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PCR based detection of gyrB2 gene from *Pseudomonas* sp. affected human clinical isolates

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ABSTRACT

Detection of virulence gene is a key component in determining the pathogenicity of any isolates because these genes act multi-functionally and multi-factorially. Gyrase specific gene primer in combination of PCR technology allows precise detection of DNA gyrase subunit B2 gene (gyrB2) from different virulent microorganisms. In the present study, forward and reverse primers with a length of 20bp for both were used for detection of gyrB2 genes in clinical isolates of *Pseudomonas* sp. collected from patients suffering from urinary tract infection (UTI). A total of 12 isolates of *Pseudomonas* sp. *viz.*, Ps1, Ps2, Ps3, Ps4, Ps5, Ps6, Ps7, Ps8, Ps9, Ps10, Ps11 and Ps12 were used in present study in which gyrB2 gene amplified in all 12 isolates and gave the expected 1130bp PCR product after visualization under gel documentation system in 1.2% agarose gel. This PCR was outstanding in the detection of gyrB2 gene in urinary tract infected patients caused by *Pseudomonas* sp. species.

Key Words: *Pseudomonas* sp., virulence gene, *gyr*B2, PCR detection, visualization.

INTRODUCTION

Molecular techniques have been resolved in recent years for detection of pathogen and their virulent genes proven promising in disease diagnosis and prophylaxis. Among them Polymerase Chain Reaction (PCR) is the most significant one in last couple of years. *Pseudomonas* species are Gram negative, catalase positive, oxidase positive, rod shaped, non-motile bacteria that are found in the environment (Nathan *et al.*, 2011) and also in the human clinical samples (Oberhofer, 1980). Urinary Tract Infection (UTI) is the commonest infection among patients in intensive care facilities across the world ant it ranks worst among patient morbidity and mortality cases in hospital acquired infections (Mittal *et al.*, 2009). Saber *et al.* (2010) described that the UTI infection in Bangladesh caused by *Pseudomonas* species are due to the virulence factors expressed by the organism. Bajaj *et al.* (1999) reported that *Pseudomonas* species caused urinary tract infection (9.64%) in maximum number of cases followed by *Staphylococcus aureus* (6.93%). Several genes responsible for virulence properties of *Pseudomonas* species but DNA *gyrase* subunit B2 gene (gyrB2) is the principle one has been reported by many researchers. DNA gyrase is a prokaryotic type II topoisomerase which cut both strands of the DNA helix simultaneously in order to manage DNA tangles and supercoils and a major target of quinolone antibacterials (Nitiss, JL., 2009). Nitiss also reported that the majority of mutations conferring resistance to quinolones arise within the quinolone resistance-determining region of GyrA close to the active site (Tyr^{122}) where DNA is bound and cleaved. However, some quinolone resistance mutations are known to exist in GyrB. Campbell *et al.* (1995) detect *Pseudomonas* sp. by PCR in infant's through16s rRNA sequencing Dauga (2002) amplified gyrB gene and performed molecular phylogenetic analysis in enterobacteriaceae.

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MATERIALS AND METHODS

Collection of bacterial isolates

Pseudomonas sp. bacterial isolates were collected from three different medical colleges and hospitals of Sylhet district, Bangladesh viz. M. A. G. Osmani

Table 1. Pseudomonas sp. solates with their isolation history.

Isolates	Collected from	Type of patient
Ps1	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps2	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps3	Ragib-Rabeya Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps4	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps5	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps6	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps7	Ibn Sina Hospital, Sylhet, Bangladesh	UTI
Ps8	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps9	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps10	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
Ps11	Ibn Sina Hospital, Sylhet, Bangladesh	UTI
Ps12	Ragib-Rabeya Medical College and Hospital, Sylhet, Bangladesh	UTI

Medical College Hospital, Ibn Sina Hospital and Ragib-Rabeya Medical College and Hospital, Bangladesh **(Table 1)**. Isolates were collected from patients suffering from urinary tract infection (UTI).

Culture condition

The bacterial isolates were streaked in nutrient agar plates from previous plate and incubate in 37ºC for overnight for appropriate colony formation. After the incubation the single colony of each plate was selected for re-isolation to a pure culture in nutrient agar plate.

DNA extraction

A total of 12 bacterial isolates were inoculated in nutrient broth culture and incubated overnight at 37°C and 120rpm in a shaker incubator. The bacterial genomic DNA was extracted by using commercial DNA extraction kit (Bio Basic Inc., 160 Torbay Road, Markham Ontario, Canada) and extracted DNA was preserved at -20°C in an ultra freezer.

PCR reaction mixture set up

The PCR was performed in 25µl reaction mixtures containing DNA template (genomic DNA of bacteria) of 1.2µl, 1µl of 25 mM $MgCl_2$, 5µl of 5x colorless reaction buffer, 0.5 µl concentration of each deoxynucleotide triphosphate (dNTP), 1.2µl of each forward primer and reverse primer **(Table 2)** and 0.15µl DNA polymerase along with its amplification buffer.The amplifications were carried out in a MultiGene gradient thermal cycler (Labnet International Inc. USA).

