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The development of *Burkholderia* bacteria as heterologous hosts†

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Covering up to 2024

Drug resistance is a serious and growing problem, and new small molecules are needed for a wide variety of clinical and agricultural applications. Natural products, encoded by biosynthetic gene clusters, have consistently been a source of chemical diversity for finely tuned interactions with a range of molecular targets of interest. However, many gene clusters are not transcriptionally active, making heterologous expression in a different host strain a useful tool to access bioactive small molecules. *Burkholderia* spp. bacteria hold promise as heterologous hosts because of their intrinsic natural product capabilities. In this review, we summarize natural products successfully isolated from *Burkholderia* spp. heterologous hosts up until 2024. We then compare the hosts that have been tested and discuss ongoing development efforts to improve access to new natural products in titers sufficient for drug development and industrial applications.

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1. Introduction

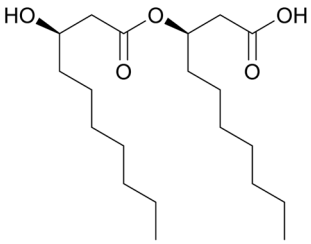
New molecules with novel mechanisms of action are desperately needed to counteract drug resistance. Natural products have historically been a promising source of unique and diverse chemical scaffolds, often already tuned to interact with specific molecular targets of interest.[1] In the post-genomic era, natural products research often takes a DNA-first approach of genome sequencing and mining to identify biosynthetic gene clusters (BGCs) that could give rise to new natural products. However, many BGCs are transcriptionally inactive under standard laboratory conditions, requiring strategies to activate BGCs and enhance natural product discovery and development.[2]

Heterologous expression entails the cloning and expression of DNA from a native producer strain into a suitable host strain. Heterologous expression provides a shortcut to pathway modification, metabolic optimization and analogue generation, and to potentially improving yields for accelerated structure elucidation and bioactivity testing.[3] Given the vast number of bacterial sources of BGCs, access to host strains that are phylogenetically close to the source organism along with a robust set of synthetic biology tools is important. Though each case is unique, heterologous expression is frequently more successful when the BGC source is close to the taxonomic classification of the host strain.[3,4]

The Burkholderiales order of bacteria are amongst the top10 most promising sources of natural products based on sequenced genomes.[5,6] Indeed, several therapeutically relevant natural products are produced by *Burkholderia* spp. bacteria, including antitumor agents rhizoxin, romidepsin and thailanstatins/spliceostatins. Rhizoxin was originally isolated in

the mid-1980s from various strains of *Rhizopus* fungi[7] before it was discovered that endosymbiotic bacteria of the *Burkholderia* genus (later renamed as a new genus, *Mycetohabitans*, of the Burkholderiaceae family) were responsible for its production.[8] As the causal agent of rice seedling blight, rhizoxin targets tubulin, resulting in it entering human clinical trials.[9] Romidepsin (FK228, Istodax®), first discovered in extracts from *Chromobacterium violaceum* in 1994, is a histone deacetylase (HDAC) inhibitor approved for the treatment of T-cell lymphomas.[10–12] Related thailandepsins were later isolated from *Burkholderia thailandensis*.[13] *B. thailandensis* also produces thailanstatins/spliceostatins,[14] spliceosome inhibitors originally isolated from *Burkholderia* sp. FERM BP-3421 (previously named *Pseudomonas* sp.) in 1996.[15] Thailanstatin A was tested pre-clinically as a payload for antibody drug conjugates.[16] These examples highlight the promise of Burkholderiales bacteria as a source of novel therapeutics that remains underexplored.

This intrinsic natural product capacity is precisely why *Burkholderia* spp. are arguably an excellent entry point for synthetic biology applications. With an existing metabolic pool of precursors for various biosynthetic classes of natural products and the ability to harbor and express large autologous BGCs, exploring heterologous production is feasible. This review aims to summarize natural products successfully isolated from *Burkholderia* spp. heterologous hosts up until 2024 (Fig. 1–7 and ESI Table S1†), which was only briefly reviewed previously.[17] We will also compare the host strains that have been tested (Fig. 8, Tables 1 and 2) and discuss ongoing host development efforts to improve access to new molecules in titers sufficient for drug development and industrial applications. This review will not tackle the use of *Burkholderia* hosts in other fields of research, such as biopolymers,[18–20] and biocontrol and bioremediation.[21–23] Readers interested in these topics are directed to the cited references.



HAA C10-C10 (**1**)
B. glumae BGR1
No titer reported

Fig. 1 Structure of C10–C10 3-(3-hydroxyalkanoyloxy)alkanoate (HAA), a precursor to rhamnolipids, produced in *Burkholderia glumae* BGR1.

The studies reviewed here were found using PubMed by searching for any publications that used *Burkholderia* spp. as a heterologous host for natural product production. Note that the taxonomy of Burkholderiales bacteria has been revised over the years. For example, some strains that are now known as *Burkholderia* were previously named as *Pseudomonas* several decades ago,[24] and many strains previously named as *Burkholderia* were recently reclassified to *e.g.*, *Paraburkholderia* or *Mycetohabitans*.[25,26] In this review, we have only included hosts that are currently classified as *Burkholderia*. Readers interested in more diverse microbial hosts are directed to other reviews.[27–32]

2. Natural products obtained by heterologous expression using *Burkholderia* hosts

2.1. Rhamnolipid precursors

Rhamnolipids are glycolipids that act as biosurfactants and are appealing as a sustainable, biodegradable and low toxicity

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alternative to current surfactants. Many native rhamnolipid producer strains are pathogenic to either humans or plants, and their production is tightly regulated, making heterologous expression an attractive strategy for downstream engineering.[33] The first step of rhamnolipid biosynthesis is catalyzed by RhlA, which esterifies two units of 3-hydroxyfatty acids of variable chain length to form a di-lipid, 3-(3-hydroxyalkanoyloxy)alkanoate (HAA). Later steps then attach either one or two rhamnose units *via* RhlB and/or RhlC.[34] The wild-type *Pseudomonas aeruginosa* PA14 produces predominantly C10–C10 HAA (**1**) (Fig. 1), while the wild-type *Burkholderia glumae* BGR1 makes mostly C12–C12 and C14–C14 HAAs. Dulcey and colleagues deleted the native *rhlA* gene from the *B. glumae* BGR1 host and expressed the *rhlA*/*rhlB* operon from *P. aeruginosa* on a pBBR1-based construct under the *P. aeruginosa* promoter P_{rhlA}. While the titer of the products after growth in nutrient broth supplemented with mannitol was not quantified, the relative titer of 3-hydroxyfatty acids were comparable to the native producer, and the distribution of products was successfully shifted from longer-to shorter-chain HAAs.[35] Of note, heterologous production succeeded using DNA sourced from a Gammaproteobacterium (*Pseudomonas*) in a Betaproteobacteria (*Burkholderia*) host.

2.2. Ribosomally synthesized and post-translationally modified peptides (RiPPs)

Most ribosomally synthesized and post-translationally modified peptides (RiPPs) that have been produced in a *Burkholderia* host are lasso peptides. The lasso peptide name derives from their lariat topology of an isopeptide-bonded macrolactam ring through which the C-terminal residues are threaded and held in place either by steric side chain interactions or disulfide bridges.[36,37] These unique peptides have garnered substantial interest due to their thermal and proteolytic stability, promising bioactivities, and amenability to structure modifications.[36,37] Lasso peptide BGCs are small, requiring as few as three genes, making them conveniently sized for DNA synthesis and expression in heterologous hosts.

The first lasso peptide isolated from a *Burkholderia* strain, aided by genome mining, was the antibiotic and RNA polymerase inhibitor capistruin (**2**) in 2008 (Fig. 2).[38,39] Originating in *B. thailandensis* E264, autologous expression and isolation was successful, but the authors also produced capistruin heterologously in *E. coli*. However, compound titers were low at only about 0.2 mg L^{-1} using a defined medium, and the maximum titer of capistruin ever reported *via* an *E. coli* host was 1.6 mg L^{-1}.[38,40] Kunakom & Eustáquio sought to express the capistruin BGC in a more closely related host, selecting the industrial strain *Burkholderia* sp. FERM BP-3421, which had already proven to be a natural product powerhouse by reaching up to 2.5 g L^{-1} of autologous thailanstatin A production after culture media optimization and pathway engineering.[41] A low-copy pRO1600 replicon and L-arabinose induction using the *araC*/P_{BAD} system led to successful production of capistruin in FERM BP-3421, but yields were only 1 mg L^{-1} in M20 defined media and rose to only 3.2 mg L^{-1} in 2S4G complex media.[42] The authors reasoned that the production of spliceostatin congeners (such as thailanstatin A) in g L^{-1} yields was likely disrupting the genetic and metabolic flux of the expression platform. Using a previously generated strain of FERM BP-3421 deficient in spliceostatins (Δ*fr9DEF*::*tet*),[43] capistruin production was improved in complex media to 13 mg L^{-1}.[42] Intriguingly, an outlier clone of the wild-type parent was also isolated that reached 116 mg L^{-1}.[42] The large but variable improvement generated questions regarding the mechanisms that might explain the differences between clones.

