

The Potential of Cyanobacteria Growth in Different Water Sources

Muluneh Menamo¹ and Zinabu Wolde^{2*}¹South Agricultural Research institute, Hawassa P.O.BOX 05, Hawassa, Ethiopia²Ethiopian Sugar Corporation Research and Training, P.O.BOX 15, Wonji, Ethiopia

Abstract

Many studies have addressed the effect of different environmental factors on growth, heterocyst frequency and N fixation of cyanobacteria. One of the factors is water quality. Different cyanobacteria strains different efficiency to adapt the quality of water. Therefore, this study was conducted to select the best combination of N-fixing cyan bacterial strains under different water sources. A factorial combination of the three *Anabaena* species strains (E10, E3 and E8) and two water sources (tap water and distilled water) were laid out in a complete randomized design in the laboratory set up. The ANOVA revealed that cyanobacteria strain E3 performed best on distilled than in tap water in terms of optical density, heterocyst frequency and total nitrogen than the other strains but no significant differences appeared between the two water sources. Thus, indicated that cyanobacteria strains are growing in different water sources and the ability to grow more than one water sources.

Keywords: Cyanobacteria; Nitrogen fixation; Water quality

Introduction

Many studies have addressed the effect of different environmental factors on growth, heterocyst frequency and N fixation of cyanobacteria [1]. Nitrogen fixation depends on photosynthesis to provide ATP for energy and carbon compounds as electron donor [1]. Consequently the duration and the rate of N fixation depends on past and current conditions that influence cyanobacteria C balance, such as moisture, temperature, light intensity, water quality and supply of assimilates. Soil pH and its mineral nutrient status also influence the availability of N through biological N-fixation. With respect to biomass productivity of cyanobacteria in open pond production systems, the determining factors include temperature, CO₂ deficiencies, inefficient mixing and light limitation [2]

Noticed that higher concentration of NaCl (130Mm) inhibits nitrogenous activity by 50% or more in this regards, [3] attributed the reduced nitrogenous activity to the decreased heterocyst frequency with increase in salt concentration. However, amongst the cyanobacterial species there is a physiological salinity tolerance range that affects nitrogen fixation [4]. For this reason the study was conducted with the objective of to know the growth performances of different strains of cyanobacteria in different water sources (distilled and tap water).

Material and Methods

Description of the study

The study was carried out at the soil microbiology laboratory at College of Agriculture, Hawassa University, during 2012. Hawassa is located at latitude of 07°03'18.6"N and the longitude of 38°30'15.6"E with the elevation of 1620 masl. It has an average annual rainfall of 1046 mm and average maximum and minimum temperature of 13.3 and 27.5°C, respectively [5].

Source of Cyanobacteria strains and the lettuce seed

Anabaena spp. of Cyanobacterial strains E3, E10 and E8 were used for the study. The E3 strain was isolated from soil sample from pigeon pea field at Ziway while E10 and E8 were from soils at Hawassa (by the lake side) and at Yirgalem, respectively. These strains were isolated at Colorado State University and obtained from soil microbiology laboratory of Hawassa University College of agriculture.

Experimental details

The experiment was conducted in the laboratory to evaluate the performances of the three different cyanobacterial strains (E3, E8 and E10) on two water sources (distilled and tap water). These strains were evaluated in terms of their growth rate, optical density and Heterocyst frequency during growth in the lab set up (in Erlenmeyer flasks) for 21 days in laboratory. The total N content was analyzed after 21 days of growth.

Evaluation of the growth of cyanobacterial strains in two water sources

The three different strains of cyanobacteria were grown in distilled and tap water in soil microbiology laboratory at Hawassa University (HU), College of agriculture (CA) in a laboratory setup consisting of 18 Erlenmeyer flasks as shown in Figure 1. The three strains of cyanobacteria (E10, E8, E3) and the two water sources were arranged in a complete randomized design (CRD), with the factorial combination of two factors (water sources and the strains), and each strain was replicated three times in each of the water sources (Table 1).

The culture, Allen Arnon (AA) media was prepared [6]. Sample of each cyanobacteria strain was transferred from the stock to fresh medium made from double distilled water. Eighteen flasks containing the Allen Arnon (AA) media were prepared using the two water sources

Cyanobacteria strains	Water sources	
	Distilled water(D)	Tap water(T)
E3	E3D (T1)	E3T (T4)
E8	E8D (T2)	E8T (T5)
E10	E10D (T3)	E10T (T6)

Table 1: Treatment combinations for cyanobacteria stains x water sources study.

