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БІОТЕХНОЛОГІЯ

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THE *MIA* MUTANTS OF *STREPTOMYCES ALBUS* J1074 ARE PRONE TO TRANSLATIONAL ERRORS AND SUSCEPTIBLE TO CERTAIN STRESSORS

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Streptomyces albus J1074 has been established by us as a convenient model to study different aspects of $tRNA^{Leu}_{UAA}$-dependent regulatory mechanisms, that take place in genus *Streptomyces*. These mechanisms are important for proper morphological and physiological transitions of streptomycete colonies, such as the onset of antibiotic production in stationary phase of growth. The genes for post-transcriptional modification of adenosine residue in 37^{th} position of $tRNA_{XXA}$ family (so called *mia* genes) were shown to be important for the aforementioned processes, most likely because they impact $tRNA^{Leu}_{UAA}$ among other tRNAs. Our results were largely consistent with what is known about *mia* mutations in the other model systems, such as yeast and enterobacteria. Nevertheless, we also revealed several differences from the model systems, such as decreased susceptibility to hydrogen peroxide. This prompted us to look deeper into the behavior of the *mia* mutants, particularly their response to different stress factors. Here we report that *S. albus mia* mutants exhibit increased mistranslation rate as compared to their parental strain. These mutants are more susceptible than the parental strain to disulfide stress inducer diamide and DNA repair stressor caffeine. In summary, although the deficiency in certain tRNA modification appears to cause identical or very similar response (such as elevated mistranslation) across all so far studied bacterial systems, it also induces species- or genus-specific effects (such as disparate effects on H_2O_2 susceptibility). These differences could be attributed to the peculiarities of organization/function of regulatory pathway governing the response to a given stress. The observed results are further discussed in the wider context of the role of tRNA modification pathway in bacterial biology.

Keywords: *Streptomyces albus*, tRNA, nonsense suppression assay, diamide, caffeine

Post-transcriptional tRNA modifications (PTTMs) have recently emerged as a novel and underappreciated layer of regulation of gene expression in pro- and eukaryotes [6]. One of the most general mechanisms by which PTTMs exert their biological effects hinges on unequal usage of synonymous codons and modification of their cognate tRNAs, leading to so called adaptive (codon-specific) translation. A good example of this phenomenon has recently been reported for *Escherichia coli* [13]. In this species proline codons (CCN) are decoded by three isoacceptor $tRNA^{Pro}$, ($tRNA_{UGG}$, $tRNA_{GGG}$, $tRNA_{CGG}$) all of which carry methylated guanine residue in 37^{th} position of the tRNA (m^1G37; adjacent to anticodon loop which occupies positions 34-36). This PTTM is introduced into tRNA by SAM-dependent methyltransferase TrmD. The *trmD* null mutant cannot be generated, pointing to the essentiality of this PTTM. The fully modified $tRNA_{UGG}$ is capable of reading all proline codons, but loss of m^1G37 specifically impairs reading of CC(C/U), leading to frameshifts. Importantly, mRNAs for many membrane proteins responsible for active export of toxic substances are enriched with CC(C/U) codon near start codons

[7]. Thus, knockdown of TrmD function renders *E. coli* highly sensitive to different antibiotics because of failure to produce respective efflux proteins. Hence, loss of certain PTTM may affect the translation of a subset of mRNAs, enriched with the codons that require modified tRNA (so called modification-tunable transcripts [1]).

The abundance and importance of adaptive translation in antibiotic-producing streptomycetes remains unknown. In related genus *Mycobacterium,* PTTM-related mechanisms play important role in response to oxidative stress [5]. Involvement of PTTM in some kind of regulation of stress responses appears to be a general theme across kingdoms, as studies of several eukaryotic models suggest [3, 4, 8, 15]. We showed recently that knockouts of genes for hypermodification of adenosine residue in 37 position of $tRNA_{XXA}$ family (controlled by *mia* genes [16]) caused distinct changes in morphological and metabolic differentiation of *Streptomyces* [11, 17]. Nevertheless, the understanding of cause-to-consequence links between the *mia* mutations and the phenotypes in *Streptomyces* remain obscure. A body of circumstantial evidence supports our initial suggestion that effects of *mia* mutations are caused by impaired PTTM of $tRNA^{Leu}_{UAA}$ (BldA). Yet there are also data portraying a wider impact of these mutation on *Streptomyces* tRNome [11], as well as data that run counter the established paradigm, such as decreased hydrogen peroxide susceptibility [12]. This makes harder to compare our data and interpret them in the wider context of what is known about biological roles of PTTMs in bacteria. We decided to address this uncertainty by asking as to whether *mia* mutants of *S. albus* exhibit some of the properties that are a hallmark of PTTM deficiency in the other bacterial systems. These include elevated mistranslation level and susceptibility to other types of chemical stresses. Our results reveal clear parallels between *Streptomyces* and other bacteria with the regard to the phenotypes being studied.

