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GENE EXPRESSION PROFILING OF MULTI-DRUG RESISTANT *E. COLI* AFTER EXPOSURE BY NANOMOLECULAR IODINE-CONTAINING COMPLEX

Abstract. Gene profiling was performed to assess the transcriptomic effect of the FS-1 drug during long-term exposure to the multidrug-resistant strain of *E. coli* ATCC® BAA-199. FS-1 was added to the E. coli culture at a concentration corresponding to 1/2 MBC for ten passages.

As a result of the effect of FS-1 on a multiresistant strain of *E. coli*, significant differential regulation of many key genes was observed. Long-term cultivation on a medium containing a sub-bactericidal concentration of FS-1 leads to substantial metabolic changes in bacteria associated with the suppression of the central pathways of energy production and amino acid synthesis. In contrast, the synthesis of nucleotides and fatty acids was activated. It was found that some key metabolic pathways are suppressed by the action of FS-1, causing a General tendency to decrease the redox potential of the cell and the production of ATP. Our research shows a significant decrease in the activity of genes involved in β-oxidation of fatty acids, CTC, glyoxylate shunt, which leads to the suppression of aerobic respiration, causing bacteria to switch to less effective anaerobic respiration. The results of our research demonstrate the suppression of oxaloacetate formation in the CPC, which is a precursor of aspartate biosynthesis, as well as the suppression of the shikimate pathway and, as a result, the reduction of tryptophan formation. Reduced production of aspartate and tryptophan probably leads to a lack of NAD^+, depriving the bacteria of NADPH. However, the lack of NADPH can be partially compensated by activation of the pentose phosphate cycle under these conditions, which serves as an essential source of NADPH and pentose sugars for nucleotide biosynthesis. The reduced redox potential of cells and the production of NADH cofactors under the action of FS-1 may affect the sensitivity of *E. coli* culture to antibiotics. Thus, a better understanding of the metabolic flow can lead to effective therapeutic or preventive measures to combat antibiotic-resistant bacteria.

Key words: *E. coli*, antibiotic resistance, FS-1, RNA, sequencing, gene expression.

Introduction. Bacterial resistance to antimicrobial agents is an urgent problem in modern society [1]. The world health organization recognizes antibiotic resistance of microorganisms as the greatest threat to human health worldwide. Patients infected with multidrug-resistant bacteria are subjected to more complicated treatment processes than those infected with susceptible pathogens [2]. The growth of

antibacterial resistance levels the achievements of modern medicine, such as cancer treatment, transplantation, surgery, etc. [3]. Pathogens can acquire resistance to antibiotics, either through mutations or by horizontal transfer of resistance genes. Bacteria, which are resistant to a particular drug, may develop the strength to other second-line antibiotics and cause multidrug-resistant infections [4,5].

Although resistant patterns are found in both Gram-positive and Gram-negative bacteria, the most significant concern is associated with Gram-negative bacteria with an acquired resistance against several or even all available antibiotics, which are reported worldwide with an increasing rate [6,7]. An *Escherichia coli* strain selected for the study represents nosocomial infection pathogens [8]. Drug-resistant *E. coli* isolates are widely detected in the environment, including water resources [9] and agricultural products [10].

Different approaches are used to combat antibiotic resistance. The most interesting, in our opinion, is based on the induction of drug resistance reversion of multidrug-resistant bacterial populations to regain susceptibility to conventional antibiotics. Some drugs of this kind inhibit specific resistance mechanisms, thereby neutralizing the evolutionary advantages of antibiotic resistance, leading to an increase in the number of antibiotic-sensitive bacteria in the population [11].

This study aimed to carry out the total gene expression profiling to assess the effects of the drug FS-1 inducing antibiotic resistance reversion in the model multidrug-resistant strain *E. coli* ATCC® BAA-199 during its cultivation with FS-1.

Materials and methods. The multidrug-resistant *E.coli* bacterial culture (ATCC® BAA-199™) was obtained from the American Type Culture Collection (https://www.lgcstandards-atcc.org/en.aspx). The drug FS-1 was added to MHB cultivation medium in a concentration of 500 μg/ml that corresponded to 1/2 of the minimal bactericidal concentration (MBC) of FS-1 calculated for the strain *E. coli* BAA-199. The bacterial culture was cultivated at 37°C for 10 days with daily re-inoculations to tubes with fresh MHB medium. Gene expression was stopped sharply by mixing the bacterial culture with RNAlater (Sigma), in a ratio of 5:1. As a negative control, the bacterial culture was cultivated for 10 days on the medium without the drug FS-1. All experiments were performed in three replicates.

