A gene cluster for the biosynthesis of moenomycin family antibiotics in the genome of teicoplanin producer *Actinoplanes teichomyceticus*

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Abstract Moenomycins are phosphoglycolipid antibiotics notable for their extreme potency, unique mode of action and proven record of use in animal nutrition without selection for resistant microflora. There is a keen interest in manipulation of structures of moenomycins in order to better understand their structure-activity relationships and to generate improved analogs. Only two almost identical moenomycin biosynthetic gene clusters are known, limiting our knowledge of the evolution of moenomycin pathways and our ability to genetically diversify them. Here we report a novel gene cluster (*tchm*) that directs production of the phosphoglycolipid teichomycin in *Actinoplanes teichomyceticus*. Its overall genetic architecture is significantly different from that of the moenomycin biosyntnesis (*moe*) gene clusters of *Streptomyces ghanaensis* and *Streptomyces clavuligerus*, featuring multiple gene rearrangements and two novel structural genes. Involvement of the *tchm* cluster in teichomycin biosynthesis was confirmed via heterologous co-expression of amidotransferase *tchmH5* and *moe* genes. Our

work sets the background for further engineering of moenomycins and for deeper inquiries into the evolution of this fascinating biosynthetic pathway.

Key words: moenomycins, teichomycin, nosokomycin, *Actinoplanes*

Introduction

Moenomycins (Fig. 1) are phosphoglycolipid natural products that directly target peptidoglycan glycosyltransferases involved in the penultimate step of bacterial cell wall biosynthesis (Kahne et al. 2005; Ostash and Walker 2010a). Despite decades of use as animal growth promoters, there have been no reports on significant resistance to moenomycins (Pfaller 2006). Uniqueness of their structures, mode of action and extremely high potency against major Gram-positive pathogens (including MRSA and VRE strains) have attracted attention to them as a model to develop new antibacterial drugs (Lovering et al. 2007; Yuan et al. 2008; Tseng et al. 2014). It is necessary to explore chemical space around the moenomycin scaffold in order to better understand structure-activity relationships and, eventually, to develop improved analogs. Recently, robust chemical tools to manipulate moenomycin have been developed (Taylor et al. 2006; Adachi et al. 2006, Gampe et al. 2013), which are supported by an understanding of the molecular mechanisms of moenomycin A action (Yuan et al. 2008; Gampe et al. 2011). Although new molecules can be produced through chemical synthesis, this approach does not provide rapid access to a range of molecules (Ostash et al. 2010b). Novel moenomycins obtained through biosynthesis would be invaluable as starting materials for chemical manipulations and biological evaluation. The biosynthetic approach hinges on the availability of diverse collections of genes, whose combinatorial expression would lead to novel phosphoglycolipids. However, only two almost identical moenomycin biosynthesis gene clusters (*moe*) have been described so far, in *Streptomyces ghanaensis* ATCC14672 (Ostash et al.,

2009) and *S. clavuligerus* ATCC27064 (Song et al., 2010), limiting the prospects for novel moenomycins via biosynthesis.

This fact prompted us to turn our attention to *Actinoplanes teichomyceticus*, which was described in late 1970s as a producer of two distinct groups of antibiotics: teichomycins A1 and A2. Teichomycin A1 (TeiA) complex was shown to fall into moenomycin family of antibiotics (Bardone et al. 1978; Borghi et al. 1984), while teichomycin A2 turned out to be a mixture of lipoglycopeptide metabolites known today as teicoplanin (Pryka et al. 1988; Li et al. 2004; Sosio et al. 2004; Horbal et al. 2014). From limited chemical degradation experiments (Bardone et al. 1978; Borghi et al. 1984) it was inferred that TeiA closely resembles other members of moenomycin family, although the exact structure of TeiA was not established.

