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Characteristics of the Saprophytic Reference Strain FA of *Claviceps paspali*

A. Řičicová, V. Pokorný and Z. Řeháček

Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

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ABSTRACT. Growth properties and morphological features of the saprophytic collection culture *Claviceps paspali* strain FA were investigated. The strain was characterized by a range of utilization of 13 carbon sources in a basic synthetic and a peptone medium. A temperature of 23 °C was more favourable for growth than 28 °C. Stages of changes of the culture cultivated for a long time on Sabouraud's medium were detected by electron microscopy. The white culture was characterized by true septated mycelium with different types of terminal parts of hyphae. Arthrospores occurred after a 14-d cultivation, small spherical conidia released individually from hyphae were quite rare. As compared with other strains of the same species, strain FA did not form spherical clusters of conidia. On the other hand, a spontaneous rupture of the surface cell wall in different parts of hyphae and release of the cytoplasm were observed. In corn-steep containing media the formation of individual, pair and chain-like forms of arthritic conidia was stimulated. Destructive autolytic changes of hyphae were detected in the medium with potato extract.

Strains of *Claviceps paspali* described by Stevens and Hall (1910) were first mentioned as producers of ergot alkaloids by Arcamone *et al.* (1960). *C. paspali* strains described by various authors were either isolates obtained from the natural material as parasites on the grass *Paspalum distichum* or *P. dilatatum* or were derived from cultures maintained in collections. In most cases the strains were investigated with respect to the production of alkaloids. A pronounced morphological variability, different forms of developmental stages and different experimental approach caused that some strains of the genus *Claviceps*, classified as different species, were considered to be synonymous by some authors: for instance the species described originally as *C. rolfsii* Stev. *et* Hall was according to Langdon (1954) synonymous with *C. paspali* Stev. *et* Hall. Oddo and Tonolo (1967) who revised parasitic *C. paspali* cultures, suggested their reclassification in a new genus *Mothesia*. *C. paspali* strain FA, an α-hydroxyethyllysergamide producer, cultivated under saprophytic conditions, morphologically differed from some other strains of identical taxonomical classification (Řičicová *et al.* 1981). Therefore, we performed more detailed nutritional and morphological studies of the strain FA maintained on a solid medium without mutagenic treatment.

MATERIAL AND METHODS

Microorganisms. Claviceps paspali STEV. *et* HALL, strain FA, derived from strain F-2056 (*Istituto Superiore di Sanità, Centro Internazionali di Chimica Microviologica*, Roma), was maintained as a saprophytic culture in test tubes (200×15 mm) containing 15 mL of Sabouraud's medium at 4 °C in the dark. It was transferred after a 2—3-week cultivation at 23 °C at the earliest and after 1 year at the latest. *C. paspali* strains MG-6 and So 7—18 (from *Paspalum distichum*) were obtained from the *Pharmaceutical Institute, University of Mainz* (FRG). Strains *C. purpurea* PLA-4 and PLO-2 were from the *Research Institute of Pharmacy and Biochemistry* (Prague), strain *C. purpurea* 14934 from the *ATCC Collection*, Rockville (USA).

Cultivation conditions and media. The basic carbon and nitrogen sources were selected on the basis of macroscopical evaluation of the growth intensity in complex and synthetic media. Solid media were inoculated with an aqueous suspension washed from the surface of agar slants with a 14-d or older culture grown at 23 °C. Utilization of C-sources for growth was evaluated in a synthetic medium and a peptone-containing medium (*Oxoid Manual* 1961) according to Shirling and Gottlieb (1966).

