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The ester-linked ferulic acid of wheat bran and sugar-beet pulp can be converted to vanillin using biological transformation. Free ferulic acid from sugar-beet pulp and from wheat bran was almost quantitatively obtained by extensive degradation of the cell-walls using enzyme mixtures complemented with specific ferulic acid esterases. The Basidiomycete Pycnoporus cinnabarinus *then converted the released ferulic acid to vanillin. The selection of stable and highly productive strains was achieved using formal genetics. The use of cellobiose as an activator of the vanillin pathway, and the sequential addition of a precursor (ferulic acid) in cultures of selected* P. cinnabarinus *strains, allowed 90 and 300 mg / L of vanillin to be obtained from ferulic acid enzymically released from wheat bran and sugar-beet pulp, respectively. This process was adapted into a two-step process involving two filamentous fungi,* Aspergillus niger *and* P. cinnabarinus, *with complementary capabilities of transformation.*

Keywords: vanillin; ferulic acid; glycanases; bioconversion; cereal brans; sugar-beet pulp

Introduction

Vanillin is one of the most universally used aromatic molecules in the food, pharmaceutical and cosmetic industries. Two commercial types exist, (i) pure vanillin obtained by chemical synthesis from guaiacol or black liquors from the paper industry, with an annual world market of 12,000 tons and a price of approximately 12 ECU/kg and (ii) a vanilla extract obtained by the ageing and alcoholic extraction of vanilla pods. The world market consists of around 1800 tons of pods, of which the vanillin content is 2–3 g/100 g, that is, 40–50 tons of pure natural vanillin. The extract also contains other aromatic compounds such as *p*-hydroxy-benzaldehyde and anisaldehyde.

Natural vanilla extract is 250 times more expensive than synthetic vanillin, because of the complexity of the culture and tedious ageing process, but the consumer demand is higher for natural products. European and American legislations agree that flavour obtained by a biological process (enzymic or microbiological) from natural products can be considered as natural (cf. Official Journal no. L 184 15/7/88 and 91/71, Official

† This paper is dedicated to the memory of Mike Coughlan.
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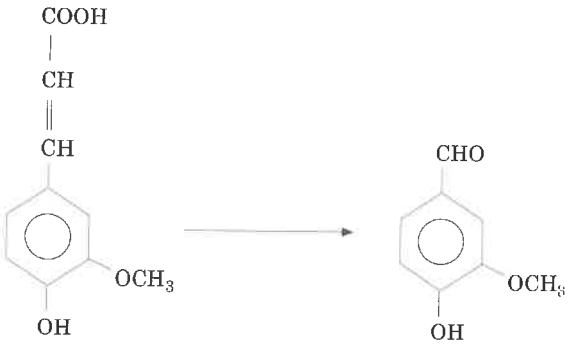

Ferulic acid Vanillin

Fig. 1 Chemical structure of ferulic acid and vanillin

Journal no. L 42 15/2/91). Thus pure vanillin obtained by a biotechnological process can be considered as natural, and has a much lower production cost. At present, there is no process for the production of natural pure vanillin, since all of the vanilla currently sold consists of a complex extracted mixture.

Ferulic acid is a potential precursor for the fungal production of vanillin (1) (**Fig. 1**). Certain monocots and dicots contain this phenolic acid (2, 3). In monocotyledonous plants, the most important sources of ferulic acid are Poaceae, especially wheat, which accumulates ferulic acid in the cell-wall polysaccharides (heteroxylans) of the grain. Brans (by products of the milling industry) are therefore suitable raw materials for production of ferulic acid (4). In dicotyledonous plants, ferulic acid is found in the Chenopodiaceae (spinach, glasswort, beet, etc.) where it is a constituent of pectic polysaccharides and is therefore concentrated in cell wall-rich materials. Thus sugar-beet pulp, the residue of sugar refinery, is another potential raw material as a source of ferulic acid (5).

Several reports have demonstrated the bioconversion of ferulic acid. Nazareth and Marvinkurve (6) reported the nonoxidative decarboxylation of ferulic acid by *Fusarium solani* to 4-vinylguaicol which was then oxidized to vanillin and vanillic acid. Similar results were obtained (7) for *Paecilomyces variotii* and *Pestalotia palmarum*. A new route involving a demethylation of ferulic acid to caffeic acid, followed by side-chain shortening to yield protocatechuic acid, was described by Tillet and Walker (8) for *Penicillium rubrum*. *Pycnoporus cinnabarinus* I-937 was selected in our studies for its ability to efficiently transform synthetic ferulic acid to vanillin [1]. Preliminary studies showed that a vanillin concentration of up to 64 mg/L, corresponding to a molar yield of 27.5%, could be obtained by this microorganism.

