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Identification of diazotrophic nostocalean cyanobacteria of north eastern region of India and evaluation for nitrogenase activity and extracellular ammonium excretion

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ABSTRACT

In this study, morphological and microscopic observations were performed along with growth measurements, evaluation of extracellular ammonium and nitrogenase activity. A total of 62 diazotrophic nostocalean cyanobacteria belonging to 12 genera viz- *Scytonema* (2), *Anabaena* (22), *Nostoc* (17), *Cylindrospermum* (2), *Anabaenopsis* (1), *Dichothrix* (1), *Tolypothrix* (2), *Calothrix* (6), *Aulosira* (4), *Microchaete* (3), *Wollea* (1) and *Rivularia* (1) which were originally isolated from different niches of north east India were procured from National fresh water cyanobacterial and microalgal repository of DBT-IBSD, Imphal, Manipur, India. Among the different nostocalean cyanobacteria encountered from the various habitats, *Anabaena* sp. BTA650, an isolate from lithophytes under running water, Moreh, Chandel, Manipur exhibited highest nitrogenase activity of 111.08±13.26 nmole C_2H_4 μg^{-1} Chl-a h^{-1} can be a potential candidate for biofertilizer formulation particularly for terraced hill rice culture condition.

1. INTRODUCTION

The North East India is a frontier region with picturesque hills and green meadows which shelters thousand of species of flora and fauna. It is connected to the rest of India by a narrow 20 km wide corridor of land. The lushness of its landscape, geographical and ecological diversity makes the North East India quite different from other parts of the subcontinent. Cyanobacteria are impressive ecosystem engineers since the beginning of the evolutionary history of planet [1]; [2]. They are often referred to as 'miniature factories' of the biological world and represent an alternative source of a variety of bioactive compounds, lipids/fatty acids, proteins, enzymes, pigments and compounds of pharmaceutical and nutraceutical value [3]; [4]. According to [5]; [6], the order Nostocales includes filamentous cyanobacteria that are capable of cell differentiation in heterocysts, akinetes or reproductive trichomes (hormogonia).

The role of nitrogen fixing cyanobacteria in enhancing soil fertility has been long known [7]; [8]. With increasing awareness of their varied potential applications, studies on cyanobacterial diversity have been reported from around the world in recent times [9]; [10]; [11]. Few reports on studies of cyanobacteria from the north eastern region of India are [12]; [13]; [14]; [15]; [16].

Diazotrophic cyanobacterial strains originally isolated from different niches of north eastern region of India were investigated morphologically and biochemically characterized for the biochemical components such as extracellular ammonium excretion, nitrogenase activity and production of chlorophyll-a.

2. MATERIALS AND METHODS

2.1 Preparation of the culture medium

BG-11(-N) non nitrogenous culture medium [17] was used for culturing of nostocalean cyanobacteria. Stock solutions of all the ingredients were prepared and stored in screw capped amber colour bottles. A volume of each ingredient was taken accordingly for one litre of the medium.

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The pH of the culture medium was maintained through the experiment between 7.0-7.5. For adjusting the pH, N/10 solutions of sodium hydroxide and hydrochloric acid were used and calibrated in pH meter (ThermoFisher).

2.2 Identification of diazotrophic nostocalean cyanobacteria

Microscopic observations and photomicrography of the pure cultures were made with the aid of trinocular research microscopes: Nikon Eclipse 80*i* (Nikon) and Carl Zeiss Axio Scope A1 coupled with Carl Zeiss Imaging Systs 32 Software Vision AxioVision 4.7.2 (Carl Zeiss) having fluorescence attachment. Identification of cultures was carried out with the help of keys as described by [18]; [19].

2.3 Partial biochemical characterization of diazotrophic nostocalean cyanobacteria

A total of 62 diazotrophic cyanobacterial strains were procured from the Freshwater cyanobacterial and microalgal repository of IBSD, Imphal, Manipur, India (A National facility created by Department of Biotechnology, Govt. of India). The strains were selected based on their fast growth rate for biochemical screening.

Extracellular ammonium excretion, nitrogenase activity and chlorophyll-a were estimated on exponential phase of growth cycle.

2.4 Preparation of inoculum

A loopful of nostocalean cyanobacterial strain was inoculated in 150 ml BG-11(-N) medium and incubated for 10 days under a light/dark cycle of 14/10 h at 28±2°C and 54-67 µmol m^{-2} s^{-1} light intensity. On 10^{th} day of incubation, nostocalean cyanobacterial strain was homogenized using sterile glass bead in shaker incubator. Then, 15 ml of homogenized biomass was again inoculated in a flask containing 150 ml of BG-11 (-N) broth medium for the biochemical estimations.

