JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Fermentation of tobacco with basidiomycetes

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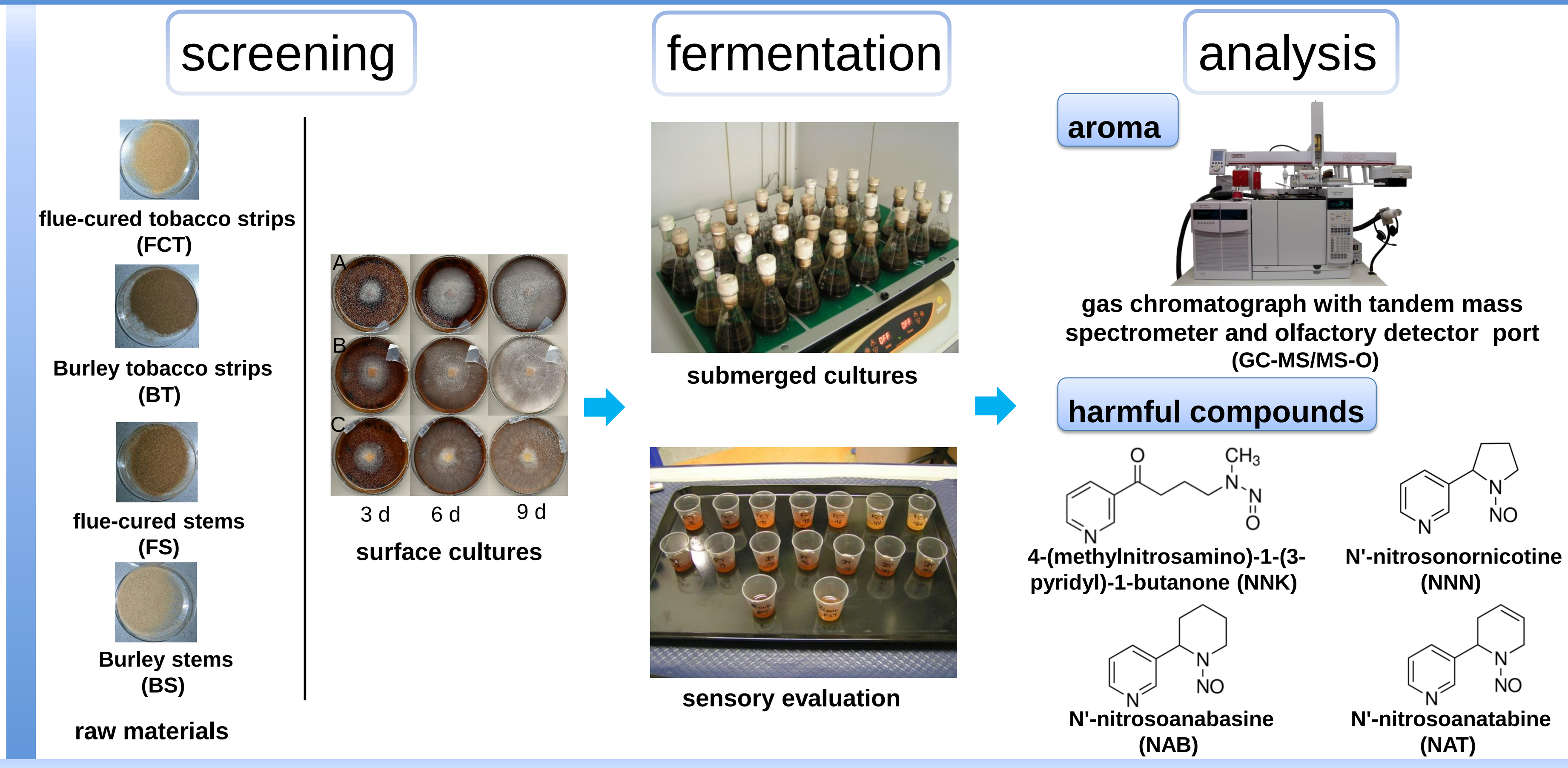
Introduction

Representing the highest developed class of fungi, basidiomycetes possess as stated in the literature a unique capability of degrading some harmful and potentially harmful constituents as well as of producing a wide range of natural flavors. It is thus an alluring idea to reduce the levels of some harmful compounds and to produce pleasant aroma attributes in a single fermentation step.

