Original article

Cloning and expression of Neisseria meningitides luxS gene

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Abstract

Neisseria meningitides is a Gram-negative bacterium which is an important causative agent of septicemia and meningitis. Numerous pathogenic bacteria contain luxS, which is required for autoinducer-2 production. Neisseria meningitides contains a functional copy of luxS that is necessary for full meningococcal virulence. Neisseria meningitides DNA was extracted and its luxS gene was amplified by nested PCR. PCR product was purified and cloned in to pQE-30 expression vector. Recombinant plasmid was transformed and mass cultured. luxS was expressed in E. coli and confirmed by western blot analysis. In this study, N. meningitides luxS was amplified, cloned and expressed successfully. The sequencing of PCR product confirmed that amplified gene was luxS. Gene was expressed and observed in SDS-PAGE. Protein was reacted by his tag monoclonal antibody through western blot analysis.

Keywords: Neisseria meningitides; luxS; auto inducer 2; cloning

INTRODUCTION

Bacterial meningitis is a serious disease accounting for an estimated 173,000 deaths worldwide per year. Neisseria meningitides is the causative agent of most cases of bacterial meningitis, together with Haemophilus influenza and Streptococcus pneumonia. In industrialized countries, annual attack peak rates of meningococcal disease average 1-3 per 100,000 of the population. The highest incidence is in children under the age of 5 years, with a secondary peak in teenagers and young adults [1].

N. meningitides is a commensally organism. The normal habitat of the meningococcus is the human nasopharynx, but its pathogenic effects lie in the ability of the organisms to traverse the epithelial lining of the respiratory tract and invade the bloodstream. The onset of disease is rapid and notoriously difficult to distinguish from other febrile illnesses [2].

N. meningitides is an obligate human pathogen, which has adapted highly to its host. The development of a polysaccharide capsule and production of highly variable cell surface proteins are examples of mechanisms evade the host immune response [3].

The luxS gene of N. meningitides is required for its pathogenecity. The enzyme LuxS is responsible for the production of autoinducer-2 [AI-2], a molecule that has been implicated in quorum sensing in many bacterial species [4]. Quorum sensing is a mechanism that allows bacteria to behave as multicellular organisms [5]. This phenomenon plays a major role in gene regulation in many environments, and importantly, convincing data suggest that many pathogens rely on these communication systems to

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promote infection [6].

Research about quorum sensing, and inhibition thereof, may provide a means of treating many common and damaging chronic infections without the use of growth-inhibitory agents, such as antibiotics, preservatives, and disinfectants, that unavoidably select for resistant organisms [7].

The luxS gene product is required for AI-2 production [8]. AI-2 differs from all other auto inducers because it can be used for interspecies cell-to-cell communication [9].

The aim of this work was cloning and expression of Neisseria meningitides luxS gene.

MATERIALS AND METHODS

Bacteria and DNA extraction

N. meningitides (PTCC 1507) was purchased from Iranian scientific and industrial Research Center and cultured in BHI medium. Bacterial DNA was extracted by phenol-chloroform method, briefly, bacterial suspension were transferred to micro tube and lysed by lysis buffer (50 mM glucose, 25 mM Tris, 5 mM EGTA) for 1 hour at 37°C and bacterial DNA was extracted by phenol-chloroform method and subjected for PCR amplification.

PCR amplification

A Nested PCR was designed based on N. meningitides luxS gene sequence with SacI and BamHI restriction site on 5´end of forward and reverse primers respectively (Nest I primers: LuxS F1 5′-ACG GCA TTT TTG GCG GAT TA-3′ and LuxS R1 5′-ATG CCC CTA CTA GAC AGT TTC-3′ and nest II primers: LuxS F2 5`- GAG CTC ATA TGT TAG GCG TTC AGC AGC CC-3′ and LuxS R2 5′-GGA TCC CTA GAC AGT TTC AAA GTC GAT-3′ were amplified 507 bp as luxS gene). All primers were synthesized by Primm Company.

