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Treska Dh. Kamil
Department of Biology, Medical Microbiology, College of Science, Cihan University, Erbil, Kurdistan Region, Iraq, sanariafj@epu.edu.iq

Sanaria F. Jarjes
Erbil Polytechnic University, sanariafj@epu.edu.iq

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Abstract

The study was carried out to detect the identity of Proteus spp. isolated from various clinical specimens in Erbil City by polymerase chain reaction (PCR) technique. Specimens were of urine, wounds swabs, burn swabs, vaginal swabs, ear swabs, eye swabs, and sputum. Fifty-one Proteus isolates, (47) Proteus mirabilis, and (4) Proteus vulgaris, were undergone PCR assay using specific primers, targeting the genes ureR and Urease C (ureC), that encode for urease enzyme as a virulence factor of Proteus species. It was found that all isolates of Proteus mirabilis yielded positive result for ureR gene with an amplicon length of 225 bp, as well as, all isolates of Proteus vulgaris exhibited positive PCR products on gel for ureC gene with an amplicon length of 263 bp. Our results indicate that ureR and ureC based PCR method seems to be an appropriate method for characterization of Proteus mirabilis and Proteus vulgaris respectively.

Keywords

Proteus mirabilis, Proteus vulgaris, Polymerase chain reaction, ureR, Urease C

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REVIEW ARTICLE

Molecular Characterization of *Proteus* spp. from Patients Admitted to Hospitals in Erbil City

Treska Dh. Kamil[1], Sanaria F. Jarjes[2]*

[1]Department of Biology, Medical Microbiology, College of Science, Cihan University, Erbil, Kurdistan Region, Iraq, [2]Department of Medical Laboratory Technology, Erbil Technical Health College, Erbil Polytechnic University, Kurdistan Region, Iraq

***Corresponding author:**
Sanaria F. Jarjes,
Department of Medical Laboratory Technology, Erbil Technical Health College, Erbil Polytechnic University, Kurdistan Region, Iraq.
E-mail: sanariafj@epu.edu.iq

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ABSTRACT

The study was carried out to detect the identity of *Proteus* spp. isolated from various clinical specimens in Erbil City by polymerase chain reaction (PCR) technique. Specimens were of urine, wounds swabs, burn swabs, vaginal swabs, ear swabs, eye swabs, and sputum. Fifty-one *Proteus* isolates, (47) *Proteus mirabilis*, and (4) *Proteus vulgaris*, were undergone PCR assay using specific primers, targeting the genes *ureR* and *Urease C* (*ureC*), that encode for urease enzyme as a virulence factor of *Proteus* species. It was found that all isolates of *Proteus mirabilis* yielded positive result for *ureR* gene with an amplicon length of 225 bp, as well as, all isolates of *Proteus vulgaris* exhibited positive PCR products on gel for *ureC* gene with an amplicon length of 263 bp. Our results indicate that *ureR* and *ureC* based PCR method seems to be an appropriate method for characterization of *Proteus mirabilis* and *Proteus vulgaris* respectively.

Keywords: *Proteus mirabilis, Proteus vulgaris*, Polymerase chain reaction, *ureR, Urease C*

INTRODUCTION

Genus *Proteus* belongs to the enterobacteriaceae family (Pal et al., 2014). There are several species of *Proteus* including five named species: *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus hauseri,* and *Proteus myxofaciens*, as well as three unnamed *Proteus* genomospecies (O'Hara et al., 2000).

Proteus is a Gram-negative, rod-shaped, facultative anaerobic, non-capsulated, non-spore forming, motile, and urease splitting bacterium (Kamel and Jarjes, 2015). It is mostly found in natural environments and under favorable conditions, are able to cause a variety of opportunistic nosocomial infections (Feglo et al., 2010) including the infections of the urinary tract, burns, skin, eyes, ears, nose, the respiratory tract, as well as gastroenteritis (Jacobsen et al., 2008).

