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HOMOSERINE LACTONE PRODUCTION BY CLINICAL ISOLATES OF *Proteus vulgaris* FROM PATIENTS WITH URINARY TRACT INFECTIONS

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ABSTRACT

Proteus vulgaris has been described as an aetiological agent in urinary tract infections. Quorum sensing regulates the expression of virulence factors in a wide variety of pathogenic bacteria.Most Gram negative bacteria produce acyl homoserine lactones. In this study, Homoserine L-lactone was synthesized by *Proteus vulgaris* through using aspartic acid which was added to the culture media at a final concentration 1%. Homoserine lactone production was checked at different intervals of incubation (after 2, 3, 4, 5, 6 and 24 hour) where 0.01% of KCN is added to show the time of Homoserine L-lactone appearance in the bacterial growth. When one drop of culture supernatant was added to fresh growth of *Proteus vulgaris* on slide, aggregation or quorum sensing was seen as indicator of HSL (Homoserine lactoneproduction) production.

Keywords: *Proteus vulgaris*, Quorum Sensing, Homoserine Lactone

INTRODUCTION

Proteus vulgaris is a rod-shaped Gram-negative chemoheterotroph bacterium. The size of individual cells varies from 0.4~0.6µm by 1.2~2.5µm. *P.vulgaris* possesses peritrichous flagella, making it actively motile. It inhabits the soil, polluted water, raw meat, gastrointestinal tracts of animals, and dust. In humans, *Proteus* species most frequently cause urinary tract infections, but can also produce severe abscesses **[1]**. Many different factors may influence the evolution and course of pathogenesis; temperature, pH, chemical compounds from the host. Moreover, numerous natural microflora should be added to host factors, which may also detect signals deriving from other bacterial species. During this process, known as

quorum sensing (QS), which is the technique in which bacteria regulate their gene expression to better serve the needs of the group rather than the individual. Numerous species of bacteria employ a mechanism of quorum sensing. This signaling process allows the cells comprising a bacterial colony to coordinate their gene expression in a cell-density dependent manner **[2]**.Quorum sensing is mediated by small diffusible molecules termed autoinducers that are synthesized intracellularly (throughout the growth of the bacteria) and released into the surrounding milieu. As the number of cells in a bacterial colony increases, so does the extracellular concentration of the autoinducer. Once a threshold concentration is reached (at which point the population is considered to be "quorate"), productive binding of the autoinducer to cognate receptors within the bacterial cells occurs, triggering a signal transduction cascade that results in population wide changes in gene expression **[3, 4]**. Thus, quorum sensing enables the cells within a bacterial colony to act cooperatively, facilitating population-dependent adaptive behavior **[2]**. QS controls genes that direct activities that are beneficial when performed by groups of bacteria acting in synchrony. Processes controlled by QS include bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion **[3, 5, 6]**.

Three major quorum-sensing circuits have been described: one used primarily by Gram-negative bacteria, one used primarily by Gram-positive bacteria, and one that has been proposed to be universal **[7]**. Most Gram negative bacteria use a certain molecule called an acyl homoserine lactone´ or AHL´ that is used as the signaling molecule. Gram positive bacteria use oligopeptides as their signaling molecule, which have been cut from a large polypeptide synthesized in the cytoplasm. These two systems are species-specific, and each Gram negative or Gram positive bacteria produce their own specific auto-inducer. Thus, these molecules enable intra-species communication. These are private, secret conversations **[8]**.

Most Gram negative bacteria produce Acyl homoserine lactones (AHLs for short) as the signaling molecule of choice, whose production depends on *S*-adenosylmethionine (SAM) as a substrate **[9]**. Each of these molecules interacts with its own specific binding protein, but with no other **[8]**. These molecules are small and can easily enter and exit the cellular membrane. At a certain threshold value of auto-inducer, the molecule binds to the binding protein and group behavior becomes expressed bacteria produce signal called autoinducer

(AI), which is secreted to environment. Every AI, reaching critical concentration causes growth of the bacterial population and induces changes in the expression of genes resulting from switching life cycle and bacterial metabolism **[6]**. In some cases of Gram-negative bacterial QS, AIs are detected by two-component histidine kinase receptors that function analogously to those described in the preceding paragraph for Gram-positive QS bacteria **[6]**. The aim of this study was to detect quorum sensing in *Proteus vulgaris* by detection the synthesis of Homoserine lactone.

