Chiang Mai J. Sci. 2023; 50(2): 1-19
https://doi.org/10.12982/CMJS.2023.014
Journal homepage : http://epg.science.cmu.ac.th/ejournal/

Research Article

Transcriptome Study of Cold Plasma Treated *Pseudomonas aeruginosa*

Kok Jun Liew[†][a], Xinhua Zhang[†,*][b,c], Xiaohong Cai [c], Dongdong Ren [d], Wei Liu [c], Zhidong Chang [c] and Chun Shiong Chong*[a]

[a] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia
[b] School of Photoelectric Engineering, Changzhou Institute of Technology, Changzhou Jiangsu, China
[c] Suzhou Amazing Grace Medical Equipment Co., Ltd, Suzhou, Jiangsu, China
[d] ENT Institute and Department of Otorhinolaryngology, Eye & ENT Hospital, Fudan University, Shanghai, China

[†]These authors have contributed this work equally as first authors.
*Author for correspondence; e-mail: zhangxh@czu.cn (X.Z.); cschong@utm.my (C.S.C.)

Received: 02 November 2022
Revised: 07 February 2023
Accepted: 09 February 2023

Abstract

Cold plasma is a disinfection technique widely used in food, agricultural, and medical industries. This work used cold plasma to sterilize *Pseudomonas aeruginosa* and cell survivability was determined. RNA sequencing was used to determine the bacterial responses at 1 minute (T1), 3 minutes (T3), and 5 minutes (T5) of cold plasma treatments. The results show that longer treatment leads to lower cell survivability. Cold plasma induced rapid cell responses in *P. aeruginosa*. Gene Ontology enrichment analysis showed that T5 had the most enriched terms compared to T1 and T3. The most affected genes were those involved in antioxidant production, transcriptional regulators, ribosome formation, transporters, chemotaxis, and cell motility. *P. aeruginosa*'s initial response (T1) to cold plasma involved the upregulation of antioxidant genes, followed by the downregulation of transcriptional regulators, transporters, chemotaxis, and cell motility as the intermediate response (T3), and the final response (T5) included heavy downregulation in ribosome formation. Previous transcriptome studies of cold plasma focused mainly on prokaryotic cells such as *E. coli* and *B. subtilis*, while studies on *P. aeruginosa* are limited. This study demonstrated the sequential response of *P. aeruginosa* against cold plasma via transcriptome analysis.

Keywords: low-temperature plasma, plasma jet, microbial disinfection, bacterial inactivation, sterilization, decontamination

1. Introduction

Plasma is the "fourth state of matter", along with solid, liquid, and gas. It is an ionized superheated gas with an equal number of positively and negatively charged particles [1]. Plasma can be classified as "hot" or "cold", depending on its characteristics. Cold plasma has low temperatures (300-600K) and has barely ionized gas molecules (e.g., 0.1–1%) [1]. It is frequently formed at atmospheric pressure or lower, resulting in a low-density environment with lower collision rates between electrons and gas molecules [1]. Cold plasma has many synonyms in the scientific community, such as "low-temperature plasma", "non-thermal plasma", "non-equilibrium plasma",

"atmospheric-pressure plasma", or combination names such as "atmospheric pressure non-equilibrium plasma" and "cold atmospheric-pressure plasma".

Cold plasma has been used in altering the surface properties of different materials for a wide range of purposes [2], preparation, modification, and regeneration of the catalytic materials [3], remediation and removal of pollutants [4], treating wastewater and sewage sludge [5], lignocellulosic biomass pretreatment [6], and promoting the formation of biofilm on plasma-treated surfaces [7]. Furthermore, cold plasma is an effective decontamination technology widely used in agricultural, food, and medical industries [8–10]. Cold plasma can eradicate microorganisms in foods, vegetables, and drugs while retaining their natural flavours, structures, and nutrients [11]. Cold plasma has been proven effective in inhibiting the growth of microorganisms on the infected skin without damaging the patient's skin while promoting the wound healing efficiency [12].

Most cold plasma-related studies focused on eliminating microorganisms in foods, dairy products, fruits, and vegetables [13,14]. The targeted microorganisms for the inactivation tests included pathogens like *Bacillus* spp., *Clostridium* spp., *Enterococcus faecalis*, *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., and *Listeria monocytogenes* [15–17]. Antimicrobial tests were conducted to assess the effects of the cold plasma against specific microorganisms by using the plate colony-counting method, assessment of cell surface damages via scanning (SEM) or transmission electron microscopy (TEM), determination of the release of DNA and protein from the cell after treatment, etc. [16]. Although some researchers had also used the transcriptome, RNA-sequencing, and DNA microarray to study the cell responses against the cold plasma, they were focused on the eukaryotic cell lines such as human, cancer, and plant but not on the prokaryotic cells. A few bacteria were studied in terms of transcriptomic responses to cold plasma treatment, including *B. cereus*, *B. subtilis,* *E. coli* and *S. aureus* [18–20].

This study used *Pseudomonas aeruginosa* for cold plasma treatment, an opportunistic pathogen commonly found on human skin. This bacterium can cause several diseases such as chronic obstructive pulmonary disease, pneumonia, sinusitis, and skin infection [21,22]. *P. aeruginosa* also causes otitis media in patients, known as middle ear infection [23]. According to research, *P. aeruginosa* is difficult to eradicate due to its wide range of antimicrobial resistance and preference in forming biofilm that can withstand higher concentration of antibiotics [21–23]. The transcriptomic responses of *P. aeruginosa* toward direct cold plasma treatment have not yet been investigated. In this study, the inactivation effects of cold plasma generated from a self-developed low-temperature air plasma jet on *P. aeruginosa* ATCC 9027 via transcriptome analyses were conducted. The transcriptional responses of *P. aeruginosa* when treated with cold plasma were investigated.

2. MATERIALS AND METHODS

2.1 Prototype Design of Low-Temperature Air Plasma Jet (LTAPJ) and Parameters used for Cold Plasma Generation

In this experiment, a self-developed device called the "Low-Temperature Air Plasma Jet (LTAPJ)" was used. This device generates plasma via atmospheric pressure plasma jet method and atmospheric air as the gas input. The major components in the device are control circuit, pulse generator circuit, high voltage converter, cathode, anode, air pump, air tubing, and nozzle. Other parameters used in this LTAPJ are: 5L/min of air flow rate, 5kV of discharge voltage, and 90–150kHz of the plasma generating frequency.

2.2 Experiment Design of *Pseudomonas aeruginosa* ATCC 9027 Treated with Cold Plasma

Pseudomonas aeruginosa ATCC 9027 was purchased from Beijing Zhongkezhijian Biotechnology Co., Ltd. The bacterium was grown on a Luria-Bertani

medium. A 16-hour grown bacterial liquid culture in Luria-Bertani medium was prepared, centrifuged, washed, and resuspended in 0.5% (*w*/*v*) saline water prior to the cold plasma's microbial inactivation test. The bacterial suspension was adjusted to OD_{600nm} of 1.0, which is approximately 10^8 cell/mL as determined by using a hemocytometer. Two mL bacterial suspension was transferred into a 5 mL Bijou bottle. Afterwards, the Bijou bottle was put underneath the device for treatment with the nozzle aimed directly at the opening. Treatment was examined at durations of 1, 3, 5, 10, 15, 20, 25, and 30 minutes while maintaining the distance between the nozzle and liquid surface at 3 cm. After treatment, the treated liquid culture was serially diluted (dilution factors of $10^0 - 10^9$) and spread onto Luria-Bertani agar. Agar plates were incubated for 24 hours at 37°C for bacterial growth and the colony-forming unit (CFU) was calculated. Liquid culture without cold plasma treatment (0 minute) was used as a reference control. Each treatment was conducted in three replicates.

2.3 Total RNA Extraction from Cold Plasma Treated *P. aeruginosa*

The treatment setup for the transcriptome study was similar to that described in Section 2.2. After cold plasma treatment, the 2 mL culture was centrifuged at 4°C, 10,000 rpm for 1 minute. The supernatant was discarded, and the cell pellet was quick-freezing in liquid nitrogen to preserve the RNA before extraction. Every treatment (0, 1, 3, and 5 minutes) was replicated at least five times. A TRIzol® reagent (Invitrogen, Waltham, MA, USA) was used for RNA extraction based on the manufacturer's protocol. The genomic DNA was removed from the extracted RNA using DNase I (Takara Bio, Kusatsu, Shiga, Japan). The quality and quantity of the extracted RNA were examined by using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and ND-2000 (NanoDrop Technologies, Wilmington, DE, USA).