Media: Sabouraud (Burton 1949), medium with ammonium citrate (Kybal *et al.* 1968), medium with potato extract (Vero *et al.* 1966), standard potato-dextrose agar (Oxoid), medium with mannitol (Agurell and Ramstad 1962), media with corn-steep liquor (Kobel *et al.* 1964), medium 31 containing molasses, medium 32 with glucose and lactose according to Moyer and Coghill, with glucose according to Harris and Rugger, medium 41 containing glycerol according to Coghill and Moyer, medium with amino acids (Köhler 1956), tyrosine media (Kocková-Kratochvílová 1954; Ettlinger *et al.* 1958; Shinobu 1958), Tresner-Danga's medium (1958), gelatin media (Waksman 1957, 1961).

Macroscopic evaluation. The colour of the aerial and substrate mycelium was evaluated according to the colour scales of Prauser (1964) and Tresner-Backus (1963).

Microscopy. Growing culture was followed at 23 °C directly on Petri dishes with the aid of a light microscope (*Zeiss*, type Nf), by prints of intact mycelium on the net by a transmission electron microscope (*Tesla* BS 613, Brno) or in a scanning microscope (*Phillips*) after previous rotation gilding.

RESULTS AND DISCUSSION

Cultivation characteristics

Utilization of carbon sources for growth. For the evaluation of the saprophytic strains of the genus *Claviceps* no taxonomical methods have so far been described and the respective media for their morphological description have not yet been described. Therefore, in order to follow the utilization of different carbon sources we selected two basic media — one synthetic, the other complex. Conditions suitable for the characterization of fungal cultures were tested and the results obtained were verified in a synthetic medium used for the evaluation of streptomycetes (Shirling and Gottlieb 1966). According to Bonns (1922), the growth characteristics of the genus *Claviceps* resembled those of actinomycetes.

The assimilation test was not influenced by inoculation with the mycelium (Řičicová *et al.* 1982) grown either in Sabouraud's medium or yeast-malt extract medium (medium 2, Shirling and Gottlieb 1966). Strain FA grew abundantly on D-glucose, D-fructose, D-mannitol, sucrose, maltose and dextrin, it did not grow on D-xylose, lactose, *myo*-inositol and cellulose, independently of the composition of the basic medium (synthetic medium or peptone-containing medium). Utilization of L-arabinose, L-rhamnose, raffinose and D-glucitol depended on the composition of the medium: in the synthetic medium the strain utilized L-arabinose, raffinose, D-glucitol, whereas in the peptone medium it did not utilize L-arabinose, on raffinose and D-glucitol the utilization was questionable to negative. In the synthetic medium the strain did not grow on L-rhamnose, in the peptone basic medium the result was questionable to negative. By applying the carbon assimilation test under submerged conditions, *i.e.* when cultivating strain FA on D-glucose, D-fructose, sucrose, maltose, D-mannitol and D-glucitol, and comparing with literature data obtained with other strains, the strain used here was identical with *C. paspali* strain TA-1 (Brar *et al.* 1968).

Selection of an artificial substrate for the evaluation of the strain on a solid medium. According to Békésy (1956) the growth of saprophytic strains of the genus *Claviceps* was typical on malt extract agar. On yeast-malt extract agar (medium M 2, Shirling and Gottlieb 1966) the culture grew sparsely and developed atypically. Cultivation on pure gelatin in the presence of glucose and Bactopeptone Difco (considered to be a suitable nitrogen source from the point of view of definability of basic components; *Difco Manual* 1953) resulted in massive growth of the mycelium, leading to a typical skin-like cover with a creamy white mycelium.

On the basis of macroscopic examination of the culture growth on media used traditionally for strains of the genus *Claviceps* (Table I) we have chosen Sabouraud's medium modified according to Burton (1949) with glucose, malt extract (*Difco*) and Bactopeptone (*Difco*) as the basic medium for strain FA. Increased glucose concentration (from 4 to 5—10 %) influenced favourably growth, the mycelium remained without lytic changes even after a 21-d cultivation. The medium was useful for preservation of the reproduction ability of the strain; this fact should be stressed particularly when comparing with the medium containing potato extract in which reproduction of the long-term stored culture was suppressed.

