ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2011. Випуск 56. С. 84–90
Visnyk of the Lviv University. Series BIology. 2011. Issue 56. P. 84–90

ГЕНЕТИКА

УДК 579.873.1:577.181.4

CHARACTERIZATION OF TEICHOMYCIN A_1 COMPLEX PRODUCED BY *ACTINOPLANES TEICHOMYCETICUS* NRRL-B16726

B. Ostash

Ivan Franko National University of Lviv
4, Hrushevskyi St., Lviv 79005, Ukraine
e-mail: bohdanostash@gmail.com

Actinoplanes teichomyceticus is an important microorganism used for industrial production of teicoplanin, a "last line defense" glycopeptide antibiotic against multidrug resistant cocci. This strain also produces a second group of antibiotics collectively known as teichomycin A_1 complex (TeiA). Here, the ability of *A. teichomyceticus* to accumulate TeiA in different media has been examined and structures of the major members of TeiA have been determined with the help of high-resolution mass spectrometry. TeiA is not produced in all tested media; moreover, different batches of nutrients (such as rapeseed and cottonseed flours) affected the ability of the strain to produce TeiA. Mass spectrometry experiments revealed that TeiA consists of 3 known and 1 novel moenomycins; the presence of other as-yet-unknown phosphoglycolipids cannot be ruled out due to their low production level and inherently complex biosynthetic pattern. It is likely that genes for early stage of moenomycin A biosynthesis are highly similar to TeiA biosynthetic genes, and their cloning can be pursued via homology-based searches.

Key words: *Actinoplanes*, teichomycin A_1, moenomycin, mass spectrometry.

Actinoplanes teichomyceticus is a representative of rare and slow-growing actinomycetes that possess motile spores in sporangium-like sacs [17]. Actinoplanetes often produce secondary metabolites not found in more common genera, such as *Streptomyces*, *Micromonospora* or *Saccharopolyspora*. *A. teichomyceticus* is not exception from this trend: it is the only known producer of teicoplanin (Fig. 1), a clinically valuable glycopeptide that is active against vancomycin- and methicillin- resistant cocci [5, 6, 14]. Much effort has been put into physiological and genetic studies on teicoplanin biosynthesis [2, 4, 7, 13–16]; however, *A. teichomyceticus* has also been shown in early works to accumulate second group of antibiotics referred to as teichomycin A_1 complex (TeiA). TeiA is believed to consist of moenomycin-like antibiotics, although this statement is based on very limited chemical degradation experiments [1]. There is growing interest in exploration of moenomycin family antibiotics as a basis for the development of a new class of antibiotics operating through inhibition of peptidoglycan glycosyltransferases [11, 12]. In this regard, it is essential to investigate all

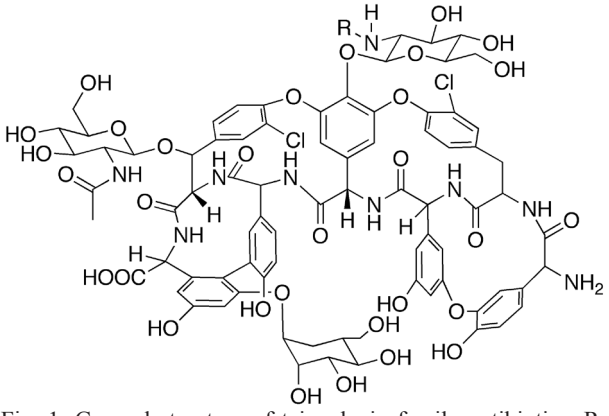


Fig. 1. General structure of teicoplanin family antibiotics, R – acyl chains of different lengths.

available natural producers of moenomycins. This would help estimate natural diversity within this family of antibiotics and predict the chemical space around phosphoglycolipid scaffold accessible through combinatorial biosynthetic approaches. To date, *A. teichomyceticus* is the only known non-streptomycete producer of moenomycins [3, 12]. Significant evolutionary distance between *Actinoplanes* and *Streptomyces* may ensure significant divergence between moenomycin biosynthetic pathways in these bacteria, and thus novel compounds and/or new genes for moenomycin biosynthesis can be discovered in TeiA producer. As a first step towards exploring the TeiA biosynthesis, I have examined the ability of *A. teichomyceticus* to produce TeiA in different media and elucidated the structures of the major components of this complex with the help of mass spectrometry approaches.