PCR reaction was included 100 ng bacterial DNA, 150 nM dNTP, 40 Pico moles each of forward and reverse primers, 1.5 mM $MgCl_2$, 1X PCR buffer and 1.25 units of Taq DNA polymerase in 50 μL final volume. PCR amplification was performed by following parameters: denaturing at 94° C for 60 sec., annealing at 59.5℃ for 60 sec. and extension at 72° C for 60 sec. Nested II PCR was done like nest I annealing temperature was 70°C [10].

Gene cloning

PCR product was electrophoresed on 1% low melting point agarose gel and DNA band was sliced under long wave UV and recovered [11] by DNA purification kit (Fermentas Cat. No k0513).

Recovered DNA was cloned into pTZ57R cloning vector via T/A cloning method. Briefly, EcoRV blunt digested pTZ57R was 3´tailed using dTTP by terminal deoxy nucleotidyl transferase [12] and ligated with 5´A tailed PCR product [13]. The reaction was transformed into E. coli DH5? strain competent cells [14] and dispensed on LB agar plates containing 100 μg/ml ampicillin, 20μg/ml X-gal and IPTG at 20mM final concentration. There were grew some white/blue colonies on agar plate and we selected white colonies containing recombinant plasmids. Recombinant plasmid was extracted [15] and digested by SacI and HindIII (for suitable gene orientation in pQE-30 we have to used HindIII enzyme with extra 36 nucleotide at N terminal of LuxS gene), electrophoresed on 1% agarose gel and released band (luxS gene) was recovered by DNA extraction kit (Fermentas Cat. No k0513) and sub cloned in SacI and HindIII digested pQE-30 expression vector and transformed in E. coli, XL1blue strain competent cells.

Gene expression and western blot analysis

Expression was performed as described previously [16] with some modifications. Briefly, recombinant pQE-30 was transformed in M15 bacterial cell and cultures containing 100 μg/ml ampicillin in the logarithmic phase (at OD600 = 0.6) were induced with 0.5 mM isopropyl-β-D-galactopyranoside (IPTG). Samples were collected before induction, 3 and 5 hours after induction. Cells were lysed in 5X sample buffer (100 mM Tris-HCl, pH 8, 20% glycerol, 4% SDS, 2% -mercaptoethanol, 0.2% bromophenol blue) and analyzed by 12% SDS-PAGE. The gel was stained with Coomassie brilliant blue R-250 [17]. The non induced control culture was analyzed in parallel (Novagen catalogue).

Gel was transferred to nitrocellulose membrane [18] and analyzed by western blot. Briefly, membrane was incubated by primary antibody [anti his-tag monoclonal antibody] diluted 1:1000 and then with secondary antibody (Sheep Anti-Mouse Ig Peroxidase-Conjugated) diluted 1:200, and finally, DAB/H_2O_2 as substrate were used to detect the antigen-antibody reaction [19].

Sequencing

PCR product was electrophoresed on low melting agarose gel, DNA band was sliced, purified by DNA purification kit (Fermentas Cat. No k0513) and sequenced by Primm Company

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RESULTS

Neisseria meningitides genomic DNA was extracted and subjected to PCR amplification and PCR product was electrophoresed on 1.5% agarose gel (figure 1).

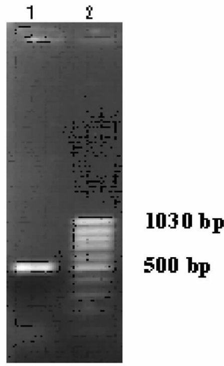

Figure 1: Electrophoresis of PCR product of Neisseria meningitides luxS gene on 2% agarose gel. Lane 1: 507 bp as PCR product of Neisseria meningitides luxS gene. Lane 2: 100 bp DNA ladder marker.

