The Journal of Antibiotics (2019) 72:785–792
https://doi.org/10.1038/s41429-019-0228-2

JARA Japan Antibiotics Research Association

The Society for Actinomycetes Japan

REVIEW ARTICLE

Albicidin, a potent DNA gyrase inhibitor with clinical potential

Saeed Mujahid Hashimi [1]

Received: 4 April 2019 / Revised: 23 July 2019 / Accepted: 14 August 2019 / Published online: 26 August 2019
© The Author(s), under exclusive licence to the Japan Antibiotics Research Association 2019

Abstract

The emergence of multiple antibiotic-resistant bacteria is a serious global problem which requires the development of new effective antimicrobial therapeutics. Albicidin produced by the sugarcane pathogen *Xanthomonas albilineans* is a potent DNA gyrase inhibitor with inhibitory effects significantly better than most DNA gyrase inhibitors. Albicidin acts primarily by inhibiting the religation of the cleaved DNA intermediate during the gyrase catalytic sequence similar to quinolones. The clinical realization of albicidin has been hampered by limited production and its unsolved structure. In this review, the relationship between albicidin and sugarcane leaf-scald disease is described. Furthermore, the biosynthesis and resistance mechanisms of albicidin are discussed. Finally, recent efforts to solve the structure and produce albicidin in a heterologous host and chemically are summarized.

Introduction

Serious infections caused by antibiotic-resistant bacteria have become a major global problem, indicating the need for further innovation in antimicrobial research and development to provide the next generation of antimicrobial drugs. The biosynthesis of secondary metabolites requires multi-step enzymatic pathways starting with intermediates of primary metabolites as precursors [1]. Genes involved in the biosynthesis of antibiotics and other secondary metabolites have been cloned and characterized from a wide variety of organisms in recent decades, revealing some of their complex genetic organization and biosynthetic mechanisms [1–3].

Toxins produced by phytopathogenic bacteria increase the severity of disease in plants. Several of the phytotoxins from pseudomonads also possess antibacterial activity [4, 5]. In many cases, the structure of toxins, the nature of intermediates in their biosynthesis and their mode of action in plant diseases are known.

Albicidin phytotoxins produced by *Xanthomonas albilineans* are key pathogenicity factors in the development of leaf scald, one of the most devastating diseases of sugarcane (*Saccharum* interspecific hybrids) [6–9]. Furthermore, albicidin inhibits the in vitro supercoiling activity of *Escherichia coli* DNA gyrase, with IC_{50} (40–50 nM) below most coumarins and quinolones by blocking the religation of the cleaved DNA intermediate during the gyrase catalytic sequence and inhibits the relaxation of supercoiled DNA by gyrase and topoisomerase IV [10]. Recently, a gene cluster spanning more than 50 kb in the genome of *X. albilineans* associated with albicidin production has been cloned [11–15]. Most importantly the chemical structure for albicidin has been solved and de nova synthesis has been achieved.

In this review, the relationship between albicidin and sugarcane leaf-scald disease will first be described. Structure and biosynthesis of albicidin will be discussed. Mechanisms of albicidin action and resistance will then be summarized.

Albicidin and sugarcane leaf-scald disease

Sugarcane leaf-scald disease

Leaf-scald disease is a major disease of sugarcane (*Saccharum* spp. hybrids), with the potential to cause severe losses of cane yield and quality in susceptible cultivars. It is a vascular disease caused by the gram-negative bacterium *X. albilineans* [9, 16]. Leaf-scald disease has been reported

✉ Saeed Mujahid Hashimi
smhashimi@iau.edu.sa

[1] Department of Basic Science, Biology Unit, Deanship of Preparatory Year and Supporting Studies, and Department of Stem Cell Research, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 34212, Saudi Arabia

in more than 50 countries [16] and continues to spread into new areas [17–19]. Symptoms including the emergence of chlorotic leaves, wilting, necrosis and sometimes rapid death of plants, often appear after a prolonged latent period [20]. In its latent period, the pathogen can remain dormant until environmental conditions are favorable for symptom expression. However, when the disease enters its acute phase, entire blocks of apparently healthy cane can die off and become completely unharvestable over a period of only a few weeks. The pathogen is mechanically transmitted during harvesting, and can also spread naturally, for example by wind-blown exudates under cyclonic conditions [16]. *X. albilineans* infects several grasses other than sugarcane. Control of the disease is based on the use of resistant varieties as this reservoir of the pathogen in weed grasses makes eradication virtually impossible [16].

Electronic microscopic examination of the structure of infected leaves revealed that the pathogen is confined to the xylem vessels or adjacent intercellular spaces during the early stages of disease development [21]. Mature chloroplasts were absent from white leaf tissue and the plastids in these tissues were proplastids, etioplasts, and vesicular forms smaller than chloroplasts [21]. Birch and Patil [7] suggest that the characteristic white pencil lines and chlorosis of emerging leaves are due to the production by *X. albilineans* of a diffusible phytotoxin which selectively blocks chloroplast differentiation.

Albicidin antibiotics and phytotoxins

Chlorosis inducing isolates of *X. albilineans* produce a family of potent antibiotics in culture. The major antimicrobial component, called albicidin, is a low molecular weight compound with several aromatic rings [7]. The compound is soluble in polar organic solvents and partially soluble in water. Albicidin is rapidly bactericidal at nanomolar concentrations against a wide range of gram-positive and gram-negative bacteria (Table 1), but it shows no cytotoxicity to cultured mammalian cells at 8 $\mu g\ ml^{-1}$ [22]. Albicidins are therefore of great interest as potential clinical antibacterial drugs. This clinical interest is further heightened by the recent discovery that malaria and toxoplasma parasites contain vestigial plastids that are essential for their survival [21].

