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Isolation and Identification of Dominant N_2 Fixing Cyanobacterial Strains from Different Locations

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ABSTRACT

Isolation of cyanobacteria dominated in the soil samples collected from different sites in Kafr El- Sheikh, and El-Dkahlia governorates isolates were successfully obtained as bacterial free cyanobacteria. They were identified cyanobacteria was carried out using the following: thallus color, thallus morphology and size of heterocyst, vegetative and reproductive cells. Heterocyst-forming cyanobacteria were also cultured as *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., and *Chroococcus* sp. *Nostoc* sp . were isolated at high frequency; *Nostoc* sp. and *Anabaena* sp. isolates representing in kafr El-Sheikh and El-Dakahlia Governorates. Less frequency of cyanobacteria belonging to different genera namely *Oscillatoria* sp. and *Chroococcus* sp. *Nostoc* spp.were recorded highest amount of fixed nitrogen and mass production with in increasing incubation period of interacellular and /or extracellular nitrogenfixation. While,the most active strains for nitrogenase activity were *Nostoc mucorum* K and *Anabaena oryzae* D.

Keywords : Cyanobacteria , Anabaena , Nostoc , Oscillatoria , Chroococcus.

INTRODUCTION

Recently, great attention has been paid to the beneficial effects of cyanobacteria in paddy soils. The amount of fixed nitrogen by these blue- green algae could supply rice plants with their needs of nitrogen. In this respect, the release of growth promoting substances during algal growth must not be neglected.

It is well known that cyanobacteria are group of photosynthetic procaryotes which, can fix atmospheric nitrogen in submerged rice fields. Biological N_2 fixation is essential for cyanobacterial process and is dependent upon an adequate population. Caynobacteria (blue – green algae) are photosynthetic prokaryote.

Gram negative bacteria, diversity ranges from unicellular to multicellular , branched filamentous , slight to intense pigmentation , autotrophic to hetrotrophic , free living to symbiotic , psychrophilic to thermophilic acidophilic to alkylotrophic , planktonic to epiphytic . Some workers have emphasized the importance of phyto plaktons (Alam *et al* ., 1989) . Cyanobacterial strains which do possess the character of nitrogen fixation adds up to value addition of these microbial strains mainly in the field of culture , food , fertilizer (Muthukumar *et al* ., 2007).

The present work was carried out to understand the diversity , isolations and identification of cyanobacteria from different locations and determination of their growth rates .

MATERIALS AND METHODS

Culturing of cyanobacteria

The following methods were applied on air-dried soil samples collected from different sites in Kafr El-Sheikh and El-Dakahlia governorates (El-Gamal *et al*., 2008), BG11 medium (Staub, 1961) and modified Watanabe medium (El-Nawawy *et al*., 1958) for isolation and culturing of cyanobacteria. Semi-solid medium as described by El- Ayouty and Ayyad (1972) were applied. About 10 g of the soil sample were also placed in flasks containing 100 ml of the sterilized liquid medium. The Petri-dishes as well as flasks were incubated at 30°C under continuous light (2500 lux) . The properties of soil for isolation cyanobacteria were determined according to Piper (1950) and Jackson (1973) are presented in Table (1).

Table 1. Physical and chemical analyses of soils .

Charactaristics	Kafr El-Sheikh- Shakh	El-Dkahlia-El-Mansoura
	Clay loam soil	Sandy loam
Physical analysis		
Sand(%)	40.0	53.30
Silt (%)	30.0	23.80
Clay(%)	30.0	22.90
Chemicalanalysismeq-1		
Ca CO_3(%)	1.11	14.00
Ca++	6.5	13.11
Mg^{++}	4.0	5.86
Na^{+}	9.0	5.90
K^{+}	2.1	2.33
CO_3^{--}	0.0	0.00
HCO_3^{-}	4.5	17.69
Cl^{-}	9.0	5.60
SO_4^{--}	8.1	3.91
E.C.(dS/m)	2.4	2.76
pH	7.7	8.11

Isolation of cyanobacteria

The following techniques were applied for dry soil samples to isolate cyanobacteria.

