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Effect of Growth Conditions on Hemolysin Production from Clinical Isolates of *Escherichia Coli*

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ABSTRACT: ***Background:*** *Escherichia coli belongs to the Enterobacteriaceae family and is found in the digestive systems of humans and various animals as normal flora.* ***Objective:*** *This study aims to identify the optimal conditions necessary for the synthesis of hemolysin by E. coli isolated from a variety of clinical contexts.* ***Methods:*** *A total of 120 specimens were collected from different clinical sources in Baghdad Hospitals. Screening for hemolysin production was done by plate and Spectrophotometric methods. Furthermore, many conditions for producing hemolysin from clinical E. coli isolates were investigated including temperature, pH, incubation time, inoculum size, culture medium, and aeration.* ***Results:*** *Six isolates of E. coli exhibited hemolysin production. Optimal conditions for hemolysin production were determined: the temperature was 37 °C with an optical density of 0.931, the pH was 7 with an optical density of 0.976, the incubation period was 24 hours with an optical density of 0.885, 1% inoculum resulting in an optical density of 0.558, brain heart infusion as the best medium with an optical density of 1.697, and a shaker method showing superior results (optical density of 1.235) compared to static aeration (optical density of 0.988).* ***Conclusions:*** *This study identified the optimal conditions necessary for the synthesis of hemolysin by E. coli. By understanding these optimal conditions, researchers may be better equipped to develop strategies for managing and controlling the spread of these pathogenic bacteria.*

KEYWORDS: *E. coli; Hemolysin; Growth conditions; Clinical specimens; Normal flora*

INTRODUCTION

Escherichia coli (*E. coli*) is a type of bacteria that exists as a beneficial symbiont in the digestive systems of humans and various animals. Thus, this bacterium is crucial in aiding digestion and synthesizing specific vitamins. Despite that, it can cause many bacterial infections, which makes this bacterium a significant concern for public health. In addition, the emergence of drug-resistant strains of *E. coli* presents a serious global issue [1]. Antibiotic resistance assists the bacterium to survive and proliferate, even in the presence of treatments that are generally effective against bacteria. The production of certain chemicals, including bacterial hemolysin is considered an essential factor for *E. coli* pathogenicity [2]. The hemolysin assists in the invasion of bacteria and hemolyze red blood cells (RBCs), which can lead to the release of hemoglobin. Hemolysins are a type of protein known for their ability to form pores and they are produced by various bacteria including *E. coli* [3]. This process culminates in the release of hemoglobin, a crucial protein involved in oxygen transport in the blood. In the case of *E. coli*, multiple types of hemolysins have been identified including α-hemolysin (HlyA), enterohemolysin (EhxA), and hemolysin E (HlyE) [4]. These proteins assist the bacteria in colonizing and invading host tissues and can cause significant damage to the host organism making *E. coli* a key focus in the fight against bacterial infections. Enterohemorrhagic *E. coli* (EHEC) is a subtype of the broader *E. coli* species, is known to produce two specific hemolysins, EhxA and HlyE. Burgos and Beutin [5] discovered a significant similarity between EhxA and HlyA, the latter being a major virulence factor found in both the chromosome and plasmid of pathogenic *E. coli* strains. EhxA is a potent virulence factor linked to severe illnesses such as Hemolytic Uremic Syndrome (HUS)

and Hemorrhagic Colitis (HC) [6]. This highlights the role of such factors in the development of life-threatening diseases. Interestingly, the HlyA secretion mechanism in uropathogenic *E. coli*, a type known to cause urinary tract infections (UTI), was the first identified example of a type-1-secretion system (T1SS) [7]. The prognosis for patients suffering from UTI and who also exhibit alpha-hemolysin secretion is generally poor [8]. This is primarily because uropathogenic *E. coli* (UPEC) strains are often associated with more severe infections. Common EHEC strains can be recognized by their high levels of EhxA, making it a useful phenotypic marker [8]. This extracellular hemolysin, produced by *E. coli*, stands as a significant virulence factor [6]. It is implicated in infections that occur outside the intestines adding to the complexity of *E. coli*'s pathogenicity.

