Higher Fungi for Generating Aroma Components through Novel Biotechnologies

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Volatiles produced by shake flask cultured mycelium of 20 basidiomycete strains were concentrated by an adsorption technique and analyzed by capillary gas–liquid chromatography (GLC), gas–liquid chromatography–olfactometry (GLC–O), and coupled capillary gas–liquid chromatography–mass spectrometry (GLC–MS). One hundred twenty-three compounds, mostly alcohols, aldehydes, ketones, esters, and phenols, were identified.

Keywords: *Basidiomycetes; volatiles; submerged cultures; adsorption; degradation; flavor*

INTRODUCTION

The potential of basidiomycetes to produce flavors is of considerable interest with regard to their biotechnological utilization, because the present supply of flavors is mainly limited to the biosynthetic capabilities of natural plants. Plant sources strongly depend on factors that are difficult to control, such as influence of weather, risk of plant diseases, sociopolitical instability of major supplying areas, and trade restrictions. The biotechnological production of flavors could represent an advantageous alternative to previous sources. Furthermore, consumers' preference for natural food additives and the stereochemical structure/activity relationship of many character impact compounds are additional driving forces for developing a biotechnology of flavors. Compared to all other microorganisms, the volatile spectrum of basidiomycetes is closest to those of plant volatiles with highly potent aliphatics (1-octen-3-ol), aromatics (anisaldehyde, benzaldehyde), and terpenoids (citronellol, linalool) as products reported (Berger et al., 1988). In this study, a total of 20 lignolytic basidiomycetes were submerged cultured, and the volatile compounds in the culture medium were analyzed.

MATERIALS AND METHODS

Microorganisms. The examined strains of basidiomycetes (Table 1) were obtained from the Centraalbureau voor Schimmelcultures (CBS), AG Baarn, The Netherlands, and the Friedrich Schiller Universität (FSU), Pilzkulturensammlung, Weimar. *Polyporus* sp. was identified by biochemical and microscopical methods.

Cultivation. Strains were inoculated (homogenized mycelium) into 350 mL of a glucose (30 g L^{-1})/ asparagine (4.5 g L^{-1})/yeast extract (3 g L^{-1}) medium and grown aerobically at 25 °C in 1 L shake flasks on a rotary shaker (INFORS, Multitron, Switzerland). The culture period was 20 days. On days 2, 3, 4, 5, 6, 8, 10, 14, 17, and 20, 30 mL of culture broth was centrifuged and stored in the refrigerator at −30 °C, and the volatile compounds of each strain were isolated and analyzed in the combined culture media. This dynamic isolation procedure allows us to obtain information on intermittently changing odor profiles and to examine the metabolism over the entire cultivation period (Abraham et al., 1993). The kinetics of formation of volatiles indicated the overall physiological status of the culture and assisted in determining adequate cultivation conditions. On each sampling date odor, glucose concentration, pH, and dry weight of the cultures were determined.

Determination of Culture Parameters. Quantitative glucose determination was done with an enzymatic diagnostic kit, based on phosphorylation and oxidation of glucose (Sigma, Germany). Dry weight was determined by removing the mycelium in the 30 mL samples by centrifugation, filtration through blauband filters (no. 589³, Schleicher and Schüll, Germany) with a filtering flask, and subsequent drying in a desiccator at 105 °C for 12 h. The pH value in the samples was determined by a pH glass electrode (GPHR 1400, Neolab, Germany). Descriptive odor assessments of the submerged cultures were obtained by a panel of six trained judges sniffing the shake flask cultured mycelia at intervals of 2 days. Gas–liquid chromatography–olfactometry (GLC–O) was done by two trained persons. Odor assessments as given in Table 2 refer to the impressions perceived under experimental conditions; some known odorous molecules eluted at concentrations below their respective recognition thresholds.

