

Micellar impact on biotic production of ergot alkaloids

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Abstract: The impact of potassium lauryl sulfate on ergot-alkaloids fermentation by some fungal strains such as *Claviceps fusiformis* MKR-122, *Claviceps africana* MKR-125, *Claviceps paspali* MKR-128 and *Claviceps purpurea* MKR-130 has been assessed. It has been found that the fungal strain of *Claviceps purpurea* MKR-130 attracts much attention for good ergot-alkaloids production. It has been found that maximum ergot-alkaloids, i.e, 1025 mg/L has been achieved from 230g glucose which is 11.413% higher in comparison to control at 6.5×10^{-5} M molar concentration of potassium lauryl sulfate under optimised parametric condations such as 27°C temperature 5.2, pH and 8 days of optimum incubation period.

(Key words : Ergot alkaloids fermentation, potassium lauryl sulfate and *Claviceps purpurea* MKR-130

Introduction

Ergot alkaloids are a class of indole derivatives having biological and medicinal activities. Over the past decades, the pharmacological activities of these compounds have been extensively studied¹ In the Middle Ages, ergot alkaloids were thought to be natural toxins with an impact on human health². It is well known that the main producers of ergot alkaloids are members of the genus *Claviceps*, which belongs to Ascomycotina in taxonomy. As a parasitic fungus, *Claviceps* can grow on grass or grain and the ingestion of the infected grass or grain by humans or animals led to the breakout of severe epidemics.^{3,4} In 1850, a clearer understanding of ergot poisoning was provided when the French scientist Tulasne

comprehensively elucidated the life cycle of *Claviceps*. The Swiss biochemist Stoll first isolated ergotamine in 1918, and the Swiss psychiatrist Maier found it to be effective for the treatment of migraines in 1926, marking the beginning of the modern study of ergot alkaloids.^{5,6} In recent years, other genera of Ascomycota including *Aspergillus*⁷ and *Penicillium*,⁸ even *Epichloë* have also been proven to produce ergot alkaloids⁹⁻¹⁰.

The fermentation process requires 5 to 7 days; while using the known processes 8 to 12 days were required for the production of peptide-type alkaloids in appreciable amounts. Due to the decreased pigment production and the low amount of mycelia, the alkaloids can be removed more easily from the culture broth.

The production of the high yields of lysergic acid alkaloids by submerged cultures of *Claviceps* species has been achieved after some 40 years of effort. The first successful processes involved the production of simple lysergic acid derivatives, and these are now well established. The products of these fermentations may be readily hydrolyzed to lysergic acid that, in turn, can be chemically converted to various pharmacologically active amides

Micelles can be defined as molecular aggregates formed by substances dispersed in a liquid phase with which they form a colloid, which is a mixture that has insoluble particles that remain permanently dispersed in another substance. The micellar state is characterized by microcapsules or bubbles of lipids (organic substances with little

or no solubility in water) that have hydrophobic and hydrophilic ends in their molecular structure and are capable of reducing the surface tension of the medium in which they are dispersed, meaning they have surfactant effect. Due to their characteristics, micelles are capable of keeping molecules inside and preventing them from being solubilized or dispersed. Today, science has found various practical applications for micelles. Micelles are widely used in industrial and biological fields for their ability to dissolve and move non polar substances through an aqueous medium, or to carry drugs which are, often, scarcely soluble in water. The carrying ability of micelles can be altered if parameters determining their size and shape are changed. However, the shape and size of the micelle are functions of the nature of the system.¹¹⁻²¹ 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.²²⁻²⁸

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 potassium lauryl sulfate on ergot alkaloids fermentation by *Claviceps purpurea* MKR-130.

Experimental

The impact of micelle, i.e., potassium lauryl sulfate on ergot alkaloids fermentation by *Claviceps purpurea* MKR-130 has been studied. The experimental results have been described in table -1.

Temperature : $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$

Assay method :

Evaluation of ergot alkaloids formed was made colorimetrically²⁹⁻³².

Sterilization :

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

Organism used : fermentation by *Claviceps*

purpurea MKR-130 was used as source of enzymes for production of ergot alkaloids.

Age of inoculum : 50h

Quantum of inoculum : 10ml

Incubation periods : 4, 8 and 10 days

Optimum incubation period : 8 days

Vegetative medium²² The composition of the vegetative medium was as follows :

Dextrose : 130.00 g; Citric acid : 20.00 g;

Yeast extract : 0.20 g; KH_2PO_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 : 5.2;

Distil water : 1000 ml.