MATERIALS AND METHODS

During a period between September to December 2022, 120 clinical specimens were collected from different clinical sources (urine, stool, wound, and blood) from Baghdad Al-Shaheed Al-Sadr Hospital, Ibn Al-Balady Children & Maternity Hospital, Ibn Alkuf Hospital for Spinal Cord Injuries and Al-Kindi Teaching hospitals. All isolates were cultured primarily in enclosed containers containing nutrient agar then sub-cultured in brain heart infusion broth bottles and incubated at 37 °C for 24 hours to enrich the bacteria, then sub-cultured on MacConkey agar, Blood agar, and Nutrient agar [9].

Phenotypic characteristics were determined by colony morphology on the culture media that differ in shape, size, odor, color, texture, opacity, and margin of colonies. Gram stain according to Brown and Smith [10] was used to detect and differentiate the form and arrangement of bacteria via microscopic examination. Catalase, oxidase, and IMVC tests were used for the identification of *E. coli* [10]. The identification of *E. coli* was confirmed by the VITEK-2 system.

Screening of *E. coli* for Hemolysin Production

1 Plate Method (Agar Medium)

From 18-hours cultures of various *E. coli* strains, bacterial suspensions in sterile saline matching 1.5×10^8 CFU/ml were prepared. The human blood agar medium was completely covered with 10 l of each suspension, and it was then incubated at 37 °C for 16 hours. The hemolysis was measured after 16 hours.

2 Spectrophotometric Method (Liquid Medium)

With slight modifications, the spectrophotometric approach published by Di Venanzio *et al.* [11] was used to determine the synthesis of hemolysin in a liquid medium. *E. coli* was cultured overnight in nutrient broth at 37 °C. Twenty µL of nutrient broth was combined with 20 µL of *E. coli* and 0.5 µL of McFarland (McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). The mixture was then incubated at 37 °C for 24 hours. Red blood cell (RBC) suspension was made by centrifuging 2 mL of blood (0.8 milliliters of RBC) three times with eight milliliters of Phosphate-buffered saline (PBS) buffer for five minutes, then adding the sediment to the PBS buffer (9.2 milliliters of PBS buffer), 1 mL of bacterial culture and one milliliter of RBC suspension, all of which were incubated at 37°C for one hour to supernatant the *E. coli* culture. According to Hertle *et al.* [12], complete hemolysis of RBCs was carried out by adding 1% Triton X-100, and the relative optical density for the specimen was compared to (OD) for complete hemolysis of RBCs. Calculation of hemolysis was performed as the following:

$$Hemolysis\% = \frac{A571(\text{specimen with hemolysin}) - A571(\text{control without hemolysin})}{A571(\text{total lysis caused by Triton x-100}) - A571(\text{control without hemolysin})} \times 100$$

The Best-growing Conditions for Producing Hemolysin from Clinical *E. coli* Isolates

1 Temperature

The *E. coli* species were dispersed in tubes filled with nutrient broth and subjected to culture conditions for 24 hours. This was performed at a range of distinct temperatures, specifically 25, 30, 37, 40, and 45 °C, to determine the appropriate temperature for hemolysin production [11]. Following the

incubation period, the proportion of hemolysin produced at each of the specified temperatures was determined and recorded.

2 pH

The nutrient broth was formulated at varying pH levels, specifically 5, 6, 7, 8, and 9. The pH was fine-tuned using an electronic pH meter, along with 1N solutions of NaOH and HCl. Once the desired pH was achieved, the media were prepared and sterilized using an autoclave. Subsequently, these media were inoculated with a suspension of *E. coli* and incubated at the optimum temperature. The media were also maintained at the most effective pH. Following these procedures, the rate of hemolysin production was evaluated for each pH level [11].

3 Incubation Time

The nutrient broth was imbued with a suspension of *E. coli* bacteria and then incubated under the optimal conditions identified earlier – these conditions pertained to both temperature and pH. The incubation periods were set at 24, 48, and 72 hours. After each respective incubation period, the degree of hemolysin production was assessed and quantified [11].

4 Inoculum Size

To discern the influence of inoculum size on the production of hemolysin, the pH of the culture medium (Nutrient broth) was fine-tuned to the optimal level. Subsequently, this medium was imbued with varying sizes of inoculum, specifically 1, 2, 4, 6, 8, and 10% of an inoculum, each possessing a concentration of 1.5 x 108 CFU/ml for the ECU1 isolate. These preparations were then incubated at the ideal temperature, for the most effective incubation duration. Upon the conclusion of these procedures, the proportion of hemolysin production was calculated for each inoculum size.

