Review

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Regulation of LaeA and Velvet Proteins on the Production of Mycotoxins and Other Fungal Secondary Metabolites

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Abstract: Fungi are rich sources of secondary metabolites of agrochemical, pharmaceutical and food importances, such as mycotoxins, antibiotics and antitumor agents. Secondary metabolites play vital roles in fungal pathogenesis, growth and development, oxidative status modulation, and adaptation/resistance to various environmental stresses. LaeA contains an *S*-adenosylmethionine binding site and displays methyltransferase activity. The members of velvet proteins include VeA, VelB, VelC, VelD and VosA, for each member with a velvet domain. LaeA and velvet proteins can form multimeric complexes such as VosA-VelB and VelB-VeA-LaeA. They belong to global regulators and are mainly impacted by light. One of their most important functions is to regulate gene expression that are responsible for secondary metabolite biosynthesis. The aim of this mini-review is to represent the newest cognition on the biosynthetic regulation of mycotoxins and other fungal secondary metabolites by LaeA and velvet proteins. In most cases, LaeA and velvet proteins positively regulated production of fungal secondary metabolites. The regulated fungal species mainly belong to the toxigenic fungi from the genera of *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Magnaporthe*, *Monascus*, and *Penicillium* for the production of mycotoxins. We can control secondary metabolite production to inhibit the production of harmful mycotoxins while promoting the production of useful metabolites by global regulation of LaeA and velvet proteins in fungi. Furthermore, the regulation by LaeA and velvet proteins should be a practical strategy in activating silent biosynthetic gene clusters (BGCs) in fungi to obtain previously undiscovered metabolites.

Keywords: global regulation; toxigenic fungi; LaeA; velvet proteins; secondary metabolites; mycotoxins; phytotoxins; biosynthetic gene cluster; biological activities; regulation mechanisms

1. Introduction

Fungal secondary metabolites are highly complex and have a rich diversity that makes fungi a treasure of bioactive secondary metabolites [1–4]. Some fungal metabolites are harmful to plants, humans and animals, which are usually called mycotoxins [5–8]. Many bioactive metabolites derived from fungi display their broad potential as agrochemicals, pharmaceuticals, cosmetics and food additives [9,10].

In recent years, it has become easier through genome mining to identify and functionally predict fungal metabolites [11]. Many strategies can regulate (i.e., promote or inhibit) the biosynthesis of secondary metabolites in fungi such as one strain-many compounds (OSMAC), global regulation, epigenetic regulation, heterologous expression, and combinatorial biosynthesis [12–14]. Among them, global regulation for secondary metabolite production has been thought to be the most practical strategy. Global regulation is a complex upper-level regulatory network in which microorganisms respond comprehensively to external environmental stimuli such as light intensity, temperature, ambient pH, redox status, carbon and nitrogen sources. LaeA and velvet proteins belong to global

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regulators, and are mainly regulated by light. Both LaeA and velvet proteins can form multimeric complexes. They are involved in fungal development and secondary metabolism [15–19].

In the past 20 years, many advances have been achieved about the regulation of LaeA and velvet proteins on fungal secondary metabolism and development. Though some reviews were published [15,20,21], many other recent achievements have not been included. In this review, we focused on the regulation of LaeA and velvet proteins on secondary metabolite production in fungi, in order to inhibit the production of mycotoxins, promote the production of useful metabolites, reveal previously undiscovered metabolites, clarify their regulation mechanisms, and accelerate their applications.

2. Regulation of LaeA on Secondary Metabolite Production in Fungi

LaeA (loss of *aflR* expression) is also called Lae1 or LAE1. It was first identified as a nuclear protein in *Aspergillus*. Compared to the velvet proteins, LaeA has no velvet domain [16]. LaeA functions as a global regulator of secondary metabolism and morphogenetic development in various filamentous fungi. LaeA protein sequence contained an *S*-adenosylmethionine binding site, so it has been proposed to have methyltransferase activity, and might linked to the remolding of chromatin structure to regulate gene transcription by lysine or arginine methylation of histone [22,23]. In most cases, the *laeA* genes in fungi positively regulated production of secondary metabolites. Only a few *laeA* genes were found to negatively regulate secondary metabolite production (Table 1) [21]. At present, the regulation studies of LaeA mainly focuses on the toxin-producing fungal species for mycotoxin control, as well as plant endophytic fungi and marine-derived fungi for mining bioactive compounds. Some examples of LaeA regulating secondary metabolite production in fungi are shown in Table 1. The structures of the metabolites are shown in Figure S1.

Overexpression of *AaLaeA* increased production of antitumor compounds including myricetin (**1**), geraniol (**2**), ergosterol (**3**) and other compounds in the endophytic fungus *Alternaria alstroemeria* by metabolomic analysis [24]. In contrast, overexpression of *AaLaeA* in another endophytic fungus *A. alstroemeria* derived from the medicinal plant *Artemisia annua* decreased production of antitumor compounds. Unfortunately, the antitumor compounds were not further identified [25]. Many species in *Alternaria* genus usually belong to the plant pathogenic fungi to produce host-specific toxins (HSTs) and cause host plant diseases [2]. *A. alternaria* was the pathogen of some plants such as tomato, apple and strawberry to produce AAL-, AM- and AF-toxins, respectively. It was found that LaeA positively regulated production of the HSTs as well as development and pathogenicity of *A. alternaria* [26]. In other instances, deletion of *laeA* in *A. alternata* greatly decreased the production of alternariol (AOH, **4**) and alternariol monomethyl ether (AME, **5**) [27]. Furthermore, overexpression of a LaeA-like global transcriptional regulator in marine-derived fungus *A. alternata* JJY–32 led to the discovery of an anti-inflammatory meroterpenoid namely tricycloalternarene O (**6**) [28].

Arthrobotrys flagrans (synonym *Duddingtonia flagrans*) was a typical nematode-trapping fungus which has been used for nematode biocontrol. Overexpression or deletion of *AfLaeA* positively regulated production of secondary metabolites and their antinematodal activity, whereas some metabolites were not produced due to the absence of *AfLaeA*. The antinematodal activity of these secondary metabolites needs further confirmation [29].

There are many examples of *Aspergillus* species regulated by LaeA to produce secondary metabolities (Table 1). Overexpression of *Az5LaeA* in *Aspergillus* sp. Z5 exhibited increased production of diorcinol (**7**). The *laeA* (*AnLaeA*) gene from *A. nidulans* was heteroexpressed in *Aspergillus* sp. Z5, and was also found to increase production of diorcinol (**7**) [30]. Introduction of *laeA* from *A. nidulans* to *Aspergillus* sp. FKI-5362 led to an increase in production of an antifungal compound MS-347a (**8**), which could inhibit the growth of broad plant pathogenic fungi including *Botrytis cinerea*, *Colletotrichum gloeosporoides*, *Leptosphaeria maculans*, and *Pyricularia oryzae* [31]. MS-347a (**8**) was previously screened to inhibit myosin light chain kinase from *Aspergillus* sp. KY52178 [32]. When *laeA* gene was deleted in *A. carbonarius*, the production of citric acid (**9**) [33] and ochratoxin A (OTA, **10**) [34,35] was greatly decreased, respectively. If the deleted mutant Δ*laeA* of *A. carbonarius* was colonized in nectarines and grapes, OTA (**10**) was significantly reduced [35]. When *A. carbonarius*

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was treated with eugenol at 0.2 μL/mL, OTA (**10**) production was decreased at 87.7%. The transcription of the clustered genes for OTA biosynthesis was significantly reduced under eugenol stress and was further confirmed by RT-qPCR analysis. The expression of LaeA was markedly downregulated by eugenol, which indicated that eugenol was probable through inhibiting LaeA expression to decrease OTA (**10**) production in *A. carbonarius* [35].

Overexpression of *llm1* encoding LaeA-like methyltransferase in *A. cristatus* led to an increase of contents of multiple secondary metabolites including terpenoids and flavonoids. Unfortunately, these metabolites were not identified [37]. With a scaled-up fermentation of the Δ*flLaeA* strain of *A. flavipes*, five metabolites, including two previously undescribed piperazine derivatives flavipamides A (**11**) and B (**12**), along with three known nonribosomal peptides asperphenamate (*N*-benzoylphenylalaniny-*N*-benzoylphenyl-alaninate, **13**), 4′-OMe-asperphenamate (**14**), cyclic Pro-Gly-Val-Gly-Try(8-OH, 3-prenyl)-Gly-Trp (**15**) were obtained by LC-MS guided isolation [38].

Aflatoxins (AFs) have been thought of as the most potent carcinogens, and some fungal species from *Aspergillus* genus are their main producers, especially *A. flavus*. LaeA was revealed to positively regulate production of alfatoxins in *A. flavus* [39–42]. Deletion of *laeA* in *A. flavus* resulted in the significant upregulation of the NAD^+-dependent histone deacetylase sirA involved in silencing secondary metabolism clusters via chromatin remodeling. Accompanying the chromatin modification, the enzymes participating in secondary metabolism, including biosynthesis of sterigmatocystin (ST, **16**), aflatoxin B1 (**17**), cyclopiazonic acid (**18**) and ustiloxin B (**19**), were drastically decreased [40,43,44]. The enzymes in the ustiloxin B (**19**) biosynthesis pathway might be indirectedly regulated by LaeA [44]. An interaction partner of LaeA namely kinetochore protein Spc105 was revealed to regulate development and secondary metabolism in *A. flavus*. Moreover, Spc105 positively regulated the production of secondary metabolites such as aflatoxins and kojic acid (**20**), and negatively regulated the production of cyclopiazonic acid (**18**). Transcriptome analysis of the Δ*spc105* mutant revealed that 23 backbone genes for secondary metabolism were differentially expressed, corresponding to 19 of the predicted 56 secondary metabolite gene clusters, suggesting a broad regulatory roles of Spc105 on secondary metabolism in *A. flavus* [45].

The production of several mycotoxins was positively regulated by LaeA in *A. fumigatus*. For examples, the deletion of *laeA* led to decreased production of gliotoxin (**21**), endocrocin (**22**), fumagillin (**23**), fumagatin (**24**), and helvolic acid (**25**) in several strains of *A. fumigatus* [46–49]. Production of cyclopiazonic acid (**18**) was increased when *laeA* was overexpressed in *A. fumisynnematus* [50]. Deletion of *laeA* gene in *A. luchuensis* mut. *kawachii* led to the reduced production of citric acid (**9**). LaeA was considered as the methyltransferase through regulating citrate exporter-encoding *cexA* gene [51].

Overexpression of *laeA* in *A. nidulans* led to an increased production of sterigmatocystin (**16**) [52] and terrequinone A (**26**) [53]. Accordingly, the deletion of *laeA* gene in *A. nidulans* reduced the production of sterigmatocystin (**16**) and norsolorinic aid (NOR, **27**) [54]. The deletion of *laeA* also led to a depressed expression of genes involved in biosynthesis of sterigmatocystin (**16**), terrequinone A (**26**) and penicillin G (benzylpenicillin, **28**) [55]. However, a contradictory example was that the deletion of *laeA* gene in *A. nidulans* also led to an increased production of sterigmatocystin (**16**) [56]. Generally, LaeA or LaeA-like methyltransferase F (LlmF) negatively regulated biosynthesis of sterigmatocystin (**16**) in *A. nidulans*.

Aspergillus niger is a biotechnologically important filamentous fungus and has been thought of as an industrial cell factory for the production of secondary metabolites with a broad spectrum of application fields covering agriculture, food, and pharmaceutical industry [57]. Deletion of *laeA* in *A. niger* decreased production of asperrubrol (**29**), atromentin (**30**) and JBIR 86 (**31**), but increased production of aspernigrin A (**32**) and BMS-192548 (**33**), which meant that LaeA positively regulated production of asperrubrol (**29**), atromentin (**30**) and JBIR 86 (**31**), and negatively regulated production of aspernigrin A (**32**) and BMS-192548 (**33**) in *A. niger* [58]. Overexpression of *laeA* gene in *A niger* led to an activation of secondary metabolite BGCs in the mutant. Three compounds including flaviolin (**34**), orlandin (**35**) and kotanin (**36**) were identified [59]. LaeA could influence the secondary metabolite profile in *A. niger* FGSC A1279 based on the genome sequencing and transcriptome

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analysis [60]. The production of ochratoxin A (OTA, **10**) in *A. niger* was decreased in the deleted mutant and increased in the overexpressed mutant. Another similar example was that deletion of *laeA* gene in *A. ochraceus* led to the reduced production of OTA (**10**) [61]. This indicated that LaeA positively regulated gene expression of OTA (**10**) BGC in *A. niger* and *A. ochraceus*. In contrast, it was found that the upregulation of gene expression of OTA BGC did not necessarily increase OTA (**10**) production in *A. niger* [62].

Kojic acid (**20**) production was inhibited in the *laeA* disruption strain of *A. oryzae*, and restored in the *laeA* complement strain, which meant that LaeA positively regulated biosynthesis of kojic acid (**20**) in *A. oryzae* [63]. In the expression system of *A. oryzae*, LaeA also showed its positive regulation on the heterologous BGCs. Overexpression of *laeA* resulted in the increased production of monocolin K (MK, **37**) and terrequinone A (TQ, **38**). The successful production of secondary metabolites belonging to different structural groups, namely MK (**37**) as a polyketide, and TQ (**38**) as a hybrid of amino acid and isoprenoid, indicated that the *laeA*-enriched *A. oryzae* was a versatile host for the heterologous expression of the biosynthetic gene clusters such as the BGC of MK (**37**) from *Monascus pilosus*, and the BGC of TQ (**38**) from *A. nidulans* [64]. For *A. pachycristatus* and *A. pseudoterreus*, the production of secondary metabolites was also positively regulated by LaeA [65,66]. Production of sterigmatocystin (**16**) and echinocandin B (ECB, **39**) was decreased in *laeA* deleted mutant of *A. pachycristatus* [65]. Overexpression of *laeA* in *A. pseudoterreus* improved itaconic acid (**40**) yield at the expense of biomass by increasing the expression of key biosynthetic pathway enzymes and attenuating the expression of genes involved in phosphate acquisition and scavenging. Increased yield was observed in optimized conditions as well as conditions containing excess nutrients that might be present in inexpensive sugar containing feedstocks such as excess phosphate or complex nutrient sources [66].

A. terrreus is the main industrial producer of lovastatin (**41**), a drug to lower cholesterol. Lovastatin (**41**) is also used as a precursor for simvastatin production. In *A. terreus*, the overexpression of *laeA* gene triggered the increase of gene transcription related to penicillin G (**28**) and lovastatin (**41**) biosynthesis [43]. It has been observed that overexpression of *laeA* gene in *A. terreus* increased the production of lovastatin (**41**) [67,68]. Chemical epigenetic modifiers 1,3-diaminopropane and spermidine also upregulated lovastatin (**41**) production and expression of lovastatin (**41**) biosynthetic genes in *A. terreus via* LaeA regulation [69]. Overexpression of *laeA* in *A. terreus* resulted in the activation of a silent secondary metabolite cluster without corresponding known metabolites. The yields of two antibacterial alkaloids dihydroisoflavipucines 1 (**42**) and 2 (**43**) reached 183 mg/L and 1.55 mg/mL, respectively. Both compounds showed obviously anti-*Vibrio* activities with the MIC values ranging from 16 to 64 μg/mL against *Vibrio anguillarum*, *V. campbellii*, *V. harveyi* and *V. vulnificus* [70].

The production of beauvericin (**44**) and bassiatin (**45**) was reduced in the *BbLaeA* disruption strain of *Beauveria bassiana*, but was increased in the overexpressed strain [71].

The production ability of oxalic acid (OA, **46**) was lost in *laeA* disruption strain of *Botryitis cinerea* [72]. Another example was that the production yield of abscisic acid (ABA, **47**) was decreased 90% in *laeA* disruption strain of *B. cinerea*. It was considered that BcLAE1 was involved in epigenetic regulation as a methyltransferase, with enhanced H3K9me3 modification and attenuated H3K4me2 modification in Δ*Bclae1* mutant of *B. cinerea* [73].

Overexpression of *laeA* in *Chaetomium globosum* CBS148.51 up-regulated expression of the chaetoglobosin BGC and resulted in the isolation of seven cytochalasans including chaetoglobosins A (**48**), B (**49**), D (**50**), E (**51**), O (**52**), V (**53**) and Z (**54**). Of them, chaetoglobosin Z (**54**) was a new cytochalasan. These cytochalasans displayed strong cytotoxic activity against the HepG 2 cell line [74]. Similarly, the production of chaetoglobusin A (**48**) in Δ*CglaeA* mutant of another *C. globosum* strain was inhibited, its *CglaeA-C* strain restored the production of chaetoglobusin A (**48**), and the strain of *CglaeA* overexpression led to an increase in chaetoglobusin A (**48**). It indicated that LaeA positively regulated the production of chaetoglobusin A (**48**) in *C. globosum* [75].

