

# Study the Role of Ph in Curli Biogenesis Gene Expression in Enterobacter Cloacae Local Isolates

Marrrib N. Rasheed<sup>1</sup>, Basima Qasim Hasan Al-Saadi<sup>1</sup>, Osama Mohammed Hasan<sup>2</sup>,  
Noor Yahy Salman<sup>1</sup>, Sara Faisal<sup>1</sup>

<sup>1</sup>Doctor at Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq, <sup>2</sup>Doctor at Basic Medical Science Department, Collage of Dentistry, Mustansiriyah University, Baghdad, Iraq

## Abstract

Enterobacter cloacae is the most common pathogenic species of the genus Enterobacter, identified in hospitalized and enfeebled patients as the etiologic agent of many infections and considered as a significant bacterial pathogen in recent years. *E. Cloacae* are typical gram negative opportunistic forms of bacteria that cause illness after another infection or injury has influenced the host immune system and are related with nosocomial infections. The infection may be caused by GIT, UTI or cross contamination of the blood. The present study focused on the existence of the main curli biogenesis gene (*csgA*) and the role of pH as an environmental factor in the gene expression (*csgA*) for curli biogenesis. In this study, urine samples from 75 patients. In Baghdad city clinically diagnosed with urinary tract infection, for gram staining, Api20System and gene expression (*csgA*) gene in Standardized pH7 and acid pH4 RT-PCR was performed with specific primers. Conventional gram staining techniques, agricultural methods and System API 20E showed good results for *E. Cloacae*. For the 75 patients, 10 (13.3%) the findings find the highest gene expression fold values for the (*csgA*) gene in pH7 while the lowest fold value for (*csgA*) at acid pH4 (0.1577) thus the modify conditions growth such as pH of bacteria *E. Cloacae* triggers gene expression changes for biogenesis process. the findings of *rpoB* gene expression, which was used as a control gene, indicated that this gene was good as a housekeeping gene.

**Keyword:** *E. Cloacae*, *csgA* gene expression, curli biogenesis gene.

## Introduction

*E. Cloacae* is also a common nosocomial pathogen responsible for bacteremia and lower respiratory, urinary and intra-abdominal infections. as well as endocarditis, septic arthritis, osteomyelitis and skin and soft tissue infections. And endocarditis, septic arthritis, osteomyelitis and diseases of the skin and soft tissue infection. The skin and gastrointestinal tract are the most

frequent places where *E. Cloacae* can be infected [1]. *E. Cloacae* are often isolated from nosocomial infections, including pneumonia, urinary tract and bloodstream infections [2]. Curli is a new class of bacterial surface structures which is expressed in *E. Cloacae*, *Escherichia coli* and *Salmonella* spp. and is specified by its ability to bind to serum protein fibronectin [3]. Curli Fimbriae is a new class of surface compositions on the surface of *E. Cloacae*, *E. coli* and *Salmonella*, which are characterized by their ability to bind with protein serum fibronectin [4]. Associated with the adhesion and assembly of bacterial cells on surfaces. As well as being an essential part of the extracellular matrix, that contributes to the formation of the biofilm. The curly cilia is a distinctive element of virulence, because it interacts with a wide range of host proteins, including cell-based intercellular proteins and link-phase proteins, thus helping bacteria

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### Corresponding author:

**Dr. Maarib.N.Rasheed**

Asistance Prof. Department of genetic engineering in institute of genetic engineering and Biotechnology for post graduate studies – university of Baghdad  
Address Iraq - Baghdad - Al-jadiriya -university of Baghdad, E-mail: marrabnaz@yahoo.com

spread to the host. Curly cilia owned toll like receptors, which activates the innate immune system, therefore this considered as a molecular type associated with the pathogen (PAMPs) [5].

### Material and Methods

#### Samples:

A total of 10 isolates from 75 urinary cultures were collected in this study in patients with UTI admitted to governmental AL-Kathumia pediatric hospitals in Baghdad, these isolates were obtained from April to September 2017. We obtained the sample and stored it. Identification of Bacteria as Enterobacter Traditional biochemical methods (API20 E, Biomerieux, France) were used to conduct cloacae. Bacterial isolates have been maintained for further analysis by inoculation of single pure isolated colony to brain heart infusion agar (BHIA) slant incubated at 37 ° C during the night, stored at 4 ° C for a few weeks [6,7].

#### The optimal pH which affected on(*csgA*) gene expression:

Approximately 0.1 mL of each strain ‘s nutrient broth culture was applied to 10 mL of BHI broth and incubated at specific pH 4 and 7 with temperature tolerance and 24-hour incubation at 37 ° C, Extraction of RNA was done using General RNA Extract-ion Kit (Disbio, China).

#### Complete RNA extraction with TRIZol:

Complete RNA was collected from all samples using the General RNAExtraction Kit (Disbio, China) using the manufacturer’s pro-protocol. Synthesis of cDNA from mRNA: By Using Wiz Script™ RT FDmix Kit, the complete RNA was reversely transcribed to complementary DNA (cDNA). The treatment was conducted in a 20µl reaction volume, as advised by the manufacturer. 20µl was the complete amount of RNA to be reversely transcribed. Thermal cycler steps of cDNA Reverse Transcriptase conditions are shown in Table 1.

