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**Thesis submitted for the degree of Ph.D. in Plant Pathology**

**Genotypic and phenotypic identification of *Azospirillum* species of  
wheat and corn fields and evaluation of effects of dominant species  
on wheat and corn yield and *Fusarium graminearum* control in  
Isfahan province**

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**June 2020**

## Abstract

*Azospirillum* bacteria are one of the most plant growth promoting rhizobacteria. They exist in the rhizosphere of many crop plants. In the research, some soil and plant roots were collected from wheat and maize rhizosphere of the plant fields in Isfahan. The samples were cultured in the tubes containing Nitrogen Free Bromothymol (NFB) semisolid media. To isolate *Azospirillum* strains, after the bacterial growth, the bacterial pellicles were streaked on NFB and Rojo Congo (RC) specific and semi-specific media to the bacterium. The bacteria were isolated based on morphological characteristics on the media and then were purified. Detection and identification of *Azospirillum* isolates was done using molecular tests by Az16S-A and Azo494-F/Azo756-R specific primers, 16S rRNA gene sequencing and standard bacteriology phenotypic tests. In PCR reaction, 646 and 263 bp fragments were amplified by Az16S-A and Azo494-F/Azo756-R primers, respectively, that confirmed the presence of *Azospirillum* bacteria. The results of the phenotypic tests based on Unweighted Pair-Group method using Arithmetic Average (UPGMA) and Jaccard's similarity coefficient (J) using NTSYS software, 2.01, classified *Azospirillum* isolates with 51% similarity in two main groups I and II. The sequencing of the 16S rRNA gene in some isolates determined at least three species of *A. brasilense*, *A. lipoferum* and *A. zeae* in rhizosphere of wheat and maize plants. The *Azospirillum* plant growth promoting characteristics (PGP) were determined by investigating the presence of *NifH* and *NifD* genes of nitrogenase. Examination of the properties of phosphate solubilization and production of chitinase, HCN, siderophores and indole acetic acid were also performed using specific tests. The amplification of 360 and 390 bp fragments by FdB260/FdB261 primers, respectively, confirmed the presence of *NifH* and *NifD* genes. Specific tests also revealed the ability of indole acetic acid production, insoluble phosphate solubilization with average value of 15 and 18.8 mg/l, respectively, and siderophore production in the isolates, and showed that some of the isolates possessed several PGP properties, while some of the isolates had one or no characteristic. To investigate the effect of *Azospirillum* isolates on the performance of wheat and maize plants, seeds of the Back Cross Roshan and Pishgam varieties of wheat, and Single Cross 704 and SC1263 varieties of maize were disinfected and treated with the bacterial isolates. The seeds were planted and after 35 days, some growth characteristics of plants were measured. *Azospirillum* Az16 was determined as the most effective growth promoting isolate. In addition, the effect of plant growth promoting *Azospirillum* isolates on *Fusarium graminearum* was also studied after inoculation of pots with pathogenic fungi. The disease severity was 63% lower in the pots treated with *Azospirillum* Az16 compared to the control plants. The genomic profilings of different *Azospirillum* isolates produced unique band patterns and showed high genetic diversity between the isolates. According to the study, the *Azospirillum* isolates of wheat and maize fields of Isfahan province have different species and genetic diversity. The band patterns amplified using rep-PCR can be somewhat useful for classification of *Azospirillum* species.

**Key words:** *Azospirillum*, Plant growth promoting, sequencing, rep-PCR, Genetic diversity