2.4 Library Preparation and RNA Sequencing

The extracted RNA samples were sent to Biozeron Biotechnology Co., Ltd (Jiading, Shanghai, China) for library preparation and sequencing. A TruSeq RNA sample preparation kit (Illumina, San Diego, CA, USA) and a RiboZero rRNA removal kit (Epicentre, Madison, WI, USA) were used for RNA-seq strand-specific library construction following the manufacturer's protocol. A Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) was used to check the quality of the prepared library. RNA sequencing was conducted at PE150 mode in a NovoSeq 6000 system (Illumina, San Diego, CA, USA).

2.5 Bioinformatics

FastQC v0.11.9 software was used to examine the quality of the raw paired-end reads generated from the sequencer. Adapter trimming and low-quality read filtering were conducted using Trimmomatic v0.36 with specific parameters (SLIDINGWINDOW:4:15 MINLEN:75) [24]. Afterwards, clean reads were mapped and aligned to the reference genome with orientation mode using Rockhopper v2.0.3 [25]. The expression level for each transcript was calculated using the fragments per kilobase of read per million mapped reads (FPKM) method to identify differentially expressed genes (DEGs) between two different samples. DEGs were further analyzed using EdgeR v3.6.3, in which the DEGs must fulfil the following criteria: (i) the logarithmic of fold change must be greater than 2 and (ii) the false discovery rate (FDR) should be less than 0.05 [26]. Gene Ontology (GO) functional enrichment analysis was carried out on the DEGs by using Goatools v0.9.9 [27].

3. Results and Discussion

3.1 Evaluation of LTAPJ Generated Cold Plasma's Microbial Inactivation Efficiency

Based on the result of CFU plate counting, the cold plasma generated from LTAPJ exhibits different efficacy in the microbial disinfection

experiment. At 1, 3, and 5 minutes of cold plasma treatment, *P. aeruginosa* can retain 100.00±7.53%, 91.11±9.85%, and 81.95±10.37% as shown in Figure 1. When the treatment exceeds 10 minutes, the cell survivability falls below 50% and achieves 100% death at 20 minutes onwards of cold plasma treatment. The effect of cold plasma is directly proportional to the treatment duration; the total number of alive bacteria decreased as the culture was exposed to the plasma for a longer duration. Similar observations concur in other studies [28–30]. For example, CFU was constantly decreased when *Psychrobacter glacincola*, *Brochothrix thermosphacta*, and *Pseudomonas fragi* cultures were treated with two different cold plasma devices for longer duration (0–11 minutes)[29]. After 0–6 minutes of cold plasma treatment, reduction of CFU was reported in 3 Gram-negative species (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), 2 Gram-positive species (*Bacillus subtilis* and *Staphylococcus aureus*), and yeast (*Saccharomyces cerevisiae*)[30].

3.2 General Statistics of the Transcriptome Data

The raw RNA sequencing data had been deposited in NCBI Sequence Read Archive (SRA) database with accession numbers SRR21111573–SRR21111576. Around 6.01 Gb from an average of 39.8 million raw reads were generated from each sample, as shown in Table 1. After adapter trimming and quality filtering using Trimmomatic v0.36 [24], each sample had 36.1–40.6 million clean reads. Approximately 43.99–85.80% of the total clean reads can be aligned to *P. aeruginosa* NCTC10332 complete genome sequence (Genbank accession number: LJ831024.1) using Rockhopper v2.0.3 [25]. DEGs analysis was conducted using edgeR package [26] for the three treatments (1, 3, and 5 minutes) compared to the untreated control experiment. Herein, treatment settings for 1 minute, 3 minutes and 5 minutes were referred to as T1, T3, and T5, respectively. The up- and downregulated genes in the T1 experiment were 62 and 22, respectively. T3 had 13 up- and 339 downregulated genes; for T5, it had 11 and 694, respectively. Collectively, the bacterial cells of *P. aeruginosa* in T1 exhibited more upregulated genes, while T3 and T5 had more downregulated genes. Overall, the identified DEGs from each treatment accounted for approximately 1.44%-12.05% of the total genes (encoded gene numbers: 5851) in the *P. aeruginosa* NCTC10332 genome.

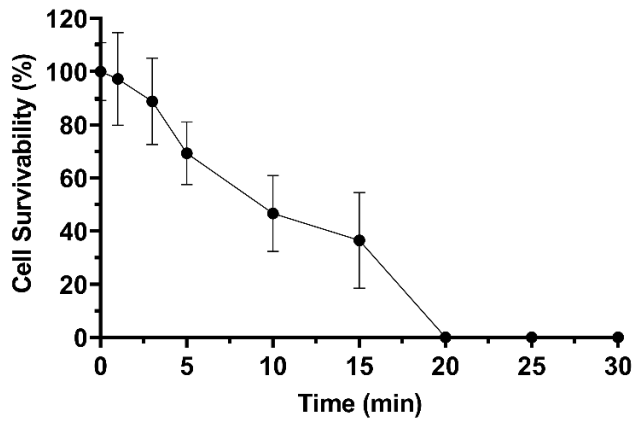

Figure 1. Cell survivability (%) of *Pseudomonas aeruginosa* after cold plasma treatment at different duration.

Table 1. Summary of RNA sequencing data.

Condition	No. of bases (Gb)	No. of raw reads	No. of clean reads	Mapped reads (%)	No. of upregulated DEGs	No. of downregulated DEGs
Control (untreated)	5.95	39,687,320	36,940,284	44.70	N/A	N/A
T1 (1 min treatment)	6.48	43,213,444	40,577,398	43.99	62	22
T3 (3 mins treatment)	5.95	38,633,458	37,726,594	72.88	13	339
T5(5 mins treatment)	5.67	37,804,842	36,112,092	85.80	11	694

3.3 Function Enrichments of the DEGs

Figure 2 shows the functional enrichment analysis of the DEGs found in *P. aeruginosa* plasma treatments following Gene Ontology. According to the findings, *P. aeruginosa* in T1 showed DEGs in GO categories of "response to stress", "ion transport", and "cellular response to stimulus", all of which classified under Biological Process (BP). Whereas T3 exhibits DEGs in Cellular Component (CC) and Molecular Function (MF). In contrast, the T5 treatment had many DEGs found in the three main GO categories (BP, CC and MF). For instance, the DEGs classified under the BP for T5 included "response to oxidative stress", "ion transport", and "gene expression". For CC, "ribosomal subunit", "plasma membrane", "organelle", etc. were mainly affected. The DEGs in the T5 setting for MF were mainly found in "transporter activity", "structural constituent of ribosome", "RNA binding", "antioxidant activity", etc.

3.4 DEGs Comparison in Depth Among T1, T3, and T5 Settings

DEGs were manually analyzed by categorizing the selected genes into five groups: "antioxidant production", "transcriptional regulator", "ribosome formation", "transporters", and "chemotaxis and cell motility". Figure 3 shows each group's total number of up- and downregulated genes in each setting (T1, T3, and T5). The T1 showed primarily upregulated genes, particularly in the antioxidant production, including 10 genes with average Log_2FC greater than 3.7. In T3, the number of upregulated genes involved in antioxidant production decreased to 7 ($Log_2FC \approx 3.0$), while there were more downregulated genes, including 37 transcriptional regulators ($Log_2FC \approx -3.2$), 24 transporters ($Log_2FC \approx -3.6$), and 16 genes of chemotaxis and cell motility ($Log_2FC \approx -3.4$). For T5, DEGs were mainly found in the downregulated portion, including 60 transporters ($Log_2FC \approx -5.5$), 56 transcriptional regulators ($Log_2FC \approx -4.3$), and 34 genes for ribosome formation ($Log_2FC \approx -4.4$). Each classified group was further explained below.

