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MINI-REVIEW

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Genome-guided discovery of diverse natural products from *Burkholderia* sp.

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Abstract *Burkholderia* species have emerged as a new source of diverse natural products. This mini-review covers all of the natural products discovered in recent years from *Burkholderia* sp. by genome-guided approaches—these refer to the use of bacterial genome sequence as an entry point for in silico structural prediction, wet lab experimental design, and execution. While reliable structural prediction based on cryptic biosynthetic gene cluster sequence was not always possible due to noncanonical domains and/or module organization of a deduced biosynthetic pathway, a molecular genetic method was often employed to detect or alter the expression level of the gene cluster to achieve an observable phenotype, which facilitated downstream natural product purification and identification. Those examples of natural product discovery from *Burkholderia* sp. provide practical guidance for future exploration of Gram-negative bacteria as a new source of natural products.

Keywords *Burkholderia* · Genome mining · Metabolic engineering · Natural product discovery

Introduction

The genus *Burkholderia* comprises more than 40 species, which inhabit diverse ecological niches such as soil and water, plant surface and rhizosphere, and animal and human respiratory tract [10, 11, 54]. Some of the best known *Burkholderia* species are the *B. cepacia* complex (Bcc) often associated with plant rhizosphere as biocontrol agents and as opportunistic human pathogens in both cystic fibrosis and immunocompromised individuals [31, 44], *B. mallei* as the causal agent of glanders in animals [15, 19], and *B. pseudomallei* as the etiological agent of melioidosis in both animals and humans [13, 28]. Driven largely by the research on *Burkholderia* pathogenicity, the complete genomes of 36 *Burkholderia* strains have so far been sequenced and the data are available in the public databases. Analysis revealed that the *Burkholderia* species have a median genome size of 7.27 Mb (ranging from 3.75 Mb to almost 10 Mb) (Table 1), ranking in the top 5 % tier among all bacterial genomes. Every *Burkholderia* genome contains not only multiple pathogenic islands but also a surprisingly large number of putative natural product biosynthetic genes clusters (ranging from 7 to 27, with an average number of 15) (Table 1), as predicted with the antiSMASH program [5, 33]. Those gene clusters are predicted to produce bacteriocins, butyrolactones, ectoines, homoserine lactones, lantipeptides, nonribosomal peptides, phenazines, phosphonates, polyketides, siderophores, terpenes, varying hybrid molecules, or structurally unclassified molecules. Statistically, the percentage content of the thiotemplate modular systems (TMS) in *Burkholderia* genomes is only second to that of actinobacteria, higher than those of bacilli, cyanobacteria, myxobacteria and fungi [35].

The availability of vast genome sequence data and genome-guided discovery technologies has resulted in the discovery of a plethora of structurally and functionally diverse natural products from *Burkholderia* sp. in recent years. Some of those small molecules have entered extensive preclinical evaluations as drug candidates. Although an even larger number of natural products from *Burkholderia* sp. have been discovered, mostly by conventional

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Table 1 Genome sizes and number of putative natural product biosynthetic gene clusters in each completely sequenced genome of *Burkholderia* sp.

Burkholderia strain	Genome size (Mb)	Number of putative natural product biosynthetic gene clusters
Burkholderia sp. 383	8.68	13
Burkholderia sp. CCGE1001	6.83	7
Burkholderia sp. CCGE1002	7.88	8
Burkholderia sp. CCGE1003	7.04	8
Burkholderia sp. KJ006	6.63	9
Burkholderia sp. RPE64	6.96	8
Burkholderia sp. Y123	8.9	10
B. ambifaria AMMD	7.53	19
B. ambifaria MC40-6	7.64	14
B. cenocepacia AU 1054	7.28	13
B. cenocepacia HI2424	7.7	13
B. cenocepacia J2315	8.06	13
B. cenocepacia MC0-3	7.97	14
B. cepacia GG4	6.47	8
B. gladioli BSR3	9.05	22
B. glumae BGR1	7.28	17
B. mallei ATCC 23344	5.84	18
B. mallei NCTC 10229	5.74	17
B. mallei NCTC 10247	5.85	16
B. mallei SAVP1	5.23	14
B. multivorans ATCC 17616	7.01	13
B. phenoliruptrix BR3459a	7.65	8
B. phymatum STM815	8.68	10
B. phytofirmans PsJN	8.21	11
B. pseudomallei 668	7.04	25
B. pseudomallei 1026b	7.23	23
B. pseudomallei 1106a	7.09	23
B. pseudomallei 1710b	7.31	27
B. pseudomallei BPC006	7.16	23
B. pseudomallei K96243	7.24	23
B. pseudomallei MSHR305	7.43	24
B. rhizoxinica HKI 454	3.75	16
B. thailandensis E264	6.72	21
B. thailandensis MSMB43[a]	6.96	16
B. thailandensis MSMB121	6.73	17
B. vietnamiensis G4	8.39	9
B. xenovorans LB400	9.73	9