2.5 Estimation of extracellular ammonium excretion

Estimation of extracellular ammonium excretion was performed as per the method described by [20] and the optical density (O.D.) of the solution was read at 640 nm in UV-spectrophotometer (UV-1800 Shimadzu).

2.6 Determination of nitrogenase activity

Nitrogenase activity determined by acetylene reduction activity (ARA) was performed as per the method of [21]. 5 ml each of the homogenized cyanobacterial samples were dispensed in 20 ml capacity vials. 10% of gas phase was taken out and same quantity was replaced by injection of acetylene gas into the airtight sample vials. The samples were incubated at 28±2ºC under 54 µmol m^{-2} s^{-1} light intensity for 90 mins. The activity was analysed in GC-FID (Chemito Ceres 800 plus gas chromatograph, ThermoScientific) with Porapak-T packed column by injection of 1 ml of the ethylene gas formed with gas tight syringe taken from the sample vials after arresting the metabolic activity of the samples by dispensing 0.5 ml of 10% TCA on completion of 90 mins incubation. Calibration was done by using 99.9% ethylene gas. Analysis condition for the GC-FID were injector temperature set at 150°C, oven temperature set at 50°C for 15 mins and detector temperature set at 200°C using nitrogen as carrier gas. The acetylene reduction activity was calculated using Chemito Chrome Card version 2.6 software.

2.7 Estimation of chlorophyll-a

Chlorophyll-a was estimated as per the method detailed by [22]. The optical density (O.D.) of the supernatant was read at 665 nm in UV-spectrophotometer (UV-1800 Shimadzu).

2.8 Statistical analysis

Statistical analysis of the data was conducted by using Microsoft office Excel 2007 for standard deviation.

3. RESULTS

All the morphological characteristics viz. filament/trichome and branching pattern, appearance of sheath, cells shape, heterocysts, akinetes shapes etc. of the nostocalean cyanobacteria were studied (Table-1). Selected photomicrographs of different nostocalean genera for morphological studies are shown (Fig-1).

Thallus behaviours of selected strains in BG-11 broth and agar plate medium are also shown (Figs 2 & 3). Nitrogenase activity and extracellular ammonium excretion exhibited in the logarithmic/exponential stage are presented in Table-2.

Anabaena sp. BTA650 showed the highest nitrogenase activity (111.08±13.26 nmole C_2H_4 μg^{-1} Chl-a h^{-1}) followed by *Calothrix* sp. BTA265 (89.23±0.00 nmole C_2H_4 μg^{-1} Chl-a h^{-1}), *Scytonema hofmanni* BTA124 (86.32±0.74 nmole C_2H_4 μg^{-1} Chl-a h^{-1}), *Rivularia* sp. BTA510 (76.73±5.39 nmole C_2H_4 μg^{-1} Chl-a h^{-1}), *Nostoc* sp. BTA197 (28.13±0.14 nmole C_2H_4 μg^{-1} Chl-a h^{-1}) and *Anabaena* sp. BTA281 (21.47±0.45 nmole C_2H_4 μg^{-1} Chl-a h^{-1}).

Anabaena sp. BTA385 showed the highest (412.05±0.11 µg ml^{-1}) and the least by *Microchaete* sp. BTA300 (4.30±0.00 µg ml^{-1}) for extracellular ammonium excretion.

4. DISCUSSION

In the present study, 62 diazotrophic nostocalean strains which were originally isolated from different niches of north east India were procured from the Freshwater cyanobacterial and microalgal repository of IBSD, Imphal, Manipur for the investigation which comprises of 12 genera viz- *Scytonema* (2), *Anabaena* (22), *Nostoc* (17), *Cylindrospermum* (2), *Anabaenopsis* (1), *Dichothrix* (1), *Tolypothrix* (2), *Calothrix* (6), *Aulosira* (4), *Microchaete* (3), *Wollea* (1) and *Rivularia* (1). Among the different nostocalean cyanobacteria, *Anabaena* sp. BTA650 isolated from lithophytes under running water, Moreh, Chandel, Manipur exhibited highest nitrogenase activity of 111.08±13.26 nmole C_2H_4 μg^{-1} Chl-a h^{-1} can be a potential candidate for biofertilizer formulation and applications in the terraced rice field condition. Not only *Anabaena* sp. BTA650 alone, *Calothrix* sp. BTA265, *Scytonema hofmanni* BTA124, *Rivularia* sp. BTA510, *Nostoc* sp. BTA197 and *Anabaena* sp. BTA281 which were selected out of the 62 strains produced 89.23±0.00 nmole C_2H_4 μg^{-1} Chl-a h^{-1}, 86.32±0.74 nmole C_2H_4 μg^{-1} Chl-a h^{-1}, 76.73±5.39 nmole C_2H_4 μg^{-1} Chl-a h^{-1}, 28.13±0.14 nmole C_2H_4 μg^{-1} Chl-a h^{-1} and 21.47±0.45 nmole C_2H_4 μg^{-1} Chl-a h^{-1} respectively which are superior or far better than earlier reports of [23]; [24]; [25]. Maximum nitrogenase activity was reported to observe at logarithmic stage along with higher frequency of heterocysts [26]. The heterocyst frequency and nitrogenase activity were comparable to many *Nostoc* species [27] suggesting its definite role in fixed nitrogen contribution to the rice field ecosystems on its mass turn over. In our investigation of diazotrophic cyanobacteria for nitrogenase activity during the logarithmic stage of growth, high rate of acetylene reduction activity was observed which can be assumed as due to high frequency of heterocyst at this stage of growth.

