Resistome in *Streptomyces rimosus* — A Reservoir of Aminoglycoside Antibiotics Resistance Genes

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Abstract—Investigation of aminoglycoside acetyltransferases in actinobacteria of the genus *Streptomyces* is an integral part of the study of soil bacteria as the main reservoir and possible source of drug resistance genes. Previously, we have identified and biochemically characterized three aminoglycoside phosphotransferases, which cause resistance to kanamycin, neomycin, paromomycin, streptomycin, and hygromycin B in the strain *Streptomyces rimosus* ATCC 10970 (producing oxytetracycline), which is resistant to most natural aminoglycoside antibiotics. In the presented work, it was shown that the resistance of this strain to other AGs is associated with the presence of the enzyme aminoglycoside acetyltransferase, belonging to the AAC(2') subfamily. Induction of the expression of the gene, designated by us as *aac(2')-If*, in *Escherichia coli* cells determines resistance to a wide range of natural aminoglycoside antibiotics (neomycin, gentamicin, tobramycin, sisomycin, and paromomycin) and increases minimum inhibitory concentrations of these antibiotics.

INTRODUCTION

Resistance to antibiotics is one of the main problems in modern medicine and represents a global risk for health care due to catastrophic spread of clinical bacterial strains with multiple antibiotic resistance. Natural environment is the largest source and reservoir of resistance — soil, aquatic, atmospheric, and zoonotic ecosystems, as well as artificial ecosystem contain elements of antibiotic resistance [1, 2]. The possibility of transfer of the resistance genetic elements between the bacteria in the mixed populations facilitates increase of horizontal transfer, as well as emergence and selection of the resistant forms of bacteria. The genes mediating resistance to antibiotics hypothetically originate from the bacteria belonging to the genus *Streptomyces*, which are producers of a wide spectrum of antibiotics and represent a significant reservoir of the genes mediating resistance to antibiotics in soil [3, 4].

Multiple drug resistance to aminoglycoside antibiotics (AG) is due to the presence in the bacterial genomes of the modifying enzymes belonging to three subclasses: aminoglycoside phosphotransferases (APH), aminoglycoside-3-N-acetyltransferases (AAC), and aminoglycoside nucleotidyltransferase (ANT). Induction of the synthesis of the enzymes modifying aminoglycosides is a strategy for survival of bacteria resistant to antibiotics [4, 5]. The APH and AAC enzymes have been identified and characterized mainly in the strains producers of aminoglycosides such as *Streptomyces griseus* (streptomycin producer), *Streptomyces kanamyceticus* (kanamycin producer), and *Streptomyces fradiae* (neomycin producer). These strains are resistant to other AGs, which are not produced by these bacteria. Existence of multiple resistance to AGs has also been demonstrated for the actinomycetes not producing AGs [6, 7].

At the same time, the primary bioinformatics analysis revealed that the genes annotated as *aph* and *aac* are

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Abbreviations: AAC, aminoglycoside acetyltransferase; AG, aminoglycoside antibiotics; APH, aminoglycoside phosphotransferase; MIC, minimal inhibitory concentration.

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widely represented in many genomes of actinobacteria of the *Streptomyces* genus. Moreover, the enzymes mediating resistance to AGs are currently poorly understood, some of them are associated with antibiotic resistance, the rest of them have other functions, including participation in communications with other soil organisms. Hence, identification and characterization of novel enzymes associated with resistance to aminoglycoside antibiotics is an important task [6, 8].

It has been established previously that the strain *Streptomyces rimosus* subsp. *rimosus* ATCC 10970 (oxytetracycline producer) exhibits resistance to the majority of natural AGs. We have identified and biochemically characterized the following aminoglycoside phosphotransferases in the *S. rimosus* ATCC 10970 strain: APH(3')-VIII, APH(3'')-Id, and AphSR2, which mediate resistance to kanamycin, neomycin, paromomycin, streptomycin, and hygromycin B. Three-dimensional structures of APH(3')-VIII and APH(3'')-Id were resolved (in apo-form and in complex with streptomycin and ADP) [9-13].