3.4.1 Antioxidant production

A total of 16 antioxidant-related DEGs were found in the transcriptome data of *P. aeruginosa* (Table 2). Ten of them are upregulated in T1 environments, including genes encoding two peroxiredoxin (Locus_Tag: AT700_ RS07510 and RS21100), two catalases (KatA and KatB; RS03530 and RS23970), a rubredoxin (RS27850), an alkyl hydroperoxide reductase contributed by subunit C and F (AhpC and AhpF; RS00710 and RS00715), and an organic hydroperoxide resistance protein (Ohr; RS10630) which regulated by a transcriptional regulator (OhrR; RS10635). These proteins play important role in regulating peroxide levels in the cells, defending against the reactive oxygen species (ROS), and protecting the cell from oxidative stress [31–33]. A gene encoded for thioredoxin-disulfide reductase (TrxB; RS21095) was upregulated. This enzyme is part

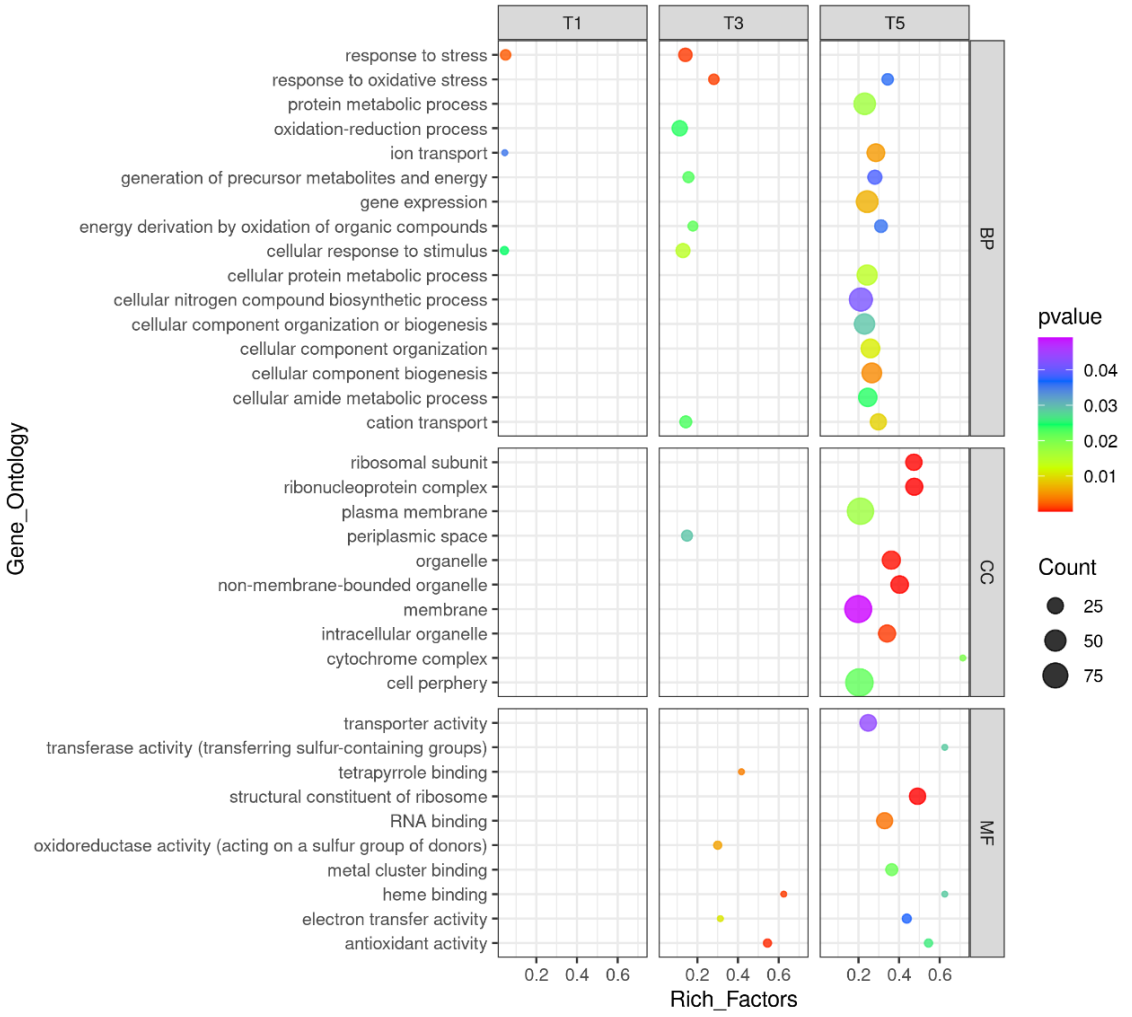

Figure 2. Gene Ontology (GO) functional enrichment analyses of cold plasma treatment of 1, 3, and 5 minutes, represent T1, T3, and T5, respectively. All the GO terms at the y-axis are classified according to the Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The rich factor at the x-axis is defined as the ratio of the DEGs number annotated in a specific GO term to all gene numbers annotated in that same GO term, the greater the rich factor, the greater the degree of enrichment. The bubble size indicates the number of DEGs, while the rainbow colour bar indicates the pvalue of the enrichment.

of the thioredoxin (Trx) system, a key antioxidant system that protects the cell from oxidative stress by regulating dithiol/disulfide balance through its disulfide reductase activity [34]. There were also antioxidant-related DEGs identified in T3 and T5. However, the total number of upregulated DEGs was lesser, and some were even downregulated (e.g. RS07130, RS10750, RS11900, RS12340, RS22690, and RS27285). The results indicated that *P. aeruginosa* produces antioxidants at the initial stage (T1). As the bacterial cell was exposed to longer cold plasma treatment, as in T3 and T5, the antioxidant

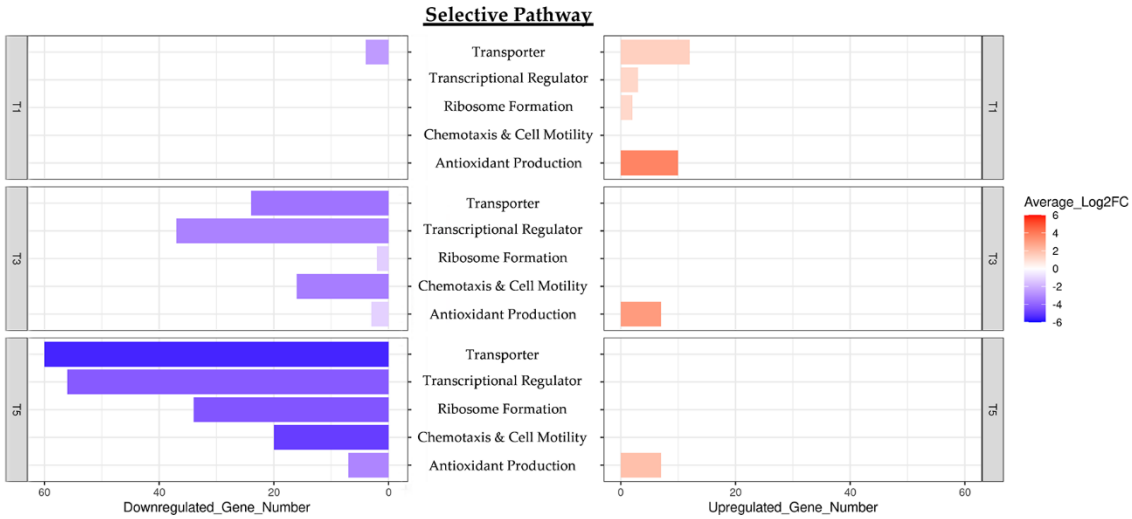

Figure 3. The gene regulation of the selected DEGs. The bar chart at the right shows the number of upregulated genes, while the left bar chart shows the number of downregulated genes. The gradient color of the bar chart indicates the average log_2FC of the selected genes in the particular functions.

Table 2. DEGs related to the antioxidant production of *P. aeruginosa* in T1, T3, and T5.

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
1.	AT700_RS00710	ahpC; peroxiredoxin	3.42	2.53	1.80
2.	AT700_RS00715	ahpF; alkyl hydroperoxide reductase subunit F	4.97	3.55	2.32
3.	AT700_RS03530	katA; catalase KatA	4.39	2.20	1.11
4.	AT700_RS07130	peroxiredoxin C	~	~	-2.83
5.	AT700_RS07510	peroxiredoxin	2.10	~	~
6.	AT700_RS10630	ohrR; organic hydroperoxide resistance protein OhrR	4.92	3.94	3.82
7.	AT700_RS10635	ohrR; hydroperoxide stress response transcriptional regulator OhrR	1.60	~	-1.54
8.	AT700_RS10750	glutathione peroxidase	~	~	-2.80
9	AT700_RS11900	trxB; thioredoxin-disulfide reductase	~	-1.37	-3.14
10.	AT700_RS12340	tpx; thiol peroxidase	~	~	-3.33
11.	AT700_RS21095	trxB; thioredoxin-disulfide reductase	4.47	2.99	1.50
12.	AT700_RS21100	peroxiredoxin	5.38	3.01	1.33
13.	AT700_RS22690	sodB; superoxide dismutase [Fe]	~	-1.11	-2.35
14.	AT700_RS23970	katB; catalase KatB	4.98	3.41	1.90
15.	AT700_RS27285	trxA; thioredoxin TrxA	~	-1.04	~
16.	AT700_RS27850	rubredoxin	1.15	~	-6.15

genes were downregulated as the bacterial cells were getting stressed, and some died. In another study, genes encoding superoxide dismutase (SodA), catalase (KatE), and alkyl hydroperoxide reductase (AhpC) in *S. aureus* were upregulated after exposure to cold plasma for 10 and 30 mins [19]. Another study on *E. coli* showed DEGs on catalase (KatG), alkyl hydroperoxide reductase (AhpC and AphF), superoxide dismutase (SodA), thioredoxin (TrxC), and glutaredoxin (GrxA) [20]. While in cold plasma-treated *B. cereus*, genes encoding for catalase and superoxide dismutase were not differentially expressed [18]. The genes regulation of antioxidants may or may not be affected by cold plasma, which could be due to different cell types, the strength of cold plasma, exposure duration, etc.

