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OVEREXPRESSION OF SGM 5' UTR MRNA REDUCES GENTAMICIN RESISTANCE IN BOTH *ESCHERICHIA COLI* AND *MICROMONOSPORA MELANOSPOREA* CELLS

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Abstract — The 16S rRNA methylases are expressed by most of the antibiotic producing bacteria in order to protect themselves against antibiotics by methylation of 16S rRNA at positions which are crucial for their action. The sgm sisomicin-gentamicin resistance gene from *Micromonospora zionensis* methylates G1405 positioned in the A site of 16S rRNA, which includes a CCGCCC hexanucleotide. The same hexanucleotide is also present 14 nucleotides in front of the ribosome binding site of sgm mRNA. The model proposed for translational regulation of sgm assumes that Sgm binds to this motif, both on 16S rRNA and on the 5' untranslated region (UTR) of its own mRNA. The 5' UTR mRNA sequence was overexpressed on 3'-truncated sgm mRNA, and the effect on gentamicin resistance conferred by Sgm was tested in *Escherichia coli* and in *Micromonospora melanosporea*. Overexpression of the sgm mRNA regulatory region decreases the resistance to gentamicin in both *E. coli* and *M. melanosporea*. This effect is likely to be due to titration of Sgm molecules by the overexpressed 5' UTR.

Key words: Sgm, 16S rRNA methylase, gentamicin resistance, regulation

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INTRODUCTION

Aminoglycoside-producing strains utilize ribosomal modification as a means of self-defense, regardless of whether they also employ drug-modifying enzymes. Some of the genes cloned from actinomycetes that produce aminoglycoside antibiotics include the *grm* gene from *Micromonospora purpurea* (Kelemen et al., 1991; Vasiljevic and Cundliffe, 1990), *sgm* from *Micromonospora zionensis* (Kojic et al., 1992) and two genes (*kamB* and *kgmB*) from *Streptomyces tenebrarius* (Holmes and Cundliffe, 1991; Skeggs et al., 1987). In each case, resistance is due to methylation of ribosomal RNA at a single site characteristic of a given phenotype, i.e. resistance to a particular group of aminoglycoside antibiotics. For example, the KgmB enzyme methylates residue G-1405 in 16S rRNA using S-adenosyl-methionine (SAM) as a co-factor (Beauclerk and Cundliffe, 1987). It was recently shown in *in vitro* methylation assays that Sgm and KgmB methylases act at the same residue, that is at G1405 within 16S rRNA (manuscript in preparation).

Both *sgm* and *kgmB* appear to be down-regulated at the posttranscriptional level by a mechanism of translational autoregulation (Kojic et al., 1996; Vajic et al., 2004). According to the model proposed, expression of the resistance genes ensures that enough methylase molecules modify 16S rRNA (the primary target), and when all ribosomes are protected unnecessary translation is prevented by binding to their own mRNA (the secondary target). A CCGCCC hexanucleotide has been identified 14 nucleotides upstream of the ribosome binding site of the *sgm,* and the same hexanucleotide is also present in the A site of 16S rRNA, i.e., the region where most of the aminoglycoside resistance methylases act (Fourmy et al., 1996, Vicens and Westhof, 2003). Moreover, Sgm methylase can down-regulate *kgmB*::*lacZ* fusions, presumably by binding to a CGCCC motif present in the 5' UTR region of *kgmB* (Vajic et al., 2004).

In the course of elucidating the Sgm's translational autoregulation by employing the *E. coli lacZ* gene and operon fusion systems, it has been noticed

that overexpression of the secondary target, i.e., the *sgm* mRNA, could alter the resistance of *E. coli* to gentamicin. The aim of the present work was to confirm this observation. Accordingly, further experiments were designed to test this possibility, both in a heterologous host such as *E. coli* and in a homologous host, *M. melanosporea.*

