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Effect of Tween Series Surfactants on Alkaloid Production by Submerged Cultures of *Claviceps* Species

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Supplementation of Tween surfactants promoted alkaloid production by submerged cultured of *Claviceps* sp. strain SD-58. Tween 80 (0.5%) exhibited the maximum (2-fold) stimulatory effect when added to the medium at the initial stage of cultivation. The stimulation of alkaloid production by Tween 80 was found to be associated with the increase in cell mass, higher consumption of nutrients and enhanced excretion of alkaloids from the cells. The results are discussed in relation to the physiology of alkaloid production in *Claviceps* sp. strain SD-58.

Earlier work from this laboratory demonstrated that alkaloid production by *Claviceps* sp. Strain SD-58 is sensitive to phosphate concentration in medium.[1,2] The lower accumulation of tryptophan and higher ATP generation due to carbon catabolic shift,[3,4] repression of chlamydospore formation[1] and inhibition and repression of phosphatases[5,6] are some of the factors in the lower production of alkaloids in high-phosphate-containing medium. The enhancement of alkaloid production by Tween surfactants is well documented. However, the mechanism by which Tween surfactants exert such effect is not clearly understood. In continuation of our work on the physiology of alkaloid production, we studied effect of the Tween series of surfactants on alkaloid production by submerged cultures of *Claviceps* sp. strain SD-58.

Materials and Method

Claviceps sp. strain SD-58 (ATCC 26019) was maintained on potato dextrose agar slopes by subculturing every two weeks at 25°C for five days, and storing at 5°C.

Inoculum preparation, composition of NL-406 medium and submerged cultivation conditions were the same as described earlier.[1,7]

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Alkaloid was estimated by the method of Allport and Cocking,[8] using elymoclavine as a standard. Sucrose and mannitol from the fermentation broth were estimated by the methods of Morris[9] and Burton,[10] respectively. The methods of Fewcett and Scott,[11] and Chaeftel *et al.*[12] were used for the estimation of ammonia and succinic acid, respectively.

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

Results and Discussion

Tween series surfactants exhibited a stimulatory effect on alkaloid production during the submerged cultivation of *Claviceps* sp. SD-58 (Table 1). Of the Tween surfactants tested, Tween 80 (0.5%) showed maximum effect on alkaloid production, giving about 140% higher alkaloid yield than that of the control. However, supplementation of the major fatty acid of each Tween surfactant to the medium (50 μg/ml) had no stimulatory effect on alkaloid production (data not shown). This suggested that the entire molecule of Tween surfactant is essential for the observed effect.

The effects of some other surface active agents on alkaloid production are shown in Table 2. Polyethylene glycol, ethylene glycol and 1, 2 propanediol stimulated alkaloid production, although to lesser extents than Tween 80. In contrast, Triton-X, 2,3 butendiol, 1,3 butendiol, 1,3 cyclohexendiol and 1,2 cyclohexendiol showed com-

Table 1. Effect of Tween surfactants on alkaloid production* by submerged cultures of *Claviceps* sp. strain SD-58.

Addition (%, v/v)	Tween surfactant			
	20	40	60	80
0.0	210	224	228	225
0.01	280	320	298	465
0.1	310	356	401	495
0.2	330	375	418	510
0.5	337	385	432	540
0.6	337	387	435	540

* Values are expressed as μg/ml after 10 days of fermentation.

plete inhibition of alkaloid production.

As illustrated in Fig. 1a, addition of Tween 80 in the early stage of the fermentation was beneficial to alkaloid production. Addition of Tween 80 also produced a parallel increase in cell mass (Fig. 1b). Converted to alkaloid production in μg/mg of dry cells, these data give values of 43.5, 38.4, 38.0 and 27.3 for the 12-day cultures to which Tween 80 had been supplemented on the 0, 3rd, 5th and 9th days, respectively. These data indicate that the increase in alkaloid production by supplementation of Tween surfactants is not only due to the increase in numbers of producing cells (Fig. 1b), but also to the increase in the efficiency of cells to produce alkaloids. The efficiency was highest when Tween 80 was added at the beginning of fermentation (43.5 μg/mg dry cells) and the lowest when Tween 80 was added on the 9th day of fermentation (27.3 μg/mg dry cells).

