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ERGOT *Claviceps purpurea* (Fries) Tulasne ALKALOID DIVERSITY AND VIRULENCE: EVOLUTION, GENETIC DIVERSIFICATION, AND METABOLIC ENGINEERING

(review)

A.A. VOLNIN✉, P.S. SAVIN

All-Russian Research Institute of Medicinal and Aromatic Plants, 7, ul. Grina, Moscow, 117216 Russia, e-mail volnin@vilarnii.ru (✉ corresponding author), savin@vilarnii.ru
ORCID:
Volnin A.A. orcid.org/000000019222536X Savin P.S. orcid.org/0000000254413471
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Abstract

Claviceps purpurea (Fries) Tulasne is a valuable source of many bioactive metabolites (alkaloids) for pharmaceutical industry and a unique plant—parasite model but also a serious adversary for plant growing, feed and livestock industries causing significant economic damage in different countries. Ergot appeared in South America in the Paleocene, the age of the genus *Claviceps* is 20.4 million years (K. Píchová et al., 2018). Intraspecific diversity and divergence of indole alkaloid production gene cluster occurred in accordance with the evolutionary "hourglass model" (M. Liu et al., 2021). Ergometrine, ergosine, ergotamine, α-ergocryptine, ergocornine, ergocristine and 8-S(-inine-) epimers are the major identified ergoalkaloids which account for approximately 50 % of the ergot alkaloid metabolome. *Claviceps* alkaloid gene clusters consist of varying numbers of genes, posses two or three copies of *dmaW*, *easE*, *easF* genes, and there are many facts of frequent gene loss and acquisition (M. Liu et al., 2021). Differences in metabolomic profiles of *C. purpurea* indole alkaloids correlate with the *lpsA* gene variability. Diversity of the ergot alkaloids is a result of sequence diversity in the *easH/lpsA* tandem-duplicated region (C. Hicks et al., 2021). The *lpsA1* and *lpsA2* genes derived from recombination events (S. Wyka et al., 2022), i.e., the *lpsA* genes are supposed to be due to reshuffling (C. Hicks et al., 2021). *C. purpurea* has a relatively large accessory genome (~ 38 %), high recombination rates ($\rho = 0.044$), and transposon-mediated gene duplication (S. Wyka et al., 2022). A transgenic yeast line is capable of producing enantiopure D-lysergic acid up to a level of 1.7 mg/l (G. Wong et al., 2022). Genetically engineered cultures of *Metarhizium brunneum* can produce 86.9 % lysergic acid and 72.8% dihydrolysirgic acid (K. Davis et al., 2020). Expression of the *trpE* and *dmaW* genes is quantitatively related to intensity of alkaloid synthesis in saprophytic *Claviceps* cultures (M. Králová et al., 2021). Pectin is the main target of CAZymes proteins responsible for cell wall degradation during *C. purpurea* and *C. paspali* infection (B. Oeser et al., 2017; H. Oberti et al., 2021). Polygalacturonase, MAP kinase, transcription factor CPTF1 (*Cptf1* gene), GTPase (*Cdc42* gene) make the main contribution to *Claviceps* virulence (B. Oeser et al., 2017; E. Tente et al., 2021). Ergot affects the auxin, ethylene, and cytokinin pathways in plants, with varying effects depending on tissue type and time after inoculation (E. Tente, 2020; Tente et al., 2021). Wheat resistance is due to mutations in DELLA proteins (E. Tente, 2020; A. Gordon et al., 2020) while rye resistance is due to pectinesterase activity, cell wall modification, and modulation of pollen tube growth (COBRA-like protein and pectinesterase inhibitor) (K. Mahmood et al., 2020).

Keywords: *Claviceps purpurea*, ergot, alkaloids, biosynthesis pathways, toxicity, virulence, genotype, gene clusters, *Claviceps*, *C. purpurea*

Recent identification of genes encoding proteins that catalyze the successive steps in alkaloid biosynthesis in fungi are due to advances in genomics and molecular biology [1]. The diversity of fungal secondary metabolites can be expanded by activating silent gene clusters in artificially cultivated strains or by replenishing existing collections of fungal cultures in order to obtain new natural biological products [1, 2].

Ergot, a parasitic fungus *Claviceps purpure*a (Fries) Tulasne, is an important source of biologically active substances for the production of drugs [3-5]. Natural ergoalkaloids, as well as their semi-synthetic derivatives, are used as pharmaceuticals in modern medicine, for example in obstetrics and gynecology [6] as inhibitors of prolactin release [7, 8], as well as for the treatment of a number of neurological diseases [9], in particular parkinsonism [[9, 10], dementia [9, 11] and hypertension [9, 12]. Recent studies point to the high promise of ergot alkaloids (metergoline derivatives) for the creation of new antimicrobial agents [13]. In Russia, with the participation of VILAR, a number of drugs based on ergot alkaloids have been developed: abergine (α-, β-ergocriptines), novocristine (dihydroergocristine), bellataminal (ergotamine), ergometrine maleate (ergometrine) [14].

The VILAR collection includes five strains of the ergot parasitic culture *Claviceps purpurea* (Fries) Tulasne (producers of α-, β-ergocriptines, ergotamine, ergotoxin, ergocristine, and ergocornam). All parasitic strains are maintained in an active state, including the stage of seasonal cultivation in the field on winter rye crops (variety Moskovskaya 12) and an intermediate in vitro stage in the form of an axenic culture. In addition, two saprophytic ergot strains (producing ergocriptine and ergotamine) have been registered, which are also maintained in a live collection [15-17].

In agriculture, ergot causes significant economic losses. Ergot is a health hazard not only for animals, but also for humans [18, 19]. In world practice, contamination of products, animal feed, and pasture crops with ergot toxins is a fairly common occurrence, and the toxic effect of ergoalkaloids on animal metabolism has been fairly well studied [20-22], including in vitro [23, 24]. In addition, not only alkaloids, but also other secondary metabolites of ergot, such as ergo pigments [25), lecanoric acid and its derivatives [24], indole-diterpenes [25-28], epipolytiodiketopiperazines [29], can have a toxic effect.

The quantitative characteristics and composition of the metabolome of ergoalkaloids can vary greatly depending on the specific conditions for the development of the fungus and its species, which makes it difficult to monitor the content of ergoalkaloids and, therefore, assess the degree of contamination of agricultural products with ergotoxins [30, 31]. In wheat and barley plants, alkaloids also penetrate the healthy grain, which is formed above and below the infected areas of the inflorescence [32].

Issues of toxicology and secondary metabolism of ergot have become more and more relevant in the last decade. Scientific reviews consider the technological and physiological features of ergot cultivation [15, 16], functional characteristics of key genes and gene clusters involved in the biosynthesis of alkaloids in different genera of the Ergot family (*Clavicepitaceae*) [30, 33-35], and discuss the identification and classification of intermediate products. synthesis [36, 37], construction of pathways for the formation of ergoalkaloids in the fungus and various heterologous organisms, strategies for optimizing the creation and use of recombinant producer strains. Ergot virulence factors have been described [32, 38, 39]. Published analytical materials (39) are refined and supplemented [40, 41].

In the present review, we have detailed and systematized the results of studying the mechanisms of alkaloid biosynthesis and ergot virulence in different members of the *Claviceps* genus, taking into account new data on the role of alkaloid biosynthesis and virulence cluster genes in the evolution and spread of ergot, paying attention to methodological aspects, including metabolic engineering. Such an integrated approach to understanding the genetic diversification of ergot, the associated diversity of alkaloids, toxins, and knowledge of the molecular mechanisms of their biosynthesis expand our understanding of the biology of the *Claviceps* genus and form the basis for solving practical problems both in agriculture

and in the creation of drugs based on ergot metabolites. .

