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SEQUENCES OF LANDOMYCIN E AND CAROTENOID BIOSYNTHETIC GENE CLUSTERS, AND MOLECULAR STRUCTURE OF TRANSCRIPTIONAL REGULATOR OF *STREPTOMYCES GLOBISPORUS* 1912

Streptomyces globisporus 1912 and its derivatives 1912-2 and 1912-4Crt are the producers of the landomycin E, carotenoids and the regulator of antibiotics biosynthesis and morphogenesis of streptomycetes. The genome DNA of two mutant strains, 1912-2, the more effective producer of the landomycin E and the regulator, and 1912-4Crt, the producer of beta-carotene and lycopene, was sequenced by Illumina. Comparative analysis of the DNA sequences using GenBank data allowed localization of 36 landomycin E biosynthetic genes lnd of S. globisporus 1912 in one cluster. Twenty of these lnd genes have been sequenced for the first time. The new regulatory responce gene lndRR and the sensor kinase gene lndY1 were proposed as the members of the putative two-component system. High identity (94–95 %) was determined for the lnd genes of the 1912 strain and those of the metagenomic clone AZ97. Seven carotenoid biosynthetic genes crt of the strain 1912-4Crt were sequenced and localized in one cluster consisting of two convergent operons from 4 and 3 crt genes. High homology (93 %) of the crt gene clusters of S. globisporus 1912 and S. griseus IFO 13350 was shown. Two non-punctual repeats (NPRs) of 21 bp were identified in the sequence of crtY gene coding lycopene cyclase. It was shown that the deletion of 117 bp including the sequence between NPRs of 96 bp and one NPR from 5`-side activated the crt gene cluster and increased the production of beta carotene (6.91 mg/l) and lycopene (3.24 mg/l) by the strain 1912-4Crt. Deletion of 86 bp was revealed in the regulatory gene lndRR resulting in the deficiency of landomycin E production in the strain 1912-4Crt. The DNA sequences of crt and lnd genes of S. globisporus 1912 were submitted to the NCBI database with accession numbers KM349312 and KJ645792, respectively.

S. globisporus 1912 produced a low-molecular-weight compound that, like A-factor, restored the landomycin E and streptomycin biosynthesis and sporulation of the defective mutants S. globisporus 1912-B2 and S. griseus 1439, respectively. The compound was purified by thin layer chromatography and HPLC. It had an absorption maximum at λ_{max} = = 245 nm and a molecular mass m/z 244. On the basis of NMR spectroscopy the chemical structure of the transcriptional regulator was elucidated as the new (L)-N-methylphenylalanyl-dehydrobutyrine diketopiperazine.

K e y w o r d s: Streptomyces globisporus, landomycin E, beta-carotene, lycopene, diketopiperazine, gene clusters, sequences.

Soil bacteria Streptomyces produces the majority of the known antibiotics, many of the carotenoids and transcriptional regulators [18–20]. The research of the new antitumor antibiotics presents an important scientific problem because of the rise of drug resistant metastatic cells from the initial tumor [4]. The

natural pigments beta-carotene and lycopene play an important role as the antioxydants and biostimulators, and are widely used in the medicine, food industry and cosmetics [3]. The transcriptional regulators of Streptomyces are presented by a large number of the different chemical compounds providing their producers with the ability to form aerial mycelia, secondary metabolites and adapt to environmental conditions [18].

The approach for solving the above mentioned problems includes the microbiological, biochemical, physical and molecular genetic methods.

Genetic control of the biosynthesis of the new antitumor antibiotic landomycin E and carotenoids, and molecular structure of the transcriptional regulator of antibiotic production and morphogenesis in *Streptomyces globisporus* 1912 were studied during last 5 years at the Department of Genetics of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (IMV NASU) [12–15].

Present paper contains the main results dedicated the genetic control of landomycin E and carotenoid biosynthesis, and molecular structure of the new transcriptional regulator of *S. globisporus* 1912, obtained in the IMV NASU.