Isolation of total RNA was performed following the developer's guidelines using the RiboPure Bacteria Kit (Ambion, Lithuania). RNA's quality and quantity were determined using the NanoDrop 2000s spectrophotometer (Thermo Scientific, USA) at optical wavelengths 260 and 280 nm. Purification of the total RNA from ribosomal 16S and 23S RNA was carried out using the MICROBExpress Bacterial mRNA Purification Kit (Ambion, Lithuania) following the developer recommendations. The efficiency of template-RNA purification was determined on the Bioanalyzer 2100 (Agilent, Germany) with the RNA 6000 Nano LabChip Kit (Agilent Technologies, Lithuania).

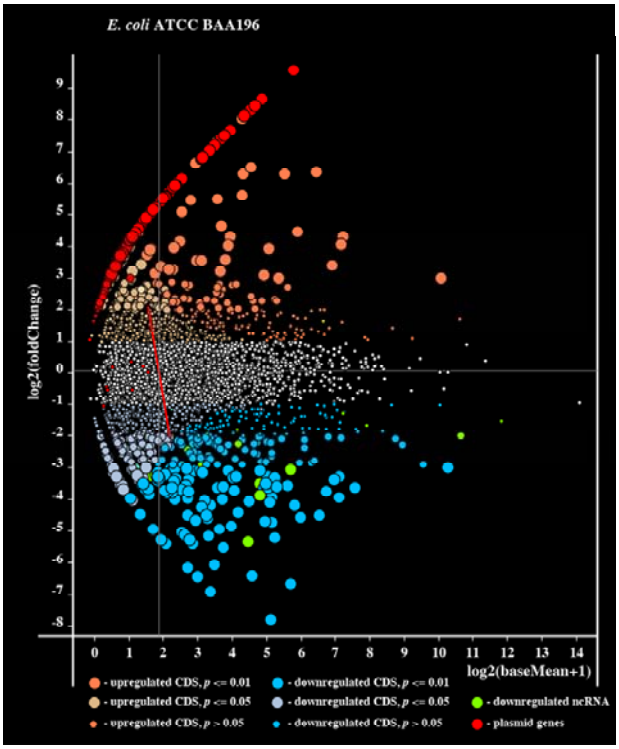
RNA fragment library was prepared by enzymatic fragmentation with the Ion Total RNA Seq Kit V2 (Life Technologies, USA). Barcoding of the obtained RNA library was carried out with the Ion Xpress RNA-Seq Barcode 01-16 Kit (Life Technologies, USA), according to the manufacturer's instructions. RNA sequencing was performed using the Ion Torrent PGM sequencer (Life Technologies, USA) with the Ion 318 Chip Kit V2.

RNA fragments obtained by sequencing were aligned against the reference genome [12] using the QIAGEN CLC Workbench 7.0.3 software package. To visualize the results and compare gene expression profiles, we applied our in-house scripts written on Python 2.5. The differential gene expression statistics were calculated using the R-3.4.4 software package DESeq2. [13]. The role of protein-coding genes in the *E. coli* metabolism was determined using the Pathway Tools 24 software [14] implemented in the EcoCyc database [15].

Results. In our previous studies, it was shown that FS-1 induced a reversion to antibiotic sensitive phenotype in drug-resistant bacteria [16]. The whole genome sequence comparison did not reveal any significant mutations in genes responsible for drug resistance. It was assumed that the experimentally determined antibiotic resistance reversion might be caused by an alternative gene expression regulation and possibly by epigenetic mechanisms [17].

For this purpose, the bacterial culture E. coli BAA-199 was grown for 10 consecutive passages with a constant content of the drug FS-1 in the medium that corresponded to half of its MBC estimated for this bacterium. Six RNA samples obtained after 10 passages with FS-1 and on the same medium without FS-1 as negative control were extracted and sequenced.