It was observed that the outlier capistruin overproducer clone had increased plasmid copy number *via* an as of yet unknown mechanism.[42] To reverse engineer high capistruin production, Fernandez and colleagues interrogated the effect of plasmid copy number. The highest recorded titers of capistruin were achieved by expressing a high-copy pBBR1 construct containing the *araC*/P_{BAD} system in the spliceostatin deficient FERM BP-3421 strain (Δ*fr9DEF*::*tet*) grown in 2S4G complex media, reaching 240 mg L^{-1}.[44] However, the bacterium displayed a growth defect that was only alleviated at the expense of capistruin production when a point mutation (nucleotide G468A, residue G159S) was introduced to reduce the plasmid copy number.[44,45] This work further succeeded in isolating two new lasso peptides predicted in the genome of *Mycetohabitans* sp. B13 by expressing the synthetic BGC in FERM BP-3421 *Δfr9DEF*::*tet*. In contrast to capistruin above, optimal yields were obtained using an expression vector containing a pBBR1 replicon with the G159S mutation. Mycetolassin-15 (**3**) and −18 (**4**) were isolated at 6 mg L^{-1} and 5 mg L^{-1}, respectively, in 2S4G medium (Fig. 2). No antimicrobial activity was detected, and the activity of mycetolassins remains unknown.[44]

Meanwhile, in their continued efforts to improve *B.* sp. FERM BP-3421 as a heterologous host, Adaikpoh and colleagues sought to determine the driving forces of autologous spliceostatin production. They discovered a pathway-specific transcriptional activator, *fr9A*, and a promoter, P_{fr9C}, that drive expression of the core polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) genes. Expressing the capistruin BGC in a new spliceostatin defective mutant strain (Δ*fr9A*) using a high-copy number, pBBR1-based plasmid and the L-arabinose-inducible *araC*/P_{BAD} system generated 112 mg L^{-1}.[46] Moreover, the authors tested a new Fr9A-regulated expression system (*ORF-1–fr9A*/P_{fr9C}) which produced about 65 mg L^{-1} of capistruin in the same growth medium, indicating that the *araC*/P_{BAD} system is superior to *ORF-1–fr9A*/P_{fr9C} at least for lasso peptide production.

Mycetohabitans spp. (previously *Burkholderia*/*Paraburkholderia*) are endophytes of *Rhizopus* fungi and thus have been investigated for specialized metabolite production with an eye to understanding this interkingdom interaction. *Mycetohabitans rhizoxinica* contains several cryptic BGCs, and expression of the burhizin-23 BGC in *E. coli* originally led to a truncated burhizin-17 product in unquantifiable yields presumably less than 0.4 mg L^{-1}, even after ribosome binding site (RBS) optimization.[47] In 2020, Bratovanov and colleagues sought the full length burhizin product, choosing the more closely related *Burkholderia gladioli* pv *agaricicola* HKI0676 as a host. Using a pBBR1-based construct and *araC*/P_{BAD}, 1 mg L^{-1} of burhizin-

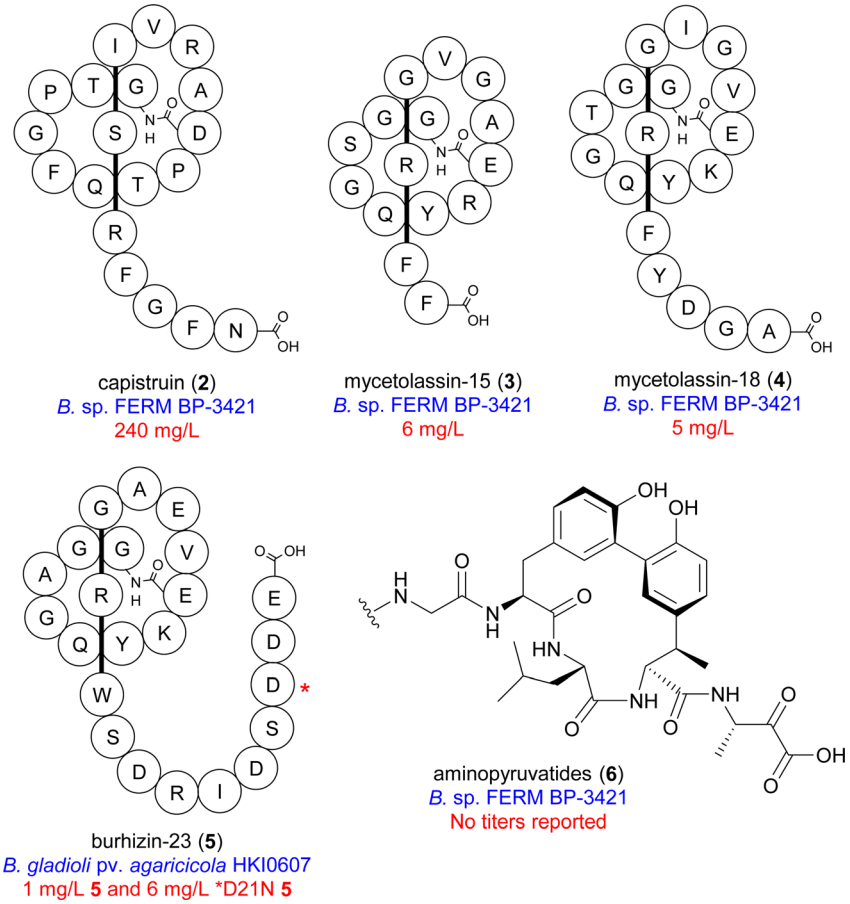

Fig. 2 Structures of the ribosomally synthesized and post-translationally modified peptides (RiPPs) produced in *Burkholderia* sp. FERM BP-3421 Δ*fr9DEF*::*tet* (**2**, **3**, **4**, and **6**) and in *Burkholderia gladioli* pv *agaricicola* HKI0676 (**5**).

23 (**5**) was obtained after culturing in M20 defined media (Fig. 2). This was increased to 6 mg L^{-1} burhizin-23 when the point mutation D21N was applied to interrogate if the conserved, negatively charged tail residues were crucial for streamlined biosynthesis.[48] It was ultimately determined that burhizin-23 and other RiPPs are not required for *M. rhizoxinica* colonization of *Rhizopus microsporus*, though an evolutionary benefit could not be ruled out.

More recently in 2024, a new type of RiPP was characterized by expressing a BGC from *B. thailandensis* E264 in *B.* sp. FERM BP-3421. The van der Donk group selected the aminopyruvatide BGC (*apy*), which encoded three metalloenzymes including a class-defining multinuclear non-heme iron-dependent oxidative enzyme.[49] Though the final natural product is not yet known because there was no colocalized pathway-specific protease, the generic endoproteinase GluC was used on the heterologously expressed precursor ApyA to determine modifications to the five C-terminal residues (**6**) (Fig. 2). Notably, only two tailoring enzymes were successfully expressed in *E. coli*, with the remaining three necessitating the use of the FERM BP-3421 host to preserve enzymatic function.[49] For gene expression in the *Burkholderia* sp. FERM BP-3421 host, a L-rhamnose inducible promoter and pBBR1-based plasmid were used.