Temperature conditions. Strain FA could grow at 25 °C in all commonly used media (including Table I). At 28 °C the culture did not grow in most media (including corn-steep medium). The mycelium intensively surface-grown at 23 °C had longer hyphae as compared with shorter and more branched hyphae of the culture cultivated at 28 °C.

The strain could be maintained for long time intervals in paraffin-sealed test tubes at 4 °C in the dark. After 8 weeks it germinated up to 2 d at 25 °C. The strain left for the same time at room temperature did not grow up to 9 d.

Macroscopic characteristics

Strain FA produced white to slightly yellowish aerial mycelium in all media (Table I). On Sabouraud's agar a rich compact growth was observed. Only on prolonged cultivation (54 d on Sabouraud's agar) could a slight

TABLE I. Colour of the aerial and substrate mycelium and morphological characteristic of *C. paspali* strain FA in complex and synthetic media

Medium	Cultivation time (d)	Colour of mycelium[a]					Individual cells or fragments after 14–28 d (size, µm)
		aerial			substrate		
		colour	A	B	colour	B	
With ammonium citrate	7	white	W a	Coo 7a	creamy	Co 5a	
	14	pale yellow	Y 2ba	Coo 7a	light yellow	Co 4a	13–17 × 3–4
	21	pale yellow	Y 2ba	Co 7a	creamy-light yellow	Co 5a-b	
Sabouraud	7	white	W a	Coo 7a	creamy	Coo 5b Co 6a	
	14	pale yellow	R 2ca	Co 7a	creamy-light yellow	Co 5a-b	17.5 × 3.5
	21	pale yellow	Y 2ba	Co 7a	light yellow	Co 5b	
With potato extract	7	white	W a	Coo 7a	creamy	Coo 5a Co 6a	
	14	pale yellow	Y 2ba	Coo 7a	dark beige	Coo 4s	mycelium
	21	pale yellow	Y 2ba	Coo 7a	dark beige	Coo 4s	
With D-mannitol	7	—[b]	—	—	creamy (indications)	Coo 5a	
	14	pale yellow	Y 2ba	Coo 7a	light brown	Coo 5s	11 × 3.5–6.5;
					brownish pink	Oc 6t	26–33 × 3.5–5.0[d]
	21	pale yellow	Y 2ba	Co 7a	yellow	Co 3a	
					brownish pink	Oc 6s	
					grey	Oc 7c	
					dark grey-brown	O 7s	
					grey-violet	Pr 7t	
With corn-steep[c]	7	white	W a	Coo 7a	creamy	Coo 5a	
	14	pale yellow	R 2ca	Co 7a	brown	Oc 4r	8–23 × 3.5–5.0
	21	pale yellow	Y 2ba-R 2ca	Co 7a	brown	Oc 4r	

[a] A — scale according to Tresner and Backus (1963), B — scale according to Prauser (1964).
[b] Insufficient growth.
[c] Kobel *et al.* (1964).
[d] Rarely.

grey to grey-pink colour of the mycelium be detected. The colour of the substrate mycelium was yellow to brown, depending on the composition of the cultivation medium and age of the culture.

Strain FA did not exhibit a significant pigmentation. A slightly violet colour in the substrate was observed only in the mannitol-containing medium (Table I). During growth in three tyrosine media (stimulating formation of pigments of melanin type) it produced a red pigment at the beginning (10 d in Shinobu's medium), and later a brown, slightly diffusing, pigment. In media according to Kocková-Kratochvílová (1954) and according to Ettlinger *et al.* (1958) a slight brown pigment was observed after 5 d, in the medium according to Tresner-Danga (1958) the pigment was orange and later brown.

A characteristic pleasant fruit odour could be detected after a 4-5-d cultivation on Sabouraud's medium.

