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1. Studies on Fermentative Biotic Production of Ergot Alkaloids

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ABSTRACT

In comparison to submerged fermentation, Claviceps fusiformis produced 3.9 times more total ergot alkaloids during solid-state fermentation. The production of Claviceps purpurea was equal, but the range of alkaloids was expanded through solid-state fermentation. The natural compounds called indole alkaloids, or ergot alkaloids, are nitrogen-containing substances. Fungi belonging to the phylum Ascomycota, including Claviceps, Epichloë, Penicillium, and Aspergillus, are the most often occurring producers. Based on their structural characteristics, ergot alkaloids are classified into three groups: peptides, lysergic acid amides, and clavines. The initial metabolic pathways that lead to the formation of the tetracyclic ergoline ring structure are shared by all of them. Different enzymes can alter the ergoline ring in different ways, producing a wide range of naturally occurring bioactive chemicals that are used as precursors or medical treatments. In the present investigation different ergot alkaloids fungus would be taken for the conversion of sugar raw material molasses to ergot alkaloids exposed to different useful molecule under optimized parameters. Ergot alkaloids were the first antimigraine drugs available. Ergot or ergot fungi refers to a group of fungi of the genus Claviceps. Ergot extract has been used in pharmaceutical preparations, including Ergot alkaloids in products such as Cafe got (containing caffeine and ergotamine or ergoline) to treat migraine headaches, and ergometrine, used to induce uterine contractions and to control bleeding after childbirth. In this paper we will discuss. Studies on Fermentative Biotic Production of Ergot Alkaloids.

KEYWORDS:

Fermentative, Biotic Production, Ergot Alkaloids, Claviceps Purpurea, Toxic Effects, Producing Species, Plants, Aspergillus, Penicillium, Metabolic Compounds

2. Introduction:

Today, ergot alkaloids have found widespread clinical use and more than 50 formulations contain natural or semisynthetic ergot alkaloids. They are used in the treatment of uterine atonia, postpartum bleeding, migraine, orthostatic circulatory disturbances, senile cerebral insufficiency, hypertension, hyperprolactinemia, acromegaly, and Parkinsonism. The broad physiological effects of ergot alkaloids are based mostly on their interactions with neurotransmitter receptors on the cells. [1] Ergot alkaloids are known to affect the nervous system and to be vasoconstrictors. Historically, ingestion of contaminated grain, particularly rye, has been implicated in epidemics of both gangrenous and convulsive ergotism, although such epidemics no longer occur in humans due to increased knowledge of the cause and to more varied modern diets. Outbreaks of ergotism in livestock do still occur, however these compounds have also been used as abortifacients. Ergot alkaloids are produced by the filamentous fungi of the genus, Claviceps (e.g., Claviceps purpurea – Ergot, Mutter Korn). [2]

Since there has always been competition between saprophytic production fermentation and parasitic culture (field production) of ergot alkaloids for product preparation, the future of fermentation, and in particular ergot alkolid fermentation, appears bright. [3] Since field production of ergot alkaloids is susceptible to a variety of environmental circumstances, such as severe weather, which results in a significant decrease in the production of valuable alkaloids, this uneconomic approach (field production) has been replaced today by submerged fermentation (lab method). [4]

Ergot alkaloids and their Biosynthesis: Ergotism:

When animals or humans eat feed or grains contaminated with ergot, the disease known as ergotism develops. The sclerotium body of the fungus Claviceps is called ergot. The fungus's alkaloids build up in the ergot, which causes disease symptoms. Throughout history, there have been multiple cases of animal and human poisoning due to the lethal characteristics of ergot alkaloids. [5] Throughout the Middle Ages, ergot poisoning occurred in both Central and Western Europe. The fungal species ergot was originally recognized in 1711, and the life cycle of Claviceps purpurea was clarified by Tulasne in 1853. [6]

Ergot alkaloid Producing Species:

Fungi belonging to the Clavicipitaceae family and order Hypocreales, including Claviceps, Epichloë, Neotyphodium, and Balansia spp., are producers of ergot alkaloids. Other fungi that are known to produce ergot alkaloids are Penicillium species and Aspergillus. Green plants belonging to the Convolvulaceae family, including Rivea, Calistigia, and Ipomoea, have also been shown to contain ergot alkaloids. [7]

Ergot alkaloids are a broad category of bioactive substances derived from many plant and fungal species. These were initially observed in the fungus Claviceps purpurea, which is the cause of rye ergot disease. For the first time, these ergot alkaloids were discovered and identified in the sclerotia that the rye plant produces on its kernels. The sclerotia of the genus Claviceps have been reported to contain a wide variety of ergot alkaloids with commercial and industrial significance.

Nevertheless, ergot alkaloids can also be produced in small amounts by a number of higher plants as well as other fungal species such Epichole, Blansia, Penicillium, and Aspergillus. Ascomycetes are fungi that may effectively manufacture alkaloids both in the wild and in a lab setting. [8] One prominent genus of Ascomycetes that is important for creating secondary metabolites of commercial value is Penicillium. Significant amounts of alkaloids, antibiotics, hormones, and mycotoxins can be produced by Penicillium species. Based on structural differences, ergot alkaloids, also known as clavines, lysergic acid amides, and ergopeptines, have been classified into three groups. Ergot alkaloids are commercially manufactured using a variety of fermentation procedures in order to manufacture medications. Industrial controls and improvements were made to the synthesis of alkaloids with the addition of various organic and inorganic materials to the fermentation medium. [10]

Review of Literature:

Ergot alkaloids are indole metabolic compounds with specific biological activities. Human recognition of ergot alkaloids is as old as the civilization. The compounds have been widely used as drugs and in medicines to cure different human diseases. Their ecological role is weakly understood but they have been involved in activities against mammals, nematodes, insects and bacteria (Ball et al., 1997). Many genera have been involved in the biosynthesis of ergot alkaloids e.g., Claviceps, Balansia, Penicillium, and Aspergillus. There are many species of genus Penicillium who have been involved in the production of ergot alkaloids e.g., P. sizovae, P. roquefortii, P. corylophilum and P. chrysogenum. Aspergillus species are also playing a significant role in the synthesis of these metabolites e.g., A. fumigatus, A. tamari and A. flavus. [11]

Ergot alkaloids are produced by a number of fungi and are useful in both medicine and agriculture (Schardl et al. 2006). These include the opportunistic human pathogen A. fumigatus (family Trichomonacide, order Eurotiales), which produces a family of simpler, clavine-type ergot alkaloids including chanoclavine-I, festuclavine, fumigaclavine B, fumigaclavine A, and fumigaclavine C. Additionally, among these fungi are plant-associated pathogens and symbionts from the family Clavicipitaceae (order Hypocreales), which usually produce lysergic acid derivatives. Advances in agriculture and medicine could result from a comprehensive understanding of the genes and enzymes involved in the manufacture of ergot alkaloids. [12]

Ergot alkaloids are physiologically active secondary metabolites that are generated by numerous species of fungi and have significant medicinal, toxicological, and pharmacological applications. Ergot alkaloids, including ergometrine, ergotamine, ergotoxine, and lysergic acid, as well as their derivatives, are widely utilized in the production of significant pharmaceuticals and in the medical field to treat a variety of illnesses. It has previously been discovered that the family Clavicipiataceae member Claviceps purpurea produces an impressive array of ergot alkaloids that are crucial for pharmacology. After infecting rye seeds, Clavicepspurpurea forms sclerotium, and these sclerotia are the repositories of secondary metabolites called alkaloids (Prabakaran, 2014). [13]

Objectives:

- To Studies on Fermentative Biotic Production of Ergot Alkaloids
- Ergot alkaloid common chemical structure
- Efficacy of some useful compounds to enhance the production of ergot alkaloids.