Albicidin-deficient (Tox$^-$) mutants of *X. albilineans* fail to cause chlorosis or any other symptoms of leaf-scald disease in inoculated sugarcane [6, 8]. Transgenic sugarcanes that express the albicidin detoxification enzyme (AlbD) block systemic disease [9, 23]. These results indicate that albicidin phytotoxins are responsible for the characteristic chlorotic symptoms in *X. albilineans* infected sugarcane and that they play an overall role in systemic disease development by weakening host defenses [23].

Table 1 Activity of albicidin against gram-positive and gram-negative bacteria

Organism	Albicidin MIC/IC_{50}	References
E. coli	0.031 $\mu g\ ml^{-1}$	[63]
E. coli DNA gyrase	50 nM	[10]
S. enteritidis	0.5 $\mu g\ ml^{-1}$	[63]
P. aeruginosa	1 $\mu g\ ml^{-1}$	[63]
S. aureus	16 $\mu g\ ml^{-1}$	[63]
M. luteus	1 $\mu g\ ml^{-1}$	[63]
B. subtilis DSM10	297 nM	[64]
S. typhimurium TA100	37 nM	[64]

Effect of albicidin on chloroplasts

It was postulated that albicidin might cause the blocked chloroplast differentiation, in diseased plants, as chloroplasts exhibit prokaryotic-like mechanisms of DNA replication [6]. Furthermore, it has been shown that plant chloroplasts have functional DNA gyrase that shows sensitivity to quinolones and as such, this is the most likely target of albicidin [24, 25]. Albicidin mutants and revertants showed a correlation between albicidin production and ability to cause disease [6]. An albicidin resistant bacterial strain of *Pantoea dispersa* (SB1403), showed a strong capacity for enzymatic detoxification of albicidin [26]. Susceptible sugarcane plants co-inoculated with *P. dispersa* showed a 98% reduction in the frequency of white pencil lines, even with a tenfold excess of *X. albilineans* inoculum. Also, the pathogen could not be reisolated and mutants of *P. dispersa* that failed to produce the detoxification enzyme were less effective in biocontrol [20, 26].

Albicidin is a potent inhibitor of DNA gyrase

Early studies suggested that albicidins block DNA replication in bacteria and sugarcane proplastids and inhibit replication of bacteriophage T4 and T7 [6, 7, 22]. The primary mode of action was shown to be a rapid and complete block of DNA synthesis. Albicidin also resulted in partial inhibition of RNA and protein synthesis, but this probably reflects decreasing cell viability [22]. Furthermore, albicidin did not appear to bind directly to DNA, as no change in the absorption spectra is observed on mixing albicidin with *E. coli* DNA [22]. The kinetics of DNA replication inhibition by albicidin closely resemble the effects of inhibition of DNA gyrase by the coumarin and quinolone antibiotics [22, 27, 28]. This selective inhibition of DNA synthesis without binding suggested a specific interaction of albicidin with an essential DNA replication protein [22].

Recent work by Hashimi et al. [10] showed that albicidin is a potent inhibitor of DNA Gyrase with minimum inhibitory concentrations less than most quinolones. Albicidin was shown to inhibit the religation of the cleaved DNA intermediate during the gyrase catalytic sequence, and also inhibit the relaxation of supercoiled DNA by gyrase and topoisomerase IV. Furthermore, *E. coli* strains harboring quinolone (GyrA S83L) and CcdB (GyrA R462C) resistant mutations showed cross-resistance to albicidin suggesting a similar mechanism of action.

Albicidin resistance mutants of *E. coli* have not demonstrated cross-resistance to inhibitors of the gyrase subunits, or to other DNA replication inhibitors [22]. This might be hampered by the fact that albicidin resistant *E. coli* arise at high frequency by mutations in *tsx*, the gene for an outer membrane protein involved in the active uptake of nucleosides from the surrounding medium [29, 30].

DNA gyrase as a drug target

DNA gyrase is known to be the target of several classes of antimicrobial agents [31]. Quinolones are synthetic compounds that target DNA gyrase A and act by stabilizing the DNA gyrase-DNA cleavage complex, thus inhibiting DNA supercoiling. Shortly after binding the Gyrase-DNA complex, quinolones induce a conformational change in the enzyme. Once the gyrase has cleaved the DNA, the quinolone traps this complex and prevents the religation of the DNA strands. Consequently, the quinolone-gyrase-DNA complex inhibits DNA replication [32].

In contrast, coumarins (coumermycin A_1 and novobiocin) are natural products of *Streptomyces* which inhibit DNA gyrase by competing with ATP for binding to the Gyrase B subunit [27, 31, 33]. Enzymatic analysis of the 43 kDa amino-terminal fragment of GyrB shows that it contains a coumarin sensitive ATPase activity. It has been shown that novobiocin is a noncompetitive inhibitor of the ATPase activity and binds a monomer of the 47 kDa GyrB fragment, whereas coumermycin which resembles a dimer of novobiocin, forms a complex with a dimer of the 47 kDa fragment [31].

Selective site-specific mutagenesis of DNA gyrase has revealed that mutations which confer resistance to quinolones map to both gyrase A and B subunits, while those conferring resistance to coumarins map to gyrase B [31]. Mutations which confer resistance to these drugs are shown in Fig. 1.