1- Sterilized 7% agarized Z- medium and modified Watanabe medium was poured in Petri dishes (10 cm in diameter). Few grams of soil samples were spread in the from of strip (1cm in broad) in each Petri dish (El-Ayouty and Ayyad,1972) and the dishes were then incubated at 30ºC under continuous light of 120 cm long white fluorescent lamps light intensity of 2500 Lux.
2- Soil sample was placed in 250 ml Erlenmeyer flasks containing 100 ml of steriled liquid medium. Then left under the previously mentioned conditions of light and temperature.

Purification of cyanobacteria

The unialgal cultures were purified as described by Pringsheim (1949) at all cases any colored growth was picked up, sub cultured and streaked several times in new agarized Watanabe medium plate. The previous procedure was repeated many times to get unialgal cultures.

Bacteria free cyanobacterial cultures

Cyano bacteria free cultures, were obtained as described by Washing (Hoshaw and Rosowski, 1973) and tested for free cyanobacteria according to Mercuric chloride treatment (Gupta *et al*., 1959) .

Selection of the most efficient N_2-fixing cyanobacterial strains

A growth curve experiment was conducted for 14 isolates to compare their growth activities and their capacities for N_2-fixation, so as to choose the most efficient strains, which were further used throughout the present investigation. Cyanobacteria isolates were cultivated as usual for 28 days, to determine cyanobacteria dry weight, and the fixed nitrogen amount.

Identification of the isolated cyanobacteria

For characterization and identification of the purified cyanobacteria isolates, 500 ml Erlenmeyer flasks each containing 250 ml of Modified Watanabe liquid medium and also plates of agarized Modified Watanabe medium were inoculated with a loopfull of 10 days old culture of each cyanobacteria isolates. Inoculated flasks and plates were incubated at 28-30ºC under continuous illumination (2500 lux) for 10 days. The identification of cyanobacteria was carried out using the following: thallus color, thallus morphology , size of heterocyst, vegetative and reproductive cells. Heterocyst-forming cyanobacteria were also cultured in nitrogen-free Z-medium (Castenholz 2015).

Preparation of standard cyanobacterial inoculum

The inoculum of the cyanobacterial identified strains were prepared by inoculating 500 ml Erlenmeyer flasks each containing 200 ml of Modified Watanabe liquid medium with a loopfull of 21 days old culture of each cyanobacteria strains. Inoculated flasks were incubated at 28-30ºC under continuous illumination (2500 lux) for 21 days.

Maintenance of cyanobacterial cultures

Stock cultures were maintained in a refrigerator at 5ºC on agar slants of Modified Watanabe Medium (El-Nawawy *et al.,* 1958). Sub culturing was carried out every six month. The purity of the cultures was tested regularly by both microscopic examination and incubation on nutrient agar. The principle idea behind that, is that bacteria could grow on organic medium. Thus, they could be easily detected and contaminated cultures were discarded .

Total nitrogen

Total nitrogen in the cyanobacteria were determined using the micro-kjeldahl method according to Jackson (1973) . Results were expressed as mg nitrogen each 100 ml culture.

Nitrogenase activity

The capacity of cyanobacteria strains to fix nitrogen was assayed by acetylene reduction technique according to Hardy *et al* . (1973). Nitrogenase activity was then calculated by the following formula :

µmole C_2H_4 /ml culture=R x (container volume (test tube) /incubation time x Inj . Vol. x D x 22.4) x 10^3 .

Where : Inj . = injecting volume – R = reading - D = The volume of the medium .

The results were presented as µ mole C_2H_4 / 100 ml culture /day .

RESULTS AND DISCUSSION

Isolation, purification and identification of cyanobacteria.

Isolation and purification of cyanobacteria dominated in the soil samples collected from different sites in Kafr El- Sheikh, and El-Dkahlia governorates (El-Gamal *et al* ., 2008) ,isolates were successfully obtained as bacterial free cyanobacteria. They were identified cyanobacteria was carried out using the following: Thallus color, thallus morphology and dimension, size of heterocyst, vegetative and reproductive cells. Heterocyst - forming cyanobacteria were also cultured as *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., and *Chroococcus* sp., (Table 2) as the most of cyanobacteria they were associated with other microorganisms, hence, these must be purified from any contaminants, they exposed to different trials of purification. However, washing, ultra violet irradiation and mercuric chloride treatments were the most effective method for obtaining cyanobacteria cultures free from bacteria, while the other methods gave some success for killing bacteria in one side and some failure in the other side, which could be lethal for cyanobacteria themselves. These isolates were examined for their morphological and cultural characteristics, according to Venkataraman (1981) & Roger and Ardales (1991), in liquid and solid watanabe medium (Staub, 1961).

