9 Vanilla

H. Korthou
Plant Science, Fytagoras BV,
Zernikedreef 9, 2333 CK Leiden, The Netherlands

R. Verpoorte
Section Metabolomics, Department of Pharmacognosy, IBL,
Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands

9.1 Introduction

Vanilla is widely used in food, beverages and cosmetics. It is produced from the beans of *Vanilla planifolia* Andrews, a member of the orchid family (Orchidaceae). The plant originates from Mexico where it was already used when the Spaniards arrived. Now it is cultured in various tropical countries, such as Madagascar, Indonesia, Uganda, Comoro, Tahiti, Papua Guinea, India and Mexico. Each of these growth sites yields vanilla with different flavour characteristics.

The total world consumption of vanilla beans is decreasing, mainly owing to the very high price ($300–500 per kilogram in 2004); in 2004 the demand was about half of the 2,200 t used in 1999 [5]. Madagascar produces more than half of the world production (1,000–1,200 t). Indonesia is the second largest producer, with some 350 t. Owing to various diseases of the plant and strong international competition, including from new production regions, Indonesian production has considerably decreased in the past few years, but the quality has increased [3].

The plant requires special growth conditions and the formation of beans requires pollination by specialised insects, which means that in most places pollination is done by hand. After 9 months of maturation, the vanilla beans undergo an elaborate processing known as curing, a process which takes about 6 months. Basically curing is a sort of fermentation process at elevated temperature, in which the beans are dried and the flavour develops, among others through the hydrolysis of the vanillin glucoside, resulting in free vanillin (Fig. 9.1), the most important flavour compound in the beans. The curing process is a highly traditional process; it is still not well understood and differs in the various producing regions. Despite various studies concerning the biosynthesis of vanillin and its role for the plant, many questions still remain. But to increase and ensure reproducible quality and to improve the efficiency of the curing process, further insight into the biochemistry of the vanillin production in the plant is required, as well as of other characteristic flavour compounds occurring in the beans.

Vanilla and vanillin are very versatile flavours, at any concentration they are acceptable, and most people enjoy the flavour, making it the world's most popular flavour. It is used in food (e.g. ice cream, various other diary products, choco-

lates and cakes), beverages (cola-type drinks), cosmetics and tobacco. There is a distinct difference between vanilla extract and vanillin, and most people prefer the extract-based products. Hoffman et al. [21] reviewed the analysis of vanilla constituents and flavours of vanilla. Improved or altered methods of curing and new cultivars may lead to a further diversification of the vanilla flavour.

Because of the large consumption of vanilla-flavoured products, vanillin is also made by other routes, such as (bio)conversion of related natural products or via synthesis. Only 0.2% of the approximately 6,000 t of vanillin used in the flavour market is derived from plants, for which vanilla is the major source [60, 61]. Most vanillin is synthetic; some several tons comes from microbial processes [38, 52]. About 60% of the vanillin goes into food and beverages, 33% into perfumes and cosmetics and 7% into pharmaceuticals [44]. The price of natural vanillin extracted from vanilla is estimated to be between $1,200 and $4,000 per kilogram [60, 61]. Natural vanillin derived from microbial production has a price of about $1,000 per kilogram [52]. Synthetic vanillin costs about $11–15 per kilogram [52, 53]. Consequently counterfeiting occurs because of the large price differences between natural vanillin and synthetic vanillin. Special analytical tools such as NMR spectrometry are applied to analyse the source from which the vanillin is derived.

Here we will review the current knowledge about the vanilla curing process, the biosynthesis of vanillin and alternative biotechnological production methods.

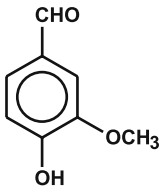


Fig. 9.1 Vanillin

9.2 The Plant

The genus *Vanilla* belongs to the family Orchidaceae, one of the largest plant families, with more than 18,500 species. The *Vanilla* Swartz genus has more than 100 species, amongst which 15 are aromatic. Three species have economic value, one of which is *Vanilla planifolia* Andrews (previously known as *V. fragrans* (Salisb.) Ames). Two other species, *V. pompona* Schiede and *V. tahitensis* J.W. Moore, are cultivated on a small scale for vanillin production. The former is more resistant against diseases, but gives pods of an inferior quality. The latter species is grown in Tahiti; it has a distinctly different flavour. It might be a man-

made hybrid. Because of its unique flavour, the beans are more expensive than those of *V. planifolia* [46]

The diseases include fungal infestations caused by, among others, *Calospora vanillae* (anthracnose, whole plant), *Fusarium* sp. (root rot, fruit rot), *Phytophthora* sp. (fruit rot), *Colletotrichum* sp. and *Gloeralla vanilliae* (root rot). Besides these fungal diseases, viral diseases also pose a serious problem, e.g. Cymbidium mosaic virus and the cucumber mosaic virus. Suboptimal growing conditions and excessive rain or drought are the major reasons for diseases [46].Vanilla is a fleshy perennial vine, and requires a tree or artificial support for its growth. Adventitious aerial roots adhere to the supporting tree. The plant can grow as high as 10–15 m, but for cultivation the plants are kept low to facilitate the hand pollination and the harvest of the beans. The plant, propagated by cuttings, needs about 2–4 years before it flowers, and can produce for a period of 5–6 years. Each plant has about ten to 20 flower clusters of 15–20 flowers each. After hand pollination eight to 12 of these flowers will develop into pods.

