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# Detection of the Ability of Salmonella Typhi Isolation from Iraqi Patients to Produce Biofilm

Menan Abdul-Razaq Abdul-Khadum , Ali Jaffar Saleem*
menan.abdulrazaq@uodiyala.eduo.iq
* College of Education for PureScience- Diyala University, alijaffar3@yahoo.com

**Abstract**
In current study, 29 clinical isolates Salmonella typhi were collected from different Private laboratories in Diyala. The results showed that 29 isolates belonged s. typhi based on phenotypic, implantation and biochemical tests, as well as molecular diagnostics of the 16SrRNA gene using PCR technology.
The ability of bacteria to produce biofilm was studied in two ways, the first was on Congo Red agar , and the biofilm formation was 41.38%, while the second method was Microtittter plate using Tryptic soya broth media fortified with 1% of glucose, and the biofilm formation was 100%, of which the strong biofilm formation was 17.24%, the moderate biofilm formation was 20.69%, and weak formation biofilm was 62.07% .
Also, some of the factors affecting biofilm production were studied, including temperature, pH, carbon and nitrogen sources using Mineral salts broth media . The results showed that the temperature of 30 °C and pH = 7 is better for biofilm formation. The results also showed that the best carbon sources for biofilm production are lactose and glucose in While the inorganic nitrogen sources were the best in biofilm formation compared to the organic nitrogen sources.

**Keywords**: Salmoella typhi ,16SrRNA gene, biofilm formation.

## Introduction

The genus salmonella is one of the genera of the intestinal bacterial family and includes about 2600 serotypes, salmonella is a gram stain-negative bacterium, classified into two types S. enterica and S. bongori (ALJobouri , 2019). They move by flagella, are non-spore-forming , contain pilli (Grimont et al., 2000) .Salmonella grows at optimum pH between 6,5-7.5 and temperature is 37C°,also it can grow at pH between 3-9.5 and temperature between 3- 52C° (ALJobouri, 2019).
Recent international statistics indicate that typhoid fever contributes to 21.6-26.9 million cases of illness and 21,600 deaths annually (WHO, 2018; Wong et al., 2019).
Most bacteria have the ability to form closed matrix aggregates or biofilm (Abbas and Ahmed, 2014), which may help them get rid of the host's immune system (Prouty *et al.*, 2002). A biofilm is defined as complex microbial assemblages that are attached to solid surfaces and adhere to them, and this activity is affected by the surrounding

environmental conditions, biofilm is important for determining chronic infection (Al-Khafaji and Al-Kaabi, 2016).

The bacterial biofilm formation is a very complex property and can be influenced by various factors, both intra- and extracellular. The formation of biofilms is usually associated with nutritional deficiency and stress, especially toxic substances, promote cells to aggregate to collectively reduce the surface area under stress( Under conditions of stationary phase stress or exposure to harmful substances) and cell aggregation, the cell surface becomes more hydrophobic (Puhm *et al*., 2022).

## METHODS AND MATERIALS

### I. SAMPLE COLLECTION AND IDENTIFICATION

During the period from September 2021 to December 2021, 75 blood specimens were collected at suspected outpatient's patients for typhoid fever in Diyala .The specimens were transplanted on MacConkey ,Blood agars also on XLD and S-S agars ,that incubated at temperature of 37C° for 24-48 hours, diagnosed on phenotypic, cultural characteristics ,microscopy with gram stain and biochemical tests: catalase and imvic (Macfaddin ,2000).

### Molecular Identification

DNA extraction from *S.typhi* isolates by Genomic DNA extraction kit (ABIO, USA). DNA preparation were then analyzed via electrophoresis thereof 1.5% agarose gel. PCR be used to amplify the entire sequences of the genes studied in this research. The specific primers (Macrogen, Korea) utilized for the expansion to these genes were shown in (table1).

PCR mixture was prepared by adding 12.5µl of GoTaq®Green master Mix (2X) promega,2µl template DNA, 1µl from each forward and reverse primers with final concentration 1 poml/µl, finally volume was completed to 25µl by adding nuclease free water by thermal cycle ,and the temperature of annealing was 58C°,and PCR products were detected in 1.5 % agarose gel for 1 hr. at 75 V, stained with ethidium bromide and visualized by transilluminator.