Genera, Cyanobacteria in the examined samples.

Out of the several isolates of cyanobacteria, from the rhizosphere soil of rice plants grown in the different locations namely, kafr El-Sheikh, and El-Dkahlia governorates, 14 isolates were obtained in pure cultures; bacteria free.

Table (2) also , showed the distribution of these isolates in the different Governorates. The 14 isolates were identified up to genera. Results showed that *Nostoc* sp. were the dominating organisms .

Nostoc sp. were isolated also at high frequency; where, 6 isolates representing in kafr El-Sheikh Governorate. less numbers of cyanobacteria belonging to different genera namely *Oscillatoria* sp. (1), *Chroococcus* sp. (1) and *Anabaena* sp. (1) were also obtained in pure cultures from kafr El-Sheikh Governorate. But in El-Dakahlia Governorate *Nostoc* sp. were isolated also at high frequency; where, 3 isolates Less numbers of cyanobacteria belonging to different genera namely *Oscillatoria* sp. (1), and *Anabaena* sp. (1).

Great variations were recorded in mass production and amount of fixed nitrogen between the different cyanobacterial genera and in some cases ecological effects are appeared. 9 instances *Nostoc* spp .isolates dominating in all governorates. Ranges of mean of *Anabaena* sp. cyanobacteria mass production (mg/100 ml-culture) were found to be in the order of 22 to 170 (1 isolate), *Nostoc* sp. 22 to 195 (3 isolates) *Oscillatoria* sp. 15 to 70 (1 isolate) El-Dakahlia governorate .

Ranges of mean of *Anabaena* sp. cyanobacteria mass production (mg/100 ml-culture) were found to be in the order of 30 to 197 (1 isolate), *Nostoc* sp. 21 to 201 (6 isolates) *Oscillatoria* sp. 27 to 92 (1 isolate) *Chroococcus* sp. 18 to 100 (1 isolate); in kafr El-Sheikh governorate.

The same trend was also obtained with rates of nitrogen fixation, where the highest amount of fixed nitrogen was within *Nostoc* sp. isolates recorded with kafr El-Sheikh isolates. Rates of nitrogen fixation ranged between 1.47 to 15.72 and 1.46 to 12.98, mg N/100ml-culture with isolates obtained from kafr El-Sheikh and El-Dakahlia ,respectively. . These results are in agreement with those obtained by El-zawawy (2016) reported that the ecological effect on the activation of growth and rates of nitrogen fixation by the different isolates of cyanobacteria.

Table 2. Numbers of the purified isolates of cyanobacteria and their activity obtained from the different locations.

Locations	Genera	No. of isolates	Range of algal activity	
			Mean dry weight,mg/100ml-culture	Mean amount of fixed nitrogen,mg/100ml-culture
Kafr El-sheikh(K)	*Anabaena* sp.	1	30 to 197	3.33 to 14.53
	Nostoc sp.	6	21 to 201	1.47 to 15.72
	Oscillatoria sp.	1	27 to 92	1.96 to 4.84
	Chroococcus sp.	1	18 to 100	1.20 to 4.94
El-Dakahlia (D)	*Anabaena* sp.	1	22 to 170	1.81 to 11.00
	Nostoc sp.	3	22 to 195	1.46 to 12.98
	Oscillatoria sp.	1	15 to 70	0.81 to 3.85
Total number of isolates		14		

Scientifc name of cyanobacterial strains

1- *Nostoc paludosum*
2- *Anabaena oryzae* (two strains)
3- *Nostoc muscorum*
4- *Nostoc pruniforme* (two strains)
5- *Nostoc verrucosum* (two strains)
6- *Nostoc entophytum*
7- *Nostoc rivulare*
8- *Nostoc viride*
9- *Chroococcus minor*
10- *Oscillatoria brevis* (two strains)

Identification of cyanobacterial species

1-*Nostoc paludosum*

Thallus punctiform; trichome straight, old one mostly entangled with a sheath; cell cylindrical or quadratic, as length as breadth or slightly smaller than breadth, 3.2-3.8 µ width and 4.1-4.5 µ length; heterocysts both terminal and intercalary, slightly broader than the vegetative cells, oblong or slightly; a kinetes oblong or oval, 4.1-6.3 µ and 5-6.3 µ length. According to Gupta and pandey (1979).

