Syst Synth Biol (2012) 6:23–30
DOI 10.1007/s11693-012-9093-z

RESEARCH ARTICLE

Gene network analysis of *Aeromonas hydrophila* for novel drug target discovery

Vijai Singh · Dharmendra Kumar Chaudhary · Indra Mani

Received: 13 April 2012 / Revised: 6 May 2012 / Accepted: 8 May 2012 / Published online: 22 May 2012

Abstract Increasing the multi-drug resistance *Aeromonas hydrophila* creates a health problem regularly thus, an urgent needs to develop and screen potent antibiotics for controlling of the infections. There are many studies have focused on interactions between specific drugs, little is known about the system properties of a full drug interaction in gene network. Thus, an attractive approach for developing novel antibiotics against DNA gyrase, an enzyme essential for DNA replication, transcription, repair and recombination mechanisms which is important for bacterial growth and cell division. Homology modeling method was used to generate the 3-D structure of B subunit of DNA gyrase (gyrB) using known crystal structure. The active amino acids in 3-D structure of gyrB were targeted for structure based virtual screening of potent drugs by molecular docking. Number of drugs and analogs were selected and used for docking against gryB. The drugs Cinodine I, Cyclothialidine and Novobiocin were found to be more binding affinity with gyrB-drug interaction. The homology of gyrB protein sequence of *A. hydrophila* resembles with other species of *Aeromonas* closely showed relationship in phylogenetic tree. We have also demonstrated the gene network interactions of gyrB with other cellular proteins which are playing the key role in gene regulation. These findings provide new insight to understand the 3-D structure of gyrB which can be used in structure-based drug discovery; and development of novel, potent and specific drug against B subunit of DNA gyrase.

Keywords *Aeromonas hydrophila* · gyrB · Docking · Phylogenetic tree · Gene network · Drug

Electronic supplementary material The online version of this article (doi:10.1007/s11693-012-9093-z) contains supplementary material, which is available to authorized users.

V. Singh (✉)
Institute of Systems and Synthetic Biology, Universite d'Evry Val d'Essonne-Genopole-CNRS UPS3201, Batiment Geneavenir 6, Genopole Campus 1, 5 rue Henri Desbruères, 91030 Evry Cedex, France
e-mail: vijaisingh15@gmail.com

V. Singh · D. K. Chaudhary (✉) · I. Mani
National Bureau of Fish Genetic Resources, Canal Ring Road, PO-Dilkusha, Lucknow 226002, India
e-mail: chaudharydk12@gmail.com

I. Mani
Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi 221005, India

Introduction

Aeromonas hydrophila is a ubiquitous pathogen involved in gastroenteritis, meningitis, skin and soft tissue infections with a variety of clinical syndromes in immunocompromised patients globally (Holmes et al. 1996; Janda and Abbott 1998). It is associated with hemorrhagic septicemia in cold blooded animals including fish, reptiles and amphibians (Janda 1991; Austin and Austin 1999). Hemorrhagic septicemia in fish fin and tail rot due to *A. hydrophila* infection resulted in high rate of mortality in aquaculture systems (Chakraborty et al. 1987; Hickman-Brenner et al. 1987; Barghouthi et al. 1989; Janda et al. 1994). *A. hydrophila* is an emergent human pathogen which caused serious health problem regularly around the globe (Janda and Abbott 1998; Abbott et al. 1998; Joseph and Carnahan 2000).

The most widely used method for controlling *A. hydrophila* infections in aquaculture is using antimicrobial drugs. Extensive use of antibiotic has resulted in rapid spread of multi-drug resistant pathogens (Rathore et al.

2006). There is an essential for controlling *A. hydrophila* infection using different antibiotics which is targeted the specific protein/enzyme of *A. hydrophila.* The unique ability of gyrase B to introduce negative supercoiling into DNA and itself controls DNA supercoiling. Gyrase catalyzes the conversion of relaxed, closed circular duplex DNA to negatively superhelical form, which is more favorable for recombination. The mechanism of supercoiling involves in wrapping of gyrase around a region of DNA, double strand breaks in that region, passing a second region of DNA through the breaking and rejoining of broken strands. The *gyrB* encodes B-subunit of DNA gyrase, a type-II DNA topoisomerase which have been reported as a suitable phylogenetic marker for bacterial identification (Yamamoto and Harayama 1996, 1998; Venkateswaran et al. 1998; Yamamoto et al. 1999, 2000).

