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Biotransformation of molasses to ergot alkaloids by *Claviceps purpurea BPS-1660* exposed to N, N-dimethyl dodecylamine (N, N-dMDA)

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Abstract : The influence of N, N-dimethyl dodecylamine (N, N-dMDA) on biotransformation of molasses to ergot alkaloids by *Claviceps purpurea* BPS-1660 has been studied. It has been found that micelle under trial has enhanced the yield of ergot alkaloids to an extent of 7.065% higher in comparison to control.

(Key words: Ergot alkaloids fermentation, N, N-dimethyl dodecylamine (N, N-dMDA) and *Claviceps purpurea* BPS-1660).

Introduction

Micelles are formed when surfactants are dissolved in water. The micelles are clusters of surfactant molecules with definite shapes and sizes. However, the shape and size of the micelle are functions of the nature of the system.[1-11] It is well known that nature provides some versatile compounds without which it would have been difficult for the life to persist. Surfactants are one such group of compounds which are used in various fields of science from electronics to biology.[12-15]

Thus, from the above brief review it is evident that there is no definite opinion regarding the influence of micelles on the production of ergot alkaloids and in view of this the author has studied the influence of N, N-dimethyl dodecylamine (N, N-dMDA) on biotransformation of molasses to ergot alkaloids by *Claviceps purpurea* BPS-1660.

Experimental

The effect of micelle, i.e., N, N-dimethyl dodecylamine (N, N-dMDA) on the fermentative production of ergot alkaloids has been studied . The experimental results have been described in table -1.

Temperature : $27^0C \pm 1^0C$

Assay method :

Evaluation of ergot alkaloids formed was made colorimetrically[16-18].

Sterilization :

At 15 lbs steam pressure for 30 min. in an autoclave

Organism used :

Claviceps purpurea BPS-1660 was used as source of enzymes for production of ergot alkaloids.

Age of inoculum : 50h

Quantum of inoculum : 10ml

Incubation periods : 6, 8 and 10 days

Optimum incubation period : 8 days

Vegetative medium[19] The composition of the vegetative medium was as follows :

Dextrose : 125.00 g; Citric acid : 15.00 g; Yeast extract : 0.15 g; KH_2PO_4: 0.60g; $MgSO_4.7H_2O$:0.35g; $FeSO_4.7H_2O$: 0.010g; $ZnSO_4.7H_2O$: 0.020g; pH : 5.4; Distil water : 1000 ml.

The pH of the medium was adjusted to 5.4 by adding requisite amount of NH_4OH solution. Now 100 ml of the vegetative medium was taken into 250 ml conical flask. The flasks were then plugged and sterilised in an autoclave at 15 lbs steam pressure for 30 minutes.

Table - 1
Biotransformation of molasses to ergot alkaloids by *Claviceps purpurea BPS-1660* exposed to N, N-dimethyl dodecylamine (N, N-dMDA)

Concentration of micelle a x 10^{-3} M	*Yield of Ergot -alkaloids in mg/litre			% of ergot alkaloids increase (+) in 8 days of incubation period
	6 days	8 days	10 days	
1.0 x 10^{-3} M (+) micelle	601	928	881	(+) 0.869
2.0 x 10^{-3} M (+) micelle	619	939	889	(+) 2.065
3.0 x 10^{-3} M (+) micelle	633	948	893	(+) 3.043
4.0 x 10^{-3} M (+) micelle	650	960	910	(+) 4.347
5.0 x 10^{-3} M (+) micelle	671	975	918	(+) 5.978
6.0 x 10^{-3} M** (+) micelle	680	985***	925	(+) 7.065
7.0 x 10^{-3} M (+) micelle	666	980	919	(+) 6.521
8.0 x 10^{-3} M (+) micelle	658	970	913	(+) 5.434
9.0 x 10^{-3} M (+) micelle	642	955	901	(+) 3.804
10.0 x 10^{-3} M (+) micelle	631	943	893	(+) 2.500
Control (-) micelle	518	920	886	—

* Mean of three observations.
** Optimum concentration of micelle used
*** Optimum yield of ergot alkaloids
(+) values indicate % increase (+) after 8 days. Experimental deviation (±) 1.5% to 3.5%

Inoculation of the vegetative medium

The vegetative medium was inoculated by transferring 5 disc of mycelium (each of 10 mm dia) from 8days old slant.