*Corresponding author: Zinabu Wolde, Ethiopian Sugar corporation Research and Training, P.O.BOX 15, Wonji, Ethiopia, E-mail: sos.zine04@gmail.com

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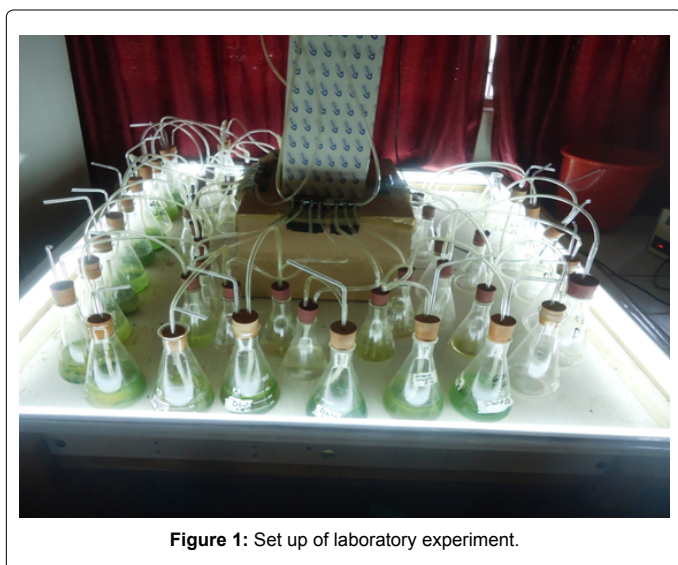


Figure 1: Set up of laboratory experiment.

each having a volume of 500ml. Each water sources was used to prepare media and each experimental unit (Erlenmeyer flasks) had a volume of 180ml from each media. Then equal amount of suspension of each strain was inoculated into each of the 180ml growing media in a 1:10 culture dilution [7]. Finally the volume of the culture was maintained to 200ml. Cultures were grown in the light box set-up prepared in the lab (Figure 1). Air was supplied for each strain for 6h day⁻¹ by using top fine air pump (model AIR-8000) and constant light intensity supplied by four fluorescent tubes of light for 12 h day⁻¹. Prior to setting this experiment, each strain was assessed under the microscope for purity or if there are predators.

The growth rate of cyanobacteria in each experimental unit was measured every day by taking absorbance (optical density) using sterile cuvettes after adjusting the spectrophotometer at 655 nm [8]. At the end of the first phase, after incubation for 21 days and analysis of results, the best performing cyanobacterial strain was selected for experiment II (Figure 1).

Data collection

Optical density (OD): Refers to biomass determination in terms of optical density at 655 nm at the end of the experiment by using the spectrophotometer (JENWAY MODEL 6300).

Heterocyst frequency: Determined by counting the number of heterocyst in a given filament and expressed by % frequency [9].

Specific growth rate: An increment in optical density during exponential phase was used to calculate the specific growth rate (μ) by the equation $\mu = (\ln x_2 - \ln x_1) / (t_2 - t_1)$ where x_1 and x_2 are the optical densities at time t_1 and t_2 [10].

Total nitrogen: After the end of the experiment (21 days) total nitrogen of each cultured strain was determined by using the kjeldahl procedure as described by [11].

Water analysis

Except pH and EC, water quality analyses were conducted at JIJE laboratory, Addis Ababa, Ethiopia, following the standard laboratory procedure, pH and EC were determined by using thermo scientific origin star five meter (USA), NO₃⁻ was measured by ultraviolet spectrometric screening methods, Na⁺ was measured by flame emission

photometric method B by azomethine-H method, CO₃⁻ and Cl⁻ were determined by titration and argentometric methods [11].

Data collected were subjected to Analysis of Variance (ANOVA) using statistical analysis software [12]. As appropriate to the design of the experiment. Mean separations were done using LSD at 5% level of probability

Result and Discussion

Water quality analysis Results of water quality analysis before culturing cyanobacteria indicated that the pH of the tap water was 6.8 while that of the distilled water was 7.2 (Table 2), and thus within the acceptable range for cyanobacteria growth as stated by [13]. However, the tap water is more saline than the distilled water. The salinity level of the tap water was 130 μscm^{-1} while the distilled water had 28 μscm^{-1} salinity. The lowest salinity level in distilled water could be due to the fact that the salinity levels decrease with the decreasing concentration of Na⁺ and Cl⁻ ions, which is relatively lower in the distilled water. Similarly, the amount of total dissolved solids (TDS) of tap water was higher than the distilled water. This could be due to higher cation and anion concentration in tap water. In addition, there was higher NO₃⁻ in the tap water than in the distilled water. These properties indicated that tap and distilled water had different quality, which may affect the growth and nitrogen fixation of cyanobacteria (Table 2).

The growth performance of cyanobacterial strains on the different water sources

Optical density (OD) and growth rate (OD DAY⁻¹): The results showed that there is statistically significant difference between the strains on optical density and growth rate ($P \leq 0.01$). However, OD and growth rate showed no statistical difference between the water sources and also there was no interaction between the two factors. The result revealed that the highest optical density was recorded from strain E3 (1.73 nm) and the lowest was recorded from E10 (1.46 nm). Similarly, the highest growth rate (0.94) was observed from E3 strain, which is statistically at par to E8. The OD of E8 and E10 were statistically at par, thus not different from each other. The OD of strain E3 is 18.4% over that of E10 strain.