There are several reports available on generation of three dimensional (3-D) structure of unknown protein using homology modeling. However, the 3-D structure of gyrB from *A. hydrophila* is not yet determined. Homology modeling has been used for generation of 3-D structures of 3-oxoacyl-acyl carrier protein synthase II of *Mycobacterium tuberculosis* (Singh and Somvanshi 2009a) and aerolysin as well as hemolysin proteins in *A. hydrophila* (Singh and Somvanshi 2009b; Singh et al. 2009). Therefore, several antibiotics and its derivative have been used against DNA gyrase for controlling the cell viability. Cyclothialidine is a potent drug against DNA gyrase which has been isolated from *Streptomyces filipinensis* (Goetschi et al. 1993). The amino substituted coumarins have synthesised and evaluated in vitro as inhibitors of DNA gyrase which is also showed antibacterials activity. Novobiocin like coumarins, 4-(dialkylamino)-methylcoumarins and 4-((2- alkylamino)ethoxy)coumarins have been discovered as gyrase B inhibitors with promising antibacterial activity (Laurin et al. 1999). However, single drug against particular protein is not very effective to control multidrug resistance *A. hydrophila*. Therefore, we need to understand the complete gene network involves in cellular activity.

The gene networks are a complex interaction of biological parts such as DNA, RNA and proteins contributed in biological process. In depth knowledge of biological process seems that we need to explore the relationship between network structure and the dynamics of genes, proteins and other biomolecules. There are number of reports available on gene and protein interaction and other gene networks which lead to target site for drug discovery (Samal and Jain 2008). It can also be helpful to repress and activate the gene networks for control of cellular activity. Some proteins serve to activate and repress other genes which are also known as transcription factors are playing key role in gene regulation. It binds with operator site on promoter region which is initiated the turn off and on process. The aim of present study is to develop homology based model of DNA gyrase B subunit in *A. hydrophila* and structure based potent drugs screening; and demonstrated the gene network of B subunit of DNA gyrase.

Materials and methods

Collection of sequences

The complete protein sequence of DNA gyrase B subunit of *A. hydrophila* and other species of *Aeromonas* were retrieved from NCBI (http://www.ncbi.nlm.nih.gov). The relatedness of sequence deposited in databases was evaluated by BLAST (Altschul et al. 1997) which is implemented in NCBI (http://www.ncbi.nim.nih.gov/blast). We performed BLASTP for searching of protein structural similarity with protein databank (PDB). The alignment was also done for query protein sequence with Protein Data Bank (PDB: 1EI1) template using CLUSTAL X.

Homology modeling for generation of 3-D structure

The crystal structure of DNA gyrase B of *Escherichia coli* was available at 2.30 Å resolution in PDB (PDB: 1EI1) and used as a template from protein data bank (http://www.rcsb.org/pdb/) to generate 3-D models. The homology modeling was used to generate the 3-D structure of gyrB using Modeller9v6 (Sali and Blundell 1993) and was visualized in PYMOL (Delano 2002). Evaluation of generated 3-D structure of gyrB was considered minimum model score and dope score. The 3-D structure of gyrB was validated with PROCHECK (Laskowski et al. 1993) which generates Ramachandran plot (RP); where the amino acid residues present in allowed, disallowed region and overall G- factor were considered.

Virtual screening of drugs by molecular docking

All drugs were taken from NCBI Pubchem compounds in SDF which is converted into 3-D structure using Open Babel 2.0.2 software. The generated 3-D structure of gyrB and drugs were used for molecular docking with AutoDock3.0.5 (Morris et al. 1998). The docking parameters were as follows: 100 docking trials, population size 150, random starting position and conformational translation step ranges of 1.5 Å, rotation step ranges 35, elitism of 1, mutation rate 0.02, cross over rate of 0.8, local search rate of 0.06 and 25 million energy evaluations. Distance-dependent function of the dielectric constant was used for the calculation of the energetic maps and all other parameters were used by default value. We selected the drugs bind to 3-D of gyrB with highest binding affinity.

Construction and analysis of phylogenetic tree

The protein sequence of gyrB of *A. hydrophila* was used to search for homologous sequences by using BLASTP and homologous sequences were retrieved from NCBI-GenBank. The retrieved sequences were aligned in CLUSTALX (Thompson et al. 1997) and using the passion correction which is implemented in MEGA4.0 (Tamura et al. 2007) software for construction of phylogenetic tree by neighbor-joining (NJ) method. Total 100 bootstrapped values were sampled to determine a measure of the support for each node on the consensus tree.

Protein–protein interaction

We used STRING database of known and predicted protein interaction (http://string-db.org/). The interactions include direct (physical) and indirect (functional) associations; they are derived from four sources: (1) genomic context, (2) High-throughput experiments, (3) conserved coexpression and (4) literature knowledge. In the STRING quantitatively integrates interaction data from these sources for a large number of organisms and transfers information between organisms. It is currently covers 5,214,234 proteins from 1,133 organisms during protein–protein interaction.

