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Original Article

Molecular Study of Urease *ureR* Gene of *Proteus mirabilis* Isolated from Urinary Tract Infections, Najaf, Iraq

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Abstract

Proteus mirabilis is considered one of the causative pathogens that leads to complicated urinary tract infection (UTI); moreover, it produces urease. Urease plays a key role as a virulence factor for *P. mirabilis*. *UreR*, a member of the AraC/XylS family of transcriptional regulators, positively activates the expression of the *ure* gene cluster in the presence of urea. Therefore, this study was designed to investigate the contribution of *ureR* to urease activity and virulence in urinary tract infections. A total of 74 clinical samples were collected from August to December 2020. The urine samples were taken from individuals with parasitic infections in their urinary tracts. After cultivating the samples on the MacConkey agar, the initial identification was performed based on traditional methods with the automated VITEK-2 compact method. Bacterial isolates were inoculated by stabbing and streaking into a slant of urease agar, which were then incubated at 37°C for 24-48 h. The polymerase chain reaction technique was used to detect the *P. mirabilis ureR* gene. The results of biochemical studies were utilized to confirm the identification of *P. mirabilis* isolates that had previously been made. All isolates had the same oxidase-negative, catalase-positive, oxidase-negative, and catalase-positive properties. They were motile, methyl red, and uric acid, catalase, citrate, and urease positive. The results of investigating the expression of the *ureR* gene in 15 isolates of *P. mirabilis* suggested that only 14 (93.3%) of the isolates produced *ureR* gene products using unique primers.

Keywords: *Proteus mirabilis*, *ureR* gene, PCR

1. Introduction

Proteus mirabilis refers to a Gram-negative, anaerobic rod rod-shaped bacterium that is a member of the Enterobacteriaceae family and consists of more than four kinds; the majority of *P. mirabilis* types that trigger illness in humans are linked to opportunistic infections (1). *Proteus mirabilis* can be found in abundance in the real world. The most prominent opportunistic infectious bacterium is *Escherichia coli.* It is an opportunistic bacterial pathogen that in proper circumstances may trigger a variety of diseases, including urinary tract infections (UTIs), especially complicated UTIs (2). It induces disease by expressing P and type 1 fimbriae is capable of flagellum-mediated motility (3, 4).

The capability of *Proteus* spp. to generate urease and alkalinize urine via hydrolyzing urea to NH_4 allows it to be successful at creating a suitable environmental condition for survival. This causes the deposition of organic and inorganic materials, resulting in the creation of struvite stones. Struvite stones are composed of struvite (magnesium ammonium phosphate) and apatite (calcium carbonate). *Proteus mirabilis* causes urinary stones and encrusts indwelling catheters increase the persistence of the infection; the formation of stones around the organism renders

antibiotic treatment ineffectual. Urease catalyzes the hydrolysis of urea into CO_2 and NH_4, increasing the local pH of the environment and intercedes the precipitation of naturally soluble polyvalent ions from the urine, especially the deposition of magnesium, ammonium, phosphate, and calcium ions, resulting in the production of struvite and carbonate hydroxyapatite crystals that make up urinary stones (5).

It is well documented that the urease gene cluster of *P. mirabilis* consists of *ureABC* and *ureDEFG*. The *ureABC* encodes the apoenzyme structural subunits, while the *ureDEFG* is responsible for encoding the proteins that facilitate the insertion of the essential nickel ions into the catalytic site (6). *UreR* is the responsible factor for activating the urease operon expression. *UreR*, a member of the AraC/XylS family of transcriptional regulators, positively activates the expression of the *ure* gene cluster in the presence of urea (7, 8).

Proteus mirabilis is considered one of the causative pathogens that leads to complicated UTIs; moreover, it produces urease. Urease plays a key role as a virulence factor for *P. mirabilis*. Therefore, this study was designed to investigate the contribution of *ureR* to urease activity and virulence in UTIs.