MATERIALS & METHODS

Bacterial Isolates

Proteus vulgaris was isolated from a patient with UTI who were admitted to four hospitals: Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, Al-Hashymia hospital and Al-Qasim hospital during the period from 4/2012 to 1/2013.Standard biochemical tests were used for detecting *P. vulgaris* strains and by Vitek system (BioMerieux, USA) **[10, 11]**.

Detection of Quorum Sensing in *P.vulgaris*

The detection of quorum sensing was carried out according to **[12, 13]**.

a) LB media were prepared and supplemented with 1% aspartic acid and distributed in six flask.

b) After incubation with *P. vulgaris*, the flasks were incubated at 37°C for intervals (2, 3, 4, 5, 6, and 24hr).

c) At the end of each interval, KCN (0.01%) was added and then after 18hr the media was filtered by Millipore filter (0.4mm) and then the filtrated was used for quorum sensing detection.

d) The quorum sensing test was done on slide through mixing one drop of supernatant and one drop of fresh bacterial growth , and then stained with gram stain and the slide was examined by microscope.

e) The positive result was scored due to the presence of aggregation bacterial cell.

Chemical Detection of Homoserine Lactone

Homoserine production was checked through the separation of supernatant from culture media, and then the supernatant was dialyzed verses LB free of KCN. After 24 hours the dialyzed homoserine containing media were inoculated by *P.vulgaris* again for 24 hour and then Brands test was used to detect methionine or homocystein synthesis through conversion of homoserine to homocystein **[12, 13]**.

Brands Test

Reaction of sodium nitroprusside with sulfhydryl componud (e.g cysteine,

homocysteine) yeilds rose or purple red complex products **[14]**.

RESULTS & DISCUSSION

To study quorum sensing in *P. vulgaris,* aspartic acid was used as the main focal metabolites for homoserine synthesis. It was observed that homoserine was accumulated in culture media after the addition of KCN in which the later will inhibit threonine synthesis, through its effect on threonine synthase enzyme. Homoserine lactone production was also checked by using brands test in order to ensure that quorum sensing occurs as a result of homoserine lactone synthesis.

Quorum sensing was carried out as mentioned in paragraph (Detection of quorum sensing in *P.vulgaris*). The presence of aggregation of *P. vulgaris* as in **Figure 1** was considered a positive result versus the negative results in the absence of homoserine lactone.

The best interval for accumulation of homoserine lactone was after 4hours of incubation in which the homoserine lactone became at maximum concentration. However, under certain condition the bacteria can form homocystein as a result of production of homocystein synthase, and the later may be used as indicator for homoserine lactone synthesis.

Bacterial virulence factors are regulated by quorum-sensing molecules which are derivatives of serine substituted by a fatty acid, i.e., acylatedhomoserine lactones, abbreviated as acyl-HSLs **[15, 16, 17]**. The quorum sensing mechanism involves two types of autoinducers: AI-1 based on homoserine lactone and AI-2 based on other molecules. The majority of signal substances in Gram-negative bacteria are substituted by fatty acid derivatives of acyl-HSL (AI-1). There is no evidence that quorum sensing receptors and AI-1 signal molecules are associated with swarming motility in *Proteus* **[18]**. Whereas, Daniels *et al*. **[19]** showed that the QS signal acylhomoserine lactone enhances swarming motility in *Serratia liquefaciens*. Among the Gram-negative bacteria, the most well studied quorum-sensing system is the LuxR-LuxI homologous system **[20]**. This quorum-sensing system is widespread among Gram-negative genera and is involved in the regulation of many host-associated phenotypes, including production of virulence factors and secondary metabolites **[20, 21]**. Two distinct mechanisms of signalling mediated by *N*-AHLs have been described. In most Gram-negative bacteria, the signal is generated by an *N*-AHL synthase of the LuxI family of proteins, and is perceived by an *N*-AHL receptor protein belonging to the LuxR

