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Investigating the Severity of Environmental Stress Conditions on Growth, the Protein Profile, and Biochemical Characteristics of *Pseudomonas aeruginosa* ATCC 27853

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Abstract

Pseudomonas aeruginosa is the main cause of nosocomial infections, and is resistant to most antibiotics, this study aimed to evaluate the effects of different stress conditions on growth, the protein profile, and biochemical characteristics of this bacterium. The cells of *P. aeruginosa* ATCC 27853 in the logarithmic phase were exposed to different stress factors such as sucrose concentration, ethanol, acid, osmotic pressure, and $CoCl_2$. Following each stress condition, the growth and the survival of bacterial cells were determined. Microscopic observation showed morphological changes in different stress conditions. *P. aeruginosa* ATCC 27853 tolerated up to pH 3, 55% (V/V) ethanol, and $CoCl_2$ up to 7% (W/V), and beyond these amounts, the bacterium lost its ability to survive. Maximum tolerance to sucrose was about 35% (W/V). The results showed that different stress conditions could not effect on the main biochemical characteristics of *P. aeruginosa* ATCC 27853. Scanning electron microscopy of the cells exposed to different stress conditions showed wide changes in the morphology of cells. In addition, upon treating to different stresses significant changes were observed in the protein profile of *P. aeruginosa* ATCC 27853 according to SDS-PAGE analysis. It can be concluded that severe environmental stresses have great effects on the growth pattern, phenotypic characteristics, and protein profile of *P. aeruginosa* ATCC 27853. If the stresses induced all at once, they will cause the death, but if they are affected slowly and for longer period, most bacteria will be able to repair the damaged parts, and the growth of will resume.

Key words: *Pseudomonas aeruginosa* ATCC 27853, Stress, Protein profile, Biochemical characteristics, Growth pattern

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1. Introduction

All living organisms respond to adverse conditions and stresses at the cellular level (Sun & Zhou, 2018). It seems that the response of eukaryotes to environmental stress is much more complex than that of bacteria (Shetty et al., 2019). These responses can be induced by many different chemical, environmental, and biological factors, including toxins, sodium arsenite, ethanol, sulfhydryl groups, hydrogen peroxide, heavy metals, amino lipid analogs, antibiotics, and viral contamination. (Macario et al., 1999).

Envelope stress responses (ESRs) are crucial to the maintenance of this barrier and function to detect and respond to perturbations in the envelope caused by environmental stresses. In addition, pathogenic bacteria are exposed to an array of challenging and stressful conditions during their lifecycle and, in particular, during infection of a host (Hews et al., 2019). In the past years, many genes and proteins related to stress have been studied, and their results have been that stress causes the inactivation of some genes or the negative regulation of their expression, and on the other hand, stress activates a number of other proteins. Therefore, molecular chaperones help other cell proteins fold correctly after translation and enter the cells where they are needed and where their function is (Diamant et al., 2001), and the study of stress responses will continue to be a topic of biological, medical, and applied research (Moat et al., 2002).

The genus *Pseudomonas* includes more than 10 species, most of which are saprophytes. Most of them, like *Pseudomonas aeruginosa*, *Pseudomonas* putida, *Pseudomonas cepacia*, *Pseudomonas* stutzeri, *Pseudomonas maltophilia*, and *Pseudomonas putrefaciens*, cause opportunistic infections in humans (Woods & Iglewski, 1983). In addition, over the last decades, there has been a dramatic global increase in multidrug-resistant (MDR) pathogens, particularly among Gram-negative bacteria. *Pseudomonas aeruginosa* is responsible for various healthcare-associated infections (Mahmood et al., 2021). Approximately 80% of *Pseudomonas* is obtained from clinical samples (Hancock et al., 1983; Woods & Iglewski, 1983). *P. aeruginosa* is a gram-negative bacterium that is remarkable for its worldwide ubiquity and extensive environmental distribution in soil, water, and plant matter, as well as its ability to cause a variety of opportunistic infections in humans. It is a major cause of morbidity and mortality in hospitalized patients and those with cystic fibrosis (CF) (Ozer et al., 2019). Pseudomonas has two types of pigments in the sample: green pigment and blue pigment. Blue pigment (pyocyanin) is a characteristic of purulent infections (Abdelaziz et al., 2022).

P. aeruginosa is considered a major health care threat due to its vast rates of emergence, acquisition, and spread of different resistance mechanisms (Bassetti et al., 2017). This consequently led to challenges in initiating appropriate targeted therapy, particularly in severe infections, leading to increased morbidity and mortality as well as prolonged hospital stays and subsequently excessive health care costs (Zilberberg et al., 2014). It is resistant to physical factors, including heat, high concentrations of salt, disinfectants, and most antibiotics. An antibiotic should not be used to treat Pseudomonas infections because the bacteria quickly become resistant to it. Some antibiotics, like fluoroquinolones, gentamicin, and imipenems, can be used (Klibanov et al., 2004; Todar, 2006). This bacterium causes between 10 and 20% of hospital infections (Botzenhart & Rüden, 1987). P. aeruginosa has a cytoplasmic membrane with a symmetric phospholipid bilayer and an asymmetric outer membrane with a phospholipid inner face and a lipopolysaccharide outer layer, which generates a permeability barrier. The outer membrane of *P. aeruginosa* contains numerous proteins, including lipoproteins and channels (Chevalier et al., 2017; Remans et al., 2010). The vast majority of individuals with cystic fibrosis (CF) will eventually become chronically infected with Pseudomonas aeruginosa (Moreau-Marquis et al., 2008; Jennings et al., 2021). *Persistence* of CF airway infections is associated with non-resolving inflammation, accelerated lung disease, and earlier mortality (Gibson et al., 2003; Jennings et al., 2021).