2.3. Polyynes

Bacterial polyynes are natural products with alternating single and triple C–C bonds. Despite their high reactivity, polyynes may be valuable biotechnological tools.[50] Cepacin A (**7**) was first isolated in 1984 from *Burkholderia cepacia* (previously *Pseudomonas cepacia*),[51] while caryoynencin (**8**) was found in extracts from *Burkholderia caryophylli* (previously *Pseudomonas caryophylli*) in 1987.[52] In 2022, Petrova and colleagues undertook the heterologous expression of these polyyne BGCs in *Burkholderia* and *Paraburkholderia* spp. hosts.[53] Using the native promoters and a pBBR1 replicon, the cepacin A BGC from *Burkholderia ambifaria* BCC0191 was successfully expressed in *B. ambifaria* BCC1105, while the caryoynencin BGC from *Burkholderia gladioli* BCC1697 was transferred to both *B. ambifaria* BCC0191 and *B. ambifaria* BCC1105. Due to the intrinsic instability of the polyyne structures (Fig. 3), no quantification of products was possible; however, relative cepacin A titers were lower than the native producer in both a metabolite induction medium and a biomimetic pea exudate medium, whereas relative caryoynencin titers were lower than the native producer in the metabolite induction medium but higher than the native producer in the biomimetic medium.[53]

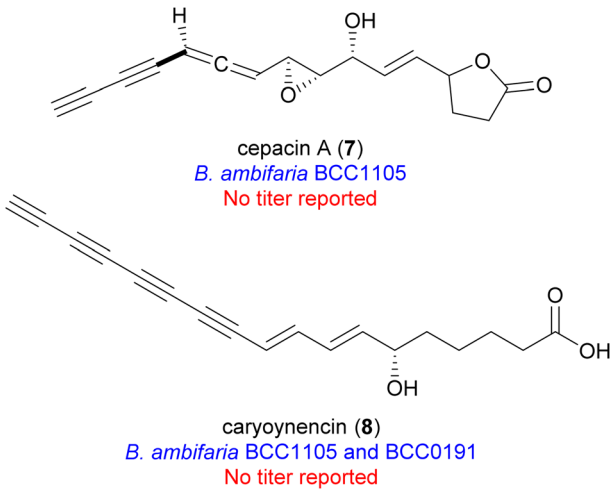

Fig. 3 Structures of the polyynes produced in *B. ambifaria* BCC1105 (**7**, **8**) and in *B. ambifaria* BCC0191 (**8**).

2.4. Polyketides (PKs)

Very few unique polyketides (PKs) have been produced in heterologous *Burkholderia* systems, with the notable exception of glutarimide-containing gladiofungins A, C and D-H. The first members of this family of *trans*-AT butanolide PKs were isolated from *B. gladioli* HK10739, a symbiont of the *Lagria villosa* beetle, in 2020 and found to be potent antifungals against both ascomycete and basidiomycete fungi representatives.[54] Chen and colleagues reported the isolation of five novel gladiofungins (D-H) (**9–13**) and two known ones (A and C) in 2023 *via* heterologous expression of the BGC from *B. gladioli* ATCC 10248 in *B. thailandensis* E264 (Fig. 4).[55] The E264 host strain had the efflux pump *oprC* replaced with a *Streptomyces* phage ϕC31 *attB* integration site for stable chromosomal localization and expression of larger BGCs.[56] The expression plasmid contained a corresponding ϕC31 *attP* site and integrase gene, a bacterial artificial chromosome (BAC) replicon functional in *E. coli*, and a constitutive P_{genta} promoter. Once again, product titers were not quantified, but it was noted that gladiofungin D-H levels were higher in the engineered E264 host than in another host tested (*Schlegelella brevitalea* DSM 7029, now *Caldimonas brevitalea*[57]) when both were cultured in CYMG complex media.[55]

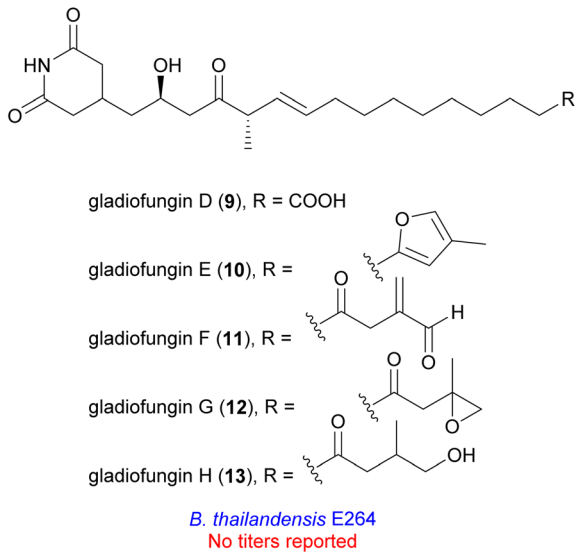

Fig. 4 Structures of the polyketide (PK) gladiofungins produced in *B. thailandensis* E264 Δ*oprC*::*attB*.

2.5. Nonribosomal peptides (NRPs)

Like PK gene clusters, nonribosomal peptide (NRP) clusters are often large and repetitive, making traditional cloning strategies difficult. For this reason, many researchers opt to use BAC vectors for maintenance in *E. coli* and to integrate heterologous BGCs into the genome of the new host instead of relying on self-replicating vectors. For example, the Bian group aimed to develop *B. gladioli* ATCC 10248 into a synthetic biology chassis by replacing the native gladiolin BGC (*gbn*) with an ϕC31 *attB* integration site. The modified host *B. gladioli Δgbn*::*attB* was then used for the production of three previously known types of lipopeptide NRPs, rhizomide A (**14**) and C8-rhizomide A (**15**), holrhizin A (**16**), and WAP-8294A1, A2 and A4 (**17–19**) (Fig. 5).[58]

The cyclic rhizomides were first isolated in 2018 by *in situ* promoter replacement in *M. rhizoxinica* HKI 454.[59] For heterologous expression, the native rhizomide BGC was cloned under the constitutive $P_{tn5\text{-}km}$ promoter in a ϕC31 integrative vector with a p15A *E. coli* replicon.[58] The cloned BGC contained a R149A mutation in the starter condensation domain,[60] leading to the production of both rhizomide A (**14**) and C8-rhizomide A (**15**) in M9 minimal media.[58] Holrhizin A (**16**), a linear lipopeptide surfactant, also derives from *M. rhizoxinica* HKI 454 and plays an important role in the colonization process of *Rhizopus* fungi by *Mycetohabitans* endosymbionts.[61] The same construct backbone and production medium as for rhizomide A were used. Products **14–16** were detected but not quantified.[58]

The WAP-8294A series of macrocyclic antibiotics are effective against methicillin-resistant *Staphylococcus aureus*, as noted upon their discovery in 1998.[62] Originally isolated from *Lysobacter* sp. WAP-8294, the BGC responsible for their expression is also found in *Lysobacter enzymogenes* Yc36, from where it was cloned. The expression strategy involved several different promoters. Notably, host choice was crucial for the detection of WAP-8294A1, A2 and A4 (**17–19**), as no products were observed in *B. thailandensis* E264 or *C. brevitalea* DSM 7029, both established Burkholderiales hosts.[58] This could be due to the Gammaproteobacterial source of the BGC. The presence of WAP-8294As was also dependent on growth medium, since a complex GBS broth had to be used. Promoters were modified iteratively to optimize titers, and the highest relative titers were obtained when *ORF5* was under P_{Apra} and *ORF3* was under P_{gbn}. In addition, two acyl-CoA ligases were overexpressed under the $P_{tn5\text{-}km}$ promoter and the pBBR1 replicon to increase relative WAP-8294As titers further, though no absolute quantification was performed.[58]

2.6. Polyketide-nonribosomal peptides (PK-NRPs)

The Bian group also expressed hybrid polyketide-nonribosomal peptides (PK-NRPs) as reported in 2023, including previously

