Mining and characterization of the PKS–NRPS hybrid for epicoccamide A: a mannosylated tetramate derivative from Epicoccum sp. CPCC 400996

Tao Zhang1*, Guowei Cai1,2, Xiaoting Rong13, Jingwen Xu1, Bingya Jiang1, Hao Wang1, Xinxin Li1, Lu Wang1, Ran Zhang1, Wenni He1* and Liyan Yu1*

Abstract

Background: Genomic analysis indicated that the genomes of ascomycetes might carry dozens of biosynthetic gene clusters (BGCs), yet many clusters have remained enigmatic. The ascomycete genus Epicoccum, belonging to the family Didymellaceae, is ubiquitous that colonizes different types of substrates and is associated with phyllosphere or decaying vegetation. Species of this genus are prolific producers of bioactive substances. The epicoccamides, as biosynthetically distinct mannosylated tetramate, were first isolated in 2003 from Epicoccum sp. In this study, using a combination of genome mining, chemical identification, genetic deletion, and bioinformatic analysis, we identified the required BGC epi responsible for epicoccamide A biosynthesis in Epicoccum sp. CPCC 400996.

Results: The unconventional biosynthetic gene cluster epi was obtained from an endophyte Epicoccum sp. CPCC 400996 through AntiSMASH-based genome mining. The cluster epi includes six putative open reading frames (epiA-epiF) altogether, in which the epiA encodes a tetramate-forming polyketide synthase and nonribosomal peptide synthetases (PKS–NRPS hybrid). Sequence alignments and bioinformatic analysis to other metabolic pathways of fungal tetramates, we proposed that the gene cluster epi could be involved in generating epicoccamides. Genetic knockout of epiA completely abolished the biosynthesis of epicoccamide A (1), thereby establishing the correlation between the BGC epi and biosynthesis of epicoccamide A. Bioinformatic adenylation domain signature analysis of EpiA and other fungal PKS-NRPSs (NRPs) indicated that the EpiA is l-alanine incorporating tetramates megasynthase. Furthermore, based on the molecular structures of epicoccamide A and deduced gene functions of the cluster epi, a hypothetic metabolic pathway for biosynthesizing compound 1 was proposed. The corresponding tetramates releasing during epicoccamide A biosynthesis was catalyzed through Dieckmann-type cyclization, in which the reductive (R) domain residing in terminal module of EpiA accomplished the conversion. These results unveiled the underlying mechanism of epicoccamides biosynthesis and these findings might provide opportunities for derivatization of epicoccamides or generation of new chemical entities.

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
**Background**
Filamentous fungi have been demonstrated to be attractive and rich repertoires for pharmaceutical drugs in human history [1, 2]. However, it is becoming challenging to acquire novel lead compounds with traditional chemical-only methodologies for high frequency of rediscovery of known molecules [3]. Thus, several approaches have been employed to activate cryptic biosynthetic pathways, including “OSMAC” approach [4, 5], co-culture [6, 7], and chemical epigenetic agents supplement [8, 9]. Additionally, genome sequencing has revolutionized natural products (NPs) exploitation endeavor, demonstrating that the capacity of filamentous fungi to yield compounds was far more than we anticipated [2, 10]. Meanwhile, we could now easily access to the sequenced genome of targeted microorganism whose metabolites have not been, or rarely, characterized. Genome-guided mining approach is usually conducted to set up the linking between secondary metabolites identification and targeted biosynthetic gene clusters (BGCs) verification [11, 12]. Also, previous studies have demonstrated that genome mining is an effective approach that facilitated exploitation for new metabolites with fascinating structures, and numerous BGCs were investigated [10, 13, 14]. Thus, the genome-guided metabolomic analysis strategy renders the bioprospecting of bioactive compounds effective and promising.

Tetramic acid derivatives bearing pyrrolidine-2, 4-dione ring system, comprise a structurally unique family of secondary metabolites (SMs) (Fig. 1) [15]. Naturally occurring tetramates have attracted increasing interest from pharmaceutical and research communities due to their diverse bioactivities including antibacterial, fungicidal, antiviral, and cytotoxic potencies [16–18]. Bio-synthetically, most tetramate derivatives are originated from polyketide synthase-nonribosomal peptide synthase (PKS−NRPSs) hybrid pathways, and these polyketide−peptide compounds are generated from Dieckmann

**Conclusion:** Genome mining and genetic inactivation experiments unveiled a previously uncharacterized PKS−NRPS hybrid-based BGC epi responsible for the generation of epicoccamide A (1) in endophyte Epicoccum sp. CPCC 400996. In addition, based on the gene cluster data, a hypothetical biosynthetic pathway of epicoccamide A was proposed.

**Keywords:** Epicoccamide, Genome mining, PKS−NRPS hybrid, Tetramates, Biosynthesis, Epicoccum

![Fig. 1](image-url) 
**Fig. 1** Representatives of naturally occurring tetramic acid derivatives. The pyrrolidine-2, 4-dione ring unit is in red.
condensation of an amino acid (or nonproteinogenic) to a polyketide-derived acyl chain [16, 19]. Recently, post-genomic analysis accelerated the biosynthetic studies on this class of SMs, and numerous tetramate-forming biocatalysts have been elucidated, as exemplified by aspyridone [20], tenellin [21], tirandamycin [15], cyclopiazonic acid [22], fusarisetin A [23], pseudoturin [24], streptolydigin [25], pyranovolin [26], oxaleimide A [27], burnettramic acid [28], and calipryridone [29]. Although these studies revealed the molecular bases for these tetramates and demonstrated that diverse arrays of fascinating biocatalysts are the key components for their structural diversification [16, 18]. However, many biosynthetic reactions still remain to be unveiled.