P. aeruginosa produces numerous virulence determinants associated with pathogenicity, including exotoxins, proteases, other enzymes, pigments, secretion systems, alginate capsules, flagella, and pili (Park & Koo, 2022). Importantly, *P. aeruginosa* possesses different mechanisms to manipulate or evade the host immune response, including a mucoid exopolysaccharide known as alginate. Remarkably, alginate slime promotes biofilm formation as it anchors the cells to the environment, which is able to protect against immune defenses. This ability of P. aeruginosa to manipulate host immunity results in a worsening of the disease in this population, associated with the formation of biofilm and other phenotypes (Chirgwin et al., 2018).

Currently, combination therapy is only recommended in limited scenarios and has been successful for a long time, but the increase in worldwide resistance necessitates the need for newer agents (Ibrahim et al., 2020). Combinations of gentamicin and carbenicillin are very effective in patients with acute *P. aeruginosa* infections. Gentamicin, tobramycin, amikacin, and colistin are effective in the treatment of Pseudomonas, but it is important to perform a sensitivity test before prescribing the antibiotics. In patients with leukopenia, several types of vaccines have been obtained, but they are still not widely used or available (Brown, 1975; Poole, 1994). In the present study, the effect of environmental stress, including osmotic pressure, pH, ethanol, and $CoCl_2$, on *P. aeruginosa* ATCC 27853 was evaluated, and morphological, biochemical, and protein profile changes of this bacterium were studied in detail.

2. Materials and Methods

2.1. Bacterial strain and chemicals

Pseudomonas aeruginosa ATCC27853 was obtained from the microbial resource centers of Boo-Ali Hospital and the Shahriari Branch's Scientific Research Center, Tehran, Iran. All chemicals used in this study were obtained from Merck Co. (Darmstadt, Germany).

2.2. Studying the effect of different stress conditions on *P. aeruginosa* ATCC 27853 Different parameters, such as ethanol, sucrose, acidity, and heavy metal $CoCl_2$, were evaluated on *P. aeruginosa* ATCC 27853. For stress treatment, *P. aeruginosa* ATCC27853 cells were grown in TSB broth to reach mid-log phase (OD600=0.4-0.5). For osmotic stress, cells were inoculated in TSB broth containing sucrose at 0.0% (control), 5, 10, 17, 24, 30, 32, and 35%. For oxidative stress, *P. aeruginosa* ATCC27853 cells were exposed to different concentrations of ethanol, including 0.0 (control), 5, 15, 25, 35, 45, 50, and 55%. For acidic stress, a variety of pHs in acidic conditions (2.5, 3.0, 4.0, 5, and 6) were employed. For the study of the effect of heavy metals, $CoCl_2$ at concentrations of 1, 3, and 7% was used. *P. aeruginosa* ATCC27853 cells were treated for 3 hours in each stress condition. Then, the cells were centrifuged at 10000 rpm for 20 min at 4°C. Then cell pellets were resuspended in phosphate buffer (pH 7.2) and cultured in differential media to evaluate the effects of each stress parameter in comparison to non-stress conditions.

2.3. Microscopic study

Morphological and structural changes upon stress conditions on *P. aeruginosa* ATCC 27853 cells were studied by the method of Cefali et al. (2002) with some modifications. The mid-log phase cells grown in Luria Bertani broth were centrifuged at 10000 rpm for 15 min, washed twice with sterile phosphate buffer saline, and immediately placed on a circular slide for drying. To check that the air-drying process of samples did not affect cell morphology (critical point overcoming), parallel scanning electron microscope (SEM) imaging was performed (Zeiss-Gena, Germany). Samples for obtaining SEM images were prepared in duplicate: the first was set by the critical point drying (CPD) protocol; the second was prepared without drying to the critical point, according to the procedure suggested by Al-Tahhan et al. (2000).

2.4. Protein profile study

A protein profile study under different stress conditions was studied using crude extract preparation. The mid-log phase cells in stressed and non-stressed conditions grown in LB medium were centrifuged at 10000 rpm for 15 min at 4

°C, immediately washed with sterile phosphate buffer saline, and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), containing 0.3 mg phenylmethanesulfonylfluoride/ml. All cells were lysed using sonication on ice by five 10-s bursts at 4 W (Cole Parmer Instrument Co., Vernon Hills, IL) and stored at -20°. Cell debris and unbroken cells were removed from all samples by centrifugation at 10000 rpm for 15 min at 4°C. The total protein concentration was determined by the Bradford method. Bovine serum albumin (BSA) was used as the standard. All experiments were performed in triplicate (Southey-Pillig et al., 2005).

2.4.1.One-dimensional gel electrophoresis and image analysis

Protein extraction using the sodium carbonate-based membrane method was performed on each of the samples. The supernatants were added to 33 mL of ice-cold 100 mM sodium carbonate and placed on a rocker at 4 °C for one hour. Then the solution was ultra-centrifuged at 30000 rpm at 4 °C for one hour. Total protein patterns in cell extracts were analyzed by one-dimensional polyacrylamide gel electrophoresis. The gels were stained with silver nitrate (Southey-Pillig et al., 2005; Wright et al., 2019).

3. Results

P. aeruginosa ATCC 27853 was examined under an optical microscope after Gram staining, as shown in Figure 1.

The biochemical characteristics of *P. aeruginosa* exposed to different ethanol concentrations are shown in Table 1. *P. aeruginosa* cells are resistant to ethanol concentrations up to 55% (V/V), and after that, no growth was observed. All biochemical tests were unaffected except nitrate reduction and gas production, which became negative after 25% (V/V) of ethanol.

Table 2 shows the effects of different pHs on *P. aeruginosa*. Maximum tolerance was observed at pH 3.0, and bacterial cell growth was inhibited at lower pHs. Tables 3 and 4 show the effects of different concentrations of sucrose and $CoCl_2$ on *P. aeruginosa* cells. Maximum tolerances to sucrose and $CoCl_2$ were about 35% (W/V) and 7% (W/V), respectively.

