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Case Report

First Report of *Arthrobacter agilis* Associated with Elm Trees in Iran

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Abstract

Elms (*Ulmusspp.*) are popular species of deciduous trees belonging to the family Ulmaceae. Elms are ornamental trees commonly used in cities and forests. During late June 2015, isolation of bacterial associated was performed for the first time on elm trees, in the Tabriz (East Azarbaijan Province, Iran). Based on morphological, biochemical and molecular characteristics, the isolates were identified as *Arthrobacter agilis*. This study provides the first report on the occurrence of this bacterium on elm trees in Iran.

Keywords: Ulmaceae; Elm trees; *Arthrobacter agilis*; Tabriz

Case Presentation

Elms are dicots that taxonomically belonging to genus *Ulmus*, family Ulmaceae, order Rosales and kingdom Plantae. These first appeared in the Miocene geological period about 20 million years ago, originating in what is now central Asia and spread all over the Northern hemisphere over time [1]. There are not precise information about all elms in whole of the world, but estimated that there are 136 million elms whit more than 10 cm diameter that 95 percent of those are belong to Europe, Asia and North America [2]. Iran, also, is one of the countries that some species of elms outspread in it, but the precise map of number and dissemination is not available yet [3]. Some special abilities, including tolerance to salt, wind and place switching, partly fast growing in any kind of soils, wealthy timber, tolerance against physical pressure and soil Compaction, and besides, considerably beauty and elegance because of high height and special form of leaves and branches cause these trees used all over the Europe, North America and other areas of world, day by day [4]. Elms are ornamental trees commonly used in cities and forests of Northwest of Iran.

The tissues of 14 elm trees were collected during 2015-16 from different areas of Tabriz city. To isolate the bacteria from branches and trunks, small sections (10 to 15 mm square) were excised from the margins and centers of these parts. These sections of tissues were sterilized in 70% alcohol for 30 seconds and were rinsed three times in sterilized distilled water for 30 seconds. The tissue pieces were cut, allowing to release bacteria into sterilized distilled water for 1 hour, then 1 ml of the diluted bacterial cell suspension streaked onto Nutrient Agar (NA) medium. The inoculated plates were incubated at 28°C for 72 hours. Fifty five bacterial strains were obtained and characterized from this work. Observations and continuation the research were made for development of well separated red, coccoid, small bacterial colonies on the nutrient agar medium that they were nine strains.

Hypersensitive reaction and pathogenicity tests were carried out tobacco (*Nicotianatabacum*) and elm young shoots, respectively. Scratched parts were inoculated with 1 loop full of 48-h old bacterial culture on nutrient agar medium. Parts injected with bacteria strains covered by parafilm. All treatments were conducted with two branches per bacterial isolate. Controls were inoculated with sterile water without bacterial colonies. The inoculated young shoots subsequently incubated at 24°C for 50 days in the humidity chamber. All of the nine selected strains were used for pathogenicity test.

Biochemical tests, such as gram reaction, catalase, oxidase, urease, lipase, gelativase, aerobic and anaerobic, as described by Schaad et al. (2001) were utilized to characterize the bacterial isolates. The amplification of 16S rDNA was performed with a final volume of 37µl containing 2.5µl of total DNA, 1.5µl of the 27F primer (5′-TCCGTAGGTGAACCTGCGG-3′), 1.5µl of the 1492R primer (5′ TCTCCGCTTATTGATATGC-3′), 12.9µl of distilled water, 18.6µl of master mix [5]. Master mix involve three components that were dNTPs, $MgCl_2$ and Tag DNA Polymerase. The conditions of PCR were as follows: 95°C for 5 min, followed by 36 cycles of denaturation at 94°C for 1min, annealing at 60°C for 1 min and extension at 72°C for 1 min, then an extra extension at 72°C for 10 min. The identification of these isolates was carried out using BLAST (Basic Local Alignment Search Tool) in NCBI, so that the most similarity of experimental sequences verified with the reference sequences in the databases. Since these nine strains were similar in Phenotypic characteristics, one strain (M7) selected for DNA sequencing.

Bacterial colonies, which were isolated from tissues, were red and coccoid-shaped on NA medium. Several isolates of purified colonies were selected for streaking on Nutrient Agar (NA) slants and stored at 4°C in refrigerator for future use. The phenotypic characteristics of the all isolates were similar to those previously described by Koch et al. [6].

All strains were Gram-positive, oxidase-positive, catalase-positive, urease-negative, gelativaze-positive and lipasse-negative. These bacterial strains weren't able to produce Hypersensitive Reaction (HR) signs on tobacco leaves. After 50 days of incubation in the humidity chamber, none the young shoots inoculated with bacterial strains have pathogenicity symptoms. Control young shoots remained symptomless, too. Thereby, hypersensitive reaction and pathogenicity test were negative ontobacco and young shoots in all the isolates, respectively (Table 1).

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Table 1: Phenotypic characteristics of M7 (*Arthrobacter agilis*) compared with *Arthrobacterflavus* +, Positive and - , Negative.

Characteristic	M7	*Arthrobacter agilis*	*A. flavus*
Colony colour	Red	Red	Yellow
Form	Coccoid	Coccoid	Rod-coccus
Gram reaction	Positive	Positive	Positive
Catalase	+	+	+
Oxidase	+	+	-
Urease	-	-	-
Lipase	-	-	+
Gelativase	+	+	+
Aerobic	-	-	+
Anaerobic	-	-	-

Some data from Reddy et al. (2000).

Results from 16S rDNA gene sequence analysis and biochemical tests indicated that all the nine bacterial strains belong to *Arthrobacter agilis* species and the sequence from this bacterium (M7 strain) has been submitted to GenBank (Accession number: KY012350). The experiments confirmed the presence of *A.agilisas* associated bacterium for the branches and trunkes of elm trees.

In the 1995, reclassification of *Micrococcus agilis* (Ali-Cohen 1889) was carried out to the genus *Arthrobacter* as *Arthrobacter agilis* comb. nov. [6]. *A. Agilis* is a psychrotropic bacterium that isolated from Antarctic sea ice [7]. Furthermore, *A.agilis* has been founded as an ice-related bacteria and isolated from water [8]. *A.agilis* promote the growth of *Medicagotruncatula*, *M. sativa* and *Pinusdevoniana* plants [9-12].

Conclusion

This result is the first record of *A.agilis* as associated bacterial with elm trees in Iran. Since the elms are the most important trees in Northwest of Iran, this study is considered as a basis for works that will describe the *A.agilis* of life cycles and will diagnose other associated bacteria to applicate the endophytic bacteria for development and growth in trees. The molecular method is a very useful tool for the rapid detection of *A.agilis* in elms. Further studies will be needed to establish if there is a connection between these trees and the presence of *A.agilis*. Also, more studies are needed to know if there are any genetic variations among the strains.

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