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Effect of Biotin on Alkaloid Production During Submerged Cultivation of *Claviceps* sp. Strain SD-58

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Addition of biotin to culture medium NL-406 significantly increased alkaloid yield during submerged cultivation of *Claviceps* sp. strain SD-58. Alkaloid yield was further enhanced by incorporating leucine in biotin-supplemented culture medium. Increased alkaloid production was associated with an increase in the lipid content of cells and in the number of chlamydospores. Biotin deficiency caused a reduction in alkaloid yield and a parallel decrease in lipid content and chlamydospore numbers.

Within the last few years, there has been a resurgence of interest in ergot alkaloids because the newer derivatives are potentially therapeutic agents for parkinsonism, acromegaly, amenorrhea-galactorrhea, suppression of postpartum lactation, and treatment of breast cancer and possibly cancer of the prostate (3, 5, 14, 16). Soon after the discovery that ergot alkaloids can be produced under saprophytic conditions, numerous investigations of their fermentative production by *Claviceps* spp. were performed (3, 11, 16). However, our understanding of the physiology of alkaloid formation is still poor. The present investigation is an extension of our previous work on the physiology of saprophytic *Claviceps* strains (9, 17, 24, 25; J. D. Desai and S. R. Shah, Folia Microbiol. (Prague), in press; H. C. Vaidya and J. D. Desai, Folia Microbiol. (Prague), in press). In contrast to Taber and Vining (23), we recently reported a close parallel between the rate of alkaloid synthesis and cell-lipid accumulation when *Claviceps purpurea* (Fr.) Tul was cultivated on different nitrogen sources (9). The addition of a higher concentration of KH_2PO_4 (0.1%) in the cultivation medium of *Claviceps* sp. strain SD-58 caused a significant reduction in the ability of cells to synthesize alkaloids with a concomitant fall in the lipid content of the cells (H. C. Vaidya, Ph.D. thesis, Sardar Patel University, Vallabh Vidyanagar, India). The role played by biotin-dependent carboxylation in lipid biosynthesis is now well established (13). The work reported here was undertaken to gain further insight into the physiology of alkaloid formation in *Claviceps* sp. strain SD-58. It deals with the effect of biotin on alkaloid production by shaken culture of *Claviceps* sp. strain SD-58.

Claviceps species strain SD-58 (ATCC 26019) from the American Type Culture Collection, Rockville, Md., was maintained on potato dextrose agar slopes by subculturing every 2 weeks, incubating at 25°C for 5 days, and storing at 5°C.

Inoculum preparation and the culture medium (NL-406) used for submerged cultivation were the same as those described by Robbers et al. (21). Culture medium NL-406 consisted of (grams per liter): mannitol, 50; sucrose, 50; succinic acid, 5.4; yeast extract (Difco Laboratories), 3; KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.3; $FeSO_4 \cdot 7H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.0044, and sufficient NH_4OH to bring the pH of the solution to 5.4. The medium was distributed in 250-ml Erlenmeyer flasks and sterilized by autoclaving. Sugars were separately sterilized and added to give 60 ml of medium per flask. Shaken (200 rpm) culture conditions were the same as those described earlier (24, 25; Desai and Shah, in press). Biotin deficiency in the fungus was created with avidin as described by Desai and Modi (6, 8).

Alkaloids in fermentation broth were estimated colorimetrically with the Van Urk reagent (26) as modified by Allport and Cocking (2), using elymoclavine as a standard. Total lipids were extracted with chloroform:methanol (2:1 [vol/vol]) and were freed from water-insoluble impurities by the method of Folch et al. (12). Numbers of chlamydospores in fermentation broths were counted as described earlier (24).

The results reported here are average values from at least three independent experiments.

Figure 1 illustrates the effect of biotin on growth and alkaloid production by *Claviceps* sp. strain SD-58. It is evident that the addition of biotin (3 μg/ml) caused about 75% stimulation in