2- *Anabaena oryzae*

Thallus green, gelatinous, memberanous. Trichomes short, straight. Cells barrel-shaped, 1 times as long as broad, 4 µ broad, 5 µ long. Heterocysts terminal and intercalary; intercalary heterocysts subspherical, 4-5 µ broad, 4-5 µ long; terminal heterocysts conical and longer than broad, 3-3.5 µ broad, 4-4.5 µ long. Akinetes 3-6 in series, sub-spherical, 5-6 µ broad, 6-7 µ long. reported from paddy fields of India, Pakistan and Iran (Naz *et al.* 2004, Shariatmadari and Riahi 2010).

3-*Nostoc muscorum*

Culture is dark green trichomes had no ramifications. They were uniseriate, single, aggregated, and showed neither polarity nor tapering. No sheath was formed. Trichomes composed of three sizes and shapes of cells; a barrel cells (5-6 x 5.5-7µ);b) granular, ellipsoidal cells (5 x 5.5-7.5µm); c) yellowish- brown rounded cells of 8.9µ in diameter. Few heterocysts were observed. They were of single occurrence with 2 position, intercalary and terminal. according to Roger and Ardales (1991)

4-*Nostoc pruniforme*

Colonies gelatinous to rubbery, up to 4 cm in diameter, blue-green, green or brown; black and crusty when dry; trichomes of many cells (typically 60–80 cells between heterocytes), sheath close fitting when visible; cells subglobose to barrel-shaped, 2–3 µ long, 3–4 µ diam.; heterocytes intercalary or terminal, globose or ellipsoid, usually 1–4 per filament, 5–7 µ long, 5–6 µ diameter ; akinetes large, spherical to elliptical and about 10 m diameter . Hormogonia in irregularly sausage-shaped, thin walled packets, with beaks at both ends, heterocyte in one or more rarely both beaks, hormogonal cells slightly smaller and more oval than vegetative cells, in contracted spiral trichomes. Spheres with numerous hormogones observed. similar to *Nostoc pruniforme* as described by Prescott (1982).

5-*Nostoc verrucosum*

Colony usually a globose flabbellate sack or lamellate crust, rubbery, dirty green to blackish, from a few mms to several cms in extent; trichomes with visible sheaths in outer parts of colony, and grading from densely aggregated at margins to more open towards the inside, often spiralled or twisted, often quite long; cells barrel-like to subglobose, L/D 0.8–1.2,(2.4–) 3.0–4.8 µm diam.; heterocytes intercalary or falsely terminal on the short trichomes, ovate (5–)6.0–7.2 µm; akinetes, when present, paired or forming short moniliform sequences in trichomes, and larger in dimensions than vegetative cells, subglobose to ovate, L/D 1.5, c. 5 µm diam. Rippka,*et al*.(1979).

6-*Nostoc entophytum*

Thallus microscopically small, yellowish green, later brownish; trichome densely coiled, cells quadrate to short barrel-shaped, 2.7-3.6 µ width, 2.7-4.1 µ length; terminal heterocysts spherical, 4.1-4.5 µ width and 4.1-5 µ length; spores spherical or laterally compressed, 3.6-7.2 µ width, and 4.1-6.5 (8) µ length. According to Gupta and pandey (1979) and El-Gamal (1995).

7-*Nostoc rivulare*

Thallus generally developed and extend on the agar surface as a thin layer, flexuous; young cells pale green, older ones brown or pale yellowish, 4.1-5.4 µ width, quadratic, oblong or barrel shaped, 3.6-5.4 µ length; heterocysts terminal or intercalary, cylindrical or oblong, 4.5-6.8µ width, 5-7.2 µ length; spores oblong or nearly spherical, 5.4-8.1 µ width, 3.6-8.6 µlength. El-Gamal (1995) described the species, thallus of variable size; trichomes loosely entangled, flexuous blue green, 4 µ breadth 4-6 length; heterocysts, 5-6 µ breadth; spores oblong 6-7(-8) µ broad, 8-9.5 µ long. According to Geitler (1932)

