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Impact of Some Parameters on the Survival and Proliferation of Foodborne Pathogens: Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Streptococcus pyogenes

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ABSTRACT

Food poisoning caused by foodborne microorganisms is a significant public health concern. This study aims to investigate the impact of several nutritional and environmental parameters on the growth of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococc pyogenes* in both model systems and food. The specific impact of temperature, pH, and UV light stress on pathogens. Applying physical stress on microorganisms is a standard method to induce cell inactivation and promote food stability. This study found that the optimal temperature for the growth of these pathogens is between 30° C and 50° C. No growth was observed beyond 50° C, indicating temperature sensitivity; low temperatures between -20° C and 4° C did not allow bacterial growth within 24 h. The pH study demonstrated that these pathogens grow best at pH 7, indicating the importance of environmental pH conditions for bacterial proliferation. Moreover, exposure to UV light led to significant bacterial cell death and DNA damage, highlighting the potential of UV light as a microbial control method. These findings help understand how physiological stress factors impact the growth and survival of foodborne pathogens, providing insights into food safety and public health strategies.

Keywords: Bacillus subtilis, Escherichia coli, Food poisoning, Growth factors, Staphyloccus aureus, Streptococc pyogenes

1. Introduction

Food poisoning occurs when water and various types of food get contaminated with infectious microorganisms, poisons, and chemicals [1]. Food poisoning involves identifying acute illness with gastrointestinal or neurological symptoms affecting two or more individuals who shared a meal within 72 h [2, 3]. Environmental stress impacts bacteria at

molecular and cellular levels, influencing growth and survival [4, 5]. Bacterial growth thrives within specific temperature and pH ranges [6, 7]. Ultraviolet radiation is effective against various microorganisms [8]. Bacteria in diverse ecosystems confront persistent challenges, necessitating adaptation to fluctuating nutrient availability and stressors [9, 10]. The selection of temperature, pH, and UV radiation as factors affecting bacterial growth is justified due to

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https://doi.org/10.70176/3007-973X.1007 3007-973X/© 2024 Al-Ayen Iraqi University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). their significant impact on bacterial physiology and survival [11, 12].

Heat treatment is a crucial method for controlling bacterial growth in various settings, including food safety and medical applications [13]. One study has demonstrated that exposing bacteria to high temperatures can effectively reduce or eliminate pathogenic microorganisms, thereby enhancing food safety and preventing infections [14].

Microorganisms exhibit diverse adaptations to pH, which is crucial for their growth and survival. Acidophiles thrive optimally at pH 1.0 to 5.5 [15]. Bacteria typically favor pH values of 4 to 8 [16]. Though slowed, microbial growth under low pH conditions remains metabolically active [17]. Staphylococcus aureus (S. aureus) can thrive in diverse food matrices due to its pH and salt tolerance [18, 19]. Enterotoxin production in S. aureus is pH-dependent, with optimal conditions between pH 4.5 and 9.6 [20]. Esherichia coli (E. coli) shows optimal survival at pH levels below neutral, with varying heat resistance depending on pH conditions [18, 21]. Notably, E. coli, Bacillus subtilis (B. subtilis) and Streptococcus pyogenes (S. pyogenes) exhibit cytoplasmic pH homeostasis across a wide external pH range [22, 23]. Comprehending how microbial physiology responds to pH is crucial to ensuring food safety and optimizing industrial processes [24].

Following the cold shock, bacterial growth significantly decelerates due to inhibited cellular processes at low temperatures [25]. However, specific genes are activated by temperature downshift, which is pivotal for cold shock response (CSR) and adaptation [26]. Bacterial growth post-cold shock exhibits variations across species and conditions [27, 28].

Recently investigated the efficacy of ultraviolet (UV) light in deactivating bacterial spores critical for food preservation [29], one study evaluated how the absorbance properties of treatment media influenced UV lethality and explored the synergistic effects of UV light combined with mild heating [30].

Due to the vital importance of this topic, this study assesses the susceptibility of *E. coli*, *S. aureus*, *S. pyogenes*, and *B. subtilis* to physiological stressors such as low and high tempretures, pH, and UV light.

2. Methodology

2.1. Culture media and chemical materials

Luria Broth (LB) (Merck, Germany), and Mueller Hinton (MH) broth (Sigma-Alorich, USA) were used. Both media were supplemented with Agar-agar (Merck, Germany), the media were prepared according to instructions of manufacture company. Additionally, Sodium hydroxide (NaOH) (Sigma-Alorich, USA) hydrochloric acid (HCl) (Merck, Germany), were used to alkali and acidify the medium.