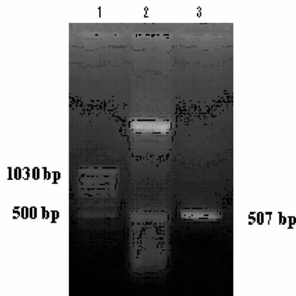

Figure 2: Elctrophoresis of digested recombinant plasmid on 2% agarose gel. Lane 1: 100 bp DNA ladder marker. Lane 2: SacI and BamHI digested recombinant plasmid. Lane 3: 507 bp as Neisseria meningitides PCR product

PCR product was ligated to pTZ57R/ T vector and transformed into E. coli DH5?. White colonies contained recombinant plasmids were selected. Recombinant plasmid was extracted and electrophoresed on 0.8% agarose gel. For confirmation, the recombinant plasmid was digested (luxS/ pTZ57R) by SacI and BamHI restriction enzymes, and released 507 bp (luxS gene), figure 2 shows digested recombinant plasmid.

Then recombinant plasmid was digested by SacI and HindIII restriction enzymes again and electrophoresed on 2% agarose gel. The cloned PCR product (luxS gene) plus 36bp, the distinct between BamHI and HindIII on plasmid multiple cloning site (543 bp) was released.

Digestion reaction was electrophoresed on LMP agarose gel, released DNA band was sliced and purified by DNA extraction kit (Fermentas cat No. k0513) and sub cloned into SacI and HindIII digested pQE-30 expression vector (for suitable orientation of gene into pQE30, we digested recombinant T/vector by SacI and HindIII restriction enzymes) and named luxs/pQE-30. There is 36 bp after stop codon that does not effect on expressed protein. Sub cloned gene was confirmed using restriction analysis with SacI and HindIII enzymes (Figure 3).

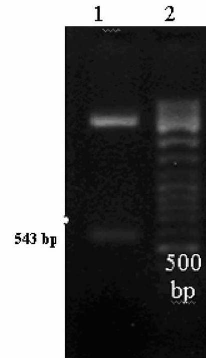

Figure 3: Electrophoresis of digested recombinant LuxS/ pQE-30 plasmid on %2 agarose gel. Lane 1: Recombinant plasmid digested with SacI and HindIII enzymes. Lane 2: 100 bp DNA ladder marker

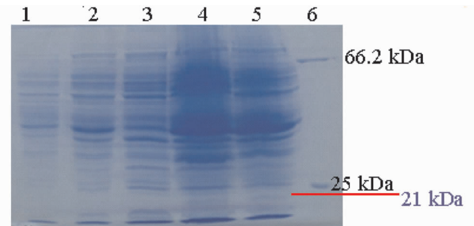

Figure 4: 12% SDS-PAGE. Lane 1: Cell lysates of M15 collected 5h after induction. Lane 2: Cell lysates of M15 containing intact pQE-30 collected 5h after induction. Lane 3: Cell lysates of M15 containing luxS/ pQE-30 collected before induction. Lane 4: Cell lysates of M15 containing luxS/ pQE-30 collected 3h after induction. Lane 5: Cell Lysates of M15 containing luxS/ pQE-30 collected 5h after induction. Lane 6: Protein molecular size marker.

SDS-PAGE

pQE-30 expression vector before ATG start codon encodes 13 amino acid, after induction by IPTG, luxS have 181 amino acid, So the recombinant plasmid must be encodes protein with approximately 21 kDa molecular weight, after induction (Figure 4).

Western blotting

Figure 5 shows reactivity of recombinant protein by immunoblotting, probes with monoclonal antibody. Western blot showed that there is higher expres-

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sion at 5 h than 3 h after induction.