5 Culture Media

As part of the experiment, three distinct types of media were utilized: Nutrient broth, Brain heart infusion broth, and Tryptic soy broth. Each broth was adjusted to the optimal pH. Subsequently, these broths were inoculated with the bacterial suspension using the most effective inoculum size. Following inoculation, the specimens were incubated for the optimal period, at the ideal temperature. At the end of these procedures, the degree of hemolysin production was measured and recorded for each type of culture medium.

6 Aeration

The experiment was extended to analyze the impact of aeration on the production of hemolysin. To this end, the optimal culture medium, Brain heart infusion was fine-tuned to the best pH. This medium was then inoculated with the bacterial suspension using the most effective inoculum size. The specimens were subsequently incubated at the optimal temperature. Two conditions were examined: one with shking at 150 rpm to simulate aeration, and one without shaking, representing a static condition. This was done for the ideal incubation duration as established. Following these procedures, the proportion of hemolysin production was assessed for each condition.

RESULTS AND DISCUSSION

Distribution of Specimen

The presence of *E. coli* was found in 83 out of 120 clinical samples, the results showed in Table 1 that a higher incidence of *E. coli* isolates was from urine 41 (49.4%), followed by wound 18 (21.7%), stool 14 (16.9%) and blood 10 (12%).

Table 1. Distribution of *E. coli* according to clinical specimens

Clinical Sources	No. (%)
Urine	41 (49.4%)
Stool	14 (16.9%)
Wound	18 (21.7%)
Blood	10 (12%)
Total	83 (100%)

E. coli is widely recognized as a prevalent causative agent in bacterial infections [4]. The findings from this study align with those reported by Naqid *et al.* [1], who found a significantly higher occurrence of *E. coli* in urine specimens (92.2%) compared to wound (3.9%) and blood (0.9%) specimens. This higher prevalence in urine could be attributed to predisposing factors for UTIs such as inadequate hygiene and low socioeconomic status [12]. These results also concord with the findings of Ghaffoori and Suleiman [13], who observed a higher prevalence of *E. coli* in urine specimens (n = 49) compared to wound specimens (n = 10). Similarly, Kadhim [14] reported a higher incidence of *E. coli* in urine specimens (72.13%) as opposed to wound specimens (16.66%). These studies collectively suggest a trend toward a heightened incidence of *E. coli* in urine specimens across different research contexts.

Isolation and Identification of *E. coli*

A series of biochemical tests were conducted to characterize the *E. coli* isolates. These tests included the oxidase test, catalase test, indole test, methyl red test, Voges-Proskauer test, and citrate utilization test. The results of these tests are presented in Table 2. It was found that the *E. coli* isolates tested positive for indole production, methyl red, and catalase. Conversely, the isolates tested negative for Voges-Proskauer, Simmons Citrate, and oxidase.

Table 2. Biochemical Test Reaction of *E. coli*

Biochemical test	Result
Oxidase Test	Negative
Catalase Test	Positive
Indole Test	Positive
MR Test	Positive
VP Test	Negative
Citrate Test	Negative

* MR: Methyl Red, VP: Voges-Proskauer

The biochemical test results from this study align with those reported by Al-Sabawi and Jwher [15], and Rhumaid *et al.* [16]. They also found that *E. coli* isolates tested positive for indole production, methyl red, and catalase, and tested negative for Voges-Proskauer, Simmons Citrate, and oxidase. These findings are further corroborated by Mahe *et al.* [17], who similarly reported that *E. coli* isolates were positive for indole production and methyl red, and negative for Voges-Proskauer and Simmons Citrate. These collective results signify a consistent pattern in the biochemical characteristics of *E. coli* across diverse studies. The catalase test is utilized to detect the presence of the enzyme catalase in bacteria. This enzyme facilitates the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. In *E. coli*, two types of catalase enzymes, namely hydroperoxidase I (HPI) and HPII, are present, both of which catalyze the degradation of peroxide [18]. The indole test reveals the ability of a bacterium to metabolize tryptophan using the enzyme tryptophanase, resulting in the production of indole. The presence of indole is indicated by a pink-to-red coloration that develops upon the addition of Kovacs' reagent. The methyl red test, significant in differentiating certain gram-negative intestinal bacteria, assesses mixed-acid fermentation. In this process, the acids produced are sufficient to reduce the pH of the MR-VP broth to 5.0 or less. In an environment with these acids, the pH indicator methyl red turns red. A negative methyl red test implies that the organism does not engage in mixed acid fermentation [10]. *E. coli*, along with other low-ratio organisms, employ the mixed acid pathway to

