Study of virulence factors of *Pseudomonas aeruginosa*

By

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B.Sc. in Microbiology, Faculty of Science, Fayoum University, 2011
M.Sc. in Microbiology, Faculty of Science, Fayoum University, 2015

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Approval Sheet

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6. Summary and Conclusion

P.aeruginosa is a Gram-negative bacterium. It is a multidrug-resistant pathogen with considerable medical importance. T3SS is a critical virulence factor and is associated with high antibiotic resistance and elevated mortality rates in animals (Sawa *et al.*, 2014). Moreover, strains with active T3SS are persistent and increase disease severity in humans; this reveals its important role in nosocomial infections (Sawa *et al.*, 2014; Hauser., 2009; Anantharajah *et al.*, 2016).

P. aeruginosa has at least three of four effectors ExoS, ExoT, ExoU, and ExoY. They interfere with the innate host immune system by disrupting the cell actin cytoskeleton, inhibiting phagocytosis, and causing cell necrosis (Hauser., 2009)

The Aim of this study is to understand the function, activity, and regulation of T3SS. In addition, comparing between regulatory pathways in
P. aeruginosa and *Y. enterocolitica* will allow the determination of species-specific or conserved regulatory pathways which is very important in the T3SS inhibitors designing process.

The results obtained in this study can be summarized in the following points:

1- In *P. aeruginosa,* T3SS injectisomes forms distinctive bright foci under fluorescence microscope that are localized at the cell membrane but have no specific pattern and needle staining showed that the needles co-

localize with the PscQ Foci which means it can be considered to represent fully assembled T3SS injectisome (Lambaki 2019; Selinger 2019).

2. - *P. aeruginosa* has a heterogeneous population which means that not all cells are showing foci for T3SS. In addition, the number of positive cells

containing T3SS foci starts at a very low number in an earlier time point (1h) then increases gradually until it reaches the peak at 3h, then decreases gradually with the increase of cell density.

3. - Mass spectrometry data showed the detected effectors are ExoS, ExoT, and ExoY which only appeared at secreting conditions (Lambaki., 2019). To investigate T3SS activity plasmid at the single-cell level and population level, pAD731 reporter was used, results showed that only in *P. aeruginosa* only 2.5% of the population are active and secreting.

4. - C-di-GMP high or low levels have no effect on T3SS activity or formation in *P. aeruginosa.* On the other hand, Results indicated that T3SS increases cell aggregation by presumably increasing the c-di-GMP level inside the cell.

5. - Results showed that high salt concentration increased the rate of growth of *P. aeruginosa* strains. Furthermore, it increased the assembly and activity of T3SS.

6. - We were not able to detect an interaction of the PscQ cytosolic component with external regulatory proteins using Co-immunoprecipitation. And no useful information was found using online bioinformatics tools.

7. After labeling of FliM cytosolic protein of C ring of T3SS core of the flagellum (homolog of PscQ protein of injectisome T3SS) with fluorophore and testing under florescence microscope to study similarities and differences of the two systems, results showed that it appeared as a positive clear signal which is one polar spot at each cell detected in all cells.

Data have also shown that different salt concentrations do not affect the assembly of the T3SS core of the flagellum. So, T3SS are regulated differently in the same species in different systems.

8. From the data in this work and other data in our lab, It was found that the assembly, activity, and regulation of T3SS in *P. aeruginosa* and *Y. enterocolitica* are very different although they belong to the same T3SS Family.

A general and collective conclusion that we can withdraw from our work, is that every T3SS is unique and have a different complex regulation mechanism, even if it exists in the same species in different systems as in the case of injectisome and flagellum or indifferent but related species such as in *P. aeruginosa* and *Y. enterocolitica*. So, the inhibitor's design should be specific to each T3SS.