2.2. Bacterial cultivation

The Gram-negative bacterial culture of *E. coli* and the Gram-positive bacterial cultures of *B. subtilis, S. aureus*, and *S. pyogenes* were utilized. These cultures were maintained at the Malaysia University of Science and Technology (MUST). The mother cultures were thawed by incubating them at 37°C for 24 h. *S. aureus* and *E. coli* were cultivated using MH agar. In contrast, *S. pyogenes* and *B. subtilis* were cultivated using LB agar [31].

2.3. The parameters

2.3.1. Heat treatment

Bacterial cultures were grown in 50 mL of broth (LB and MH) at 37°C for 24 h. After incubation, each culture were diluted to 10^{-6} and 0.1 mL spreaded onto agar plates. The plates were then incubated at various temperatures (30°C, 37°C, 50°C, and 80°C) for 24 h.

2.3.2. Cold treatment

Cold treatment was conducted following the protocol outlined by Lee [26]. Bacterial cultures were grown in 50 mL of broth (LB and MH) at 37°C for 24 h. After incubation, 1 mL of the bacterial culture were diluted to 10^{-6} before spreading 0.1 mL onto agar plates. The plates were then incubated at different temperatures (4°C, 0°C, -4°C, and -20°C) for 24 h.

2.3.3. pH treatment

The pH treatment procedure followed method described by Eschlbeck [16]. Bacterial cultures were grown in 50 mL of broth (LB and MH) at 37° C for 24 h. Subsequently, 1 mL of each bacterial culture was diluted to 10^{-6} mL, and then 0.1 mL spreaded onto agar plates. The agar plates were adjusted to pH 2 with HCl and pH 10 with NaOH, while another set of plates was maintained at pH 7 as a control. All plates were incubated for 24 h at 37° C.

2.3.4. UV treatment

UV treatment was conducted following the protocols outlined by Kodoth [29]. Bacterial cultures were grown in 50 mL of broth (LB and MH) at 37° C for 24 h. Subsequently, 1 mL of bacterial culture was diluted to 10^{-6} mL, and then 0.1 mL spreaded onto agar plates. The agar plates were exposed to UV light for 6, 12, and 24 h [30]. This method evaluates the effectiveness of UV light exposure on bacterial growth inhibition over varying durations.

2.3.5. Colony Forming Unites (CFU) count

Each bacterial species was cultured on three agar plates per treatment, and the number of growth colonies was counted. The average colony count per agar plate was calculated for each treatment. This approach ensures an accurate assessment of bacterial growth under different experimental conditions [32].

2.3.6. Statistical analysis

The data were presented as mean \pm standard deviation. To determine statistical significance, a one-way analysis of variance (ANOVA) was conducted on the replicates, with a *p*-value of \leq 0.05 being considered significant.

3. Results

An adequate understanding of the factors that influence the growth of foodborne pathogens in foods would offer significant advantages by coordinated approach to preservation the foods against pathogenicity of foodborn bacteria.

3.1. Heat treatment

The colonies grown of *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes* was assessed under varying heat stress conditions. All bacterial strains exhibited growth at 30°C for 24 h (Fig. 1). Optimal growth occurred at 37°C (Fig. 1), reflecting typical body temperature conditions conducive to bacterial growth [33]. However, no bacterial growth was observed at 50°C and 80°C.

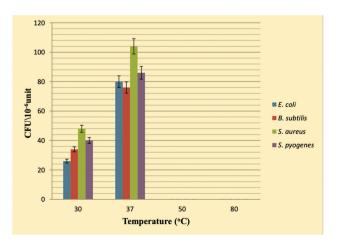


Fig. 1. Effect of heat treatment on the growth of *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes*.

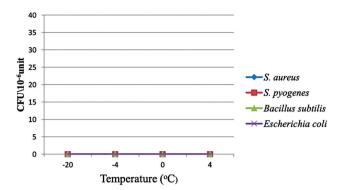


Fig. 2. Effect of cold treatment on the growth *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes*.

3.2. Cold treatment

The colonies grown of *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes* under different cold stress conditions were examined. Fig. 2 illustrates no growth at temperatures of -20° C, -4° C, 0° C, and 4° C at all.