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Cladosporium fulvum was the non-obligate biotrophic fungal tomato pathogen. Deletion of *laeA* in *C. fulvum* led to the increased production of the mycotoxin cladofulvin (**55**), which meant that LaeA negatively regulated biosynthesis of cladofulvin (**55**) in this fungus [76].

T-toxin (**56**) was a host selective phytotoxin produced by maize pathogen *Cochliobolus heterostrophus*. Deletion of *Chlae1* decreased production of T-toxin (**56**) in *C. heterostrophus* [77].

Coprinoferrin (**57**) was an acylated tripeptide hydroxamate consisting of tandem aligned N^5-hexanoyl-N^5-hydroxy-L-ornithine with modifications of *N*-acetyl and *C*-carboxamide. Knockout of *laeA* in the mushroom fungus *Coprinopsis cinerea* upregulated the biosynthesis of a novel siderophore namely coprinoferrin (**57**), which indicated that LaeA negatively regulated production of coprinoferrin (**57**) [78]. The unique chemical properties made coprinoferrin (**57**) an iron (III) binder (siderophore), which helped iron acquisition from the environment and promoted hyphal growth as well as fruiting body formation in *C. cinerea*. In addition, coprinoferrin (**57**) could be chemically synthesized from *N*-Boc-L-glutamic acid 5-benzyl ester [79].

Daldinia eschscholzii was an endophytic fungus from the guts of mantis (*Tenodora aridifolia*). Replacement of the native promoter of the global regulator LaeA-like gene of *D. eschscholzii* by a strong gpdA promoter led to the generation of two novel cyclopentenone metabolites, named dalestones A (**58**) and B (**59**). Both dalestones inhibited the gene expression of TNF-α and IL-6 in LPS-induced RAW264.7 macrophages [80].

Deletion of *DsLaeA* resulted in enhanced production of dothistromin (**60**) in the pine needle pathogen *Dothistroma septosporum*, and increased expression of the regulatory gene *DsAflR* in dothistromin (**60**) biosynthetic pathway [81].

Fusarium fujikuroi (teleomorph: *Gibberella fujikuroi*) is the pathogen of rice bakanae disease to produce a series of secondary metabolites such as bikaverin (**61**), fusaric acid (**62**), gibberellins, fusarins, and fusarubins. Among them, fusaric acid (**62**) and fusarins belong to the harmful mycotoxins [82]. LaeA positively regulated production of some metabolites in *F. fujikuroi*. For examples, deletion of *laeA* in *F. fujikuroi* led to decreased production of gibberellins A3 (**63**) and A4 (**64**), fusarin C (**65**), fumonisins B1 (**66**), B2 (**67**), B3 (**68**) and B4 (**69**), deoxynivalenol (**70**), and 15-acetyl deoxynivalenol (**71**) [83]. The similar results were subsequently confirmed. Deletion of *lae1* gene led to reduced production of fusaric acid (**62**), fusarinolic acid (**72**), and dehydrofusaric acid (**73**) in *F. fujikuroi* strain [84]. Furthermore, deletion of *lae1* led to decreased production of gibberellins, fumonisins and fusarin C (**65**). Overexpression of *lae1* led to increased production of gibberelins in another *F. fujikuroi* strain [85]. However, LaeA also negatively regulated production of some metabolites in *F. fujikuroi*. The production of bikaverin (**61**) was increased in the deletion mutant of *F. fujikuroi* [83]. Another example was that deletion of *lae1* gene in *F. fujikuroi* led to upregulation of gibepyrone BGC expression as well as increased production of gibepyrones A (**74**), B (**75**), C (**76**), D (**77**), E (**78**), and F (**79**) [86].

LaeA positively regulated mycotoxin production of the following phytopathogenic *Fusarium* species. Deletion of *FglaeA* in *F. graminearum* led to a dramatic reduced production of trichothecenes and zearalenone (**80**). Overexpression of *FglaeA* caused the increased production of trichothecenes and zearalenone (**80**). This indicated that FgLaeA positively regulated production of phytotoxins of *F. graminearum* [87]. For the fungus *F. oxysporum*, deletion of *laeA* caused the decreased production of beauvericin (**44**) and fusaric acid (**62**), which contributed to virulence on plant hosts such as tomato plants [88]. For the fungus *F. oxysporum* f.sp. *niveum*, the deletion of *FoLae1* gene led to depressed conidiation and reduced production of fusaric acid (**62**) and bikaverin (**70**). In addition, all of these alterations in the deleted mutants were restored in the corresponding complementation strains. [89]. For the fungus *F. verticillioides*, the deletion of *laeA* reduced production of fusaric acid (**62**), fusarin C (**65**), bikaverin (**70**), and fumonisins [90].

Ganoderic acids (GAs) are lanosterol-type triterpenoids produced by *Ganoderma* species that possess multiple bioactivities including anti-cancer, anti-inflammatory, antioxidant, and anti-HIV activities [91]. When a methyltransferase-like laeA gene was deleted in *G. lingzhi*, the production of ganoderic acids was reduced. RT-qPCR analysis further revealed that the transcription levels of genes

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involved in the biosynthesis of garnoderic acids were drastically lower in the Δ*laeA* strain. In contrast, constitutive overexpression of *laeA* resulted increased concentration of GAs [92].

Magnaporthe oryzae causes blast disease, the most serious disease of cultivated rice affecting global rice production. *MolaeA* negatively regulated sporulation and melanin biosynthesis, and positively regulated production of penicillin G (or called benzylpenicillin, **28**) [93]. Metabolomic profiling analysis showed that overexpression of *MolaeA* led to increased biosynthesis of secondary metabolites in *M. oryzae*. Unfortunately, these metabolites have not been identified [94].

Some *Monascus* species can produce edible pigments, with their structures bearing a highly oxygenated pyranoquinone bicyclic core and a quaternary carbon center. However, the mycotoxin citrinin (**81**) produced by some *Monascus* strains restricts application of the pigments [95]. Monacolin K (**37**), a cholesterol-lowering agent, was increased for 3 times when *laeA* was overexpressed in *Monascus pilosus*. In addition, the pigment production was also remarkably increased [96]. The production of monacolin K (**37**) was also increased when *laeA* was overexpressed in *M. purpureus* [97]. For another *Monascus* species, the deletion of *MrlaeA* in *M. ruber* exhibited drastically reduced production of toxin citrinin (CIT, **81**) and six pigments including rubropunctamine (**82**), monascorubramine (**83**), monascin (**84**), rubropunctatin (**85**), ankaflavin (**86**), and monascorubrin (**87**) [98].

The *laeA* gene from *Aspergillus nidulans* was heteroexpressed in the fungus *Penicillium* sp. LC1-4. Overexpression of *AnLaeA* caused an increased production of a bioactive compound quinolactacin A (**88**). It indicated that heteroexpressed of AnLaeA in fungi was a simple and effective method to explore metabolic potential [30]. LaeA could also positively regulate production of antibacterial pseurotins in *Penicillium* sp. Deletion of *laeA* gene in *Penicillium* sp. strain MB inhibited production of the members with 1-oxa-7-aza-spiro[4,4] non-2-ene-4,6-dione skeleton. Among these deduced compounds, pseurotins A (**89**), B (**90**), C (**91**), D (**92**), and E (**93**) displayed obvious antibacterial activity. It was why cheese rind bacterial communities assembled with the *laeA* deletion mutant of *Penicillium* sp. strain MB had significantly higher bacterial abundances than the wild-type strain [99].

To date, secondary metabolite production of *Penicillium* species have been found to be positively regulated by LaeA. Overexpression of *PbrLaeA* led to the discovery of four compounds including fumigatin chlorohydrin (**94**), iso-fumitatin chlorohydrin (**95**), spinulosin (**96**) and pyranonigrin F (**97**) in the fungus *P. brocae* HDN-12-143. Among them, iso-fumitatin chlorohydrin (**95**) was a new compound. Both fumigatin chlorohydrin (**94**) and iso-fumitatin chlorohydrin (**95**) exhibited cytotoxic activity against HL-60 with IC_{50} values of 18.63 μM and 24.83 μM, respectively [100]. Overexpression of *PclaeA* in *P. chrysogenum* gave rise to 25% increase production of penicillin G (benzylpenicillin, **28**). *PclaeA* knock-down mutants exhibited drastically reduced production and biosynthesis gene expression of penicillin G (**28**) [22]. Deletion of *laeA* in *P. chrysogenum* decreased production of penicillin G (**28**) [101]. However, epigenetic modifiers 1,3-diaminopropane (1,3-DAP) and spermidine completely restored the levels of penicillin G production in the *laeA* knock-down mutant. This indicated that LaeA in *P. chrysogenum* might act epigenetically on the expression of secondary metabolite genes by heterochromatin reorganization, which should be studied in detail [102]. Small reduction of penicillin G (**28**) was also reported in another Δ*PclaeA* mutant of *P. chrysogenum* [103]. The full-length *laeA* gene namely *Pci-laeA* with the sequence as 1,340 bp including an ORF of 1,284 bp encoding 427 amino acids was cloned from *P. citrinum*. The predicted molecular mass of Pci-LaeA was 48.72 kDa with an estimated theoretical isoelectric point of 6.96. Pci-LaeA had a conserved *S*-adenosylmethionine binding site and a potential MlcR (a pathway specific regulator in mevastatin biosynthesis) binding site [104]. When *laeA* gene was deleted in *P. citrinum*, production of compactin (also named ML-236B, mevastatin, **98**) was suppressed [105]. Comparative transcriptome analysis revealed that the function loss of *PdLaeA* in *P. digitatum* resulted in the reduced expression of several secondary metabolite gene clusters [106].

Sorbicillinoids are important hexaketide metabolites derived from fungi. They have a variety of biological activities with unique structural features to make them attractive candidates for developing new pharmaceutical and agrochemical agents [107,108]. Overexpression of *laeA* gene in the marine-derived fungus *P. dipodomyis* YJ-11 induced metabolic variations to afford a series of

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sorbicillinoids including two new ones named 10,11-dihydrobislongiquinolide (**99**) and 10,11,16,17-tetrahydrobislongiquinolide (**100**), as well as four known analogues, bislongiquinolide (**101**), 16,17-dihydrobislongiquinolide (**102**), sohirnone A (**103**), and 2′,3′-dihydrosorbicillin (**104**). This indicated that regulation of LaeA is a useful strategy in activating silent gene clusters in fungal strains to obtain previously undiscovered compounds [109].

The mycotoxin patulin (**105**) is produced in the colonized tissue by *P. expansum* during storage of apples. Deletion of *laeA* in *P. expansum* led to a decrease of patulin (**105**) production, which positively regulated patulin gene expression and patulin biosynthesis. Loss of LaeA affected the colonization of *P. expansum* in apple fruits. The Δ*laeA* strains showed reduced virulence at all stages of apple maturity, and the disease severity was reduced by up to 22% in more mature fruits [110,111]. It demonstrated that patulin metabolism modulated by LaeA contributed in part to pathogenicity of *P. expansum* [110].

The LaeA in *P. oxalicum* played important roles in asexual development, expression of secondary metabolite gene clusters, and extracellular glycoside hydrolase synthesis. Deletion of laeA gene led to decreased production of secondary metabolites. Unfortunately, these differential metabolites have not been identified [112]. Four (i.e., cluster_1, cluster_5, cluster_14, and cluster_26) of the 28 secondary metabolic gene clusters were significantly downregulated in Δ*laeA* mutant compared with wild type strain (WT) of *P. oxalicum*. The LaeA was speculated as the putative methyl-transferase. Histone H2B lysine 122 and lysine 130 were considered as the putative targets of LaeA [113]. Another example was that the disruption of *PrlaeA* in *P. roqueforti* led to a substantial reduction in the production of the three metabolites roquefortine C (**106**), mycophenolic acid (**107**), and andrastin A (**108**). However, deletion of *PrlaeA* had little impact on asexual development [114].

Disruption of *laeA* in *Pestalotiopsis microspore* led to the decreased production of pestalotiollide B (PB, **109**) [115]. Similarly, deletion of *PoLaeA1* in *Pleurotus ostreatus* decreased the intracellular polysaccharide (IPS) content by about 28-30% as well as cellulose activity, which provided new insights into the regulation of polysaccharide biosynthesis and cellulose production in filamentous fungi [116]. *PoLAE1* also positively regulated tenuazonic acid (TeA, **110**) production of rice blast pathogen *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*) [117].

LaeA positively regulated secondary metabolite production of the following *Trichoderma* species. Overexpression of *TalaeA* in *T. afroharzianum* led to production of two new antifungal polyketides: (1*R*,3*E*,5*E*)-1-(3,5-dihydroxy- 2,4-dimethylphenyl)-1-hydroxyhepta- 3,5-dien-2-one (**111**) and (1*R*,3*E*,5*E*)-1-(3,5-dihydroxy- 2,4-dimethylphenyl)-1-methoxyhepta- 3,5-dien-2-one (**112**). Both compounds showed strong antifungal activity on plant pathogenic fungi *Botrytis cinerea*, *Colletotrichum lagenarium* and *Fusarium oxysporum* f.sp. *nicotianae* [118]. Deletion of *Tllae1* in *T. longibrachiatum* reduced the production of peptaibols to a large degree. The peptaibols belonged to antimicrobial peptides and were named as trichokonins (TKs) which were mainly classified into 20-aa trichokonin A (TKA) and 12-aa trichokonin B (TKB). Overexpression of *Tllae1* in *T. longibrachiatum* led to 2-fold increased production of petaibols. Overexpression of *laeA* gene in *T. reesei* led to the increased production of sorbicillinoids, which were not identified. If the *laeA* gene in another *T. reesei* strain was deleted, the production of sterigmatocystin (**16**) was decreased [119]. *T. reesei* had a potential to produce terpenoids. If *lae1* gene along with major hemi-cellualse genes were deleted, the production of sesquiterpenoid ophiobolin F (**113**) in *T. reesei* was increased to 1187.06 mg/L by using the modified chassis [120].

Deletion of *VmLaeA* in apple canker pathogen *Valsa mali* led to greatly reduced virulence with lesion length reduced by 48% on apple twigs. The toxicity of secondary metabolites produced by *VmLaeA* deletion mutant (Δ*VmlaeA*) was markedly decreased in comparison with the wild type strain. Unfortunately, these toxic metabolites have not been identified [121].

Table 1. Some examples of LaeA regulating secondary metabolite production in fungi.