8-*Nostoc viride*

Colonies macroscopic, forming mucilaginous, amorphous, dark green. Trichomes constricted, narrowed at the ends, freely irregularly coiled, sometimes forming very densely coiled groups of intensely flexuous filaments inside the colony, 4-5 µ wide. Mucilage colourless or

slightly yellowish. Cells barrel-shaped up to cylindrical, usually longer than wide, rarely square, particularly in the terminal parts of trichomes; apical cells sometimes slightly larger, oval. Cell content homogeneous, bluegreen. Heterocytes terminal and intermediate, distinctly longer than vegetative cells, rounded, oval, ovoid or barrel-shaped in intermediate position, terminal heterocytes 4.5-6.2 µ wide, 5.2-8 µ long, intercalary heterocytes 6-8.5 µ wide, 6.5-11 µ long. Akinetes in long rows between heterocytes or also in trichomes without heterocytes. Ripe akinetes oval, with granular content, with smooth surface, slightly brownish epispore, 6-9 µ wide, 10-12.5 µ long. Reproduction by hormogonia formation not observed. Disintegration of trichomes in solitary cells was commonly observed. According to Bornet and Flahault (1887).

9-*Chroococcus minor*

Thallus slimy-gelatinous, dirty blue green; cells spherical, 3-4 µ in diameter, irregularly scattered, singly; sheath thin and colourless, hardly visible Desikachary (1959).

10-*Oscillatoria brevis*

Culture dark green, trichome solitary, straight, not constricted at the cross-wall. Cells 2.5-3.1 µ length 4-7.1 µ and 4-8.2 µ width. Apex attenuated, cross-walls granulated according to El-Gamal (1995).

Determination of cyanobacteria growth rates:

Nitrogen fixation

Results in Table(3) showed gradual increases in cyanobacteria fixed-nitrogen (mg N/100 ml-culture) the experiment proceed, where the highest cyanobacteria fixed-nitrogen were recorded with all strains at the log phase lies between 14-28 days of inocubation period.

The efficiency of cyanobacterial strains to fix the atmospheric nitrogen is one of the most important parameters used for selection of cyanobacterial isolates for preparing cyanobacteria inoculants.

Results in Table (3) indicated that all strains varied in their capacity in the extracellular-nitrogen secreted where, the lowest nitrogen content was for strain *Oscillatoria brevis* D. On the other hand, the superior the extracellular-nitrogen secreted were for the strains *Nostoc muscorum* k and *Nostoc entophytum* K respectively. Extracellular-nitrogen secreted amount by cyanobacterial isolates increased gradually with increasing incubation period. The highest values of extracellular-nitrogen secreted were recorded at 28 days of growth *Nostoc muscorum* k and *Nostoc entophytum* K recorded 4.12, 2.55 mg N/100ml liquid culture respectively against 0.64 mg N/100ml culture the lowest one for *Oscillatoria brevis* D.

These indicated that all strains varied in their capacity in the intracellular -nitrogen secreted where, the lowest intracellular-nitrogen content was for strain *Oscillatoria brevis* D. On the other hand, the superior the intracellular -nitrogen secreted were for the strains *Nostoc muscorum* k and *Nostoc entophytum* K respectively. Intracellular-nitrogen secreted amount by cyanobacterial strains increased gradually with increasing incubation period. The highest values of intracellular-nitrogen secreted were recorded at 28 days of growth *Nostoc muscorum* k and *Nostoc entophytum* K recorded 11.60, 12.11 mg N/100ml liquid culture respectively against 3.21 mg N/100ml culture the lowest one for *Oscillatoria brevis* D.

The total nitrogen fixation where, the lowest nitrogen content was for strain *Oscillatoria brevis* D. On the other hand, the superior fixed nitrogen amounts were for the strains *Nostoc muscorum* k and *Nostoc entophytum* K respectively. Nitrogen fixed amount by cyanobacterial strains increased gradually with increasing incubation period. The highest values of the fixed nitrogen were recorded at 28 days of growth., *Nostoc muscorum* k and *Nostoc entophytum* K recorded 15.72, 14.66 mg N/100ml liquid culture respectively against 3.85 mg N/100ml culture the lowest one for *Oscillatoria brevis* D. These results are in agreement with those obtained by Roger and Kulasooriya, (1980) it could be concluded that the cyanobacteria strains of *Nostoc muscorum* k. and *Nostoc entophytum* K were the most efficient strains in biomass production and nitrogen fixation capacity and therefore, these two strains were selected for preparing cyanobacterial inoculants as biofertilizer applied for the complete the followed experiments in the current study. On the other hand, Simpson's index is a measure of diversity, which takes into account both richness and evenness, although it gives more weight to the more abundant species in a sample. Generally, cyanobacteria increased the frequency of heterocysts during unfavourable environment and nutrient deficiency. Nutrient accumulation may have different forces on the ecosystem at different period.