Evolution, distribution and role of ergot toxic metabolites. Molecular genetic technologies make it possible to study the biology of ergot at the level of gene clusters, genomes, transcriptomes, metabolomes, using a wide arsenal of tools. For the analysis of the ergot genome, pyrosequencing is used by the method of single/paired-end pyrosequencing) (42), paired-end sequencing (PEs) [43, 44], Sanger sequencing [45], shotgun pyrosequencing and mate-pair sequencing [46]. Thus, PEs were used to analyze the transcriptome during signaling interactions of *C. purpurea* with the host *Secale cereale* [47] and sequencing the genome of *Claviceps paspali* [48], single-end multiplexed sequencing was used to analyze the wheat transcriptome upon infection with *C. purpurea* (49).

The study of the evolutionary history of the genus *Claviceps* has recently begun [43, 50]. It has been established that the center of origin for ergot in the Paleocene was South America [50]. Four sections are classified according to ecological, morphological, and metabolic features: *Citrinae*, *Paspalorum*, *Pusillae*, and *Claviceps* [43, 50], which split in the *Paleocene* and *Eocene* [50]. The speciation of *Pusillae* occurred during the Eocene, Oligocene, and Miocene and was associated with warm-season herbs from the subfamilies *Panicoideae*, *Aristidoideae*, *Chloridoideae*, *Micrairoideae*, *Arundinoideae*, and *Danthonioideae* [43, 50] with subsequent spread from South America to Africa [43, 50]. The age of the genus *Claviceps* is estimated at 20.4 Myr [50], and its division and further expansion into North America is associated with the ecological features of new host plants (transition from parasitizing Cyperaceae sedges to cold-resistant plants from the subfamilies *Pooideae*, *Bambusoideae*, and *Oryzoideae* [syn: *Ehrhartoideae*] [50-52]. This was followed by the worldwide spread of *Claviceps* [43, 50].

Citrinae, *Paspalorum*, and *Pusillae* are characterized by a narrow range of distribution and low toxicity, while the section *Claviceps* is considered to be evolutionarily more successful and adaptable, since it has the widest range of host plants and range [43, 50, 53]. Currently, the classifications and systematic position of natural parasitic strains of ergot continue to be refined [54-56].

The ergot population in the US differs from European isolates [57]. It is claimed that in the USA there are no subpopulations of this parasitic fungus, formed depending on the host plant [54, 57, 58]. In the US, there are moderate levels of genotypic diversity (H = 3.43-4.23) and gene diversity (H_{exp} = 0.45-0.57) [59]. There is genetic differentiation between North American populations from different host plants (22%), but it is geographically mixed. The standardized association index ranged from 0.007 to 0.122 for four groups (two regions and two hosts, Kentucky bluegrass and perennial ryegrass) [59]. Three genetic lines of *C. purpurea* have previously been identified [60], reflecting ecological differentiation and adaptation [53, 61, 62]. Lines designated as G1, G2, and G3 ecotypes have been differentiated based on conidial morphology, alkaloid profiles, RAPD (random amplified polymorphic DNA) markers, and the ability or inability of sclerotia to maintain buoyancy [53, 61, 63]. At present, the ecotypes of *C. purpurea* G1, G2, and G3 are described as three independent species, the *C. purpurea sensu stricto*, *C. humidiphila*, and *C. spartinae*, respectively [53, 60].

The reasons for the evolutionary success and high adaptability of the section *Claviceps* are not completely clear [43]. To some extent, this can be explained by the production of a large number of toxic metabolites, which limits the consumption of plants affected by the fungus by vertebrates and invertebrates and is important for the mutualistic relationship between the parasite and the host, which provides plant protection from phytophages [50]. However, this issue remains the subject of debate [40].

For the divergence of of the indole alkaloid cluster (EAS) genes, phylogenetic analysis and analysis of DNA polymorphism revealed a correspondence to the evolutionary hourglass model (HGM) in the intraspecific diversity of ergot [44]. The description is borrowed from the ontogenesis when morphological divergences on the middle stages of embryonic development are more conservative than those at earlier and later stages [44, 64, 65]. Thus, the rate of evolution of the genes for the early stages of synthesis of the *dmaW* and *easF* alkaloids is much higher than that for the genes for the intermediate stages *easA*, *easC*, *easD*, and *easE* [44]. This pattern, supported by genomic studies, is consistent with the hourglass model [44, 66]. The HGM model suggests developmental constraints [44, 67]. Metabolic pathways for the synthesis of alkaloids have been viewed as unusually inefficient due to the fact that many intermediates accumulate in excess of what is needed to form the final products [44, 68]. However, this redundancy of intermediates is likely to serve as a factor in the stabilization of the biosynthetic pathway and hinder the selection of variants with changes in the corresponding genes [44].

The variety of *Claviceps* alkaloids has been deribed from three main processes. i.e., the acquisition of genes, the loss of genes, and a change in the sequence of genes for the biosynthesis of alkaloids [35, 46].

Changes in the architecture and plasticity of the genome can shape the direction of the evolutionary process of fungi and their adaptability [43]. Presumably, it is the secondary metabolites of ergot that serve as the factors of the primary influence on the diversification and promotion of the species into new ecological niches and help maintain its global distribution and a wide range of hosts [42]. The composition of the alkaloid synthesis gene cluster and unique polymorphisms indicate that *C. purpurea* is currently undergoing a process of adaptation resulting in a wide variety of peptide alkaloids [45]. Thus, the evolution of ergot is directed from specialized genomes (*Citrinae* and *Paspalorum*) to adaptive ones (*Pusillae* and *Claviceps*). This is facilitated by the joint localization of the transposed elements around the effectors. It is also suggested that in section *Claviceps* there was a loss of the repeat-induced point mutation (RIP), which led to unrestricted tandem gene duplication that corresponds to an increase in the potential for expansion of the range of hosts and speciation [43]. The results show the absence of gene duplication in sections *Citrinae* and *Paspalorum*, probably due to the presence of RIP-like mechanisms, which corresponds to the high host specificity and low species diversity of *Paspalorum* [43]. It is not clear whether such rearrangements gave *Claviceps* an advantage in moving to new hosts and new climatic conditions when separating sections and leaving South America, or were a consequence of this event [43].

C. purpurea showed significantly high recombination rates ($\rho = 0.044$), a relatively large accessory genome (38%), and transposon-mediated gene duplication [42]. It is important to note that the total content of transposable elements in the ergot genome is relatively low (8.8%), the genome size does not vary [42], and recombination is not the main duplication factor in *C. purpurea* [42]. Pseudogenization and neofunctionalization can also be significant processes. Due to the absence of RIP mutations, the increase in the number of transposable elements is probably controlled by high recombination rates [42].

We believe that the “hourglass” model in the evolutionary trajectory of the ergot alkaloid productivity gene cluster most likely indicates that parasitic strains that are promising in terms of alkaloid bioproduction can be weakened by natural selection and lost.

Identification of ergot strains. SSR (simple sequence repeats, short simple repeats, microsatellites) markers have been developed for rapid

identification of *C. purpurea* strains. In addition to distinguishing *C. purpurea* isolates, these SSRs can differentiate isolates of three other *Claviceps* species, the *C. pusilla*, *C. paspali*, and *C. fusiformis* [69]. For genotyping, multilocus sequence typing (MLST) can be used in combination with quantitative real-time polymerase chain reaction (qPCR) [70].

Some of the alkaloids are specific to certain strains of *C. purpurea* and can be used as chemotaxonomic markers for identification [71].