Isolation of Volatile Compounds. GLC extracts were prepared from the centrifuged and combined culture media by Lewatit 1064 adsorption; cleanup of Lewatit 1064 (Bayer, Germany) was carried out by extraction with acetone for 24 h in a Soxhlet extractor, subsequent heating for 3 h at 103 °C, and cooling to ambient temperature in a desiccator. After this, the material was conditioned for 1 h in methanol and then, after the methanol was removed, transferred to 100 mL of deionized water. The culture medium was adjusted to pH 7.8 with $NaHCO_3$ solution, and 50 μg of methyl decanoate, dissolved in methanol, was added as an internal standard. One gram of cleaned Lewatit 1064 was added to the culture medium in a 500 mL gastight shaking flask of a rotary shaker, and the suspension was shaken for 1 h at 250 rpm. Lewatit was separated by sedimentation and decanting from the supernatant culture medium. Sedimented Lewatit was washed with 50 mL of water, and the adsorbed components were eluted three times with 50 mL of a mixture of pentane/dichloromethane (2:1). Combined eluates were dried over anhydrous sodium sulfate and concentrated to a volume of 1 mL using a Vigreux column.

Gas Chromatographic Conditions. The concentrates were analyzed by means of capillary gas–liquid chromatography using a Carlo Erba Fractovap Series 2150 gas chromatograph coupled to a Shimadzu CR 5A integrator. Chromatographic conditions were as follows: CW 20M capillary column (25 m × 0.32 mm i.d., Leupold, Germany), injection volume, 1 μL; splitless, injection port, 225 °C; flame ionization detector, 225 °C, temperature program, 40 °C for 3 min and then to 210 °C at a rate of 3 °C min^{-1}; carrier gas, hydrogen, flow rate, 4.6 mL min^{-1}. Identification of the odorous compounds was carried out by GLC–olfactometry (GLC–O) and GLC–MS. The retention indices, odors and mass spectra of the sample components were compared to those of reference samples. GLC–O was carried out using a Carlo Erba Fractovap Series 2150 gas chromatograph and a CW 20M megabore capillary column (25 m × 0.53 mm i.d., Leupold), with an outlet splitter (Valco effluent splitter, split ratio 1:1) to a heated (230

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Table 1. Origin of Basidiomycete Strains Used in This Study

no.	basidiomycete strains		origin
1	*Armillaria mellea*	(Vahl:Fr.) Kummer	CBS 100.12
2	*Grifola frondosa*	(Dickson:Fr.) S.F. Gray	CBS 480.63
3	*Ganoderma applanatum*	(Pers.) Pat.	CBS 250.61
4	*Hericium erinaceus*	(Bull.:Fr.) Pers.	CBS 260.74
5	*Kuehneromyces mutabilis*	(Schaeffer:Fr.) Singer & A.H. Smith	FSU K 01-6
6	*Lentinula edodes*	(Berk.) Pegler	FSU A 20-5
7	*Lentinula edodes*	(Berk.) Pegler	FSU A 20-8
8	*Meripilus giganteus*	(Pers.:Fr.) Karsten	CBS 581.86
9	*Pholiota squarrosa*	(Weigel:Fr.) Kummer	CBS 570.87
10	*Pholiota populnea*	(Weigel:Fr.) Kummer	CBS 264.32
11	*Pholiota populnea*	(Pers.:Fr.) Kuyper & Tjall	CBS 382.82
12	*Pleurotus sapidus*	FSU	FSU P226-1
13	*Nigroporus durus*	(Jungh.) Murrill	CBS 313.36
14	*Polyporus* sp.	Abraham	self-isolated
15	*Polyporus tumulosus*	Cooke & Massee ex Cooke	CBS 332.49
16	*Polyporus umbellatus*	(Pers. Fr.) Fr.	CBS 483.72
17	*Sarcodontia setosa*	(Pers.) Donk	CBS 261.59
18	*Trametes hirsuta*	(Wulfen:Fr.) Pilat	CBS 282.73
19	*Tyromyces floriformis*	(Quelet) Bond. & Singer	CBS 232.53
20	*Tyromyces sambuceus*	(Lloyd) Imazeki	CBS 492.76