In this study the genes encoding aminoglycoside acetyltransferase in the genome of *S. rimosus* ATCC 10970 have been identified, which potentially could mediate resistance of the strain to AGs. We have identified the enzyme designated as AAC(2')-If, which belongs to the subfamily described above. Cloning of the gene encoding this enzyme in the *Escherichia coli* cells allowed to establish that this gene determines resistance to a broad spectrum of natural AGs. Minimal inhibitory concentrations (MICs) for aminoglycoside antibiotics against the *E. coli* cells expressing *aac(2')-If* were determined.

MATERIALS AND METHODS

Bacterial strains, vectors, media, and cultivation conditions. The following strains were used in the study: *S. rimosus* subsp. *rimosus* ATCC 10970 [14], *E. coli* DH5a (F⁻, Φ 80 ΔlacZΔM15, Δ(lacZYA-argF), U169) (Promega, USA) [15], *E. coli* BL21(DE3) (F⁻, dcm, ompT, hsdS(r$_{\bar{B}}$m$_{\bar{B}}$), gal λ (DE3)) (Novagen, USA) [16], and *E coli* NiCo21(DE3) (can::CBD fhuA2 [lon] ompT gal (λ DE3) [dcm] arnA::CBD slyD::CBD glmS6Ala ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int:: i21 Δnin5) (New England Biolabs, USA) [17]. Expression vectors pET16b and pET32a (Novagen) [16] containing His-Tag at the N-terminal region for isolation and purification of recombinant proteins were used for cloning.

S. rimosus ATCC 10970 strain was cultivated in a liquid YEME medium containing 25% sucrose [18]. Luria broth (L-broth) was used for growing *E. coli*, solid growth media contained 2% (*w/v*) agar [19]. Medium was supplemented with ampicillin (100 μg/ml) to ensure selective growth of the cells containing plasmids.

DNA manipulations. Genomic DNA from the *S. rimosus* ATCC 10970 strain was isolated according to the

Table 1. Sequences of primers used in the study

Name of primer	Primer sequence 5'→3'*
AAC-SrN1	tcgtcatatgatgaccgacgcacacccccct
AAC-SrC1	agccggatccctaccagacgtccccact
AAC-SrN2	tcgcggatccatgaccgacgcacacccccct
AAC-SrC2	ccgcaagcttctaccagacgtccccac

* The sites for restriction endonucleases are underlined.

protocol from the Kieser et al. manual [18]. Isolation of plasmid DNA, preparation of *E. coli* competent cells, transformation, and analysis of recombinant plasmids were carried out using standard procedures [19]. DNA fragment encoding AAC(2')-If of *S. rimosus* was amplified from the genomic DNA using a Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Lithuania) with a PTC-0150 minicycler (MJ Research Inc., USA). The following oligonucleotides were designed for amplification: AAC-SrN1 and AAC-SrC1 − for cloning of the *aac(2')-If* into a pET16b plasmid through the restriction sites of endonucleases NdeI and BamHI and oligonucleotides: AAC-SrN2 and AAC-SrC2 − for cloning of the *aac(2')-If* into a pET32a plasmid through restriction sites of endonucleases BamHI and HindIII. Nucleotide sequencers of the primers used in the study (Sintol, Russia) are presented in Table 1.

Expression of the *aac(2')-If* gene from *S. rimosus* ATCC 10970 strain in *E. coli*. *E. coli* cells containing plasmids pET16b:*aac(2')-If* and pET32a:*aac(2')-If* were grown on a shaker in a liquid growth medium (L-broth) with ampicillin at 37°C to optical density 0.6 at 625 nm (~2 h), next expression was induced by addition of iso-propyl-D-thiogalactoside (IPTG) to final concentration 0.5 and 1.0 mM. Next cultivation was continued at 28°C for 18 h followed by precipitation of the cell with centrifugation (3000*g*, 10 min, 4°C) and storage in a freezer at −20°C. Cells were defrosted and re-suspended in a sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 5% glycerol, 2% 2-mercaptoethanol, 0.1% SDS, and 0.001% bromophenol blue followed by heating at 95°C for 10 min. A fraction of soluble proteins was analyzed with electrophoresis in a 12.5% SDS-PAAG using molecular mass marker proteins SM0441 (Fermentas, Lithuania). Soluble protein fractions from the strains *E. coli* BL21(DE3) and NiCo21(DE3) containing plasmids pET16b and pET32a without inserts were used as controls.