Amplification condition

PCR reaction was optimized with the following parameters: an initial denaturation step of 94°C for 4 min; a denaturation step of 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 90 s; and a final extension step of 72°C for 10 min. 35 serial cycles of reaction was performed.

RESULTS AND DISCUSSION

Amplified PCR products were detected by agarose gel electrophoresis of each of amplification mixture in 1.2% agarose gels in 0.5% Tris-borate-EDTA buffer. Gel was stained with ethidium bromide solution (10mg/ml) for 20 minutes followed by destaining in distill water for 10 minutes. The position of each band on gel then visualized and documented in a gel documentation system. GyrB2 gene has been amplified in all isolates of *Pseudomonas* sp., *viz.*, Ps1, Ps2, Ps3, Ps4, Ps5, Ps6, Ps7, Ps8, Ps9, Ps10, Ps11 and Ps12. The sizes of the amplification products obtained by the PCR were identical to those predicted from the target gyrB2 primers **(Figure 1)**.

Table 2. Primer used for present study.

Primer	Sequence (5′ to 3′)	PCR product length	Pathogen
GyrB2-F	TCCGGCGGTCTGCACGGCGT	1130bp	*Pseudomonas* sp.
GyrB2-R	TTGTCCGGGTTGTACTCGTC		

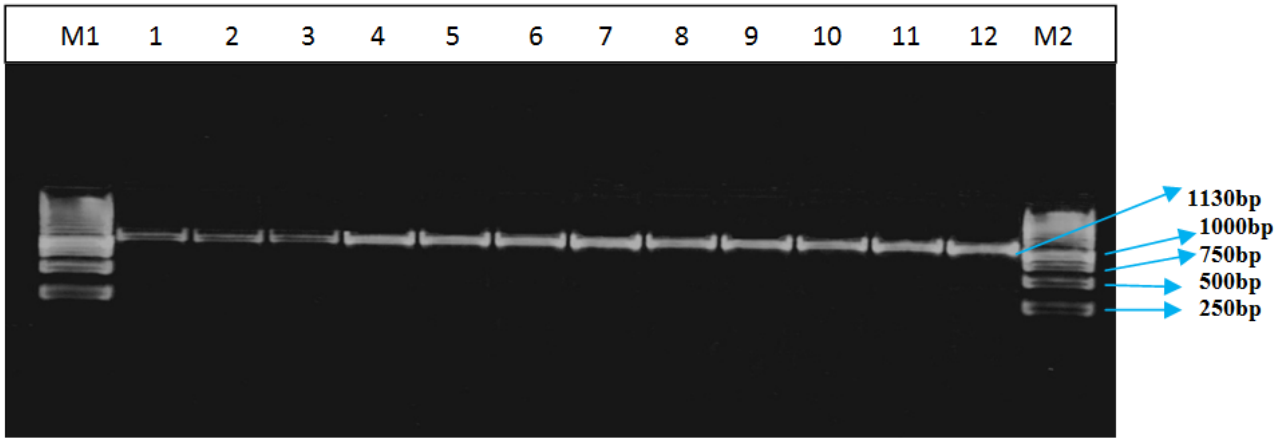

Figure 1: PCR amplification of gyrB2 gene in *Pseudomonas* sp. with expected product length of 1130bp in 1.2% agarose gel.
M1 and M2 = 1kb DNA ladder
1-12= *Pseudomonas* sp. isolates
1=Ps1, 2=Ps2, 3=Ps3, 4=Ps4, 5=Ps5, 6=Ps6, 7=Ps7, 8=Ps8, 9=Ps9, 10=Ps10, 11=Ps11 and 12=Ps12.

Pseudomonas sp. has been reported as a common pathogen for human associated with pneumonia and other diseases. Turner *et al.* (2009) described that the pathogenicity of *Pseudomonas aeruginosa* depend on the expression of a diverse set of genes. Bradbury (2010) determined virulence factors of *Pseudomonas aeruginosa* by PCR and find out virulent genes that contribute pathogenicity of *Pseudomonas sp.*. The gyrB gene is a single-copy gene, present in all bacteria which encode the ATPase domain of DNA gyrase, an enzyme essential for DNA replication (Huang, 1996). Earlier studies reported that gyrase subunit B2 gene contribute the major virulence properties of many bacterial species and has been used as a molecular tool for identification of bacterial species (Coenye and LiPuma, 2002) and phylogenetic analysis (Yamamoto and Harayamapcr, 1995; Shen *et al.*, 2006). Podschun and Ullmann (1998) reported the contribution of gyrB2 gene in progression of enterobactericeae associated disease. In present study all collected (12) isolates were positive for gyrB2 gene revealing the distribution and virulence properties of these gene in *Pseudomonas* species and confirms the detection of gyrB2 gene product of 1130bp using PCR as a taxonomic marker.

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