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NATURAL PRODUCTS - REVIEW

# 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

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**Abstract** *Penicillium chrysogenum* is an excellent model fungus to study the molecular mechanisms of control of expression of secondary metabolite genes. A key global regulator of the biosynthesis of secondary metabolites is the LaeA protein that interacts with other components of the velvet complex (VelA, VelB, VelC, VosA). These components interact with LaeA and regulate expression of penicillin and PR-toxin biosynthetic genes in *P. chrysogenum*. Both LaeA and VelA are positive regulators of the penicillin and PR-toxin biosynthesis, whereas VelB acts as antagonist of the effect of LaeA and VelA. Silencing or deletion of the *laeA* gene has a strong negative effect on penicillin biosynthesis and overexpression of *laeA* increases penicillin production. Expression of the *laeA* gene is enhanced by the *P. chrysogenum* autoinducers 1,3 diaminopropane and spermidine. The PR-toxin gene cluster is very poorly expressed in *P. chrysogenum* under penicillin-production conditions (i.e. it is a near-silent gene cluster). Interestingly, the downregulation of expression of the PR-toxin gene cluster in the high producing strain *P. chrysogenum* DS17690 was associated with mutations in both the *laeA* and *velA* genes. Analysis of the *laeA* and *velA* encoding genes in this high penicillin producing strain revealed that both *laeA* and *velA* acquired important mutations during the strain improvement programs thus altering the ratio of different secondary metabolites (e.g. pigments, PR-toxin) synthesized in the high penicillin producing mutants when compared to the parental wild type strain. Cross-talk of different secondary metabolite pathways has also been found in various *Penicillium* spp.: *P. chrysogenum* mutants lacking the penicillin gene cluster produce increasing amounts of PR-toxin, and mutants of *P. roqueforti* silenced in the PR-toxin genes produce large amounts of mycophenolic acid. The LaeA-velvet complex mediated regulation and the pathway cross-talk phenomenon has great relevance for improving the production of novel secondary metabolites, particularly of those secondary metabolites which are produced in trace amounts encoded by silent or near-silent gene clusters.

**Keywords** LaeA protein · Velvet complex · Filamentous fungi · *Penicillium chrysogenum* · *Aspergillus nidulans* · Global regulators · Secondary metabolites · Penicillin biosynthesis · PR-toxin biosynthesis · Cross-talk · Gene clusters

## Introduction

Filamentous fungi play very important roles in nature [100]. They form part of the ecological systems for degradation of plant and animal decaying material. On the other hand fungi contribute to the maturation processes of cheese and other fermented products for the food industry and play a key role in the pharmaceutical industry as producers of antibiotics, antitumor agents, anticholesterolemics and immunomodulators [24, 66, 101].

An outstanding group of secondary metabolites produced by a few fungal species are the β-lactam antibiotics [1]. In this article we focus our attention on the characterization of the Velvet complex of *Penicillium chrysogenum* and its role on the biosynthesis of penicillin and PR-toxin, two metabolites of this fungus which are known to be regulated by LaeA and other components of the velvet complex.

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*P. chrysogenum* is an excellent model system to study the molecular mechanisms of control of secondary metabolites biosynthesis because of the availability of its complete genome sequence and detailed proteome information [44, 45, 84, 91].

## The penicillin biosynthetic pathway and the penicillin gene cluster

The penicillin biosynthetic pathway starts in the cell cytosol with the non-ribosomal formation of the tripeptide (L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV) from L-α-aminoadipic acid, L-cysteine and L-valine. This first step is catalyzed by the ACV synthetase [99], encoded by the *pcbAB* gene [26, 63]. In the second step the tripeptide LLD-ACV, in which the valine has the D-configuration, is converted to isopenicillin N (IPN) by the IPN synthase [81], encoded by the *pcbC* gene. IPN is the first bioactive β-lactam compound of the pathway. Then, IPN enters the peroxisome [72], presumably by an MFS transporter [30], and the L-α-aminoadipyl side chain is replaced by a hydrophobic side chain in a reaction catalyzed by the IPN acyltransferase (IAT), encoded by the *penDE* gene [2, 4, 65]. The IAT is located inside the peroxisomal matrix [35, 72]. This transacylation reaction requires previous activation of the side chain that is achieved by specific peroxisomal aryl-CoA or acyl-CoA synthetases [54, 57, 94]. In recent years considerable advances have been made on our knowledge of the enzymology and the molecular mechanisms of the four penicillin biosynthetic enzymes [reviewed by 69].

In *P. chrysogenum, Penicillium nalgiovense*, *Aspergillus nidulans* and *Aspergillus oryzae,* three of the genes responsible for penicillin biosynthesis (*pcbAB, pcbC* and *penDE*) are clustered with other ORFs forming an amplifiable 56.8 kb DNA unit [32, 90], which is present in several copies in high penicillin producing strains [31, 32, 38, 90]. Two of the penicillin biosynthetic genes, *pcbAB* and *pcbC*, are transcribed from divergent (bidirectional) promoter regions. The control of expression of genes from promoter regions containing divergent promoters has been associated with reorganization of the heterochromatin structure that allows easier interaction of those divergent promoter regions with the RNA polymerase and the transcriptional factors [34, 43].

The accumulated evidence in several filamentous fungi shows that the regulation of many pathways for secondary metabolites, such as antibiotics, is frequently associated with cellular differentiation processes [15, 98]. Several studies have provided insights of other factors that link production of secondary metabolites and morphological differentiation processes [52].

## Pathway-specific and global regulators

The biosynthesis of penicillin and some other secondary metabolites is regulated by global regulators that integrate signals for response to nitrogen, carbon, and phosphate limitation. In some secondary metabolite gene clusters, there is a specific regulator that controls the adjacent genes, e.g., AflR in the aflatoxin gene cluster in *Aspergillus parasiticus* [19] and *A. nidulans* [29]. However, in many other cases there are no specific cluster-situated regulators and it is unclear how the expression of these clusters is controlled. Furthermore, evidence for regulators located in a cluster that control the expression of a different cluster has been reported recently [9].

Regulation of those clusters of secondary metabolites that lack cluster-situated specific regulators, seems to be mediated by global regulators that influence many genes, such as PacC, NreA, CreaA, CPCR1 and FKH1, among others [21, 27, 28].

Two of these global regulators are LaeA and VelA, commonly designated as core components of the velvet complex. This multicomponent complex was initially identified in *A. nidulans* and *Aspergillus fumigatus*. This protein complex affects heterochromatin rearrangements and, therefore, the study of the velvet complex has great importance for understanding the expression of genes encoding secondary metabolite gene clusters.