Preparation of the production medium

The composition of the production medium employed throughout the investigation was as follows :

Sugar : 200.00 g; Citric acid : 20.00 g; Yeast extract : 0.15 g; KH_2PO_4: 0.55g; $MgSO_4.7H_2O$:0.55g; KCl : 0.15 g; $FeSO_4.7H_2O$: 0.010g; $ZnSO_4.7H_2O$: 0.008g; pH : 5.4; (Adjusted by NH_4OH solution)
Distil water to make up 1000 ml.

Now from the above composition 100 ml of the production medium was taken into 250 ml conical flask. The flasks were plugged and sterilized in an autoclave.

100 ml of vegetative medium was taken into 250 ml flasks. The flasks were plugged with non-absorbent cotton and were sterilized in an autoclave at 15 lbs steam pressure for 30 minutes and were left for cooling at room temperature. Now, two flasks were inoculated each with 5 discs (each disc of 10 mm dia). of 8 days old *Claviceps purpurea* B.P.S. 1660. The flasks were kept on a rotary shaker (230 rpm) at 27 ± 1^0C for 8 days. After 8 days the content of the flask was homogenised in a sterile mixer for 10 seconds. This is the vegetative stage I. For second vegetative stage the flasks were taken each containing 100 ml of the same vegetative medium. Now, each flask was inoculated with 10ml of inoculum from vegetative stage I. These flasks were put on a rotary shaker operating at 230 rpm for 50 hours. After 50 hours 10 ml of the inoculum was used to inoculate the production medium.

99-flasks, each containing 100 ml of the production medium was taken for production stage. These were arranged into 11 sets, each set consisting of 9-flasks.

Now, M/10 solution of N, N-dimethyl docecylamine (N, N-d MDA) was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml of this solution was added in the flasks from 1st to 10th set respectively. Remaining one set was kept as control and it contained no (N, N-d MDA). Each flask was inoculated with 10 ml of the inoculum from IInd vegetative stage. The flasks were kept on a rotary shaker operating at 230 rpm at temperature 27 ± 1^0C. Colorimetric analysis were carried out after 6, 8 and 10 days of incubation period.

Results and Discussion

The influence of N, N-dimethyl docecylamine (N, N-d MDA)

The data recorded in the table -1 shows that like the micelle N-dimethyl docecylamine (N, N-d MDA) has given significant yield of ergot alkaloids.

The maximum yield of ergot alkaloids, i.e. 985 mg/litre in the presence of 6.0×10^{-3}M concentration of N, N-dimethyl docecylamine (N, N-d MDA) has been observed which is 7.065% higher in comparison to control fermenter flasks, i. e., 920 mg/litre in 8 days of optimum incubation period. It has been found that the gradual addition of N, N-dimethyl docecylamine (N, N-d MDA) to the production of ergot alkaloids by submerged fermentation from 1.0×10^{-3}M to 6.0×10^{-3}M gradually increases the production of ergot alkaloids and a maximum yield of ergot alkaloids has been obtained at 6.0×10^{-3}M concentration of N, N-dimethyl docecylamine (N, N-d MDA).

It has been found that further addition of the micelle N-N-dMDA from concentrations 7.0×10^{-3}M to 10×10^{-3}M has caused continuous fall of ergot alkaloids production in the order 6.521%, 5.434%, 3.804% and 2.500% respectively. However it is interesting to note that the production of ergot alkaloids exposed to any experimental concentrations of N, N-dimethyl docecylamine (N, N-d MDA), i.e., from 1.0×10^{-3}M to 10×10^{-3}M has been found higher in comparison to control flasks, i. e. 920 mg/litre in 8 days of optimum incubation period.

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