BRIEF REPORT

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Formation of Conidia in a Saprophytic Strain *Claviceps paspali* Producing Simple Lysergic Acid Derivatives

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ABSTRACT. A non-mutant saprophytic strain *C. paspali* which forms conidia both on a solid medium and during submerged fermentation is described. Conidiation proceeded in parallel with culture growth and production of alkaloids. The effect of composition of culture media on the intensity of conidiation is described.

Sporulation of saprophytic cultures of *Claviceps* contributes to their stability and substantially facilitates the preparation of high-production mutants. Unlike the saprophytic cultures of *Claviceps purpurea* and *C. fusiformis* which commonly form conidia during both surface and submerged cultivation, cultures of *C. paspali* reproduce mostly through vegetative mycelium and they can be made to form conidia only with difficulty (*for review see* Řeháček 1986). However, the ability to form conidia on solid media was observed in a *C. paspali* strain producing paspalic acid (Kobel *et al.* 1964) and in *C. paspali* strains (Segal and Germanier 1974; Mercantini *et al.* 1967) producing simple derivatives of lysergic acid. Recently, Pertot *et al.* (1986) described a strain of *C. paspali* with mutation-induced conidiation.

We studied the strain *Claviceps paspali* MG-6, especially its conidiation on a solid medium, production of simple derivatives of lysergic acid, and the effect of composition of culture media on sporulation intensity.

The saprophytic strain *Claviceps paspali* (Stevens et Hall) MG-6 was isolated from the grass *Paspalum dilatatum* in the vicinity of Rome by Prof. H. Rochelmayer (*Institute of Pharmacy, University of Mainz*). The strain was maintained on a solid Sabouraud medium (Burton 1949) containing (g/L): peptone 10, glucose 40, malt extract Difco 26, tap water, pH 5.8 after sterilization. Sporulation medium SP (Gaberc-Porekar *et al.* 1983) contained (g/L): sucrose 300, casein-peptone 10, KH_2PO_4 0.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.007, $ZnSO_4.7H_2O$ 0.006, tap water, NH_4OH to pH 6.2. Solid media contained 2 % agar.

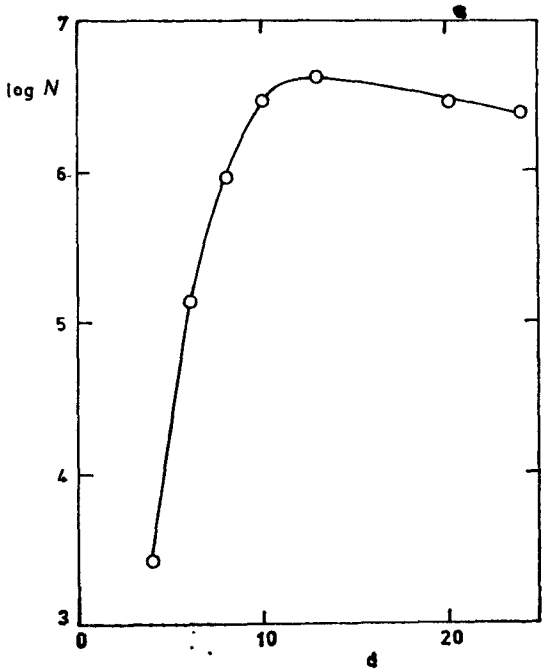

FIG. 1. Time course of conidiation of *C. paspali* on agar medium SP; *N* — number of conidia per cm^2 of agar medium.

Sabouraud slant cultures were washed off into 60 mL medium SP in Erlenmeyer flasks (300 mL) and incubated for 6 d in the dark at 24 ± 1 °C on a rotary shaker (frequency 4 Hz, stroke eccentricity 55 mm). The resulting vegetative inoculum was used to inoculate (10 %) fermentation medium MP (Řičicová *et al.* 1982) or medium D (Kobel *et al.* 1964) which were then culti-

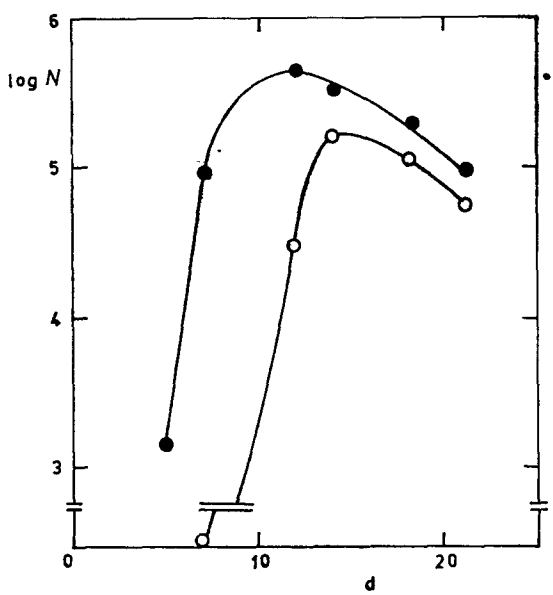

FIG. 2. Time course of conidiation of submerged cultures of *C. paspali* in media MP (*open symbols*) and D (*closed symbols*; *N* — number of conidia per mL.