Analysis of resistance to aminoglycoside antibiotics. Antimicrobial activity against the *E. coli* BL21(DE3) cells expressing AAC(2')-If was defined as a MIC, which was determined using two methods: disk diffusion method and method of linear dilution. *E. coli* BL21(DE3) cells containing the pET32a plasmid were used as a control.

For the disk diffusion method [20] dishes with LB-agar containing 10 µg/ml IPTG were inoculated by spreading bacterial cultures at concentration 10^5-10^6 CFU/ml onto them. Paper disks containing AG were placed onto the dishes with LB-agar and incubated at 37°C for 18 h, and next, diameters of the zones around the disks without bacterial growth were measured.

MICs of aminoglycoside antibiotics were determined with the method of dilution in a broth according to recommendations of the Clinical and Laboratory Standards Institute (CLSI) [21]. *E. coli* cells were grown in 2 ml of Mueller−Hinton Broth (MHB) up to optical density of 0.3 at 625 nm to adjust to the 0.5 McFarland standard (a measure of culture density corresponding to cell concentration 1.5×10^8 CFU/ml), and next were diluted to final density 10^5-10^6 CFU/ml. Aliquots of cell culture (100 µl) were added to the tubes containing double dilutions of antibiotics in 2 ml of MHB. To induce AAC(2')-If expression, IPTG (100 µM) were added to the tubes. Following cultivation for 18 h at 25°C MIC values were determined as the lowest concentrations of AGs that caused total inhibition of the cell growth (determined spectrophotometrically at 625 nm).

Bioinformatics analysis. Sequences of aminoglycoside acyltransferases (AAC) from the strain of *S. rimosus* ATCC 10970 were obtained from the NCBI database. The program Blastp [22] was used to compare these sequences with the AAC sequences known from the literature; ClustalW algorithm was used for comparing the AAC sequences with the sequences of the proteins in the PDB database with known 3D structures − SAS [23]; multiple alignments of amino acid sequences were carried out using ClustalW algorithm [24].

Statistical analysis was carried out using Student's *t*-test.

RESULTS

Bioinformatics analysis of aminoglycoside acetyltransferases from *S. rimosus* ATCC 10970 strain. In the genome of the ATCC 10970 strain [14] 13 N-acetyltransferase genes from the GNAT family have been annotated with gene loci numbers: SRIM_009260; SRIM_011135; SRIM_012310; SRIM_018100; SRIM_020380; SRIM_020760; SRIM_025200; SRIM_029930; SRIM_030810; SRIM_033455; SRIM_038510; SRIM_040130; SRIM_040160. All identified N-acetyltransferases contain NAT_SF domains and coenzyme A binding sites.

Comparison of amino acid sequences of all N-acetyltransferase enzymes identified in *S. rimosus* with the sequences of the previously known aminoglycoside acetyltransferases from the families AAC(1), AAC(3), AAC(2'), and AAC(6') using programs BLAST and SAS revealed that only one of them (SRIM_030810) exhibits the degree of identity of 52.1% with the AAC(2')-Ic from *Myco-*

bacterium tuberculosis (GenBank: CCP42991.1, PDB ID: 1m44) and 50.0% − with the AAC(2')-Id from *Mycolicibacterium smegmatis* (GenBank: AIU12332.1, PDB ID: 7crm).

Currently, only one subclass AAC(2')-I have been identified in the AAC(2') subfamily. The gene encoding AAC(2')-Ia was found in the chromosome of the opportunistic pathogenic bacteria *Providencia stuartii*, AAC(2')-Ib was identified in *Mycolicibacterium fortuitum*, AAC(2')-Ic − in *M. tuberculosis* and *Mycobacterium bovis*, AAC(2')-Id − in *M. smegmatis*, and AAC(2')-Ie − in *Mycobacterium leprae*. The enzymes of AAC(2') subfamily are capable of both N- and O-acetylation of numerous aminoglycoside substrates such as kanamycin, paromomycin, gentamycin, amikacin, and tobramycin [25-27].

The degree of identity (similarity) of amino acid sequences between the SRIM_030810 (AAC(2')-Sr) and AAC(2')-Ia, AAC(2')-Ib, AAC(2')-Ic, AAC(2')-Id, AAC(2')-Ie is 36.5 (64.9)%; 51.7 (80.1)%; 51.2 (78.5)%; 48.2 (72.8)%, and 48.6 (74.6)%, respectively. Alignment of the sequences (Fig. 1) shows that AAC(2')-Sr contains all conserved amino acids [28] characteristic for the sequences of the enzymes from the AAC(2') subfamily, hence, we denoted this enzyme as AAC(2')-If.

Cloning of the *aac(2')-If* gene into *E. coli* and analysis of expression. In the first stage the *aac(2')-If* gene was cloned in composition of the pET16b expression vector. To examine expression of the *aac(2')-If* gene in *E. coli* the strains BL21(DE3) and NiCo21(DE3) containing the recombinant pET16b:*aac(2')-If* plasmid were cultivated in the liquid LB medium with IPTG induction, next cells were precipitated by centrifugation, and analyzed with the help of electrophoresis 12.5% SDS-PAGE. Analysis of the electropherogram revealed that the *aac(2')-If* gene is not expressed in *E. coli* in composition of the pET16b expression vector.

Thereby, the *aac(2')-If* was cloned in composition of the pET32a vector containing the sequence of thioredoxin gene at the N-terminal region. The obtained results (Fig. 2) showed that in the cells of the *E. coli* BL21(DE3) and NiCo21(DE3) strains there was an additional protein fraction with molecular mass of around 37 kDa, which corresponded to the calculated molecular mass of the AAC(2')-If protein together with the mass of a linker protein in the pET32a containing thioredoxin. Maximum expression of the *aac(2')-If* gene was observed in the *E. coli* BL21(DE3) strain.

Investigation of the spectrum and level of resistance of *E. coli* BL21(DE3) containing the pET32a:*aac(2')-If* plasmid to aminoglycoside antibiotics. To determine the spectrum of resistance of *E. coli* BL21(DE3) expressing the *aac(2')-If* gene to AGs the standard disk diffusion method was used. It was established that expression of the *aac(2')-If* gene ensures resistance of the *E. coli* BL21(DE3) cells to the wide spectrum of natural AGs: neomycin, gentamycin, tobramycin, sisomicin, and paromomycin (Table 2, Fig. 3). The obtained results are

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AAC(2')-Sr  -----------MTDAHPLLAHTAELDARTRAAAKALLHDVFE---------GDMTDEDW   39
AAC(2')-Ia  ------------MGIEYRSLHTSQLTLSEKEALYDLLIEGFE---------GDFSHDDF   38
AAC(2')-Ib  MPFQDVSAPVRGGILHTARLVHTSDLDQETREGARRMVIEAFE---------GDFSDADW   51
AAC(2')-Ic  ----------MHTQVHTARLVHTADLDSETRQDIRQMVTGAFA---------GDFTETDW   41
AAC(2')-Id  MLTQHVSEARTRGAIHTARLIHTSDDLQETRDGARRMVIEAFRDPSGDSDFTDDFTDDDW   60
AAC(2')-Ie  ---------MDTHHVHTARLVHTADLDGETLRRLQQMVTDAFA---------GDFDETDW   42


AAC(2')-Sr  DHALGGVHALVWEGEELIGHASVVQRQMVHAG-----RPLRCGYVEGVGVRADRRGRGHG   94
AAC(2')-Ia  AHTLGGMHVMAFDQQKLVGHVAIIQRHMALDN-----TPISVGYVEAMVVEQSYRRQGIG   93
AAC(2')-Ib  EHALGGMHAFICHHGALIAHAAVVQRRLLYRD-----TALRCGYVEAVAVREDWRGQGLA  106
AAC(2')-Ic  EHTLGGMHALIWHHGAIIAHAAVIQRRLIYRG-----NALRCGYVEGVAVRADWRGQRLV   96
AAC(2')-Id  DHALGGMHALISHHGALIAHGAVVQRRLMYRGPDGRGHALRCGYVEAVAVREDRRGDGLG  120
AAC(2')-Ie  EHALGGMHALIWRHGTIIAHAAVVQRRLFYHG-----NALRCGYLEGVAVRKDCRGRGLV   97


AAC(2')-Sr  AAMMTALERVVRDAYDLGALSASDGAADFYAARGWQLWRGPSYTLAPG-GLERTEEEDGG  153
AAC(2')-Ia  RQLMLQTNKIIASCYQLGLLSASDDGQKLYHSVGWQIWKGKLFELKQG-SYIRSIEEEGG  152
AAC(2')-Ib  TAVMDAVEQVLRGAYQLGALSASDTARGMYLSRGWLPWQGPTSVLQPA-GVTRTPEDDEG  165
AAC(2')-Ic  SALLDAVEQVMRGAYQLGALSSSARARRLYASRGWLPWHGPTSVLAPT-GPVRTPDDDGT  155
AAC(2')-Id  TAVLDALEQVIRGAYQIGALSASDIARPMYIARGWLSWEGPTSVLTPTEGIVRTPEDDRS  180
AAC(2')-Ie  HALLDAIEQVIRGAYQFGALSSSDRARRVYMSRGWLPWLGPTSVLAPT-GVIRTPDDDGS  156


AAC(2')-Sr  IYVLPGAV----PLDLTGDLACDWRSGDVW                              179
AAC(2')-Ia  V----MGWKADGEVDFTASLYCDFRGGDQW                              178
AAC(2')-Ib  LFVLPVGLPAGMELDTTAEITCDWRDGDVW                              195
AAC(2')-Ic  VFVLPIDI----SLDTSAELMCDWRAGDVW                              181
AAC(2')-Id  LFVLPVDLPDGLELDTAREITCDWRSGDPW                              210
AAC(2')-Ie  VFVLPVGI----NPDTSSGLMCDWRAGNVW                              182
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Fig. 1. Alignment of the AAC(2')-If (AAC(2')-Sr) sequence with AAC(2')-Ia *P. stuartii*, AAC(2')-Ib *M. fortuitum*, AAC(2')-Ic *M. tuberculosis*, AAC(2')-Id *M. smegmatis*, and AAC(2')-Ie *M. leprae*. Conserved amino acids characteristic for AAC(2')-I are highlighted in gray.

Table 2. Investigation of the spectrum of resistance of *E. coli* BL21(DE3) containing pET32a:*aac(2')-If* plasmid to aminoglycoside antibiotics

Antibiotics		BL21(DE3) pET32a	BL21(DE3) pET32a:*aac(2')-If*
Name	Concentration, µg/disk	Diameter of clear zone, mm*	
Kanamycin	30	21 ± 0.5	21 ± 0.5
Neomycin	30	20 ± 0.5	15 ± 0.5[#]
Paromomycin	10	17 ± 0.5	7 ± 0.5[#]
Streptomycin	10	22 ± 0.5	22 ± 0.5
Gentamycin	10	22 ± 0.5	15 ± 0.5[#]
Tobramycin	10	26 ± 0.5	17 ± 0.5[#]
Sisomicin	10	25 ± 0.5	19 ± 0.5[#]
Amikacin	30	24 ± 0.5	24 ± 0.5

Note. * Average results of four independent measurements ± standard deviations are presented.

[#] Significant difference from the control (BL21(DE3) pET32a), $p < 0.01$.

in agreement with the results of bioinformatics analysis, according to which this gene is assigned to the AAC(2') subfamily.

