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Identification of fatty acids in submerged cultures of *Claviceps* species

(Claviceps; fatty acids; gas chromatography–mass spectrometry; chemotaxonomy)

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1. SUMMARY

Fatty acids were identified in the submerged mycelium of *Claviceps purpurea, Claviceps paspali, Claviceps fusiformis* and *Claviceps* sp. SD-58 by gas chromatography–mass spectrometry (GC–MS) and used for a chemotaxonomical study. A close relatedness was found between *Claviceps* sp. SD-58 and *C. fusiformis*. The composition of fatty acids in high-production mutants differed substantially from that of the parent strains. Fatty acid analysis is thus probably only applicable in the taxonomy of non-mutated isolates of *Claviceps* cultured under standard conditions.

2. INTRODUCTION

Fatty acids (FA) from the lipid fraction of the mycelium of pyrenomycetes of the genus *Claviceps*, and their synthesis, have attracted the attention of a number of authors [1–11]. Special interest has centered on the relationship between the synthesis of FA and the formation of ergot alkaloids and on the occurrence of ricinoleic acid [5–8]. The results of FA analyses can be used for evaluation of the relatedness of organisms, both prokaryotic and eukaryotic. *Claviceps* spp. are known to contain only 7 FA from the C_{14}–C_{18} range [5,6,9]. A recent study [12] showed that ricinoleic acid is present in different species of *Claviceps* and thus not specific to *C. purpurea*. The aim of the present work was to characterise FA in the mycelium of submerged cultures of common species of the genus *Claviceps* and, at the same time, to characterise the relatedness of the strains under study.

3. MATERIALS AND METHODS

3.1. Strains and cultivation conditions

Experiments were carried out with the strains *C. purpurea* 129, *C. purpurea* CP/7/5/35 [13], *C. purpurea* PLA4, *C. fusiformis, Claviceps* sp. SD-58, *C. paspali* MG-6, *C. paspali* FA (CCMF-731). All strains are deposited in the collection of the Institute of Microbiology, Czechoslovak Academy of Sciences; their description and cultivation procedure has been published previously [12]. *C. purpurea* CP/7/5/35 was obtained by a 4-stage mutagenic improvement of *C. purpurea* 129 using ultra-violet (UV) light. It produces a mixture of clavines (predominantly agroclavine, elymoclavine

Table 1

Percentage content of fatty acid as methyl esters in *Clavicepa*

Fatty acid [a]	*C. purpurea* [c] CP/7/5/35	*C. purpurea* [d] CP/7/5/35	*C. purpurea* PLA4	*C. purpurea* 129	*C. paspali* MG-6	*C. paspali* FA	*C. fusiformis*	*Clavicepa* Sp. SD-58
7:0	0.70	9.21	–	–	5.97	0.76	4.42	2.89
i-7:0	0.40	–	–	0.70	0.43	–	–	0.03
ai-7:0	1.34	–	–	0.23	1.37	0.30	0.33	0.30
7:1	0.55	–	–	–	0.52	–	0.27	0.06
8:0	2.50	6.45	–	0.28	3.99	0.64	0.15	0.27
i-8:0	1.03	0.51	–	0.14	0.34	0.19	–	0.18
8:1	0.42	5.76	–	0.11	0.09	1.28	0.30	0.12
8:1	1.16	0.18	–	–	0.06	–	–	0.09
9:0	1.16	0.05	0.38	0.93	0.06	–	0.12	1.40
ai-9:0	0.25	0.67	–	–	0.18	–	–	–
9:1	0.29	0.78	0.11	–	0.06	–	0.24	–
$br_{4,x}$-9:0	2.36	–	–	–	–	–	–	–
10:0	0.90	0.05	0.07	0.07	0.06	–	–	0.18
i-10:0	0.52	–	–	–	–	–	–	–
br_x-10:0	0.33	–	–	–	–	–	–	–
10:1	0.22	–	–	–	0.06	–	–	–
$br_{4,x}$10:0	5.53	0.23	0.26	–	–	–	0.03	–
11:0	–	–	–	–	–	–	–	0.15
12:0	0.15	–	0.30	0.11	0.09	0.11	0.09	0.09
i-12:0	0.22	–	–	–	0.06	0.11	–	–
12:1	0.17	–	–	–	0.03	0.08	–	–
13:0	0.26	–	–	–	–	–	0.06	–
ai-13:0	0.31	–	–	–	–	– –	–	
13:1	0.05	–	–	–	–	–	–	–
13:2	2.36	–	0.45	–	–	–	–	–
14:0	3.50	1.15	0.98	1.28	0.85	1.74	1.09	2.04
i-14:0	0.64	0.18	0.07	–	–	0.11	–	–
14:1	1.11	0.16	0.19	0.15	0.12	0.19	0.09	0.15
15:0	0.66	2.49	0.11	0.64	2.56	0.49	0.64	0.58
ai-15:0	–	0.16	–	–	–	–	–	–
16:0	12.89	10.60	16.18	19.12	16.75	19.55	19.69	17.64
16:1	4.61	5.29	15.05	7.18	5.21	7.43	6.73	8.21
16:2	0.11	0.32	0.37	0.21	–	0.31	0.09	0.12
17:0	0.35	1.68	0.19	1.76	0.15	0.70	1.30	0.33
i-17:0	0.29	4.38	0.26	0.27	2.47	0.54	1.61	0.48
ai-17:0	2.34	5.99	0.11	3.11	3.78	0.98	1.54	1.70
17:1	0.72	0.46	0.07	–	0.24	0.43	0.36	0.36
17:1	–	0.58	–	0.25	–	–	1.76	0.91
18:0	10.13	6.96	13.96	12.57	14.33	16.81	13.94	13.69
18:1	10.11	17.57	16.36	21.32	14.11	26.75	15.07	20.34
18:2	6.47	12.38	15.63	20.35	18.91	12.34	14.32	10.16
HO-18:1 [b]	1.90	0.48	3.24	0.07	0.30	0.20	4.39	4.11
19:0	0.95	–	0.38	0.21	0.03	–	0.30	0.27
i-19:0	0.22	–	0.87	–	0.06	–	0.15	–
ai-19:0	0.70	–	0.90	0.94	0.49	–	0.70	1.76
19:1	0.29	1.52	0.30	1.24	0.37	0.11	0.24	0.52
19:1	0.52	–	0.53	0.29	0.18	–	0.33	0.39
20:0	4.60	0.35	2.41	2.06	0.31	0.31	3.18	3.37
i-20:0	1.57	0.21	–	0.12	0.40	–	1.67	3.07
i-20:1	2.52	–	–	–	–	–	–	–
20:1	4.24	–	0.34	0.15	–	–	0.21	0.15
21:0	1.77	0.37	1.69	0.23	2.44	2.61	0.30	0.18
i-21:0	0.09	0.46	3.76	0.31	1.46	2.83	0.36	0.12
21:0	0.09	1.61	3.01	0.86	2.16	1.21	0.36	0.12

