Molecular Cloning, Expression of *minD* Gene from *Lactobacillus acidophilus* VTCC-B-871 and Analyses to Identify *Lactobacillus rhamnosus* PN04 from Vietnam *Hottuynia cordata* Thunb.

Tu Hoang Khue Nguyen · Vinh Thi Thanh Doan ·
Ly Dieu Ha · Huu Ngoc Nguyen

Received: 31 July 2012 / Accepted: 28 February 2013 / Published online: 16 March 2013
© Association of Microbiologists of India 2013

Abstract The *minD* gene encoding an inhibitor cell division MinD homolog from *Lactobacillus acidophilus* VTCC-B-871 was cloned. We showed that there were 97 % homology between *minD* genes of *L. acidophilus* VTCC-B-871 and *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* Lc705. Based on the analysis of the DNA sequence data from the *L. rhamnosus* genome project and sequenced *minD* gene of *L. acidophilus* VTCC-B-871, a pair of primers was designed to identified the different *minD* genes from *L. acidophilus* ATCC 4356, *L. rhamnosus* ATCC 11443. Besides, the polymerase chain reaction product of *minD* gene was also obtained in *L. rhamnosus* PN04, a strain was isolated from Vietnamese *Hottuynia cordata* Thunb. In addition, we performed a phylogenetic analysis of the deduced amino acid sequence of MinD homologs from *L. acidophilus* VTCC-B-871 with the other strains and compared the predicted three-dimension structure of *L. acidophilus* VTCC-B-871 MinD with *Escherichia coli* MinD, there are similarity that showed evolution of these strains. The overexpression of *L. acidophilus* VTCC-B-871 MinD in *E. coli* led to cell filamentation in IPTG and morphology changes in different sugar stresses, interestingly. The present study is the first report characterizing the *Lactobacillus* MinD homolog that will be useful in probiotic field.

Keywords Cell division inhibitor · Morphology change · *Lactobacillus* · Comparative analyses · *Hottuynia cordata* Thunb.

Introduction

In *Escherichia coli*, the proper placement of the cell division site generates two equally sized daughter cells which are maintained by the MinC, MinD and MinE proteins encoded by the *min* locus. In this system, the MinC and MinD protein acts as the inhibitors of cell division by blocking septum formation at all potential division sites (polar and mid sites) [1]. The MinE protein gives topological specificity to the MinCD division inhibitors by restricting its activity to polar division sites, thus ensuring that separation is limited to the proper division site at midcell. MinE binds to the trailing edge of MinD and stimulating its ATP hydrolysis, which results in the release of MinD, and thus MinC and MinE, from the membrane [2–4]. In the other hand, *Bacillus subtilis* contains MinCD homologues and DivIVA acts topologically, but not MinE [5]. It was also noticed that the entire nucleotide sequences of the *Streptomyces* genomes of *Streptomyces coelicolor* and *Streptomyces avermitilis* have recently reported, and this strain carried the MinD homolog, but not MinC or MinE [6, 7]. The MinD homolog harbored by *Streptomyces lavendulae* ATCC25233 has also been characterized and this strain did not carry MinC and MinE [8]. Since the genus *Streptomyces* consists of filamentous bacteria, *minD* in *S. lavendulae* ATCC 25233 may have a role other than cell division.

Lactic acid bacteria are one of the most commonly used probiotics. The role of prebiotics in improving human health has attracted global attention and the research is

T. H. K. Nguyen (✉) · V. T. T. Doan · H. N. Nguyen
School of Biotechnology, International University, Hochiminh City National University, Quarter 6, Linh Trung Ward, Thu Duc District, Hochiminh City, Vietnam
e-mail: nhktu@hcmiu.edu.vn

L. D. Ha
Department of Reference Substances, Institute for Drug Quality Control, Hochiminh City, Vietnam

mostly focused on the strains belonging to *Lactobacillus* [9]. The survival of *Lactobacillus* probiotics was usually lower than the amounts noted in probiotic label in their products. Therefore, to find out the roles of Min system in *Lactobacillus* that made a wide genus with the different survival rates that relate to the cell division are necessary. From the stated reasons, we cloned and tested whether the *Lactobacillus acidophilus* MinD protein is functional in *E. coli* cells by overexpression. By analysis the *minD* gene from *L. acidophilus* VTCC-B-871, the *minD* genes from *L. acidophilus* ATCC 4356 and *L. acidophilus* ATCC 11443 *Lactobacillus rhamnosus* PN04, a strain isolated and identified from Vietnamese *Hottuynia cordata* Thunb. were identified from which a method for determination of *L. acidophilus* and *L. rhamnosus* will be applied so far.