MATERIALS AND METHODS

Strains, growth conditions, and plasmids used in this study

E. coli strain NM522, *Micromonospora melanosporea* DSM43126 and *Streptomyces lividans* TK21 were used. Growth media were prepared as described in Sambrook et al. (1989) and Hopwood et al. (1985). In short, the *E. coli* strain was grown in LB (Luria-Bertani) medium. The *Micromonospora* strain was grown on mM plates, and trypticase soy broth (TSB) supplemented with manitol (3% final concentration) was used for growth in liquid medium. The *Streptomyces lividans* strain was grown on NE plates and YEME liquid medium. R2YE supplemented with MRS liquid medium was used for regeneration of *Micromonospora* protoplasts. Ampicilin at concentrations of 50 μg/ml and 30 μg/ml gentamicin were used for selection of transformants in *E. coli.* In *Micromonospora*, plasmids were selected at 30 μg/ml gentamicin and 40 μg/ml nosiheptide. Isopropyl-β-D-thiogalactopyranoside (IPTG) was used for transcriptional induction, its concentrations being as cited in the text. All plasmids used in this work, with relevant characteristics and details of their constructions, are listed in Table 1.

Transformation procedures and recombinant DNA techniques

Restriction endonuclease digestions, ligations and transformation of *E. coli* strains were performed

Table 1. Plasmids used in this study.

Plasmid	Genes and characteristics of interest	Source or reference
pUC19	Amp^r, high copy number plasmid	Yanisch-Perron et al., 1985
pUF1	Amp^r, pUC19, *sgm-lacZ* fusion preceded by the regulatory sequence under control of inducible $P_L$tl promoter.	Kojic et al., 1996
pFΔR	Amp^r, pUC19, plasmid carries *sgm-lacZ* fusion without the regulatory sequence under control of $P_L$tl .	This study
pULL	Amp^r, pUC19, *Pvu*I- *Eco*RI α-fragment of β-galactosidase under control of $P_L$tl .	This study
pUF6KS2	Amp^r, Gm^r, pUC19, *sgm-lacZ* fusion preceded by the regulatory sequence under control of the inducible $P_L$tl and *sgm* gene under control of constitutive P_{kan}.	This study
pUFΔRKS2	Amp^r, Gm^r, pUC19, plasmid carries *sgm-lacZ* fusion without the regulatory sequence under control of $P_L$tl. and *sgm* gene under control of constitutive P_{kan} .	This study
pMZ1	Cryptic plasmid from *Micromonospora zionensis*	Oshida et al., 1986
pBMZ13	Gm^r, pMZ1, *sgm* gene under control of its own promoters.	Vukov and Vasiljevic, 1998
pMRSP1	Nh^r, pIJ486, N-terminal region of *sgm* under control of P1 promoter from pMZ1	This study
pMRSP29	Nh^r, pIJ486, N-terminal region of *sgm* under control of P29 promoter from pMZ1.	This study

according to standard protocols (Sambrook et al., 1989; Hopwood et al., 1985). The *Micromonospora melanosporea* strain was transformed using transformation procedures described previously (Kojic et al., 1991).

Determination of minimal inhibitory concentrations (MIC)

Equal masses of micelial fragments were resuspended in 1 ml of TSB medium. 100 µl from each suspension was streaked on mM medium plates containing different concentrations of antibiotics. Lists of antibiotics and concentrations used are given in Table 2. Cell growth in liquid media was monitored by reading OD at 550 nm.

RESULTS

Overexpression of 3'-truncated sgm mRNA lowers resistance to gentamicin conferred by Sgm in E. coli