Materials and methods. *Strains and media.* The initial wild-type strain *S. globisporus* 1912 was isolated from a soil sample from Armenia and stored in the Ukrainian Collection of Microorganisms at the Institute of Microbiology and Virology of NASU as the strain *S. globisporus* Ac-2098 [htpp://www.imv.kiev.ua/images/doc/catalog/UCM_catalog.pdf].

The following three derivatives of this strain were used in present study. The mutant strain 1912-2 was isolated by action of nitrosoguanidine on the spores and mycelium fragments of the strain 1912 [15]. It produces about 200 mg/L of the landomycin E and the new transcriptional regulator of antibiotic biosynthesis and morphogenesis in streptomycetes of diketopiperazine nature. The strain 1912-B2 is defective in landomycin E and regulator biosynthesis [12]. The strain 1912-4Crt is the spontaneous mutant of 1912, producing beta-carotene (7.0 mg/L) and lycopene (3.24 mg/L). The strains *S. griseus* 773 and 1439, the producer of streptomycin, and the antibiotically and A-factor inactive mutant, respectively, were also used in this study [1, 6]. The mentioned streptomycetes were grown on liquid and solid minimal and corn-soy media [15].

Purification of the A-factor and the new transcriptional regulator. The A-factor (2S)-isocapryloyl-(3R)-oxymethyl-γ-butyrolactone) and the unknown regulator were extracted from solid media with chloroform-acetone (2:1) and separated by thin layer chromatography on silica gel 60 F254 (Merck, Darmstadt, Germany).

Both regulators were further purified by HPLC using an HPLC/MS (Agilent Technologics, Germany) system with UV detector and a Zorbax Hypersyl ODS reversed phase column.

Absorption and NMR spectra of the regulator were measured at 125.707 MHz (^{13}C NMR) and 300.141 MHz (^{1}H NMR) on Varian INOVA 500 and Mercury 300 spectrometers, respectively. LC/MS was performed using a liquid chromatograph, Agilent Technologies 1200.

Isolation of chromosomal DNA. Chromosomal DNA of 1912-2 and 1912-

4Crt was prepared according to the standard protocol of the Kirby method, dissolved in TE buffer, pH 8.0 and stored at 4 °C [7]. The A_{260}/A_{280} index of the DNA preparations was equal to 2.1–2.3, and agarose gel electrophoresis showed one compact and localized band of DNA.

DNA sequencing was carried out in BaseClear B.V. (Leiden, Netherlands) by the Illumina technology.

Results. *Structure elucidation of the new transcriptional regulator.* The strain 1912-2 produced the compound with Rf 0.4, which is absent in the extract of agar culture of the mutant strain 1912-B2. This compound, like the A-factor, restored the sporulation and antibiotic biosynthesis in *S. griseus* 1439 and *S. globisporus* 1912-B2 (Fig. 1).

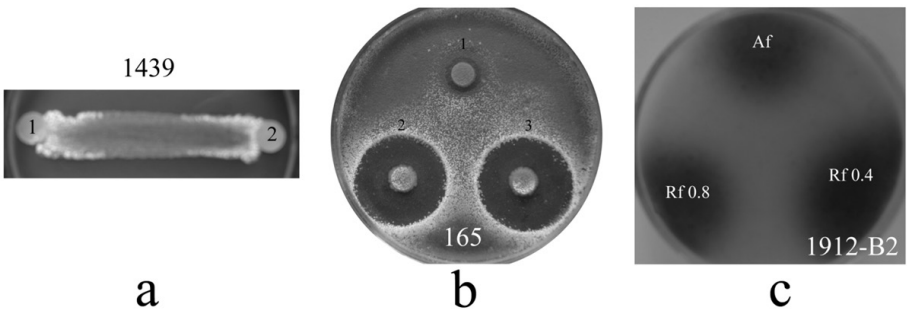


Fig. 1. Biological activity of (L)-N-methylphenylalanyl-dehydrobutyrine diketopiperazine (MDD): (a) restoration of sporulation of *S. griseus* 1439 by A-factor (1) and MDD (2); (b) restoration of streptomycin biosynthesis in *S. griseus* 1439 by A-factor (1) and MDD (2). Agar blocks cut out from sporulating (1, 2) and non-sporulating (3) areas of an agar culture of strain 1439 (a) were puted on the lawn of a streptomycin-sensitive culture *S. levoris* 165. Zones of growth inhibition were generated by streptomycin. (c) Restoration of landomycin E biosynthesis in *S. globisporus* 1912-B2 by A-factor (1), MDD (3) and a compound with Rf 0.8 (2)

