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Experimental Studies and *in silico* Analysis on Quarum Sensing and System Simulation in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is the most common pathogenic bacterium present in human microbial flora, biofilms, soil and water associated and support self microbial communications and survilance. The present study shows estimated protein concentration present in two days grown in Mullar hinton broth was resulted around 32.5 mg/ml. The studies in protein interaction on X-ray film with aqueous fruit and vegetable extracts showed good results with apple, straw berry, cucumber, grape, orrange, papaya and cabbage more effectively and least interaction was showed in spinach, red spinach and menthi. The quantification studies showed band similar to standard (Albumin) at 67 kDa. Several proteins like LuxQ, LuxU, albumin and LuxP were involved in quorum sensing as per the previous studies conducted by KEGG database. The proteins were submitted to string database for understanding system simulation and the network constructed shows involvement of other proteins like pspF, rhoN, cheA, Hmp etc. Ligand-receptor docking results for the selected autoinducers of QS with receptors showed good activity of HMP protein with Ridaforolimus showing energy value of –134.9 kcal/mol. The results showed that autoinducer like Ridaforolimus plays key role in the mechanism of quorum sensing in P. aeruginosa.

Keywords: *Quorum sensing, system simulation, P. Aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa is the most common gram-negative and rod-shaped bacterium associated with serious illnesses [1, 2]. The extracellular accumulation of autoinducers signals produce cell-cell communication or Quorum Sensing (QS) and alter coordinate behavior and gene expression in *P. aeruginosa* [3]. Quorum sensing is an association and growth process of microbial communication that collectively controls a group of behaviors in a colony. *P. aeruginosa* has employed several interconnected QS systems like, lasRl [4], rhlRl [4], PQS [5], LuxI [6], LuxR [6], HSL [7], OmpK and *qscR* [8]. These molecules dock with auto inducers and stabilize several receptors and show several bioprocesses like DNA binding, enabling dimerization and transcription of QS target genes.

Systems simulation is a combination of techniques and methods used to generate numeric models or displaying complex interaction within system/s [9, 10]. These models provide good solution in solving problems of complexity of the system and understand behavior of the systems.

The regulations of expression of genes present in a system by several messengers or autoinducers are controlled by mechanisms of QS systems [11]. Multicellular systems like *Homo sapiens* contain several thousands of microbes that produce molecules to control and regulate several biological processes. Developing several chemical modifications of autoinducers in a system involves regulating the biological function of QS system. The present work was conducted on *P. aeruginosa*, which is the proposed model to control and inhibit QS system in formation of biofilm and several virulence factors.

MATERIALS AND METHODS

Screening of Microorganisms in Growth Media

The media like nutrient broth and Muller Hinton agar media were selected for screening

of *P. auriginosa*. In the present study, *P.auriginosa* was isolated from soil samples. The colony was identified using standard biochemical identification methods. The isolated strain was used for conducting further work for QS.

Biochemical Protein Identification and Estimation

Biochemical identification and estimation for protein was done by Ninhydrin method. For 5 ml of the isolated sample, 1 ml of the ninhydrin solution was to be added. The solution was heated gently at 80–100ºC for 4–7 min, cooled to room temperature and recorded the absorbance using calorimeter at the wavelength of 570 nm [12]. The sample solution should be compared and measured based on the standard graph (Table 1 and Figure 1).

Protein Purification and Quantification

Protein purification was conducted using **c**olumn chromatography. Quantification of the pure product of protein was conducted using SDS PAGE [13].

Protein Interaction Analysis in Fruit and Vegetable Juices

An X-ray film has to be taken and the experiment should be conducted in a dark room [14]. Mix one drop of aqueous extract of fruits and vegetables respectively with one drop of purified protein sample of *P. aeruginosa.* Place a drop of mixed sample on X-ray film and leave for 10 min. Finally, wash the film with water gently and observe the film for interaction.

***In Silico* Protein-Protein Interaction**

In silico protein-protein interaction was done with string database [14].

Docking

The docking was conducted using iGEMDOCK version 2.1. The receptors (from string data) and ligands (from Drug Bank related to QS) are presented in Figures 2 and 3.

Table 1: *Standard Values.*

x	y
100	1.96
90	1.93
80	1.90
70	1.87
60	1.53
50	1.41
40	1.27
30	1.15
20	1.00
10	0.4
0	0

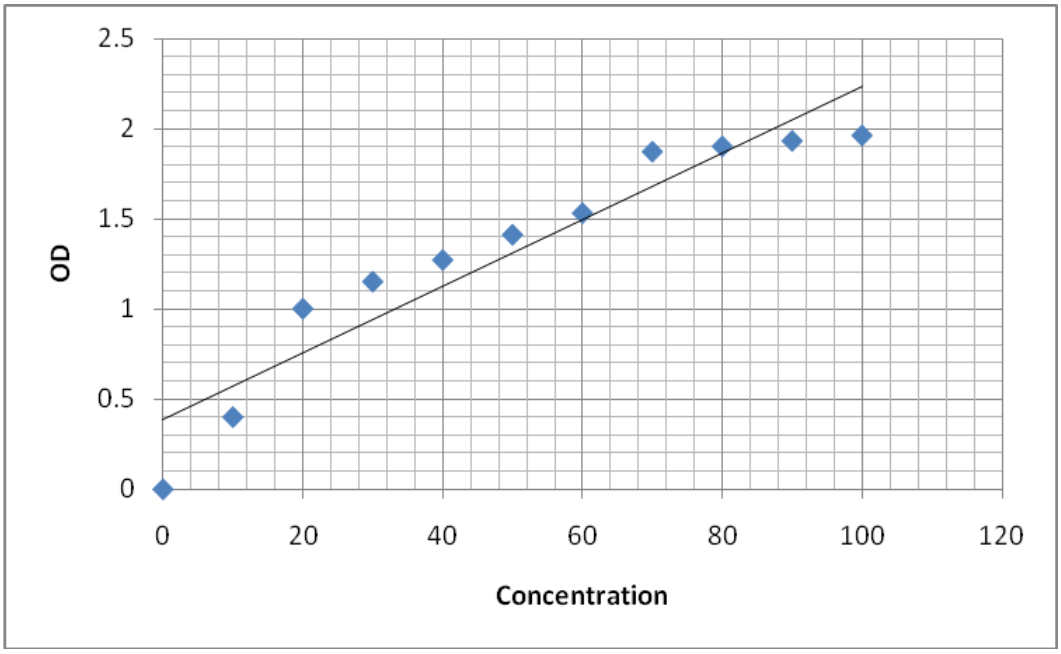

Fig. 1: *Standard Graph.*

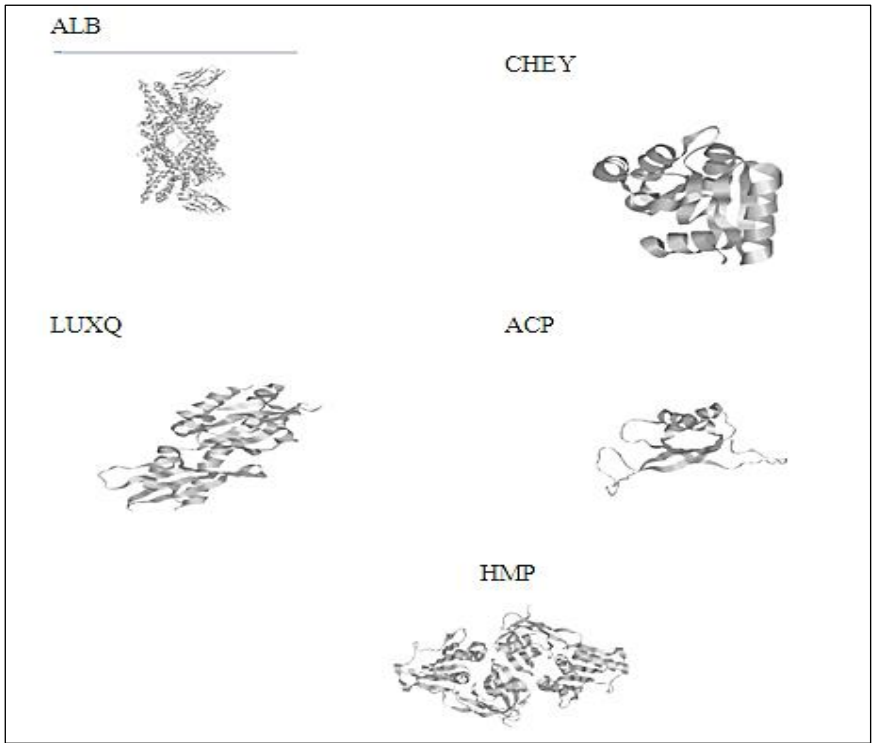

Fig. 2: *Selected Receptors.*

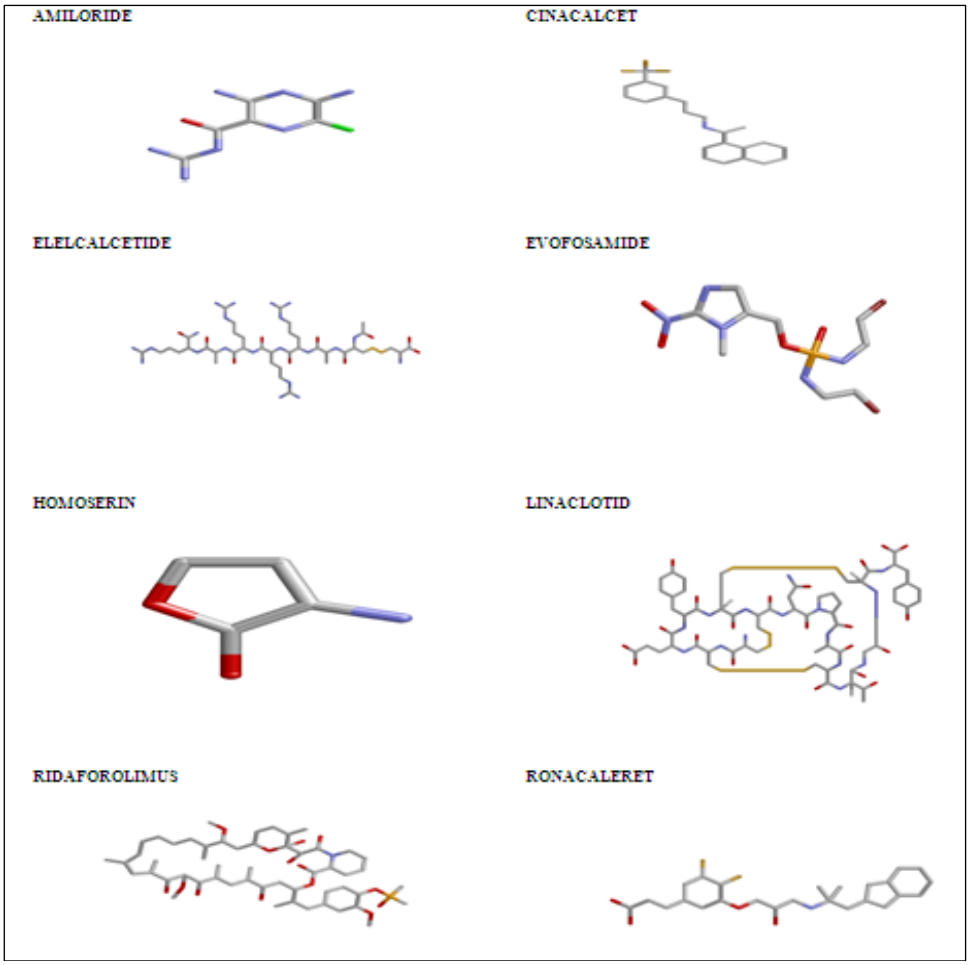

Fig. 3: *Selected Ligands in QS.*

RESULTS AND DISCUSSIONS

Microorganisms were screened in the broth and the proteins were separated using protein separation technique. The biochemical protein test was conducted using Ninhydrin method. The protein detection with conversion to thick purple color in both Candida and Pseudomonas represents presence of more proteins and are presented in Figure 4. A specific inhibitor was isolated using ageinst tyrosine-specific protein kinase from fermentation broth of Pseudomonas species [15]. The purification of samples from OM protein composition of Pseudomonas aeruginosa showed 73 and 49 kD protein staining bands [16, 17].

Estimated protein concentration is shown in Figure 5. The OD value for the protein sample was measured as 1. The OD when cutted in the standard plot was obtained in the experimentation. The protein concentration for the isolated protein resulted around 32.5 mg/ml.

The activity of the protein interaction was measured based on the clear transperent zone that was degraded gelatin present on X-ray film. The protein interaction with fruit and vegetable aqueous extracts showed good results with apple, straw berry, cucumber, grape, orrange, papaya and cabbage more effectively; and least shown in spinach, red spinach and menthi (Figure 6). Previous studies have showed that antibiotics like azithromycin (AZM), ceftazidime (CFT), and ciprofloxacin (CPR) decrease the expression of a range of QS-regulated virulence factors based on DNA microarrays, virulence factor assays and reverse transcription-PCR studies [18].

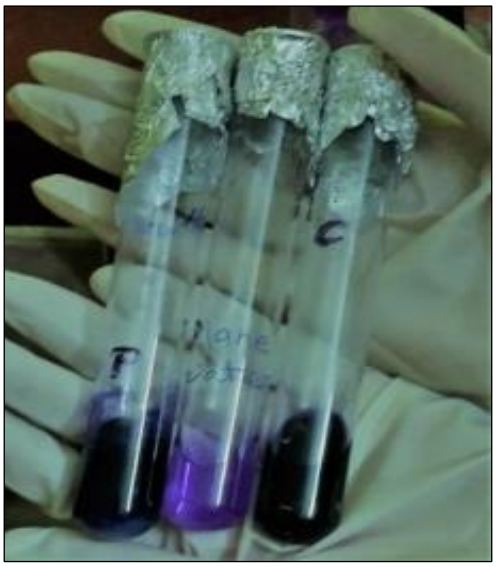

Fig. 4: *Protein Test for Positive Report.*

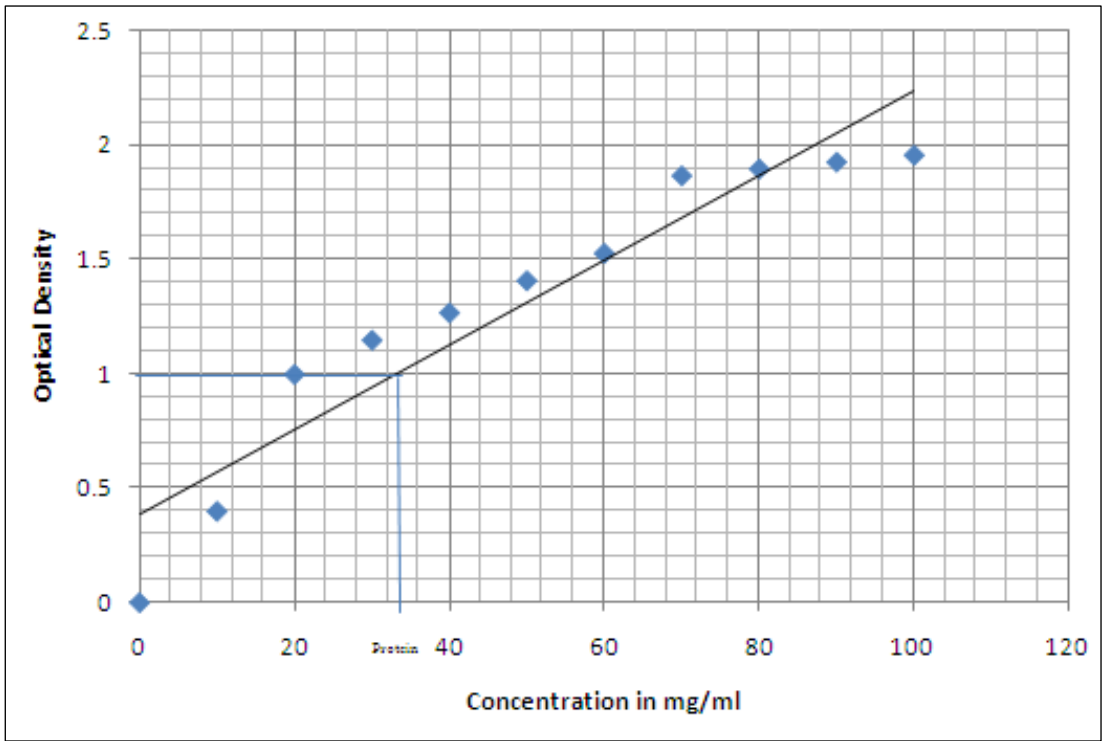

Fig. 5: *Protein Estimation.*

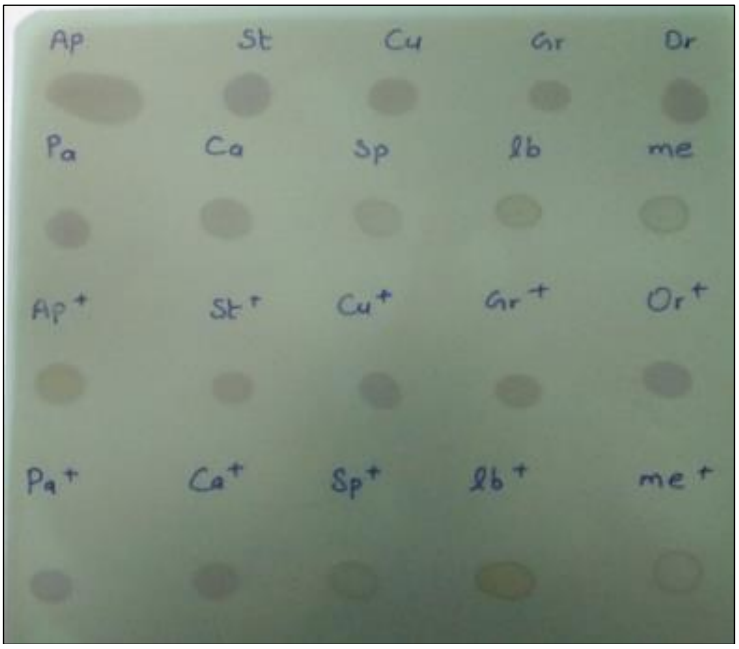

Fig. 6: *X-Ray Protein Interaction.*

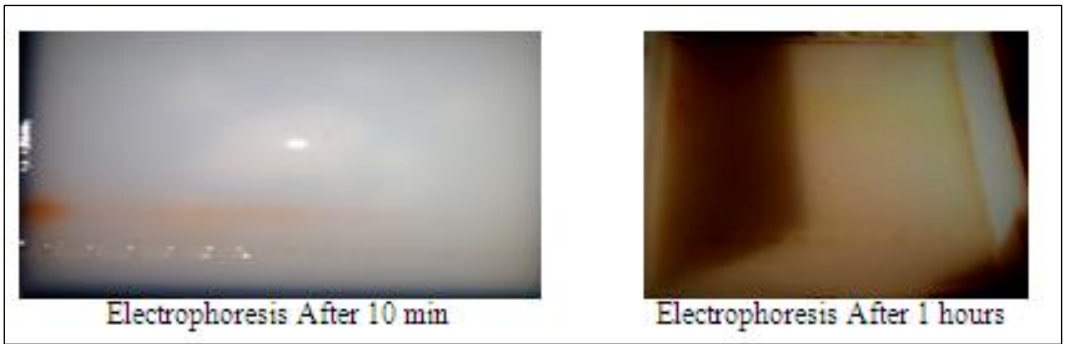

Fig. 7: *Protein Quantification.*

The isolated and purified samples were runned for quantification in gel electrophoresis and resulted in banks similar to albumin (Figure 7). The band observed were shown similar to albumin (67 kDa). Albumin has a great role in quorum sensing due to increase of binding ability of nonspecific lipid molecules [19].

The proteins involved in quarum sensing were retrived from KEGG database and has been reported in Figure 8.

Figure 9 shows that several proteins like LuxQ, LuxU, albumin and LuxP are involved in quorum sensing. The proteins were submitted to string database and the network constructed shows involvement of other proteins like pspF, rhoN, cheA, Hmp etc. Previous reports have also shown that QS involves PQS, Rh1 and Las systems.

In the natural conditions, bacteria release several kinds of micromolecules and autoinducers including metabolic end products, several primary and secondary metabolites such as antibiotics and siderophores (iron chelators), and some cell-to-cell signaling molecules. The small signaling molecules make proteins depend on the action of diffusible signal molecules and leads to cell-cell communications as quorum sensing [20].

Ligand-Receptor Docking Results

The docking was conducted using iGEMDOCK ad the reports are provided in Table 2. The results show good activity of HMP protein with Ridaforolimus (autoinducer) showing energy value of –134.9 kcal/mol. Active site and the docking poses of the best molecule (HMP vs. Ridaforolimus) has been shown in Figure 10.

Ridaforolimus-induced AKT activation is a possible mechanism of resistance to mTOR inhibitors act as molecularly targeted therapies and cytotoxic agents [21].

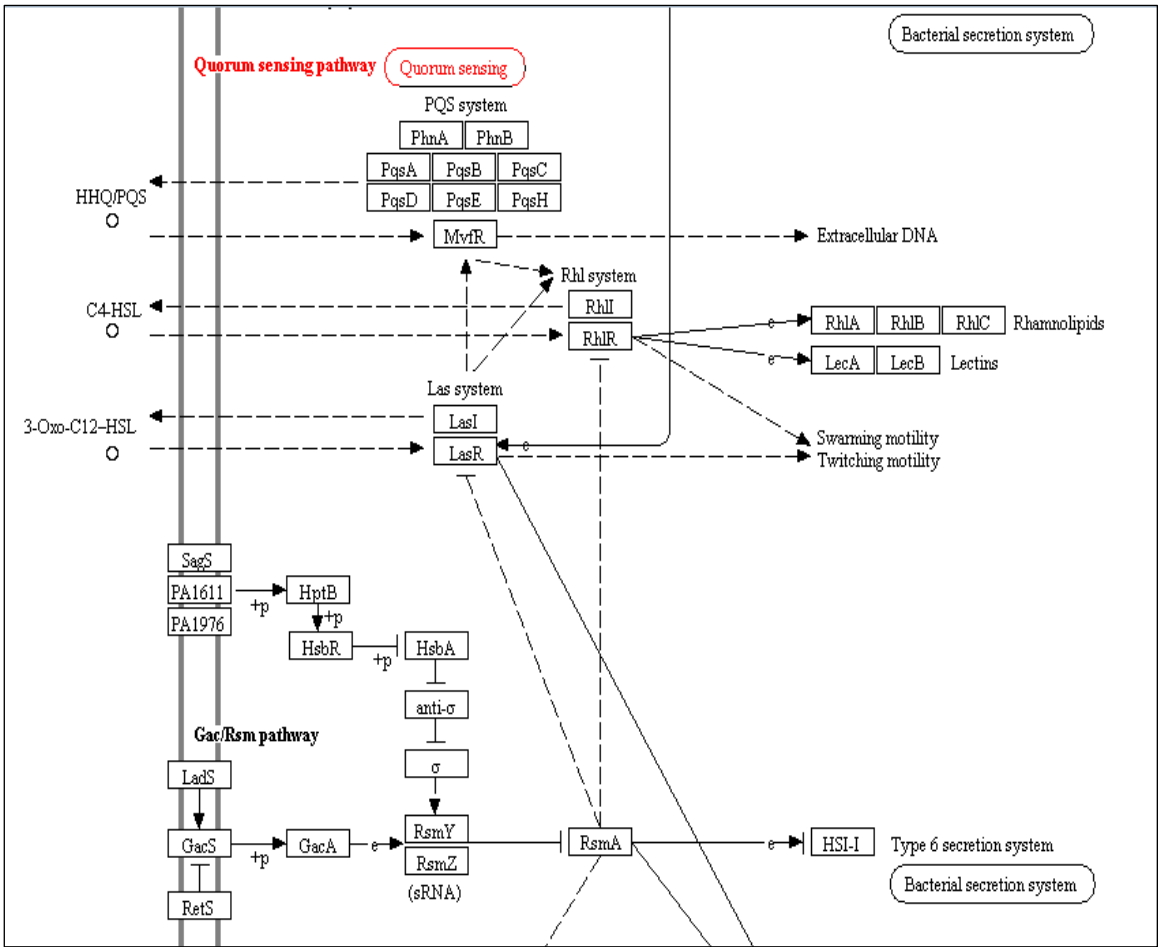

Fig. 8: *Mechanism of Quarum Sensing from KEGG Database.*

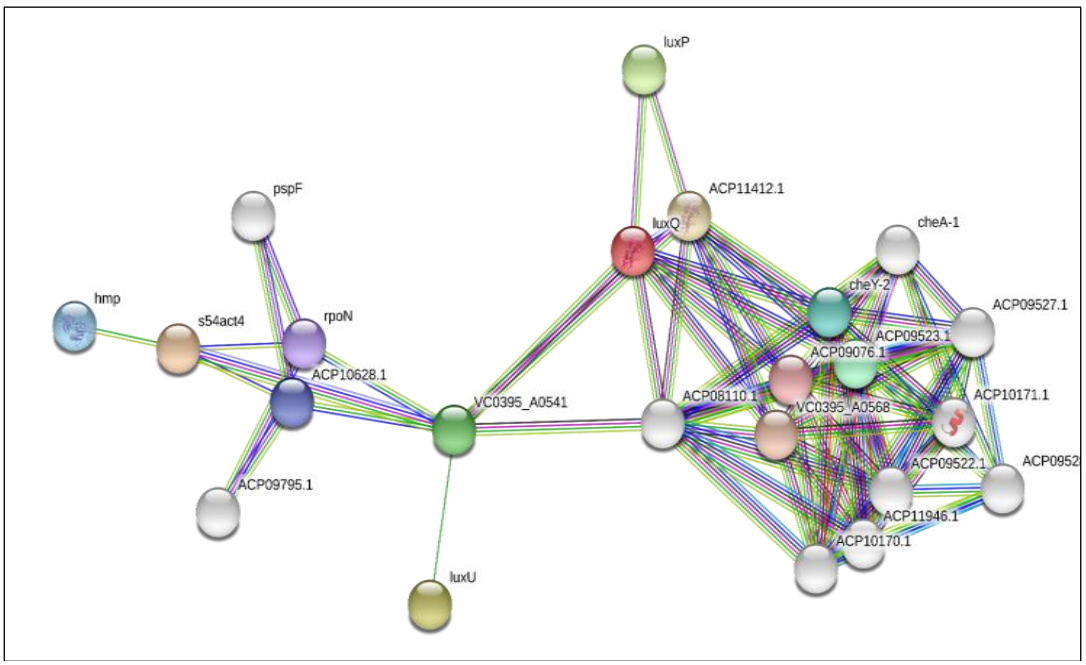

Fig. 9: *String Report Involving in QS.*

Table 2: *Docking Results.*

Protein	Amiloride	Cinacalcet	Ronacaleret	Ridaforolimus	Linaclotid	Evofosamide	Homoserin	Elelcalcetide
ALB	–85.22	–86	–108.4	–109	–80.5	–87.3	–62.55	–89.6
CHEY	–94.3	–71.22	–87	–105.1	–114.8	–76.47	–64.4	–130.7
LUXq	–85.9	–83.1	–99.5	–125.6	–124.3	–76.3	–54.8	–130.7
ACP	–83.4	–69.7	–79.9	–124.51	–117.2	–68.5	–56.1	–120.3
HMP	–91.1	–86.88	–98.8	–134.9	–121.85	–101	–56.9	–112.9

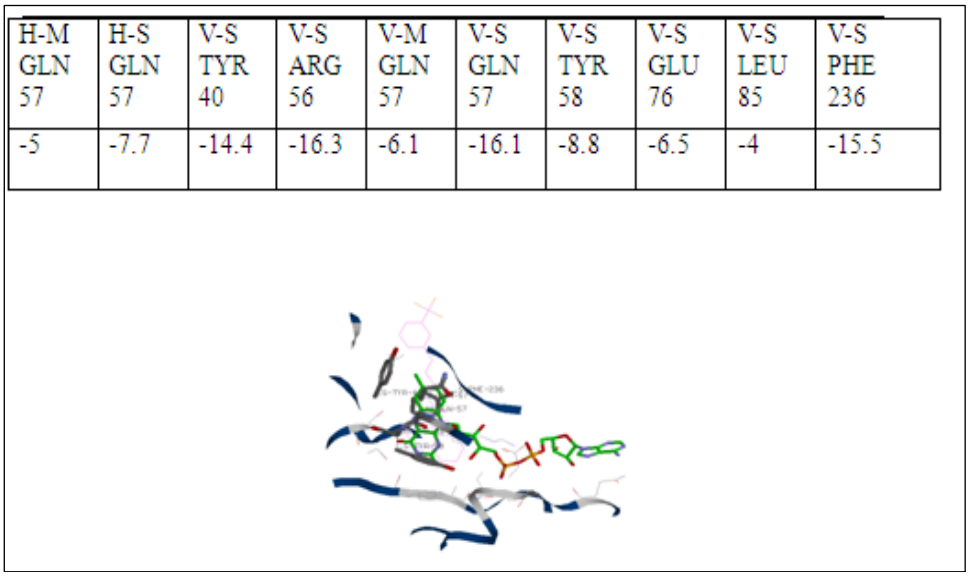

Fig. 10: *HMP vs. Ridaforolimus (Active Site).*

CONCLUSION

The information elucidated by the studies on role of quorum sensing is the useful process in designing strategies of expression in inter- and intra-cellular signaling systems. The results showed good activity of HMP protein with Ridaforolimus showing energy valur of –134.9 kcal/mol. Further process of mechanism studies can provide better results in the understanding of QS.

CONFLICT OF INTEREST

No conflict of interest.

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