Herein, we report identification and characterization of the genes responsible for the production of TeiA by *A. teichomyceticus*. There is a high similarity between individual genes from *tchm* and *moe* clusters, although their overall genetic organization is different. Also, two *tchm* genes, having no counterparts in the *moe* cluster, were revealed and are likely involved in moenomycin lipid moiety production. Expression of the amidotransferase gene *tchmH5*, a homologue of *moeH5*, in a *moeH5*-deficient heterologous host confirmed involvement of TchmH5 in moenomycin production and revealed its altered donor substrate specificity. Finally, we provide initial LC-MS-based evidence for the structure of major components of the TeiA complex. Our work offers new opportunities for the biosynthetic derivatization of moenomycins.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains used in this study are listed in Table 1. *Escherichia coli* strains were grown in Luria Bertani (LB) medium and antibiotics were added to cultures,

when required, at the following concentrations per milliliter: ampicillin 65 µg, kanamycin 50 µg, apramycin 50 µg, hygromycin 120 µg. Medium components and antibiotics were purchased from Sigma-Aldrich (St. Louis, MO, USA).

For conjugation, *S. ghanaensis* and *S. coelicolor* strains were grown on oatmeal or on MS-medium for vigorous sporulation (Kieser et al. 2000). Selection of the exconjugants was performed on the same media supplemented with appropriate antibiotics, when required. For DNA isolation, exconjugants were grown in 25 mL liquid tryptic soy broth (TSB) medium.

Recombinant DNA techniques

Isolation of genomic DNA from *S. ghanaensis*, *S. coelicolor*, and *A. teichomyceticus,* and plasmid DNA from *E. coli* was carried out using standard protocols (Kieser et al. 2000). Restriction enzymes and molecular biology reagents were used according to recommendation of suppliers Thermo Scientific (Schwerte, Germany), Promega (Madison, USA), NEB (England).

Genome sequencing and annotation

Genome sequencing was performed using an Illumina platform. Small (300 bp) and large (3000 bp) insert shotgun sequencing libraries were prepared from high-molecular mass genomic DNA of *A. teichomyceticus* NRRL-B16726. Reads were assembled using the Newbler assembler v2.6 (Roche). The initial Newbler assembly consisted of 1000 contigs in 284 scaffolds. Some of the gaps were closed by primer walking using specially designed PCR primers. Protein coding genes and ribosomal binding sites were identified using Prodigal v2.60.[34] For annotation, BLAST searches against the NCBI non-redundant protein database were performed. Non-coding genes were predicted using tRNAscan-SE v1.3.1and RNAmmer v1.2 (Lowe and Eddy 1997; Lagesen et al. 2007).

Identification of the *tchm* gene cluster

To find the *tchm* biosynthetic gene cluster in the *A. teichomyceticus* genome, a search a homolog of the MoeO5 protein was performed. As a result, gene *tchmO5* was identified. Comparison of the other genes in the vicinity of *tchmO5* to the *moe* cluster revealed 18 ORFs that are located on two scaffolds and might be responsible for teichomycin production. These two scaffolds do not overlap. Thus, to fill the gap between them, we performed sequencing of the fragment that was obtained after amplification using primers scaffold51-54Forw and scaffold51-54Rev (Table 2), one of which was complementary to the end of scaffold 51 and another to scaffold 54. As a result of the analysis, we closed the hole between the two scaffolds and identified all genes that might constitute the *tchm* cluster. The accession number to the *tchm* gene cluster sequence is KU726098.

Plasmid construction

A 2.05 kb fragment containing the *tchmH5* gene was amplified from the chromosomal DNA of *A. teichomyceticus* using primers tchmH5Forw and tchmH5Rev (Table 2), then hydrolysed with *Kpn*I and *Eco*RI and cloned into the respective site of pSETPmoeE5. As a result, plasmid pSETmoeEtchmH5 (Table 1) was obtained. A 2.4 kb *Xba*I/*Eco*RI fragment, containing the *tchmH5* gene fused with the *moeE5p* promoter, was retrieved from pSETmoeE5Asp and cloned into the respective site of pOOB47a (Ostash et al. 2013). This yielded pOOBPmoetchmH5 (Table 1).

The apramycin resistance gene (*aac(3)IV*) in pOOBPmoetchmH5 was replaced with the hygromycin cassette (pHYG1) that was amplified using primers P1Am-Hyg-up and P2Am-Hyg-rp (Table 2) and the λRed recombination process (Gust et al. 2002). This gave plasmid pOOBPmoetchmH5-hyg (Table 1).