Fungus	Overexpression/ Deletion of *laeA*	Positive/Negative Regulation	Production of Secondary Metabolites	Ref.
Alternaria alstroemeria	Overexpression	Positive	Increased production of myricetin (**1**), geraniol (**2**), ergosterol (**3**) and other compounds determined by metobolomic analysis.	[24]
Alternaria alstroemeria	Overexpression	Negative	Decreased production of the antitumor compounds via controlling the transcription of *AaFla1*.	[25]
Alternaria alternata	Deletion	Positive	Decreased production of alternariol (**4**) and alternariol monomethyl ether (**5**).	[27]
Alternaria alternata	Overexpression	Positive	Increased production of the anti-inflammatory meroterpenoid tricycloalternarene O (**6**).	[28]
Arthrobotrys flagrans	Overexpression and deletion	Positive	Increased production of the secondary metabolites by overexpression of *AflaeA*, and decreased production of the secondary metabolites by deletion of *AflaeA*.	[29]
Aspergillus sp. Z5	Overexpression	Positive	Increased production of diorcinol (**7**).	[30]
Aspergillus sp. FKI-5362	Overexpression	Positive	Increased production of MS-347a (**8**).	[31]
Aspergillus carbonarius	Deletion	Positive	Decreased production of citric acid (**9**).	[33]
Aspergillus carbonarius	Deletion	Positive	Decreased production of ochratoxin A (**10**).	[34]
Aspergillus carbonarius	Deletion	Positive	Decreased production of ochratoxin A (**10**) in Δ*laeA* strain colonized in nectarines and grapes.	[35]
Aspergillus carbonarius	Inhibtion of LaeA	Positive	Decreased production of ochratoxin A (**10**) by treatment with eugenol through inhibiting LaeA expression	[36]
Aspergillus cristatus	Overexpression	Positive	Increased production of multiple secondary metabolites including terpenoids and flavonoids.	[37]
Aspergillus flavipes	Deletion	Negative	Increased production of flavipamides A (**11**) and B (**12**), asperphenamate (**13**), 4′-OMe-asperphenamate (**14**), and cyclic Pro-Gly-Val-Gly-Try(8-OH, 3-prenyl)-Gly-Trp (**15**).	[38]
Aspergillus flavus	Deletion	Positive	Decreased production of sterigmatocystin (**16**).	[43]
Aspergillus flavus	Deletion	Positive	Decrease production of aflatoxins.	[39]
Aspergillus	Deletion	Positive	Decreased production of aflatoxin B1 (**17**).	[40]

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flavus				
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxins.	[41]
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxins, cyclopiazonic acid (**18**) and ustiloxin B (**19**).	[44]
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxins.	[42]
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxins and kojic acid (**20**).	[45]
Aspergillus fumigatus	Deletion	Positive	Decreased production of gliotoxin (**21**) and endocrocin (**22**).	[43]
Aspergillus fumigatus	Deletion	Positive	Decreased production of several mycotoxins including gliotoxin (**21**).	[46]
Aspergillus fumigatus	Deletion	Positive	Decreased production of gliotoxin (**21**), fumagillin (**23**), fumagatin (**24**) and helvolic acid (**25**).	[47]
Aspergillus fumigatus	Deletion	Positive	Decreased production of gliotoxin (**21**).	[48]
Aspergillus fumigatus	Deletion	Positive	Decreased production of endocrocin (**22**)	[49]
Aspergillus fumisynnematus	Overexpression	Positive	Increased production of cyclopiazonic acid (**18**).	[50]
Aspergillus luchuensis mut. *kawachii*	Deletion	Positive	Decreased production of citric acid (**9**).	[51]
Aspergillus nidulans	Overexpression	Positive	Increased production of terrequinone A (**26**).	[53]
Aspergillus nidulans	Deletion	Positive	Decreased production of sterigmatocystin (**16**) and norsolorinic aid (**27**).	[54]
Aspergillus nidulans	Deletion	Positive	Decreased production of streigmatocystin (**16**) and penicillin G (**28**).	[43]
Aspergillus nidulans	Deletion	Positive	Depressed expression of genes involved in biosynthesis of sterigmatocystin (**16**), terrequinone A (**26**) and penicillin G (**28**).	[55]
Aspergillus	Deletion	Negative	Increased production of sterigmatocystin (**16**).	[56]

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nidulans				
Aspergillus nidulans	Overexpression	Positive	Increased production of sterigmatocystin (**16**).	[52]
Aspergillus niger	Deletion	Positive	Decreased production of asperrubrol (**29**), atromentin (**30**) and JBIR 86 (**31**).	[58]
Aspergillus niger	Deletion	Negative	Increased production of aspernigrin A (**32**) and BMS-192548 (**33**).	[58]
Aspergillus niger	Overexpression	Positive	Increased production of flaviolin (**34**), orlandin (**35**) and kotanin (**36**).	[59]
Aspergillus niger	Overexpression and deletion	Positive	Decreased production of OTA (**10**) in the deleted mutant, but increased production of OTA (**10**) in the overexpressed mutant.	[62]
Aspergillus ochraceus	Deletion	Positive	Decreased production of OTA (**10**).	[61]
Aspergillus oryzae	Deletion	Negative	Increased production of kojic acid (**20**).	[63]
Aspergillus oryzae	Overexpression	Positive	Increased production of monacolin K (**37**) and terrequinone A (**38**)	[64]
Aspergillus pachycristatus	Deletion	Positive	Decreased production of sterigmatocystin (**16**) and echinocandin B (**39**).	[65]
Aspergillus pseudoterreus	Overexpression	Positive	Increased production of itaconic acid (**40**).	[66]
Aspergillus terreus	Overexpression	Positive	Increased production of lovastatin (**41**).	[67]
Aspergillus terreus	Overexpression	Positive	Increased production of dihydroisoflavipucines 1 (**42**) and 2 (**43**).	[70]
Beauveria bassiana	Overexpression and deletion	Positive	Decreased production of beauvericin (**44**) and bassiatin (**45**) in the *BbLaeA* disruption strain, but increased production in the overexpressed strain.	[71]
Botrytis cinerea	Deletion	Positive	Decreased production of oxalic acid (**46**).	[72]
Botrytis cinerea	Deletion	Positive	Decreased production of abscisic acid (**47**).	[73]
Chaetomium globosum	Overexpression	Positive	Increased production of seven cytochalasans including chaetoglobosins A (**48**), B (**49**), D (**50**), E (**51**), O (**52**), V (**53**) and Z (**54**).	[74]

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Chaetomium globosum	Overexpression and deletion	Positive	Decreased production of chaetoglobusin A (**48**) in Δ*CglaeA* mutant, restored production of chaetoglobusin A (**48**) in *CglaeA-C* strain, and increased production of chaetoglobusin A (**48**) in *CglaeA-OE* strain.	[75]
Cladosporium fulvum	Deletion	Negative	Increased production of cladofulvin (**55**).	[76]
Cochliobolus heterostrophus	Deletion	Positive	Decreased production of T-toxin (**56**).	[77]
Coprinopsis cinerea	Deletion	Negative	Increased production of coprinoferrin (**57**).	[78]
Daldinia eschscholzii	Replacement of a strong *pgdA* promoter	Positive	Induced production of dalestones A (**58**) and B (**59**)	[80]
Dothistroma septosporum	Deletion	Negative	Increased production of dothistromin (**60**).	[81]
Fusarium fujikuroi	Deletion	Negative	Increased production of bikaverin (**61**).	[83]
Fusarium fujikuroi	Deletion	Positive	Decreased production of gibberellins A3 (**63**) and A4 (**64**), fusarin C (**65**), fumonisins B1 (**66**), B2 (**67**), B3 (**68**) and B4 (**69**), deoxynivalenol (**70**), and 15-acetyl deoxynivalenol (**71**).	[83]
Fusarium fujikuroi	Deletion	Positive	Decreased production of fusaric acid (**62**), fusarinolic acid (**72**), and dehydrofusaric acid (**73**)	[84]
Fusarium fujikuroi	Deletion and overexpression	Positive	Deletion of *laeA* led to decreased production of gibberellins, fumonisins and fusarin C (**65**). Overexpression of *laeA* led to increased production of gibberelins.	[85]
Fusarium fujikuroi	Deletion	Negative	Increased production of gibepyrones A (**74**), B (**75**), C (**76**), D (**77**), E (**78**), and F (**79**)	[86]
Fusarium graminearum	Deletion and overexpression	Positive	Deletion of *FglaeA* led to a dramatic reduced production of trichothecenes and zearalenone (**80**). Overexpression of *FglaeA* caused the increased production of trichothecenes and zearlenone (**80**).	[87]
Fusarium oxysporum	Deletion	Positive	Decreased production of beauvericin (**44**) and fusaric acid (**62**)	[88]
Fusarium oxysporum f.sp. *niveum*	Deletion	Positive	Decreased production of bikaverin (**61**) and fusaric acid (**62**)	[89]
Fusarium verticillioides	Deletion	Positive	Decreased production of bikaverin (**61**), fusaric acid (**62**), fusarin C (**65**) and fumonisins.	[90]

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Ganoderma lingzhi	Deletion and overexpression	Positive	Decreased production of ganoderic acids in the deleted mutant, and increased ganoderic acids in the overexpressed mutant.	[92]
Magnaporthe oryzae	Overexpression	Positive	Decreased production of melanin, and increased production penicillin G (**28**)	[93]
Magnaporthe oryzae	Overexpression	Positive	Increased production of secondary metabolites.	[94]
Monascus pilosus	Overexpression	Positive	Increased production of monacolin K (**37**) and unidentified pigments.	[96]
Monascus purpureus	Overexpression	Positive	Increased production of monacolin K (**37**).	[97]
Monascus ruber	Deletion	Positive	Decreased production of citrinin (**81**) and six pigments rubropunctamine (**82**), monascorubramine (**83**), monascin (**84**), rubropunctatin (**85**), ankaflavin (**86**), and monascorubrin (**87**).	[98]
Penicillium sp. LC1-4	Overexpression	Positive	Increased production of quinolactacin A (**88**).	[30]
Penicillium sp. MB	Deletion	Positive	Inhibited production of the members with the 1-oxa-7-aza-spiro[4,4] non-2-ene-4,6-dione skeleton, including pseurotins A (**89**), B (**90**), C (**91**), D (**92**), and E (**93**).	[99]
Penicillium brocae HDN-12-143	Overexpression	Positive	Increased production of fumigatin chlorohydrin (**94**), iso-fumitatin chlorohydrin (**95**), spinulosin (**96**) and pyranonigrin F (**97**)	[100]
Penicillium chrysogenum	Overexpression and deletion	Positive	Overexpression of *PclaeA* gene led to increased production of penicillin G (**28**). Deletion of *PclaeA* led to decreased production of penicillin G (**28**).	[22]
Penicillium chrysogenum	Deletion	Positive	Decreased production of penicillin G (**28**).	[101]
Penicillium chrysogenum	Deletion	Positive	Decreased production of penicillin G (**28**).	[102]
Penicillium chrysogenum	Deletion	Positive	Small reduction of penicillin G (**28**)	[103]
Penicillium citrinum	Deletion	Positive	Decreased production of compactin (**98**).	[105]
Penicillium digitatum	Deletion	Positive	Reduced expression of several secondary metabolite BGCs.	[106]
Penicillium dipodomyis YJ-11	Overexpression	Positive	Increased production of sorbicillinoids including 10,11-dihydrobislongiquinolide (**99**), 10,11,16,17-tetrahydrobislongiquinolide (**100**), bislongiquinolide (**101**), 16,17-dihydrobislongiquinolide (**102**), sohirnone A (**103**), and 2′,3′-dihydrosorbicillin (**104**).	[109]

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Penicillium expansum	Deletion	Positive	Decreased production of patulin (**105**).	[110, 111]
Penicillium oxalicum	Deletion	Positive	Decreased production of secondary metabolites.	[112]
Penicillium oxalicum	Deletion	Positive	Four of the 28 secondary metabolite BGCs were significantly downregulated.	[113]
Penicillium roqueforti	Deletion	Positive	Decreased production of roquefortine C (**106**), mycophenolic acid (**107**), and andrastin A (**108**).	[114]
Pestalotiopsis microspore	Deletion	Positive	Decreased production of pestalotiollide B (**109**).	[115]
Pleurotus ostreatus	Deletion	Positive	Decreased production of the intracellular polysaccharide (IPS).	[116]
Pyricularia oryzae	Deletion and overexpression	Positive	Deletion of *PoLAE1* reduced the production of tenuazonic acid (**110**). Overexpression of *PoLAE1* led to increased production of tenuazonic acid (**110**).	[117]
Trichoderma afroharzianum	Overexpression	Positive	Induced production of (1*R*,3*E*,5*E*)-1-(3,5-dihydroxy- 2,4-dimethylphenyl)-1-hydroxyhepta-3,5-dien-2-one (**111**) and (1*R*,3*E*,5*E*)-1-(3,5-dihydroxy- 2,4-dimethylphenyl)-1-methoxyhepta-3,5-dien-2-one (**112**).	[118]
Trichoderma longibrachiatum	Deletion and overexpression	Positive	Deletion of *Tllae1* reduced the production of peptaibols. Overexpression of *Tllae1* led to 2-fold increased production of petaibols.	[122]
Trichoderma reesei	Overexpression	Positive	Increased production of sorbicillinoids.	[123]
Trichoderma reesei	Deletion	Positive	Decreased production of sterigmatocystin (**16**).	[119]
Trichoderma reesei	Deletion	Negative	Increased production of ophiobolin F (**113**)	[120]
Valsa mali	Deletion	Positive	Decreased production of toxic metabolites.	[121]

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3. Regulation of Velvet Proteins on Secondary Metabolite Production in Fungi

The velvet proteins (or called velvet family proteins) included VeA (velvet A), VelB (velvet like B), VelC (velvet like C), VelD (velvet like D) and VosA (viability of spores A). These five proteins all contain the velvet and transactivation domains. They are highly conserved in dimorphic and filamentous fungi [40,124–126]. They mainly play important roles in fungal development, asexual sporulation, sexual development, secondary metabolism, and stress tolerance [127]. It has been revealed that LaeA and velvet proteins formed the multimeric complexes such as VelB-VeA-LaeA, VelB-VosA, and VelB-VelB in fungi. The heterotrimeric VelB-VeA-LaeA complex controls sexual development and secondary metabolism in response to light [128–132]. The following is the research progress of velvet proteins on the regulation of secondary metabolite production in fungi.

3.1. Regulation of VeA on Secondary Metabolite Production in Fungi

The VeA (also called VelA, Ve1, and Vel1) proteins usually positively regulated production of secondary metabolites in fungi. Most of the regulated metabolites were polyketides. Some examples of VeA regulating secondary metabolite production in fungi are shown in Table 2. The structures of the metabolites are shown in Figure S1.

Disruption of the *AcveA* gene in *Acremonium chrysogenum* resulted in reduction of cephalosporin C (**114**) production, which meant AcVeA positively regulated cephalosporin C (**114**) biosynthesis in *A. chrysogenum* [133].

Deletion of *veA* gene in *Alternaria alternata* greatly reduced sporulation and production of alternariol (AOH, **4**) and alternariol monomethyl ether (AME, **5**) [27]. The production of both AOH (**4**) and AME (**5**) in *A. alternata* was significantly stimulated by blue light. The disruption of *AaVeA* resulted in a marked decrease of AOH (**4**) and AME (**5**) production under blue light illumination [134].

Aspergillus carbonarius was the pathogen of grape Aspergillus rot [135]. The fungus could produce ochratoxin A (OTA, **10**). Deletion of *veA* in *A. carbonarius*, the production of OTA (**10**) was almost disappeared [34]. Deletion of *veA* in *A. carbonarius* resulted in significant reduction of OTA (**10**) production. During both *in vitro* growth and infection of grapes, non-mycotoxigenic strains could outcompete the wild-type strain. OTA (**10**) defective Δ*veA* mutant was considered as the potential biocontrol agent [136].

VeA affected the biosynthesis of mycotoxins in *Aspergillus flavus*. Deletion of *veA* in *A. flavus* decreased the production of cyclopiazonic acid (**18**), aflatrem B (**115**), and aflatoxins [137]. Deletion of *veA* also decreased the production of asparasone A (**116**) [138] and aflatoxin B1 (**17**) in *A. flavus* [139]. VepN contained a septin-type guanine nucleotide-binding domain, representing a conserved protein family from yeast to humans belonging to the P-loop GTPase superfamily. It was found that the global regulation gene *veA* positively regulated *vepN* to influence aflatoxin production, morphological development, and pathogenicity in *A. flavus* [140].

Both deletion and overexpression of *veA* in *Aspergillus fumigatus* led to decreased production of gliotoxin (**21**). The RNA sequencing data provided evidence supporting this pattern. It was possible that both deletion and over-expression of *veA* downregulated *fumR* transcription, suggesting that *veA* influences the activation of the fumagillin gene cluster through regulation of *fumR* [141]. The similar regulation pattern was also observed in *A. fumigatus* to produce other secondary metabolites. Both deletion and overexpression of *veA* in *A. fumigatus* decreased production of fumagillin (**23**), gumitremorgin G (**117**), fumigaclavine C (**118**) and glionitrin A (**119**) [142].

The deletion of *veA* in *A. nidulans* suppressed the production of sterigmatocystin (**16**). The *veA* deletion mutant produced less penicillin G (**28**) than the regular strain. The *veA* gene was also required for sexual development [143]. The deletion of *veA* gene in *A. nidulans* reduced productin of sterigmatocystin (**16**) and norsolorinic aid (NOR, **27**) [54]. VeA was thought to be involed in the penicillin G (**28**) biosynthesis *via* repression of the expression of *acvA* gene, which led to reduced penicillin production in *A. nidulans* [144]. Further investigaton was that VeA repressed the expression of cryptic orsellinic acid (**120**) BGC in *A. nidulans* through histone 3 acetylation. Deletion of *veA* led

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to increased production of orsellinic acid (**120**), F9775A (**121**) and F9775B (**122**) in *A. nidulans* [145]. Further investigation revealed that *A. nidulans* VeA was a multi-phosphorylated protein and hypothesized that at least four specific amino acids (T167, T170, S183 and Y254) underwent reversible phosphorylation to trigger development and sterigmatocystin (**16**) biosynthesis. Double mutation of T167 to valine and T170 to glutamic acid exerted the largest effects with regards to sexual development and *veA* gene expression [146]. In the dark, VeA entered the nucleus of *A. nidulans*, formed VelB-VeA-LaeA heterotrimeric complex, and controlled sexual development and enhanced sterigmatocystin (**16**) production [128]. The downstream transcription factor regulatory gene of *veA* was revealed as *mtfA* in *A.nidulans*. Deletion of *mtfA* could decrease the expression of the genes in the penicillin gene cluster, reducing penicillin production. In this case, overexpression of *mtfA* enhanced the transcription of penicillin BGC, increasing penicillin production more than 5 fold with respect to the control. However, it was detrimental for the expression of terrequinone BGC either deletion or overexpression of mtfA. In addition to its effect on secondary metabolism, mtfA also affected asexual and sexual development in *A. nidulans*. Deletion of *mtfA* resulted in a reduction of conidiation and sexual stage [147]. Another example about downstream transcription factor regulatory gene of *veA1* was revealed as *sclB* in *A. nidulans*. Deletion of *sclB* also decreased production of aspernidines [148].