°C) sniffing port. Injection volume was 1 μL in the splitless mode (for 1 min). The same chromatographic conditions were used. Mass spectrometry was performed on a Hewlett-Packard 5989A mass spectrometer (quadrupole), coupled to a HP 5890 Series II gas chromatograph. The system was equipped with a 25 m × 0.32 mm CW 20M capillary column (Leupold). Chromatographic conditions were the same as above, with helium as carrier gas. The ionization energy was 70 eV.

RESULTS AND DISCUSSION

Table 2 lists the neutral volatile compounds isolated from the combined culture media. The concentration levels and the odor assessments of isolated compounds are given.

Alcohols. A total of 40 different alcohols was generated, most of them in a range up to 100 μg L^{-1}. The most abundant ones were higher alcohols; most of the strains produced 1-butanol, 2-methyl-1-propanol, and 3-methyl-1-propanol. *Sarcodontia setosa*, producing up to 24 mg L^{-1} 3-methyl-1-butanol, was the most effective alcohol-generating strain. This odor impression is reported in many fungi guide books as a taxonomic criterion for *S. setosa*. 1-Octen-3-ol and 3-octanol are typical eight-carbon cleavage products of lipoxygenase-catalyzed degradation of fatty acids and occur regularly in the late phase of cultivation, indicating an increasing contact of enzyme and substrate as a result of increased cell lysis. The corresponding aliphatic aldehydes could be found in rare cases only and in small amounts. In their natural habitats the survival of wood-destroying fungi depends on pronounced abilities to enzymatically attack genuine lignins. In synthetic nutrient media their ecologic situation is frequently reflected by a preference of aromatic compounds in anabolic and catabolic routes. Thus, it is not surprising that a lot of compounds with an aromatic structure were found, among them some alcohols. 2-Phenylethanol is generated by *Grifola frondosa* up to 800 μg L^{-1}, and 4-methoxybenzenemethanol by *Pleurotus sapidus* up to 750 μg L^{-1}. The corresponding aromatic aldehydes could be found occasionally: good examples were phenylmethanol and benzaldehyde (*S. setosa, Hericium erinaceus*), 2-phenylethanol and phenylacetaldehyde (*Pholiota squarrosa*), and 4-methoxybenzenemethanol and 4-methoxybenzaldehyde (*P. sapidus*).

Ketones. Twelve ketones were generated by the strains examined; most of them were aliphatic ones.

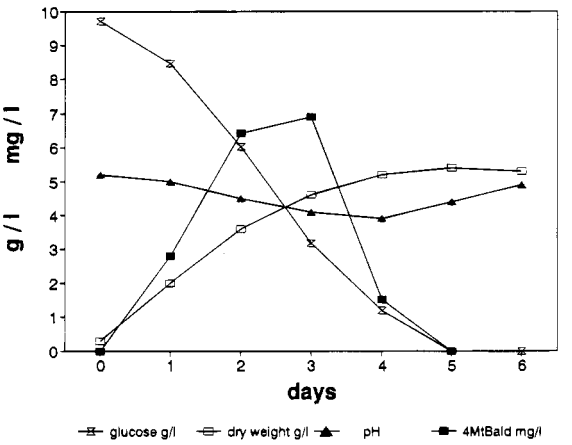

Figure 1. Degradation of 4-methoxybenzaldehyde by *P. sapidus*.

3-Hydroxy-2-butanone, 3-octanone, 4-methyl-3-penten-2-one, and 1-phenylethanone were preferentially generated but in small absolute amounts (670 μg L^{-1} 3-hydroxy-2-butanone by *H. erinaceus*, 560 μg L^{-1} 3-octanone by *Nigroporus durus*, 350 μg L^{-1} 4-methyl-3-penten-2-one by *Ganoderma applanatum*, 740 μg L^{-1} 1-phenylethanone by *Trametes hirsuta*).