To determine whether overexpression of Sgm's secondary target might effect Sgm-conferred gentamicin resistance in *E. coli*, three vectors were constructed, each harboring *sgm* under control of the P_{kan} promoter, while the 5' UTR of *sgm* was expressed from the inducible $P_L$tl promoter (Fig. 1). pUF6KS2 expresses the *sgm-lacZ* fusion, which contains the regulatory hexanucleotide preceding the RBS of *sgm*, while pUFΔRKS2 is characterized by deletion of the hexanucleotide. The pULLKS2 plasmid was used as a negative control; it contains only the α-fragment of β-galactosidase without 5' UTR sequences and the N-terminal region of *sgm*. The growth rate of *E. coli* NM522 cells containing plasmids pUF6KS2, pUFΔRKS2, and pULLKS2 was measured under conditions of increasing concentrations of gentamicin and induction of transcription from $P_L$tl with IPTG. Cells were grown in overnight cultures in the presence of 50 µg/ml ampicilin. Batches of liquid cultures with increasing concentrations of gentamicin and in the absence or presence of 1 mM IPTG were inoculated with 1% of overnight cultures and cell growth was monitored after 16 h of growth (Fig. 2). It was observed that cells with the pUF6KS2 plasmid, which overexpress the *sgm* secondary target, show a marked reduction of growth rate in the presence of gentamicin. Cells carrying pUFΔRKS2, which do not express the *sgm* secondary target, showed the same growth rate in the presence of gentamicin as observed for the control strain with pULLKS2.

Correlation between induction of secondary target transcription and sensitivity to gentamicin

Correlation between induction of transcription of secondary target (5' UTR plus) and sensitivity to gentamicin in *E. coli* cells was further tested by measuring growth rates of cells with pUF6KS2, pUFΔRKS2, and pULLKS2 throughout the course of the experiment. Cells were grown in LB medium

Table 2. Resistance of *Micromonospora melanosporea* clones to gentamicin and nosiheptid.

	Gm 5 +Nh20	Gm 10 +Nh20	Gm20 +Nh20	Gm50 +Nh20	Gm100 +Nh20	Gm200 +Nh20	Gm300 +Nh20	Gm400 +Nh20
M. melanosporea +pBMZ13 +pMRSP1	+	+	+	-	-	-	-	-
M. melanosporea +pBMZ13 +pMRSP29	+	+ / -	-	-	-	-	-	-
	Gm 5	Gm10	Gm20	Gm50	Gm100	Gm200	Gm300	Gm400
M. melanosporea + pBMZ13	+	+	+	+	+	+	+	+

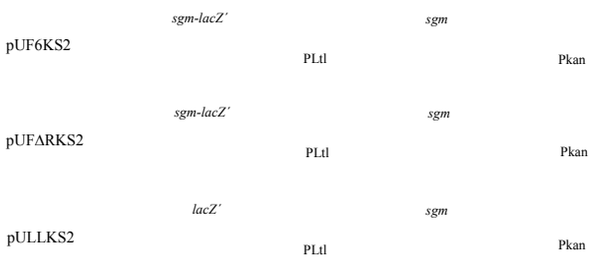

Fig. 1. Plasmid system for analysis of the influence of Sgm secondary target overexpression on E. coli gentamicin resistance. Only relevant plasmid regions for this study are shown. Shaded boxes indicate sgm and lacZ gene regions, arrows the direction of transcription, triangle the RBS region of sgm, and circles the hexanucleotide on the mRNA target.

secondary target into *Micromonospora melanosporea* and examined its effect on Sgm-conferred gentamicin resistance in this bacterium.

Influence of sgm secondary target overexpression on gentamicin resistance in Micromonospora melanosporea

Micromonospora melanosporea does not produce any antibiotic; it is sensitive to aminoglycosides including gentamicin and thus suitable for testing the effect of overexpression of the *sgm* regulatory region on mRNA in a homologous background. *M. melanosporea* was transformed with pBMZ13, a low copy plasmid that contains *sgm* under control of its own promoter (Vukov and Vasiljevic, 1998).