Syiem *et al*. (2011) reported that *Nostoc* sp. of Meghalaya showed enzyme assays reflecting nitrogenase activity of 6.98 nmole C_2H_4 μg^{-1} Chl-a h^{-1} and [24] also reported nitrogenase activity of 12±0.6 nmole C_2H_4 μg^{-1} Chl-a h^{-1} from *Nostoc* ANTH which clearly indicates that our studied strains would be more useful as source of biofertilizer. Present finding is also in support by another previous report of [25] in which nitrogenase activity was 50±8.00 nmole C_2H_4 μg^{-1} Chl-a h^{-1} as exhibited by *Anabaena cylindrica* of their study. Species of *Nostoc*, *Anabaena*, *Tolypothrix*, *Aulosira*, *Cylindrospermum*, *Scytonema*, *Westiellopsis* and several other genera are widespread in Indian rice field soils and are known to contribute significantly to their fertility [28]; [29].

For extracellular ammonium excretion, BTA385 showed the highest value (412.05±0.11 µg ml^{-1}) which was also at par with the previous finding of [16] during the logarithmic stage of growth for *Anabaena fuellebornii* Schmidle (ammonium excretion-96.90±0.00 µg ml^{-1}).

In aquatic environments that lack direct photosynthesis, cyanobacteria often form thick microbial mats, making cyanobacteria crucial in such ecosystem. These mats also contain the primary producers at the basis of the microbial food web. The role of cyanobacteria in the sustained fertility of flooded/irrigated rice field soils is well established [30]; [31]. Many photosynthetic free-living strains of cyanobacteria capable of fixing nitrogen have been isolated and used in biofertilizer consortia in south-east Asian countries and the potential impact of these organisms on agriculture through their use as biofertilizers, soil conditioners, plant growth regulators and soil health ameliorators is well recognized [32]; [33]; [34]. The role of N_2-fixing cyanobacteria in maintenance of the fertility of rice fields has been well substantiated and documented all over the world. The cyanobacteria used as biofertilizers consist of *Nostoc*, *Anabaena*, *Tolypothrix*, *Plectonema*, *Aphanothece*, *Cylindrospermum*, *Aulosira* and *Scytonema*. Nitrogen fixing cyanobacteria are being used as nitrogen biofertilizers in rice fields in countries where rice is the major staple diet [35]. Cyanobacterial N_2-fixation in the oceans contributes significantly to the global N budget [36]; [37]; [38]. In addition, cyanobacteria are key contributors to global nitrogen fixation, and many produce unique secondary metabolites [39]. The ability of diazotrophic cyanobacteria to fix atmospheric nitrogen make them unique in their ability to independently secure their carbon and nitrogen requirements. All this attributes make our studied diazotrophic Nostocalean strains to be unique for application as biofertilizers and formulations.

Table 1: Morphological characteristics of the diazotrophic nostocalean cyanobacteria.

SN	Name of the strains	Taxonomical features				
		Filament/ trichome &branching pattern	**Sheath**	**Cell shape**	**Heterocyst**	**Akinete**
1	*Scytonema bohneri* Schmidle BTA106	Blackish green, false branched	Colourless	Rectangular	Rectangular	-
2	*Anabaena oryzae* Fritsch BTA109	Straight	Not distinct	Barrel	Barrel	Ellipsoidal
3	*Scytonema hofmanni* Ag. ex Born. et Flah. BTA124	Irregularly bent, false branched	Thick hyaline	Cylindrical	Rectangular	-
4	*Anabaenopsis raciborskii* Wolosz. BTA125	Short	Not distinct	Cylindrical	Spherical	Ellipsoidal
5	*Anabaena circinalis* Rabenhorst ex Born. et Flah. BTA129	Circinate	Not distinct	Barrel	Subspherical	Cylindrical
6	*Nostoc spongiaeforme* Agardh ex Born. et Flah. BTA131	Flexuous	Diffluent	Partly cylindrical and partly barrel-shaped	Spherical	Oblong
7	*Tolypothrix distorta* Kutzing ex Born. et Flah. BTA187	Richly false branched	Thin	Cylindrical	Cylindrical	-
8	*Nostoc* sp. Ag. ex Born. et Flah. BTA197	Flexuous	Diffluent	Partly cylindrical and partly spherical	Spherical	Oblong
9	*Nostoc commune* Vaucher ex Born. et Flah. BTA210	Flexuous, entangled	Lamellated	Barrel	Spherical	-
10	*Aulosira pseudoramosa* Bharadwaja BTA213	Irregularly bent, false branched	Thick hyaline	Cylindrical	Rectangular	-