The deletion of *veA* in *Aspergillus niger* suppressed the production of ochratoxins A (OTA, **10**), α (OTα, **123**) and β (OTβ, **124**). The *veA* gene acted as the positive regulator of conidia production, OTA (**10**) biosynthesis, and oxidative stress tolerance in *A. niger*, regardless of light conditions. Darkness promoted conidial production and OTA (**10**) biosynthesis in the wild-type strain of *A. niger* [149]. The deletion of *veA* in *A. oryzae* also decreased production of kojic acid (**20**) [150]. Disruption of *veA* significantly reduced the production of echinocandin B (**39**) and sterigmatocystin (**16**) in *A. pachycristatus* [65]. Deletion of *veA* gene in *A. parasiticus* reduced aflatoxin BGC gene expression and aflatoxisome development [151].

CgVeA in *Chaetomium globosum* was thought of as a light signaling responsive regulator. It was involved in regulation of chaetoglobusin A (**48**) biosynthesis. Deletion of *CgveA* caused an obvious decrease in chaetoglobusin A (**48**) production from 51.32 to 19.76 mg/L under dark condition. In contrast, *CgveA* overexpression resulted in a dramatic increase in chaetoglobusin A (**48**) production, reaching 206.59 mg/L under illumination, which was higher than that noted in darkness. The RT-qPCR results confirmed that CgVeA, as a light responsive regulator, positively regulated chaetoglobusin A (**48**) biosynthesis by controlling the expression of core genes of the chaetoglobusin A (**48**) biosynthetic gene cluster and other relevant regulators [152].

Deletion of *veA* in tomato fungal pathogen *Cladosporium fulvum* led to increased production of the pigment cladofulvin (**55**), which meant that VeA negatively regulated biosynthesis of cladofulvin (**55**) in this fungus [76].

Deletion of *veA* in *Fusarium fujikuroi* led to decreased production of gibberellins A3 (**63**) and A4 (**64**), fusarin C (**65**), fumonisins B1 (**66**), B2 (**67**), B3 (**68**) and B4 (**69**), deoxynivalenol (**70**), and 15-acetyl deoxynivalenol (**71**). However, the production of bikaverin (**61**) was increased in the deletion mutant [83]. The similar results were confirmed later. Deletion of *vel1* led to reduced production of fusaric acid (**62**), fusarinolic acid (**72**), and dehydrofusaric acid (**73**) in *F. fujikuroi* strain [84]. Deletion of *vel1* led to decreased production of gibberellins, fumonisins and fusarin C (**65**). Overexpression of *lae1* led to increased production of gibberelins in another *F. fujikuroi* strain [85]. Deletion of *vel1* gene in *F. fujikuroi* led to upregulation of gibepyrone BGC expression as well as increased production of gibepyrones A (**74**), B (**75**), C (**76**), D (**77**), E (**78**), and F (**79**) [86].

Deletion of *veA* in *Fusarium graminearium*, the causal agent of Fusarium head blight, led to reduced production of deoxynivalenol (vomintoxin or DON, **68**) [153], and also led to decreased production of trichothecenes [154].

Overexpression of *FnveA* in *Fusarium nematophilum*, the antitumor activity of the crude extract was increased on A549 cancer cells. Unfortunately, the antitumor compounds were not identified [155].

Deletion of *veA* in *Fusairum oxysporum* caused the decreased production of beauvericin (**44**) and fusaric acid (**62**), which contributed to virulence on plant hosts such as tomato plants [88]. Deletion

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of *FoVel1* gene in *F. oxysporum* f.sp. *niveum* led to depressed conidiation and reduced production of bikaverin (**61**) and fusaric acid (**62**). In addition, all of these alterations in the deleted mutants were restored in the corresponding complementation strains. [89].

Deletion of *Ffvel1* in *Fusarium verticillinoides* led to decreased production of gibberellins (**63**) and A4 (**64**), fusarin C (**65**), fumonisins B1 (**66**), B2 (**67**), B3 (**68**) and B4 (**69**), deoxynivalenol (**70**), and 15-acetyl deoxynivalenol (**71**). However, the production of bikaverin (**61**) was increased in the deletion mutant. The regulation mechanisms of *vel1* on the above metabolite production should be similar to those of *laeA* in this fungus [83]. Deletion of *veA* in maize pathogen *Fusarium verticillioides* led to decreased production of fusarin C (**65**), fumonisins B1 (**66**), B2 (**67**) and B3 (**68**) [156]. Further investigation showed that VeA was necessary for causing symptom and mycotoxin synthesis in maize seedlings by *F. verticillioides* [157].

Deletion of *mve1* gene in *Mycosphaerella graminicola* decreased production of melanin. The Δ*mve1* mutant displayed albino phenotype with significant reduction in melanin biosynthesis and less production of aerial mycelia on agar plates [158].

Deletion of *veA* gene in *Neurosopora crassa* decreased both asexual conidiation and carotenoid production [159]. Further investigation showed that the production of siderophore coprogen (**125**) was also decreased in Δ*veA* mutant of *N. crassa* [160].

Deletion of *veA* gene in *Penicillium chrysogenum* decreased the production of penicillin G (**28**) [101,161]. Small reduction of penicillin G (**28**) was also reported in another Δ*PcvelA* mutant of *P. chrysogenum* [103]. *P. citrinum* is well-known to produce compactin (also called ML-236B or mevastatin, **98**). This polyketide exhibited a potent inhibitory activity on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Compactin (**98**) was industrially converted into pravastatin by microbes. Currently, pravastatin has been widely used as a pharmaceutical drug for the treatment of hypercholesterolemia [161]. Deletion of *veA* in *P. citrinum* led to suppressed production of compactin (**98**), and overexpression of *veA* led to increased production of compactin (**98**). It indicated that *veA* is the key regulation factor of compactin (**98**) biosynthesis [105]. Overexpression of *veA* in *P. citrinnum* led to the compactin (**98**) production to be increased [162].

Penicillium expansum is the causal agent of apple blue mould disease [163]. It produces the mycotoxins citrinin (**81**) and patulin (**105**). The disruption of *veA* in *P. expansum* drastically reduced the production of citrinin (**81**) and patulin (**105**) on synthetic media, associated with a marked down-regulation of all genes involved in the biosynthesis of the two mycotoxins. Moreover, the null mutant Δ*PeveA* strain was unable to produce patulin (**105**) on apples [127]. Deletion of *veA* in *P. expansum* led to the production of patulin (**105**) in Δ*veA* mutant to be completely blocked [164,165]. The Δ*veA* mutants also exhibited reduced growth and conidiation when exposed to stressors, including cell membrane stress, oxidative stress, osmotic stress, and different pH values, which indicated that patulin (**105**) contributed to fungal anti-stress ability. Non-mycotoxigenic strain Δ*veA* of *P. expansum* showed its biocontrol capability against postharvest pathogen of pome fruit during postharvest handling and storage [165].

Deletion of *veA* gene in *Pestalotiopsis microspora* led to increased production of pestalotiollide B (**103**). The *veA* gene appeared to negatively regulate the biosynthesis of pestalotiollide B (**103**) [115].

Table 2. Some examples of VeA regulating secondary metabolite production in fungi.

Fungus	Overexpression/ Deletion of *veA*	Positive/Negative Regulation	Production of Secondary Metabolites	Ref.
Acremonium chrysogenum	Deletion	Positive	Decreased production of cephalosporin C (**114**).	[133]
Alternaria alternata	Deletion	Positive	Decreased production of alternariol (**4**) and alternariol monomethyl ether (**5**).	[27]
Alternaria alternata	Deletion	Positive	Decreased production of alternariol (**4**) and alternariol monomethyl ether (**5**).	[134]
Aspergillus carbonarius	Deletion	Positive	Production of ochratoxin A (**10**) was decreased to almost zero.	[34]
Aspergillus carbonarius	Deletion	Positive	Decreased production of ochratoxin A (**10**).	[136]
Aspergillus flavus	Deletion	Positive	Decreased production of cyclopiazonic acid (**18**), aflatrem B (**115**), and aflatoxins.	[137]
Aspergillus flavus	Deletion	Positive	Decreased production of asparasone A (**116**).	[138]
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxin B1 (**17**).	[139]
Aspergillus flavus	Deletion and overexpression	Positive	Decreased production of aflatoxins in deletion muatant, and increased production of aflatoxins in overexpression mutant.	[140]
Aspergillus fumigatus	Deletion	Positive	Decreased production of gliotoxin (**21**).	[141]
Aspergillus fumigatus	Overexpression	Negative	Decreased production of gliotoxin (**21**).	[141]
Aspergillus fumigatus	Deletion	Positive	Decreased production of fumagillin (**23**), gumitremorgin G (**117**), fumigaclavine C (**118**) and glionitrin A (**119**).	[142]
Aspergillus fumigatus	Overexpression	Negative	Decreased production of fumagillin (**23**), gumitremorgin G (**117**), fumigaclavine C (**118**) and glionitrin A (**119**).	[142]
Aspergillus nidulans	Deletion	Positive	Decreased production of sterigmatocystin (**16**) and penicillin G (**28**).	[143]
Aspergillus nidulans	Deletion	Positive	Decreased production of penicillin G (**28**).	[144]
Aspergillus	Deletion	Positive	Decreased production of sterigmatocystin (**16**) and norsolorinic aid (**27**)	[54]

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nidulans				
Aspergillus nidulans	Deletion	Negative	Increased production of orsellinic acid (**120**), F9775A (**121**) and F9775B (**122**).	[145]
Aspergillus nidulans	Deletion	Positive	Decreased production of aspernidines	[148]
Aspergillus niger	Deletion	Positive	Decreased production of ochratoxins A (**10**), α (**123**) and β (**124**).	[149]
Aspergillus ochraceus	Deletion	Positive	Decreased production of ochratoxin A (**10**).	[61]
Aspergillus oryzae	Deletion	Positive	Decreased production of kojic acid (**20**).	[150]
Aspergillus pachycristatus	Deletion	Positive	Decreased production of sterigmatocystin (**16**) and echinocandin B (**39**).	[65]
Aspergillus parasiticus	Deletion	Positive	Decreased production of sterigmatocystin (**16**).	[166]
Aspergillus parasiticus	Deletion	Positive	Reduced aflatoxin BGC gene expression and aflatoxisome development	[151]
Chaetomium globosum	Deletion and overexpression	Positive	Decreased production of chaetoglobusin A (**48**) in *veA* deleted mutant, and increased production of chaetoglobusin A (**48**) in *veA* overexpressed mutant.	[152]
Cladosporium fulvum	Deletion	Negative	Increased production of cladofulvin (**55**).	[76]
Cochliobolus heterostrophus	Deletion	Positive	Decreased production of T-toxin (**56**).	[77]
Fusarium fujikuroi	Deletion	Negative	Increased production of bikaverin (**61**).	[83]
Fusarium fujikuroi	Deletion	Positive	Decreased production of gibberellins A3 (**63**) and A4 (**64**), fusarin C (**65**), fumonisins B1 (**66**), B2 (**67**), B3 (**68**) and B4 (**69**), deoxynivalenol (**70**), and 15-acetyl deoxynivalenol (**71**).	[83]
Fusarium fujikuroi	Deletion	Positive	Decreased production of fusaric acid (**62**), fusarinolic acid (**72**), and dehydrofusaric acid (**73**)	[84]
Fusarium fujikuroi	Deletion	Positive	Decreased production of gibberellins, fumonisins and fusarin C (**65**).	[85]
Fusarium fujikuroi	Deletion	Negative	Increased production of gibepyrones A (**74**), B (**75**), C (**76**), D (**77**), E (**78**), and F (**79**)	[86]
Fusarium graminearium	Deletion	Positive	Reduced production of deoxynivalenol (**69**).	[153]

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Fusarium graminearium	Deletion	Positive	Decreased production of trichothecenes.	[154]
Fusarium nematophilum	Overexpression	Positive	Increased production of antitumor compounds.	[155]
Fusarium oxysporum	Deletion	Positive	Decreased production of beauvericin (**44**) and fusaric aicd (**73**)	[88]
Fusarium oxysporum f.sp. *niveum*	Deletion	Positive	Decreased production of bikaverin (**61**) and fusaric acid (**62**)	[89]
Fusarium verticillioides	Deletion	Positive	Decreased production of fusarin C (**65**) and fumonisins B1 (**66**), B2 (**67**) and B3 (**68**).	[156]
Mycosphaerella graminicola	Deletion	Positive	Decreased production of melanin.	[158]
Neurospora crassa	Deletion	Positive	Decreased production of carotenoids.	[159]
Neurospora crassa	Deletion	Positive	Decreased production of siderophore coprogen (**125**) and carotenoids.	[160]
Penicillium chrysogenum	Deletion	Positive	Decreased production of penicillin G (**28**).	[101]
Penicillium chrysogenum	Deletion	Positive	Decreased production of penicillin G (**28**).	[161]
Penicillium chrysogenum	Deletion	Positive	Small reduction of penicillin G (**28**)	[103]
Penicillium citrinum	Deletion and overexpression	Positive	Decreased production of compactin (**98**) in the deletion mutant, and increased production of compactin (**98**) in the overexpressed mutant.	[105]
Penicillium citrinum	Overexpression	Positive	Increased production of compactin (**98**).	[162]
Penicillium expansum	Deletion	Positive	Decreased production of citrinin (**81**) and patulin (**105**).	[127]
Penicillium expansum	Deletion	Positive	Blocked production of patulin (**105**).	[164]
Penicillium expansum	Deletion	Positive	Lost production of patulin (**105**).	[165]
Pestalotiopsis microspora	Deletion	Negative	Increased production of pestalotiollide B (**103**)	[115]

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3.2. Regulation of VelB on Secondary Metabolite Production in Fungi

VelB (also called Vel2) mainly coordinates with other members such as LaeA, VeA and VosA to regulate the production of fungal secondary metabolites in fungi as VelB lacked the site of nuclear localization signal (NLS) [128]. Some examples of VelB regulating secondary metabolite production in fungi are shown in Table 3. The structures of the metabolites are shown in Figure S1.

Deletion of *velB* in *Aspergillus flavus* abolished aflatoxin production and sclerotial formation either under illumination or in darkness. VelB may have a dual role and likely coordinate with FluG to modulate its functions [167]. Similar result was reported by Eom et al. that inactivation of *velB* led to decreased production of aflatoxin B1 (**17**) in *A. flavus* [139].

Knock out of *velB* gene in *Aspergillus nidulans* led to reduced content of sterigmatocystin (**16**) under illumination. However, the production yield of sterigmatocystin (**16**) in deletion mutant was almost the same as that of wild type strain, which indicated that the mycelial growth rate of deletion mutant was bigger than that of wild type strain [128]. Further investigation showed that the deletion of *velB* in *A. nidulans* resulted in decreased mRNA levels of *vadJ* throughout the life cycle. Conversely, the deletion of *vadJ* resulted in elevated production of sexual fruiting bodies and sterigmatocystin (**16**). This indicated that *velB* was necessary for proper coordination *via vadJ* to regulate sterigmatocystin (**16**) production [168].

Deletion of *velB* gene in *Aspergillus ochraceus* led to drastically reduced production of ochratoxin A (OTA, **10**) [61].

The deletion of *BcvelB* led to increased conidiation and melanin biosynthesis in *Botrytis cinerea*. Expression of melanin biosynthesis gene cluster was also up-regulated [169].

CsVelB positively regulated melanin production of *Colletotrichum siamense* [170].

Deletion of *ClvelB* gene in *Curvularia lunata* led to the decrease of the production of conidia and the phytotoxin methyl 5-hydroxymethylfuran-2-carboxylate (**126**). The Δ*ClvelB* mutant was impaired in colonizing the host tissue. However, deletion of *ClvelB* gene led to the increase in aerial hyphae and melanin production [171].

Deletion of *vel2* gene in *F. fujikuroi* led to upregulation of gibepyrone BGC expression as well as increased production of gibepyrones A (**74**), B (**75**), C (**76**), D (**77**), E (**78**), and F (**79**) [86].