Materials and Methods

Plasmids, Bacterial Strains, Growth Conditions

The pUC19 and pGEM-T vectors used for molecular cloning and *E. coli* JM109, BL21(DE3)pLysS were purchased by Promega. The pET28 (a+) used for overexpression was purchased by Novagen. *L. rhamnosus* GG, *L. rhamnosus* ATCC 11443, *L. acidophilus* ATCC 4356, *L. acidophilus* VTCC-B-871 purchased by Vietnam type culture collection (VTCC). *E. coli* JM109 was used as a host to clone *Lactobacillus minD* genes. *E. coli* BL21(DE3)pLysS was used as an expression strain. *Lactobacillus* strains were grown on MRS for 72–96 h at 30 °C. *E. coli* strains were grown in Luria–Bertani for 18–24 h at 37 °C with shaking at 200 rpm. When required, antibiotics were added to media in the following concentrations: 100 µg of ampicillin/ml, 10 µg of chloramphenicol/ml, 50 µg of kanamycin/ml for *E. coli*.

DNA and RNA Isolation

Genomic DNA was isolated from *Lactobacillus* strains that had been grown for 72–96 h in MRS. The samples were incubated in MRS according to standard protocols. Total RNA was purified according to manufacturer's instructions (Takara).

Isolation of the Homologous DNA *minD* Probe from *Lactobacillus rhamnosus* GG

Genomic DNA from *L. rhamnosus* GG was amplified by PCR using a sense primer OMR1(5'-GAATGCGACCGGG GCGGCTGACGGTGCGA-3') and an anti-sense primer OMR2 (5'-TCAACGGCACGCTATCACCTAGTAACCG GC-3') which was homologous to sequences between 391

and 739 nt of the *minD* gene (Gene ID: 8422477). The PCR was done under the following conditions: an initial 2 min at 95 °C; then, 29 cycles of 1 min at 95 °C followed by 30 s at 55 °C; and 30 s at 72 °C, finally, an extension period of 30 s at 72 °C. A PCR product of 349 bp corresponding to *minD* fragment was ligated into the pGEM-T vector and introduced into *E. coli* JM109 from the TA Cloning kit (Promega).

Cloning, Sequencing and DNA Analysis

The genomic DNA from *L. acidophilus* VTCC-B-871 strain was digested with restriction enzymes supplied by Takara (Japan). The digestion was followed as instructions of the company. Southern hybridization was performed by using a Hybond-N+ (Amersham Biosciences) membrane. Probe labeling, hybridization and detection were performed with AlkPhos Direct Labeling and Detection System (Amersham Biosciences) according to the protocol supplied by the manufacturer.

The cloning *minD* from *L. acidophilus* was performed [10]. DNA sequencing was performed with the ABI PRISM 310 genetic analyzer using the BigDye terminator cycle sequencing ready reaction kit according to the manufacturer's protocols. The *Lactobacillus minD* genes was determined and analyzed using Fasta. The protein molecular mass, pI were calculated on an ExPASy Proteomics Server. The sequence data obtained in this study has been submitted to the DDBJ.

Overexpression Studies of MinD and Light Microscopy

The *L. acidophilus minD* was amplified by PCR with a sense primer BHE1 (5'-CATATGGGGACAGCGTTAGT AGTGACTTC-3') (the *NdeI* site is underlined) and an antisense BHE2 (5'-CTCGAGGATGGCGATGGAACAA TTTTGAC-3') (the *XhoI* site is underlined). The amplified *minD* was subcloned into pGEM-T vector and then was checked by DNA sequencing. The *minD* fragment was cut out from pGEM-T vector by *NdeI* and *XhoI* double-digestion and inserted into the same sites of pET-28(a+) to produce pET-28(a+)/*minD*. *E. coli* BL21(DE3)pLysS transformed with pET-28(a+)/*minD* was grown in LB medium supplemented with appropriate antibiotics at 37 °C to OD₆₀₀ = 0.5, after which 0.5 mM IPTG or 1 % glucose, 1 % saccharose, 1 % manitol was added to culture to induce at 28 °C from 5 to 24 h. Light microscopy was used to observed the morphological changes in *E. coli*.