Teichomycin production and analysis

Spores of *A. teichomyceticus* were grown as a preculture in a liquid seed medium (g/L: glucose 30, yeast extract 5, peptone 5, K_2HPO_4 4, KH_2PO_4 2, $MgSO_4 \times 7H_2O$ 0.5, pH 7.2) at 30°C and 200 rpm for 5 days. 3 ml of the obtained seed broth were used to inoculate 300-ml flasks containing 50 ml of seed medium and were kept growing further for 5 days at 30°C and 200 rpm. The production culture was centrifuged for 10 min at 5000 rpm, supernatant was discarded and the cell pellets were washed two times with distilled water. TeiA extraction from the cells was performed by stirring them with 10 ml of methanol overnight. After centrifugation at 5000 rpm for 5 min, the supernatant was transferred into a fresh tube and concentrated *in vacuo*, enriched for moenomycin-like compounds using Waters Sep-Pak C18 SPE cartridge, and subjected to LC/MS analysis. LC/MS experiments were performed using a Phenomenex Gemini 5µ C18 100A column (100 mm x 2.0 mm) at a flow rate of 0.5 mL/min (solvent A: water, 5 mM ammonium formate; solvent B: 35:65 water:acetonitrile modified with 5 mM ammonium formate). Samples were monitored in negative mode on Agilent 6520 LC/QTOF and Bruker Esquire 3000 ESI-MS spectrometers with a linear gradient over 20 min (0-100 % B from 2 min to 18 min). Selected mass-peaks were subjected to MS/MS experiments as described (Ostash et al. 2007).

Moenomycin production and analysis

Spores of *S. coelicolor* or *S. ghanaensis* strains, containing plasmids for moenomycin production, were inoculated as seed cultures into liquid TSB medium and were grown for 2 days at 200 rpm and 30°C. Afterwards, 5 % of the preculture was inoculated into 300 ml flasks containing 50 ml of TSB medium. Flasks were incubated at 30°C and 220 rpm for 5 days. Moenomycin was extracted from biomass as previously described (Ostash et al. 2013). Obtained samples were directly used for the LC-MS or MS^2.

Results

Identification of the teichomycin biosynthetic gene cluster

At the beginning of this project there were no available gene clusters for moenomycin biosynthesis from non-streptomycete species, and no genome sequences from genus *Actinoplanes*. We therefore generated draft genome sequence of *A. teichomyceticus* using Illumina sequencing (for details see Materials and methods). As a result, contigs that cover 98% of the genome were generated. Using several available assemblers, 1000 contigs were composed into 284 scaffolds. The quality was enough to mine for secondary metabolic gene clusters and other genes of interest. We reasoned that TeiA, like all moenomycins, is composed of glycoside- and isoprene-derived moieties bridged by 3-phosphoglyceric acid (3-PG) (Bardone et al. 1978; Ostash et al. 2009). Thus, TeiA gene cluster should encode a homologue of the MoeO5 protein that catalyzes the first dedicated step in moenomycin biosynthesis – transfer of farnesyl-PP onto a 3-PG moiety (Ostash et al. 2009; Ostash et al. 2007). Indeed, we were able to identify a gene, *tchmO5*, whose translation product showed 55% identity and 67% similarity to MoeO5. Further analysis (for details see Materials and Methods) turned up 18 ORFs in the vicinity of *tchmO5*, most of which showed varying degree of similarity to genes within *moe* cluster of *S. ghanaensis*.

Annotation and analysis of the teichomycin gene cluster

The G+C content of the *tchm* cluster is 73.4%, typical for the *Actinoplanes* genus (Yamamura et al. 2012); it did not differ from the overall GC content of *A. teichomyceticus* NRRL-B16726 genome (72.7%) and its flanking regions (in average 71.8%). The putative functions of the predicted ORFs and their similarity to *moe* clusters from *S. ghanaensis* and *S. clavuligerus* are summarized in Table 3.