The pH of the medium was adjusted to 5.2 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.

Inoculation of the vegetative medium :

The vegetative medium was inoculated by transferring 5 disc of mycelium (each of 10 mm dia) from 8 days old slant. Preparation of the production medium The composition of the production medium employed throughout the investigation was as follows :

Sugar : 230.00 g; Citric acid : 30.00 g;

Yeast extract: 0.30 g; KH_2PO_4 : 0.70g;

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.70g;

KCl : 0.10 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.010g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$

: 0.008g; pH : 5.2; (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* MKR-130. The flasks were kept on arotary shaker (230 rpm) at

Table - 1
Micellar impact on biotic production of ergot alkaloids

Concentration of micelle used A x 10 ^{-x} M	*Yield of Ergot -alkaloids in mg/litre			% of ergot alkaloids increased after 8 days
	4 days	8days	10days	
Control	515	920	890	-
0.5 × 10 ⁻⁵ M	518	922	892	(+) 0.217%
1.0 × 10 ⁻⁵ M	520	925	895	(+) 0.543%
1.5 × 10 ⁻⁵ M	525	930	899	(+) 1.086%
2.0 × 10 ⁻⁵ M	530	935	904	(+) 1.630%
2.5 × 10 ⁻⁵ M	532	938	907	(+) 1.956%
3.0 × 10 ⁻⁵ M	535	940	910	(+) 2.173%
3.5 × 10 ⁻⁵ M	540	950	915	(+) 3.260%
4.0 × 10 ⁻⁵ M	544	955	918	(+) 3.804%
4.5 × 10 ⁻⁵ M	550	960	920	(+) 4.347%
5.0 × 10 ⁻⁵ M	555	970	925	(+) 5.434%
5.5 × 10 ⁻⁵ M	560	985	930	(+) 7.065%
6.0 × 10 ⁻⁵ M	565	995	937	(+) 8.152%
6.5 × 10 ⁻⁵ M**	570	1025***	940	(+) 11.413%
7.0 × 10 ⁻⁵ M	568	1022	938	(+) 11.086%
7.5 × 10 ⁻⁵ M	566	1020	935	(+) 10.869%
8.0 × 10 ⁻⁵ M	560	1009	930	(+) 9.673%
8.5 × 10 ⁻⁵ M	556	995	925	(+) 8.152%
9.0 × 10 ⁻⁵ M	551	980	920	(+) 6.523%
9.5 × 10 ⁻⁵ M	548	970	915	(+) 5.434%
10.0 × 10 ⁻⁵ M	540	950	910	(+) 3.260%

* Each value represents mean of three observations ** Optimum concentration of micelle used

*** Optimum yield of ergot alkaloids (+) values indicate % increase in the yield of ergot alkaloids after 8 days. Experimental deviation (±) 1.5-3%

27 ± 1°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. 189-flasks, each containing 100 ml of the production medium was taken for production stage. These were arranged into 21 sets, each set consisting of 9-flasks.

Now, M/1000 solution of potassium lauryl sulfate was prepared and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 10.0 ml of this solution was added in the flasks from 1st to 20th set respectively. Remaining one set was kept as control and it contained no potassium lauryl sulfate. 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°C. Colorimetric analysis were carried out after 4, 8 and 10 days of incubation period.

Results and Discussion

The influence of potassium lauryl sulfate on biotic production of ergot alkaloids. The data recorded in the table - 1 shows that micellar efficacy of potassium lauryl sulfate on production of ergot alkaloids by fermentation has been found stimulatory but not too significant. It has been found that there is a gradual and mild increase in the production of ergot alkaloids by fermentation with the stepping up of the micelle potassium lauryl sulfate till the maximum yield

of ergot alkaloids, i.e., 1025mg/litre was obtained at 6.5×10^{-5} M concentration of potassium lauryl sulfate which is 11.413% higher in comparison to control fermenter flasks, i.e; 920mg/litre in 8 days of optimum incubation period. It has also been recorded that production of ergot alkaloids exposed to potassium lauryl sulfate, i.e; at 6.5×10^{-5} M the yield of ergot alkaloids has been found a bit more in comparison to control fermentor flasks.

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