# Detection of *luxS* Gene in *Serratia marcescens* and *rpoS* Gene in *Enterobacter cloacae* Isolates Using PCR Reaction

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## Abstract

*luxS* and *rpoS* genes were involved in the regulation of some biological processes, which interfere with virulence of *Serratia marcescens* and *Enterobacter cloacae* bacterial species sequentially. *luxS* have an important activity in Quorum Sensing (QS) processes, but *rpoS* interfere with tolerance and resistance processes toward stress conditions correlating with its virulence. The study aimed to detect *rpoS* presence in *E. cloacae* and *luxS* gene presence in *S. marcescens* separately isolated from 16 leukaemia patients (4 of *S. marcescens* and 12 of *E. cloacae*), who characterised with bacteraemia by using primers designed for this purpose by using PCR technique. The results showed that all of the bacterial isolates of *S. marcescens* contain *luxS* gene, while no one of *E. cloacae* isolates show positive results for *rpoS* gene. The study concluded that *S. marcescens* has high virulence as a result of *luxS* presence, which controls Quorum sensing and prodigiosin formation and affecting host immunity. Moreover, the negative results of *rpoS* gene can be explained according to two possibilities: the gene can be found in the isolates with a copy differ from the registered sequences of the *rpoS* gene, or the gene did not present in those isolates with presence of alternatives offset its activities. [DOI: 10.22401/JUNS.21.1.18]

Keywords**:** *rpoS* gene, *Enterobacter cloacae*, *luxS* gene, *Serratia marcescens.*

## 1.Introduction

*luxS* gene correlates with Quorum sensing (QS) in many bacterial species [1]. The product of this gene expression is S-ribosylhomocysteine lyase (LuxS) enzyme, which is responsible for the production of Autoinducer -2 (Al-2) that involved in QS in many bacteria [2]. Previous studies indicated the importance of this gene in the regulation of QS pathway in *S. marcescens*, which expressed as a result to response for bacterial density [3]. So that it's involved in Al-2 dependent Quorum sensing [4], which include Pfs and LuxS enzymes that are responsible for the biological manufacturing of signal molecules, contribute to the virulence induction of human opportunistic pathogen *Serratia* [5]. LuxS enzyme plays a regulatory role in the metabolic activities of *Serratia* by regulation of production of some primary metabolites in addition to prodigiosin (one of the secondary metabolites) [6], which regulate by *luxS* gene in some *S. marcescens* strains. Moreover, any mutation in *luxS* leads to reduce its virulence factors like prodigiosin and haemolysin production [3, 7]. According to previous studies and what concluded by coulthurst *et al* [2] study, *luxS* interferes with prodigiosin production and regulate antibiotic production in *Serratia* spp. with a significant difference in its virulence factors. Many of cellular pathways developed in bacteria in order to tolerate severe stress conditions and one of those pathways was *rpoS* gene responsible for RNA polymerase sigma S (sigma 38) factor which interfere with virulence of many pathogens, particularly in stationary phase [8-12]. In *E. cloacae*, this gene considered one of regulatory genes through response to starvation, as well as its considered to be one of virulence factors such as regulation of hydrogen peroxide catalase II (HPII), which give it the ability to resist $H_2O_2$, In addition to regulation of toxins secretion from *Serratia* strains [10]. Since *rpoS* involved in the controlling of some virulence genes in pathogenic bacteria to enable those bacteria to resist stressed environments supplied by the host [13], which lead to the controlling of number of genes involved in cellular viability maintenance in stationary phase like oxidative stress, hyper-osmolality, starvation and

reduced PH [13- 15]. In addition to that, the incidence of some changes in gene regions can lead to the formation of more virulent strains [11]. The study aimed to detect the presence of *luxS* gene in *Serratia marcescens* and *rpoS* gene in *Enterobacter cloacae* investigate its importance as virulence factors in the studied isolates.

## 2.Materıals And Methods

### 2.1 Bacterial Isolates

The study performed on 4 bacterial isolates of *S. marcescens* and 12 bacterial isolates of *E. cloacae* from 33 bacterial isolates, which isolated from 60 blood samples of leukaemia patient from National Center of Haematology Researches and Therapy and from Baghdad Teaching Hospital from April to October (2016), who were suffered from some clinical signs and diagnosed with bacteraemia. The bacterial isolates characterised with Api-20E.

### 2.2 Polymerase chain reaction (PCR) of *luxS* and *rpoS*

#### 2.2.1. DNA Extraction

Genomic DNA was extracted from the bacterial isolates using DNA extraction kit. (Promega, USA) and DNA purity was measured by NanoDrop device (Thermo scientific, USA), then the extracted DNA were subjected to electrophoresis to ensure its purity and integrity.

