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Screening the antimicrobial effect of ferrocene-boronic acid on *Pseudomonas aeruginosa* using proteomics and metabolomics approach

Engin KOÇAK [1] *, Ceren ÖZKUL [2], Gülce TAŞKOR ÖNEL [3], Emirhan NEMUTLU [4,5], Sedef KIR [4], Meral SAĞIROĞLU [2]

1 Department of Analytical Chemistry, Faculty of Gülhane Pharmacy, Health Sciences University, 06100, Ankara, Turkey.
2 Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey.
3 Department of Basic Pharmaceutical Science, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan, Turkey.
4 Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey.
5 Drug and Cosmetic R&D and Quality Control Laboratory (HUNİKAL), Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey.
* Corresponding Author. E-mail: kengin@hacettepe.edu.tr (E.K.); Tel. +90-546-720 23 52.

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ABSTRACT: Antibiotic resistance is one of the most serious global problems around the world. *Pseudomonas aeruginosa* is gram-negative bacteria and plays important role in local and systemic infections. In our work, we tried to understand mode of antimicrobial action of ferroccene- boronic acid over *Pseudomonas aeruginosa* via metabolomics and proteomics analysis. In proteomics analysis, we found that ferrocene-boronic acid effects various antimicrobial targets like ATP-dependent DNA helicase RecQ, Transcription-repair-coupling factor and Primasome assembly protein PriA. In metabolomics analysis, the ferrocene-boronic acid induced various metabolites involved in pyrimidine metabolism, lipid and fatty acid metabolism. Moreover, various polyamines like spermine and spermidine, which are very important for antibiotic resistance, pathogenesis and bacterial biofilm formation were decreased by ferroceneboronic acid. We believe that our results will contribute further studies regarding organometallic compounds in microbiology filelds.

KEYWORDS: Metabolomics; proteomics; boronic acid; *Pseudomonas aeruginosa*.

1. INTRODUCTION

In recent years, bacterial antibiotic resistance has been considered one of the most serious public health problem worldwide [1]. *Pseudomonas aeruginosa (P. aeruginosa)* is a gram negative environmental bacteria, which may also cause multiple infections in human both local and systemic. It is also considered as a major causative agent of nosocomial infections associated with high morbidity and mortality. As an environmental pathogen, *P. aeruginosa* has high capability to develop resistance to antimicrobials, which limits the treatment options in clinical setting [2, 3]. Both intrinsic and acquired mechanisms play a role in *P. aeruginosa* antibiotic resistance involving overexpression of efflux pumps, low outer membrane permeability, antibiotic-inactivating enzymes such as AmpC β-lactamases [4]. Omics technologies offer a great opportunity to understand antibiotic-bacteria relationship at gene, protein and metabolite level and may lead to new antibiotic discovery method. This is especially important to find new antimicrobial targets and treatment methods. Proteomics and metabolomics, which are the subtitles of post genomics area and give essential information about phenotype, have emerged as key tool to clarify mode of action of natural or synthetic antimicrobial agents.

Organometallic compounds have been developed and used in medicinal chemistry for a long time [5]. Today various organometallic compounds like cisplatin are using as chemotherapeutic agents for various cancer types. Moreover, organometallic compounds have been studied in microbiology field to overcome antibiotic resistance problem [6-8]. Ferrocene based organometallic compounds have withdrawn attention

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for clinical applications because of their anticancer and antimicrobial properties [6, 9-11]. Boronic acid and its derivatives are very important in medicinal chemistry. They have unique physicochemical properties and intensively investigated for cancer therapy, antimicrobial agents, and drug delivery systems [12-15]. Several studies showed that boronic acid could provide an alternative approach to inhibit beta lactamases [16]. Firstly, Kiener et. al. reported that boronic acids may act as broad-spectrum inhibitors of beta-lactamases [17]. Beesley et. al showed that boronic acids behaved as reversible competitive inhibitors of class C Beta-lactamases [18]. Boronic acids form a dative bond with the catalytic Ser residue in the enzyme active site. The boron atom in this covalent adduct has a tetrahedral geometry thus mimicking the transtition state formed during the hydrolysis of β-lactams. Vaborbactam, which is a cyclic boronic acid based antimicrobial drug, has been used with meropenem against antibacterial resistant pathogens [19, 20].

In this study, we focused on antimicrobial activity of ferrocene-boronic acid (FBA) on *P. aeruginosa* via proteomics and metabolomics. Antimicrobial studies showed that FBA is effective on the *P. aeruginosa*. We used proteomics and metabolomics approaches to understand mode of action of FBA at molecular level. Our results showed that FBA effects DNA replication and also various metabolic processes in *P. aeruginosa*. We believe that our results will contribute further studies regarding ferrocene-boronic acids and its derivatives in antimicrobial field.