Table 2. Effects of some surface active agents other than Tween surfactants on alkaloid production (μg/ml) by submerged cultures of *Claviceps* sp. strain SD-58.

Addition (0.5%, v/v)	Alkaloid produced (μg/ml)				
	Cultivation time (days)				
	2	4	6	8	10
None	15	18	35	108	224
Polyethylene glycol	25	48	131	261	531
Ethylene glycol	21	39	110	252	512
1,2 Propandiol	22	37	111	251	470
Tween 80	30	51	128	272	541

Addition of Tween 80 caused about 15–20% increase in the consumption of major nutrients from the medium and about 50% reduction in the intracellular accumulation of alkaloids (Table 3). The increase in nutrient consumption on Tween 80 supplementation may be attributed to the higher uptake of nutrients due to the change in permeability of cells and/or stimulation of the metabolism of nutrients. The change in permeability of fungal cells on exposure to Tween surfactants is well documented.[13–15] On the other hand, the increase in cell mass (Fig. 1b) and in the efficiency of cells for alkaloid synthesis upon Tween 80 addition during fermentation suggests the stimulation of metabolic activities of *Claviceps* sp. SD-58.

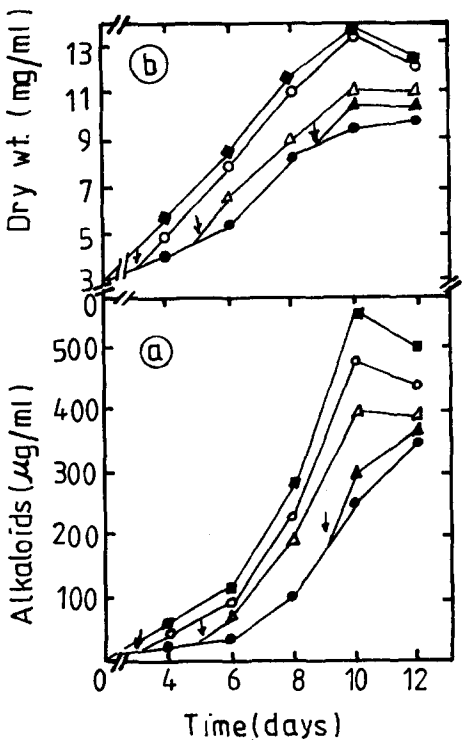

Fig. 1. Effect of Tween 80 on alkaloid production (a) and growth (b) during the submerged cultivation of *Claviceps* species strain SD-58.
Arrows indicate the time of Tween 80 (0.5% v/v) supplementation during the fermentation. Control (●) was not supplemented with Tween 80.

Table 3. Effect of Tween 80 on nutrient consumption and intracellular alkaloid level during the submerged cultivation of *Claviceps* sp. strain SD-58.

	Without Tween 80	With Tween 80
Nutrient consumption (g/flask)		
Sucrose	1.49	1.90
Mannitol	2.50	2.90
Succinic acid	0.19	0.24
Ammonium ion	0.09	0.14
Intracellular alkaloid (μg/ml)	390	190

Fermentation was carried out in NL-406 medium without (control) and with 0.5% (v/v) Tween 80 as described in the text. The amounts of residual nutrients after 10 days of fermentation were determined and subtracted from the initial values to calculate consumption. The intracellular alkaloid was calculated on the basis of 4 μl water/mg dry cells.[20]

The change in cell permeability of *Claviceps* sp. SD-58 on supplementation of Tween-80 was also evident in the leakage of alkaloids from the cells into the medium (Table 3). The excretion of alkaloid from the cells may relieve the inhibition of alkaloid synthesis leading to higher alkaloid production. The accumulation of alkaloids in the cells has been found to cause inhibition and repression of alkaloid synthesis.[16,17] The inhibition of anthranilate synthetase,[17] dimethylallyltryptophan synthetase[18] and chanoclavine cyclase[19] by ergot alkaloids, has also been documented.

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