3.4.2 Transcriptional regulator

Table 3 shows the DEGs list of the transcriptional regulators (TRs) in the three treatment settings compared to the control. In T1, only 3 upregulated DEGs were identified, and no downregulation of TRs was identified. As the duration of the treatments increased to 3 and 5 minutes, the total number of DEGs increased. Where T3 consisted of 37 and T5 had 56 downregulated TRs. TRs act as activators or repressors in regulating the transcription processes of different pathways [35]. LysR family TRs, for example, regulate a wide range of genes involved in virulence, metabolism, quorum sensing, and motility [36]. Table 3 shows a total of 10 LysR TRs (RS00295, RS02380, RS02635, RS09115, RS12240, RS15840, RS19230, RS20955, RS21260,

Table 3. DEGs related to the transcriptional regulator of *P. aeruginosa* in T1, T3, and T5.

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
1.	AT700_RS00295	LysR family transcriptional regulator	~	~	-2.08
2.	AT700_RS00370	tagR; type VI secretion system-associated regulator TagR	~	~	-3.33
3.	AT700_RS01815	TetR/AcrR family transcriptional regulator	~	-1.01	-2.81
4.	AT700_RS02030	pilG; twitching motility response regulator PilG	~	-2.69	~
5.	AT700_RS02035	pilH; twitching motility response regulator PilH	~	-3.66	~
6.	AT700_RS02170	TetR/AcrR family transcriptional regulator	~	~	-3.07
7.	AT700_RS02380	LysR family transcriptional regulator	~	-11.20	~
8.	AT700_RS02555	Lrp/AsnC family transcriptional regulator	~	-2.96	~
9.	AT700_RS02570	AsnC family transcriptional regulator	~	~	-3.21
10.	AT700_RS02595	nirQ; transcriptional regulator NirQ	~	~	-2.67
11.	AT700_RS02630	dnr; transcriptional regulator Dnr	~	-3.79	-5.56
12.	AT700_RS02635	LysR family transcriptional regulator	~	-3.43	-12.39
13.	AT700_RS03010	response regulator transcription factor	~	~	-11.90
14.	AT700_RS03575	pchR; pyochelin biosynthesis transcriptional regulator PchR	~	~	-2.73
15.	AT700_RS03730	bfiR; two-component system response regulator BfiR	~	-2.77	-4.89
16.	AT700_RS05335	narL; two-component system response regulator NarL	~	-3.31	-4.80
17.	AT700_RS06740	response regulator transcription factor	~	~	-4.74
18.	AT700_RS07470	MarR family transcriptional regulator	~	-3.79	-4.76

Table 3. (Continued).

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
19.	AT700_RS07815	regulatory protein NosR	~	-2.91	~
20.	AT700_RS08180	MerR family transcriptional regulator	~	-5.11	~
21.	AT700_RS08450	hybrid sensor histidine kinase/response regulator	~	~	-3.13
22.	AT700_RS08960	IclR family transcriptional regulator	~	-10.55	~
23.	AT700_RS09115	LysR family transcriptional regulator	~	~	-2.96
24.	AT700_RS10035	TetR/AcrR family transcriptional regulator	~	~	-4.75
25.	AT700_RS10635	ohrR; hydroperoxide stress response transcriptional regulator OhrR	1.60	~	-1.54
26.	AT700_RS11535	pfeR; two-component system response regulator PfeR	~	-2.91	~
27.	AT700_RS12240	LysR family transcriptional regulator	~	-1.43	-2.49
28.	AT700_RS12390	heavy metal response regulator transcription factor	~	-2.80	~
29.	AT700_RS12610	response regulator	~	-3.61	~
30.	AT700_RS13325	AraC family transcriptional regulator	~	~	-3.14
31.	AT700_RS13410	gntR; LacI family DNA-binding transcriptional regulator GntR	~	~	-3.33
32.	AT700_RS13620	metalloregulator ArsR/SmtB family transcription factor	~	~	-2.74
33.	AT700_RS13710	ptxS; transcriptional regulator PtxS	~	~	-3.35
34.	AT700_RS13750	Cro/Cl family transcriptional regulator	~	~	-2.31
35.	AT700_RS14900	cmrA; AraC family transcriptional regulator CmrA	~	~	-11.89
36.	AT700_RS15815	TetR/AcrR family transcriptional regulator	~	-3.01	~
37.	AT700_RS15840	LysR family transcriptional regulator	~	~	-2.77
38.	AT700_RS15885	GlxA family transcriptional regulator	1.14	~	-2.37
39.	AT700_RS16385	helix-turn-helix transcriptional regulator	~	-3.03	~
40.	AT700_RS17605	GntR family transcriptional regulator	~	~	-3.14
41.	AT700_RS17915	chemotaxis response regulator protein-glutamate methylesterase	~	~	-4.91
42.	AT700_RS17940	fleN; flagellar synthesis regulator FleN	~	-3.27	-5.26
43.	AT700_RS18435	XRE family transcriptional regulator	~	~	-2.80
44.	AT700_RS18805	MarR family winged helix-turn-helix transcriptional regulator	~	-1.05	-1.73
45.	AT700_RS18815	TetR family transcriptional regulator	~	-3.62	-4.87
46.	AT700_RS19230	LysR family transcriptional regulator	~	~	-2.56
47.	AT700_RS19255	ddaR; transcriptional regulator DdaR	~	-3.49	-5.66
48.	AT700_RS19400	phoP; two-component system response regulator PhoP	~	~	-4.69
49.	AT700_RS20485	YebC/PmpR family DNA-binding transcriptional regulator	~	-1.29	-2.99
50.	AT700_RS20575	hda; DnaA regulatory inactivator Hda	~	-1.18	-2.99
51.	AT700_RS20810	csrA; carbon storage regulator CsrA	~	~	-3.23

Table 3. (Continued).

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
52.	AT700_RS20955	LysR family transcriptional regulator	~	-2.89	~
53.	AT700_RS21185	oruR; ornithine utilization transcriptional regulator OruR	~	~	-5.72
54.	AT700_RS21260	LysR family transcriptional regulator	~	-3.04	~
55.	AT700_RS21355	GntR family transcriptional regulator	~	~	-2.12
56.	AT700_RS21520	mucC; alginate biosynthesis regulator MucC	~	~	-4.91
57.	AT700_RS21525	sigma factor AlgU regulator MucB	~	-2.81	-4.81
58.	AT700_RS21570	response regulator	~	~	-5.81
59.	AT700_RS22640	FeoC-like transcriptional regulator	~	-3.51	-5.79
60.	AT700_RS23045	GlxA family transcriptional regulator	~	-1.33	~
61.	AT700_RS23185	ptsN; PTS IIA-like nitrogen regulatory protein PtsN	~	-2.77	~
62.	AT700_RS23780	TraR/DksA family transcriptional regulator	~	-3.96	-6.35
63.	AT700_RS24595	cbrB; two-component system response regulator CbrB	~	-3.00	~
64.	AT700_RS24795	fur; ferric iron uptake transcriptional regulator	~	-2.73	-4.69
65.	AT700_RS24915	AraC family transcriptional regulator	~	~	-4.61
66.	AT700_RS25495	desT; TetR family transcriptional regulator DesT	~	~	-5.22
67.	AT700_RS27000	carbon storage regulator	~	-3.31	~
68.	AT700_RS27460	rnk; nucleoside diphosphate kinase regulator	~	-1.49	-3.36
69.	AT700_RS27530	glnK; P-II family nitrogen regulator	~	~	-3.14
70.	AT700_RS27990	betI; transcriptional regulator BetI	~	-1.45	-3.38
71.	AT700_RS28135	helix-turn-helix transcriptional regulator	~	~	-5.51
72.	AT700_RS28305	pycR; LysR family transcriptional regulator PycR	~	~	-5.13
73.	AT700_RS28885	DeoR family transcriptional regulator	~	~	-5.11
74.	AT700_RS30375	quorum-sensing transcriptional regulator RsaL	1.06	~	-2.54

and RS28305) were downregulated in T3 and T5, in turn affecting the aforementioned pathways. Similar results were observed for other TRs like TetR, AcrR, AsnC, and AraC. Most of them were also downregulated in T3 and T5. This result indicates that prolonged cold plasma treatment on *P. aeruginosa* culture severely affected the cell transcription processes. Other studies have also found that cold plasma affects TRs. For example, *E. coli* showed upregulation of SoxS, SoxR, and OxyR which are responsible for oxidative stress response [20]. However, these TRs were not differentially expressed in this study. In another study, the cold plasma treatment on *S. aureus* showed upregulation of Rbf, an AraC family TR responsible for biofilm formation [19]. DEGs of TRs such as MarR, GntR, Crp, and Spx were found in the transcriptome of cold plasma-treated *B. cereus* [18].