The plant grows best in humid, tropical conditions; drought can easily kill the plant. Direct sun should also be avoided for growth sites, but full shade is also detrimental for the plant; therefore, vanilla is often planted between small shade-giving trees such as bananas and coffee, which should reduce the full sunshine to about one third to half of its intensity. Also artificial nets are used to create the right growth conditions. The plant grows well from sea level to altitudes of more than 760 m at a temperature ranging from 20 to 30 °C [46].

9.3 Vanillin

Vanillin is the most widely appreciated flavour compound in the world. Its odour threshold for humans is 11.8 x 10^{-14} M [6]. It has the unique characteristic that even at very high dose the flavour is still pleasant. Vanillin has various activities. The antimicrobial effects on the fungi *Aspargillus flavus*, *A. niger*, *A. ochraeus* and *A. parasiticus* and the bacteria *Escherichia coli*, *Bacillus subtilus* and *Staphylococcus aureus* were reviewed by Tipparaju et al. [57]. This makes vanillin a potential food preservative for a wide variety of products like diary products, soft drinks and fruit juices [13, 61]. In several studies the antioxidant, antimutagenic, anticlastogenic and anticarcinogenic activities of vanillin were demonstrated [12, 27, 54].

9.4 Biosynthesis

The biosynthesis of vanillin has been extensively studied both in the plant and in plant cell cultures (Scheme 9.1). There are some contradictions between the results of these studies, and consequently several questions about the biosynthetic

pathway remain unanswered. At least all studies support the involvement of the shikimate pathway and the phenylalanine (phenylpropanoid) pathway. The first question is at what stage the C_3 side chain is oxidised to yield the aldehyde function. This could be before or after the formation of the typical 3-methoxy, 4-hydroxy substitution pattern in the aromatic ring. It is also unknown whether vanillin is derived from the lignan precursors having an alcohol function in the C_3 part of the phenylpropanoid, or from the cinnamic acid type (acid group). Zenk [63] showed that labelled ferulic acid was incorporated into vanillin. However, Kanisawa et al. [26] proposed that the major pathway would go via 4-coumaric acid glucoside, which is the precursor for *p*-hydroxybenzaldehyde glucoside, the central intermediate for the biosynthesis of the glucosides A and B as well as vanillin (Scheme 9.1). But their hypothesis left the possibility that the more oxidised compounds such as ferulic acid glucoside also can be converted into the corresponding aldehyde.

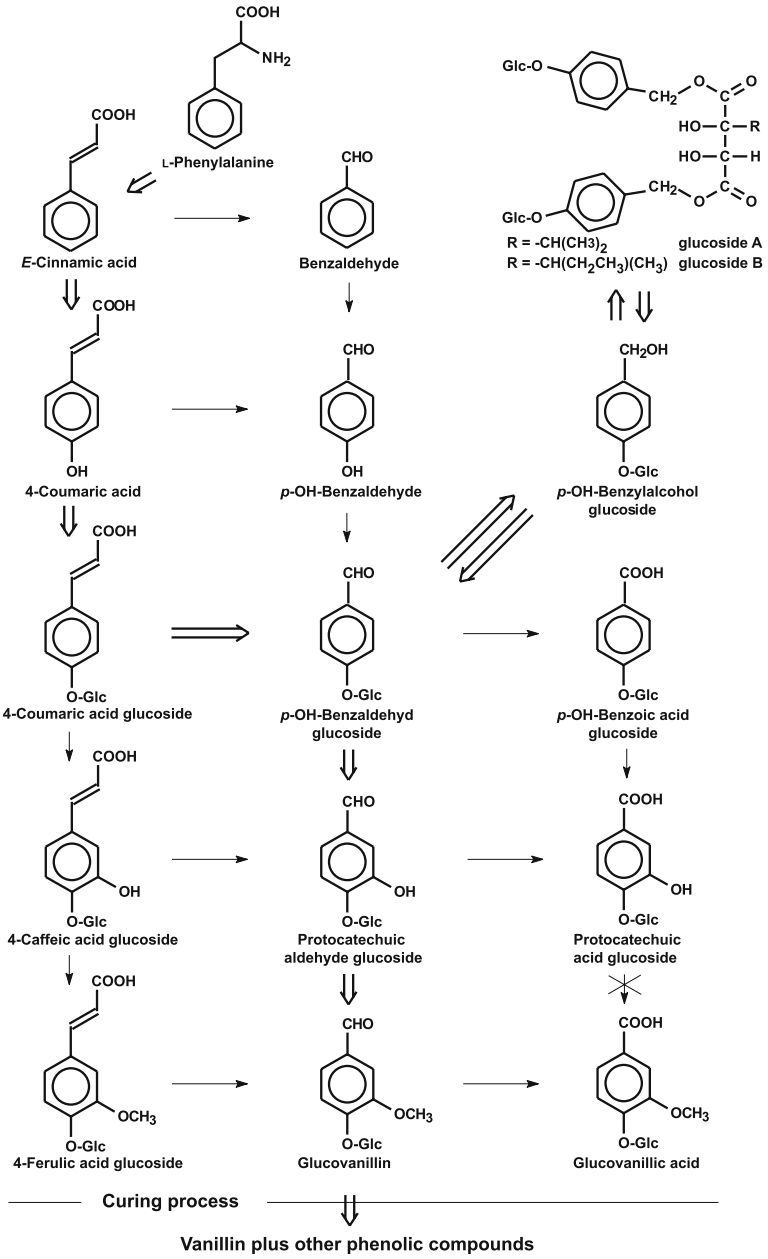
Various experiments in *Vanilla* plant cell cultures, however, gave different results [14–18, 30, 51]. This might be due to the fact that different biosynthetic pathways operate in the beans and in the cell culture. In fact most of the work in cell cultures showed only conversion of non-glucosylated products. Ferulic acid feeding resulted in increased vanillin levels. The fact that the *V. planifolia* cell cultures do not produce vanillin in any significant amount means that the results from studies using vanilla cell cultures for elucidation of the pathway should be considered with caution. Finally, it cannot be excluded that different pathways may contribute to the vanillin production in the beans. Scheme 9.1 shows that vanillin can be formed through different ways in a complex network of compounds.

