Acta Bot. Croat. 53, 39–47, 1994.

CODEN: ABCRA 2
ISSN 0365–0588

UDC 547.94:582.282,19 = 20
Original scientific paper

EFFECT OF ANTIFOAMS ON THE BIOSYNTHESIS OF ERGOT ALKALOIDS BY HIGH-PRODUCTIVE STRAIN OF *CLAVICEPS PASPALI* F–2057

SREĆKO MATOŠIĆ, MILENA MEHAK, JAGODA ŠUŠKOVIĆ, ZORAN GOLOB

(Faculty of Food Technology and Biotechnology. University of Zagreb)

Received December 13, 1993

The effect of several surfactans i. e. commercial antifoams on biosynthesis of ergot alkaloids was studied. Addition of some surfactants of polyglycole structure and Tweens to submerged cultures of high-productive strain of *C. paspali* F-2057 caused a change in alkaloid synthesis intensity. Pluronik (polyetoxypolypropoxy polymer) added in the range of 0.25 to 0.75% markedly stimulated the production of ergot alkaloids. The Pluronik-supplemented culture reached maximal alkaloid yields one or two days earlier than the control. Production of alkaloids increased twice. The maximal yield achieved was 5.35 gL^{-1} with the process productivity amounting to 17.2 $mgL^{-1}h^{-1}$.

Introduction

The biosynthesis of ergot alkaloids is regulated genetically as part of programs for culture differentiation and development and is controlled, like the synthesis of some primary metabolites, by common mechanisms. Permanent changes favouring alkaloid synthesis can be induced by manipulating (mutation by chemical mutagens or by irradiation with UV light) the strain genetic structure (Reháček 1980 and 1983, Kren et al. 1986, Didek-Brumec et al. 1987).

However, temporary changes and regulation in the biosynthesis of ergot alkaloids can be induced by manipulation of cultivation conditions and composition of medium. Biosynthesis of alkaloids depends on mechanisms of control of tryptophan biosynthesis. Both, the biosynthesis of alkaloids and the biosynthesis of tryptophan are inhibited in the culture of *C. purpurea* by

inorganic phosphate at concentrations that are optimal for the culture growth or do not inhibit the growth (Pažoutova et al. 1981, 1984). Incrased concentrations of inorganic phosphate, however, do not inhibit alkaloid synthesis in the culture of *C. paspali* (Matošić et al. 1983).

Aeration of the cultivation liquid has an opposite effect on the alkaloid production. A decreased respiration is one of the possible ways of increasing alkaloid formation. However opposite opinion has also been expressed (Rehaček 1980). Desai et al. (1986) and Mizrahi and Miller (1969) increased the production of alkaloids twice by adding Tween 80 to the cultivation medium containing a submerged culture of *C. paspali.* Dimethylsulfoxide increases the alkaloid yield by 50%. Both these surfactants affect permeability of the cytoplasmic membrane. By disturbing the permeation barrier, the cells increase the liberation of alkaloids from the region of their synthesis and thus prevent possible feedback inhibition (Rehaček 1980). Surfactants do not affect the biosynthetic pathway (Mizrahi and Miller 1968).

Increase of alkaloid formation by the addition of Tween 80 is accompanied by a shift in the organic acid and amino acid level in the cell pool (Rehaček 1971). From this it follows that the alkaloid biosynthesis is regulated by the level at the intracellular precursors of the primary metabolites. An increased concentration of numerous metabolites in the cell pool can also be attained by increasing the medium osmolarity. High osmolarity may favour production of alkaloids if in this way the carbon source may serve this function in addition to its nutritional role (Tonolo 1967, Rehaček 1991). In this regard, NaCl may spare some of the sugar requirement (Puc 1977).

The effect of different commercial surfactants, used in fermentation industry on biosynthesis of ergot alkaloids, was the subject of investigation presented in this paper.

Materials and Methods

The *microorganism Claviceps paspali* F–2057 from the Collection of the Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, was used in this study.