Proteus expresses several virulence factors involved in pathogenesis such as adhesions, swarming motility, urease, hemolysin, proteases, and lipopolysaccharide endotoxins (Armbruster and Mobley, 2012). Urease is an important virulence factor in the pathogenicity of *Proteus* and urease production is a prominent characteristic of the genus *Proteus* (Mohammed et al., 2014). Furthermore, the gene clusters encoding this enzyme have been cloned from numerous bacterial species including *Proteus* species (Mobley and Belas, 1995). The urease gene cluster includes three structural genes, *ureA, ureB,* and *Urease C* (*ureC*), besides four accessory genes, *ureD, ureE, ureF,* and *ureG,* and a positive transcriptional activator, *ure*R.

The *ureA*, *ureB*, *ureC*, *ureD*, *ureE*, *ureF*, *ureG,* and *ure*R genes on *ure* operon are governed the production of urease enzyme. Previous studies pointed out *ureC* as a major gene, it encodes the large subunit responsible for the production of urease enzyme of the *Proteus*, and it is highly conserved among all species, so it regarded as a diagnostic feature of this bacteria (Schabereiter-Gurtner et al., 2001; Li and Mobley, 2002; Shoket et al., 2014).

Transcription of the structural genes of urease is urea-inducible, where *ure*R acts as a regulator of urease activity and stimulate expression of urease genes in the presence of urea (Poore and Mobley, 2003).

For the importance of *Proteus* species as an opportunistic pathogens that can cause infections in different anatomical sites of the human body, this study is concerned with molecular characterization of *Proteus* spp., isolated from various clinical specimens of patients admitted to hospitals in Erbil City/Kurdistan region of Iraq, using polymerase chain reaction (PCR) by detection of urease enzyme genes, *ure*R and *ureC*, which regarded as a diagnostic feature of these bacteria.

MATERIALS AND METHODS

Proteus Isolates

Fifty-one *Proteus* isolates, (47) *P. mirabilis*, and (4) *P. vulgaris*, were obtained from a previous study (Kamil and Jarjes, 2019). They were isolated from patients with symptomatic infections (urinary tract infections, wounds, burns, respiratory tract infections, and vaginitis) whom admitted to different hospitals in Erbil City, during the period from 1st October 2018 till 1st April 2019, and they were identified phenotypically depending on cultural and morphological characteristics, as well as, biochemical characteristics by Vitek 2 system.

Molecular Detection of *Proteus* Isolates

All isolates of *P. mirabilis* and *Proteus vulgaris* were subjected to molecular characterization by detecting of specific genes, *ure*R and *ureC* respectively.

Bacterial DNA extraction

Bacterial DNA was extracted from all isolates using the DNA extraction kit (GeNet Bio, Korea) according to the information of the supplying company. The concentration and purity of extracted DNA were measured using Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) at absorbance (260/280 nm), at ratio 1:8 as pure DNA, and done according to Desjardins and Conklin (2010). The DNA templates were kept in (−20°C) until used for PCR amplification.

Primers and PCR amplification

The primers were provided by GENEXIZ (South Korea) in lyophilized form. The PCR method based on *ure*R gene for identification of *P. mirabilis* was carried out, as described by Zhang et al. (2013), using forward primer sequences (5`-GGTGAGATTTGTATTAATGG-`3) and that of reverse (5`ATAATCTGGAAGATGACGAG-`3) with product size 225 bp. The PCR conditions were as illustrated in Table 1. On the other hand, *ureC* was employed for the identification of *P. vulgaris* isolates by PCR. Table 2 clarifies the primer sequences and PCR conditions which are used to amplify a portion of the coding region of *ureC* gene, as described by AL-Imam and AL-Rubaii (2016).

Detection of amplified products

PCR products were assessed by electrophoresis in 1.2% agarose gel stained with safe dye (Biolabs-USA). The gel was prepared by dissolving 1.2 g of agarose powder in 100 ml of Tris-acetate-EDTA Buffer (TAE) in microwave, allowed to cool to 50°C, and then a Safe Dye at the concentration of 5 μL/100 ml was added (Sambrook and Russell, 2001). UV Trans-illuminator (San. Gabriel, USA) was used to observe the DNA bands, and then gel was photographed with a digital camera. 100 bp DNA ladder (Gene dire) was used as molecular marker.