ferment sugars, resulting in a low ratio of CO_2 to H_2 gas produced during fermentation. This process yields acidic by-products (primarily lactic and acetic acids), neutral fermentation products such as ethanol, as well as CO_2 and H_2 for each mole of glucose fermented. The substantial amount of acids generated prompts a significant drop in the pH of the culture medium. When the culture medium turns red upon the addition of the indicator methyl red, it indicates a pH of 4.4 or lower due to glucose fermentation. This color change signals a positive result for the methyl red (MR) test [19]. The Voges-Proskauer test established that the organism was incapable of leveraging the butylene glycol pathway, and hence, did not produce acetoin. The citrate utilization test further demonstrated that the organism could not use citrate as its sole carbon source, likely due to the absence of a citrate transporter. This inability to utilize citrate consequently prevented the medium from undergoing a color shift from green to blue.

Screening of *E. coli* Isolates for Hemolysin Production

1 Plate Method

According to hemolysin production, 6 isolates of *E. coli* were hemolysin produced, as shown in Tables 3 & 4 and Table 5. The results in Table 3 showed that 6 (7.23 %) out of 83 *E. coli* isolates were hemolysin producers and 77 (92.77%) were non-producers, higher incidence of hemolysin production was in *E. coli* isolates isolated from stool 2 (14.3%) and wound 2 (11.1), and lower in urine 1 (2.4 %) and blood 1 (10%).

Table 3. Hemolysin Production by *E. coli* Isolates

Clinical Sources	No. (%)	Positive	Negative
Urine	41 (49.4%)	1 (2.4 %)	40 (97.6 %)
Stool	14 (16.9%)	2 (14.3%)	12 (85.7%)
Wound	18 (21.7%)	2 (11.1)	16 (88.9%)
Blood	10 (12%)	1 (10%)	9 (90%)
Total	83 (100%)	6 (7.23 %)	77 (92.77 %)

Table 4. Hemolysin Production by plate method by the clinical *E. coli* isolates grown on blood agar after 24 h

***E. coli* Isolates No.**	**Diameter of Hemolytic Zone (mm)**	**Clinical Source**
Ec 1	25	UTI
Ec 2	16	Stool
Ec 3	15	Wound
Ec 4	13	Blood
Ec 5	12	Stool
Ec 6	10	Wound

The findings from this study contrast with those reported by Hussein and Al-Hasany [20], who found that 42.6% of *E. coli* isolates from urine were hemolysin producers. In contrast, none of the urine isolates in this study were hemolysin producers. When considering hemolysin production from stool specimens (14.3%), the results from this study are comparable to those reported by Mare *et al.* [21], who found that 18.2% of the stool strains were hemolysin producers. They emphasized the need for continually updating laboratory protocols to ensure accurate and current diagnostic procedures. Bírošová *et al.* [22], reported that out of 200 *E. coli* isolates (across various clinical sources including urine specimens, and vaginal and rectal swabs), all but one strain exhibited a hemolytic phenotype.

Table 5. Hemolysin Production by *E. coli* Isolates by liquid medium

***E. coli* Isolates No.**	**Absorbance of hemolysin (OD-571 nm)**
Ec 1	0.998
Ec 2	0.925
Ec 3	0.724
Ec 4	0.513
Ec 5	0.918
Ec 6	0.828

Meher Rizvi et al [23] reported that out of the 103 *Escherichia coli* isolates they studied, 56 were hemolytic and 47 were non-hemolytic. Moreover, the majority (66% or 35 strains) of hemolytic *E. coli* were of extra intestinal origin, while the remaining 44% (or 21 strains) were of intestinal origin. The results from this study align closely with those of Vaish *et al.* [24], who found that 9% of *E. coli* strains produced hemolysin. Since it was discovered that (50%) of these isolates are hemolysin-producing, some strains of *E. coli* bacteria particularly those isolated from infections outside the human digestive canal produce hemolysin. Hemolysin production according to earlier research is between 16.6 and 41% [25]. It was discovered that most of the strains of *E. coli* bacteria present as a natural microflora in the human intestine are non-producing hemolysin, but once they settle in the urinary tract, they can produce it. This indicates that hemolysin produced from strains of *E. coli* bacteria is important in the virulence of the bacteria especially in infections outside the intestines of various animals. Alpha-hemolysin production by some strains of *E. coli* bacteria that cause infections outside the intestine is an important component of this bacteria's virulence. Hemolysin binds to protein-fatty bodies found on the surface of the target cell, creating membrane holes in the host cells [26]. Therefore, hemolysin serves as a route for bacteria to access nutrients from host cells. For instance, "iron" plays a determining role in the development of numerous bacterial diseases.