Out of 18 identified *tchm* genes, 16 have orthologues in the *moe* cluster, although the overall genetic architectures of *tchm* and *moe* clusters are quite different (Fig. 2). The *tchm* cluster lacked homologues of *moeA4-B4-C4* genes, those that form *moe* 2 cluster in *S. ghanaensis*, and is composed only of a single set of genes that are located on one locus; no putative A-ring biosynthesis genes were found elsewhere in the NRRL-B16726 genome. In addition, there are no homologues of *moeB5**, *A5**, *S5* and *R5* in the *tchm* cluster and there are two extra genes coding for a putative prenylsynthetase (*tchmZ*) and a prenylcyclase (*tchmY*). Like the *moe* cluster, *tchm* lacks cluster-situated regulatory genes. In contrast to the *moe* genes, the *tchm* genes do not contain TTA codons, which are known to limit moenomycin production at the translational level, in their coding sequences (Makitrynskyy et al., 2013). In general, $tRNA^{Leu}_{UAA}$ (encoded by *bldA* gene) is an important and widespread regulatory switch in *Streptomycetes* (Chater and Chandra, 2008; Chandra and Chater 2008; Liu et al. 2013). It is likely that the *tchm* cluster is not subject to direct *bldA* control.

The *tchm* cluster encodes proteins involved in isoprenoid metabolism. Namely, TchmO5 and TchmN5 are homologous to prenyltransferases MoeO5 and MoeN5, whose roles in moenomycin biosynthesis are well established (Ostash et al. 2009). According to sequence (BLASTP) and structural homology (HHPred) alignment programs, the two others, TchmY and TchmZ, are homologous to isoprenoid biosynthesis enzymes of C2- and C1-like superfamilies, respectively. The former includes prenylcyclases that catalyze a cationic cyclization cascade converting linear prenyl chains to fused ring compounds (Sato 2013). The representatives of the latter family are known as trans-isoprenyl diphosphate synthases that catalyze head-to-tail (HT) (1'-4) condensation reactions and synthesize various chain length (C10 to C50) linear isoprenyl diphosphates from precursors isopentenyl diphosphate and dimethylallyl diphosphate (Wang and Ohnuma 2000). We therefore suggest that TchmY performs terminal cyclization of the moenocinyl chain in teichomycin, leading to diumycinol chain (Fig. 1, unit z) and, correspondingly, cyclized isomers of moenomycins, such as AC326-α (Ostash

and Walker, 2010a; Ostash et al. 2010b; He et al. 2000). The prenylpyrophosphate synthase TchmZ could provide a pool of activated isoprene units specifically for moenomycin production pathway.

Almost the entire complement of carbohydrate modification and transfer genes of *moe* cluster have counterparts in *tchm*. Notable differences are the absence of genes for: i. conversion of N-acetyl-D-glucosamine (GlcNAc) into chinovosamine (*moeR5*, *moeS5*); ii. A-ring biosynthesis (*moeA4-C4*).

The conversion of moenomycins with a free carboxyl group on the terminal galacturonic acid (nosokomycins, Fig. 1) into a diverse set of compounds featuring a carboxamide, an A-ring, or amino acids, is carried out by single glutamine amidotransferase MoeH5 (Ostash et al. 2013). There are two putative genes for asparagine synthetase in the *tchm* cluster, *tchmF5* and *tchmH5*. The translation product of the former is similar to MoeF5, which is involved in carboxyamidation of the first carbohydrate unit in moenomycins. Interestingly, the second protein, TchmH5, resembles MoeF5 and MoeH5 almost to the same degree, implying that duplication of ancestral MoeF5 might be the evolutionary origin of MoeH5 in moenomycin biosynthesis. In comparison to MoeF5 that poses a typical Ntn-type asparaginase domain for glutamine hydrolysis with absolutely conserved cysteine Cys_1, the Ntn domain of MoeH5 is significantly truncated that is an additional evidence that the latter has evolved from the former (Ostash et al., 2013). A higher degree of similarity between MoeF5 and TchmF5 indirectly suggests that TchmH5 functions as MoeH5, namely it controls conversion of nosokomycin into final products. Both TchmH5 and TchmF5 contain an N-terminal Ntn-type asparaginase domain for glutamine hydrolysis and a C-terminal AsnB-like asparagine synthetase domain. All conserved amino acids, including the Cys1 residue absolutely required for activity, are present in the Ntn domain of TchmH5 and TchmF5 (Fig. S1). Thus, both TchmF5 and TchmH5 proteins appear to be typical glutamine amidotransferases that can use only glutamine as an amide nitrogen donor to transfer to the appropriate moenomycin intermediate (Zalkin 1993; Zalkin and Smith, 1998). In contrast, MoeH5 contains a truncated N-domain conferring this