In this review, we describe the release of ferulic acid from raw materials with the subsequent bioconversion of the released ferulic acid to vanillin. This could lead to a biotechnological production of natural vanillin in two steps, the first using enzymes (from GRAS (generally regarded as safe) microorganisms) to liberate ferulic acid from the cell wall matrix, and the second using GRAS microorganisms for the biotransformation of this precursor to vanillin.

Materials and Methods

Chemicals

Sugar-beet pulp and wheat bran were obtained from Agro-Industrie Recherches et Développement (Bazancourt, France). Ferulic acid, vanillin and the other phenolic compounds used were obtained as described previously (9). Oat spelt xylan was obtained from Fluka Chemical Co.

Sugar and linkage analysis

The composition of both wheat bran and sugar-beet pulp was determined as described previously (4, 10). Feruloylated oligosaccharides were fractionated by liquid chromatography (11) and their structures confirmed by total sugar analysis and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) (12).

Enzymes

Aspergillus niger ferulic acid esterases (FAE)-I and -II were purified from a commercial pectinase preparation (13). *A. niger* FAE-III was purified from strain CBS120.49 grown on oat spelt xylan for 4 d (14). *A. niger* CinnAE was purified from strain CS180 grown on sugar-beet pulp for 4.5 d (15). Commercial enzyme preparations were obtained from Novo-Nordisk A/S (10). *Trichoderma viride* xylanase was either obtained from Novo-Nordisk A/S as Novozyme 431 or purchased from Megazyme (Bray, Ireland). *A. niger* xylanase 1 (16), *Talaromyces emersonii* xylanases (VII, IX and X (17)), and *Ps. fluorescens* xylanase (XYLA, (18)) were purified as described previously.

Enzyme assays

Feruloyl esterase activity against methyl ferulate (MFA) and xylanase activity were determined as described previously (19). Protein was determined using Coomassie-Plus reagent, with bovine serum albumin used as a protein standard.

Fungal strains

Pycnoporus cinnabarinus. MUCL-39532 and I-937, and *A. niger* I-1472 were maintained on malt agar slants. Culture conditions and growth measurements have been described previously (9, 20).

HPLC analysis

Phenolics were determined by C_{18} reverse phase HPLC (enzymic release from cell walls (10, 19) and biotransformation (9). Oligomeric sugars were determined by high performance anion-exchange chromatography

(HPAEC) using a Carbopak PA1 column (Dionex, Sunnyvale, U.S.A.) (10, 19).

Isolation of ferulic acid

The preparation of adsorbents (activated charcoal, Amberlite XAD), and the binding and elution of ferulic acid from the adsorbents were performed as previously described (21).

Results and Discussion

Release of ferulic acid from raw materials

Sugar-beet pulp and wheat bran were chosen as raw materials because they are especially rich sources of ferulic acid. The chemical composition of cereal brans and sugar-beet pulp are shown in **Table 1**. The ferulic acid content was ~0.5–1 g/100 g for wheat bran and sugar-beet pulp. Some diferulic acids were also detected (data not shown). The main sugars were galacturonic acid (sugar-beet pulp), xylose (brans), arabinose (sugar-beet pulp, brans) and glucose (brans and sugar-beet pulp). These sugars are monomeric constituents of cellulose, pectins (in sugar-beet pulp) and heteroxylans (in cereal brans). The presence of methanol and acetic acid is typical for beet pectins since galacturonic residues may be methyl- and acetyl-esterified. Wheat bran and sugar-beet pulp contain significant amounts of ferulic acid, covalently-linked to sugar (arabinose and galactose) residues. These residues are in turn

Table 1 Chemical composition of the raw materials(as g/100 g of dry weight)

Component	Wheat bran[a]	Sugar-beet pulp[b]
Uronic acid	6.6[c]	21.1[d]
Rhamnose	0.0	2.4
Arabinose	9.6	20.9
Xylose	16.5	1.7
Mannose	1.3	1.1
Galactose	1.2	5.1
Glucose	11.0	21.1
Proteins	15.8	11.3
Starch	18.6	0.0
Methanol	—	1.8
Acetic acid	—	3.9
Ferulic acid	0.5	0.8
Lignin	2.6	—

[a] Ralet *et al.* (4).
[b] Micard *et al.* (10).
[c] Glucuronic acid.
[d] Galacturonic acid.