2. RESULTS

2.1. Antimicrobial susceptibility

The determined MIC value of FBA against *P. aeruginosa* ATCC 27853 was 16 µg/mL. Thus the ½ MIC value (8 µg/mL) was used for metabolomics and proteomics studies.

2.2. Proteomics analysis

Digested tryptic peptides were analyzed in LC-qTOF-MS (Agilent 6530) system and raw data were performed by using Maxquant. 713 common proteins were identified between control and FBA treated groups. Multi scatter plot analysis was used to observe reproducibility of proteomics analysis of control and FBA treated groups. Pearson correlation coefficients were bigger than 0.929 for both groups. Principal component analysis (PCA) was used to show effect of FBA on overall proteome and reproducibility of biological replicates in both groups (Figure 1). In present work, student's t-test were applied and it was found that 132 proteins were differentially expressed ($p<0.05$).

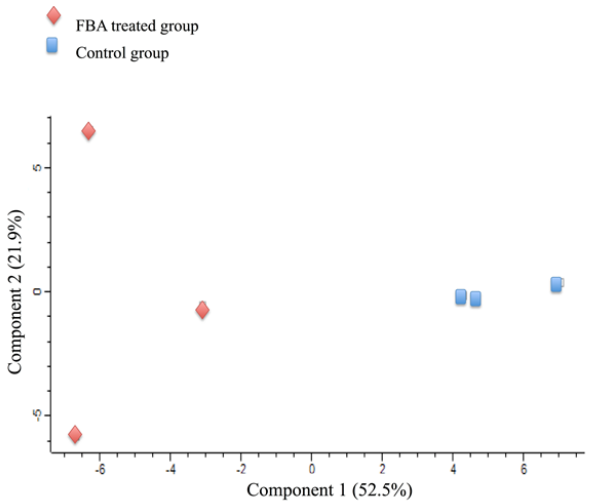

Figure 1. PCA score plot of proteome of control and FBA treated groups.

Differentially expressed proteins were classified by their functionallity using gene ontology approach to understand systematic effect of FBA on cell biology. Firstly, significantly altered proteins were classified according to their location in cells. Results showed that 64% of the proteins locates in cellular part (cytoplasmic part), 24% of the proteins are membrane proteins, 8% of them locates into protein containing complex and 4% of the proteins cullestered in organelles (Figure 2A).

In addition, we classified proteins according to their molecular functions (Figure 2B). We observed that the half of the differentially expressed proteins have catalytic activity (50%). These proteins are important for biochemical reactions in cells. Binding proteins (23.7%) bind to nucleic acid, RNA or organic compounds. Transporter activity proteins (19.7%) are very important in bacterial metabolism. Especially transporter proteins are very important in drug resistance mechanism.

We also classified functionally for biological processes in which proteins are involved (Figure 2C). Results showed that FBA is very effective on metabolic process (60%). Localization (12.3%) and cellular process (9.2%) are other FBA induced systems.