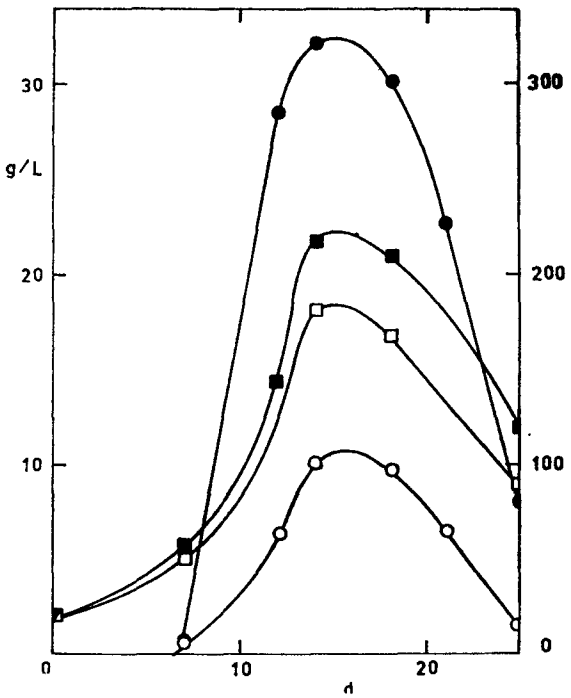

FIG. 3. Time course of submerged cultivation of *C. paspali*; *squares*: dry mass (g/L), *circles*: total alkaloids (mg/L); *closed symbols*: medium D, *open symbols*: medium MP.

vated under the above conditions. The number of spores was determined in a Bürker chamber, dry mass was assayed gravimetrically in 5 mL of culture. Total alkaloids were determined in the fermentation broth colorimetrically with the van Urk reagent (Robbers *et al.* 1972). Qualitative analysis of a mixture of alkaloids was carried out by TLC (Řeháček *et al.* 1971*b*).

Culture of *C. paspali* grew on solid media in a continuous white layer on which appeared, after 15 d, ochre-coloured droplets of honeydew containing elongated conidia (Plate 1A). The culture produced no pigment into the medium. On the SP medium it formed sphacelial, abundantly branched hyphae whose tips carried conidia and honeydew (Plate 1B). The most intensive formation of conidia was observed between cultivation days 4 and 11 on solid medium SP (up to 4×10^6 conidia per cm^2). After 13 d the formation of conidia practically ceased (Fig. 1), with isolated germination of newly formed conidia taking place (Plate 1C). Sporulation on Sabouraud medium was much poorer than on medium SP. For this reason cultures from a solid SP medium were used for preparing submerged vegetative inoculum.

Submerged cultures of strain MG-6 in medium SP (seed cultures) were composed of poorly branched, septated, sphacelial hyphae. Under these conditions the cultures grew intensively but produced no conidia or alkaloids. In production medium D the mycelium of strain MG-6 was characterized by poorly branched sphacelial hyphae (Plate 1D), in later developmental phases also by sclerotial cells. After 4 d of cultivation intensive formation of conidia set in, with a maximum in a 12-d-old culture (concentration of conidia 430/μL). The subsequent drop in the number of conidia was apparently due to

their germination. The morphology of the culture grown on medium MP did not substantially differ from that of a culture grown on medium D but the onset of conidiation in medium MP was delayed by 5 d (Fig. 2). In production media the production of alkaloids and biomass growth proceeded in parallel, maximum growth and alkaloid production being attained around cultivation day 14 (Fig. 3). The dominant component of the produced alkaloid mixture was 2-hydroxyethyllysergamide, with a lower concentration of ergine, erginine, ergometrine, ergometrinine, chanoclavine-I and elymoclavine.

Like *C. paspali* strains described by Kobel *et al.* (1964), Segal and Germanier (1974) and Mercantini *et al.* (1967), the saprophytic strain *C. paspali* MG-6 is capable of forming conidia on a solid medium. However, strain MG-6 is the only known non-mutant strain forming conidia in a submerged culture as well. According to Pertot *el al.* (1986) the number of conidia in a submerged culture of a mutant sporulating strain of *C. paspali* increased until the end of cultivation whereas in strain MG-6 the number of conidia after 12 d decreased owing to germination. We cannot exclude the possibility that the germination of conidia was induced by a decrease in the alkaloid concentration in the medium of an ageing culture. The inhibitory effect of alkaloids on conidia germination of the production strain was described by Lingappa and Lingappa (1967). The number of conidia in production cultures of strain MG-6 increased nearly in parallel with the intensity of culture growth and production of alkaloids. A similar pattern including the maximum number of conidia was found by Pažoutová *et al.* (1977) in the strain *C. purpurea* 129.

Opinions concerning the relationship between culture conidiation and alkaloid production differ (*cf.* Pažoutová *et al.* 1977). Until 1977 it was assumed that strains forming conidia under conditions of submerged cultivation are unsuitable for production of ergot alkaloids. The strain *C. paspali* MG-6 is another example of a sporulating production strain of *Claviceps*. As seen from the comparison of submerged cultures of strain MG-6 growing on media SP, MP and D, the number of conidia is directly proportional to the alkaloid production.

The actual production capacity of strain MG-6 is hard to estimate because of the reutilization of extracellular alkaloids after 15 d. The initial rate of alkaloid production between days 7 and 10 is analogous to that found in another high-production strain of *Claviceps*.

Claviceps paspali MG-6 was biochemically studied in detail. Ergometrine (0.1 mmol/L) was found to stimulate alkaloid production at the beginning of cultivation (Řeháček *et al.* 1971*a*, 1972); it also affected the activity of a number of enzymes of primary metabolism (Sajdl 1973). In this strain the produced alkaloids seem to play an autoregulatory role.

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