Culture media: Medium from maintenance of the culture was potato infusion-glucose-agar. Seed cultures were prepared in the medium (gL^{-1} tap water): succinic acid neutralized with ammonia to pH 5.2 50; mannitol 40; KH_2PO_4 1: $MgSO_4 \cdot 7H_2O$ 0.3. The production media (Matošić et al. 1984) contained (gL^{-1} tap water):

(A) succinic acid neutralized with ammonia to pH 5.2 50; mannitol 60; KH_2PO_4 1. $MgSO_4 \cdot 7H_2O$ 0.3.

(B) mannitol 200; bacto peptone 90; KH_2PO_4 1; $MgSO_4 \cdot 7H_2O$ 0.3; pH 6.4.

Media were sterilized for 30 min./110 °C.

Surfactants added to the media were commercial antifoams for fermentation processes, and are commercially known as: Tween 60, Tween 80, PEG, 500, PEG 150, Kontramin 24, Span 80, Pluronik L61 SCU, Etopon LSP, Concentrations used were (% V/V):0.01; 0.05; 0.10; 0.25; 0.50; 0.75.

Cultivation: culture was maintained on agar plates, and 2–weeks old single colony was used for inoculating the medium in each seed flask. All

fermentation experiments were carried out in two stages: a seed-stage fermentation for 6 days; a production-stage fermentation for 14 days: inoculated with 10% of the seed culture homogenized in a Waring blendor (14 000 min^{-1}). Cotton-wool-plugged 500 ml Erlenmeyer flasks containing 100 ml of culture media were incubated at 24 °C on a rotary shaker with a 6 cm stroke.

Analytical determination: alkaloids were determined spectrophotometrically, with reference to a standard solution of ergometrin base, by van Urk reagent (B a n k s et al. 1974). Biomass dry weight was determined by filtration and drying at 105 °C.

Results and Discussion

Several commercial surfactants were expected to improve the biosynthesis of ergot alkaloids. Unfortunately because of the complexity of metabolism of microorganism and the polymer structure of surfactants, empirical way is the only possible way to choose the appropriate antifoam for each single fermentation process. Two of surfactants chosen and used in this work were silicones (Etopan, Kontramin) and six others were different polyglycols. The results in Table 1 and Figures 1, 2, 3 and 4 show that Tweens Polyethyleneglycole 1500, Span and Pluronik (polyetoxypolypropoxy polymers) promote the rate of alkaloid production. Two different effects were obtained: shorter time of

Table 1. Productivity of the process of alkaloid biosynthesis in relation to addition of surfactants to the medium

	Surfactants (concentration %)		Maximal alkaloid yields			Process productivity ($mg \cdot L^{-1} \cdot h^{-1}$)	Biomass dry weight ($g \cdot L^{-1}$)
			Days of cultivation	Concentration ($g \cdot L^{-1}$)	% of control		
	Control	(∅)	14		100	7.9(1)	15.5(1)
(A)	Tween 60	(0.75)	14	3.23	112	9.6	23.0
	Tween 80	(0.75)	14	4.00	148	11.9	14.3
	Etopon LSP	(0.01)	0	0	0	0	0
	PEG 500	(0.01)	0	0	0	0	10.1
	PEG 1500	(0.25)	13	3.18	120	10.2	20.2
	Kontramin 24	(0.01)	14	1.65	62	4.9	15.9
	Span 80	(0.75)	14	5.30	195	15.8	21.2
	Pluronik L61SCU	(0.25)	13	5.40	214	17.3	10.3
(B)	Tween 60	(0.75)	16	5.20	179	13.5	35.7
	Tween 80	(0.75)	14	4.06	150	12.1	19.6
	PEG 1500	(0.75)	12	3.25	130	11.3	24.5
	Span 80	(0.75)	14	3.10	115	9.2	20.7
	Pluronik L61SCU	(0.75)	12	5.00	195	17.3	15.6