Aldehydes. Four aliphatic and seven aromatic aldehydes were found in the cultures; the dominating one was benzaldehyde. A reason for the small number and amounts of aldehydes could be the rapid oxidation by different oxidizing fungal enzyme systems, as they have particulary been reported for white rot fungi (Odier and Artraud, 1992). In the combined culture medium of *P. sapidus* 13 mg L^{-1} 4-methoxybenzaldehyde was quantified, but this must be looked at as an equilibrium concentration. A more detailed study of *P. sapidus* showed that this basidiomycete is able to degrade its own product, 4-methoxybenzaldehyde, at a rate of 5.5 mg L^{-1} day^{-1} (Figure 1). This is an excellent example for nearly simultaneous generation and degradation of a volatile compound by a basidiomycete within a few days, for the resulting significant changes of the aroma profile during the cultivation, and for the difficulty to estimate the highest obtainable amount of a given volatile with common methods (Hanssen and Sprecher, 1981). *S. setosa* generated 1.1 mg L^{-1} 2-methylpentanal which, with its fruity odor, contributed to the total odor impression. 2,4-Dihydroxy-3,6-dimethylbenzaldehyde (*Polyporus* sp., *T. hirsuta, Tyromyces floriformis*) and

Table 2. Concentration Levels and Odor Assessments of 123 Neutral Volatile Compounds Isolated from Culture Media of Submerged Cultured Mycelia of 20 Basidiomycete Strains