Other agents which target DNA gyrase include microcin B17, CcdB, simocyclinones, cyclothialidine, cinodine and clerocidin [31, 34, 35]. Microcin B17, CcdB, and clerocidin inhibit the supercoiling assay by stabilizing the Gyrase-DNA complex [31, 36]. In contrast, simocyclinones inhibit an early catalytic step of the gyrase by interfering with enzyme-DNA binding [34]. Cyclothialidine inhibits the ATPase activity of gyrase by competing for the ATP binding site [37]. The precise mode of action of these agents with gyrase is incompletely understood, although resistance to some of these compounds has been mapped to a specific unit of gyrase.

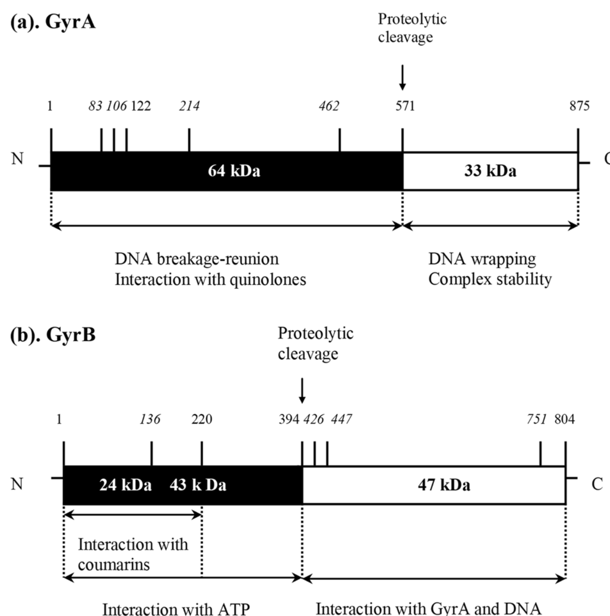

Fig. 1 Domain organization of *E. coli* DNA gyrase. The **a** GyrA (97 kDa) and **b** GyrB (90 kDa) proteins are represented as linear blocks with proposed domain boundaries indicated. Key amino acids referred to in the text are also shown. Amino acid 122 in GyrA is the active-site tyrosine. Amino acids whose mutation leads to drug resistance are numbered in italics. Quinolone-resistance mutations map to GyrA (86–106) and GyrB (426 and 447). Mutation at GyrB (136) confers coumarin resistance, a mutation at GyrB (751) confers Microcin B17 resistance and mutations at GyrA (214 and 462) confer resistance to the F plasmid protein CcdB. Adapted from [31, 68–70]

Structure and function of the albicidin biosynthetic pathway

Transposon mutagenesis revealed that at least two gene clusters spanning more than 60 kb in the genome *X. albilineans* are involved in albicidin production [38, 39]. Subsequently, three genes required for albicidin biosynthesis were identified, cloned, and sequenced from two Queensland strains of *X. albilineans* [14, 40, 41]. A study with *X. albilineans strain* Xa23R1 from Florida revealed that three gene clusters, containing a total of 22 open reading frames (ORFs), are involved in albicidin biosynthesis [11, 42]. Figure 2 shows the major albicidin biosynthetic cluster containing 19 ORFs.

Nonribosomal peptide and polyketide synthases

Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are structurally and mechanistically related to fatty acid synthases, all of which catalyze the

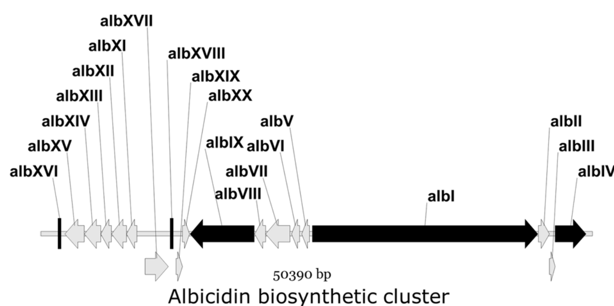

Fig. 2 Physical map and genetic organization of the DNA region containing the albicidin gene cluster involved in albicidin production. The location and direction of the 19 open reading frames (ORFs) identified in the gene cluster are shown by thick arrows. The three polyketide synthase and nonribosomal peptide synthase genes are shown by black and thick arrows. Modified from [11] to show the arrangement of genes involved in albicidin biosynthesis

synthesis of biopolymers in the absence of a nucleic acid or other templates [43].

In an NRPS the modules can be further subdivided into three functional domains: the adenylation domain (A) activates amino acids, a peptidyl carrier protein (PCP) attaches the growing polypeptide and a condensation domain (C) that catalyzes the formation of the peptide bond [43]. By analogy, a PKS module consists of three domains: the acyltransferase domain activates acyl-CoA; the acyl carrier protein (ACP) domain tethers the growing polyketide and the ketosynthase domain (KS) forms the bond. Each NRPS or PKS system also has a domain for loading a starter unit onto the first PKS/NRPS module and a chain-terminating thioesterase (TE) domain [44].

The NRPS substrate-binding pockets are so highly specific for their substrates that predictive models based upon consensus signature motifs for known substrates have been determined. Substrate specificity is determined at the binding pocket, consisting of a stretch of about 100 amino acid residues between highly conserved motif A4 and A5 [45]. Based on sequence analysis of known A domains, in relation to the crystal structure of the GrsA substrate-binding pocket, models have been developed to predict substrate specificity from 8 or 10 amino acids lining the pocket [46, 47].