Protein structure accession number

The homology model of 3-D structure of DNA gyrase B subunit of *A. hydrophila* subsp. hydrophila ATCC 7966 was submitted to Protein Model Database (http://mi.caspur.it/PMDB/) and assigned under PM0075580.

Results and discussion

DNA gyrase B subunit (NCBI accession number: ABK37959) of *A. hydrophila* was used for generation of molecular model using known 3-D crystal structure. The N-terminal region of gyrB of *A. hydrophila* contains ATP binding site which was confirmed from NCBI conserved domain database. It was resembled with DNA gyrase B subunit of *E. coli*. There are few drugs earlier reported against the inhibition of gyrase activity. Sometimes due to mutations in the active amino acid residues, bacteria showed the resistance to antibiotics. There is an important requirement to develop; screen and potent target based specific antibiotics for controlling of *A. hydrophila* infection. Total 25 isolates of *A. hydrophila* have been selected for antibiotic sensitivity assay. All these isolates were resistant to Cephalothin, Ampicillin, Novobiocin and Nitrofurazone while sensitive to Gentamicin (80 %), Co-trimaxazole (92 %), Chloramphenicol and Ciprofloxacin (Rathore et al. 2006).

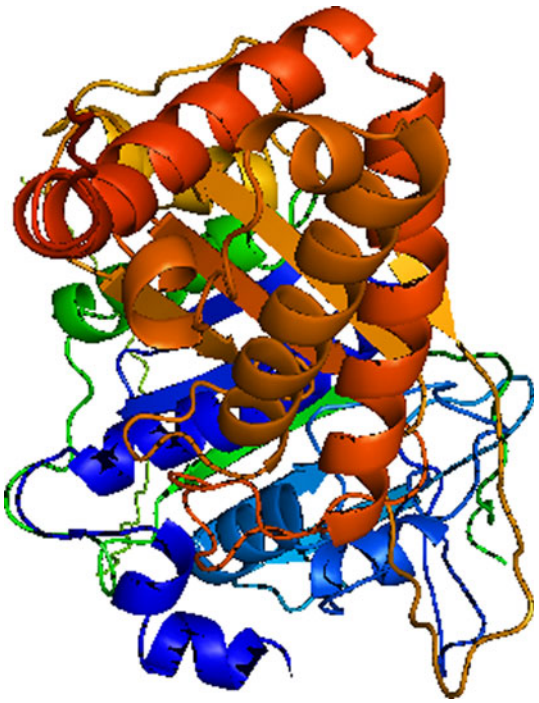

Fig. 1 The 3-D structure of DNA gyrase B subunit of *Aeromonas hydrophila* showing $\alpha + \beta$ sheets

However, there is major difficulty for selection of suitable antibiotics for controlling of infection. Here, we were performed the homology modeling for generation of conserved protein DNA gyrase B subunit which showed 36 % identity with crystal structure of DNA gyrase B of *E. coli*. Both the protein sequences of gyrB of *A. hydrophila* and *E. coli* (PDB: 1EI1) were aligned. Total 5 models were generated by Modeller9v6 and the Gibbs free energy of gyrB of *A. hydrophila* was almost similar with template. The 3-D structure of gyrB of *A. hydrophila* was shown (Fig. 1) and it restrains alpha helix rich in the structure. Ramachandran plot (RP) for gyrB of *A. hydrophila* was shown (Supplementary Fig. S1) in the allowed and disallowed regions the amino acid residues were 80.9 and 1.6 % respectively. All these above properties of gyrB showed the good quality of 3-D structure.

There is recent report on the homology modeling has been used to fabricate the 3-D structure of aerolysin and hemolysin of *A. hydrophila* using known protein crystal structure (Singh and Somvanshi 2009b; Singh et al. 2009). The 3-D structure of gyrB of *A. hydrophila* was taken for screening of suitable antibacterial drugs based on highest binding affinity. The gyrB specific antibacterial drugs such as common name, IUPAC name, molecular formula, molecular weight and 2-D structure of each drugs used in this study were given (Supplementary Table 1). Eight suitable drugs were selected for molecular docking against whole 3-D structure of gyrB and three drugs Cinodine I,

Table 1 The interaction energy (kcal/mol) of DNA gyrase B and drugs obtained from the molecular docking