#### 2.2.2 Primers

Specific primers were used for detection of *rpoS* and *luxS* genes, which designed with primer 3 program (as indicated in Table (1)) and supplied by Alpha DNA as lyophilized powder then dissolved with a specific volume of sterilized distilled water according to manufacturer recommendations to form stock solution for each primer with (100µM) concentration then its stored at -20°C.

***Table (1)***
***Primers sequences and the product size for each one.***

| *Primer name* | *Gene ID** | *Direction* | *Nucleotide sequence* | *Product size (bp)* |
|---|---|---|---|---|
| *luxS* | 3278137 | F** | TCATGGCATACCATCACGG → | 360 |
| | | R | TCCAGAATGTGCTTGGCGAT← | |
| *rpoS* | 9124132 | F | →AACGGTATTGGCCTGCCTTT | 354 |
| | | R | ←TGCCCCTGATGCGATAACTG | |

**** Gene ID obtained from GenBank/NCBI.***
***** F: Forward; R: Reverse.***

#### 2.1.1 PCR Reaction mixture

The PCR mixture purchased from BioNEER composed of Buffer solution, dNTPs and Taq DNA polymerase, which mixed like what described in Table (2). While the program used for PCR reaction of *luxS* and *rpoS* genes described in Table (3).

***Table (2)***
***PCR mixture components and its volumes.***

| *Component* | *The volume of each component in µl* | *The concentration* |
|---|---|---|
| Distilled water | 15 | - |
| Master mix | 5 | - |
| Primer forward | 1 | 1 µM |
| Primer reverse | 1 | 1 µM |
| DNA template | 3 | Depending on the isolate* |

****As indicated in Table (4).***

*Table (3)*
*The program used in PCR Reaction of luxS and rpoS genes.*

| Steps | Temperature | | Time | |
|---|---|---|---|---|
| | rpoS | luxS | rpoS | luxS |
| Initial denaturation | 95°C | 95°C | 5 Min. | 5 Min. |
| Denaturation | 95°C | 95°C | 45 Sec. | 45 Sec. |
| Annealing | 57°C | 56°C | 45 Sec. | 40 Sec. |
| Number of cycles: 35 | | | | |
| Extension | 72°C | 72°C | 1 Min. | 25 Sec. |
| Final Extension | 72°C | 72°C | 10 Min. | 10 Min. |

**2.1.2 Gel electrophoresis**

Electrophoresis device was supplied with 1xTBE-buffer (Promega, USA) and Agarose gel plate prepared with 1% concentration of Agarose (BIOBASIC, Canada) with using of safe DNA loading dye (BIOland, USA) then 5 µl of PCR product of *luxS* or *rpoS* and (100 bp) DNA ladder (BioNEER, Korea) was added to gel wells carefully before supplying of 50 volts of voltage for 5 minutes then 100 volts for 40 minutes. After that the gel plate was checked for the presence of bands using Desktop gel imager UV-trans-illuminator (SCOPE-21, Japan) under 312 nm UV light, then the molecular size of each band was detected according to the distances in comparison with DNA ladder.

**2.1. DNA extraction and purity results**

The concentrations and purity of the isolated genomic DNA which extracted from *S. marsescens* and *E. cloa*cae bacterial isolates were described in Table (4). The extracted DNA was undergone electrophoresis to ensure DNA purity and integrity, and the results shown in Fig.(2, 3).

*Table (4)*
*The concentrations and purity of the genomic DNA of S. marsescens and E. cloacae bacterial isolates.*

| Con(ng.ml$^{-1}$) | 280/ 260 | Abs 280 | Abs 260 | Sample |
|---|---|---|---|---|
| 72.6 | 1.78 | 0.818 | 1.453 | *E. cloacae* |
| 63.2 | 1.29 | 1.36 | 1.76 | *E. cloacae* |
| 186.5 | 1.77 | 2.105 | 3.729 | *E. cloacae* |
| 43.1 | 1.90 | 0.453 | 0.862 | *E. cloacae* |
| 87.4 | 1.60 | 1.091 | 1.749 | *E. cloacae* |
| 80.3 | 1.68 | 1.56 | 2.63 | *E. cloacae* |
| 62.1 | 1.29 | 1.30 | 1.68 | *E. cloacae* |
| 85.7 | 2.25 | 0.432 | 0.976 | *E. cloacae* |
| 72.5 | 1.78 | 0.813 | 1.451 | *E. cloacae* |
| 60.0 | 1.21 | 1.32 | 1.60 | *E. cloacae* |
| 86.4 | 1.60 | 1.08 | 1.73 | *E. cloacae* |
| 60.5 | 1.34 | 1.31 | 1.76 | *E. cloacae* |
| 80.1 | 1.58 | 1.58 | 2.50 | *S.marcescens* |
| 56.8 | 1.30 | 1.23 | 1.60 | *S.marcescens* |
| 48.3 | 1.89 | 0.83 | 1.57 | *S.marcescens* |
| 64.1 | 1.20 | 1.40 | 1.69 | *S.marcescens* |

