

## Studies on ergot alkaloids fermentation exposed to aluminium dodecyl sulfate

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**Abstract** : Some of the unique characteristics of reverse micelles MC's make them very useful for biotechnological application. The characteristic size of micelles toward the pharmaceutical uses varies from 10 to 80 nm. The influence of aluminium dodecyl sulfate on ergot alkaloids fermentation 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 6.737% higher in comparison to control when 200 g sugar solution is allowed to ferment at 27°C temperature, 5.4 pH and 8 days of optimum incubation period.

**(Key words):** Ergot alkaloids fermentation, aluminium dodecyl sulfate and *Claviceps purpurea* BPS-1660).

### Introduction

Ergot alkaloids are a class of indole derivatives produced by the genera of *Ascomycota* including *Claviceps*, *Aspergillus*, *Penicillium*, and *Epichloa*. Many natural and semi-synthetic ergot alkaloids exhibit valuable pharmacological activities and have been widely used in the therapy of human CNS disorders. Owing to the development of genome sequencing technology, the gene clusters involved in the biosynthesis of ergot alkaloids have been identified from these fungi. In this article, we briefly introduce the pharmacological activities and possible mechanisms of action of some ergot alkaloids<sup>1-3</sup>. Then we summarize the recent progress in the functional characterization of the key genes and gene clusters involved in the biosynthetic pathways of ergot alkaloids from different genera. Particularly, we summarize and discuss the constructions of ergot alkaloid biosynthetic pathways in different heterologous

hosts and the optimization strategies performed on the recombinant strains, which provide references for producing ergot alkaloids and the derivatives in cell factories by synthetic biology in the future<sup>6-10</sup>.

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.<sup>11-21</sup> 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.<sup>22-27</sup>

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 aluminium dodecyl sulfate on ergot alkaloids fermentation by *Claviceps purpurea* BPS-1660.

### Experimental

The effect of micelle, i.e., aluminium dodecyl sulfate on the fermentative production of ergot alkaloids has been studied. The experimental results have been described in table -1.

Temperature : 27°C ± 1°C

#### Assay method :

Evaluation of ergot alkaloids formed was made colorimetrically<sup>28-30</sup>.



Table - 1

## Studies on ergot alkaloids fermentation exposed to aluminium dodecyl sulfate

Concentration of micelle $a \times 10^{-3} \text{ M}$	*Yield of Ergot -alkaloids in mg/litre			% of ergot alkaloids increase (+) in 8 days of incubation period
	6 days	8 days	10 days	
Control	609	935	890	—
$1.0 \times 10^{-3} \text{ M}$	618	942	896	(+) 0.748%
$2.0 \times 10^{-3} \text{ M}$	622	950	899	(+) 1.604%
$3.0 \times 10^{-3} \text{ M}$	630	960	915	(+) 2.673%
$4.0 \times 10^{-3} \text{ M}$	638	969	920	(+) 3.636%
$5.0 \times 10^{-3} \text{ M}^{**}$	645	998***	926	(+) 6.737%
$6.0 \times 10^{-3} \text{ M}$	639	970	921	(+) 3.743%
$7.0 \times 10^{-3} \text{ M}$	632	965	916	(+) 3.208%
$8.0 \times 10^{-3} \text{ M}$	620	955	901	(+) 2.139%
$9.0 \times 10^{-3} \text{ M}$	614	940	895	(+) 0.534%
$10.0 \times 10^{-3} \text{ M}$	611	938	891	(+) 0.320%