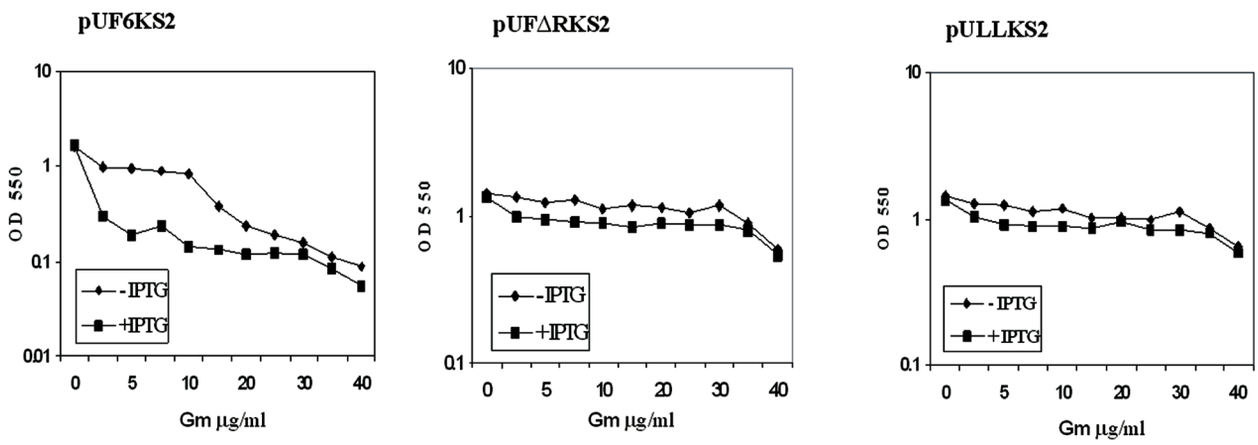

Fig. 2. Growth curve of *E. coli* NM522 cells transformed with pUF6KS2, pUFΔRKS2, and pULLKS2 measured under noninducing conditions (- IPTG) or in the presence of 1 mM IPTG (+ IPTG).

containing 5 μg/ml of gentamicin and induced with 0, 0.01 mM, 0.1 mM and 1 mM IPTG. The results clearly demonstrate that only overexpression of the regulatory sequence (construct pUF6KS2) causes dose-dependent reduction in the growth rate (Fig. 3). Correlation between induction of the secondary target and sensitivity to gentamicin was confirmed in this way. These results therefore suggest that the 5' UTR sequence, when overexpressed along with the *sgm* gene, presumably exerts its negative effect on the establishment of Sgm-conferred gentamicin resistance in *E. coli* cells by titrating the Sgm molecules. These observations also raised the question as to whether the same is true in the homologous background. To learn the answer, we next introduced a

The *sgm* secondary target was expressed *in trans* from plasmids expressing this regulatory region under the control of two different promoters: either P1, which confers resistance to 30 μg/ml kanamycin when cloned into pIJ486, the *Streptomyces* promoter-probe plasmid; or the P29 promoter isolated from the pMZ1 plasmid, which confers resistance to 400 μg/ml kanamycin when tested in the same vector (Kojic et al., 1994). The constructed plasmids containing the *sgm* secondary target under control of the P1 and P29 promoters were named pMRSP1 and pMRSP29, respectively. Resistance to gentamicin was measured in *M. melanosporea* cells cotransformed with either pBMZ13 plus pMRSP1 or pBMZ13 plus pMRSP29. As a control, a strain con-