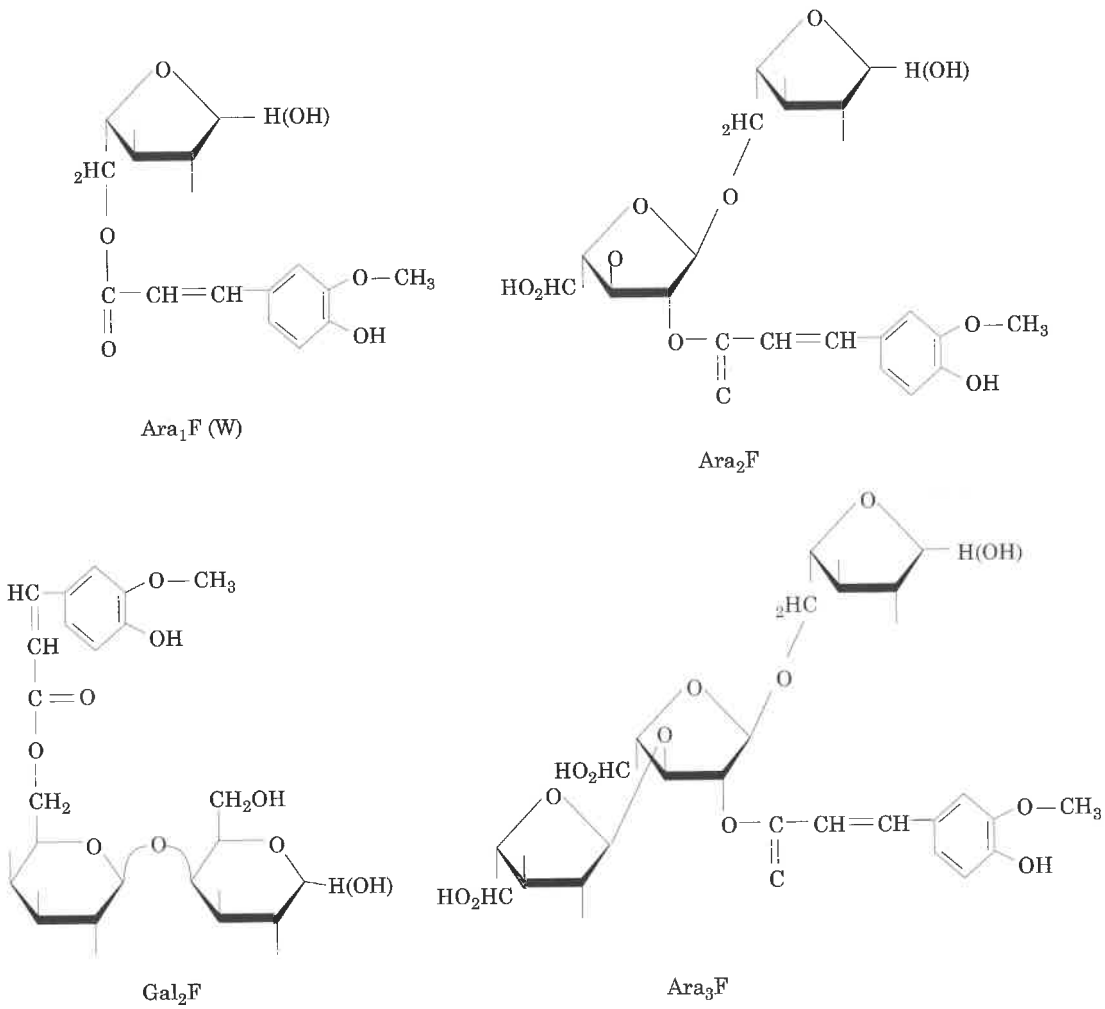

Fig. 2 Structure of some feruloylated oligosaccharides from sugar-beet pulp and wheat bran, F = ferulic acid; Ara = arabinose; Gal = galactose; W = wheat

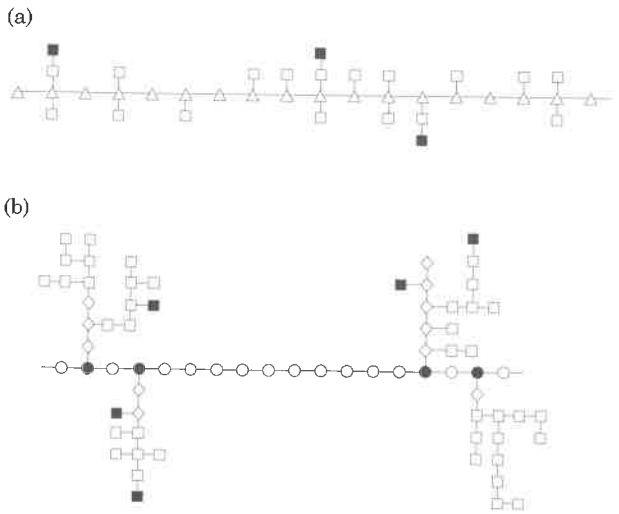

Fig. 3 Schematic structure of (a) wheat bran heteroxylans, (△) = xylose; (□) = arabinose; (■) = ferulic acid, and (b) sugar beet pectins, (○) = galacturonic acid; (●) = rhamnose; (□) = arabinose; (◇) = galactose; (■) = ferulic acid