### II. TABLE (1): PRIMER AND AMPLIFIED PCR PRODUCTS USED IN STUDY

| *a) Gene* | **Primer sequences (5'- 3')** | **size(bp)** | **Reference** |
|---|---|---|---|
| *16SrRNA* | F: CCTGGACAAAGACTGACGCT<br>R: CGCTTCTCTTTGTATGCGCC | 523 | *B. Al-Oqaili,2019* |

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**Detection of Biofilm** :

Congo Red agar was present as stated in (Freeman et al., 1989). As this medium is used to detect the ability to produce biofilm bacteria, also the Micro-titter plate method was used to quantitatively detect the biofilm as stated in (Kerkeni et al., 2016).

Some factors temperature, pH, carbon sources and nitrogen sources that affect biofilm production were studied using Mineral salt broth medium prepared according to the method( Beck, 1971; Al-Sa'adi, 2014). Biofilm production was detected using Microtitter plate using the method (Kerkeni et al., 2016)

## RESULTS AND DISCUSSIONS

### Identification of *S. typhi*

Bacteria isolates have been diagnosed in two ways: the first is phenotypic by microscopic ,it's gram negative and cultural characteristics (on XLD agar showed red color with or without black center for $H_2S$ production ,and on S-S agar showed pale yellow for not fermentation lactose with a black center for producing $H_2S$, in addition of biochemical tests, the second is molecular detection by polymerase chain reaction (PCR).

The results of the current study showed 29 (38%) isolation of *S.typhi* depending on phenotypic and culture characteristics. Also genetically diagnosed by *16SrRNA* gene and the isolates of *S.typhi* possessed the *16S rRNA* gene 100%. (Fig.1).

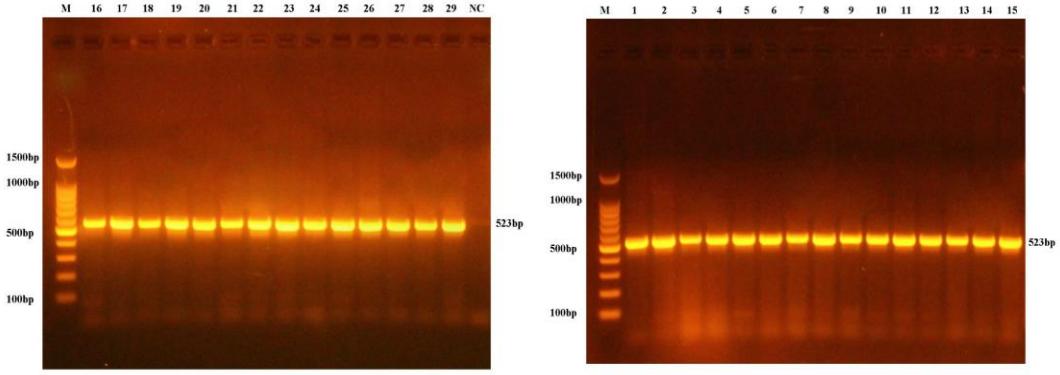


**Fig.1**: Agaros gel electrophoresis (1.5 agaros,100 V for 1h ,for *16SrRNA* gene, DNA ladder (100-1500 bp).

This gene contains conserved regions that overlap with variable regions ,there are 9 variable regions, that are useful in determining genus and orders bacteria because *16SrRNA* is present in all bacterial species and also has little variable( Suardana,2014 ;Srinivasan et al ,2015).

The results showed that the percentage of biofilm productivity was 41.38%, while the percentage of non-productive bacteria was 58.62% (17 isolates) out of 29 isolates.

As for the second method, it was using Micro-titter plate using Tryptic soya broth medium fortified with 1% of glucose, and the percentage of biofilm productivity was 100%, of which the strong biofilm was 17.24% (5 isolates), the moderate biofilm was 20.69% (6 isolates), and the bacteria The weak membrane was 62.07% (18 isolates).