Isolation and Identification of *Lactobacillus rhamnosus* from *Hottuynia Cordata* Thunb.

Hottuynia Cordata Thunb. samples were collected in the Southern of Vietnam. No specific permits were required for

the described field studies. The leaves were incubated in MRS for 72–96 h at 30 °C. The culture was used to spread onto MRS agar that was incubated in MRS for 72–96 h at 30 °C. The purified colonies were tested by microscopic examination with gram stain and catalase negative [11]. The isolated strains were identified by biochemical characterization based on the ability of the isolates to utilize different carbon sources, which determined by API CHL 50 system (bioMérieux, Lyon, France) and 16S rRNA sequencing analysis.

Phylogenetic Analyses, Protein Homology Modelling and Analysis

Phylogenetic analyses were performed on the MinD deduced amino acid sequences that were previously reported. Protein sequences were aligned with ClustalW software and clustered by using the un-weighted pair group method for the arithmetic mean. The tertiary structures of the deduced amino acid sequences of MinD were predicted by homology modelling using the Swiss-Model Server [12, 13] and MinD from *E. coli* (PDB: 3q9l) was used as template. The structural parameters and prediction quality of the modeled structures were evaluated using the program QMEAN4 with respect to score obtained for high-resolution experimental structures solved by X-ray crystallography [14].

Results and Discussion

Analysis of Genomic DNA of *Lactobacillus acidophilus* VTCC-B-871

L. rhamnosus GG genome was used as a template to prepare a 349 bp-DNA probe for Southern blotting and colony hybridization. A 4.0 kb *HindIII*-*PvuII* fragment was cloned from *L. acidophilus* VTCC-B-871 chromosomal DNA. A *minD* gene of 798 bp was sequenced and was deposited in the DDBJ database under accession no. AB725356. The MinD protein encoded by *L. acidophilus* VTCC-B-871 consists of 265 amino acids with a calculated pI of 5.07 and Mw of 28857.17 kDa. Having 100 % identity, the protein exhibits highest similarity to MinD (EHJ35458) from *L. rhamnosus* ATCC 21052 and 99 % identity to MinD (YP_00317015, gene ID: 8422477) from *L. rhamnosus* GG.

By comparison of nucleotide sequences of the *minD* gene from *L. acidophilus* VTCC-B-871 with *L. rhamnosus* strains, there are 779/798 (98 %) identity to *L. rhamnosus* ATCC 8530 and 777/798 (97 %) identity to Lc 705 and strain GG. Interestingly, the analyses the nucleotide sequence showed that there was 100 % similarity of the 40

nt at 5' end and 40 nt at 3' end of *minD* gene in *L. acidophilus* VTCC-B-871 and *L. rhamnosus* ATCC 8530, Lc 705 as well as GG. Clearly, *minD* gene will be identified easily and used in the identification of *L. acidophilus* or *L. rhamnosus*.

Conservation of MinD Proteins from Various Bacteria and Phylogenic Analysis

Figure 1. shows the alignment of amino acid sequences of MinD proteins from *Lactobacillus* strains with 15 strains listed in Fig. 1. The Walker A and B motifs and the two Asp residues (Asp³⁸, Asp⁴⁰) located between them are conserved in all sequences, suggesting that the *Lactobacillus* MinD protein possess an ATPase activity like that of *E. coli* MinD. Although *L. acidophilus* and *L. rhamnosus* strains are gram positive, the consensus nucleotide binding sequence of the Walker A motif is known as G/A-X-X-G-X-G-K-T/S that overlap with Walker A in gram negative *E. coli*, and *Neisseria gonorrhoea* [15] and that is distinguishable with *Streptomyces* which are gram-positive and the seventh Lys is replaced by Ala or Thr [8]. The results elucidated the relationship of *minD* from gram negative and gram positive. The protein or deduced amino acid MinD sequences from 15 strains were used to generate phylogenetic trees. Clustal alignment used for phylogenetic analysis allowed to determine the location of amino acids expected to have a catalytic role in MinD (Fig. 1). The combination with the tree analysis showed the early separation between amino acid sequences from the aligned strains can be explained in terms of the evolution of MinD for different purposes (Fig. 2). On this background, the results of the phylogenetic analyses based both on amino acid sequence similarities as well as their structural features would be strengthen the phylogenetic analysis and to establish a relationship between the genes encoding MinD with their three-dimensional structures involved in ATP binding (Fig. 2). By the comparison, the MinC and MinE homologs might have been eliminated in the process of the evolution.