1 – Average value
A – Medium A
B – Medium B

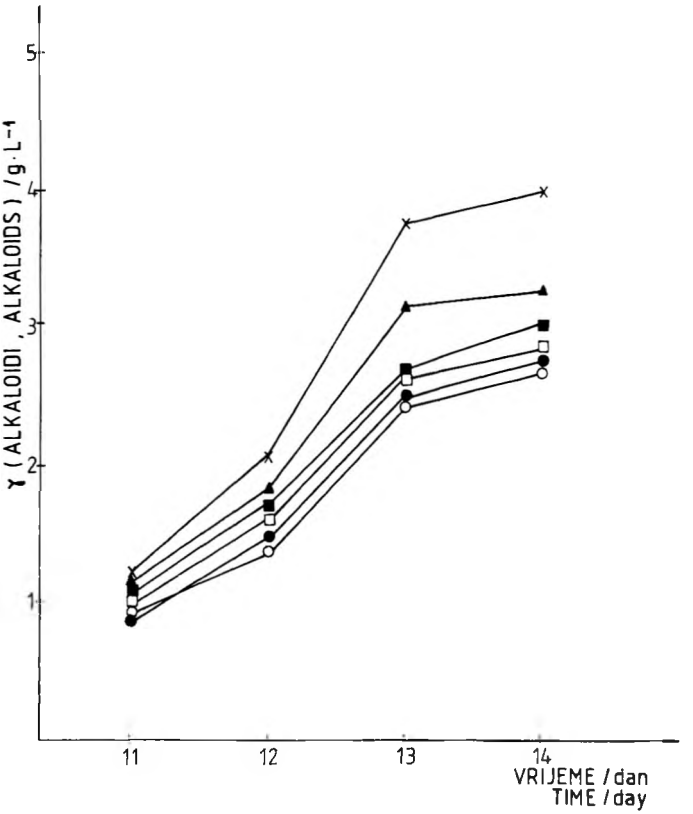

Fig. 1. Course of the process of alkaloid biosynthesis in relation to the concentration of surfactant Tween 80 added to the medium (cultivation in medim A). Concentrations of Tween 80(%): ○ – 0; ● – 0.05; □ – 0.10; ■ – 0.25; ▲ – 0.50; x – 0.75.

process duration when PEG 1500 and Pluronik were added to the medium (Figure 3, Table 1); and increase of maximal yields with five surfactants (Tween 60, 80; PEG 1500; Span; Pluronik) added to the fermentation medium. The maximal yields were reached in Pluronik-supplemented cultures (5.40 gL^{-1}), with the process productivity which amounted to 17.3 $mgL^{-1}h^{-1}$, or more than twice as much as control (Table 1).

Silicone antifoams used in experiments completely inhibit metabolism and growth of *C. paspali*, which generally agrees with the suggestion of Smith and Davis (1980), that silicones seem to provide good antifoam action with bacteria, while polyglycols are more effective with fungi. Inhibition of alkaloid biosynthesis caused by PEG 500 added to the fermentation medium, could be explained by a lower molecular weight of PEG 500 molecules in relation to PEG 1500 polymer (Table 1).

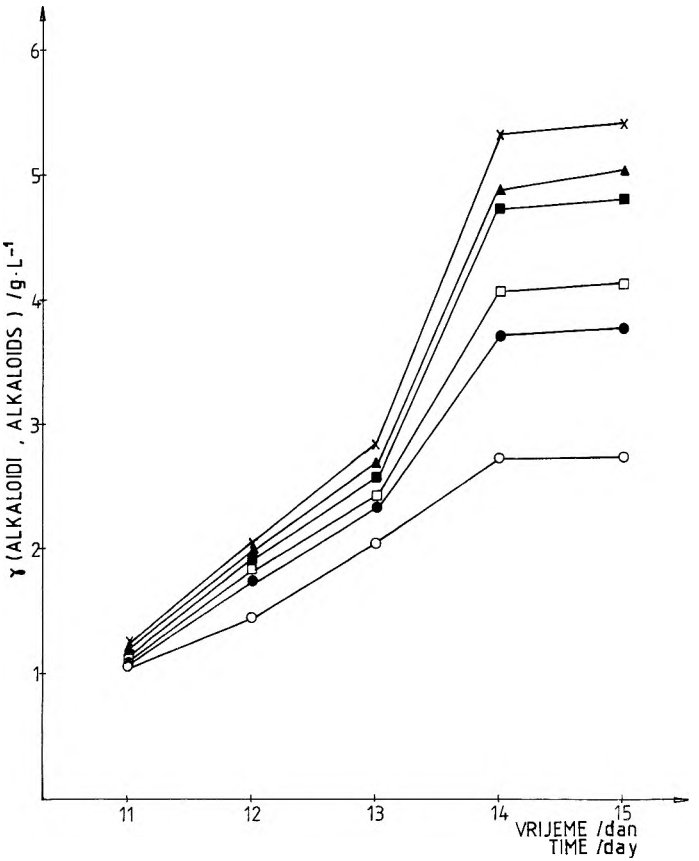

Fig. 2. Course of the process of alkaloid biosynthesis in relation to the concentrations of surfactants Span 80 added to the medium (cultivation in medium A). Concentrations of Span 80%: ○ – 0; ● – 0.05; □ – 0.10; ■ – 0.25; ▲ – 0.50; x – 0.75.