Deletion of *FgvelB* in *Fusarium graminearum* led to decreased production of deoxynivalenol (DON, **68**) [172]. Production of trichothecenes and zearalenone (**80**) in *FgvelB*-deleted strain of *F. graminearum* was also dramatically reduced compared with the wild strain [173]. A similar example is that the deletion of *FpvelB* led to notable differences in growth, conidiation, virulence and deoxynivalenol (**68**) production in *F. pseudograminearum*. Furthermore, FpVelB positively regulated another secondary metabolite BGC associated with pathogenesis by modulating the expression of the *PKS11* gene. FpVelB regulated pathogen virulence by influencing deoxynivalenol (**68**) production in *F. pseudograminearum* [174].

Deletion of *velB* in *Neurospora crassa* led to reduced biosynthesis of light-dependent carotenoids [160].

Penicillium expansum is the pathogen of apple blue mold disease and the main producer of patulin (**105**). The Δ*PevelB* mutant colonized apples, but at a lower rate than the wild type and complemented strains. Conidiation was significantly reduced in theΔ*PevelB* strain. Under light conditions, the Δ*PevelB* strain showed a reduced level of spore viability. Deletion of the *velB* gene strongly inhibited the production of mycotoxins chaetoglobusin A (**48**), citrinin (**81**), and patulin (**105**) on synthetic media or in planta, but increased the production of fumarylalanine (**127**). In addition, the genes involved in siderophore biosynthesis, ergosterol biosynthesis, and nitrate assimilation were also upregulated in the Δ*PevelB* strain. This indicated that VelB was involved in the development, pathogenicity, and secondary metabolism of *P. expansum* [175].

Deletion of *veA* in *Pestalotiopsis microspora* led to a decrease in production of pestalotiollide B (**103**). The *velB* gene appeared to stimulate the biosynthesis of pestalotiollide B (**103**) [115].

Table 3. Some examples of VelB regulating secondary metabolite production in fungi.

Fungus	Overexpression/ Deletion of *velB*	Positive/Negative Regulation	Production of Secondary Metabolites	Ref.
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxins.	[167]
Aspergillus flavus	Deletion	Positive	Decreased production of aflatoxin B1 (**17**).	[139]
Aspergillus nidulans	Deletion	Positive	Decreased production of sterigmatocystin (**16**).	[128]
Aspergillus nidulans	Deletion	Negative	Increased production of sterigmatocystin (**16**).	[168]
Aspergillus ochraceus	Deletion	Positive	Reduced production of ochratoxin A (**10**).	[61]
Aspergillus oryzae	Deletion	Negative	Increased production of kojic acid (**20**).	[150]
Botrytis cinerea	Deletion	Negative	Increased production of melanin.	[169]
Colletotrichum siamense	Deletion	Positive	Decreased production of melanin.	[170]
Curvularia lunata	Deletion	Positive	Decreased production of methyl 5-hydroxymethylfuran-2-carboxylate (**126**).	[171
Fusarium fujikuroi	Deletion	Positive	Decreased production of gibberellins, fumonisins and fusarin C (**65**).	[85]
Fusarium fujikuroi	Deletion	Negative	Increased production of gibepyrones A (**74**), B (**75**), C (**76**), D (**77**), E (**78**), and F (**79**)	[86]
Fusarium graminearum	Deletion	Positive	Decreased production of deoxynivalenol (**68**).	[172]
Fusarium graminearum	Deletion	Positive	Decreased production of trichothecenes and zearalenone (**80**).	[173]
Fusarium pseudograminearum	Deletion	Positive	Decreased production of deoxynivalenol (**68**).	[174]
Neurospora crassa	Deletion	Positive	Reduced biosynthesis of carotenoids.	[160]

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Penicillium chrysogenum	Deletion	Negative	Increased production of penicillin G (**28**).	[176]
Penicillium Expansum	Deletion	Positive	Blocked production of patulin (**105**).	[164]
Penicillium expansum	Deletion	Positive	Decreased production of chaetoglobusin A (**48**), citrinin (**81**) and patulin (**105**).	[175]
Penicillium expansum	Deletion	Negative	Increased production of fumarylalanine (**127**).	[175]
Pestalotiopsis microspora	Deletion	Positive	Decreased production of pestalotiollide B (**103**).	[115]

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3.3. Regulation of VelC, VelD and VosA on Secondary Metabolite Production in Fungi

In velvet proteins, the functions of VelC, VelD and VosA in fungi have been seldom studied for their regulation on secondary metabolism. Some examples of VelC, VelD and VosA regulating secondary metabolite production in fungal species of the genera *Aspergillus* and *Penicillium* are shown in Table 4. The structures of the metabolites are shown in Figure S1.

3.3.1. Regulation of VelC

The VelC (also called Vel3 and VE-3) protein belongs to the velvet family of regulators involved in the control of development and secondary metabolite production in fungi [177]. Deletion of *velC* gene in *Aspergillus oryzae* led to decreased production of kojic acid (**20**) [150]. Deletion of *velC* gene in *Penicillium expansum* led to markedly decreased production of patulin (**105**) [164]. For the above two fungal species, VelC positively regulated secondary metabolite production.

3.3.2. Regulation of VelD

The VelD protein was also called Vel4. Deletion of *velD* gene in *Aspergillus flavus* led to decreased production of aflatoxin B1 (**17**) [139]. Another example was that deletion of *velD* gene in *A. oryzae* led to decreased production of kojic acid (**20**) [150]. It indicated that VelD positively regulated secondary metabolite production in *A. flavus* and *A. oryzae*.

3.3.3. Regulation of VosA

The regulation of VosA on secondary metabolite production was studied in detail in *Aspergillus nidulans*. The deletion of *vosA* in *A. nidulans* resulted in the lack of trehalose (**128**) in spores, a rapid loss of the cytoplasm, organelles and viability of spores, and a dramatic reduction in tolerance of conidia to heat and oxidative stress [178]. RNA-seq-based genome-wide expression analysis demonstrated that the loss of *vosA* in *A. nidulans* led to elevated expression of sterigmatocystin (**16**) biosynthetic genes and a slight increase in sterigmatocystin (**16**) production in ascospores. Moreover, the deletion of *vosA* caused upregulation of additional gene clusters associated with the biosynthesis of other secondary metabolites including asperthecin (**129**), microperfuranone (**130**), and monodictyphenone (**131**) [179]. VosA in *A. nidulans* could interact with the downstream target SclB to negatively regulated production of secondary metabolites including emericellamides A (**132**), C (**133**) and D (**134**), austinol (**135**) and dehydroaustinol (**136**) [125]. The second instance was that VosA-repressed *dnjA* gene negatively regulated metabolism in *Aspergillus* species. The deletion of *dnjA* caused increased production of sterigmatocystin (**16**) and aflatoxin B1 (**17**) in *A. nidulans* and *A. flavus*, respectively [180]. The third instance was that the VosA-VelB-repressed *mcrA* gene negatively regulated sterigmatocystin (**16**) production in *A. nidulans*. The conidia of Δ*mcrA* mutant contained more amounts of sterigmatocystin (**16**) [181]. The fourth instance was that VosA-VelB targeted gene *vidD*, which was required for proper fungal growth, development, and sterigmatocystin (**16**) production in *Aspergillus nidulans* [182]. Furthermore, transcriptomic, protein-DNA interaction, and metabolomics studies of VosA, VelB and WetA in *A. nidulans* played interdendent, overlapping, and distinct roles in governing morphological development and metabolic remodeling in the conida, leading to the production of vital conidia suitable for fungal proliferation and dissemination. The related secondary metabolites regulated by VosA, VelB and WetA in *A. nidulans* asexual spores included sterigmatocystin (**16**), austinol (**135**), dehydroaustinol (**136**), norsolorinic acid (**137**), nidurufin (**138**), versiconol (**139**), and emericellamids A (**132**), C (**133**), D (**134**), E (**140**), and F (**141**) [183]. In addition, Vos-VelB could activate putative C_6 transcription factor VadZ to regulate development and sterigmatocystin (**16**) production in *A. nidulans* [184].

Table 4. Some examples of VelC, VelD and Vos A regulating secondary metabolite production in fungi.

Overexpression/ Deletion	Fungus	Positive/Negative Regulation	Production of Secondary Metabolites	Ref.
Deletion of *velC*	*Aspergillus Oyzae*	Positive	Decreased production of kojic acid (**20**).	[150]
Deletion of *velC*	*Penicillium Expansum*	Positive	Decreased production of patulin (**105**).	[164]
Deletion of *velD*	*Aspergillus flavus*	Positive	Decreased production of aflatoxin B1 (**17**).	[139]
Deletion of *velD*	*Aspergillus oyzae*	Positive	Decreased production of kojic acid (**20**).	[150]
Deletion of *vosA*	*Aspergillus nidulans*	Positive	Lost production of trehalose (**128**) in spores.	[178]
Deletion of *vosA*	*Aspergillus nidulans*	Negative	Slightly increased production of sterigmatocystin (**16**) in ascospores, and upregulation of the BGCs associated with the biosynthesis of other secondary metabolites, including asperthecin (**129**), microperfuranone (**130**), and monodictyphenone (**131**).	[179]
Deletion of *vosA*	*Aspergillus nidulans*	Negative	Increased production of emericellamides A (**132**), C (**133**) and D (**134**), austinol (**135**) and dehydroaustinol (**136**)	[125]
Deletion of *vosA*	*Aspergillus nidulans*	Negative	Increaseed production of sterigmatocystin (**16**).	[184]
Deletion of *vosA*	*Aspergillus oyzae*	Negative	Increased production of kojic acid (**20**).	[150]

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4. Conclusions

LaeA and velvet proteins could obviously regulate the production of fungal secondary metabolites by responding to the light conditions under which fungi are grown. We can manipulate fungal secondary metabolite production to inhibit the production of harmful mycotoxins while promoting the production of useful metabolites [160]. However, we only know a little about the regulation mechanisms between LaeA/velvet proteins and secondary metabolite BGC expression, which should be studied in detail in the future [11,14,70,185].

In summary, it should be an effective strategy to promote or inhibit production of secondary metabolites through global regulation of LaeA and velvet proteins in fungi. Some cryptic BGCs for secondary metabolite production are possibly activated by LaeA and velvet proteins through the regulatory networks. It is beneficial for the excavation of bioactive compounds from fungi. Furthermore, some non-mycotoxigenic fungal strains obtained by deletion or overexpression of *laeA* or velvet protein encoding genes could be used as the biocontrol agents by applying in plants to reduce mycotoxin contamination [165].

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Structures of the compounds **1–141** identified from fungi through regulation of LaeA and velvet proteins.

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References

1. Takahashi, J.A.; Teles, A.P.C.; Bracarense, A.A.P.; Gomes, D.C. Classical and epigenetic approaches to metabolite diversification in filamentous fungi. *Phytochem. Rev*. **2013**, *12*, 773–789.
2. Lou, J.; Fu, L.; Peng, Y.; Zhou, L. Metabolites from *Alternaria* fungi and their bioactivities. *Molecules* **2013**, *18*, 5891–5935.
3. Tian, J.; Lai, D.; Zhou, L. Secondary metabolites from *Acremonium* fungi: diverse structures and bioactivities. *Mini-Rev. Med. Chem*. **2017**, *17*, 603–632.
4. Han, P.; Zhang, X.; Xu, D.; Zhang, B.; Lai, D,; Zhou, L. Metabolites from *Clonostachys* fungi and their biological activities. *J. Fungi* **2020**, *6*, 229.
5. Brase, S.; Encinas, A.; Keck, J.; Nising, C.F. Chemistry and biology of mycotoxins and related fungal metabolites. *Chem. Rev*. **2009**, *109*, 3903–3990.
6. Pitt, J.I.; Miller, J.D. A concise history of mycotoxin research. *J. Agric. Food Chem*. **2017**, *65*, 7021−7033.
7. Li, P.; Su, R.; Yin, R.; Lai, D.; Wang, M.; Liu, Y.; Zhou, L. Detoxification of mycotoxins through biotransformation. *Toxins* **2020**, *12*, 121.
8. Xu, D.; Xue, M.; Shen, Z.; Jia, X.; Hou, X.; Lai, D.; Zhou, L. Phytotoxic secondary metabolites from fungi. *Toxins* **2021**, *13*, 261.
9. Sonowal, S.; Gogoi, U.; Buragohain, K.; Nath, R. Endophytic fungi as a potential source of anti-cancer drug. *Arch. Microbiol*. **2024**, *206*, 122.
10. Sun, Z.; Wu, Y.; Long, S.; Feng, S.; Jia, X.; Hu, Y.; Ma, M.; Liu, J.; Zeng, B. *Aspergillus oryzae* as a cell factory: research and applications in industrial production. *J. Fungi* **2024**, *10*, 248.
11. Jia, X.; Song, J.; Wu, Y.; Feng, S.; Sun, Z.; Hu, Y.; Yu, M.; Han, R.; Zeng, B. Strategies for the enhancement of secondary metabolite production *via* biosynthesis gene cluster regulation in *Aspergillus oryzae*. *J. Fungi* **2024**, *10*, 312.
12. Hewage, R.T.; Aree, T.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. One strain-many compounds (OSMAC) method for production of polyketides, azaphilones, and an isochromanone using the endophytic fungus *Dothideomycete* sp. *Phytochemistry* **2014**, *108*, 87–94.