enzyme with the ability to use a wider set of amide donor substrates (Ostash et al. 2013).

The *tchm* gene cluster, similarly to the *moe* cluster, contains four genes for two putative ABC transport systems that might be involved in moenomycin production or extrusion.

Overexpression of *tchmH5* in *S. ghanaensis* dH5 and *S. coelicolor* M1152 moeno38-6$^+$

Our initial bioinformatic analysis (*vide supra*) pointed to the possibility that TchmH5 can be involved in the last step of teichomycin biosynthesis and it may have narrower donor substrate specificity compared to MoeH5. To test this hypothesis, we attempted to complement *moeH5* deficiency in two strains, namely *S. ghanaensis* dH5 (Ostash et al., 2013) and *S. coelicolor* M1152 moeno 38-6$^+$, with the *tchmH5* gene (Lopatniuk et al. 2014). The dH5 and M1152 moeno 38-6$^+$ strains accumulated nosokomycin A and A2, respectively, as the final products of the moenomycin pathway (Fig. 1).

For the complementation experiments, we constructed plasmids pOOBPmoetchmH5 and pOOBPmoetchmH5-hyg (Table 1) where *tchmH5* was placed under the control of the NDP-hexose epimerase gene *moeE5* promoter (*moeE5p*) (Horbal et al. 2013). The pOOBPmoetchmH5 and pOOBPmoetchmH5-hyg were transferred into *S. coelicolor* M1152 moeno 38-6$^+$ and *S. ghanaensis* dH5, respectively.

We assessed moenomycin production in the recombinants and compared it to the control strains. LC-MS analysis of extracts from *S. coelicolor* M1152 38-6$^+$ pOOBmoetchmH5$^+$ clearly showed that the strain produced a new compound, in comparison to extracts from *S. coelicolor* M1152 38-6$^+$, that had a molecular mass of 1500.6 Da and HPLC retention time similar to that of nosokomycin B_2 (Fig. 3a). MS/MS analysis of *m/z* 1500.6 detected several characteristic mass peaks routinely observed during fragmentation of moenomycins (Fig. S2). Therefore, based on

HPLC mobility, molecular mass and fragmentation data, we postulate that this compound is nosokomycin B_2. We detected no mass peaks that would correspond to glycine- or other aminoacid-decorated moenomycins in M1152 38-6^+ pOOBmoetchmH5^+ extracts. These data are in agreement with the suggestion that TchmH5 can produce only carboxamide in the presence of ammonia and nosokomycin (Fig. 3b).

We have found no new compounds in the extracts of *S. ghanaensis* dH5 carrying pOOBPmoetchmH5-hyg, although the parental compound to be converted by TchmH5, nosokomycin A, was abundantly produced. At this point, we cannot explain why TchmH5 did not convert nosokomycin A into its carboxamide form, nosokomycin B, as it did in the heterologous host strain M1152 38-6^+ which produced nosokomycin A_2. The latter differs from nosokomycin A in its C-ring, which comes in the form of a GlcNAc residue in nosokomycin A_2, and as chinovosamine (Chi), a reduced version of GlcNAc, in nosokomycin A. Although a minor difference, this may account for the inability of TchmH5 to recognize nosokomycin A as an acceptor substrate. This suggestion is in line with the fact that the *tchm* cluster lacks homologues of *moeR5* and *S5* genes, which are involved in the conversion of UDP-GlcNAc into UDP-Chi (Ostash et al., 2009). Our experimental finding may reflect extensive co-evolution of different stages of the teichomycin pathway, so that downstream enzymes are able to accept only certain substrates.