3.4.3 Ribosome formation

A ribosome is a macromolecular machine in charge of decoding mRNA sequences and protein synthesis [37]. In prokaryotes, these

two functions are harbored by the ribosome's 30S small and 50S large subunits, and they are formed by smaller peptides, so called ribosomal proteins [37]. Each ribosomal protein regulates genes at different levels, and detail studies of these ribosomal proteins are available [38,39]. Table 4 shows the genes encoding ribosomal proteins in *P. aeruginosa* identified as DEGs. Except for T1 and T3 settings with a low DEG number of ribosomal proteins, all the cells' ribosomal proteins were downregulated in T5, with the Log_2FC ranging from -2.17 to -5.38. This observation indicated that prolonged cold plasma treatment can eventually bring the protein synthesis function of a cell to a halt. At the time of writing, most studies had merely emphasized the effects of cold plasma on the ribosomal proteins' regulation. A recent study showed 23 ribosomal proteins encoded genes downregulated in cold plasma-treated *S. aureus* [40]. A proteomics study of cold plasma-treated *P. aeruginosa* also showed that many ribosomal proteins were downregulated [41], coinciding with the finding of this transcriptomics study.

3.4.4 Transporters

Regulation of transporter genes in cold plasma-treated *P. aeruginosa* varied among the three treatment settings (Table 5). T1 had more upregulated transporter genes than the downregulated transporter genes. For instance, a cation transporter (RS06895), two MFS transporters (RS22630 and RS28785), a multidrug efflux RND transporter (RS12540), and numerous ABC transporters for different substrates were upregulated. This upregulation of transporters related genes is a response of the *P. aeruginosa* to oxidative stress, which is used as a signal in the expression of the efflux system, contributing to antibiotic resistance [42]. As the cold plasma treatment duration increased to 3 and 5 minutes, 24 and 60 transporter genes were downregulated in T3 and T5, respectively. These extreme transporter downregulations indicated that the cell could not bring nutrients into the cell or maintain ion equilibrium between the cell and the environment. Moreover, the downregulation of these transporter genes was likely due to the irreversible damage of translation system in the cell, involving ribosome as shown earlier. In cold plasma-treated *E. coli*, most iron-mediating transporters (FepA, FtnB, FepC, DppC, NapF) were downregulated. In contrast, transporters for substrates such as amino acids, carbohydrates, nitrate-nitrite, and multidrug were upregulated [20]. Whereas for *S. aureus*, only drug efflux pump protein and metal ions transporters were DEGs after cold plasma treatment [19,40]. Some transporters in *B. cereus* were identified as DEGs but were not further investigated [18].

3.4.5 Chemotaxis and cell motility

Chemotaxis is a prokaryotic mechanism that allows cells to respond to changing environmental conditions. It involves signaling, flagellar assembly, and movement by flagella. Table 6 shows the complete list of DEGs related to the chemotaxis and the flagellar assembly. In T1, none of the genes were DEGs, indicating that short cold plasma treatment will not affect the chemotaxis pathway and flagellar and pilus assembly of the cells. However, as the treatment time increased, some genes related to this pathway were downregulated. In T3, there were 16 DEGs, and T5 had 20 DEGs. These gene downregulations affect the motility of the cells. The finding indicates that prolonged cold plasma treatment could immobilize the cells of *P. aeruginosa*. Similar findings were reported by Joshi et. al., where genes related to cell motility in *E. coli* were downregulated when the bacteria were exposed to the cold plasma-treated solution [20]. This might be due to the oxidative stress, acid stress, and starvation suffered by the cells [20]. Mols et. al. reported that repression of flagella-related genes was found after 5 minutes of cold plasma-treated *B. cereus* [18]. Another study on cold plasma-treated *P. aeruginosa* also demonstrated 23 differentially expressed proteins (DEPs) in its proteomics data, which supported the transcriptome findings of this study [41].

Table 4. DEGs related to ribosome formation of *P. aeruginosa* in T1, T3, and T5.

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
1.	AT700_RS02900	rpsU; 30S ribosomal protein S21	~	~	-5.38
2.	AT700_RS03375	rpsG; 30S ribosomal protein S7	~	~	-4.60
3.	AT700_RS03395	rplC; 50S ribosomal protein L3	~	~	-4.63
4.	AT700_RS03400	rplD; 50S ribosomal protein L4	~	~	-4.88
5.	AT700_RS03405	rplW; 50S ribosomal protein L23	~	~	-4.82
6.	AT700_RS03410	rplB; 50S ribosomal protein L2	~	~	-4.66
7.	AT700_RS03420	rplV; 50S ribosomal protein L22	~	~	-4.56
8.	AT700_RS03440	rpsQ; 30S ribosomal protein S17	~	~	-4.76
9.	AT700_RS03460	rpsN; 30S ribosomal protein S14	~	~	-4.58
10.	AT700_RS03465	rpsH; 30S ribosomal protein S8	~	~	-4.75
11.	AT700_RS03470	rplF; 50S ribosomal protein L6	~	~	-4.65
12.	AT700_RS03475	rplR; 50S ribosomal protein L18	~	~	-4.81
13.	AT700_RS03490	rplO; 50S ribosomal protein L15	~	~	-4.52
14.	AT700_RS03500	rpmJ; 50S ribosomal protein L36	~	~	-4.76
15.	AT700_RS03515	rpsD; 30S ribosomal protein S4	~	~	-4.55
16.	AT700_RS03580	pchE; pyochelin non-ribosomal peptide synthetase PchE	~	~	-3.24
17.	AT700_RS03585	pchF; pyochelin non-ribosomal peptide synthetase PchF	~	~	-3.06
18.	AT700_RS06030	rpsP; 30S ribosomal protein S16	~	~	-5.03
19.	AT700_RS06035	rimM; ribosome maturation factor RimM	~	~	-4.97
20.	AT700_RS06755	type B 50S ribosomal protein L31	1.35	~	-2.17
21.	AT700_RS06760	ykgO; type B 50S ribosomal protein L36	1.08	-1.10	-2.53
22.	AT700_RS09565	rmf; ribosome modulation factor	~	~	-2.50
23.	AT700_RS09970	rpmF; 50S ribosomal protein L32	~	-1.42	-4.63
24.	AT700_RS11260	rplT; 50S ribosomal protein L20	~	~	-4.54
25.	AT700_RS23025	rpsI; 30S ribosomal protein S9	~	~	-4.91
26.	AT700_RS23230	ribosome-associated protein	~	~	-2.31
27.	AT700_RS23710	rpsT; 30S ribosomal protein S20	~	~	-4.58
28.	AT700_RS24670	rpsO; 30S ribosomal protein S15	~	~	-4.75
29.	AT700_RS24695	rimP; ribosome maturation factor RimP	~	~	-4.62
30.	AT700_RS25295	prmA; 50S ribosomal protein L11 methyltransferase	~	~	-4.93
31.	AT700_RS25705	rplI; 50S ribosomal protein L9	~	~	-4.73
32.	AT700_RS25715	rpsR; 30S ribosomal protein S18	~	~	-4.75
33.	AT700_RS25815	rsgA; small ribosomal subunit biogenesis GTPase RsgA	~	~	~
34.	AT700_RS26310	rpmE; 50S ribosomal protein L31	~	~	-5.14
35.	AT700_RS27240	rbbA; ribosome-associated ATPase/putative transporter RbbA	~	~	-5.33

Table 5. DEGs related to transporters of *P. aeruginosa* in T1, T3, and T5.