covalently attached to heteroxylan and pectin components of the cell wall. The exact location of the feruloyl groups has been extensively studied through enzymic and acid hydrolysis followed by NMR analysis of the highly purified feruloylated oligosaccharides (11, 12). The main conclusions (**Fig. 2**) are that, in sugar-beet pulp, approximately 50% of the ferulic acid is linked to O-2 of arabinose residues and 50% to O-6 of galactose residues, and that in wheat brans, ferulic acid is linked to O-5 of arabinose residues. Schematic structures of the cell-wall polysaccharides bearing this substituent are indicated in **Fig. 3**. Sugar-beet pulp contains pectic substances in which 'hairy' regions and 'smooth' regions are present (22). Rhamnose, which is part of the (rhamnogalacturonic) backbone of pectin, bears side-chains mainly composed of arabinose and galactose residues. Wheat bran contains large amounts of heteroxylans in which xylose forms the backbone (xylans) on which arabinose residues are attached; feruloyl groups are found linked to these arabinose residues. Some microorganisms secrete a range of ferulic acid esterases (FAE), each with different specificities (13, 15, 23). The activities depend on the nature of the sugar and the linkage with ferulic acid, as well as on the length of the oligosaccharide moiety (**Table 2**). Four inducible enzymes from *Aspergillus niger* strains have been identified (15, 24, 25). FAE I acts preferentially on O-2 feruloylated arabinose, and also on O-6 feruloylated galactose (26). FAE II and FAE III have highest activity on O-5 feruloylated arabinose. Another enzyme, active on sugar-beet pulp, has been isolated and termed cinnamoyl esterase (CinnAE); it shows highest activity on O-2 feruloylated arabinose (15). From these studies, it is clear that different enzymes are needed depending on the origin of the substrate (cereal or sugar-beet pulp), since the nature of the linkages between ferulic acid and the sugar moiety depends on the source (27). The direct action of pure ferulic acid esterases on the raw materials is much less than on the soluble substrates, implying that an extensive degradation of the cell wall polysaccharide is first required. A thorough screening of commercial enzyme preparations was conducted (see below) and some containing adequate 'pectinase' and 'xylanase' activities were found. However, the amount of ferulic acid esterases in the complexes was always limiting and it was necessary to complement the preparations with these esterases. Wheat bran and sugar-beet pulp showed different degradabilities and yields of ferulic acid.

Commercial preparations and 'monocomponent' enzymes, both from Novo Nordisk A/S, have been tested (10, 27, 28) for their abilities to release ferulic acid and sugars. The best preparation was 'SP 584', a pectinolytic preparation devoid of cellulolytic activities; however the ferulic acid esterases activities were found to be limiting in this preparation. With low amounts of enzymes, very little free ferulic acid was obtained; about 60% of the total amount of ferulic acid of the sugar-beet pulp could be released by using much more SP 584. The addition of CinnAE increased this yield to 95% (**Table 3**). The degradation of the sugar-beet pulp by SP 584 leaves a residue particularly rich in cellulose which can be further degraded into cellobiose and glucose by selected cellulolytic preparations (29).

On a laboratory scale, 95% of the total ferulic acid was released from wheat bran with FAE III and endoxylanase from *Trichoderma viride* (24). The yield of ferulic acid was highly dependent on the source of the xylanase (19), as shown in **Fig. 4**. The reaction proceeded via feruloylated oligosaccharides, which are very efficient substrates for the esterase. However, the overall solubilization and the yield of monomeric sugars was rather low.

Separation of ferulic acid

After enzymic degradation of the raw materials, free ferulic acid is released in a complex mixture of neutral and acidic sugars, methanol, acetic acid, *etc.* It therefore has to be isolated from compounds, which could inhibit the bioconversion. It has been demonstrated on a laboratory scale, that some adsorbents (such as resins and activated carbon) have a very high specificity for

Table 2 Characteristics of the different ferulic acid esterases

Enzyme	Origin	Molar mass (kDa)	Preferred substrate	Reference
FAE I	*Aspergillus niger*	132.0	O-2 feruloylated arabinose O-6 feruloylated galactose	27
FAE II	*Aspergillus niger*	29.0	O-5 feruloylated arabinose	13
FAE III	*Aspergillus niger*	36.0	O-5 feruloylated arabinose	27
CinnAE	*Aspergillus niger*	150.0	O-2 feruloylated arabinose	25