Ergot alkaloids and their producers. At present, the production of ergot alkaloids with various pharmacological activities is based mainly on fermentation using saprophytic cultures [9, 17, 36]. Approximately 60% of commercially produced ergoalkaloids are obtained by submersible cultivation of specially created mutant or recombinant strains of *C. purpurea* or heterologous organisms on liquid nutrient media, the remaining 40% are obtained by cultivation of ergot in the field [9, 72, 73].

The main problems in the production of ergoalkaloids are a wide variety of synthesized alkaloids, which complicates the subsequent extraction and purification from by-products and increases the cost of production [9, 36], as well as the instability and tendency to degradation of saprophytic strains during cultivation and storage [9, 36, 72, 73]. Saprophytic strains lose the sclerotio-like cellular morphology of the mycelium with an irreversible loss of the ability to produce alkaloids [72, 73]. Modern methods for the chemical synthesis of D-lysergic acid include long multistage reactions under harsh conditions and are not always enantioselective [3, 9, 74, 75].

The parasitic stage of the ergot life cycle is characterized by the formation of sclerotia, the only resting vegetative organ of the fungus, in which alkaloids are synthesized [76]. Ergot sclerotia collected in different geographical areas can serve as a valuable starting material for the selection of highly productive strains [77].

For the cultivation of ergot, the alternation of the parasitic stage of the life cycle and the axenic culture (re-sowing of sclerotium on a nutrient medium in vitro with the possibility of obtaining saprophytic mycelium with sclerotia-like morphology and purple pigmentation) is extremely important [40]. Morphological selection in axenic culture makes it possible to obtain a plectenchymatic form of mycelium, which resembles the early sclerocial stage of rye infection, producing lysergic acid and peptide alkaloids under submerged cultivation conditions [40, 78, 79]. Lysergic acid is also produced in vitro by isolates of parasitic strains selected in atypical habitats [40, 80]. However, this is a rare phenomenon: selection based on morphological features requires the cultivation of a large number of sclerotia in axenic culture, the detected mycelium is poorly separated, and the resulting isolates are extremely sensitive to cultivation conditions, which makes the process very time consuming and depends on a large number of different factors [17, 40].

UV mutagenesis [7, 16, 81-83] or chemical mutagens [7, 16], in particular nitrous acid [7, 84] or N-methyl-N'-nitro-N-nitrosoguanidine [7, 85] are applicable to obtain auxotrophic mutant strains of ergot or mutants with altered production of ergoalkaloids [7].

Among the methods for obtaining recombinant ergot-producing strains, the CRISPR/Cas9 genomic editing technology [7, 86, 87], polyethylene glycol (PEG)-mediated transformation [7, 88], and agrobacterial transformation using *Agrobacterium tumefaciens* (ATMT) [27] should be mention. Improvement of genetic engineering methods based on homologous recombination (HR) [89-91] makes it possible to obtain designer lines of ergot [8, 47] and heterologous organisms [73], including those with increased synthesis of target alkaloids [8, 73].

Methods for sampling, isolation, purification, detection, and quantification of ergot alkaloids in food, feed, plant materials, and animal tissues have been

extensively described, standardized, and validated [92-94]. Liquid chromatography with fluorescence (LC-FLD) or mass spectrometric (LC-MS/MS) detection is used for quantification [92, 94]. Less commonly used liquid chromatography with UV detection (LC-UV) [92) and enzyme-linked immunosorbent assay (ELISA) [92, 94, 95]. Express methods are also used, e.g., quantitative analysis with Van Urk reagent (colorimetric non-selective quantification of indole alkaloids) [40, 94] and thin layer chromatography [40].

The diversity and synthesis biology of ergot alkaloids is important to consider when they are found in agricultural products [96]. Quantitative comparison of ergoalkaloid production in 13 *Claviceps* species from natural communities and grass and cereal agrocenoses in Europe, North America, New Zealand and South Africa [25] drew the authors to the conclusion that doses and mechanisms of toxicity of ergot secondary metabolites must be reconsidered. According to various sources, in *C. purpurea s. l.* the average accumulation of alkaloids in sclerotia varies from 0.01-1.3 mg/g [61, 96-98] to 2.88-7.26 mg/g [99], but sometimes reaches 5-10 mg/g [61, 96, 97, 100].

Mass spectra of 67 peptide alkaloids of ergot were obtained [71]. The main identified ergoalkaloids were ergometrine, ergosine, ergotamine, α-ergocryptine, ergocornine, ergocristine and their 8-S(-inine-) epimers, which accounted for at least 50% of the total isolated alkaloid metabolome [25, 71, 95]. Matrix-assisted laser desorption ionization mass spectrometric imaging (MALDI-MSI) assessed the distribution of two representative alkaloids (ergocristine and ergometrine) produced by different strains of *C. purpurea* upon rye infection. Ergometrine shows a relatively even distribution throughout the sclerotium, while ergocristine is concentrated in the proximal region [101].

Cluster of alkaloid bioproduction genes. The cluster organization of alkaloid biosynthesis genes in ergot was first reported in 1999, and in particular, the importance of the *dmaW* gene for biosynthesis was shown [8, 102]. Clusters of genes for the biosynthesis of ergoalkaloids have been found in various fungi [30], for example, in *Clavicipitaceae* [30, 103, 104], in particular, in *Claviceps* [30, 102, 103], *Epichloe* [20, 30], *Periglandula* [3], *Metarhizium brunneum* [86, 105], *Neotyphodium lolli* [106], *Balansia cyperi*, *Balansia obtecta* [30]; in *Aspergillus* [107, 108], in particular, in *Aspergillus fumigatus* [107, 110], *A. leporis*, *A. homomorphus*, *A. hancockii* [111] and *A. japonicus* [112, 113]; in *Clavulinopsis fusiformis* [106]; in *Arthroderma benhamiae* [114, 115]; in *Penicillium camemberti* and P*enicillium biforme* [116].

Ergoalkaloids are represented by three main classes [34, 36, 37]. In clavine-type alkaloids (ergoclavines), the structural framework is formed by the aldehyde hanoklavin-I. Hanoklavin-I, as well as some other ergoclavins, such as agroclavin and elimoclavin, also serve as intermediates in the synthesis of ergoamides and ergopeptides. The second class is the enantiomers of lysergic acid and ergoamides: D-lysergic acid and its amides, including ergometrine. Lactam alkaloids and ergopeptides are the most complex compounds, representing the most numerous and diverse class of ergoalkaloids [34, 36, 37]. The pharmacological effect of ergot alkaloids is attributed to the molecular similarity between their ergoline backbone and the monoamine neurotransmitters adrenaline, dopamine and serotonin [9, 117, 118]. All ergoalkaloids have the common structure of a tetracyclic system (ergoline) containing an indole nucleus, in which rings A and B are formed from tryptophan, and rings C and D are formed as a result of cyclization of dimethylallyl pyrophosphate and tryptophan [25, 36, 72].

The alkaloid synthesis gene cluster in ergot is represented by 12-14 genes [8, 44], the *cloA*, *dmaW*, *easA*, *easC*, *easD*, *easE*, *easF*, *easG*, *easH*, *lpsA*, *lpsB*, *lpsC*; the *lpsA* gene has two homologues, *lpsA1* and *lpsA2*, the *easH* gene

is presented as *easH1*, and the reduced pseudogene *easH2* [8, 44-46]. The alkaloid biosynthetic cluster genes are responsible for the functioning of all the enzymes necessary for the formation of biosynthetic end products, ergotamine and ergocriptine, from tryptophan in some strains of *C. purpurea* [44, 46]. Two additional genes *easP* and *easO* were found in *C. paspali* [44].