_Epicoccum_ genus is distributed widespread, which has been frequently isolated as endophytes residing in medicinal plants [30] and found to live in association with other organism [31, 32]. Species from this genus are prolific producers of bioactive secondary metabolites, including epicolorazines, (dithio)diketopiperazines, triornicin, flavipins, epicoconigrones, epicocecolides, epicocconone, and epicolactone dimers (beetleane A, epicoane A) [14, 31, 33–35]. The metabolites isolated from _Epicoccum_ strains, cover a diverse array of bioactivities including strong inhibitors of liver fibrosis, cytotoxic and antimicrobial activities, making them potential drug candidates [31]. The epicoconamides, as biosynthetically distinct tetramic acid glycoside, were first isolated in 2003 from a jellyfish-derived culture of _E. purpurascens_ and later identified in _Epicoccum_ sp. [36] However, the genetic basis of this metabolite has been elusive and needs to be investigated. Recently, Li and coworkers characterized a gene cluster _bua_ involving in the burnettramic acid generation from _Aspergillus burnettii_, which revealed that the PKS–NRPS hybrid BuaA and the mannoyltransferase BuaB functioned in the formation of proline-incorporating NRPS hybrid BuaA and the mannoyltransferase BuaB from this metabolite has been elusive and needs to be investigated. Recently, Li and coworkers characterized a gene cluster _bua_ involving in the burnettramic acid generation from _Aspergillus burnettii_, which revealed that the PKS–NRPS hybrid BuaA and the mannoyltransferase BuaB functioned in the formation of proline-incorporating NRPS hybrid BuaA and the mannoyltransferase BuaB.

**Materials and methods**

**Strains, plasmids, and culture**
The fungus _Epicoccum_ sp. CPCC 400996 was isolated from the medicinal plant, deposited in the China Pharmaceutical Culture Collection Center (CPCC), and used as the parental strain. The strain was routinely cultured on potato dextrose medium (PDA; BD company) under conditions at 28 °C. _Escherichia coli_ DH5α (GenScript) was used for DNA molecular cloning. _Agrobacterium tumefaciens_ strain AGL-1 was employed as helper strain during _A. tumefaciens_-mediated transformation of fungal _Epicoccum_. The induction medium (IM) utilized for coculture experiments was prepared as described elsewhere [37]. The strains, plasmids, primers, and derived mutants are listed in Table 1 and preserved in 15% glycerol at −80 °C. Large scale fermentation of _Epicoccum_ cultures were performed using rice medium (80 g/100 mL, autoclaved at 121 °C for 30 min) and cultivated at 28 °C for 30 days.

**Sequencing and bioinformatic analysis**
The genomic DNA (gDNA) of strain _Epicoccum_ sp. CPCC 400996 was extracted using CTAB extraction buffer denoted previously [37]. The gDNA was recovered followed by chlorform extraction, precipitation with isopropanol and further washing with 75% alcohol. The precipitated gDNA was dissolved in 1 mL deionized water, digested with RNase, and purified by chloroform extraction. Then the precipitated gDNA was dried and redissolved in 300 μL deionized water. The shotgun genome sequencing was performed at Shanghai Majorbio Bio-pharm Technology Co. (Shanghai, China) on an Illumina Hiseq 2000 system. Sequence assembly was performed with SOAP denovo 1.05 [38]. Gene annotations were then conducted with GenomeMatcher [39] for local BLAST searching and analyzed via FGENESH platform (www.softberry.com). Protein sequences were compared with the BlastP program in the NCBI database. AntiSMASH database was carried out for bioinformatic mining of metabolic clusters [40]. PKS/NRPS analysis online platform was used for in silico prediction of PKS/NRPS domain organization [41].

**General techniques for DNA manipulation**
For constructing the disruption cassette of the gene _epiA_, the homologous arms of the _epiA_ was amplified from gDNA of strain CPCC 400996 using primer sets (_epiA_-Up-F/R and _epiA_-Dn-F/R, respectively) listed in Table 1. PCR amplifications were carried out on a Veriti 96 Well
Thermal Cycler (Applied Biosystems) using PrimeSTAR®GXL DNA polymerase (Takara). The two PCR amplicons were ligated into a pEASY®-Blunt vector (Transgen) and confirmed through DNA sequencing. The downstream amplicon epiA-Dn was recovered by restriction enzymes digestion using XhoI and ApaI (New England Biolabs), then ligated into simultaneously linearized with XhoI/ApaI digested pAg1-H3 vector to create the plasmid H3-epiA-Dn (Table 1). In addition, the upstream amplicon epiA-Up was recovered by SpeI/Pacl (New England Biolabs) double enzyme digestions, and then ligated into linearized vector H3-epiA-Dn to create the plasmid H3-ΔepiA. The H3-ΔepiA was transformed into A. tumefaciens cells. DNA restriction endonucleases were employed following the instructions as recommended by the manufacturer. Primers employed to amplify the genes are listed in Table 1.

### Generating Epicoccum-derived deletant ΔepiA

The gene deletion of epiA was performed by A. tumefaciens-mediated target gene disruption system, as previously described for *Pseudogymnoascus destructans* [42]. The deletant strain was obtained by replacing targeted gene sequence with the selective hygromycin marker in wild type strain of CPCC 400996. The *A. tumefaciens*-mediated transformation (ATMT) of Epicoccum sp. was performed with minor modifications. The *Agrobacterium* cells were transformed with disruption plasmid H3-ΔepiA by electroporation. The co-cultivation experiments were conducted at 30 °C for 72 h. The randomly selected transformants were then inoculated onto selective plates comprising of cefotaxime (250 µg/mL) and hygromycin (150 µg/mL). The mutants were transferred into PDB selection broth to verify the genotype by diagnostic PCRs following the extraction of gDNA. Primers used for diagnostic PCR verification are listed in Table 1. The comparison in amplicons size between the target gene replaced by the hygromycin selection marker and the native gene promoted us to verify whether the mutants exhibited the correct gene deletion.