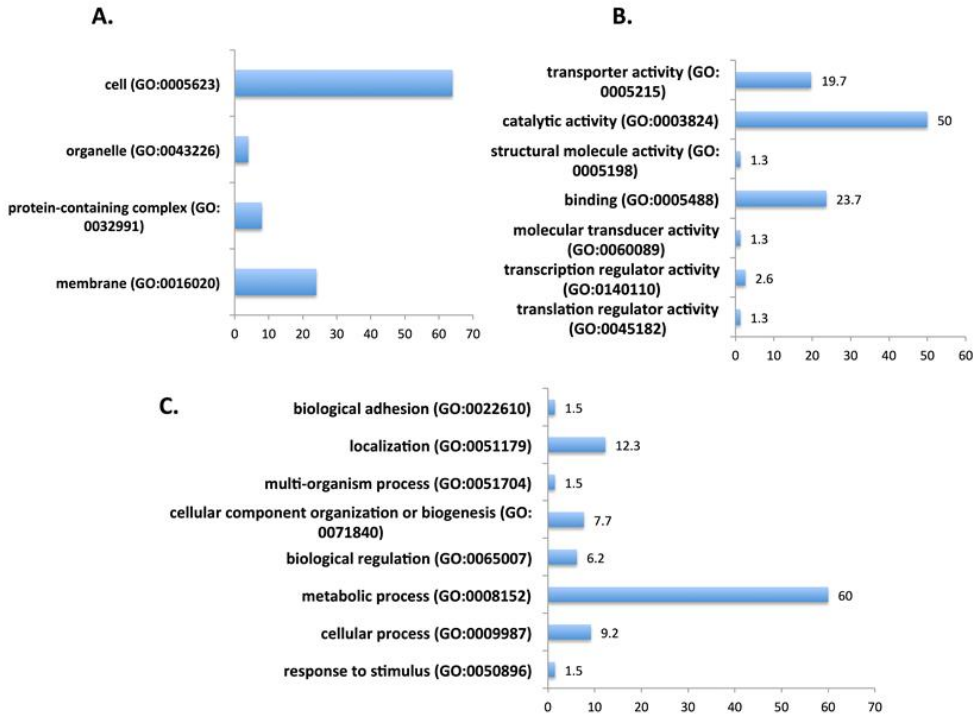

Figure 2. Functional classification of altered proteins between control and FBA treated groups.

2.3. GC/MS based metabolomics analysis

To further investigate the effects of FBA over *P. aeruginosa* at the metabolite level, cell metabolites were extracted and GC–MS was performed to acquire the metabolite profile of *P. aeruginosa* under FBA stress. In PCA analysis was applied to observe effect of FBA on global metabolome structure of *P. aeruginosa* as well as proteomics analysis (Figure 3).

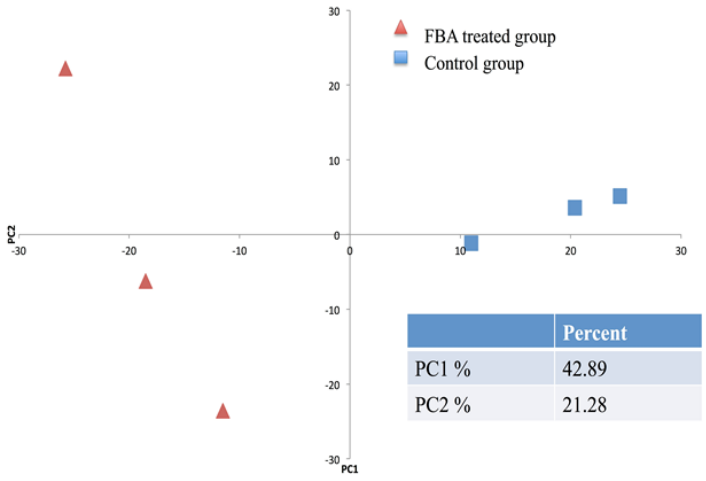

Figure 3. PCA scopre plot of metabolome of control and FBA treated groups.

In identification process, 71 metabolites were identified. Two sample t-test was carried out to determine differentially expressed metabolites. Altered metabolites were listed in Table 1 with fold change.

Table 1. Differentially altered metabolites between control (c) and and FBA treated groups (t).

Metabolites	Fold change (c/t)
5-Methylcytosine	4.74
Cysteamine	1.56
Heptadecanoic acid	6.50
Maltotriose	0.68
Spermidine	3.48
Allantoin	0.07
Xanthosine	7.56
Maltotriose	0.12
Tryptamine	7.13
5-Methylcytosine	4.74
L-Isoleucine	10.24
Myo-Inositol	0.13
Spermine	8.64
1-Aminocyclopropane-1-carboxylic acid	5.82
Lactitol	7.23
Phosphoethanolamine	6.14
Stearic acid	2.75
N-Acetyl-DL-serine	4.35
Erythronic acid lactone	5.32

3. DISCUSSION

3.1. Proteomics analysis

In present work, we used LC-MS based proteomics approach to observe differentiation of proteome structure of Pseudomonas under FBA stress. In proteomics analysis, reverse phase liquid chromatography has been used to separate peptides with gradient system which usually contains water and acetonitrile as mobile phase. Peptides separate according to their polarity in gradient elusion, in which acetonitrile phase increase by the time. In present work, we follow classic proteomics metodology and used reverse phase liquid chromatography to separate peptides.

Firstly, we evaluated our proteomics results in terms of reproducibility via multi scatter plot analysis. Results showed high reproducibility between biological replicates for control and FBA treated groups. Also we used PCA analysis to observe general effect of FBA over pseudomonas at protein level. PCA analysis showed that FBA is a very effective over *P. aeruginosa* and main parameter for differentiation of proteome structure in control and FBA treated groups. According to two sample t-test, it was found that expression levels of 132 proteins are altered under FBA stress.

Altered proteins were classified functionally to understand FBA effect on cell structure. Firstly, we evaluated proteins in terms of cellular component. As expected most of the proteins locate in cellular part. As well, various membrane proteins were altered by the effect of FBA.