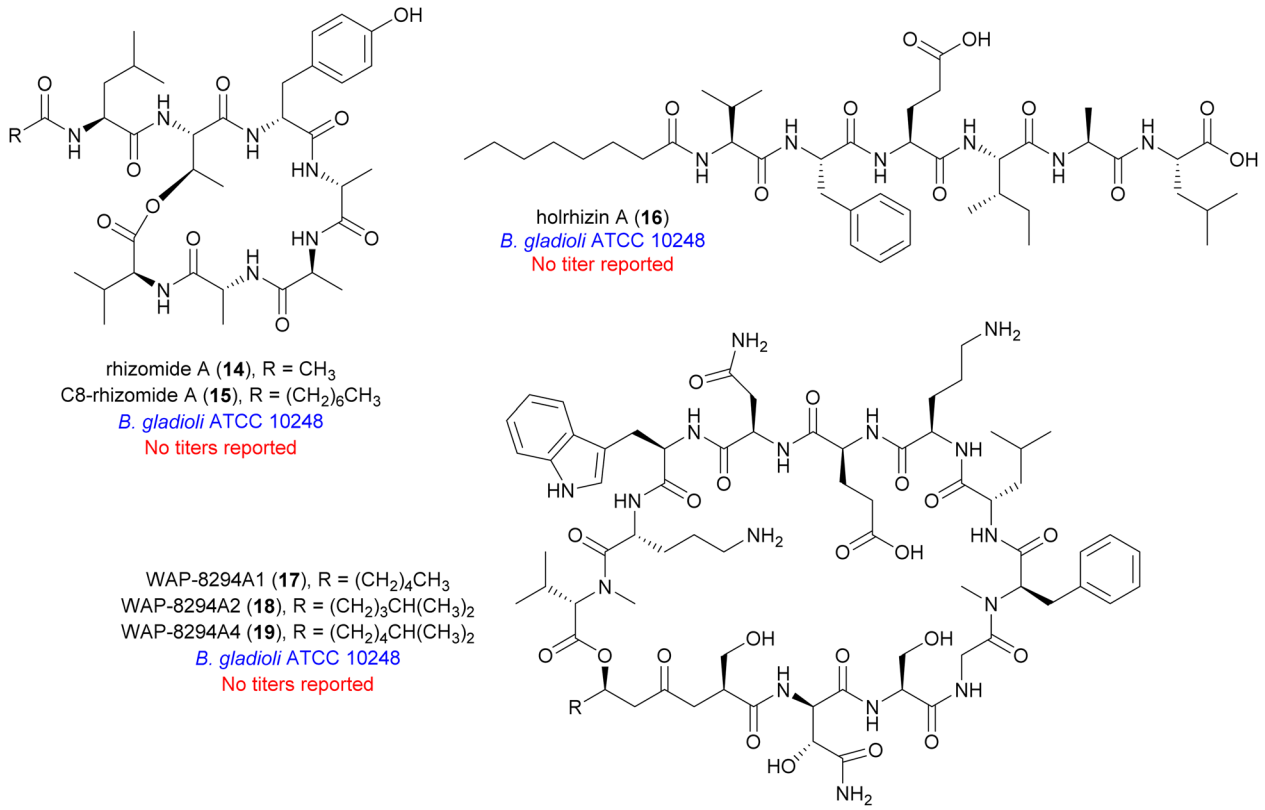

Fig. 5 Structures of the nonribosomal peptides (NRPs) produced in *B. gladioli* ATCC 10248 *Δgbn::attB*.

known thailandamide A (**20**), glidobactin A (**21**), luminmycin E (**22**), rhizoxins (**23**, **24**, **28**, and **29**), and disorazol F_2 (**30**), in their engineered *B. gladioli Δgbn::attB* heterologous host using M9 minimal medium (Fig. 6).[58] All of the explored BGCs derived from Betaproteobacteria except for the disorazol gene cluster, which is of myxobacterial origin. It should also be noted that of these compounds, glidobactin A and luminmycin E are derived from a *cis*-AT PKS-NRPS assembly line, whereas thailandamide A, rhizoxins, and disorazol F_2 are encoded by *trans*-AT PKS-NRPSs.

The antibiotic thailandamide A (**20**) was first reported in 2008 from *B. thailandensis* E264 during a study of *trans*-AT PKS-NRPS pathway evolution by the Piel group.[63] It inhibits fatty acid biosynthesis in both Gram-positive and Gram-negative bacteria.[64] The thailandamide A BGC was successfully expressed in the *B. gladioli Δgbn::attB* host using a ϕC31 integrative BAC vector and the constitutive $P_{tn5\text{-}km}$ promoter, which was notably unsuccessful in the alternative host *C. brevitalea* DSM 7029.[58]

Glidobactin A (**21**), an antitumor natural product that also shows broad antifungal activity, was reported in 1988 from extracts of *C. brevitalea* DSM 7029 (previously *Polyangium brachysporum*).[65] A linear congener, luminmycin E (**22**), discovered in 2014, had already been heterologously produced in *E. coli* Nissle.[66] Both **21** and **22** were produced with transcription driven from a P_{tet} promoter.[58]

Rhizoxins are a class of phytotoxins originally reported from *Rhizopus chinensis* in 1984,[7] and the family was later expanded with the discovery of the WF-1360 complex of antitumor antibiotics, derived from *Rhizopus* sp. No. F-1360.[67] As mentioned in the Introduction, the true rhizoxin producers are endophytic *Mycetohabitans* spp. bacteria.[8,68] Rhizoxins M1 (**23**), M2 (**24**), WF-1360B (**28**) and WF-1360F (**29**) were detected after heterologous expression of the corresponding BGC from *M. rhizoxinica* HKI 454 using the native promoters.[58]

Disorazol F_2 (**30**) is a potent antitumor natural product reported in 1994.[69] The disorazol BGC from the myxobacterium *Sorangium cellulosum* So ce12 (Myxococcota phylum) was expressed using a P_{tet} promoter.[58,70] Nonetheless, expression in *B. gladioli Δgbn::attB* (Pseudomonadota phylum) was fruitful. Heterologous titers of **20–24** and **28–30** were not reported (Fig. 6).[58]

Rhizoxins and disorazols have also been isolated from an engineered strain of *B. thailandensis* E264 that lacks three key efflux transporters (Δ*BAC::attB*) to render it susceptible to several antibiotics for synthetic biology purposes. The minimal disorazol BGC was cloned from its native *S. cellulosum* So ce12 into a construct containing a p15A replicon and likewise allowing for ϕC31 integration into the host genome. The highest titers of disorazol F_2 (**30**) (38.3 mg L^{-1}) were obtained in M9 minimal media after replacement of all four promoters driving *disABCD* expression with stronger host elements.[56] Though its production was not quantified, Wang and colleagues also endeavored to express the rhizoxin and shuangdaolide BGCs in the engineered E264 host; the former was successful, with masses corresponding to rhizoxins M1, M2, Z1, Z2 and/or S2 (**23–27**), while no peak corresponding to shuangdaolide could be detected.[56] This was likely due to the increasing