In total, 4407 genes were identified on the chromosome of *E. coli* BAA-199 [12]. A confident differential expression ($p \leq 0.05$) was identified for 522 genes, of which 322 genes were negatively regulated by the drug FS-1 (figure).



Differential gene regulation in *E. coli* BAA196 culture grown with FS-1 compared to the negative control culture. Each cycle represents one gene plotted in accordance with the calculated average expression level (axis X, baseMean) and fold change (axis Y, foldChange) represented by base 2 logarithms. Up- and downregulated genes encoding proteins (CDS), non-coding regulatory RNA (ncRNA), and the genes located on the plasmid are depicted by cycles of different color and size (depending on the estimated confidence) as described in the figure legend. The red trend line links the average baseMean and foldChange values calculated for up- and downregulated genes.

Using the services STRING [18] and KEGG [19], gene clustering was performed by their functions and GO terms. Metabolic pathways associated with the genes regulated in *E. coli* by exposure to FS-1 in the medium are summarized in Table 1. To determine the metabolic pathways, the *Escherichia coli* K-12 substr. MG1655 genome was used as a reference culture.

Metabolic pathways of *E. coli* induced by FS-1

#term ID*	Term description	Observed gene count	Background gene count	False discovery rate
eco00620	Pyruvate metabolism	19	52	0.00038
eco01230	Biosynthesis of amino acids	18	116	2.33e-08
eco00020	Citrate cycle (TCA cycle)	16	27	2.88e-05
eco00630	Glyoxylate and dicarboxylate metabolism	16	41	0.00075
eco00190	Oxidative phosphorylation	12	43	0.0357
eco00010	Glycolysis / Gluconeogenesis	8	43	0.00014
eco01212	Fatty acid metabolism	9	21	0.0112
eco00030	Pentose phosphate pathway	5	30	0.00041
eco00230	Purine metabolism	8	91	0.0094

Note: * - Pathway ID according to KEGG.

Pyruvate occupies the key metabolic node linking carbohydrate catabolism with energy production and biosynthesis and also represents the main switch between respiratory and enzymatic metabolism. In *Escherichia coli*, pyruvate dehydrogenase (PDHC) and pyruvate formate lyase (PFL) catalyze the main pathway of conversion of pyruvate to Acetyl-CoA. Also, these enzymes initiate the aerobic respiration and anaerobic fermentation, respectively [20].

The pyruvate dehydrogenase (PDHC) complex consists of three subunits encoded by genes *aceE*, *aceF*, and *lpd*. Our results do not show any significant change in the expression level of *aceE* (1.2-fold change) and *aceF* (1.4-fold change) compared to the negative control. However, there was a strong negative regulation of *lpd* for −2.8 fold change. Lpd-lipoamide dehydrogenase catalyzes the transfer of electrons to the final acceptor-NAD+. This complex of reactions is the connecting bridge between glycolysis and the Krebs cycle. Since the expression of one of the main components of the PDHC complex was suppressed in our studies, we assumed that pyruvate could be dissimilated via pyruvate:formate lyase (PFL) pathway. Significant activation of *pflB* gene (by 7.4 fold) that encods a pyruvate-formate lyase subunit confirms this statement. There is also clear evidence of 2 to 9 fold activation of the genes involved in anaerobic respiration (*tdcE, narK, narH, narI, napB, nirB, nirD, nrfA*) [21].

Additionally to the activation of the PHDC complex, a significant increase of expression of various transhydrogenase was observed; particularly, the soluble (encoded by *sthA*) and membrane-bound (encoded by *pntAB*) pyridine nucleotide transhydrogenases. By converting NADH to NADPH, both these transhydrogenases provide 35-45% of the NADPH required for biosynthetic pathways of *E. coli* [22].

Besides, significant repression of *aldA* and *lldD* genes (by −445 and −39 fold, respectively) was observed. These genes are strongly inhibited in anaerobic conditions [23].

Acetyl-CoA synthetase (*acs*) catalysis synthesis of Acetyl-CoA from acetate using ATP [24] that is one of two alternative pathways by which *E. coli* can assimilate acetate to Acetyl-CoA. In our studies, the activity of the *acs* gene was suppressed almost 32 fold change under the action of FS-1.