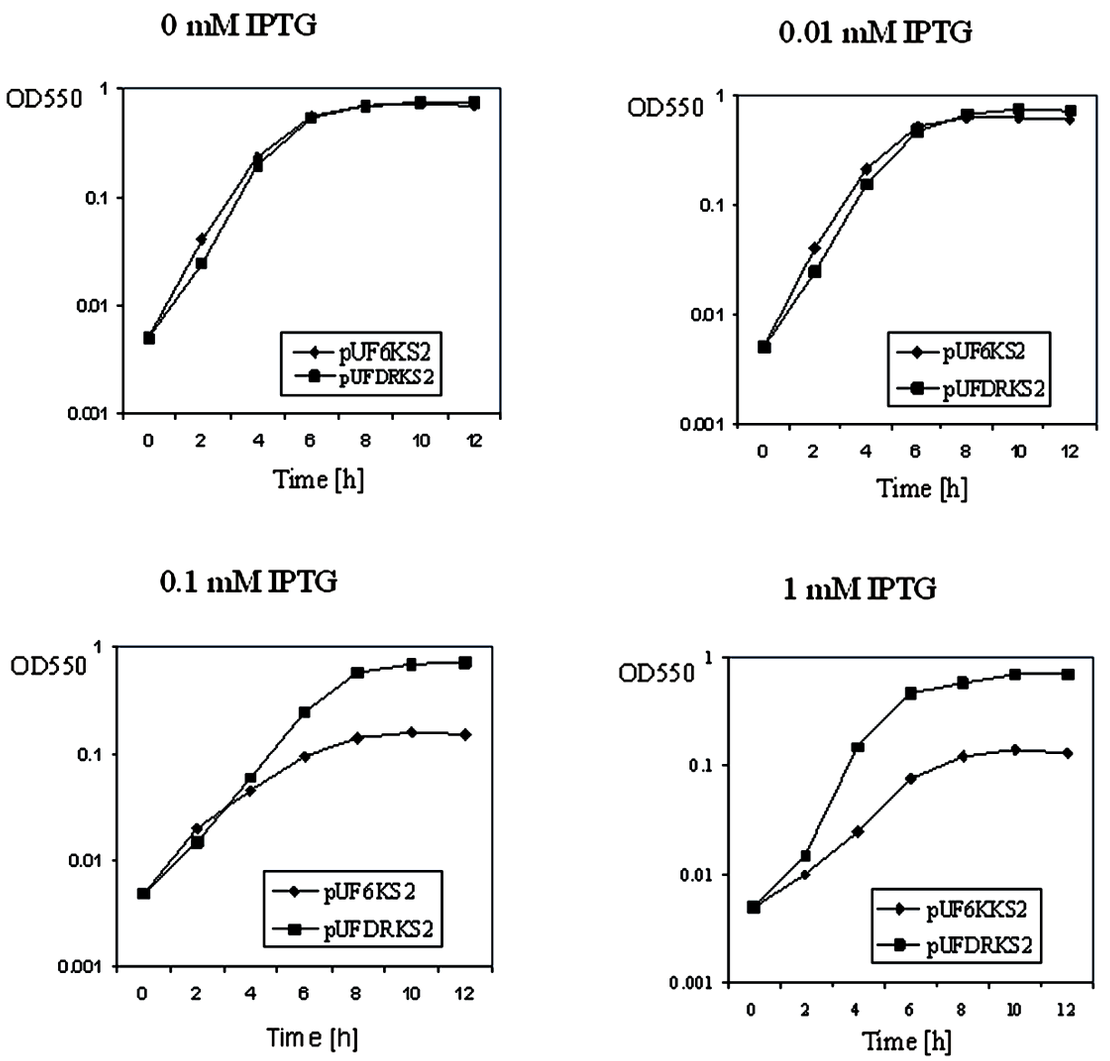

Fig. 3. Growth curve of *E. coli* NM522 cells transformed with pUF6KS2 and pUFΔRKS2 under conditions when transcription from PLtl was induced with 0, 0.01 mM, 0.1 mM, and 1 mM IPTG.

taining the pBMZ13 plasmid was used. Growth was monitored on mM plates with increasing concentrations of gentamicin (Table 2). Nosiheptid was added to the media to ensure the presence of pMRSP1 and pMRSP29, plasmids that have gene conferring resistance to this antibiotic, as a selective marker. Perhaps surprisingly, the results showed that expression of 5' UTR under the weak P1 promoter was sufficient to cause a dramatic decrease of gentamicin resistance, and that the effect was further potentiated by expression under the strong P29 promoter, when reduction of gentamicin resistance was almost complete. Thus, the presence of *sgm* regulatory sequence expressed under the P1 promoter reduces the level of resistance to 20 μg/ml gentamicin, whereas expression from the stronger P29 promoter reduces resistance even more (5-10 μg/ml). Hence, the results clearly indicate that resistance to gentamicin is reduced when the secondary target is overexpressed in *M. melanosporea*, as was observed in *E. coli*.