Therefore, the growth of thirty basidiomycetes on four tobacco samples (flue-cured tobacco strips, flue-cured stems, Burley tobacco strips, and Burley stems) as sole carbon source was evaluated. Generation of natural aroma and remediation of Tobacco Specific Nitrosamines (TSNA) and ammonia by fermentation of tobacco with some basidiomycetes were performed subsequently.

Workflow



Results

1. *Screening*

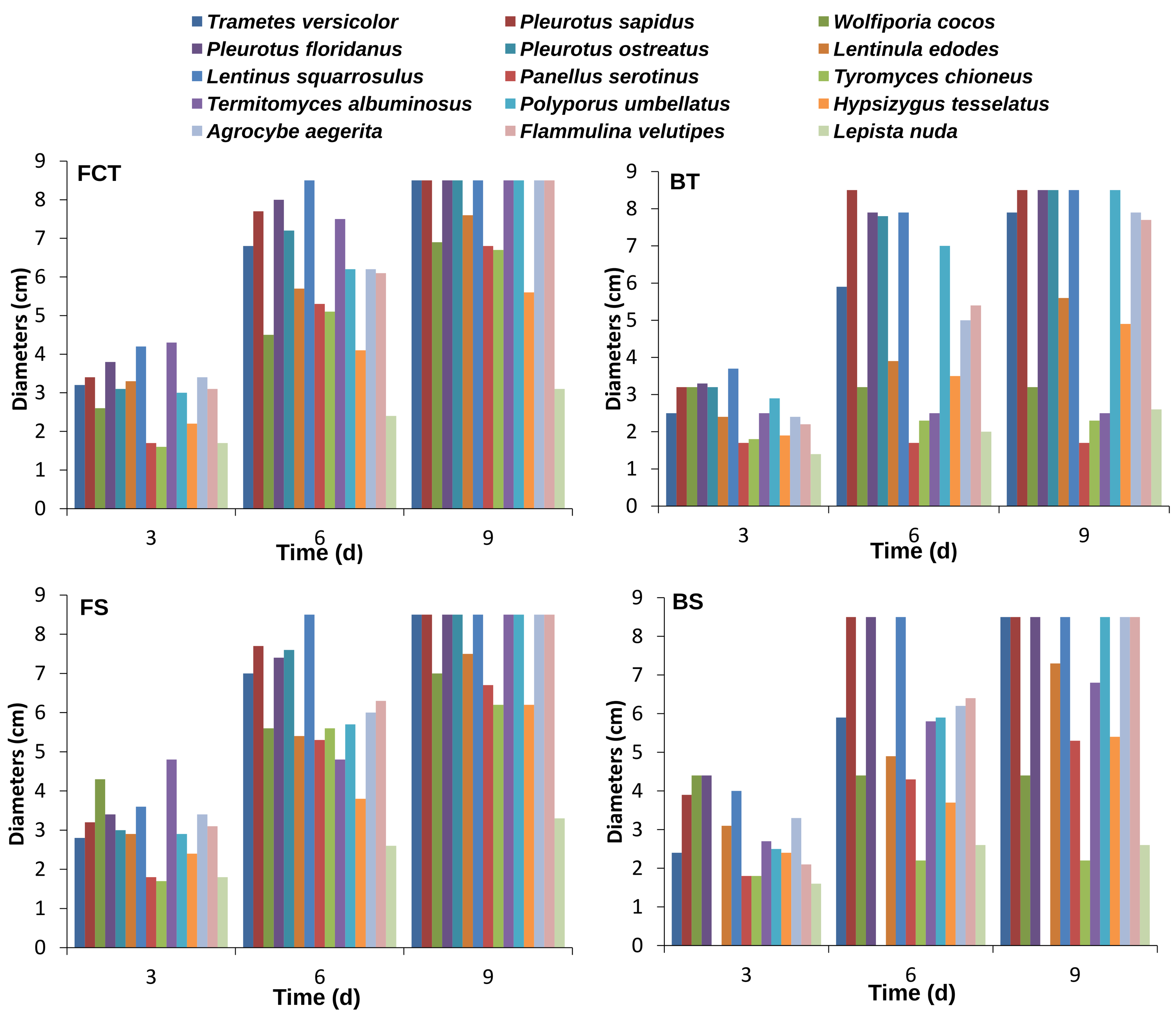