There were 9 genes regulated by the presence of FS-1 in the medium, which were involved in metabolism of fatty acids. Six of these genes, *fadA*, *fadE*, *fadJ*, *fadB*, *fadD* and *fadI*, were negatively regulated. The analysis showed that all these inhibited genes are involved in β-oxidation of fatty acids – a metabolic process of degradation of fatty acids to $FADH_2$, NADH, and Acetyl-CoA. Catabolic β-oxidation is followed by oxidation of Acetyl-CoA in the Krebs cycle that serves as one of the main sources of energy producing ATP molecules by oxidative phosphorylation. Other 3 genes, *accA, accD* and *fabB*, were no more than 2.5 fold positively regulated. They participate in initiation and prolongation of fatty acid synthesis, i.e. in anabolic processes of the bacterium; particularly, in synthesis of cell membrane fatty acids.

Glycolytic processes of energy production and precursor biosynthesis may be carried out in *E. coli* through the glycolysis to the tricarboxylic acid cycle (TCA), Entner Doudoroff pathway (EDP), Embden–Meyerhof–Parnas pathway (EMPP) and the oxidative pentose phosphate pathway (PPP) [25]. The TCA is central not only to energy metabolism, but also plays a significant role in anabolism being an important source of precursors for the synthesis of such compounds as amino acids, carbohydrates, fatty acids, etc. Out of the 16 genes involved in TCA, 15 genes (*sdhC, sdhD, sdhA, sdhB, sucC, sucD, sucA, sucB, gltA, icd, acnA, acnB, fumC, fumA, lpdA*) were inhibited. In addition to this, 5 of 8 glycolysis genes, *acs, agp, aldB, glpX, tpiA*, were downregulated by FS-1. The obtained data shows a general inhibition of both, TCA and glycolysis, which in turn may lead to a decrease in amino-acid biosynthesis activity and oxidative phosphorylation, which should be bypassed somehow by the bacterium. Indeed, many biosynthetic pathways sourced from TCA and glycolysis were inhibited or downregulated by FS-1. They include the biosynthesis of amino acids lysine/methionine (*thrA*, −21 fold), phenylalanine/tyrosine (*pheA*, −4.9 fold), threonine (*thrB*, −7.5 fold; *thrC*, −5.2 fold), valine/isoleucine (*ilvs*, *ilvD*, *ilvE*, in average −2 fold), asparagine (*asnA*, *asnB* and *iaaA*, in average −2 fold), and glutamine (*glnA*, −3.7 fold). Besides, the genes involved in the shikimate pathway, during which the chorismate precursor of tryptophan is formed, were also suppressed. In general, this may indicate a decrease in the anabolism of the culture.

There was a general suppression of the TCA associated glyoxylate shunt caused by the drug FS-1 that could be expected as this pathway links TCA with b-oxidation of fatty acids, and both these pathways were strongly downregulated. Out of 16 shunt-related genes, 15 were strongly inhibited. Another function of the glyoxylate shunt is the replenishment of intermediate tricarboxylic and dicarboxylic acids, which

are intermediates of the Krebs cycle. Krebs cycle is functionally linked with the oxidative phosphorylation (in the electron transport chain) where electrons are transferred from donor compounds to acceptor compounds during redox reactions, and the energy is produced in the form of ATP molecules [26]. *E. coli* genome contains a cluster of three cytochrome oxidase enzymes – cytochrome bo oxidase (*CyoABCD*), cytochrome bd-I oxidase (*CydABX*), and cytochrome bo-II oxidase (*AppCD*). These enzymes function as major terminal oxidases in the aerobic respiratory chain of *E. coli* that generates the proton motive force (PMF) [27,28,29]. In our studies, *cyoABCD* cytochromes and all related genes mentioned above were strongly inhibited. Notably, the expression of *cyoABCD* genes encoding cytochrome *o* oxidase was downregulated −19.6, −8.0, 6.0 and −64 fold, respectively. Gene *cyoE* that is necessary for the functioning of the cytochrome complex was also suppressed by −7.0 fold.

Another cluster of genes showing a significant suppression by FS-1 was that one encoding NADH: ubiquinone oxidoreductase I (NDH-1) biosynthesis. NDH-1 is a NADH dehydrogenase that catalyzes the transfer of electrons from NADH to a pool of quinones in the cytoplasmic membrane, generating the proton electrochemical gradient, which is also a part of both the aerobic and anaerobic respiratory chain of the cell.

