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Short Communication

Innate immunity to recombinant QseC, a bacterial adrenergic receptor, may regulate expression of virulence genes of avian pathogenic *Escherichia coli*

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ABSTRACT

Certain bacterial pathogens rely on a membrane bound sensor kinase, QseC, to coordinate their virulence gene expression in a process called quorum sensing. The present study evaluated the effect of host immunity to a recombinant QseC protein, on the virulence gene expression of avian pathogenic *Escherichia coli* (APEC) of O78 serogroup (APECO78). For this purpose, we constructed a plasmid expressing QseC protein which is 50 kDa in size and stimulated avian macrophage-like cells (AMCs) with the native form of QseC protein at different concentrations. The cell culture medium of QseC-stimulated AMCs was then used to investigate its effect on APECO78 growth rate and virulence gene expression. Growth curve analysis of APECO78 indicated that growth rate of APECO78 in Luria Bertani (LB) broth containing the culture medium of stimulated AMCs was significantly lower and was impeded at entering the exponential phase. The expression of virulence genes of APECO78 such as *aufA*, *fliC*, *fimH*, *fyuA*, *iucC*, *iutA*, *msbB* and *vat* were also significantly down-regulated. On the other hand, APECO78 grown in LB containing the cell culture medium of non-stimulated AMCs did not exhibit these changes. Additionally, stimulation with QseC effectively induced interferon gamma (IFN-γ), Toll-like receptor 4 (TLR-4) and Toll like receptor 15 (TLR-15) expression in AMCs. To summarize, our results demonstrated that recombinant QseC protein could be immunogenic and induces host immunity that regulates selective, yet major, virulence gene expression of APECO78 bacteria. Thus, present data provide evidence that QseC, a bacterial functional analog of adrenergic receptor, holds a promise as one of the vaccine candidates against APEC infections.

1. Introduction

Quorum sensing (QS) is a cell-to-cell signaling mechanism used by various species of bacteria to coordinate the gene expression in response to chemical hormone-like molecules called autoinducers (Hughes and Sperandio, 2008; Nealson et al., 1970). The bacterial signals are produced and used for communication between bacteria and the eukaryotic host that modulates host cell-signal transduction http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2667375/-R4 (Telford et al., 1998). These signals are sensed by membrane-embedded sensory histidine kinases which are widely present in bacteria, archaea and eukaryotes, and are useful for signal transduction (Grebe and Stock, 1999). Of relevance, QseC is an important sensory kinase and functions as a bacterial adrenergic receptor (Clarke et al., 2006). It acts as a bifunctional sensor kinase/phosphatase that controls the phosphorylation

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state of QseB, and thereby maintains optimal gene expression in bacteria (Hadjifrangiskou et al., 2011; Kostakioti et al., 2009). Thus, QseC-mediated quorum sensing plays a significant role in bacterial virulence and disease progression.

In a recent review, it has been discussed that bacterial quorum sensing mechanisms could be exploited to manipulate their virulence (LaSarre and Federle, 2013). Despite few concerns in developing therapeutics against the QS system, some promising developments have been reported to inhibit QS signaling (LaSarre and Federle, 2013). At present, the studies have neither reported on the immunogenicity of QseC sensory kinase protein nor have investigated the effect of QseC-induced host immunity on bacterial virulence mechanisms. With this research in mind, the present study evaluated the effect of innate immunity to a recombinant QseC protein, on the virulence gene expression of a strain of avian pathogenic *Escherichia coli* (APEC) belonging to O78 serogroup (APECO78).

2. Materials and methods

2.1. Bacterial strains, plasmids, media, chemicals and growth conditions

The plasmid pQE30 (Qiagen Inc., Valencia, CA) was used as a cloning as well as an expression vector. *Escherichia coli* M15 (Qiagen) was used as the host bacteria to express QseC protein. The *Escherichia coli* strain APECO78, a pathogenic *Escherichia coli* isolated from the ovaries of a hen diagnosed with egg layer peritonitis was used to investigate the virulence gene expression. All strains were grown at 37 °C in Luria-Bertani (LB) broth (Difco, Sparks, MD, USA). Antibiotics were purchased from IBI Scientific (Peosta, IA, USA) whereas enzymes and corresponding reaction buffers were purchased from Promega (Madison, WI, USA).

