УДК 579.873.1:577.181.4

K. S. Marakasova, B. O. Ostash, V. O. Fedorenko

Ivan Franko National University of Lviv, Grushevsky str., 4,
Lviv, 79005, Ukraine; e-mail: v_fedorenko@franko.lviv.ua

NEGATIVE REGULATION OF MOENOMYCIN A BIOSYNTHESIS IN *STREPTOMYCES GHANAENSIS* ATCC14672

Members of the soil-dwelling prokaryotic genus Streptomyces produce around two-thirds of all known antibiotics. Moenomycin A is a potent antibacterial drug against many Gram-positive pathogens, including vancomycin-resistant pathogens. Studying of moenomycin A biosynthesis regulation is of great importance because of need of new tools for combinatorial biosynthesis. In this work we examined the effects of known pleiotropic regulator DasR on moenomycin biosynthesis under heterologous conditions. DasR controls several pivotal cellular processes, including the transport of N-acetylglucosamine (GlcNAc), ubiquitous metabolite and an important source of carbon and nitrogen in soil ecosystems. Gene dasR was inactivated in S. coelicolor and moenomycin biosynthetic gene cluster was expressed in the mutant and parent strains. Moenomycin production increased twofold in dasR-deficient strain, confirming the involvement of DasR in regulation of phosphoglycolipid production.

K e y w o r d s: streptomycetes, moenomycin A, regulator DasR, N-acetylglucosamine.

Streptomycetes are complex multicellular Gram-positive soil bacteria, perhaps best known for their ability to produce over two-thirds naturally derived antibiotics. Moenomycin A (MmA) is produced by *Streptomyces ghanaensis* ATCC14672. Moenomycin A (MmA) is a member of the phosphoglycolipid family of antibiotics, which are the only natural products known to directly target the extracellular peptidoglycan glycosyltransferases involved in bacterial cell wall biosynthesis. The emergence of resistance to existing antibiotics represents a significant threat to public health. New antibiotics with activity against resistant bacterial strains are desperately needed. The structural and biological uniqueness of MmA make it an attractive starting point for the development of new antibacterial drugs.

The entire MmA biosynthetic (*moe*) gene cluster from the producer *Streptomyces ghanaensis* ATCC14672 was cloned and sequenced [1]. The regulation of *moe* cluster gene expression is unclear because it appears to lack dedicated regulatory genes. The aim of this work is to find genes that can regulate MmA biosynthesis. Particularly we focused our attention on gene *dasR*, encoding a protein involved in regulation of transport and metabolism of N-acetylglucosamine (GlcNAc). The latter is a breakdown product of chitin, widely represented polymer in soils. Two molecules of GlcNAc are also present in the MmA, and, therefore, it is logically to suppose that DasR might somehow be implicated in regulation of MmA production. We addressed this question through expression of MmA gene cluster and subsequent analysis of MmA production levels in *dasR*-deficient and parent strains of *S. coelicolor* M145.

Materials and methods

Plasmids and *Streptomyces* strains used in this work are listed in Table. *E. coli* ET12567 (pUB307) was used to perform intergeneric conjugation from *E. coli* to *Streptomyces* strains. *Sarcina lutea* was used as a test-culture for antibiotic activity test.

Table

Plasmids and *Streptomyces* strains

Plasmid/Strain	Genotype/description	Source/reference
moeno38-5	Contains major portion of *moe* cluster 1, $Ap^rHyg^rKm^r$	Ostash B., Lviv University
cosmid SC7E4	Contains a fragment of *S. coelicolor* genome with insertion of modified Tn5 transposon into *dasR* coding sequence; $Ap^rAm^rKm^r$	Dyson P.J., University of Wales Swansea
S. ghanaensis ATCC14672	Wild-type producer of MmA	ATCC
S. coelicolor M145	$SCP1^-$, $SCP2^-$; model strain, produces actinorhodin and undecylprodigisin	M. Bibb, John Innes Centre
S. coelicolor M145Δ *dasR*	*dasR* mutant, Am^r	This study
M145Δ *dasR* moeno38-5$^+$	*S. coelicolor* M145Δ *dasR* carrying moeno38-5	This study
M145 moeno38-5$^+$	*S. coelicolor* M145 carrying moeno38-5	This study