MICs of AGs were determined using the method of double dilutions of antibiotics in liquid medium.
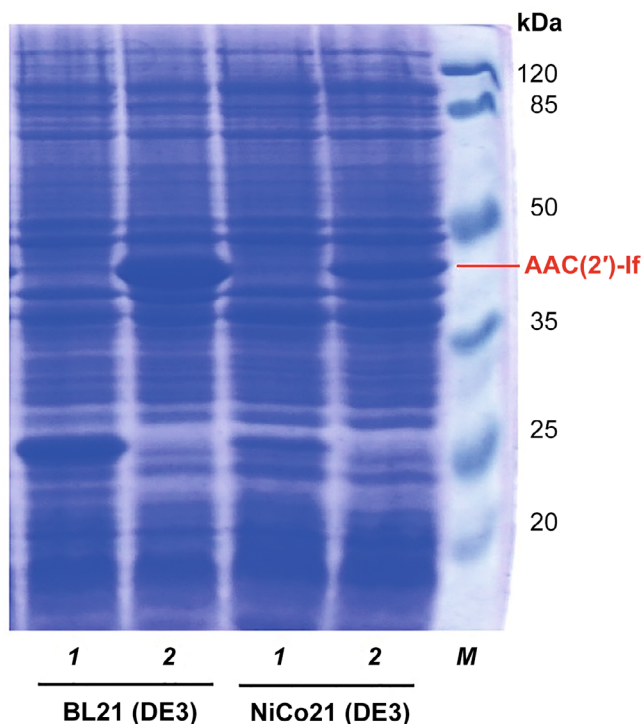
Fig. 2. Electropherogram of the soluble fraction of AAC(2')-If proteins in *E coli* BL21(DE3) and NiCo21(DE3) containing plasmids. Lanes: *1*) pET32a, *2*) pET32a:*aac(2')-If*; *M*) molecular mass markers SM0441.
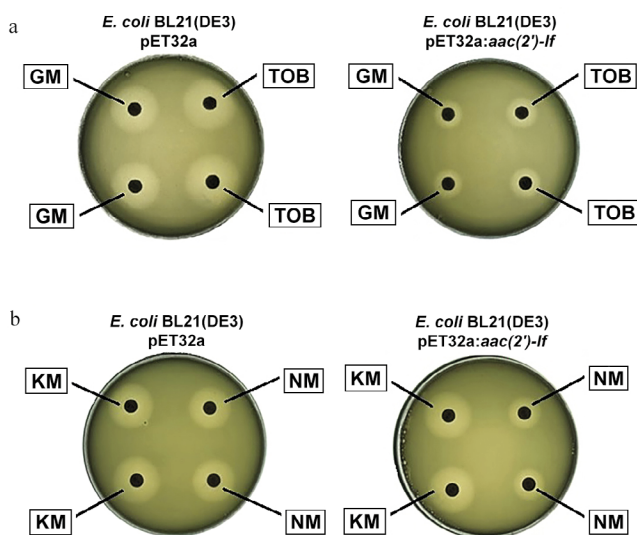
Fig. 3. Analysis of the spectrum of resistance of the cells of *E. coli* BL21(DE3) expressing *aac(2')-If* gene to AGs using standard disk diffusion method: a) to gentamycin and tobramycin; b) to kanamycin and neomycin. Designations: KM, kanamycin; NM, neomycin; GM, gentamycin; TOB, tobramycin.

The obtained results (Table 3) demonstrate that induction of the *aac(2')-If* gene expression results in 20-fold increase of MIC of gentamycin − from 4 to 80 µg/ml. Similar changes were observed for sisomicin, paromomycin, tobramycin (15-16-fold increase of MICs), and neomycin (7.5-fold increase of MIC). Hence, the identified AAC(2')-If enzyme induced in the *E. coli* cells is active *in vivo* and effectively protects these cells against the specified antibiotics.

Hence, both examinations indicate that the identified in this study AAC(2')-If enzyme from the *S. rimosus* ATCC 10970 imparts resistance to the bacteria to AGs (gentamycin, tobramycin, neomycin, paromomycin, and sisomicin), and do not affect resistance to kanamycin and streptomycin.

Based on the obtained results, it could be suggested that gentamycin, tobramycin, neomycin, paromomycin, and sisomicin are substrates of the AAC(2')-If enzyme identified in this study.

DISCUSSION

Three APH enzymes (APH(3')-VIII, APH(3'')-Id, and AphSR2) were identified in our previous studies that define resistance to AGs: kanamycin, neomycin, paromomycin, streptomycin, and hygromycin B. However, it has been shown previously that the *S. rimosus* ATCC 10970 strain is resistant to all natural AGs. It has been suggested based on this fact that the resistance to AGs in this strain could be due to not only APHs, but also due to the presence of other enzymes such as AAC.

Table 3. Minimum inhibitory concentration (MIC) aminoglycoside antibiotics

AG antibiotic	*E. coli* strain; MIC, µg/ml*	
	BL21(DE3) pET32a	BL21(DE3) pET32a:*aac(2')-If*
Gentamycin	4 ± 1	$80 \pm 2^{\#}$
Sisomicin	4 ± 1	$64 \pm 2^{\#}$
Paromomycin	16 ± 2	$250 \pm 5^{\#}$
Tobramycin	8 ± 2	$120 \pm 4^{\#}$
Neomycin	16 ± 2	$120 \pm 4^{\#}$
Kanamycin	16 ± 2	16 ± 2
Streptomycin	16 ± 2	16 ± 2

Note. * Average results of four independent measurements $\pm$ standard deviations are presented.