13. Moon, H.; Han, K.-H.; Yu, J.-H. Upstream regulation of development and secondary metabolism in *Aspergillus* species. *Cells* **2023**, *12*, 2.
14. Xue, M.; Hou, X.; Fu, J.; Zhang, J.; Wang, J.; Zhao, Z.; Xu, D.; Lai, D.; Zhou, L. Recent advances in search of bioactive secondary metabolites from fungi triggered by chemical epigenetic modifiers. *J. Fungi* **2023**, *9*, 172.
15. Brakhage, A.A. Regulation of fugnal secondary metabolitsm. *Nat. Rev. Microbiol*. **2012**, *11*, 21–32.
16. Jain, S.; Keller, N. Insights to fungal biology through LaeA sleuthing. *Fungal Biol. Rev*. **2013**, *27*, 51–59.
17. Fanelli, F.; Geisen, R.; Schmidt-Heydt, M.; Logrieco, A.F.; Mule, G. Light regulation of mycotoxin biosynthesis: new perspectives for food safety. *World Myctotoxin J*. **2016**, *9*, 129–145.
18. Lind, A.L.; Smith, T.D.; Saterlee, T.; Calvo, A.M.; Rokas, A. Regulation of secondary metabolism by the velvet complex is temperature-responsive in *Aspergillus*. *G3-Genes Genom. Genet*. **2016**, *6*, 4023–4033.
19. Palonen, E.K.; Raina, A.; Brandt, A.; Meriluoto, J.; Keshavarz, T.; Soini, J.T. Transcriptomic complexity of *Aspergillus terreus* velvet gene family under the Influence of butyrolactone I. *Microorganisms* **2017**, *5*, 12.
20. Bayram, O.; Braus, G.H. Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. *FEMS Microbiol. Rev*. **2012**, *36*, 1–24.
21. Martin J.F. Key role of LaeA and velvet complex proteins on expression of β-lactam and PR-toxin genes in *Penicillium chrysogenum*: cross-talk regulation of secondary metabolite pathways. *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 525–535.
22. Kosalkova, K.; Garcia-Estrada, C.; Ullan, R.V.; Godio, R.P.; Feltrer, R.; Teijeira, F.; Mauriz, E.; Martin, J.F. The global regulator LaeA controls penicillin biosynthesis, pigmentation and sporulation, but not roquefortine C synthesis in *Penicillium chrysogenum*. *Biochimie* **2009**, *91*, 214–225.
23. Zhao, Z.; Gu, S.; Liu, D.; Liu, D.; Chen, B.; Li, J.; Tian, C. The putative methyltransferase LaeA regulates mycelium growth and cellulose production in *Myceliophthora thermophile*. *Biotechnol. Biofuels Bioprod*. **2023**, *16*, 58.
24. Yin, X.; Han, L.; Zheng, W.; Cai, L.; Qin, M.; He, Z.; Kang, J. Global regulatory factor AaLaeA upregulates the production of antitumor substances in the endophytic fungus *Alternaria alstroemeria*. *J. Basic Microbiol*. **2022**, *62*, 1402–1414.
25. Feng, C.; Zheng, W.; Han, L.; Wang, J.; Zha, X.; Xiao, Q.; He, Z.; Kang, J. AaLaeA targets AaFla1 to mediate the production of antitumor compound in *Alternaria alstroemeria*. *J. Basic Microbiol*. **2024**, *64*, 68–80.
26. Takao, K.; Akagi, Y.; Tsuge, T.; Harimoto, Y.; Yamamoto, M.; Kodama, M. The global regulator LaeA controls biosynthesis of host-specific toxins, pathogenicity and development of *Alternaria alternata* pathotypes. *J. Gen. Plant Pathol*. **2016**, *82*, 121–131.
27. Estiarte, N.; Lawrence, C.B.; Sanchis, V.; Ramos, A.J.; Crespo-Sempere, A. LaeA and VeA are involved in growth morphology, asexual development, and mycotoxin production in *Alternaria alternata*. *Int. J. Food Microbiol*. **2016**, *238*, 153–164.
28. Liu, G.; Liu, D.; Li, Z.; Jiao, J.; Hou, X.; Zhang, X.; Che, Q.; Zhu, T.; Li, D.; Zhang, G. Overexpression of transcriptional regulator and tailoring enzyme leads to the discovery of anti-inflammatory meroterpenoids from marine-derived fungus *Alternaria alternata* JJY-32. *Front. Mar. Sci*. **2022**, *9*, 1015684.
29. Zhang, Y.; Wang, X.; Ran, Y.; Zhang, K.-Q.; Li, G.-H. AfLaeA, a global regulator of mycelial growth, chlamydospore production, pathogenicity, secondary metabolism, and energy metabolism in the nematode-trapping fungus *Arthrobotrys flagrans*. *Microbiol. Spectr*. **2023**, *11*, e00186-23.
30. Khan, I.; Xie, W.-L.; Yu, Y.-C.; Shen, H.; Xu, Y.; Wang, J.-Q.; Debnath, S.C. Xu, J.-Z.; Zheng, D.-Q.; Ding, W.-J.; Wang, P.-W. Heteroexpression of *Aspergillus nidulans laeA* in marine-derived fungi triggers upregulation of secondary metabolite biosynthetic genes. *Mar. Drugs* **2020**, *18*, 652.
31. Honma, S.; Kimishima, A.; Kimishima, A.; Honsho, M.; Kojima, H.; Tokiwa, T.; Nishitomi, A.; Kato, S.; Kondo, N.; Araki, Y. Re-discovery of MS-347a as a fungicide candidate through a new drug discovery platform with a multidrug-sensitive *Saccharomyces cerevisiae* screening system and the introduction of a global secondary metabolism regulator, *laeA* gene. *Biosci. Biotechnol. Biochem*. **2024**, *88*, 824–829.
32. Nakanishi, S.; Ando, K.; Kawamoto, I.; Matsuda, Y. MS-347a, a new inhibitor of myosin light chain kinase from *Aspergillus* sp. KY52178. *J. Antibiot*. **1993**, *46*, 1775–1781.
33. Linde, T.; Zoglowek, M.; Lubeck, M.; Frisvad, J.C.; Lubeck, P.S. The global regulator LaeA controls production of citric acid and endoglucanases in *Aspergillus carbonarius*. *J. Ind. Microbiol. Biotechnol*. **2016**, *43*, 1139–1147.
34. Crespo-Sempere, A.; Marin, S.; Sanchis, V.; Ramos, A.J. VeA and LaeA transcriptional factors regulate ochratoxin A biosynthesis in *Aspergillus carbonarius*. *Int. J. Food Microbiol*. **2013**, *166*, 479–486.
35. Maor, U.; Barda, O.; Sadhasivam, S.; Bi, Y.; Levin, E.; Zakin, V.; Prusky, D.B.; Sionov, E. Functional roles of LaeA, polyketide synthase, and glucose oxidase in the regulation of ochratoxin A biosynthesis and virulence in *Aspergillus carbonarius*. *Mol. Plant Pathol*. **2021**, *22*, 117–129.
36. Jiang, N.; Wang, L.; Jiang, D.; Wang, M.; Liu, H.; Yu, H.; Yao, W. Transcriptomic analysis of inhibition by eugenol of ochratoxin A biosynthesis and growth of *Aspergillus carbonarius*. *Food Control* **2022**, *135*, 108788.
37. Wang, Y.; Chen, Y.; Zhang, J.; Zhang, C. Overexpression of *llm1* affects the synthesis of secondary metabolites of *Aspergillus cristatus*. *Microorganisms* **2022**, *10*, 1707.

38. Liu, Y.; Li, P.; Qi, C.; Zha, Z.; Meng, J.; Liu, C.; Ha, J.; Zhou, Q.; Luo, Z.; Wang, J.; et al. Cryptic piperazine derivatives activated by knocking out the global regulator LaeA in *Aspergillus flavipes*. *Bioorg. Med. Chem.* **2024**, *103*, 117685.
39. Kale, S.P.; Milde, L.; Trpp, M.K.; Frisvad, J.C.; Keller, N.P.; Bok, J.W. Requirement of LaeA for secondary metabolism and sclerotial production in *Aspergillus flavus*. *Fungal Genet. Biol*. **2008**, *45*, 1422–1429.
40. Amaike, S.; Keller, N.P. Distinct roles for VeA and LaeA in development and pathogenesis of *Aspergillus flavus*. *Eukaryot. Cell* **2009**, *8*, 1051–1060.
41. Chang, P.-K.; Scharfenstein, L.L.; Ehrlich, K.C.; Wei, Q.; Bhatnagar, D.; Ingber, B.F. Effects of laeA deletion on *Aspergillus flavus* conidial development and hydrophobicity may contribute to loss of aflatoxin production. *Fungal Biol*. **2012**, *116*, 298–307.
42. Yao, G.; Yue, Y.; Fu, Y.; Fang, Z.; Xu, Z.; Ma, G.; Wang, S. Exploration of the regulatory mechanism of secondary metabolism by comparative transcriptomics in *Aspergillus flavus*. *Front. Microbiol*. **2018**, *9*, 1568.
43. Bok, J.W.; Keller, N.P. LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryot. Cell* **2004**, *3*, 527–535.
44. Lv, Y.; Lv, A.; Zhai, H.; Zhang, S.; Li, L.; Cai, J.; Hu,Y. Insight into the global regulation of laeA in *Aspergillus flavus* based on proteomic profiling. *Int. J. Food Microbiol*. **2018**, *284*, 11–21.
45. Zhi, Q.-Q.; He, L.; Li, J.-Y.; Li, J.; Wang, Z.-L.; He, G.-Y.; He, Z.-M. The kinetochore protein Spc105, a novel interaction partner of LaeA, regulates development and secondary metabolism in *Aspergillus flavus*. *Front. Microbiol*. **2019**, *10*, 1881.
46. Bok, J.W.; Balajee, S.A.; Marr, K.A.; Andes, D.; Nielsen, K.F.; Frisvad, J.C.; Keller, N.P. LaeA, a regulator of morphogenetic fungal virulence factors. *Eukaryot Cell* **2005**, 4, 1574–1582.
47. Keller, N.; Bok, J.; Chung, D.; Perrin, R.M.; Shwab, E.K. LaeA, a global regulator of *Aspergillus* toxins. *Med. Mycol*. **2006**, *44*, S83–S85.
48. Perrin, R.M.; Fedorova,N.D.; Bok, J.W.; Cramer, R.A.; Wortman, J.R.; Kim, H.S.; Nierman, W.C.; Keller, N.P. Transcriptional regulation of chemical diversity in *Aspergillus fumigatus* by LaeA. *PLoS Pathog*. **2007**, *3*, e50.
49. Lim, F.Y.; Hou, Y.; Chen, Y.; Oh, J.-H.; Lee, I.; Bungi, T.S.; Keller, N.P. Genome-based cluster deletion reveals an endocrocin biosynthetic pathway in *Aspergillus fumigatus*. *Appl. Environ. Microbiol*. **2012**, *78*, 4117–4125.
50. Hong, E.J.; Kim, N.K.; Lee, D.; Kim, W.G.; Lee, I. Overexpression of the laeA gene leads to increased production of cyclopiazonic acid in *Aspergillus fumisynnematus*. *Fungal Biol*. **2015**, *119*, 973–983.
51. Kadooka, C.; Nakamura, E.; Mori, K.; Okutsu, K.; Yoshizaki, Y.; Takamine, K.; Goto, M.; Tamaki, H.; Futagami, T. LaeA controls citric acid production through regulation of the citrate exporter-encoding cexA gene in *Aspergillus luchuensis* mut. *kawachii*. *Appl. Environ. Microbiol.* **2020**, *86*, e01950–e01919.
52. Grau, M.F.; Entwistle, R.; Oakley, C.E.; Wang, C.C.C.; Oakley, B.R. Overexpression of an LaeA-like methyltransferase upregulates secondary metabolite production in *Aspergillus nidulans*. *ACS Chem. Biol*. **2019**, *14*, 1643–1651.
53. Bouhired, S.; Weber, M.; Kempf-Sontag, A.; Keller, N.P.; Hoffmeister, D. Accurate prediction of the *Aspergillus nidulans* terrequinone gene cluster boundaries using the transcriptional regulator LaeA. *Fungal Genet. Biol*. **2007**, *44*, 1134–1145.
54. Shaaban, M.I.; Bok, J.W.; Lauer, C.; Keller, N.P. Suppressor mutagenesis identifies a velvet complex remediator of *Aspergillus nidulans* secondary metabolism. *Eukaryot. Cell* **2010**, *9*, 1816–1824.
55. Reyes-Dominguez, Y.; Bok, J.W.; Berger, H.; Shwab, E.K.; Basheer, A.; Gallmetzer, A.; Scazzocchio, C.; Keller, N.; Strauss, J. Heterochromatic marks are associated with the repression of secondary metabolism clusters in *Aspergillus nidulans*. *Mol. Microbiol*. **2010**, *76*, 1376–1386.
56. Palmer, J.M.; Theisen, J.M.; Duran, R.M.; Grayburn, W.S.; Calvo, A.M.; Keller, N.P. Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. *PLoS Genet*. **2013**, *9*, e1003193.
57. Yu, R.; Liu, J.; Wang, Y.; Wang, H.; Zhang, H. *Aspergillus niger* as a secondary metabolite factory. *Front. Chem*. **2021**, *9*, 701022.
58. Niu, J.; Arentshorst, M.; Nair. P.D.; Dai, Z.; Baker, S.E.; Frisvad, J.C.; Nielsen, K.F.; Punt, P.J.; Ram, A.F. Identifification of a classical mutant in the industrial host *Aspergillus niger* by systems genetics: LaeA is required for citric acid production and regulates the formation of some secondary metabolites. *G3-Genes Genom. Genet*. **2016**, *6*, 193–204.
59. Wang, B.; Li, X.; Tabudravu, J.; Wang, S.; Deng, H.; Pan, L. The chemical profile of activated secondary metabolites by overexpressing LaeA in *Aspergillus niger*. *Microbiol. Res*. **2021**, *248*, 126735.
60. Wang, B.; Lv, Y.; Li, X.; Lin, Y.; Deng, H.; Pan, L. Profiling of secondary metabolite gene clusters regulated by LaeA in *Aspergillus niger* FGSC A1279 based on genome sequencing and transcriptome analysis. *Res. Microbiol*. **2018**, *169*, 67–77.
61. Wang, G.; Zhang, H.; Wang, Y.; Liu, F.; Li, E.; Ma, J.; Yang, B.; Zhang, C.; Li, L.; Liu, Y. Requirement of LaeA, VeA, and VelB on asexual development, ochratoxin A biosynthesis, and fungal virulence in *Aspergillus ochraceus*. *Front. Microbiol*. **2019**, 10, 2759.

62. Zhang, M.; Yang, Y.; Li, L.; Liu, S.; Xue, X.; Gao, Q.; Wang D.; Zhang, Y.; Zhang, J. LaeA regulates morphological development and ochratoxin A biosynthesis in *Aspergillus niger*. *Mycotoxin Res*. **2022**, *38*, 221–229.
63. Oda, K.; Kobayashi, A.; Ohashi, S.; Sano, M. *Aspergillus oryzae laeA* regulates kojic acid synthesis genes. *Biosci. Biotechnol. Biochem*. **2011**, *75*, 1832–1834.
64. Sakai, K.; Kinoshita, H.; Nihira, T. Heterologous expression system in Aspergillus oryzae for fungal biosynthetic gene clusters of secondary metabolites. *Appl. Microbiol. Biotechnol*. **2012**, *93*, 2011–2022.
65. Lan, N.; Yue, Q.; An, Z. Bills, G.F. Apc.LaeA and Apc.VeA of the velvet complex govern secondary metabolism and morphological development in the echinocandin-producing fungus *Aspergillus pachycristatus*. *J. Ind. Microbiol. Biotechnol*. **2020**, *47*, 155–168.
66. Pomraning, K.R.; Dai, Z.; Munoz, N.; Kim, Y.-M.; Gao, Y.; Deng, S.; Lemmon, T.; Swita, M.S.; Zucker, J.D.; Kim, J.; et al. Itaconic acid production is regulated by LaeA in *Aspergillus pseudoterreus*. *Matab. Eng. Commun*. **2022**, *15*, e00203.
67. Zhgun, A.A.; Nuraeva, G.K.; Eldarov, M.A. The role of LaeA and LovE regulators in lovastatin biosynthesis with exogenous polyamines in *Aspergillus terreus*. *Appl. Biochem. Microbiol*. **2019**, *55*, 639–648.
68. Barrios-Gonzalez, J.; Perez-Sanchez, A.; Bibian, M.E. New knowledge about the biosynthesis of lovastatin and its production by fermentation of *Aspergillus terreus*. *Appl. Microbiol. Biotechnol*. **2020**, *104*, 8979–8998.
69. Zhgun, A.A.; Nuraeva, G.K.; Dumina, M.V.; Voinova, T.M.; Dzhavakhiya, V.V.; Eldarov, M.A. 1,3-Diaminopropane and spermidine upregulate lovastatin production and expression of lovastatin biosynthetic genes in *Aspergillus terreus via* LaeA regulation. *Appl. Biochem. Microbiol*. **2019**, *55*, 243–254.
70. Zhang, B.; Wen, S.; Qin, X.; Wang, Z.; Yao, G. Activating biosynthesis by overexpressing LaeA and anti-Vibrio activity of dihydroisoflavipucines of marine-derived *Aspergillus terreus*. *Mycosystema* **2023**, *42*, 562–569.
71. Yin, M.; Xiao, D.; Wang, C.; Zhang, L.; Dun, B.; Yue, Q. The regulation of BbLaeA on the production of beauvericin and bassiatin in *Beauveria bassiana*. *World J. Microbiol. Biotechnol*. **2022**, *38*, 1.
72. Schumacher, J.; Simon, A.; Cohrs, K.C.; Traeger, S.; Porquier, A.; Dalmais, B.; Viaud, M.; Tudzynski, B. The VELVET complex in the gray mold fungus *Botrytis cinerea*: impact of BcLAE1 on differentiation, secondary metabolism, and virulence. *Mol. Plant-Microbe Interact.* **2015**, *28*, 659–674.
73. Wei, Z.; Shu, D.; Sun, Q.; Chen, D.; Li, Z.; Luo, D.; Yang, J.; Tan, H. The BcLAE1 is involved in the regulation of ABA biosynthesis in *Botrytis cinerea* TB-31. *Front. Microbiol*. **2022**, *13*, 969499.
74. Jiang, T.; Wang, M.; Wang, M.; Li, L.; Si, J.; Song, B.; Zhou, C.; Yu, M.; Wang, X.; Zhang, Y.; Ding, D.; Zou, Z. Overexpression of the global regulator LaeA in *Chaetomium globosum* leads to the biosynthesis of chaetoglobosin Z. *J. Nat. Prod*. **2016**, *79*, 2487–2494.
75. Cheng, M.; Zhao, S.; Lin, C.; Song, J.; Yang, Q. Requirement of LaeA for sporulation, pigmentation and secondary metabolism in *Chaetomium globosum*. *Fungal Biol*. **2021**, *125*, 305–315.
76. Griffiths, S.; Saccomanno, B.; De Wit, P.J.G.M.; Collemare, J. Regulation of secondary metabolite production in the fungal tomato pathogen *Cladosporium fulvum*. *Fungal Genet. Biol*. **2015**, *84*, 52–61.
77. Wu, D.; Oide, S.; Zhang, N.; Choi, M.Y.; Turgeon, B.G. ChLae1 and ChVel1 regulate T-toxin production, virulence, oxidative stress response, and development of the maize pathogen *Cochliobolus heterostrophus*. *PLoS Pathog*. **2012**, *8*, e1002542.
78. Tsunematsu, Y.; Takanishi, J.; Asai, S.; Nakazawa, T.; Watanabe, K. Genomic mushroom hunting decrypts coprinoferrin, a siderophore secondary metabolite vital to fungal cell development. *Org. Lett*. **2019**, *21*, 7582–7586.
79. Hayakawa, I.; Isogai, T.; Takanishi, J.; Asai, S.; Ando, C.; Tsutsumi, T.; Watanabe, K.; Sakakura, A.; Tsunematsu, Y. Synthesis and biological evaluation of coprinoferrin, an acylated tripeptide hydroxamate siderophore. *Org. Biomol. Chem*. **2024**, *22*, 831–837.
80. Zhou, Z.-Z.; Zhu, H.-J.; Yang, C.-L.; Liu, Y.-J.; Jiang, N.; Xiao, Y.-S.; Shi, L.-Y.; Jiao, R.-H.; Ge, H.-M.; Tan, R.-X. Dalestones A and B, two anti-inflammatory cyclopentenones from *Daldinia eschscholzii* with an edited strong promoter for the global regulator *LaeA*-like gene. *Chin. J. Nat. Med*. **2019**, *17*, 387–393.
81. Chettri, P.; Bradshaw, R.E. LaeA negatively regulates dothistromin production in the pine needle pathogen *Dothistroma septosporum*. *Fungal Genet. Biol.* **2016**, *97*, 24–32.
82. Janevska, S.; Tudzynski, B. Secondary metabolism in *Fusarium fujikuroi*: strategies to unravel the function of biosynthetic pathways. *Appl. Microbiol. Biotechnol*. **2018**, *102*, 615–630.
83. Wiemann, P.; Brown, D.W.; Kleigrewe, K.; Bok, J.W.; Keller, N.P.; Humpf, H.U.; Tudzynski, B. FfVel1 and FfLae1, components of a velvet-like complex in *Fusarium fujikuroi*, affect differentiation, secondary metabolism and virulence. *Mol. Microbiol*. **2010**, *77*, 972–994.
84. Niehaus, E.-M.; Von Bargen, K.W.; Espino, J.J.; Pfannmuler, A.; Humpf, H.-U.; Tudzynski, B. Characterization of the fusaric acid gene cluster in *Fusarium fujikuroi*. *Appl. Microbiol. Biotechnol*. **2014**, *98*, 1749–1762.