Finally, the genes included in the *sdhCDAB* operon encoding quinone oxidoreductase (SQR) catalyze the oxidation of succinate to fumarate, the process accompanying the reduction of ubiquinone to ubiquinol, were also suppressed. SQR plays an important role in cellular metabolism and binds the TCA cycle to the chain of respiratory electron transportation.

Global profiling of the *E. coli* transcriptome revealed 5 genes (*prsA, pgi, gnd, pgl glpX*), related to the oxidative pentose phosphate pathway (PPP) induced by the action of the drug FS-1. It should be noted that PPP is not used by *E. coli* for energy production, but sources numerous biosynthetic pathways, which may compensate to some extent the potent TCA inhibition. In PPP, glucose is phosphorylated to glucose-6-phosphate, which then is oxidized to ribulose-5-phosphate forming two reduced NADPH molecules. The alternative glycolytic pathway used by *E. coli* for energy production is EDP and EMPP. Two key genes of the EDP pathway, phosphogluconate dehydratase *edd* and keto-hydroxyglutarate-aldolase *eda*, were 1.8 and 1.6 fold upregulated by the treatment with FS-1. Regulation of the critical enzymes of the EMPP pathway, 6-phosphofructokinase subunits *pfkAB*, was insignificant.

While the synthesis of many amino acids was suppressed, there was an explicit activation of genes involved in purine biosynthesis: *hpt, prs, purT, purF, purC, guaA, guaB, purL*. Moreover, almost all genes involved in purine biosynthesis were positively induced by the drug FS-1. Synthesis of nucleotides may be activated because of the need to replace damaged nucleotides in DNA and RNA, or to intensify the synthesis of such bioactive compounds as NAD(P) needed to cope with the increased redox potential. Finally, a strong up-regulation of many genes located on the large virulence plasmid was observed (figure). The most expressed plasmid genes were those associated with plasmid mobilization and conjugation that implies strong stress posed on the bacterial cell.

Conclusion. Gene expression profiling of a multidrug-resistant *E. coli* strain demonstrated a significant differential regulation of many key genes resulted from the exposure of the bacterium to FS-1. It may be concluded that long-term passivation on a medium containing a sub-bactericidal concentration of the drug FS-1 leads to profound metabolic changes in the bacterium associated with downregulation of the central pathways of energy production and synthesis of amino acids. In contrast, the synthesis of nucleotides and fatty acids was activated. Thus, some key metabolic pathways are suppressed under the action of FS-1, causing a general tendency of decreasing the redox potential of the cell and ATP production. The significant reduction of the pathways of β-oxidation of fatty acids, TCA and glyoxylate shunt leads to suppression of the aerobic respiration, forcing bacteria to switch to a less effective anaerobic respiration.

In living organisms, NAD molecules, which are essential for bacterial cells' redox sustainability, are synthesized *de novo* from amino acids aspartate and/or tryptophan. This study showed a suppression of synthesis of oxaloacetate in TCA that is a precursor of aspartate biosynthesis, as well as suppression of the shikimate pathway leading to tryptophan biosynthesis. Suspended production of aspartate and tryptophan likely causes the lack of NAD, while the strong suppression of the Krebs deprives bacteria of NADPH. However, the shortage of the latter bioactive compound may be partially compensated by activation of the oxidative pentose phosphate pathway, which serves as an essential source of NADPH and pentose sugars for nucleotides biosynthesis strongly activated at this condition.

Reduced cell redox potential and decreased production of NADH cofactors may be critical mechanisms of the increased susceptibility of the FS-1 treated culture of *E. coli* to antibiotics, many of which act through elevation of the oxidative stress. Thus, a better understanding of the metabolic flow can lead to effective therapeutic or preventive measures to overcome antibiotic-resistant bacteria.

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ҚҰРАМЫНДА НАНОМОЛЕКУЛА ИОДЫ БАР КЕШЕН ӘСЕРІНЕН КЕЙІНГІ *E. COLI* МУЛЬТИРЕЗИСТЕНТТІК ШТАММ ГЕНДЕРІ ЭКСПРЕССИЯСЫН ПРОФИЛИРЛЕУ

Аннотация. Микроорганизмнің антибиотикалық тұрақтылығы әлемде адам денсаулығына қауіп төндіреді. Микробқа қарсы препараттарды шамадан тыс қолдану төзімді микроорганизмдердің дамуы мен таралуына қатысты қауіпті ұлғайтады. Осыған байланысты олардың микробқа қарсы тұрақтылық механизмдерін зерттеу қазіргі қоғамның өзекті мәселесі болып саналады.