Table 3. Mean amounts of fixed-nitrogen by cyanobacteria strains (mg N/100 ml-culture) .

Cyanobacterial strains	Culture age days											
	7			14			21			28		
	Intra cellular	Extra cellular	Total	Intra cellular	Extra cellular	Total	Intra cellular	Extra cellular	Total	Intra cellular	Extra cellular	Total
Nostoc paludosum D	1.43	0.34	1.77	2.34	0.46	2.8	5.34	1.11	6.45	10.99	1.99	12.98
Anabaena oryzae D	1.54	0.27	1.81	1.67	0.36	2.03	3.2	0.95	4.15	8.67	2.33	11.00
Anabaena oryzae K	1.97	0.36	2.33	3.58	0.66	4.24	8.21	1.32	9.53	11.43	3.10	14.53
Nostoc muscorum k	2.93	0.369	3.299	4.94	1.00	5.94	8.49	1.88	10.37	11.60	4.12	15.72
Nostoc pruniforme K	2.35	0.39	2.74	3.387	0.68	4.067	6.4	1.51	7.91	9.76	3.11	12.87
Nostoc pruniforme D	1.65	0.3	1.95	3.07	0.54	3.61	6.28	1.2	7.48	9.64	3.22	12.86
Nostoc verrucosum K	2.59	0.53	3.12	3.6	0.68	4.28	5.1	0.73	5.83	6.11	1.11	7.22
Nostoc verrucosum D	0.88	0.166	1.46	2.52	0.37	2.89	2.6	0.48	3.08	4.23	0.99	5.22
Nostoc entophytum K	2.79	0.36	3.15	4.66	0.79	5.45	6.88	1.17	8.05	12.11	2.55	14.66
Nostoc rivulare K	1.2	0.27	1.47	1.79	0.47	2.26	3.61	0.67	4.28	4.99	1.22	6.21
Nostoc viride K	2.12	0.32	2.44	2.88	0.47	3.35	3.39	0.92	4.31	5.99	1.77	7.76
Chroococcus minor K	100	0.2	1.2	1.98	0.37	2.35	3.41	0.48	3.89	4.23	0.71	4.94
Oscillatoria brevis K	1.77	0.19	1.96	2.37	0.39	2.76	2.65	0.47	3.12	4.11	0.73	4.84
Oscillatoria brevis D	0.67	0.14	0.81	2.00	0.23	2.23	2.33	0.46	2.79	3.21	0.64	3.85

Biomass production :

Results in Table(4) showed gradual increases in cyanobacteria biomass dry weight as the experiment proceed, where the highest cyanobacteria dry weights were recorded with all strains at the log phase lies between 14-28 days of inocubation period . Indicated that all strains varied in their capacity in biomass production secreted where, the lowest biomass was for strain *Oscillatoria brevis* D. On the other hand, the biomass were the strains *Nostoc muscorum* k and *Nostoc entophytum* K respectively biomass amount by cyanobacterial strains increased gradually with increasing incubation period. The highest values of biomass were recorded at 28 days of growth *Nostoc muscorum* k and *Nostoc entophytum* K recorded 201, 198 mg/100ml liquid culture respectively against 70 mg/100ml culture the lowest one for *Oscillatoria brevis* D. These results are in agreement with those obtained by (El-Zawawy, 2016 and Taha 2000) who found that cyanobacteria exhibited the highest dry weight with increasing the incubation period**.**

Table 4. Mean dry weight of the cyanobacteria strains (mg/100ml-culture) .