S no.	Drugs	Binding energy (kcal/mol)	Docked energy (kcal/mol)	Inter molecular energy (kcal/mol)	Torsional energy (kcal/mol)	Internal energy (kcal/mol)	RMSD
1.	Albamycin	−04.45	−02.26	−06.94	2.49	04.68	133.15
2.	Coumermycin	−07.13	19.55	−10.87	3.74	30.42	149.55
3.	Cyclothialidine	−16.24	−16.20	−18.53	2.49	02.53	123.77
4.	Cinodine I	−11.51	−09.14	−17.11	5.60	07.97	145.20
5.	Novobiocin	−08.46	−05.79	−10.64	2.18	04.85	125.65
6.	Clerocidin	−08.67	07.95	−13.96	5.29	21.91	131.25
7.	Nalidixic acid	−00.59	−00.81	−01.22	0.62	00.41	138.00
8.	Cathomycin	−03.02	−03.94	−04.57	1.56	00.63	136.03

Cyclothialidine and Novobiocin were found highest binding affinity. On the basis of docking, the interaction of drugs with gyrB shown several energy such as docking energy, inter molecular energy, torsional energy, RMSD and internal energy were given (Table 1).

In the present study, total 10 docking experiment were performed with the entire 3-D structural protein of gyrB which is considered lowest free energy of docked complex with hydrogen bonds. The docking energy of the Cinodine I, Cyclothialidine and Novobiocin were −09.14, −16.20 and −05.79 kcal/mol, respectively. The design and synthesis of a series of novel 2, 3-dihydroisoindol-1-ones structurally related to cyclothialidine 2 with DNA gyrase showed inhibition activity (Lübbers et al. 2007). The fragment-based design of potent DNA gyrase inhibitors has been reported. Using the virtual screening and NMR spectroscopy analysis identified the binding of two low-molecular weight fragments (2-aminobenzimidazole and indolin-2-one) to the 24 kDa N-terminal fragment of DNA gyrase B. In silico optimization of indolin-2-one led to the discovery of potent DNA gyrase inhibitors (Oblak et al. 2005).

In the present study, several amino acids residue in 3-D structure of gyrB during the interaction with drug were given in Table 2. Amino acid residues such as Asn75, Arg137, Arg77, Asn138, Ser303, Val141, Glu143 and Tyr302 in 3-D structure of gyrB of *A. hydrophila* were observed with interaction of Cinodine I. The drug was bound with these amino acids of gyrB and three hydrogen bond (HB) were generated between UNK1:O-Ser303:OG, UNK1:O-Tyr302 UNK1:H-Arg77:O atoms with distance these 2.163, 2.163 and 2.163 Å respectively (Fig. 2a). In second interaction, amino acid residues viz. Phe105, Arg672, Asp106, Asp107, Thr595, Lys111 and Val670 in gyrB of *A. hydrophila* were observed with interaction of Cyclothialidine. The drug was bound with these amino acids of gyrB and three HB were formed between drug and amino acids UNK0:H—Phe105:O, UNK0:H—Val670:O, UNK0:H-Arg672:O atoms with distance 2.175, 2.175 and 2.175 Å respectively (Fig. 2b). The amino acid residues viz. Lys111, Thr595, Lys104, Phe105, Glu673, Asp107, Arg672 in gyrB of *A. hydrophila* were observed in the interaction with Novobiocin which was bound with these amino acids of gyrB and single hydrogen bond (HB) was formed in between UNK1:H—Glu673:OE1 atom with 2.24 Å distance (Fig. 3). The amino acids of the 3-D structure of aerolysin of *A. hydrophila* have been targeted by saponin drug which could be useful for prevention of oligomerization on the surface of RBCs. Molecular docking has been performed against the aerolysin with the saponin and its analogs which showed to be potent for inhibition of oligomerzation (Singh and Somvanshi 2009b).

The 3-oxoacyl-acyl carrier protein synthase II of *M. tuberculosis* catalyses the initiation of fatty acid synthesis pathway by condensation of acyl CoA and mycolic acid during the elongation phase. The homology modeling has been used to generate and validate 3-D protein structure of 3-oxoacyl-acyl carrier protein synthase II. Thiolactomycin, Thiophenone and multidrug Cerulenin Isoniazed have been found potent for inhibition against *M. tuberculosis* docking experiment (Singh and Somvanshi 2009a). As we know that the evolutionary relationship of *A. hydrophila* based on DNA gyrase protein which is also present in other bacterial pathogen.

