Detection of *Proteus mirabilis* using a newly designed GST-B primer for the Glutathione S-Transferase B gene

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ABSTRACT

Proteus mirabilis is an opportunistic pathogen causing urinary tract infections, which is the most important species among other members of this genus. *P. mirabilis* can be conventionally identified based on biochemical tests such as lactose fermenting, swarming phenomenon oxidase, and indole tests. In this study biochemical tests of 12 *Proteus* spp. isolates (6 *P. mirabilis*, 4 *P. vulgaris*, and 2 *P. penneri*) were studied. However, some strains exhibited different phenotypic characteristics, which may lead to misidentification. The aim of this study was to design a PCR primer that can be used specifically to identify *P. mirabilis*. The GST-B primer detected all 6 isolates of *P. mirabilis*. Suggesting that molecular identification is more reliable than conventional examination and the GST-B primer can be used to detect *P. mirabilis* specifically. For further studies, GST-B primers can be used to multiply the GST enzyme and study its functions.
Keywords: *Proteus*; *mirabilis*; *gst*B; Gene; Primer.

1. Introduction

Proteus mirabilis is an expedient pathogenic bacterium causing UTI, which is the most important species among other members of this genus. On standard laboratory conditions, *Proteus* spp. produce circular symmetry colonies with regularly spaced concentric terraces or zones, which are distinguished by their colony shape [1]. This pattern of growth occurs because of the periodic repetition of alternating phases: swarming and consolidation [2, 3]. Members of this genus are opportunistic pathogens that are implicated in complicated urinary tract infections, bacteremia, and frequently, nosocomial infections. Among these members, *Proteus mirabilis* is the most isolated species responsible for 90% of all *Proteus* infections in humans, followed by *P. vulgaris*, *P. penneri*, and *P. hauser* [4-6]. *P. mirabilis* and other species which belong to this genus can be distinguished from other bacterial genera by swarming phenomenon, but the distinction between *P. mirabilis* and other *Proteus* spp. remains a challenge [7]. Although *P. mirabilis* differs from other species of *Proteus* by a negative indole test, some studies [8-10] revealed the presence of indole-positive *P. mirabilis*. Moreover, indole-negative strains of *P. penneri* were detected [11-14]. Glutathione S-transferase (GST) is a multifunctional enzyme that aids cellular detoxification. There are two forms of GSTs homodimers and heterodimers [15]. These enzymes have a variety of roles in bacterial development [16]. It has been found that the GST of *P. mirabilis* reduces the antibacterial action of β-lactam medicines [17]. According to previous studies [18, 19], the GST of *P. mirabilis* has 203 amino acid residues and its main structure has a low degree of similarity (17-26%) with the equivalent enzyme from diverse sources. In addition, they revealed that the GST of *P. mirabilis* has a molecular mass of 22856 Da. This study aimed to design PCR primers for molecular identification of *P. mirabilis* based on a specific sequence of the glutathione S-transferase (*gst*B) gene.

2. Material and Method

Bacterial samples (6 *P. mirabilis*, 4 *P. vulgaris*, and 2 *P. penneri*) were obtained from the Microbiology Laboratory of Pharmacy Department in Osol Aldeen University College. All culture media used in this study were purchased from Lab M (UK). All PCR primers in this study were provided from BM laboratory systems (Ankara, Turkey).

Biochemical tests

Traditional biochemical tests such as motility, indole, methyl red (MR), Voges Proskauer (VP), citrate, catalase, urease, oxidase, and lactose fermentation were used to identify bacterial samples.

Molecular identification

DNA extraction

A genomic DNA purification kit (Promega) was used to extract DNA. All overnight bacterial cultures were centrifuged for 3 minutes at 13000rpm, then the supernatants were discarded. Each bacterial sample was

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re-suspended in a mixture containing 480 µL of 50 mM EDTA and 120 µL of 2% lytic enzyme, mixed vigorously, and incubated at 37oC for 1.5 h. Supernatants were removed after centrifuging them at 13000 rpm for 3 min then re-suspended in 600 microliters of lysis solution for nuclei and incubated at 80oC for 10 min. 200 microliters of protein there was also added precipitation solution. and incubated at -20oC for 10 min. Centrifuged and supernatants were transferred into new sterile Eppendorf tubes including 600 microliters of isopropanol (invert mixing until thread-like DNA strands form). Centrifuged at 13000 rpm for 3 minutes then discarded and left to dry on a clean absorbent paper. 600 µL of 70 % ethanol was added then gently invert mixed and centrifuged at 13000 rpm for 3 min. Again, poured off and left to dry on a clean absorbent paper. Finally, 100 µL of rehydration DNA solution was added and incubated at a temperature of 65oC for 1 h.