Materials and methods

Strain *A. teichomyceticus* NRRL-B16726 was used throughout this work. *A. teichomyceticus* was incubated on dried oatmeal plates at 30°C for 7 days to obtain sporulated lawn. The spores (average titer – 5.8×10^8 ml^{-1}) were inoculated into various liquid media (30 ml per 300 ml shaken flask) to study TeiA accumulation. Following media were tested: TM1 (g/l: glucose – 20, soy flour – 10, peptone (BD Biosciences, MI, USA) – 5, yeast extract (BD Biosciences, MI, USA) – 5, $MgSO_4\times7H_2O$ – 0,5, NaCl – 1,2, $CaCl_2$ – 0,1, $CaCO_3$ – 5), TM2 (g/l: glucose – 20, glycerol (Malincrodt Chemicals, NJ, USA) – 10, yeast extract (BD Biosciences, MI, USA) – 5, $MgSO_4\times7H_2O$ – 0,5, NaCl – 0,1, $CaCl_2\times7H_2O$ – 0,1), TM3 (g/l: glucose – 10, malt extract (EMD Biochemicals, CA, USA) – 20, cottonseed flour Pharmamedia ProFlo (Protein Traders, OH, USA) – 10, yeast extract (BD Biosciences, MI, USA) – 5, L-proline (Sigma, USA) – 0,5), TM4 (g/l: glucose – 20, dextrin (Sigma, USA) – 80, soybean flour (Sigma, USA) – 10, rapeseed meal (various sources: Difco, MI, USA; crude flour of Ukrainian origin; Roth, Germany) – 20, yeast extract (BD Biosciences, MI, USA) – 5, peptone (BD Biosciences, MI, USA) – 5, $MgSO_4\times7H_2O$ – 0,5, NaCl – 1,2, $CaCl_2$ – 0,1, $CaCO_3$ – 5), TSB (Difco). After 5–7 days of growth, mycelia were spun down, washed with water and extracted with 5 ml of methanol. The methanol extract was dried in SpeedVac (Eppendorf, Germany), reconstituted in water and used for solid phase extraction and purification of moenomycins as described [9, 10]. Thin layer chromatography of TeiA has been performed on silica gel-coated aluminum plates (Merck, NJ, USA), mobile phase: 30% NH_4OH:2-propanol (3:7). *Bacillus cereus* was used for biochromatography of TeiA, as described [1, 9]. Agilent ESI-MS 1100 and ESI-qTOF 1450 machines (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, USA) were used to analyze the components of TeiA according to established procedures [10, 18]. Cosmid moeno38-5 [8] was used for transfer of *S. ghanaensis* moenomycin biosynthetic gene cluster into *A. teichomyceticus* according to described intergeneric conjugation protocol [2].

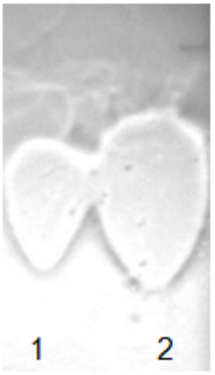


Fig. 2. Biochromatography of teichomycin A1 complex of *A. teichomyceticus* after methanol extraction and solid-phase purification (2). 1 – standard (moenomycin A, 2 mcg).