Sequence analysis of the *X. albilineans* biosynthetic region showed that it contains one large multifunctional fused PKS-NRPS gene (designated *xabB/AlbI*) and two small NRPS genes (designated *albIV* and *albIX*) [11, 14]. *xabB* (syn. *albI*) encodes an enzyme of 6879 amino acids (aa) with several domains involved in polyketide and peptide synthesis. The PKS region of XabB is divided into three modules (Fig. 3). The module designated PKS-1 contains acyl-CoA ligase and acyl carrier protein (ACP1) domains. The module designated PKS-2 contains ®-ketoacyl synthase (KS1) and ®-ketoacyl reductase (KR) domains followed by two consecutive ACP domains (ACP2 and ACP3). The module designated PKS-3 contains a KS domain (KS2) followed by a PCP domain (PCP1) [11, 14].

The PKS module of XabB is connected to four NRPS modules, by the PCP1 domain. The first three NRPS domains contain the general order of C, A and PCP domains typical of such enzymes [48] and NRPS-4 contains only a single C domain. The NRPS region of XabB contains a duplicated region corresponding to NRPS-1 and NRPS-3. This indicates that two identical amino acids are added to the growing chain by NRPS-1 and NRPS-3 separated by an amino acid that is added by NRPS-2 [11].

AlbIV forms one NRPS which contains a single A domain followed by a PCP domain. NRPS AlbIX contains two NRPS modules, the first module containing an A and a PCP domain while the second module contains a C, A and PCP domain. Interestingly the A and PCP domains of both NRPS modules in AlbIX are identical, implying that they load the same amino acid onto the growing albicidin chain. At the end of the polypeptide is a TE domain responsible for terminating the growing chain [11].

Modifying and regulatory enzymes

Once the polypeptide chains have been released, they frequently undergo further enzymatic modification by ancillary enzymes (e.g., methyltransferases, hydroxylases and glycosyl transferases). This subsequent modification is generally required for the final product to be biologically active [49]. Most tailoring enzymes are dedicated to the biosynthetic pathway itself and are encoded by genes that are clustered with the core PKS and NRPS genes [48, 50].

The major biosynthetic gene cluster of albicidin contains two methyltransferases (*xabC* and *xabD*) that utilize *S*-adenosyl-methionine as a co-substrate for *O*-methylation of small molecules [51–53]. Evidence from insertional mutagenesis and complementation proves that *xabC* is involved in albicidin biosynthesis in *X. albilineans* [41]. There are four other ORFs designated *xabJ* (possible alpha/beta fold hydrolase COG0596, with 39% similarity over 260 aa to GenBank Accession AA054683), *xabK* (probable benzoyl-CoA oxygenase OG3396, with 54% similarity over 426 aa to AAN39376), *xabL* (probable dienelactone hydrolase COG4188, with 58% similarity over 278 aa to ZP_00172356) [54] and *albV* (probable carbamoyl transferase, with 46% similarity over 441 aa to AAG02370 from *Streptomyces verticillus* [11].

The production of antibiotics is associated with tight regulation of expression in the producing organism. This regulation involves switching on the biosynthetic genes during the correct phase of growth and controlling the export of the antibiotic. The biosynthetic gene cluster of albicidin contains one regulatory gene (designated *albVIII*)

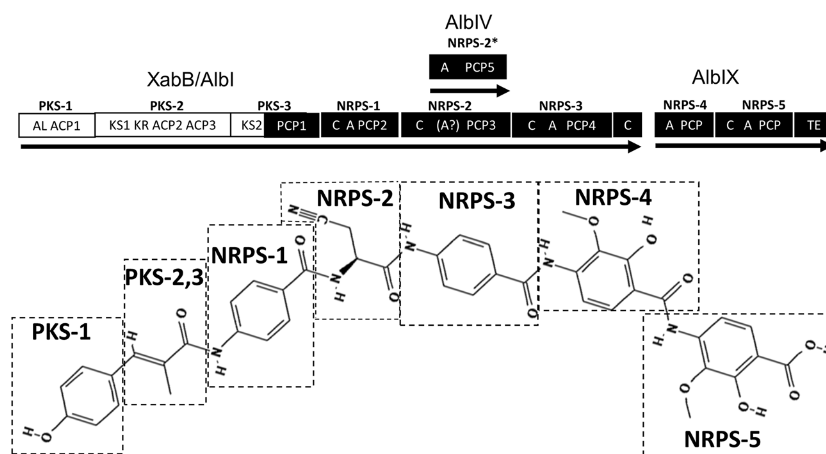

Fig. 3 Model for the synthesis of albicidin by the three polyketide synthase (PKS) modules and the seven nonribosomal peptide synthase (NRPS) modules identified in albicidin biosynthetic cluster. Boxes with dotted lines around the chemical structure of albicidin indicate the stepwise synthesis by PKSs and NRPSs. Abbreviations: A adenylation, ACP acyl carrier protein, AL acyl-CoA ligase, C condensation, KR ®-ketoacyl reductase, KS ®-ketoacyl synthase, NRPS nonribosomal peptide synthase, PCP peptidyl carrier protein, PKS polyketide synthase, TE thioesterase. The question mark in the NRPS-2 domain indicates that this A domain is incomplete. Modified from [11]

[11]. Analysis of *albVIII* showed it is most similar to the syringomycin regulatory gene *syrP* from *Pseudomonas syringae*. Searches of protein sequence databases demonstrated that s*yrP* was most similar to histidine kinases such as the CheA regulatory protein of *E. coli*. Site-directed insertional mutagenesis of the *syrP* gene [55], exhibited an unusual pleiotropic phenotype including a failure to produce syringomycin in liquid media in contrast to the production of elevated levels of the toxin on agar media. Furthermore, the *syrP* mutant was relieved of the suppression of toxin production that accompanies inorganic phosphate concentrations of >1 mM on agar media [55].