By changing the media composition (namely by replacing ammonia nitrogen by amino nitrogen of peptone) the effect of surfactants on the intensity of alkaloid synthesis also changed. The positive effect of Span diminished, but the effect of Tween 60 increased from 12% to 79%. Probably, the addition of Tween 60 to the medium, as can be seen in Figure 4, supports the growth of microorganism by facilitating transport of metabolites into the cells, with simultaneous increase of the intensity of alkaloid synthesis. There are some possible explanations of the effect of antifoams i. e. surfactants on the biosynthesis of ergot alkaloids.

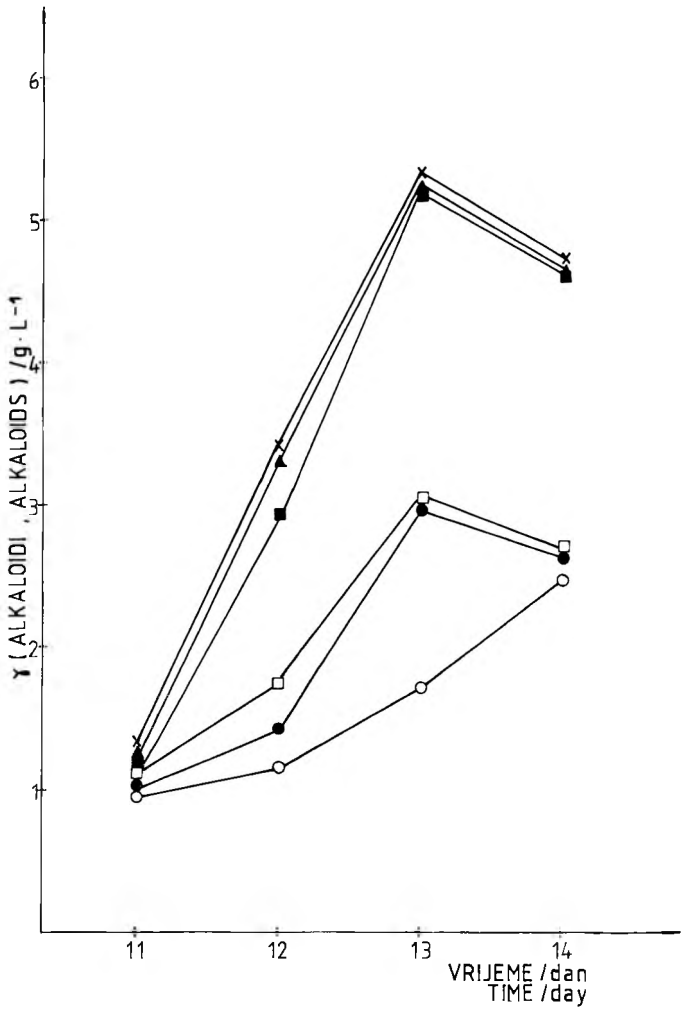

Fig. 3. Course of the process of alkaloid biosynthesis in relation to the concentrations of surfactant Pluronik L61SCU added to the medim (cultivation in medium A). Concentrations of Pluronik L61SCU (%): ○ – 0; ● – 0.05; □ – 0.10; ■ – 0.25; ▲ – 0.50; x – 0.75.

