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Novel Aromas of Fungal Origin

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Abstract

Studies dealing with the *in vitro* production of alcohols, esters, aromatic compounds, lactones by species belonging to the genera *Aspergillus, Ceratocystis, Fusarium, Phellinus, Penicillium, Thielaviopsis, Trametes, Mycoacia* and *Trichoderma* are reviewed. Elsewhere, conversions of β ionone and isophorone into new tobacco flavourings by *Lasiodiplodia theobromae* and a selected strain of *Aspergillus niger* are reported. Potential uses of filamentous fungi for aroma and flavour production is discussed.

Keywords: aromas, fungal

1. Aroma and Biotechnology

Natural plant products such as fruits, flowers and herbs, animal products such as musk products of the Maillard reaction and products from chemical synthesis are the main sources of aromas and flavors. Currently, biotechnology appears as an explorative way of aroma production, involving enzymology, bioconversion, tissue and cell culture and, tentatively the use of new microorganisms. Here, we will lay stress on the experimental utilization of filamentous fungi. Numerous species of fungi may be considered as potential aroma-producing microorganisms through the culture of their mycelia. The fungal odors have been considered as criteria for identification by different authors for example by Badcock (1939) who has listed the odorous properties of numerous species. some wood rotting species provide several interesting examples for the production of alcohols, esters, aromatic compounds, terpenes, lactones, etc. Their ability to convert complex molecules into new flavourings will be examined. Optimization of the production and potentiality of use will be discussed.

2. Rapid Review of Volatiles Identified in Fungal Cultures

Aroma production by microorganisms has been the subject of numerous reviews. The following information is extracted from a recent report (Latrasse et al., 1985).

Alcohols and esters

The following alcohols: 3 and 2-methylbutanol, butanol, isobutanol, pentanol, hexanol, 3-oxtanol, 1-octen-3-ol and phenylethanol are generally present in the fungal cultures. They are derived partly from the metabolism of the following amino-acids: leucine, isoleucine, valine, phenylalanine according to Ehrlich's pathway. When 1-octen-3-ol is abundant the culture has the typical "Champignon de Paris" topnote. Esters are also frequently present (butyl, amyl and octyl acetates) and these are considered to result form a detoxification process where acetic acid and higher alcohols are converted. Except in a few cases, acetic acid and short-chained fatty acids are generally not utilized by fungi for which isobutanol is also toxic. Cultures of *Thielaviopsis basicola* and those of some species belonging to the genus *Ceratocystis* give off diverse fruity odors due to their richness in esters (Table 1). The type of aromas depends on the nature of the carbon and nitrogen sources as we will see later.

Table 1. Alcohols and esters identified in the fruity smelling cultures of some species belonging to the genus *Ceratocystis* and *Thielaviopsis*.

	Ceratocystis				*Thielaviopsis*
	coerulescens	*fimbriata* var. *platini*	*moniliformis*	*major*	*basicola*
ethyl acetate	abund.	abund.	abund.	abund.	abund.
ethanol	abund.	minor	abund.	abund.	abund.
propyl acetate		minor			minor
isobutanol	abund.	minor	abund.	abund.	trace
isobutyl acetate	minor	major	abund.	minor	minor
isoamyl alcohol	abund.	trace	minor	minor	trace
isoamyl acetate			minor		trace

Aromatic compounds

The production of aromatic compounds (Fig. 1, Tables 2,3) in non agitated liquid medium or on malt extract agar medium has been obtained from some