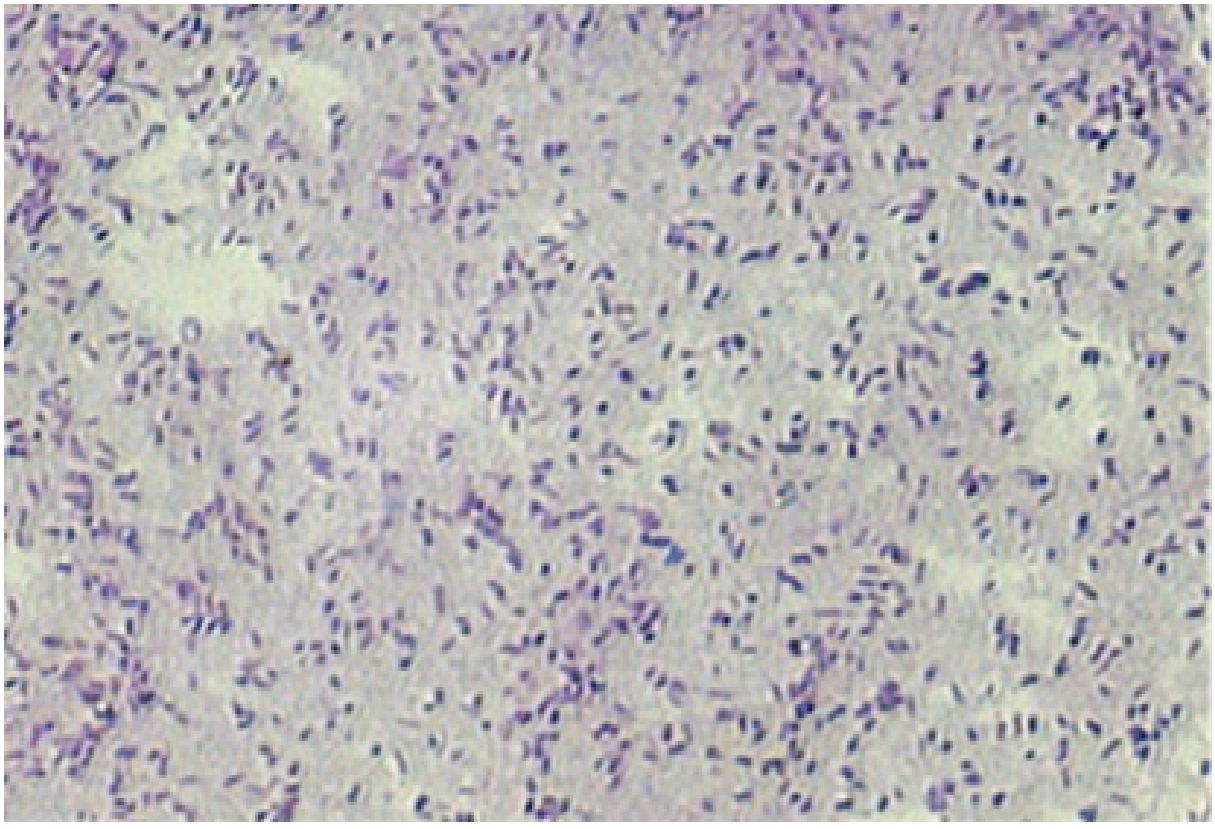

Fig. 1. Gram staining of *P. aeruginosa* ATCC 27853

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Table 1. Biochemical characteristics of *Pseudomonas aeruginosa* ATCC 27853 under ethanol stress

	Ethanol						
Culture Medium	**5%**	**15%**	**25%**	**35%**	**45%**	**50%**	**55%**
Citrate	+	+	+	+	+	-	-
Lysine decarboxylase	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-
Motility	+	+	+	+	+	-	-
Nitrate Reduction	+	+	-	-	-	-	-
Gas Production	+	+	-	-	-	-	-
(TSI) Glucose	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	-	-

Note: - indicates non-growth. + indicates growth.

Table 2. Biochemical characteristics of *Pseudomonas aeruginosa* ATCC 27853 under different pHs

	Acidic pH				
Culture Medium	**pH 2.5**	**pH 3**	**pH 4**	**pH 5**	**pH 6**
Citrate	-	-	+	+	+
Lysine decarboxylase	-	-	-	-	-
Urease	-	-	-	-	-
MR	-	-	-	-	-
VP	-	-	-	-	-
Indole	-	-	-	-	-
Motility	-	-	+	+	+
Nitrate reduction	-	-	+	+	+
Gas Production	-	-	+	+	+
Glucose(TSI)	-	-	Alkaline	Alkaline	Alkaline

Note: - indicates non-growth. + indicates growth.

Table 3. Biochemical characteristics of *Pseudomonas aeruginosa* ATCC 27853 under different sucrose concentrations

	Sucrose Concentrations						
Culture Medium	**5%**	**10%**	**17%**	**24%**	**30%**	**32%**	**35%**
Citrate	+	+	+	+	+	+	+
Lysine decarboxylase	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	-	-	-
Gas Production	+	+	+	+	-	-	-
Glucose (TSI)	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline

Note: - indicates non-growth. + indicates growth.

Table 4. Biochemical characteristics of *Pseudomonas aeruginosa* under different $CoCl_2$ concentrations

	$CoCl_2$ Concentrations		
Culture Medium	**1%**	**3%**	**7%**
Citrate	+	+	+
Lysine decarboxylase	-	-	-
Urease	-	-	-
MR	-	-	-
VP	-	-	-
Indole	-	-	-
Motility	+	+	+
Nitrate Reduction	+	+	+
Gas Production	+	+	+

Figure 2 shows the effects of different pH values on total proteins in *P. aeruginosa* cell extracts. As shown by the decreasing pH values up to pH 3, the overall protein bands decrease significantly.