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
1.	AT700_RS00705	ABC transporter permease	~	-4.10	~
2.	AT700_RS01365	sulfate ABC transporter ATP-binding protein	~	~	-2.30
3.	AT700_RS01370	cysW; sulfate ABC transporter permease subunit CysW	1.49	~	-1.30
4.	AT700_RS01375	cysT; sulfate ABC transporter permease subunit CysT	~	~	-2.41
5.	AT700_RS01380	sulfate ABC transporter substrate-binding protein	1.75	~	~
6.	AT700_RS01440	polyamine ABC transporter substrate-binding protein	~	-3.54	~
7.	AT700_RS01610	autotransporter domain-containing protein	~	~	-10.49
8.	AT700_RS02235	inorganic phosphate transporter	~	~	-10.81
9.	AT700_RS02695	DMT family transporter	-1.15	~	~
10.	AT700_RS03620	RhtX/FptX family siderophore transporter	1.12	-1.20	-2.77
11.	AT700_RS03675	mexI; multidrug efflux RND transporter permease MexI	~	~	-3.07
12.	AT700_RS03680	mexH; multidrug efflux RND transporter periplasmic adaptor MexH	~	~	-3.01
13.	AT700_RS03685	mexG; multidrug efflux RND transporter inhibitory subunit MexG	~	~	-3.02
14.	AT700_RS03995	peptidase domain-containing ABC transporter	~	~	-2.91
15.	AT700_RS04405	ABC transporter ATP-binding protein	~	~	-3.08
16.	AT700_RS04585	transporter substrate-binding domain-containing protein	~	~	-11.54
17.	AT700_RS05030	tauA; taurine ABC transporter substrate-binding protein	1.58	~	-1.67
18.	AT700_RS05065	MetQ/NlpA family ABC transporter substrate-binding protein	1.61	~	-1.70
19.	AT700_RS05345	NarK/NasA family nitrate transporter	-3.70	-5.84	-7.65
20.	AT700_RS05350	narK2; nitrate/nitrite transporter NarK2	-4.23	-7.05	-7.63
21.	AT700_RS05385	ABC transporter substrate-binding protein	~	~	-2.92
22.	AT700_RS05950	nagE; N-acetylglucosamine-specific PTS transporter subunit IIBC	1.47	~	~
23.	AT700_RS06375	mexJ; multidrug efflux RND transporter periplasmic adaptor subunit MexJ	~	-2.82	~
24.	AT700_RS06720	ABC transporter permease	~	~	-12.33
25.	AT700_RS06725	ABC transporter ATP-binding protein	~	~	-11.19
26.	AT700_RS06895	cation transporter	2.30	~	~
27.	AT700_RS07115	MFS transporter	~	~	-5.87
28.	AT700_RS07435	MFS transporter	~	-3.57	-5.77
29.	AT700_RS07800	ABC transporter ATP-binding protein	~	-3.26	~
30.	AT700_RS08480	sugE; quaternary ammonium compound efflux SMR transporter SugE	~	~	-5.81
31.	AT700_RS08645	glycine betaine ABC transporter substrate-binding protein	~	~	-11.60
32.	AT700_RS08685	ABC transporter ATP-binding protein/permease	~	~	-2.70

Table 5. (Continued).

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
33.	AT700_RS09880	lipoprotein-releasing ABC transporter permease subunit	~	~	-4.75
34.	AT700_RS09890	lipoprotein-releasing ABC transporter permease subunit	~	~	-4.87
35.	AT700_RS09910	biopolymer transporter ExbD	~	~	-5.00
36.	AT700_RS10475	multidrug/biocide efflux PACE transporter	~	-4.90	~
37.	AT700_RS10590	ABC transporter ATP-binding protein	~	~	-4.84
38.	AT700_RS11015	formate/nitrite transporter family protein	~	~	-11.79
39.	AT700_RS12530	oprN; multidrug efflux RND transporter outer membrane subunit OprN	~	~	-1.63
40.	AT700_RS12540	mexE; multidrug efflux RND transporter periplasmic adaptor subunit MexE	1.43	~	~
41.	AT700_RS12970	metal ABC transporter ATP-binding protein	~	~	-6.07
42.	AT700_RS14095	ABC transporter substrate-binding protein	1.04	~	-2.27
43.	AT700_RS15720	multidrug efflux SMR transporter	~	~	-5.98
44.	AT700_RS16100	microcin C ABC transporter permease YejB	~	~	-4.84
45.	AT700_RS16955	SulP family inorganic anion transporter	~	~	-11.18
46.	AT700_RS18520	glutamate/aspartate ABC transporter substrate-binding protein	~	~	-2.83
47.	AT700_RS18525	amino acid ABC transporter permease	~	~	-2.92
48.	AT700_RS18650	MFS transporter	~	-2.92	-4.94
49.	AT700_RS18745	aitP; CDF family iron/cobalt efflux transporter AitP	~	-3.89	~
50.	AT700_RS18800	MFS transporter	~	~	-2.08
51.	AT700_RS18820	MFS transporter	~	-3.00	~
52.	AT700_RS18950	amino acid ABC transporter permease	-1.23	~	-12.25
53.	AT700_RS18955	amino acid ABC transporter ATP-binding protein	~	-3.58	
54.	AT700_RS19380	dctA; C4-dicarboxylate transporter DctC	~	-1.35	-3.01
55.	AT700_RS22045	MFS transporter	~	-10.33	~
56.	AT700_RS22310	inorganic phosphate transporter	~	-1.30	~
57.	AT700_RS22630	MFS transporter	1.85	~	~
58.	AT700_RS22645	feoB; Fe(2+) transporter permease subunit FeoB	~	-2.74	-4.83
59.	AT700_RS23140	mlaE; lipid asymmetry maintenance ABC transporter permease subunit MlaE	~	-2.71	-4.94
60.	AT700_RS23145	ABC transporter ATP-binding protein	~	~	-4.89
61.	AT700_RS23160	lptC; LPS export ABC transporter periplasmic protein LptC	~	~	-5.20
62.	AT700_RS23375	ABC transporter substrate-binding protein	~	-3.06	~
63.	AT700_RS23380	ABC transporter permease subunit	~	~	-6.84
64.	AT700_RS23385	ABC transporter permease subunit	~	-3.02	-5.15
65.	AT700_RS24030	cdrB; two-partner secretion system transporter CdrB	~	~	-6.45

Table 5. (Continued).

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
66.	AT700_RS24190	MFS transporter	~	~	-2.89
67.	AT700_RS24365	iron ABC transporter substrate-binding protein	~	~	-3.20
68.	AT700_RS24825	lactate permease LctP family transporter	1.40	~	-2.84
69.	AT700_RS25335	urtA; urea ABC transporter substrate-binding protein	~	~	-11.71
70.	AT700_RS25480	MFS transporter	~	~	-6.55
71.	AT700_RS26835	ABC transporter ATP-binding protein	~	~	-4.60
72.	AT700_RS27165	iron ABC transporter permease	~	~	-4.92
73.	AT700_RS27235	ABC transporter permease	~	-2.86	~
74.	AT700_RS27240	rbbA; ribosome-associated ATPase/putative transporter RbbA	~	-3.38	-5.33
75.	AT700_RS27245	HlyD family efflux transporter periplasmic adaptor subunit	~	-4.04	-6.53
76.	AT700_RS27330	LysE family transporter	~	~	-12.23
77.	AT700_RS28000	choV; choline ABC transporter ATP-binding protein	~	-2.81	~
78.	AT700_RS28620	zinc ABC transporter substrate-binding protein	~	~	-2.69
79.	AT700_RS28655	MetQ/NlpA family ABC transporter substrate-binding protein	~	~	-3.17
80.	AT700_RS28785	MFS transporter	1.04	~	~

3.5 Detailed DEGs Comparison Revealed the Sequential Response of *P. aeruginosa* against the Cold Plasma

Based on the results above, it was clearly shown that *P. aeruginosa* responded differently in the three treatments, which were classified into the initial response (T1 setting), intermediate response (T3), and final response (T5) of the cells against the cold plasma in this study (Figure 4). *P. aeruginosa* produced many antioxidants during the first minute of cold plasma treatment to overcome the oxidative stress caused by ROS. As the treatment continued, bacterial cells got stressed and encountered high cell death, therefore, this leads to downregulation of antioxidant genes. In contrast, ROS affect the transcription process, substrate and ions transportation, and the cell movement in T3. This situation worsened when the cold plasma treatment lasted 5 minutes. The protein synthesis which depends on ribosome availability, was heavily affected.