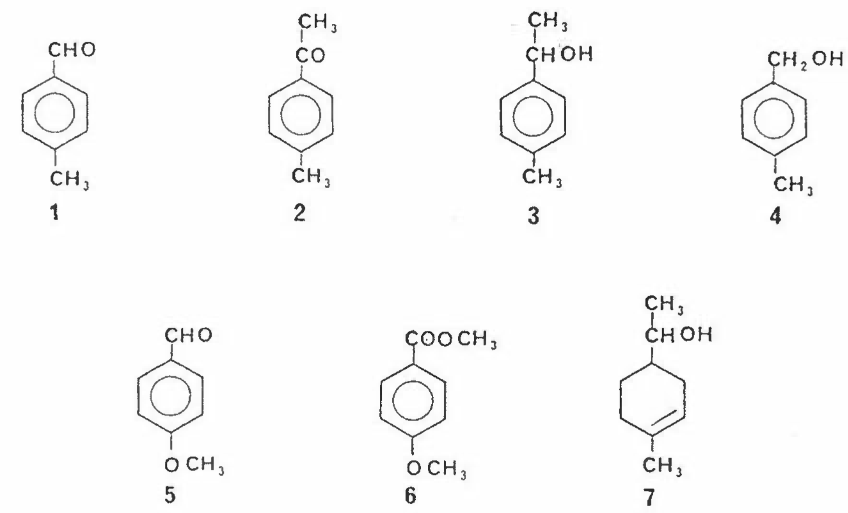


Figure 1. Aromatic compounds identified in cultures of species belonging to the genera *Phellinus, Mycoacia, Trametes* and *Lentinus*.
1 : p. tolualdehyde, 2 : p. methylacetophenone, 3 : p. dimethylbenzyl alcohol, 4 : p. methylbenzylalcohol, 5 : anisaldehyde, 6 : methylanisate, 7 : α-4 methylcyclo-3-hexen-ethyl alcohol.

Table 2. Aromatic compounds identified in cultures of some species belonging to the genus *Phellinus*.

		Phellinus		
Compounds	Odor	*igniarius*	*laevigatus*	*tremulae*
methyl benzoate	fruity	major	major	major
ethyl benzoate	fruity	X		X
methylsalicylic acid ester		X	X	X
benzyl alcohol		X	X	X
phenylethanol	rose	X	X	X

X Compound identified in the culture of the considered fungus

wood rotting species belonging to the genus *Phellinus, Trametes, Lentinus* and *Mycoacia uda*. Cultures of *M. uda* give off an almond-like aroma mainly due to p. tolualdehyde. Its production has been optimized by cultivation in a medium composed of malt extract (20 g/l), potato extract (200 ml), glucose (10 g/l), magnesium sulfate (2 g/l) and calcium carbonate (2 g/l).

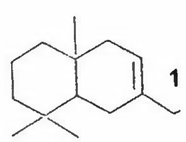
Table 3. Aromatic compounds identified in the cultures of *Mycoacia uda, Trametes odorata, T. suaveolens* and *Lentinus lepideus.*

Compounds	Odor	*Mycoacia uda*	*Trametes odora* & *T. suaveolens*	*Lentinus lepideus*
methylanisate	anise		X	X
anisaldehyde			X	
anisic acid			X	
p tolualdehyde	almond	X		
methylacetophenone	fruity	X		
p α dimethylbenzyl alcohol	herbaceous	X		
p methylbenzyl alcohol		X		
methylphenylacetate	honey	X	X	
methyl p methoxycinnamate			X	
methyl cinnamate			X	

X Compound identified in the culture of the considered fungus

Terpenes

Several wood rotting species belonging to the genus *Ceratocystis* and *Trametes* produce volatile terpenes (Table 4) when cultured in liquid media containing potato extract and glucose. For a given species, this production depends highly on the strain and the amino-acid. Thus, sesquiterpene production by *Lentinus lepideus* is optimized in the presence of asparagine (0.1%), glutamine (0.1%) and leucine (0.15%). Cultures of *Ceratocystis moniliformis* give off a citrus-like aroma (presence of geraniol and citronellol) when growing in a galactose and urea containing medium (glucose equivalent : 2.5% — nitrogen equivalent : 0.1%). A sesquiterpene hydrocarbon, thujopsene, and a possessing apple-like aroma, nerolidol, (Fig. 2) have been identified in sporulated cultures of *Penicillium decumbens.*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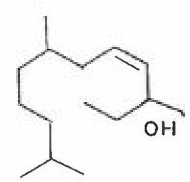

Figure 2. Thujopsene (1) and neurolidol (2), two terpenes identified in the sporulated cultures of *Penicillium decumbens.*

Monoterpene biosynthesis by *C. moniliformis* has been studied with the aid of labelled precursors such as mevalonate, L-leucine and acetate. Although the incorporation of the markers has been very low, biosynthesis by fungi was found to be similar to biosynthesis in higher plants.