[a] Draft genome sequence obtained by the authors' group. Included here as an exception, as it is relevant to the thailanstatin group of natural products [30] covered in this mini-review

natural product chemistry approach, this mini-review only covers those discovered by genome-guided approaches—these refer to the use of bacterial genome sequence as an entry point for in silico structural prediction, wet lab experimental design and execution—to be in compliance with the theme of this special issue of JIMB. In addition, this mini-review does not elaborate on the technical details of genome mining and genome-guided discovery approaches because such topics are covered by other articles published in this special issue of JIMB or elsewhere [8, 12, 55, 63].

Betulinan/terferol analogues

Betulinans A–C (Fig. 1) are benzoquinone compounds isolated from the fruiting bodies of *Lenzites betulina* and the culture of a fungus belonging to the Order of *Chaetothyriales* [16, 29]. Terferol (Fig. 1) is a terphenyl compound discovered from the culture of *Streptomyces* sp. in a screening for eukaryotic phosphodiesterase (PDE) inhibitors [36, 37].

The Brady group identified a cryptic gene cluster BTH-II0204-207 in the *B. pseudomallei* K96243 genome and a highly similar gene cluster in the *B. thailandensis* E264

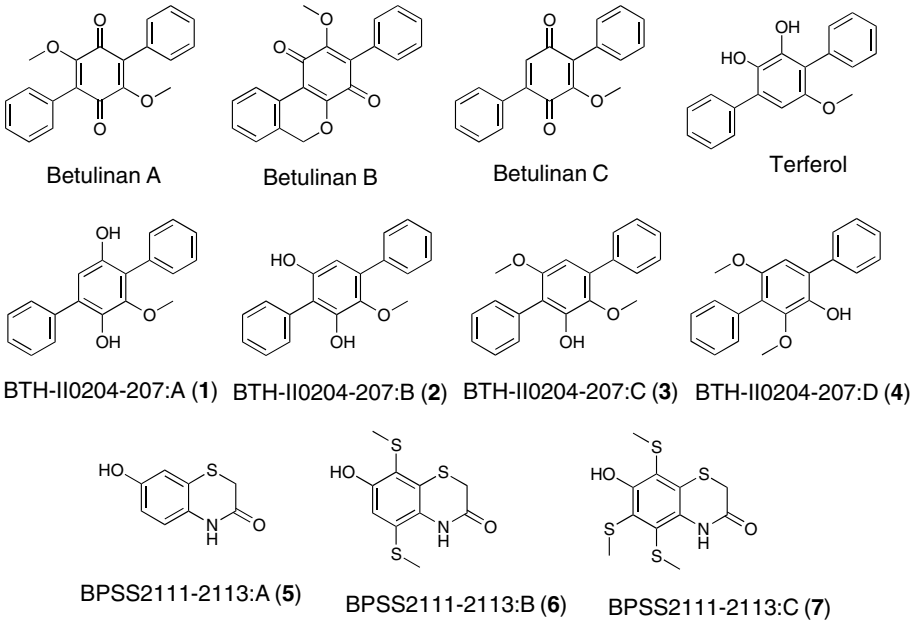


Fig. 1 Structures of betulinans A–C, terferol, BTH-II0204-207:A–D (**1**–**4**) and BPSS2111-2113:A–C (**5**–**7**). Only natural products discovered from *Burkholderia* sp. through genome-guided approaches are given a *bold numerical number* in *parenthesis* while reference compounds are not. This rule applies to all figures in this mini-review

genome. They PCR-amplified the gene clusters from respective genomic DNA, cloned under the control of an IPTG-inducible P_{tac} promoter, and introduced the constructs into *Pseudomonas aeruginosa* by conjugation for heterologous expression. Multiple new peaks appeared in the HPLC trace of ethyl acetate extract of the transformed *P. aeruginosa* culture induced by IPTG, which led to the discovery of four betulinan/terferol analogues, named BTH-II0204-207:A–D (**1**–**4**) (Fig. 1) [3]. These compounds differ from betulinans or terferol by either the position or number of methyl substituents found on each metabolite.