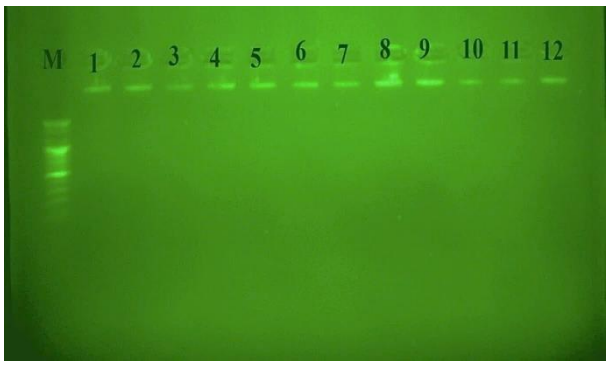


***Fig.(2): Isolated DNA electrophoresis from Enterobacterclacae.***

**** M: Marker (100bp ladder).***

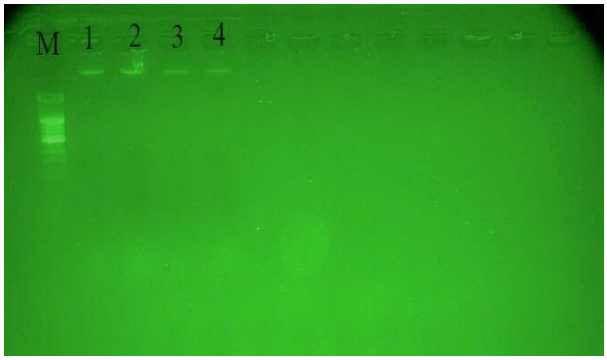


***Fig.(3): Isolated DNA electrophoresis from Serratia marcescens.***

**** M: Marker (100bp ladder).***

### 2.3.Detection of *luxS* gene in *Serratia marcescens*:

The results of *luxS* gene detection in *S. marcescens* showed that all of the isolated bacteria from leukaemic patients (4 isolates) who suffering from bacteraemia contain *luxS* gene as presented in Fig.(4).


***Fig.(4): PCR reaction product of luxS gene from Serratia marcescens.***

**** M: Marker (100bp ladder).***

### 2.4. Detection of *rpoS* gene *Enterobacter cloacae*

The results that obtained from PCR reaction for detection of *rpoS* genes in *E. cloacae*, which are displayed in Fig.(5) elucidated that no one of *E. cloacae* isolates shows positive result to *rpoS* gene

## 3. Discussion

The study aimed to detect the presence of *luxS* gene in *S. marcescens* isolated from leukaemia patients' blood samples and the study indicated that all of the isolates show positive results for the presence of this gene. This agrees with Coulthurst *et al* [2] study, which indicted that all of *S. marcescens* 274 and *S. marcescens* 39006 strains contain this gene, which gives it the ability to produce Al-2 activity. Instead of the differences in the downstream region of this gene between *S. marcescens* strains, which caused by unknown reasons that lead to differences in gene regulatory control on different characteristics depending on the strains such as variations in prodigiosin production control between *S. marcescens* 274 and 39006. Moreover, *luxS* gene mutations in *S. marcescens* 274 only can lead to significant differences in its virulence [2]. Its role in virulence proved by many studies; since Sun *et al* [6] indicated that Al-2 production (one of QS inducers) was controlled by *luxS* gene transcription level in *S. marcescens*. and Coulthurst *et al* prove that *S. marcescens* 274 and 39006 strains produce significant amounts of Al-2 activity during activation of a chromosomal copy of the gene in both strains that prove the responsibility of the gene on Al-2 production in both strains. Nevertheless, it's decreased when bacteria enter into the stationary phase in *S.marcescens*. [2,6] In addition, another previous study revealed that the gene has an importance in Quorum Sensing regulation which plays an important regulatory role in the metabolic activities of *S.marcescens* by regulation of production of some primary metabolites as well as prodigiosin (from secondary metabolites), [6] this pigment regulated by QS and *luxS* in some strains of *Serratia* and considered to be an antibiotic and play an important role in virulence and its production controlled by complex process and depend on environmental processes, Since QS mechanism enable the bacteria to regulate gene expression as a response to bacterial density so that any mutation can lead to reduce prodigiosin and haemolysin production as well as virulence [2, 3, 7] In spite of inability of our