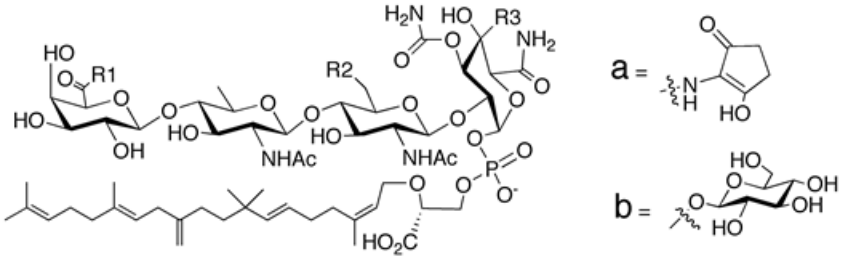
Results and discussion

At the first stage of the work, the ability of *A. teichomyceticus* to produce TeiA in different media has been examined. Several media known to give high titers of teicoplanin [4, 13, 15] were tested; of these, only TM3 and TM4 also caused the accumulation of teichomycins, as evident from biochromatography results (extract from TM3 is

shown on Fig. 2 as an example). However, there was batch-to-batch 50-fold variation in ability of *A. teichomyceticus* to produce TeiA when different rapeseed meals were used to prepare TM4. Moreover, production in TM3 varied greatly (10-fold) in spite of the fact that the same media components were used throughout the work. The production of antibiotics has long been known to be a variable trait [17], especially in wild type strains, which can be a highly heterogenous population (e.g., some cells may have spontaneously lost TeiA genes, or ability to upregulate them etc.). $TeiA_1$ variability in TM3 reflects the need for clonal selection of *A. teichomyceticus* in order to isolate stable and high-producing clones. TeiA was viewed for a long time as a minor component of all antibiotically active substances produced by *A. teichomyceticus* [1, 13], although no detailed work has been devoted to TeiA. It can be concluded that production of TeiA is greatly stimulated by certain nutritional factors, whose identity can be a subject of future research.

The TeiA moved as a single spot during thin layer chromatography (Fig. 2) because of close structural relatedness of different moenomycins [12]. To analyze the chemical composition of TeiA, a preparative thin layer chromatography has been used to purify the entire complex, which was analyzed by a set of liquid chromatography and mass spectrometry approaches. The results presented below are a summary of a series of independent fermentations in TM3 and purifications of TeiA complex. The structures of moenomycins were inferred from the accurate mass and collision-induced dissociation spectra produced in tandem mass spectrometry experiments. These parameters were compared to reference values for known moenomycins which are extremely well defined [10, 18].

The strain accumulated three known moenomycins, namely nosokomycins A and D, and compound 6 (Fig. 3, Table 1), all of which were identified in *S. ghanaensis* fermentation broths [10, 12]. Of these compounds, nosokomycin A (1485.6 Da) was a major one, and it was examined in a tandem mass spectrometry experiment to confirm its identity. The expected pattern of fragmentation of 1485.6 Da mass peak (Fig. 4) supported the proposed structure, also providing an evidence that the other peaks belong to moenomycin family. Besides known compounds, a new moenomycin A analog, referred to as teichomycin A1-1 (Table 1, Fig. 3) has been identified. It is the simplest naturally occurring moenomycin ever discovered, that combines the absence of any modification around terminal carbohydrate ring, branching glucose unit and methyl group on the hexose directly attached to phosphate group. From the biosynthetic point of view, teichomycin A1-1 can be considered a shunt product on the way to nosokomycin A. In addition to the four



	R1	R2	R3
nosokomycin A	OH	b	CH_3
compound 6	a	OH	H
nosokomycin D	NH_2	OH	CH_3
teichomycin A1-1	OH	OH	H

Fig. 3. Structures of moenomycins isolated from *A. teichomyceticus* as judged from mass spectrometry experiments.

aforementioned moenomycins, *A. teichomyceticus* appeared to accumulate several other novel moenomycins (1599.6 Da, 1151.6 Da); their structural elucidation was not pursued because of extremely low production level.