### Table 1 Strains, plasmids and oligonucleotides used in this study

| Strains | Description | Source |
|---------|-------------|--------|
| E. coli Trans T1 | lacZΔ4 recA1 deoR F′ – mcrA Δ (mrr-hsdRMS-mcrBC) g80 lacZΔM15Δ araD1396 (ara-leu7697 galU galK) | Transgen |
| T1-ΔepiA | E. coli cell carrying disruption cassette ΔepiA | This study |
| Agrobacterium tumefaciens (AGL-1) | Carrying pCAMBIA3301 plasmid, KanR | [42] |
| AGL-1-ΔepiA | AGL-1 cell carrying ΔepiA | This study |
| Epicoccum sp. CPCC 400996 | Wild type strain | |
| Δ epiA-5 | epiA deletant of CPCC 400996 | This study |
| Δ epiA-11 | epiA deletant of CPCC 400996 | This study |

| Plasmids | Description | Source |
|----------|-------------|--------|
| pAg1-H3 | Vector for targeted gene replacement containing HYG gene as selection marker | [59] |
| H3-epiA-Dn | 3′ prime arms of epiA gene cloned into pAg1-H3 | This study |
| H3-ΔepiA | 5′ & 3′ prime arms of epiA gene cloned into pAg1-H3 | This study |

| Oligonucleotides | Sequence (5′–3′) | Purpose |
|-----------------|-----------------|---------|
| Z01 (epiA-Up-F) | CGACTAGTTCTAGTCTAGCGTATGACG | 5′ prime arm amplification |
| Z02 (epiA-Up-R) | CATTAAAAATGATCAGAACCATT | 3′ prime arm amplification |
| Z03 (epiA-Dn-F) | ATCTCGAGTCAGAACCCTAACCAATGAC | In deletion region characterization |
| Z04 (epiA-Dn-R) | ATGGGGCCCTGAGCCCCACCCCTGCGG | epiA 5′ prime characterization |
| Z05 (epiA-In-F) | ATTCATGAAAGAAGAGAGAGGAG | epiA 3′ prime characterization |
| Z06 (epiA-In-R) | GGAAACCAAGTCCGACG | Hyg+ gene characterization |
| Z07 (cha-epiA-UF) | GGGGCTACAATCTTTCG | |
| Z08 (cha-epiA-UR) | GATACAGACACACCCTT | |
| Z09 (cha-epiA-DF) | GTAAGGGGGACCACTTTCG | |
| Z10 (cha-epiA-DR) | AGCACATCAACCCACAA | |
| Z11 (cha-hph-F) | GGGCAAGGAATTAGATGAGT | |
| Z12 (cha-hph-R) | TGCAATAGGTCAGTCTT | |
Chemicals and chemical analysis
High performance liquid chromatography (HPLC) analyses were conducted on an Agilent 1290 system with an Agilent-SBC18 column (4.6 × 250 mm, 5 μm). Thin Layer Chromatography (TLC) was carried out on silica gel GF254 plates from Qingdao marine chemical company, China. The medium pressure liquid chromatography was performed on Combi Flash Rf 200 (Teledyne Isco, Lincoln NE, USA) with a SEPAFLASH® Flash silica gel column (330 g, Santai Technologies, China). Nuclear Magnetic Resonance (NMR) data was acquired using Bruker AVIII-600 spectrometer (150 MHz for 13C NMR and 600 MHz for 1H NMR, Bruker Corporation, Karlsruhe, Germany). High resolution electrospray ionization mass spectra (HRESIMS) were recorded on a Thermo LTQ Orbitrap XL Mass Spectrometer (Thermo Fisher Scientific, USA).

Scale-up production, isolation of metabolite epicoccamide A
Seed cultures of the strain CPCC 400996 were cultivated in 500 ml flasks (×5) each containing 200 mL of liquid medium (PDB, BD company) at 30 °C on a rotary shaker (220 rpm). After 96 h, 5 mL of the seed culture was used to inoculate into 500 mL erlenmeyer flask (×50) with a rice fermentation medium (rice 100 g/flask, deionized H2O 100 mL/flask). After 30 days cultivation, the cultures were harvested, extracted 2 times with methanol (3 × 30 L) for 30 min each under an ultrasonic bath (10% power intensity). The total methanol solutions were concentrated under reduced pressure to afford the dried extract (60.5 g). Then the methanol extract was dispersed in aqueous (1 L) and extracted by using petroleum ether (3 × 1 L) and ethyl acetate (3 × 1 L) successively. The ethyl acetate residue (5.7 g) was subjected to silica gel column chromatography eluting with a petroleum ether and ethyl acetate mixed solvent system in a step gradient (100:0–0:100, v/v) to afford five fractions (Fr.1–Fr.5). Fr.4 (5.4 g) was further chromatographed on Sephadex LH20 and ODS-MPLC (RP-C18) subsequently. The purification of sub-fractions was carried out on semi preparative HPLC [YMC-Pack ODS-A, 10 mm × 250 mm, 5 μm, flow rate: 3.5 mL/min, 55% acetonitrile-water (v/v)] to obtain epicoccamide A (1) (9.8 mg, tR 14.0 min).

Results
Bioinformatic analysis of the biosynthetic gene cluster epi
We utilized a genome sequencing strategy to investigate the biosynthetic potential of the fungus Epicoccum sp. CPCC 400996. The Illumina HiSeq 2500 sequencing of the strain produced ~4148 million bases in total. Assembly of the unpaired shotgun sequencing reads promoted us to obtain 514 contigs, which includes 30.92 million nonredundant bases. After careful bioinformatic analyses of the genome with AntiSMASH platform (https://fungismash.secondarymetabolites.org), we obtained two biosynthetic gene clusters in the genome of the strain CPCC 400996 that carry glycosyltransferase encoding gene. Of particular, one gene cluster caught our attention, which was somewhat similar to the BGC of the burnetramic acid and was designated as the epi cluster (Fig. 2; Table 2). Six putative open reading frames (epiA–epiF) altogether were acquired based on bioinformatic analysis of the continuous DNA region. The protein EpiA has the

| Protein | Bua homologues (coverage/identity) | Eqx homologues (coverage/identity) | Putative functions |
|---------|-----------------------------------|-----------------------------------|-------------------|
| EpiA    | BuaA (97%/53%)                    | EqxS (86%/38%)                    | PKS-NRPS hybrid   |
| EpiB    | BuaB (89%/40%)                    | ND                                | Glycosyltransferase|
| EpiC    | BuaC (95%/57%)                    | EqxC (92%/43%)                    | Trans-enoyl reductase|
| EpiD    | ND                                | ND                                | hypothetical protein|
| EpiE    | BuaG (93%/48%)                    | ND                                | Cytochrome P450    |
| EpiF    | ND                                | EqxD (98%/48%)                    | N-methyltransferase|

Table 2 Putative functions of ORFs within the BGC epi involved in epicoccamide A biosynthesis

Fig. 2 Genetic organization of the epicoccamide A biosynthetic locus found in the Epicoccum sp. CPCC 400996 genome. The PKS – NRPS module abbreviations are; KS, ketosynthase; AT, malonyl-CoA transferase; MET, methyltransferase; DH, dehydratase; KR, ketoreductase; ER, enoyl reductase; ACP, acyl carrier protein; SAM, S-adenosyl-L-methionine; C, condensation domain; A, adenylation domain; T, thiolation domain; R, reductive domain. Predicted functions of individual ORFs are summarized in Table 2.
domain organization of KS−AT−DH−MET−KR−ACP−C−A−T−R as verified by in silico analysis. BlastP alignment illustrated that the EpiA showed 53% identity to hybrid PKS−NRPS synthetase BuaA in A. burnettii, followed by the fusaridione A synthetase FdsS, fusarisetin A synthetase Fsa1, equisetin synthetase Eqxs, pyrrolocin synthetase PrlS, pyrichalasin H synthase PyiS, and illicicolin H IccA, members of fungal tetramer-forming PKS−NRPS hybrids.