For a general review of the biosynthesis of C_6C_1 compounds, see Mustafa and Verpoorte [39].

9.5 Enzymes

Only a few steps of the biosynthesis of vanillin are known down to the level of the enzymes and the genes. Particularly the glucosidases involved in the formation of vanillin from its glucoside have been studied extensively. As vanillin is almost completely stored in the form of a glucoside, the role of the glucosidase is crucial for the quality of the final product, as a high level of vanillin is required for good quality. Concerning the glucosidases, different results have been reported.

Kanisawa et al. [26] reported that two glucosidases are present in vanilla pods. A non-specific enzyme occurs in both leaves and beans, whereas a specific vanillin glucosidase was detected only in the beans. Using the *p*-nitrophenyl-glucoside (NPG) assay for detecting activity, Odoux et al. [42] and Ranjoanisafy [47] (cited in Odoux and Havkin-Frenkel [41]) purified and characterised a glucosidase from the beans. The enzyme with a molecular mass of 201 kDa con-



Scheme 9.1 Proposed pathway for vanillin biosynthesis in *Vanilla planifolia* beans according to Kanisawa et al. [26]. The *thick arrows* represent the most likely pathway

sisted of four subunits (50 kDa each). Because no specific enzyme assay was used, the occurrence of a highly specific vanillin glucoside hydrolysing enzyme, as reported by Kanisawa et al. [26], cannot be excluded.

Havkin-Frenkel (cited in Odoux and Havkin-Frenkel [41]) found a series of glucosyl hydrolases in green vanilla beans. The enzymes included α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase and β-mannosidase. The β-glucosidase showed maximum activity at 50 °C; the α-galactosidase and the β-galactosidase had optima at 55 and 60 °C, respectively, temperatures which are similar as those during the curing process.

Dignum et al.[9] followed glucosidase activity during the curing process using the NPG assay. They could not detect glucosidase activity anymore after the autoclaving step, though vanillin glucoside was still hydrolysed in the beans. The presence of a non-NPG-assay-detectable glucosidase can thus not be excluded. The glucosidase activity measured in green beans was also strongly dependent on the type and pH of the incubation buffer used. The highest activity was obtained at pH 8 with a [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane propane buffer. Freezing of the green beans caused a dramatic loss of glucosidase activity, and the enzyme extract lost much of its activity when stored at -20 °C; storage at -80 °C particularly in the presence of glycerol gave better results. Dignum et al. [10] measured the kinetic properties of the glucosidase from the green beans for several glucosides occurring in vanilla. For vanillin glucoside the V_{max} was 9.4 IU mg^{-1} protein and K_m was 5.1 mM; values in the same range were found for the glucosides of vanillic acid, *p*-hydroxybenzaldehyde and ferulic acid, Also for the synthetic substrate NPG, a similar activity was found; however, for creosol, and guaiacol glucosides a much higher K_m was found, whereas for 2-phenylethanol and *p*-cresol glucosides no activity was detected. Hanum [20] reported a much higher V_{max} and a lower K_m for vanilla glucosidase.

The enzyme is localised in the placental tissue of the beans, i.e. where the vanillin glucoside is also found [41, 42]. The enzyme seems to be localised in the cytoplasm. Though not proven, it is hypothesised that the enzyme and the glucoside are in different cellular compartments, similar to the case for many other secondary metabolite glucosides in other plants (e.g. see Geerlings et al. [19]). The compartmentation is part of the plant defence. Once a cell has been destroyed by, e.g., an insect, the glucosidase will come together with the glucoside, resulting in the formation of a toxic aglycone. A common defence response in plants, a phytoanticipin present in the plant cell is converted immediately into a highly toxic and reactive compound after attack by a microorganism or an insect. During the curing process when the cell integrity is destroyed, the vanillin glucoside will diffuse through the bean and in contact with the enzyme it will be converted into vanillin.