Microscopic characteristics

Sabouraud's medium. Strain FA formed an abundant growth (Plate 1A) of mycelium with cell septae located at unequal distance from each other (10—40 μm). Basic, sparsely branched mycelium of the culture produced at certain places synnematic strands formed by up to four parallel filaments during later cultivation. Lateral weaker filaments grew from thicker hyphae, their adhesion could be frequently observed (Plates 1C, D). The lateral protrusion of the hypha (Plate 1D) could be sometimes longer but never further branched. The thickness of the filaments (minimum 1.4 μm, maximum 4.8 μm) depended on the age of the culture and relative humidity inside the Petri dish. In the electron microscope the hyphae had a smooth, electron-dense surface. A pronounced segmentation of the furrowed electron-transparent inner part of a coiled hypha (9-30-d old) is detailed on Plate 2A. The terminal part of a 54-d thickened hypha, in the orifice of which a protruding inner part with a different optical density is clearly seen, is illustrated in Plate 2E.

Significant morphological structures on Sabouraud's medium. At a time of macroscopically visible growth, curled to regularly broken hyphae of 3.5—4.0 μm in diameter, indicating the formation of arthrospores (Plate 1B) were observed. Terminal hyphae were slightly constricted without formation of a spontaneously fragmenting mycelium. Individual elongated thallic arthrospores of 13.6—18.7 × 3.5—3.7 μm in size were formed within a 10—14 d cultivation. Thicker and longer hyphae and fragments appeared even in older cultures. The presence of individual separated cells of the size identical with mycelial fragments was stimulated by sucrose.

Plate 2B illustrates a long terminal segment not exceeding the diameter of the hypha, conically narrowed at both electron-dense tips, with two irregular circular centres situated at both poles of the fragment. Similar, but less distinct, centres could also be seen in non-segmented terminals of certain hyphae. The surface of the hyphae was in some cases covered with a monolayered wall forming a hyphal sheath. The surface layer was electron-dense. This envelope of the terminal part of the hypha ruptured either longitudinally or transversely (Plate 3A, B). The inner part of the hypha having a different electron density, was visible below the ruptured outer coat. Terminal regions

of some hyphae in the mature culture were swollen in a club-like fashion (Plate 3C).

At the margin of the growing mycelium terminal club-like structures (12.5—14.8 × 7.4—10.3 μm) located on the non-septated electron-transparent hypha could occasionally be seen (Plate 4). This filament was usually very long (180 μm on Plate 4C) and highly refractile so that only a single fragment could be seen in the electron microscope. It is possible that after coiling of the hyphal terminus, rupture of the outer layer and excretion of its content the terminal club-like structure was formed.

Release of the inner content of hyphae below the terminal part is a repeated phenomenon visible in different variations both in the optical (Plate 4B, D) and the electron microscope (Plate 4A) during a 9—12 d cultivation at 23 °C. In most cases the released matter forms fanshaped structures around the rupture, from which it was released. The matter is composed of particles about 1 μm in size as determined in the scanning microscope (Plate 4A). The cytoplasm at the apex (Plate 1E) or below it was released spontaneously without any physical treatment, as compared with similar changes in *Mucor rouxii* (Bartnicki-Garcia and Lippman 1972). After tearing up or breakage of the terminal hyphal part sharp stumps of hyphae remained at the site of excreted cytoplasm. Residues of released inner organelles can be usually seen in orifices of the broken apices (Plate 4E). During cultivation on solid Sabouraud's medium the appearance of hyphae changed after 9—10 d, due to the presence of the exudate or liquid covering parts of the hyphae visible both with naked eye and in the light microscope. It is known that strains of the genus *Claviceps* release extracellular polysaccharides during the period of maximal growth (Buck *et al.* 1978) that might be contained in this liquid.