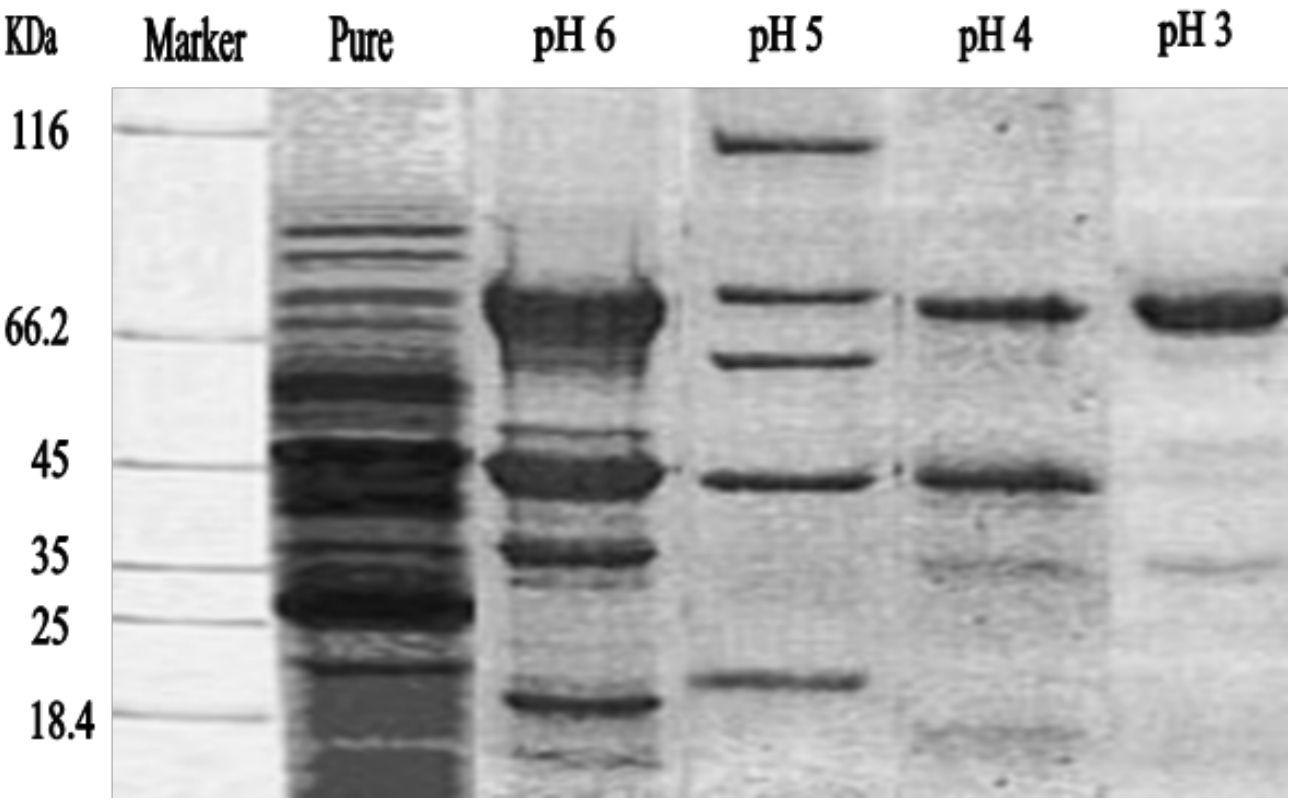

Fig. 2. Total protein profile of *Pseudomonas aeruginosa* ATCC 27853 at different pHs

The protein profile of *P. aeruginosa* is significantly affected by increasing concentrations of ethanol and $CoCl_2$, as shown in Figures 3 and 4, respectively.