Table 1

Moenomycins isolated from *A. teichomyceticus*

Name	Rt[1], min	Exact mass $[M-H]^-$	
		Calcd	Obsvd
Nosokomycin A	9,4	1485,6164	1485,6184
Compound 6	9,1	1404,5856	1404,5872
Nosokomycin D	9,1	1322,5801	1322,5807
Teichomycin A1-1	9,7	1309,5484	1309,5478

[1] conditions of liquid chromatography described in [12]

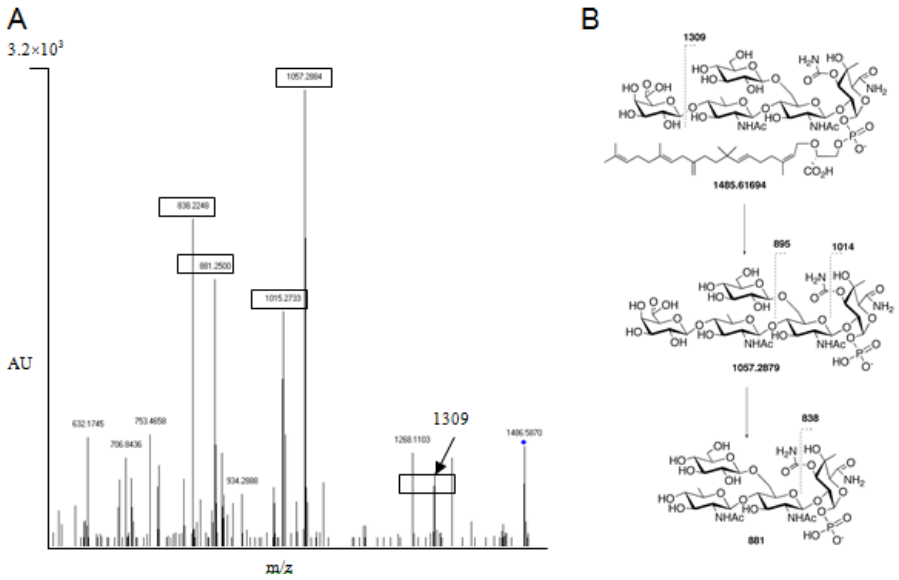


Fig. 4. Tandem mass spectrometry of 1485.6 mass peak. Collision-induced dissociation (CID) spectrum of 1485.6 (A) and expected fragmentation pattern of nosokomycin A (B). Fragments observed in CID of 1485.6 (and its 1486.6 isotope) that correspond to the expected fragments of nosokomycin A are shown in black rectangle.

The teichomycin complex produced by *A. teichomyceticus* turned out to resemble flavomycin complex of *S. ghanaensis* [12] pointing to genetic relatedness of the underlying biosynthetic pathways. Although notable variation within moenomycin family antibiotics is observed around terminal sugar decoration [12], pharmacophore parts of moenomycins and TeiA (first two sugars attached to lipid-phosphoglycerate unit) are the same. Hence, genes controlling first steps should be functionally interchangeable between *S. ghanaensis* and *A. teichomyceticus*. However, due to differences in codon usage in these two genera [17], functionally identical genes still might exhibit significant difference at the nucleotide level. To assess the degree of identity of moenomycin biosynthetic genes (*moe*) between the two microorganisms, a conjugative transfer of non-replicative cosmid moeno38-5 (carrying *moe* genes of *S. ghanaensis* [8]) has been attempted. The rationale behind this experiment was as follows: this cosmid can only be maintained in *A. teichomyceticus* cell if the homologous recombination between the cosmid and TeiA producer

genome can occur, which in its turn requires at least 70–80% of identity between the recombining DNAs [2]. Succesfull generation of *A. teichomyceticus* moeno38-5^{+} transconjugants would imply high degree of similarity between *moe* cluster and TeiA biosynthetic genes. In spite of numerous attempts, the generation of such transconjugants haven't met with success. Hence, it can be assumed that TeiA biosynthetic gene cluster does not carry long fragments of significant homology to *moe* cluster. This result encourages further investigations of TeiA biosynthesis, as genes for its production are apparently different and may have certain advantages over *moe* genes for chemoenzymatic and biocombinatorial synthesis of novel moenomycins. PCR approaches based on highly conserved regions of prenyl synthase *moeO5*, a key gene for moenomycin biosynthesis [9, 10], have therefore greater promise for identification of genes for TeiA biosynthesis.