11	*Nostoc spongiaeforme* Agardh ex Born. et Flah. BTA227	Flexuous	Diffluent	Partly cylindrical and partly spherical	Spherical	Oblong
12	*Nostoc* sp. BTA229	Highly coiled	Distinct at periphery	Subspherical	Spherical	-
13	*Anabaena* sp. BTA230	Curved	Distinct at periphery	Barrel	Spherical	-
14	*Tolypothrix byssoidea* (Berk.) Kirchner BTA 257	Irregulary false branched	Thin	Barrel	Rectangular	-
15	*Calothrix* sp. BTA265	Single and slightly bent	Lamellated and distinct	Broader than the length and tapering	Half spherical	-
16	*Anabaena doliolum* Bharadwaja BTA280	Straight	Not distinct	Barrel	Barrel	Ellipsoidal
17	*Anabaena* sp. BTA281	Straight	Not distinct	Barrel	Barrel	Ellipsoidal
18	*Anabaena bergii* Ostenfeld BTA284	Coiled	Thick	Spherical	Subspherical	Elongated
19	*Anabaena oryzae* Fritsch. BTA293	Straight	Not distinct	Barrel	Barrel	Ellipsoidal
20	*Anabaena circinalis* Rabenhorst ex Born. et Flah. BTA296	Circinate	Not distinct	Barrel	Subspherical	Cylindrical
21	*Microchaete* sp. BTA300	Single and bent	Distinct	Cylindrical	Subspherical	-
22	*Anabaena iyengarii* Bharadwaja BTA302	Not distinct	Barrel	Barrel	Ellipsoidal	Ellipsoidal
23	*Nostoc ellipsosporum* (Desm.) Rabenh. ex Born. et Flah. BTA313	Not distinct	Cylindrical	Subspherical	Ellipsoidal	Ellipsoidal
24	*Anabaena* sp. BTA320	Not distinct	Cylindrical	Spherical	Ellipsoidal	Ellipsoidal
25	*Nostoc ellipsosporum* (Desm.) Rabenh. ex Born. et Flah. BTA332	Not distinct	Cylindrical	Subspherical	Ellipsoidal	Ellipsoidal
26	*Nostoc spongiaeforme* Agardh ex Born. et Flah. BTA347	Diffluent	Partly cylindrical and partly spherical	Spherical	Oblong	Oblong
27	*Anabaena circinalis* Rabenhorst ex Born. et Flah. BTA376	Not distinct	Barrel	Subspherical	Cylindrical	Cylindrical
28	*Nostoc commune* Vaucher ex Born. et Flah. BTA384	Lamellated	Barrel	Spherical	-	-
29	*Anabaena* sp. BTA385	Not distinct	Barrel	Subspherical	Cylindrical	Cylindrical
30	*Nostoc calcicola* Brebisson ex Born. et Flah. BTA394	Distinct at periphery	Barrel	Subspherical	Subspherical	Subspherical
31	*Anabaena iyengarii* Bharadwaja BTA397	Not distinct	Barrel	Barrel	Barrel	Barrel
32	*Microchaete loktakensis* Bruhl et Biswas BTA425	Broad with lamellae	Cylindrical	Spherical	-	-
33	*Anabaena* sp. BTA428	Distinct at periphery	Cylindrical	Oblong	Ellipsoidal	Ellipsoidal
34	*Calothrix* sp. BTA431	Thick gelatinous	Barrel at the base	Subspherical	-	-
35	*Calothrix* sp. BTA437	Distinct	Barrel at the base	Subspherical	-	-
36	*Nostoc* sp. BTA439	Distinct	Subspherical	Spherical	-	-
37	*Aulosira* sp. BTA472	Lamellated	Cylindrical	Rectangular	-	-
38	*Anabaena orientalis* Dixit. BTA496	Not distinct	Quadrate	Cylindrical	Ellipsoidal	Ellipsoidal
39	*Nostoc commune* Vaucher ex Born. et Flah. BTA504	Lamellated	Barrel	Spherical	-	-
40	*Rivularia* sp. BTA510	Thin lamellated	Longer than broad at base and broader than length at apex	Conical	-	-
41	*Aulosira prolifica* Bharadwaja BTA514	Thin	Cylindrical	Ellipsoidal	-	-
42	*Anabaena* sp. BTA545	Not distinct	Cylindrical	Spherical	Oblong	Oblong
43	*Calothrix clavata* West, G.S. BTA550	Very thin	Discoid	Hemi-spherical	-	-
44	*Anabaena* sp. BTA650	Not distinct	Barrel	Barrel	Spherical	Spherical