Precursor synthesis genes

Polyketide and polypeptide antibiotic biosynthesis begin with activation of starter units followed by elongation of the antibiotic backbone by the large PKS and NRPS enzymes. The albicidin biosynthetic cluster contains several genes which may have a role in the production of precursor molecules. Genes designated *ubiC* and *pabAB* show similarity to PHBA (*para*-hydroxybenzoate) synthase and PABA (*para*-aminobenzoate) synthase respectively [56, 57]. Furthermore, these albicidin precursors might be activated by another gene *xabE* (syn. AlbVII) which showed similarity to benzoate CoA ligase.

Resistance genes

Antibiotic biosynthetic genes are often clustered with one or more genes conferring resistance to the antibiotic in bacteria [1]. The major albicidin biosynthetic cluster contains two genes for self-protection. *albF* is an ABC transporter involved in the active efflux of albicidin. Expression of *albF* in *E. coli* increased albicidin resistance by 30–50 fold [54]. Another gene *albG* shows similarity to quinolone-resistance gene *qnr*. Expression of albG in *E. coli* conferred significant albicidin resistance. Furthermore, the addition of purified AlbG to the in vitro DNA gyrase supercoiling assay conferred albicidin resistance suggesting an interaction with the DNA gyrase subunits [10].

Resistance to albicidin is conferred by genes present in other bacteria. AlbD produced by *Pantoea dispersa* is an endopeptidase that directly cleaves the peptide bond of albicidin and subsequently rendering it inactive [58]. While, AlbA synthesized by *Klebsiella oxytoca* binds to albicidin and completely inhibits its activity [59, 60].

X. albilineans DNA gyrase

As albicidin is a potent inhibitor of DNA gyrase, the albicidin producer *X. albilineans* would require resistance mechanisms for self-protection. In vitro supercoiling assays with purified DNA gyrase subunits of *X. albilineans* showed a 25-fold resistance to albicidin and ciprofloxacin in comparison to the *E. coli* DNA gyrase [61]. However, when the *X. albilineans* DNA gyrase A subunit was substituted with *E. coli* DNA gyrase A in the assay, this combination was as sensitive as the *E. coli* DNA gyrase AB suggesting that resistance to albicidin is conferred by the DNA gyrase A subunit.

Structure of albicidin

Structural analysis of albicidin has shown that it consists of *p*-aminobenzoic acids and cyanoalanine with a chemical formula $[M+H]C_{44}H_{39}O_{12}N_6$ and molecular weight of 843.8260 Da [56]. It consists of the nonproteinogenic α-amino acid β-cyano-L-alanine (Cya-3), the aromatic *δ*-amino acids *p*-aminobenzoic acid (pABA-2 and pABA-4) and 4-amino-2-hydroxy-3-methoxybenzoic acid (pMBA-5 and pMBA-6). The main structure is linked with 3-(4-hydroxyphenyl)-2-methyl acrylic acid at the N-terminal (Fig. 3).

Strategies for improving the production of albicidin

Albicidin is a potent antibiotic that with minimum inhibitory concentrations significantly less than many current DNA gyrase inhibitors. However, albicidin production is limited from *X. albilineans* and other strategies are needed to improve the production of albicidin to realize its clinical potential.

Heterologous production

Heterologous biosynthesis of compounds in bacteria relying on transforming the complete biosynthetic cluster into a host bacterium can be used to increase production. Since the complete genome of *X. albilineans* has been sequenced and the albicidin biosynthetic cluster has been identified, heterologous expression of albicidin would be feasible. Recently, the complete biosynthetic cluster of albicidin was transferred to *Xanthomonas axonopodis* pv. Vesicatoria [62]. Albicidin production was increased sixfold compared to *X. albilineans* suggesting a promising strategy for engineering overproduction. The advantage of the heterologous host offers a fast-growing bacterium which is easily amenable to genetic modification compared to *X. albilineans*. Further modifications to improve albicidin production includes: (i) addition of constitutive promoters to upregulate transcription (ii) the use of codon optimization to improve translation and (iii) addition of albicidin efflux pumps to improve albicidin secretion.

Total synthesis of albicidin

Another strategy to improve albicidin production is chemical synthesis. In 2015, a convergent total synthesis approach was used to synthesize albicidin [63]. Three different fragments of albicidin were synthesized in this strategy; (i) an N-terminal coumaric acid, (ii) a central tripeptide and (iii) C-terminal dipeptide. The central tripeptide was coupled to the C-terminal peptide by BTC-mediated coupling resulting in a pentapeptide. Finally, quantitative BTC-mediated coupling to coumaric acid and global allyl deprotection resulted in albicidin. Antimicrobial activity of the synthesized albicidin was shown to be in accordance with that of natural albicidin (IC_{50}: 40 nM).

The structure and chemical synthesis of albicidin open the possibility of synthesis of albicidin derivatives with improved biological activity. Albicidins were produced with N-terminal acylation to improve antimicrobial activity [64]. Fourteen derivatives were synthesized with variable cinnamoyl, phenylpropanoyl, and benzoyl N-terminal residues. Derivatives with substitutions in the *para*-position of benzoyl N-terminal group were shown to be the most significant in terms of activity while short N-acetylated derivatives showed significantly reduced activity.

Modification to the central amino acid of albicidin with various amino acids was performed to determine the effect of charge, chirality, and steric bulk on antimicrobial activity [65]. It was found that charged amino acids reduce albicidin activity while uncharged amino acids retain activity. Threonine was found to be the most promising in increasing albicidin activity.