Table 1 (continued)

Fatty acid [a]	*C. purpurea* [c] CP/7/5/35	*C. purpurea* [d] CP/7/5/35	*C. purpurea* PLA4	*C. purpurea* 129	*C. paspali* MG-6	*C. paspali* FA	*C. fusiformis*	*Claviceps* Sp. SD-58
22:0	1.29	0.58	0.60	1.37	-	0.11	1.76	2.07
22:1	0.64	-	-	0.10	0.09	0.05	0.06	0.15
23:0	0.07	-	-	0.24	-	-	0.21	0.12
24:0	1.03	0.18	0.87	1.03	-	0.56	0.97	1.43
24:1	0.59	-	-	-	- -	-	0.06	
25:0	0.03	-	-	-	-	-	0.15	-
26:0	0.08	-	-	-	-	-	-	-
26:1	0.09	-	-	-	-	-	0.39	-

[a] First number, number of carbon atoms in the chain; second number, number of double bonds; 1, isoacid; ai, anteisoacid; br_x, $br_{4,x}$, methyl groups on C_4 and C_x;
[b] Ricinoleic acid
[c] Cultivated on sucrose-containing media (CS2).
[d] Cultivated on maltose-containing media (CMG).

and chanoclavine-I) in about the same composition, but in a higher yield, than the parent strain 129. To assess the effect of external conditions on the composition of FA, strain CP/7/5/35 was cultivated, not only on the universally employed medium CS2 [12] but also on medium CMG, i.e., medium CS2 in which 100 g/l sucrose is replaced with 95 g/l maltose and 5 g/l glucose.

3.2. Analytical methods

Methyl esters of mycelial fatty acids were prepared and analysed as described previously [12] using GC–MS on a HP 5995 instrument (Hewlett Packard, USA) with capillary fused silica column 60 m long with a SP-2330 phase (Supelco, USA). The quantity and composition of the alkaloid mixture was determined by high-performance liquid chromatography [14].

3.3. Numerical analysis of FA data

Mathematical evaluation of FA analysis was performed using the areas of individual peaks, expressed as % of the total area of all peaks (Table 1). Similarities were calculated with the aid of coefficient S_0 (based on the degree of overlap of 2 superimposed traces) [15]. Clustering was achieved by the mean linkage method [16] on an EC 1040 computer using a programme set up by one of the authors (V.K.).