Research Methodology:

All the research was accessed from four most popular search engines i.e., PubMed, Scopus, Web of Science and Google Scholar. The papers from the standard scientific journals were only included, in which the researches on clinical trials were mainly focused in the present research.

Result and Discussion:

Man has always looked to nature for medicinal remedies. Fungi and other microorganisms are a great source of compounds with potential medicinal use. The relatively recent discovery of penicillin signaled the beginning of a new age for fungi. Six of the twenty prescription medications that were the best-selling in 1995 included ingredients that came from fungus. [14]

Figure 2.1 Ergots (The Blackened Claw-Like Objects) of Claviceps Purpurea on Wild Oat, Avena Fatua. (Source: www.davidmoore.org.uk)

Ergot, which can develop in the ovary of rye Secale cereale, is the dried sclerotium of the fungus Claviceps purpurea. A mature grain head may have one or more of its kernels replaced by these sclerotia, which are rigid, dark-colored masses that resemble horns (Fig. 1). Ergot can infect other wild and cultivated cereals (barley, wheat, oats), as well as other grasses, however it most commonly targets rye. In order to allow the ergots to infiltrate the food chain, these sclerotia—which also contain fungal toxins—are collected with the grain. [15]

Ergotism, or the widespread contamination by ergot, was a common occurrence in the Middle Ages. Consuming rye bread tainted with C. purpurea led to limb gangrene, neurological dysfunction, and even death. Nonetheless, ergot has a place in medicine; midwives have been using it to lessen postpartum hemorrhage since 1582. It has been discovered that ergotamine, an ergot alkaloid, can help relieve migraine headaches.

Ergot was most likely originally employed as an oxytocic medication in medicine. By giving three sclerotia, Adam Loncier of Germany recorded the first instance of ergot inducing labor contractions in 1582. At the time, it was the most successful medication for this purpose; it caused a quick and abrupt cesarean birth that took less than three hours to complete. However, ergot was ultimately determined to be inappropriate for this use since there were significant variances in the active components, making it impossible to deliver an accurate dosage. Severe side effects from ergot included intense nausea and vomiting. And it was Hosack of New York who said in 1822 that many stillbirths were caused by uterine rupture, which killed the mother. By the end of the 1800s, ergot was essentially no longer used as an oxytocic. Only in the 20th century was ergot shown to be effective in treating migraine attacks. Ergotamine, an ergot alkaloid, would be primarily involved in this. [16,17]

Ergot Alkaloids:

Geographical location affects the production of these chemicals because C. purpurea is the primary producer in Europe. Furthermore, a variety of factors influence the production of extraassimal amino acids (EAs), including the type of fungi and plants, the concentration of fungus, temperature, humidity, and nutrients. However, climatic conditions have the greatest impact on EA production due to its preference for wet soils and rainfall. The main products made from seeds and grains that have been shown to contain these harmful substances are rye, barley, triticale, oats, and millet, with rye, triticale, and barley being the most affected.

There are currently around 80 known EAs, which can be categorized into three main groups: peptide-type (which feature an extra cyclic tripeptide linked through an amide bond to the lysergic acid), simple lysergic acid amines, and clavinet-type (hydroxyl- and dehydro-derivatives of 6, 8-dimethylergoline). As seen in Figure 2, all EAs have an ergoline ring as their primary structural element and a nitrogen atom at position 6 (which can be methylated in some configurations). The key structural differences are in the ergoline ring substitution at position C8, as well as the presence of a double bond between C8 and C8 or C9 and C10. [18]

