

## Biotic production of ergot alkaloids by *Claviceps purpurea* AK-169 exposed to N, N-dimethyl dodecylamine

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**Abstract:** The efficacy of N, N-dimethyl dodecylamine on biotransformation of molasses to ergot alkaloids by *Claviceps purpurea* AK-169 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 and *Claviceps purpurea* AK-169).

### Introduction

For many years, ergot alkaloids have been considered both a problem to be mitigated and a potential medical cure. These compounds have been primarily studied in the medical / pharmaceutical<sup>1</sup> and agricultural fields<sup>2</sup>. Depending on one's perspective, the impact that ergot alkaloids have had on the progress of human medicine and livestock production can be either positive or negative. The dose or concentration of ergot alkaloid exposure is paramount. This can determine whether these compounds are implicated in the morbidity and mortality of individuals with St. Anthony's Fire, or whether they can be used to treat migraines and post-partum bleeding; it can determine whether they are used to maximize plant resistance and persistence, or whether they constitute an animal welfare concern for grazing livestock<sup>3,4</sup>. The ethics of ergot alkaloid use is debated to this day, but there is no debating the impact of these compounds. Many of the positive and negative issues associated with ergot alkaloids have specific conditions with regional implications,

but that does not diminish the magnitude of impact that these compounds have had on humans, livestock, and plants globally.

Research evaluating ergot alkaloids can be both basic and applied. Many types of research perspectives are necessary in understanding ergot alkaloids' functions and how these findings might be applied. The focus of this Special Issue concerns original research and review articles that highlight benefits and detriments, and successes and failures involving ergot alkaloids around the world with deference to regional distinctions. Research models range from fungus, to plant, to mammal; and the ergot alkaloids produced by both *Claviceps* and *Epichloë* spp. of fungi are included in this Special Issue. All submissions focus on ergot alkaloids' effects (positive or negative) in different contexts. There is a benefit to this shared interest, even if the issues with ergot alkaloids do not directly overlap.

Significant advancements in the manipulation of plant-endophyte symbioses have been made in recent years to optimize the profile and concentration of the secondary compounds produced<sup>5</sup>. Eady<sup>6</sup> has reviewed the complexities plant breeders encounter when selecting a desired plant-endophyte symbiont, with New Zealand ryegrass as a model. This is a balance between selecting a source of ergot alkaloids that permit greater plant persistence, and inhibiting ergot alkaloid production that results in mycotoxicosis in grazing livestock in combination with desired

plant traits. This makes the understanding of ergot alkaloid production paramount. The potential of using various-omics technologies to study ergot alkaloid production has been demonstrated in this Special Issue. In addition to traditional selection processes, Florea et al.<sup>7</sup> demonstrate the use of CRISPR technology to create a non-transgenic strain of *Epichloë* fungus without the genes necessary to produce ergot alkaloids. Fungi that produce ergot alkaloids can be endophytic and parasitic. Ergot contamination of cereal crops in Canadian provinces has become an issue of increasing concern. Hicks et al.<sup>8</sup> evaluate diversity in genes related to ergot alkaloid production in Canadian strains of the parasitic *Claviceps purpurea* to better characterize and understand the variation of ergot alkaloid content. Also looking at Canadian strains of *C. purpurea*, Liu et al.<sup>9</sup> evaluated the evolution patterns of gene clusters associated with different classes of ergot alkaloid production. Work of this caliber is critical to better understand this evolving issue.

Historically, human interactions with ergot alkaloids have been defined by large-scale poisonings through the consumption of contaminated grains<sup>1</sup>. Incidents of human ergot poisonings are increasingly rare due to improvements in crop management, grain screening and cleaning<sup>10</sup>, and the regulation of safe quantities in food and feed<sup>11</sup>. However, there are still areas in the world where this can be an issue<sup>12</sup>, and there is also still interest in the pharmaceutical potential of ergot alkaloids. Their most prominent use has been the treatment of migraines and controlling post-partum bleeding in the 18<sup>th</sup> and 19<sup>th</sup> centuries. In a current review of the past gynecological and obstetric uses of ergot alkaloids, Smakosz et al.<sup>13</sup> defined a potential role for the application of ergot alkaloids in modern obstetrics. In addition to clinical uses of ergot alkaloids, research assessing the sustainable production of ergot alkaloids in desirable formulations is needed. Shahid et al.<sup>14</sup> have developed a response surface methodology to select strains of *Penicillium citrinum* for their

ability to produce ergot alkaloids in culture. Many researchers that study ergot alkaloids can relate to the challenges associated with obtaining purified forms of desired ergot alkaloids in any quantity.