Cyanobacterial strains	Culture age (days)			
	7	14	21	28
Nostoc paludosum D	22	35	100	195
Anabaena oryzae D	22	50	82	170
Anabaena oryzae K	30	59	92	197
Nostoc muscorum k	72	65	98	201
Nostoc pruniforme K	34	60	95	190
Nosto cpruniforme D	25	54	85	179
Nostoc verrucosum K	38	60	80	177
Nostoc verrucosum D	20	30	66	130
Nostoc entophytum K	52	75	99	198
Nostoc rivulare K	21	43	69	132
Nostoc viride K	33	59	92	180
Chroococcus minor K	18	35	51	100
Oscillatoria brevis K	27	36	45	92
Oscillatoria brevis D	15	32	50	70

Nitrogenase activity :

Strains of cyanobateria were isolated and efficiency levels of the N_2-fixation differed between the strains (Table 5).

Table 5. Nitrogenase activity of cyanobacterial strains.

Cyanobacterial strains	Nitrogenase activity (nmoles C_2H_4/ml/day)
Nostoc paludosum D	21.60
Anabaena oryzae D	99.60
Anabaena oryzae K	69.60
Nostoc muscorum k	170.88
Nostoc pruniforme K	28.80
Nostoc pruniforme D	57.60
Nostoc verrucosum K	17.04
Nostoc verrucosum D	25.44
Nostoc entophytum K	12.96
Nostoc rivulare K	38.40
Nostoc viride K	24.00
Chroococcus minor K	20.88
Oscillatoria brevis K	12.72
Oscillatoria brevis D	38.40

The strains reduced acetylene actively at the rate of 12.72 – 170.88 N moles C_2H_4/ml/day. The most active strains *Nostoc muscorum* k and *Anabaena oryzae* D were selected for further experiments. These results are similar to those reported by El-Sawah,(2018) that the ability of cyanobacteria fix N_2 and the most active cyanobacteria for nitrogenase activity strains *Anabaena oryzae* and *Nostoc* spp **.**

REFERENCES

Alam, M.J. ; Habib,M. and Begum, M. (1989). Effect of water quality and dominance genera of phytoplankton on the abundance of available genera . Pak. J. Sci. Ind. Res. 32 : 194-200.

Bornet, J. B. E. and Flahault, C. G. M. (1887). Révision des Nostocacées hétérocystées. Annales des Sciences Naturelles Botanique. 7:51- 129.

Castenholz, Richard,W. (2015). Bergey,s Manual of Systematic Bacteriology " General characteristics of the Cyanobacteria. pp.475.

Desikachary, T. V. (1959). "Cyanophyta". New Delhi: Indian Council of Agricultural Research. 686 pp

El-Ayouty, E.Y. and Ayyad, M.A. (1972). "Studies on blue-green algae of the Nile Delta 1-Description of some species in a wheat field". Egypt. J.Bot., 15:283-321.

El-Gamal A.D. (1995). Systematical studies on the algae isolated from some cultivated areas and laboratory studies on the effect of light, temperature and humidity on three selected soil algae. Ph.D. Thesis, Fac. Sci., Al-Azhar Univ., Cairo, Egypt.

El-Gamal, A.D.; Ghanem, N.A.E.; El-Ayouty, E.Y. and Shehata, E.F. (2008). Studies on soil algal flora in Kafr El- Sheikh Governorate, Egypt. Egyptian J. Phycol. 9: 1-23.

El-Nawawy, A. S.; Lotfi, M. and Fahmy, M. (1958) .Studies on the ability of some blue-green algae to fix atmospheric nitrogen and their effect on growth and yield of paddy. Agric. Res. Rev. 36(2): 308-320.

El-Sawah,A.M.M.A.(2018).Studiesondifferentbiofertilizers mixtures and their effect on growth of some economicalplants.Ph.D.Thesis,Fac.Agric., Mansoura Univ.,Egypt,pp.134.

El-Zawawy, H.A.H. (2016). Microbiological and Ecological Studies on The Activity of Cyanobacteria in Different Types of Soil. Ph.D Thsis Fac. Agric. Al- Azhar Univ.,Egypt.

Geitler, L. (1932). Cyanophyceae. In Rabenhorst's Kryptogamen flora. Akademische Verlagsgesellschaft, Leipzig, pp. 1-1196.

Gupta,K.K. and Pandy,D.J.(1979).Potential use of cyanobacterial species in bioremediation of industrial effuents. Arch.Microbiol., 195.

Gupta, R.S.; Maheshwari, K.L. and Sen Gupta, S.R. (1959). "A method for obtaining algal cultures free from associated microorganisms". Curr. Sci., 25-91.