The *dmaW*, *easF*, and *easC* genes are expressed at the four initial stages of ergot alkaloid biosynthesis, in addition, the *easE* gene is responsible for ergoline C-ring closure, followed by the formation of tetracyclic clavines, which requires the activity of the *easD*, *easA*, *easG*, and *cloA* genes. Later stages are synthesis of lysergic acid amides, dihydroalkaloids and complex peptides with the participation of the *lpsA-C* and *easH* genes (Fig. 1) [34, 35, 44].

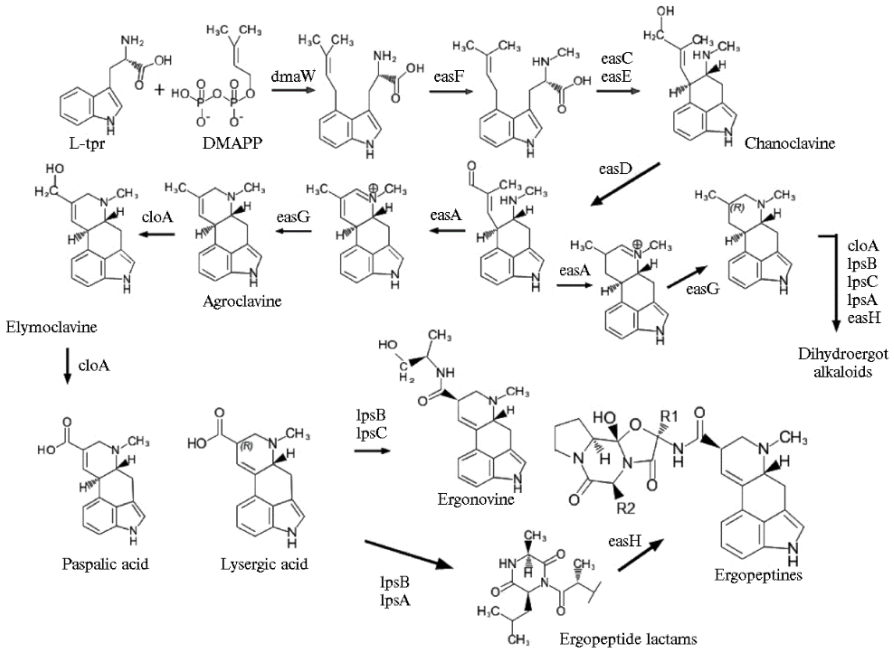

Fig. 1. Metabolic pathway for the biosynthesis of ergoalkaloids in *Claviceps* spp. (with addendum by S. Robinson and C. Young, edited by M. Liu) [34, 35, 44].

The results of studies have shown the presence of a different number of indole cluster genes in representatives of *Claviceps* (44,119). The presence of two or three copies of the *dmaW*, *easE*, and *easF* genes has been established, as well as a generally high frequency of gene acquisition and loss [44]. Homologues of nine cluster genes were found in *C. fusiformis* (44). In some *C. paspali* isolates, the *easG* gene may be absent [55] and *easE* may be non-functional [44]. In *C. paspali*, the alkaloid biosynthesis gene cluster is unstable and subject to partial elimination, and isolates capable of producing the predicted but not yet identified alkaloids have been identified [55]. Some strains of *C. africana* have eight genes for alkaloid biosynthesis (*cloA*, *easH2*, *lpsB*, and *lpsC* are missing), *C. lovelessii* has ten genes, in particular, *lpsC* and *easH2* are missing, and *easH1* and *lpsB* carried mutations leading to the appearance of stop codons [44]. In some strains of *C. maximensis* and *C. citrina* (*Citrinae*), in the absence of production of certain alkaloids, the same genes were not detected [44]. Only representatives of *Claviceps* had *lpsC* and *easH2*, although representatives of *C. perihumidiphila*, *C. ripicol*a, and *C. arundinis* lacked *lpsC*, and *C. capensis*, *C. cyperi*, *C. humidiphila*, and *C. monticola* had a partially identical *lpsC* gene sequence. Three strains of *C. purpurea* and three strains of *C. quebecensis* did not contain *easH2* [44].

Ways of biosynthesis of alkaloids and their genetic control. The metabolomic profiles of ergot alkaloids consist of complex mixtures of minor stereoisomers, constitutional isomers and transition products [45]. Changes in the operation of the alkaloid biosynthesis gene cluster can lead to the accumulation of various intermediates and by-products of the metabolic pathway and affect the rate of accumulation of alkaloids, especially those whose synthesis is regulated by feedback intermediates [8, 34, 35, 120]. The metabolic pathway for the biosynthesis of ergoalkaloids has several branch points. An optimized biosynthetic pathway excluding such points is shown in Figure 2 [9] as an example of the formation of D-lysergic acid from tryptophan. The accumulation of intermediate and final products in different species and strains of ergot is not the same: clavine derivatives, dihydro derivatives are formed, lysergic acid isomerization and lysergine amides, peptide alkaloids and their epimers are synthesized [34, 121, 122].

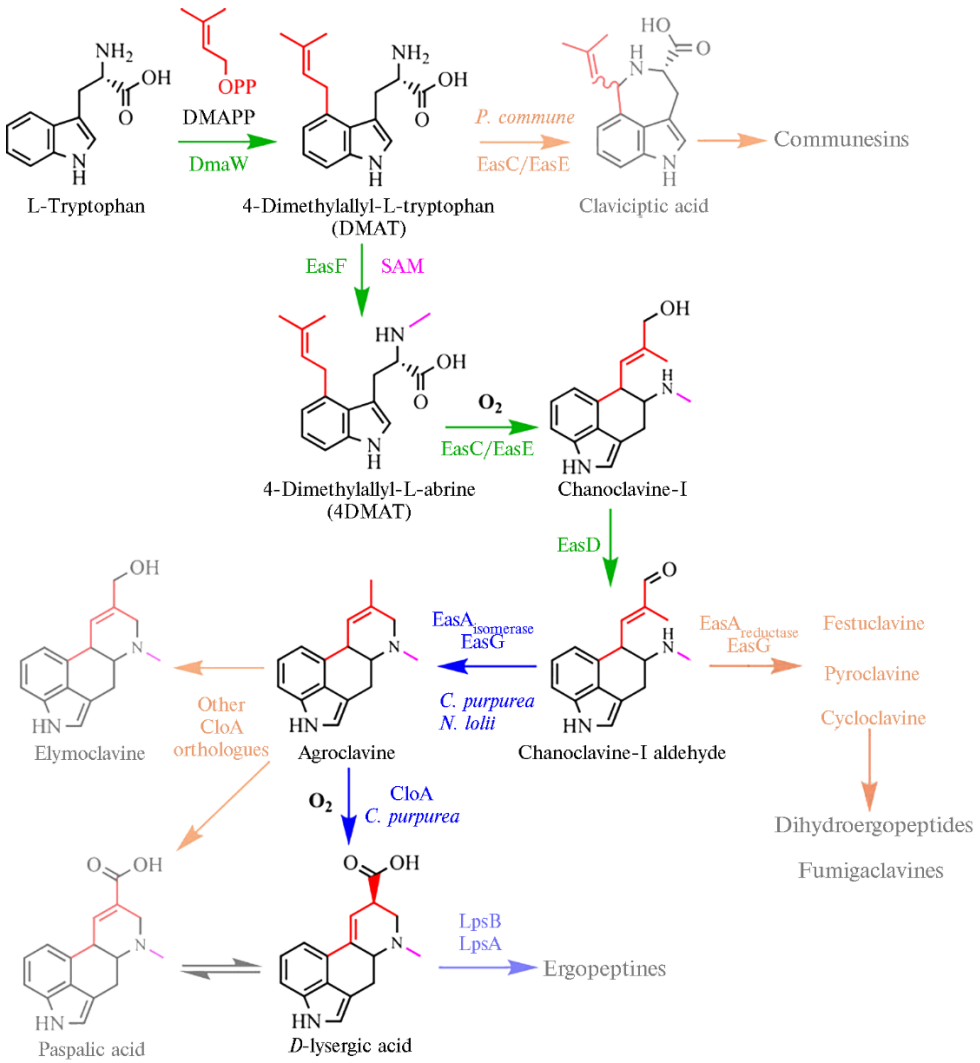

Fig. 2. A reconstructed optimal pathway for the biosynthesis of D-lysergic acid from tryptophan, excluding branch points [9].