study to detect strain type of the isolates according to the obtained results, indeed; the strains colours were red or reddish pink, which demonstrates prodigiosin production like what appears since *S. maecescens* 274 and 39006 strains produce prodigiosin and all of these strains contain *luxS* gene, which improves the results obtained by PCR detection of the gene, but Coulthurst *et al* [2] prove *luxS* rule in the regulation of prodigiosin production in 274 strain but not in 39006 strain.

*rpoS* was an important factor in controlling of cells and assisting in bacterial persistence under stress condition and contribute to the virulence by many effects, Our study accomplished in the presence of *rpoS* gene *E. cloacae* isolated from blood specimens from leukaemia patients. PCR results obtained by specific primers for *rpoS*, elucidated that no one of *E. cloacae* isolates appeared positive results. This result agree somewhat with Hussain and Alammar [8] study, which elucidated high possibility for the negative result of *rpoS* gene in *E. cloacae* because 31% only of *Enterobacter* isolates have *rpoS* gene (21 positive isolates from 75 isolates) which include 28% from *E. cloacae* and 56% of *E. sakazakii*. That study represented little probability for positive results and it accomplished in the same zone of our study, in addition to the differences in the gene appearance probability according to the differences in the studied numbers of isolates. Since that study included high significant number (84 isolates) in comparison to our study isolates' number (12 isolates), but no isolate of our study show positive result. Martinez-Garcia demonstrated that bacterial DNA, which fails to correlate with its specific primer of the gene in PCR reaction show a negative result, but it didn't indicate the absence of the gene also the isolates which show a negative result appeared to have less consensual sequences with *E. coli rpoS* sequence as this gene considered to be widespread and conserve in Enterobacteriaceae so that the isolate which lacks the gene show less persistence under thermal and osmolality conditions. [10,12] Moreover, When Martinez- Garcia *et al* [10] create *E. coli rpoS* probe to detect the presence of the gene in Enterobacteriaceae including *E. cloacae*, he found that *rpoS* gene in *E. cloacae* similar to the gene in *E. coli*, since he studied the presence of the gene in *E. cloacae* CET 863 strain and he found that the identity percent was 80% between *E. coli rpoS* and *E. cloacae rpoS*. While some Enterobacterceae members show negative results in both PCR and southern analysis in both *Proteus Vulgaris* and *Providencia Stuartii*. According to the absence of studies, which explain the reason for this negative result in addition to the inability to explain this reason, according to the results obtained from the study so that we can establish some hypotheses which must be studied later to explain this result. The hypotheses include the absence of the gene in the studied isolates strains according to inability to detect the strain from study results, in addition to presence of many strains follow *E. cloacae*, since the studied strains by Martinez-Garcia *et al* [10] and Navarro-Liorens *et al* [16] show positive results for *rpoS* gene involving CETC 960 (free mutation wild-type strain), ATCC 13047 strain and RC10-RC1 strains. So that it can be subrogated by other factors to perform its functions in resistance to some stress conditions, for example: some *E. coli* strains which lack *rpoS* gene can adapt to stress conditions which include hyper osmolality by addition of mobile factor IS10 to ost-BA operon promoter [17], or the negative results can be resulted from differences in gene sequence in the studied isolates from the registered sequences which lead to inability to detect the gene, as well as can be resulted from the absence of the plasmid containing the gene since it's portable on pBEcl plasmid [10] or the mutations which can happened in the downstream region of the gene that effect on the ability of the primer to link to its complement sequence, for example, when Martinez- Garcia *et al* [10] induce addition mutation in *rpoS* gene in *E. cloacae*, which lead to replace of *rpoS* wild-type gene with not working allele and the resulted strain named with *E. cloacae* E1. Few studies obtained on *E. cloacae* ability to produce virulence-related characteristics [8]. As it's considered to be an opportunistic pathogen responsible for Bacteremia [10] and it can enter in extraintestinal infections, because of the

presence of virulence-related features like adhesion ability and invasion on Eukaryotic cells. While it has the ability to chelate the iron outside gastrointestinal tract so that it can live and spread inside the host and resist the environmental stresses like the effect accomplished by *rpos* [8].

