BRIEF REPORT

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Screening for Strains of the Genus *Mortierella*, Showing Elevated Production of Highly Unsaturated Fatty Acids

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ABSTRACT. The content of oligounsaturated fatty acids was investigated in 13 strains of filamentous fungi of the genus *Mortierella*. *M. isabellina* producing up to 500 mg/L of γ-linolenic acid and *M. verticillata* containing up to 300 mg/L of arachidonic acid were found to be perspective producers of these acids. In *M. parvispora* dihomo-γ-linolenic acid (100 mg/L) could be detected.

Oligounsaturated (formerly incorrectly "polyunsaturated") fatty acids (PUFA) were detected as components of cell lipids of plants, animals and microorganisms. These microbial acids have recently attracted pronounced attention. Already in 1981 Suzuki *et al.* (1981) performed an extensive screening of PUFA, especially of γ-linolenic (γ-18:3; 6,9,12-18:3 or 18:3 ω−6) acid. Of several tens of fungal strains the genus *Mortierella* was selected and studied in more detail (Shimizu *et al.* 1988, 1989*c*; Yamada *et al.* 1987; Šajbidor *et al.* 1988).

Strains containing increased amounts of the γ-18:3 acid (Hansson and Dostálek 1988; Šajbidor *et al.* 1988) and also of other PUFA, particularly of arachidonic (20:4 ω−6 or 5,8,11,14–20:4; Totani and Oba 1987; Yamada *et al.* 1987) and of eicosapentaenoic ("EPA"; 20:5 ω−3 or 5,8,11,14,17-20:5; Shimizu *et al.* 1988, 1989*c*) acids were detected. All these PUFA, including dihomo-γ-linolenic (20:3ω−6 or 8,11,14,20:3; Yamada *et al.* 1988; Shimizu *et al.* 1988, 1989*b*) acid, are not only important precursors in the biosynthesis of prostaglandins and related compounds but also decrease the risk of arteriosclerosis and have favorable properties in human nutrition (vitamin F).

Therefore, we decided to perform a rapid screening of potentially suitable strains

of the genus *Mortierella* overproducing PUFA.

Thirteen strains of filamentous fungi of the genus *Mortierella* obtained from the *Culture Collection of Fungi, Department of Botany, Charles University*, Prague: *Mortierella isabellina* No. 1109, No. 224, No. 1108, No. 1098, *M. ramanniana* No. 209, No. 1022, No. 472, No. 202, No. 210, *M. verticillata* No. 206, *M. nana* No. 1103, *M. parvispora* No. 199 and *M. vinacea* No. 1097.

Each strain was cultivated both in synthetic medium A (according to Kamisaka *et al.* 1988), containing (in g/L of distilled water): glucose 30, $CH_3COONa.3H_2O$ 5, K_2HPO_4 3, $(NH_4)_2SO_4$ 1.5, $MgSO_4.7H_2O$ 0.3, NaCl 0.1; in mg/L: $FeSO_4.7H_2O$ 10, $CaCl_2.2\ H_2O$ 1.2, $ZnSO_4.7H_2O$ 1, $MnCl_2.4\ H_2O$ 1, $CuSO_4.5\ H_2O$ 0.2, thiamin-HCl 2, biotin 0.02 (pH 5.7) and in a complex medium B (malt extract 20 g, 1 L distilled water, pH 5.7). 500-mL Erlenmeyer flasks containing 50 mL of the media A or B were inoculated with 2 mL of a spore suspension obtained by washing of a 7-d culture grown on wort agar slants in 200 × 17 mm test tubes. Spores from two test tubes were always washed with 50 mL of sterile water and the spore suspension was shaken for 1 min. The cultivation was for 7 d at 28 °C on a reciprocal shaker (frequency of strokes 1.7 Hz). After 7 d the mycelium was filtered and washed with distilled water. Dry mycelial mass was determined in one-half of the samples and the second half was used in further procedure.

The homogenized mycelium was extracted with a $CHCl_3$–MeOH mixture, the total lipids were weighed and after saponification and esterification the originating fatty acid methyl esters (FAME) were determined. Gas chromatography was done at the Sigma 3B (*Perkin Elmer*, USA) apparatus. A capillary fused silica column Supelcowax 10 (*Supelco*, USA) 30 m × 0.32 mm (0.25-μm film) was used. Carrier gas hydrogen, 370 mm/s. Injector and detector temperature was 250 °C. Column temperature was programmed from 150 to 240 °C at 4 K/min.

Production of biomass and fatty acids is presented in Tables I and II. In the synthetic medium A a lower total biomass production was found (as compared with the complex medium B), whereas the difference in production of the γ-18:3 acid is not so pronounced. The highest quantity of γ-18:3 was produced by *M. ramanniana* strain 210 in the synthetic medium (360 mg/L), whereas in the complex medium the highest amount was produced by *M. isabellina* strain 224 (505 mg/L). As compared with literature data (Šajbidor *et al.* 1988; Hansson and Dostálek 1988) in which 170 mg/L and 437 mg/L of the γ-18:3, respectively, were described (glucose as carbon source), the results described here were not significantly different.