Further closer investigation on the bilateral sequences of the epiA facilitated the discovery of the auxiliary genes encoding tailoring enzymes for tetracetic acid late stage modification. The gene products conclude one glycosyltransferase (EpiB), one LovC-like trans-ensyl reductase (EpiC), one cytochrome P450 monoxygenase (EpiE), one methyltransferase (EpiF), and one hypothetical protein (EpiD) that are in accord with the structural features of glycosylated tetracetic acid derivatives. Although the biosynthetic gene cluster epi is homologous to the bua in A. burnettii, the majority of coding gene products exhibit >50% sequence identical [28]. Nevertheless, compared to the biosynthetic gene cluster bua, the epi lacks two genes that code cytochrome P450 (buaD) and proline hydroxylase (buaE), but instead one more methyltransferase coding epiC was discovered. Additionally, BlastP alignment indicated that EpiF exhibited 47−49% identities to EqxD, Fsa4, and PynC, respectively, and the three methyltransferases are believed to catalyze the methyl group incorporation at N position during biosynthesis of tetramates equisetin [43], fusarisetin [27], and pyranonigrin [44]. Since tetramate glycoside epicocamides have been isolated from two Epicoccum strains, we speculated that the fungus could produce epicocamides and the gene cluster epi might be able to responsible for the biosynthesis of these molecules.

Structure elucidation of epicoccamide A from Epicoccum sp. CPCC 400996

Compound 1 was purified as yellow oil, which had a molecular formula of C_{29}H_{51}NO_{9} as verified by ESI MS (m/z 556.76, calculated for C_{29}H_{51}NO_{9} [M−H]−). On the basis of 1H, 13C NMR and HMBC data, along with the analysis of literature data [36], Compound 1 was suggested as an epicoccamide derivative—epicoccamide A. The NMR data were shown as follows, in 1H NMR data (in DMSO, 600 MHz): δ_{H} 3.87 (1H, brs, H-4), 1.23 (3H, d, J=6.3 Hz, H-5), 2.88 (3H, s, H-6), 3.50 (1H, m, H-8), 1.58 (1H, m, H-9), 1.41 (1H, m, H-9), 1.20−1.29 (m, H-10−H-20), 1.50 (2H, m, H-21), 3.39 (1H, m, H-22), 3.74 (1H, m, H-22), 1.08 (3H, d, J=6.7Hz, H-23), 4.33 (1H, br.d, H-1′), 3.60 (1H, m, H-2′), 3.23 (1H, m, H-3′), 3.29 (1H, m, H-4′), 3.00 (1H, m, H-5′), 3.45 (1H, m, H-6′) and 3.67 (1H, m, H-6′). The 13C NMR data (in DMSO, 151 MHz): δ_{C} 172.3 (C-1), 100.2 (C-2), 189.8 (C-3), 62.2 (C-4), 14.4 (C-5), 26.0 (C-6), 194.5 (C-7), 35.2 (C-8), 33.0 (C-9), 26.6 (C-10), 28.8−29.2 (C-11−C-19), 25.6 (C-20), 29.2 (C-21), 68.4 (C-22), 16.9 (C-23), 100.2 (C-1′), 70.6 (C-2′), 73.7 (C-3′), 67.2 (C-4′), 77.5 (C-5′) and 61.4 (C-6′), which were similar to epicoccamide derivatives (Additional file 1: Figs. S1−S2). According to the above analyses, compound 1 was composed of three moieties as a tetracatic acid, an alphatic chain, and a hexose. The structures of the latter two moieties were verified by 1H−1H COSY, HMBC and ESI MS (Additional file 1: Table S1, Figs. S3−S6) analysis. Furthermore, the teramid acid moiety was determined by HMBC correlations from H-5 (δ_{H} 1.23) to C-3 (δ_{C} 189.8) and C-4 (δ_{C} 62.2), and from H-6 (δ_{H} 2.88) to C-1 (δ_{C} 172.3) and C-4 (δ_{C} 62.2), coupled with ESI MS/MS (−) data (Additional file 1: Fig. S7) at m/z 126.08 for the molecular fragment resulting from the α-cleavage of the teramid acid moiety. Thus, compound 1 was identified as epicoccamide A and the structure was shown in Fig. 1.

Characterization of the BGC epi responsible for the yielding of epicoccamide A

To verify the participation of EpiA in the yielding of epicoccamide A, disruption of the PKS−NRPS megasynthetic coding gene epiA was conducted. We performed a gene inactivation experiment to supply the evidence that epiA is responsible for epicoccamide A biosynthesis. Prior to ATMT experiment, we tested two antibiotics including hygromycin B and G418. Hygromycin concentration of 100 µg/mL could completely inhibit the growth of the parental strain and was used as following selective antibiotic. A selective cassette HygR (pTrpC-HygR-tTrpC) consisting of the promoter coding gene pptrpC, hygromycin resistance gene (HygR), and the terminator coding gene tTrpC was constructed by conventional restriction enzymes digestion and ligation strategy. The knockout plasmid construction was performed based on the scaffold of pAg1-H3 by replacing one of the two nearly continuous fragments from the region of the gene epiA on bilateral of the HygR selective cassette.