4. Conclusions

Cold plasma generated from the LTAPJ device was proved to have disinfection ability against the cells of *Pseudomonas aeruginosa*. The longer the treatment duration, the lower its cell survivability. Furthermore, transcriptome analysis revealed the sequential response of *P. aeruginosa* against the cold plasma, including upregulation of antioxidant genes in the beginning, followed by downregulation of transcriptional regulators, transporters, and chemotaxis and cell motility in prolonged treatment. Genes involved in ribosome formation were also downregulated after prolonged treatment, affecting the translation process that could lead to low cell survivability.

Table 6. DEGs related to the chemotaxis and cell motility of *P. aeruginosa* in T1, T3, and T5.

No.	Locus_Tag	Gene Functions	DEGs (log_2FC)		
			T1	T3	T5
1.	AT700_RS02030	pilG; twitching motility response regulator PilG	~	-2.69	~
2.	AT700_RS02035	pilH; twitching motility response regulator PilH	~	-3.66	~
3.	AT700_RS02040	chemotaxis protein CheW	~	-3.42	-5.27
4.	AT700_RS02045	pilJ; chemotaxis chemoreceptor PilJ	~	-2.76	~
5.	AT700_RS09905	MotA/TolQ/ExbB proton channel family protein	~	~	-4.81
6.	AT700_RS10020	PilZ domain-containing protein	~	~	-3.11
7.	AT700_RS10275	methyl-accepting chemotaxis protein	~	-10.46	-10.46
8.	AT700_RS10540	methyl-accepting chemotaxis protein	~	-2.84	-5.09
9.	AT700_RS17395	methyl-accepting chemotaxis protein	~	-3.32	-5.34
10.	AT700_RS17890	chemotaxis protein CheW	~	~	-3.02
11.	AT700_RS17910	flagellar motor protein	~	~	-5.71
12.	AT700_RS17915	chemotaxis response regulator protein-glutamate methylesterase	~	~	-4.91
13.	AT700_RS17935	fliA; RNA polymerase sigma factor FliA	~	-2.86	-4.82
14.	AT700_RS17935	fliA; RNA polymerase sigma factor FliA	~	-2.86	-4.82
15.	AT700_RS17940	fleN; flagellar synthesis regulator FleN	~	-3.27	-5.26
16.	AT700_RS17945	flhF; flagellar biosynthesis protein FlhF	~	-3.04	-4.80
17.	AT700_RS17980	fliP; flagellar type III secretion system pore protein FliP	~	~	-3.15
18.	AT700_RS17985	fliO; flagellar biosynthetic protein FliO	~	~	-5.99
19.	AT700_RS18000	fliL; flagellar basal body-associated protein FliL	~	-2.97	-5.10
20.	AT700_RS19800	fliF; flagellar M-ring protein FliF	~	~	-4.89
21.	AT700_RS19825	flagellar protein FliT	~	~	-4.77
22.	AT700_RS19840	fliD; flagellar filament capping protein FliD	~	~	-3.18
23.	AT700_RS19940	flgD; flagellar hook assembly protein FlgD	~	-1.23	~
24.	AT700_RS26275	pilO; type 4a pilus biogenesis protein PilO	~	-2.86	~
25.	AT700_RS26280	pilN; type 4a pilus biogenesis protein PilN	~	-3.17	~
26.	AT700_RS26285	pilM; type IV pilus assembly protein PilM	~	-2.72	~
27.	AT700_RS26425	methyl-accepting chemotaxis protein	~	~	-5.57

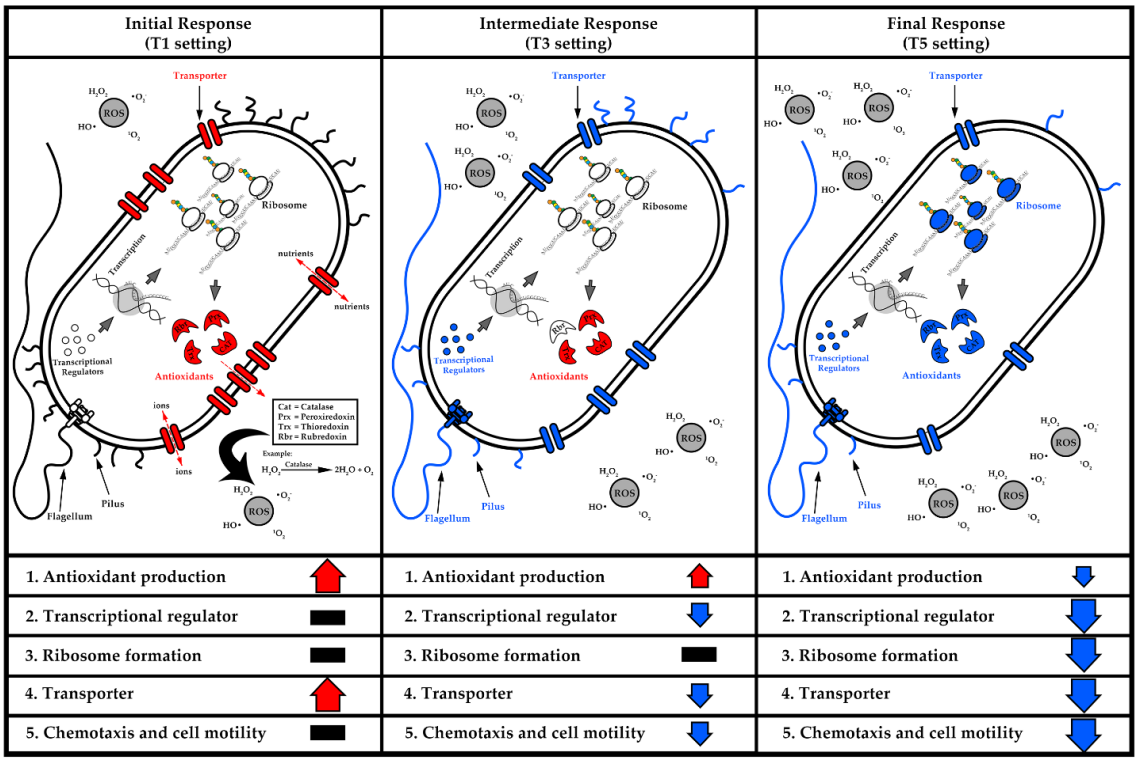

Figure 4. The sequential transcriptomic responses of *P. aeruginosa*. Initial, intermediate, and final responses of the cells were represented by T1, T3, and T5 treatment, respectively. In the image, red indicates the gene upregulation of the respective cellular components, while blue shows the gene downregulation. The up and down arrows at the bottom of the figure show the relative number of identified DEGs. Larger arrows indicate more DEGs. The black minus bars show that the respective cellular component is unaffected by the treatment.

ACKNOWLEDGEMENTS

This study was supported by Professional Development Research University Grant (grant number: 06E00) and External Grant (grant number: 4B779) from Universiti Teknologi Malaysia. This study was also supported by the Gusu Innovation and Entrepreneurship Leading Talents Project (ZXL2018192), and the Suzhou 2020 Novel Coronavirus Emergency Prevention and Control Technology Project, and the 2020 Jiangsu Province Industry-University-Research Cooperation Project (BY2020506). K.J.L. is a Researcher of Universiti Teknologi Malaysia under the Post-Doctoral Fellowship Scheme for the Project: "Transcriptome Analysis of Low Temperature Air Plasma Jet Treated Microorganisms".

CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DATA AVAILABILITY

The raw data for RNA sequencing of *Pseudomonas aeruginosa* ATCC 9027 in this study are publicly available in NCBI Sequence Read Archive

(SRA) under accession numbers SRR21111573–SRR21111576. The data can also be accessed via BioProject accession number PRJNA870582 and BioSample accession numbers SAMN30379855–SAMN30379858.

References

[1] Lazra Y., Dubrovin I., Multanen V., Bormashenko E., Bormashenko Y. and Cahan R., *Microorganisms*, 2020; **8(5)**: 704. DOI 10.3390/microorganisms8050704.

[2] Baniya H.B., Guragain R.P. and Subedi D.P., Cold Atmospheric Pressure Plasma Technology for Modifying Polymers to Enhance Adhesion: A Critical Review; in Mittal K.L., ed., *Progress in Adhesion and Adhesives, Volume 6*, Wiley, 2021: 269-307.DOI 10.1002/9781119846703.ch19.