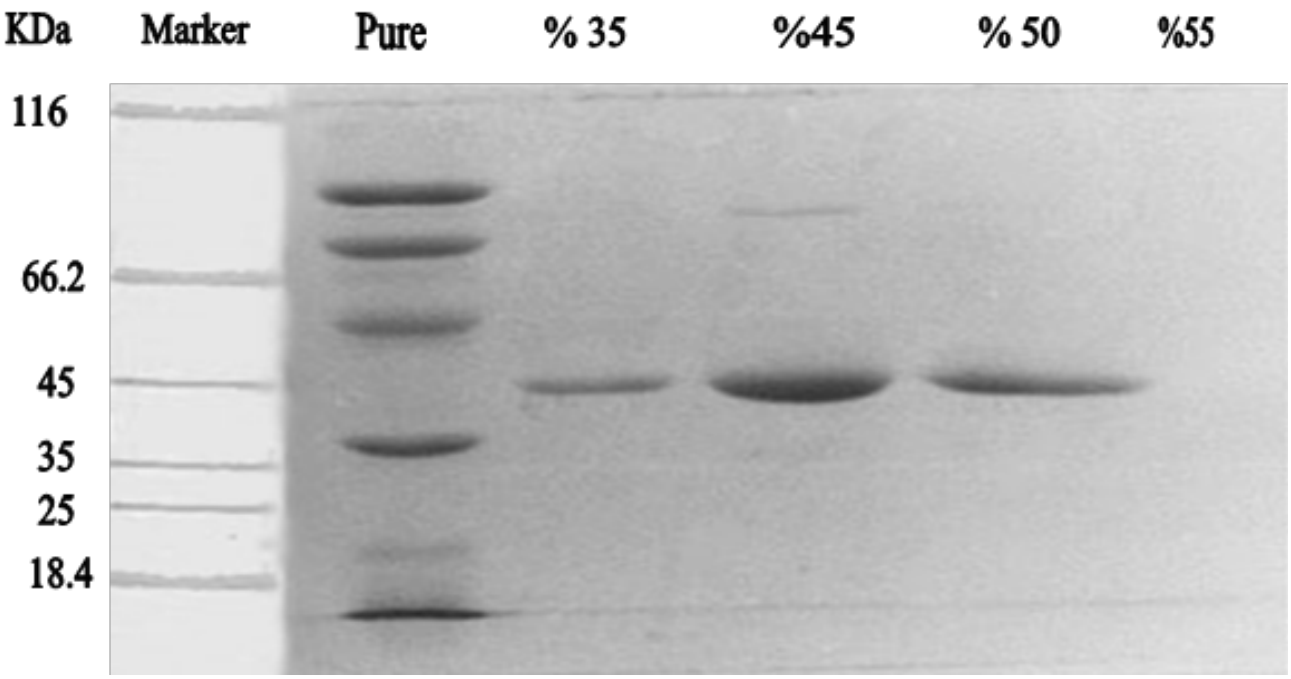

Fig. 3. Total protein profile of *P. aeruginosa* ATCC 27853 at different ethanol concentrations

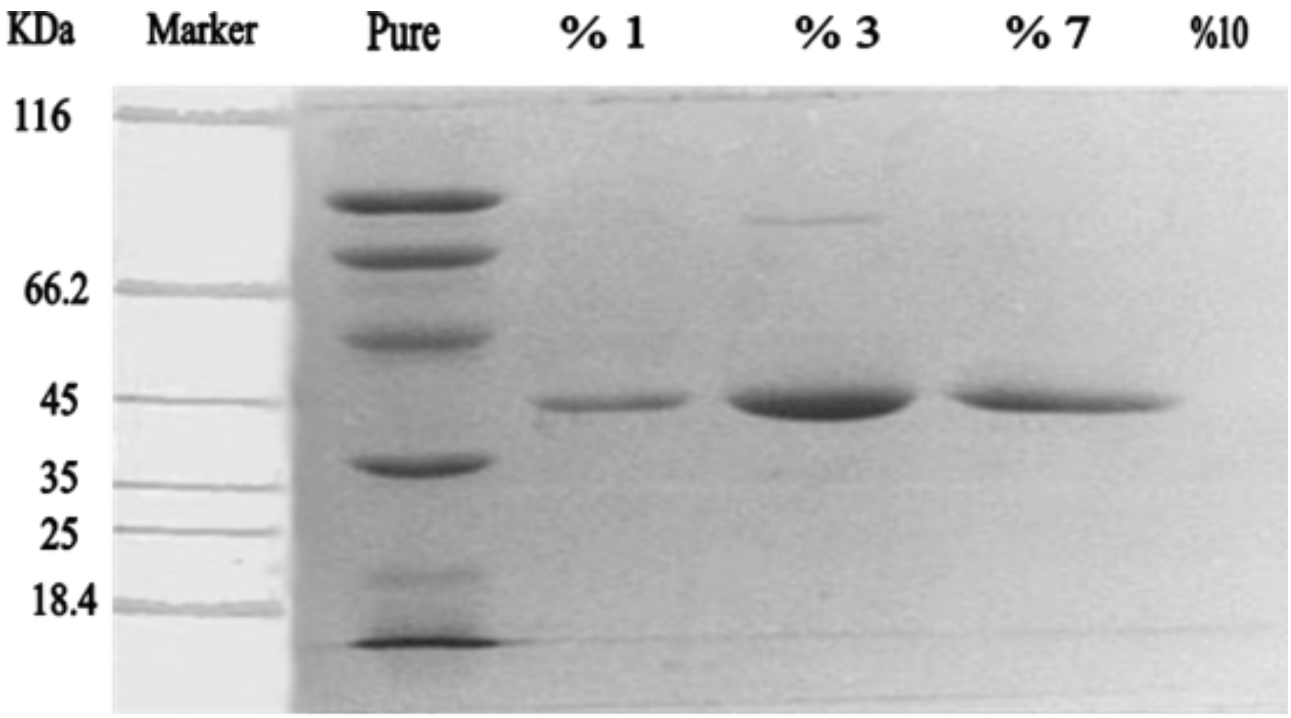

Fig. 4. Total protein profile of *P. aeruginosa* ATCC 27853 at different CoCl*2* concentrations

Total protein concentrations vary upon exposure to different concentrations of sucrose, as shown in Figure 5.