## The model velvet complex in *Aspergillus nidulans*

The core of the velvet complex in *A. nidulans* is composed of three subunits VelA (initially name VeA), VelB and LaeA [5]. This complex is involved in the stress signaling cascade and affects production of several secondary metabolites and also participates in the fungal response to light. Of these three components LaeA plays a key role in the regulation of secondary metabolism through heterochromatin reorganization [11]. An even more complex regulation network was established when the multisubunit velvet complex was shown to link the differentiation and secondary metabolism processes [5, 8, 16, 51, 55, 80, 86]. The founding member of this complex was VelA [48]. Under dark conditions, this protein forms a heterotrimeric complex with VelB, and the global regulator of secondary metabolism LaeA [5, 10]. A third velvet protein, VosA, also interacts in the dark with VelB, and it is proposed that the heterodimer VosA-VelB represses asexual spore formation and controls spore maturation and trehalose biosynthesis [6, 74]. Shuffling of VelB between the VelB-VosA dimer and VelA-LaeA complexes is controlled by the LaeA protein, which therefore, plays a key role in the dynamics of complexes regulating secondary

**Table 1** Genes of *P. chrysogenum* encoding LaeA and the core components of the velvet complex

| *P. chrysogenum* genes | Proteins | Similarity to the *A. nidulans* homologues (%) |
|---|---|---|
| Pc16g14010 | LaeA | 75 |
| Pc13g13200 | VelA | 61 |
| Pc22g22320 | VelB | 63 |
| Pc22g11790 | VelC | 58 |
| Pc22g06890 | VosA | 66 |

metabolism and development in *A. nidulans* [6]. In contrast, the function of VelC, the fourth member of the velvet superfamily, is still unclear [7]. The LaeA and other components of the Velvet complex are conserved in different fungi although there are still important differences in the percentage of conserved amino acids (Table 1) [15]. The sequence differences suggest a significant degree of plasticity that was confirmed in heterologous complementation experiments.

## The *P. chrysogenum laeA* gene

The *laeA* gene of *P. chrysogenum* (Pc-*laeA*) was first isolated and characterized by Kosalková et al. [56]. It encodes a protein with 61 % amino acid identity to the orthologous protein of *A. nidulans*. The *laeA* gene is expressed well in *P. chrysogenum* during the growth phase in cultures in complex media under conditions of penicillin production. Transcripts of *laeA* are already present in batch cultures at 24 and 48 h of cultivation. This gene contains an intron (nucleotide positions 390–445) as shown by RT–PCR studies. The deduced LaeA protein sequence contains an S-adenosylmethionine binding site and the protein has been proposed to have methyltransferase activity [40, 56].

The *laeA* gene is present as a single copy both in the wild type *P. chrysogenum* NRRL 1951 strain and in improved penicillin production strains *P. chrysogenum* Wis 54-1255 and ASP-78. Unlike the penicillin gene cluster (*pcbAB-pcbC-penDE*) which is amplified in tandem in a 56.8 kb region in the high production strains [31, 32, 73], the *laeA* gene is located outside of the amplifiable region and is present in an identical restriction fragment in all the strains tested [56], indicating that this chromosomal region has not been reorganized during strain improvement.

Overexpression of the *laeA* gene in *P. chrysogenum* was achieved by locating the *laeA* gene under the control of the *Aspergillus awamori gdh* promoter. Interestingly, overexpression of this gene resulted in a fourfold increase in the transcript level of the *pcbC* and *penDE* genes in the transformed strain (Fig. 1). This led to an increased production of 20 % in penicillin as compared to the parental strain.

Studies on the effect of *laeA* inactivation on pigment production, penicillin biosynthesis and differentiation were performed using a mutant silenced in *laeA*. For this purpose a transformant containing the RNAi interference system carrying an exonic fragment of *laeA* was constructed [89]. Northern analysis revealed that expression of the *laeA* gene in this silenced transformant was drastically reduced. The silenced transformant lacks the typical green pigment of *P. chrysogenum* and also it lacks the brown pigment that is observed on the reverse side of colonies growing on solid medium.

There was a reduction of about 50 % in the sporulation of the silenced transformants indicating that *laeA* has also an important role in the control of asexual differentiation.

The silenced transformant showed a drastically reduced production of penicillin, 59 and 47 % production with respect to the parental strain at 72 and 96 h of cultivation [56]. Interestingly, there was no reduction of the

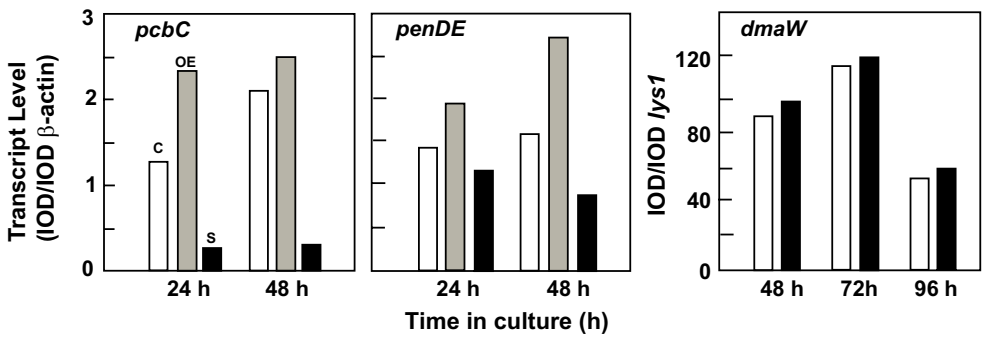


**Fig. 1** Transcription studies in a *laeA* mutant of *P. chrysogenum*. Transcription of the penicillin biosynthesis genes *pcbC* and *penDE* and the roquefortine *dmaW* gene of *P. chrysogenum* in the parental Wis54-1255 strain (*white bars*; control, C), the *laeA* overexpressing strain (*gray bars*, overexpressed OE), and the *laeA* silenced mutant (*black bars*, silenced S). Data are standardized with respect to the expression of the actin gene expression in the case of *pcbC* and *penDE* and in relation to *lys1* in the case of *dmaW* [56]

production of roquefortine C, another metabolite produced in small amounts by *P. chrysogenum* [36]. It has been reported that in *A. nidulans* different secondary metabolites are regulated either positively or negatively by LaeA to a different extent. Therefore, it is possible that the lack of regulation of the roquefortine biosynthesis is due to the low level expression of the roquefortine C gene cluster, probably due to its localization site in the chromosome. Differences in expression have been associated with the localization of the secondary metabolite gene cluster in the genome [77].

The distinct effect of LaeA on penicillin and roquefortine C biosynthesis was confirmed by transcriptional studies using probes internal to the *pcbC* and *penDE* genes that encode two enzymes of the penicillin biosynthesis pathway, and also the *rpt* (synonimus *dmaW*) gene that encodes the dimethylallyltriptophan synthase, the first enzyme of the roquefortine C biosynthesis [36, 67].

The transcriptional studies in the *laeA* silenced mutant showed differences in the effect of LaeA on the early and late genes of the pathway: expression of the *pcbC* gene is drastically reduced, by a factor of fivefold at 48 h, whereas the expression of *penDE* is reduced in the order of twofold (Fig. 1). Indeed, expression of *penDE* occurs from a different promoter regulated by a different set of transcriptional factors [64]. Other secondary metabolites, still not identified chemically, were also regulated by LaeA as shown by HPLC studies. Some of these are regulated in a positive manner, whereas others are down regulated in the *laeA* mutant [56]. Some of those unknown yellow secondary metabolites have been identified recently as members of the sorbecillinoids family [84].

Genes for primary metabolism do not seem to be significantly affected by LaeA. The transcription of *lys1* gene, that encodes homocitrate synthase, the first enzyme of the lysine pathway, was tested in the *laeA* mutant as compared to the parental strain. There was no difference of expression in the *laeA* mutant. A likely explanation is that primary metabolism genes do not require the same transcriptional factors as do the secondary metabolism, which interact with LaeA.