45	*Microchaete* sp. BTA673	Lamellated	Broader than length and somewhat uniform throughout	Subspherical	-	-
46	*Cylindrospermum* sp. BTA674	Not distinct	Cylindrical	Conical	Oblong bigger than heterocyst	Oblong bigger than heterocyst
47	*Nostoc* sp. BTA676	Lamellated	Barrel	Spherical	-	-
48	*Calothrix marchica* Lemmermann BTA680	Thin	Broad at base and taper as hair	Subspherical	-	-
49	*Nostoc calcicola* Brebisson ex Born. et Flah. BTA698	Distinct at periphery	Barrel	Subspherical	Subspherical	Subspherical
50	*Anabaena oryzae* Fritsch BTA791	Not distinct	Barrel	Barrel	Ellipsoidal	Ellipsoidal
51	*Wollea* sp. BTA797	Distinct	Barrel	Subspherical	-	-
52	*Anabaena orientalis* Dixit BTA809	Not distinct	Quadrate	Cylindrical	Ellipsoidal	Ellipsoidal
53	*Anabaena torulosa* (Carm.) Lagerh ex Born. et Flah. BTA815	Not distinct	Barrel	Subspherical	Subcylindrical	Subcylindrical
54	*Nostoc calcicola* Brebisson ex Born. et Flah. BTA818	Distinct at periphery	Barrel	Subspherical	Subspherical	Subspherical
55	*Nostoc hatei* Dixit BTA822	Not distinct	Barrel	Spherical	Spherical	Spherical
56	*Calothrix marchica* Lemmermann BTA828	Thin	Broad at base and taper as hair	Subspherical	-	-
57	*Aulosira* sp. BTA829	Not distinct	Cylindrical	Rectangular	-	-
58	*Dichothrix* sp. BTA833	Distinct	Taper at end	Basal conical	-	-
59	*Cylindrospermum* sp. BTA866	Not distinct	Cylindrical	Conical	Oblong bigger than heterocyst	Oblong bigger than heterocyst
60	*Anabaena* sp. BTA893	Distinct at periphery	Barrel	Spherical	Spherical	Spherical
61	*Anabaena* sp. BTA914	Not distinct	Barrel	Subspherical	Subspherical	Subspherical
62	*Nostoc commune* Vaucher ex Born. et Flah. BTA917	Lamellated	Barrel	Spherical	-	-

BTA= Biotechnological Algae

Table 2: Partial biochemical characterization of diazotrophic nostocalean cyanobacteria.