Sequencing

PCR product was sequenced and deposited in GenBank at accession number DQ123817

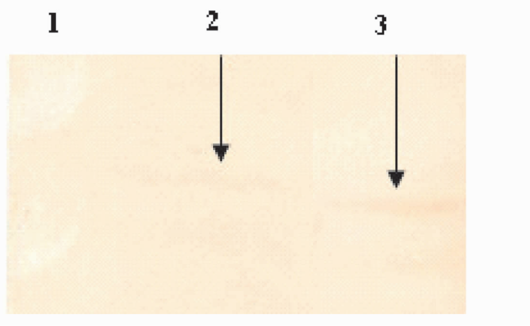

Figure 5: Immunoblotting. Lane 1: Cell lysates containing pQE-30 5h after induction. Lane 2: Cell lysates containing luxS/ pQE-30 3h after induction. Lane 3: Cell lysates containing luxS/ pQE-30 collected 5h after induction.

DISCUSSION

The luxS-dependent quorum sensing system could be an attractive target for antibiotics since the structure of the biosynthetic enzyme for the signaling molecule is known and a role in virulence has been demonstrated, notably in E. coli [20, 21], Vibrio cholera [22] and Streptococcus pyogenesis [23]. However, many details of the quorum sensing system remain to be elucidated, including the physiological response induced by AI-2 in the large number of bacteria containing the luxS gene, and therefore likely to produce AI-2. Additionally, an identical, or very similar, signaling molecule appears to be produced by a wide variety of bacterial species.

The observation of cross-species induction of luminescence in Vibrio harveyi demonstrates that the signaling molecule from one species of bacterium is able to cause a physiological response in other species [24, 25].

A recent report investigating the role of luxS in a serogroup B strain of N. meningitides showed that a luxS mutant was attenuated in an infant rat model of infection for bacteraemia [8]. However Dove et al suggested that AI-2 may be a metabolic by-product and not a cell-to-cell signaling molecule in N. meningitides [26].

To investigate the effect of the autoinducer 2 (AI-2) on protein expression in Neisseria meningitides, a luxS mutant of strain MC58 was grown in the presence and absence of in vitro-produced AI-2, and differential protein expression was assessed by two-dimensional differential gel electrophoresis. N. meningitides did not show a global response to AI-2 signaling activity [27].

LuxS/AI-2 regulates a host of physiological activities including virulence, biofilm (especially mixed biofilm) formation, motility, toxin and antibiotic production, luminescence, and ABC transporter expression. DNA microarray analyses have shown that inactivation of the luxS gene affects the expression of 2-10% of all bacterial proteins. Mutation of the luxS gene interferes with biofilm formation and the release of virulence factors. Many studies showed luxS mutants as either or defective much attenuated pathogens [26].

It is possible that the luxS negative strains were rendered virulent by AI-2 produced by indigenous bacteria in the hosts [26].

The luxS gene is present in a wide variety of bacterial species including significant human pathogens (e.g. H. influenza, H. pylori, S. aureus and S. pneumonia) and is highly conserved. Thus, inhibitors or antagonists against AI-2/LuxS should act as broad spectrum antibiotics. LuxS is not present in humans, so the agents should have little toxicity. Finally, LuxS is a metalloenzyme. Potent metal-chelating inhibitors against many metalloenzymes have been relatively easy to come by.

There are already ample examples of inhibiting type 1 quorum sensing as an antibacterial strategy. Another attractive target is the nucleosidase Pfs; its inhibition would block the production of AI-2 and result in the accumulation of toxic SAH in bacteria [28].

In this study, luxS of Neisseria meningitides that was standard strain of Iran, successfully cloned and expressed in pQE-30 expression vector. Result of expression was production of recombinant protein with approximately 21 kDa molecular weight, which can be used in future studies.

We propose that auto-inducers may provide a class of unconventional antibiotics. They would not kill the bacteria but protect of their virulent, allowing the host's immune system sufficient time to destroy the pathogen. Inhibition of biofilm formation would make pathogens more sensitive to conventional antibiotics. This approach should be less likely to result in the development of drug resistance due to selective pressure.

Conclusion: The luxS of Iranian standard strain Neisseria meningitides was cloned and expressed

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successfully.

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