DISCUSSION

Our interest in regulation of the *sgm* gene derives from the fact that we were unable to detect Sgm protein synthesis in an *E. coli* minicell system,

despite the fact that *E. coli* cells containing the cloned gene were gentamicin resistant (Kojic et al., 1992). The Sgm protein has a limited number of intracellular target sites, and it is anticipated that relatively few enzyme molecules are sufficient for complete modification of the target (i.e., 16S rRNA). Sgm, like many other components involved in translation, is autoregulated at the post-transcriptional level (for a review, see Kozak, 2005, and references therein). Autoregulation of the *sgm* has been proved *in vitro* using gene and operon *sgm-lacZ* fusions (Kojic et al., 1996). The results presented in this paper show a relationship between the expression of *sgm* mRNA (secondary target) and gentamicin resistance in *E. coli* and *M. melanosporea* strains. It is known that the resistance level conferred by RNA-modifying methylases is not gene dosage dependent, so a small number of molecules is enough to render ribosomes resistant to antibiotics. However, the results presented in this paper clearly demonstrate that an excess of the secondary target can titrate down the amount of Sgm methylase and thus reduce the number of available molecules necessary to obtain a high level of resistance. Experiments with the *E. coli* system show a direct correlation of sensitivity to gentamicin with the level of induction of the $P_L$tl promoter by IPTG (Fig. 3). This correlation was not so obvious in *M. melanosporea* cells, where two constitutive promoters of different strength were used – P1, a relatively weak promoter, and P29, a relatively strong one. Despite the fact that expression of the secondary target could not be controlled by induction of transcription (as it is in *E. coli*), experiments with the *Micromonospora* system also show that the stronger promoter, i.e., one that results in more of the secondary target in the cell, lowers resistance more than the weaker promoter. Moreover, the MIC of gentamicin with the P29 promoter was diminished almost to the MIC value of the *M. melanosporea* wild-type strain (3 μg/ml, as reported in Kojic et al., 1999). Such a strong effect in the *Micromonospora* strain can be attributed to structural specificity of *Micromonospora* 16S rRNA. The 16S rDNA sequences of numerous *Micromonospora* strains have been determined (Koch et al., 1996), and it was shown that all key nucleotides in the A site are conserved, although one interesting feature is discernible in sequences of *M. melanosporea* and *M. purpurea*. Namely, these two strains have C-1409 changed to G, so that the stem beginning with the 1409-1491 base pair is disrupted. The importance of this basepair for paromomycin resistance in *E. coli* was demonstrated by mutagenesis of 16S rRNA and by chemical probing (De Stasio et al., 1989). However, *M. melanosporea* and *M. purpurea* are sensitive to paromomycin, like all other tested *Micromonospora* strains (Matkovic et al., 1984).

The obtained results should also be discussed in the light of specific binding of methylase to the 30S subunit and its own mRNA. Strong decrease of gentamicin resistance upon overexpression of 5' UTR in *E. coli* might not be surprising, but the similar effect in *M. melanosporea* was certainly unexpected. In the latter case, it would hardly be anticipated that the situation is similar to that in *E. coli*, since it would suggest that the binding of methylase is, even in the homologous system, stronger to its own mRNA than to 30S ribosomal subunits. Unfortunately, specificity of binding could not be confirmed in electrophoretic mobility shift assays with purified Sgm and either the mRNA or the 16S rRNA oligonucleotide model (Ilic-Tomic and Vasiljevic, unpublished results), leading to the conclusion that some other components of translational machinery are involved in recognition of both targets.

In any case, the results presented here raise the interesting question as to whether the expression of 5' UTR in the producer *Micromonospora zionensis* affects resistance of this organism to gentamicin. If so, this would seem to be at odds with the fact that 16S rRNA is the primary target of Sgm; hence, the enzyme should bind to it with much higher affinity than to its own mRNA. In *M. zionensis*, *sgm* is transcribed from two promoters (Kojic et al., 1992), so it is formally possible that *M. zionensis* regulates the establishment of its resistance by changing the availability of 5' UTR via activation of the P2 promoter. Conceivably, by activating the P2 promoter (located 350 nt upstream of the start codon), *M. zionensis* might trap the 5' UTR responsible for autoregulation in a secondary structure of the longer mRNA, thereby rendering it less available for Sgm.