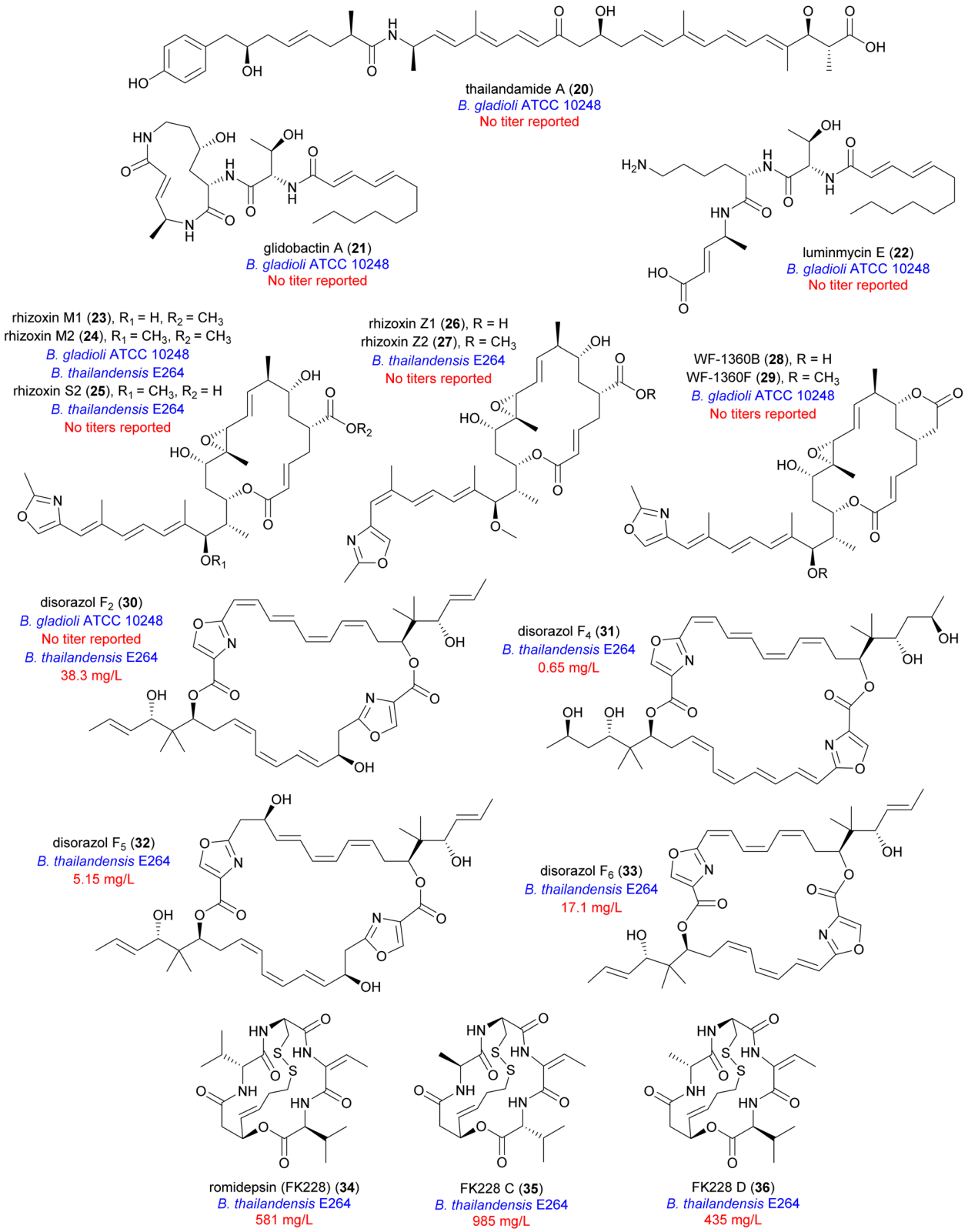

Fig. 6 Structures of the hybrid polyketide-nonribosomal peptides (PK-NRPs) produced in *B. gladioli* ATCC 10248 *Δgbn::attB* (**20**, **21**, **22**, **23**, **24**, **28**, **29**, **30**), *B. thailandensis* E264 Δ*BAC::attB* (**23**, **24**, **25**, **26**, **27**, **30**, **31**, **32**, **33**) and *B. thailandensis* E264 Δ*tdp::attB* (aka KOGC1) (**34**, **35**, **36**).

phylogenetic differences between the shuangdaolide source, *Streptomyces* sp. B59 (Actinomycetota phylum), and the *Burkholderia* host (Pseudomonadota phylum).

In a follow-up paper, the disorazol-producing *B. thailandensis* E264 Δ*BAC::attB* mutant was further engineered *via* domain inactivation and module deletion to generate new-to-nature

disorazols F_4, F_5 and F_6 (**31–33**) with variable tailoring and macrocycle sizes (Fig. 6). The highest titers of disorazol F_6 (17.1 mg L^{-1}) were achieved when PKS module 6 was deleted at both flanking acyl carrier protein-ketosynthase linkers (ΔM6C). Disorazols F_4 (dehydratase DH1 inactivation) and F_5 (DH7 inactivation) reached 0.65 mg L^{-1} and 5.15 mg L^{-1}, respectively.[71]

Perhaps the most famous *Burkholderia*-encoded PK-NRP is romidepsin (FK228) (**34**),[10] approved and marketed as Istodax® in 2011 for the treatment of peripheral and cutaneous T-cell lymphomas (Fig. 6).[72,73] Current industrial supply is provided by the native producer *C. violaceum* No. 968 (Betaproteobacteria class, Neisseriales order) with titer estimates approximating 19 mg L^{-1}, though proprietary industrial titers may be higher. In 2018, a new source was found in *B. thailandensis* MSMB43. Titers of romidepsin from this source peaked at 168.5 mg L^{-1} after optimization by fed-batch fermentation, use of M8 defined medium, and recombinant expression of the thailandepsin regulator *tdpR* from *B. thailandensis* E264 under the IPTG-inducible P_{lac} promoter using a pBBR1 replicon.[74] New romidepsin derivatives were isolated in significant titers in 2023 following heterologous production of a hybrid combinatorial BGC in *B. thailandensis* E264 Δ*tdp::attB* (aka KOGC1). The wild-type strain was modified by replacing the autologous thailandepsin BGC (*tdp*) with an ϕC31 *attB* site. Synthetic BGCs were prepared by recombineering modules of the romidepsin and thailandepsin BGCs derived from *C. violaceum* No. 968 and *B. thailandensis* E264, respectively, before introduction to the KOGC1 host.[75] It was this strain that showed the highest reported titer of romidepsin to date, at 581 mg L^{-1} in M9 minimal media. Additional engineering provided six new-to-nature derivatives, two of which, FK228 C (**35**) and D (**36**), reached up to 985 and 453 mg L^{-1}, respectively, and displayed stronger cytotoxicity than the parent compound.[75]

2.7. Polyketide-nonribosomal peptide-polyunsaturated fatty acids (PK-NRP-PUFAs)

The final case study we present is that of the discovery of megapolipeptins A (**37**) and B (**38**), unique hybrid molecules that are part PK-NRP and part polyunsaturated fatty acid (PUFA) reported in 2024 (Fig. 7). The orphan BGC was found in the genomes of *Paraburkholderia megapolitana* strains but appeared to be silent under laboratory conditions, and previous work led to the hypothesis that yields would be low even if the cluster could be activated.[76,77] Therefore, the *mgp* BGC was cloned from *P. megapolitana* RL18-039-BIC-B,[77] using a CRISPR-Cas9 strategy[78] into a BAC vector under *araC*/P_{BAD} control and transferred into a spliceostatin deficient strain of *B.* sp. FERM BP-3421 (Δ*fr9A*) for integration *via Pseudomonas* phage ϕCTX *attB*.[79] When the strain was grown in complex 2S4G media, 0.6 and 1.5 mg L^{-1} were obtained for megapolipeptins A and B, respectively. No antimicrobial activity was detected and the bioactivity of megapolipeptins remains to be identified.[77]

3. Comparison of *Burkholderia* hosts and their development

Based on large-scale studies that tested the expression of tens of BGCs, the success rate of heterologous expression only reaches up to ~30% when using one host.[3,80–83] The choice of host can impact success. Although exceptions exist, it has been shown that, in general, the greater the DNA sequence identity between source strain and host, the higher the success rate in terms of the amount and number of products detected.[4] Thus, to discover Burkholderiales natural products *via* heterologous expression, *Burkholderia* hosts are advantageous. In choosing *Burkholderia* hosts (Table 1), several factors need to be considered, including genetic tractability, the availability of genetic tools such as vectors and gene "parts" for BGC refactoring (*e.g.*, promoters), and lack of virulence. Virulence is of particular concern in this case because some *Burkholderia* species are known to be pathogenic to humans and other animals (*e.g.*, *Burkholderia mallei*/*pseudomallei*, and *B. cepacia* complex)[84–89] or to plants and mushrooms (*B. glumae*, *B. gladioli*).[90–96] Another factor is the host's intrinsic genetic and metabolic abilities to maintain and express large and/or complex BGCs which can be

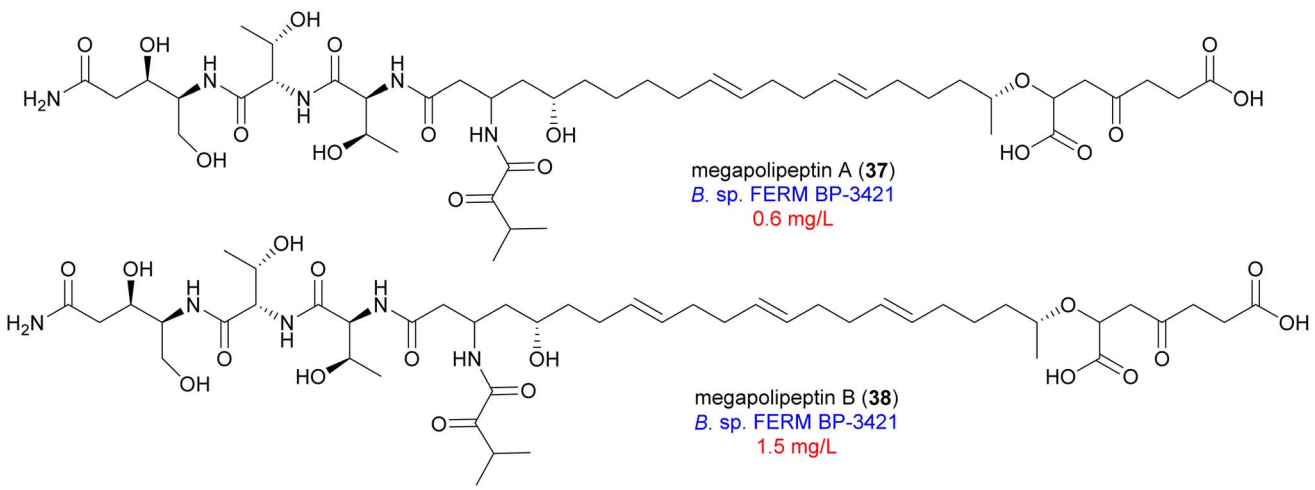

Fig. 7 Structures of the hybrid polyketide-nonribosomal peptide-polyunsaturated fatty acids (PK-NRP-PUFAs) produced in *Burkholderia* sp. FERM BP-3421 Δ*fr9A*.