The BTH-II0204-207 gene cluster contains four genes that encode a single module nonribosomal peptide synthetase (NRPS), a dehydratase, an isomerase, and a methyltransferase. It was predicted that the NRPS module condenses two phenylalanine units to initiate the biosynthesis of BTH-II0204-207:A through a mechanism that is similar to the biosynthesis of the fungal metabolite terrequinone [3, 6]. Although the methyltransferase is assumed to account for the varied methylation patterns of the betulinan/terferol analogues, the exact roles of the predicted dehydratase and isomerase enzymes remain unknown; it is also not clear how the amino groups are removed.

Bioactivity assay indicated that BTH-II0204-207:A is a potent PDE4 inhibitor [3]. Noteworthy, the first selective PDE4 inhibitor for the treatment of chronic obstructive pulmonary disease (COPD), roflumilast (DALIRESP®), was approved by the US Food and Drug Administration (FDA) in 2011 [51]. Betulinans, terferol, and their congeners have thus been regarded as promising drug candidates targeting PDE4-related disease such as COPD, some types of brain tumors, and other inflammatory diseases [20, 27, 34, 43].

During the same course of study, the Brady group heterologously expressed another small cryptic gene cluster BPSS2111-2113 from *B. pseudomallei* K96243 and isolated three non-betulinan compounds named BPSS2111-2113:A–C (**5**–**7**) (Fig. 1). These compounds share a 7-hydroxy-2*H*-benzo[*b*][1,4]thiazin-3(4*H*)-one core structure but BPSS2111-2113:B–C have two to three methyl mercapto substitutions, respectively. The biosynthetic origin or bioactivities of those compounds was not investigated [3].

Capistruin

Lasso peptides are ribosomally synthesized bioactive peptides consisting of 16 to 21 amino acids featuring a C-terminal tail that threads through an N-terminal macrolactam ring to form a tightly knotted structure [21, 32]. This structural conformation endows the peptides resistance to proteolysis and varying bioactivities including inhibition of HIV replication, inhibition of bacterial RNA polymerase, and induction of mitochondrial permeability and the subsequent loss of cytochrome c [14, 42, 57]. Lasso peptides are classified depending on the presence (class I) or absence (class II) of four conserved cysteine residues, which are involved in the formation of two intramolecular disulfide bonds [47]. The best studied lasso peptide is microcin J25 (MccJ25) (Fig. 2) from *Escherichia coli* whose biosynthetic gene cluster contains four genes, *mcjABCD*, encoding a precursor protein McjA, two processing enzymes McjB and McjC, and an export and immunity protein McjD, respectively [1, 45, 48, 49].

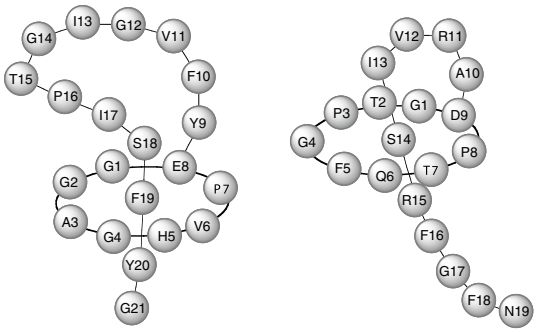
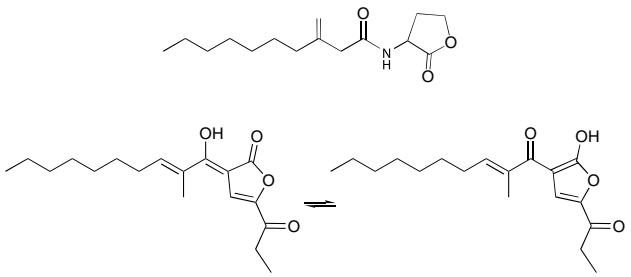


Fig. 2 Structures of microcin J25 and capistruin (**8**) drawn with 1-letter amino acid abbreviations and residue numbers due to their large sizes and a lasso confirmation



Malleilactone (**9**, left) and its tautomer burkholderic acid (**10**, right)