In summary, the findings presented here clearly indicate the need for a more precise understanding of the regulatory interplay between Sgm methylase and its primary and secondary targets.

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REFERENCES

Beauclerk, A. A. D., and *E. Cundliffe* (1987). Site of action of two ribosomal RNA methylases responsible for resistance to aminoglycosides. *J. Mol. Biol.* **193**, 661-671.

De Stasio, E. A., Moazed, D., Noller, H. F., and *A. E. Dahlberg* (1989). Mutations in 16S ribosomal RNA disrupt antibiotic-RNA interactions. *EMBO J.* **8,** 1213-1216.

Fourmy, D., Recht, M. I., Blanchard, S .C., and *J. D. Puglisi* (1996). Structure of the A site of *Escherichia coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic. *Science* **274**, 1367-71.

Holmes, D. J. and *E. Cundliffe* (1991). Analysis of a ribosomal RNA methylase gene from *Streptomyces tenebrarius* which confers resistance to gentamicin. *Mol. Gen. Genet.* **229**, 229-237.

Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P. Ward, J.M., and *H. Schrempf* (1985). *Genetic Manipulation of Streptomycetes. A Laboratory Manual*. John Innes Foundation, Norwich, UK.

Kelemen, G. H., Cundliffe, E., and *I. Financsek* (1991). Cloning and characterization of gentamicin-resistance genes from *Micromonospora purpurea* and *Micromonospora rosea. Gene* **98**, 53-60.

Koch, C., Kroppenstedt, R. M., and *E. Stackebrandt* (1996). Intrageneric relationships of the actinomycete genus *Micromonospora*. *Int. J. Syst. Bacteriol.* **46,** 383-387.

Kojic, M., Milojevic, N., and *B. Vasiljevic* (1999). Gentamicin-resistance determinants confer background dependent hygromycin B resistance. *Microb. Drug Resist*. **5**, 177-182.

Kojic, M., Topisirovic, L., and *B. Vasiljevic* (1991). Efficient transformation of *Micromonospora melanosporea* protoplasts by *Streptomyces* plasmid. *Current Microbiol.* **174**, 7868-7872.

Kojic, M., Topisirovic, L., and *B. Vasiljevic* (1992). Cloning and characterization of an aminoglycoside resistance determinant from *Micromonospora zionensis. J. Bacteriol.* **174**, 7868-7872.

Kojic, M., Topisirovic, L., and *B. Vasiljevic* (1996). Translational autoregulation of the *sgm* gene from *Micromonospora zionensis*. *J. Bacteriol.* **178**, 5493-5498.

Kojic, M., Vukov, N., Topisirovic, L., and *B. Vasiljevic* (1994). Isolation and mapping of the promoters of the plasmid pMZ1 from *Micromonospora zionensis. Arch. Biol. Sci*. Belgrade **46**, 17-21.

Konstantinovic, M., Maksimovic, V., Nikcevic, G., and *V. Glisin* (1991). Hybrid $P_L$tl promoter with dual regulation control. *DNA Cell Biol.* **10**, 389-395.

Kozak, M. (2005) Regulation of translation via mRNA structure in prokaryotes and eukaryotes. *Gene* **361**, 13–37.

Matkovic, B., Piendl, W., and *A. Böck* (1984). Ribosomal resistance as a wide-spread self-defence in aminoglycoside-producing *Micromonospora* species. *FEMS Microbiol. Lett.* **24,** 273-276.

Miljkovic, M., and *B. Vasiljevic* (2002). *In trans* regulation of the *sgm::lacZ* fusion. *Arch. Biol. Sci.* Belgrade **54**, 1P-2P.

Moazed, D., and H. F. Noller (1987). Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature* **327**, 389-394.

Oshida, T., Takeda, K., Yamagushi, T., Ohshima, S., and *Y. Ito* (1986). Isolation and characterization of plasmids from *Micromonospora zionensis* and *Micromonospora rosaria. Plasmid* **16**, 74-76.