HPLC and LC/ESIMS of the purified regulator gave a clear molecular ion signal at *m/z* 245 $(M + H)^+$. NMR spectroscopy afforded the molecular formula of $C_{14}H_{16}N_2O_2$ and the molecular structure of the new compound *N*-methylphenylalanyl-dehydrobutyrine diketopiperazine (MDD) (Table 1, Fig. 2 and 3). It is known that A-factor binds to the regulatory domain of the receptor proteine ArpA of *S. griseus* NRBC 13350 [5, 19]. *S. globisporus* 1912-2 contained *arpA* gene of 831 nucleotides, 92 % of which have identity with the sequence of the SGR_3731 gene encoding the ArpA protein in *S. griseus* NRBC 13350.

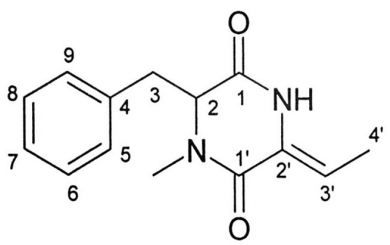
The high identity between the ArpA of 1912-2 and SGR_3731 genes explains the similar biological activity of A-factor and MDD. Both regulators bind to the receptor protein ArpA, causing its dissociation and consequently activation of transcription of the genes of the AdpA regulon, which control aerial mycelium formation as well as the biosynthesis of antibiotics and pigments.

Complete sequnence of landomycin E biosynthetic gene cluster. S. globisporus 1912 produced the red-orange antitumor antibiotic landomycin E, a member of the angucycline family antibiotics, occupied a prominent place among polyketides [9]. Landomycin E induces apoptosis in a G0-G1 phase of

Table 1

NMR measurements (^{1}H, ^{13}C, HSQC, HMBC, COSY, NOE) of the regulator

	^{13}C (125 MHz)	atom type	^{1}H (300 MHz)	HMBC	COSY	NOESY
1	165.4	C_q	–			
1’	158.7	C_q	–			
4	134.8	C_q	–			
5, 9	129.6	2 CH	7.02 (m)	7	6/8	
6, 8	128.0	2 CH	7.20 (m)	4	5/9	
2’	127.2	C_q	–			
7	126.8	CH	7.20 (m)			
3’	111.6	CH	5.38 (q, *J*)	1’		4’
2	62.7	CH	4.36 (m)	1, 1’, 3, 4	3	
3	36.7	CH_2	3.15, 3.01, ABX, J_{AB} , J_{AX} , J_{BX}	1, 2, 4, 5/9	2	
NMe	32.2	CH_3	2.97 s	1’, 2		—
4’	10.6	CH_3	1.41 d, *J*	2’, 3’		3’, NH
		NH	9.74 s br			