RESULTS

Detection of *ureR* Gene among *P. mirabilis* Isolates by PCR Technique

Results of molecular identification shown in Figures 1-3, indicated that all *P. mirabilis* isolates were positive for the presence of *ure*R gene at 225bp, except isolates number 16, 31, 35, and 44 that produced unclear bands on agarose. These isolates were undergone a second run on gel electrophoresis and the results are shown in Figure 4. In general, all isolates of *P. mirabilis* (100%), exhibited positive PCR products on gel electrophoresis for *ure*R gene at 225 bp.

Detection of *ureC* Gene among *P. vulgaris* Isolates by PCR Technique

The results of the PCR for the isolates of *P. vulgaris* showed that all the (4) isolates (100%) were positive for the presence of *ureC* gene at 263 bp [Figure 5].

DISCUSSION

In a previous study, (47) *P. mirabilis*, and (4) *P. vulgaris* were isolated from patients admitted to different hospitals in Erbil City and identified phenotypically (Kamil and Jarjes, 2019). In this study, for more confirmation to the identity of these isolates, species specific primers have been used to amplify urease gene, (*ureR*) from *P. mirabilis* isolates and (*ureC*) from *P. vulgaris*, that responsible for the production of urease enzyme and regarded as a diagnostic feature of these species using PCR.

Urease is a hallmark of infections with *Proteus* species and it is considered as one of the most important virulence factor of *Proteus*. Many studies demonstrate the high ability of *Proteus* species to produce urease (Jones and Mobley, 1988; Mobley and Chippendale, 1990 and Jones et al., 2007).

Table 1: Primer sequences of *Proteus mirabilis ureR* gene and polymerase chain reaction conditions used in this study

Functional category	Primers detail				Reference
	Primer Sequence (5´ – 3´) (Oligonucleotide)		Amplicon size (bp)	Cycling program	
ureR	F	5´ GGTGAGATTTGTATTAATGG 3´	225	94°C–4 min: 1 cycle 94°C–40 s: 40 cycles 58°C–1 min: 40 cycles 72°C–20 s; 40 cycles 72°C –10 min: 1 cycle	Zhang et al. (2013)
	R	5´ ATAATCTGGAAGATGACGAG 3´			

Urea is generated in humans following the breakdown of amino acids and is evenly distributed throughout the body. *Proteus* spp. are able to utilize urea as a nitrogen source through the activity of the urease enzyme, which splits urea into ammonia and carbon dioxide (Mobley et al., 1995). This process raises the local pH that can interfere with host function, as well as, resulting in direct tissue damage at the sites of infection (Nielubowicz and Mobley, 2010).

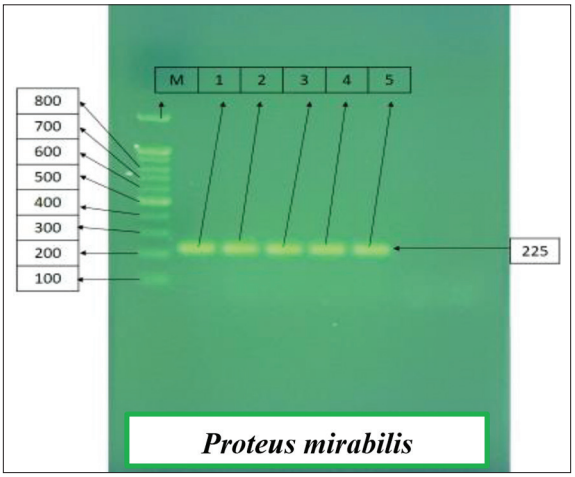

Figure 1: Agarose (1.2%) gel electrophoresis of PCR product of amplified *ureR* gene of *P. mirabilis* isolates (amplicon with 225bp). Lane M: DNA marker (100 bp); Lane 1- 5: Amplicon of *P. mirabilis* (5 isolates)

A wide distribution of *ure*R among *P. mirabilis* was detected in several studies such as (Mobley et al., 1995; Lu et al., 2000 and Lee and Deininger, 2000). Furthermore, Mobley and Chippendale (1990) revealed that all *P. mirabilis* isolated from various clinical sources generated a high quantity of urease compared with other bacteria and there was a conformity between phenotypic and molecular detection of urease activity. These data suggest that *ure*R governs the inducibility of *P. mirabilis* urease.