A. *teichomyceticus* produces moenomycins

Guided by the bioinformatic analysis as well as results of the *tchmH5* heterologous expression, we suggested that *A. teichomyceticus* should accumulate, as a major compound, either nosokomycin B_2 or its derivative with a diumycinol chain (see Fig. 1 for the structures). Indeed, careful LC-MS analysis of the biomass extracts showed that the strain accumulated a compound with molecular mass of 1500.6 Da (Fig. 4). Its chromatographic mobility, CID pattern (see Fig. S3) and accurate mass

(calcd: 1500.6278; obsvd: 1500.6263) is consistent with the structure of known member of the moenomycin family NoB_2, or its diumycinol-carrying derivative. We observed two other moenomycin-like products in the extracts. The first one corresponds to NoA_2 or its diumycinol-carrying derivative (accurate mass: calcd: 1501.6118; obsvd: 1501.6105; see also Fig. S4), a precursor of the 1500.6 Da compound featuring a free carboxyl group on its B-ring and 1484.6 Da. The second one had LC mobility, accurate mass (calcd: 1484.6329; obsvd: 1484.6344) and a CID pattern (Fig. S5) consistent with the structure of nosokomycin B, or its diumycinol-bearing derivative. The major product of 1500.6 Da (NoB_2) and NoB differ in the structure of their C-rings, which is N-acetylglucosamine (GlcNAc) in the former, while the latter carries its reduced form, Chi. It was rather unexpected to observe NoB in *A. techomyceticus* extracts, since the *tchm* cluster evidently has no genes (homologous to *moeR5* and *moeS5*) for conversion of GlcNAc into Chi. Nevertheless, we previously observed production of Chi-bearing moenomycins in the absence of a *moeR5moeS5* gene pair in the heterologous strain *S. lividans* (Ostash et al., 2007). Probably, there are *moeR5moeS5* homologs elsewhere in *A. techomyceticus* genome, that could, with low efficiency, lead to the production of Chi. We also note that the production level of the compound with molecular mass 1484.6 Da is much lower as compared to major expected product with mass 1500.6 Da (see Fig. 4). No other moenomycin-like compounds were detected in the extracts. In spite of extensive experimentation with fermentation conditions, the production of nosokomycin B_2 (or its cyclized isomer) was either too low or too irreproducible to obtain reasonable quantities for NMR analysis and verification of the structure of the lipid chain.

Discussion

Pathogenic bacteria in both clinical and community settings is a major global health problem of humankind (Paphitou 2013; Fair and Tor 2014). Nowadays, it is obvious that emergence of the resistance to a new antibiotic introduced into a wide use is inevitable and in most cases, that is only a matter of time. Therefore, there is

an urgent and permanent need in new drugs, especially antibiotics that have new modes of action and uncommon targets. Moenomycins are representatives of a small group of phosphoglycolipids that act through direct inhibition of peptidoglycan glycosyltransferases (PGTs) involved in bacterial cell wall formation and are active against vancomycin- and methicillin-resistant Gram-positive pathogens (Ostash and Walker 2010a). Despite that they are widely used in animal nutrition, no resistance to these antibiotics was detected thus they represent a group of highly active and promising drugs (Pfaller 2006). Therefore, there is a need to investigate chemical space around this group of antibiotics in order to get new improved analogs.

