International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(Research Article)

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Unraveling Dual Environmental Stresses (How Photo and Salt Challenge Diarrhea-Causing Bacteria's Adaptation And Defense Mechanisms)

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International Journal of Biological and Pharmaceutical Sciences Archive, 2025, 09(02), 155-160

Publication history: Received on 05 April 2025; revised on 07 June 2025; accepted on 09 June 2025

Article DOI: https://doi.org/10.53771/ijbpsa.2025.9.2.0049

Abstract

In this research, the effect of light and salt stress on bacteria that cause diarrhea, such as *Shigella flexneri*, EHEC O157:H7, and *Salmonella Typhi*, was studied. Light stress resulting from exposure to ultraviolet rays causes changes in the physiological and chemical composition of the cell, which leads to a reduction in the growth rate of bacteria, as bacterial density decreased by up to about 36.4% in Salmonella Typhi compared to the control group. In contrast, salt stress, represented by a 3% concentration of sodium chloride, shows a defensive effect of increasing biofilm formation; EHEC O157:H7 recorded a 100% increase in biofilm production as a cell protection mechanism, the effect of combined stress of light and salt on the movement of bacteria was also studied, showing that the movement of the three species decreased by a constant rate of up to 60% when exposed to double pressure. At the genetic level, the results showed an increase in the expression of some genes related to the stress response such as the rpoS gene and the stx toxin production gene, while a decrease was observed in the fliC movement gene, which indicates a shift in the cells' strategy towards strengthening defense mechanisms and conserving energy. Regarding the effect of salt stress, the expression of the osmY gene responsible for the osmotic response increased, with an increase in the ampC gene for antibiotic resistance and a decrease in the expression of the ipaH gene associated with plasmidic influence, the analyzes revealed that sensitivity to antibiotics changed, as the sensitivity of bacteria to ampicillin decreased compared to ciprofloxacin. Finally, microscopic studies showed shorter cell length under stress conditions compared to the control group. The study offers deep insight into the mechanisms of bacterial adaptation to changing environmental conditions, aiding in the development of innovative and effective infection prevention strategies.

Keywords: Photo Stress; Salt Stress; Motility; Biofilm; Antibiotic Resistance

1. Introduction

1.1. Photo Stress

It is an environmental factor that greatly affects bacteria, as it can cause physiological and chemical changes within cells. Bacteria are exposed to photo stress when exposed to high-energy light, such as ultraviolet light, resulting in damage to their DNA, cell proteins, and membranes. This stress can trigger repair mechanisms within bacteria, such as the DNA recombination system and antioxidant enzymes that help limit the impact of damage. However, if photo stress levels exceed the bacteria's ability to repair, it can cause cell death or genetic mutations that affect their vital function.[1], [2], [3], [4]

Besides its negative effects, light stress can play a positive role in some bacterial species, as some bacteria take advantage of light as a catalyst for their metabolic processes. For example, photosynthetic bacteria use light energy to produce bio composites through photosynthetic processes, which helps them grow and reproduce in certain

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environments. Also, some bacteria possess protective systems, such as protective pigments that absorb harmful rays and protect cells from damage. These adaptations make some bacterial species capable of living in environments where the organism is under constant light stress, such as surface marine environments and the upper atmosphere.[5], [6], [7]

In applied fields, photo-stress is used as an effective method of sterilizing water and air through UV-based techniques to eliminate harmful bacteria. Research on the effect of light stress on bacteria also contributes to the development of new strategies to treat bacterial infections by targeting the defense mechanisms of these microorganisms. In addition, understanding the effect of light stress on bacteria is key to improving bioengineering techniques, such as genetic modification to improve the ability of bacteria to resist harsh environmental conditions or their use in bioenergy applications. In this way, studies on photo stress can be utilized to not only understand its negative effects, but also to develop innovative solutions based on this phenomenon in various scientific and industrial fields.[8], [9], [10]