Hardy, R.W.F. ; R.C.Burs and R.D.Holsten (1973). Application of the acetylene – ethylene assay for measurement of nitrogen fixation . Soil Biol. Biochem.,5:47-81.

Hoshaw, R.W. and Rosowski, J.R. (1973). "Methods Of Microscopic Algae". In Stein, J. R., ed., Hand Book Of Phycological Methods, Cambridge Univ. press. London, New York, Sidney.

Jackson, M.L. (1973)."Soil Chemical Analysis, Constable and CO2". Agric. Exper. Mad. Wisconsin.USA.P: 183-187.

Muthukumar,C.;Muralitharan,G.;Vijayakumar R.;Panneerselvam , A.and Thajuddin, N. (2007).Cyanobacterial biodiversity from different freshwater ponds of Thanjavur. Tami Nadu (India). Acta Bat.Malacitana,32:17-25.

Naz, S.; Ul-Hasan, M. and Shameel, M.(2004). Taxonomic study of Anabaena Bory Nostocophyceae, Cyanophyta) from northern areas of Pakistan. -Pak. J. Bot., 36 (2): 283-295.

Piper, C. S. (1950). "Soil and plant analysis Inter. Science Publisher, Inc. New York, USA".

Prescott, G.W.(1982). Agae of western Great lakes AreaW.M.C. Brown company publishers, Dubuque, lowa, USA.

Pringsheim, E.G.(1949). "Pure culture of algae, their preparation and Maintenance". Cambridge. Univ.

Rippka, R.; Deruelles, J. ; Waterbury, J.B.; Herdman, M.; Stanier R.Y. (1979) . Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J. of Gen. Microbiol., 111, pp. 1-61.

Roger, P.A. and Ardales, S. (1991). "Blue-Green algae collection". International Rice Research Institute, IRRI, Pub. Manila, Philippines.

Roger, P.A. and Kulasooriya, S.A. (1980) . Blue-green algae and rice. Book 112 p.

Shariatmadari,Z.and Riahi,H.(2010). New records of heterocystous cyanophyta from paddy field of Iran. Boil.Fert.Soil,8:229-234.

Staub, R. (1961). "Ernahrungsphysiologisch aurakologische untersuchengen an der planktischen blualg Oscillatoria rubescence D. C". Scheweiz. Zeitschr Hydrobiologie, 23:82-198.

Taha, M.T. (2000). "Biotechnological application of cyanobacteria in the constitution of a soil model of a biofortified farming system". M.Sc. Thesis, Fac. Sci., Azhar Univ., Egypt.

Venkataraman, G.S. (1981). "The cultivation of algae". Indian Council Agric. Res., New Delhi, India.

عزل وتعريف سلالات السيانوبكتيريا المثبته للنتروجين من مواقع مختلفه .

عايدة حافظ عفيفى عامر[1] ، فتحى إسماعيل على حوقه[1] ، محمود محمد جاب الله[2] و أحمد السيد عبدالرحمن أبو العطا[1]

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تم جمع عينات تربه من مواقع مختلفه في محافظتى كفر الشيخ والدقهليه وذلك لعزل السيانوبكتيريا حيث تم الحصول على عزلات السيانوبكتيريا نقيه ثم تعريفها وذلك طبقا شكل و لون الثالوس وحجم الهتيروسست بالإضافه إلى الخلايا الخضريه و التكاثريه . كانت الهتيروسست عند تنميتها تنتمى لأجناس السيانوبكتيريا مثل أنابينا و نوستوك و أوسيلاتوريا و وكذلك كرووكوكس .وكانت معظم الأنواع تتبع جنسى النوستوك و الأنابينا و ذلك في محافظتى كفر الشيخ والدقهليه بينما كانت أقل الأنواع تنتمى إلى جنسى الأسيلاتوريا و الكرووكوكس . وقد سجلت أنواع جنس النوستوك أعلى قيمه في النيتروجين المثبت ونمو الخلايا وذلك بزيادة فترة التحضين في حالة النيتروجين المثبت داخل وخارج الخلايا . بينما سجلت سلالتى النوستوك مسكورم (المعزوله من أراضى كفر الشيخ) وأنابينا أوريزا (المعزوله من أراضى الدقهليه) أعلى نشاط لإنزيم النيتروجينيز.