3.3. pH treatment

The effect of pH stress on the growth of *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes* bacteria was evaluated across three pH levels: pH 2, pH 7, and pH 10. Fig. 3 demonstrates growth at pH 7 only and no growth at pH 2 and 10.

3.4. UV treatment

The bacterial cultures of *E. coli*, *B. subtilis*, *S. aureus* and *S. pyogenes* were exposed to UV for time duration of 12 and 24 h. The colonies number drops under UV, as shown in (Fig. 4).

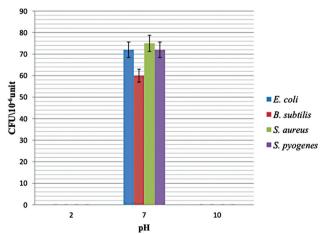


Fig. 3. Effect of pH treatment on growth *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes*.

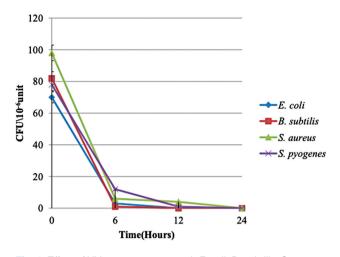


Fig. 4. Effect of UV treatment on growth *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes*.

4. Discussion

Environmental changes profoundly affect bacterial growth and survival, prompting a stress response. This adaptive mechanism involves gene regulation and protein activity, supporting cellular defense and homeostasis restoration. Additionally, it enhances resistance against diverse environmental stresses, a phenomenon known as cross-protection [34].

4.1. Effect of temperature on bacterial growth

E. coli optimally grows at 37°C and are thermal generalist within their growth spectrum. It can be rapidly evolved to be adapted to higher temperatures such as 42°C. E. coli has an extensive temperature range for growth, from 8°C to 49°C, with an optimum of 37°C [35]. Increasing the temperature above 40°C or below 20°C results in a progressively slower growth rate until the growth stops at the determined temperature of 49°C, or the minimum of 8°C. Previous study has shown that a rapid transfer of an exponential culture of the mesophilic E. coli from a temperature within the regular physiological range (i.e., from approximately 20°C to 38°C) to temperatures below 20°C has induced a cold shock response. E. coli SUBE01 lost viability upon heat shock and another remarkable aspect was to evaluate the possible positive effect on the high temperature stressed E. coli SUBE01 growth at critical (45°C) and above critical (47°C) [36].

A study examined *E. coli* growth in three different media (nutrient, Luria-Bertani (LB), and minimal agar) at varying temperatures. Optimal growth occurred at 37° C across all media. In comparison, growth was inhibited at 45° C, evidenced by lower OD₆₀₀ and colony numbers, potentially due to reactive oxygen species [37]. *Bacillus subtilis* exhibited

growth after 24 h of incubation at 37°C and 40°C. but no growth was observed at 50°C and 80°C. These findings align with studies by El-Gayar [38], that investigated the impact of temperature on cell morphology, staining behavior, and growth rate. Previous report indicates that transferring growing cultures of B. subtilis from 37°C to 15°C results in increased optical density and viable count, with cells regaining typical morphology, suggesting recovery from thermal stress. Subcultures of these recovered cells continue to grow well at 15°C. In contrast, cultures transferred to 12°C show growth. However, without recovery from stress, cultures previously at 15°C still grow at 12°C without structural alterations seen in cells with a 37°C history [39]. Another study found that Bacillus spp. grown at 48–53°C produced up to 10^2 CFU/mL, eliminating turbidity and CFUs at 54°C, indicating a critical temperature threshold of 53°C [36].

S. aureus displays a broad temperature range for growth (6.5–46°C), with optimal growth occurring between 30–37°C, and can survive briefly at extreme temperatures ($<6.5^{\circ}$ C, $>46^{\circ}$ C) [31, 40].

S. pyogenic virulence factor expression is influenced by various factors, including osmolality, temperature, pH, and growth medium, with pre-warming the medium to 37°C recommended for optimal growth [41]. *S. pyogenes* can resist temperatures up to 50°C for 30 min but is susceptible to 55°C thermal stress [41].