Fig. 3 Structures of an *N*-acyl homoserine lactone of *Pseudomonas aeruginosa*, malleilactone (**9**) and its tautomer burkholderic acid (**10**)

The Marahiel group identified a gene cluster homologous to the *E. coli mcjABCD* operon in the genome of *B. thailandensis* E264 and predicted this strain to produce a class II lasso peptide. They went on to optimize the fermentation conditions for elevated gene expression and successfully isolated the predicted peptide, named capistruin (**8**) (Fig. 2) [25]. Mass spectrometric and NMR structural studies confirmed the lasso structure of capistruin. Heterologous expression of *capABCD* in *E. coli* was sufficient to produce the matured capistruin peptide. Capistruin consists of 19 amino acids and is maturated from a 47-amino acid precursor CapA. CapB and CapC are protease and synthetase/cyclase, respectively, for processing CapA, and CapD is an export and immunity protein. It was proposed that during capistruin maturation, CapB cleaves off the first 28-amino acid leader peptide on CapA to free the N-terminal glycine (G1), and CapC activates the side-chain carboxyl group of aspartic acid (D9) to promote the condensation (C) reaction to form a 9-residue macrolactam ring. The tight threading of the 10-residue C-terminal tail through the macrolactam ring in mature capistruin suggests the CapA precursor has to be folded correctly beforehand, so that the ring is enclosed around the C-terminal tail [26].

Capistruin was found to possess moderate antimicrobial activities against representative *Burkholderia*, *E. coli* and *Pseudomonas* strains, with MIC values in the range of 12–50 μM [25].

Malleilactone/burkholderic acid

When surveying the genomes of *Burkholderia* sp., the Brady group and the Hertweck group independently identified a cryptic gene cluster commonly present in the genomes of *B. mallei* ATCC 23344, *B. pseudomallei* K96243 and *B. thailandensis* E264. This gene cluster encodes a noncanonical hybrid polyketide synthase (PKS)-NRPS pathway that contains unusual domains and module organization, from which no putative compound structure could be predicted. After having performed promoter exchanges in *B. thailandensis* E264 to activate this enigmatic pathway either by rhamnose induction (the Brady group) or by a constitutively expressed promoter design (the Hertweck group), both groups observed a significantly elevated production of an unknown compound during HPLC profiling of culture extracts of the engineered strains, which eventually led them to discover the elusive yellow pigment compound, named malleilactone (**9**) by the Brady group [4] or burkholderic acid (**10**) by the Hertweck group [17] (Fig. 3). During the course of compound purification, the Hertweck group noticed that the burkholderic acid was highly unstable until a methylation reaction was performed to convert the labile compound into a stable methylated derivative. Isotope labeling and NMR structural studies finally solved the structure of this mysterious compound. Malleilactone/burkholderic acid appeared to exist naturally as tautomers.

In preliminary assays the Brady group found malleilactone to possess weak to moderate activities against some Gram-positive bacteria and human cell lines [4]. Independently, the Hertweck group found burkholderic acid and its methylated derivative to have no antimicrobial activity and only weak to moderate cytotoxicity [17]. Malleilactone/burkholderic acid and its tautomeric butenolide bear significant structural similarity with bacterial quorum sensing molecules such as A-factor and *N*-acyl homoserine lactone (AHL) (Fig. 3); they are likely involved in microbial communication.

Because of the unusual domains and module organization of the deduced hybrid PKS-NRPS pathway for malleilactone/burkholderic acid biosynthesis, the elucidation of the compound structure retrospectively assisted the Hertweck group to propose a biosynthetic model. In this model, one longer aliphatic chain is built from a short-chain fatty acid that is activated by a CoA ligase BurJ. This C8 intermediate is loaded onto an acyl carrier protein (ACP) on BurF and elongated by two PKS cycles to