Protein Homology Modelling and Comparisons of Protein Structures

Once the tertiary structure of MinD was predicted, these results strongly support the notion that there is a close relationship between the tertiary structure of MinD and the lifestyle of the microorganisms. Comparative analyses of three-dimensional structures have been utilized for different purposes in searching for putative biological functions, drug design, protein–protein interaction studies [14]. However, to our knowledge, the study that uses a comparative analysis of protein structure in combination with a

		Walker A	↓ ↓	Walker B	
L. wel	1	MGEAIVITSEKGGVGKTTSTAN	-12-	CLIDMDIG-70-	DYDYILIDCPAG 124
B. sub	1	-GEAIVITSEKGGVGKTTTSAN	-12-	CLVDTDIG-70-	EFDYVIIDCPAG 124
L. san	1	MGEAIVITSEKGGVGKTTTSAN	-12-	CLMDLDIG-70-	QFDYVLIDCPAG 124
L. aVT1	1	MGTALVITSEKGGVGKTTTSAN	-12-	VLLDLDIG-70-	DFDFIILDSPAG 124
L. rha	1	MGTALVITSEKGGVGKTTTSAN	-12-	VLLDLDIG-70-	DFDFIILDSPAG 124
L. cas	1	MGTALVITSEKGGVGKTTSSAN	-12-	VLLDLDIG-70-	DFDFIILDSPAG 124
E. amyl	1	MARIIVITSEKGGVGKTTSSAA	-12-	VVIDFDIG-70-	DFDFIVCDSPAG 124
E. pyr	1	MARIIVITSEKGGVGKTTSSAA	-12-	VVIDFDIG-70-	DFDFIVCDSPAG 124
P. ana	1	MARIIVITSEKGGVGKTTSSAA	-12-	VVIDFDIG-70-	AFDFIVCDSPAG 124
E. coli	1	MARIIVITSEKGGVGKTTSSAA	-12-	VVIDFDIG-70-	DFEFIVCDSPAG 124
N. gor	1	MAKIIWITSEKGGVGKTTTSAS	-12-	AVIDFDVG-72-	DFEFIVCDSPAG 126
S. cel	1	MSKVYVITSEKGGVGKTTTIAN	-12-	IAIDFDVG-70-	GFEYICDSPAG 124
R. le	2	MGKVIWITSEKGGVGKTTSTAA	-12-	VVDFDVG-70-	YFDWICDSPAG 124
W. suc	1	MGIVITVITSEKGGVGKTTTCAN	-12-	VAVDFDIG-70-	EFDVLLDSPAG 124
S. coe	148	GGTVVTVSGAKGGVGATLTAVQ	-12-	ALLMDLQ-70-	EYEVVVDCGAG 271
		***** *		* *	*

Fig. 1 Alignment of conserved motifs in MinD from *Lactobacillus* strains and other species. Alignment was carried out with the Clustal W program. Listed proteins are from the following strains: L. wel, *Listeria welshimeri*; B. sub, *Bacillus subtilis*; L. san, *Lactobacillus sanfranciscensis*; L. aVT1, *Lactobacillus acidophilus* VTCC-B-871; L. rha, *Lactobacillus rhamnosus*; L. cas, *Lactobacillus casei*; E. amyl, *Erwinia amylovora*; E. pyr, *Erwinia pyrifoliae*; P. ana, *Pantoea*

ananatis; E. coli, *Escherichia coli*; N. gor, *Neisseria gonorrhoeae*; S. cel, *Sorangium cellulosum*; R. le, *Rhizobium leguminosarum*; W. suc, *Wolinetella succinogenes*; S. coe, *Streptomyces coelicolor*. The conserved motifs (Walker A and B) and the two Asp residues are indicated by bars and arrow heads, respectively. Asterisks below the sequences show the conserved residues in all sequences