Herein, we report for the first time, the identification and experimental verification of the teichomycin biosynthetic gene cluster from *A. teichomyceticus*. Analysis of the *tchm* cluster revealed the presence of 18 ORFs, 16 of which have orthologs within the *S. ghanaensis moe* cluster (Ostash et al. 2009). Despite a high level of similarity between individual genes within the clusters, their overall genetic organization is strikingly different because of multiple rearrangements and the presence of 2 additional *tchm* genes (Fig. 2). Furthermore, A-ring and chinovosamine biosynthetic *moe* genes have no counterparts in the *tchm* cluster. We suggest that the relative simplicity of genetic organization of the latter cluster reflects an ancestral (or at least early) state, which has evolved into a more complex and rearranged pathway encoded by the *moe* cluster of *S. ghanaensis* and related *Streptomycetes* (Ostash et al. 2009; Song et al. 2010). Comparative analysis of *moe* and *tchm* clusters lends several lines of support to this conjecture. First, unlike the split *moe* cluster, all *tchm* genes form a single cluster which contains fewer genes than the *S. ghanaensis moe* cluster. Second, there is collinearity between *tchm* gene organization and the order of participation of respective proteins in TeiA biosynthesis. That is, all *tchm* genes for early steps of TeiA production (up to trisaccharide; *tchmO5-E5-GT1-F5-M5-R5-GT5-G4*) are located together, while in the *moe* cluster, the same genes are interspersed with those involved in later stages of moenomycin synthesis. Finally, the *tchm* cluster appears

not to be subject to direct translational control, which governs *moe* cluster expression (Makitrynskyy et al. 2013). At the time of writing of this paper more *Actinoplanes* genomes have become available and their screening with the antiSMASH online service revealed a *tchm* gene cluster in the genome of *A. subtropicus* as well.

The involvement of the *tchm* locus in TeiA biosynthesis was confirmed via heterologous expression of the *tchmH5* amidotransferase gene. Furthermore, we revealed that the substrate specificity of TchmH5 is different from that of MoeH5. Namely, while the latter is capable of transferring amide-donor groups as diverse as ammonia, A-ring, and glycine (Ostash et al. 2013), TchmH5 catalyzes only carboxiamidation using glutamine as an amide nitrogen donor; it appears to recognize only a certain type of acceptor substrate, that is, NoA_2 (Fig. 3b). TchmH5 would be an interesting tool in the context of experiments aimed to simplify the composition of moenomycins produced by *S. ghanaensis*. Finally, in this work, we provided initial clues about the structure of principal components of the TeiA complex. Taking into account the *tchm* cluster analysis and LC-MS data, we suppose that TeiA in fact is either nosokomycin B_2 or its derivative carrying a diumycinyl instead of a moenocinyl chain. Summing up obtained data, we anticipate that characterization and further study of the *tchm* genes open the way for genetic manipulations of the cluster to obtain moenomycins with improved biological properties.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no competing interests.

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Table 1 Strains and plasmids used in this work.

Table 2 Primers used in this work.

Table 3 Putative ORFs of the teichomycin biosynthetic gene cluster and their putative functions.

Fig. 1 Structures of moenomycin family antibiotics mentioned in the text. Moenomycin A (MmA) is the founding member of the family.

Fig. 2 Genetic organization of the teichomycin (*tchm*) biosynthetic gene cluster of *A. teichomyceticus* and its comparison with moenomycin (*moe*) gene clusters. Homologues in the *tchm* and *moe* clusters are attached with lines and *tchm* genes that do not have counterparts in the *moe* cluster are denoted with red color.

Fig. 3 Nosokomycin production profiles of the *S. coelicolor* M1152 moeno38-6^{+} strain and its derivative carrying *tchmH5* gene under the control of *moeE5p* promoter. Extracted ion chromatograms are represented for different moenomycin derivatives. NoA_2 – nosokomycin A_2; NoB_2 – nosokomycin B_2; $MoeG_2$ – moenomycin G_2 (a)**.** Schematic reaction of nosokomycin A_2 conversion into nosokomycin B_2 catalyzed by TchmH5 protein (b).

Fig. 4 Moenomycin production profile of *A. teichomyceticus* NRRL-B16726. Extracted ion chromatograms showing the presence of moenomycins in the methanol extracts from NRRL-B16726. Abbreviated names of the compounds and their masses (in Da) are given to the right (see Fig. 1 for structures). Red arrow indicates peak corresponding to NoA_2 (1501.6 Da) that overlaps with 1501.6 Da isotope of NoB_2.