Next, after A. tumefaciens-mediated transformation experiment, of 19 putative HygR Epicoccum transformants examined, the mutants including ΔepiA-9 and ΔepiA-11 were verified by diagnostic PCR characterization. The primer pairs resided within the deleted region of the epiA, yielding a 613-bp PCR ampiclon for wild type (WT) strain but not for the selected mutants (Fig. 3A, Bi). The mutants that had no amplicons were further characterized for ΔepiA::HygB+ integration at the homologous site by using four additional primers pairs. With one HygR specific primer set, both mutants had two amplicons of 3256 bp and 3191 bp in size, respectively, while...
the control WT gave no amplicons (Fig. 3A, Bi). Further verification with one primer set located in deletion region of epiA, two amplicons with 2741 bp and 2162 bp in size were amplified from WT, whereas no amplicons were recovered from both mutants (Fig. 3A, Biii). The thus-obtained crude extracts from ΔepiA-9 and ΔepiA-11, and parental strain were analyzed for its metabolites profile by high performance liquid chromatography (HPLC). At the same HPLC condition, the peak of epicoccamide A at 10 min that confirmed it is the target compound along with the evidence of m/z 556 [M-H]−. However, as shown in Fig. 3C, the mutant strains ΔepiA-9 and ΔepiA-11 completely abolished the production of 1. These results further corroborated with our prediction regarding the function of the gene cluster epi, confirming that the gene epiA is responsible for epicoccamide A biosynthesis in Epicoccum sp.

**Investigation on the biosynthesis of metabolite epicoccamide A**

Epicoccamides are unique glycosylated tetramic acid derivatives featuring a linear aliphatic chain attached to tetramate and mannose moieties. The genetic base for the biosynthesis of burnettramic acid was well determined previously, and the biosynthesis of the epicoccamide A should be highly similar to that of burnettramic acid [34]. In the light of the molecular structures of epicoccamide A and proposed gene functions of the gene cluster epi, a biosynthetic pathway of epicoccamide A was envisioned (Fig. 4). Domains of the megasynthase EpiA are programmed and generate polyketide chains with varied methylation and reduction modifications and NRPS modules are in charge of fusing the polyketide chain to an l-alanine and an offloading R domain for tetramate cyclization and chain release. Of this compound, acetate is extended seven times with seven unities of malonate by PKS domains, programmed C-methylation occurs following the first two extensions derived from an intact methylmalonate precursor, and cycles of full reduction occur after the first two extensions. The following steps involve the methyltransferase EpiF for installation of the methyl group into the nitrogen position of tetramate ring and glycosyltransferase EpiB to accomplish the β-D-mannose transferring reaction. However, in the burnettramic acid pathway, the polyketide chain of intermediate burnettramic acid aglycones occur multiple hydroxylations oxygenated by the cytochrome P450 BuaG, followed by BuaB-catalyzed mannosyltransferring for the generation of final product burnettramic acid. In contrast, the intermediate epicoccamide aglycone was N-methylated to afford the N-methyl modified intermediates, a feature that is lacking in burnettramic acid. Additionally, it is important to note that cytochrome P450 EpiE exhibited higher identities (>40%) to P450s including HimC,
BuaG, and AscH, which participated in hydroxylations or multiple oxidations of the polyketide chain during himeic acid A, and burnettramic acid biosynthesis, respectively [34, 45]. We believed that novel and further oxygenated derivatives of epicoccamides catalyzed by P450 EpiE would be mined from *Epicoccum* sp.

**EpiA** is quite an unconventional fungal PKS−NRPS megasynthase, as it is only the first fungal PKS−NRPS that has been exhibited to specifically recognize and activate the amino acid of L-alanine. We next phylogenetically analyzed the adenylation domains of characterized PKS-NRPSs from biosynthetic pathways of fungal tetramates. Similar analyses were performed on embedded adenylation domains in NRPSs for cyclic peptides including cyclosporin A and emericellamides [46, 47]. Notably, in this phylogenetic dendrogram, EpiA, EasA-A4, and EasA-A5 clustered in a distinct branch with CcsA-A11 of cyclosporin A formation in *Tolypocladium inflatum*, which are more inclined to acquire L-alanine as precursors (Fig. 5). Additionally, the 10 signature residues for the adenylation domains of the alanine-incorporating EpiA indicated that positions 235, 236, and 517 are conserved (D_{235}L_{236}K_{517}), while others are flexible (Additional file 1: Table S1). We believe their significance for alanine might be uncovered with increasing alanine-incorporating PKS-NRPSs.

**Discussion**

Using a targeted gene deletion, we have identified the *epi* family genes encoding the biosynthetic pathway for the tetramate derivative epicoccamide A in the fungal strain CPCC 400996. Interestingly, during our research on epicoccamides biosynthesis, Kwon and coworkers also characterized the genetic BGC (*epc*) of epicoccamide in *E. nigrum* KACC 40,642 [48]. It is worth noting that both *epi* and *epc* gene clusters are syntenic and highly identical, and this further demonstrated our proposal that the cluster *epi* is responsible for biosynthesizing epicoccamides [48]. Since the study of the tetramate acid-generated epicoccamides identified from *E. purpurascens* two decades ago, the underlying genetic base and biochemical transformation of this class of compounds remain to be characterized [36, 46]. With genome sequences becoming accessible due to plummeting costs, genomics-based methods are now commonplace and might hold the key to lead a renaissance in the field of secondary metabolites discovery [4, 13, 49, 50]. The understanding to link secondary metabolites to their corresponding BGCs and vice versa, along with ever-increasing understanding of biosynthetic machineries, has inspired the field of genome
mining-guided secondary metabolites exploitation for the rational discovery of new chemical entities [3, 51]. In this report, genome guided bioinformatic analysis of the fungus CPCC 400996 revealed the localization of a novel PKS-NRPS hybrid biosynthetic gene cluster. Additionally, the gene cluster epi also includes one glycosyltransferase encoding gene epiB and one N-methyltransferase encoding gene epiF, which implies that this gene cluster might be the candidate for epicoccamides biosynthesis in the fungus Epicoccum sp. CPCC 400996 (Fig. 2). Taken together, a genome-guided secondary metabolites mining approach was performed to discover the compound epicoccamide A in the producer of Epicoccum sp. strain CPCC 400996.