Roeling et al. [50] studied the possibility that microorganisms may be involved in the hydrolysis of the glucoside during the curing process; however, they could not find any evidence for the presence of specific microorganisms growing on the beans after the killing step.

For the biosynthesis of vanillin, several other enzymes are of interest. First of all, phenylalanine ammonia lyase (PAL); this enzyme converts phenylalanine into the cinnamic acid type of compounds, the first intermediates in the vanillin biosynthesis after the primary metabolism. PAL activity could be detected in green beans, but after scalding this activity is lost. The chain shortening enzyme (CSE), responsible for the conversion of a C_6C_3 compound into a C_6C_1 compound, was found to be localised in the cytosol of cells of the placental trichomes in the green beans [23].

Peroxidases might play a role in decomposition of vanillin and in flavour generation during the curing process. Their activity is high in green beans and the enzymes also remain active during the curing. The same applies for proteases, which might be a reason for not recovering glucosidase activity from the beans in the curing process.

9.6 Curing

Dignum et al. [10] studied the curing process in Bali (Indonesia). The curing process starts with the so called killing or scalding. After harvesting, the green beans are thrown into hot water (60–70 °C) for 1–2 min. After that they are stored in boxes for 2–5 days; this phase of the process is called autoclaving, a confusing name as the temperature never goes over 50 °C. After this step the beans are spread in the sun on blankets and wrapped up again during the night in boxes; this phase (sunning and sweating) goes on for about 2 weeks. As the beans are everyday in the hands of the labourers, they sort the beans during this process into different quality classes. In the next phase (2–4 weeks) the beans are dried further; at the end of this step they have reached a water content of 25–40%. The final stage consists of storing the beans for several months in small bundles in a sealed box, or in plastic vacuum bags. After this stage the beans are ready for use. The total curing process may last as long as 6 months.

Dignum et al. [8, 10] followed this process in detail on a production site in Bali to measure the various parameters of the processing in order to mimic these in a laboratory model curing system. The parameters are summarised in Table 9.1. On the basis of the observations, a model curing system was set up to study different parameters under controlled conditions and the effect on some enzymes and the vanillin production.

The curing process is an essential step for the production as the flavour develops gradually during the process, in part due to enzymatic conversion of the vanillin glucoside, in part due to other enzymatic and chemical reactions, including oxidations. Further knowledge of these chemical and biochemical processes is thus of great importance for optimising the production of high-quality vanilla beans.

Table 9.1 Parameters of laboratory curing processes under traditional Indonesian conditions [10]

Stage	Temperature (°C)	Relative humidity (%)	Time
Scalding (killing)	70		1.5 min
Autoclaving	60	95	3 h
	55	95	3 h
	50	95	3 h
	45	95	3 h
Sunning/sweating	40	70	1 h
	47.5	62.5	3 h
	55	55	2 h
	50	95	6 h
	42.5	95	12 h
Slow drying	30	80	3 weeks

9.7 Chemistry

More than 250 compounds have already been identified in vanilla beans, representing a broad variety of classes of natural products such as monoterpenoids, fatty acids and various esters thereof, benzoic acid derivatives, hydrocarbon ketones and alcohols, phenylpropanoids and other phenolics [8, 21]. Some of these phenolics occur also as glucoside [8, 25, 26]. Major components in cured beans, besides vanillin (0.3–2%), are *p*-hydroxybenzaldehyde (0.2%), *p*-hydroxybenzylmethyl ether (0.02%) and acetic acid (0.02%). In green beans glucovanillin, bis[4-(*β*-D-glucopyranosyloxy)benzyl-2-isopropyltartrate] (glucoside A) and bis[4-(*β*-D-glucopyranosyloxy)benzyl-2-(2-butyl)tartrate] (glucoside B) are the major compounds [8, 25, 26] .

More than 95% of the volatile components are present at very low level (below 10 ppm) [21]. For a review on the various compounds identified in vanilla, see Dignum et al. [8]. For studies on the specificity of the glucosidase(s) in vanilla a series of glucosides occurring in green beans have been synthesised [11, 31, 40]. Glucovanilline can also be produced by feeding vanillin to plant cell cultures [28, 55]—an almost 50% yield of glucosylation was obtained from *V. planifolia* cell cultures [62].

Synthetic vanillin is a major intermediate in the production of various chemicals, including medicines and herbicides. Because of the large difference in price between vanillin from a natural origin and synthetic vanillin, counterfeiting is not uncommon. As natural and synthetic vanillin are chemically identical, isotope ratios of hydrogen (D/H) and carbon ($^{13}C/^{12}C$) isotopes are used to

determine the source of vanillin. Such analyses can be done by means of mass spectrometry or NMR. The latter has the advantage that the position-specific incorporation is determined (site-specific natural isotope fractionation NMR spectrometry). This highly specific method enables the differentiation of vanillin of all known sources [49].