Table 1 Strains and plasmids used in this work

Bacterial strains and plasmids	**Description**	**Source or reference**
A. teichomyceticus	Producer of teicoplanin and teichomycin	NRRL-B16726
S. coelicolor M1152 moeno38-6^{+}	Producer of nosokomycin A2	Lopatniuk et al., 2014
S. ghanaensis dH5	Producer of nosokomycin A	Ostash et al., 2013
E. coli DH5α	Host for routine subcloning experiments	Thermo Scientific
E. coli ET12567 pUZ8002	(dam-13::Tn9 dcm-6), pUZ8002^{+} (ΔoriT), used for conjugative transfer of DNA	Kieser et al., 2000
pOOB47a	Derivative of pKC1139E containing *ermEp-moeH5* fusion, Am^{r}	Ostash et al., 2013
pSETmoeEtchmH5	Derivative of pSETPmoeE5 containing *tchmH5* gene under the control of *moeE5* promoter	This work
pOOBPmoetchmH5	Derivative of pOOB47a containing *tchmH5* gene under the control of *moeE5p*	This work
pOOBPmoetchmH5-hyg	Derivative of pOOBPmoetchmH5 containing *tchmH5* gene under the control of *moeE5p*	This work

Table 2 Primers used in this work

Primer	**Sequence 5'-3'**	**Purpose**
tchmH5Forw	TTTGGTACCAGGAGGATCATCGACCGGTACGCGGAC	*tchmH5* amplification
tchmH5Rev	TTTGAATTCCAGGTAGGTGGAGCAGCGATAG	
scaffold51-54Forw	ACGGCATCGTCCGCGACGTGG	amplification of the region between scaffolds 51 and 54
scaffold51-54Rev	TCGACCACCACCAGCTCCCAC	
P1Am-Hyg-up	GTGCAATACGAATGGCGAAAAGCCGAGCTCA TCGGTCAGCCCGTAGAGATTGGCGATCCC	replacement of the apramycin resistance gene with hygromycin
P2Am-Hyg-rp	TCATGAGCTCAGCCAATCGACTGGCGAGCGGC ATCGCATCAGGCGCCGGGGGCGGTGTC	

Table 3 ORFs of the teichomycin biosynthetic gene cluster and their putative functions

Protein	**Protein homologue**	**Identity/similarity with *S. ghanaensis* protein, % %**	**Identity/similarity with *S. clavuligerus* protein, % %**	**Putative function**
TchmD5	MoeD5	46/61	48/64	ABC transporter-like protein
TchmJ5	MoeJ5	48/61	46/60	ABC transporter ATP-binding protein
TchmE5	MoeE5	53/63	49/60	UDP-glucose 4-epimerase
TchmF5	MoeF5	49/60	56/64	Asparagin synthetase
TchmGT1	MoeGT1	54/66	54/66	Glycosyltransferase
TchmY	-	-	-	Prenylcyclase
TchmO5	MoeO5	55/67	47/58	Prenylsynthetase
TchmK5	MoeK5	85/91	82/89	SAM, methyl-cobalamin-dependent methyltransferases
TchmGT5	MoeGT5	55/66	57/67	Glycosyltransferase
TchmGT4	MoeGT4	56/66	52/64	Glycosyltransferase
TchmM5	MoeM5	63/75	63/75	Carbamoyltransferase
TchmN5	MoeN5	49/62	48/61	Prenyltransferase
TchmH5	MoeH5	23/35	-	Asparagine synthetase (glutamine-hydrolyzing)
TchmGT2	MoeGT2	66/76	66/78	Glycosyltransferase
TchmGT3	MoeGT3	54/67	-	Glycosyltransferase
TchmZ	-	-	-	Polyprenylsynthetase
TchmX5	MoeX5	52/69	57/70	Putative ABC transporter membrane protein
TchmP5	MoeP5	60/73	61/72	ABC transporter ATPase
-	MoeB5*	-	-	Nonfunctional acyl CoA ligase
-	MoeA5*	-	-	Aminolevulinate synthase
-	MoeR5	-	-	Hexose-4,6-dehydratase
-	MoeS5	-	-	Hexose-4-ketoreductase
-	MoeA4	-	-	Putative acyl-CoA ligase
-	MoeB4	-	-	Amide synthetase
	MoeC4	-	-	Aminolevulinate synthase