Standard genetic techniques for *E. coli* and *Streptomyces* and for DNA manipulations were used as described by Sambrook *et al.* [2] and Kieser *et al.* [3]. *Streptomyces* strains were grown in liquid TSB media for MmA production. *Streptomyces* strains were grown on rich (R2YE) and minimal medium (MM) for estimate the effect of N-acetylglucosamine on the production of the pigmented antibiotics. *E. coli* and *Sarcina* strains were grown in LB supplemented with appropriate antibiotics. Oatmeal medium was used to obtain spores of streptomycetes and to plate intergeneric matings. Where needed, streptomycete strains were incubated in the presence of antibiotics: kanamycin (Km, 50 μg ml^{-1}), apramycin (Am, 50 μg ml^{-1}) or hygromycin (Hyg, 100 μg ml^{-1}). MmA was extracted by stirring the biomass (1 g, wet weight) with 3 ml of methanol for 12 h. The extract was concentrated *in vacuo* and diluted to the final volume of 300 μl. For the antibiotic diffusion assay, paper discs (∅ 5mm, Whatman) were impregnated with a portion of the extract and dried at 37 °C for 1 h. Discs were placed on the plates with 0.7% soft agar containing *S. lutea*. The plates were incubated at 4 °C for 1 h and then at 37 °C for 17 h. The productivity of the strains was referred back to the equal weight of the dry biomass.

Results and discussion

Bioinformatics research has revealed the presence of two genes homologous to GntR-type regulator *dasR* within the genome of *S. ghanaensis* ATCC14672. Probably, DasR is involved in control of the *Streptomyces* sugar phosphotransferase system, responsible for import of several carbon sources, most notably N-acetylglucosamine, the monomer of chitin. In literature, binding of *S. coelicolor* DasR protein to the consensus

sequence (DasR-responsive element (*dre*)) is well documented [4]. We have detected two putative *dre* elements within *S. ghanaensis* ATCC14672 genome. These elements are located within promoters upstream of two genes involved in MmA's carbohydrate portion assembly (*moeE5:* TTGGTCCGGACA, *moeGT5:* TCGGTCGGGCCC).

For knock-out of *dasR* gene in *S. coelicolor* M145 a cosmid SC7E4.1 (tab.) has been used. Phenotype of the generated mutant was confirmed via PCR analysis. We have confirmed the effect of N-acetylglucosamine on the production of the pigmented antibiotics of mutant grown on rich (R2YE) and minimal medium (MM) agar plates with/without N-acetylglucoseamine. For heterologous expression of MmA biosynthesis gene cluster, *S. coelicolor* M145 Δ*dasR* (*moeno*38-5) and *S. coelicolor* M145 (*moeno*38-5) strains were constructed. Antibiotic activities of generated mutants were tested with the help of test-culture *Sarcina lutea*. Our data show that in liquid medium *S. coelicolor* Δ*dasR* strain produces two times more moenomycin as compared to initial strain (M145). Thus DasR regulator seems to be involved in regulation of MmA biosynthesis.

Our study showed that *S. coelicolor* DasR could be involved in regulation of MmA biosynthesis. This is also supported by bioinformatic evidence for presence of two *dasR*-like ORFs and two putative *dre* sequences within *moe* cluster of *S. ghanaensis*. Probably DasR binds *dre* sequences in this way repressing MmA biosynthesis. It will be interesting to investigate the role of DasR in MmA producer, *S. ghanaensis* ATCC14672, which contains two genes highly homologous to *S. coelicolor dasR*. We suppose that DasR is not a single global negative regulator involved in MmA biosynthesis and more extensive search will turn up other repressors and activators of MmA production. Their rational manipulation will form a basis for improvement of MmA titers in native and heterologous producers.

REFERENCES

1. *Ostash B., Saghatelian A., and Walker S.* A Streamlined Metabolic Pathway for the Biosynthesis of Moenomycin A // Chemistry & Biology. — 2007. — 14, № 3. — P. 257—267.

2. *Sambrook J. and Russel D.W.* Molecular Cloning. A Laboratory Manual. — 3rd ed. — Cold Spring Harbor Laboratory, NY, 2001. — 2713 p.

3. *Kieser T., Bibb M., Buttner M.J., Chater K.F. and Hopwood D.A.* Practical *Streptomyces* genetics. — Norwich: The John Innes Foundation, 2000. — 613 p.