In this study, the phylogenetic tree was constructed using *gyrB* sequence of *A. hydrophila* and other bacteria. Total 3 major clades like *Enterobacteriaceae, Vibrio* and *Aeromonas* species were observed in phylogeny of *gyrB* protein sequences. *A. hydrophila* and *A. salmonicida* were observed in same clades; it indicates the similar antibacterial drugs such as Cinodine I, Cyclothialidine and Novobiocin can be used to inhibit the gyrase activity (Supplementary Fig. S2). *Vibrio* species were also closed with *Aeromonas* species; the same drugs might be helpful for inhibition of gyrase activity. The phylogeny indicates the *gyrB* is a stable and potential drug target for *Aeromonas* species especially *A. hydrophila* and *A. salmonicida*. The phylogenetic relationships of *Aeromonas* species have been

Table 2 The summary of the active amino acid residues and hydrogen bond formed between the drugs and DNA gyrase B obtained through molecular docking

S. No.	Protein designation	Active amino acids in gyrB	Drugs	Interaction of gyrB and drugs	Distance of hydrogen bonds (Å)
1.	gyrB	Asn75, Arg137, Arg77, Asn138, Ser303, Val141, Glu143, Tyr302	Cinodine I	UNK1:O—Ser303:OG	2.163
				UNK1:H—Arg77:O	2.163
				UNK1:O—Tyr302	2.163
2.	gyrB	Phe105, Arg672, Asp106, Asp107, Thr595, Lys111, Val670	Cyclothialidine	UNK0:H—Phe105:O	2.175
				UNK0:H—Val670:O	2.175
				UNK0:H—Arg672:O	2.175
3.	gyrB	Lys111, Thr595, Lys104, Phe105, Glu673, Asp107, Arg672	Novobiocin	UNK1:H—Glu673:OE1	2.240

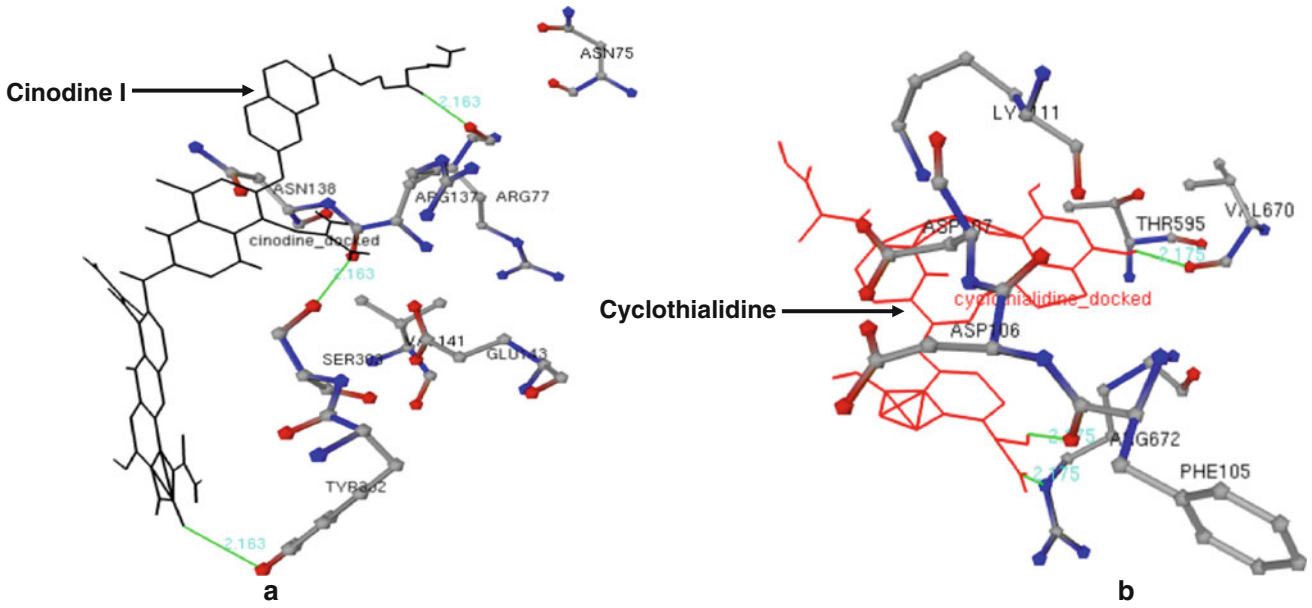

Fig. 2 The interaction of high affinity potent antibacterial drugs with gyrB of *A. hydrophila* showing the hydrogen bonds. **a** Cinodine I and **b**: Cyclothialidine