Although medical applications focus on ergot alkaloids' positive effects in humans, animal agriculture has historically and consistently viewed ergot alkaloids as a problem to be solved. Further, changing environmental conditions cause the ever-changing fungal production of ergot alkaloid profiles and concentrations. This necessitates routine surveys of grains and grasses. In this Special Issue, these are exemplified by the on-farm monitoring of ergot alkaloid levels in Kentucky horse pastures described by Lea and Smith<sup>15</sup>, as well as the ergot alkaloids found in Slovenian feed grains, as described by Babic et al.<sup>16</sup>. Research of this nature is ongoing globally and contributes greatly to the mitigation of large-scale problems as well as the identification of future areas in need of research.

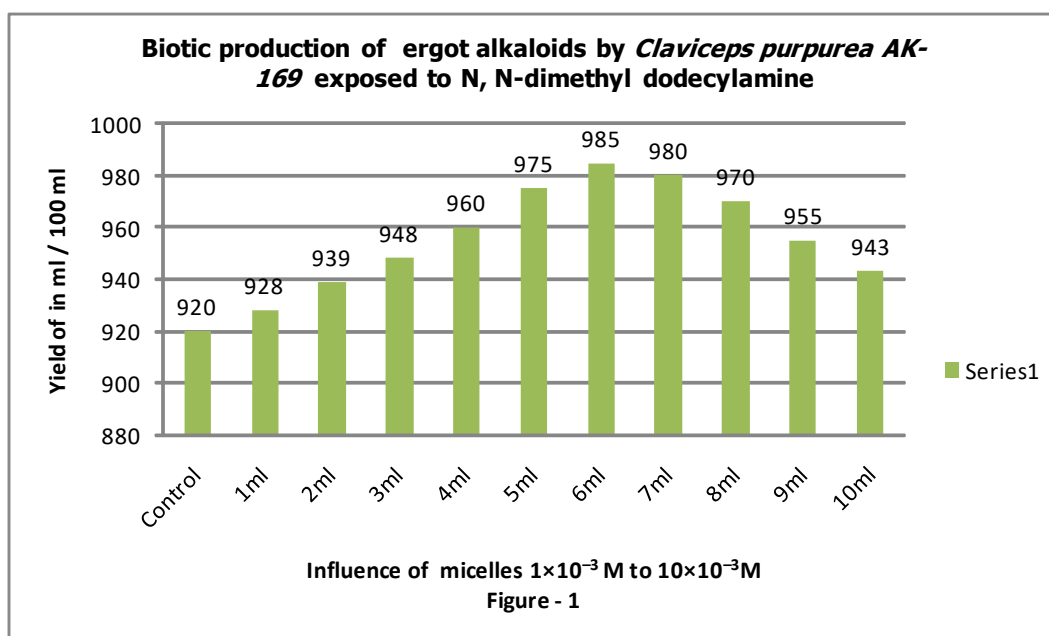
The variation of the content and concentration of ergot alkaloids is further complicated by livestock exposed to ergot alkaloids that demonstrate varied responses to the toxins. Poole et al.<sup>17</sup> and Wilbanks et al.<sup>18</sup> have studied various aspects, including genetics, that may make cattle more resistant to consumed ergot alkaloids. Ault-Seay et al.<sup>19</sup> used advanced-omics technologies to look at the rumen microbial and host metabolomes to provide a whole-animal characterization of impacts of ergot alkaloids. Mote and Filipov<sup>20</sup> reviewed the use of interactomics to provide a systemic understanding of the pathologies caused by ergot alkaloids that cause fescue toxicosis. A very specific pathology associated with ergot alkaloids and ergotism is a chronic vasoconstriction. Yonpaim et al.<sup>21</sup> looked at the acute exposure of ergot alkaloids on vasoactivity in ovine vasculature, and Valente et al.<sup>22</sup> evaluated prolonged ergot alkaloid exposure on

**Table - 1**  
**Biotic production of ergot alkaloids by *Claviceps purpurea* AK-169**  
**exposed to N, N-dimethyl dodecylamine**

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

\* 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 7 days. Experimental deviation (±) 1.5-3%



the vasoactivity of bovine vasculature. Both studies<sup>21,22</sup> respectively evaluated aspects related to the ability of ergot alkaloids to interact with adrenergic and serotonergic receptors<sup>23</sup>, and both papers concluded that receptor-mediated treatments for ergot alkaloid-induced vasoconstriction could be explored as potential therapies. From a systemic evaluation of ergot alkaloids' impact on the whole animal or microbiome, to the study of a specific symptom, there is much yet to be learned about how ergot alkaloids disrupt mammalian physiology.