85. Niehaus, E.-M.; Rindermann, L.; Janevska, S.; Munsterkotter, M.; Guldener, U.; Tudzynski, B. Analysis of the global regulator Lae1 uncovers a connection between Lae1 and the histone acetyltransferase HAT1 in *Fusarium fujikuroi*. *Appl. Microbiol. Biotechnol*. **2018**, *102*, 279–295.
86. Janevska, S.; Arndt, B.; Niehaus, E.-M.; Burkhardt, I.; Rosler, S.M.; Brock, N.L.; Humpf, H.-U.; Dickschat, J.S.; Tudzynski, B. Gibepyrone biosynthesis in the rice pathogen *Fusarium fujikuroi* is facilitated by a small polyketide synthase gene cluster. *J. Biol. Chem*. **2016**, *291*, 27403–27420.
87. Kim, H.-K.; Lee, S.; Jo, S.-M.; McCormick, S.P.; Butchko, R.A.E.; Proctor, R.H.; Yun, S.-H. Functional roles of FgLaeA in controlling secondary metabolism, sexual development, and virulence in *Fusarium graminearum*. *PLoS One* **2013**, *8*, e68441.
88. Lopez-Berges, M.S.; Hera, C.; Sulyok, M.; Schafer, K.; Capilla, J.; Guarro, J.; Di Pietro, A. The velvet complex governs mycotoxin production and virulence of *Fusarium oxysporum* on plant and mammalian hosts. *Mol. Microbiol*. **2013**, *87*, 49–65.
89. Sun, Y.; Zhang, X.; Chai, Z.; Li, Y.; Ren, Z.; Wang, M.; Ma, Z.; Wang, Y.; Feng, J. Involvement of *FoVEL1* and *FoLAE1* in conidiation, virulence and secondary metabolism of *Fusarium oxysporum* f.sp. *niveum*. *J. Integr. Agric*. **2024**, *23*, https://doi.org/10.1016/j.jia.2024.01.029.
90. Butchko, R.A.E.; Brown, D.W.; Busman, M.; Tudzynski, B.; Wiemann, P. Lae1 regulates expression of multiple secondary metabolite gene clusters in *Fusarium verticillioides*. *Fungal Genet. Biol*. **2012**, *49*, 602–612.
91. Ahmad, M.F.; Wahab, S.; Ahmad, F.A.; Ashraf, S.A.; Abullais, S.S.; Saad, H.H. *Ganoderma lucidum*: A potential pleiotropic approach of ganoderic acids in health reinforcement and factors influencing their production. *Fungal Biol. Rev*. **2022**, *39*, 100–125.
92. Luo, Q.; Li, N.; Xu, J.-W. A methyltransferase LaeA regulates ganoderic acid biosynthesis in *Ganoderma lingzhi*. *Front. Microbiol*. **2022**, *13*, 1025983.
93. Saha, P.; Ghosh, S.; Roy-Barman, S. *MoLAEA* regulates secondary metabolism in *Magnaporthe oryzae*. *mSphere* **2020**, *5*, e00936-19.
94. Subba, P.; Saha, P.; Karthikkeyan, G.; Bismwas, M.; Prasad, T.S.K.; Roy-Barman, S. Metabolite profiling reveals overexpression of the global regulator, *MoLAEA* leads to increased synthesis of metabolites in *Magnaporthe oryzae*. *J. Appl. Microbiol*. **2022**, *132*, 3825–3838.
95. Qin, X.; Xie, B.; Zong, X.; Yu, X.; Feng, Y. Selective production, relationship and controversy between *Monascus* pigments and citrinin. *Food Biosci*. **2023**, *56*, 103233.
96. Lee, S.S.; Lee, J.H.; Lee, I. Strain improvement by overexpression of the *laeA* gene in *Monascus pilosus* for the production of *Monascus*-fermented rice. *J. Microbiol. Biotechnol*. **2013**, *23*, 959–965.
97. Zhang, C.; Zhang, H.; Zhu, Q.; Hao, S.; Chai, S. Li, Y.; Jiao, Z.; Shi, J.; Sun, B. Wang, C. Overexpression of global regulator LaeA increases secondary metabolite production in *Monascus purpureus*. *Appl. Microbiol. Biotechnol*. **2020**, *104*, 3049–3060.
98. Liu, Q.; Cai, L.; Shao, Y.; Zhou, Y.; Li, M.; Wang, X.; Chen, F. Inactivation of the global regulator LaeA in *Monascus ruber* results in a species-dependent response in sporulation and secondary metabolism. *Fungal Biol*. **2016**, *120*, 297–305.
99. Tannous, J.; Cosetta, C.M.; Drott, M.T.; Rush, T.A.; Abraham, P.E.; Giannone, R.J.; Keller, N.P. LaeA-regulated fungal traits mediate bacterial community assembly. *mBio* **2023**, *14*, e00769-23.
100. Wang, L.; Zhang, X.; Zhang, K.; Zhang, X.; Zhu, T.; Che, Q.; Zhang, G.; Li, D. Overexpression of global regulator PbrlaeA leads to the discovery of new polyketide in fungus *Penicillium brocae* HDN-12-143. *Front. Chem*. **2020**, *8*, 270.
101. Hoff, B.; Kamerewerd, J.; Sigl, C.; Mitterbauer, R.; Zadra, I.; Kürnsteiner, H.; Kück, U. Two components of a *velvet*-like complex control hyphal morphogenesis, conidiophore development, and penicillin biosynthesis in *Penicillium chrysogenum*. *Eukaryot. Cell* **2010**, *9*, 1236–1250.
102. Martin, J.; Garcia-Estrada, C.; Kosalkova, K.; Ullan, R.V.; Albillos, S.M.; Martin, J.-F. The inducers 1,3-diaminopropane and spermidine produce a drastic increase in the expression of the penicillin biosynthetic genes for prolonged time, mediated by the LaeA regulator. *Fungal Genet. Biol*. **2012**, *49*, 1004–1013.
103. Veiga, T.; Nijland, J.G.; Driessen, A.J.M.; Bovenberg, R.A.L.; Touw, H.; Van den Berg, M.A.; Pronk, J.T.; Daran, J.M. Impact of velvet complex on transcriptome and penicillin G production in glucose-limited chemostat cultures of a β-lactam high-producing *Penicillium chrysogenum* strain. *OMICS* **2012**, *16*, 320–333.
104. Xing, W.; Deng, C.; Hu, C.-H. Molecular cloning and characterization of the global regulator LaeA in *Penicillium citrinum*. *Biotechnol. Lett*. **2010**, *32*, 1733–1737.
105. Baba, S.; Kinoshita, H.; Nihira, T. Identification and characterization of *Penicillium citrinum* VeA and LaeA as global regulators for ML-236B production. *Curr. Genet.* **2012**, *58*, 1–11.
106. Zhu, C.; Wang, Y.; Hu, X.; Lei, M.; Wang, M.; Zeng, J.; Li, H.; Liu, Z.; Zhou, T.; Yu, D. Involvement of LaeA in the regulation of conidia production and stress responses in *Penicillium digitatum*. *J. Basic Microbiol*. **2020**, *60*, 82–88.
107. Meng, J.; Wang, X.; Xu, D.; Fu, X.; Zhang, X.; Lai, D.; Zhou, L.; Zhang, G. Sorbicillinoids from fungi and their bioactivities. *Molecules* **2016**, *21*, 715.

108. Hou, X.; Zhang, X.; Xue, M.; Zhao, Z.; Zhang, H.; Xu, D.; Lai, D.; Zhou, L. Recent advances in sorbicillinoids from fungi and their bioactivities (covering 2016–2021). *J. Fungi* **2022**, *8*, 62.
109. Yu, J.; Han, H.; Zhang, X.; Ma, C.; Sun, C.; Che, Q.; Gu, Q.; Zhu, T.; Zhang, G.; Li, D. Discovery of two new sorbicillinoids by overexpression of the global regulator LaeA in a marine-derived fungus *Penicillium dipodomyis* YJ-11. *Mar. Drugs* **2019**, *17*, 446.
110. Kumar, D.; Barad, S.; Chen, Y.; Luo, X.; Tannous, J.; Dubey, A.; Matana, N.G.; Tian, S.; Li, B.; Keller, N.; Prusky, D. LaeA regulation of secondary metabolism modulates virulence in *Penicillium expansum* and is mediated by sucrose. *Mol. Plant Pathol*. **2017**, *18*, 1150–1163.
111. Kumar, D.; Tannous, J.; Sionov, E.; Keller, N.; Prusky, D. Apple intrinsic factors modulating the global regulator, LaeA, the patulin gene cluster and patulin accumulation during fruit colonization by *Penicillium expansum*. *Front. Plant Sci*. **2018**, *9*, 1094.
112. Zhang, X.; Zhu, Y.; Bao, L.; Gao, L.; Yao, G.; Li, Y.; Yang, Z.; Li, Z.; Zhong, Y.; Li, F.; et al. Putative methyltransferase LaeA and transcription factor CreA are necessary for proper asexual development and controlling secondary metabolic gene cluster expression. *Fungal Genet. Biol.* **2016**, *94*, 32–46.
113. Zhang, X.; Yang, Y.; Wang, L.; Qin, Y. Histone H2B lysine 122 and lysine 130, as the putative targets of *Penicillium oxalicum* LaeA, play important roles in asexual development, expression of secondary metabolite gene clusters, and extracellular glycoside hydrolase synthesis. *World J. Microbiol. Biotechnol*. **2024**, *40*, 179.
114. Marcano, Y.; Montanares, M.; Gil-Duran, C.; Gonzalez, K.; Levican, G.; Vaca, I.; Chavez, R. Pr*laeA* affects the production of roquefortine C, mycophenolic acid, and andrastin A in *Penicillium roqueforti*, but it has little impact on asexual development. *J. Fungi* **2023**, *9*, 954.
115. Akhberdi, O.; Zhang, Q.; Wang, D.; Wang, H.; Hao, X.; Liu, Y.; Wei, D.; Zhu, X. Distinct roles of velvet complex in the development, stress tolerance, and secondary metabolism in *Pestalotiopsis microspora*, a taxol producer. *Genes* **2018**, *9*, 164.
116. Zhang, G.; Yan, P.; Leng, D.; Shang, L.; Zhang, C.; Wu, Z.; Wang, Z. Functional roles of LaeA-like genes in fungal growth, cellulase activity, and secondary metabolism in *Pleurotus ostreatus*. *J. Fungi* **2022**, *8*, 902.
117. Yun, C.-S.; Motoyama, T.; Osada, H. Regulatory mechanism of mycotoxin tenuazonic acid production in *Pyricularia oryzae*. *ACS Chem. Biol*. **2017**, *12*, 2270–2274.
118. Ding, Z.; Wang, X.; Kong, F.D.; Huang, H.-M. Zhao, Y.-N.; Liu, M.; Wang, Z.-P.; Han, J. Overexpression of global regulator Talae1 leads to the discovery of new antifungal polyketides from endophytic fungus *Trichoderma afroharzianum*. *Front. Microbiol*. **2020**, *11*, 622785.
119. Karimi-Aghcheh, R.; Bok, J.W.; Phatale, P.A.; Smith, K.M.; Baker, S.E.; Lichius, A.; Omann, M.; Zeilinger, S.; Seiboth, B.; Rhee, C.; et al. Functional analyses of *Trichoderma reesei* LAE1 reveal conserved and contrasting eoles of this regulator. *G3-Genes Benomes Genet.* **2013**, *3*, 369–378.
120. Xiao, M.; Wang, Y.; Wang, Y.; Yan, X.; Zhu, Z.; Tian, E.; Yang, C.; Ma, E.; Zou, G.; Zhou, Z.; Wang, P. Repurposing the cellulase workhorse *Trichoderma reesei* as a ROBUST chassis for efficient terpene production. *Green Chem*. **2024**, *25*, 7362–7371.
121. Feng, Y.; Yin, Z.; Wu, Y.; Xu, L.; Du, H.; Wang, N.; Huang, L. LaeA controls virulence and secondary metabolism in apple canker pathogen *Valsa mali*. *Front. Microbiol*. **2020**, *11*, 581203.
122. Shi, J.-C.; Shi, W.-L.; Zhou, Y.-R.; Chen, X.-L.; Zhang, Y.-Z.; Zhang, X.; Zhang, W.-X.; Song, X.-Y. The putative methyltransferase *TlLAE1* is involved in the regulation of peptaibols production in the biocontrol fungus *Trichoderma longibrachiatum* SMF2. *Front. Microbiol*. **2020**, *11*, 1267.
123. Seiboth, B.; Karimi, R.A.; Phatale, P.A.; Linke, R.; Hartl, L.; Sauer, D.G.; Smith, K.M.; Baker, S.E.; Freitag, M.; Kubicek, C.P. The putative protein methyltransferase LAE1 controls cellulase gene expression in *Trichoderma reesei*. *Mol. Microbiol*. **2012**, *84*, 1150–1164.
124. Rohrig, J.; Yu, Z.; Chae, K.-S.; Kim, J.-H.; Han, K.-H.; Fischer, R. The *Aspergillus nidulans* velvet-interacting protein, VipA, is involved in light-stimulated heme biosynthesis. *Mol. Microbiol*. **2017**, *105*, 825–838.
125. Thieme, K.G.; Gerke, J.; Sasse, C.; Valerius, O.; Thieme, S.; Karimi, R.; Heinrich, A.K.; Finkernagel, F.; Smith, K.; Bode, H.B.; et al. Velvet domain protein VosA represses the zinc cluster transcription factor SclB regulatory network for *Aspergillus nidulans* asexual development, oxidative stress response and secondary metabolism. *PLoS Genet.* **2018**, *14*, e1007511.
126. Yu, W.; Pei, R.; Zhang, Y.; Tu, Y.; He, B. Light regulation of secondary metabolism in fungi. *J. Biol. Eng*. **2023**, *17*, 57.
127. Assaf, C.E.H.; Snini, S.P.; Tadrist, S.; Bailly, S.; Naylies, C.; Oswald, I.P.; Lorber, S.; Puel, O. Impact of *veA* on the development, aggressiveness, dissemination and secondary metabolism of *Penicillium expansum*. *Mol. Plant Pathol*. **2018**, *19*, 1971–1983.
128. Bayram, O.; Krappamann, S.; Ni, M.; Bok, J.W.; Helmstaedt, K.; Valerius, O.; Braus-Stromeyer, S.; Kwon, N.-J.; Keller, N.P.; Yu, J.-H.; Braus, G.H. VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. *Science* **2008**, *320*, 1504–1506.
129. Aleklett, K.; Boddy, L. Fungal behaviour: a new frontier in behavioural ecology. *Trends Ecol Evol*. **2021**, *36*, 787–796.

