

Efficacy of brucine on ergot alkaloids fermentation

Santosh Kumar Singh* and Ved Prakash Sahay**

*University Department of Chemistry, S. K. M. University, Dumka

**Department of Chemistry, S. P. College, Dumka (Jharkhand)

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Abstract: The efficacy of brucine on ergot-alkaloids fermentation by some fungal strains such as *Claviceps fusiformis* VD-118, *Claviceps africana* VD-110, *Claviceps paspali* VD-98 and *Claviceps purpurea* VD-74 has been assessed. It has been found that the fungal strain of *Claviceps purpurea* VD-74 is a good ergot-alkaloids producer. It has been found that maximum ergot-alkaloids, ie, 1030 mg/L has been achieved from 225g glucose which is 11.351% higher in comparison to control at 6.0×10^{-5} M molar concentration of brucine under optimised parametric condations such as 27°C temperature 5.2, pH and 7 days of optimum incubation period.

(Key words : Ergot alkaloids fermentation, brucine and *Claviceps purpurea* VD-74

Introduction

Ergot alkaloids are widely used for therapy of acute migraine headaches and include ergotamine and dihydroergotamine, both of which act by causing vasoconstriction of the carotid artery beds. Ergot alkaloids have multiple side effects, but have little effect on the liver and have not been clearly linked to instances of clinically apparent acute liver injury.

Brucine, is an alkaloid closely related to strychnine, most commonly found in the *Strychnos nux-vomica* tree. Brucine poisoning is rare, since it is usually ingested with strychnine, and strychnine is more toxic than brucine. In synthetic chemistry, it can be used as a tool for stereospecific chemical syntheses¹⁻⁵.

While brucine has been shown to have good anti-tumor effects, on both hepatocellular

carcinoma⁶ and breast cancer,⁷ its narrow therapeutic window has limited its use as a treatment for cancer. Brucine is also used in traditional Chinese medicine as an anti-inflammatory and analgesic agent,⁸ as well as in some Ayurveda and homeopathy drugs⁹.

The influence of alkaloids specially on microbes and microbial process have not been studied extensively¹⁰. Since some alkaloids¹¹⁻¹⁹ are known to be produced during some fermentation process it is obvious that such alkaloids being toxic are not toxic to the microbes involved in the fermentation bioprocess.

Thus, from the above brief review it is evident that there is no definite opinion regarding the influence of brucine on the production of ergot alkaloids and in view of this the authors have studied the influence of brucine on ergot alkaloids fermentation by *Claviceps purpurea* VD-74.

Experimental

The effect of brucine on ergot alkaloids fermentation by *Claviceps purpurea* VD-74 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 colorimetrically²⁰⁻²².

Sterilization :

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

** For Correspondence : E-mail : vpsahay121@gmail.com

Table - 1
Efficacy of brucine on ergot alkaloids fermentation

Concentration of brucine used A x 10 ^{-x} M	*Yield of Ergot -alkaloids in mg/litre			% of ergot alkaloids increased after 7 days
	5days	7days	9days	
Control	519	925	889	- -
0.5 × 10 ⁻⁵ M	521	930	893	0.540
1.0 × 10 ⁻⁵ M	525	936	896	1.189
1.5 × 10 ⁻⁵ M	530	945	902	2.162
2.0 × 10 ⁻⁵ M	545	955	905	3.243
2.5 × 10 ⁻⁵ M	550	963	909	4.243
3.0 × 10 ⁻⁵ M	556	972	915	4.108
3.5 × 10 ⁻⁵ M	560	981	920	6.054
4.0 × 10 ⁻⁵ M	569	989	930	6.918
4.5 × 10 ⁻⁵ M	570	996	936	7.675
5.0 × 10 ⁻⁵ M	579	1008	946	8.972
5.5 × 10 ⁻⁵ M	586	1019	950	10.162
6.0 × 10 ⁻⁵ M**	580	1030***	956	11.351
6.5 × 10 ⁻⁵ M	573	1009	952	9.081
7.0 × 10 ⁻⁵ M	569	998	950	7.891
7.5 × 10 ⁻⁵ M	555	983	996	6.270
8.0 × 10 ⁻⁵ M	550	975	930	5.405
8.5 × 10 ⁻⁵ M	540	969	927	4.756
9.0 × 10 ⁻⁵ M	536	961	922	3.891
9.5 × 10 ⁻⁵ M	540	955	910	3.243
10.0 × 10 ⁻⁵ M	536	950	911	2.702

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

*** Optimum yield of ergot alkaloids (+) values indicate % increase in the yield of ergot alkaloids after 7 days. Experimental deviation (±) 1.5-3%

Organism used : fermentation by *Claviceps purpurea* VD-74 was used as source of enzymes for production of ergot alkaloids.

Age of inoculum : 50h

Quantum of inoculum : 10ml

Incubation periods : 5, 7 and 9 days

Optimum incubation period : 7 days

Vegetative medium²³ The composition of the vegetative medium was as follows :

Dextrose : 125.00 g;

Citric acid : 15.00 g;

Yeast extract : 0.15 g;

KH₂PO₄ : 0.60g;

MgSO₄·7H₂O:0.35g;

FeSO₄·7H₂O : 0.010g;

ZnSO₄·7H₂O : 0.020g;

pH :5.2;

Distil water : 1000 ml.

The pH of the medium was adjusted to 5.2 by adding requisite amount of NH₄OH 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 7 days old slant.

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

Sugar : 225.00 g; Citric acid : 25.00 g;
Yeast extract: 0.25 g; KH_2PO_4 : 0.65g;
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.65g;
 KCl : 0.15 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 7 days old *Claviceps purpurea* VD-74. The flasks were kept on a rotary shaker (230 rpm) at $27 \pm 1^\circ\text{C}$ for 7 days. After 7 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 21 sets, each set consisting of 9-flasks.

Now, M/1000 solution of brucine 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 brucine.

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 $27 \pm 1^\circ\text{C}$. Colorimetric analysis were carried out after 5, 7 and 9 days of incubation period.

Results and Discussion

The influence of brucine.



The data recorded in the table - 1 shows that efficacy of brucine 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 brucine till the maximum yield of ergot alkaloids, i.e., 1030 mg/litre was obtained at $6.0 \times 10^{-5}\text{M}$ concentration of brucine which is 11.351% higher in comparison to control fermenter flasks, i.e; 925mg/litre in 7 days of optimum incubation period. It has also been recorded that production of ergot alkaloids exposed to brucine., i.e; at $6.0 \times 10^{-5}\text{M}$ the yield of ergot alkaloids has been found a bit more in comparison to control fermentor flasks.

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