6-Benzyl-3-eth-(Z)-ylidene-1-methyl-piperazine-2,5-dione

Fig. 2. Molecular structure of MDD

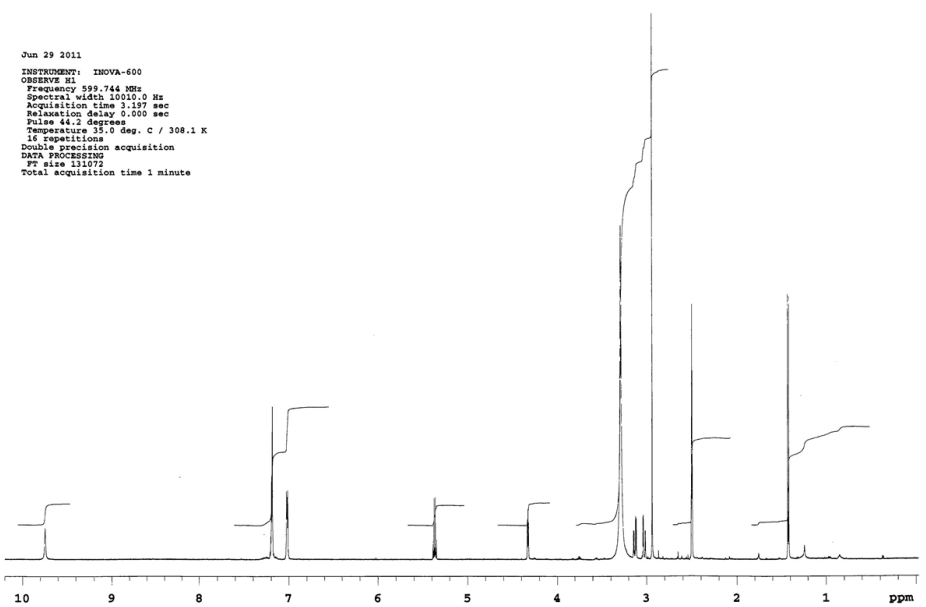


Fig. 3. NMR spectrum of MDD

cancer cells, including those resistant to doxorubicin [8].

The biosynthesis of landomycin E is encoded by the *lnd*-cluster which consists of 27 structural and 3 regulatory genes [2]. The complete sequences of 14 *lnd* genes of *S. globisporus* 1912 were established earlier [17].

In the Department of Genetics of microorganisms of IMV NASU the determination of the sequences of all *lnd* genes of the landomycin E cluster from *S. globisporus* 1912 was finished. It included 20 previously unsequenced genes, and the cause of the lack of *lnd* genes expression in mutant strain 1912-4Crt was cleared up [14].

The localization of the *lnd* genes in the assembled genome was identified with the BLAST program (www.ncbi.nlm.nih.gov/blast) using the information about sequences of *lnd* and *lan* genes available in GenBank. The sequences of the last 20 *lnd* genes of the landomycin E biosynthetic gene cluster from *S. globisporus* 1912 were identified and localized in the contig 220/23 on the basis of high identity (94–95 %) with the *lnd* genes from the metagenomic clone AZ97 (GenBank HQ828984). Comparison of the above mentioned *lnd* genes and *lan* genes, encoding biosynthesis of the landomycin A in *S. cyanogenus* S136 (GenBank AF080235), showed a lower degree of homology (identity 80–85 %).

Characteristics of the *lnd* and *lan* genes from the three different clusters are presented in the Table 2. The genetic organization of the landomycin E biosynthetic gene cluster from *S. globisporus* 1912 is presented in the Fig. 4. The new response regulatory gene *lndRR* was identified.

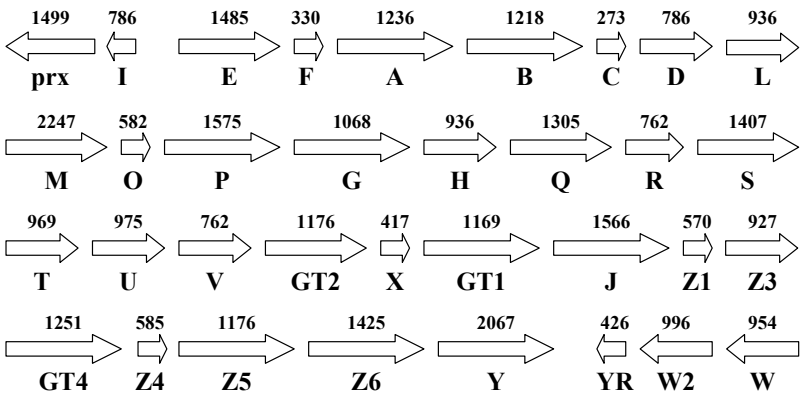


Fig. 4. Organization of the landomycin E biosynthetic gene cluster of *S. globisporus* 1912

The genes *lndRR* and *lndY1* belong to a putative two-component system involved in the regulation of landomycin E biosynthesis. The gene *lndRR* in the strain 1912-4Crt has a deletion of 86 bp, from 31702 to 31787 bp (GenBank KJ645792) resulting in a strain deficient in landomycin E biosynthesis.

