Algal Research 26 (2017) 56–64

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Phytohormones and free volatile fatty acids from cyanobacterial biomass wet extract (BWE) elicit plant growth promotion

Manickam Gayathri, Sumathy Shunmugam, Nooruddin Thajuddin, Gangatharan Muralitharan*

Department of Microbiology, Centre of Excellence in Life Sciences, Bharathidasan University, Palkalaiperur, Tiruchirappalli 620 024, Tamilnadu, India

ARTICLE INFO

Keywords:
Cyanobacteria
Multi-criteria decision analysis
Geometric analysis of interactive aid
Phytohormones
Plant growth promotion
Volatile organic compounds

ABSTRACT

The present study highlights the importance of cyanobacteria in plant growth promotion and screened the effect of biomass wet extract (BWE 1% and BWE 10%) of twenty cyanobacterial strains on the morphometric parameters of *Pisum sativum* L. seedlings after germination. Cyanobacterial BWE treatment significantly increased the radicle, plumule and total seedling length along with percentage of response than the control treatment with water. At BWE 1% treatment, *Scytonema bohneri* MBDU 104 showed 73.3% of the total seedling response, whereas *Dolichospermum spiroides* MBDU 903 showed increased total seedling length of 5.69 cm among other strains tested. All the tested strains varied among themselves at different parameters. To identify the most potent isolate, all the tested morphometric parameters were evaluated using a multi – criteria decision analysis (MCDA) of PROMETHEE-GAIA software. The isolate *Scytonema bohneri* MBDU 104 was selected as the potent plant growth promoting cyanobacteria and its BWE was purified using preparative – HPLC and analysed by LC-MS/MS for identifying possible plant growth elicitors. Phytohormones like indole-3 acetic acid (IAA), indole-3 butyric acid (IBA) and cinnamic acid along with free volatile fatty acids were identified and implicated for plant growth promotion by cucumber seed germination bioassay.

1. Introduction

With growing worldwide population and environmental damage due to rapid increase in industrialization, there will soon be greater demand to provide food for all people. To meet this global requirement, the world needs to greatly improve the agricultural productivity in a sustainable and eco-friendly manner. It is necessary to replace many existing agricultural practices and one best alternative is to use naturally occurring plant growth promoting bacteria (PGPB) in the agricultural field instead of all chemical growth promoters. PGPB include rhizobacteria [1], symbiotic rhizobia [2] and cyanobacteria, also known as blue-green algae [3,4]. These organisms promote plant growth and development directly either through nitrogen-fixation process, phosphate solubilization or modulating plant hormone levels or indirectly through minimizing the effects of phytopathogens on plant growth and development by acting as biocontrol agents [5,6].

The communication among the plants and their related microbes is of great interest, since these paved the way for new agricultural practices [7,8]. Cyanobacteria are the common inhabitant of the rice field ecosystem which fix the atmospheric nitrogen as well as maintain the soil texture and improve crop productivity [9]. Cyanobacterial diversity in diverse rice rhizosphere regions of India showed that genus belonging to *Nostoc* and *Anabaena* comprised 80% of isolates in addition to the existence of few heterocystous genera such as *Hapalosiphon*, *Westiellopsis* and *Calothrix* and non-heterocystous genera including *Phormidium* and *Oscillatoria* [10]. It is well evident that these cyanobacterial strains release varied quantities of phytohormones (auxins, gibberellins, cytokinins), polypeptides, aminoacids [11], polysaccharides [12] for plant growth and development [13,14] together with ammonia and small nitrogenous polypeptides during active growth of the cells, and other secondary metabolites after death and decomposition [15].