Determining the Optimum Growing Conditions for Producing Hemolysin from Clinical *E. coli* Isolates

1 Temperature

The chosen *E. coli* isolates (EcU1) were cultured under various temperatures (25, 30, 37, 40, and 45 °C) to ensure the ideal temperature that facilitates hemolysin production. It was determined that the peak production of hemolysin occurred at a temperature of 37 °C, which was associated with an optical density of 0.931, as depicted in Figure 1.

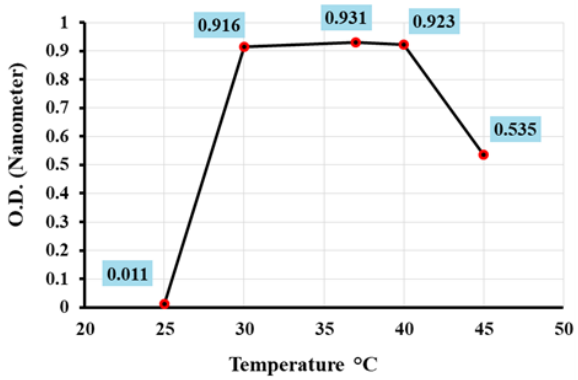

Figure 1. Effect of temperature on hemolysin production from clinical isolates of *E. coli* (EcU1)

A local study by Al-Shammary *et al.* [27] on variable bacteria S. aureus concluded that the best hemolysin production was at the temperature of 35-40 °C. Much pathogenicity of bacteria is related to the expression of particular genes, which are required for growth and continued existence under changing ecological conditions.

A study by Vaish *et al.* [24] showed that hemolysin activity was at 35 °C, they noticed hemolysin production was detected by the presence of a zone of complete clearance of erythrocytes around the colony as observed against transmitted light. Variable studies on different bacteria that produce hemolysins showed that bacterial cells cultured at 37 °C gave 10-fold less lysis compared with those grown at 30 °C because of altering in LPS structure and decreasing the levels of the outer membrane protein ShlB (Serratia hemolysin B protein) at 37 °C, also these results indicated a significantly increased in expression of shlA(Serratia hemolysin A protein) gene in 30 °C.

2 pH

A variety of media with pH values (5, 6, 7, 8, and 9) were used to determine the best pH for producing hemolysin. It appeared that the best pH for hemolysin production was 7 with an optical density of 0.976, as shown in Figure 2.

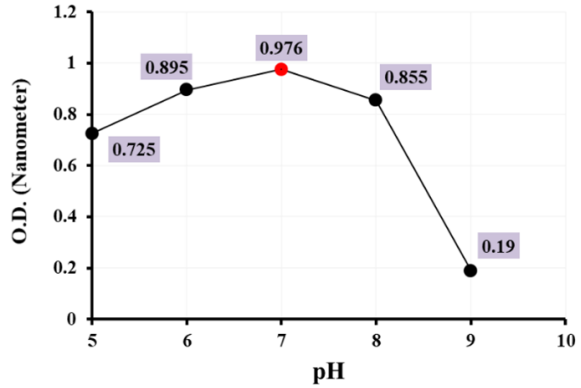

Figure 2. Effect of pH on hemolysin production from *E. coli* (EcU1)

The current study agreed with a local study conducted by Mare *et al.* [21], who worked on Diarrheagenic *Escherichia coli*, they showed that hemolysin production and hemolytic activity on blood agar was at pH 7.4. A local study by Al-Shammary *et al.* [27] study on different bacteria concluded that the best hemolysin production was in the pH near neutrality pH 7-7.5. A local study was carried out by Razzaq *et al.* [28], they detected hemolytic activity of hemolysin purified by ammonium sulfate at pH 6.8, which was approximate to the current result. Naskar *et al.*, 2023 [29] examined the hemolysin production produced by uropathogenic *E. coli* at pH 7.4 which was approximate to the current result.

PH can alter the physiology of microorganisms such as the optimal activity of enzymes, which in turn influences the success of the biological therapy process. pH values that are very high or low may result in a total loss of enzyme function. The best pH value for one enzyme may vary depending on many factors, including the source of the enzyme, even though the enzymes have good activity at physiological pH levels, which are around 7.0, another factor that also varies of best pH value for enzyme depending on are: existence of materials, enzyme immobilization, mutations in enzymes, environmental motivations such as temperature, pH and nutrient availability [30]–[32].