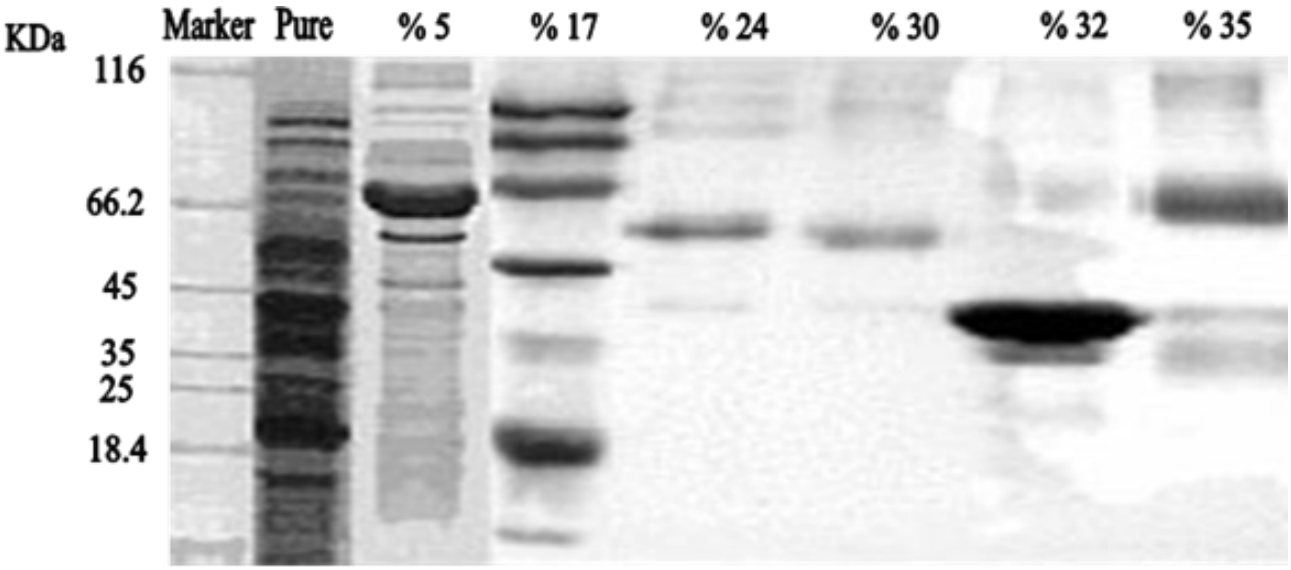

Fig. 5. Total protein profile of *P. aeruginosa* ATCC 27853 at different sucrose concentrations.

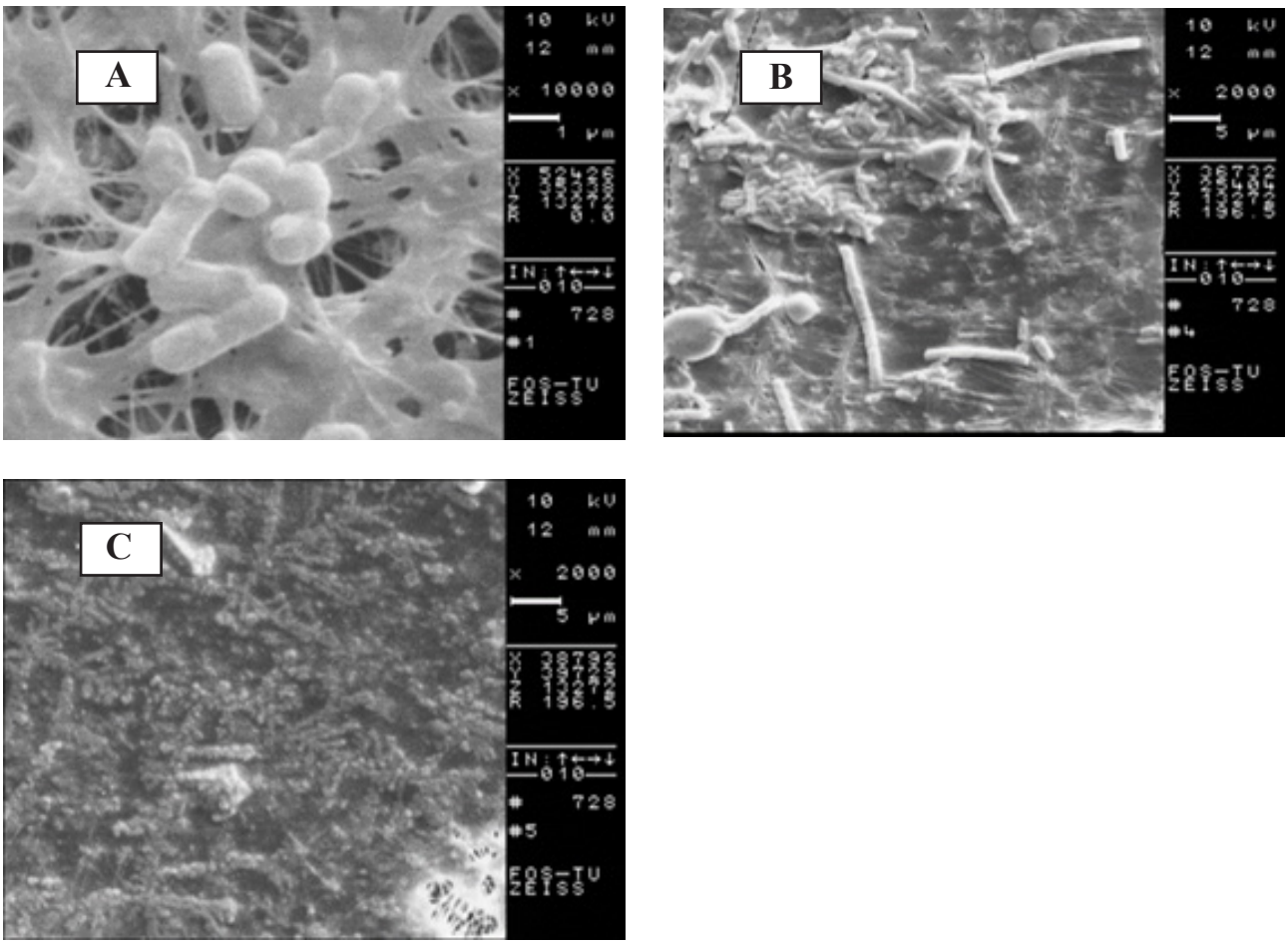

Fig. 6. Scanning electron micrographs of *P. aeruginosa* ATCC27853 under different stress conditions. (A) Normal cells without stress conditions; (B) Treated with ethanol at a concentration of 50% (V/V); and (C) treated with sucrose at a concentration of 30% (W/V).

4. Discussion

In this study, different stress conditions, including temperature, osmotic, acidic, ethanol, and heavy metals, were evaluated on *P. aeruginosa* ATCC27853. Each stress condition affects the cell size, colony morphology, and protein profile of this bacterium. As we know, heat shock protein formation is not limited to temperature changes and is concerned with general responses to all stress conditions. Also, heat shock proteins play an important role in pathogenicity and enable bacteria to survive inside macrophages.