Though, the beneficial effects of cyanobacteria by treating with crude extracts on some vegetables, herbaceous and crops plants including *Solanum lycopersicum*, *Cucurbita maxima*, *Cucumis sativus* [16], *Oryza sativa* [17], *Triticum aestivum* [18,3], *Zea mays* [12,19], *Phaseolus* sp., *Cucumus melo* and *Lactuca sativa* [20] have been well established already, only few studies have characterized the chemical constituents responsible for plant growth promotion. In addition, little information is available for the selection and screening of most suitable plant growth promoting cyanobacterial strains that thrive on the rice field ecosystems. The aim of this study was to screen and select potential plant growth promoting cyanobacteria collected from varied rice field ecosystem of Tiruchirappalli and Thanjavur (rice bowl of Tamilnadu)

* Corresponding author.
E-mail address: drgm@bdu.ac.in (G. Muralitharan).

http://dx.doi.org/10.1016/j.algal.2017.06.022
Received 30 September 2016; Received in revised form 16 June 2017; Accepted 27 June 2017

Table 1
Cyanobacterial strains with their GenBank Accession numbers and source of isolation tested in this study for plant growth promoting activity.

S·No	Cyanobacterial strains	GenBank Accession no.	Source of isolation
1	*Scytonema bohneri* MBDU104	KU213647	Rice field, Poondi, Thanjavur 10.86° N, 78.93° E
2	*Calothrix* sp. MBDU901	KU213657	Rice field, Alakkudi, Thanjavur 10.76° N, 79.05° E
3	*Nostoc* sp.MBDU624	KX002066	Rice field, Mathur, Tiruchirappalli 10.71° N, 78.72° E
4	*Nostoc spongiaeforme* MBDU704	KX002067	Rice field, Tirukattuppalli 10.84° N, 78.95° E
5	*Nostoc commune* MBDU101	KU213644	Rice field, Poondi, Thanjavur 10.86° N, 78.93° E
6	*Anabaena* sp. MBDU902	KX002072	Rice field, Alakkudi, Thanjavur 10.76 N, 79.05 E
7	*Nostoc muscorum* MBDU702	KU213653	Rice field, Tirukattuppalli 10.84° N, 78.95° E
8	*Nostoc* sp. MBDU804	KX002070	Rice field, Budalur, Thanjavur, 10.47° N, 78.58° E
9	*Nostoc microscopicum* MBDU102	KU213645	Rice field, Poondi, Thanjavur 10.86° N, 78.93° E
10	*Dolichospermum spiroides* MBDU903	KX002071	Rice field, Alakkudi, Thanjavur 10.76 N, 79.05 E
11	*Nostoc Punctiforme* MBDU621	KU213648	Rice field, Mathur, Tiruchirappalli, 10.71° N, 78.72° E
12	*Calothrix brevissima* MBDU801	KU213651	Rice field, Budalur, Thanjavur, 10.47° N, 78.58° E
13	*Calothrix* sp. MBDU701	KU213652	Rice field, Tirukattuppalli 10.84° N, 78.95° E
14	*Nostoc sphericum* MBDU622	KU213649	Rice field, Mathur, Tiruchirappalli, 10.71° N, 78.72° E
15	*Aphanothece stagnina* MBDU803	KX002069	Rice field, Budalur, Thanjavur, 10.47° N, 78.58° E
16	*Nostoc linckia* MBDU623	KU213650	Rice field, Mathur, Tiruchirappalli, 10.71° N, 78.72° E
17	*Anabaena variabilis* MBDU103	KU213646	Rice field, Poondi, Thanjavur 10.86° N, 78.93° E
18	*Nostoc sp.* MBDU001	KU213655	Cycas sp., Tiruchirappalli, 11.02° N,79.00° E
19	*Nostoc commune* MBDU703	KU213656	Rice field, Tirukattuppalli 10.84° N, 78.95° E
20	*Nostoc commune* MBDU802	KX002068	Rice field, Budalur, Thanjavur, 10.47° N, 78.58° E

districts and to characterize the molecular elicitors through LC-MS/MS analysis.

2. Materials and methods

2.1. Isolation and purification of cyanobacterial strains

Twenty cyanobacterial isolates were collected from varied rice fields of Tiruchirappalli and Thanjavur district, Tamilnadu, India including a symbiotic *Nostoc* sp. from *Cycas* (Table 1). The non-axenic cyanobacterial isolates were purified by serially diluting the sample and by plating on to sterile BG-11_0 (without N_2, N_2-fixing) agar medium [21]. The plates were incubated up to 10 days at 25 ± 2 °C under continuous light (50 μmol $m^{-2} s^{-1}$). Individual colonies developed on the surface of the plates were picked using sterile inoculation loop and subcultured into 50 mL of BG-11_0 medium and incubated at 25 ± 2 °C under continuous light (50 μmol $m^{-2} s^{-1}$). This process was repeated several times until axenic cultures were obtained. The axenicity of cyanobacterial cultures were confirmed by microscopy and by plating in LB agar. Individual axenic cultures were grown in BG-11_0 medium and kept under continuous light (50 μmol $m^{-2} s^{-1}$) at 25 ± 2 °C. The cultures were periodically shaken by hand.