Several studies referred to use of *ure*R-based molecular method for identification of *P. mirabilis* such as Poore and Mobley (2003), Zhang et al. (2013) who designed a species specific primers depending on the conserved *ure*R sequence of *P. mirabilis* to identify this species using PCR, a 225-bp DNA product was amplified from this species and detected on an agarose gel. As well as, Adnan et al. (2014) who presented *ure*R as a high discriminatory power for identification of *P. mirabilis* using the PCR technology. Alatrash and Al-Yasseen (2017) also showed that all *P. mirabilis* isolated from patients with urinary tract

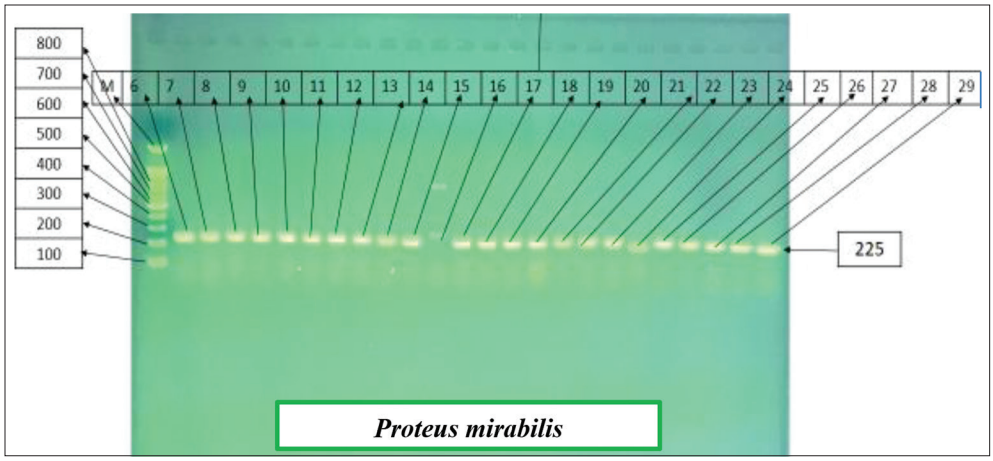

Figure 2 : Agarose (1.2%) gel electrophoresis of PCR product of amplified *ureR* gene of *P. mirabilis* isolates (amplicon with 225bp). Lane M: DNA marker (100 bp); Lane 6- 29: Amplicon of *P. mirabilis* (24 isolates)

Table 2: Primer sequences of *Proteus vulgaris ureC* gene and polymerase chain reaction conditions used in this study

Functional category	Primers detail				Reference
	Primer sequence (5´ – 3´) (Oligonucleotide)		Amplicon size (bp)	Cycling program	
ureaseC	F	5´CGTTTGCGATGGCAAGTACAAGTAAG 3´	263	94°C–4 min: 1 cycle 94°C–30s: 30 cycles 62°C 30 min: 30 cycles 72°C–60 s: 30 cycles 72°C–10 min: 1 cycle	AL-Imam and AL-Rubaii (2016)
	R	5´GCAAATTGAGTGACTTTGGCTGGACC 3´			