Жұмыста ұзақ уақыт өсіру барысында ФС-1 препаратының әсерін бағалау үшін *Escherichia coli* ATCC® BAA-199 мультирезистенттік штамм гендерін профилирлеу нәтижесі келтірілген. ФС-1 препараты 10 пассаж бойы минималды бактерицидтік концентрация (МБК) 1/2 мөлшеріне сәйкес келетін дозада қолданылды. РНҚ-ны оқшаулау және тазарту әзірлеушілердің ұсыныстарына сәйкес коммерциялық жинақтарды қолдану арқылы жүзеге асырылды. РНҚ фрагменттерінің кітапханасы Ion Total RNA Seq Kit V2 (Life Technologies, АҚШ) арқылы ферментативті шектеу негізінде дайындалды. РНҚ оқшаулау және тазарту кітапханаларды кодтауда коммерциялық жиынтық арқылы өндіруші нұсқауларына сәйкес Ion Xpress RNA-Seq Barcode 01-16 (Life Technologies, АҚШ) жиынтығын қолдану арқылы жүзеге асырылды. РНҚ секвенирлеу Ion Torrent PGM секвенаторында (Life Technologies, АҚШ) жүргізілді. Секвененрлеу барысында алынған РНҚ фрагменттерін құрастыру және туралау QIAGEN CLC Workbench 7.0.3 бағдарламалық қамтамасыз ету пакеті арқылы анықтамалық геном негізінде жасалды. Гендер экспрессиясының деңгейі бағалау жағдайы R-3.4.4 бағдарламалық қамтамасыз ету барысында енгізілген DESeq2 пакеті арқылы жүргізілді. *E. coli* метаболизміндегі ақуызды кодтайтын гендер рөлі EcoCyc деректер базасына ендірілген Pathway Tools 24 бағдарламалық қамтамасыз ету көмегі арқылы анықталды.

ФС-1 препаратының мультирезистенттік *E. coli* штамына әсері нәтижесінде көптеген негізгі гендердің маңызды дифференциалды реттелуі байқалды. ФС-1 препаратының суббактерицидтік концентрациясы бар ортада ұзақ уақыт өсіру нуклеотидтер мен май қышқылдарының синтезі белсенді өндіріс пен аминқышқылдар синтезінің орталық жолдарын басуға байланысты бактериялардың метаболикалық өзгеруіне әкеледі. ФС-1 әсерінен метаболизмнің кейбір негізгі жолдары басылып, жасушаның тотығу – қалпына келтіру потенциалының төмендеуіне және АТФ түзілуіне ықпал ететіндігі анықталды. Зерттеулерімізде май қышқылдарының β-тотығуымен, ЦТК, глиоксилат шунтымен айналысатын гендер белсенділігінің едәуір төмендегенін көрсетті, бұл аэробты тыныс алуды басады, бактериялардың анаэробты тыныс алу тиімділігін төмендетеді. Зерттеулеріміздің нәтижесінде Аспартат биосинтезінің алғышарты болып саналатын ЦТК-да оксалоацетат түзілісін басуды, сонымен қатар шикимат жолының тежелуін және соның нәтижесінде триптофан түзілісі төмендейді. Аспартат пен триптофан өндірісінің төмендеуі НАДФ бактериясынан айырылып, $НАД^+$ жетіспеуіне әкелуі мүмкін. Алайда НАДФ жетіспеушілігі нуклеотидті биосинтез үшін НАДФ пен пентозды қанттың маңызды көзі болып саналатын пентозды фосфат циклінің активтенуі арқылы ішінара өтеледі. ФС-1 әсерінен жасушалардың тотығу потенциалының төмендеуі және НАД коэффициенттерінің өндірілуі *E. coli* өсіндісінің антибиотиктерге сезімталдығының жоғарылауына әсер етуі мүмкін. Осылайша метаболизм ағынын жете түсіну антибиотикке төзімді бактериялармен күресудің тиімді терапиялық немесе профилактикалық шараларына әкелуі мүмкін.