In our results we observed that one protein, ATP-dependent DNA helicase (RecQ), was differentially expressed in nucleus. RecQ proteins are important factors for maintaining genomic stability, DNA replication, recombination and repair machinery [21, 22]. In our work, we observed that expression level of RecQ was decreased in FBA treated groups. This is one of the most critical points of this work. Decreasing expression level of RecQ means FBA effects DNA replication like quinolone antibiotics. In Table 2. change of expression level of RecQ was showed. Also we found that expression levels of some membrane proteins were altered. Porin D(oprD) is a membrane protein and down regulated in FBA treated groups. Studies showed that oprD is important regulator of outer membrane permeability. One of the main resistance

mechanism of *P. aeruginosa* to carbapenems is reduction of outer membrane permeability and decreased production of oprD which subsequently cause the decrease intake of the antibiotics [23, 24]. In our present study, we observed down regulation of oprD in FBA treated group, which indicates that it can be a possible resistance mechanism to FBA. Moreover, we found that another outer membrane protein, ompD, increased in FBA tretated group. OmpD is important regulator in efflux pump system and also very important for cell to cell communication, antibiotic susceptibility and promoting virulence [25, 26]. Increasing expression levels of these proteins shows developing resistance also confirmed the above-mentioned hyphothesis that *p. ruginosa* apparently developed resistance to FBA by reducing its intake to the cell.

Proteins were also evaluated according to their molecular functions (Figure 2B). The results showed that most of the altered proteins have catalytic function in biological reactions. Adenylate cyclase, cyaA, is very important enzyme and key regulator in various cellular processes. This protein involves in the synthesis of cyclic AMP which regulates many cellular processes and also resistance machinery against the antibiotics [27]. In present work, we found expression level of cyaA increases in the FBA treated groups. This could be another resistance mechanism against FBA.

Binding proteins are another group, which is induced by FBA. We found that FBA induced expression level of aruC, rpsI, mfd, pyrG and pchE. Among these proteins transcription-repair coupling protein (mfd)is very important for DNA repair system [28]. Our experiments showed that FBA decreased mfd level and induced DNA repair machinery (Table 2).

Another DNA binding protein, Exoenzyme S synthesis regulatory protein (ExsA), is transcriptional activator of the type III secretion system Type III secretion system is the major virulence determinant of *P. aeruginosa* strains which mainly involves in translocating bacterial toxins directly to eukaryotic host cells [29, 30]. We observed that expression level of ExsA increased in FBA treated groups. Thus, FBA stress conditions likely to cause an increased virulence in *P. aeruginosa*.

We also investigated altered proteins with biological processes. As expected most of the altered proteins involves in primary metabolic, biosynthetic, catabolic, nitrogen compound, organic substance metabolic processes. We also found FBA induces expression level of various proteins, which are involved in response to stimulus processes. We observed that FBA decreased priA (Figure 4C). PriA is multifunctional protein and very important for DNA replication, DNA repair and response to stress conditions [31]. Various studies showed that priA is an essential regulator for cell proliferation and important target for antimicrobial agents [32, 33]. This result is another evidence for effect of FBA on DNA replication and repair process.

Table 2. Expression level of antimicrobial targets, which induced by FBA.

Gene symbol	Protein name	Fold change (Log2)
RecQ	ATP-dependent DNA helicase RecQ	-5.57
mfd	Transcription-repair-coupling factor	-5.16
PriA	Primasome assembly protein PriA	-4.85

3.2. Metabolomics analysis

GC-MS based metabolomics approach has been used frequently in metabolomics analysis. GC-MS system is stable, reproducible and data process system is more reliable than LC-MS based metabolomics approaches [34].

In our work, we tried to understand effect of FBA on metabolome profile of *P. aeruginosa*. PCA showed that FBA effects metabome profile as well as proteome profile. We found differentially altered metabolites and investigated these metabolites individually**.**

In our work, we observed FBA decreased 5-Methylcytosine level was decreased by FBA treatment. This metabolite is involved in pyrimidine metabolism. Pyrimidines are essential building blocks of DNA nucleic acids and RNA. Pyrimidine metabolism is very important for DNA replication, cell cycle, proliferation and survival [35, 36].

In metabolomics analysis, results represented that FBA induces various metabolites in purine metabolism. Purines are essential strucutes for DNA replication, cell survival and growth as well as pyrimidines [37]. In proteomics analysis we found that FBA affects DNA replication and in metabolomics analysis showed similar result.

Fatty acid metabolism is essential processes for cell proliferation and survival [38]. In recent years, fatty acid metabolism emerged as a desirable target for antibiotic discovery. Inhibitors of microbial fatty acid

biosynthesis have been evaluated as potent antimicrobial agents. In our work, we found FBA affects fatty acid metabolism and decreased stearic and and heptadecanoic acid.