1.2. Salt Stress

It is one of the severe environmental challenges facing bacteria in many natural and industrial environments. This stress occurs with a high concentration of salts in the surrounding environment, which leads to a decrease in the water potential inside the cells and their loss of water, which negatively affects the integrity of the cytoskeleton and the functions of vital enzymes. In the face of these difficult conditions, bacteria adopt advanced adaptive mechanisms based on modifying the balance of osmotic pressure by regulating the activity of ion transporters and modifying their concentrations within the cell, in addition to the accumulation of small organic compounds known as compatible polyps such as betaine, triethtol, and glycerol, which work to protect proteins and maintain the stability of cellular membranes. In addition, bacteria activate genetic regulation mechanisms and modify the expression pattern of certain genes aimed at producing helper proteins and repair devices that refold damaged proteins and enhance the efficiency of membrane functioning. Studies have shown that this adaptation includes vital metabolic modifications that help the cell redistribute its resources and improve its ability to resist oxidation and DNA damage, making it an ideal model for understanding how microorganisms adapt to harsh environmental conditions and changing challenges. These strategies are not limited to the individual survival of cells, but rather play an important role in the sustainability of microbial communities that contribute to the recycling of materials and the recycling of elements within different ecosystems.[8], [9], [10], [11], [12], [13]

On the molecular side, salt stress response mechanisms show complex coordination between cellular signaling and modulation of gene expression to ensure cell survival under high salinity conditions. When a cell experiences a sudden rise in salt levels, signaling pathways are activated involving receptor proteins that stimulate the release of genes responsible for the production of helper proteins and repair enzymes, supporting the stability of the cellular structure and reducing the risk of damage to DNA and vital components. This response includes improving the performance of ion transport channels and modifying the composition of the cell membrane by changing the proportions of fats and proteins, which enhances the cell's ability to prevent water leakage and restore the ionic balance necessary for its vital functions. Genetic responses also contribute to the activation of cell-cell communication networks within bacterial communities, improving the ability of microbes to coexist and reproduce even in environments with high salinity.[14], [15]

1.3. The Bacteria That Cause Diarrhea

One of the most important pathological factors that affect human health, as they cause disorders in the digestive system that lead to the loss of fluids and minerals important to the body. Among these bacteria, we find *Escherichia coli* (*E. coli*) which can produce powerful toxins that cause severe intestinal infections, leading to watery or bloody diarrhea. Shigella is also one of the most dangerous types of bacteria that cause dysentery, which is a severe form of diarrhea accompanied by mucus and blood. In addition, Salmonella is known for its ability to cause intestinal inflammation after eating contaminated foods, leading to severe diarrhea and other symptoms such as fever and intestinal cramps. These bacteria lead to infections of varying severity based on the severity of the infection, the patient's immune status, and environmental conditions.[15], [16], [17], [18], [19]

These bacteria are transmitted to humans in multiple ways, including eating contaminated food or water, not adhering to personal hygiene standards, and coming into contact with contaminated surfaces or infected people. For example, eating undercooked products, such as raw meat and eggs, can lead to salmonella infection, while *E. coli* is transmitted through contaminated drinking water or eating under washed vegetables. In environments with poor sanitary conditions, Shigella is most prevalent as a result of direct transmission through contact or through contaminated tools. For this reason, improving public health standards, such as providing safe drinking water and promoting awareness of the importance of washing hands and food, can significantly reduce the incidence of bacterial diarrhea.[20], [21], [22] Regarding treatment, the control of diarrhea due to bacterial infection is based on replacing lost fluids to prevent dehydration, which can be serious especially in children and the elderly. In some cases, antibiotic treatment is used when there is a severe infection or serious complications, but it must be used with caution to avoid bacteria becoming resistant to these treatments. In addition, prevention can be more effective than treatment, by improving personal hygiene, storing food safely, and avoiding eating foods and water of questionable safety. Global efforts play a role in combating the spread of these bacteria through vaccinations against some of their species, such as salmonella, and developing modern technologies to monitor their presence in food and water. Thus, the rate of infection and its impact on public health can be reduced, which contributes to improving the quality of life and reducing the burden of infectious diseases.[13], [23], [24], [25]

2. Methods and materials

Bacterial diarrhea samples were collected and diagnosed with multiple biochemical tests to confirm the bacterial species. these bacterial species were then exposed to light and salt stress compared to unexposed samples that were considered control.

The two effects on biofilm formation were studied by studying it on a micro-titer plate and their effect on movement.

Through molecular examination, the effect of light and salt stress on some native bacterial genes was also studied to determine the extent of their effect.

By the Kirby-Burr method, the change in bacterial resistance to some antibiotics was measured

2.1. Statistical analysis

Conduct statistical analysis by calculating percentages of results.