## 4. References


[1] Joyner Jessica, Wanles David, SinGalliano Christopher D., Lipperin K., "Use of Quantitative Real-Time PCR for Direct Detection of *Serratia marcescens* in Marine and other Aquatic Environments", APPL. ENVIRON. MICROB. 80 (5), 1679-1683, 2014.

[2] Coulthurst Sarah J., Kurz C. Leopold, Salmond George P. C., "*luxS* Mutants of *Serratia* Defective in Autoinducer-2-Dependent Quorum Sensing Show Strain-Dependent Impacts on Virulence and Production of Carbapenem and Prodigiosin", MICROBIOLOGY+. 150, 1901-1910, 2004.

[3] Martinez Katrina C., Teves Franco, Gandmadamba Ma, Reina Suztt B., "Sequence Analysis of Putative *luxS* gene Involved in Prodigiosin Biosynthesis from Philippine Local Stains of *Serratia marcescens*", INT. RES. J. BIOL. SCI. 2 (4), 13-19, 2013.

[4] Houdt Rob Van, Givskov Micheal, Michiels Chris W., "Quorum Sensing in *Serratia*", FEMS MICROBIOL REV. 37, 407-424, 2007.

[5] chauder Stephen, Shokat Kevan, Surette Michael G., Bassler Bonnie L., "The *luxS* Family of Bacterial Auto-inducers: Biosynthesis of A novel Quorum Sensing Signal Molecule", MOL BIOL+. 41 (2), 463-476, 2001.

[6] Sun Shu-Jing, Liu Yu-Chen, Zhu Hu, "The dependence of Quorum Sensing in *Serratia marcescens* JG on The transcription of *luxS* gene", ARCH. MICROBIAL. 197 (5), 715-721, 2015.

[7] De Keersmacker, Sigrid C. J., Sonck Kathleen, Vanderleyden Jos, "Let *luxS* speak up in Al-2 Signalling", TRENDS in Microbiology. 14 (3), 114-119, 2006.

[8] Hussain Mahdi, Alammar Muheel, "Molecular Study of Some Virulence Factors Encoding genes of *Enterobacter* spp. Isolated from Different Clinical Specimens", Magazin of AL-kufa University for Biology. 5 (2), 1-13, 2013.

[9] Chen Guozhu, "Studies on the Control of gene Expression of *rpoS* and its Regulon in *E. Coli*", PhD, thesis, McMaster University, Hamilton, Ontario, 2003.

[10] Martinez-Garcia Esteban, Tormo Antonio, Navarro-Liorens Juana Maria, "Further Studies on *rpoS* in *Enterobacteria*: Identification of *rpoS* in *Enterobactor cloacae* and *Kluyeracryo crescens*", ARCH MICROBIAL. 175, 395-404, 2001.

[11] Kotewicz Michael L., Li Baoguang, Levy Dan D., Leclerc J. Eugene, Shifflet Andrew W., Cebula Thomas A., "Evolution of Multi-gene Segments in The Muts-RpoS Intergenic Region *Salmonella enteric* Serovar Typhimurium LT2", MICROBIOLOGY+. 148, 2531-2540, 2002.

[12] Dong Tao, Schellhorn Herb E., "Global Effect of *rpoS* on gene Expression in Pathogenic *Escherichia Coli* 0157:H7 Strain EDL 933", BMC GENOMICS. 10 (349), 1-17, 2009.

[13] Kazmierczak Mark J., Wiedmann Martin, Boor Kathryn J., "Alternative Sigma Factors a", MICROBIOL MOL BIOL R. 69 (4), 227-543, 2005.

[14] Hengge-Aronis Regine, "Signal Transduction and Regulatory Mechanisms Involved in Control of the Sigma (*rpoS*) Subunit of RNA Polymerase", Microbiology Reviews. 66 (3), 373-395, 2002.

[15] Abid Intidhaar Naeem, "Bacteriological and Molecular Study of *Enterobactor* spp. Isolated from Different Clinical Specimens", PhD, thesis, University of Kufa, Iraq, 2013.

[16] Navarro-Lioréns J. M., Martinez-Garcia, Esteban, Tormo Antonio, "*Enterobacter cloacae rpos* Promoter and gene Organization", ARCH MICROBIAL. 179, 33-41, 2002.

[17] Stoeble Daniel M., Dorman Charles J., "The effect of Mobile Element IS10 on Experimental Regulatory Evolution in *Escherichia coli*", MOL BIOL EVOL. 27 (9), 20105-20112, 2010.