A recent study used LC–MS/MS bioactivity-guided spectral networking analysis of albicidin extracts from *X. albilineans* to identify eight different natural albicidins with differential activity against gram-positive and gram-negative bacteria [66].

The albicidin biosynthetic cluster also contains an *O*-Carbamoyl-Transferase (Alb15) which carbamoylates albicidin at the N-terminal resulting in carbamoyl-albicidin [67]. DNA gyrase supercoiling assays showed that carbamoyl-albicidin was six times more potent in inhibiting bacterial gyrase activity when compared to albicidin. In vivo assays showed that carbamoyl-albicidin was differential in its effects on gram-negative bacteria, while it showed similar activity to albicidin in gram-positive strains [67].

Conclusion

Albicidin is a potent DNA gyrase inhibitor with concentrations significantly less than current DNA gyrase inhibitors used clinically. Recent studies have identified the complete biosynthetic cluster of albicidin, produced albicidin in heterologous hosts, solved the structure, and chemically synthesized albicidin and its derivatives. These recent advancements should help to realize the clinical potential of albicidin as new antibiotics are urgently needed to combat multi-antibiotic-resistant bacteria.

Acknowledgements The author would like to thank Professor Robert Birch for his helpful comments on the paper.

Compliance with ethical standards

Conflict of interest The author declare that he has no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Martin JF, Liras P. Organization and expression of genes involved in the biosynthesis of antibiotics and other secondary metabolites. Annu Rev Microbiol. 1989;43:173–206.
2. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism - from biochemistry to genomics. Nat Rev Microbiol. 2005; 3:937–47.
3. Martin JF. Clusters of genes for the biosynthesis of antibiotics - Regulatory genes and overproduction of pharmaceuticals. J Ind Microbiol. 1992;9:73–90.
4. Bender CL, Rangaswamy V, Loper J. Polyketide production by plant-associated Pseudomonads. Annu Rev Phytopathol. 1999; 37:175–96.
5. Gross DC. Molecular and genetic analysis of toxin production by pathovars of *Pseudomonas syringae*. Annu Rev Phytopathol. 1991;29:247–78.
6. Birch RG, Patil SS. Evidence that an albicidin-like phytotoxin induces chlorosis in sugarcane leaf scald disease by blocking plastid DNA-replication. Physiol Mol Plant Pathol. 1987; 30:207–14.
7. Birch RG, Patil SS. Evidence that an albicidin-like toxin induces chlorosis in sugarcane leaf scald. Phytopathology. 1985; 75:1313–1313.
8. Birch RG. Correlation between albicidin production and chlorosis induction by *Xanthomonas albilineans*, the sugarcane leaf scald pathogen. Physiol Mol Plant Pathol. 1987;30:199–206.
9. Zhang LH, Xu JL, Birch RG. Engineered detoxification confers resistance against a pathogenic bacterium. Nat Biotechnol. 1999;17:1021–4.
10. Hashimi SM, Wall MK, Smith AB, Maxwell A, Birch RG. The phytotoxin albicidin is a novel inhibitor of DNA gyrase. Antimicrob Agents Chemother. 2007;51:181–7.
11. Royer M, Costet L, Vivien E, Bes M, Cousin A, Damais A, et al. Albicidin pathotoxin produced by *Xanthomonas albilineans* is encoded by three large PKS and NRPS genes present in a gene cluster also containing several putative modifying, regulatory, and resistance genes. Mol Plant Microbe. 2004;17:414–27.
12. Huang GZ, Zhang LH, Birch RG. Analysis of the genes flanking xabB: a methyltransferase gene is involved in albicidin biosynthesis in *Xanthomonas albilineans*. Gene. 2000;255:327–33.
13. Huang GZ, Zhang LH, Birch RG. Albicidin antibiotic and phytotoxin biosynthesis in *Xanthomonas albilineans* requires a phosphopantetheinyl transferase gene. Gene. 2000;258:193–9.
14. Huang GZ, Zhang LH, Birch RG. Multifunctional polyketide-peptide synthetase essential for albicidin biosynthesis in *Xanthomonas albilineans*. Microbiology. 2001;147:631–42.