We next phylogenetically analyzed the EpiA R domain with the NRPS R or R' domains from fungal metabolic pathways and the corresponding Dieckmann cyclases or TE domains involved in bacteria-derived tetramates [15]. Four paradigms have been proposed to decipher the releasing mechanism of tetramate derivatives. These conclude the following conditions: (i) the action of a hybrid PKS-NRPS megasynthase TE carrying module involved in bacterial polycyclic tetramic macrolactams (PTMs) biosynthesis, exemplified as the HSAF [52], FtbB, and SGR-814, for which the terminal TE module of Orf6 in the HSAF biosynthetic pathway (carrying peptide ligase and protease activities) catalyzes tetramate generation [52]; (ii) the action of a reductive (R) domain locating in terminal module within the fungal PKS-NRPS hybrids as highlighted by tenellin [17], equisetin [16], fusaridione A [53], and burnettramic acid [28], in which the dissected R domain of FsdS has been demonstrated in vitro to catalyze Dieckmann-type cyclization for releasing corresponding tetramates; (iii) the action of Dieckmann cyclases as reflected by the actinomycete-derived metabolites including tirandamycin [15], kirromycin [54], and α-lipomycin [55], in which TrdC family cyclases could in vitro catalyze the cyclization of tetramate scaffolds; and (iv) chemistry performed in generating bacteria-derived spiro-linked tetramates including pyrroindomycins [56] and chlorothricins [57], these include a phylogenetically unique two gene ensemble PyrD3/PyrD4 and ChlD3/ChlD4. In this phylogenetic tree, EpiA R domain clustered in a separate branch including NRPS R or R' domains involved in fungal tetramates biosynthesis (Fig. 6). This suggested that it might support the proposal that the releasing mechanism or cyclizations are conserved and unique during biosynthesis of fungal tetramates.

Investigation of the biosynthetic gene cluster epi revealed neighboring genes that are highly homologous to reported bua cluster in A. burnettii genome. These two gene clusters contained a deduced 5-membered heterocycle formation encoding genes together with other
Putative tailoring ORFs conserved among the previously reported biosynthetic pathways of tetramate derivatives (Fig. 7). A more detailed cluster blast bioinformatic analysis was carried out on other fungal tetramates including equisetin from *Fusarium heterosporum* ATCC 74,349 [14], himeic acid from *Aspergillus japonicus* MF275 [13], fusarisetin A from *Fusarium* sp. FN080326 [23], Sch210971/2 from *Hapsidospora irregularis* BP-2511 [58], and pyranoviolin A from *A. violaceofuscus* CBS 115,571 [26]. Significantly, all these TETases genes are related to a double-gene ensemble that catalyzes the formation of the tetramate moiety. The ensemble includes genes that encode a PKS-NRPS hybrid and a trans- enoyl reductase (ER). The prevalence of these TETases-encoding gene clusters across these fungi has promoted the conclusion that they might encode for yielding of a group of structurally related tetramic acids that have a common metabolic origin (Fig. 7). Comparisons of these fungal tetramates encoding gene clusters will contribute us to elucidate the biosynthetic mechanisms of this unique class of secondary metabolites. The genetic investigations of these fascinating biocatalysts will facilitate us to rationally generate novel epicoccamide derivatives with structural diversification and improve pharmacological property.

**Concluding remarks**

In conclusion, we identified a previously uncharacterized PKS–NRPS hybrid based biosynthetic gene cluster of epicoccamide A (1) from *Epicoccum* sp. CPCC 400996, which enables us to investigate the genetic mechanism of epicoccamide A biosynthesis. The gene cluster *epi* for generating the compound (1) was verified by gene inactivation experiment in the native producer. Based on the gene cluster data, a putative metabolic pathway of epicoccamide A was proposed.

*Fig. 6* Unrooted phylogenetic tree of NRPS R domains or proteins catalyzing Dieckmann cyclization during tetramates biosynthesis. NRPS R domains involved in fungal tetramates metabolic pathways; TrdC family cyclases involved in actinomycete-derived tetramates; TEs involved in bacterial polycyclic tetramic macrolactams (PTMs) biosynthesis; PyrD3, and ChlD3 involved in pyrrolidine-2,4-dione ring formation during pyrroindomycin and chlorothricin synthesis, respectively.
These findings might provide the feasibility of using them for further investigation of epicoccamides biosynthesis. Our study demonstrates that genome mining targeted strategy would accelerate the discovery of secondary metabolites, and also provide opportunities for derivatization of epicoccamides via manipulating biosynthetic pathway.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12934-022-01975-2.

**Additional file 1:** Table S1. NMR Data for epicoccamide A (1). Table S2. Comparison of the signature residues for amino-acid selection by adenylation (A) domains of fungal PKS-NRPS (NRPS). **Figure S1.** The \( ^1 \)H NMR spectrum of compound 1. **Figure S2.** The \( ^{13} \)C NMR spectrum of compound 1. **Figure S3.** The \( ^1 \)H-\( ^1 \)H COSY spectrum of compound 1 (in DMSO). **Figure S4.** The HMQC spectrum of compound 1 (in DMSO). **Figure S5.** The HMBC spectrum of compound 1 (in DMSO). **Figure S6.** HRESIMS spectrum of compound 1. **Figure S7.** ESI MS/MS (-) spectrum of compound 1.

**Acknowledgements**

We are grateful to Dr. Seogchan Kang (University Park, PA, USA) for providing Agrobacterium tumefaciens AGL-1 strain. Prof. Gang Liu (IMCAS, Beijing) is thanked for the generous gift of plasmid pAg1-H3.

**Author contributions**

TZ and LY obtained funding support. TZ conceived the study, supervised the project, conducted experiments and bioinformatic analysis. GC and WH isolated the compound and elucidated the structures of the compound. XR, JX, BJ, HW, XL, LW, and RZ advised on the interpretation of partial data in Figs. 3, and 6. TZ wrote the original draft. TZ, WH, and LY edited the draft manuscript. All authors read and approved the final manuscript.

**Funding**

This work was financially supported by the National Natural Science Foundation of China (No. 31872617, 32141003), the CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-1-055, 2019-I2M-1-005), and the central level, scientific research institutes for basic R & D fund business (3332018097).

**Availability of data and materials**

All data generated during this study are included in this article, and all material is available upon request.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors approved publication.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China. 2Medical Research...
References

1. Bills GF, Gloer JB. Biologically active secondary metabolites from the fungi. Microbiol Spectr. 2016. https://doi.org/10.1128/microbiolspec.FUNK-0009-2016.