## Effect of *laeA* and *velA* in the high producing strain *P. chrysogenum* P2

Following the work of Kosalková et al. [56] on the *laeA* gene other researchers proceeded with the identification of additional components of *P. chrysogenum* velvet complex. Hoff et al. [40] used in those studies a P2*niaD* strain, derived from the P2 Panlabs strain, which is a high penicillin producing strain with 5–6 copies of the amplified 56.8 kb DNA region (containing the penicillin gene cluster and ancillary biosynthetic genes). For this purpose they cloned the *P. chrysogenum velA* gene by similarity with the orthologous *velA* gene of *A. nidulans* described previously [51]. The *P. chrysogenum velA* is similar to the *A. nidulans* orthologue, but lacks certain domains, for example the NLS (nuclear localization sequence) domain is not clearly present in this particular protein. Mutants disrupted in the *velA* gene were obtained and tested for differences in penicillin production. The *velA* mutant showed a reduced production of penicillin, as occurs also with the *laeA* mutant, indicating that both, VelA and LaeA, are positive regulators of penicillin biosynthesis.

The genes *pcbC* and *penDE* were drastically reduced in their expression in the *velA* mutant [40]. Pairwise comparisons of gene expression in the P2*niaD* parental strain and the *velA* mutant revealed that several genes of primary metabolism were also affected to a different extent; the main differences were observed in genes related to cell wall precursor biosynthesis and differentiation, particularly to spore formation. Regarding secondary metabolism, other than penicillin biosynthesis, there were some genes that were overexpressed in the *velA* mutant while others were downregulated; this means that *velA* has also an important role in regulation of secondary metabolism, either repressing or enhancing gene expression of these secondary metabolism genes.

Hoff and coworkers [40] also studied the role of *laeA* in the P2*niaD* strain. This gene was 100 % identical to that cloned by Kosalková et al. [56] in *P. chrysogenum* Wis54-1255. Mutants in *laeA* were obtained in *P. chrysogenum* P2*niaD* by standard gene replacement procedures. These mutants were totally defective in LaeA, unlike from the silenced mutants of Kosalková et al. [56]; their penicillin production level was drastically reduced from 5 g/l to about 0.5 g/l, i.e., a reduction of 90 % in the penicillin titre. These results confirm the previous observation obtained with the silenced mutant. In conclusion, both *laeA* and *velA* act as positive regulators of penicillin biosynthesis. In addition, complementation of the deleted *laeA* mutant with an intact copy of the wild type restored penicillin production to normal level.

## Effect of VelA on sporulation and differentiation

It is known that *velA* in *A. nidulans* has an effect on sporulation but this effect is distinct in different fungi. In *P. chrysogenum* the comparison of the parental strain with the *velA* mutant revealed that, in the parental strain the conidiation is light-dependent whereas in the *velA* mutant the number of spores is similar in both dark and light conditions. On the other hand the *laeA* mutant of *P. chrysogenum* P2*niaD* shows a very clear reduction in conidiation. Microscopical

observation revealed that the parental strain *P. chrysogenum* P2*niaD* forms chains of spores, whereas the *laeA* mutant forms only one spore in each phialide, which means that the reduction of conidiation affects the formation of conidia but not the number of conidiophores.

## Heterologous complementation studies: LaeA and VelA plasticity

As mentioned above there are significant differences in the amino acid sequences of both the LaeA and the VelA proteins in different fungi. However, complementation of *P. chrysogenum* mutants using the *Fusarium fujikuroi laeA* and *velA* genes, showed that there is a heterologous complementation of the effect of the disruption of these genes on production of penicillin [40].

The conservation of amino acids between *P. chrysogenum* and *F. fujikuroi* LaeA proteins is of 33 % and that of VelA is of 38 %. Heterologous complementation suggests that despite the differences in the amino acid sequences the catalytic sites (e.g. methyltransferase) or, protein-to-protein interacting motifs of these proteins appear to be functional in heterologous multicomponent complexes.

Surprisingly, in contrast to the previous work [40, 56, 62] on the important role of LaeA and VelA, Veiga et al. [93] described in another high producing strain derived from the DSM culture collection that the deletion of *velA* and *laeA* genes does not have too much effect on penicillin production. The reason is that these authors performed their experiments under glucose limited continuos culture conditions, and therefore, these results suggest that the LaeA and VelA effect is greatly influenced by the culture conditions, particularly by glucose limitation. These results point to a possible involvement of the CreA regulator in the LaeA mediated control of gene expression.

## Additional components of the velvet complex

Recently, Kopke et al. [55] described the characterization of three additional components of the velvet complex in *P. chrysogenum*, namely PcVelB, PcVelC, and PcVosA. Using yeast two-hybrid analysis and bimolecular fluorescence complementation these authors demonstrated that all velvet proteins are part of an interacting-protein network.

Studies using single and double disrupted mutants indicate that several velvet subunits have opposing roles in the regulation of penicillin biosynthesis. LaeA was shown to act as a positive regulator, in agreement with the initial observation of Kosalková et al. [56], while PcVelB represses this biosynthetic pathway [55]. In addition, PcVelB and PcVosA promote conidiation, while PcVelC has an inhibitory effect. Further genetic analyses showed that light-dependent spore formation depends not only on PcVelA but also on PcVelB and PcVosA.

## Novel insight in the velvet complex of *P. chrysogenum*

### Effect of diamines on *laeA* expression and penicillin biosynthesis

The biosynthesis of fungal secondary metabolites is frequently induced by plant-derived external elicitors, e.g., alginate in *P. chrysogenum* [37], that form part of an attack/defense cross-talk between plant and fungi. Secondary metabolism appears to be also regulated by endogenous inducers, which may work in a way similar to that of bacterial autoinducers.

Several autoinducer molecules have been studied in relation to the formation of secondary metabolites; jasmonic acid in plants and some basidiomycetes secondary metabolites [78] and conidiogenone in *Penicillium cyclopeum* [82].

A few years ago Jorge Martín and coworkers [62] described that an autoinducer isolated from the culture broth of *P. chrysogenum* and *Acremonium chrysogenum* was able to stimulate the biosynthesis of penicillin. This autoinducer was purified and identified by NMR and mass spectrometry as 1, 3-diaminopropane and the genes for the biosynthesis of 1, 3-diaminopropane were identified in *P. chrysogenum* genome. Further studies showed that 1,3 diaminopropane and spermidine are able to activate the expression of the penicillin biosynthesis genes *pcbC* and *penDE,* and therefore, the effect of these diamines is due to an increase of the transcription of these genes or to the increase of the stability of the messenger RNAs. *In P. chrysogenum* no inducing effect was exerted by γ-butyrolactone, jasmonic acid, or the penicillin precursor δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine.

It is unclear if the effect of the diaminopropane autoinducer is specifically related to the transcription of a single secondary metabolite gene cluster or is a more general phenomenon related to heterochromatin-associated expression of genes for many secondary metabolites.

Diaminopropane induced transcription of the *pcbAB*, *pcbC*, and *penDE* genes and penicillin biosynthesis when added at inoculation time, but its effect was smaller if added at 12 h and it had no effect when added at 24 h, as shown by Northern analysis and *lacZ* reporter quantification of promoter activity. Addition of pure 1, 3-diaminopropane stimulated the production of penicillin by about 100 % as compared to the titres of the control culture [62].