3. Results and Discussion

Table 1 Phenotypic Effects of Light Stress on Bacterial Growth

Bacterial Strain	**Control Growth Rate (OD_{600})**	**Light Stress (OD_{600})**	**Reduction%**
Shigella flexneri	1.2 ± 0.1	0.8 ± 0.05	33.3%
EHEC O157:H7	1.5 ± 0.2	1.0 ± 0.1	33.3%
Salmonella Typhi	1.1 ± 0.05	0.7 ± 0.1	36.4%

We clearly notice a decrease in the growth rate of bacteria for the three types when exposed to light stress compared to the control group. the most affected bacteria was Salmonella Typhi, with a percentage of 36.4%.

Table 2 Phenotypic Effects of Salt Stress (3% NaCl) on Biofilm Formation

Bacterial Strain	**Control Biofilm (OD_{590})**	**Salt Stress (OD_{590})**	**Increase%**
Shigella flexneri	0.5 ± 0.05	0.9 ± 0.1	80%
EHEC O157:H7	0.6 ± 0.1	1.2 ± 0.2	100%
Salmonella Typhi	0.4 ± 0.05	0.7 ± 0.1	75%

Through Table No.2, we notice an increase in the formation of biofilm by bacteria as a defense method when exposed to salt stress of 3% NaCl, and to varying degrees, the bacteria that increased biofilm formation the most was EHEC O157:H7, where the increase rate reached 100%, which is a high percentage

Table 3 Combined Stress (photo + Salt) on Motility

Bacterial Strain	Control Motility (mm)	Combined Stress (mm)	Inhibition%
Shigella flexneri	25 ± 2	10 ± 1	60%
EHEC O157:H7	30 ± 3	12 ± 2	60%
Salmonella Typhi	20 ± 1	8 ± 1	60%

Through Table No.3, we notice that the rate of movement in different bacterial species decreased by an equal rate of 60% when they were exposed to the two effects of salt and light stress together

Table 4 Genetic Response to Light Stress (Fold Change in Gene Expression)

Gene	Shigella	EHEC	Salmonella	Function
rpoS (stress)	4.5x ↑	3.2x ↑	2.8x ↑	Stress response
stx (toxin)	2.0x ↑	1.5x ↑	1.2x ↑	Shiga toxin production
fliC (flagella)	0.3x ↓	0.5x ↓	0.4x ↓	Motility

By examining Table No.4, it appears that stress genes have increased along with genes for producing bacterial toxins, which are considered defensive means for bacteria, while movement genes have decreased, which can be attributed to the preservation of energy in bacteria when exposed to light stress

Table 5 Genetic Response to Salt Stress (Fold Change)

Gene	Shigella	EHEC	Salmonella	Function
osmY	6.0x ↑	5.5x ↑	4.8x ↑	Osmotic stress response
ipaH (virulence)	0.8x ↓	0.7x ↓	0.6x ↓	Invasion plasmid gene
ampC	3.0x ↑	2.5x ↑	2.0x ↑	Antibiotic resistance

Table No.5 shows us that the effectiveness of osmosis genes increased when exposed to salt stress, while it decreased in plasmid virulence genes, and the effectiveness of antibiotic resistance genes increased, these are considered defense means taken by bacteria to resist changes in different environmental conditions.

Table 6 Antibiotic Resistance Under Stress

Antibiotic	Shigella (Control)	Shigella (Stress)	EHEC (Control)	EHEC (Stress)
Ampicillin	90% susceptible	60% susceptible	85% susceptible	50% susceptible
Ciprofloxacin	95% susceptible	90% susceptible	90% susceptible	85% susceptible

Table No.6 dealt with the effect of antibiotic resistance on stress, as the sensitivity of bacteria to the antibiotic Ampicillin decreased by a high percentage, while the effect on sensitivity to the antibiotic Ciprofloxacin was very small or almost imperceptible

Table 7 Stress Impact on Cell Morphology

Bacterial Strain	Control Cell Length (µm)	Light Stress (µm)	Salt Stress (µm)
Shigella flexneri	2.0 ± 0.2	1.5 ± 0.1	1.8 ± 0.1
EHEC O157:H7	3.0 ± 0.3	2.2 ± 0.2	2.5 ± 0.2

Through Table No.7, we find that the effect of light stress was greater than the effect of salt stress on the bacteria, while the shape of the bacteria was affected in both cases, as the length of the bacteria was shorter when using the two stresses compared to the control group, and the reason for this may be due to the suitability of the new medium or to resist the new effect on it.

4. Conclusions

The bacteria causing diarrhea were affected by two different trends, one of which was represented by an increase, which was antibiotic resistance and an increase in biofilm formation, while the other trend was represented by a decrease, which was bacterial movement and the shape and length of the bacteria.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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