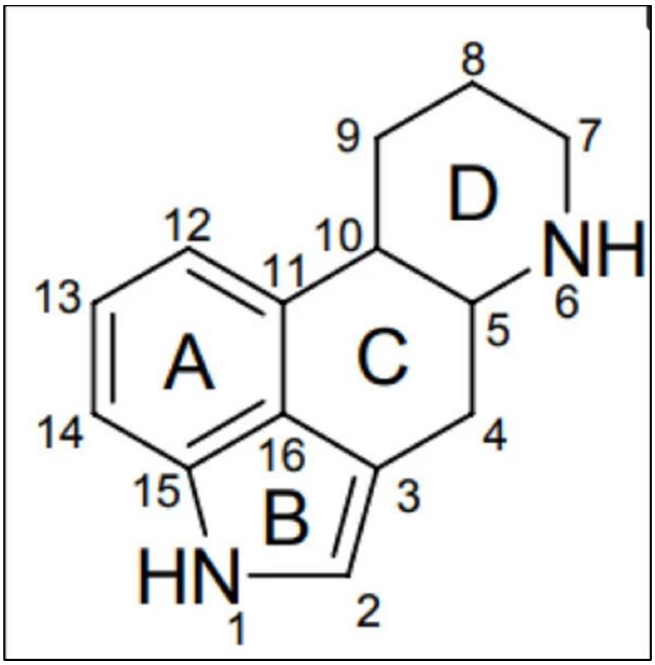

Figure 2.2 Ergot Alkaloid Common Chemical Structure

Ergometrine, ergosine, ergotamine, ergocornine, ergokryptine, and ergocristine and their -inine derivatives are the most significant ergot alkaloids (EAs). EFSA produced a scientific opinion on ergot alkaloids in food and feed, describing the clavine type as the most prevalent and dangerous EAs. The ergoline ring's C8 position is epimerized to form the C8 (S)-configuration, which results in the suffix -inine. The suffix -ine is associated with the (R)-configuration.

Although the epimerization of ergot alkaloids is still not fully understood, there are a number of known influencing factors. This process can be impacted by variables such as temperature, humidity, light, pH, and solvent properties. Numerous studies show that the epimerization process can be minimized by using aluminum foil or amber glass, non-protic solvents, and temperatures of −20 °C or lower. [19]

Toxicity and Mechanisms of Action:

The effects of consuming ergot alkaloids can range from acute to chronic disorders and in some cases result in death, depending on the quantity and frequency of administration. Given that these substances are known to interact with dopaminergic, serotonergic, and adrenergic receptors, these effects may appear in a variety of ways (Figure 3). Excessive consumption of EAs can lead to vasoconstriction, which is characterized by cramps, swelling, red markings, necrosis, loss of extremities, and death. This effect is mediated by the interaction of α-adrenergic receptors. Central nervous system symptoms, including hallucinations, giddiness, formication, nausea, paralysis, psychosis, dementia, dizziness, pins and needles, limb seizures, and death, are brought on by interactions with serotoninergic and dopaminergic receptors. [20]