To cater for the large demand for vanillin, besides different synthetic methods also biotechnological processes have been developed. Synthetic vanillin has a major drawback that products containing this compound cannot be labelled as containing a natural flavour. On the other hand, biotechnological products can be labelled as natural.

For the synthesis several processes have been described using different natural starting materials, such as coniferin, eugenol, guaiacol and lignin (for reviews, see [22, 44, 48, 60, 61].

9.8 Biotechnological Production of Vanillin

To produce natural vanillin at a lower price, various biotechnological approaches have been explored, such as plant cell cultures and bioconversion of natural compounds by means of microorganisms or isolated enzymes.

The production of fine chemicals by means of large-scale plant cell cultures is feasible [59]. But although *V. planifolia* cell cultures have been studied extensively, no economically feasible vanillin production has resulted from this (for reviews, see [22, 44, 48, 61]. *Capsicum frutescens* cell cultures were able to produce some vanilla flavour compounds upon being fed various precursors [48]. The amount of vanilla flavour compounds could be enhanced by treating the cultures with methyl jasmonate [56]. Cell suspension cultures of *Capsicum annuum* were able to produce vanillin after being fed with exogenous ferulic acid [24]. Next to plant cell cultures, it was shown that crude enzyme extracts from plants could be used for bioconversions of readily available precursors. Enzymes from soybean are able to convert isoeugenol into vanillin after addition of powdered activated carbon and peroxide [34]. A soybean lipoxygenase can produce vanillin from esters of coniferyl alcohol [35]. A vanillyl alcohol oxidase with broad specificity was obtained from *Penicillium*; this enzyme can convert vanillylamine (e.g. obtainable from the hydrolysis of capsaicin) or creosol [58].

Various microorganisms (e.g. *Bacillus fusiformis*, *Pseudomonas fluorescens*, *Pseudomonas acidovorans*, *Penicillium simplicissimum*, *E. coli*, *Corynebacterium glutamicum*, *Saccharomyces cerevisiae*, *Pycnoporus cinnabarinus*, *A. niger*) are able to convert fed natural phenylpropanoids precursors, such as ferulic acid, eugenol, isoeugenol, coniferyl alcohol, vanillyl alcohol and vanillylamine-isorhapotin (a stilbene), into vanillin [1, 2, 29, 38, 44, 48, 52, 60, 61, 64]. All these precursors have the same aromatic substitution pattern as vanillin; thus, they only require a chemical modification in the aliphatic carbon side chain. Priefert et al. [44] distinguished four different mechanisms for the shortening of

the side chain of ferulic acid: non-oxidative decarboxylation, side chain reduction, and coenzyme A (CoA) dependent as well as independent deacetylation. In all cases the toxic and highly reactive aldehyde formed is rapidly converted to the corresponding alcohol or acid. Rabenhorst and Hopp [45] using an *Amycolatopsis* species and Mueller et al. [37] using *Streptomyces setonii* were able to obtain high yields of vanillin (more than10 g l^{-1}) in the conversion of ferulic acid. The molar yields were about 75%. In both cases the bacterial species used had a high tolerance for vanillin. Muheim and Lerch [38] reported that the *Streptomyces setonii* strain mentioned could be the basis of an economical microbial production of vanillin from ferulic acid with a production of more than 6.4 g l^{-1}. The major bottleneck for these processes is the high price of ferulic acid. Eugenol is a much cheaper alternative ($9 per kilogram) [44], but so far the reported vanillin yields are lower than for ferulic acid conversion. When ferulic acid can be obtained from agricultural by-products for a low cost, it might be an interesting alternative for the production of vanillin. Ferulic acid is the most abundant hydroxycinnamic acid in the plant world, since it is an important structural component of the plant cell wall. Feruloyl esterases from a wide range of microorganisms can be used to release ferulic acid from the plant [36]. Cheap agricultural by-products like sugar beet pulp and maize bran are a good source for ferulic acid that could be released by the filamentous fungus *Pycnoporus cinnabarinus* [4, 32]. Interestingly, addition of a culture filtrate of the fungus *A. niger* resulted in direct biotransformation of autoclaved maize bran into vanillin [32]. A novel strain of *Bacillus fusiformis* was described that produced high amounts of vanillin from isoeugenol [64]. The cost of vanillin from a microbial production was estimated to be $1,000 per kilogram [52].

High-rate bioconversion of eugenol to ferulic acid was reported for an *E. coli* XL1-blue strain expressing the *vaoA* gene from *Penicillium simplicissimum* encoding vanillyl alcohol oxidase, which converts eugenol to coniferyl alcohol, together with the genes *calA* and *calB* encoding coniferyl dehydrogenase and coniferyl aldehyde dehydrogenase of *Pseudomonas*. This transgenic bacterial strain was able to convert eugenol to ferulic acid (14.7 g l^{-1}) with a molar yield of 93.3% [43]. The enzyme 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) from *Pseudomonas fluorescens* converted ferulic acid CoA into vanillin. This gene in combination with 4-hydroxycinnamoyl-CoA ligase was overexpressed in *E. coli*; this strain is capable of converting ferulic acid into vanillin.