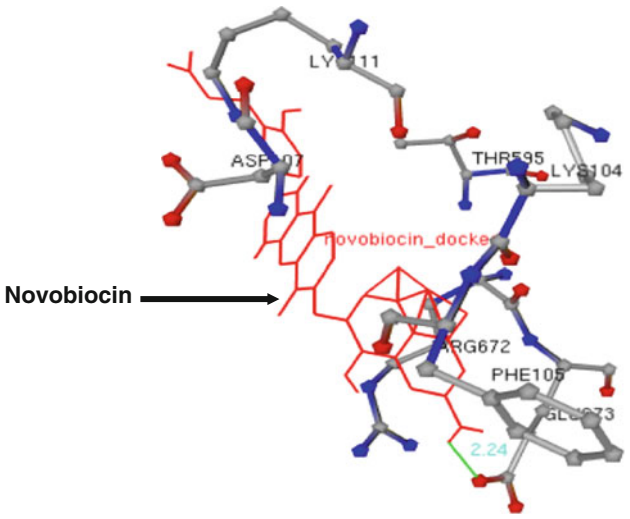

Fig. 3 The interaction of high affinity potent Novobiocin antibacterial drugs with gyrB of *A. hydrophila* showing the hydrogen bonds

reported using the sequences of *gyrB*. The nucleotide sequences of *gyrB* has been determined from 53 *Aeromonas* strains including some new strains, which were also characterized using 16S rDNA regions (Yanez et al. 2003). *A. bestiarum* and *A. salmonicida* have been reported for phylogenetic relationships on the basis of 70 strains using *rpoD* sequence. Whereas, the sequences of gyrB has already been proven for determining the phylogenetic relationship. Nucleotide sequences of *rpoD* and *gyrB* showed that both genes are similar substitution rates and a similar number of variable positions (Soler et al. 2004). The phylogenetic tree has been constructed using the aerolysin and hemomysin protein sequences of *A. hydrophila* and showed the homology with *Aeromonas* and other pathogenic bacteria (Singh and Somvanshi 2009b; Singh et al. 2009). In the above point, we have observed one protein targeted by single drug; sometime there is problem of drug activity because of high rate of mutation in gyrB.

Therefore, we further move forward to understand the gene regulation.

In the present study, we have shown role of DNA gyrase B in cellular system and also how to interact with other proteins/enzymes. B subunit; DNA gyrase negatively supercoils closed circular double- stranded DNA in an ATP-dependent manner and also catalyzes the inter conversion of other topological isomers of double-stranded DNA rings including catenanes and knotted rings. In the *gyrB* gene network, we found closely related proteins such as *gyrA*, *dnaN*, *parC*, *recF* and *dnaA* with >0.9 score (Table 3). We showed herein combinatorial influence of these newly constructed gene network of *A. hydrophila* that can use for regulation of *gyrB* gene for controlling cell physiology. Moreover we have shown protein–protein interaction in *gyrB* gene network (Fig. 4). In the Protein–protein interactions (PPI) occur when the two or several proteins/enzymes bind to each other often to carry out their biological function. Many of the molecular processes in cell such as DNA replication are carried out by large molecular machines that are built from a large number of protein components organised by their protein–protein interactions. There is a global analysis of 2,709 interactions between proteins of *Saccharomyces cerevisiae* has been performed and facilitated the establishment of a single large network of 2,358 interactions among 1,548 proteins (Schwikowski et al. 2000). The first step is needed to specifically define PPI which is commonly involved as physical interactions with molecular docking between proteins within a cellular systems. In several PPI repositories, it is a straightforward process to obtain all the proteins that interact with a given query protein which builds a corresponding network of molecular interactions (De Las Rivas and Montanillo 2010). To illuminate the architecture and dynamics of large scale genetic regulatory networks of cells is an important goal in systems and synthetic biology. The system level dynamical properties of the gene network of *Escherichia coli* that regulates metabolism and show how its design leads to biologically useful cellular properties (Samal and Jain 2008). It is a new approach for targeting any gene regulation for the cellular mechanism by knowing the expected proteins involve in gene network.

Table 3 Protein–protein interaction in gene network

Functional proteins	Score	Potential functions
gyrA	0.999	DNA gyrase, A subunit
dnaN	0.995	DNA polymerase III, beta subunit; DNA polymerase III is a complex, multichain enzyme responsible for most of the replicative synthesis in bacteria
parC	0.990	DNA topoisomerase IV
recF	0.987	DNA replication and repair protein RecF; The recF protein is involved in DNA metabolism; it is required for DNA replication and normal SOS inducibility
dnaA	0.977	Chromosomal replication initiation protein; Plays an important role in the initiation and regulation of chromosomal replication. Binds to the origin of replication
rpoD	0.875	RNA polymerase sigma factor RpoD; Sigma factors are initiation factors that promote the attachment of RNA polymerase to specific initiation sites and are then released
rpoB	0.741	DNA-directed RNA polymerase subunit beta; DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates
dnaJ	0.738	Chaperone protein DnaJ; Participates actively in the response to hyperosmotic and heat shock by preventing the aggregation of stress-denatured proteins and by disaggregating proteins, also in an autonomous, dnaK-independent fashion
mnmE	0.726	tRNA modification GTPase TrmE; Exhibits a very high intrinsic GTPase hydrolysis rate. Involved in the addition of a carboxymethylaminomethyl (cmnm) group at the wobble position (U34) of certain tRNAs, forming tRNA-cmnm(5)s(2)U34
rpmH	0.706	50S ribosomal protein L34