Acknowledgements. *Author thanks Prof. S. Walker (Harvard Medical School, Boston) for the access to high-resolution MS-MS machine. This work has been supported by grant Bg-01F of the Ministry of Education, Science, Youth and Sport of Ukraine and by the scholarship of Cabinet of Ministers of Ukraine (to B. O.; 2009–2010).*

REFERENCES

1. *Bardone M. R., Paternoster M., Coronelli C.* Teichomycins, new antibiotics from *Actinoplanes teichomyceticus*-Nov-Sp.2. Extraction and chemical characterization // J. Antibiot. 1978. Vol. 31. P. 170–177.
2. *Ha H. S., Hwang Y. I., Choi S. U.* Application of conjugation using phiC31 *att/int* system for *Actinoplanes teichomyceticus*, a producer of teicoplanin // Biotechnol. Lett. 2008. Vol. 30. P. 1233–1238.
3. *Huber G*. Moenomycin and related phosphorus containing antibiotics. In: Hahn, F. E. (ed). *Antibiotics*, Springer-Verlag, New York, USA, 1979.
4. *Jung H.-M., Kim S.-Y., Prabhu P.* et al. Optimizaton of culture conditions and scale-up to plant scales for teicoplanin production by *Actinoplanes teichomyceticus* // Appl. Microbiol. Biotechnol. 2008. Vol. 80. P. 21–27.
5. *Jung H.-M., Jeva M., Kim S.-Y.* et al. Biosynthesis, biotechnological production, and application of teicoplanin: current state and perspectives // Appl. Microbiol. Biotechnol. 2010. Vol. 82. P. 57–69.
6. *Kahne D., Leimkuhler C., Lu W., Walsh C.* Glycopeptide and lipoglycopeptide antibiotics // Chem. Rev. 2005. Vol. 105. P. 425–448.
7. *Li T. L. H. F., Haydock S. F., Mironenko T.* et al. Biosynthetic gene cluster of the glycopeptide antibiotic teicoplanin: characterization of two glycosyltransferase and the key acyltransferase // Chem. Biol. 2004. Vol. 11. P. 107–109.
8. *Makitrinskyy R., Rebets Yu., Ostash B.* et al. Genetic factors that influence moenomycin production in streptomycetes // J. Ind. Microbiol. Biotechnol. 2010. Vol. 37. P.559–566.
9. *Ostash B., Saghatelian A., Walker S.* A streamlined metabolic pathway for the biosynthesis of moenomycin A // Chem. Biol. 2007. Vol. 14. P. 257–267.
10. *Ostash B., Doud E. H., Lin C.* et al. Complete characterization of the seventeen step moenomycin biosynthetic pathway // Biochem. 2009. Vol. 48. P. 8830–8841.
11. *Ostash B., Doud E., Fedorenko V*. The molecular biology of the moenomycins: towards novel antibiotics based on inhibition of bacterial peptidoglycan glycosyltransferases // Biol. Chem. 2010. Vol. 391. P. 499–504.
12. *Ostash B., Walker S.* Moenomycin family antibiotics: chemical synthesis, biosynthesis, and biological activity // Nat. Prod. Rep. 2010. Vol. 27. P. 1594–1617.

13. *Parenti F., Beretta G., Berti M., Arioli V.* Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. Description of the producer strain, fermentation studies and biological properties // J. Antibiot. 1978. Vol. 31. P. 276–283.
14. *Somma S., Gastaldo L., Corti A.* Teicoplanin, a new antibiotic from *Actinoplanes teichomyceticus* nov. sp. // Antimicrob. Agents Chemother. 1984. Vol. 26. P. 917–923.
15. *Song J. M., Park J. T., Lee H. S.* et al. Production of teicoplanin from *Actinoplanes teichomyceticus* ID9303 by adding proline // Biosci. Biotechnol. Biochem. 2008. Vol. 72. P. 1635–1637.
16. *Sosio M., Kloosterman H., Bianchi A.* et al. Organization of the teicoplanin gene cluster in *Actinoplanes teichomyceticus* // Microbiol. 2004. Vol. 150. P. 95–102.
17. *Vobis G.* The genus *Actinoplanes* and related genera // Prokaryotes. 2006. Vol. 3. P. 623–653.
18. *Zehl M., Pitternauer E., Rizzi A., Allmaier G.* Characterization of moenomycin antibiotic complex by multistage MALDI-IT/RTOF-MS and ESI-IT-MS // J. Am. Soc. Mass. Spectrom. 2006. Vol. 17. P. 1081–1090.