SN	Name of the strains	Extracellular ammonium excretion ($\mu g\ ml^{-1}$)	Chlorophyll-a ($\mu g\ ml^{-1}$)	Nitrogenase activity (nmole $C_2H_4\ \mu g^{-1}$ Chl-a h^{-1})
1	*Scytonema bohneri* BTA106	56.55±0.02	0.71±0.01	13.67±0.18
2	*Anabaena oryzae* BTA109	77.55±0.01	2.75±0.00	3.28±0.00
3	*Scytonema hofmanni* BTA124	106.05±0.01	0.19±0.10	**86.32±0.74**
4	*Anabaenopsis raciborskii* BTA125	55.95±0.00	4.89±0.06	7.30±0.12
5	*Anabaena circinalis* BTA129	62.40±0.01	3.18±0.03	3.28±0.01
6	*Nostoc spongiaeforme* BTA131	85.50±0.00	8.76±0.01	3.10±0.04
7	*Tolypothrix distorta* BTA187	105.60±0.02	0.82±0.00	10.54±0.05
8	*Nostoc* sp. BTA197	44.10±0.06	0.99±0.11	**28.13±0.14**
9	*Nostoc commune* BTA210	78.92±0.01	2.12±0.01	2.17±0.09
10	*Aulosira pseudoramosa* BTA213	75.75±0.01	0.88±0.01	1.19±0.04
11	*Nostoc spongiaeforme* BTA227	184.95±0.01	1.42±0.00	2.85±0.12
12	*Nostoc* sp. BTA229	169.95±0.01	3.93±0.00	1.98±0.18
13	*Anabaena* sp. BTA230	130.35±0.02	5.43±0.08	0.92±0.15
14	*Tolypothrix byssoidea* BTA257	181.50±0.00	2.10±0.09	3.16±0.19
15	*Calothrix* sp. BTA265	46.95±0.01	0.07±0.06	**89.23±0.00**
16	*Anabaena doliolum* BTA280	65.25±0.02	0.74±0.02	21.34±0.02
17	*Anabaena* sp. BTA281	150.95±0.01	0.95±0.03	**21.47±0.45**
18	*Anabaena bergii* BTA284	27.15±0.01	3.78±0.04	16.03±0.03
19	*Anabaena oryzae* BTA293	26.10±0.01	3.16±0.07	3.92±0.01
20	*Anabaena circinalis* BTA296	35.55±0.00	0.70±0.00	9.71±0.18
21	*Microchaete* sp. BTA300	4.30±0.00	3.63±0.05	1.08±0.05
22	*Anabaena iyengarii* BTA302	4.90±0.00	2.41±0.03	4.81±0.09
23	*Nostoc ellipsosporum* BTA313	9.40±0.00	4.66±0.02	1.61±0.03
24	*Anabaena* sp. BTA320	5.10±0.00	4.92±0.07	2.23±0.15
25	*Nostoc ellipsosporum* BTA332	75.70±0.00	ND	ND

26	*Nostoc spongiaeforme* BTA347	12.49±0.01	2.74±0.03	3.96±0.05
27	*Anabaena circinalis* BTA376	19.50±0.00	1.67±0.04	2.12±0.06
28	*Nostoc commune* BTA384	46.80±0.01	1.63±0.01	3.07±0.04
29	*Anabaena* sp. BTA385	412.05±0.11	1.74±0.03	5.04±0.08
30	*Nostoc calcicola* BTA394	147.00±0.06	4.10±0.04	6.03±0.00
31	*Anabaena iyengarii* BTA397	69.75±0.01	1.36±0.05	5.86±0.00
32	*Microchaete loktakensis* BTA425	144.90±0.06	6.84±0.08	1.42±0.04
33	*Anabaena* sp. BTA428	318.50±0.02	6.73±0.04	0.85±0.08
34	*Calothrix* sp. BTA431	242.55±0.10	3.23±0.09	7.34±0.00
35	*Calothrix* sp. BTA437	19.65±0.09	3.75±0.05	0.27±0.05
36	*Nostoc* sp. BTA439	263.40±0.03	4.87±0.08	1.77±0.04
37	*Aulosira* sp. BTA472	257.10±0.05	2.83±0.00	2.74±0.09
38	*Anabaena orientalis* BTA496	181.50±0.02	5.67±0.03	1.43±0.18
39	*Nostoc commune* BTA504	207.30±0.01	1.46±0.06	4.61±0.00
40	*Rivularia* sp. BTA510	265.20±0.08	0.71±0.08	**76.73±5.39**
41	*Aulosira prolifica* BTA514	325.50±0.01	3.70±0.05	2.49±0.05
42	*Anabaena* sp. BTA545	144.90±0.16	3.86±0.07	1.45±0.04
43	*Calothrix clavata* BTA550	327.00±0.05	6.44±0.05	0.66±0.00
44	*Anabaena* sp. BTA650	221.25±0.03	0.18±0.03	**111.08±13.26**
45	*Microchaete* sp. BTA673	257.85±0.18	3.55±0.07	3.41±0.02
46	*Cylindrospermum* sp. BTA674	373.95±0.02	3.34±0.05	2.80±0.18
47	*Nostoc* sp. BTA676	280.80±0.01	0.68±0.00	15.15±0.04
48	*Calothrix marchica* BTA680	251.85±0.04	2.37±0.07	2.16±0.07
49	*Nostoc calcicola* BTA698	329.55±0.17	0.63±0.00	14.94±0.18
50	*Anabaena oryzae* BTA791	387.90±0.02	1.65±0.04	10.23±0.03
51	*Wollea* sp. BTA797	315.60±0.18	1.70±0.04	1.79±0.07
52	*Anabaena orientalis* BTA809	319.50±0.19	1.84±0.07	5.65±0.00
53	*Anabaena torulosa* BTA815	330.00±0.18	2.59±0.04	3.03±0.01
54	*Nostoc calcicola* BTA818	335.85±0.09	3.25±0.03	6.91±0.06
55	*Nostoc hatei* BTA822	320.85±0.00	1.97±0.05	0.98±0.18
56	*Calothrix marchica* BTA828	346.50±0.12	4.94±0.05	6.40±0.00
57	*Aulosira* sp. BTA829	312.15±0.07	1.30±0.15	13.07±0.00
58	*Dichothrix* sp. BTA833	268.35±0.13	1.25±0.16	8.00±0.01
59	*Cylindrospermum* sp. BTA866	300.15±0.04	2.52±0.17	10.24±0.04
60	*Anabaena* sp. BTA893	295.35±0.01	1.65±0.09	9.09±0.00
61	*Anabaena* sp. BTA914	208.35±0.09	5.76±0.16	0.37±0.18
62	*Nostoc commune* BTA917	55.05±0.00	4.62±0.18	1.04±0.00

