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

Treska Dh. Kamil¹, Sanaria F. Jarjes²*

¹Department of Biology, Medical Microbiology, College of Science, Cihan University, Erbil, Kurdistan Region, Iraq. ²Department 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: sanariaf@epu.edu.iq

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, 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, *ureR*.

The *ureA*, *ureB*, *ureC*, *ureD*, *ureE*, *ureF*, *ureG*, and *ureR* 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 *ureR* 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/Kurdish region of Iraq, using polymerase chain reaction (PCR) by detection of urease enzyme genes, *ureR* 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, ureR 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 ureR gene for identification of P. mirabilis was carried out, as described by Zhang et al. (2013), using forward primer sequences (5ˊ-GGTGAGATTGTGATTAATGG-3ˊ) and that of reverse (5ˊ-ATAATCTGGGAAATGACGAG-3ˊ) 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 ureR gene at 225 bp, 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 ureR 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

<table>
<thead>
<tr>
<th>Functional category</th>
<th>Primer Sequence (5ˊ – 3ˊ) (Oligonucleotide)</th>
<th>Amplicon size (bp)</th>
<th>Cycling program</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ureR</td>
<td>F  5ˊ- GGTGAGATTGTGATTAATGG 3ˊ</td>
<td>225</td>
<td>94°C–4 min: 1 cycle</td>
<td>Zhang et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>R  5ˊ- ATAATCTGGGAAATGACGAG 3ˊ</td>
<td></td>
<td>94°C–40 s: 40 cycles</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58°C–1 min: 40 cycles</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C–20 s: 40 cycles</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>72°C–10 min: 1 cycle</td>
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</table>
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).

A wide distribution of *ureR* 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 *ureR* governs the inducibility of *P. mirabilis* urease.

Several studies referred to use of *ureR*-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 *ureR* 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 *ureR* 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 infections.

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

<table>
<thead>
<tr>
<th>Functional category</th>
<th>Primers detail</th>
<th>Amplicon size (bp)</th>
<th>Cycling program</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ureaseC</td>
<td>F 5’CGTTTGCGATGGAATGACAGTAAG 3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R 5’GCAAATTTGAATGACTTTTGCGACC 3’</td>
<td>263</td>
<td>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</td>
<td>AL-Imam and AL-Rubaii (2016)</td>
</tr>
</tbody>
</table>
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 Limanskii 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.

REFERENCES