Detection of 16SrRNA gene of Proteus spp.

The 16SrRNA gene of all bacteria samples was amplified using 16SrRNA Forward and Reverse primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-CTACGGCTACCTTGTTACGA-3', respectively. PCR reaction mixtures (each sample individually) and PCR conditions were set as described by [20].

PCR Primer Design for gstB gene

The National Center for Biotechnology Information (NCBI) Pick Primers tool was used to design primers for the glutathione transferase gene of *P. mirabilis* using the GenBank U38482.1. For manufacturing, the designed PCR primer sequences were sent to BM laboratory systems (Ankara, Turkey).

Amplification of gstB gene

The designed primers were prepared as described by the manufacturer and diluted to obtain 10 pmol/µL. The PCR mixture was prepared to contain 5X master mix, 1 µL GST-B Forward and 1 µL GST-B Reverse primers, 3 µL DNA, and 15 µL Milli Q water. Moreover, the PCR program was set to start with 94°C for 5 minutes, then 30 cycles (each cycle has 94°C for 1 minute of denaturation, heating to an annealing point of 59°C for 1 min and an elongation temperature of 72°C for 1.5 min) and ended with a final extension temperature of 72°C for 7 min.

Agarose gel electrophoresis

A gel electrophoresis device was used to monitor the amplified gene by running 5 µL of PCR products on 45 minutes on a 2 percent agarose gel with ethidium bromide TAE buffer at 120V and 90W.

Detection of optimal annealing temperature

Gradient PCR technique was used to detect the optimal annealing temperature between 55-65oC. Briefly, a DNA sample of one *P. mirabilis* isolate was selected randomly and eight identical PCR reaction mixtures (same as described previously) of the selected DNA were prepared.

3. Results

Biochemical test

Results of biochemical tests are shown in **Table 1**.

Molecular detection of Proteus spp. based on 16SrRNA gene

All bacterial samples showed a positive PCR product of 1500bp for the 16SrRNA gene as shown in **Figure 1**.

Primers design

The study showed that the 20-nucleotide primers (GST-B Forward 5'-TCTGCCGAGCCAAGATAACC-3' and GST-B Reverse 5'-GACGGGTTGTGTGATGTTCG-3') were specific for the *gst*B gene of *P. mirabilis*. Since the GST-B Forward primer binds between 1818 and 1837 nucleotides, while the GST-B Reverse primer binds between 1932 and 1913 nucleotides in the *gst*B gene. Furthermore, the designed primers contain 55% of the G-C bond DNA with a melting temperature of 59°C.

Amplification of the gstB gene

The amplified *gst*B gene was observed in all strains of *P. mirabilis*, while it was not observed in any strain of *P. vulgaris* and *P. penneri* **Figure 2**.

Optimal annealing temperature

Gradient PCR technique showed that the GST-B primers had an annealing temperature between 57-61°C, but the optimal binding was observed at 59°C **Figure 3**.

Table1. Results of biochemical tests

Tests	*P. mirabilis*	*P. vulgaris*	*P. penneri*
	6	4	2
Motility (swarming)	+	+	+
Indole	V (+1)*	+	+
Methyl Red (MR)	+	V (+3)	+
Voges Proskauer (VP)	-	-	-
Citrate	V (+2)	-	-
Catalase	+	+	+
Urease	+	+	+
Oxidase	-	-	-
Lactose Fermentation	-	-	-
V= Variable (Number of positive tests)			

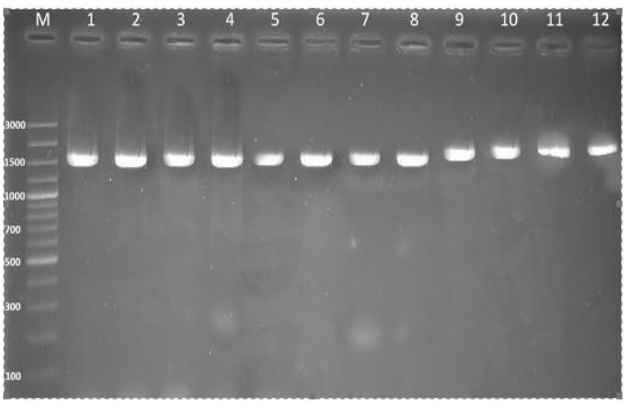

Figure 1: Molecular detection of Proteus spp. by 16SrRNA gene.