family of transcriptional regulators. The *N*-AHL autoinducers bind to their cognate LuxR-type proteins only on reaching a critical threshold concentration. Autoinducer binding controls the transcriptional activity of the LuxR protein in regulating the expression of target genes, which can include *luxI*. This establishes a positive feedback loop for *N*-AHL synthesis, although it must be noted that positive feedback is not a universal feature of *N*-AHL-mediated quorum-sensing systems. In some Gram-negative bacteria such as *Vibrio* spp., *N*-AHL synthesis is directed by a LuxM synthase (unrelated to LuxI) and perception of the signal involves a cytoplasmic membrane-associated sensor kinase. To date, *N*-AHL-dependent quorum-sensing circuits have been identified in a wide range of Gram-negative bacteria, where they regulate various functions including bioluminescence, plasmid conjugal transfer, biofilm formation, motility, antibiotic biosynthesis, and the production of virulence factors in plant and animal pathogens **[22, 23]**. AHLs produced by bacteria could serve as potential biomarkers in the management of bacterial diseases and, thus, monitoring them in biological samples may be a significant analytical tool for the investigation of such diseases **[24]**. Specifically, altered bacteria-host interaction has been implicated in several health imbalances and disorders. For instance, there are several reports referred to the role of bacteria in gastrointestinal disorders such as inflammatory bowel disease and irritable bowel syndrome **[25]**. The observation that quorum sensing is linked to virulence factor production and biofilm formation suggests that many virulent Gram-negative organisms could potentially be rendered nonpathogenic by inhibition of their quorum-sensing systems. Research into quorum sensing, and inhibition thereof, may provide a means of treating many common and damaging chronic infections without the use of growth-inhibitory agents, such as antibiotics, preservatives, and disinfectants, that unavoidably select for resistant organisms **[20]**.

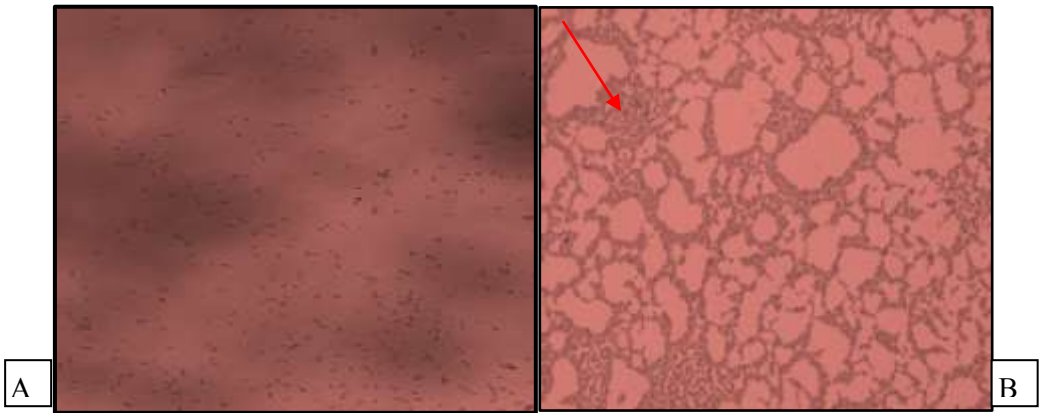

Figure (1): Detection of Quorum Sensing in *P. vulgaris* (100x)
A: control (absence of homoserine); B: positive result (⟶)

CONCLUSION

Quorum sensing was carried out through the production of homoserine lactone by *Proteus vulgaris* isolates where, it was found that there was an aggregation of the bacterial cells after addition of the supernatant that likely occurs as a result for the presence of homoserine lactone.

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