Complete sequence of the carotenoid biosynthetic gene cluster.

Genetic study of the representatives of different species of *Streptomyces* showed the presence of the carotenoid biosynthetic gene clusters in their genomes in the functionally inactive state. Activation of the transcription of these

Table 2

Characterization of landomycin A and E biosynthetic genes

Gene	Strain and gene size, bp			Gene localization		Gene product
	S136	AZ97	1912-2	Contig	Length, bp	
prx	–	–	1499	478	2542 – 4035	endopeptidase
*lndI**	–	786	786	“	1081 – 1866	two-component regulatory system
lndE	1476	1485	1485	220/23	495 – 1979	oxygenase
lndF	330	330	330	“	2018 – 2347	cyclase
lndA	1275	1236	1236	“	2410 – 3645	ketosynthase
lndB	1221	1218	1218	“	3642 – 4859	chain length factor
lndC	270	273	273	“	4950 – 5222	acyl carrier protein
lndD	786	786	786	“	5271 – 6056	polyketide ketoreductase
lndL	960	936	936	“	6095 – 7030	cyclase
lndM	2250	2238	2247	“	7033 – 9279	oxygenase-reductase
lndO	582	582	582	“	9342 – 9923	reductase
lndP	1575	1575	1575	“	10011 – 11585	decarboxylase
lndElndG	1068	1068	1068	“	11969 – 13036	NDP-hexose synthetase
lndH	981	987	936	“	13084 – 14019	NDP-hexose 4,6-dehydrogenase
lndQ	1305	1305	1305	“	14112 – 15416	NDP-hexose 3,4-dehydratase
lndR	762	762	762	“	15413 – 16174	4-keto-reductase
lndS	1410	1407	1407	“	16207 – 17613	NDP-hexose 2,3-dehydratase
lndT	966	969	969	“	17613 – 18581	oxidoreductase
lndU	648	975	975	“	18578 – 19552	unknown
lndV	762	762	762	“	19655 – 20416	reductase
lndGT2	1122	1176	1176	“	20797 – 21972	glycosyl transferase
lndX	444	417	417	“	21969 – 22385	isobutyryl-CoA mutase
lndGT1	1173	1169	1169	“	22480 – 23469	glycosyl transferase
lndJ	1554	1563	1566	‘	23787 – 25352	transporter
lndZ1	579	570	570	“	25404 – 25973	NDP-hexose 3,5-epimerase
lndZ3	981	951	927	“	25985 – 26911	NDP-hexose 4-ketoreductase
lndGT4	1254	1251	1251	“	26970 – 28220	glycosyltransferase
lndZ4	603	585	585	“	28251 – 28835	putative reductase
lndZ5	1194	1158	1176	“	28832 – 30007	oxygenase
lndZ6	717	1500	1445	“	30185 – 31629	unknown
lndRR	–	–	780	“	31721 – 32500	two-component regulatory system
lndY1	–	–	723	“	32595 – 33317	sensor kinase
lndY2	–	–	762	“	33836 – 34597	kinase
lndYR	–	–	426	“	34653 – 35078	GntR-like regulator
lndW2	–	–	996	“	35104 – 36099	ABC transporter
lndW	–	–	954	“	36096 – 37049	ATP-ase of ABC transporter

The break between contigs 478 (*lndI*) and 220/23 (*lndE*) consists of 6 bp (5`-CGTCCG-3`).

cryptic *crt* genes in *S. coelicolor* A3(2) and *S. griseus* IFO 13350 requires induction of a stress-responsible sigma factor by illumination the culture with blue light [19] or increasing copy number of a *crtS* gene [10].