Table 4. Terpenes identified in cultures of some fungal wood-rotting species.

Compounds	Odor	*Ceratocystis* *variospora*	*Ceratocystis* *moniliformis*	*Phellinus igniarius* and *P. tremulae*	*Penicillium decumbens*	*Trametes odorata*
linalol	floral, rose	X	X	X		
geraniol	rose	X	X			X
nerol	rose		X			X
citronellol	rose	X	X			X
α terpineol			X			
geranial		X	X			
neral		X	X			
geranyl acetate		X				
citronellol acetate		X				
nerolidol	rose, apple				X	
thujopsene					X	

Collins and Halim, 1971 — Lanza et al., 1966 — Lanza and Palmer, 1977 — Collins and Halim, 1972a — Halim et al., 1975 — Halim and Collins, 1971 — Sastry et al., 1980b (reported by Latrasse et al., 1985)
X Compound identified in the culture of the considered fungus

Production of γ lactones by Fusarium poae

It has been recently studied by Sarris and Latrasse (1985): *Fuscarium poae* (Peck) Wollenw. is a parasitic imperfect fungus and a weak pathogen toward carnations and cereals. Its colonies produce a very particular odor which is repulsive to some but pleasant to others. In the quoted study, *F. poae* was grown on solid and in liquid malt media and the resulting colonies have produced a noticeable fruity smell with a predominant canned peach-like aroma. The strong fruity odor is mainly due to lactones: γ penta-, γ hexa-, γ-hepta, γ octa, γ nona-, γ and δ deca-, γ undeca-, γ dodeca- and cis-6-dodecen-4-olide (Fig. 3, 4, 5). They were identified after gas chromatography and mass spectrometry by comparison of retention data and odors with those of authentic samples. Cis-6-dodecen-4-olide is the most important lactone