Materials and Methods

Strains and plasmids, used in this work, are listed in Table. Tryptic soy broth (Merck), oatmeal and soy-mannitol agars [9, 10] were used to grow *Streptomyces* (at 30 °C) and plate matings. *Escherichia coli* strains were grown in liquid or agar LB medium, supplemented with appropriate antibiotic (if needed) at 37 °C. Genomic DNA from *Streptomyces* and plasmid DNA from *E. coli* were isolated using standard protocols [3]. Molecular biology enzymes were used according to recommendation of suppliers (Thermo).

Stop codon suppression tests were carried out with the help of plasmids pSETGUSTAG and pSETGUSTAG286. They are derivatives from plasmid pSETGUS, where the 2^{nd} and 286^{th} codons of *gusA* gene ORF, respectively, are replaced with TAG stop codon. Spectrophotometric analysis of β-glucuronidase activity of *gusA*$^+$-strains was measured as described in [14]. Activity of GusA enzyme was calculated in Units/g. The rates of stop codon suppression are given in percentages, while the β-glucuronidase activities of respective pSETGUS$^+$-strains were taken as 100 %.

Disc diffusion assay of *S. albus* susceptibility to diamide was carried out as follows. Approximately 10^7 spores of ΔmiAB or SAM2 were added to 15 mL of soft GYM [10] agar (1 %). The resulting mixture was overlaid onto Petri plates, containing 10 ml of GYM medium (2 % agar). After solidification, disks with 10 μL of 0.1M diamide were put on the surface of the test plates. The diameter of sterile zone was measured after 48 h of incubation at 30 °C. For the test of caffeine susceptibility, serial dilutions of liquid *S. albus* cultures (grown for 48 h in TSB) were plated on GYM medium with caffeine concentrations of 0 mM, 5 mM, and 10 mM. The lawns of *S. albus* strains were plated under the same conditions.

Plasmids and bacterial strains, used in this work

Strain	Relevant characteristics	Source
S. albus SAM2	Derivative of J1074, deletion of pseudo $attB^{\varphi C31}$ site	[2]
S. albus Δ*miaA*	Deletion of *miaA* (*XNR_1074*) in SAM2	[11]
S.albus Δ*miaB*	Deletion of *miaB* (*XNR_1078*) in SAM2	[11]
S. albus Δ*miaAB*	Deletion of *miaA* and *miaB* in SAM2	[12]
E. coli ET12567 pUZ8002	Strain for conjugative transfer of coresident plasmids; *dam-13::Tn9 dcm-6 hsdM;* harbors conjugative plasmid pUZ8002; Cm^R, Km^R	[9]
pSETGUS	φC31-based vector carrying *gusA* fusion to thiostrepton-inducible promoter *tipAp*; Am^R	[14]
pSETGUSTAG	pSETGUS with TAG codon in second position of the gene *gusA*; Am^R	[14]
pSETGUSTAG286	pSETGUS with TAG codon in 286th position of *gusA* gene; Am^R	[14]

Cm^R – resistance to chloramphenicol, Km^R – to kanamycin, Am^R – to apramycin

Results and Discussion

Mistranslation of stop codons is elevated in *mia* mutants of *S. albus*, as compared to the parental strain. We used two reporter constructs to elucidate the influence of *mia* mutations on mistranslation. Plasmids pSETGUSTAG and pSETGUSTAG286, both containing stop codon TAG in *gusA* gene ORF, as well as control plasmid pSETGUS, with unchanged *gusA*, were used. Schemes of the above mentioned *gusA* fusion constructs are shown on Fig. 1.

