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ORIGINAL ARTICLE

Diversity of antagonistic bacteria isolated from medicinal plant *Peganum harmala* L.

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Abstract The antimicrobial activity of plant extract of *Peganum harmala*, a medicinal plant has been studied already. However, knowledge about bacterial diversity associated with different parts of host plant antagonistic to different human pathogenic bacteria is limited. In this study, bacteria were isolated from root, leaf and fruit of plant. Among 188 bacterial isolates isolated from different parts of the plant only 24 were found to be active against different pathogenic bacteria i.e. *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecium*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. These active bacterial isolates were identified on the basis of 16S rRNA gene analysis. Total population of bacteria isolated from plant was high in root, following leaf and fruit. Antagonistic bacteria were also more abundant in root as compared to leaf and fruit. Two isolates (EA5 and EA18) exhibited antagonistic activity against most of the targeted pathogenic bacteria mentioned above. Some isolates showed strong inhibition for one targeted pathogenic bacterium while weak or no inhibition for others. Most of the antagonistic isolates were active against MRSA, following *E. faecium*, *P. aeruginosa*, *E. coli* and *E. faecalis*. Taken together, our results show that medicinal plants are good source of antagonistic bacteria having inhibitory effect against clinical bacterial pathogens.

1. Introduction

Medicinal plants are potential source of natural products that play an important role in preventing different human diseases. According to a survey of World Health Organization (WHO), 70–80% of the world population especially from developing countries rely on natural products of medicinal plants for their health care (Akerele, 1993). These natural products are either produced by plants or their associated microbes. Several previous studies have reported the beneficial effects of plant associated microbes. These microbes generally bacteria, are present in the phyllosphere, rhizosphere or reside inside the plant in a mutualistic relationship (Strobel et al., 2004). In this mutualism, bacteria play an important role in plant growth promotion by increasing nutrients uptake and mineral solubilization. Furthermore, this interaction helps in protecting host plant against different pathogens (El-Deeb et al., 2013).

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In recent years, bioactive metabolites from medicinal plants have gained global attention. Bioactive metabolites are produced by medicinal plant or associated microbes. These bioactive metabolites are involved in symbiotic association with the host plant (Strobel, 2003). Bacteria produce bioactive metabolites exhibiting activities against phytopathogens as well as against bacteria, fungi, viruses, protozoans affecting humans and animals (Strobel et al., 2004).

The Kingdom of Saudi Arabia is so wide having more diverse flora as variation in climate and height of different area. Different medicinal plants and their extract have antibacterial, antifungal, anti-inflammatory activity and are used here by local people in kingdom in traditional medicine especially in remote area (Abulafatih, 1987; Al-Said, 1993; Ageel et al., 1986; Bokhari, 2009; Saadabi, 2006). Hermal (*Peganum harmala* L.) is a medicinal plant in the kingdom planta used in folk medicines due to insecticidal activity (Rharrabe et al., 2007), inhibition of reproduction (Nath et al., 1993; Adday, 1994), antimicrobial activity, and in cure of different diseases such as gastrointestinal, hypertension, cardiac, nervous system disorders, diabetes (Moloudizargari et al., 2013). The plant extract also shows *in vivo* and *in vitro* cytotoxicity in cancer cell line, rats and mouse model (Lamchouri et al., 2013).

In some previous studies in the kingdom planta many medicinal plants were used for antimicrobial studies (Alamri and Moustafa, 2012) but little is known about the distribution and isolation of bacteria from medicinal plants (El-Deeb et al., 2013). Therefore, the present study was designed to isolate and screen bacteria from *P. harmala* against human pathogenic bacteria. Furthermore, these potential bacteria were identified using 16S rRNA gene and phylogenetic analysis was performed.

2. Materials and methods

2.1. Plant collection and isolation of bacteria

P. harmala samples were collected in March 2014, from Taif region, Saudi Arabia. After collection, plant specimens were placed in a sterile bag and transferred to laboratory within 24 h for bacterial isolation. These plant samples were washed with sterile distilled water to remove soil and root, leaf and fruit of plant were separated. Bacteria were isolated from each part after cutting 1.0 g of tissue from each part mentioned above. These plant tissue samples were ground using sterile mortar and pestle. This homogenate was used to make serial dilutions using autoclaved distilled water and 0.1 ml aliquots were plated out on two different isolation media, 1/2 Tryptic soy agar and 1/2 R2A agar (HIMEDIA) supplemented with amphotericin B 25 μg/ml to inhibit fungal growth. These plates were then incubated at 28 °C for 1–2 weeks. Bacterial colonies were selected based on morphological features such as size, color and appearance. For pure culture, colonies were re-streaked and stocks were maintained in 15% (v/v) glycerol solution and stored at −80 °C for further use.

2.2. Screening of bacteria for antibacterial potential

Bacteria isolated from different parts of plants were screened for antibacterial activity using deferred antagonistic assay. In brief bacterial isolates were grown at 28 °C and 24 h grown culture of bacterial isolates was then overlaid with 0.1% soft agar mixed with test strains. All test strains were diluted to final concentration A_{600} = 0.1. Plates were again incubated at 28 °C for 24 h and the zone of inhibition was documented. The test strains of bacteria (*Escherichia coli* ATCC 8739, MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 27270 and *Pseudomonas aeruginosa* ATCC 27853) were pregrown in LB broth at 37 °C.

2.3. DNA extraction and PCR analysis

The selected strains were subjected to extraction of genomic DNA for 16S rRNA gene analysis for identification of antagonistic bacterial strains. Genomic DNA of selected bacteria was extracted using commercial genomic DNA extraction kit (Thermo Scientific, Waltham, USA). The 16S rRNA gene was amplified from the extracted DNA using bacterial universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Amplifications were performed with the following thermal cycle: one cycle of 95 °C for 5 min followed by 30 cycles of 94 °C for 1 min, an annealing of 58 °C for 50 s and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. The PCR products were separated by agarose gel electrophoresis and purified using PCR purification kit (Thermo Scientific, Waltham, USA) according to the manufacturer's instructions and were sequenced by Macrogen (Seoul, Korea).

2.4. Phylogenetic analysis of antagonistic bacteria

For taxonomic identification of the antagonistic bacteria, the 16S rRNA gene sequences of all isolates were compared with sequences of matched type strains obtained from National Centre for Biotechnology Information (NCBI) and using the EzTaxon database (http://www.eztaxon.org/; Chun et al., 2007). The closest type species match was recorded along with the percent sequence similarity (Table 1). Multiple alignments were performed by using the CLUSTAL_X program (Thompson et al., 1997) and gaps were edited by using Bio Edit (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree were constructed by using a neighbor-joining method (Saitou and Nei, 1987) in the MEGA4 Program (Tamura et al., 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985).

2.5. Nucleotide sequence numbers

The nucleotide sequences obtained for 24 bacterial strains in this study have been deposited in the GenBank database under accession numbers KR812389 to KR812412.

3. Results

3.1. Isolation of antagonistic bacteria from plant

A total of 188 bacteria forming morphologically different colonies were isolated from roots, leaf and fruit of plant. All the isolated strains from different parts of the plant mentioned above were cultured on 1/2 TSA and 1/2 R2A. Bacterial count

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