4. RESULTS

The occurrence and relative proportions of FA in the mycelium of submerged cultures of *Clavi-*

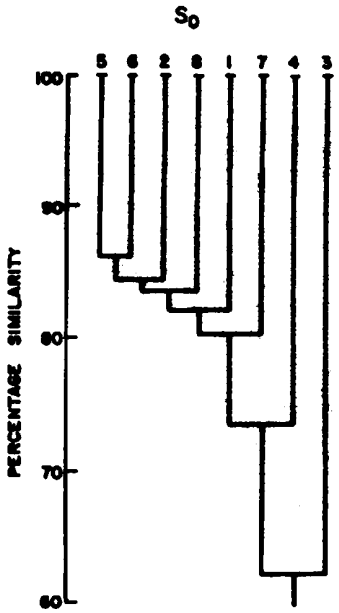

Fig. 1. Clustering of *Claviceps* strains using the mean linkage algorithm with S_0 coefficient. (1) *C. purpurea* PLA 4; (2) *C. purpurea* 129 (3) *C. purpurea* CP/7/5/35; (4) *C. purpurea* CP/7/5/35 (Cultivated on CMG medium); (5) *C. fusiformis*; (6) *Claviceps* sp. SD-58; (7) *C. paspali* MG-6; (8) *C. paspali* FA.

ceps are summarised in Table 1. Most of the cultures contained both saturated and unsaturated FA, with chain lengths of 7–26 carbon atoms. In all cases the major components of FA were hexadecanoic, hexadecenoic, octadecanoic, octadecenoic and octadecanedienoic acids. Ricinoleic acid was found in all strains [12], albeit only in traces in some of them.

The broadest spectrum of fatty acids was found in the mutant strain *C. purpurea* CP/7/5/35 cultivated on medium CS2 (C-source sucrose). The strain contained 59 FA, of which 24 had a relative proportion above 1%. The lowest number of FA was found in non-mutant strains of *C. purpurea* PLA4 (33 FA, 10 FA above 1%) and *C. paspali* FA (31 FA, 10 FA above 1%). The richest in unsaturated FA were strains *C. purpurea* PLA4 and *C. purpurea* 129, the richest in saturated FA was *C. purpurea* CP/7/5/35.

Statistical evaluation of similarity of FA profiles in the form of a dendrogram is given in Fig. 1.

5. DISCUSSION

The dendrograms illustrating the possible relatedness of individual strains indicate that strain *Claviceps* sp. SD-58 belongs to the species *C. fusiformis*. The identicality of the two strains was also assumed by Waiblinger et al. [6]. As shown in Table 1, two strains are similar in the content of unsaturated FA (*C. fusiformis*, 45.2% *Claviceps* sp. SD-58, 45.7%) and in the level of ricinoleic acid (4.39%; 4.11%). Of interest is also the finding of non-trace amounts of docosanoic (22 : 0) and tetracosanoic (24 : 0) acids in the two strains.

Comparison of our data from strain *Claviceps* sp. SD-58 with the results of Waiblinger et al. [6] indicates identical contents of hexadecanoic (16 : 0) and octadecanoic acids (18 : 0) on the one hand, and substantial differences in the levels of hexadecenoic (16 : 1), octadecenoic (18 : 1) and octadecane dienoic (18 : 2) acids, on the other. These differences were attributed to varying behaviour of the strain, different cultivation conditions, and also to the more sensitive method used here.

The relatedness between other strains under study cannot be unambiguously characterised, since the conclusions often vary accordng to the method used for data processing. However, a special position is occupied by *C. purpurea* CP/5/7/35, which is conspicuously dissimilar from all the other strains, including *C. purpurea* 129, from which it was obtained by mutagenic treatment [13]. This may be due to the fact that in this strain, the mutation, in addition to affecting the intensity of clavine synthesis, also alters lipid biosynthesis. The total number of FA in *C. purpurea* CP/7/5/35 increased to 59 (the parent strain contained 36 FA), the number of non-trace FA (1% of total level) increasing from 12 to 24. These findings suggest a lowering of the specificity of enzymes of FA synthesis brought about by mutagenic treatment. One can also speculate that, owing to the changes in composition of FA, the transport of individual alkaloids through the cell membrane may have changed and resulted in a consecutive increase in alkaloid production. Another interesting feature of strain *C. purpurea* CP/7/5/35 is the occurrence of the branched fatty acids 9 : 0 and 10 : 0. The levels of octadecene (18 : 1) and octadecanediene (18 : 2) acids were lower than in the parent strain while the level of ricinoleic acid increased several times. On another C-source (maltose), strain CP/7/5/35 exhibited similar changes, but its mycelium lacked the 9 : 0 and 10 : 0 branched acids, the level of 7 : 0, 8 : 0 and 8 : 1 acids was markedly increased and the content of ricinoleic acid dropped substantially. Nevertheless, even on a different C-source, the strain exhibited similar differences from its parent strain.

The dendrogram indicates a relatively close relation between the 2 strains of *C. paspali* studied, which contained almost identically low proportions of ricinoleic acid. Somewhat surprising is the similarity of *C. purpurea* 129 to strains of *C. fusiformis*. *C. purpurea* 129, obtained by mutation improvement, probably underwent (analogous to *C. purpurea* CP/7/5/35) a deviation from the phenotype typical of the species *C. purpurea*. This is also documented by the fact that, in contrast to the majority of *C. purpurea* strains, *C. purpurea* 129 alkaloid production consists solely of clavines.

The results show that changes in the intensity of

formation of ergot alkaloids caused by genetic treatments are usually accompanied by changes in the synthesis of FA. We may thus assume that analysis of FA can be used for the taxonomy of the pyrenomycete *Claviceps* only in naturally occurring isolates cultivated under standard conditions.

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