2. Materials and Methods

2.1. Samples Collection and Identification

A total of 74 clinical samples were collected from August to December 2020. The urine samples were taken from individuals who had parasitic infections in their urinary tracts. After cultivating on MacConkey agar, biochemical tests VITEK 2 compact gadget was used for the final identification.

2.2. Phenotypic Detection of Urease

Bacterial isolates were inoculated by stabbing and streaking into a slant of urease agar, which were then incubated at 37°C for 24-48 h.

2.3. DNA Extraction

DNA was extracted employing the wizard of G-BIOSCIENCES' GET™ plasmid miniprep kit (G-BIOSCIENCES, USA) according to the manufacturer's instructions for high yield and accuracy. An Ultraviolet (UV) transilluminator was used to detect DNA using gel electrophoresis. The polymerase chain reaction (PCR) technique was employed to detect the *P. mirabilis ureR* gene, the forward and reverse primers are shown in table 1. This primer was created by Alpha DNA Company in Canada. The PCR condition for the amplification of *ureR* is presented in table 2. Validated amplified products were run on a 1% agarose gel electrophoresis to assess the magnitude of the PCR items. The gel was stained for 1.5 h at 85 v with 5 mg/mL ethidium bromide. A single band identified at the target point on UV light transilluminator bands was photographed using a gel documentation system. A 1,000-bp ladder was used to determine the molecular weights of amplified components (Bioneer, Korea).

Table 1. Primers used in this study

Primer	Sequence (5'-3')	Size (bp)
ureR	F-CCGGAACAGAAGTTGTCGCTGGA- R-GGGCTCTCCTACCGACTTGATC-	359

Table 2. PCR program of *ureR* primer applied in the thermocycler

Gene name	Temperature (°C)/Time (min)					Cycles number
	Initial denaturation	Cycling conditions			Final extension	
		Denaturation	Annealing	Extension		
ureR	94/2	94/1	58/1	72/1	72/5	30

3. Results and Discussion

Cultural morphology, microscopic characteristics, and biochemical tests were used to identify bacterial isolates collected from clinical samples. The colonial morphology of *P. mirabilis* isolates was employed to assess their cultural identity. The colonies of *P. mirabilis* were grown on blood agar and nutrient agar swarm in waves and, under microscopic examinations, could be seen individually, in pairs, or in minute chains. The results of biochemical studies were utilized to confirm the identification of *P. mirabilis* isolates that had previously been made. All isolates had the same oxidase-negative and catalase-positive properties. They were motile, methyl red, and uric acid, catalase, citrate, and urease positive. The isolates were able to brew glucose, which was in agreement with the results of studies by Al-Muhanna, Banoon (9) and Al-Kraety (10).

3.1. Molecular Detection of *ureR* Gene Isolated from *Proteus mirabilis*

The results of investigating the expression of the *ureR* gene in 15 isolates of *P. mirabilis* suggested that only 14 (93.3%) of the isolates produced *ureR* gene products using unique primers (Table 1). The PCR identification of these isolates is depicted in figure 1.

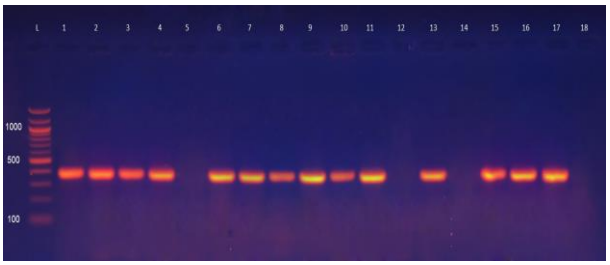

Figure 1. PCR amplification productions of *Proteus mirabilis* isolates magnified with *ureR* gene primers yielding a 359-bp product

Figure 1 shows that *P. mirabilis* isolates containing the *ureR* gene generated positive PCR generations on a gel, with the whole isolates containing that gene. D'Orazio, Thomas (11) recorded identical results when they used PCR to validate the existence of the *urease* gene in *P. mirabilis*.

Authors' Contribution

All authors made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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