Individual spherical structures (conidia) of smaller size (1.4—6.8 μm) were sporadically released even after a long-term cultivation (3—55 d; Plate 2C). Conidia with smooth surface are produced at hyphal apices that become later dry and their residues remain attached to conidia (Plate 2D). Due to the small size of these morphological forms detected in the electron microscope, we tried to separate them from mycelial fragments by filtration of the suspension obtained by washing the surface of the grown culture through *Schleicher-Schuell* 589[3] membranes. The cells passing through the filter were unequally developed and their size was 1.2—6.8 μm. Their viability was verified by transferring the filtrate to the solid and liquid medium where rare colonies with typical white mycelium appeared. On Sabouraud's medium we have never observed accumulation of these unicellular forms.

A minor portion of the population grown on Sabouraud's medium formed hyphae lacking the surface compact electron-dense layer, some of them changing their shape.

At contact zones of the mycelia of a 10—14-d-old culture (Plate 5A) non-segmented hyphae forming spherical structures 6.0—6.6 μm in size (Plate 5B) were detected. Coiling of the hypha even outside this visible zone to a structure of 9.6 × 7.2 μm in size is detailed in the scanning microscope picture (Plate 5C).

In a culture followed for a long time (1 month and more) hyphae 1.7 μm in diameter with spherical elements of 1.7 μm situated at the site of electron-dense septa occurred at contact zones of the mycelia (Plate 5D, E, F). The presence of these elements in hyphae was emphasized by the temperature

effect of the electron beam. Their visibility in the hyphae is enhanced by the fact that their surface is not completely electron-dense. In their size these spherical structures are identical with the spherical conidia illustrated in Plate 2.

After a long-term cultivation of the FA strain (35—55 d) the surface growth turned grey and deformations of the mycelium induced by exhaustion of nutrients and drying of the substrate could be observed in the microscope. In the electron microscope the hyphae remained smooth but their mean size varied considerably.

Traditionally used media. As compared with some authors we cultivated strain FA on Sabouraud's medium without any difficulties and losses of reproducibility caused by cultivation of the *Claviceps paspali* strains on potato agar (Mizrahi and Miller 1968). A decreased reproduction after serial transfers on potato medium led us to investigate their growth in the electron microscope. On standard PDA medium the growth was significantly less than on Sabouraud's medium. A fine-grained structure could be observed in hyphae of the 8-d mycelium. In the electron microscope hyphae were covered with an uneven layer of the exudate (or liquid), below which their contours disappeared completely. In the predominating part of a three-week population hyphae with damaged surface of the cell wall were detected (Plate 6E). Irregular particles penetrate here from the inside of the hyphae to the surface and, simultaneously, the filamentous type of the mycelium caused by autolytic processes disappears (Plate 6F, G). Only rare hyphae were terminated by a single club about 10 μm in diameter, which was spherical or ovoid (22 × 10 μm).

The growth of strain FA was favourably influenced by compounds contained in the medium with natural potato extract. In addition to hyphae identical with those on Sabouraud's medium and typical terminal cells (Plate 6C) we observed simultaneously a large number of hyphae with released plasma in the terminal part of hyphae. In the scanning microscope perforations could be seen in hyphae of the mycelium (9 d; Plate 6B). However, they could not be observed in hyphae of a younger (*e.g.* 6-d) culture (Plate 6A). Similarly to the previous media the mycelium was covered with a layer of polysaccharides. A portion of a 20-d population consisted of hyphae without distinct contours passing to irregular structures composed of residues of small electron-dense elements (Plate 6D). The mycelium is apparently subjected here to destructive changes. Cessation of growth on potato-extract-containing media is probably caused by limited nutrition leading to autolysis, during which the cell wall is damaged and the hyphae lose their rigidity and shape.