Fig. 1 **Growth of basidiomycetes on different types of substrates** (10 g/L)

Most of the strains grew well, and FCT and FS proved to be the most promising substrates (*Fig. 1*). A series of volatiles, like *p*-anisaldehyde, benzaldehyde, *β*-damascenone, linalool, and 1-octen-3-ol were emitted from the surface cultures.

2. *Submerged fermentation*

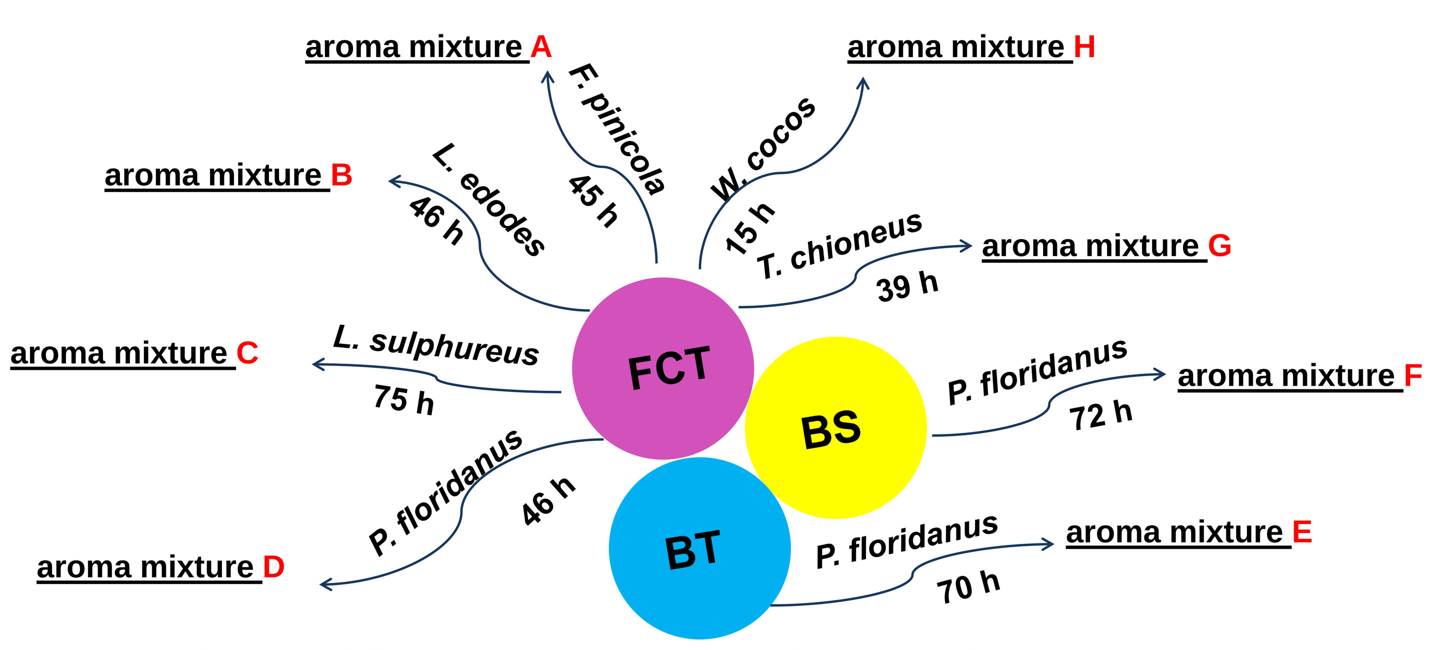
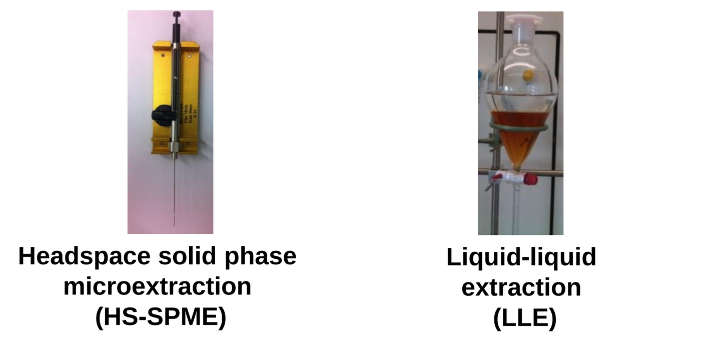