All experiments were replicated three times and results are presented as mean ±SD

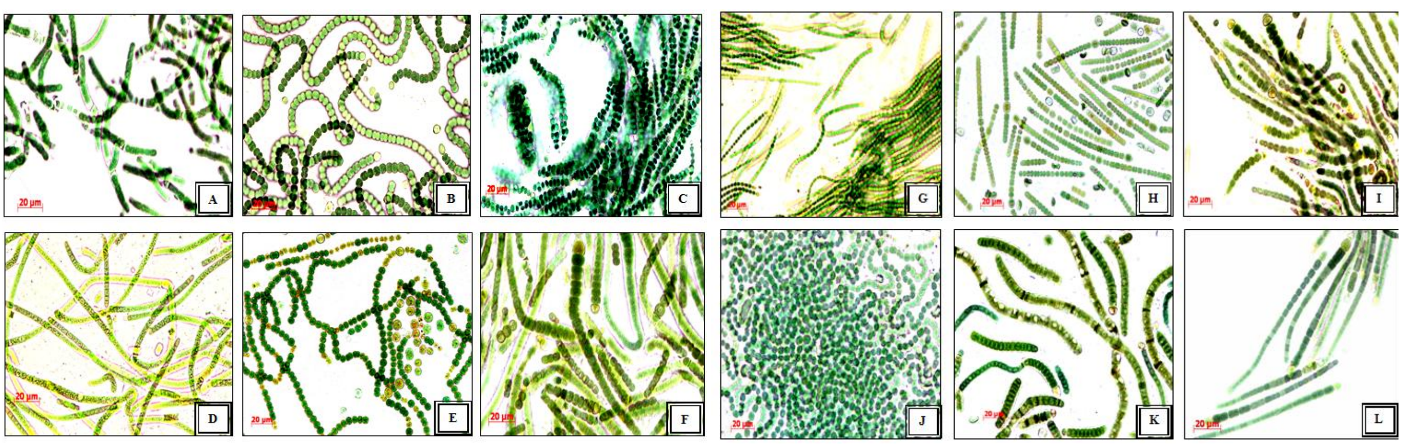

Fig 1: Photomicrograph of some selected diazotrophic nostocalean strains
A. *Scytonema bohneri*; **B.** *Nostoc* sp.; **C.** *Nostoc spongiaeforme*; **D.** *Tolypothrix byssoidea*; **E.** *Anabaena* sp.; **F.** *Microchaete* sp.; **G.** *Anabaena oryzae*; **H.** *Anabaena* sp.; **I.** *Calothrix* sp.; **J.** *Aulosira pseudoramosa*; **K.** *Scytonema hofmanni*; **L.** *Rivularia* sp.

