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Detection of *Sphingomonas paucimobilis* and antibacterial activity of *Prosopis farcta* extracts on it

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Abstract

Six isolates of *Sphingomonas paucimobilis* were isolated from 120 hospital workers hands in Erbil city/Iraq by using VITEK2 Compact system, then further confirmed by PCR technique and by detecting specific gene TDP-glucose pyrophosphorylase (320bp) for *S. paucimobilis* ATCC 31461 and all local isolates. The effect of 17 antibiotics against isolated bacteria was studied and the results showed that all isolates were 100% sensitive to Piperacillin/Tazobactam, Imipenem, Meropenem, Ciprofloxacin, and Levofloxacin, while it was 83.33% resistant to Cefoxitin and Cefepime. The minimum inhibitory concentration (MIC) of *Prosopis farcta* pods extracts against *S. paucimobilis* isolate (S.p4) was 1000 μg/ml for methanol and ethanol extracts and 1200 μg/ml of watery extract. Moreover, the inhibitory effect of the above plant extracts (sub MIC) was studied against bacterial plasmid and the results showed three plasmid DNA bands when treated with 1000 μg/ml for watery extract and missing one band after treating with 800 μg/ml for both methanol and ethanol extracts. Also, the results of inhibitory effects of mentioned plant extracts on total protein by using SDS-PAGE showed the differences in protein banding pattern which 32 bands was formed when treated with 1000 μg/ml for watery extract and it was decreased to 16 bands when treated with 800 μg/ml for both methanol and ethanol extract.

Keywords: Antibacterial plant activity; Plasmid profile; Protein; *Sphingomonas paucimobilis*; TDP-Glucose pyrophosphorylase gene

1. Introduction

The species *Sphingomonas paucimobilis* within the genus Sphingomonas which belong to family *Sphingomonadaceae*. This bacteria was first discovered as an agent in humans in 1977 and named *Pseudomonas paucimobilis*. While, in 1990 it was renamed as *S. paucimobilis* in accordance with phylogenetic data [1]. It is a gram-negative, strictly aerobic, slightly motile, non-fermentative, oxidase positive opportunistic pathogen that rarely causes infections in humans, and forms yellow-pigmented colony in blood agar [2]. *S. paucimobilis* occurs widely in both natural and nosocomial environments, including several clinical specimens, hospital water supplies, temperature probe respirators, stocked distilled water, blood, removes, hospital dialysis equipment, patients with meningitis, septicemia, bacteremia, peritonitis, wound infections, respiratory therapy equipment, and laboratory instruments. It is also can cause nosocomial and community-acquired infection [3–5]. Indeed, it has been implicated as a

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causative agent of infections in immunocompromised patients and healthcare-associated infections [6]. The bacteria Sphingomonas produce extracellular biopolymers including certain gellan-related exopolysaccharides and the species *S. paucimobilis* elaborate a gellan-related polysaccharide that forms a gel if deacetylated [7,8]. The exopolysaccharides (EPS) gellan is synthesizes a gelling agent with applications in the food, pharmaceutical, and other industries, the mentioned EPS is composed of a repeating linear tetrasaccharide unit consisting of D-glucose, D-glucuronic acid and L-rhamnose in a 2:1:1 ratio, respectively, with glycerate and acetate substituents [9,10].

The major problem in medical microbiology science is increasing development of drug resistance in pathogenic bacteria as well as the appearance of an desirable side effects which more dangerous than the disease itself and the search ongoing for new antibacterial compounds with improved activity to replace the antibiotics [11,12]. Moreover, many sttudies found that different plant species have been used for treating many bacterial diseases in different countries [13–15]. The *Prosopis farcta* (Dwarf mesquite) is a very popular medical plant, belong to family Leguminosea which is a little prickly spiny shrub and this plant is native of United States, Africa, Asia (Turkey, Iran and Iraq) and has some medicinal properties such as anti-inflammatory effects, treating gastric ulcers, fetus abortion, dysentery, arthritis, larynx inflammation, heart pains, and asthma [16–18]. Also it used to treat diabetes [13], it is antitumour activity and antioxidant capacity [19,20] antiparasitic and antimicrobial activity [21], increase high-density lipoprotein cholesterol, decrease low-density lipoprotein cholesterol and hepatoprotective potential [22,23].

2. Material and method

Six isolates of *S. paucimobilis* were isolated from 120 worker hands in different hospitals in Erbil city, Iraq. All specimens were examined microbiologically and the subcultures that yielded a pure growth of yellow colonies of non-spore-forming, oxidase positive, Gram-negative, rod-shaped bacteria were identified by the automated VITEK 2 Compact system (Biomerieux, France) and further confirmed by PCR. Also, the antimicrobial susceptibility of the isolates was studied by the same above system.

2.1. Preparation of plant extracts

Pods and seeds of *P. farcta* were obtained from the local market in Erbil city. It was identified and classified [24] in the Education Salahaddin University Herbarium (ESUH), Erbil-Iraq. The samples were washed, dried, grinded, and the plant extracts were prepared by soaking 250 g of plant powder in 1.5 L of each of water, ethanol, and methanol separately for 5 days at room temperature. After that, each extract was filtered by using Whatman filter paper No. 1, then each of the filtrate extracts was dried by using a rotary evaporator at 60 C°. The dried extract was stored at 4 °C until use.

2.2. Antibacterial activity P. farcta pods extract against isolated bacteria by using MIC

The antibacterial activity of aqueous, ethanol and methanol extracts of *P. farcta* pods against the most resistant isolate of *S. paucimobilis* was studied by using MIC containing different concentration of the *P. Farcta* extracts (100, 200, 400, 600, 800, 1000 and 1200)µg/ml separately for each extract [25].

2.3. Detection of S. paucimobilis local isolates on molecular level

All the molecular techniques used in this study depended on Sambrook and Russell [26]. Plasmid DNA was extracted and purified from the 5 ml overnight culture of the selected isolates of the *S. paucimobilis* grown in LB broth medium containing 100 µg/ml Ampicillin using a plasmid DNA purification kit, according to the manufacturer's instructions (Qiagen, Germany). For DNA extraction, G-spin™ Genomic DNA extraction kit was used. The kit utilizes silica-based membrane technology in the form of a convenient spin column. The genomic DNA purified and DNA was extracted from isolates and used for detection of isolated bacteria by using PCR technique as mentioned by (INTRON Biotechnology, South Korea) and the purified DNA immediately stored at −20 °C. For DNA amplification, the sequence of the TDP-glucose pyrophosphorylase gene (GenBank: AY247402.1) was obtained from the National Centre for Biotechnology Information (NCBI) database. Specific primers were designed generate fragments about 320 bp (F- 5′-3′ TAT CCC CTG TCC GTG CTG AT, R- 5′-3′ GGA TCG TCG ACA TGG TAG GC). PCR was performed with DreamTaq Green PCR master mix (Fermentas, Germany) according to the manufacturer's instructions. The 50 µl reaction mixtures were composed of 25 µl master mix, 2 µl (100 ng) DNA templates, 1 µl for each forward and reverse specific primer (10 pmol), and the volume was completed by adding 21 µl of nuclease free water. The PCR cycling

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