Table 1 Current *Burkholderia* hosts and their stage of development[a]

Heterologous host	Genome modification(s)	DNA transfer methods used	Expression tools used	Biosynthetic range tested	Source BGC range tested	Best titer	Virulence	Ref.
Burkholderia glumae BGR1	Rhamnolipid *rhlA*$^{-}$ mutant	Conjugation	pBBR1 replicon, BGC P_{rhlA}	Rhamnolipid precursors	Gammaproteobacteria	NR	Rice pathogen	35
Burkholderia gladioli pv *agaricicola* HKI0676	None	Conjugation	pBBR1 replicon, L-arabinose inducible *araC*/P_{BAD}	RiPPs	Betaproteobacteria	6 mg L^{-1} burhizin-23 (**5**) D21N	Plant and mushroom pathogen	48
Burkholderia ambifaria BCC1105 and BCC0191	None	Conjugation	pBBR1 replicon, BGC promoters	Polyynes	Betaproteobacteria	NR	*B. cepacia* complex, previously used as a biopesticide (BCC0191)	53
Burkholderia thailandensis E264	PK-NRP thailandepsin Δ*tdp::attB* mutant (KOGC1), efflux Δ*BAC::attB* and Δ*oprC::attB* mutants	Conjugation electroporation	ϕC31 integrative vectors, constitutive P_{genta}, E264 autologous promoters	PKs PK-NRPs	Betaproteobacteria Myxococcia	985 mg L^{-1} FK228 C (**35**)	Low virulence to humans and animals	55, 56, 71 and 75
Burkholderia gladioli ATCC 10248	PK gladiolin Δ*gbn::attB* mutant	Conjugation electroporation	ϕC31 integrative vectors, constitutive $P_{tn5\text{-}km}$, P_{pra}, P_{Kan}, and P_{tet}, autologous P_{gbn}	NRPs PK-NRPs	Betaproteobacteria Gammaproteobacteria Myxococcia	NR	Plant pathogen	58
Burkholderia sp. FERM BP-3421	PK-NRP spliceostatin Δ*fr9DEF::tet,* Δ*fr9A,* and ΔP_{fr9C} mutants	Conjugation electroporation mimicry by methylation	pRO1600, pBBR1 and pBBR1 (Rep G159S) replicons, ϕCTX integrative vectors, L-arabinose inducible *araC*/P_{BAD}, L-rhamnose inducible *rhaRS*/P_{rhaB}, autologous constitutive P_{S7}, autologous *ORF-1–fr9A*/P_{fr9C} system	RiPPs PK-NRP-PUFAs	Betaproteobacteria	240 mg L^{-1} capistruin (**2**)	Unknown permissive growth temperature <35 °C	42, 44, 46, 49 and 77

[a] NR, not reported.

Table 2 Genomic and natural product information for current *Burkholderia* hosts. BGC type was determined using antiSMASH 8.0.1,[121] and the BGC number was manually curated to account for superclusters (BGCs that fall next to each other and are counted as one region)[a]

Heterologous host	Genome availability (accession #s)	Genome size (organization)	# of BGC regions	# of BGCs	BGC types	Isolated or detected products	Ref.
Burkholderia glumae BGR1	Yes (CP001503–CP001508)	7.28 Mbp (two chromosomes, four plasmids)	17	21	PKS, NRPS, PKS-NRPS, RiPP, terpene, phosphonate, homoserine lactone, arylpolyene, phenazine, other	Toxoflavin, fervenulin, reumycin	92 and 114
Burkholderia gladioli pv *agaricicola* HKI0676	Sequenced but not publicly available	N/A	N/A	N/A	PKS, NRPS, PKS-NRPS, RiPP, terpene, other	Haereogladin A, burriogladin A, icosalide A1, gladiobactin, gladiofungin A, toxoflavin, caryoynencin, sinapigladioside	122
Burkholderia ambifaria BCC0191	Yes (CP142981–CP142983)	7.62 Mbp (two chromosomes, one plasmid)	22	25	PKS, NRPS, PKS-NRPS, RiPP, terpene, phosphonate, homoserine lactone, butyrolactone, arylpolyene, phenazine, other	Cepacin, pyrrolnitrin, burkholdines	89 and 123
Burkholderia thailandensis E264	Yes (CP000085 and CP000086)	6.72 Mbp (two chromosomes)	20	24	PKS, NRPS, PKS-NRPS, RiPP, terpene, homoserine lactone, other	Rhamnolipids, hydroxyalkylquinolines, bactobolins, thailandamide, thailandepsins, thailandenes, malleilactone A, capistruin, burkholdacs, bis-bukrholdacs, acybolins, *N*-acyl homoserine lactones, *N*-acyl anthranilates, terphenyl	38, 63, 106, 118, 120 and 124–134
Burkholderia gladioli ATCC 10248	Yes (CP009319–CP009323)	8.90 Mbp (two chromosomes, three plasmids)	23	26	PKS, NRPS, PKS-NRPS, RiPP, terpene, phosphonate, homoserine lactone, other	Burriogladiodins, haereogladiodins, gladiofungins	55, 116 and 135
Burkholderia sp. FERM BP-3421	Yes (CP117779–CP117782)	7.73 Mbp (two chromosomes, two plasmids)	31	33	PKS, NRPS, PKS-NRPS, RiPP, terpene, phosphonate, homoserine lactone, ectoine, other	Spliceostatins/thailanstatins, selethramide, romidepsin, aminopyrrolnitrin	105, 115 and 136

[a] N/A, not applicable (we were unable to run the analysis because the genome is not publicly available).

predicted from autologous BGCs and known natural products (Table 2).

The proposed development continuum of heterologous hosts described by de Lorenzo and colleagues starts with recombinant DNA (rDNA) hosts, moving to synthetic biology (SynBio) chassis and arriving at standardized SynBio chassis (Fig. 8).[97] Here we will discuss where along this roadmap each of the six current hosts falls, what criteria they have already met, and what the future of *Burkholderia* chassis development should prioritize. It should be noted that in line with the "one host does not fit all" mindset, there may be different expression platforms that better support certain natural product classes or BGC sources, and we hope to provide clarity on what is currently known about each strain's unique qualifications.

3.1. Comparison of *Burkholderia* hosts to other common hosts

There are many hosts, both prokaryotic and eukaryotic, that have been used for the heterologous expression of a wide variety of molecules of interest.[32] For bacterial natural product discovery, the most prevalent host strains are gram-negative *E. coli* and gram-positive *Streptomyces* spp., though another notable gram-negative host is *Pseudomonas putida*.[31,32]

Though there are several advantages to using *E. coli* as a host, namely its rapid growth rate, wealth of available genetic tools, and existing metabolic models,[29] it also has limitations that are not yet fully understood. For example, despite the tendency to use *E. coli* for heterologous expression of lasso peptides regardless of the source taxa,[3] *Burkholderia* sp. FERM BP-3421 is a better host for at least some Burkholderiales RiPP BGCs, reflecting the close phylogenetic relationship between source DNA and host (Table 1 and ESI Table S1†).[44] *P. putida* is another γ-Proteobacterium that has seen significant investment in development for the production of industrially relevant chemicals as well as natural products.[98,99] Of note, *P. putida* displays a natural tolerance for stress,[100] and it has been engineered for improved endurance[101] and genome streamlining.[31] Although we are not aware of any side by side comparisons between *Burkholderia* hosts and *P. putida*, the latter may serve as inspiration for the further development of *Burkholderia* hosts.