Interestingly, our studies showed a clear control of *laeA* gene expression by the diamines 1,3 diaminopropane and

spermidine [62]; this means that the transcript level of *laeA*, and therefore of the penicillin biosynthetic genes, is under the control of these diamines. Furthermore, the diamines reverse the effect of *laeA* silencing in *P. chrysogenum.* It is not entirely clear how the diamines revert the effect of *laeA* silencing but it is known that the amino groups of 1,3-diaminopropane and other diamines are charged positively and interact with nucleic acids in the cells [83, 92]. Polyamines are known to modulate binding of certain proteins (e.g., estrogen receptors) to Z-DNA-forming regions [88]. This raises the question of whether there are DNA structures similar to Z-DNA in the promoters of some secondary metabolite genes. Polyamines alter the specific DNA–protein interactions, particularly that of heat shock proteins and activator proteins induced by heat shock. Therefore, it is likely that 1, 3-diaminopropane modulates the expression of penicillin biosynthesis genes by interacting with the DNA and other activator proteins in the promoters of the *pcbAB*, *pcbC*, and *penDE* genes.

Control of *laeA* gene expression by other regulators has been described. AflR, a regulator of the $Zn_2$-$Cys_6$ class, located in the sterigmatocystin gene cluster, represses expression of the *laeA* gene and subsequently the biosynthesis of sterigmatocystin, penicillin and lovastatin [10]. In conclusion, certain transcriptional factors and/or autoinducers regulate expression of *laeA* in different fungi and, therefore, subsequentially control secondary metabolites biosynthesis.

## Changes in the Velvet complex during the strain improvement programs

Interestingly, there were some significant differences in the nucleotide sequences of the *velA* and *laeA* genes of the high producing strain of DSM as compared to other *P. chrysogenum* strains, resulting in amino acid differences of the encoded proteins. For example, the absence in the VelA protein of a sequence for protein degradation, and also changes in the S-adenosylmethionine binding site of LaeA. Since the velvet complex is a multisubunit structure, the impact of strain improvement programs on the core component of the Velvet complex were studied comparing the wild type *P. chrysogenum* NRRL1951 with the *P. chrysogenum* orthologs of relevant genes in high producing strains: LreA, LreB, FphA, KapA, VelB, and VosA were all identical in the genomes of strains NRRL1951 (wild type), Wisconsin 54-1255 and the high producing strain DS17690. However, important mutations were found in Pc16g14010 and Pc13g13200 genes that encode LaeA and VelA, respectively [84].

During the strain improvement programs the *laeA* gene acquired a nucleotide substitution at position 850 (T to C) which resulted in a substitution of a lysine for glutamic acid ($Lys^{284}Glu$). A second mutation was introduced during development of the high producing strain DS17690 and caused a $Gly^{338}Ser$ substitution. In addition, a non-sense mutation was found in the *velA* gene; a C to T substitution at nucleotide position 943 formed a stop codon resulting in the formation of a truncated VelA protein (from 562 to 315 amino acids) [84]. These results indicate that the velvet complex has been drastically mutated in the strain improvement programs, and this likely has impacted the expression of several secondary metabolite encoding genes. This explains the lack of yellow `pigment in the penicillin overproducing strain and changes in other secondary metabolites. Surprisingly, when the transcriptomes in *laeA* and *velA* disrupted mutants of *P. chrysogenum* DS17690 were compared with the transcriptome of the reference strain, only 23 genes were differentially transcribed between the reference strain and both *laeA* and *velA* mutants. Eleven of these 23 genes, located in two different clusters, are downregulated in both mutants and appear to encode proteins related to secondary metabolites. One of these set of genes encode proteins of unknown function and the second set, corresponding to genes Pc12g06310 to Pc12g06370, includes a gene for aristolochene synthase [93]. Aristolochene is a precursor of the PR-toxin (see below) and the entire pathway for biosynthesis of the PR-toxin of *P. chrysogenum* has been identified recently [39]. It is important to note that the genes known to be expressed differentially in the *laeA* and *velA* mutants of DS17690 strain, correspond exactly to the PR-toxin cluster (Fig. 2).

## The biosynthesis of PR-toxin in *P. chrysogenum*: expression of a near-silent gene cluster is up-regulated by LaeA and VelA

The PR-toxin is a bicyclic sesquiterpene belonging to the eremophilane terpenoid class [95]. The PR-toxin molecule, containing 17 carbon atoms derives from three molecules of isopentenyldiphosphate and an acetyl group. Four other compounds related to PR-toxin named eremofortines A,B,C and D, were proposed to be intermediates in the biosynthesis of PR-toxin [71] and this was supported by recent evidence on the enzymes encoded by the PR-toxin gene cluster [39].

Aristolochene is a non-oxygenated 15-carbon molecule that is formed by direct cyclization of farnesyl-diphosphate. The aristolochene synthase was isolated from *P. roqueforti* and it was shown to convert in vitro farnesyl-diphosphate to aristolochene [41, 79]. The aristolochene synthase was later purified from *A. terreus* [17]. The *ari*1 gene encoding this enzyme was initially cloned from *P. roqueforti* [17, 18, 79].

Until recently it was unknown whether a PR-toxin gene cluster exists in *P. chrysogenum*, since this fungus does not produce a significant amount of PR-toxin, although might synthesize traces of it [33]. The silencing of the PR-toxin (*prx*) genes decreases PR-toxin biosynthesis in *P. roqueforti* but resulted in a large overproduction of mycophenolic

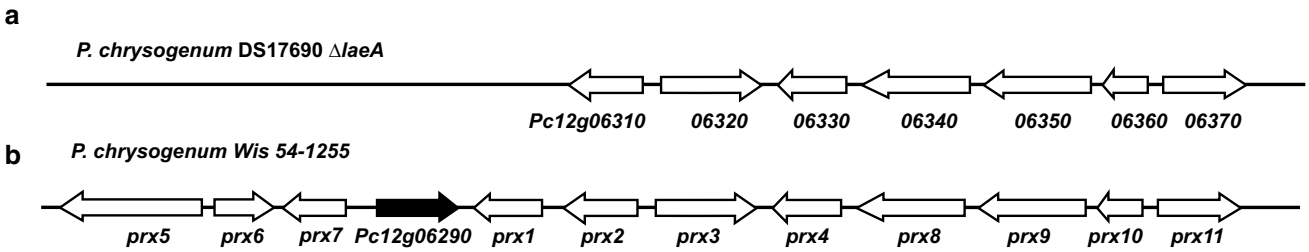


**Fig. 2** PR-toxin genes in *P. chrysogenum* strains. Comparative alignment of: **a** the gene cluster (Pc12g06310 to Pc12g06370) described to be under regulated in the *laeA* knock-out mutant derived from the high producing strain DS17690 [93] and, **b** the PR-toxin gene cluster (*prx1* to *prx11*) of *P. chrysogenum* Wis54-1255 [39], where Pc12g06290 corresponds to a pseudogene. Note that the genes in A and B are the same and corresponds to those of the PR-toxin gene cluster. The *prx2* gene encodes the aristolochene synthase