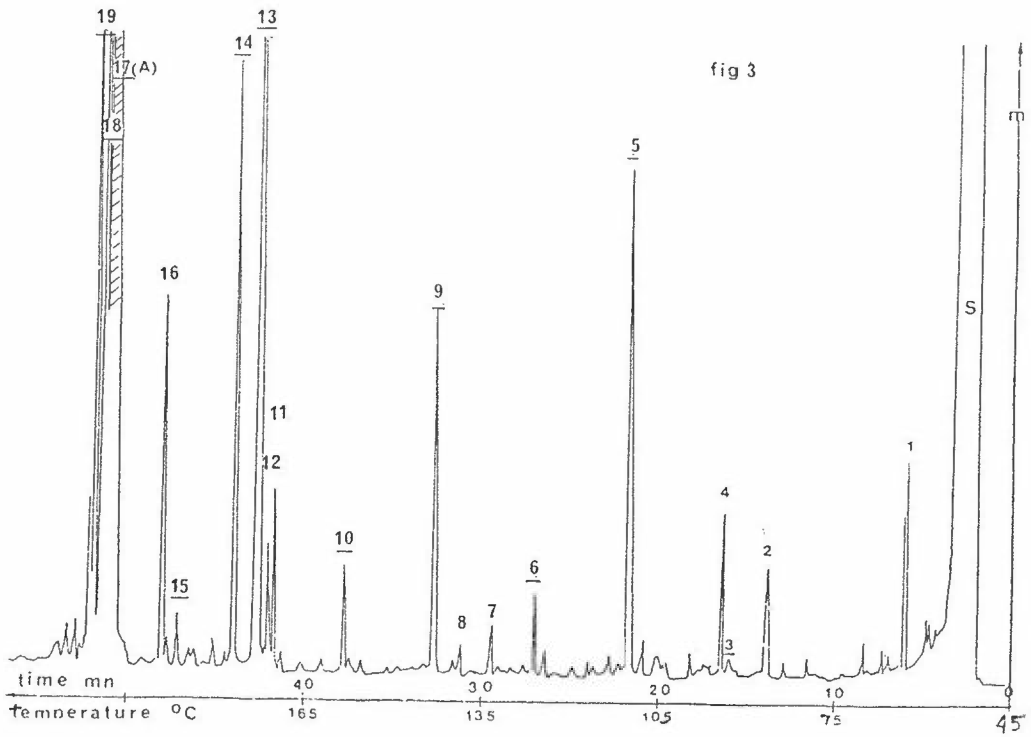

Figure 3. Chromatogram of the lactones identified in a culture of *Fusarium poae*.
3 : γ penta-, 5 : γ hexa-, 6 : γ hepta-, 9 : γ octa-, 10 : γ nona-, 13 : γ deca-, 14 : δ deca-, 15 : γ undeca-, 17 : cis-6-dodecen-4-olide, 18 : di-unsaturated γ dodeca-, 19 : γ dodecalactone.

(about 2 mg/l) and is responsible for the predominant canned peach-like aroma (Table 5).

Production of pentyl-6-α pyrone by Trichoderma viride

T. viride is a very common soil fungus. It has been intensively studied for its saprophytic potential and its parasitic and antagonistic behaviour towards other fungi. When grown on a non-agitated medium containing potato extract-glucose (10 g/l), $CaCo_3$ (0.2 g/l), $MgSO_4$, $7H_2O$ (0.2 g/l), it sporulates after 3–4 days of growth at ambient temperature, giving dark green conidia which give off a coconut-like aroma due to pentyl-6-pyrone (Fig. 6). The high yield of this metabolite may be of interest for the flavour industry.

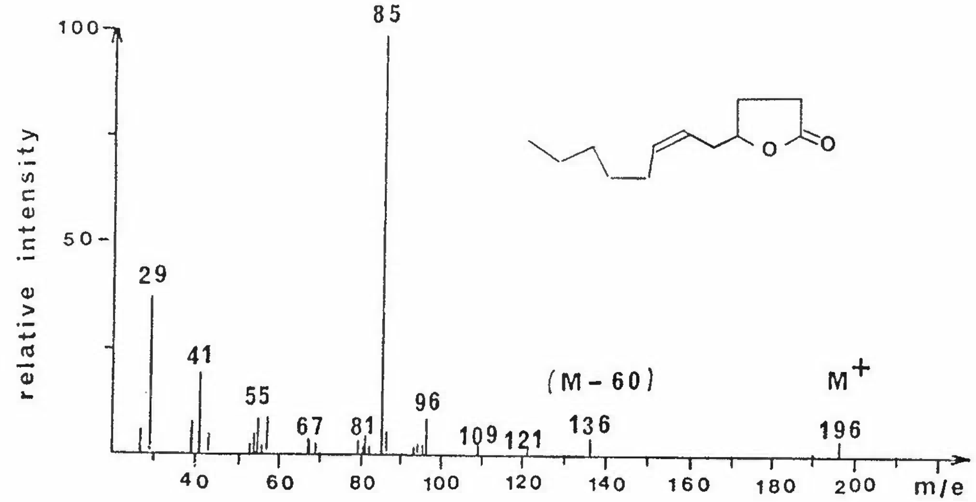

Figure 4. Mass spectra of cis-6-dodecen-4-olide.