Түйін сөздер: *E. coli*, антибиотикорезистенттік, ФС-1, РНҚ, секвенирлеу, гендер экспрессиясы.

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ПРОФИЛИРОВАНИЕ ЭКСПРЕССИИ ГЕНОВ МУЛЬТИРЕЗИСТЕНТНОГО ШТАММА *E. COLI* ПОСЛЕ ВОЗДЕЙСТВИЯ НАНОМОЛЕКУЛЯРНЫМ ЙОД-СОДЕРЖАЩИМ КОМПЛЕКСОМ

Аннотация. Антибиотикорезистентность микроорганизмов является угрозой здоровья человечества во всем мире. Чрезмерное использование противомикробных препаратов привело к тревожному увеличению развития и распространения устойчивых микроорганизмов. В связи с этим изучение механизмов их устойчивости к противомикробным препаратам является актуальной проблемой современного общества.

В данной работе приведены результаты профилирования генов мультирезистентного штамма *Escherichia coli* ATCC® BAA-199, для оценки действия препарата ФС-1 при длительном культивировании. Препарат ФС-1 использовали в дозе, соответствующей 1/2 минимальной бактерицидной концентрации (МБК) в течение 10 пассажей. Выделение и очистку РНК проводили при помощи коммерческих наборов в соответствии с рекомендациями разработчиков. Библиотеку фрагментов РНК готовили путем ферментативной рестрикции с помощью Ion Total RNA Seq Kit V2 (Life Technologies, США). Баркодирование библиотеки осуществляли с использованием набора Ion Xpress RNA-Seq Barcode 01-16 (Life Technologies, США) в соответствии с инструкциями производителя. Секвенирование РНК проводили на секвенаторе Ion Torrent PGM (Life Technologies, США). Сборку и выравнивание фрагментов РНК, полученных во время секвенирования, проводили на основе эталонного генома с использованием пакета программного обеспечения QIAGEN CLC Workbench 7.0.3. Оценку уровней экспрессии генов проводили с использованием пакета DESeq2 имплементированного в программное обеспечение R-3.4.4. Роль белок-кодирующих генов в метаболизме *E. coli* была определена с помощью программного обеспечения Pathway Tools 24, внедренного в базу данных EcoCyc.

В результате воздействия препарата ФС-1 на мультирезистентный штамм *E.coli* наблюдалась значительная дифференциальная регуляция многих ключевых генов. Длительное культивирование на среде, содержащей суббактерицидную концентрацию препарата ФС-1, приводит к глубоким метаболическим изменениям в бактериях, связанных с подавлением центральных путей продукции энергии и синтеза аминокислот, в тоже время синтез нуклеотидов и жирных кислот был активирован. Установлено, что некоторые ключевые метаболические пути подавляются под действием ФС-1, вызывая общую тенденцию снижения окислительно-восстановительного потенциала клетки и продукции АТФ. В наших исследованиях показано существенное снижение активности генов участвующих в β-окислении жирных кислот, ЦТК, глиоксилатном шунте, что приводит к подавлению аэробного дыхания, заставляя бактерии переключаться на менее эффективное анаэробное дыхание. В результатах наших исследований демонстрируется подавление образования оксалоацетата в ЦТК, который является предшественником биосинтеза аспартата, а также угнетение шикиматного пути и, как следствие, снижение образования триптофана. Снижение продукции аспартата и триптофана, вероятно, приводит к нехватке $НАД^+$, лишая бактерии НАДФ. Однако нехватка НАДФ может быть частично компенсирована активацией в данных условиях пентозофосфатного цикла, который служит важным источником НАДФ и пентозных сахаров для биосинтеза нуклеотидов. Снижение окислительно-восстановительного потенциала клеток и продукции кофакторов НАД под действием ФС-1 могут влиять на повышение чувствительности культуры *E. coli* к антибиотикам. Таким образом, лучшее понимание метаболического потока может привести к эффективным терапевтическим или профилактическим мероприятиям по борьбе с устойчивыми к антибиотикам бактериями.

Ключевые слова: *E. coli*, антибиотикорезистентность, ФС-1, РНК, секвенирование, экспрессия генов.

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