E. coli has been genetically engineered to convert shikimate into vanillin by introducing the genes encoding a shikimate dehydrogenase yielding 3-dehydroshikimic acid, a dehydratase converting this into protocatechuic acid and a catechol-*O*-methyltransferase converting this acid into vanillic acid. Finally a reductase yielded vanillin [33, 44]. The various patents for biotechnological production of vanillin were reviewed by Priefert et al. [44] and Daugsch and Pastore [7].

The HCHL encoding gene (see above) has been overexpressed in various plant cells and plants by Walton and co-workers (tobacco, *Datura stramonium*). In none of these systems could vanillin be detected; however, various benzoic

acid derivatives were found, including vanillic acid glucoside [61]. This might be due to a lack of ferulic acid as a substrate in the plant cells and/or due to the toxic aldehydes being immediately converted into the corresponding acids or alcohols, similar to what is found in experiments on feeding vanillin to various cell cultures [62]. Genetic engineering of vanilla plants to overexpress this enzyme is not likely to be very successful, as the plants already contain a very high level of vanillin (2–6%) in the producing tissues.

Whether genetically engineered organisms will be successful for the production of vanillin not only depends on the economic feasibility of the process, but also on the acceptance by the public of GMO-produced vanillin.

9.9 Conclusions

Vanillin is the most important flavour compound in vanilla, and is often used to replace the extract. Vanillin can be obtained from vanilla beans, but because of the high costs of the beans, various other production methods have been developed. By far the cheapest production method is chemical synthesis, but vanillin made in this way cannot be labelled as natural. Of course this provokes counterfeiting and necessitates advanced quality control methods (NMR). It also initiates many studies in alternative natural production methods. These include microbial production and genetic engineering of microorganisms and plants. Microbial production of vanillin has been achieved, but the price is high ($1,000 per kilogram). Genetic engineering might be possible to either increase vanillin production in vanilla or introduce the pathway into other plants. However, public opinion against GMOs will be a major hurdle for this approach, besides the fact that the vanillin biosynthetic pathway is not known and no transformation–regeneration system for vanilla has been developed yet. In any case, all these methods only focus on vanillin, whereas the flavour of vanilla is more than only vanillin. Therefore, improvement of the agricultural practices and the curing system might be a more important strategy, also as it would offer farmers in the developing countries higher yields of better quality and thus higher incomes. To improve agricultural practice and yields, more knowledge about the pest and disease resistance of the plant and about the regulation of flowering, fruit ripening and vanillin biosynthesis is required. Further studies on vanilla are thus of great interest.

References

1. Achterholt S, Rabenhorst J, Steinbuechel A and Preifert H (2003) Process for the preparation of vanillin and suitable microorganisms. US Patent 2,003,092,143
2. Achterholt S, Rabenhorst J, Steinbuechel A and Preifert H (2004) Process for the preparation of vanillin and suitable microorganisms. US Patent 2,004,203,123

3. Bernard F (2005) Vanilla in Indonesia. In: Vanilla: The first international congress. Allured, Carol Stream, pp 33–40
4. Bonnina E, Brunel M, Gouy Y, Lesage-Meessen L, Asther M, Thibault J (2001) *Aspergillis niger* I-1472 and *Pycnoporus cinnabarinus* MUCL39533, selected for the biotransformation of ferulic acid to vanillin, are able to produce cell wall polysaccharide-degrading enzymes and feruloyl esterases. Enzyme Microb Technol 28:70–80
5. Brownell R (2005) The commercial survival of natural vanilla. In: Vanilla: The first international congress. Allured, Carol Stream, pp 1–3
6. Buccellato (2005) The various uses of vanilla in perfumery. In: Vanilla: The first international congress. Allured, Carol Stream, pp 67–70
7. Daugsch A, Pastore G (2005) Obtencao de vanilina: Oportunidade biotecnologica. Quim Nova 28:642–645
8. Dignum M, Kerler J, Verpoorte R (2001a) Vanilla production: technological, chemical and biosynthetic aspects. Food Res Int 17:199–219
9. Dignum M, Kerler J, Verpoorte R (2001b) Alpha-glucosidase and peroxidase stability in crude enzyme extracts from green beans of *Vanilla planifilolia* Andrews. Phytochem Anal 12:174–179
10. Dignum M, Kerler J, Verpoorte R (2002) Vanilla curing under laboratory conditions. Food Chem 79:165–171
11. Dignum M, van der Heijden R, Kerler J, Winkel C, Verpoorte R (2004) Identification of glucosides in green beans of *Vanilla planifolia* Andrews and kinetics of vanilla beta-glucosidase. Food Chem 85:199–205
12. Durant S, Karran P (2003) Vanillins–a novel family of DNA-PK inhibitors. Nucleic Acids Res 31:5501–5512
13. Fitzgerald DJ, Stratford M, Gasson MJ, Narbad A (2004) The potential application of vanillin in preventing yeast spoilage of soft drinks and fruit juices. J Food Prot 67:391–395
14. Funk C, Brodelius P (1990a) Influence of growth regulators and an elicitor on phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. Phytochemistry 29:845–848
15. Funk C, Brodelius P (1990b) Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. II Effects of precursor feeding and metabolic inhibitors. Plant Physiol 94:95–101
16. Funk C, Brodelius P (1990c) Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. III Conversion of 4-methoxycimnnamic acids into 4-hydroxybenzoic acids. Plant Physiol 94:102–108
17. Funk C, Brodelius P (1992) Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. IV Induction of vanillinic acid formation. Plant Physiol 99:256–262
18. Funk C, Brodelius P (1994) *Vanilla planifolia* Andrews: in vitro biosynthesis of vanillin and other phenylpropanoids derivatives. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Medicinal and aromatic plants VI, vol 26. Springer, Berlin Heidelberg New York, pp 377–402
19. Geerlings A, Martinez-Lozano Ibanez M, Memelink J, van der Heijden R, Verpoorte R (2000) The strictosidine ß-D-glucosidase gene from *Catharanthus roseus* is regulated coordinately with other terpenoid-indole alkaloid biosynthetic genes and the encoded enzyme is located in the endoplasmic reticulum. J Biol Chem 275:3051–3056
20. Hanum T (1997) Changes in vanillin and activity of β-glucosidase and oxidases during post harvest processing of vanilla beans (*Vanilla planifolia*). Bull Teknol Ind Pangan 8:435–443