The highest OD and growth rate observed from strain E3 could be attributed to its genetic efficiency and high nitrogen fixation capacity. In earlier work, [14] reported that strain E3 recorded higher OD and growth rate in river water compared to other *Anabaena* strains. Similarly, [15] reported that the same strain had high OD and growth rate in river water as compared to other strains investigated. The differences observed in growth of the strains could be attributed to the difference in their inherent physiological efficiency [4].

Though it is not statistically significant, relatively the highest OD

Parameters	Water sources	
	Tap	Distilled
pH	6.8	7.2
EC(μscm^{-1})	130	28
NO ₃ ⁻ N(mgL ⁻¹)	0.28	0.21
Na (mgL ⁻¹)	24	12.6
B (mgL ⁻¹)	6	0.78
Co ₃ ²⁻ (mgL ⁻¹)	0	0
Cl ⁻ (mgL ⁻¹)	15	2.4
TDS (mgL ⁻¹)	67	11

Table 2: Chemical properties of tap and distilled water.

growth parameters				
	Optical density at (655nm)	Growth rate (ODDAY ⁻¹)	Heterocyst frequency (H.F) (%)	Total N(mgl ⁻¹)
Strains				
E3	1.73 ^a	0.94 ^a	3.09 ^a	45.61 ^a
E8	1.52 ^b	0.74 ^a	2.09 ^b	13.08 ^b
E10	1.46 ^b	0.32 ^b	1.43 ^c	9.18 ^c
LSD 5%	0.14	0.27	0.107	0.627
water source				
Distilled water	1.62	0.70	2.24	22.6
Tap water	1.51	0.63	2.17	22.58
LSD (5%)	NS	NS	NS	NS
CV (%)	6.96	32.12	3.78	2.15

Means followed the same letters in the same column are not significantly different at $p \leq 0.05$ probability

Table 3: Growth performance of cyanobacteria on different water sources.

and growth rate 1.62 and 0.70 OD day⁻¹, respectively, were recorded from the distilled water. However, there were differences between the water sources in salt concentration, and the tap water had relatively higher salt concentration (Table 2). This has not significantly affected growth of the strains perhaps because it was in a tolerable level. Higher salt concentration has been reported to reduce specific growth rate of cyanobacteria strain by reducing carbohydrate production of the strains [9]. [1] Also claimed the susceptibility of N-fixing cyanobacteria to ionic stress and they indicated that *Anabaena* spp. L-31 reduced its growth by 50% within five days of exposure to higher salinity.

Heterocyst frequency (HF) and the total N: Heterocyst frequency and the total N content of *Anabaena* spp. showed significant differences ($P \leq 0.001$) between the cyanobacteria strains. The maximum heterocyst frequency and total N (3.09% and 45.61 mgL⁻¹, respectively) were recorded from strain E3 while the minimum heterocyst frequency and total N (1.43% and 9.18 mgL⁻¹, respectively) were recorded in E10 cyanobacteria strain (Table 3). However, no statistically significant difference was observed between the different water sources and there was no interaction effect on HF and total N between water sources and the cyanobacterial strains.

The highest heterocyst frequency and the highest total N recorded from E3 strain could probable be attributed to the fact that this strain had higher growth rate (optical density), a result in line with earlier report [14]. In support of the current result, [15] claimed that E3 produced highest heterocyst frequency and total N (3.2%, 46.41 mgL⁻¹, respectively).

The heterocyst frequency and the total nitrogen were positively correlated with the optical density ($r=0.65085^{***}$, 0.65085^{***} , respectively). Similarly, the heterocyst frequency was highly positively correlated with total N ($r=0.94472^{***}$) (Appendix table 1) (Table 3).

Summary and Conclusion

A laboratory study was conducted to identify the best N fixing cyanobacteria strain under two water sources. The experiment comprised six treatments made up of factorial combinations of two water sources and the three strains of *Anabaena* spp (E3, E10, and E8) laid out in complete randomized design (CRD) with three replication. The three strains of *Anabaena* spp (E3, E10 and E8) in the two water sources (distilled and tap water) were determined by measuring optical density, growth rate, total N and heterocyst frequency.

The results indicated significant difference in optical density,

growth rate, total N and heterocyst frequency between the three strains of *Anabaena* spp. The highest optical density (1.73), growth rate (0.94 OD DAY⁻¹), heterocyst frequency (3.09%) and total nitrogen (46.62 mgL⁻¹) were recorded from E3 strain.

Though not statistically different, highest mean was recorded on all parameters from distilled water than on tap water. Also the interaction between the two water sources and the strains of *Anabaena* spp were not significant. Hence strain E3 and tap water were selected for mass culturing of cyanobacteria in raceway.

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