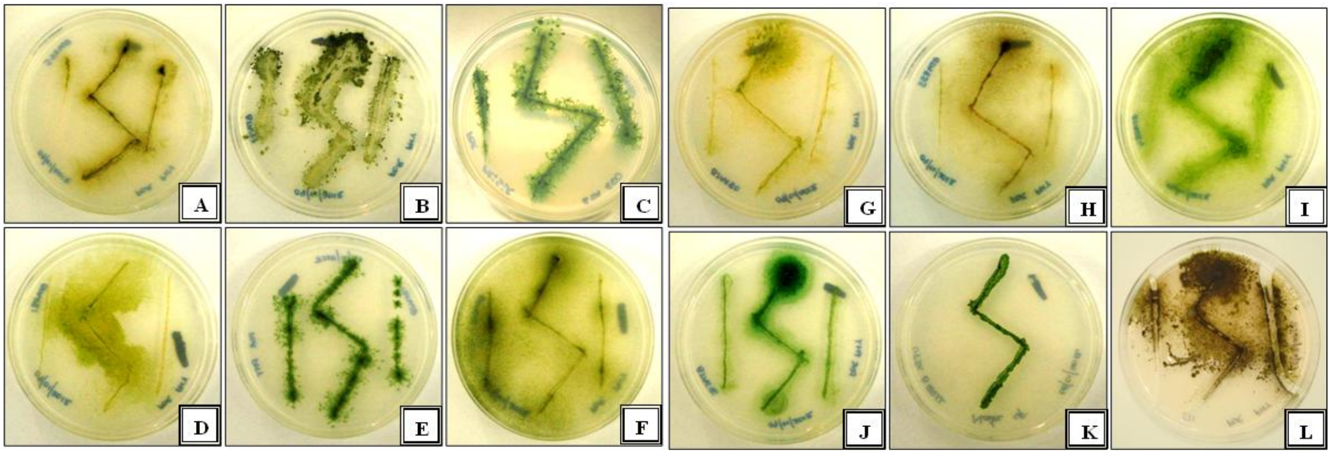

Fig. 2: Cultural behaviour of some selected diazotrophic strains on agar plate medium
A. *Calothrix* sp.; **B.** *Nostoc muscorum*; **C.** *Anabaena* sp.; **D.** *Anabaena* sp.; **E.** *Scytonema hofmanni*; **F.** *Rivularia* sp.; **G.** *Anabaena doliolum*; **H.** *Dichothrix* sp.; **I.** *Anabaena bergii*; **J.** *Nostoc commune*; **K.** *Nostoc* sp.; **L.** *Nostoc carneum*.

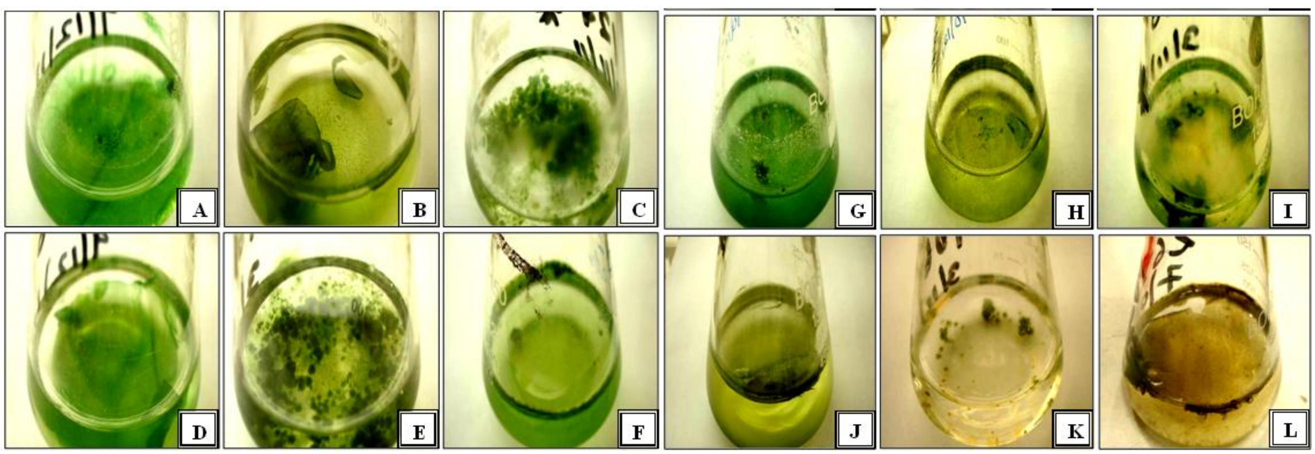

Fig. 3: Cultural behaviour of some selected diazotrophic strains in broth medium
A. *Anabaena* sp.; **B.** *Anabaena bergii*; **C.** *Scytonema hofmanni*; **D.** *Nostoc commune*; **E.** *Tolypothrix distorta*; **F.** *Anabaena* sp.; **G.** *Anabaena* sp.; **H.** *Nostoc calcicola*; **I.** *Anabaena doliolum*; **J.** *Nostoc muscorum*; **K.** *Scytonema bohneri*; **L.** *Calothrix* sp.

5. CONCLUSION

Among the various diazotrophic nostocalean cyanobacteria considered for the study, *Anabaena* sp. BTA650, an isolate of lithophytes under running water, Moreh, Chandel, Manipur exhibited highest nitrogenase activity of 111.08±13.26 nmole C_2H_4 μg^{-1} Chl-a h^{-1} which could be a potential candidate for biofertilizer formulation and application in the terraced hill rice field condition. In addition to this, *Calothrix* sp. BTA265, *Scytonema hofmanni* BTA124, *Rivularia* sp. BTA510, *Nostoc* sp. BTA197 and *Anabaena* sp. BTA281 also stand as promising candidates for biofertilizer formulation to enhance soil fertility and also minimized energy budget on nitrogen based chemical fertilizers production.

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