In some rare cases the carotenoid producing mutants can appear in a spontaneous manner in *S. globisporus* 1912 [11] and *S. albus* J1074 [16]. Complete

sequence of the carotenoid biosynthetic gene cluster in the strains 1912-2 and 1912-4Crt, defective and active producers of carotenoids, correspondingly, will give answer on the mechanism of activation of the carotenoid biosynthesis.

The genomes of the strains 1912-2 and 1912-4Crt were sequenced by Illumina. Identification of the *crt* genes in the contigs was carried out by means of the BLAST tools using the sequences of *crt* genes of different *Streptomyces species* present in GenBank (Table 3). The sizes of *crt* genes of streptomycetes are approximately equal with the exception of the smaller length *crtI* of *S. coelicolor* A3(2) and *crtB* of *S. albus* J1074.

Table 3

Characteristic of the carotenoid biosynthetic genes of *Streptomyces*

NN	Strain (GenBank access N)	*crt* gene, size (bp) and identity with 1912 *crt* gene (%)						
		crtE	***crrtI***	***crtB***	***crtV***	***crtU***	***crtT***	***crtY***
1	*S. coelicolor* A3(2) (AL939104.1)	1572 (68)	996 (76)	1179 (75)	1011 (75)	1569 (74)	741 (69)	1218 (69)
2	*S. griseus* NRBC 13350 (AF272737.1)	1278 (84)	1524 (92)	1029 (92)	1017 (95)	1554 (93)	729 (94)	1242 (93)
3	*S. avermitilis* MA-4680 (AB070934.1)	1140 (66)	1542 (78)	1029 (75)	1080 (75)	1503 (76)	732 (70)	1347 (69)
4	*S. albus* J1074 (NC_020990)	1239 (68)	1542 (78)	963 (75)	999 (75)	1560 (74)	– (72)	1209 (72)
5	*S. fulvissimus* DSM 40593 (NC_021177.1)	1251 (74)	1518 (86)	1034 (83)	1023 (82)	1563 (85)	714 (80)	1230 (77)
6	*S. globisporus* 1912 (KM349312)	1206	1518	1029	1005	1554	717	1239

Products of the *crt* genes: *crtE* – geranylgeranyl pyrophosphate synthase, *crtI* – phytoene synthase, *crtB* – phytoene dehydrogenase, *crtV* – methylesterase, *crtU* –dehydrogenase, *crtT* – methyltransferase, *crtY* – lycopene cyclase.

There is a considerably greater difference between the homology of *crt* genes. All *crt* genes of *S. globisporus* 1912 have very high identity with the corresponding *crt* genes of *S. griseus* NRBC 13350 (92–95 %). There is also similarity between all *crt* gene clusters in organization and direction of transcription. These clusters are presented by two convergent operons (Fig. 5).

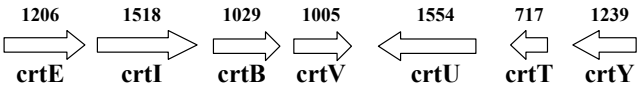


Fig. 5. Organization of the carotenoid biosynthetic gene cluster of *S. globisporus* 1912

The main result of our study was the discovery of the deletion of 117 bp in the *crtY* gene coding lycopene cyclase. The length of *crtY* in 1912-2 strain with the silent *crt* gene cluster was 1239 bp, whereas in 1912-4Crt strain, producing beta-carotene and lycopene, it was reduced to 1122 bp. The *crtY* gene in the strain 1912-2 has two non-punctual repeats (NPRs) of 21 bp, flanking the sequence of 96 bp. The deletion begins from 669 bp and last to 785 bp. Both repeats are not identical.

NRP from 3`-side of the (+) DNA strand contained four base substitutions (underlined letters) (Table 4). The first 6 bp from the 5`- side are the same in both NRPs (GGGGCG) and may be the site for site-specific recombination resulting in the deletion of the NRP from 3`-side and the flanking sequence of 96 bp. The rearranged *crtY* gene in the strain 1912-4Crt has 1122 bp and only one NRP with the overlapping stop-start codon TGATG [CATCA in the (+) DNA strand].