Surfactants probably, by wetting and lowering surface tension of the medium, affect the permeability of cytoplasmic membrane. By disturbing permeation barrier, the cells increase the liberation of alkaloids from the region of their synthesis and thus prevent possible feedback inhibition. Surfactants do not affect the biosynthetic pathway but promote the utilization

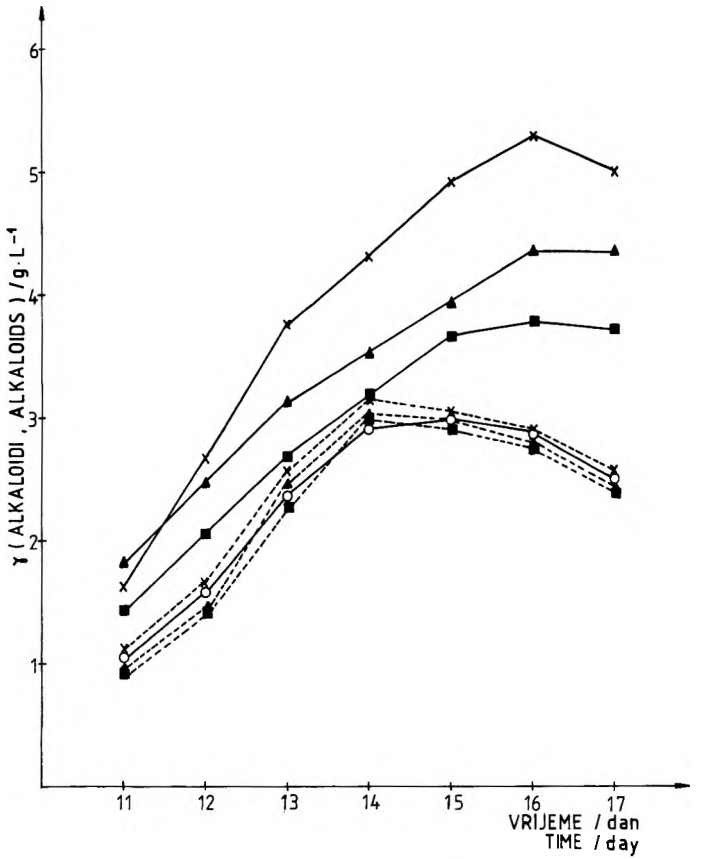

Fig. 4. Effect of surfactant Tween 60 on biosynthesis of ergot alkaloids during the cultivation *C. paspali* F–2057 in medium A (-----) and medium B(——).
Concentrations of Tween 60(%): o – 0; ■ – 0.25; ▲ – 0.50; x – 0.75.

of metabolites (M i z r a h i and M i l l e r 1969). The alkaloids formation by addition of surfactants is also accompanied by a shift in organic and amino acid level in the cell pool, which are intracellular precursors of alkaloid biosynthesis (R e h a č e k 1971, 1980).

C o n c l u s i o n

Addition of several polyglycols and Tweens to submerged cultures of high productive strain of *Claviceps paspali* F–2057 caused a temporary change in alkaloid biosynthesis intensity. Two different effects were obtained: (1) shortening of process duration and (2) increasing the maximal yields of alkaloids. The maximal yields reached in the Pluronik-supplemented cultures of *C. paspali* increased twice in relation to control.

References

Banks, G. T, P. G. Mantle, C. A. Szczyrbak 1974: Large scale production of clavine alkaloids by *Claviceps fusiformis.* J. gen. Microbiol. *82.* 345–361.

Desai, J. D, H. C. Patel, A. J. Desai 1986: Effect of Tween series surfactants on alkaloid prodiction by submerged culture of *Clavicepss*species. J. Ferment. Tehnol. *6,* 499–501.

Didek-Brumec M., A. Puc, H. Sočič, M. Alačevič, 1987: Isolation and characterisation of a high-yielding *Claviceps purpurea* strain producing ergotoxines. Prehrambeno-tehnol. biotehnol. rev. *25,* 103–109.

Kren. V., S. Pažoutova, P. Smedera, V. Rilka, Z. Rehaček, 1986: High – producing mutant of *Claviceps purpurea* 59 accumulating secoclavines. FEMS Microbiology Letters, *37,* 31–34.

Matošič, S., J. Kunčič, G. Tamburašev, 1983: Influence of concentration of phosphorus on biosynthesis of ergot alkaloids in culture of *Claviceps paspali.* Prehrambeno-tehnol. rev *21,* 151–154.

