts showing antimicrobial activity, which inhibits the growth of different types of Gram-positive and -negative bacteria [18], yeasts [47] and fungi [23]. Ferulic acid is also used as a cross-linking agent with polysaccharides to formulate new gels in food processing [8, 29].

Coniferyl alcohol is a known commercial source of vanillin. The enzyme released by fungi, laccase, has the ability to dechlorinate the chlorinated phenols. Cho et al. suggested that the addition of coniferyl alcohol with fungal laccase enhances the abolition of chlorophenols from waste water within a short period [6].

Coniferyl aldehyde is a chemoattractant for the targeting of insects. It has pesticidal and antibacterial activity to kill and control populations of insects and arachnids [7].

Vanillin is widely used in a broad range of flavors for food, confectionary and beverage production (approximately 60 %), as a fragrance component in perfumes and cosmetics (approximately 33 %), for pharmaceuticals (approximately 7 %) and in various medical fields. It is also used as flavoring in pharmaceutical preparations; it has been utilized for the production of herbicides, antifoaming agents and drugs [36, 53].

Vanillic acid is the starting material in the chemical synthesis of oxygenated aromatic chemicals such as vanillin, one of the most important flavor molecules [53]. Vanillic acid is the derivative of benzoic acid, is used as a flavoring agent, and may be useful in the treatment of ulcerative colitis and intestinal inflammatory disorders [21].

Protocatechuic acid is believed to be a possible growth inducer and apoptosis inhibitor for neural stem cells [13].

## Conclusion

This review aims to confer information regarding biosynthesis and metabolism of various value-added products obtained from and enzymes involved in eugenol biotransformation pathways. To date, many of the compounds are produced by conventional methods, such as chemical synthesis or extraction from natural resources. An interesting route for natural synthesis is based on microbial transformation or biotransformation. It has been shown that the production of aromatic compounds for the food and pharmaceutical industry by the use of enzymes or microbes has many advantages over conventional techniques [39]. In the production of value-added compounds, recombinant strains have advantages over wild strains for better yield. However, products obtained via genetic engineering are less appreciated by consumers, especially in Europe [36]. In spite of the fact that in recombinant strains the genes being transferred occur naturally in other species, there remain unknown consequences in altering the natural state of an organism through foreign gene expression. Such alterations could change the organism's metabolism, growth rate, and response to external environmental factors. The potential health risks to humans could include the possibility of exposure to new allergens in genetically modified foods (GMOs), in addition to the transfer of antibiotic-resistant genes to gut flora. The attitude towards usage of genetically modified products differs depending upon people's level of education and varying interpretations of the steps actually involved to obtain the product. When it comes to genetically modified foods, those who feel strongly that the development of GMOs is against nature or religion have called for clear labeling rules so that they can select which items to purchase. Prior to permitting commercial use of GMOs, governments perform risk assessments to determine the possible consequences of their use, but difficulties in estimating the impact of commercial GMO use makes regulation of these organisms a challenge [33]. According to FDA and European legislation, products obtained by enzyme or microbial process are considered natural [44]. Biotechnological processes are also usually environmental friendly and thus are less damaging to the environment than chemical processes. Nature provides a rich supply of resources for microbiologists interested in the exploitation of microbes and all possibilities related to it for the production of natural value-added compounds.

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