\* 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%

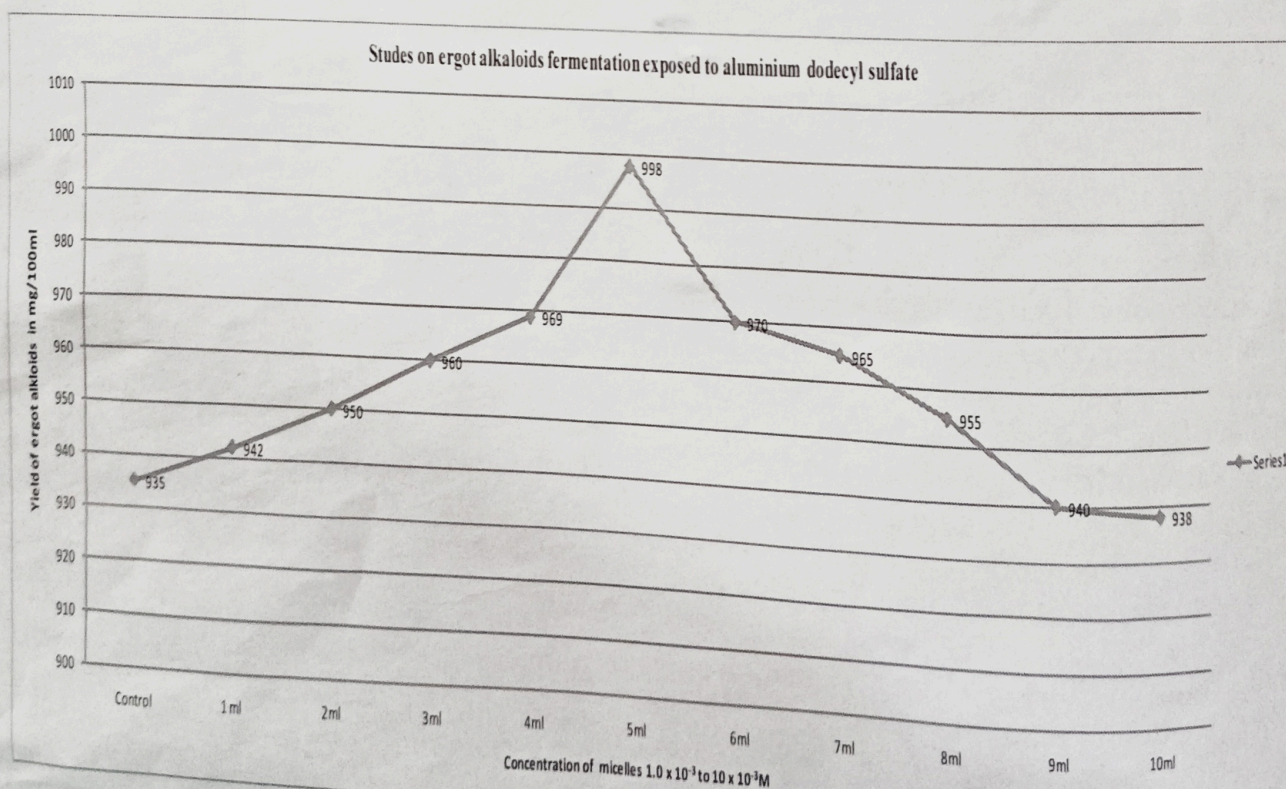


Figure-1



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<sup>11</sup> The composition of the vegetative medium was as follows :

Dextrose : 125.00 g; Citric acid : 15.00 g; Yeast extract : 0.15 g;  $\text{KH}_2\text{PO}_4$  : 0.60g;

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  : 0.35g;

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  : 0.010g;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  : 0.020g;

pH : 5.4; Distil water : 1000 ml.

The pH of the medium was adjusted to 5.4 by adding requisite amount of  $\text{NH}_4\text{OH}$  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.

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  $27 \pm 1^\circ\text{C}$  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 or

production stage.

These were arranged into 11 sets, each set consisting of 9 flasks. Now, M/10 solution of aluminium dodecyl sulfate 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 aluminium dodecyl sulfate. Each flask was inoculated with 10 ml of the inoculum from 11th vegetative stage. The flasks were kept on a rotary shaker operating at 230 rpm at temperature  $27 \pm 1^\circ\text{C}$ . Colorimetric analysis were carried out after 6, 8 and 10 days of incubation period.

## Results and Discussion

### The influence of aluminium dodecyl sulfate

The data recorded in the table -1 & Figure -1 shows that the micelle aluminium dodecyl sulfate has given significant yield of ergot alkaloids.

The maximum yield of ergot alkaloids, i.e. 998 mg/litre in the presence of  $5.0 \times 10^{-3}\text{M}$  concentration of aluminium dodecyl sulfate has been observed which is 6.737% higher in comparison to control fermenter flasks, i.e., 935 mg/litre in 8 days of optimum incubation period. It has been found that the gradual addition of aluminium dodecyl sulfate to the production of ergot alkaloids by submerged fermentation from  $1.0 \times 10^{-3}\text{M}$  to  $6.0 \times 10^{-3}\text{M}$  gradually increases the production of ergot alkaloids and a maximum yield of ergot alkaloids has been obtained at  $5.0 \times 10^{-3}\text{M}$  concentration of aluminium dodecyl sulfate. It has been found that further addition of the aluminium dodecyl sulfate from concentrations  $6.0 \times 10^{-3}\text{M}$  to  $10 \times 10^{-3}\text{M}$  has caused continuous fall of ergot alkaloids production in the order 3.743%, 3.208%, 2.139%, 0.534% and 0.320% respectively.

However it is interesting to note that the production of ergot alkaloids exposed to any experimental concentrations of aluminium



dodecyl sulfate, i.e., from  $1.0 \times 10^{-3} \text{M}$  to  $10 \times 10^{-3} \text{M}$  has been found higher in comparison to control

flasks, i. e., 935 mg/litre in 8 days of optimum incubation period.

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