Fig. 2 The phyogenic tree was used by the un-weighted pair group method using the arithmetic mean and clustering the three-dimensional structures of MinD. **a** and **c** Three-dimensional structure of MinD from *Lactobacillus acidophilus* VTCC-B-871 and *S. coelicolor* respectively, predicted by homology modelling using the Swiss-Model Server. **b** Three-dimensional structure of MinD from *E. coli* (PDB: 3q9l)

phylogenetic analysis to explore the evolution of lifestyle. Using Swiss-model server, the structure of *L. acidophilus* was predicted, using the template of *E. coli* (Fig. 2) with the final total energy of -8877.295 kJ/mol. The quality of the structure prediction was estimated by QMEAN4 (Table 1). The structures showed the alpha helix and beta

sheet in *L. acidophilus* MinD and *E. coli* MinD occurring in the region of 2–249 amino acids of *L. acidophilus* (Fig. 2) with the ligand models of 2 ATP and 1Mg^{2+} molecules while *Streptomyces* MinD showed modelling homology in the region of 149–245 amino acids with no ligand model in this structure that corresponds to the sequences aligned in

Table 1 QMEAN4 data for model quality estimation

Scoring function term	Raw score	Z-score
C-beta interaction energy	−138.89	0.13
All-atom pairwise energy	−6408.44	−0.7
Solvation energy	−26.27	−0.06
Torsion angle energy	−55.07	−0.85
QMEAN4 score	0.721	−0.90

the Fig. 1. Although *E. coli* MinD exhibited a dimer that indicated the self-interaction [4] and *L. acidophilus* MinD predicted in monomer, the monomers of these structures are highly similar.

The results meant the comparative analysis can be an important tool for studying the proteins of microorganisms but also for the evolution of microorganisms and their proteins, since structural differences may reflect other important properties such as substrate specificity and others that can not be inferred from the analysis of amino acid sequences only. Therefore, *minD* gene might also participate in the evolution in microorganisms.

Study the *minD* Genes in *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus rhamnosus* ATCC 11443

To find out the relation, the MinD homologs were identified in *L. acidophilus* ATCC 4356 and *L. rhamnosus* ATCC 11443. As discussed above, a pair of primers with a sense primer OMS (5'-ATGGGGACAGCGTTAGTAGTGACTTC-3') and an antisense OMS1 (5'-GATGGCGATGGAACAATTTTGAC-3') was designed from *L. acidophilus* VTCC-B-871 to identify *minD* gene in *L. acidophilus* ATCC 4356 and *L. rhamnosus* ATCC 11443. After isolation, a *minD* gene from *L. acidophilus* ATCC 4356 was sequenced. A *minD* gene of 798 bp was sequenced and deposited in the DDBJ under accession no. AB725355. Similarly, a *minD* gene of 798 bp from *L. rhamnosus* ATCC 11443 was deposited in the DDBJ under accession no. AB725357. With the understanding of *minD* in sequences, the role of the survival of different strains will be discovered.

Isolation and Identification of *Lactobacillus rhamnosus* from *Hottuyia Cordata* Thunb.

To make a sure of the *minD* existence and aid for identification, an isolation of *L. rhamnosus* from *H. cordata* Thunb. was done and checked by biochemical tests using ABI 50CHL and 16S rRNA sequencing. By using the API 50CHL (BioMerieux), a isolated strain showed the result of *L. rhamnosus* (Data not shown). By Blast search, the 16S rRNA sequence of *L. rhamnosus* shows 99 % identity to

L. rhamnosus NT10. The isolated strain was named *L. rhamnosus* PN04 and the 16S rRNA sequence was deposited in DDBJ (accession number: AB738399). Using the OMS sense and OMS1 antisense primers, a PCR product of *minD* gene was detected, indeed (Fig. 3). The results also pointed the relationship between *L. acidophilus* and *Lactobacillus rhamnosus*.

Overexpression of *minD* Gene and Light Microscopy

To test whether the *L. acidophilus* MinD protein is functional in *E. coli* cells, the *E. coli* BL21(DE3)plysS was introduces with plasmid pET-28(a+) containing *minD*. After expression, cells transformed with the pET-28(a+) exhibited the normal rod-shaped morphology (Fig. 4a), while the same strain transformed with pET-28(a+)/*minD* exhibited a mixed phenotype of long filaments (Fig. 4b). The result of filamentous phenotype may have occurred because *Lactobacillus* MinD enhanced MinC-mediated inhibition of cell division at all potential division sites in *E. coli* cells. Indeed, it has also been reported that the overexpression of *Neisseria* MinD in *E. coli* cells leads to filamentation [15]. This result indicated that *Lactobacillus* MinD is functional across species. The cells transformed with the pET-28(a+)/*minD* were also tested to grow in glucose, saccharose and manitol. Interestingly, under the saccharose stress, the cells become long and curled shape (Fig. 4c). The IPTG inducer was used in pET vector system because of T7 promoter. However, under the sugar stresses, the morphology was changeable. The hypothesis was posed whether the interaction between MinD and sugar. The