In present work, we found that FBA induces phosphoethanolamine, which is sphingolipid and involved in lipid metabolism. Sphingolipids are essential structure in cell membrane. In recent years, studies showed that sphingolipids play a role in the pathogenesis [39]. Our results showed that FBA affects lipid metabolism as well fatty acids.

We also observed that FBA decreased spermine and spermidine level in cells. These metabolites are involved in various cellular pathway like glutation, beta alanine pathways. In previous works, results showed that level of spermine and other polyamines are very important for cell. They are also involved in microbial pathogenesis, biofilm formation, toxin activity, and antibiotic resistance [40]. Spermine and spermidine, which effects outer membrane permeability and drug uptake, mediate antibiotic resistance. In our work, FBA inhibits synthesis of spermine and spermidine and effects outer membrane based resistance mechanism.

4. CONCLUSION

In the present work, for the first time antimicrobial effect of FBA over *P. aeruginosa* were investigated via proteomics and metabolomics approaches. We observed that FBA affects DNA replication and causes DNA damage at protein and metabolite level like mechanism of quinolone antibiotics. Also we found that FBA induces fatty acid metabolism. In addition, we observed resistance mechanism against FBA via membrane and efflux proteins.

5. MATERIALS AND METHODS

5.1. Antimicrobial activity

P. aeruginosa ATCC 27853 was cultured on Tryptic Soy Agar. In order to confirm the minimum inhibitory concentration (MIC) FBA against *P. aeruginosa*, standard broth microdilution test was performed according to the method reported by Clinical Laboratory Standards Institute (CLSI) [41]. Piperacillin/tazobactam was used as reference compound for broth microdilution. For proteomics experiments, *P. aeruginos*a ATCC 27853 was cultured on Mueller Hinton Broth (MHB) and incubated under 37^{o}C until the log phase achieved. The bacterial suspension was prepared in 50 mL flasks with MHB containing the 1/2 MIC value of FBA (8 µg/mL) in order to obtain an initial concentration of 5x10^{5} cfu/mL bacteria. As control experiments the same concentration of bacterial culture was also prepared without adding FBA. Flasks were incubated at 37°C for 20 h. Experiments were performed in triplicate. After the incubation period, the control and treated bacterial cultures were normalized according to OD600 value [42]. Normalized samples were then centrifuged and washed with Phosphate Buffered Saline (PBS). The pellet was resuspended in PBS and the bacterial cells were disrupted using a lysis buffer containing 500 µg/mL lysozyme**.**

5.2. Proteomics analysis

Total protein amount of lysis extracts of treated and untreated groups were determined by using Bio-Rad DC assay. Chloroform (Sigma)/methanol (Sigma)/water co-solvent system was used for protein extraction. Firstly, 400 µL methanol was added to 100 µL cell lysates and then 100 µL chloroform was added and finally 300 µL water added to mixtures. Proteins were separated as a pellet between organic and aqueous phases. Upper liquid phase (aqueous) was removed and then 400 µL methanol was added. Proteins were accumulated at the bottom of tube by centrifugated 15000 rpm for 5 min. Supernatant was removed and proteins pellet was dried at room temperature. Extracted proteins were suspended in 100 mM amonnium bicarbonate (Sigma) solution, which contained 20% methanol (v/v). Proteins were incubated with 200 mM dithiothreitol (DTT) (Sigma) at 56^{o}C for 20 min. After reduction, proteins were incubated with 100 mM iodoacetamide (sigma) at room temperature. Proteins were digested by Trypsin (1:100 (w/w) at 37^{o}C for 16 hours incubation. The tryptic peptides were dissolved in acetonitrile that contained 0.1 % formic acid**.**

We used optimized proteomics methods in our previous work [43]. The peptides were analyzed using LC-qTOF-MSTOF (Agilent 6530) for analysis. They were separated in C18 column (Zorbax C18 column 150 x 2.1 mm, 1.8 µm) at 55^{o}C. Mobile phase contained 0.1% formic acid in water (A) and 0.1% formic acid in

acetonitrile (B) and flow rate was adjusted 200 µL/min. Peptides were eluted with a gradient of 3%-50% mobile phase B over 130 minutes followed by 50%-90% mobile phase B for10 minutes. They were ionized in the positive mode of ESI source. The capillary voltage was adjusted at 4000 V with a drying temperature of 350 °C. Auto MS-MS data of peptides were recorded between 300 and 1400 m/z.

The most intense six ions were selected for MS/MS analysis. Ion charge states were +2, +3 and +4. The fragmentation energy was adjusted to 45 V. 20 µg protein was loaded to LC-MS system for each analysis. Three technical replicates were analyzed for treated and untreated groups.