The use of *Streptomyces* spp. hosts for natural product discovery is often dictated by the abundance of natural product BGCs in members of this well-studied group. Indeed, the Streptomycetales order is the most biosynthetically diverse, with Burkholderiales following in third place based on available genomes.[5] Because of their prolific arsenals, significant effort has optimized *Streptomyces* spp. hosts and they have been widely applied.[102–104] In comparison, *Burkholderia* hosts are in their developmental infancy, but some (*i.e. B.* sp. FERM BP-3421 and *B. thailandensis* E264) have shown significant promise for high yield production of drug leads.[41,75]

3.2. *Burkholderia* host development status

3.2.1. rDNA host requirements. The requirements for rDNA hosts are exogenous DNA uptake, no virulence, and genetic tools (Fig. 8).[97] Electroporation and conjugation from *E. coli* are two common methods of DNA transfer into Pseudomonadota (previously Proteobacteria). For the hosts described here, conjugation from various *E. coli* strains seems to be the preferred method for DNA transfer into *Burkholderia* as it helps bypass the host's innate restriction-modification systems and enable the transfer of large plasmids.[53,55,56,58,71,77] Moreover, electroporation protocols have been reported for *B. gladioli* ATCC 10248,[58] *B. thailandensis* E264,[75] and *B.* sp FERM BP-3421,[42] though plasmid size often limits success. Finally, for *B.* sp. FERM BP-3421, DNA transfer efficiency was improved by identifying restriction-modification systems and harnessing the host's DNA methyltransferases in a mimicry-by-methylation strategy.[105]

Regarding virulence, *B. thailandensis* is a low virulence species often used as a model for the *B. pseudomallei* human pathogen to which it is closely related.[106] *B. thailandensis* is rarely pathogenic to humans or animals;[107] however, infections in humans with strains identified as *B. thailandensis* have been documented.[108–111] *B. glumae* BGR1 is pathogenic to rice plants and other crops causing grain and seedling rot.[90–93] *B. gladioli* is also a plant pathogen,[94,95] but it is isolated less frequently than *B. glumae*.[93] *B. gladioli* pv *agaricicola* primarily infects mushrooms.[96] *B. ambifaria* belongs to the *B. cepacia* complex that includes opportunistic human pathogens but also some biocontrol strains.[86–88] *B. ambifaria* BCC0191 was used commercially in the USA as a biopesticide but was later withdrawn due to safety concerns.[89] *B.* sp. FERM BP-3421 (ref. 105) is closely related to *Burkholderia rinojensis* A396 which has been

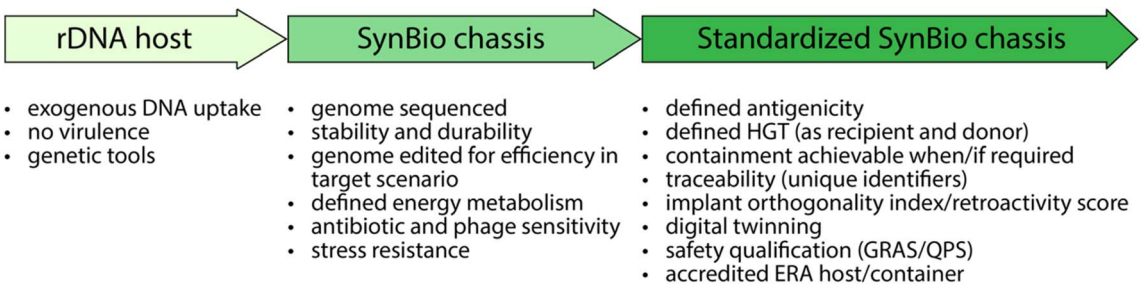

Fig. 8 Schematic of host development timeline and stages (adapted from ref. 97). rDNA, recombinant DNA. SynBio, synthetic biology. HGT, horizontal gene transfer. GRAS, generally regarded as safe, as defined by the US FDA. QPS, qualified presumption of safety, as defined by the European Food Safety Authority. ERA, environmental risk assessment.

investigated as a biocontrol agent effective against plant, insect and mite pests[112] and as a herbicide. The herbicidal activity is in part due to romidepsin production.[113] Although the plant pathogenicity or biocontrol potential of *B.* sp. FERM BP-3421 is unknown, this strain does not grow at 37 °C (permissive growth temperature up to 35 °C),[15] which reduces the potential pathogenicity to humans.

In terms of genetic tools, replicative vectors based on the broad host range pBBR1 replicon are the most popular to express smaller BGCs such as those encoding RiPPs and polyynes.[35,44,46,48,49,53] Moreover, a study from Fernandez and colleagues probed the effect of plasmid copy number on bacterial growth and lasso peptide production using the FERM BP-3421 host. They showed that reducing the copy number of the pBBR1 replicon *via* a point mutation alleviates a growth defect and can impact product titers.[44] In contrast, integrative vectors were frequently used for larger assembly line BGCs (Table 1 and ESI Table S1†). Site-specific integration was based either on *int*/*attP* of *Streptomyces* phage ϕC31 after introduction of the corresponding *attB* site in the genome of the host[55,56,58,71,75] or *int*/*attP* of *Pseudomonas* phage ϕCTX[79] without host modification.[77]

Native BGC promoters were used only in a few instances.[35,53,56,75] Mostly, BGCs were modified to contain either constitutive[55,58] or inducible promoters.[42,44,46,48,49,77] Three studies compared natural product titers using different promoters. Adaikpoh and colleagues showed that the L-arabinose inducible *araC*/P_{BAD} system outperformed the native spliceostatin BGC promoter P_{fr9C}, whereas P_{fr9C} outperformed the native P_{S7} ribosomal protein promoter for RiPP capistruin production in *B.* sp. FERM BP-3421.[46] Bai and colleagues compared the ability of different promoters to drive the transcription of the *waps* genes and observed the highest titers of WAP-8294As (**17–19**) when the combination P_{Apra}-*ORF5*-P_{gbn}-*ORF3* was used, where P_{Apra} is a constitutive promoter from an apramycin resistance gene and P_{gbn} is an autologous promoter from the gladiolin BGC.[58] Wang and colleagues compared six native promoters for PK-NRP disorazol F_2 production, identifying P46 from a DUF4148 domain-containing protein as resulting in the highest titers.[56]

For a detailed report on the synthetic biology tools available for engineering *Burkholderia* spp., see the review by Adaikpoh and colleagues.[17] For a comprehensive look at cloning techniques for any host, see the review by Seshadri and colleagues.[32]

3.2.2. SynBio chassis progress. All strains meet the first requirement for a SynBio chassis for having their genomes sequenced (Fig. 8 and Table 2), though the genome of *B. gladioli* pv *agaricicola* HKI0676 was not publicly available at the time of writing.[89,105,106,114–116] In terms of genome editing, no modifications were made to *B. ambifaria*[53] and *B. gladioli* pv *agaricicola* HKI0676,[48] and the only modification made to *B. glumae* BGR1 was deletion of its autologous copy of *rhlA* to support the production of rhamnolipid precursors (**1**).[35] In contrast, three *Burkholderia* spp. hosts have undergone some level of generalizable genome optimization, placing them more firmly on the roadmap of SynBio chassis development, *B. thailandensis* E264, *B. gladioli* ATCC 10248, and *B.* sp. FERM BP-3421. These three host strains have also been shown to successfully produce more than one biosynthetic class of natural product, some from diverse source taxa (Table 1). *B. gladioli* ATCC 10248 was only tested with known NRPs and PK-NRPs thus far, whereas *B. thailandensis* E264 was used to produce known and new analogues of PKs and PK-NRPs, and *B.* sp. FERM BP-3421 was used to produce known and new RiPPs and new PK-NRP-PUFAs (Table 1).

B. thailandensis E264 contains several autologous BGCs of interest and is therefore often used as the source for natural product discovery (Table 2). As a host, some key mutations have been made to this strain that have enabled its success. First, the autologous thailandepsin BGC was deleted and replaced with a ϕC31 *attB* integration site to support site-specific integration of heterologous constructs (strain KOGC1). The introduction of recombineered hybrid BGCs (thailandepsin and romidepsin) from two different bacterial sources into KOGC1 enabled the production of up to 581 mg L^{-1} romidepsin (FK228, **34**), the best titer reported to date, and up to 985 mg L^{-1} of FK228 C (**35**).[75] It is important to note though that thailandepsins and romidepsin are structurally related, so the host was heterologously making something very similar to its autologous products, possibly explaining the high titers.