130. Yu, Z.; Streng, C.; Seibeld, R.F.; Igbalajobi, O.A.; Ingelfinger, J.; Fischer, R. Genome-wide analyses of light-regulated genes in *Aspergillus nidulans* reveal a complex interplay between different photoreceptors and novel photoreceptor functions. *PLoS Genet*. **2021**, *17*, e1009845.
131. Ma, X.; Lu, L.; Zhang, Y.; Fang, M.; Shao, K.; Sun, X.; Yao, F.; Wang, P. Velvet family members regulate pigment synthesis of the fruiting bodies of *Auricularia cornea*. *J. Fungi* **2023**, *9*, 412.
132. Moon, H.; Lee, M.-K.; Bok, I.; Bok, J.W.; Keller, N.P.; Yu, J.-H. Unraveling the gene regulatory networks of the global regulators VeA and LaeA in *Aspergillus nidulans*. *Microbiol. Spectr*. **2023**, *11*, e00166-23.
133. Dreyer, J.; Eichhorn, H.; Friedlin, E.; Kurnsteiner, H.; Kuck, U. A homologue of the *Aspergillus* velvet gene regulates both cephalosporin C biosynthesis and hyphal fragmentation in *Acremonium chrysogenum*. *Appl. Environ. Microbiol*. **2007**, 73, 3412–3422.
134. Wang, L.; Wang, M.; Jiao, J.; Liu, H. Roles of Aa*VeA* on mycotoxin production *via* light in *Alternaria alternata*. *Front. Microbiol*. **2022c**, *13*, 842268.
135. Torres-Palazzolo, C.; Ferreyra, S.; Hugalde, I.P.; Kuhn, Y.; Combina, M.; Ponsone, M.L. Recent advances in biocontrol and other alternative strategies for the management of postharvest decay in table grapes. *Int. J. Food Microbiol*. **2024**, *420*, 110766.
136. Llobregat, B.; Gonzalez-Candelas, L.; Ballester, A. Ochratoxin A defective *Aspergillus carbonarius* mutants as potential biocontrol agents. *Toxins* **2022**, *14*, 745.
137. Duran, R.M.; Cary, J.W.; Calvo, A.M. Production of cyclopiazonic acid, aflatrem, and aflatoxin by *Aspergillus flavus* is regulated by *veA*, a gene necessary for sclerotial formation. *Appl. Microbiol. Biotechnol*. **2007**, *73*, 1158–1168.
138. Cary, J.W.; Harris-Coward, P.Y.; Ehrlich, K.C.; Mavungu, J.D.D.; Malysheva, S.V.; Saeger, S.D.; Dowd, P.F.; Shantappa, S.; Martens, S.L.; Calvo, A.M. Functional characterization of a *veA*-dependent polyketide synthase gene in *Aspergillus flavus* necessary for the synthesis of asparasone, a sclerotium-specific pigment. *Fungal Genet. Biol*. **2014**, *64*, 25–35.
139. Eom, T.-J.; Moon, H.; Yu, J.-H.; Park, H.-S. Characterization of the velvet regulators in *Aspergillus flavus*. *J. Microbiol*. **2018**, *56*, 893–901.
140. Xu, J.; Jiang, M.; Wang, P.; Kong, Q. The gene *vepN* regulated by global regulatory factor *veA* that affects aflatoxin production, morphological development and pathogenicity in *Aspergillus flavus*. *Toxins* **2024**, *16*, 174.
141. Dhingra, S.; Andes, D.; Calvo, A.M. VeA regulates conidiation, gliotoxin production, and protease activity in the opportunistic human pathogen *Aspergillus fumigatus*. *Eukaryot. Cell* **2012**, *11*, 1531–1543.
142. Dhingra, S.; Lind, A.L.; Lin, H.-C.; Tang, Y.; Rokas, A.; Calvo, A.M. The fumagillin gene cluster, an example of hundreds of genes under *veA* control in *Aspergillus fumigatus*. *PLoS One*, **2013**, *8*, e77147.
143. Kato, N.; Brooks, W.; Calvo, A.M. The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by *veA*, a gene required for sexual development. *Eukaryot. Cell* **2003**, *2*, 1178–1186.
144. Sprote, P.; Brakhage, A.A. The light-dependent regulator velvet A of *Aspergillus nidulans* acts as a repressor of the penicillin biosynthesis. *Arch. Microbiol*. **2007**, *188*, 69–79.
145. Bok, J.W.; Soukup, A.A.; Chadwick, E.; Chiang, Y.-M.; Wang C.C.C.; Keller, N.P. VeA and MvlA repression of the cryptic orsellinic acid gene cluster in *Aspergillus nidulans* involves histone 3 acetylation. *Mol. Microbiol*. **2013**, *89*, 963–974.
146. Rauscher, S.; Pacher, S.; Hedtke, M.; Hniemeyer, O.; Fischer, R. phosphorylation code of the *Aspergillus nidulans* global regulator VelvetA (VeA) determines specific functions. *Mol. Microbiol*. **2016**, *99*, 909–924.
147. Ramamoorthy, V.; Dhingra, S.; Kincaid, A.; Shantappa, S.; Feng, X.; Calvo, A.M. The putative C_2H_2 transcription factor MtfA is a novel regulator of secondary metabolism and morphogenesis in *Aspergillus nidulans*. *PLoS One* **2013**, *8*, e74122.
148. Wang, G.; Ran, H.; Fan, J.; Keller, N.P.; Liu, Z.; Wu, F.; Yin, W.-B. Fungal-fungal cocultivation leads to widespread secondary metabolite alteration requiring the partial loss-of-function VeA1 protein. *Sci. Adv*. **2022**, *8*, eabo6094.
149. Zhang, J.; Chen, H.; Sumarah, M.W.; Gao, Q.; Wang, D.; Zhang, Y. *veA* gene acts as a positive regulator of conidia production, ochratoxin A biosynthesis, and oxidative stress tolerance in *Aspergillus niger*. *J. Agric. Food Chem*. **2018**, *66*, 13199–13208.
150. Sano, M. Regulation of kojic acid production in *Aspergillus oryzae*. *JSM Mycotoxins* **2022**, *72*, 39–42.
151. Chanda, A.; Roze, L.V.; Kang, S.; Artymovich, K.A.; Hicks, G.R.; Raikhel, N.V.; Calvo, A.M.; Linz, J.E. A key role for vesicles in fungal secondary metabolism. *Proc. Natl. Acad. Sci. U. S. A*. **2009**, *106*, 19533–19538.
152. Wang, Z.; Zhao, S.; Zhang, K.; Lin, C.; Ru, X.; Yang, Q. *CgVeA*, a light signaling responsive regulator, is involved in regulation of biosynthesis and conidia development in *Chaetomium globosum*. *Synth. Syst. Biotechnol*. **2022**, *7*, 1084–1094.
153. Jiang, J.; Liu, X.; Yin, Y.; Ma, Z. Involvement of a velvet protein FgVeA in the regulation of asexual development, lipid and secondary metabolisms and virulence in *Fusarium graminearum*. *PLoS One* **2011**, *6*, e28291.

154. Merhej, J.; Urban, M.; Dufresne, M.; Hammond-Kosack, K.E.; Richard-Forget, F.; Barreau, C. The velvet gene, *FgVe1*, affects fungal development and positively regulates trichothecene biosynthesis and pathogenicity in *Fusarium graminearum*. *Mol. Plant Pathol*. **2012**, *13*, 363–374.
155. Qin, M.; Li, Y.; Cai, L.; Yin, X.; He, Z.; Kang, J. Overexpression of the global regulator FnVeA upregulates antitumor substances in endophytic *Fusarium nematophilum*. *Can. J. Microbiol*. **2022**, *68*, 531–541.
156. Myung, K.; Li, S.; Butchko, R.A.; Busman, M.; Proctor, R.H.; Abbas. H.K.; Calvo, A.M. *FvVE1* regulates biosynthesis of the mycotoxins fumonisins and fusarins in *Fusarium verticillioides*. *J. Agric. Food Chem*. **2009**, *57*, 5089–509.
157. Myung, K.; Zitomer, N.C.; Duvall, M.; Glenn, A.E.; Riley, R.T.; Calvo, A.M. The conserved global regulator VeA is necessary for symptom production and mycotoxin synthesis in maize seedlings by *Fusarium verticillioides*. *Plant Pathol*. **2012**, *61*, 152–160.
158. Choi, Y.-E.; Goodwin, S.T. *MVE1*, Encoding the velvet gene product homolog in *Mycosphaerella graminicola*, is associated with aerial mycelium formation, melanin biosynthesis, hyphal swelling, and light signaling. *Appl. Environ. Microbiol*. **2011**, *77*, 942–953.
159. Bayram, O.; Krappmann, S.; Seiler, S.; Vogt, N.; Braus, G.H. *Neurospora crassa ve-1* affects asexual conidiation. *Fungal Genet. Biol*. **2008**, 45, 127–138.
160. Bayram, O.S.; Dettmann, A.; Karahoda, B.; Moloney, N.M.; Ormsby, T.; McGowan, J.; Cea-Sanchez, S.; Miralles-Duran, A.; Brancini, G.T.P.; Luque, E.M.; et al. Control of development, secondary metabolism and light-dependent carotenoid biosynthesis by the velvet complex of *Neurospora crassa*. *Genetics* **2019**, *212*, 691–710.
161. Neto, R.N.M.; Gomes, E.D.; Weba-Soares, L.; Dias, L.R.L.; Da Silva, L.C.N.; De Miranda, R.D.M. Biotechnological production of statins: metabolic aspects and genetic approaches. *Curr. Pharm. Biotechnol*. **2019**, *20*, 1244–1259.
162. Cao, S.; Zheng, Y.; Zhang, H.; Huang, Y.; Liao, G.; Hu, C. On investigation of *veA* gene in regulating mevastatin biosynthesis, conidia development in *Penicillium citrinum*. *J. Southwest China Normal Univ*. (*Nat. Sci. Ed*.) **2016**, *41*, 67–73.
163. Luciano-Rosario, D.; Keller, N.P.; Jurick, W.M. *Penicillium expansum*: biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. *Mol. Plant Pathol*. **2020**, *21*, 1389–1526.
164. Li, B.; Chen, Y.; Zong, Y.; Shang, Y.; Zhang, Z.; Xu, X.; Wang, X.; Long, M.; Tian, S. Dissection of patulin biosynthesis, spatial control and regulation mechanism in *Penicillium expansum*. *Environ. Microbiol*. **2019**, *21*, 1124–1139.
165. Llobregat, B.; Gonzalez-Candelas, L.; Ballester, A. Exploring the biocontrol capability of non-mycotoxigenic strains of *Penicillium expansum*. *Toxins* **2024**, *16*, 52.
166. Calvo, A.M.; Bok, J.; Brooks, W.; Keller, N.P. *veA* is required for toxin and sclerotial protudction in *Aspergillus parasiticus*. *Appl. Environ. Microbiol*. **2004**, *70*, 4733–4739.
167. Chang, P.-K.; Scharfenstein, L.L.; Li, P.; Ehrlich, K.C. *Aspergillus flavus* VelB acts distinctly from VeA in conidiation and may coordinate with FluG to modulate sclerotial production. *Fungal Genet. Biol*. **2013**, *58*, 71–79.
168. Zhao, Y.; Lee, M.-K.; Lin, J.; Moon, H.; Park, H.-S.; Zheng, W.; Yu, J.-H. The putative sensor histidine kinase VadJ coordinates development and sterigmatocystin production in *Aspergillus nidulans. J. Microbiol*. **2021**, *59*, 746–752.
169. Yang, Q.; Chen, Y.; Ma, Z. Involvement of BcVeA and BcVelB in regulating conidiation, pigmentation and virulence in *Botrytis cinerea*. *Fungal Genet. Biol*. **2013**, *50*, 63–71.
170. Gao, J.; Zhou, S.; Tang, W.; Wang, J.; Liu, H.; Zhang, Y.; Wang, L.; Li, X.; Liu, Z. The velvet proteins CsVosA and CsVelB coordinate growth, cell wall integrity, sporulation, conidial viability and pathogenicity in the rubber anthracnose fungus *Colletotrichum siamense. Microbiol. Res*. **2023**, *268*, 127290.
171. Gao, J.-X.; Yu, C.-J.; Wang, M.; Sun, J.-N.; Li, Y.-Q.; Chen, J. Involvement of a velvet protein ClVelB in the regulation of vegetative differentiation, oxidative stress response, secondary metabolism, and virulence in *Curvularia lunata*. *Sci. Rep*. **2017**, *7*, 46054.
172. Jiang, J.; Yun, Y.; Ma Z. FgVELB is associated with vegetative differentiation, secondary metabolism and virulence in *Fusarium graminearum*. *Fungal Genet. Biol*. **2012**, 49, 653–662.
173. Lee, J.; Myong, K.; Kim, J.; Kim, H.; Yun, S.; Lee, Y. FgVelB globally regulates sexual reproduction, mycotoxin production and pathogenicity in the cereal pathogen *Fusarium graminearum. Microbiology* **2012**, *158*, 1723–1733.
174. Wu, Y.; Han, S.; Wang, Y.; Li, Q.; Kong, L. Velvet family protein FpVelB affects virulence in association with secondary metabolism in *Fusarium pseudograminearum*. *Cells* **2024**, *13*, 950.
175. Tahtah, N.; Zetina-Serrano, C.; Rocher, O.; Naylies, C.; Lippi, Y.; Khoury, A.E.; Atoui, A.; Jamin, E.L.; Oswald, I.P.; Lorber, S. Implication of VelB in the development, pathogenicity, and secondary metabolism of *Penicillium expansum*. *Postharvest Biol. Technol*. **2023**, *195*, 112121.

 doi:10.20944/preprints202407.1280.v1

176. Kopke, K.; Hoff, B.; Bloemendal, S.; Katschorowski, A.; Kamerewerd, J.; Kuck, U. Members of the *Penicillium chrysogenum* velvet complex play functionally opposing roles in the regulation of penicillin biosynthesis and conidiation. *Eukaryot. Cell* **2013**, *12*, 299–310.
177. Essadik, I.; Boucher, C.; Bobee, C.; Cabet, E.; Gautier, V.; Lalucque, H.; Silar, P.; Chapeland-Leclerc, F.; Ruprich-Robert, G. Mutations in *Podospora anserina MCM1* and *VelC* trigger spontaneous development of barren fruiting bodies. *J. Fungi* **2024**, *10*, 79.
178. Ni, M.; Yu, J.-H. A novel regulator couples sporogenesis and trehalose biogenesis in *Aspergillus nidulans*. *PLoS One* **2007**, *2*, e970.
179. Kim, M.-J.; Lee, M.-K.; Pham, H.Q.; Gu, M.J.; Zhu, B.; Son, S.-H.; Hahn, D.; Shin, J.-H.; Yu, J.-H.; Park, H.-S.; et al. The velvet regulator VosA governs survival and secondary metabolism of sexual spores in *Aspergillus nidulans*. *Genes* **2020**, *11*, 103.
180. Son, Y.-E.; Cho, H.-J.; Chen, W.; Son, S.-H.; Lee, M.-K.; Yu, J.-H.; Park, H.-S. The role of the VosA-repressed *dnjA* gene in development and metabolism in *Aspergillus* species. *Curr. Genet*. **2020**, *66*, 621–633.
181. Lee, M.-K.; Son, Y.-E.; Park, H.-S.; Alshannaq, A.; Han, K.-H.; Yu, J.-H. Velvet activated McrA plays a key role in cellular and metabolic development in *Aspergillus nidulans*. *Sci. Rep*. **2020**, *10*, 15075.
182. Son, Y.-E.; Park, H.-S. Unveiling the functions of the VosA-VelB target gene *vidD* in *Aspergillus nidulans*. *Mycology* **2021**, *49*, 258–266.
183. Wu, M.-Y.; Mead, M.E.; Lee, M.-K.; Neuhaus, G.F.; Adpressa, D.A.; Martien, J.I.; Son, Y.-E.; Moon, H.; Amador-Noguez, D.; Han, K.-H.; et al. Transcriptomic, protein-DNA interaction, and metabolomic studies of VosA, VelB, and WetA in *Aspergillus nidulans* asexual spores. *mBio* **2021**, *12*, e03128-20.
184. Zhao, Y.; Lee, M.-Y.; Lim, J.; Moon, H.; Park, H.-S.; Zheng, W.; Yu, J.-H. The velvet-activated putative C_6 transcription factor VadZ regulates development and sterigmatocystin production in *Aspergillus nidulans*. *Fungal Biol*. **2022**, *126*, 421–428.
185. Lyu, H.-N.; Liu, H.-W.; Keller, N.P.; Yin, W.-B. Harnessing diverse transcriptional regulators for natural product discovery in fungi. *Nat. Prod. Rep*. **2020**, *37*, 6–16.