2. Keller NP. Fungal secondary metabolism: regulation, function and drug discovery. Nat Rev Microbiol. 2019;17:167–80.

3. Malit JJL, Leung HYC, Qian PY. Targeted large-scale genome mining and candidate prioritization for natural product discovery. Mar Drugs. 2022. https://doi.org/10.3390/md20060398.

4. Machado H, Tuttel RN, Jensen PR. Omics-based natural product discovery and the lexicon of genome mining. Curr Opin Microbiol. 2017;39:136–42.

5. Pan R, Rai A, Chen J, Zhang H, Wang H. Exploring structural diversity of microbial secondary metabolites using OSMAC strategy: a literature review. Front Microbiol. 2019;10:294.

6. Zhuang L, Zhang H. Utilizing cross-species co-cultures for discovery of novel natural products. Curr Opin Biotechnol. 2021;69:252–62.

7. Bertrand S, Bohni N, Schnee S, Schumpp O, Gindro K, Wolfender JL. Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. Biotechnol Adv. 2014;32:1180–204.

8. Toghuoe RMM, Sahal D, Boyom FF. Recent advances in inducing endophytic fungal specialized metabolites using small molecule elicitors including epigenetic modifiers. Phytochemistry. 2020;174:112338.

9. Cichewicz RH. Epigenome manipulation as a pathway to new natural product scaffolds and their congeners. Nat Prod Rep. 2010;27:11–22.

10. Medema MH, de Rond T, Moore BS. Mining genomes to illuminate the specialized chemistry of life. Nat Rev Genet. 2021;22:553–71.

11. Zhang T, Cai G, Rong X, Wang Y, Gong K, Liu W, Wang L, Pang X, Yu L. A combination of genome mining with an OSMAC approach facilitates the discovery of and contributions to the biosynthesis of melleolides from the Basidiomycete Armillaria tabescens. J Agric Food Chem. 2022;70:12430–41.

12. Zhang T, Pang X, Zhao J, Guo Z, He W, Cai G, Su J, Cen S, Yu L. Discovery and activation of the cryptic cluster from Aspergillus sp. CPC040735 for asperphenalonenol biosynthesis. ACS Chem Biol. 2022;17:1524–33.

13. Bauman KD, Butler KS, Moore BS, Chekan JR. Genome mining methods to discover bioactive natural products. Nat Prod Rep. 2021;38:2010–29.

14. Chi LP, Li XM, Li L, X, Wang BG. Cytophatic thiodiketopiperazine derivatives from the deep sea-derived fungus Epicoccum nigrum sp. Org Biomol Chem. 2003;1:507–10.

15. Gui C, Li Q, Mo X, Qin X, Ma J, Ju J. Discovery of a new family of Deckmann cyclases essential to tetramic acid and pyridone-based natural products biosynthesis. Org Lett. 2015;17:628–31.

16. Mo X, Guldner TAM. Synthetic strategies for tetramic acid formation. Nat Rev Microbiol. 2020;18:44579–8.

17. Lahilari R, Hiji M. Screening, identification and evaluation of potential biocontrol fungal endophytes against Rhizoctonia solani ACGs on potato plants. EMBS Microbiol Lett. 2010;311:1152–9.

18. Cao PR, Zheng YL, Zhao QY, Wang XB, Zhang H, Zhang MH, Yang T, Gu YC, Yang MH, Kong LY. Beetleane A and epicoine A: two carbon skeletons produced by Epicoccum nigrum. Org Lett. 2021;23:3274–7.

19. Harwoko H, Lee J, Hartmann R, Mändi A, Kurtatt T, Muller WEG, Feldbrugge M, Kalcheuer R, Ancheeva E, Delatos G, Frank M, Liu Z, Proske P. Azacoccines F-H, new flavipin-derived alkaloids from an entomophagous fungus Epicoccum nigrum MK140709. Fiteroterapia. 2020;106:104698.

20. Zhang Y, Liu S, Che Y, Liu X. Epicoccins A-D, epipolythiodioxopiperazines from a Cordycys-colonizing isolate of Epicoccum nigrum. J Nat Prod. 2007;70:1522–5.

21. Wright AD, Ostergaard C, Konig GM. Epicoccamides, a novel secondary metabolite from a jellyfish-derived culture of Epicoccum purpurascens. Org Biomol Chem. 2003;1:507–10.

22. Zhang T, Zuo Y, Jia X, Liu J, Gao H, Song F, Liu M, Zhang L. Cloning and characterization of the gene cluster required for beauvericin biosynthesis in Fusarium proliferatum. Sci China Life Sci. 2013;56:628–37.

23. Li H, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen L, Yi S, Yang H, Wang J, Wang J. De novo assembly of human genomes with massively parallel short read sequencing. Genome Res. 2010;20:265–72.

24. Ohtsubo Y, Ikeoka-Ohtsubo W, Nagata Y, Tsuda M. GenoMatcher: a graphical user interface for DNA sequence comparison. BMC Bioinf. 2008;9:376.

25. Bnin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Winkel GP, Medema MH, Weber T. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res. 2021;49:W29–35.

26. Bachmann BO, Ravel J. Chapter 8. Methods for in silico prediction of PKS-NRPS gene required for the biosynthesis of cyclopiazonic acid in Aspergillus oryzae. Fungal Genet Biol. 2008;45:1608–15.

27. Kato N, Nogawa T, Hirota H, Jang JH, Takashashi S, Ahn JS, Osada H. A new enzyme involved in the control of the stereochemistry in the decalin formation during equisetin biosynthesis. Biochem Biophys Res Commun. 2015;460:210–5.

28. Mayya S, Grundmann A, Li X, Li SM, Turner G. Identification of a hybrid PKS-NRPS required for pseurotin biosynthesis in the human pathogen Aspergillus fumigatus. ChemBioChem. 2007;8:1736–43.

29. Olano C, Gomez C, Perez M, Palomino M, Pineda-Lucena A, Carballo JR, Brana AF, Mendez C, Salas JA. Deciphering biosynthesis of the RNA polymerase inhibitor streptolydigin and generation of glycosylated derivatives. Chem Biol. 2009;16:1031–44.

30. Wei X, Chen L, Tang JW, Matsuda Y. Discovery of pyravelobin A and its biosynthetic gene cluster in Aspergillus visitaeformus. Front Microbiol. 2020;11:562083.