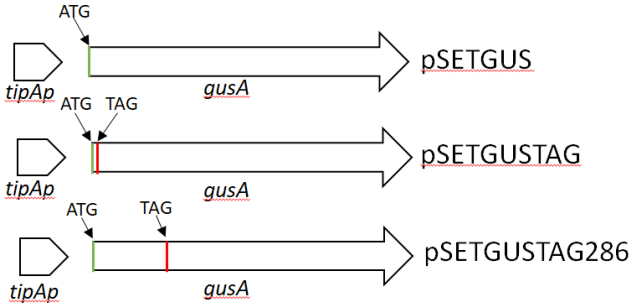

Fig. 1. Schemes of *gusA* constructs in plasmids pSETGUS, pSETGUSTAG and pSETGUSTAG286. *tipAp* – thiostrepton-inducible promoter; *gusA* – β-glucuronidase gene; ATG – start codon in *gusA* ORF; TAG – stop codon, introduced in 2nd and 286th position of *gusA* ORF, respectively

S. albus parental strain (SAM2) and its *mia* mutants, individually carrying each of the aforementioned plasmids, were generated. GusA activity was measured at 48 h of growth in TSB, as described in Methods. We noticed elevated levels of β-glucuronidase activity for some of the *mia* mutants, containing TAG-versions of *gusA* gene, compared to the respective SAM2 strains. We calculated the rates of TAG-codon suppression in pSETGUSTAG- and pSETGUS-TAG286-containing *mia* mutants and SAM2. Results are summarized in Fig. 2 and given in percentages, for every case the GusA-activity of respective $pSETGUS^+$-strains is taken as 100 %. Mutant Δ*miaAB*, carrying the plasmid pSETGUSTAG, as well as Δ*miaA* with plasmid pSET-GUSTAG286, demonstrated higher rates of stop codon misreading, as compared to controls. An increased stop codon readthrough could be explained by elevated translational infidelity, described previously for some *mia* mutants. We have also noticed that the position of TAG codon in ORF of *gusA* gene affected the level of suppression. Thus, for Δ*miaAB* strain TAG suppression is more prominent if the stop codon is in 2nd position rather than 286th, while for Δ*miaA* mutant the opposite effect was observed. It was already shown by us that Δ*miaAB* and Δ*miaA* mutants differ in the degree of the mutant phenotypes [12].

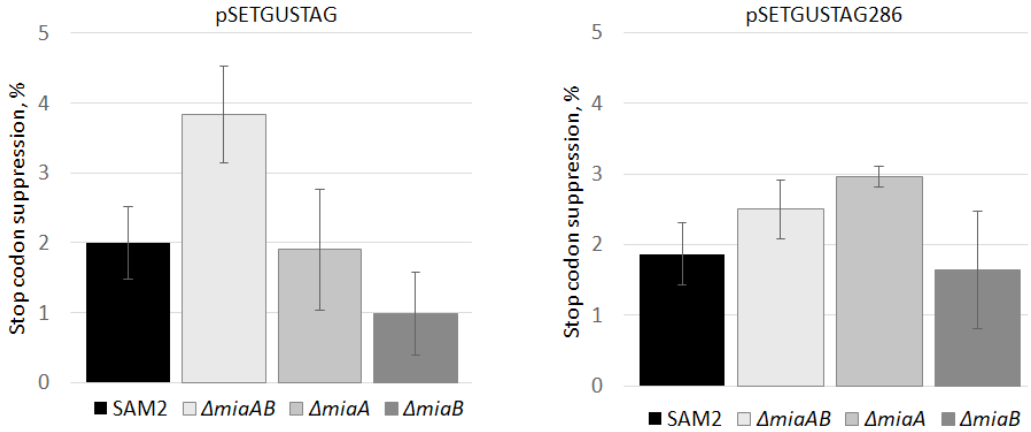

Fig. 2. Rates of stop codon suppression for *S. albus* SAM2, Δ*miaAB*, Δ*miaA* and Δ*miaB* strains. pSETGUSTAG carries stop codon in 2nd and pSETGUSTAG286 – in 286th position of the gene *gusA*. Percentages are calculated against respective pSETGUS+ strains. Represented results are given as mean values of three repeats with standard errors

The *mia* mutants exhibited sensitivity towards caffeine and diamide. Diamide is known as a powerful oxidant of thiols in proteins. Caffeine is a purine alkaloid used worldwide. It is quite toxic compound and can cause DNA damage in microorganisms. We used two tests to observe *mia* mutants' response to this kind of chemical stresses. Susceptibility to diamide was tested using paper disks with 10μL of 0.1M solution, as described in Methods. The result of this assay is summarized in Fig. 3. Our data demonstrates that Δ*miaAB* strain is more susceptible to diamide than the control strain SAM2.