Fig. 2 **Basidiomycete/substrate combinations (for A – H *cf. Fig. 3*)**
(*F. pinicola* = *Fomitopsis pinicola*; *L. sulphureus* = *Laetiporus sulphureus*)

3. *Analysis*

Aroma analysis



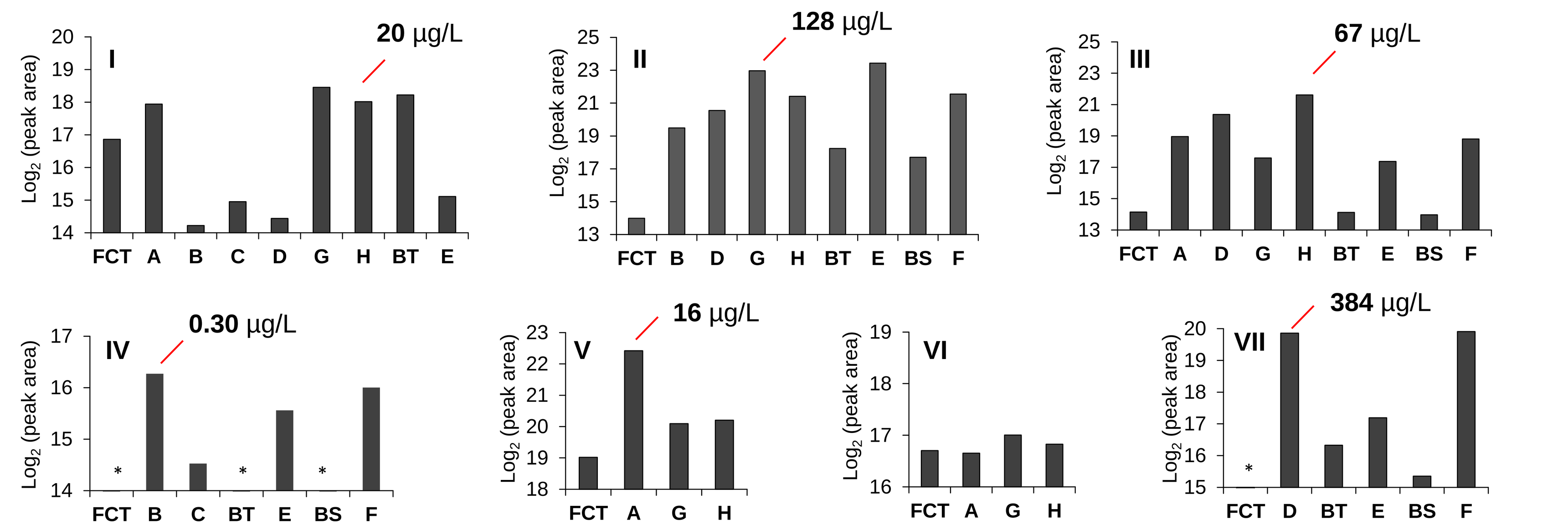


Fig. 3 **Changes of the concentrations of some odor-active compounds in tobacco fermented with basidiomycetes and in non-fermented tobacco**
(**I** 6-methyl-5-hepten-2-one; **II** benzaldehyde; **III** linalool; **IV** *p*-tolualdehyde; **V** *β*-damascenone; **VI** *β*-ionone; **VII** *p*-anisaldehyde;
* compounds detected only in the fermented tobacco)