4. *Rigali S., Nothaft H., Noens E.E., Schlicht M., Colson S., Мьller M., Joris B., Koerten H.K., Hopwood D.A., Titgemeyer F., van Wezel G.P.* The sugar phosphotransferase system of *Streptomyces coelicolor* is regulated by the GntR-family regulator DasR and links N-acetylglucosamine metabolism to the control of development // Mol. Microbiol. — 2006. — 61, № 5. — P. 1237—1251.

Е. С. Маракасова, Б. О. Осташ, В. А. Федоренко

Львовский национальный университет имени Ивана Франко, ул. Грушевского, 4, Львов, 79005, Украина; e-mail: v_fedorenko@franko.lviv.ua

НЕГАТИВНАЯ РЕГУЛЯЦИЯ БИОСИНТЕЗА МОЕНОМИЦИНА А В ШТАММЕ *STREPTOMYCES GHANAENSIS* ATCC14672

Реферат

Проведен биоинформатический анализ генома *S. ghanaensis* ATCC14672. В геноме *S. ghanaensis* обнаружены два гена, гомологичных гену *dasR* в *S. coelicolor*,

продукт которого является членом семейства регуляторов GntR. DasR-плейотропный регулятор, который негативно регулирует биосинтез антибиотиков, метаболизм N-ацетилглюкозамина и спрообразование. В кластере генов биосинтеза моеномицина А идентифицированы вероятные последовательности *dre*, с которыми может связываться белок DasR. Получено нокаут гена *dasR* в штамме *S. coelicolor* M145. Для штамма дикого типа M145 характерно увеличение синтеза пигментированных антибиотиков актинородина и ундецилпродигиозина на минимальной среде с добавлением N-ацетилглюкозамина. Такого эффекта не наблюдалось в штамме с нокаутом гена *dasR* (*S. coelicolor* M145Δ *dasR)*. Осуществлена гетерологическая экспрессия кластера генов биосинтеза моеномицина А в штаммах *S. coelicolor* M145 Δ *dasR* и *S. coelicolor* M145. Анализ антибиотической активности этих штаммов показал, что ген *dasR S. coelicolor* M145 негативно регулирует экспрессию генов биосинтеза моеномицина А. Синтез моеномицинов в штамме с нарушенным геном *dasR* был вдвое выше, чем в штамме *S. coelicolor*, который содержит функциональный ген *dasR*.

К л ю ч е в ы е с л о в а: стрептомицеты, моеномицин А, регулятор DasR, N-ацетилглюкозамин.

К. С. Маракасова, Б. О. Осташ, В. О. Федоренко

Львівський національний університет імені Івана Франка, вул. Грушевського, 4, Львів, 79005, Україна; e-mail: v_fedorenko@franko.lviv.ua

НЕГАТИВНА РЕГУЛЯЦІЯ БІОСИНТЕЗУ МОЕНОМІЦИНУ А У ШТАМІ *STREPTOMYCES GHANAENSIS* ATCC14672

Реферат

Проведено біоінформативний аналіз геному *S. ghanaensis* ATCC14672. У геномі *S. ghanaensis* виявлено два гени, гомологічні до гена *dasR S. coelicolor*, продукт якого належить до родини регуляторів GntR. DasR-плейотропний регулятор, задіяний у негативній регуляції біосинтезу антибіотиків, метаболізму N-ацетилглюкозаміну та спороутворення. У межах кластера генів біосинтезу моеноміцину А ідентифіковано імовірні послідовності *dre*, з якими може зв'язуватись білок DasR. Отримано нокаут гена *dasR* в штамі *S. coelicolor* M145. Для штаму дикого типу M145 характерне зростання синтезу пігментованих антибіотиків актинородину та ундецилпродигіозину на мінімальному середовищі при додаванні N-ацетилглюкозаміну. Натомість такого зростання не спостерігали у штамі з нокаутом гена *dasR* (*S. coelicolor* M145Δ *dasR)*. Здійснено гетерологічну експресію кластера генів біосинтезу моеноміцину А у штамах *S. coelicolor* M145 та *S. coelicolor* M145Δ *dasR*. Аналіз антибіотичної активності цих штамів виявив, що ген *dasR S. coelicolor* M145 негативно регулює експресію генів біосинтезу моеноміцину А. Синтез моеноміцинів у штамі зі зруйнованим геном *dasR* удвічі вищий порівняно зі штамом *S. coelicolor* M145, що містить функціональний ген *dasR*.

К л ю ч о в і с л о в а: стрептоміцети, моеноміцин А, регулятор DasR, N-ацетилглюкозамін.