In contrast with media rich in polysaccharides and poor with respect to nitrogenous compounds, we used media containing corn-steep and a synthetic medium with amino acids (Köhler 1956) forming the main components of corn-steep. Even in these media the strain did not exhibit pronounced differences in the colour of the surface growth. On Kobel's medium the yellowish, very short mycelium cultivated at 23 °C grew laterally as compared with the culture cultivated at 28 °C. Growth of the strain was characterized by similar morphological features as that on Sabouraud's medium. However, the surface of thick hyphae of mature culture grown at 23 °C was covered with electron-dense spots that occurred only rarely on

TABLE II. Unicellular forms of *C. paspali* strain FA in media with corn-steep

Medium	Mycelium	Unicellular forms	Size, μm
Kobel *et al.* (1964)	compact, short, creamy white to pale yellowish	rare or clustered, spherical and even elongated	1.4—3.1 × 0.8—2.9
With molasses	rich, filamentous, white (exudate)	individual ovoidal cells with smooth surface	2.0 × 1.1
With glucose and lactose (No. 32)[a]	compact, short, white	rarely released from terminal part of hypha, paired on hypha, chained	2.5—3.2 × 1.4—2.2
With glucose[a]	long filamentous, individual flat colonies, white	spherical with surface cell wall residues	3.5 × 2.6
With glycerol (No. 41)[a]	short, rare colonies, white	terminal, not released	3.8 × 3.2
With amino acids[a]	rich, filamentous, white	paired on hypha	—

[a] Köhler (1956).

the hyphae of the mycelium cultivated on Sabouraud's medium. Fragments of hyphae are seen on Plate 7B. In a three-week culture we demonstrated accumulation of unicellular forms of $1.7-2.0 \times 0.8$ μm.

Morphological control of individual colonies isolated (diameter 3.5—15 mm after a 14-d cultivation) revealed a club with spotted ornamentation in the largest of them (16.2—10.8 μm). We assume that this could be a non-deformed, fully developed form of the population cultivated on Kobel's medium (Plate 7A). It was found microscopically that the culture cultivated at 28 °C contains residues of hyphae and a deformed irregular structure corresponding in size to the above-mentioned terminal clubs.

The occurrence of unicellular forms was stimulated on Kobel's medium and other rich nitrogenous media (Table II); these were also detected, although in minimal numbers, on Sabouraud's medium. Pictures of the culture cultivated in the medium with glucose and lactose (no. 32) revealed swelling of hyphal apices followed by separation of the terminal thallic conidium (Plate 7C). Plate 7D shows four conidia in a chain that are still connected by a lyzed layer.

Comparison of strain FA with other strains of C. paspali

Cejp (1957) ascribed only a minor taxonomical value to about 20 species of the genus *Claviceps*. He did not consider morphological features of a parasitic culture as sufficient criterion for species discrimination. The author stressed the classification of strains in the so-called races differing biochemically, namely by different content of alkaloids and their composition. Skalický and Starý (1962) supplemented the classification of parasitic cultures by chemotaxonomy of alkaloids and divided the cultures into the physiological, phenological, geographical and climatic races. Kybal and Brejcha (1955) used qualitative levels of alkaloids in sclerotia for the division of *Claviceps purpurea* into three groups of biological races, similarly to Kobel *et al.* (1964) who divided strains of *C. paspali* into chemical races. Strains of *C. paspali* producing different alkaloids were summarized by Willamann and Li (1970).

C. paspali strain FA appeared to be a high-producing culture producing alkaloids under submerged conditions (Řičicová *et al.* 1982), as compared with some other unimproved producers of derivatives of lysergic acid or peptide alkaloids (*C. paspali* strain MG-6, *C. purpurea* strains PLA-4, PLO-2 *etc.*). As within a period of more than 10 years we could reproduce maximum yields of about 2000 μg total alkaloids in 1 mL of fermentation liquid, we assume that strain FA was maintained on Sabouraud's medium using a procedure that did not lead to degeneration. According to Békésy (Gröger 1958) there exist strains cultivated for 4—5 years saprophytically without degenerative changes.