3 Incubation Time

In this investigation, the best incubation duration was found to be 24 hours with an optical density of 0.885, as shown in Figure 3. This study evaluated the best incubation times that might be used to estimate hemolysin production by *E. coli*.

A local study by Abdulwahhab and Khalaf [33] on different bacteria *S. marscecens* showed that the best incubation period was 24 hrs., at this time the absorbance at 571 nm for hemolysin production was 3.0. However, Maarib *et al.* [34] on the same bacteria concluded that the optimum incubation time was 48hrs. A study by Stonsaovapak 2000 [35] showed that the production of hemolysin (produced from Vibrio parahaemolyticus) could be detected after 6 or 8 hrs. of incubation. At these same temperatures, bacterial strains produced comparable cell numbers by 12 hrs.; However, the greatest hemolysin production occurred at 35 °C. This indicates that temperature, rather than cell numbers determines the total hemolysin produced during a controlled incubation period.

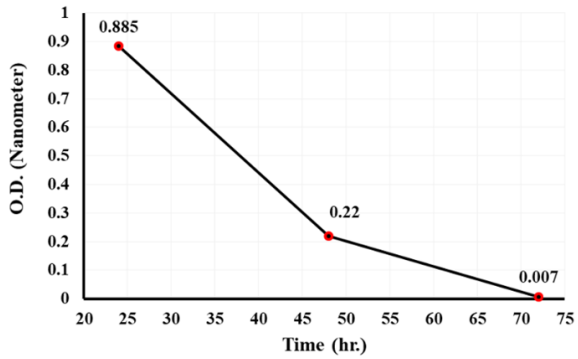

Figure 3. Effect of Incubation Time on hemolysin production from *E. coli* (EcU1)

4 Inoculum Size

The current study studied how inoculum size affected hemolysin production. Different inoculum sizes including 1, 2, 4, 6, 8, and 10% of (1.5108) CFU/ml, were used for growing *E. coli* (EcU1) isolate. The best inoculum for hemolysin production, as determined by the optimal inoculum was 1%, as shown in Figure 4, with an optical density of 0.558.

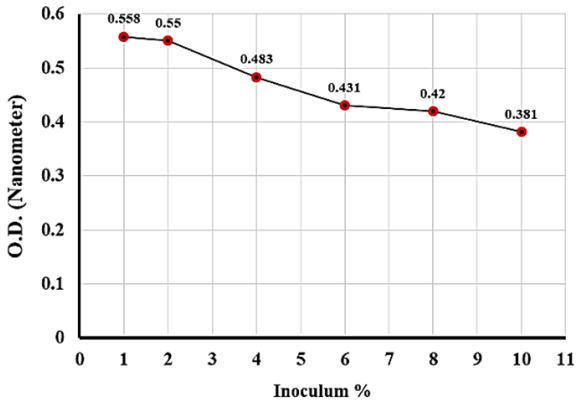

Figure 4. Effect of Inoculum on hemolysin production from *E. coli* (EcU1)

The current study similar to the result reported that a greater inoculum size of 11% (v/v) was found to reduce protease generation more than a smaller inoculum of 1% (v/v) by Mabrouk *et al.*, [36]. Therefore, a stronger protease production may not necessarily be indicated by larger inoculum sizes. The increase in protease production with lower inoculum sizes was due to the higher surface area to volume ratio, which led to improved protease synthesis. An extremely tiny inoculum size indicates an insufficient number of bacteria, which lowers the amount of released protease. On the other hand, larger inoculum sizes may cause or contribute to a lack of oxygen and nutrient depletion in the culture media [37].

There are various ideas for expression of the inoculum effect, these ideas include population diversity and quorum sensing, as well as the theory, which suggests that the effect of inoculum influence and changes in the growth/no growth border about inoculum size are dependent on the time it takes for a particular inoculum size to achieve growth under the specific environmental conditions [37]. In the two-stages inoculum system, there was also a significant interaction between the primary and secondary inoculums [37].

5 Media Culture

It investigated how culture medium affected hemolysin production. Tryptic soy broth (TSB), Brain Heart Infusion broth (BHI), and Nutrient broth (NB) were used to incubate *E. coli* (EcU1). As demonstrated in Figure 5, the best media for hemolysin production was brain heart infusion with an optical density of 1.697.