Recorded MS/MS data was processed with Maxquant (Maxplank instutitute). *P. aeruginosa* database was downladed from UniprotKB in fasta format. Peptide and proteins were identified by matching of recorded MS-MS with in silico MS-MS. In matching process, 20 ppm mass tolerance was used for first and main search. Carboamidomethylation on cysteine, oxidation on methionine and acetylation on -N units of proteins were chosen as fixed and variable modifications. Two missed cleavages were allowed. In identification process false discovery rate (FDR) value was selected as 0.01 for reliable identification.

Label free quantification algorithm in Maxquant (MLFQ) was used for semi-quantification between FBA treated and untreated groups.

For statistical data analysis, the R-based Perseus program was used. Reproducibility of technical replicates in treated and untreated groups were investigated by multi scatter plot analysis. Principal component analysis showed overall effect of FBA on to find significantly expressed proteins, two-sample t test was performed with number of randomizations to 250, and FDR to 1% to find differentially expressed proteins. Differentially expressed proteins were classified functional in Panther gene list platform.

5.3. Metabolomics analysis

GC–MS based metabolomic analysis was performed as previously described [44,45]. 850 µL methanol:water mixture (9:1 v/v) was added to 150 µL cell lysate. Metabolites were separated from proteins by centrifugation at 15000 rpm for 5 min. The whole supernant were evaporated under vacuum centrifuge at 4°C. For derivatization, the dried samples were methoxylated using 20 µL of methoxyamine hydrochloride (20 mg/mL) in pyridine for 90 min at 30°C and derivatized using 80 µL of N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) and 1% trimethylchlorosilane (TMCS) for 30 min at 37°C. The derivatized samples were transferred into silanized vials for the analysis and 2 µL was injected Oven temperature was fixed at 70 °C for one minute, then increasing up to 325°C by 10 °C/min rate and held for 10 minutes at 325°C. Total analysis time was 37.5 minutes. Data acquisition was performed in full scan mode with mass range between 50-650 m/z.

The MS-DIAL software was used for peak exaction, peak alignment, deconvolution analysis, peak identification, quantification and normalization. In MS-DIAL settings, mass range was between 0-1000 Da. Minimum peak height for peak detection was 1000 amplitude. Retention time tolerance was 0.5 min. Identification score cut off was 70%. For metabolite identification we used GC/MS all public Kovats retention index database (15302 records) (http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/). For statistical analysis Excel macro program, which was developed by Riken center for sustainable resource science(http://www.prime.psc.riken.jp/Metabolomics_Software/StatisticalAnalysisOnMicrosoftExcel/index.html). Two sample t-test was used differentially expressed metabolites and pricipal also principal component analysis was used to determine effect of FBA on global metabolome structure of pseudomonas.

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REFERENCES

[1] Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Microbiol Spectr. 2016; 4(2): 201-208. [CrossRef]

[2] Hassan KS, Al-Riyami D. Infective Endocarditis of the Aortic Valve caused by Pseudomonas aeruginosa and Treated Medically in a Patient on Haemodialysis. Sultan Qaboos Univ Med J. 2012; 12(1): 120-123. [CrossRef]

[3] Lu CW, Hao JL, Liu XF, Liang LL, Zhou DD. Pseudomonas aeruginosa endophthalmitis caused by accidental iatrogenic ocular injury with a hypodermic needle. J Int Med Res. 2017; 45(2): 882-885. [CrossRef]

[4] Lister, PD, Wolter, DJ, Hanson ND. Antibacterial-Resistant Pseudomonas aeruginosa: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin Microbiol Rev. 2009; 22(4): 582-610. [CrossRef]

[5] Gasser G, Ott I, Metzler-Nolte N. Organometallic anticancer compounds. J Med Chem. 2011; 54(1): 3-25. [CrossRef]

[6] Forti KM, Bernard F, Santiago-Collazo G, Garcia W, Vera JL, Meléndez E. Para-Substituted Functionalised Ferrocene Esters with Novel Antibacterial Properties. J Clin Diagn Res. 2018; 12(2): 1-4. [CrossRef]

[7] Albada B, Metzler-Nolte N. Highly Potent Antibacterial Organometallic Peptide Conjugates. Acc Chem Res. 2017; 50(10): 2510-2518. [CrossRef]

[8] Patra M, Gasser G, Metzler-Nolte N. Small organometallic compounds as antibacterial agents. Dalton Trans. 2012; 41(21): 6350-6358. [CrossRef]

[9] Janka V, Žatko D, Ladislav V, Pál P, Janka P, Gabriela M. Some ferrocenyl chalcones as useful candidates for cancer treatment. In Vitro Cell Dev Biol Anim. 2015; 51(9): 964-974. [CrossRef]