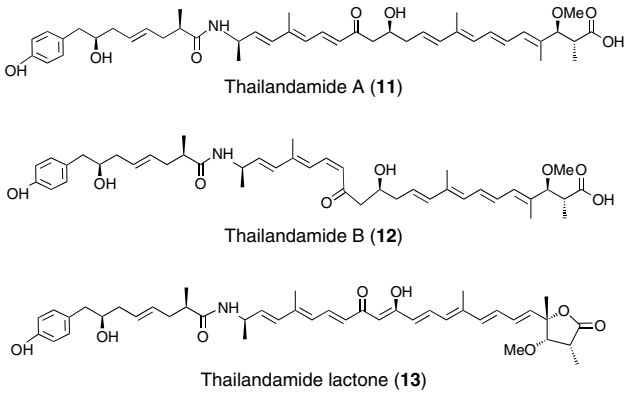


Fig. 4 Structures of thailandamide A (**11**), thailandamide B (**12**), and thailandamide lactone (**13**)

form a C12 intermediate covalently linked to the last ACP on BurF. Meanwhile, a shorter aliphatic chain is initiated by a propionyl unit derived from methionine, followed by one cycle of PKS extension of a hydroxymalonyl moiety to form a C5 intermediate covalently linked to an ACP on BurA. Although it is uncertain about how the transamination, decarboxylation and desulfurization of the methionine might occur, it is likely that a C domain on BurF is responsible for bringing together the two enzyme-bound aliphatic chains by forming an ester bond. Finally, a reductase (Red) domain at the C-terminal of BurF cleaves the ACP-bound intermediate, which further undergoes a spontaneous cyclocondensation to afford malleilactone/burkholderic acid [17].

Thailandamides and thailandamide lactone

When surveying the bacterial genomes for *trans*-acyltransferase (AT) PKSs, the Piel group, in collaboration with the Hertweck group, identified a giant cryptic gene cluster in the genome of *B. thailandensis* E264, which encodes a 17-module hybrid *trans*-AT PKS-NRPS pathway [41]. The authors predicted two slightly different versions of a complicated long chain polyene product using two distinctive prediction methods, which led to the discovery of thailandamide A (**11**), its congener thailandemide B (**12**), and thailandemide lactone (**13**) (Fig. 4) [22, 23, 41]. Those compounds are elusive because they are produced in minute amounts only in the early growth phase, and they undergo rapid heat- and light-induced decomposition. The Hertweck group had overcome those technical difficulties by manipulating the bacterial quorum sensing regulation to upregulate the *tha* gene cluster expression, by optimizing the cultivation conditions, by scaling up the fermentation volume, and by performing all purification steps in total darkness within a few hours. Eventually, they obtained enough compounds for structure elucidation and bioactivity assays.