In papers concerning the effect of composition of the cultivation medium on production of PUFA only the role of various carbon sources (saccharides, acetate, glycerol) or of added plant oils was investigated. The authors found that glucose is the best carbon source and the stimulatory effect of plant oils on the increased PUFA content has not yet been fully clarified (Shimizu *et al.* 1989*a*, *b*). In our experiments we used a synthetic and a complex medium in which glucose and malt extract (a complex of saccharides), respectively, served as carbon sources.

Table I. Production of biomass and fatty acids (FA) by strains of the genus *Mortierella* growing in the medium A

Strain[a]		Biomass	FA	Fatty acids[b] composition, %							γ-18:3
		g/L	g/L	14:0	16:0	16:1	18:0	18:1	18:2	γ-18:3	mg/L
M. isabellina	1098	8.6	4.0	0.8	25	1.5	6.5	47	11.4	7.6	300
	1088	9.1	2.9	0.7	24	1.1	6.3	45	12.0	11.6	340
	224	9.6	2.3	0.9	25	1.7	4.5	49	9.5	9.2	215
	1109	9.4	3.2	0.8	29	1.5	4.7	51	10.3	7.6	240
M. ramanniana	472	9.4	2.2	0.9	24	0.6	6.2	38	16.3	14.4	315
	210	11.1	3.3	0.9	23	0.5	8.3	44	12.6	10.8	360
	1022	10.3	1.5	0.9	24	0.7	6.8	35	15.4	16.3	240
	209	9.0	2.1	1.1	24	0.6	7.8	38	15.1	13.1	280
	202	9.2	1.4	1.0	35	1.7	5.3	40	7.1	9.5	135
M. vinacea	1097	8.5	2.9	0.6	21	1.0	6.3	55	9.0	6.6	195
M. nana	1103	8.5	2.1	0.9	32	0.7	21.7	31	8.8	4.3	90

[a] *M. verticillata* 206 and *M. parvispora* 199 do not grow on the medium A.
[b] *First number*, number of carbon atoms in the chains; *second number*, number of double bond(s).

Table II. Production of biomass and fatty acids (FA) by strains of the genus *Mortierella* growing in medium B

Strain		Biomass	FA	Fatty acids composition, %							γ-18:3
		g/L	g/L	14:0	16:0	16:1	18:0	18:1	18:2	γ-18:3	mg/L
M. isabellina	1098	17.8	3.7	0.9	22	1.6	4.9	46	12.6	11.5	430
	1088	17.7	2.1	0.7	20	1.2	6.1	46	10.2	15.1	315
	224	17.9	5.1	0.8	24	2.0	4.9	48	10.2	9.9	505
	1109	20.0	4.2	1.7	20	1.7	4.3	49	12.1	11.9	495
M. ramanniana	472	22.8	1.7	1.1	20	0.5	5.8	40	15.7	16.8	285
	210	14.6	0.9	1.0	19	0.5	5.7	42	14.5	17.6	160
	1022	18.3	1.3	1.2	23	0.7	7.0	38	16.6	13.8	185
	209	16.1	0.5	0.8	20	0.7	7.9	35	18.7	16.4	75
	202	14.7	1.4	1.3	34	1.9	6.1	37	7.9	11.9	165
M. vinacea	1097	14.2	3.0	1.1	25	1.1	5.8	48	10.2	8.9	270
M. nana	1103	18.8	2.3	1.0	25	1.0	10.0	42	11.9	8.3	195
M. verticillata	206[a]	6.6	3.6	5.6	24	0.7	12.2	33	5.6	7.7	275
M. parvispora	199[b]	13.5	2.3	4.9	22	–	21.5	23	8.8	6.5	150

[a,b]These strains have different FA composition (%), *viz.* for *a* 20:3 (2.1), 20:4 (8.2), *i.e.* 295 mg/L and for *b* 20:3 (4.3), 20:4 (9.1), *i.e.* 215 mg/L.

In the synthetic medium the strains produced a higher ammount of the γ-18:3 acid with respect to the biomass formed. *M. isabellina* strain 224 was exceptional in this respect. In the complex medium a high total production of γ-18:3 was obtained (*e.g. M. isabellina* strain 224–505 mg/L; see Table II). It is clear that in the rich complex medium some strains produce high biomass quantities without a simultaneously increased production of PUFA. Therefore, it will be necessary to search for such carbon sources and their quantities as would support PUFA production. In two strains, *i.e. M. verticillata* 206 and *M. parvispora* 199, that could not be cultivated in the synthetic medium, higher PUFA homologs, *viz.* dihomo-γ-linolenic and arachidonic acids, were demonstrated. The above strains are potential sources of these PUFA, whose significance has already been mentioned above. The yields of arachidonic acids referred to in the present communication were higher (200–300 mg/L) than those described by Yamada *et al.* (1987), *i.e.* 4–160 mg/L.

It can be concluded that strains of the genus *Mortierella* are promising producers of not only the γ-18:3 acid but also of highly desirable dihomo-γ-linolenic and arachidonic acids.

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