It is believed [37] that the early stages of the biosynthesis of ergoalkaloids are conservative. Later stages are controlled by unique genes, which cause modifications and provide a large variety and species specificity of alkaloids in different

taxonomic groups of ergot [37, 114, 123].

Tryptophan synthesis in *C. purpurea* (as well as in other fungi) involves five steps carried out by three enzymatic complexes controlled by four different genes [8, 124, 125]. The most important is the anthranilate synthase complex, which consists of two subunits, AAS-I (α-subunit encoded by the *TrpE* gene) and AAS-II (a trifunctional peptide containing the β-subunit of anthranilate synthase, phosphoribosyl anthranilate isomerase and indole-3-glycerol phosphate synthase, *TrpC* gene) [8, 124]. While the α-subunit, which contains different sites for substrate binding (chorismate) and feedback inhibition by tryptophan, provides for the synthesis of anthranilic acid directly from chorismate in the presence of a large amount of ammonia, the β-subunit, together with AAS-I, catalyzes the conversion of chorismate to anthranilate in the presence of glutamine [8, 126].

The biosynthesis of alkaloids in *Claviceps* species begins with the prenylation of L-tryptophan by dimethylallyl diphosphate (DMAPP), which leads to the formation of 4-(γ,γ)-dimethylallyltryptophan (4-DMAT) [8, 127]. The 4-(γ,γ)-dimethylallyltryptophan synthase (DMATS) gene *dmaW* encodes the enzyme of the first stage of the biosynthetic pathway [109], DMAT synthase limits the rate of ergoline formation and is positively regulated by tryptophan, negatively by the intermediates agroclavine and elimoclavine [8, 36, 127]. In the promoter region of the active *dmaW* gene, binding sites were identified for CreA [8, 128], a regulatory protein involved in the repression of carbon catabolite, nitrogen metabolism regulator AREA [8, 129], transcription regulation factor PacC which is modulated depending on pH and is associated with virulence in *Aspergillus* [8, 130] as well as phosphorus deficiency-induced transcription factor NUC-1 [8, 102, 131]. The next step in the biosynthesis of ergoalkaloids is catalyzed by DMAT-N-methyltransferase (*easF* gene) [8, 132]. The subsequent reactions leading to the formation of the simplest clavin, chanoclavin-I, are catalyzed by chanoclavin-I synthase (*easE* gene) [8] and bifunctional catalase/decarbosylase (*easC* gene) [8, 106, 133]. The *easE* gene contains two exons and one intron and is 1503 bp long; the protein it encodes consists of 483 amino acid residues [36]. The protein region formed by amino acid residues 14-161 has been identified as a flavin adenine dinucleotide (FAD) binding domain, suggesting that EasE may function in a FAD-dependent manner [36, 110]. Ergot alkaloids of the lysergic and peptide classes are formed from the aldehyde hanoklavin-I as a common precursor [122]. The oxidation of hanoklavin-I to the aldehyde hanoklavin-I in the presence of NAD^+ is catalyzed by chanoklavin-I dehydrogenase encoded by the *easD* gene [134].

After five conserved steps in the formation of the chanoclavin-I aldehyde, the ergot alkaloid biosynthetic pathway branches. From the chanoclavin-I aldehyde, either festuclavin (the dihydroalkaloid pathway) or agroclavine (the D-lysergine pathway) is synthesized [34]. This process is regulated by the *easA* gene, which encodes flavin-dependent oxidoreductase [8, 34, 108], and by the *easG* gene which encodes reductase [8]. Festuclavin is formed in *C. africana*, agroclavin in *C. paspali*, *C. purpurea*, and *C. fusiformis*. Then agroclavine in *C. purpurea* is converted via elimoclavine into D-lysergic acid, and in *C. paspali* it is converted into paspalic acid [8, 34].

The enzyme CLOA (clavine oxidase, *cloA* allele, cytochrome P450-dependent monooxygenase) plays a key role in the oxidation of its substrate agroclavine to D-lysergic acid [135]. This is a process requiring cumulative six-electron oxidation and double bond isomerization [34, 136]. Deletion of *cloA* blocks the conversion of elimoclavin to D-lysergic acid (the mutant accumulates agroclavin, elimoclavin, and chanoclavin in significant amounts, but not ergopeptides). CLOA acts as a critical enzyme and links biosynthetic pathways for two different groups of ergot alkaloids [137]: agroclavine is oxidized to paspalic acid via elimoclavine by

cytochrome P450-dependent monooxygenase in the presence of NADPH and oxygen, and paspalic acid can spontaneously isomerize to D-lysergic acid [36, 137].

D-lysergic acid is converted to lysergic acid amides and ergopeptides with the participation of four lysergyl peptide synthetases (LPS), the trimodular LPS1 (LpsA1) with three domains with catalytic properties (*lpsA1* gene), monomodular LPS2 (LpsB, *lpsB* gene), monomodular LPS3 (LpsC, *lpsC* gene) and trimodular LPS4 (LpsA2, *lpsA2* gene). The LPSB/LPSC complex catalyzes the formation of ergometrine, the LPSB complex with LPS1 or with LPS4 mediates the assembly of ergopeptides [8, 138-141]. The biosynthesis of ergoamides and ergopeptides is initiated by the LpsB enzyme. D-lysergic acid, after being recognized as a substrate, is activated to form AMP ester and binds to the carrier protein LpsB. LpsA or LpsC compete for binding to LpsB [141]. LpsC can add one amino acid with the production of ergoamides, LpsA can add three amino acid residues with progressive elongation of the molecule and the formation of ergopeptams which are converted into ergopeptides by the monooxygenase EasH (*easH1* gene) [8, 142] into ergopeptides through oxidation followed by spontaneous cyclization [34]. Differences in the metabolomic profiles of *C. purpurea* alkaloids correlated with changes in the lpsA gene [45]. The study of the *lpsA1* deletion mutant led to the assumption that LpsA1 is responsible for the formation of phenylalanine-containing ergotamine and ergocristine ergopeptides [45, 140].

Biochemical analysis of extracts of *C. purpurea* sclerotia after infecting four cereal crops divided the isolates into two classes based on the content of aliphatic hydrophobic residues (I) and phenylalanine-containing alkaloids (II) in metabolomic profiles [45]. Ergotamine and ergocristine were the predominant sclerotia ergopeptides in class II, ergocryptine and ergocornine in class I. Both ergocornine and ergocryptine were found in both class I and II in samples from all four hosts, however class II sclerotia accumulated ergocornine and ergocryptine in much greater numbers [45]. Ergot alkaloid profiles specific to each of these classes are the result of nucleotide sequence variability in the tandemly duplicated *easH/lpsA* region [45, 103, 137].

In the ergoline biosynthetic cluster, the *lpsA1* and *lpsA2* genes were the result of a recombination event [42]. The presence of mobile transposable elements (TEs) similar to DNA transposons of the MULE and TcMar families and found in the intergenic space of the lpsA genes strongly suggests that TE-mediated transposition or mutations associated with TE inserts potentially contribute to the gene *lpsA* variability [45]. Highly polymorphic *lpsA1/lpsA2* intergenic spaces, rich in repetitive elements, associated with different strains of C. purpurea are closely related to divergent *lpsA2*. Domain-specific and highly variable *lpsA1/lpsA2* regions found in strain comparisons suggest that *lpsA* genes are likely to undergo recombinational shuffling [45, 143, 144].