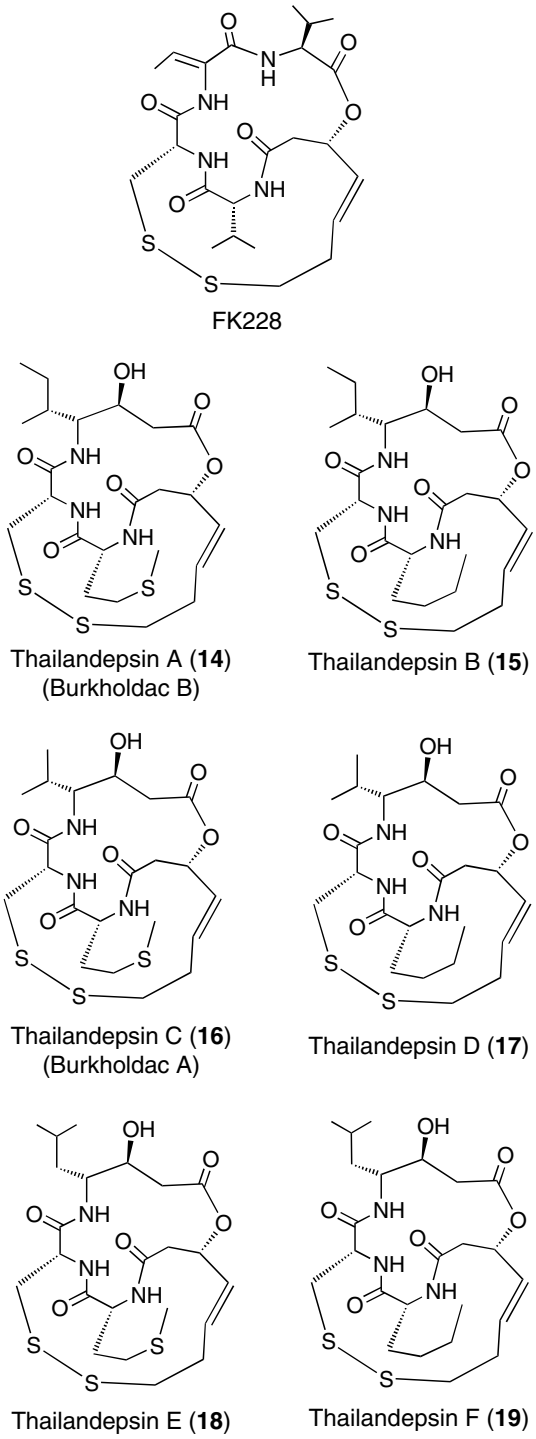


Fig. 5 Structures of FK228, thailandepsin A (burkholdac B; **14**), thailandepsin B (**15**) thailandepsin C (burkholdac A; **16**), thailandepsin D (**17**), thailandepsin E (**18**), and thailandepsin F (**19**)

The *tha* gene cluster responsible for the biosynthesis of thailandamides contains 18 ORFs, which include *thaG-HNOPQ* coding for a multimodular PKS-NRPS pathway, *thaF* for a tandem AT protein, *thaA* for a LuxR-type

pathway regulator, and genes for discrete PKS domain proteins, tailoring enzymes and other functionally unknown proteins [22]. A model for the biosynthesis of thailandamides was proposed, in which an NRPS module on ThaH is predicted to directly activate a D-Ala substrate [23]. Thailandamide lactone showed moderate antiproliferative activities against human tumor cell lines [22].

Thailandepsins/burkholdacs

The genome of *B. thailandensis* E264 contains another cryptic gene cluster (BTH_I2369-2357) that resembles the FK228 biosynthetic gene cluster in *Chromobacterium violaceum* No. 968 [9, 46, 60]. Two groups independently worked on this gene cluster around the same time frame. The Brady group employed an overexpression of transcriptional factor approach to elevate the production of natural products, which led to their discovery of burkholdac A (**16**) and burkholdac B (**14**) (Fig. 5) [2]. Our group used a gene knockout approach to disrupt the production of natural products as shown by the disappearance of several HPLC peaks, which led to the discovery of thailandepsin A (**14**) and thailandepsin B (**15**) (Fig. 5) [59]. Burkholdec B and thailandepsin A refer to the same compound. Subsequently our group further discovered thailandepsins C–F (**16**–**19**) (Fig. 5) by supplementing amino acid precursors to the fermentation medium of *B. thailandensis* E264 [58]. Burkholdacs/thailandepsins all have a bicyclic depsipeptide framework but differ from each other by having different amino acid side chains. They are natural structural analogues of FK228 [52, 53], an FDA approved anticancer drug for the treatment of refractory cutaneous T-cell lymphoma [56] and peripheral T-cell lymphoma [61]. FK228 is a potent inhibitor of class I human histone deacetylases (HDACs); differential inhibition of HDACs in cancer cells leads to a cascade of chromatin remodeling, tumor suppressor gene reactivation, apoptosis, and cell death [18, 53].

The *tdp* gene cluster responsible for the biosynthesis of thailandepsins contains 13 ORFs, which include *tdpABC-$_1$DE$_1$C$_2$E$_2$* coding for a seven-module hybrid NRPS-PKS pathway, *tdpH* for a disulfide oxidase, *thaR* for an AraR-type pathway regulator, and genes for tailoring enzymes, self-resistance and other functionally unknown proteins. A model for the biosynthesis of thailandepsins was proposed, in which a yet-to-be-identified discrete protein provides the necessary *trans*-AT activity for biosynthesis [59].

Our group and collaborators conducted extensive enzyme assays and showed that thailandepsins are potent HDAC inhibitors, particularly toward HDAC1–3 which belong to class I human HDACs, with half-maximal enzyme inhibition (IC_{50}) values in the single to sub nM range [58, 59]. Cell-based antiproliferative assays indicated that thailandepsins A and B possess a broad spectrum of growth inhibition activities, and has reached half-maximal growth inhibition (GI_{50}) for over 90% of the tested NCI60 cell lines even at the lowest concentration (10^{-8} M) used in the screening. Most interestingly, thailandepsin A demonstrated differential activities toward certain types of cell lines at total growth inhibition (TGI) and half-maximal lethal inhibition (LC_{50}) levels, particularly for those derived from colon, melanoma and renal cancers. The efficacy and long-term toxicity of thailandepsin A and thailandepsin B are being investigated in animal models.