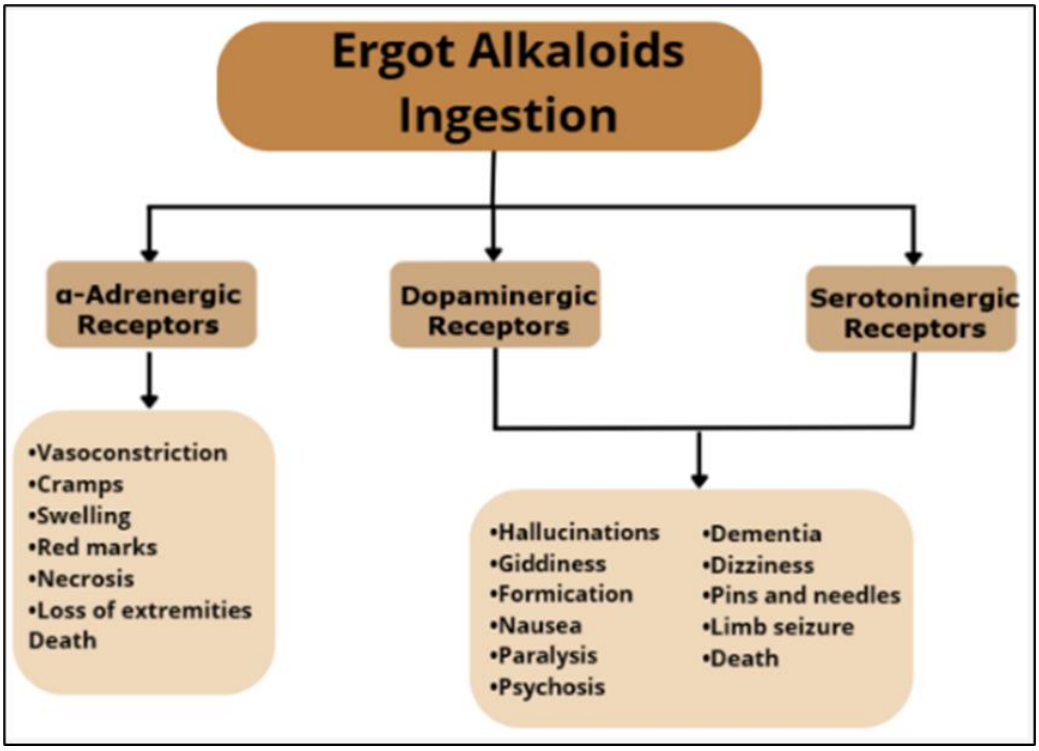

Figure 2.3 Effects of Excessive Ingestion of Ergot Alkaloids.

Ergotism, the term for intoxication caused by EAs, has been around since the Middle Ages, when people were intoxicated after consuming tainted bread, flour, and grains. The intense agony brought on by the vasoconstriction effect and the neurotoxic effects of these intoxications led to them being referred to as St. Anthony's fire or Holy Fire. Ergotism manifests in two forms: gangrenous and convulsive. Gangrenous causes tingling sensations in peripheral tissues, which can result in limb loss. Convulsive ergotism is characterized by tingling followed by delirium, epileptic-type seizures, and hallucinations. [21]

Diseases in Livestock Observed after Consumption of Plants Infected by Endophytic Fungi of the Genus Epichloë

A recent taxonomic revision has expanded the genus Epichloë to include the asexual Neotyphodium species, which is known to be generated from sexual Epichloë species. Ergot alkaloids are found in a variety of grasses infected by endophytic fungus of the genus Epichloë (Table 1). In response to symptoms seen in cattle fed tall fescue grass, the first theories on the role of ergot alkaloids in livestock health issues that were unrelated to the ingestion of sclerotia (ergots) of the Claviceps species were put forth in the 1940s. The illness is described as causing lameness in the winter, mainly in the left hind foot, which can result in foot loss. It was discovered that "the tall fescue contained some poisonous principle which will cause lameness or shedding of feet similar in all respects to the results from feeding ergot" because the disease struck in the winter, even though mature Claviceps sclerotia are present in the plant in late spring, and because typical signs of toxicity were observed even when the cattle were fed hay that contained no seeds. Following this initial

report, "fescue foot" was noted in a number of places, including Colorado, where cattle were fed "giant" fescue, and Kentucky, where cattle were grazing on meadows known as "Kentucky 31." The vasoconstrictive qualities of tall fescue grass extracts from a farm where lameness was seen were then shown. After that, the symptoms were artificially induced in steers given tall fescue hay. Visual inspection of the grass did not disclose any ergots, but analysis of the extract showed the presence of chemicals comparable to those obtained with extracts of ergots generated by Claviceps purpurea on rye. A study on the toxicity of tall fescue fodder that described the incidence and severity of fescue foot in other nations revealed significant variation in the syndrome's frequency and severity. [22]

Table 2.1. Plants Infected with Endophytic Fungi of The Genus Epichloë, Which Are Well-Known for Their Toxicity to Horses and Cattle and For the Presence of Their Main Alkaloids.