31. Sato M, Dander JE, Sato C, Chung YS, Gao SS, Tang MC, Hang L, Winter JM, Garg NK, Watanabe K, Tang Y. Collaborative biosynthesis of maleimide- and succinimide-containing natural products by fungal polyketide megasyntheses. J Am Chem Soc. 2017;139:5317–20.

32. Li H, Gilchrist CLM, Lacey HJ, Crombie A, Vuong D, Pitt JJ, Lacey E, Choow TH, Piggott AM. Discovery and heterologous biosynthesis of the burnettamides: rare PKS-NRPS-derived bolaamphiphilic pyrrolizidine derivatives from an Australian fungus, Aspergillus burnettii. Org Lett. 2019;21:1287–91.

33. Guo Y, Contessi FJ, Wang X, Ghidinelli S, Tomby DS, Andersen TE, Mortensen UH, Larsen TO. Biosynthesis of calipyridine A represents a fungal 2-pyridone formation without ring expansion in Aspergillus californicus. Org Lett. 2022;24:804–8.

34. Perveen I, Raza MA, Iqbal T, Naz I, Sehar S, Ahmed S. Isolation of antiscarc and antimicrobial metabolites from Epicoccum nigrum: endophyte of Fendula sinala. Microb Pathog. 2017;110:214–24.

35. Braga RM, Padilla G, Araujo WL. The biotechnological potential of Epicoccum spp.: diversity of secondary metabolites. Crit Rev Microbiol. 2018;44:759–78.

36. Wright AD, Ostergaard C, Konig GM. Epicocamides, a novel secondary metabolite from a jellyfish-derived culture of Epicoccum purpurascens. Org Biomol Chem. 2003;1:507–10.
43. Sims JW, Fillmore JP, Warner DD, Schmidt EW. Equisetin biosynthesis in *Fusarium heterosporum*. Chem Commun. 2005;14:186–8.
44. Awakawa T, Yang XL, Wakimoto T, Abe I. Pyranonigrin E. A PKS-NRPS hybrid metabolite from *Aspergillus niger* identified by genome mining. ChemBioChem. 2013;14:2065–9.
45. Hashimoto M, Kato H, Katsuki A, Tsukamoto S, Fuji i I. Identification of the biosynthetic gene cluster for himeic acid A: a ubiquitin-activating enzyme (E1) inhibitor in *aspergillus japonicus* MF275. ChemBioChem. 2018;19:535–9.
46. Yang X, Peng P, Yin Y, Bushley K, Spatafora JW, Wang C. Cyclosporine biosynthesis in *Tolypocladium inflatum* benefits fungal adaptation to the environment. mBio. 2018. https://doi.org/10.1128/mBio.01211-18.
47. Chiang YM, Szewczyk E, Naya k T, Davidson AD, Sanchez JF, Lo HC, Ho HY, Simitian H, Kuo E, Praseuth A, Watanabe K, Oak ley BR, Wang CCC. Molecular genetic mining of the *aspergillus* secondary metabolome: discovery of the emencellamide biosynthetic pathway. Chem Biol. 2008;15:S27–32.
48. Choi EH, Park SH, Kwon HJ. Genetic localization of epiconcamide biosynthetic gene cluster in *Epicoccum nigrum*. J Appl Biol Chem. 2022;65:159–66.
49. Gao SS, Zhang T, Garcia-Borras M, Hung YS, Billingsley JM, Houk KN, Hu Y, Tang Y. Biosynthesis of heptacyclic duclauxins requires extensive redox modifications of the phenalenone aromatic polyketide. J Am Chem Soc. 2018;140:6991–7.
50. Scherlach K, Hertweck C. Mining and unearthing hidden biosynthetic potential. Nat Commun. 2021;12:3864.
51. Kang HS. Phylogeny-guided (meta)genome mining approach for the targeted discovery of new microbial natural products. J Ind Microbiol Biotechnol. 2017;44:285–93.
52. Lou L, Qian G, Xie Y, Hang J, Chen H, Zaleta-Rivera K, Li Y, Shen Y, Dussault PH, Liu F, Du L. Biosynthesis of HSAF, a tetramic acid-containing macro lactam from *xrobacter enzymogenes*. J Am Chem Soc. 2011;133:643–5.
53. Kakule TB, Sardar D, Lin Z, Schmidt EW. Two related pyrrolidinedione synthetase loci in *Fusarium heterosporum* ATCC 74349 produce divergent metabolites. ACS Chem Biol. 2013;8:1549–57.
54. Weber T, Laiple KJ, Pross EN, Textor A, Grond S, Welzel K, Pelzer S, Vente A, Wohleb er W. Molecular analysis of the kinomycin biosynthetic gene cluster revealed beta-alanine as precursor of the pyridone moiety. Chem Biol. 2008;15:175–88.
55. Blhmayer C, Welle E, Hofmann C, Welzel K, Vente A, Breitling E, Muller M, Gla ser S, Bechthold A. Biosynthetic gene cluster for the polyenoyltetramic acid alpha-lipomycin. Antimicrob Agents Chemother. 2006;50:2113–21.
56. Wu Q, Wu Z, Qu X, Liu W. Insights into pyrroindomycin biosynthesis reveal a uniform paradigm for tetramate/tetronate formation. J Am Chem Soc. 2012;134:17342–5.
57. Jia XY, Tian ZH, Shao L, Qu XD, Zhao QF, Tang J, Tang GL, Liu W. Genetic characterization of the chlorothricin gene cluster as a model for spirito- tetronate antibiotic biosynthesis. Chem Biol. 2006;13:575–85.
58. Kakule TB, Zhang S, Zhan J, Schmidt EW. Biosynthesis of the tetramic acids Sch210971 and Sch210972. Org Lett. 2015;17:2295–7.
59. Guan F, Pan Y, Li J, Liu G. A GATA-type transcription factor AcAREB for nitrogen metabolism is involved in regulation of cephalosporin biosynthesis in *Acremonium chrysogenum*. Sci China Life Sci. 2017;60:958–67.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
• fast, convenient online submission
• thorough peer review by experienced researchers in your field
• rapid publication on acceptance
• support for research data, including large and complex data types
• gold Open Access which fosters wider collaboration and increased citations
• maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions