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Comparative Molecular docking analysis of DNA Gyrase subunit A in *Pseudomonas aeruginosa* PAO1

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Abstract:
Pseudomonas aeruginosa is an opportunistic bacterium known for causing chronic infections in cystic fibrosis and chronic obstructive pulmonary disease (COPD) patients. Recently, several drug targets in *Pseudomonas aeruginosa* PAO1 have been reported using network biology approaches on the basis of essentiality and topology and further ranked on network measures *viz.* degree and centrality. Till date no drug/ligand molecule has been reported against this targets.In our work we have identified the ligand /drug molecules, through Orthologous gene mapping against *Bacillus subtilis* subsp. subtilis str. 168 and performed modelling and docking analysis. From the predicted drug targets in PA PAO1, we selected those drug targets which show statistically significant orthology with a model organism and whose orthologs are present in all the selected drug targets of PA PAO1.Modeling of their structure has been done using I-Tasser web server. Orthologous gene mapping has been performed using Cluster of Orthologs (COGs) and based on orthology; drugs available for *Bacillus* sp. have been docked with PA PAO1 protein drug targets using MoleGro virtual docker version 4.0.2.Orthologous gene for PA3168 gyrA is BS gyrAfound in *Bacillus subtilis* subsp. subtilis str. 168. The drugs cited for *Bacillus* sp. have been docked with PA genes and energy analyses have been made. Based on Orthologous gene mapping andin-silico studies, Nalidixic acid is reported as an effective drug against PA3168 gyrA for the treatment of CF and COPD.

Keywords: *Pseudomonas aeruginosa*, CF, COPD, *In-silico*, MoleGro virtual docker, Orthology,LigandScout, Nalidixic Acid, Ciprofloxacin, Novobiocin, Norvaline.

Background:
Pseudomonas aeruginosa (PA) is a gram negative, rod-shaped and opportunistic bacterium known for causing chronic infections in cystic fibrosis and chronic obstructive pulmonary disease (COPD) patients **[1].** Its mode of action involves adherence to tissue surface using its pili, flagellum and exo-S and replication to form a mass of cells. The bacterium gradually synthesizes biofilm for its prolonged attachment with host tissues and by its virulent factors causes severe tissue damage. The bio films protect these bacteria from adverse environmental factors, hence raised a serious problem for medical care in industrialised societies, especially for immune-compromised patients and the elderly **[2].** PA is capable of acquiring resistance genes and hence, shows multiple drug resistance. The increasing number of multi drug resistant PA (MDRPA) strains has rendered many existing drugs as ineffective, including the most powerful anti-pseudomonal beta-lactams **[1].**

The capacity of PA to resist multiple front-line antibiotics makes the eradication of the organism nearly impossible **[3].** This has rendered an urgent need of discovery of new drugs and drug/ligand molecules for treating infections caused by *Pseudomonas aeruginosa*. Recently, several drug targets for PA PAO1 are predicted using network biology **[4].** A protein-

protein interaction network shows all possible interactions between proteins of an organism. These interactions are weighed and are considered on the basis of datasets available for an organism. Hubs are identified using network measures *viz*. degree and centrality. Disruption of these hubs by drug/ligand molecules will cause disruption of essential pathways in the organism and will help in the treatment of infections. To address the issue of new drug/ligand discovery we have employed the Orthologous gene mapping approach to identify the suitable ligand molecule for the DNA Gyrase A target in PA POA1 and structure modelling subsequently, docking with various ligands **[5].** Homologous sequences are orthologous if they were separated by a speciation event. Two organisms that are very closely related are likely to display very similar DNA sequences between orthologs **[5].** *Pseudomonas aeruginosa* PAO1 is closely related to *Bacillus subtilis* and shows statistically significant orthologs with it. Hence, a drug against *Bacillus subtilis* could be effective against *Pseudomonas aeruginosa* as shown by our analysis.

DNA Gyrase is an enzyme that influences all metabolic processes involving DNA by regulating negative supercoiling of bacterial DNA and is essential for replication **[6]**. The enzyme gets inhibited by two classes of antimicrobials. This shows that its composition is from reversibly associated subunits **[7].** Inhibition of GyraseA subunit affects breakage and rejoining of DNA, thereby, affecting metabolic pathways. Here, we have modelled the structures of predicted drug targets of PA PAO1 using I-Tasser. Following this, Orthologous gene mapping is done and a set of drugs that have been used for *Bacillus subtilis*, are docked with PA proteins. The energies obtained on docking with PA PAO1 proteins are comparable with *Bacillus subtilis* subsp. subtilis str. 168 as control. These drugs could be effective in overcoming PA multi drug resistant (MDRPA) problems as the drugs are directed against hubs found in the network.

Methodology:

In this study, primary focus is to identify the suitable drug molecules against DNA Gyrase A PA PAO1. Recently, several drug targets *viz.* PA0004gyrB, PA3168 gyrA, PA3482 metG, PA3834valS, PA3987 leuS, PA4238 rpoA, PA4268 rpsL, PA4269 rpoC, and PA4967 parE have been reported on the basis of network biology approch. We have selected PA0004 gyrB, PA3168 gyrA and PA3987 leuS, drug targets on the basis of finding statistically significant (orthologs; similar function in divergent species) in COG (cluster of orthologs) database. We collected orthogus genes in model organism *Bacillus subtilis* subsp. subtilis str. 168 and their corresponding known drug molecules. Drugs available for *Bacillus* sp. have been docked with proteins of PA PAO1and the ligands have been tested for drug-likeness, toxicity and other pharmacological properties. The results have been analyzed in terms of energies or 'poses' to give the best five poses which bind satisfactorily to the target protein. These molecules could be analysed *in-vitro* and *in-vivo* for confirmation and evaluation of its properties.

Retrieval of drug target and orthologous mapping

From reported drug targets in PA PAO1, we have selected PA0004, PA3168 and PA3987 for hypothesis of drug / ligand on the basis of statistically significant orthology in close model organisms. Orthologous genes have been found using Cluster of Genes (COG) database and the e-value of 1e-5 has been used for statistical significance **[8].** All three targets of PA PAO1 show orthology with three distinct genes of *Bacillus subtilis* subsp. subtilis str. 168 were selected.

Sequence analysis (MSA) and Phylogenetic analysis

Sequence alignment has been performed for finding the similarity and identity between PA PAO1 and *Bacillus subtilis* subsp. Subtilisstr. 168 using ClustalW/MSA-Emboss Needle- and ClustalW2-Phylogeny softwares **[9].** BSgyrB, BSgyrA and BSleuS genes from *Bacillus subtilis* subsp. subtilis str. 168 have exhibited orthology with PA3168, PA0004 and PA3987 genes of *Pseudomonas aeruginosa* PA PAO1 **[10].**

Ligand structure generation, modelling and docking

Drugs against *Bacillus subtilis* genes have been searched from literature and the structures have been downloaded from Pub Chem in a 2-D format **[11].** For refining the structures based on alignment and hydrogen bonding, the 2-D structures have been modified to 3-D structures using Marvin Sketch tool (3D structure of ligand shows better binding efficiency with the receptor proteins, as compared to 2D structures). The protein structures of BS gyrB, BS gyrA, BSleuS, PA0004, PA3168 and PA3987 have been modelled using the I-Tasser web server, a package of standalone computer programs which is used for protein structure prediction, refinement and structure-based functional annotations **[12].**

Docking has been performed using Mole Gro virtual docker tool where the largest cavity in the target protein is identified on the basis of cavity volume. This could be done online *viz* castP software **[13].** The docking is represented by MolDock scoring function which has been derived from the PLP (Piecewise Linear Potential) scoring functions **[14].** Docking of ligand / drug has been performed in the largest cavity based on cavity volume, and the results obtained are saved. From the docking results, energy vs. conformations graph, the MolDock score representing the binding energy and the hydrogen bonding energy score are of particular importance for analysis. These docking result as .MVD file format have been loaded on the same workplace containing the ligand and the target cavity to visualise and analyse the docking in various poses or conformations of ligand as in **Figure 1(A) & (B).**

Study of Ligand-Substrate Interactions

Interactions of different poses of ligands have been investigated on LigandScout 3.0.3 software package**.** Pharmacophores are an ensemble of universal chemical features that characterises a specific mode of action of a ligand in the active site of the macromolecule in 3-D space necessary to ensure the optimal interactions with a specific biological target and to trigger (or block) its biological response. In other words it is the 3D arrangement of functional groups that enable a compound to exert particular biological effects. Pharmacophores corresponding to all the drugs have been studied and comparative differences of interactions between drug-drug targets have been identified. The optimal interactions between drug-drug targets and Chemical features including hydrogen bonding, charge interactions and hydrophobic areas are depicted in **Figure 1(C) & Figure 1(D).**

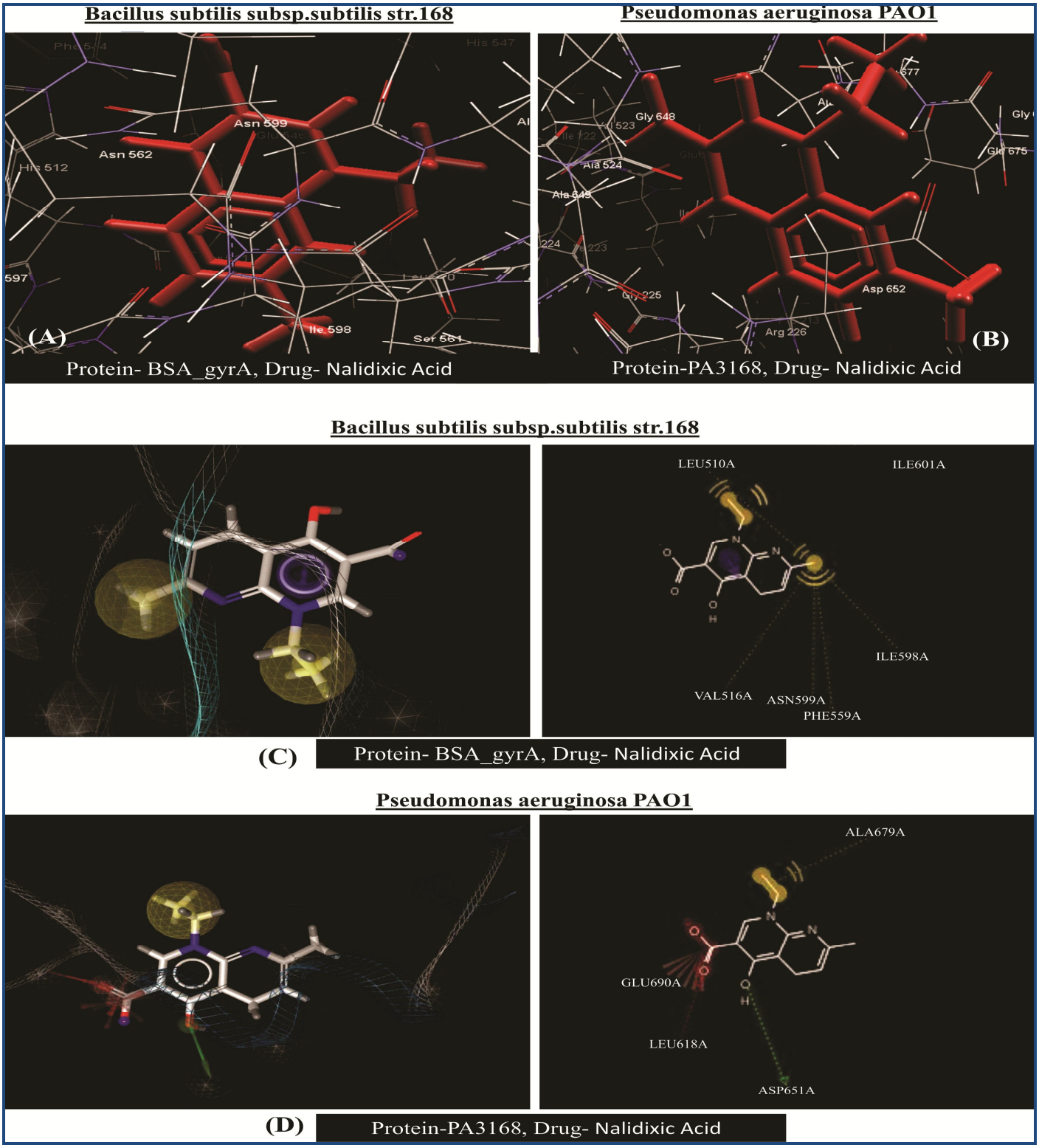

Figure 1: Snapshots of docking and interaction between DNA Gyrase subunit A protein receptor and their respective drug Nalidixic acid from Pseudomonas aeruginosa and Bacillus subtilis. **(A)** and **(B)** Red color represents ligands or drug molecules and wireform and amino acid residue represent protein structure; **(C)** The interaction of ligands with Bacillus subtilis subsp. subtilis str. 168 proteins**; (D)** The interaction of ligands with Pseudomonas aeruginosa PAO1 proteins.

Discussion:

BSgyrB, BSgyrA and BSleuS genes from *Bacillus subtilis* subsp. subtilis str. 168 showed orthology with PA0004, PA3168 and PA3987 genes of *Pseudomonas aeruginosa* PAO1. The identity and similarity as obtained by ClustalW / MSA Orthologous gene mapping method are summarised as follows: 2-hydroxyquinoline, Nalidixic Acid, Oxolinic Acid, Norfloxacin and Ciprofloxacin are known to affect BS as found from

literature **[15-21].** These have been docked with BSgyrA gene of *Bacillus subtilis* subsp. subtilis str. 168. The energies (docking score) for these ligands have been found as -79.8034,-88.5147,-107.328,-111.648 and-122.139 kJ/mol respectively with BSgyrA **Table 1 (see supplementary material).** For BSgyrB, the drugs found are 2 hydroxyquinoline, Novobiocin, Oxolinic Acid. The energies after docking have been found as -75.4863,153.154 and -107.975 kJ/mol respectively. Further, Norvaline has been used as a drug for BSleuS. The energy obtained after docking is -61.046 kJ/mol. Since, *Pseudomonas aeruginosa* PAO1 protein PA0004 showed orthology with BSgyrB gene, the drugs *viz.* 2-hydroxyquinoline, Oxolinic Acid, Novobiocin have been docked with PA0004. The energies obtained after docking are -65.0716,-102.641,-115.071 kJ/mol. Similarly, for PA3168, 2-hydroxyquinoline, Nalidixic Acid, Oxolinic Acid, Norfloxacin, Ciprofloxacin drugs have been used.

The energies after docking have been found as -52.9323,-86.9967, -72.9273, -72.4611, -76.2732 kJ/mol.For PA3987, Norvaline has been docked and MolGro Docking energy was found to be -50.1084kJ/mol as given in **Table 2 (see supplementary material).** The drug Nalidixic acid is known to target Bacterial DNA Gyrase along with other antibiotics agaisnt *Bacillus* sp. could be used to control the growth of orthologous close organism like PA PAO1. While conducting docking experiment, Nalidixic Acid has shown -88.5147 kJ/mol MolGro docking score with BSgyrA, which is comparable with PA PAO1 protein PA3168 (docking score -86.9967 kJ/Mol). Hence, it can be used for targeting PA3168 protein in PA PAO1, which is an essential protein and once disrupted, can cause elimination of the pathogen. However, other drugs used against *Bacillus subtilis* subsp. str168 have not shown comparable energy scores in PA PAO1. Also, the results obtained for other drug target proteins, PA0004 and PA3987, have not shown comparable energies in PA PAO1.

Conclusion:

Orthologous gene mapping of *Pseudomonas aeruginosa* PAO1genes namely PA0004, PA3168 andPA3987 showed orthology with BSgyrB, BSgyrA and BSleuS genes of *Bacillus subtilis* subsp.subtilis str. 168 respectively. Drugs have been found for PA3168 (DNA Gyrase subunit A), PA0004 (DNA Gyrase subunit B), and PA3987 (leucyl tRNA synthetase) based on orthologous genes found in *Bacillus subtilis* subsp. subtilis str.168. Nalidixic Acid is reported as an excellent inhibitor for Gyrase A in *Bacillus* sp. and have been found to show comparable energies and hydrogen bonding levels in PA PAO1 orthologous gene (statistically significant) PA3168 while the docking results of target PA3168 drug target of PA PAO1 with other drug molecules namely 2-hydroxyquinoline, Oxolinic Acid, Norfloxacin and Ciprofloxacin cited for BS gyr A of *Bacillus subtilis* subsp. subtilis str.168 were not comparable. Thus, our study suggests that Nalidixic acid drug molecule could be a potential DNA Gyrase A inhibitor in PA PAO1, which could be validated by *in-vitro* experiments. Further, the computer aided drug discovery process along with genomic information of drug targets may enhance our understanding towards in-sight of mechanism of drug- target interactions and their binding patterns.

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Supplementry material:

Table 1: Identity and Similarity (in percentage) between *Bacillus subtilis* and *Pseudomonas aeruginosa* PAO1usingClustalW / MSA Orthologous gene mapping approach

Protein name	Identity	Similarity
BSgyrB with PA0004	38.4 %	26.3%
BSgyrA with PA3168	48.0 %	65.8 %
BSleuS with PA3987	42.6 %	58.5 %

Table 2: Output of MoleGro virtual docker showing Cavity Volume, Mol Dock Score and H bond. for different drug targets of Pseudomonas aeruginosa and its orthologs in Bacillus subtilis.

Bacteria strain	Protein	Drug(Ligand)	Cavity Number	Cavity Volume Unit- Å^3	Mol dock Score(energy) Arbitrary unit	HBond Arbitrary unit
Bacillus subtilis subsp. subtilis str. 168	BS_gyrA	2-hydroxyquinoline	[00]	3509.25	-79.8034	-1.16373
		Nalidixic Acid				
		Oxolinic Acid				
		Norfloxacin				
		Ciprofloxacin				
	BS_gyrB	2-hydroxyquinoline	[00]	237.056	-75.4863	
		Novobiocin			-153.154	
		Oxolinic Acid			-107.975	
	BS_leuS	BS_leuS	BS_leuS	BS_leuS	BS_leuS	BS_leuS
Pseudomonas aeruginosa PAO1	PA3168	2-hydroxyquinoline	[00]	987.648	-52.9323	-2.5
		Nalidixic Acid			-86.9967	0.00
		Oxolinic Acid			-72.9273	-2.53097
		Norfloxacin			-72.4611	0.00
		Ciprofloxacin			-76.2732	-2.47654
	PA0004	2-hydroxyquinoline	[00]	640	-65.0716	-0.76023
		Novobiocin			-115.071	-3.79048
		Oxolinic Acid			-102.641	-1.63591
	PA3987	Norvaline	[00]	1948.07	-50.6014	-2.60045