Strain FA is evaluated as a reference saprophytic strain producing higher amounts of α-hydroxyethyllysergamide; characteristic properties of this strain were compared with those of other cultures of *C. paspali*, none of which had been designated as a typical strain. By comparing growth properties with those of some related saprophytic cultures designated *C. paspali* (MG-6, So 7—18) it was found that the slowly growing strain FA exhibited slightly different requirements for temperature: it grew more intensively at 23 °C than at 28 °C. Under the cultivation conditions used it was characterized by only a rare occurrence of the exudate on flat, slightly radial, furrowed,

TABLE III. Unicellular forms of *C. paspali* strains FA, MG-6 and So 7—18 on Sabouraud's medium

Strain	Cultivation time (d)	Amount	Unicellular forms	
			size, μm	predominating type
FA	14—26	0	—	—
	28	rare	17.5 × 3.5	arthrospores
MG-6	14—28	rich	10—13.5 × 3.5 and 16.5 × 5.0	oblong conidia
So 7—18	10	rare	6.5—10 × 3.5	oblong conidia
	28	rare	10 × 3.5 and 20—33 × 3.5	

creamy white colonies with tough skin-like surface and straight up to finely denticulate margin (Plate 1A). Formation of the exudate on the peptone medium (*Oxoid Manual* 1961) was stimulated in the presence of glucose, sucrose, mannitol and fructose, *i.e.* of sources on which the strain grew most abundantly. The exudate occurred only on single colonies and later than for instance in *C. paspali* strain MG-6, which was characterized by a pronounced formation of macroscopic exudate.

According to the carbon assimilation test strain FA differed from the comparable strain MG-6 by utilization of mannitol for growth in a synthetic medium, from strain So 7—18 only by intensity of utilization of certain sources. The difference between these cultures was most pronounced in the morphological properties: strain FA did not form spherical clusters of elongated cells as observed in strains MG-6 (Plate 7E), So 7—18 (Table III) or conidia typical of cultures of *C. purpurea* PLA-4 or PLO-2 (Table IV). Loveless (1964) who followed the shape and size of conidia of different strains of *C. paspali* in the parasitic stage — when honeydew on the host plant is formed, described their size as 6.5—12 × 2.5—5.0 μm. This size of conidia corresponded roughly to that of elongated oval cells observed here in the

TABLE IV. Unicellular forms of *C. purpurea* strains PLA-4, PLO-2 and ATCC 14934 on a solid medium[a]

Strain	Medium	Cultivation time (d)	Size, μm	
			max.[b]	min.
PLA-4	with ammonium citrate	12	14.5 × 4	5 × 3
		22	8 × 2.5	4 × 2.5
PLO-2	with ammonium citrate	14	16.5 × 3	4.5—5 × 3
ATCC 14934	with corn-steep	21	12.5 × 3	6 × 3

[a] Always high numbers.
[b] Predominating type of conidia.

saprophytically cultivated strain MG-6 (10.0—13.5 × 3.5 μm and 16.5 × × 5.0 μm). In strain FA cultivated on Sabouraud's medium distinct unicellular forms were not observed. The macroscopical appearance of colonies on Sabouraud's agar did not show any significant differences that would indicate that a mixture of cultures was involved. However, we observed compatibility and incompatibility in individual colonies. As compared with the autolytic process occurring in media containing potato extract, all corn-steep-containing media stimulated formation of unicellular forms of the type of arthritic conidia of more or less regular size and shape. It follows that under the conditions used strain FA resembles Kobel's culture of *C. paspali* isolated from sclerotia (Kobel *et al.* 1964).

With respect to the criteria suggested for the study of strains of the genus *Claviceps* (Skalický and Starý 1962) and description of saprophytically maintained culture, whose history we had not known, it was found that the reference strain FA of *C. paspali* is an organism, the polymorphous character of which is significantly influenced by nutritional and temperature conditions. This polymorphous character of strain FA, however, is stabilized to a certain degree in comparison with other strains of this species, as indicated by reproducibility of the yield of alkaloids produced under submerged conditions.

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The Plates will be found at the end of the issue.