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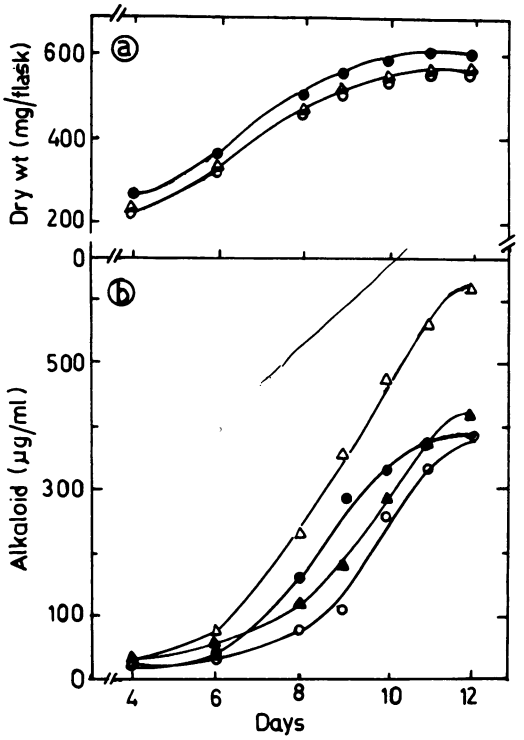

FIG. 1. Effect of biotin on growth (a) and alkaloid production (b) by *Claviceps* sp. strain SD-58. Cultures were grown in NL-406 (○) and NL-406 plus 1 μg (●), 2 μg (▲), and 3 μg (△) of biotin per ml.

alkaloid yield without affecting the growth. Further addition of biotin failed to increase alkaloid yield (data not shown). The increase in alkaloid production by biotin was associated with an increase in lipid content of the cells (Table 1). Biotin deficiency in this culture caused about 45% reduction in alkaloid yield and 65% reduction in the cell-lipid accumulation. Addition of biotin to biotin-deficient cultures restored the alkaloid yield to the level in control cultures. Approximately a 16.5% increase in alkaloid yield was obtained by adding leucine to NL-406 medium. Interestingly, when biotin was supplied along with leucine in the culture medium, the alkaloid yield was more than double that of the control. The cell-lipid accumulation and chlamydospore numbers followed the pattern of alkaloid yields. A reduction in lipid content due to biotin deficiency has been reported (4, 7, 13). The involvement of acetyl-coenzyme A carboxylase, a biotin-dependent enzyme in biogenesis of mevalonic acid and one of the precursors for alkaloid synthesis, has been documented (10, 13). Active participation of methylcrotonyl-coenzyme A carboxylase, a biotin-dependent enzyme (13), in the synthesis of alkaloids by augmenting the supply of mevalonic acid can be suggested from the stimulatory effect of leucine plus biotin on alkaloid production. Neujahr and Bjork (15) demonstrated the operation of a major biotin-dependent and a minor independent pathway for the synthesis of mevalonate in *Blakeslea trispora*. A correlation between the activity of acetyl-coenzyme A carboxylase and alkaloid synthesis has been demonstrated (18, 20, 28). The increase in chlamydospore numbers with increasing alkaloid production is consistent with observations made by Rehacek et al. (19), Vaidya and Desai (24), Voricek et al. (27), and Spalla (22).

TABLE 1. Effect of biotin, avidin, and leucine on lipid content, number of chlamydospores, and alkaloid yield during the submerged cultivation of *Claviceps* sp. strain SD-58[a]

Additions	Lipid content (mg/100 ml)	Chlamydospores (× 10^6/ml)	Alkaloid yield (μg/ml)
None (control)	14.3	1.9	351.08
Biotin	27.9	3.3	615.80
Avidin	4.9	1.4	197.90
Avidin + biotin	12.9	2.2	362.23
Leucine	16.0	2.4	409.10
Leucine + biotin	23.5	3.8	712.00

[a] Cells were cultivated in NL-406 medium with the indicated supplements. Biotin (180 μg), avidin (5 U), and leucine (60 mg) were added to each flask. After 12 days of submerged cultivation, lipid content of cells, chlamydospore numbers, and alkaloid yield were determined.

The data lead to the conclusion that the biotin level in the medium plays an important role in the expression of the *Claviceps* sp. strain SD-58 genotype. Cell-lipid accumulation parallels alkaloid yield, and there is no evident competition for their synthesis. The failure to observe any effect on the growth of culture, despite a significant increase in alkaloid yield when biotin or leucine was added, rules out an indirect effect and indicates that biotin and leucine have a role in alkaloid biogenesis. Thus, the results substantiate our earlier hypothesis that a common regulatory moment(s) participates in the synthesis of alkaloid and lipid (7), and they suggest further the involvement of biotin-dependent steps. However, the present study does not allow us to speculate on the mechanism by which chlamydospore formation is stimulated in response to the biotin level in the medium.

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