15. Pieretti I, Royer M, Barbe V, Carrere S, Koebnik R, Cociancich S, et al. The complete genome sequence of *Xanthomonas albilineans* provides new insights into the reductive genome evolution of the xylem-limited Xanthomonadaceae. BMC Genom. 2009;10:616.
16. Ricaud C, Ryan CC. Leaf scald. In Ricaud C, Egan BT, Gillaspie Jr AG and Hughes CG, editors. Disease of sugarcane: major diseases. (Amsterdam: Elsevier Science Publishers B.V); 1989. p. 39–53.
17. Grisham MP, Legendre BL, Comstock C. 1st report of leaf scald, caused by *Xanthomonas albilineans*, of sugarcane in louisiana. Plant Dis. 1993;77:537–537.
18. Comstock JC. Detection of the sugarcane leaf scald pathogen, *Xanthomonas albilineans*, using tissue blot immunoassay, ELISA, and isolation techniques. Plant Dis. 1992;76:1033–5.
19. Irvine JE, Amador JM, Gallo MI, Riess CM, Comstock JC. 1st report of leaf scald, caused by *Xanthomonas albilineans*, of sugarcane in mexico. Plant Dis. 1993;77:846.
20. Zhang LH, Birch RG. Mechanisms of biocontrol by *Pantoea dispersa* of sugar cane leaf scald disease caused by *Xanthomonas albilineans*. J Appl Microbiol. 1997;82:448–54.
21. Birch RG. *Xanthomonas albilineans* and the antipathogenesis approach to disease control. Mol Plant Pathol. 2001;2:1–11.
22. Birch RG, Patil SS. Antibiotic and process for the production thereof. US Patent 4525354; 1985.
23. Zhang LH, Birch RG. The gene for albicidin detoxification from *Pantoea dispersa* encodes an esterase and attenuates pathogenicity of *Xanthomonas albilineans* to sugarcane. Proc Natl Acad Sci USA. 1997;94:9984–9.
24. Wall MK, Mitchenall LA, Maxwell A. Arabidopsis thaliana DNA gyrase is targeted to chloroplasts and mitochondria. Proc Natl Acad Sci USA. 2004;101:7821–26.
25. Evans-Roberts KM, Mitchenall LA, Wall MK, Leroux J, Mylne JS, Maxwell A. DNA gyrase is the target for the quinolone drug ciprofloxacin in *Arabidopsis thaliana*. J Biol Chem. 2016;291:3136–44.
26. Zhang L, Birch RG. Biocontrol of sugar cane leaf scald disease by an isolate of *Pantoea dispersa* which detoxifies albicidin phytotoxins. Lett Appl Microbiol. 1996;22:132–6.
27. Gellert M, Odea MH, Itoh T, Tomizawa J. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc Natl Acad Sci USA. 1976;73:4474–78.
28. Gellert M, Mizuuchi K, Odea MH, Nash HA. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc Natl Acad Sci USA. 1976;73:3872–6.
29. Birch RG, Pemberton JM, Basnayake WV. Stable albicidin resistance in *Escherichia coli* involves an altered outer-membrane nucleoside uptake system. J Gen Microbiol. 1990;136:51–58.
30. Fsihi H, Kottwitz B, Bremer E. Single amino acid substitutions affecting the substrate specificity of the *Escherichia coli* K-12 nucleoside-specific Tsx Channel. J Biol Chem. 1993; 268:17495–503.
31. Maxwell A. DNA gyrase as a drug target. Trends Microbiol. 1997;5:102–9.
32. Hawkey PM. Mechanisms of quinolone action and microbial response. J Antimicrob Chemother. 2003;51:29–35.
33. Gellert M, Mizuuchi K, Odea MH, Itoh T, Tomizawa JI. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc Natl Acad Sci USA. 1977;74:4772–6.
34. Flatman RH, Howells AJ, Heide L, Fiedler H-P, Maxwell A. Simocyclinone D8, an Inhibitor of DNA gyrase with a novel mode of action. Antimicrob Agents Chemother. 2005;49:1093–100.
35. Nakanishi A, Oshida T, Matsushita T, Imajoh-Ohmi S, Ohnuki T. Identification of DNA gyrase inhibitor (GyrI) in *Escherichia coli*. J Biol Chem. 1998;273:1933–8.
36. Gatto B, Richter S, Moro S, Capranico G, Palumbo M. The topoisomerase II poison clerocidin alkylates non-paired guanines of DNA: implications for irreversible stimulation of DNA cleavage. Nucl Acids Res. 2001;29:4224–30.
37. Nakada N, Gmunder H, Hirata T, Arisawa M. Mechanism of Inhibition of DNA Gyrase by cyclothialidine, a Novel DNA gyrase Inhibitor. Antimicrob Agents Chemother. 1994;38:1966–73.
38. Rott PC, Costet L, Davis MJ, Frutos R, Gabriel DW. At least two separate gene clusters are involved in albicidin production by *Xanthomonas albilineans*. J Bacteriol. 1996;178:4590–6.