[10] Wu C, Ye H, Bai W, Li Q, Guo D, Lv G. New potential anticancer agent of carborane derivatives: selective cellular interaction and activity of ferrocene-substituted dithio-o-carborane conjugates. Bioconjug Chem. 2011; 22(1): 16-25. [CrossRef]

[11] Ferle-Vidović A, Poljak-Blazi M, Rapić V, Skare D. Ferrocenes (F168, F169) and fero-sorbitol-citrate (FSC): potential anticancer drugs. Cancer Biother Radiopharm. 2000; 15(6): 617-624. [CrossRef]

[12] López-Cabrera Y, Castillo-García EL, Altamirano-Espino JA, Pérez-Capistran T, Farfán-García ED, Trujillo-Ferrara JG. Profile of three boron-containing compounds on the body weight, metabolism and inflammatory markers of diabetic rats. J Trace Elem Med Biol. 2018; 50: 424-429. [CrossRef]

[13] Ryu JH, Lee GJ, Shih YV, Kim TI, Varghese S. Phenylboronic acid-polymers for biomedical applications. Curr Med Chem. 2018; 37(26): 6797-6816. [CrossRef]

[14] Wang W, Liao L, Zhang X, Lei F, Zhang Y, Liu G. An Intelligent Nanoscale Insulin Delivery System. Molecules. 2018; 23(11); 2933-2945. [CrossRef]

[15] Windsor IW, Palte MJ, Lukesh JC, Gold B, Forest KT, Raines RT. Sub-picomolar Inhibition of HIV-1 Protease with a Boronic Acid. J Am Chem Soc. 2018; 140(43): 14015-14018. [CrossRef]

[16] Zhou J, Stapleton P, Haider S, Healy J. Boronic acid inhibitors of the class A β-lactamase KPC-2. Bioorg Med Chem. 2018; 26(11): 2921-2927. [CrossRef]

[17] Kiener, PA, Waley SG. Reversible Inhibitors of Penicillinases. Biochemical Journal. 1978; 169(1): 197-204. [CrossRef]

[18] Beesley T, Gascoyne N, Knotthunziker V, Petursson S, Waley SG, Jaurin B, Grundstrom T. The Inhibition of Class-C Beta-Lactamases by Boronic Acids. Biochemical Journal. 1983; 209(1): 229-233. [CrossRef]

[19] Patel TS, Pogue JM, Mills JP, Kaye KS. Meropenem-vaborbactam: a new weapon in the war against infections due to resistant Gram-negative bacteria. Future Microbiol. 2018; 13(1): 971-983. [CrossRef]

[20] Wu G, Cheon E. Meropenem-vaborbactam for the treatment of complicated urinary tract infections including acute pyelonephritis. Expert Opin Pharmacother. 2018; 19(13): 1495-1502. [CrossRef]

[21] Hishida T, Han YW, Shibata T, Kubota Y, Ishino Y, Iwasaki H, et al. Role of the Escherichia coli RecQ DNA helicase in SOS signaling and genome stabilization at stalled replication forks. Genes Dev. 2004; 18(15): 1886-1897. [CrossRef]

[22] Shereda RD, Bernstein DA, Keck JL. A central role for SSB in Escherichia coli RecQ DNA helicase function. J Biol Chem. 2007; 282(26): 19247-19258. [CrossRef]

[23] Farra A, Islam S, Strålfors A, Sörberg M, Wretlind B. Role of outer membrane protein OprD and penicillin-binding proteins in resistance of Pseudomonas aeruginosa to imipenem and meropenem. Int J Antimicrob Agents. 2008; 31(5): 427-433. [CrossRef]

[24] Fusté E, López-Jiménez L, Segura C, Gainza E, Vinuesa T, Viñas M. Carbapenem-resistance mechanisms of multidrug-resistant Pseudomonas aeruginosa. J Med Microbiol. 2013; 62(9): 1317-1325. [CrossRef]

[25] Santiviago CA, Fuentes JA, Bueno SM, Trombert AN, Hildago AA, Socias LT. The Salmonella enterica sv. Typhimurium smvA, yddG and ompD (porin) genes are required for the efficient efflux of methyl viologen. Mol Microbiol. 2002; 46(3): 687-698. [CrossRef]

[26] Pilonieta MC, Erickson KD, Ernst RK, Detweiler CS. A protein important for antimicrobial peptide resistance, YdeI/OmdA, is in the periplasm and interacts with OmpD/NmpC. J Bacteriol. 2009; 191(23): 7243-7252. [CrossRef]

