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"Research Note"

A NEW REPORT OF N FIXATION BY TWO SPECIES OF CYANOBACTERIA*

A. AHMADI MOGHADAM** AND B. NOWRUZI

Department of Biology, Faculty of Sciences, Shahid Bahonar University, Kerman, I. R. of Iran
Emails: ahmadimoghadam@yahoo.com & bahare77biol@yahoo.com

Abstract – Cyanobacteria were collected and identified in five paddy fields with different soil textures of Golestan province in Iran. It was determined that *Nostoc ellipsosporum* and *Nostoc muscorum* were dominant in all sites among 20 species. To assess the ability of atmospheric nitrogen fixation by the species, their pure cultures were provided by growing on BG-11 solid culture medium. Subsequently, each species was grown on Allen liquid culture medium for 21 days and the growth curves were obtained by measuring thallus chlorophyll amount at 1.5 day intervals. The amounts of N fixed by each species were measured by the Indophenol method and by measuring ethylene amounts produced via the acetylene reduction process using gas chromatography. The results showed significantly higher rates in N fixation, cellular doubling time and growth rate in *N.ellipsosporum* with larger heterocysts than *N.muscorum*. Therefore, *N.ellipsosporum* with a higher ability of N and ethylene production is suggested as the superior biological fertilizer.

Keywords – Heterocystous cyanobacteria, N fixation, acetylene reduction

1. INTRODUCTION

The role of heterocystous cyanobacteria in N fixation in paddy fields has been appreciated for a long time [1]. It has been indicated that these microorganisms can provide N into these ecosystems up to 60-90 Kg yr^{-1} [2, 3]. Regarding the diversity of cyanobacteria, especially in paddy fields, and the evaluation of the ability of each species to fix atmospheric N can lead to identification of the most proper species as biofertilizers [3-5]. Cyanobacteria were collected, purified, and identified from five paddy fields in Golestan province, Iran. *Nostoc ellipsosporum* and *Nostoc muscorum*, which were identified as the most common species of these ecosystems, were morphologically compared and their growth rate and the ability of atmospheric N fixation were measured during their logarithmic and stationary phases based on the assessment of acetylene conversion into ethylene, using gas chromatography.

2. MATERIALS AND METHODS

In 2003, samples of soils were collected from five paddy fields in Golestan province [6, 7] and were grown in BG-11 [8] liquid culture in a growth chamber at 28 C with 1500-2000 lux light intensity [8]. The species were identified according to the keys [9]. The results showed that *Nostoc muscorum* and *Nostoc ellipsosporum* were common in all sites among 23 identified species [7, 10]. Heterocysts of *N. ellipsosporum* were larger than *N. muscorum* [11]. To evaluate the N fixation abilities of these species, firstly the growth curves of the species were prepared, and species were inoculated in Allen liquid culture media [8] with pH 7.4. The chlorophyll concentrations and duplication times of two species were

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**Corresponding author

measured [12, 13]. In this study the ethylene amounts produced via the reduction of acetylene by the species were measured in logarithmic and stationary phases of their growth by using gas chromatography (Shimadzo 16 A). The measurements were carried out from day 7 to day 11 at 2 day intervals in three replicates of each species [14, 3, 4]. Ethylene amounts detected were measured by injecting 1 ml volumes of different concentrations of ethylene. In addition, to ensure the results obtained by gas chromatography, total N amounts in the biomasses of the species were measured in each species by the Indophenol method [15] at 4 day intervals during a 23 day period.

3. RESULTS

The results showed a delay phase in the *N.ellipsosporum* growth curve from day 1 to day 3, a logarithmic phase from day 3 to day 11, and a stationary phase from day 11 until day 19. The growth curve of *N.muscorum* was different and showed its delay phase from day 1 to day 5, logarithmic phase from day 5 to 13, and stationary phase from day 13 to day 19. According to the equations, doubling times were calculated 3.244 days for *N.muscorum* and 3.33 days for *N.ellipsosporum* [12]. Comparison analysis of the N amounts fixed in *N.muscorum* by the one-way ANOVA and Dunken test showed significant differences between the logarithmic and stationary phases of *N.moscorum*, while these amounts were different at all times in *N.ellipsosporum* except for days fifteen and nineteen (Figs. 1 and 2). In addition, total amounts of N were higher in *N.ellipsosporum* than *N.muscorum*, both in logarithmic and stationary phases. The results of the ANOVA and Dunken test of the amounts of ethylene produced in *N.muscorum* showed no significant difference between days nine and eleven (Figs. 3 and 4). Calculation of total areas under the curves showed higher amounts of ethylene produced in *N.ellipsosporum* than in *N.*muscorum (Figs. 5 and 6).

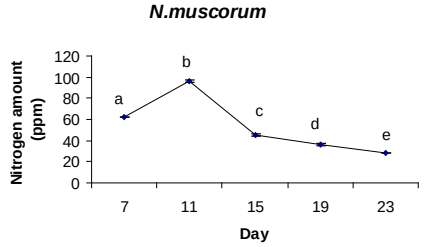

Fig. 1. Representing N amounts in *N.ellipsosporum* at logarithmic and stationary phases measured by Indophenol method. Each data is the mean of three replicates. Data with same letters are not significantly different at 95%

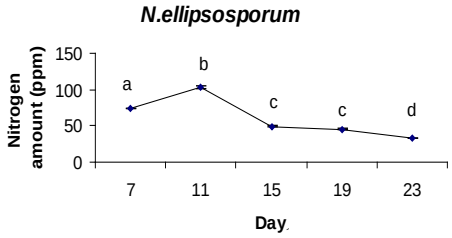

Fig. 2. Representing ethylene amounts derived from ethylene concentrations in *N.muscorum* in logarithmic phase of growth. Each data is the mean of three replicates. Data with same letters are not significantly different at 95%