Table 3 Release of ferulic acid from sugar-beet extract by different enzymes

Enzyme dosage		Ferulic acid released
SP-584 (g/l extract)	CinnAE[a] (U/l extract)[b]	(% of alkali-extractable)
0.1	—	60.0
1.0	—	93.8
—	20	25.5
0.1	20	94.0

[a] Kroon *et al.* (15).
[b] One unit of activity is the amount of enzyme releasing 1 nmole of caffeic acid from methyl caffeate (1mM) per min at 37 °C and pH 6.0.

ferulic acid and can be used very efficiently in order to purify ferulic acid after desorption with ethanol or another natural agent. The best results have been obtained using a chemically-activated carbon (21, 30); the binding was relatively selective for ferulic acid

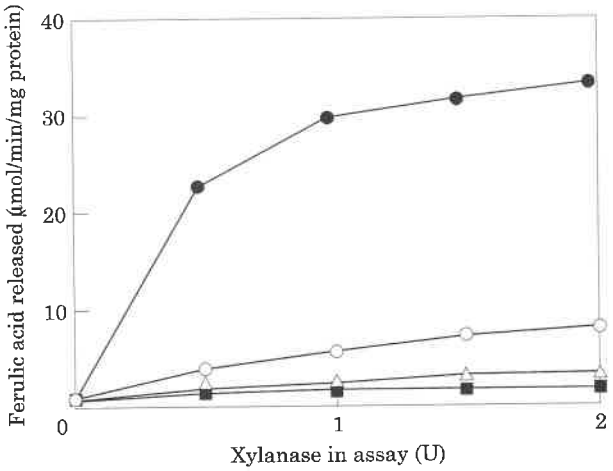

Fig. 4 Release of ferulic acid from wheat bran in the presence of FAE-III from *Aspergillus niger* and of different sources of xylanases (●) = *Trichoderma viride*; (■) = *Talaromyces emersonii*; (○) = *Aspergillus niger*; (△) = *Pseudomonas fluorescens*