21. Hoffman P, Harmon A, Ford P, Zapf M, Weber A, King S, Grypa R, Philander E, Gonzalez L, Lentz K (2005) Analytical approaches to vanilla quality and authentication. In: Vanilla: The first international congress. Allured, Carol Stream, pp 41–49
22. Hrazdina G (2006) Aroma production by tissue cultures Agric Food Chem 54:1116–1123
23. Joel DM, French JC, Graf N, Kourtiva G, Dixon AD, Havkin-Frenkel D (2003) A hairy tissue produces vanillin. Isr J Plant Sci 51:157–159
24. Kang SM, Jung HY, Kang YM, Min JY, Karigar CS, Yang JK, Kim SW, Ha YR, Lee SH and Choi MS (2005) Biotransformation and impact of ferulic acid on phenylpropanoid and capsaicin levels in *Capsicum annuum* L.cv.P1482 cell suspension cultures. J Agric Food Chem 53:3449–3453
25. Kanisawa T (1993) Flavor development in vanilla beans. Kouryou 180:113–123
26. Kanisawa T, Tokoro K, Kawahara S (1994) Flavor development in the beans of Vanilla planifolia. In: Kurihara K, Suzuki N, Ogawa H (eds) Olfaction taste XI, Proceedings of the international symposium. Springer, Berlin Heidelberg New York, pp 268–270
27. Keshava C, Keshava N, Whong WZ, Nath J, Ong TM (1998) Inhibition of methotrexate-induced chromosomal damage by vanillin and chlorophyllin in V79 cell. Teratogen Carcinogen Mutagen 17:313–326
28. Kometani T, Tanimoto H, Nishimura T, Osaka S (1993) Glucosylation of vanillin by cultured plant cells. Biosci Biotechnol Biochem 57:1157–1161
29. Krings U: Berger RG (1998) Biotechnological production of flavours and fragrances. Appl Microbiol Biotechnol 49:1–8
30. Labuda IM, Goers KA, Keon KA (1993) Microbial bioconversion process for the production of vanillin. In: Schreier P, Winterthaler P (eds) Progress in flavour precursor studies: analysis, generation, biotechnology. Proceedings of the international conference, Wuerzburg. Allured, Carol Stream, pp 477–482
31. Leong G, Uzio R, Derbesy M (1989) Synthesis, identification and determination of glucosides presnt in green vanilla beans. Flavour Fragrance J 4:163–167
32. Lesage-Meessen L, Lomascolo A, Bonnin E, Thibault JF, Buleon A, Roller M, Asther M, Record E, Ceccaldi BC and Asther M (2002) A biotechnology process involving filamentous fungi to produce natural crystalline vanillin from maize bran. Appl Biochem Biotechnol 102–103:141–153
33. Li K and Frost JW (1998) Synthesis of vanillin from glucose. J Am Chem Soc 120:10545–10546
34. Li YH, Sun ZH, Zao LQ, Xu Y (2005) Bioconversion of isoeugenol into vanillin by crude enzyme extracted from soybean. Appl Biochem Biotechnol 125:1–10
35. Markus PH, Peters ALJ, Roos R (1992) Process for the preparation of phenylaldehydes. Eur Patent Appl EP 0 542 348 A2
36. Mathew S, Abraham TE (2004) Ferulic acid: an antioxidant found naturally in plant cell walls and feruloyl esterases involved in its release and their applications. Crit Rev Biotechnol 24:59–83
37. Mueller B, Muench T, Muheim A, Wetli M (1998) Process for the production of vanillin. Patent Appl EPO885968
38. Muheim A, Lerch K (1999) Towards a high-yield bioconversion of ferulic acid to vanillin. Appl Biochem Biotechnol 51:456–461
39. Mustafa NK, Verpoorte R (2005) Chorismate derived C6C1 compounds in plants. Planta 222:1–5