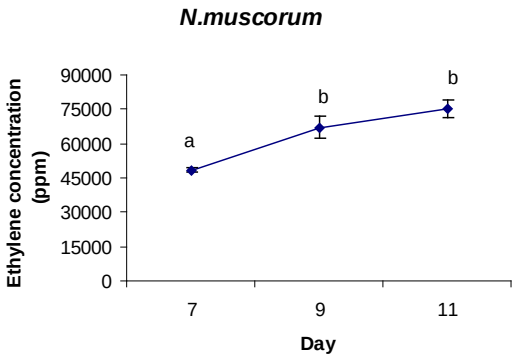

Fig. 3. Representing ethylene amounts derived from ethylene concentrations in *N.ellipsosporum* in logarithmic phase of growth. Each data is the mean of three replicates. Data with same letters are not significantly different at 95%

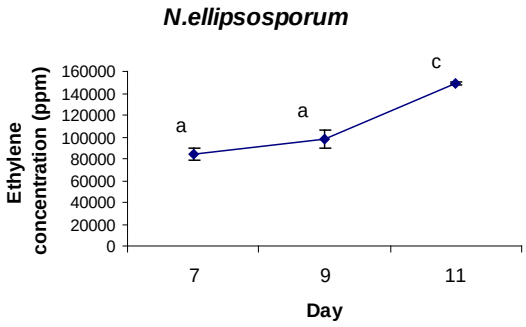

Fig. 4. Representing ethylene amounts derived from ethylene concentrations in *N.muscorum* in logarithmic phase of growth. Each data is the mean of three replicates. Data with same letters are not significantly different at 95%

Fig. 5. Representing the peaks of conversion rates of acetylene to ethylene in *N.muscorum* at days 7, 9 and 11. Data on the peaks are retention times [min] 2.4 and 11.2 for ethylene and acetylene respectively

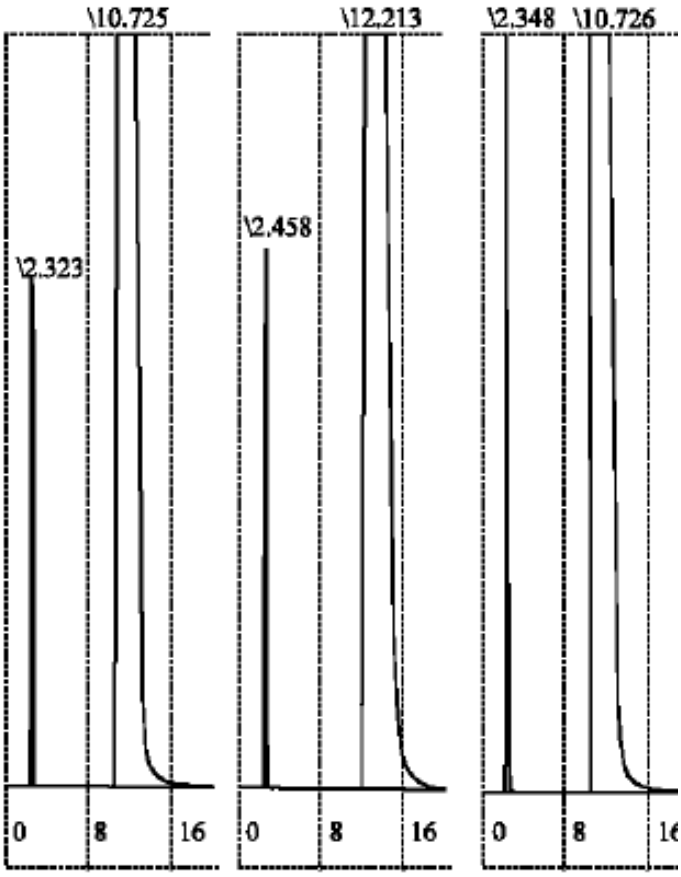

Fig. 6. Representing the peaks of conversion rates of acetylene to ethylene in *N.ellipsosporum* at days 7, 9 and 11. Data on the peaks are retention times [min] 2.4 and 11.2 for ethylene and acetylene respectively

4. DISCUSSIONS

Since the species were transferred from solid culture media into liquid culture media prior to the measurements, both species showed delay phases in the liquid culture media. Meanwhile, regarding their growth curves and duplication times it can be noted that *N.ellipsosporum* has a shorter delay phase during which necessary enzymes are provided, and a longer logarithmic phase and longer doubling time than *N.muscorum* [12]. The results of measuring total N in both species indicated an increase in nitrogen. It can be postulated that these features are probably due to the accumulation of toxic by-products and the scarcity of nutrients in the media. This condition caused the death of many cells that release some nutrients in the media, which can be the reason for the increase in cell counts at this stage [14, 1, 5]. Release of nitrogenous compounds in natural environments by cyanobacteria causes enrichment and improvments in soils. Hence, in the availability of cyanobacteria and the presence of other nutrients a favorable condition will be provided for wheat and rice plants. Higher N amounts and ethylene produced by *N.ellipsosporum* can be referred to its larger heterocysts. However, the relationship between the size of the heterocysts and the N fixation rate should be examined. The ability of *N.muscorum* and *N.ellipsosporum* employed in this research to fix different N amounts with different growth rates both give advantages for them to be applied as biofertilizers. *N.ellipsosporum* with a higher ability in the N fixation, and *N.muscorum* with a larger duplication time can each be applied to fields of different environmental conditions including different seasonal periods.

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