Piendl, W., Böck A., and *E. Cundliffe* (1984). Involvement of 16S ribosomal RNA in resistance of the aminoglycoside-producers *Streptomyces tenjimariensis, Streptomyces tenebrarius,* and *Micromonospora purpurea. Mol. Gen. Genet.* **197**, 24-29.

Sambrook, J., Fritsch, E. F., and *T. Maniatis* (1989). *Molecular Cloning: A Laboratory Manual* (2nd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Skeggs, P. A., Holmes, D. J., and *E. Cundliffe* (1987). Cloning of aminoglycoside-resistance determinants from *Streptomyces tenebrarius* and comparison with related genes from other actinomycetes. *J. Gen. Microbiol.* **133**, 915-923.

Thompson, J., Skeggs, P. A., and *E. Cundliffe* (1985). Methylation of 16S ribosomal RNA and resistance to the aminoglycoside antibiotics gentamicin and kanamycin determined by DNA from the gentamicin producer *Micromonospora purpurea. Mol. Gen. Genet.* **201**, 168-173.

Vajic, S., Anastasov, N., and *B. Vasiljevic* (2004). The *kgmB* gene, encoding ribosomal RNA methylase from *Streptomyces tenebrarius*, is autogenously regulated. *Arch. Microbiol.* **182**, 475-481.

Vasiljevic, B., and *E. Cundliffe* (1990). Cloning of *grm,* a genta-

micin resistant gene from *Micromonospora purpurea. J. Cell. Biochem.* **14A**, 122-122.

Vicens, Q., and *E. Westhof* (2003). RNA as a drug target: The case of aminoglycosides. *Chem. BioChem.* **4**, 1018-1023.

Vukov, N., and *B. Vasiljevic* (1998) Analysis of plasmid pMZ1 from *Micromonospora zionensis. FEMS Microbiol. Lett.* **162**: 317-323.

Ward. J. M., Janssen, G. R., Kieser, T., Buttner, M. J., and *M. J. Bibb* (1986). Construction and characterization of a series of multi-copy probe plasmid vectors for *Streptomycetes* using aminoglycoside phosphotransferase gene from Tn5 as indicator. *Mol. Gen. Genet.* **203**, 468-478.

Yanish-Perron, C., Vieira, J., and *J. Messing* (1985). Improved M13 phage cloning vectors and host strains: Nucleotide sequence of the M13mp18 and pUC19 vectors. *Gene* **33**, 103-119.

ОВЕРЕКСПРЕСИЈА 5' UTR MRNK SGM ГЕНА СМАЊУЈЕ РЕЗИСТЕНЦИЈУ НА ГЕНТАМИЦИН У ЋЕЛИЈАМА *ESCHERICHIA COLI* И *MICROMONOSPORA MELANOSPOREA*

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16S rRNK метилазе су експримиране у већини бактерија које производе антибиотике да би се заштитиле од дејства антибиотика путем метилације 16S rRNK на позицијама које су битне за њихово дејство. Ген *sgm* који је одговоран за резистенцију на сисомицин и гентамицин у сој *Micromonospora zionensis*, метилује G1405 у оквиру A места 16S rRNA где се налази и CCGCCC хексануклеотид. Исти хексануклеотид се налази и 14 нуклеотида испред места везивања рибозома на *sgm* информационој RNK. Предложени модел транслационе регулације *sgm* гена претпоставља да се Sgm протеин везује за овај мотив како на 16S rRNK, тако и на 5' нетранслирајућем региону (UTR) сопствене информационе RNK. 5' UTR секвенца је оверекспримирана на *sgm* информационој RNK са скраћеним 3' крајем и тестиран је ефекат на гентамицинску резистенцију у ћелијама *E. coli* и *Micromonospora melanosporea*. Оверекспресија ове регулаторне секвенце доводи до смањења резистенције у оба тестирана соја највероватније због титрације Sgm молекула од стране 5' UTR-а.