Thailanstatins

FR901464 was the first identified natural product which inhibits eukaryotic pre-mRNA splicing [38–40]. The genetic basis for FR901464 biosynthesis in *Pseudomonas* sp. No. 2663 has been decoded [62]. FR901464 is biosynthesized by a hybrid PKS-NRPS pathway that features a *trans*-AT PKS system complemented by three discrete ATs. Using the deduced FR9 proteins as templates to search the GenBank, our group identified a cryptic gene cluster in the genome of *B. thailandensis* MSMB43 that resembles that of FR901464. We obtained the bacterial strain from the US Centers for Diseases Control (CDC), cultivated it in nine different media and used RT-PCR to detect a cultivation condition of Medium 9 in which a regulatory gene of the cryptic gene cluster (*tstA*) is adequately expressed. Consequently, three new compounds, named thailanstatins A (**20**), B (**21**)

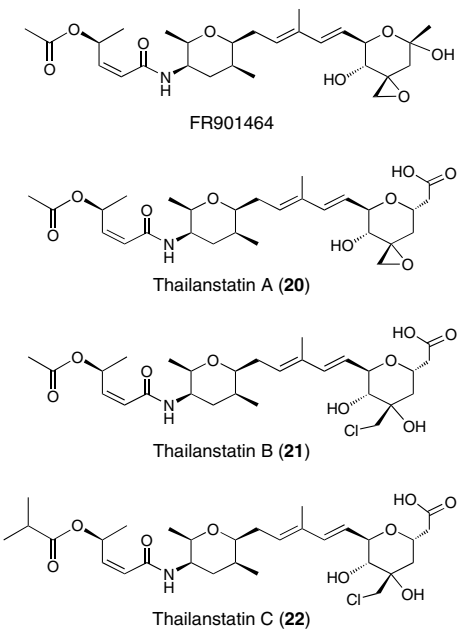


Fig. 6 Structures of FR901464, thailanstatin A (**20**), thailanstatin B (**21**), and thailanstatin C (**22**)

and C (**22**), were isolated from the fermentation broth of *B. thailandensis* MSMB43 (Fig. 6). Thailanstatins belong to the FR901464-family of microbial products that have a heavily decorated pyran ring at one end and an acetyl group or a dimethyl acetyl group at the other end. They differ from FR901464 by lacking an unstable hydroxyl group and by having an extra carboxyl moiety. Those differences endow thailanstatins with a significantly greater stability than FR901464 under physiologically relevant conditions [30].

This gene cluster contains 15 ORFs, which include *tstC-DEFGHI* coding for a nine-module hybrid PKS-NRPS pathway, *tstBJO* for three discrete AT enzymes, *tstA* for a LuxR-type pathway regulator, and genes for tailoring enzymes, self-resistance and other functionally unknown proteins. A model for the biosynthesis of thailanstatins was proposed [30].

In vitro assays showed that thailanstatins inhibit pre-mRNA splicing as potently as FR901464, with IC_{50} values in the single to sub μM range. Cell-based assays indicated that thailanstatins also possess potent antiproliferative activities in representative human cancer cell lines, with GI_{50} values in the single nM range [30]. Thailanstatins also showed promising application as potential glaucoma therapeutic agents due to their modulation activity against the glucocorticoid receptor splicing process [24].

Conclusions and perspective

Development of new and powerful bacterial molecular genetic tools in conjunction with vast bacterial genome data in the public domain has evidently catapulted the discovery of an amazing array of natural products from *Burkholderia* sp. (Figs. 1, 2, 3, 4, 5, 6). The structural diversity and functional diversity of those compounds firmly support *Burkholderia* sp. as a new source of bioactive natural products. Among those *Burkholderia* strains explored, *B. thailandensis* E264 is unequivocally the champion from which 21 putative biosynthetic gene clusters were predicted with the antiSMASH program [5, 33] (Table 2) and many interesting natural products have been discovered, including betulinan/terferol analogues [3], capistruin [25], malleilactone/burkholderic acid [4, 17], thailandamides and thailandamide lactone [22, 23, 41], and thailandepsins/burkholdacs [2, 58, 59] (Fig. 7). Furthermore, all discussed examples of genome-guided natural product discovery covered in this mini-review have occurred within the last 5 years (2008–present). It is thus almost a certainty that many more interesting natural products will be discovered from *Burkholderia* sp. with or without genome-guided approaches.