compound	odor assessment	strain no.			
		0–10 μg/L concn level	10–100 μg/L concn level	100–1000 μg/L concn level	>1 mg/L concn level
alcohols					
1-butanol	amyl alcohol like	1, 6, 7, 8, 9, 10, 11, 13, 19, 20	2, 4, 15, 16, 17		
1-pentanol	fusel oil		4, 13, 14		
1-hexanol	fruity	6, 7, 18, 20	4	13, 17	
1-heptanol	fatty, faint	19			
1-octanol	orange-like	15		13	
2-propanol	alcohol		14		
2-methyl-1-propanol	pungent	4, 8, 11	2, 3, 6, 7, 10, 18, 20	1, 5, 12, 13, 16, 17	15
2-butanol		13			
2-buten-1-ol		1, 4			
2,3-butanediol	fatty	10, 11, 13	4	14	
2-methyl-1-butanol	pungent		14		
3-methyl-1-butanol	characteristic pungent	6, 10	2, 3, 7, 11, 12, 18	1, 5, 13, 16, 20	4, 15, 17
3-methyl-2-buten-1-ol	pungent	6, 7, 14, 18	1, 4		
3-methyl-3-buten-1-ol	pungent	1, 3, 5, 6, 7, 13	4, 14, 18		
2-pentanol		13, 15, 18	12		
2-methyl-3-pentanol	pungent		15		
2-methyl-2-pentanol	pungent	18			
3-methyl-1-pentanol	wine-like, pungent	6, 7	1, 4, 14, 17		
4-methyl-pentanol	pungent		4		
3-hexanol		1, 4			
2(*E*)-hexen-1-ol	fruity, wine-like	1, 4			
3-methyl-1-hexanol	pungent	1	4		
2-methyl-3-hexanol	pungent		4, 17		
2-heptanol	pungent, herbaceous	13	17		
4-octanol	fatty	1			
3-octanol	nutty, sweet	4, 12, 16, 18	14, 17	15	13
1-octen-3-ol	mushroom, sweet	5, 12, 13, 16	14, 15		17
4,5-octanediol		1			
2(*E*)-nonen-1-ol		13			
2-butoxyethanol		2, 4, 7, 8, 10	5		
2,6-dimethyl-7-octen-1-ol	mossy	4, 6, 7, 17, 19	2		
3,7-dimethyl-1,7-octanediol		14			
2-furanmethanol	cooked sugar	4, 15	13, 17		
2-(2-butoxyethoxy)ethanol	musty	1, 3, 6, 7	4		
phenylmethanol	faint, aromatic	1, 5, 6, 8, 9, 15, 16, 18	2, 4, 14, 19, 20	17	
2-phenoxyethanol	dandelion, burnt	1, 2, 4, 19, 20			
4-hydroxybenzenemethanol	sweet	1			
2-phenylethanol	rose-like	1, 9, 12	2, 5, 6, 7, 14, 18, 20	4, 13	
3-phenylpropanol	floral	5, 14, 20	5, 13		
4-methoxybenzenemethanol	floral	5, 19	12	18	
ketones					
3-hydroxy-2-butanone	butter, fatty	5, 13	1, 15, 16, 17	4, 14	
3-penten-2-one	fruity	19	1, 13		
2-pentanone	wine-like	13			
3-hydroxy-3-methylbutanone			4		
4-methyl-3-penten-2-one	green	7, 10, 19	1, 2, 3, 5, 15, 16, 20		
3-hexanone		4			
2-heptanone	banana		2		
3-octanone	fruity, lavender		14, 15, 16	13, 17	
1-octen-3-one	mushroom	2	1, 4, 6, 9, 12, 14		
3-octen-2-one		1			
1-phenylethanone	meal	1, 2, 3, 4, 9, 13, 17, 18			
4-hydroxy-4-methyl-2-pentanone		1, 8, 10, 11, 18, 19	2, 13		
aldehydes					
hexanal	fatty, green	18			
heptanal	oily, fatty		4		
2-methylpentanal	fruity				17
2-methyl-2-butenal	green	4			
benzaldehyde	bitter almond	1	3, 5, 12, 13, 14, 18, 19	4, 17	
phenylacetaldehyde	harsh, hawthorn	9, 17			
2-furancarboxaldehyde	sweet	3, 4, 19, 20	17		
3-phenyl-2-propenal	pungent, spicy		4		
4-methoxybenzaldehyde	anise	5	8		12
2,4-dihydroxy-3,6-dimethylbenzaldehyde	sweet, phenolic	14, 18, 19			
2-hydroxy-4-methoxy-6-methylbenzaldehyde	sweet	17			
esters					
methyl butanoate	ethereal	13, 17, 18	1, 2	4	
ethyl hexanoate	fruity, apple		13		
methyl 3-hydroxybutanoate	fruity	14, 15			
methyl 3-methylbutanoate	fruity		17		

Table 2 (Continued)