Стаття: надійшла до редакції 17.02.11

доопрацьована 22.02.11

прийнята до друку 23.02.11

ХАРАКТЕРИСТИКА ТЕЙХОМІЦИНУ A_1, КОМПЛЕКСУ АНТИБІОТИКІВ, ЩО ПРОДУКУЄТЬСЯ ШТАМОМ *ACTINOPLANES TEICHOMYCETICUS* NRRL-B16726

Б. Осташ

Львівський національний університет імені Івана Франка
вул. Грушевського, 4, Львів 79005, Україна
e-mail: bohdanostash@gmail.com

Actinoplanes teichomyceticus є промислово важливим мікроорганізмом, що використовується для виробництва тейкопланіну – антибіотика "останньої лінії оборони" у лікуванні інфекцій, викликаних полірезистентними коками. Цей штам також продукує іншу групу метаболітів, т.зв. комплекс тейхоміцину A_1 (TeiA). У цій роботі вивчено здатність *A. teichomyceticus* накопичувати TeiA в різних рідких середовищах, а також встановлено хімічну будову основних компонентів TeiA методами мас-спектрометрії. Виявлено, що TeiA не накопичується у всіх досліджених середовищах; поживні компоненти середовищ різного походження (такі, як рапсове та бавовникове борошно) суттєво впливали на рівень продукції TeiA. Показано, щоTeiA складається з трьох відомих і одного нового моеноміцину. Не виключено наявність інших моеноміцинів у складі TeiA, оскільки його вичерпний аналіз ускладнено низьким рівнем продукції та розгалуженим характером біосинтезу. Імовірно, що гени ранніх етапів біосинтезу моеноміцинів і TeiA є гомологічними, а виявлення останніх можливе за допомогою використання гомологічних зондів чи праймерів на основі генів *S. ghanaensis*.

Ключові слова: *Actinoplanes*, тейхоміцин A_1, моеноміцин, мас-спектрометрія.

ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2011. Випуск 56

ХАРАКТЕРИСТИКА ТЕЙХОМИЦИНА A_1, КОМПЛЕКСА АНТИБИОТИКОВ, ПРОДУЦИРУЕМОГО ШТАММОМ *ACTINOPLANES TEICHOMYCETICUS* NRRL-B16726

Б. Осташ

Львовский национальный университет имени Ивана Франко
ул. Грушевского, 4, Львов 79005, Украина
e-mail: bohdanostash@gmail.com

Actinoplanes teichomyceticus используется для производства тейкопланина – антибіотика "последней линии обороны" против полирезистентных кокков. *A. teichomyceticus* также продуцирует вторую группу метаболитов, известных как комплекс тейхомицина A_1 (TeiA). В этой работе изучена способность *A. teichomyceticus* накоплять TeiA в различных жидких средах, а также установлено химическое строение основных компонентов TeiA с помощью высокораздельной масс-спектрометрии. Показано, что TeiA не накопляется во всех использованных средах; питательные компоненты разного происхождения (такие, как рапсовая или хлопковая мука) значительно влияли на уровень продукции TeiA. Установлено, что TeiA состоит из трех известных и одного нового моеномицина. Не исключено наличие других, до сих пор неописанных, моеномицинов в составе TeiA, так как полный анализ комплекса усложнен низким уровнем продукции и разветвленным характером биосинтеза. Вероятно, гены ранних этапов биосинтеза моеномицинов и TeiA гомологичны; идентификация последних возможна с помощью гомологичных зондов или праймеров на основе генов *S. ghanaensis*.

Ключевые слова: *Actinoplanes*, тейхомицин A_1, моеномицин, масс-спектрометрия.