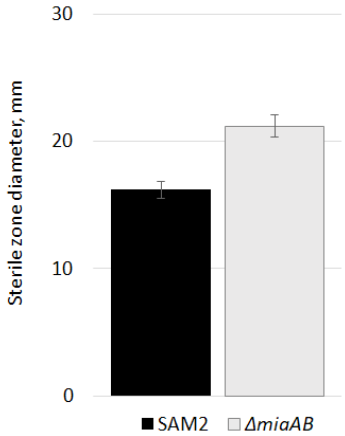

Fig. 3. Diameters of *S. albus* growth inhibition zones (in mm), caused by 10 μL of 0,1 M diamide. SAM2 – *S. albus* SAM2, Δ*miaAB* – *S. albus* Δ*miaAB*. Results represent the mean values of 11 repeats, error bars are standard error

The next assay was performed to test susceptibility to caffeine. We plated *S. albus* strains on GYM medium, supplemented with 5 mM and 10 mM of caffeine. For the spot test, strains were grown in liquid TSB for 48 h and 10 μL of cultures, together with the same amounts of 10^{-4}, 10^{-5} and 10^{-6} dilutions, were plated and incubated for 48 h, as it is shown on Fig. 4, A. As we can see, increased concentration of caffeine has negative effects on the growth of all *S. albus*

strains. With approximately equal concentration of CFUs in 1 mL of culture for SAM2, Δ*miaA* and Δ*miaAB*, only SAM2 showed visible growth on 10^{-4} dilution on 10 mM caffeine. For the lawns cultivation, results were similar (Fig. 4, B). Caffeine had negative effect on all strains, but Δ*miaA* and Δ*miaAB* showed a considerable delay in vegetative and aerial mycelium formation in presence of 5 mM and 10 mM caffeine, in comparison to control strain SAM2.

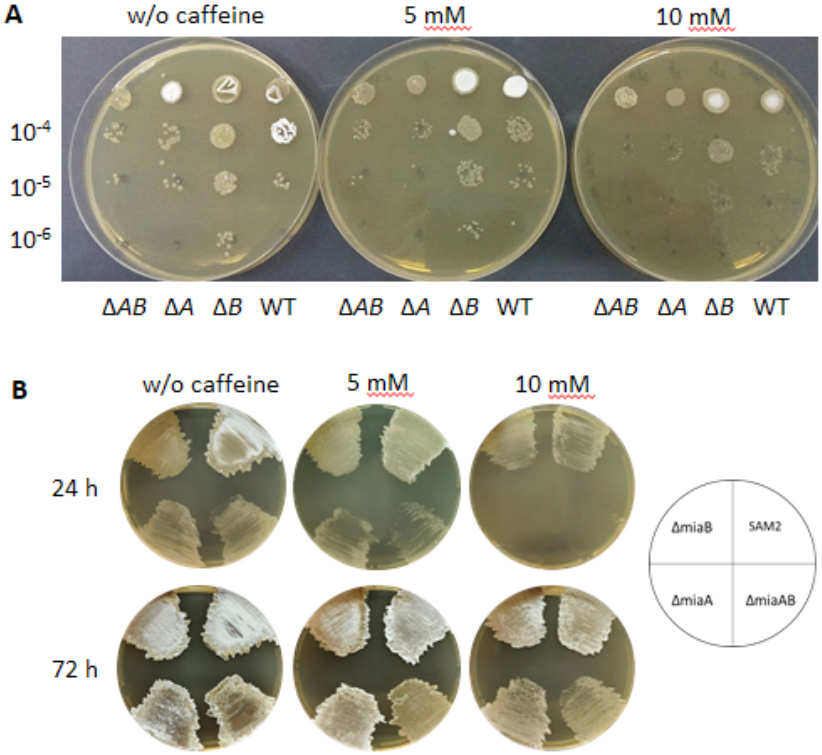

Fig. 4. Susceptibility of *S. albus* strains to caffeine. **A.** Liquid TSB cultures of *S. albus* SAM2, Δ*miaAB*, Δ*miaA* and Δ*miaB* were spotted on GYM medium, supplemented with 5 mM and 10 mM of caffeine. Plates were incubated for 72 h before taking pictures. Numbers to the left label serial dilutions. **B**. *S. albus* strains were plated on GYM medium, supplemented with 5 mM and 10 mM of caffeine, as it is shown on scheme to the right. Photos were taken on 24 h and 72 h of growth