Fig. 3 The PCR product of *minD* gene from isolated *Lactobacillus rhamnosus*. From left to right: 1, λ /HindIII marker; 2, PCR product. The arrow shows the PCR product

Fig. 4 Morphology of *Escherichia coli* harboring the *minD* gene from *Lactobacillus acidophilus*. *Escherichia coli* BL21(DE3)plysS cells harboring pET 28(a+)/*minD* and pET 28(a+) were analyzed by light microscopy. **a** *Escherichia coli* BL21(DE3)plysS cells harboring

pET 28(a+). **b** *Escherichia coli* BL21(DE3)plysS cells harboring pET 28(a+)/*minD* in IPTG. **c** *Escherichia coli* BL21(DE3)plysS cells harboring pET 28(a+)/*minD* in saccharose. The scale bar is 5 µm

results were the first reports in the morphological differentiation of *E. coli* carrying *minD* gene of *Lactobacillus*.

Acknowledgments Thanks to the grant supplied by the National Foundation of Science and Technology Development of Vietnam (Nafosted) and the support of Hochiminh City International University by which this work has been fulfilled.

References

1. Park KT, Wu W, Lovell S, Lutkenhaus J (2012) Mechanism of the asymmetric activation of the MinD ATPase by MinE. *Mol Microbiol* 85(2):271–281
2. Zhou H, Schulze R, Cox S, Saez C, Hu Z, Lutkenhaus J (2005) Analysis of *minD* mutations reveals residues required for *minE* stimulation of the *minD* ATPase and residues required for *minC* interaction. *J Bact* 187(2):629–638
3. Ma L, King GF, Rothfield L (2004) Positioning of the MinE binding site on the MinD surface suggests a plausible mechanism for activation of the *Escherichia coli* MinD ATPase during division site selection. *Mol Microbiol* 54(1):99–108
4. Lutkenhaus J, Sundaramoorthy M (2003) MinD and role of the deviant Walker A motif, dimerization and membrane binding in oscillation. *Mol Microbiol* 48(2):295–303
5. Stahlber H, Kutejova E, Muchova K, Gregorini M, Lustig A, Muller SA, Olivieri V, Engel A, Wilkinson AJ, Barak I (2004) Oligomeric structure of *Bacillus subtilis* cell division protein DivIVA determined by transmission electron microscopy. *Mol Microbiol* 52(5):1281–1290
6. Bently SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D et al (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* 417: 141–147
7. Ikeda H, Ishikawa J, Hanomoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S (2003) Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nat Biotechnol* 21:526–531
8. Nguyen HKT, Kumagai T, Matoba Y, Suzuki T, Sugiyama M (2008) Molecular cloning and functional analysis of *minD* gene from *Streptomyces lavendulae* ATCC 25233. *J Biosci Bioeng* 106(3):303–305
9. Chithra M, Muralikrishna G (2012) Prebiotic activity of purified xylobiose obtained from ragi (*Eleusine coracana*, Indaf-15) Bran. *Indian J Microbiol* 52(2):251–257
10. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratories, New York
11. Seema P, Avishek M, Arun G (2012) Potentials of exopolysaccharides from lactic acid bacteria. *Indian J Microbiol* 52(1):3–12
12. Arnold K, Bordoli L, Kopp J, Schwede T (2006) The swiss-model workspace: a web-based environment for protein structure homology modeling. *Bioinformatics* 22:195–201
13. Schwede T, Kopp J, Guex N, Peitsch MC (2003) Swiss-model: an automated protein homology-modeling server. *Nucleic Acids Res* 31:3381–3385
14. Benkert P, Biasini M, Schwede T (2011) Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics* 27(3):343–350
15. Parti RP, Biswas D, Helgeson S, Michael FS, Cox A, Dillon JA (2011) Attenuated virulence of *min* operon mutants of *Neisseria gonorrhoeae* and their interactions with human urethral epithelial cells. *Microbes Infect* 13(6):545–554