2.2. Characterization of cyanobacteria

Cyanobacterial isolates were characterized morphologically for generic assignments [21] using bright field (Optika, Italy) and confocal laser scanning microscope (CLSM) (LSM 710, Carl Zeiss, Germany) (Fig. 1). Total genomic DNA extraction and 16S rRNA gene amplification with a primer set of (A2F 5′-AGAGTTTGATCCTGGCTCAG-3′, S17R 5′-GGCTACCTTGTTACGAC-3′) were done with described methodology [22]. All PCR reactions were carried out in a 50 μL volume containing 1 μL (100 pmol) of each primer, 1 μL of 0.2 mM of dNTPs, 1 μL (100 ng) of cyanobacterial DNA and 1.25 U of Dream Taq DNA polymerase (Thermo scientific, USA) using Veriti 96-well thermal cycler (Applied Biosystems, USA). The PCR cycle for 16S rRNA primer was 94 °C for 5 min as an initial denaturation followed by 30 cycles of denaturation at 92 °C for 45 s, annealing at 58 °C for 1 min, extension at 72 °C for 1 min with a final extension at 72 °C for 7 min. The amplified and purified PCR product (High pure PCR product purification kit, Roche, Germany) was sequenced using an automated DNA sequencer (Applied Biosystem, USA). The sequences for all the isolates were submitted to the prokaryotic rRNA submission portal of GenBank and obtained the listed accession numbers in the Table 1. Sequence alignment and phylogenetic tree construction were carried out as described previously [22].

2.3. Preparation of cyanobacterial biomass wet extract

Two different concentrations of biomass wet extract (BWE 10% and BWE 1%) were prepared from all the tested cyanobacterial strains and used in the following experiments. Fresh biomass of 5 g and 0.5 g suspended in 5 mL and 0.5 mL of distilled water, respectively were ultrasonicated at 150 W for 5 mins on ice [23]. The resulting slurry was centrifuged at 10,000*g* for 30 min at room temperature. The supernatant was collected and made up to 50 mL using distilled water. The supernatant collected from 5 g and 0.5 g of fresh biomass in 50 mL of distilled water (*w/v*) is considered as BWE 10% and BWE 1% respectively [24].

2.4. Plant materials

Commercially available seeds of *Pisum sativum* L. var. Bonneville and *Cucumis sativus* L. var. Poinsette were used in this study.

2.5. Screening of cyanobacteria for growth promoting activity

All the tested cyanobacterial strains were screened for their plant growth promoting potential using seed germination assay following the method of Shende et al. [25]. The seeds of *P. sativum* L. were surface-sterilized in 70% ethanol for 30 s followed by 0.1% $HgCl_2$ solution for 5 min. Finally, they were rinsed several times with sterile distilled water before use [11]. After disinfection, germination of *P. sativum* L. seeds was carried out separately by spreading 10 seeds on filter papers placed in glass petri-dishes containing 15 mL of BWE 10% or BWE 1%. Petri dishes containing seeds with 15 mL of distilled water served as a control. Each treatment was carried out at three independent times ($n = 3$). Seeds of *P. sativum* L. were allowed to germinate on moist filter paper at 25 °C and 8 h light (15 mmol $m^{-2} s^{-1}$ warm fluorescent light) / 16 h dark cycle for 8–10 days. The morphometric parameters of seeds such as percentage of germination responses, including radicle, plumule and total seedling responses, radicle length, plumule length and total seedling length were analysed. The formulas used to calculate percentage of germination responses are as follows:

1. % of radicle response = (No. of radicle formed / Total no. of