

## Correlation between nitrogen fixation rate and alginate productivity of an indigenous *Azotobacter vinelandii* from Iran

Nosrati R<sup>1,4</sup>, Owlia P<sup>1,2\*</sup>, Sadari H<sup>1,2</sup>, Olamaee M<sup>3</sup>, Rasooli I<sup>1,4</sup>, Akhavian Tehrani A<sup>5</sup>

<sup>1</sup>Molecular Microbiology Research Center (MMRC), Shahed University, Tehran, I.R. Iran. <sup>2</sup>Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, I.R. Iran. <sup>3</sup>Department of Soil Science, Faculty of Water and Soil, Gorgan University, Gorgan, I.R. Iran. <sup>4</sup>Department of Biology, Faculty of Science, Shahed University, Tehran, I.R. Iran. <sup>5</sup>Department of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, I.R. Iran.

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### ABSTRACT

**Background and Objectives:** *Azotobacter vinelandii*, a gamma-proteobacterium, is an obligate aerobic free-living gram-negative soil bacterium capable of fixing nitrogen. Oxygen transfer rate into the cell is reduced by the increase of alginate concentrations during the course of *A. vinelandii* cultivation. This phenomenon provides a low intracellular oxygen concentration needed for nitrogenase activity. The aim of this study was to design a simple strategy to explain the alginate production, cell growth and nitrogenase activity correlation in *A. vinelandii* under aerobic conditions.

**Materials and Methods:** Thirty-five different soil samples were taken from the rhizosphere of agricultural crops of Iran. Enrichment and isolation strategies were employed for microbial isolation. Physiological and biochemical characteristics were determined. Molecular identification was performed using selective *nifH-gJ* primers. Alginate production and nitrogenase activity assay by each isolate of *Azotobacter* were carried out. Bacterial growth, alginate production and Nitrogenase activity were conducted by time-coursed quantitative measurements.

**Results:** Total of 26 isolates were selected after enrichment, isolation, and screening. The isolate was identified by molecular tests as *A. vinelandii*. The highest alginate productions of 1.02 g/l and 0.91g/l were noted after 4 days in 8 isolates, cell biomass of which were estimated 4.88-5.26 g/l. Six of 8 isolates were able to fix atmospheric N<sub>2</sub> on nitrogen-free medium. Rates obtained in isolates were in the range of 12.1 to 326.4 nmol C<sub>2</sub>H<sub>4</sub> h<sup>-1</sup> vial<sup>-1</sup>.

**Conclusions:** Nitrogen fixation and alginate production yielded significant and positive Pearson's correlation coefficient of R<sup>2</sup> = 0.760, p ~ 0.02. Finally association between bacterial growth, alginate production and nitrogenase activity almost noticeable yielded significant and positive Pearson's correlation coefficient R<sup>2</sup> = 0.723, p ~ 0.04.

**Keywords:** Alginate, *Azotobacter vinelandii*, Nitrogenase, Nitrogen fixation

### INTRODUCTION

*Azotobacter vinelandii* is a gamma-proteobacterium belonging to the family *Pseudomonadaceae*. It is

an obligate aerobic free-living gram-negative soil bacterium capable of fixing nitrogen directly from the atmosphere that helps the plants for better grain production (1). The nitrogenase enzyme complex that catalyzes dinitrogen reduction to ammonium is composed of two highly conserved proteins: the iron (Fe) protein (encoded by the *nifH* gene) and the molybdenum iron (MoFe) protein (encoded by the *nifDK* genes) (2). Evolutionarily conserved amino acid sequences within the *nifH* gene have been oppressed to design PCR primers to detect the genetic potential for

\* Corresponding author: Prof. Parviz Owlia  
Address: Molecular Microbiology Research Center, Shahed University, P.O. Box 14155-7435, Tehran, I.R. Iran.  
Tel: +98-21-88964792  
Fax: +98-21-88966310  
E-mail: owlia@yahoo.com.