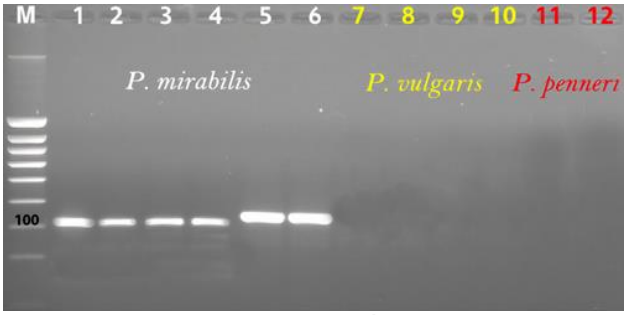

Figure 2: Detection of gstB gene.

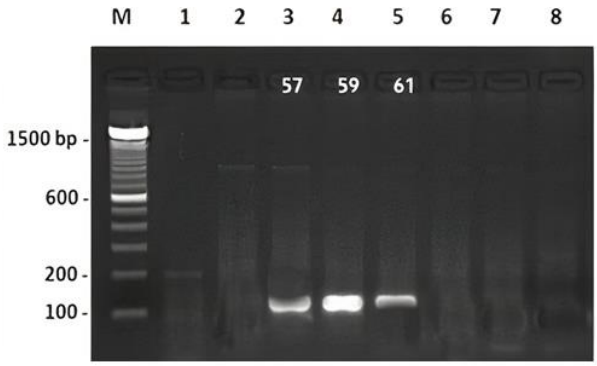

Figure 3: Detection of the optimal annealing temperature using the gradient PCR technique.

4. Discussion

Proteus spp. cause UTIs, nosocomial infections, and other miscellaneous infections such as wound infections, pneumonia, respiratory tract infection, septicemia, and otitis. *Proteus* spp. is responsible for 1–2 % of UTIs in healthy women and 20–45 % of catheter-associated UTIs. These microorganisms are responsible for 5 % of nosocomial infections resulting from improper sterilization systems by hospital staff [21-23]. Furthermore, *P. mirabilis* is the most often isolated species from patients with indwelling catheters or anatomical problems of the urinary tract [24, 25].

The distinction between *P. mirabilis* and other *Proteus* spp. usually depends on the indole test. However, indole-positive isolates of *P. mirabilis* have been detected in previous studies [9, 10, 26]. Our study detected 1 indole-positive *P. mirabilis* out of 6 isolates, indicating that the indole test may not be reliable for distinguishing *P. mirabilis* from *Proteus* spp.

All isolates in this study were confirmed as members of the genus *Proteus* based on the 16srRNA of this genus, in spite there were slight variations in the phenotypic properties of these isolates. This result was consistent with the previous study [20].

The PCR primer design showed that the *gst*B gene of *P. mirabilis* has a specific sequence in comparison with other corresponding enzymes from different sources. This was demonstrated when only *P. mirabilis* isolates showed a positive band on gel electrophoresis. Suggesting that GST-B primers can be used to detect and distinguish *P. mirabilis* from other *Proteus* spp. For further studies, GST-B primers can be used to multiply the GST enzyme and study its functions.

5. Conclusion

The design of the two oligonucleotide primers is crucial to the PCR. It is critical to take great caution while designing PCR primers. For effective PCR, several factors like primer length, percent GC content, and 3' sequence must be tuned. Certain of these factors may be easily tuned by hand, while others need the use of commercially available computer programs. When constructing primers and PCR reaction conditions, the increasing usage of information from the internet and sequences stored in gene databases are useful starting points. This study revealed that the *gst*B gene of *P. mirabilis* has a specific sequence compared to other *Proteus* spp., which aids in the detection of *P. mirabilis*.

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