Metabolic engineering of alkaloid biosynthesis. In a *C. purpurea* strain, overexpression of two genes of interest involved in alkaloid biosynthesis was described. These are the *trpE* gene for anthranilate synthase with *S76L* mutation generated to overcome inhibition by excessed tryptophan [8, 145] and the *dmaW* gene encoding dimethylallyltryptophan synthase, an enzyme involved in biosynthesis of the key intermediate of alkaloid production in ergot. Both manipulations led in a significant (up to 7-fold) increase in ergot alkaloids in submerged cultures [8].

Metabolic engineering of ergot may use the reconstruction of target biosynthetic pathways in convenient heterologous hosts, for example, in *Aspergillus nidulans* [104, 146]. Reconstruction of the ergot metabolic pathway with enzymes directing metabolic flux to desired branch points in a heterologous system ensures that there is no variation in the profile of ergot alkaloids produced [9,

136].

Significant progress has been made in the construction of pathways for the synthesis of ergoalkaloids in various heterologous hosts, such as *Aspergillus nidulans*, *Aspergillus fumigatus*: important intermediates and end products or new derivatives of ergot alkaloids have been obtained [112, 123, 136, 146].

A transgenic yeast line carrying eight genes responsible for the synthesis of D-lysergic acid (*dmaW*, *easF*, *easC*, *easE*, *easD*, *easA*, *easG*, and *cloA*) was developed (see Fig. 2) and enantiopure D-lysergic acid synthesized in a bioreactor riched in concentrations up to 1.7 mg/l [9].

Using genomic editing (CRISPR/Cas9), recombinant cultures of *Metarhizium brunneum* were obtained [86]. Their relative yield of D-lysergic acid (86.9%) and dihydrolysergic acid (72.8%) was much higher than that of engineered strains of *Neosartorya fumigata* (2.6 and 2.0%, respectively) [86]. For *C. purpurea*, a CRISPR/Cas9 genomic editing system has been developed with an editing efficiency of 50 to 100%. It successfully knocked out three target genes that are closely related to uridine biosynthesis (*ura5*), hyphal morphology (*rac*) and ergoalkaloids production (*easA*), which made it possible to obtain a uridine auxotrophic mutant (Δ*ura5*), a mutant with an altered phenotype in axenic culture (Δrac) and a mutant that did not produce alkaloids (Δ*easA*) [87].

We draw attention to the importance of several critical points in the biosynthetic pathway that can affect the amount of ergoalkaloids produced in vitro. These points are the synthesis of tryptophan from precursors and its involvement in the biosynthetic chain at the first stages of the metabolic pathway; the synthesis of clavines; the stage of agroclavine-D-lysergic acid; and three branch-points to target components, including synthesis of clavin derivatives, dihydro derivatives of alkaloids, isomerization of D-lysergic acid and stage D-lysergic acid—ergopeptides.

Ergot virulence and interaction with the host plant. When developing methods to reduce damage from damage by herb and ensure toxicological safety, it is important to take into account not only the ability of the fungus to synthesize ergoalkaloids, but also its virulence and mechanisms of interaction with the host plant. As already noted, ergoalkaloids played a significant role in the spread of ergot and the expansion of the range of this ascomycete. *C. purpurea* infects a number of economically important crops, including rye, wheat and barley [19, 49, 147]. Ergot infects cereals during flowering, infecting the tissues of unfertilized inflorescences of female plants and replacing seeds with sclerotia [53, 147]. Thus, cross-pollinating cereals that exhibit open flowering, such as rye, are particularly at risk of infection [49, 148]. Ergot is also infectious for hybrid forms of barley or wheat that have received sensitivity as a by-product of selection [49, 149, 150]. *C. purpurea* infections affect hexaploid soft wheat (*Triticum aestivum* L.) and tetraploid durum wheat *T. turgidum* subsp. *durum* (Desf.) Husn. [151, 152]. Sensitivity to *C. africana* has been described in sorghum, sensitivity to *C. africana* in millet [39, 153, 154]. The interaction of the fungus with the host can vary from antagonistic to mutualistic [155, 156]. Ergot is considered a biotrophic organism [47], but necrotrophic properties have been suggested [49].

The main quantitative indicators of ergot virulence are the amount of honeydew, which characterizes the efficiency of infection at the conidiospore stage, the size of the sclerotia and the total number of sclerotia per spike (the effectiveness of parasitism and the level of production of ascospores) [152, 157, 158]. A positive linear relationship has been noted between the size of sclerotia and the number of ascospore-containing stromas produced [159].

In some cases, *C. purpurea* exhibits weak host specificity [61, 160]. Wild isolates parasitizing on *Dactylis* representatives, when infected rye, did not cause

the usual sphacelial conidia formation but supported the growth of thin sclerotia, sometimes two per inflorescence, with a low alkaloid content, however, the sclerotia acquired their usual appearance after two cycles of axenic (in vitro) and parasitic (on rye) cultivation with successive selection of plectenchymatous axenic mycelium [40]. In the axenic culture, the unpigmented mycelium was nonpathogenic, but the pigmented (purple) plectenchymatous form showed the ability to parasitize rye inflorescences [40]. The host specificity of ergot is not the same in different phases of fungal development: at the sphacelial stage and during the formation of honeydew, the specificity is wider, and the formation of mature sclerotia is more limited by the range of hosts [161, 162].

It has been suggested that sclerotia and ascospores may be the primary inoculum [59, 163, 164]. Differences have been found in the relative contribution of ascospores and conidia to the spread of ergot [59]. Analysis of *C. purpurea* populations from different areas showed that isolates collected from one seed head accounted for 66% of genetic variability. This is considered as an indication of the infection of a significant part of the seed heads bearing multiple sclerotia with ascospores, but not with conidia. At the same time, most of the clonal isolates (they had identical multilocus genotypes) were also collected from the same seed head, indicating a role for conidia (paddy) in secondary infections within the seed heads [59].

Ergot is a homozygous organism [40]. Ascospores (stage of sexual reproduction) are dispersed mainly by wind [59, 164]. Conidia (the vegetative stage of the cycle) are introduced to healthy inflorescences by rain or irrigation, mechanically (by plant-to-plant contact) or insects, which can lead to multiple cycles of infection during flowering [19, 59, 165, 166]. For North American isolates, under a wide host range [167], earlier-flowering plants (e.g., rye) that become infected with ergot in early spring have been shown to be able to serve as a reservoir of conidia and a source of infection for late-flowering plants [59, 164]. .

Ergot infestation is reduced by immediate pollination of female inflorescences, closed flowering (cleistogamy) and physiological resistance [39]. Pollen sterility is one of the main factors facilitating ergot infection [102, 168]. Gametocidal treatment of rye increases the productivity of ergot when cultivated in planta in the field [169]. It is widely believed that *C. purpurea* causes infection by mimicking the growth of pollen tubes [39, 102]. At this stage, growth of *C. purpurea* occurs mainly intercellularly, but invasive hyphae are also found that are completely covered by the host's plasma membrane [47, 102].