[3] Di L., Zhang J., Zhang X., Wang H., Li H., Li Y., et al., *J. Phys. D Appl. Phys.*, 2021; **54**: 333001. DOI 10.1088/1361-6463/ac0269.

[4] Aggelopoulos C.A., *Chem. Eng. J.*, 2022; **428**: 131657. DOI 10.1016/j.cej.2021.131657.

[5] Gururani P., Bhatnagar P., Bisht B., Kumar V., Joshi N.C., Tomar M.S., et al., *Environ. Sci. Pollut. R.*, 2021; **28(12)**: 65062–65082. DOI 10.1007/s11356-021-16741-x.

[6] Sidana A. and Yadav S.K., *J. Clean. Prod.*, 2021; **335**: 130286. DOI 10.1016/j.jclepro.2021.130286.

[7] Farber R., Dabush-Busheri I., Chaniel G., Rozenfeld S., Bormashenko E., Multanen V., et al., *Int. Biodeter. Biodegr.*, 2019; **139**: 62–69. DOI 10.1016/j.ibiod.2019.03.003.

[8] Tabares F.L. and Junkar I., *Molecules*, 2021; **26(1)**: 1903. DOI 10.3390/molecules26071903.

[9] Xiang Q., Huangfu L., Dong S., Ma Y., Li K., Niu L., et al., *Crit. Rev. Food Sci.*, 2021: 1–19. DOI 10.1080/10408398.2021.2002257.

[10] Mravlje J., Regvar M., Starič P., Mozetič M. and Vogel-Mikuš K., *Plants*, 2021; **10(5)**: 851. DOI 10.3390/plants10050851.

[11] Ucar Y., Ceylan Z., Durmus M., Tomar O. and Cetinkaya T., *Trends Food Sci. Tech.*, 2021; **114**: 355–371. DOI 10.1016/j.tifs.2021.06.004.

[12] Joseph A., Rane R. and Vaid A., Atmospheric Pressure Plasma Therapy for Wound Healing and Disinfection: A Review; in Kumar P., and Kothari V., eds., *Wound Healing Research*, Springer, Singapore, 2021: 621–641. DOI 10.1007/978-981-16-2677-7_19.

[13] Mao L., Mhaske P., Zing X., Kasapis S., Majzoobi M. and Farahnaky A., *Trends Food Sci. Tech.*, 2021; **116**: 146–175. DOI 10.1016/j.tifs.2021.07.002.

[14] Wang S., Liu Y., Zhang Y., Lü X., Zhao L., Song Y., et al., *LWT-Food Sci. Technol.*, 2022; **153**: 112573. DOI 10.1016/j.lwt.2021.112573.

[15] González-González C.R., Labo-Popoola O., Delgado-Pando G., Theodoridou K., Doran O. and Stratakos A.C., *LWT-Food Sci. Technol.*, 2021; **149**: 111898. DOI 10.1016/j.lwt.2021.111898.

[16] Qian J., Ma L., Yan W., Zhuang H., Huang M., Zhang J., et al., *Food Microbiol.*, 2022; **101**: 103891. DOI 10.1016/j.fm.2021.103891.

[17] Mai-Prochnow A., Murphy A.B., McLean K.M., Kong M.G. and Ostrikov K.K., *Int. J. Antimicrob. Ag.*, 2014; **43(6)**: 508–517. DOI 10.1016/j.ijantimicag.2014.01.025.

[18] Mols M., Mastwijk H., Nierop Groot M. and Abee T., *J. Appl. Microbiol.*, 2013; **115(3)**: 689–702. DOI 10.1111/jam.12278.

[19] Xu Z., Wei J., Shen J., Liu Y., Ma R., Zhang Z., et al., *Appl. Phys. Lett.*, 2015; **106**: 213701. DOI 10.1063/1.4921754.

[20] Joshi S.G., Yost A., Joshi S.S., Addya S., Ehrlich G. and Brooks A.D., *Adv. Biosci. Biotechnol.*, 2015; **6(2)**: 49-62. DOI 10.4236/abb.2015.62006.

[21] Azam M.W. and Khan A.U., *Drug Discov. Today*, 2019; **24(1)**: 350–359. DOI 10.1016/j.drudis.2018.07.003.

[22] Fothergill J.L., Neill D.R., Loman N., Winstanley C. and Kadioglu A., *Nat. Commun.*, 2014; **5(1)**: 1–9. DOI 10.1038/ncomms5780.

[23] Sun P.P., Won J., Choo-Kang G., Li S., Chen W., Monroy G.L., et al., *npj Biofilms Microbiomes*, 2021; **7**: 1–10. DOI 10.1038/s41522-021-00219-2.

[24] Bolger A.M., Lohse M. and Usadel B., *Bioinformatics*, 2014; **30(15)**: 2114–2120. DOI 10.1093/bioinformatics/btu170.

[25] McClure R., Balasubramanian D., Sun Y., Bobrovskyy M., Sumby P., Genco C.A., et al., *Nucleic Acids Res.*, 2013; **41(14)**: e140. DOI 10.1093/nar/gkt444.

[26] Robinson M.D., McCarthy D.J. and Smyth G.K., *Bioinformatics*, 2010; **26(1)**: 139–140. DOI 10.1093/bioinformatics/btp616.

[27] Klopfenstein D.V., Zhang L., Pedersen B.S., Ramírez F., Vesztrocy A.W., Naldi A., et al., *Sci. Rep.*, 2018; **8(1)**: 10872. DOI 10.1038/s41598-018-28948-z.

[28] Svarnas P., Spiliopoulou A., Koutsoukos P.G., Gazeli K. and Anastassiou E.D., *Plasma*, 2019; **2(2)**: 77–90. DOI 10.3390/plasma2020008.

[29] Zhang Y., Wei J., Yuan Y., Chen H., Dai L., Wang X., et al., *Innov. Food Sci. Emerg.*, 2019; **52**: 394–405. DOI 10.1016/j.ifset.2019.01.019.

[30] Sharkey M.A., Chebbi A., McDonnell K.A., Staunton C. and Dowling D.P., *Biointerphases*, 2015; **10(2)**: 029507. DOI 10.1116/1.4916928.

[31] Perkins A., Nelson K.J., Parsonage D., Poole L.B. and Karplus P.A., *Trends Biochem. Sci.*, 2015; **40(8)**: 435–445. DOI 10.1016/j.tibs.2015.05.001.

[32] Tehrani H.S. and Moosavi-Movahedi A.A., *Prog. Biophys. Mol. Bio.*, 2018; **140**: 5–12. DOI 10.1016/j.pbiomolbio.2018.03.001.

[33] Lesniak J., Barton W.A. and Nikolov D.B., *EMBO J.*, 2002; **21(24)**: 6649–6659. DOI 10.1093/emboj/cdf670.

[34] Lu J. and Holmgren A., *Free Radical Bio. Med.*, 2014; **66**: 75–87. DOI 10.1016/j.freeradbiomed.2013.07.036.

[35] Pis Diez C.M., Juncos M.J., Dujovne M.V. and Capdevila D.A., *Int. J. Mol. Sci.*, 2022; **23**: 2179. DOI 10.3390/ijms23042179.

[36] Sheehan L.M., Budnick J.A., Blanchard C., Dunman P.M. and Caswell C.C., *Mol. Microbiol.*, 2015; **98(2)**: 318–328. DOI 10.1111/mmi.13123.

[37] Lafontaine D.L.J. and Tollervey D., *Nat. Rev. Mol. Cell Bio.*, 2001; **2**: 514–520. DOI 10.1038/35080045.

[38] Zhou X., Liao W.-J., Liao J.-M., Liao P. and Lu H., *J. Mol. Cell Biol.*, 2015; **7(2)**: 92–104. DOI 10.1093/jmcb/mjv014.

[39] Wilson D.N. and Nierhaus K.H., *Crit. Rev. Biochem. Mol.*, 2005; **40(5)**: 243–267. DOI 10.1080/10409230500256523.

[40] Liao X., Liu D. and Ding T., *Appl. Environ. Microbiol.*, 2020; **86(5)**: e02216-19. DOI 10.1128/AEM.02216-19.

[41] Liu Y., Wang J., Liu C., Chen G., Cai Z., Sang X., et al., *LWT-Food Sci. Technol.*, 2022; **169**: 113981. DOI 10.1016/j.lwt.2022.113981.

[42] da Cruz Nizer W.S., Inkovskiy V., Versey Z., Strempel N., Cassol E. and Overhage J., *Pathogens*, 2021; **10(9)**: 1187. DOI 10.3390/pathogens10091187.