A multitude of odor-active compounds including **β-acetylpyridine, p-anisaldehyde, benzaldehyde, β-damascenone, 2-decanone, geranylacetone, β-ionone, linalool, (Z)-linalool oxide, methyl benzoate, 6-methyl-2-heptanone, 6-methyl-5-hepten-2-ol, 6-methyl-5-hepten-2-one, methyl 4-methoxybenzoate, methyl 3-methylpentanoate, methyl palmitate, methyl 2-phenylacetate, nerolidol, 2-nonanone, (Z)-3-nonen-1-ol, octanal, 1-octanol, 3-octanone, 1-octen-3-ol, 2-pentanone, 3-pentanone, piperonal, 2-phenylacetaldehyde, and p-tolualdehyde** were identified by GC-MS/MS-O combined with HS-SPME in fermented tobacco samples. Some of the odorants generated by the fungi were semi-quantified by internal standard after LLE (*Fig. 3*).

Other tobacco constituents

Sample No.	Tobacco Specific Nitrosamines (TSNA) (ng/g)						Ammonia $N\text{-}NH_3$ (%)	Total alkaloids (%)
	NAB	NAT	NNK	NNN	Reduction (%) NNK	Reduction (%) Total		
FCT	< LOQ	< LOQ	19.55 ± 0.59	< LOQ			0.08 ± 0.007	1.37 ± 0.007
A	4.53 ± 0.17	< LOQ	0.93 ± 0.18	3.01 ± 0.08	95.2	56.7	< LOQ	0.98 ± 0.007
B	7.13 ± 0.71	< LOQ	< LOQ	< LOQ	100	63.5	0.03 ± 0.000	0.73 ± 0.007
C	1.02 ± 0.16	< LOQ	2.68 ± 0.28	6.39 ± 0.58	86.3	48.4	0.020 ± 0.000	0.67 ± 0.028
D	11.96 ± 0.021	< LOQ	7.49 ± 0.18	9.45 ± 0.18	61.7	0.51	< LOQ	0.55 ± 0.007
G	0.16 ± 0.007	2.78 ± 0.40	2.54 ± 0.31	10.72 ± 0.31	87.0	17.1	0.030 ± 0.000	1.05 ± 0.007
H	2.69 ± 0.22	< LOQ	2.29 ± 0.007	< LOQ	88.3	74.5	0.08 ± 0.000	0.73 ± 0.007
BT	332.6 ± 3.91	4707.13 ± 121.86	2756.15 ± 36.84	7756.94 ± 35.54			0.330 ± 0.000	2.26 ± 0.021
E	303.13 ± 2.09	3798.10 ± 98.44	2141.04 ± 30.58	5717.81 ± 114.05	22.3	23.1	0.110 ± 0.000	2.09 ± 0.021
BS	139.78 ± 7.69	2735.35 ± 34.34	2889.25 ± 76.69	4928.00 ± 45.36			0.130 ± 0.000	0.87 ± 0.007
F	109.85 ± 1.58	1432.56 ± 27.70	1994.14 ± 7.84	3688.29 ± 8.42	31.0	32.4	< LOQ	0.61 ± 0.021

Conclusions and outlook

Surprisingly, most of the screened basidiomycetes were able to grow well on tobacco substrates as the sole carbon and nitrogen source. Interesting aroma impressions were perceived after fermentation. Subsequently, the responsible compounds were identified by HS-SPME combined with GC-MS/MS-O and some of them were semi-quantified after LLE. Meanwhile, NNK, ammonia, and total alkaloids were degraded during fermentation. Taken together, fermentation of tobacco by basidiomycetes may present an interesting option for the future improvement of tobacco derived products.