**Table 2** Examples of secondary metabolites, primary metabolites and biological processes regulated by LaeA and the velvet complex in a variety of filamentous fungi

| Metabolite/process | Fungi | References |
|---|---|---|
| Secondary metabolites | | |
| Penicillin, streigmatocystin<br>Terrequinone<br>Orsellinic acid | *Aspergillus nidulans* | Bok and Keller [10]<br>Bouhired et al. [13]<br>Bok et al. [12] |
| Aflatoxin | *Aspergillus flavus* | Kale et al. [49] |
| Penicillin, PR-toxin | *Penicillium chrysogenum* | Kosalková et al. [56] |
| Mevastatin | *Penicillium citrinum* | Zheng et al.[102] |
| Azaphylones/red rice production | *Monascus pilosus* | Lee et al. [58] |
| Ochratoxin | *Aspergillus carbonarius* | Crespo-Sempere et al. [22] |
| Secondary metabolism | *Fusarium verticilloides* | Butchko et al. [14] |
| Enzymes | | |
| Cellulases | *Trichoderma reesei* | Karimi Aghcheh et al. [50] |
| Endoglucanases | *Aspergillus carbonarius* | Linde et al. [59] |
| Organic acids | | |
| Kojic acid | *Aspergillus oryzae* | Oda et al. [76] |
| Cyclopiazonic acid | *Aspergillus fumisynnematus* | Hong et al. [42] |
| Citric acid | *Aspergillus niger* | Niu et al. [75] |
| Citric acid | *Aspergillus carbonarius* | Linde et al. [59] |
| Pathogenicity/development | | |
| Virulence/fumagillin | *Aspergillus fumigatus* | Sugui et al. [87]<br>Dhingra et al. [25] |
| Pathogenicity | *Aspergillus flavus*<br>*Cladosporium fulvum* | Amaike and Keller [3]<br>Chang et al. [20]<br>Griffiths et al. [46] |
| Differentiation/secondary metabolism | *Botrytis cinerea* | Yang et al. [97]<br>Schumacher et al. [85] |
| Mycotoxins/virulence | *Fusarium oxysporum* | López-Berges et al. [61] |
| Secondary metabolism/virulence | *Fusarium graminearum* | Jiang et al. [47]<br>Kim et al. [53] |
| T-toxin/virulence | *Cochliobolus heterostrophus* | Wu et al. [96] |

acid, an antitumor compound synthesized by a different pathway [23], suggesting a cross-talk of PR-toxin and mycophenolic acid pathways.

The *P. chrysogenum* eleven gene cluster (Pc12g0_6260 to Pc12g0_6370) that includes the above mentioned *prx* genes and a 14-TMS drug/H+ antiporter, has been reported to be very poorly expressed in *P. chrysogenum* under conditions of penicillin production (strongly aerated cultures) [91], i.e., it behaves as a near-silent gene cluster [68]. We found that this near-silent gene cluster is able to produce PR-toxin in *P. chrysogenum* under static culture conditions on hydrated rice medium. Of particular note, the production of PR-toxin was 2.6-fold higher in *P. chrysogenum npe10*, a penicillin nonproducing strain deleted in the 56.8 kb amplifiable

region that includes the *pen* gene cluster, than in the Wisconsin 54-1255 parental strain from which the *npe10* mutant strain derives [39]. Similar results were reported by Harris et al. [38] using a mutant deleted only in the three penicillin biosynthetic genes, derived from the high penicillin producing strain DS17690. To further investigate this cross-talk phenomenon we used a *P. chrysogenum* mutant lacking only the IPN synthase (*pcbC gene*). This mutant does not produce penicillin but again produces increased levels of PR-toxin. These results provide another example of cross-talk between secondary metabolite pathways in this fungus.

Cross-regulation of secondary metabolite biosynthetic pathways is well known in *Streptomyces* [60, 70, 94] but only a few examples have been described in filamentous fungi [9, 68].

The cross-regulation of secondary metabolites opens a new interesting approach to improve biosynthesis of secondary metabolites in filamentous fungi. Deletion or silencing of some specific gene clusters or more specifically of some positive or negative regulatory genes may improve the biosynthesis of other secondary metabolites.

## A wealth of secondary metabolites in different fungi are regulated by LaeA and the velvet complex

**I**n this review we have concentrated in the analysis of the regulation of penicillin and PR-toxin biosynthesis by LaeA and other components of the velvet complex in *P. chrysogenum.* In addition, in the last few years several other filamentous fungi have been studied and the number of fungal metabolites described to be regulated by LaeA has increased greatly (Table 2). These metabolites include secondary metabolites such as mevastatin, cyclopiazonic acid; pigments such as the azaphilones involved in red rice production, primary metabolites such as citric acid, and enzymes such as endoglucanases or cellulases. Complex developmental processes and plant/animal pathogenicity are also regulated by LaeA. These complex differentiation or pathogenicity effects are the result of integration of the regulatory effect of LaeA and the velvet components on different biosynthetic processes.

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## References

1. Aharonowitz Y, Cohen G (1992) Penicillin and cephalosporin biosynthetic genes: structure, organization, regulation, and evolution. Annu Rev Microbiol 46:461–495
2. Álvarez E, Cantoral JM, Barredo JL, Díez B, Martín JF (1987) Purification to homogeneity and characterization of the acyl-CoA: 6-APA acyltransferase of *Penicillium chrysogenum*. Antimicrob Ag Chemother 31:1675–1682
3. Amaike S, Keller NP (2009) Distinct roles for VeA and LaeA in development and pathogenesis of *Aspergillus flavus*. Eukaryot Cell 8:1051–1060
4. Barredo JL, van Solingen P, Díez B, Alvarez E, Cantoral JM, Kattevilder A, Smaal EB, Groenen MAM, Veenstra AE, Martín JF (1989) Cloning and characterization of the acyl–coenzyme A:6– aminopenicillanic–acid–acyltransferase gene of *Penicillium chrysogenum*. Gene 83:291–300
5. Bayram ÖS, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon NJ, Keller NP, Yu JH, Braus GH (2008) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320:1504–1506
6. Bayram ÖS, Valerius O, Park HS, Irniger S, Gerke J, Ni M, Han KH, Yu JH, Braus GH (2010) LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. PLoS Genet 6:e1001226
7. Bayram ÖS, Braus GH (2012) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol Rev 36:1–24
8. Bayram ÖS, Palmer JM, Keller N, Braus GH, Bayram Ö (2015) One Juliet and four Romeos: VeA and its methyltransferases. Front Microbiol 6:1
9. Bergmann S, Funk AN, Scherlach K, Schroeckh V, Shelest E, Horn U, Hertweck C, Brakhage AA (2010) Activation of a silent fungal polyketide biosynthesis pathway through regulatory cross talk with a cryptic nonribosomal peptide synthetase gene cluster. Appl Environ Microbiol 76:8143–8149
10. Bok JW, Keller NP (2004) LaeA, a regulator of secondary metabolism in *Aspergillus* spp. Eukaryot Cell 3:527–535
11. Bok JW, Chiang YM, Szewczyk E, Reyes-Domínguez Y, Davidson AD, Sánchez JF, Lo HC, Watanabe K, Strauss J, Oakley BR, Wang CC, Keller NP (2009) Chromatin-level regulation of biosynthetic gene clusters. Nat Chem Biol 5:462–464
12. Bok JW, Soukup AA, Chadwick E, Chiang YM, Wang CC, Keller NP (2013) VeA and MvlA repression of the cryptic orsellinic acid gene cluster in *Aspergillus nidulans* involves histone 3 acetylation. Mol Microbiol 89:963–974
13. Bouhired S, Weber M, Kempf-Sontag A, Keller NP, Hoffmeister D (2007) Accurate prediction of the *Aspergillus nidulans* terrequinone gene cluster boundaries using the transcriptional regulator LaeA. Fungal Genet Biol 44:1134–1145
14. Butchko RA, Brown DW, Busman M, Tudzynski B, Wiemann P (2012) Lae1 regulates expression of multiple secondary metabolite gene clusters in *Fusarium verticillioides*. Fungal Genet Biol 49:602–612
15. Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459
16. Calvo AM (2008) The VeA regulatory system and its role in morphological and chemical development in fungi. Fungal Genet Biol 45:1053–1061
17. Cane DE, Kang I (2000) Aristolochene synthase: purification, molecular cloning, high-level expression in *Escherichia coli*, and characterization of the *Aspergillus terreus* cyclase. Arch Biochem Biophys 376:354–364
18. Caruthers JM, Kang I, Rynkiewicz MJ, Cane DE, Christianson DW (2000) Crystal structure determination of aristolochene synthase from the blue cheese mold, *Penicillium roqueforti*. J Biol Chem 275:25533–25539