compound	odor assessment	strain no. 0–10 μg/L concn level	10–100 μg/L concn level	100–1000 μg/L concn level	>1 mg/L concn level
methyl benzoate	fruity	2, 4, 5, 6, 8, 9, 14, 15, 17, 18, 19			
methyl 4-methoxybenzoate	floral, hyacinth		12		
methyl 2-methoxybenzoate	sweet, floral		12		
methyl 4-hydroxybenzoate		18			
methyl phenylacetate	honey	13, 15	17		
methyl 4-methoxyphenylacetate	sweet	18			
methyl 2,4-dihydroxy-3,6-dimethylbenzoate	oakmoss	1, 2, 18	17		14
methyl 2,4-dihydroxy-6-methylbenzoate	sweet, mossy	18			14
methyl 2-hydroxy-4-methoxy-6-methylbenzoate	earthy, mossy			14	
methyl 2,4-dimethoxybenzoate	sweet, phenolic			14	
methyl 3,6-dichloro-2-methylbenzoate	sweet	5			
methyl 2-furancarboxylate	mushroom	13, 15	2		
methyl 3-furancarboxylate	mushroom	13, 16, 18, 19	15		
methyl 3-pyridinecarboxylate		17	2		
ethyl 2-pyridinecarboxylate		9			
lactones					
4-butanolide	faint, butter	12, 17, 20		13	
4-pentanolide		18, 20	17	13	
2-hydroxy-3,3-dimethyl-4-butanolide		13, 15, 20			
3-methyl-4-pentanolide				13	
4-hexanolide		12, 16, 20			13
4-heptanolide	coconut, sweet	1, 16, 20		13	
2-hepten-4-olide		13, 20			
4-octanolide	coconut, fatty		16, 20	15	13
2-octen-4-olide	fruity		20		13
4-nonanolide	fatty, coconut	20		13	
4-decanolide	peach	13		20	
4-methyl-5-hexanolide	fruity	11	10	16	
5-hexanolide				13	
phenols					
phenol	phenol	6, 14, 20			
4-methylphenol	smoky, phenolic		14		
3-methoxy-5-methylphenol	woody, tary			14	
3-methoxy-2,5-dimethylphenol	oakmoss				14
3-hydroxy-5-methylphenol	sweet, smoky			14	
4,5-dimethoxy-2-methylphenol	sweet, phenolic		14		
2-methoxyphenol	sweet, phenolic	12			
4-methoxyphenol	sweet	12			
3,5-dimethoxyphenol	smoky	5, 16			
2,3-dimethylphenol	smoky			17	
5-methoxy-2,3-dimethylphenol	sweet	12, 18			14
2-methoxy-4-(2-propenyl)phenol	clove-like	8, 16	4		
miscellaneous					
(*Z*)-furan linalooloxide	burnt	3, 5, 10, 11			
(*E*)-furan linalooloxide	burnt	3, 5, 10, 11			
linalool	floral	3			
(*Z*)-pyran linalooloxide	herbaceous	5			
(*E*)-pyran linalooloxide	herbaceous	5			
1-methylisochinoline		4, 17, 18			
acetic acid	acetic acid	10, 12, 14			
3-methylbutanoic acid	fatty, butter	10, 11	12		
α-copaene				10	
4-methyl-5-thiazole	sweet	1, 2, 3, 5, 10, 11, 17			
d-cadinene		10, 11			
farnesol	flowery	14	13		
7β-hydroxydihydrodrimenine				14	
nerolidol	rose, floral	14			
2-hydroxy-4-methoxy-6-methylbenzoic acid					14

2-hydroxy-4-methoxy-6-methylbenzaldehyde (*S. setosa*) are typical volatile compounds of oakmoss extracts. They were generated in amounts up to 10 μg L^{-1}.

Esters. Nineteen esters could be identified, 15 of them with an aromatic skeleton; 4 were aliphatic esters. There was a great difference of ratios of the number and the concentration of aliphatic alcohols (34) and aliphatic esters (4), compared to aromatic alcohols (7) and aromatic esters (15). It appears that aromatic alcohols were preferentially transformed into esters, and aliphatic alcohols remained unchanged. The reason could be again the activity of oxidizing enzyme systems, which oxidize aromatic alcohols to aromatic carboxylic acids, which are then esterified. Methyl benzoate was generated by 11 strains in concentrations up to 10 μg L^{-1}. The most interesting strains with regard to ester generation were *Polyporus* sp. and *S. setosa*. The latter generated methyl 3-methyl-pentanoate (95 μg L^{-1}), methyl phenylacetate (85 μg L^{-1}), and methyl 2,4-dihydroxy-3,6-dimethylbenzoate (65 μg L^{-1}). *Polyporus* sp. produced typical oakmoss ester compounds, such as methyl 2,4-dihydroxy-3,6-dimethylbenzoate (1.4 mg L^{-1}), methyl 2,4-dihydroxy-6-methylbenzoate (1.1 mg L^{-1}), methyl 2-hydroxy-4-methoxy-6-methylbenzoate (550 μg L^{-1}), and methyl 2,4-dimethoxybenzoate (240 μg L^{-1}).