The delay was especially pronounced at 24 h. On the first day of growth on 10 mM caffeine Δ*miaA* and Δ*miaAB* strains were unable to form vegetative mycelium, but Δ*miaB* and SAM2 exhibited more abundant growth. Strain Δ*miaA* formed aerial mycelium on 72 h day of growth only on medium without caffeine. The strain with deletion of both *miaA* and *miaB* genes showed the highest susceptibility to caffeine. Mutant with *miaB* deletion showed the level of susceptibility to caffeine similar to that of the parental SAM2. We assume, therefore, that deletion of *miaA* gene in *S. albus* results in higher sensitivity to caffeine, and additional deletion of *miaB* pronounces this effect.

We report that *mia* mutations in *Streptomyces* impact translation fidelity and susceptibility to different stressors. Considering our data and the fact that Mia proteins modify a large set of tRNAs, we assume that codon mistranslation in *S. albus* depends not only on the position and nature of the codon, but also on the other unique features of the dynamic translational machinery. Results of bioassays show that deletion of *miaA* is the main reason of mistranslation and higher susceptibility to caffeine and diamide. As one can see, Δ*miaB* strain did not display elevated mis-

translation rate under our experimental conditions, and also this strain showed similar phenotype to the parental strains while growing on caffeine-supplemented GYM agar. Probably, *miaB* plays less important role in the response to studied stressors, as opposed to *miaA*. Indeed, posttranscriptional modifications of tRNA affect bacterial homeostasis and, since for *Streptomyces* there is a little information about *mia*-dependent pathways, it is our goal to elucidate this issue deeper.

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MIA МУТАНТИ *STREPTOMES ALBUS* J1074 СХИЛЬНІ ДО ПОМИЛОК У ПРОЦЕСІ ТРАНСЛЯЦІЇ ТА ПІДВИЩЕНОЇ ЧУТЛИВОСТІ ДО СТРЕСОВИХ ЧИННИКІВ

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Streptomyces albus J1074 використаний нами як зручна модель для вивчення різних аспектів $tRNA^{Leu}_{UAA}$-залежних регуляторних механізмів, притаманних представникам роду *Streptomyces*. Ці механізми важливі для належного перебігу морфологічних і фізіологічних змін у колоніях стрептоміцетів, таких як початок продукції антибіотиків протягом стаціонарної фази росту. Показано, що гени посттранскрипційної модифікації залишку аденозину в 37-й позиції родини тРНК з антикодоном XXA (т. зв. гени *mia*) є важливими для вищезазначених процесів, швидше за все, тому, що спричинюють найсильніший вплив на $tRNA^{Leu}_{UAA}$. Наші результати значною мірою узгоджуються з тим, що відомо про *mia* мутації в інших модельних об'єктах, таких як дріжджі й ентеробактерії. Тим не менше, ми також виявили кілька відмінностей, зокрема, зниження сприйнятливості до перекису водню. Це спонукало нас глибше вивчити властивості *mia* мутантів, особливо їхню реакцію на різні стресові фактори. Виявлено, що *mia* мутанти *S. albus* демонструють підвищений рівень супресії стоп-кодону TAG, порівняно з батьківським штамом. Ці мутанти є чутливішими до індуктора дисульфідного стресу діаміду та до кофеїну, що пошкоджує механізми репарації ДНК. У підсумку, відсутність певної модифікації тРНК, найвірогідніше, спричиняє однакову чи дуже подібну відповідь у всіх досліджуваних бактерій (наприклад, підвищення рівнів помилок під час трансляції), проте і викликає специфічні для виду чи роду ефекти (наприклад, різний вплив на чутливість до H_2O_2). Ці відмінності можна пояснити особливостями організації/функції регуляторного шляху, що координує відповідь на певний стресовий фактор. Отримані результати обговорено в ширшому контексті ролі модифікацій тРНК у біології бактерій.

Ключові слова: *Streptomyces albus*, тРНК, тест на супресію нонсенс-мутацій, діамід, кофеїн