40. Negishi I, Ozawa T (1996) Determination of hydroxycinnamic acids, hydroxybenzoic acids, hydroxybenzaldehydes, hydroxybenzyl alcohols and their glucsides y high-performance liquid chromatography. J Chromatogr A 756:129–136
41. Odoux E, Havkin-Frenkel D (2005) Hydrolysis of glucovanillin by β-glucosidase during curing of vanilla been (*Vanilla planifolia* Andrews). In: Vanilla: The first international congress. Allured, Carol Stream, pp 95–100
42. Odoux E, Chauwin A, Brillouet JM (2003) Purification and characterization of vanilla bean (*Vanilla planifolia* Andrews) β-glucosidase. J Agric Food Chem 51:3168–3173
43. Overhage J, Steinbuechel A and Priefert H (2003) Highly efficient biotransformation of eugenol to ferulic acid and further conversion to vanillin in recombinant strains of *Escherichia coli*. Appl Environ Microbiol 69:6569–6576
44. Priefert H, Rabenhorst J, Steinbuechel (2001) Biotechnological production of vanillin. Appl Microbiol Biotechnol 56:296–314
45. Rabenhorst J, Hopp R (2000) Process for the preparation of vanillin and suitable microorganisms. DE Patent 19,532,317
46. Ranadive AS (2005) Vanilla cultivation. In: Vanilla: The first international congress. Allured, Carol Stream, pp 25–32
47. Ranjoanisafy X (1998) Purification et étude de quelques propriétés de la β-glucosidase de la vanilla. Implications dans la préparation de la vanilla. Master's thesis, Ensia-Siarc, Montpellier
48. Rao SR and Ravishankar GA (2000) Vanilla flavour: Production by conventional and biotechnological routes. J Sci Food Agric 80:289–304
49. Remaud GS, Martin YK, Giles G, Martin GJ (1997) Detection of natural vanilla flavors and extracts: Application of the SNIF-NMR to vanillin and p-hydroxybenzaldehyde. J Agric Food Chem 45:859–866
50. Roeling WFM, Kerler J, Braster M, Apriyantono A, Stam H, Van Verseveld HW (2001) Microorganisms with a taste for vanilla: Microbial ecology of traditional Indonesian vanilla curing. Appl Environ Microbiol 67:1995–2003
51. Romagnoli LG, Knorr D (1988) Effects of ferulic acid treatment on growth and flavor development of cultured *Vanilla planifolia* cells. Food Biotechnol 2:93–104
52. Schrader J, Etschmann MMW, Sell D, Hilmer J-M, Rabenhorst J (2004) Applied biocatalysis for the synthesis of natural flavour compounds—current industrial processes and future prospects. Biotechnol Lett 26:463–472
53. Serra S, Fuganti C, Brenna E (2005) Biocatalytic preparation of natural flavours and fragrances. Trends Biotechnol 23:193–199
54. Sinigaglia M, Reguly ML, de Andrade HH (2004) Effect of vanillin on toxicant-induced mutation and mitotic recombination in proliferating somatic cells of *Drosophila melanogaster*. Environ Mol Mutagen 44:394–400
55. Sommer J, Schroeder C, Stoeckigt J (1997) In vivo formation of vanillin glucoside. Plant Cell Tissue Organ Cult 50:119–123
56. Suresh B, Ravishankar GA (2005) Methyl jasmonate modulated biotransformation of phenylpropanoids to vanillin related metabolites using *Capsicum frutescens* root cultures. Plant Physiol Biochem 43:125–131
57. Tipparaju S, Ravishankar S, Slade PJ. (2004) Survival of *Listeria monocytogenes* in vanilla-flavored soy and dairy products stored at 8 degrees C. J Food Prot 67:378–382

58. Van den Heuvel RRH, Fraaije MW, Laane C, van Berkel WJH (2001) Enzymatic synthesis of vanillin. J Agric Food Chem 49:2954–2958
59. Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1:13–25
60. Walton NJ, Narbad A, Faulds CB, Williamson G (2000) Novel approaches to the biosynthesis of vanillin. Curr Opin Biotechnol 11:490–496
61. Walton NJ, Mayer MJ, Narbad A (2003) Vanillin. Phytochemistry 63:505–515
62. Yuana, Dignum MJW, Verpoorte R (2002) Glucosylation of exogenous vanillin by plant cell cultures. Plant Cell Tissue Organ Cult 69:177–182
63. Zenk MH (1965) Biosynthesis von Vanillin in *Vanilla planifolia* Andr. Z Pflanzenphysiol 53:404–414
64. Zhao LQ, Sun ZH, Zheng P, Zhu LL (2005) Biotransformation of isoeugenol to vanillin by novel strain of *Bacillus fusiformis*. Biotechnol Lett 27:1505–1509