Cells exposed to increasing ethanol concentrations showed significant changes in their protein profiles. In 50% (V/V) ethanol concentration, the total protein concentration of *P. aeruginosa* cells decreased, as shown in Figure 5(B). In addition, the ability of *P. aeruginosa* to reduce nitrate and produce gas ceased after 25% (V/V) of ethanol concentration, as shown in Table 1. Motility of cells was stopped after 45% (V/V) of ethanol concentration due to the very low ability of growth cells. By decreasing the pH values, the ability of *P. aeruginosa* cells decreases significantly, and below pH 4, no growth was observed by this bacterium. Polyacrylamide gel electrophoresis showed only 2 and 1 bands at pH 4 and pH 3, respectively (Figure 2). Scanning electron micrograph of P. aeruginosa cells treated with 50% (V/V) presented in Figure 6(B), as shown, wide morphological changes are seen in comparison to control Figure 6(A). The cells became more elongated and pleomorphic.

Changes in biochemical characteristics of *P. aeruginosa* ATCC27853 upon increasing concentrations of sucrose are shown in Table 3. The ability of nitrate reduction and production of gas were negative after 30% (W/V) of sucrose concentration. Also, the total protein profile in cell-free extract was significantly reduced after 24% sucrose concentration (Figure 5). Electron micrographs of the cells treated with sucrose concentration at 30% (W/V) are shown in Figure 6(C). The cells became very small and appeared as cocci due to shrinking in response to increasing osmolarity.

The effects of different concentrations of $CoCl_2$ on the biochemical characterizations of *P. aeruginosa* ATCC27853 are shown in Table 4. As shown in the table, no significant changes were observed in the biochemical characteristics of this bacterium. After 7% (W/V) CoCl2, no growth was observed.

In a study by da Cruz Nizer et al. (2021), the oxidative stress response in *P. aeruginosa* was studied. They concluded that genes are often differently expressed under oxidative stress conditions, and the pathways and proteins employed to sense and respond to oxidative stress influence the pathogenicity and virulence of *P. aeruginosa.* Expression of detoxifying enzymes, mainly catalases, can be considered one of the main responses under oxidative stress, and their synthesis is controlled by several systems, such as quorum sensing (QS) and diverse transcriptional regulators.

The physiological responses of *P. aeruginosa* PAO1 to oxidative stress in controlled microaerobic and aerobic cultures were studied by Sabra et al. (2002). They concluded that *P. aeruginosa* PAO1 prefers microaerobic conditions for growth and expresses its virulence factors. PAO1 can create such growth conditions by at least two mechanisms: (i) blockage of the transfer of oxygen and (ii) formation of a polysaccharide capsule. It is suggested that the blockage of oxygen transfer may play an important role in the defense of this pathogen against reactive oxygen species.

5. Conclusions

In general, environmental stresses showed effects on the growth pattern, biochemical profile, and protein profile of *P. aeruginosa* ATCC 27853. If these stresses are very intense and are induced all at once, they will cause the death of bacteria, but if they are affected slowly and over a longer period, most bacteria will be able to repair the damaged parts, and the growth of bacteria will resume. The induction of environmental stresses will activate many genes and, on the other hand, cause the inactivation of a number of others, so that the activated genes expression enables the bacteria to survive in new conditions or develop new capabilities. With the effects of a number of stresses, the protein patterns change.

Some proteins are lost or their expression reduced, and some are produced or their expression increased compared to non-stress conditions, as can be seen in polyacrylamide gel electrophoresis experiments.

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Conflict of Interest

No conflict of interest was declared.

References

Abdelaziz, A. A., Kamer, A. M. A., Al-Monofy, K. B., & Al-Madboly, L. A. (2022). A purified and lyophilized *Pseudomonas aeruginosa* derived pyocyanin induces promising apoptotic and necrotic activities against MCF-7 human breast adenocarcinoma. Microbial Cell Factories, 21(1), 262.

Al-Orphaly, M., Hadi, H. A., Eltayeb, F. K., Al-Hail, H., Samuel, B. G., Sultan, A. A., & Skariah, S. (2021). Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in the Middle East and North Africa *Region.* Msphere, 6(3), e00202-21.

Al-Tahhan, R. A., Sandrin, T. R., Bodour, A. A., & Maier, R. M. (2000). Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. Applied and *Environmental Microbiology,* 66(8), 3262-3268.

Bassetti, M., Carnelutti, A., & Peghin, M. (2017). Patient specific risk stratification for antimicrobial resistance and possible treatment strategies in gram-negative bacterial infections. Expert Review of Anti-Infective Therapy, 15(1), 55-65.

Bodey, G. P., Bolivar, R., Fainstein, V., & Jadeja, L. (1983). Infections caused by *Pseudomonas aeruginosa*. Reviews of Infectious Diseases, 5(2), 279-313.

Botzenhart, K., & Rüden, H. (1987). *Hospital infections* caused by Pseudomonas aeruginosa. Basic Research and Clinical Aspects of *Pseudomonas aeruginosa*, 39, 1-15.

Brown, M. R. W. (1975). Resistance of *Pseudomonas aeruginosa*. John Wiley and Sons Ltd.

Cefali, E., Patane, S., Arena, A., Saitta, G., Guglielmino, S., Cappello, S., ... & Allegrini,M. (2002). Morphologic variations in bacteria under stress conditions: Near-field optical studies. Scanning: The Journal of Scanning Microscopies, 24(6), 274-283.

Chevalier, S., Bouffartigues, E., Bodilis, J., Maillot, O., Lesouhaitier, O., Feuilloley, M. G., ... & Cornelis, P. (2017). Structure, function and regulation of *Pseudomonas aeruginosa* porins. FEMS Microbiology Reviews, 41(5), 698-722.