19. Chang PK, Skory CD, Linz JE (1992) Cloning of a gene associated with aflatoxin B1 biosynthesis in *Aspergillus parasiticus*. Curr Genet 21:231–233
20. Chang PK, Scharfenstein LL, Ehrlich KC, Wei Q, Bhatnagar D, Ingber BF (2012) Effects of laeA deletion on *Aspergillus flavus* conidial development and hydrophobicity may contribute to loss of aflatoxin production. Fungal Biol 116:298–307
21. Cepeda-García C, Domínguez-Santos R, García-Rico RO, García-Estrada C, Cajiao A, Fierro F, Martín JF (2014) Direct involvement of the CreA transcription factor in penicillin biosynthesis and expression of the *pcbAB* gene in *Penicillium chrysogenum*. Appl Microbiol Biotechnol 98:7113–7124
22. Crespo-Sempere A, Marín S, Sanchis V, Ramos AJ (2013) VeA and LaeA transcriptional factors regulate ochratoxin A biosynthesis in *Aspergillus carbonarius*. Int J Food Microbiol 166:479–486
23. Del Cid A, Gil-Durán C, Vaca I, Rojas-Aedo JF, García-Rico RO, Levicán G, Chávez R (2016) Identification and Functional Analysis of the Mycophenolic Acid Gene Cluster of *Penicillium roqueforti*. PLoS One 11:e0147047
24. Demain AL (2014) Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biotechnol 41:185–201
25. Dhingra S, Lind AL, Lin HC, Tang Y, Rokas A, Calvo AM (2013) The fumagillin gene cluster, an example of hundreds of genes under veA control in *Aspergillus fumigatus*. PLoS One 8:e77147
26. Díez B, Gutiérrez S, Barredo JL, van Solingen P, van der Voort Lucia HM, Martín JF (1990) The cluster of penicillin biosynthetic genes. J Biol Chem 265:16358–16365
27. Domínguez-Santos R, Martín JF, Kosalková K, Prieto C, Ullán RV, García-Estrada C (2012) The regulatory factor PcRFX1 controls the expression of the three genes of β-lactam biosynthesis in *Penicillium chrysogenum*. Fungal Genet Biol 49:866–881
28. Domínguez-Santos R, García-Estrada C, Kosalkova K, Prieto C, Santamarta I, Martín JF (2015) PcFKH1, a novel regulatory factor from the forkhead family, controls the biosynthesis of penicillin in *Penicillium chrysogenum*. Biochimie 115:162–176
29. Fernandes M, Keller NP, Adams TH (1998) Sequence-specific binding by *Aspergillus nidulans* AflR, a C6 zinc cluster protein regulating mycotoxin biosynthesis. Mol Microbiol 28:1355–1365
30. Fernández-Aguado M, Martín JF, Rodríguez-Castro R, García-Estrada C, Albillos SM, Teijeira F, Ullán RV (2014) New insights into the isopenicillin N transport in *Penicillium chrysogenum*. Metab Eng 22:89–103
31. Fierro F, Barredo JL, Díez B, Gutiérrez S, Fernández FJ, Martín JF (1995) The penicillin gene cluster is amplified in tandem repeats linked by conserved hexanucleotide sequences. Proc Natl Acad Sci USA 92:6200–6204
32. Fierro F, García-Estrada C, Castillo NI, Rodríguez R, Velasco-Conde T, Martín JF (2006) Transcriptional and bioinformatic analysis of the 56.8 kb DNA region amplified in tandem repeats containing the penicillin gene cluster in *Penicillium chrysogenum*. Fungal Genet Biol 43:618–629
33. Frisvad F, Smedsgaard J, Larsen T, Samson R (2004) Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Study Mycol 49:201–242
34. García I, González R, Gómez D, Scazzocchio C (2004) Chromatin rearrangements in the *prnD-prnB* bidirectional promoter: dependence on transcription factors. Eukaryot Cell 3:144–156
35. García-Estrada C, Vaca I, Fierro F, Sjollema K, Veenhuis M, Martín JF (2008) The unprocessed preprotein form IATC103S of the isopenicillin N acyltransferase is transported inside peroxisomes and regulates its self-processing. Fungal Genet Biol 45:1043–1052
36. García-Estrada C, Ullán RV, Albillos SM, Fernández-Bodega MÁ, Durek P, von Döhren H, Martín JF (2011) A single cluster of coregulated genes encodes the biosynthesis of the mycotoxins roquefortine C and meleagrin in *Penicillium chrysogenum*. Chem Biol 18:1499–1512
37. Gang L, Casqueiro J, Gutiérrez S, Kosalková K, Castillo N-I, Martín JF (2001) Elicitation of penicillin biosynthesis by alginate in *Penicillium chrysogenum* exerted on *pcb*AB, *pcb*C and *pen*DE genes at transcriptional level. J Microbiol Biotechnol 11:812–818
38. Harris DM, van der Krogt ZA, Klaasen P, Raamsdonk LM, Hage S, van den Berg MA, Bovenberg RAL, Pronk JT, Daran JM (2009) Exploring and dissecting genome-wide gene expression responses of *Penicillium chrysogenum* to phenylacetic acid consumption and penicillin G production. BMC Genom 10:75
39. Hidalgo PI, Ullán RV, Albillos SM, Montero O, Fernández-Bodega MÁ, García-Estrada C, Fernández-Aguado M, Martín JF (2014) Molecular characterization of the PR-toxin gene cluster in *Penicillium roqueforti* and *Penicillium chrysogenum*: cross talk of secondary metabolite pathways. Fungal Gen Biol 62:11–24
40. Hoff B, Kamerewerd J, Sigl C, Mitterbauer R, Zadra I, Kurnsteiner H, Kück U (2010) Two components of a *velvet*-Like complex control hyphal morphogenesis, conidiophore development, and penicillin biosynthesis in *Penicillium chrysogenum*. Eukaryot Cell 9:1236–1250
41. Hohn TM, Plattner RD (1989) Purification and characterization of the sesquiterpene cyclase aristolochene synthase from *Penicillium roqueforti*. Arch Biochem Biophys 272:137–143
42. Hong EJ, Kim NK, Lee D, Kim WG, Lee I (2015) Overexpression of the laeA gene leads to increased production of cyclopiazonic acid in *Aspergillus fumisynnematus*. Fungal Biol 119:973–983
43. Ishida C, Aranda C, Valenzuela L, Riego L, Deluna A, Recillas-Targa F, Filetici P, López-Revilla R, González A (2006) The UGA3eGLT1 intergenic region constitutes a promoter whose bidirectional nature is determined by chromatin organization in *Saccharomyces cerevisiae*. Mol Microbiol 59:1790–1806
44. Jami MS, Barreiro C, García-Estrada C, Martín JF (2010) Proteome analysis of the penicillin producer *Penicillium chrysogenum*: characterization of protein changes during the industrial strain improvement. Mol Cell Proteom 9:1182–1198
45. Jami MS, García-Estrada C, Barreiro C, Cuadrado AA, Salehi-Najafabadi Z, Martín JF (2010) The *Penicillium chrysogenum* extracellular proteome. Conversion from a food-rotting strain to a versatile cell factory for white biotechnology. Mol Cell Proteom 9:2729–2744
46. Griffiths S, Saccomanno B, de Wit PJ, Collemare J (2015) Regulation of secondary metabolite production in the fungal tomato pathogen *Cladosporium fulvum*. Fungal Genet Biol 84:52–61
47. Jiang J, Yun Y, Liu Y, Ma Z (2012) FgVELB is associated with vegetative differentiation, secondary metabolism and virulence in *Fusarium graminearum*. Fungal Genet Biol 49:653–662
48. Käfer E (1965) Origins of translocations in *Aspergillus nidulans*. Genetics 52:217–232
49. Kale SP, Milde L, Trapp MK, Frisvad JC, Keller NP, Bok JW (2008) Requirement of LaeA for secondary metabolism and sclerotial production in *Aspergillus flavus*. Fungal Genet Biol 45:1422–1429
50. Karimi Aghcheh R, Németh Z, Atanasova L, Fekete E, Paholcsek M, Sándor E, Aquino B, Druzhinina IS, Karaffa L, Kubicek CP (2014) *The VELVET a orthologue VEL1 of Trichoderma reesei* regulates fungal development and is essential for cellulase gene expression. PLoS One 9(11):e112799
51. Kato N, Brooks W, Calvo AM (2003) The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is