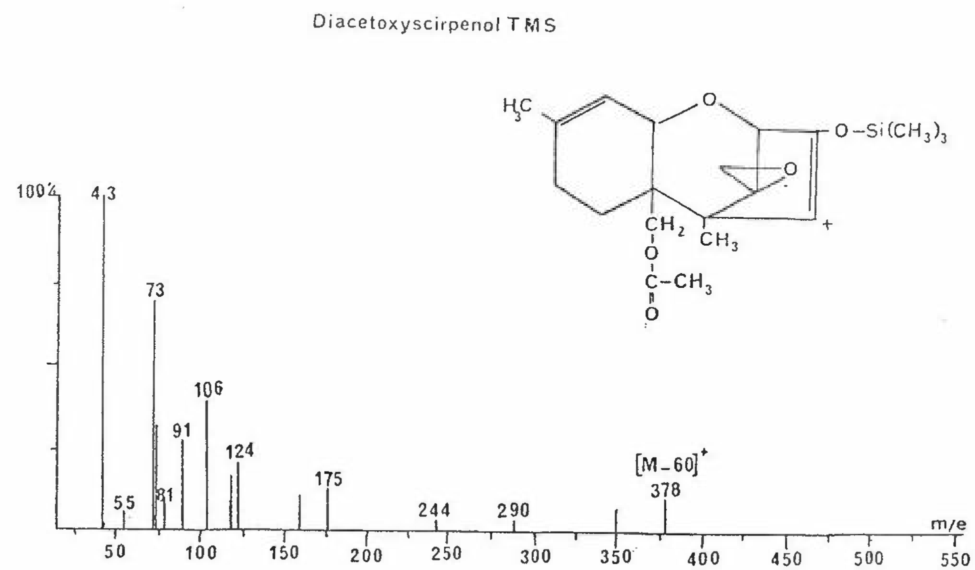

Figure 5. Mass spectra of TMS diacetoxyscirpenol, a mycotoxin found in a culture of *Fusarium poae* on peach pulp.

Table 5. Lactones identified in the culture of *F. poae*.

Compound	Fig. 3 peak	Blank broth	Cultured	Abund.	Identification MS	GC	GC odor comments
C5 alcohols	1		x	+ x	as		
furfural	2	x	x +	x	as		
γ pentalactone	3	x	tr	x	as		
benzaldehyde	4	x	x	+	x	as	
γ hexalactone	5		x	++	x	as	
γ heptalactone	6		x	+	x	g	
unknown	7		x	+			
thieno-2-3-c′pyridine	8	x	x	tr	x		
γ oxtalactone	9		x	++	x	as	coconut-like
γ nonalactone	10		x	+	x	as	coconut-like
hydrocarbon	11		x	+	x		
o-dimethylphtalate	12	x	x	+	x		
γ decalactone	13		x	+++	x	as	fruity, peachy
δ decalactone	14		x	++	x	as	creamy
γ undecalactone	15		x	+	x	g	
o-diethylphtalate	16	x	x	++	x		
cis-6-dodecen-4-olide	17		x	+++	x		canned peach-like
di-unsaturated γ dodecalactone	18		x	++	x		
γ dodecalactone	19		x	++	x	as	peachy

GC odor comments: odors sniffed at the sniffing port of the chromatograph.

as: authentic sample used for GC checking; g: graphically GC checked; abund.: abundance scale; tr: trace.

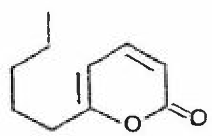

Figure 6. Pentyl-6-α pyrone: the compound responsible for the coconut-like aroma of the sporulated culture of *Trichoderma viride*.

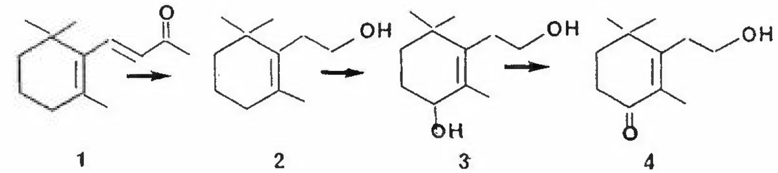

Figure 7. Conversion of β ionone by *Lasiodiplodia theobromae* into new tobacco flavourings. 1 : β ionone, 2 : β cyclohomogeraniol, 3 : hydroxy-4 β-cyclohomogeraniol, 4 : oxo-4 β cyclohomogeraniol.