39. Wall MK, Birch RG. Genes for albicidin biosynthesis and resistance span at least 69 kb in the genome of *Xanthomonas albilineans*. Lett Appl Microbiol. 1997;24:256–60.
40. Huang GZ, Zhang LH, Birch RG. Albicidin antibiotic and phytotoxin biosynthesis in *Xanthomonas albilineans* requires a phosphopantetheinyl transferase gene. Gene. 2000;258: 193–9.
41. Huang GZ, Zhang LH, Birch RG. Analysis of the genes flanking *xabB*: a methyltransferase gene is involved in albicidin biosynthesis in *Xanthomonas albilineans*. Gene. 2000;255: 327–33.
42. Vivien E, Megessier S, Pieretti I, Cociancich S, Frutos R, Gabriel DW, et al. *Xanthomonas albilineans* HtpG is required for biosynthesis of the antibiotic and phytotoxin albicidin. Fems Microbiol Lett. 2005;251:81–89.
43. Williams GJ. Engineering polyketide synthases and nonribosomal peptide synthetases. Curr Opin Struct Biol. 2013;23:603–12.
44. Cane DE, Walsh CT. The parallel and convergent universes of polyketide synthases and nonribosomal peptide synthetases. Chem Biol. 1999;6:R319–25.
45. Conti E, Stachelhaus T, Marahiel MA, Brick P. Structural basis for the activation of phenylalanine in the non-ribosomal biosynthesis of gramicidin S. Embo J. 1997;16:4174–83.
46. Challis GL, Ravel J. Coelichelin, a new peptide siderophore encoded by the S*treptomyces coelicolor* genome: structure prediction from the sequence of its non-ribosomal peptide synthetase. Fems Microbiol Lett. 2000;187:111–4.
47. Stachelhaus T, Mootz HD, Marahiel MA. The specificity-conferring code of adenylation domains in nonribosomal peptide synthetases. Chem Biol. 1999;6:493–505.
48. Marahiel MA, Stachelhaus T, Mootz HD. Modular peptide synthetases involved in nonribosomal peptide synthesis. Chem Rev. 1997;97:2651–73.
49. Cane DE, Walsh CT, Khosla C. Biochemistry—harnessing the biosynthetic code: combinations, permutations, and mutations. Science. 1998;282:63–68.
50. Hopwood DA. Genetic contributions to understanding polyketide synthases. Chem Rev. 1997;97:2465–97.
51. Haydock SF, Dowson JA, Dhillon N, Roberts GA, Cortes J, Leadlay PF. Cloning and sequence analysis of genes involved in erythromycin biosynthesis in *Saccharopolyspora erythraea* - Sequence similarities between EryG and a family of S-adenosylmethionine-dependent methyltransferases. Mol Gen Genet. 1991;230:120–8.
52. Ingrosso D, Fowler AV, Bleibaum J, Clarke S. Sequence of the D-aspartyl L-isoaspartyl protein methyltransferase from human erythrocytes—common sequence motifs for protein, DNA, RNA, and small molecule S-adenosylmethionine-dependent methyltransferases. J Biol Chem. 1989;264:20131–9.
53. Kagan RM, Clarke S. Widespread occurrence of 3 sequence motifs in diverse S-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. Faseb J. 1994;8:A1291–1291.
54. Bostock JM, Huang G, Hashimi SM, Zhang L, Birch RG. A DHA14 drug efflux gene from *Xanthomonas albilineans* confers high-level albicidin antibiotic resistance in *Escherichia coli*. J Appl Microbiol. 2006;101:151–60.
55. Zhang JH, Quigley NB, Gross DC. Analysis of the *syrP* gene, which regulates syringomycin synthesis by *Pseudomonas syringae* pv syringae. Appl Environ Micro. 1997;63:2771–8.
56. Cociancich S, Pesic A, Petras D, Uhlmann S, Kretz J, Schubert V, et al. The gyrase inhibitor albicidin consists of p-aminobenzoic acids and cyanoalanine. Nat Chem Biol. 2015;11:195.
57. Hashimi SM, Birch RG. Functional analysis of genes for benzoate metabolism in the albicidin biosynthetic region of *Xanthomonas albilineans*. Appl Microbiol Biotechnol. 2010;87:1475–85.
58. Vieweg L, Kretz J, Pesic A, Kerwat D, Grätz S, Royer M, et al. The Albicidin resistance factor AlbD Is a serine endopeptidase that hydrolyzes unusual oligoaromatic-type peptides. J Am Chem Soc. 2015;137:7608–11.
59. Rostock L, Driller R, Grätz S, Kerwat D, von Eckardstein L, Petras D, et al. Molecular insights into antibiotic resistance—how a binding protein traps albicidin. Nat Commun. 2018;9:3095.
60. Sikandar A, Cirnski K, Testolin G, Volz C, Brönstrup M, Kalinina OV, et al. Adaptation of a bacterial multidrug resistance system revealed by the structure and function of AlbA. J Am Chem Soc. 2018;140:16641–9.
61. Hashimi SM, Huang G, Maxwell A, Birch RG. DNA gyrase from the albicidin producer *Xanthomonas albilineans* has multiple-antibiotic-resistance and unusual enzymatic properties. Antimicrob Agents Chemother. 2008;52:1382–90.
62. Vivien E, Pitorre D, Cociancich S, Pieretti I, Gabriel DW, Rott PC, et al. Heterologous production of albicidin: a promising approach to overproducing and characterizing this potent inhibitor of DNA gyrase. Antimicrob Agents Chemother. 2007;51:1549–52.
63. Kretz J, Kerwat D, Schubert V, Grätz S, Pesic A, Semsary S, et al. Total synthesis of albicidin: a lead structure from *Xanthomonas albilineans* for potent antibacterial gyrase inhibitors. Angew Chem Int Ed. 2015;54:1969–73.
64. Kerwat D, Grätz S, Kretz J, Seidel M, Kunert M, Weston JB, et al. Synthesis of Albicidin derivatives: assessing the role of N-terminal acylation on the antibacterial activity. ChemMedChem. 2016;11:1899–903.
65. Grätz S, Kerwat D, Kretz J, von Eckardstein L, Semsary S, Seidel M, et al. Synthesis and antimicrobial activity of albicidin derivatives with variations of the central cyanoalanine building block. ChemMedChem. 2016;11:1499–502.
66. von Eckardstein L, Petras D, Dang T, Cociancich S, Sabri S, Grätz S, et al. Total synthesis and biological assessment of novel albicidins discovered by mass spectrometric networking. Chem – A Eur J. 2017;23:15316–21.
67. Petras D, Kerwat D, Pesic A, Hempel B-F, von Eckardstein L, Semsary S, et al. The O-carbamoyl-transferase Alb15 is responsible for the modification of Albicidin. ACS Chem Biol. 2016;11:1198–204.
68. Morais Cabral JH, Jackson AP, Smith CV, Shikotra N, Maxwell A, Liddington RC. Crystal structure of the breakage-reunion domain of DNA gyrase. Nature. 1997;388:903–6.
69. Gellert M, Fisher LM, O'Dea MH. DNA gyrase: purification and catalytic properties of a fragment of gyrase B protein. Proc Natl Acad Sci USA. 1979;76:6289–93.
70. Maxwell A, Ali JA, Bates AD, Cullis PM, Howells AJ, Jackson AP et al. DNA gyrase— structure and mechanism. J Cell Biochem. 1993:150-150.