controlled by *veA*, a gene required for sexual development. Eukaryot Cell 2:1178–1186
52. Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3:937–947
53. Kim HK, Lee S, Jo SM, McCormick SP, Butchko RA, Proctor RH, Yun SH (2013) Functional roles of FgLaeA in controlling secondary metabolism, sexual development, and virulence in *Fusarium graminearum*. PLoS One 8(7):e68441
54. Koetsier MJ, Jekel PA, van den Berg MA, Bovenberg RA, Janssen DB (2009) Characterization of a phenylacetate-CoA ligase from *Penicillium chrysogenum*. Biochem J 417:467–476
55. Kopke K, Hoff B, Bloemendal S, Katschorowski A, Kamerewerd J, Kück U (2013) Members of the velvet complex play functionally opposing roles in the regulation of penicillin biosynthesis an Conidiation. Eukaryotic Cells 12:299–310
56. Kosalková K, García-Estrada C, Ullán RV, Godio RP, Feltrer R, Teijeira F, Mauriz E, Martín JF (2009) The global regulator LaeA controls penicillin biosynthesis, pigmentation and sporulation, but not roquefortine C synthesis in *Penicillium chrysogenum*. Biochimie 91:214–225
57. Lamas-Maceiras M, Vaca I, Rodríguez E, Casqueiro J, Martín JF (2006) Amplification and disruption of the phenylacetyl-CoA ligase gene of *Penicillium chrysogenum* encoding an aryl-capping enzyme that supplies phenylacetic acid to the isopenicillin N acyltransferase. Biochem J 395:147–155
58. Lee SS, Lee JH, Lee I (2013) Strain improvement by overexpression of the laeA gene in *Monascus pilosus* for the production of monascus-fermented rice. J Microbiol Biotechnol 23(7):959–965
59. Linde T, Zoglowek M, Lübeck M, Frisvad JC, Lübeck PS (2016) The global regulator LaeA controls production of citric acid and endoglucanases in *Aspergillus carbonarius*. J Ind Microbiol Biotechnol 43:1139–1147
60. Liu G, Chater KF, Chandra G, Niu G, Tan H (2013) Molecular regulation of antibiotic biosynthesis in streptomyces. Microbiol Mol Biol Rev 77:112–143
61. López-Berges MS, Hera C, Sulyok M, Schäfer K, Capilla J, Guarro J, Di Pietro A (2013) The velvet complex governs mycotoxin production and virulence of *Fusarium oxysporum* on plant and mammalian hosts. Mol Microbiol 87:49–65
62. Martín J, García-Estrada C, Rumbero A, Recio E, Albillos SM, Ullán RV, Martín JF (2011) Characterization of an autoinducer of penicillin biosynthesis in *Penicillium chrysogenum*. Appl Environ Microbiol 77(16):5688–5696
63. Martín JF (2000) α-Aminoadipyl-cysteinyl-valine synthetases in β-lactam producing organisms. From Abraham's discoveries to novel concepts of non-ribosomal peptide synthesis. J Antibiot 53:1008–1021
64. Martín JF (2000) Molecular control of expression of penicillin biosynthesis genes in fungi: regulatory proteins interact with a bidirectional promoter region. J Bacteriol 182:2355–2362
65. Martín JF, Ingolia TD, Queener SW (1990) Molecular genetics of penicillin and cephalosporin antibiotic biosynthesis. In: Leong SA, Berka R (eds) Molecular industrial mycology. Marcel Dekker, New York, pp 149–195
66. Martín JF, García-Estrada C, Zeilinger S (eds) (2014) Biosynthesis and molecular genetics of fungal secondary metabolites. Springer, New York
67. Martín JF, Liras P, García-Estrada C (2014) Roquefortine and prenylated indole alkaloids. In: Martín JF, García-Estrada C, Zeilinger S (eds) Biosynthesis and molecular genetics of fungal secondary metabolites. Springer, New York, pp 111–128
68. Martín JF, Liras P (2015) Novel antimicrobial and other bioactive metabolites obtained from silent gene clusters. In: Sanchez S, Demain AL (eds) Antibiotics, current innovations and future trends. Caister Academic Press, Norfolk
69. Martín JF, Liras P (2016) Insights into the structure and molecular mechanisms of β-lactam synthesizing enzymes in fungi. In: Brahmachari G, Demain AL, Adrio JL (eds) Biotechnology of microbial enzymes. Elsevier, NewYork, pp 215–241
70. Martínez-Burgo Y, Álvarez-Álvarez R, Rodríguez-García A, Liras P (2015) The pathway-specific regulator ClaR of *Streptomyces clavuligerus* has a global effect on the expression of genes for secondary metabolism and differentiation. Appl Environ Microbiol 81:6637–6648
71. Moreau S, Lablache-Combier A, Biguet J (1980) Production of eremofortins A, B, and C relative to formation of PR toxin by *Penicillium roqueforti*. Appl Environ Microbiol 39:770–776
72. Müller WH, van der Krift TP, Krouwer AJ, Wosten HA, van der Voort LH, Smaal EB, Verkleij AJ (1991) Localization of the pathway of the penicillin biosynthesis in *Penicillium chrysogenum*. EMBO J 10:489–495
73. Newbert RW, Barton B, Greaves P, Harper J, Turner G (1997) Analysis of a commercially improved *Penicillium chrysogenum* strain series: involvement of recombinogenic regions in amplification and deletion of the penicillin biosynthesis gene cluster. J Ind Microbiol Biotechnol 19:18–27
74. Ni M, Yu JH (2007) A novel regulator couples sporogenesis and trehalose biogenesis in *Aspergillus nidulans*. PLoS One 2:e970
75. Niu J, Arentshorst M, Nair PD, Dai Z, Baker SE, Frisvad JC, Nielsen KF, Punt PJ, Ram AF (2015) Identification 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 (Bethesda) 6:193–204
76. Oda K, Kobayashi A, Ohashi S, Sano M (2011) *Aspergillus oryzae laeA* regulates kojic acid synthesis genes. Biosci Biotechnol Biochem 75:1832–1834
77. Palmer JM, Keller NP (2010) Secondary metabolism in fungi: does chromosomal location matter? Curr Opin Microbiol 13:431–436
78. Plett JM, Daguerre Y, Wittulsky S, Vayssières A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C, Martin F (2014) Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc Natl Acad Sci USA 111:8299–82304
79. Proctor RH, Hohn TM (1993) Aristolochene synthase. Isolation, characterization, and bacterial expression of a sesquiterpenoid biosynthetic gene (Ari1) from *Penicillium roqueforti*. J Biol Chem 268:4543–4548
80. Purschwitz J, Müller S, Fischer R (2009) Mapping the interaction sites of *Aspergillus nidulans* phytochrome FphA with the global regulator VeA and the White Collar protein LreB. Mol Genet Genom 281:35–42
81. Ramos FR, López-Nieto MJ, Martín JF (1985) Isopenicillin N synthetase of *Penicillium chrysogenum*, an enzyme that converts delta-(L-α-aminoadipyl)-L-cysteinyl-D-valine to isopenicillin N. Antimicrob Ag Chemother 27:380–387
82. Roncal T, Cordobés S, Sterner O, Ugalde U (2002) Conidiation in *Penicillium cyclopium* is induced by conidiogenone, an endogenous diterpene. Eukaryot Cell 1:823–829
83. Ruiz Herrera J (1994) Polyamines. DNA methylation and fungal differentiation. Crit Rev Microbiol 20:143–150
84. Salo OV, Ries M, Medema MH, Lankhorst PP, Vreeken RJ, Bovenberg RA, Driessen AJ (2015) Genomic mutational analysis of the impact of the classical strain improvement program on β-lactam producing *Penicillium chrysogenum*. BMC Genom 16:937