Table 4

Non punctual repeats in crtY gene of Streptomyces

Strain	Non punctual repeat sequences
S. globisporus 1912-2	5`- GGGGCGTGCGGAAGTCCATCA.... ....GGGGCGGGCGGGAGTCGAACA – 3`
S. coelicolor A3(2)	5`- GGGGCACGCGGAAGTCCATCA.... ....GGGAAGCGGCCGTGAGTCGAA –3
S. griseus NRBC 13350	5`- GGGACGTGCGGAAGTCCATCA.... ....GGGGCGGACGGGAGTCCAACA – 3`
S. avermitilis MA-4680	5`- GGGGTGTGCGGAAGTCCATGA.... ....CGAGCGGGCGCGAGTCGAAGA – 3`
S. albus J1074	5`- GTGCGGTGCGGAAGTCCATCA.... ....GGGTAGCGGGCGCGAGTCGAA – 3`
S. fulvissimus DSM 40593	5`- GCGGTGTGCGGAAGTCCATCA.... ....GGGGCGGGCGGGAGTCGAAGA – 3`

Therefore we can propose the hypothesis that the spontaneous deletion of 117 bp in the *crtY* gene leads to activation of the carotenoid production in the mutant strain 1912-4Crt. The output of the carotenoids after culture growing in the shake flasks in corn-meal medium was high, 6.91 mg/l of beta-carotene and 3.24 mg/l of lycopene. The level of the produced lycopene was increased to 50.9 mg/l in the selected mutant 1912-7Hp, the spontaneous derivative of 1912-4Crt. These strains present technological interest as possible candidates for further improvement of the carotenoid production.

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СІКВЕНСИ КЛАСТЕРІВ ГЕНІВ БІОСИНТЕЗУ ЛАНДОМІЦИНУ Е І КАРОТИНОЇДІВ І МОЛЕКУЛЯРНА СТРУКТУРА РЕГУЛЯТОРА ТРАНСКРИПЦІЇ *STREPTOMYCES GLOBISPORUS* 1912

Резюме

Streptomyces globisporus 1912 і його похідні 1912-2 та 1912-4Crt є продуцентами ландоміцину Е, каротиноїдів і регулятора біосинтезу антибіотиків і морфогене-

зу стрептоміцетів. Геномна ДНК двох мутантних штамів 1912-2 – більш активного продуцента ландоміцину Е і регулятора, і 1912-4Crt – продуцента бета-каротину і лікопіну, сіквенована за допомогою Illumina. Порівняльний аналіз послідовностей ДНК з використанням даних GenBank дозволив локалізувати 36 генів біосинтезу ландоміцину Е *S. globisporus* 1912 в одному пучку. Двадцять із цих генів сіквеновано вперше. Новий регуляторний ген lndRR і ген сенсорної кінази lndY1 запропоновані як члени передбачуваної двокомпонентної системи. Показана висока ідентичність (94–95 %) генів lnd штаму 1912 і метагеномного клону AZ97. 7 генів біосинтезу каротиноїдів crt штаму 1912-4Crt сіквеновані і локалізовані в одному пучку, який складається із двох конвергентних оперонів із 4-х і 3-х crt-генів. Показана висока гомологія (93 %) пучків генів crt *S. globisporus* 1912 і *S. griseus* IF0 13350. Ідентифіковано два не пунктуальні повтори (NPRs) із 21 н.п. у послідовності гена лікопін циклази crtY. Показано, що делеція із 117 п.н., яка включає послідовність із 96 п.н. між двома NPRs та один NPR із 5`-кінця, активує кластер генів crt і продукцію бета-каротину (6,91 мг/л) і лікопіну (3,24 мг/л) штамом 1912-4crt. Делеція із 86 п.н. виявлена в регуляторному гені lndRR, яка викликає недостатність біосинтезу ландоміцину Е у штамі 1912-4crt. Послідовності ДНК генів crt і lnd *S. globisporus* 1912 включені в базу даних NCBI під номерами доступу KM 349312 і KJ645792 відповідно.