Several loci of partial ergot resistance have been identified in wheat [152, 157, 170, 171]. Thus, resistance genes were found in chromosome 6B in the Kenya Farmer variety and in chromosomes 1B, 3B, 4B, and 5B in the Carleton variety [172]. Two ergot resistance QTLs were identified in hexaploid wheat cv. Robigus (located on chromosomes 2A and 4B, QCp.niab.2A and QCp.niab.4B) and two in cv. Solstice (chromosomes 4D and 6A, QCp.niab.6A and QCp .niab.4D) [171]. Four ergot resistance loci (QCp.aafc.DH-1B, QCp.aafc.DH-2A, QCp.aafc.DH-5A, QCp.aafc.DH-5B) were detected [n chromosomes 1B, 2A, 5A and 5B in the variety in Greenshank durum wheat [152], QCp.aafc.DH-2A (significantly reduces honeydew production) and QCp.aafc.DH-5B (reduces the total number of sclerotia per ear) contribute the most to resistance [152]. Male-sterile sorghum lines show little but consistent resistance to *C. africana* [173]. In sorghum, nine loci have been identified that affect the percentage of infection with this fungus, of which QTLs located on the chromosomes SBI-01, SBI-02, SBI-06, SBI-07, and SBI-08 make the greatest contribution to resistance [174]. In barley, genetic variability in ergot resistance has been described. Laurier, Maskot and Sabina have been reported to be the most resistant to infection (less than 0.1% sclerotia), while

Albany, Leger, Symko and Morrison are the most susceptible [149]. Partial resistance of wheat to ergot is associated with dwarfism gene alleles located at the *Rht* loci encoding DELLA proteins (*Rht-B1* and *Rht-D1*, chromosomes 4B and 4D, respectively) [32, 171]. *Rht-B1b* and *Rht-D1b* are mutations that determine the loss of sensitivity of DELLA to gibberellic acid [32]. A decrease in the amount of honeydew, as well as in the size and weight of sclerotia, was found in lines carrying mutant alleles of dwarfism and semi-dwarfism *Rht-D1b*, *Rht-D1c*, *Rht-B1c* [32]. The association between *Rht-B1b* and *Rht-D1b* and ergot resistance points to a role for gibberellic acid in *C. purpurea* infection [32].

Reprogramming of the hormonal pathways of the host plant and differential expression of auxin, ethylene, and cytokinin depending on tissue type and time after *C. purpurea* inoculation have been established [32, 49, 57]. An increase in the content of hyberrellic acid, auxin, and dihydrozeatin-type cytokinins (DHZ) has been found [32]. Gibberellic acid-mediated degradation of DELLA proteins and suppression of jasmonic acid signaling pathways have been shown to increase the incidence of ergot infection in wheat [175]. When infected with ergot, wheat genes associated with auxin become most differentially expressed in the early stages of infection. The *AUX/IAA* (auxin/indole-3-acetic acid) family genes encode known transcriptional repressors of auxin response genes, while the *GH3* (glycoside hydrolase 3) gene family encodes auxin conjugating enzymes that regulate the auxin pool through negative feedback. Both *AUX/IAA* and *GH3* are responsible for the early response to auxin. Suppression of auxin signaling by upregulation of the *AUX/IAA* genes and binding of excess auxin by GH3 family proteins serves as a direct host plant response to *C. purpurea* infection [49, 175]. Among the genes associated with ethylene, the highest activation was found in the 1-aminocyclopropane-1-carboxylate oxidase (ACO) and 1-aminocyclopropane-1-carboxylate synthase (ACS) genes. A number of genes for the biosynthesis and signaling pathways of jasmonic acid (JA) were expressed differently in response to infection. Thus, for 12-oxophytodienoate reductase (OPR) and allene oxide synthase (AOS), which catalyze the first stage of JA formation, it was noted that in the case of OPR, expression increased, AOS increased only in one gene, and the rest were suppressed [49]. Cytokinins are also involved in *C. purpurea* wheat infection with activation of cytokinin oxidase/dehydrogenase and cytokinin glycosyltransferase in plant tissues [49]. Mechanisms regulating the metabolism of gibberellic acid (GA) are induced upon infection: the gibberellin-2-beta-oxidase gene is activated at an early stage of infection, the GA receptor gene GID1 is also activated within 24 h after infection, and then it is suppressed within 48 and 72 h [49]. Salicylic acid, cytokinin, and auxin are involved in the interaction between C. purpurea and *Brachypodium distachyon* [176, 177]. Other protective mechanisms were also active upon wheat infection with *C. purpurea* (in particular, there was a steady increase in the expression of chitinase genes) [49].

Rye showed significant differences in ergot resistance [178-180]. It is believed that the main contribution to the resistance of rye is due to pectinesterase activity and metabolic processes of cell wall modification and pollen tube growth [180]. A comparative analysis of the transcriptome in rye hybrids sensitive (DH372) and moderately resistant (Helltop) to ergot showed that 12 and 8 genes, respectively, are activated differently in hybrids in response to infection [180]. Among them, six genes (*XLOC_059237*, *XLOC_1003867*, *XLOC_118963*, *XLOC_1220465*, *XLOC_1387037* and *XLOC_386424*) cell wall modification and pectinesterase metabolic pathways (180). COBRA-like protein (*XLOC_1432429*) and a putative pectinesterase inhibitor (*XLOC_118963)* show the highest differential expression [180]. In cell wall modification pathways, three genes *XLOC_1343481*, *XLOC_1343482*, and *XLOC_145869* are associated with polygalacturonase [180]. Polygalacturonase is

known as a pathogenicity factor in the interaction between *C. purpurea* and rye [180, 181], and its inhibition is one of the main mechanisms of plant resistance to fungal pathogens [182]. It has been shown that the activity of both *C. purpurea* polygalacturonases (*cppg1* and *cppg2* genes) decreases upon infection of transgenic plants with a high degree of pectin methylesterification [183, 184]. The defense responses associated with the cell wall are of decisive importance for the basal resistance of plants to fungal pathogens [180, 185]. It is also interesting to note that a COBRA-like protein, which is involved in the response of rye plants to *C. purpurea* infection, mediates the directed growth of pollen tubes in *Arabidopsis thaliana* [186]. Knockout of the COBRA-like protein gene caused gametophytic male sterility [186]. Mutant ergot strains with attenuated virulence induce the expression of other rye defense-related genes [47], in particular the chitinase gene (*Sc2Loc00083431.2*), a gene with high homology to the fungal xylanase inhibitor gene (*Sc4Loc00580338.2*), and a putative resistance protein (*Sc4Loc01458017.2*) [47].

In ergot, there are significant differences in virulence due to the rate of mycelium growth [157, 187, 188]. Hyphae elongation is determined by polysaccharide metabolism [189]. Deletion of the *Mid1* gene leads to a decrease in growth rates and a complete loss of virulence. At a certain stage of the infectious process, these signs correlate [190]. During cultivation, in some species of *Claviceps*, the mycelium grows faster than in others: for example, in the Japanese isolate of *C. sorgicola*, the rate is on average 1 mm/day [191], in the Indian isolates of *C. africana* and *C. sorghi*, 0.1 mm/day [192].

Ergot secretes plant cell wall degrading enzymes, signaling molecules and effector compounds [177, 193]. Approximately 90% of the most highly expressed ergot genes are genes that code for proteins involved in growth and development [47]. The *C. purpurea* genome contains more than 400 genes encoding putative infection development effectors; many of these genes are clustered and highly redundant. It has been suggested [47] that the high redundancy of genes in the clusters of effector compound synthesis serves as a mechanism preventing gene loss [47]. Functional analysis of some ergot effector metabolites showed that at least one of them accumulates in the plant apoplast [47]. Ergot has an extensive effector network influencing the course of infection, but a significant contribution to the virulence of any particular effector compound has not yet been established [47]. Ergot has enzymes (CAZymes) that break down the plant cell wall [47, 194]. Ten of the most expressed *C. purpurea* genes are the CAZyme protein genes [47]. *C. paspali* isolates secrete CAZyme-like proteins [195] main target of which is pectins [47, 195]. Signaling components include MAP kinases [47, 181, 196, 197], NADPH oxidases generating ROS (reactive oxygen species) (*Cpnox2* gene; controls the infection process and reduces damage in the host) [198], as well as cytokinins necessary for the successful development of infection [199, 200]. Cytokinins are required to establish and maintain plant-fungal interactions [199, 200]. Enhancement of absorptive activity and influx of nutrients to the foci of infection is the most important role of ergot cytokinins [177). Virulence-reducing cytokinin deficiency can be achieved by deleting the isopentenyltransferase gene, which is also involved in the regulation of translation [177, 200]. However, it is important to bear in mind that alternative pathways for cytokinin activation have been established [177]. The ergot polygalacturonase gene (virulence factor in the interaction of *C. purpurea* and rye) [47] *cppg1* (*cp6977*), the MAP kinase *cpmk1* gene (*cp1700*), and the superoxide dismutase *cpsod1* gene (*cp7438*) belong to the group of genes with a high level of expression, but the role superoxide dismutase is not significant in the development of infection [201].