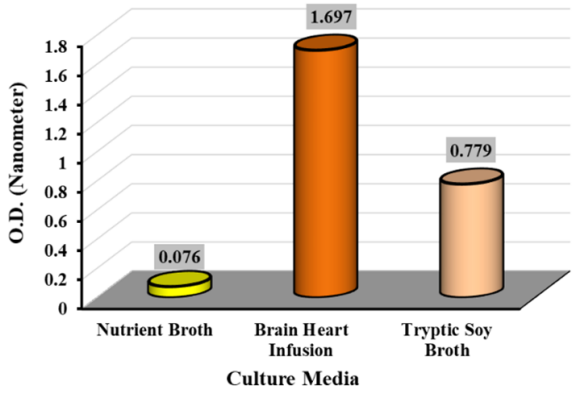

Figure 5. Effect of Culture Media on hemolysin production from *E. coli* isolate (EcU1)

The current results agreed with Rahim and Aziz [38] study on different bacteria Vibrio fluvialis who concluded that BHI media was the best medium for hemolysin production but disagreed with Abdulwahhab and Khalaf 2022 [33] study on *S. marscecens* bacteria showed that best culture medium for hemolysin production was the Nutrient broth.

6 Aeration

According to the optimum aeration of hemolysin production, the results showed that the shaker with an optical density of 1.235 was better than static aeration with an optical density of 0.988, as shown in Figure 6.

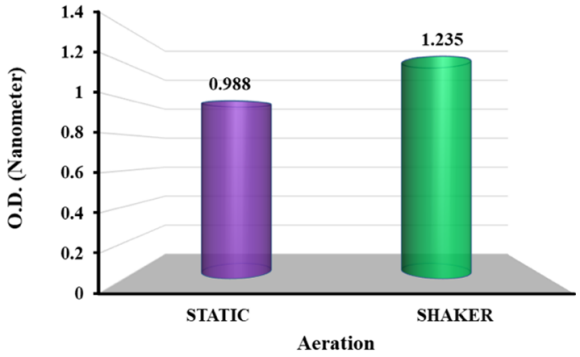

Figure 6. Effect of Aeration on hemolysin production from *E. coli* isolate (EcU1)

Mantzouridou *et al.* [39] elucidated that aeration serves dual purposes in the fermentation process. It not only provides necessary oxygen for cellular growth but also aids in eliminating exhaust gases produced during fermentation. Nonetheless, an increased aeration rate might lead to a decrease in the volume of the fermentation broth. Oxygen supply is essential for microbial growth during aerobic fermentation [39]. Kamble *et al.*. [40] demonstrated that aeration impacts the concentration of dissolved oxygen in the culture medium, thereby enhancing both growth and enzyme production. Zotta *et al.*, [41] further explained that in aerobic fermentation, the presence of oxygen influences enzyme secretion, which can be attributed to the metabolic activities occurring within the organism. Similarly, Elmansy *et al.*, [42] cited various studies reporting that the production of amylase by Bacillus spp. is significantly influenced by the presence of dissolved oxygen. Therefore, introducing air to the fermentation medium under sterile conditions using a compressor could be a promising approach as it enhances efficiency by combining agitation with aeration [42]. The current results disagreed with another study on variable bacteria by Abdulwahhab and Khalaf [33] showed that static aeration was better than a shaker. In addition, disagreed with Benedict and Schultz [43] who studied the Listeria monocytogenes strain hemolysin activity, which showed that hemolysin activity was consistently greater in static cultures than in aerated cultures for reasons unknown.

CONCLUSION

An incubation period of 24 hours with an optical density of 0.885, and 1% bacterial inoculum were the conditions for producing hemolysin with an optical density of 0.558. The optimal culture medium was the heart and brain marrow medium to produce hemolysin with an optical density of 1.697. Vibrating ventilation with an optical density of 1.235 was better than static ventilation with an optical density of 0.988.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Eman Khadum Juda: Performed the experiments, and draft writing. Khawlah Jebur Khalaf: Conceptualization, methodology, and investigation. Mounir M Salem-Bekit: Validation, and writing review.

FUNDING

None.

DATA AVAILABILITY STATEMENT

Data is available in the article.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

The Ethics Committee of Mustansiriyah University/College of Science approved this study. The reference number for ethical approval was No. 256 on 12/1/2023.

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