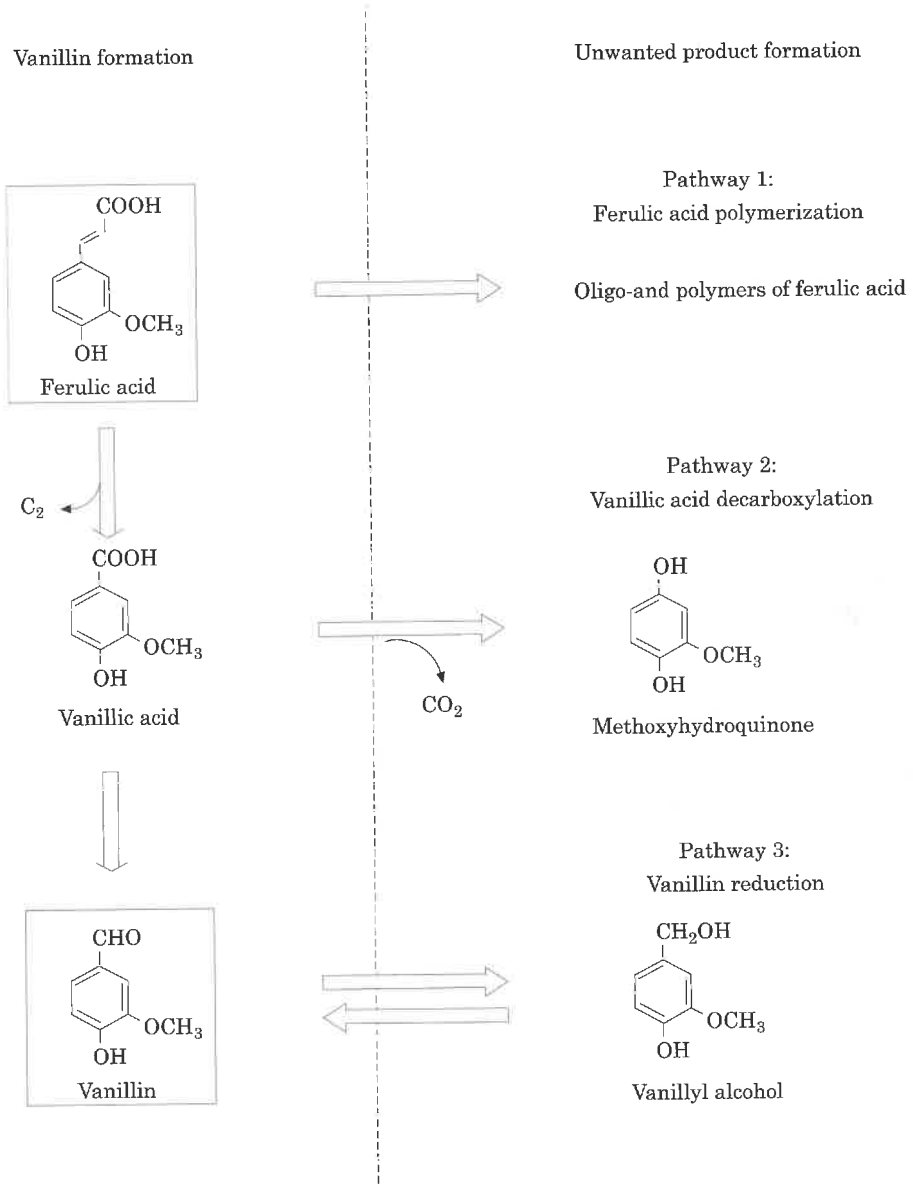

Fig. 5 Metabolic pathways showing conversion of ferulic acid to vanillin by *Pycnoporus cinnabarinus*

which could be recovered by elution with organic solvent. Using ethanol as eluant, ferulic acid was obtained from sugar-beet pulp with a purity of approximately 50%.

Conversion of ferulic acid into vanillin

The pathways of the transformation of ferulic acid by *Pycnoporus cinnabarinus* I-937 have been identified (20). Three major divergent routes lower the yield of vanillin (**Fig. 5**). The first yielded ferulic acid oligo/polymers via a laccase activity, by-passing the route to vanillin. The two other routes occurred via the ferulic acid propenoic chain degradation to yield vanillic acid. In the former, vanillic acid was subsequently reduced to vanillyl alcohol via vanillin. In the latter case, vanillic acid was decarboxylated to methoxyhydroquinone. A means to favour the production of vanillin from ferulic acid was therefore investigated.

Pycnoporus cinnabarinus I-937 was shown to express laccase activity leading to polymerization and insolubilization of ferulic acid (31). This fungus is heterothallic and is fertile only by the pairing of two compatible haploid mycelia given a secondary dikaryotic mycelium (i.e. I-937 strain) characterized by the presence of clamp connections. To overcome these difficulties, the selection by formal genetics of laccase-deficient strains from various *P. cinnabarinus* (Mycothèque de l'Université de Louvain-la-Neuve, Belgium) allowed us to obtain new monokaryotic cell lines which were more productive and genetically stable. The monokaryotization has been carried out by collecting and isolating single haploid sexual spores (basidiospores). The isolation of more than 500 monokaryons derived from seven different *P. cinnabarinus* parent strains yielded four stable laccase-negative strains (**Table 4**).

The selected laccase deficient strains were tested for vanillin production in the presence of ferulic acid. Ferulic acid metabolism by *P. cinnabarinus* laccase-deficient strains essentially occurred via a propenoic chain degradation to vanillic acid which was subsequently either reduced to vanillin (75 mg/L) and vanillyl alcohol (3 mg/L) or oxidatively decarboxylated to methoxyhydroquinone (130 mg/L. Cellobiose, which could be derived from the cellulosic fraction of sugar-beet pulp and wheat bran after enzymic degradation, was used as a fermentation feedstock to channel the flow of vanillic acid through the reductive pathway leading to vanillin. When cellobiose was used as carbon source and supplemented after 3 d of growth, a relatively high accumulation of vanillin (459 mg/L) with a small amount of methoxyhydroquinone (10 mg/L) was obtained (**Table 5**).

Table 4 Selection of monokaryotic laccase-deficient lines of *P. cinnabarinus* from laccase-positive dikaryotic parents

Strain Number	Isolation type	Nuclear type	Laccase assays
MUCL 30555	MS[a]	DK[c]	(+)(−)
MUCL 38467	SS 2[b]	MK[d]	(−)
MUCL 38466	SS 5	MK	(−)
MUCL 39532	SS 23	MK	(−)
MUCL 38620	MS	DK	(+)
MUCL 38625	SS 4	MK	(−)

[a] MS = mass spore came from vegetative mycelium.
[b] SS = single haploid sexual spore came from fruiting bodies.
[c] DK = dikaryon.
[d] MK = monokaryon.
(−) = laccase-deficient character; (+) = low laccase-positive character.

Table 5 Vanillin production

Compounds (mg/mL)	One step process[a]	Two step process[b]	
	Pycnoporus cinnabarinus	*Aspergillus niger*	*Pycnoporus cinnabarinus*
Vanillic acid	290	920	
Vanillin	459	0	560
Vanillyl alcohol	210	0	41
Methoxyhydroquinone	10	95	275

[a] From ferulic acid in a process involving laccase-deficient-strains of *Pycnoporus cinnabarinus*.
[b] Combining ferulic acid transformation to vanillic acid by *Aspergillus niger* and vanillic acid transformation to vanillin by *Pycnoporus cinnabarinus* strains.

