Standard Strategy for Comparison of the Nitrogen Fixation Rate in Different Samples

Rahim Nosrati[1]; Parviz Owlia*[1]; Horieh Saderi[2]

1-Molecular Microbiology Research Center, Shahed University, Tehran, Iran

2-Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran

Rahim_nosrati@yahoo.com

Background & Objectives: Azotobacter, a gamma-proteobacterium, is an obligate aerobic free-living gram-negative soil bacterium capable of fixing nitrogen. There are several Methods for quantitative assay of nitrogen fixation. The discovery that the nitrogenase enzyme responsible for N2-fixation also reduced C2H2 to C2H4 provided the Acetylene Reduction Assay (ARA) for the quantification of the N2-fixation process. ARA is simpler and faster than the other Methods and it provides a highly sensitive and inexpensive way to quantify nitrogenase activity in N2-fixing samples. This study aims at designing a standard strategy for comparison of the rates of N2 fixation in different samples.

Methods: Five ml of the Azotobacter broth medium in 12 ml vials was inoculated with ~104CFU/ml of each isolate and incubated for 48-96 h at 28 ºC. Once visible growth was observed, each vial was sealed with rubber stopper. By means of a disposable plastic syringe, 10% of air from the head space (7 ml) was removed and an equal amount of acetylene was injected into vials. Gas samples (0.7 ml) were removed after 24 h incubation, and were assayed for ethylene production with a gas chromatograph in triplicate. The rate of nitrogen fixation was calculated and values were obtained nmoles C2H4 h−1 vial−1.

Results: Among 8 isolates, only six were found to be able to fix atmospheric N2. Amounts of acetylene reduced by isolates in samples were quite different. Rates obtained in isolates were in the range of 12.1 to 326.4 nmol C2H4 h−1 vial−1 while cell numbers were adjusted to 107 CFU/ ml.

Conclusion: Due to probable association between bacterial cell numbers and nitrogenase activity, we should estimate cell numbers of samples or final concentration of bacterial suspension adjusted according toCFU/ ml during the course of ARA simultaneously.

Keywords: Azotobacter; Nitrogenase; Nitrogen Fixation; Acetylene Reduction Assay