Another strategy for *B. thailandensis* E264 development has involved sequential deletion of efflux transporters to make it more sensitive to some antibiotics, facilitating further engineering. For example, the efflux mutant Δ*oprC::attB* was able to aid in the discovery of new gladiofungins D-H (**9–13**),[55] while the triple efflux mutant Δ*BAC::attB* enabled both improved yield of disorazol F_2 (**30**)[56] and engineering of new disorazols (**31–33**).[71] This disorazol work is notable for its success in generating myxobacterial products in a *Burkholderia* host. These advances make it clear that *B. thailandensis* E264 is well on its way to SynBio chassis development. One concern is with regards to the low (but not completely absent) virulence of this strain to humans and animals (LD_{50} of E264 in mice has been reported as 3×10^7 colony forming units) which would need to be addressed by *e.g.*, identifying and removing virulence factors such as malleilactone.[117–120]

B. gladioli ATCC 10248 has been minimally modified but widely tested for natural product production. Bai and colleagues replaced the gladiolin BGC with an ϕC31 *attB* integration site, allowing them to produce 14 compounds (6 lipopeptide NRPs and 8 hybrid PK-NRPs) in this host.[58] While none of these were new molecules and no titers were quantified, their BGCs came from a range of Beta- and Gammaproteobacterial and myxobacterial sources, highlighting the versatility of their host. Thus, *B. gladioli Δgbn::attB* shows promise in its ongoing synthetic biology development. However, its plant pathogenicity would need to be addressed (Table 1).

Burkholderia sp. FERM BP-3421 has been the subject of iterative and ongoing engineering aimed at facilitating its use by improving DNA transfer efficiency[105] and product titers.[42,44,46,77] The wild-type strain produces up to 6 g L^{-1} of autologous spliceostatin congeners, and deletion of the corresponding PKS-NRPS core machinery by replacement with a tetracycline-selectable marker (Δ*fr9DEF::tet* mutant) improved

yields of heterologous capistruin (**2**) 4-fold in complex media.[42,43] Follow-up work generated two more spliceostatin deficient strains, mutants Δ*fr9A* and ΔP_{fr9C}. Although capistruin levels were not better than those previously reported, these are markerless strains, facilitating their use as hosts.[46] The Δ*fr9A* mutant was then used for heterologous expression of an orphan BGC from *P. megapolitana* resulting in the discovery of megapolipeptins **37** and **38**.[77] Although megapolipeptins isolated titers were modest at 2.1 mg L^{-1} combined, the heterologous platform enabled the discovery of natural products from an otherwise silent BGC. FERM BP-3421 has also been used to produce known capistruin at up to 240 mg L^{-1} and to discover new RiPPs mycetolassins **3** and **4**,[44] and the aminopyruvatides (**6**) (ESI Table S1†).[49] This strain has only been reported to express Betaproteobacterial BGCs to date but studies are ongoing to test other sources. The plant pathogenicity or biocontrol potential of FERM BP-3421 has not been explored, but the inability of this strain to grow at 37 °C reduces the chance of pathogenicity to humans.

3.3. Outlook on development, challenges and opportunities

From the six hosts discussed above, only three strains, *B. thailandensis* E264, *B. gladioli* ATCC 10248, and *B.* sp. FERM BP-3421, advanced to generalizable genome editing to either facilitate construct integration, improve antibiotic sensitivity or improve product titers. A side-by-side empirical comparison of these three hosts in terms of success rate and product titers using the same BGCs would be helpful in exposing their strengths and weaknesses.

None of the currently available *Burkholderia* spp. hosts have yet reached the status of a bona-fide, SynBio chassis or a standardized SynBio chassis en route to regulatory approval as a synthetic biology agent (Fig. 8).[97] In addition to further genome and tool development, a SynBio chassis would still require knowledge regarding stability and durability (robustness), global energy metabolism, antibiotic and phage sensitivity, and stress resistance mechanisms.

Resilience in the environment relies on population diversity. Genotypic and phenotypic heterogeneity within clonal populations can serve either as a division of labor or as a bet-hedging strategy to increase the chance of survival following environmental changes.[137,138] For example, in *B. thailandensis* E264 it has been shown that a reversible, RecA-mediated recombination of homologous insertion sequences results in the amplification of 157 genes. A single copy of the DNA region is preferred during planktonic growth, whereas two or more copies are advantageous in biofilms.[139] We also observed phenotypic heterogeneity with FERM BP-3421. When testing it as a host to produce lasso peptide capistruin, we isolated low and high producer clones, although the exact mechanism remains unresolved.[42] Understanding the reasons for heterogeneity will help generate more stable hosts.[140] To start, approaches previously used in other hosts could be applied to *Burkholderia* spp. such as the deletion of mobile elements (transposases, insertion sequences) and of prophages such as done for the development of *C. brevitalea* DSM 7029 by Liu and colleagues.[141]

Further, metabolic models would be helpful to predict changes in metabolism upon the introduction of a new pathway, helping to optimize product yields and predict the impact of genome reduction.[142,143] Sensitivity to antibiotics and phages is a requirement as a preventive tool if emergency clearance is needed. *B. thailandensis* E264 is the only of the three developed hosts that was engineered to remove efflux transporters and render it susceptible to several antibiotics. Similar engineering could be performed in other hosts. Moreover, phage defense systems could be targeted to increase phage sensitivity.

The expression of heterologous genes may induce a stress response or metabolic burden that can manifest as decreased growth and genetic instability.[144,145] Some bacterial hosts, such as *P. putida*, are naturally stress tolerant,[100] and it was shown that endurance can be further engineered by *e.g.* removing phage-related functions and flagella-related genes that deplete ATP and NAD(P)H.[146,147] The deletion of mobile elements and flagella-related genes also improved the performance of the Burkholderiales host *C. brevitalea* DSM 7029.[141] Tolerance to fermentation stresses such as the final product, pH, and salt, can also be engineered or evolved.[101,148,149] More broadly, the removal of non-essential genes can also improve chassis efficiency, with efforts to minimize and streamline genomes showing promise.[150] However, care should be taken that the modification of complicated and intertwined regulatory or metabolic processes does not impact downstream small molecule production.

In terms of a standardized SynBio chassis, the most important aspect is safety qualifications. If synthetic biology platforms are to become valuable industrial tools, they must clear rigorous regulatory hurdles. For some sectors, this will require recognition by either the US FDA as "generally regarded as safe" (GRAS) or the European Food Safety Authority as "qualified presumption of safety" (QPS). In most cases, environmental risk assessments will be necessary, which may be expedited by defining a limited number of chassis for specific applications and then modifying them only by genetic implants.[97] Living microbes being deployed as synthetic biology agents will also require barcoding for detection and containment measures, and digital twinning can be implemented in a manner analogous to software development to keep a record of strain information, modifications, and safety measures.[97,151] Efforts to improve chassis biosafety should include the removal of known virulence factors, the monitoring of horizontal gene transfer events, and continued pathogenicity testing.

4. Conclusions

New drugs with unique mechanisms of action are desperately needed in our constant arms race against drug resistance, and natural products provide diverse chemical scaffolds to reach molecular targets of interest. Despite our ability to mine thousands of BGCs from a wealth of genomic data, many are not transcriptionally active in the laboratory. Heterologous

expression presents new opportunities for pathway modification, metabolic optimization, analogue generation, and yield improvement by employing an engineered host strain. Due to their autologous bioactive natural product arsenals (Table 2), *Burkholderia* spp. bacteria have recently emerged as promising heterologous hosts, particularly for the expression of BGCs from other Burkholderiales bacteria. In this review, we have summarized natural products successfully isolated from *Burkholderia* spp. heterologous hosts up until 2024 (ESI Table S1†) and discussed ongoing host development efforts (Table 1) to improve access to natural products in titers sufficient for drug development and industrial applications. Empirical comparison of the three most developed hosts would help expose their strengths and weaknesses to select candidate(s) to move to standardized SynBio chassis development and clear safety hurdles.

5. Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

6. Conflicts of interest

There are no conflicts to declare.

7. Acknowledgments

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