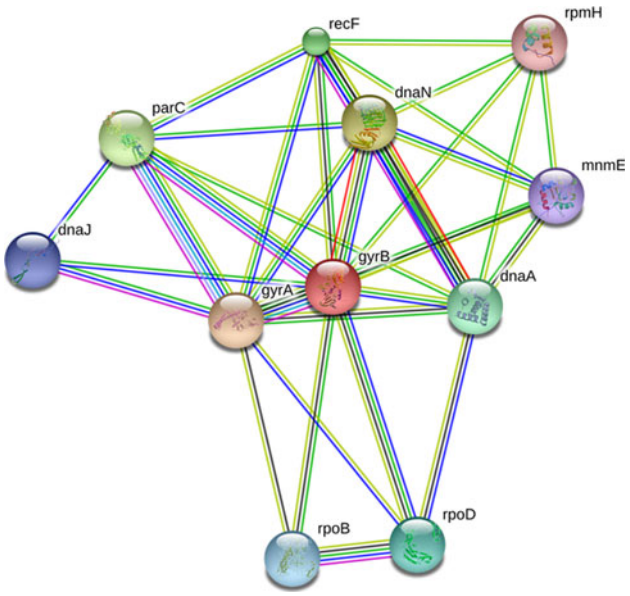

Fig. 4 Protein–protein Interaction of DNA gyrase B subunit (gyrB) of *A. hydrophila* with other proteins cellular activity in gene network

Conclusions

The present work was carried out to develop the 3-D structure of B subunit of DNA gyrase of *A. hydrophila*. The 3-D structure based screening for appropriate drugs was performed using docking and three antibacterial drugs such as Cinodine I, Cyclothialidine and Novobiocin could be found highest binding affinity with gyrB. The phylogeny of gyrB of *A. hydrophila* indicates that homologous gyrB protein may serve as a better target for the same drug which can also inhibit the growth of other bacteria. This study provides a new insight to control superfluous use of drugs in vitro trials. Construction of novel gyrB gene network of *A. hydrophila* plays a key role in gene regulation which provides a new conceptual framework for understanding the functional mechanisms of drugs and their cellular targets.

Acknowledgments Authors are grateful to Anand Kumar Singh, Pritee Singh and Reena for providing the suggestions, encouragement and fruitful discussion during preparation of the manuscript.

Conflict of interest There is no competing interest.

References

Abbott SL, Seli LS, Catino M Jr, Hartley MA, Janda JM (1998) Misidentification of unusual *Aeromonas* species as members of the genus *Vibrio*: a continuing problem. J Clin Microbiol 36:1103–1104

Altschul SF, Thomas LM, Alejandro AS, Jinghui Z, Zheng Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Austin B, Austin DA (1999) Bacterial fish pathogens: diseases in farmed and wild fish. Praxis Publishing, Chichester

Barghouthi S, Young R, Olson MO, Arceneaux JE, Clem LW, Byers BR (1989) Amonabactin a noval trypophan or phenylalanine-containing phenolate siderophore in *Aeromonas hydrophila*. J Bacteriol 171:1811–1816

Chakraborty T, Huhle B, Hof H, Bergbauer H, Goebel W (1987) Marker exchange mutagenesis of aerolysin determinant in *Aeromonas hydrophila* demonstrates the role of aerolysin in *Aeromonas hydrophila* associated systematic infection. Infect Immunol 55:2274–2280

De Las RivasJ, Montanillo C (2010) Protein–protein interactions essentials: key concepts to building and analyzing interactome networks. PLoS Comput Biol 6:e1000807

Delano WL (2002) The PYMOL molecular graphics system. DeLano Scientific, Palo Alto

Goetschi E, Angehrn P, Gmuender H, Hebeisen P, Link H, Masciadri R, Nielsen J (1993) Cyclothialidine and its congeners: a new class of DNA gyrase inhibitors. Pharmacol Therap 60(2):367–380

Hickman-Brenner FW, Mvdonald KL, Steiferwatl AG, Faning FR, Brenner DJ, Farmer JJ (1987) *Aeromonas veronii*, a new ornithine decarboxylase positive species the may cause diarrhea. J Clin Microbiol 25:900–906

Holmes P, Niccolls LM, Sartory DP (1996) The ecology of mesophilic *Aeromonas* in the aquatic environment. In: Austin B et al (eds) The genus *Aeromonas*. Wiley, New York, pp 127–150