In the biotransformation of ferulic acid to vanillin, another limiting step is the degradation of the ferulic acid propenoic chain into vanillic acid. To improve vanillin production, a new two step strategy was developed, with two filamentous fungi exhibiting complementary ability of transformation (9, 31). The first step involved *Aspergillus niger* I-1472; ferulic acid metabolism by this fungus (**Table 5**) essentially occurred via the propenoic chain degradation to yield a high concentration of vanillic acid (920 mg/L) which was subsequently decarboxylated to a lesser extent to methoxyhydroquinone (95 mg/L). In the second step, vanillic acid could be transformed to vanillin (560 mg/L) by laccase-deficient *P. cinnabarinus* (Table 5). The use of a two step process increased vanillin production 9-fold when compared to the initial single step process (1). In all these investigations, sequential additions of precursors were performed because of the relatively high toxicity of phenolic compounds. The aromas of the fermentation broth have been evaluated for their organoleptic properties. Although the predominant sensation is vanilla, in the presence of ferulic acid from sugar-beet pulp and wheat bran, a slight odour of fruity/phenolic and flower/orange was detected, respectively. In contrast, in presence of synthetic ferulic acid a pear/phenolic odour was present.

These results show that agricultural by products can be converted to vanillin, together with minor related compounds to give a more complex flavour, using enzymes and microorganisms. The release of ferulic acid from cereal brans and from sugar-beet pulp is achieved using ferulic acid esterases and polysaccharide-degrading en-

zymes, and the ferulic acid is bioconverted to vanillin using either *P. cinnabarinus* or a combination of *A. niger* and *P. cinnabarinus*.