Genome-guided natural product discovery is a smart way of discovery. It is particularly suitable for small

Table 2 Putative natural product biosynthetic gene clusters predicted in the genome of *B. thailandensis* E264 and compounds actually identified

B. thailandensis E264 genome	Gene cluster #	Category of predicted gene cluster	Identified natural product
Chromosome I	1	Bacteriocin	
	2	Type I PKS	
	3	NRPS	
	4	Terpene	
	5	NRPS	Thailandepsins
	6	NRPS	
	7	Bacteriocin	Capistruin
Chromosome II	1	Bacteriocin	
	2	Other	Betulinan/terferol analogues
	3	Homoserine lactone	
	4	Other	
	5	NRPS-type I PKS-homoserine lactone	Bactobolins[a]
	6	Homoserine lactone	
	7	*Trans*-AT PKS-type II PKS-NRPS	Thailandamides
	8	Bacteriocin	
	9	NRPS	
	10	Phosphonate	
	11	Other	
	12	NRPS-type I PKS-*trans*-AT PKS	Burkholderic acid
	13	Terpene	
	14	PKS-terpene	

[a] Bactobolins were discovered with a conventional natural product approach [7, 50], which are thus not elaborated in this mini-review

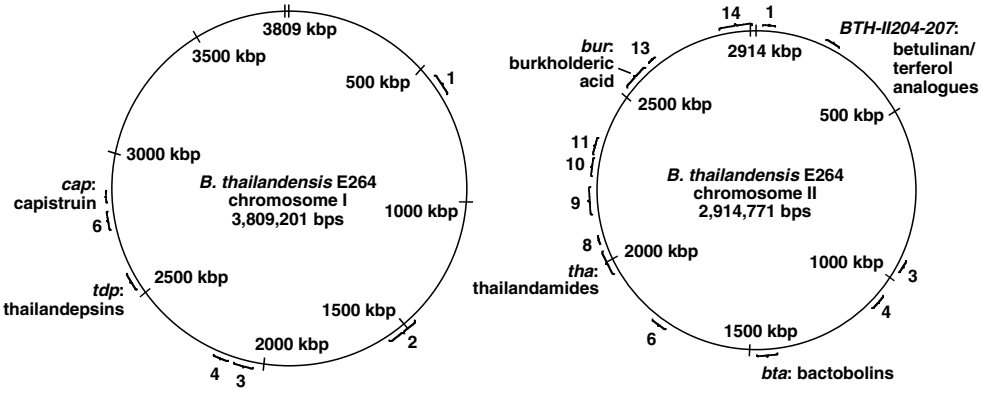


Fig. 7 Schematic depiction of the *B. thailandnsis* E264 genome consisting of chromosomes I and II. Twenty one predicted natural product biosynthetic gene clusters with the antiSMASH program [5, 33] are marked with either a numerical number (uncharacterized) or a gene cluster designation followed by compound name(s)

laboratories with very limited manpower and resources, because it does not require a large and sophisticated production and screening facility and resources that are otherwise necessary for the conventional high-throughput natural product discovery programs. Nevertheless, there are challenges for genome-guided natural product discovery in *Burkholderia* sp. and in microorganisms in general. Firstly, for those using characterized natural product biosynthetic gene cluster sequences as templates to search and identify cryptic gene clusters, there is a good chance to discover natural products that have a similar molecular scaffold as the existing compounds, as seen in the cases of capistruin [25], thailandepsins [58, 59], and thailanstatins [30]. Secondly, the inherent instability and often extremely low yield of many microbial natural products, such as the malleilactone/burkholderic acid [4, 17], the thailandamides [22, 23, 41] and thailanstatins [30], always remains a technical barrier and hampers the pace of discovery. Thirdly, the irregularity of deduced biosynthetic pathways, such as in the case of malleilactone/burkholderic acid [4, 17], often prevents a reliable structural prediction. Lastly, for bacteria species classified as category B (or A) agents, such as *B. mallei* and *B. pseudomallei*, access to their metabolic potential is often restricted due to a lack of BSL-3 facility by most natural product researchers.

Overall, those examples of successful natural product discovery from *Burkholderia* sp. can provide a very useful guidance for future exploration of other Gram-negative bacteria as a new source of natural products. Future efforts should focus on isolating and sequencing more microbial species from diverse environments, on developing more effective and versatile bacterial genetic tools for a wide range of microbial isolates, and on adopting more bioactivity assay methods to reveal the best possible uses of newly discovered natural products.

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