Janda JM (1991) Recent advances in the study of the taxonomy, pathogenicity, and infectious syndromes associated with the genus *Aeromonas*. Clin Microbiol Rev 4:397–410

Janda JM, Abbott SL (1998) Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clin Infect Dis 27:332–344

Janda JM, Abbott SL, Cheung WK, Hanson DF (1994) Biochemical identification of citrobacteria in the clinical laboratory. J Clin Microbiol 32:1850–1854

Joseph SW, Carnahan AM (2000) Update on the genus *Aeromonas*. ASM News 66:218–223

Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structure. J Appl Crystallogr 26:283–291

Laurin P, Ferroud D, Schio L, Klich M, Dupuis-Hamelin C, Mauvais P, Lassaigne P, Bonnefoy A, Musicki B (1999) Structure-activity relationship in two series of aminoalkyl substituted coumarin inhibitors of gyrase B. Bioorg Medicinal Chem Lett 9: 2875–2880

Lübbers T, Angehrn P, Gmünder H, Herzig S (2007) Design, synthesis, and structure-activity relationship studies of new phenolic DNA gyrase inhibitors. Bioorg Medicinal Chem Lett 17:4708–4714

Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comp Chem 19:1639–1662

Oblak M, Grdadolnik SG, Kotnik M, Jerala R, Filipič M, Šolmajer T (2005) In silico fragment-based discovery of indolin-2-one analogues as potent DNA gyrase inhibitors. Bioorg Medicinal Chem Lett 15:5207–5210

Rathore G, Singh V, Kumar G, Swaminathan TR, Mahanta PC (2006) Antibiotic Sensitivity and characterization of *Aeromonas hydrophila* isolated from fish and water samples. J Ecophysiol Occup Health 6:41–43

Sali A, Blundell TL (1993) Comparative protein modeling by satisfaction of spatial restrains. J Mol Biol 234:779–815

Samal A, Jain S (2008) The regulatory network of *E. coli* metabolism as a Boolean dynamical system exhibits both homeostasis and flexibility of response. BMC Syst Biol 2:21

Schwikowski B, Uetz P, Fields S (2000) A network of protein–protein interactions in yeast. Nat Biotechnol 18(12):1257–1261

Singh V, Somvanshi P (2009a) Homology modeling of 3-oxoacyl-acyl carrier protein synthase II (KAS II) from *Mycobacterium tuberculosis* H37Rv and molecular docking for exploration of drugs. J Mol Model 15:453–460

Singh V, Somvanshi P (2009b) Inhibition of oligomerization of aerolysin from *Aeromonas hydrophila*: homology modeling and docking approach for exploration of hemorrhagic septicemia. Lett Drug Des Discov 6(3):215–223

Singh V, Somvanshi P, Rathore G, Kapoor D, Mishra BN (2009) Gene cloning, expression and homology modeling of hemolysin gene from *Aeromonas hydrophila*. Protein Expr Purif 65:1–7

Soler L, Yanez MA, Chacon MR, Aguilera-Arreola MG, Catalan V, Figueras MJ, Martınez-Murcia AJ (2004) Phylogenetic analysis of the genus *Aeromonas* based on two housekeeping genes. Int J Syst Evol Microbiol 54:1511–1519

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTALX windows interface: flexible strategies

for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Venkateswaran K, Dohmoto N, Harayama S (1998) Cloning and nucleotide sequence of the gyrB gene of *Vibrio parahaemolyticus* and its application in detection of this pathogen in shrimp. Appl Environ Microbiol 64:681–687

Yamamoto S, Harayama S (1996) Phylogenetic analysis of *Acinetobacter* strains based on the nucleotide sequences of gyrB genes and on the amino acid sequences of their products. Int J Syst Bacteriol 46:506–511

Yamamoto S, Harayama S (1998) Phylogenetic relationships of *Pseudomonas putida* strains deduced from the nucleotide sequences of gyrB, rpoD and 16S rRNA genes. Int J Syst Bacteriol 48:813–819

Yamamoto S, Bouvet PJM, Harayama S (1999) Phylogenetic structures of the genus *Acinetobacter* based on gyrB sequences: comparison with the grouping by DNA–DNA hybridization. Int J Syst Bacteriol 49:87–95

Yamamoto S, Kasai H, Arnold DL, Jackson RW, Vivian A, Harayama S (2000) Phylogeny of the genus *Pseudomonas*: intrageneric structure reconstructed from the nucleotide sequences of gyrB and rpoD genes. Microbiol 146:2385–2394

Yanez MA, Catalan V, Apraiz D, Figueras MJ, Martınez-Murcia AJ (2003) Phylogenetic analysis of members of the genus *Aeromonas* based on gyrB gene sequences. Int J Syst Evol Microbiol 53:875–883