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References

1 Gross, B., Asther, M., Corrieu, G. and Brunerie, P. (1991) Production de vanilline par bioconversion de précurseurs benzéniques. European Patent No. 0453368A1.
2 Harris, P. J. and Hartley, R. D. Phenolic constituents of the cell walls monocotyledons. *Biochemical Systematics and Ecology*, **8**, 153–160 (1980)
3 Hartley, P. J. and Harris, P. J. Phenolic constituents of the cell walls of dicotyledons. *Biochemical Systematics and Ecology*, **9**, 189–203 (1981)
4 Ralet, M. C., Thibault, J.-F. and Della Valle, G. Influence of extrusion-cooking on the properties of wheat bran. *Journal of Cereal Science*, **11**, 249–259 (1990)
5 Rombouts, F. M. and Thibault, J.-F. Feruloylated pectic substances from sugar-beet pulp. *Carbohydrate Research*, **154**, 177–188 (1986)
6 Nazareth, S. and Marvinkurve, S. Degradation of ferulic acid via 4-vinylguaiacol by *Fusarium solani* (Mart.) *Canadian Journal of Microbiology*, **32**, 494–497 (1986)
7 Rahouti, M., Seigle-Murandi, F., Steiman, R. and Eriksson, K. E. Metabolism of ferulic acid by *Paecilomyces variotii* and *Pestalotia palmarum*. *Applied and Environmental Microbiology*, **55**, 2391–2398 (1989)
8 Tillet, R. and Walker, J. Metabolism of ferulic acid by *Penicillium sp*. *Archives of Microbiology*, **154**, 206–208 (1990)
9 Lesage-Meessen, L., Delattre, M., Haon, M., Thibault, J.-F., Colonna-Ceccadi, B., Brunerie, P. and Asther, M. A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. *Journal of Biotechnology*, **50**, 107–113 (1996)
10 Micard, V., Renard, C. M. G. C. and Thibault, J.-F. Enzymatic saccharification of sugar-beet pulp. *Enzyme and Microbial Technology*, **19**, 162–170 (1996)
11 Ralet, M.-C., Thibault, J.-F., Faulds, C. B. and Williamson, G. Feruloylated oligosaccharides from cell-wall polysaccharides. Part I. Isolation and purification from sugar-beet pulp. *Carbohydrate Research*, **263**, 227–241 (1994)
12 Colquhoun, I. J., Ralet, M.-C., Thibault, J.-F., Faulds, C. B. and Williamson, G. Feruloylated oligosaccharides from cell-wall polysaccharides. Part II. Structure identification of feruloylated oligosaccharides from sugar-beet pulp. *Carbohydrate Research*, **263**, 243–256 (1994)
13 Faulds, C. B. and Williamson, G. Ferulic acid esterase from *Aspergillus niger*: purification and partial characterization of two forms from a commercial source of pectinase. *Biotechnology and Applied Biochemistry*, **17**, 349–359 (1993)
14 Faulds, C. B. and Williamson, G. Purification and characterization of a ferulic acid esterase (FAE-III) from *Aspergillus niger*: specificity for the phenolic moiety and binding to microcrystalline cellulose. *Microbiology*, **140**, 779–787 (1994)
15 Kroon, P. A., Faulds, C. B. and Williamson, G. Purification and characterisation of a novel ferulic acid esterase induced by growth of *Aspergillus niger* on sugar-beet pulp. *Biotechnology and Applied Biochemistry*, **23**, 255–262 (1996)
16 Hughes, M. M. (1993) PhD thesis, University College, Galway, Ireland.
17 Tuohy, M. G., Laffey, C. D. and Coughlan, M. P. Characterization of the individual components of the xylanolytic enzyme system of *Talaromyces emersonii*. *Bioresource Technology*, **50**, 37–42 (1994)
18 Hall, J., Hazlewood, G. P., Huskisson, N. S., Durrant, A. J. and Gilbert, H. J. Homology of a xylanase and cellulase from *Pseudomonas fluorescens* subsp. *cellulosa*: internal signal sequence and unusual protein processing. *Molecular Microbiology*, **3**, 1211–1219 (1989)
19 Bartolome, B., Faulds, C. B., Tuohy, M., Gilbert, H., Hazlewood, G. and Williamson, G. Influence of different xylanases on the activity of ferulic acid esterase on wheat bran. *Biotechnology and Applied Biochemistry*, **22**, 65–73 (1995)
20 Falconnier, B., Lapierre, C., Lesage-Meessen, L., Yonnet, G., Brunerie, P., Colonna-Ceccaldi, B., Corrieu, G. and Asther, M. Vanillin as a product of ferulic acid biotransformation by the white-rot fungus *Pycnoporus cinnabarinus* I-937: Identification of metabolic pathways. *Journal of Biotechnology*, **37**, 123–132 (1994)
21 Couteau, D. and Mathaly, P. Purification of ferulic acid by adsorption after enzyme release from a sugar-beet pulp extract. *Industrial Crops and Products*, **6**, 237–252 (1997)
22 Renard, C. M. G. C., Crepeau, M.-J. and Thibault, J.-F. Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydrate Research*, **275**, 155–165 (1995)
23 Brezillon, C., Kroon, P. A., Faulds, C. B., Brett, G. and Williamson, G. Novel ferulic acid esterases are induced by growth of *Aspergillus niger* on sugar-beet pulp. *Applied Microbiology and Biotechnology*, **45**, 371–376 (1996)
24 Faulds, C. B. and Williamson, G. Release of ferulic acid from wheat bran by a ferulic acid esterase (FAE-III) from *Aspergillus niger*. *Applied Microbiology and Biotechnology*, **43**, 1082–1087 (1995)
25 Kroon, P. A. and Williamson, G. Release of ferulic acid from sugar-beet pulp using arabinanase, arabinofuranosidase and an esterase from *Aspergillus niger*. *Biotechnology and Applied Biochemistry*, **23**, 263–267 (1996)
26 Ralet, M.-C., Faulds, C. B., Williamson, G. and Thibault, J.-F. Feruloylated oligosaccharides from cell-wall polysaccharides. Part III. Degradation of feruloylated oligosaccharides from wheat bran and sugar-beet pulp by ferulic acid esterases from *Aspergillus niger*. *Carbohydrate Research*, **263**, 257–269 (1994)
27 Micard, V., Renard, C. M. G. C. and Thibault, J.-F. Studies on enzymic release of ferulic acid from sugar-beet pulp. *Lebensmittel-Wissenschaft und-Technology*, **27**, 59–66 (1994)
28 Micard, V., Renard, C. M. G. C. and Thibault, J.-F. End products of enzymic saccharification of beet pulp, with a special attention to feruloylated oligosaccharides *Carbohydrate Polymers*, **32**, 283–292 (1997)
29 Micard, V., Renard, C. M. G. C. and Thibault, J.-F. Enzymic degradation of a cellulose-rich residue from sugar-beet pulp: influence of pretreatments. *Lebensmittel-Wissenschaft und-Technology*, **30**, 284–291 (1997)
30 Grant, T. M. and King, C. J. Mechanism of irreversible adsorption of phenolic compounds by activated carbons. *Industrial and Engineering Chemistry Research*, **29**, 264–271 (1990)
31 Lesage-Meessen, L., Delattre, M., Haon, M. and Asther, M. (1995) Procédé d'obtention d'acide vanillique et de vanilline par bioconversion de microorganismes filamenteux. World Patent No. PCT/FR 95/01173