4.2. Effect of pH on bacterial growth

In one study, *E. coli* exhibited optimal growth at pH values between 4.6 and 9.5, with the highest growth rates observed within the pH 5.5 to 7.5. Notably, *E. coli* demonstrated remarkable survival even after exposure to pH 2.5 for extended periods [21]. This acid tolerance is attributed to evolutionary adaptations, as evidenced by *E. coli's* ability to thrive in highly acidic environments such as the stomach [16]. *B. subtilis*, similarly, displays a broad pH tolerance, maintaining cytoplasmic pH stability within a narrow range (pH 7.3 to 7.6) across environmental pH levels ranging from 6.0 to 9.0. Under acidic conditions, *B. subtilis* exhibits an acid tolerance response, inducing proteins that enhance survival in extreme acidity [42].

The growth characteristics of *S. aureus* are influenced by pH and temperature interactions. *S. aureus* exhibits an optimal growth pH of 6.5, with growth inhibition observed at a pH above 7.5 [43]. Acid stress significantly impacts *S. aureus* viability, as demonstrated by its sensitivity to pH 3.5 [44].

S. pyogenes, commonly found in the oral cavity and pharynx, exhibit limited tolerance to acidic

environments, rendering it susceptible to acid stress [45]. This sensitivity underscores the ecological niche preferences of *S. pyogenes*, which rarely encounters highly acidic conditions in its natural habitats [46, 47]. These findings emphasize bacteria's diverse adaptive strategies to thrive under varying pH conditions [48]. Acid tolerance mechanisms, such as regulatory gene networks and protein adaptations, play crucial roles in bacterial survival and growth in acidic environments [49]. Understanding these adaptive responses is vital for elucidating bacterial pathogenesis and designing targeted interventions to combat bacterial infections [50, 51].

4.3. Effect of UV on bacterial growth

UV radiation is known to kill bacteria by disrupting their genetic material. This inhibits their growth and reproduction, making UV radiation an effective tool in the disinfection process. Its antimicrobial effect is primarily due to the way it affects the pyrimidine bases of microbial nucleic acids. A study conducted on *E. coli* found that even after a 99.9% inactivation by UV irradiation, exposure to fluorescent light induced pyrimidine dimers in DNA. This highlights the effectiveness of UV radiation in preventing the growth and spread of harmful microorganisms. All bacteria were killed in 24 h under UV. UV radiation to produce a spectrum of radiation is used in the disinfection process [34].

The effectiveness of UV radiation against *B. subtilis* spores was assessed by Taylor et al. [52] across different wavelengths (172 nm, 222 nm, and 254 nm), resulting in a 2-log reduction of spores. In the "ADAPT" space experiment, shielding demonstrated protection against extraterrestrial UV radiation, limiting survival chances of even highly UV-resistant strains of *B. subtilis* in outer space [53].

Previous research exposed MRSA to UV radiation for varying durations (5 to 30 sec), with longer exposure times correlating with increased growth inhibition, peaking at 30 sec [54]. UV radiation was toxic to multidrug-resistant bacteria, although surviving colonies retained their resistance traits [55]. Some strains of *S. aureus* showed sunlight sensitivity dependent on the growth phase, with exponentially growing cells exhibiting higher sensitivity [56].

UVC doses ranging from 5 to 15 sec suppressed microbial growth on agar, with varying responses among different bacterial species [57]. *S. pyogenes* was notably less resistant to prolonged UV exposure than *E. coli* [58]. UV is considered from the applications that aim to control microbial growth and disinfection [55].

5. Conclusion

This work has demonstrated that growth by S. aureus, S. pyogenes, B. subtilis and E. coli is influenced by various environmental and nutritional factors, including temperature, pH and UV. The influence of incubation temperature ranges from -20° C to 80° C for 24 h and affects different pH (2, 7 and 10) and UV light at different times (6, 12 and 24 h). The idea is determining how the microorganism behave in food. Suggestions suggest that additional primary and interactive effects must be characterised in various foods. Adequate characterisation of the factors influencing S. aureus, S. pyogenes, B. subtilis and E. coli growth in foods should then allow a more rational means of modifying formulation and processing parameters better to protect consumers from the potential for food poisoning. Identifying interactions between the various factors affecting the bacteria is of particular interest. This would optimise anti-bacterial activity by manipulating multiple environmental and nutritional parameters. In this manner, the microbiological safety of specific foods can be more accurately predicted, thereby allowing a more rational means of optimising both safety and production considerations. Hopefully, future research will be directed towards determining the various primary and interactive effects that can be manipulated to control S. aureus, S. pyogenes, B. subtilis and E. coli on other pathogens in foods.

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