Two virulence factors were of particular interest in terms of the interaction between *C. purpurea* and rye. These are a transcription regulation factor encoded

by the ergot gene *Cptf1* (homolog of the yeast *Ap1* gene) and a small GTPase encoded by the C*pcdc42* gene. Deletions of these genes reduce ergot virulence [47, 202, 203]. The small GTPase (*Cdc42* gene) is involved in the organization of the cytoskeleton in fungi. In the wild strain of *C. purpurea*, the expression of the heterologous GTP-ase gene of *Colletotrichum trifolii* (Ct*cdc42*) had a significant effect on the vegetative differentiation of ergot. Expression of the dominantly active Ct*cdc42* (DA) allele led to the loss of conidia and aberrant cell shape, while the dominant-negative (DN) allele of *Ctcdc42* (with such mutations, the altered product disrupts the ratio of wild-type proteins during co-expression of genes) stimulated branching and conidiogenesis. The deletion of the Cp*cdc42* gene was not lethal and resulted in a phenotype comparable to that of negative transformants; the DeltaCp*cdc42* mutants were non-pathogenic and did not cause symptoms of the disease (invasive growth stopped at an early stage) [203].

Ergot mutant lines, auxotrophic for tryptophan, are non-infectious for rye, probably due to reduced production of plant auxins, which are synthesized from indole-3-glycerol phosphate via tryptophan-dependent and tryptophan-independent biosynthetic pathways and help the fungus to colonize the host plant [7].

It is worth emphasizing once again that the definition of parasitic fungi as plant mutualists or pathogens remains very vague, with new data published periodically supporting both the first and second statements [204]. It has been experimentally shown that infection with ergot in red fescue (*Festuca rubra*), which forms a symbiosis with the endophytic fungus *Epichloe festucae*, reduces the damage of plants by aphids (*Sitobion* sp.) by 4.5 times [204, 205]. At the same time, plants containing a symbiotic endophyte were more susceptible to *Claviceps* infection (45% of E^+ plants vs. 31% of E^- plants) [204]. The presence of *Epichloe occultans* in Lolium multiflorum and *Epichloe gansuensis* in *Achnatherum inebrians* correlated with a decrease in the incidence of *C. purpurea* infection, suggesting that, in some plant species, members of the genus *Epichloe* are likely to mediate protection against *C. purpurea* [204, 206-208]. In these cases, the growth of resistance to pathogens may be the result of increased host immunocompetence or direct competition between ergot and an endophytic strain of *Epichloe* [204, 209, 210]. The absence of differences in the number and taxonomic composition of the mycobiota between plants infected and not infected with ergot partially supports the hypothesis of the benefit of this pathogen for plants [211].

Studies of 25 years of ergot susceptibility in major crops across Canada have shown that rye is most affected by ergot, followed by bread and durum wheat, followed by barley and oats [212]. An interesting fact was that the incidence and severity of outbreaks changed annually, and over time the infection spread more and more, but its harmfulness and degree of damage did not change [212].

An important subject of study remains the relationship between ergot virulence and ergo alkaloid content [213, 214]. In rye genotypes less susceptible to ergot, the content of ergoalkaloids is reduced [213], but this was not affected by gametocidal treatment of rye [169]. A negative relationship has been found between the mass of one sclerotium and the accumulation of ergoalkaloids in winter rye and spring wheat [214]. In Russia, two varieties of wheat (Novosibirskaya 18 and T-66) immune to ergot, and 13 relatively resistant (with a lesion of no more than 5.2% and an admixture of sclerotia in the grain of no more than 0.3%), have been identified. In rye, 10 varieties demonstrate relative tolerance with damage varying from 5.8 to 33.0% and grain contamination by sclerotia of 0.3 to 1.4% [214]. Genotypes have been revealed in which resistance to ergot damage is potentially combined with the absence of accumulation of ergoalkaloids in sclerotia: these are the varieties of winter rye Rumba, Symphoniya, Harmoniya and spring wheat Epos [214].

Hybrid breeding in rye has been shown to increase grain yield while enhancing ergot susceptibility associated with cytoplasmic male sterility (CMS), which is maternally inherited [215, 216]. The close relationship between the presence of fertile pollen and susceptibility to ergot poses a breeding problem [213, 215, 217].

Thus, *C. purpurea* is, on the one hand, the most important producer of a large number of biologically active compounds (alkaloids) and a unique model of the parasite-host system [218-221]. On the other hand, it is a pathogen that causes significant economic damage to grain producers, feed and livestock industries around the world [222, 223]. While medical and biotechnological research has focused on the beneficial effects of ergot alkaloids [224] and other secondary metabolites and how they are produced [224-228], the hazards of ergoalkaloids remain an acute and worrying global problem in the crop, animal and food industries [229-232]. Its severity is exacerbated by the fact that *Claviceps* alters the profiles and intensity of alkaloid production under changing environmental conditions [231].

In recent years, the management of symbioses of plants and endophytes to optimize the profile and concentration of produced secondary metabolites (including those toxic to humans and farm animals) can be considered as a promising approach [231, 233]. Phylogenetic studies and studies of the evolutionary variability of ergot remain significant [234]. The revealed patterns can be useful in obtaining new highly productive recombinant saprophytic strains-producers of *C. purpurea*, in breeding ergot-resistant varieties, and in optimizing plant protection techniques.

An analysis of publications indicates that different plants show a similar response to *C. purpurea* infection, but resistance mechanisms (for example, in rye and wheat) appear to be different. With respect to historically and evolutionarily determined hosts with open flowering (for example, rye), *C. purpurea* is a biotroph, and partner interactions are mutualistic. In the case of atypical hosts (eg wheat) and new breeding forms sensitive to ergot, necrotrophy and classical parasitism of *C. purpurea* are possible.

Thus, here, we reviewed data on gene clusters that regulate the mechanisms of virulence and biosynthesis of alkaloids in the ergot pathogen *Claviceps purpurea* in the context of evolutionary variability, speciation, and identification of *Claviceps* strains, as well as in view of the achievements and prospects of genetic engineering. The ergot pathogen *C. purpurea* is a very variable and adaptable. This provides for the wide variety of synthesized alkaloids and the wide range of host plants. As a result, there is an abundance of strains of the fungus. The pathways for the biosynthesis of ergot alkaloids have several branch-points with accumulation of intermediate metabolites. In commercial farming, such a redundancy requires additional purification, and in wild strains, it increases toxicity to farm animals and humans. Knowledge of the regularities and genetic control of secondary metabolism in *C. purpurea* is important for the effective technologies for production of ergoalkaloids. The ultimate practical goal of ongoing genomic, transcriptomic, and metabolomic studies of *C. purpurea* and the *C. purpurea*—host plant system is to reduce the toxicity and virulence of the fungus, and limit the spread of ergot to new areas and new plants, including by creating resistant breeding forms.

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