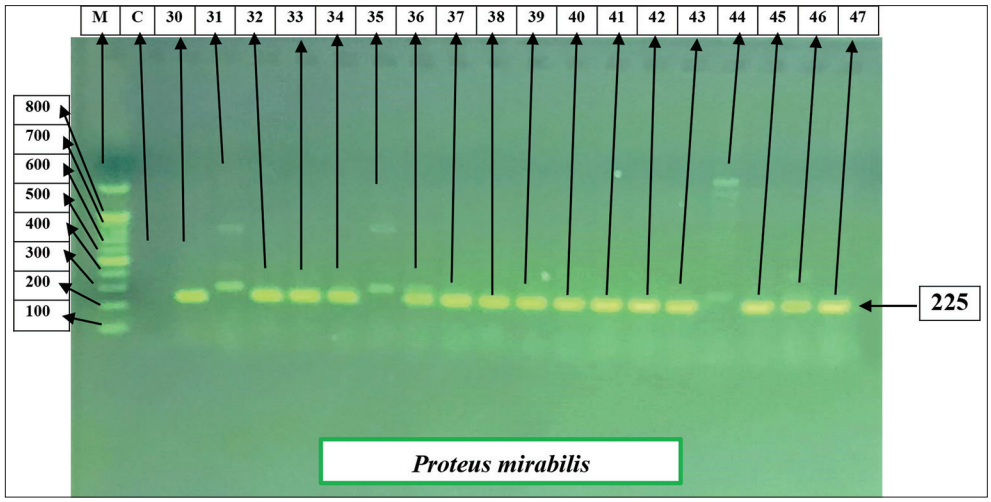

Figure 3: Agarose (1.2%) gel electrophoresis of polymerase chain reaction product of amplified *ureR* gene of *Proteus mirabilis* isolates (amplicon with 225 bp). Lane M: DNA marker (100 bp); Lane C: Control; Lane 30-47: Amplicon of *P. mirabilis* (18 isolates)

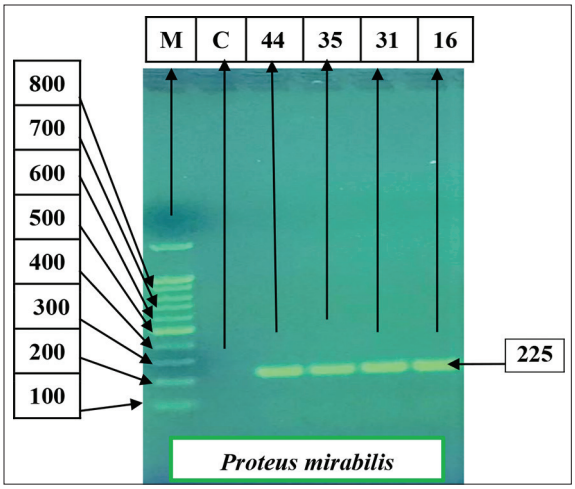

Figure 4: Agarose (1.2%) gel electrophoresis of polymerase chain reaction product of amplified *ureR* gene of *Proteus mirabilis* isolates (amplicon with 225bp). Lane M: DNA marker (100 bp); Lane C: control; Lane 1-4: Amplicon of selected *P. mirabilis* isolates

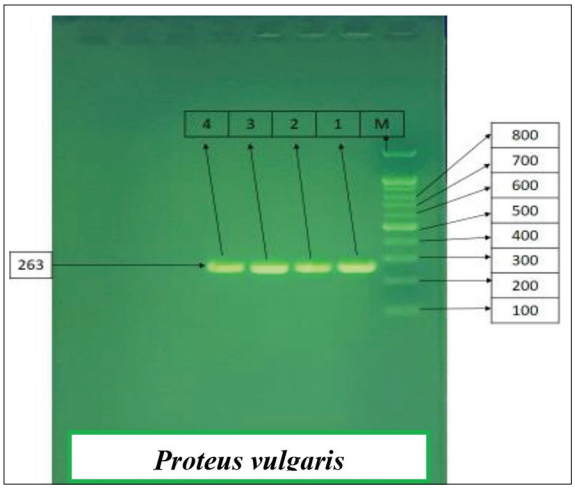

Figure 5: Agarose (1.2%) gel electrophoresis of polymerase chain reaction product of amplified *urease C* gene of *Proteus vulgaris* isolates (amplicon with 263bp). Lane M: DNA marker (100 bp); Lane 1-4: Amplicon of *P. vulgaris*

infections were able to produce urease and possess *ureR* that encode to urease by appearing of amplicon with molecular weight 359 bp when electrophoresed on 1% agarose gel. Furthermore, according to Latif et al. (2017), the results of PCR amplification to specific *ureR* primers indicated that (100%) of *P. mirabilis* isolates gave positive result at 225bp. In this study, the results were compatible to their findings as (100%) of the *P. mirabilis* isolates yielded *ureR* amplicon products with 225 bp.

On the other hand, all isolates of *P. vulgaris* were positive for the presence of *ureC* gene at 263 bp. Our result was compatible with Limanskiĭ et al. (2005); AL-Saadi et al. (2015) and AL-Imam and AL-Rubaii (2016), who used species – specific primers for this gene to detect *P. vulgaris,* and the result were excellent with primer *ureC* at 263 bp, which gave positive result for all samples.

CONCLUSION

In this study, *ureC* based PCR method at 263bp can be used for specific detection of *Proteus vulgaris*, also *ureR* based PCR method at 225bp is sufficient for fine characterization of *P. mirabilis*.

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