$^{\#}$ Significant difference from the control (BL21(DE3) pET32a), $p < 0.01$.

Analysis of the *S. rimosus* ATCC 10970 genome [14] allowed us to identify 13 genes encoding N-acetyltransferases from the GNAT family that contain NAT_SF domain and coenzyme A binding site. Based on similarities of amino acid sequences of the identified N-acetyltransferases with the sequences of the previously described aminoglycoside acetyltransferases only one of them was assigned to the known subfamily AAC(2').

The enzymes of the AAC(2') subfamily were previously identified in the opportunistic pathogen *P. stuartii* and in mycobacteria [25-27]. Multiple alignment of the sequences demonstrated that the AAC(2')-Sr contains all amino acids typical for the sequences of the enzymes from the AAC(2') subfamily [28], hence, we denoted this enzyme as AAC(2')-If. The *aac(2')-If* gene was cloned in *E. coli*. Evaluation of resistance to AGs showed that the induction of the AAC(2')-If enzyme synthesis determined resistance of *E. coli* to a wide spectrum of natural AGs: neomycin, gentamycin, tobramycin, sisomicin, and paromomycin, which is in agreement with the results of bioinformatics analysis, according to which this enzyme was assigned to the AAC(2') subfamily.

Origin of AAC could be traced to the environmental microbial species, which represent a large reservoir for the new and emerging enzymes mediating resistance, which are currently poorly understood. Induction of the synthesis of enzymes modifying AGs exemplifies the strategy of survival of bacteria resistant to antibiotics. AACs belong to the moonlighting-proteins, i.e., these enzymes display polyfunctional properties. In particular, AAC in mycobacteria partially contribute to AG resistance, and also could exert other functions such as facilitating acetylation of the cell wall proteins and of peptidoglycan, which explains broad substrate specificity of AAC-enzymes [26].

For the first time the term 'resistome' was introduced by D'Costa et al. [29] in 2006 during investigation of the genes mediating resistance to antibiotics of soil bacteria of the *Streptomyces* genus — producers of the majority of antibiotics. Much earlier one of the authors of this paper investigated transmission and mechanisms of action of the antibiotic resistance genes within the collection of different species of the *Streptomyces* genus [30]. The *S. rimosus* ATCC 10970 strain (producer of oxytetracycline) attracted attention of the researchers as a bright representative with total resistance to all natural AGs [31]. At the same time the hypothesis on the origin and evolution of the genes mediating resistance to antibiotics has been discussed widely, which was later summarized in the work by Davies and Davies [32].

Later, the term 'resistome' have been used for characterization of microbiomes in different ecological systems: soil, aquatic reservoirs, air, plants, livestock, food products, and humans. Resistome of the human intestine is considered as the most important source of drug resistance genes [33, 34].

It is known that the antibiotic resistance genes are easily transferred between the groups of bacteria of different microbiomes with the help of mobile genetic element and other systems of genetic material transfer including extracellular vesicles [2, 35, 36].

In the presented work a new AAC enzyme was identified in the *S. rimosus* ATCC 10970 strain, which displays similarities with the mycobacterial enzymes belonging to the AAC(2') subfamily. It should be noted that the important issues of resistome interactions between each other, which result in horizontal transfer of resistance genes, require further investigations. Exchange of the antibiotic resistance genes between soil bacteria and clinical pathogens is possible via the direct transfer between the soil bacteria and human pathogens or via indirect transfer through the human intestine microbiota, which emphasized clinical significance of soil resistome [37].

CONCLUSIONS

Summarizing the data obtained by the authors in the previously published studies [9-13] and in the present study it could be stated that we identified two APH enzyme and the AAC(2')-If enzyme in the *S. rimosus* ATCC 10970 strain not producing AGs, which determine resistance of the strain to all natural AGs.

The presented study opens new possibilities for investigation of transmission and features of expression of the genes determining natural resistance of actinobacteria of the *Streptomyces* genus to AGs.

Isolation of the recombinant protein AAC(2')-If has been suggested for future studies followed by elucidation of the 3D structures, which could help to reveal catalytically essential structural motifs and comparison of the obtained spatial structures with the known structures of aminoglycoside acetyltransferases from other bacterial species (including pathogens).

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