S. globisporus 1912 продукує низькомолекулярну сполуку, яка, подібно до А-фактора, відновлює біосинтез ландоміцину Е і стрептоміцину та споруляцію у дефектних мутантів *S. globisporus* 1912-B2 і *S. griseus* 1439 відповідно. Сполука очищена за допомогою тонкошарової хроматографії і ВЕРХ. Вона має максимум абсорбції при λ_{max} = 245 нм і молекулярну масу m/z 244. На основі ЯМР-спектроскопії встановлена хімічна структура транскрипційного регулятора як (L)-N-метилфенілаланіл-дегідробутирін дікетопіперазин.

Ключові слова: Streptomyces globisporus, ландоміцин Е, бета-каротин, лікопін, дікетопіперазин, пучки генів, сіквенси генів.

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СИКВЕНСЫ КЛАСТЕРОВ ГЕНОВ БИОСИНТЕЗА ЛАНДОМИЦИНА Е И КАРОТИНОИДОВ И МОЛЕКУЛЯРНАЯ СТРУКТУРА РЕГУЛЯТОРА ТРАНСКРИПЦИИ *STREPTOMYCES GLOBISPORUS* 1912

Резюме

Streptomyces globisporus 1912 и его производные 1912-2 и 1912-4Crt являются продуцентами ландомицина Е, каротиноидов и регулятора биосинтеза антибиотиков и морфогенеза стрептомицетов. Геномная ДНК двух мутантных штаммов 1912-2 – более активного продуцента ландомицина Е и регулятора, и 1912-4Crt – продуцента бета-каротина и ликопина, сквенирована с помощью Illumina. Сравнительный анализ последовательностей ДНК с использованием данных GenBank дал возможность локализовать 36 генов биосинтеза ландомицина Е *S. globisporus* 1912 в одном пучке. Двадцать из этих генов сиквенировано впервые. Новый ответственный регуляторный ген *lndRR* и ген сенсорной киназы *lndY1* предложены в качестве членов предвиден-

ной двухкомпонентной системы. Показана высокая идентичность (94–95 %) генов lnd штамма 1912 и метагеномного клона AZ97. 7 генов биосинтеза каротиноидов *crt* штамма 1912-4Crt сиквенированы и локализованы в одном пучке, состоящем из двух конвергентных оперонов из 4-х и 3-х *crt*-генов. Показана высокая гомология (93 %) пучков генов *S. globisporus* 1912 и *S. griseus* IF0 13350. Идентифицировано два не пунктуальных повтора (NPRs) из 21 п.н. в последовательности гена ликопин циклазы *crtY*. Показано, что делеция из 117 н.п., включающая последовательность из 96 п.н. между двумя NPRs и один NPR из 5`-конца, активирует кластер генов *crt* и продукцию бета-каротина (6,91 мг/л) и ликопина (3,24 мг/л) штаммом 1912-4Crt. Делеция из 86 н.п. обнаружена в регуляторном гене *lndRR*, вызывающая недостаточность биосинтеза ландомицина Е в штамме 1912-4Crt. Последовательности ДНК генов *crt* и *lnd S. globisporus* 1912 включены в базу данных NCBI под номерами доступа KM349312 и KJ645792 соответственно.

S. globisporus 1912 продуцирует низкомолекулярное вещество, восстанавливающее, подобно А-фактору, биосинтез ландомицина Е и стрептомицина, а также споруляцию у дефектных мутантов *S. globisporus* 1912-B2 и *S. griseus* 1439 соответственно. Вещество очищено с помощью тонкослойной хроматографии и ВЭЖХ. Оно имеет максимум поглощения при λ_{max} = 245 нм и молекулярную массу m/z 244. На основании ЯМР-спектроскопии установлена химическая структура регулятора транскрипции как новый (L)-N-метилфенилаланил-дегидробутирин дикетопиперазин.

Ключевые слова: Streptomyces globisporus, ландомицин Е, бета-каротин, ликопин, дикетопиперазин, пучки генов, сиквенсы генов.

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