85. Schumacher J, Simon A, Cohrs KC, Traeger S, Porquier A, Dalmais B, Viaud M, Tudzynski B (2015) The VELVET Complex in the Gray Mold Fungus *Botrytis cinerea*: impact of BcLAE1 on differentiation, secondary metabolism, and virulence. Mol Plant Microbe Interact 28:659–674
86. Spröte P, Brakhage AA (2007) The light-dependent regulator velvet A of *Aspergillus nidulans* acts as a repressor of the penicillin biosynthesis. Arch Microbiol 188:69–79
87. Sugui JA, Pardo J, Chang YC, Müllbacher A, Zarember KA, Galvez EM, Brinster L, Zerfas P, Gallin JI, Simon MM, Kwon-Chung KJ (2007) Role of laeA in the regulation of *alb1, gliP,* conidial morphology, and virulence in *Aspergillus fumigatus*. Eukaryot Cell 6:1552–1561
88. Thomas T, Thomas TJ (1993) Structural specificity of polyamines in modulating the binding of estrogen receptor to potential Z-DNA forming sequences. J Recept Res 13:1115–1133
89. Ullan RV, Godio RP, Teijeira F, Vaca I, García-Estrada C, Feltrer R, Kosalková K, Martín JF (2008) RNA-silencing in *Penicillium chrysogenum* and *Acremonium chrysogenum*: validation studies using β-lactam genes expression. J Microbiol Meth 75:209–218
90. van den Berg MA, Westerlaken I, Leeflang C, Kerkman R, Bovenberg RA (2007) Functional characterization of the penicillin biosynthetic gene cluster of *Penicillium chrysogenum* Wisconsin 54–1255. Fungal Genet Biol 44:830–844
91. van den Berg MA, Albang R, Albermann K, Badger JH, Daran JM, Driessen AJ, García-Estrada C, Fedorova ND, Harris DM, Heijne WH, Joardar V, Kiel JA, Kovalchuk A, Martín JF, Nierman WC, Nijland JG, Pronk JT, Roubos JA, van der Klei IJ, van Peij NN, Veenhuis M, von Döhren H, Wagner C, Wortman J, Bovenberg RA (2008) Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. Nat Biotechnol 26:1161–1168
92. van Dam L, Korolev N, Nordenskio L (2002) Polyamine-nucleic acid interactions and the effects on structure in oriented DNA fibers. Nucleic Acids Res 30:419–428
93. Veiga T, Nijland JG, Driessen AJM, Bovenberg RAL, Touw H, van den Berg MA, Pronk JT, Daran JM (2012) Impact of velvet complex on transcriptome and penicillin G production in glucose-limited chemostat cultures of a b-lactam high-producing *Penicillium chrysogenum* strain. OMICS 16:320–333
94. Wang L, Tian X, Wang J, Yang H, Fan K, Xu G, Yang K, Tan H (2009) Autoregulation of antibiotic biosynthesis by binding of the end product to an atypical response regulator. Proc Natl Acad Sci USA 106:8617–8622
95. Wei RD, Schnoes HK, Hart PA, Strong FM (1975) The structure of PR-toxin, a mycotoxin from *Penicillium roqueforti*. Tetrahedron 31:109–114
96. Wu D, Oide S, Zhang N, Choi MY, Turgeon BG (2012) ChLae1 and ChVel1 regulate T-toxin production, virulence, oxidative stress response, and development of the maize pathogen *Cochliobolus heterostrophus*. PLoS Pathogol 8:e1002542
97. Yang Q, Chen Y, Ma Z (2013) Involvement of BcVeA and BcVelB in regulating conidiation, pigmentation and virulence in *Botrytis cinerea*. Fungal Genet Biol 50:63–71
98. Yu JH, Keller N (2005) Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 43:437–458
99. Zhang J, Demain AL (1992) ACV synthetase. Crit Rev Biotechnol 12:245–260
100. Zeilinger S, Martín JF, García-Estrada C (2015) Fungal secondary metabolites in the OMICS Era. In: Zeilinger S, Martín JF, García-Estrada C (eds) Biosynthesis and molecular, vol II. Springer, New York
101. Zeilinger S, Martín JF, García-Estrada C (eds) (2015) Biosynthesis and molecular genetics of fungal secondary metabolites, vol II. Springer, New York
102. Zheng Y, Cao S, Huang Y, Liao G, Hu C (2014) Overexpression of LaeA enhances mevastatin production and reduces sporulation of *Penicillium citrinum*. Wei Sheng Wu Xue Bao 54:1438–1445