The collection of papers in the Global Impact of Ergot Alkaloids ([https://www.mdpi.com/journal/toxins/special\\_issues/ergot\\_alkaloid](https://www.mdpi.com/journal/toxins/special_issues/ergot_alkaloid)) (accessed on 17 February 2022) Special Issue highlights the rich diversity of research and the complexity of the problems centered around ergot alkaloids. Although many specific issues related to accidental or intentional consumption of ergot alkaloids can be localized to a certain geographic region, the problems, challenges, and fascination with ergot alkaloids is global.

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 authors have studied the influence of N, N-dimethyl dodecylamine on ergot alkaloids fermentation by *Claviceps purpurea* AK-169

### Experimental

The influence of micelles on the production of ergot-alkaloids has been studied. The experimental results have been described in table-1. The vegetative medium was prepared. The composition of the vegetative medium was same as described.

**Temperature :** 27°C + 1°C

**Assay method :** Evaluation of ergot alkaloids formed was made colorimetrically<sup>25-27</sup>.

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

### Organism used :

*Claviceps purpurea* AK-169 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>28</sup> The composition of the vegetative medium was as follows :

Dextrose 150.00g ± 10g,

citric acid 20.00g ± 2g ,

Yeast Extract - 0.20g ± .02g

KH<sub>2</sub>PO<sub>4</sub> 0.75g ± .02g, MgSO<sub>4</sub>·7H<sub>2</sub>O

0.50g ± .02g, FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.015g ± .002g

ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.025g ± .002g, pH 5.

Distilled water 1000 ml.

100ml of vegetative medium was taken into 250ml conical flasks. The conical flasks were plugged with non-absorbent cotton wool plugs and were sterilized in an autoclave at 15 lbs steam pressure for 30 minutes and were left for cooling to room temperature. Now, two conical flasks were inoculated each with 5 discs (each disc of 10mm dia.) of 8 days old *Claviceps purpurea* AK-169. The conical flasks were kept on a rotary shaker (230 rpm) at 27 ± 1°C for 8 days. After 8 days the content of the conical flasks were taken each containing 100 ml of the same vegetative medium. Now, each flask was inoculated with 10 ml of inoculum from vegetative stage I. These conical 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 sodium tetradecyl 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 conical flasks from 1st to 10th set respectively. Remaining 9-flasks were kept as control and it contained no micelles. Each conical flask was inoculated with 10 ml of the inoculum from II<sup>nd</sup> vegetative stage. The flasks were kept on a rotary shaker operating at 230 rpm at temperature  $27 \pm 1^\circ\text{C}$  for 8 days. Colorimetric analysis<sup>24</sup> were carried out after 6, 8, and 10 days of incubation period.

The experimental procedure followed for the study of the remaining three micelles, viz. sodium hexadecyl sulfate, N, N-dimethyl dodecylamine (N, N-dMDA) and Dioctadecyl dimethylammonium chloride (DODAC) are entirely the same as described above except for the fact that in place of sodium tetradecyl sulfate other micelles in trial was added to the medium.

### 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 micelles Sodium Tetradecyl Sulfate (STS) and Sodium Hexadecyl Sulfate (SHC), the micelle N, N-dimethyl docecylamine (N, N-

d MDA) has also given significant yield of ergot alkaloids.

The maximum yield of ergot alkaloids, i.e. 985 mg/litre in the presence of  $6.0 \times 10^{-3}\text{M}$  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  $1.0 \times 10^{-3}\text{M}$  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  $6.0 \times 10^{-3}\text{M}$  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  $7.0 \times 10^{-3}\text{M}$  to  $10 \times 10^{-3}\text{M}$  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  $1.0 \times 10^{-3}\text{M}$  to  $10 \times 10^{-3}\text{M}$  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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