Lactones. Fourteen lactones were identified; nearly all of them occurred in the cultures of *N. durus*, *Polyporus umbellatus*, and *Tyromyces sambuceus*. According to former examinations, especially *N. durus* was able to generate various lactones up to concentrations of 3.5 mg L^{-1} (4-octanolide) (Gatfield, 1988). A pure enzymatic cyclization seems to be unlikely, because all

of the lactone-generating basidiomycetes decreased the pH of the nutrient medium to a value between 2.0 and 3.0, which would be sufficient to chemically catalyze the cyclization of 4/5-hydroxycarboxylic acids (Gatfield, 1993).

Phenols. *Polyporus* sp. generated phenols in larger amounts, most of them being typical oakmoss compounds, such as 3-methoxy-2,5-dimethylphenol (1.7 mg L^{-1}), 5-methoxy-2,3-dimethylphenol (1.1 mg L^{-1}), 3-methoxy-5-methylphenol (630 μg L^{-1}), 3-hydroxy-5-methylphenol (350 μg L^{-1}), 4-methylphenol (70 μg L^{-1}), and 4,5-dimethoxy-2-methylphenol (80 μg L^{-1}). With the exception of *S. setosa*, other strains did not accumulate significant amounts of phenolics.

Miscellaneous. Monoterpenes and sesquiterpenes were generally identified in low concentrations. *Kuehneromyces mutabilis* generated the four furanoid and pyranoid linalool oxides [(*Z*)-furan linalool oxide, (*E*)-furane linalool oxide, (*Z*)-pyrane linalool oxide, and (*E*)-pyrane linalool oxide] in concentrations up to 10 μg L^{-1}. Linalool was found in a concentration of 8 μg L^{-1} in cultures of *Lentinula edodes*. Nerolidol was identified in cultures of *Polyporus* sp. (260 μg L^{-1}); α-copaene (*Pholiota populnea*, 250 μg L^{-1}), 7β-hydroxydihydrodrimenine (*Polyporus* sp., 410 μg L^{-1}), farnesol (*N. durus*, 50 μg L^{-1}, *Polyporus* sp., 8 μg L^{-1}), and δ-cadinene (*Pholiota populnea* no. 202, no. 11, 10 μg L^{-1}) occurred also in low concentrations.

Conclusion. Microorganisms, at present, play a minor role as an industrially used source of volatile flavors, although many microbial systems were found to deliver valuable flavor constituents. Possible reasons may be competition by other natural sources, complicated downstreaming of the volatiles, and legal issues. A number of flavor compounds, such as 4(*R*)-decanolide, 5(*R*)-decanolide, certain fatty acids, methyl ketones, and carboxylic acid esters, are being produced by microorganisms on an industrial scale (Janssens et al., 1992). The data of this study show that there is a considerable overlap of the spectra of volatile metabolites of basidiomycetes and higher plants. Aliphatic alcohols as well as aliphatic and aromatic carbonyls, as described above, are common constituents of fruit/vegetable flavors and certain essential oils. While phenolics constitute a major group of fungal volatiles, the volatile products of the mevalonate pathway occur less frequently. In view of the more than 30 000 basidiomycete species known worldwide, the potential for the formation of volatile flavors can hardly be overestimated. However, submerged cultured fungi do not differentiate specialized storage compartments for the accumulation of volatile products. This may explain the usually low stationary concentrations found in the nutrient media. A number of strains, for example *S. setosa*, *Polyporus* sp., *P. sapidus*, and *N. durus*, accumulated certain volatiles in concentrations >1 mg L^{-1}. In most cases, however, the concentrations did not reach levels that would justify an immediate industrial application. The choice of more appropriate culture conditions, the supplementation of metabolic precursors, the development of more suitable bioreactor designs, and, probably most important, the continuous in situ removal of volatiles from the producer cell open ways to the study of the biochemistry of volatile natural metabolites in suitable model systems and to high-yielding bioprocesses.

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