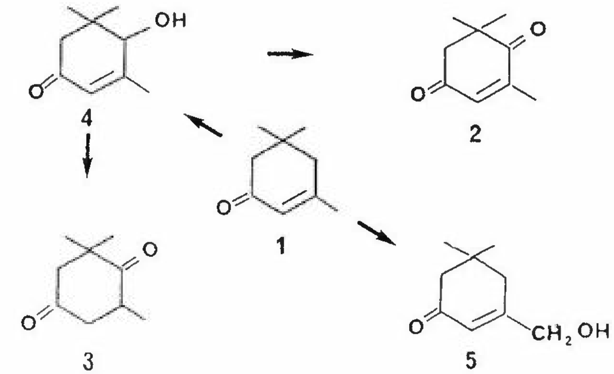

Figure 8. Conversion of isophorone by *Aspergillus niger* JTS into new tobacco flavourings. 1: isophorone, 2: 3,5,5 trimethyl 2-cyclohexen-1-4 dione, 3: 3,5,5 trimethyl cyclohexane 1-4-dione, 4: (S) 4-hydroxy 3,5,5 trimethyl 2-cyclohexen 1-one, 5: 3-hydroxymethyl 5,5 dimethyl 2-cyclohexen 1-one.

3. Conversion of Complex Molecules into New Flavourings

The method of production of new flavourings from complex molecules is different in each of the above cases. Here, a sufficient biomass is first obtained by cultivation in a sugar medium. As the sugar is consumed, the precursor to be converted is introduced by small quantities as the unique carbon source allowing the growth of the microorganism. The precursor and the corresponding metabolites are often complex molecules which cannot be currently synthesized. Conversion of β ionone by *Lasiodiplodia theobromae* ATCC 28570 leads to a tobacco smelling essential oil in which β-cyclohomogeraniol is the major metabolite (Krasnobajew and Helminger, 1982) (Fig. 7) β ionone is introduced into the culture when glucose has been entirely consumed. By a similar pathway, isophorone is converted into new tobacco flavourings by *Aspergillus niger* JTS (Fig. 8).

Table 6. Quantitative variations in the production of terpenes in the cultures of *Ceratocystis coerulescens* according to the strains used (Hanssen and Sprecher, 1981).

Strain	Monoterpenes	Sesquiterpenes
Dav. 774	t	t
Dav. 431	50	5
Dav. 700	90	390
Dav. 451	260	t
Dav. 390	550	40
Dav. 704	640	780
Dav. 765 M	1 310	2 010
Dav. 705	2 340	3 680
Dav. 50	4 410	170

t : trace : the numbers indicate chromatographic peak areas.

4. Discussion — Conclusion

Optimization of aroma production by fungi depends on physical, biochemical and physiological factors. pH, temperature and aeration are the most important physical factors which determine the conditions of growth. pH generally decreases during growth but the opposite has been observed during growth of *F. poae* the lactone-producing fungus. The quality and the intensity of the aroma may change drastically with the variation of pH. Thus, the odors given off by cultures of *C. variospora* change according to the mixture sugar/acid used. Mixtures of glucose/- , fructose/- and arabinose/acetic acid have allowed the formation of banana-, strawberry- and citrus-like odors.

The type of aromas produced varies according to the nature of the carbon and nitrogen sources used. Thus, production of lactones and terpenes by *C. moniliformis* occurs with the utilization of glycerol and galactose respectively. With the same fungus, use of leucine, methionine and glycine corresponds respectively with the formation of overripened banana-, apple- and citrus-like aromas.

The inoculum age may also influence the level production. With *M. uda* for instance, the use of 20 day old inoculum and 2 week old cultures is advised.

Cloning, as like for higher plants, is a very discriminative genetic factor as it was demonstrated with the terpene production by *C. coerulescens* (Hanssen and Sprecher, 1981) (Table 6). Production of volatiles happens during the growth or occurs when fungi have sporulated for example in the cases of *Penicillium roqueforti, P. decumbens* or *T. viride*.

Potential uses of mycelia as seasonings or flavourings have been already studied in fermentors for mushrooms as *Morella, Cantharellus, Lactarius* and *Agaricus* species. Numerous filamentous fungi however produce mycotoxins such as the trichothecenes by the *Fusarium* species. Diacetoxyscirpenol for instance was identified in a culture of *F. poae* on peach pulp. The presence of these mycotoxins forbids the "*in situ*" use of such fungi.

In conclusion, the potential uses of aroma and flavour production have to be screened by research teams including toxicologists, mycologists, microbiologists, flavor analysts and involves access to fungi collections and isolation of wild strains. These considerations confirm that the biotechnological hopes in this field enter an explorative area.

Acknowledgments

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