**Table (1): cDNA synthesis program of *rpoB* and *csgA* genes for mRNA.**

	Step 1	Step 2	Step 3	Step 4
Temperature	25 °C	42°C	85 °C	4 °C
Time	10 min	30 min	5 min	∞

#### Real time qRT PCR study the expression of *rpoB* and *csgA* genes $\Delta\Delta Ct$

The 2<sup>-DDCt</sup> method has been used to compare the transcript levels between various samples [8-10]. The interest gene CT has been normalized to that of the internal control gene. The variance between the *rpoB* ((internal control gene) and the *csgA* (interest gene) cycle threshold (Ct) values was Analysed by using the qPCR software, QIAGEN Real- time PCR system ((Rotor Gene Q, Germany). The rates of gene expression and the alteration of the fold were quantified by the threshold cycle (Ct) and the  $\Delta\Delta Ct$  was used to assess the fold of gene expression.

**Table (2): Primers used in the study.**

Primers		Primer sequence
rpoB	F	5' - GCA ACT TGT TGT CGC GGA TT- 3'
	R	5' - TCG ACC GTC GTC GTA AGC T - 3'
csgA	F	5'-ATT GCA ATC GTA GTT TCT GG -3'
	R	5'ATW GAY CTG TCA TCA GAG CCC TGG - 3'

**Table (3): Thermal profile of *rpoB* and *csgA* gene expression.**

Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	5 minutes	1
Denature	95 °C	15 seconds	40
Anneal	60 °C	60 seconds	

#### Gene expression of *csgA* in Different environmental factors

Volume equal to 0.1 mL of a nutrient-broth culture overnight of each strain Was added to 10 mL of BHI broth, a shaking incubated at different pH. The extraction of RNA complete by RNA extraction kit was carried Out in the same steps, then cDNA synthesis for mRNA was completed. RT master Mixed and programs used under standardized conditions.

#### Results and Discussion

Enterobacter species, especially Enterobacter cloacae, are important nosocomial pathogens that are responsible for approximately 13.20 percent of UTI Iraqi patients according to previous study [11]. Several surface structures of bacteria, including curli, flagella, pili and exopolysaccharide; Play roles in different aspects of development of biofilms [12]. Biofilms can be a problem in the food industry and in hospital environments Curli allow the adherence of Salmonella enteritidis to Teflon and stainless steel, which can contribute to the creation

of biofilms and contamination of surfaces frequently used in the food industry [13]. the strain's ability to form a biofilm relied on *csgA*. These researchers indicated curli were essential during the attachment process in the initial stages of biofilm growth [14]. *RpoB* rRNA expression has been shown to be strongly dependent on the physiological state of the bacterial cell in bacteria, one of the most widely used housekeeping genes in accordance with gene expression results., The essential principle for the use of housekeeping genes in molecular experiments is that their expression in the cells or tissues under investigation remains constant [15]. Variation of total change in *RpoB* rRNA expression was analyzed. In various researchers using the 2-Ct value and the 2-Ct ratio, the expression fold for the *rpoB* gene was 1, 0.933 under variable PH conditions , respectively pH7 and pH 4. little variability in the gene expression fold makes the gene *RpoB* a reliable reference gene as shown in (Table 4).

**Table (4) Fold expression of *rpoB* gene for pH factor.**

factor	Mean ct of <i>rpoB</i>	Factor 2 – $\Delta$ ct	Experimental /control	Fold
Control temp.37c°, pH7	30.5	6.5 E.q	6.5Eq/ 6.5 E.q	1
pH 4	30.6	6.1 Eq	6.1 E.q/6.5 E.q	0.933

RT-qPCR expression for *csgA* gene

The expression *csgA* is highly regulated. *E. Cloacae* isolates were efficiency. Biofilm- forming , significantly correlated with the *csgA* and *csgD* gene mRNA expression rates. The curli protein fimbriae emerged as tangled fibers and the curli-proficient strain formed mature biofilms [16]. Various environmental factors, including incubation period, incubation temperature and pH-effect on virulence expression [15]. By using Relative quantitative RT- PCR assay, we analyzed the mRNA expression of *csgA* genes by comparing

bacterial growth with different pH factors as a variable factor for each sample, we studied the role of pH on *csgA* biofilm gene expression. The Ct values of gene amplification were reported from the quantitative RT PCR software. Relative quantification used to measure the gene expression fold change [8]. This depends on the standardization of Ct values determining the *rpoB* gene, which is the difference between the mean Ct values of each case's *csgA* cDNA amplification replica and the *rpoB* gene.

**Table (5) Fold of *csgA* expression depending on 2 – $\Delta$ ct method for pH4.**

groups	Means of <i>csgA</i>	Means of <i>rpoB</i>	$\Delta$ ct	2- $\Delta$ ct	Experimental/controls	Fold
control	29.28	30.5	-1.22	2.32	2.32 /2.32	1
pH4	32.05	30.6	1.45	0.366	0.366/ 2.32	0.1577

The results in table (5) showed gene expression folding for the *csgA* gene by comparing bacterial growth with different pH factors for each sample as variable factor. The gene expression fold in pH4 was in (0.1577) times lower than normal. These findings suggest a substantial decrease in the expression of the *csgA* gene at pH 4 relative to control (Tm37, pH7). This suggests that acidity is an important factor in bacterial pathogenicity as pH has been identified as a regulator for the expression of virulent genes and is likely to be able to express curli and Suggest that this virulent factor could play a major role in the pathogenesis and invasiveness of *E. Cloacae* [16]. suggests that curli fimbriae expression in *E. Cloacae* plays an significant role in the development of biofilms Which is the first stage of bacterial pathogenicity.

**Acknowledgement** : This research was supported by the Department of Genetic Engineering, Institute of Genetic Engineering, and Postgraduate Biotechnology.

**Ethical Clearance-** Taken from Institute Research Ethics committee.

**Source of Funding-** Self

**Conflict of Interest** -nil

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