Chirgwin, M. E., Dedloff, M. R., Holban, A. M., & Gestal, M. C. (2019). Novel therapeutic strategies applied to *Pseudomonas aeruginosa* infections in cystic fibrosis. Materials, 12(24), 4093.

da Cruz Nizer, W. S., Inkovskiy, V., Versey, Z., Strempel, N., Cassol, E., & Overhage, J.(2021). Oxidative stress response in *Pseudomonas aeruginosa*. Pathogens, 10(9), 1187.

Diamant, S., Eliahu, N., Rosenthal, D., & Goloubinoff, P. (2001). Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. Journal of Biological Chemistry, 276(43), 39586-39591.

Gupta, S., & Pandey, S. (2019). ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (Phaseolus vulgaris) plants. Frontiers in Microbiology, 10, 1506.

Hancock, R. E., Mutharia, L. M., Chan, L., Darveau, R. P., Speert, D. P., & Pier, G. (1983). *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. Infection and Immunity, 42(1), 170-177.

Hews, C. L., Cho, T., Rowley, G., & Raivio, T. L. (2019). Maintaining integrity under stress: envelope stress response regulation of pathogenesis in gram-negative bacteria. Frontiers in Cellular and Infection Microbiology, 9, 313.

Ibrahim, D., Jabbour, J. F., & Kanj, S. S. (2020). Current choices of antibiotic treatment for *Pseudomonas aeruginosa* infections. Current Opinion in Infectious Diseases, 33(6), 464-473.

Jennings, L. K., Dreifus, J. E., Reichhardt, C., Storek, K. M., Secor, P. R., Wozniak, D. J., Hisert, K. B., & Parsek, M. R. (2021). *Pseudomonas aeruginosa* aggregates in cystic fibrosis sputum produce exopolysaccharides that likely impede current therapies. Cell Reports, 34(8), 108782.

Klibanov, O. M., Raasch, R. H., & Rublein, J. C. (2004). Single versus combined antibiotic therapy for gram-negative infections. Annals of Pharmacotherapy, 38(2), 332-337.

Macario, A. J., Lange, M., Ahring, B. K., & De Macario, E. C. (1999). Stress genes and proteins in the archaea. Microbiology and Molecular Biology Reviews, 63(4), 923-967.

Moat, A. G., Foster, J. W., & Spector, M. P. (Eds.). (2002). Microbial physiology. John Wiley & Sons.

Mutharia, L. M., Nicas, T. I., & Hancock, R. E. (1982). Outer membrane proteins of *Pseudomonas aeruginosa* serotype strains. The Journal of Infectious Diseases, 146(6), 770-779.

Ozer, E. A., Nnah, E., Didelot, X., Whitaker, R. J., & Hauser, A. R. (2019). The population structure of Pseudomonas aeruginosa is characterized by genetic isolation of exoU+ and exoS+ lineages. Genome Biology and Evolution, 11(7), 1780-1796.

Park, Y., & Koo, S. H. (2022). Epidemiology, molecu-

lar characteristics, and virulence factors of carbapenem-resistant *Pseudomonas aeruginosa* isolated from patients with urinary tract infections. Infection and Drug Resistance, 141-151.

Poole, K. (1994). Bacterial multidrug resistance—emphasis on efflux mechanisms and *Pseudomonas aeruginosa*. Journal of Antimicrobial Chemotherapy, 34(4), 453-456.

Price, C. W., Fawcett, P., Cérémonie, H., Su, N., Murphy, C. K., & Youngman, P. (2001). Genome-wide analysis of the general stress response in Bacillus subtilis. Molecular Microbiology, 41(4), 757-774.

Remans, K., Vercammen, K., Bodilis, J., & Cornelis, P. (2010). Genome-wide analysis and literature-based survey of lipoproteins in *Pseudomonas aeruginosa*. Microbiology, 156(9), 2597-2607.

Rosen, R., Büttner, K., Schmid, R., Hecker, M., & Ron, E. Z. (2001). Stress-induced proteins of Agrobacterium tumefaciens. FEMS Microbiology Ecology, 35(3), 277-285.

Sabra, W., Kim, E. J., & Zeng, A. P. (2002). Physiological responses of *Pseudomonas aeruginosa* PAO1 to oxidative stress in controlled microaerobic and aerobic cultures. Microbiology, 148(10), 3195-3202.

Shetty, P., Gitau, M. M., & Maróti, G. (2019). Salinity stress responses and adaptation mechanisms in eukaryotic green microalgae. Cells, 8(12), 1657.

Southey-Pillig, C. J., Davies, D. G., & Sauer, K. (2005). Characterization of temporal protein production in *Pseudomonas aeruginosa* biofilms. Journal of Bacteriology, 187(23), 8114-8126.

Sun, S., & Zhou, J. (2018). Molecular mechanisms underlying stress response and adaptation. Thoracic Cancer, 9(2), 218-227.

Speert, D. P. (1993). *Pseudomonas aeruginosa*-phagocytic cell interactions. In *Pseudomonas aeruginosa* as an opportunistic pathogen (pp. 163-181). Springer.

Todar, K. (2006). Todar's online textbook of bacteriology. University of WisconsinWoods, D. E., & Iglewski, B. H. (1983). Toxins of *Pseudomonas aeruginosa*: new perspectives. Reviews of Infectious Diseases, 5(Supplement_4), S715-S722.

Wright, B. W., Kamath, K. S., Krisp, C., & Molloy, M. P. (2019). Proteome profiling of *Pseudomonas aeruginosa* PAO1 identifies novel responders to copper stress. BMC Microbiology, 19, 1-13.

Zilberberg, M. D., Shorr, A. F., Micek, S. T., Vazquez-Guillamet, C., & Kollef, M. H. (2014). Multi-drug resistance, inappropriate initial antibiotic therapy and mortality in Gram-negative severe sepsis and septic shock: a retrospective cohort study. Critical Care, 18(6), 1-13.