[27] Donovan GT, Norton JP, Bower JM, Mulvey MA. Adenylate cyclase and the cyclic AMP receptor protein modulate stress resistance and virulence capacity of uropathogenic Escherichia coli. Infect Immun. 2013; 81(1): 249-258. [CrossRef]

[28] Roberts J, Park JS. Mfd, the bacterial transcription repair coupling factor: translocation, repair and termination. Curr Opin Microbiol. 2004; 7(2): 120-125. [CrossRef]

[29] Marsden AE, King JM, Spies MA, Kim OK, Yahr TL. Inhibition of Pseudomonas aeruginosa ExsA DNA-Binding Activity by N-Hydroxybenzimidazoles. Antimicrob Agents Chemother. 2016; 60(2): 766-776. [CrossRef]

[30] Lakkis C, Fleiszig SM. Resistance of Pseudomonas aeruginosa isolates to hydrogel contact lens disinfection correlates with cytotoxic activity. J Clin Microbiol. 2001; 39(4): 1477-1486. [CrossRef]

[31] Windgassen TA, Wessel SR, Bhattacharyya B, Keck JL. Mechanisms of bacterial DNA replication restart. Nucleic Acids Res. 2018; 46(2): 504-519. [CrossRef]

[32] Van Eijk E, Wittekoek B, Kuijper EJ, Smits WK. DNA replication proteins as potential targets for antimicrobials in drug-resistant bacterial pathogens. J Antimicrob Chemother. 2017; 72(5): 1275-1284. [CrossRef]

[33] Huang YH, Huang CC, Chen CC, Yang KJ, Huang CY. Inhibition of Staphylococcus aureus PriA Helicase by Flavonol Kaempferol. Protein J. 2015; 34(3): 169-172. [CrossRef]

[34] Ozkan E, Nemutlu E, Beksac MS, Kir S. GC-MS analysis of seven metabolites for the screening of pregnant women with Down Syndrome fetuses. J Pharmaceut Biomed. 2020; 188: 113427. [CrossRef]

[35] Moffatt BA, Ashihara H. Purine and pyrimidine nucleotide synthesis and metabolism. Arabidopsis Book. 2002: 1(18); 1-20. [CrossRef]

[36] Samant S, Lee H, Ghassemi M, Chen J, Cook JL, Mankin AS. Nucleotide biosynthesis is critical for growth of bacteria in human blood. PLoS Pathog. 2008; 4(2): 1-10. [CrossRef]

[37] Quéméneur L, Gerland LM, Flacher M, Ffrench M, Revillard JP, Genestier L. Differential control of cell cycle, proliferation, and survival of primary T lymphocytes by purine and pyrimidine nucleotides. J Immunol. 2003; 170(10): 4986-4995. [CrossRef]

[38] Yao J, Rock CO. Bacterial fatty acid metabolism in modern antibiotic discovery. Biochim Biophys Acta Mol Cell Biol Lipids. 2017; 1862(11): 1300-1309. [CrossRef]

[39] Heung LJ, Luberto C, Del Poeta M. Role of sphingolipids in microbial pathogenesis. Infect Immun. 2006; 74(1): 28-39. [CrossRef]

[40] Johnson L, Mulcahy H, Kanevets U, Shi Y, Lewenza S. Surface-localized spermidine protects the Pseudomonas aeruginosa outer membrane from antibiotic treatment and oxidative stress. J Bacteriol. 2012; 194(4): 813-826. [CrossRef]

[41] CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard -Tenth Edition.CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.

[42] Corte L, Tiecco M, Roscini L, De Vincenzi S, Colabella C, Germani R. FTIR Metabolomic Fingerprint Reveals Different Modes of Action Exerted by Structural Variants of N-Alkyltropinium Bromide Surfactants on Escherichia coli and Listeria innocua Cells. Plos One. 2015; 10(1): 1-10. [CrossRef]

[43] Kocak E, Altinoz S. Evaluation of regular UPLC/MS system for experimental and clinical proteomics. J Res Pharm. 2020; 24(4): 582-592. [CrossRef]

[44] Eylem C C, Yilmaz M, Derkus B, Nemutlu E, Camci C, Yilmaz B, Turkoglu M A, Aytac B, Ozyurt N, Emregul, E Untargeted multi-omic analysis of colorectal cancer-specific exosomes reveals joint pathways of colorectal cancer in both clinical samples and cell culture. Cancer Letters. 2020; 469: 186-194. [CrossRef]

[45] Kocak E, Ozkul C. Metabolic response of Escherichia coli to subinhibitory concentration of ofloxacin. J Res Pharm. 2020; 24(4): 593-601. [CrossRef]