Grass: Common name	Grass: Latin name	Endophyte	Syndromes or symptoms in grazing livestock	Major alkaloids affecting livestock
Tall fescue	*Lolium arundinaceum (= Schedonorus arundinaceus = Festuca arundinacea)*	*Epichloë coenophiala (= Neotyphodium coenophialum = Acremonium coenophialum)*	Fescue toxicosis, Fescue foot, Summer slump, Fat necrosis [1]	Ergovaline [2]
Perennial ryegrass	*L. perenne*	*E. festucae* var. *lolii (= N. lolii = A. lolii)*	Ryegrass staggers	Lolitrem B [3], ergovaline
Perennial ryegrass	*L. perenne*	*E. festucae* var. *lolii* x *E. typhina*	Ergot alkaloid toxicity	Ergovaline
Fine fescues	*Festuca* spp.	*E. festucae*	Grazing deterrence	Lolitrem B [3], ergovaline
Drunken horse grass	*Achnatherum inebrians (= Stipa inebrians)*	*E. gansuensis* var. *inebrians (= N. gansuense* var. *inebrians)*	Stupor	Ergonovine, ergine
Sleepy grass	*Ach. robustum (= S. robusta)*	*Epichloë* sp.	Stupor	Ergonovine, ergine
Sleepy grass [4]	*Ach. robustum*	*E. funkii (= N. funkii)*	None reported	Chanoclavine I

1: The disease's symptoms are influenced by outside conditions. For example, summer slump syndrome manifests in the summer, while fescue foot is seen during the chilly winter. There is less research on fat necrosis; 2. In forage grass, ergovaline makes up over 80% of the ergopeptides, whereas in seeds, it makes up over 50%. Due to Claviceps sclerotia (ergots) contaminating plant material, ergotamine, ergosine, ergocryptine, ergocornine, and ergocristine were found in seeds; 3: Lolitrem B and related indole-diterpene alkaloids are known to cause staggers, although ergovaline's toxic role in endophyte-infected ryegrass is less well-documented; 4 Despite being referred to as "sleepy grass," Ach. robustum plants containing E. funkii do not exhibit the same toxic effects as those containing the other, as of yet unidentified, Epichloë species that are found close to Cloudcroft, New Mexico. USA. [23]

Livestock staggers have been linked to perennial ryegrass, and endophytic fungus have been identified within the grass. Nevertheless, until it was shown that the symptoms could not be induced by consuming sclerotia found in the seed heads, the sclerotia of Claviceps purpurea were thought to be the cause of ryegrass staggers. Staggers were seen in sheep that were allowed to graze the base of the ryegrass plant, but no symptoms were seen in sheep that were prevented from doing so. Before the identification of lolitrem B, which seems to be the primary tremorgenic mycotoxin in Epichloë festucae var. lolii = Neotyphodium lolii-infected perennial ryegrass, a number of tremorgenic mycotoxins of various fungal origins were considered to be the cause of ryegrass staggers. These plants also contained ergot alkaloids, yet ergot alkaloid poisoning signs are rarely observed in cattle fed endophyte-infected ryegrass.

It is commonly acknowledged that lethal levels of lolitrem B are achieved before toxic levels of ergovaline because ergovaline typically accounts for 10% to 15% of lolitrem B concentrations. Because of this, lolitrem B has been the subject of the majority of research on endophyte-infected ryegrass. Ergot alkaloids are poorly understood, and interactions between ergovaline and lolitrem B are especially unknown. [24,25]

Conclusion:

Since the beginning of farming, rye and other plants have been plagued by ergot, a fungal infection. It was discovered that ergot alkaloids, one of its ingredients, had beneficial medical qualities. It was known that ergot may induce gangrene in the limbs of people who consumed contaminated bread. However, it was eventually found to have a first-aid benefit as a potent oxytocic that aided in birthing, and more recently, its derivatives were employed to treat migraines. Due to the lengthy history of ergot and ergotism, ergot alkaloids were first used in medications after centuries of grass infection and ancient people—primarily the impoverished—becoming intoxicated throughout the Middle Ages. Despite the fact that medications like sumatriptan have replaced ergot derivatives in the treatment of migraines, their historical significance ensures their continued existence. However, there are further uses for the ergot alkaloids, such as treating Parkinson's disease symptoms, which may imply that they will continue to be the active ingredients in many popular medications for some time to come.

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