Matošič, S., V. Bašič, M Lišnjič, 1984: Biosynthesis of ergot alkaloids by means of fungus *Claviceps paspali* Stevens and Hall. Acta Bot. Croat. *43,* 59–64.

Mizrahi, S., G. Miller, 1968: Effect of glycol on the production of ergot alkaloids by *Claviceps paspali.* Biotechnol. Bioeng. *10,* 102–103.

Mizrahi, A., G. Miller, 1969: Role of glycols and Tweens in production of ergot alkaloids by *Claviceps paspali* J. Bacteriol. *97,* 1155–1159.

Pažoutova S., J. Votruba, Z. Rehaček, 1981: A mathematical model of growth and alkaloid production in the submerged culture of *Claviceps purpurea.* Biotechnol. Bioeng. *23,* 2837–2849.

Pažoutova, S., Z. Rehaček, 1984: Phosphate regulation of phosphatases in submerged cultures of *Claviceps purpurea* producing clavine alkaloids. Appl. Microbiol. Biotechnol. *20,* 389–392.

Puc, A., H. Sočič, 1977: Carbohydrate nutrition of *Claviceps purpurea* for alkaloid production related to the osmolality of media. European J. Appl. Microbiol. *4,* 283–287.

Rehaček, U., S. C. Basappa, 1971: Effect of Tween 80 on alkaloid-producing cultures of *Claviceps paspali.* Folia microbiol. *16,* 110–113.

Rehaček, Z. 1980: Ergot alkaloids and their biosynthesis. Advances in Biochemical Engineering (A. Fiechter ed.), Springer – Verlag, Berlin, Heidelbarg, New York, *14,* 33–60.

Rehaček, Z. 1983: New trends in ergot alkaloids biosynthesis. Process Biochemistry, January/Febraury, 22–23.

Rehaček, Z. 1991: Physiology of ergot alkaloid synthesis. Biotechnology – Current Progress (P. N. Cheremisinoff. L. M. Ferrante, eds.). Technomic Publish. Comp. Lancaster, Basel, *1,* 275–276.

Smith, R. V., P. J., Davis, 1980: Induction of xenobiotic monooxigenases. Advances in Biochemical Engineering (A. Fichter ed.). Springer – Verlag, Berlin, Heilderberg, New York *14,* 62–100.

Tonolo, A., 1967: Some considerations on the production of alkaloids in artifical by cultures by species of *Claviceps.* Tul. Ann. Ist. Super. Sanita *3.* 613–628.

SAŽETAK

UTJECAJ TVARI ZA SPREČAVANJE PJENJENJA NA BIOSINTEZU ERGOT-ALKALOIDA PRI KULTIVACIJI VISOKO PRODUKTIVNOG SOJA *CLAVICEPS PASPALI* F–2057

Srećko Matošić, Milena Mehak, Jagoda Šušković, Zoran Golob

(Prehrambeno-biotehnološki fakultet Sveučilišta u Zagrebu)

Proučavan je utjecaj nekoliko različitih površinski aktivnih tvari, odnosno komercijalnih tvari za sprečavanje pjenjenja fermentacijskih hranjivih podloga na biosintezu ergot-alkaloida. Dodatak površinski aktivnih tvari poliglikolne strukture i tweena u hranjivu podlogu pri kultivaciji visoko produktivnog soja *Claviceps paspali* mijenja intenzitet biosinteze alkaloida. Dodatak pluronika

(polietoksipolipropoksi polimera) u koncentraciji od 0.25 do 0.75% znakovito potiče proizvonju ergot-alkaloida. Pri kultivaciji uz dodatak pluronika maksimalni prinosi alkaloida se postižu jedan ili dva dana ranije nego pri kontrolnoj kultivaciji, a proizvodnja alkaloida je bila udvostručena. Maksimalni postignuti prinosi iznose 5,40 gL^{-1}, pri proizvodnosti procesa 17,3 $mgL^{-1}h^{-1}$.

Prof. dr. Srećko Matošić
Mr. Milena Mehak
Mr. Jagoda Šušković
Zoran Golob, dipl. inž.
Zavod za biokemijsko inženjerstvo
Prehrambeno biotehnološki fakultet
Sveučilište u Zagrebu
Pierottijeva 6,
41 000 Zagreb, Hrvatska (Croatia)