The results of the study conducted by the researcher Abd (2019) in Al-Najaf showed that the percentage of biofilm-producing *S.typhi* isolates was 93.75%, of which 31.25% were strong formed, 62.5% were moderate forming, and 6.25% were non-productive. Also, in a study conducted by Verstraeten et al. (2008), the percentage of bacterial production of the biofilm was 34.9%, of which 11.6% were strong , 20% were moderate, 3.3% were weak, and 65.1% were non-productive, so the results of both studies do not agree with the results of The current study .

The formation of the biofilm helps the cell to tolerance various factors such as low pH, nutrient deficiencies, and host defenses, thus continue infection (Sharma *et al*., 2014). The process of detecting biofilm formation depends on many factors, including the detection method used, incubation conditions, and temperature (Soto *et al*., 2007).

The results showed the effect of temperature on biofilm production, the highest productivity was at a temperature of 30 C°, while the productivity decreased at a temperature of 25 C° and 39 C°. In the study of Al-Sa'adi (2014), the results of biofilm formation were at different temperatures (30-45), as the maximum biofilm formation was at a temperature of 35C°.

The results showed that the maximum production of biofilm at pH = 6,7,8, and decreased at pH = 3,4,5,9,10. The biofilm is a means to protect bacteria from the surrounding environmental factors. The pH affects the production of biofilm through its effect on changing the properties of the nutrient medium and the solubility and available of nutrients. It turns out that pH = 7, which is close to the pH of body fluids, enhances virulence factors and biofilm formation, and thus the bacteria will be more effective in the occurrence of infection.

In a study conducted by researchers Roy *et al*. (2021) Salmonella enterica serotype Kentucky, the productivity of biofilm was at a temperature of 25-42 C° and at a pH between 6-8, but the productivity of the optimum biofilm was at a temperature of 37 C° and at pH = 7. Ramli *et al*. (2012) reported that the maximum biofilm formation achieved was at pH=7.2, indicating that biofilm formation may decrease significantly when the medium is more acidic or basic.

Carbon sources (glucose, lactose, fructose, sucrose, and starch) were used to study biofilm productivity, and the results showed that glucose and lactose are the best sources of carbon for biofilm production, as they gave high biofilm productivity compared to other carbon sources (fructose, sucrose, and starch). ) which had low productivity at pH = 7 and a temperature of 37C°. In the study of the researcher Mahdi *et al*. (2020), the biofilm productivity was high when 1% glucose sugar was added to *Staphylococcus aureus* medium, and biofilm productivity was moderate for

*Pseudomonas aeruginosa*, as glucose stimulates multicellular aggregation and biofilm formation. Abd *et al*.(2014) mentioned that the carbon source has an effect on the growth of bacteria, especially in production of enzymes and biofilms, biofilm formation associated with an increased concentration of carbon sourses, as it has been proven that *P. aeruginosa* biofilms can be dispersed by adding simple carbon sources. When using nitrogen organic sources and under the same conditions, the results showed that the productivity was higher when using peptone, yeast extract, ammonium chloride, sodium nitrate, while the productivity decreased when adding casein and tryptone. The results of the researcher Al-Sa'adi (2014) study indicated a high biofilm formation in the presence of yeast extract and peptone (organic sources) compared to the inorganic sources NH4Cl and NaNO3.Nitrogen is one of the main sources of growth, as nitrates play an important role in the growth of *P.aeruginosa* and the formation of biofilms. Nitrate also contributes to the regulation and expression of virulence factors in *P.aeruginosa* (Abd *et al.*, 2014).Microorganisms differ in their needs for nitrogen sources according to their nature and growth requirements. In general, organic nitrogen sources support growth and metabolism more than inorganic ones (Zbar, 2011).

**Conclusion:**

*S. typhi* isolates have ability to produce a biofilm, which makes it resistant to the host's immune defenses such as antibodies, phagocytic cells and antibiotic resistance. Glugose and lactose are the better of carbon sourse for biofilm formation ,the temperature of 30°C was the best in the productivity of biofilm , and the pH 7 was better for the productivity of biofilm. Inorganic nitrogen sources are the best in biofilm productivity compared to organic nitrogen sources.

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