2.2. Cloning of qseC into pQE30 expression vector and purification of QseC protein

The *qseC* was amplified by polymerase chain reaction (PCR) using *QseC*F and *QseC*R primers and APCEO78 DNA as the template. The amplified fragment was restricted with *Bam*HI/*Hind*III enzymes and ligated to the equally digested pQE30. The resultant plasmid was designated as pQseC. *E. coli* M15 (Qiagen, Germany) cells were transformed by electroporation with pQseC and the cells were allowed to recover in LB broth (37 °C, 1 h at 1000 rpm) without selection and plated on LB agar containing ampicillin (100 micrograms/ml) and kanamycin (25 micrograms/ml). The transformed *E. coli* M15 containing the recombinant pQseC was designated as MQseC. Expression and purification of QseC protein was performed using QIAexpressionist™ Kit (Qiagen, Valenica, CA, USA) according to manufacturer's instructions. The expression of recombinant 6xHistidine-tagged (His-tagged) QseC protein was confirmed by a chromogenic method as described in the QIAexpress® Detection Kit manual (Qiagen). Purified QseC in imidazole buffer was dialyzed against a cell culture friendly Dulbecco's phosphate-buffered saline (DPBS; Sigma, Saint Louis, MO, USA).

2.3. Cell culture and treatment

An avian macrophage-like cell line, HD11 was received from the Animal Diagnostic Laboratory (Pennsylvania State University). The avian macrophage-like cells (AMCs) were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies Grand Island, NY) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. For the QseC treatment experiment, cells were maintained in an antibiotic- and serum-free medium. To investigate the effect of the culture medium of AMC treated with QseC on bacterial growth, AMCs were treated with 0.5, 1, 2.5 and 5 μg/ml of QseC protein in the native form. Precisely, AMCs (1×10^6 cells/ml) were added in triplicate into the wells of 6-well tissue culture plates together with 500 μL of DMEM alone or DMEM containing the QseC protein. The plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 48 h. The culture medium from the cells treated with different concentrations of QseC was collected, filtered using 0.22 μm syringe filter and stored at −20 °C until use. The experiments were carried out in triplicate.

2.4. Bacterial growth curves

The effect of different concentrations of QseC protein on APECO78 bacterial growth was determined by adding 1/100 volume of an overnight culture of APECO78 into 20 ml of LB broth containing 0.5, 1, 2.5 and 5 μg/mL of QseC protein in the native form. The cultures were incubated at 37 °C with shaking at 250 rpm and the optical density at 600 nm (OD_{600}) was determined every 1.5 h for 12 h. The graph was plotted as O.D *versus* time point on *Y* and *X* axes, respectively. Similarly, the effect of host immunity to different concentrations of QseC protein on APECO78 bacterial growth curve was examined. For this purpose, 1/100 volume of an overnight culture of APECO78 was added to 20 ml of LB broth containing 1 ml of AMC culture medium collected after treatment with different concentrations of QseC protein (mentioned earlier). The growth curve analysis was performed as described above. The experiments were carried out in triplicate.

2.5. Real time RT-PCR (qRT-PCR)

Regulation of virulence gene expression of APECO78 incubated with the cell culture medium of QseC-stimulated (2.5 μg/ml) and non-stimulated AMCs was examined using a quantitative real-time reverse-transcription PCR (qRT-PCR). Also, IFN-γ, TLR-4 and TLR-15 mRNA levels in QseC-stimulated (2.5 μg/ml) and non-stimulated AMCs were quantified using the same technique. Primer sets for all the genes can be found in the Supplemental Table. Real-time quantitative PCR was performed using the PerfeCTa® SYBR® Green SuperMix, Low ROX™ (Quanta Biosciences, Gaithersburg, MD) according to the manufacturer's instructions. Total RNA was purified using an RNeasy