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Isolation and Characterization of Heterotrophic Bacteria from Blue-Green Algae with Multiple Plant Growth Promoting Traits

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Abstract

Blue-green algae (BGA) are unique photosynthetic prokaryotes, which can survive and function under extreme conditions. BGA are associated with heterotrophic bacteria, which together play a distinct role in soil and plant development. Despite the high diversity of the heterotrophic bacteria associated with beneficial BGA, little is known about their potential ecological role. Thus, the present study aimed to obtain and identify BGA and their cultivable associated heterotrophic bacteria (AHB) as well as *in vitro* characterization of the obtained AHB isolates. Two nitrogen-fixing BGA were isolated from desert soil and identified as *Anabaenopsis circularis* strain AnHu and *Nostoc* sp. strain NoHu. Based on the colony morphology, 51 and 12 bacterial isolates were isolated from AnHu and NoHu, respectively, and screened for plant growth-promoting activities (PGPAs). Ten promising AHB isolates that displayed multifunctional ability were further identified using 16S rRNA gene sequence. The analysis revealed that the 6 and 4 identified AnHu- and NoHu-AHB isolates, were affiliated to 4 and 2 different phyla, respectively, indicating high diversity of cultivable AHB. The identified AHB isolates showed identical sequences with NCBI strains known for their functionality as plant growth promoters (PGPs) under harsh environmental conditions. Our results suggest that BGA-AHB are useful as an opulent source for PGPs in Egyptian agricultural arid soil.

Keywords: Blue-green algae (BGA); Associated heterotrophic bacteria (AHB); *Anabaenopsis circularis*; *Nostoc sp*.; plant growth; arid area.

1. Introduction

Blue-green algae (BGA) are considered the oldest form of life on Earth. They are ubiquitous microorganisms that can be found in almost all environments. BGA possess an incredible ability to survive and function in harsh condition. In addition to oxygenic photosynthesis, many BGA display nitrogen fixation [1]. They play environmental and ecological key roles in building up and improving soil fertility, enhancing plant development and yield, and increasing nutrient availability via, for instance, nitrogen fixation, phosphorus and phosphate solubilization, contributing to bioremediation and maintaining a sustainable ecosystem. However, the ecological importance of BGA along with their remarkable features are widely recognized, particularly, concerning being a natural resource for sustainable agriculture and environment [2].

Thanks to the variation in morphological, physiological, as well as, genetic traits, BGA releases a variety of compounds including mono and polysaccharides, vitamins, hormones, polypeptides, organic acids, and other different secondary metabolites. On one hand, these released metabolites support the presence of heterotrophic bacteria in association with BGA [3]. On the other hand, these associated heterotrophic bacteria (AHB) have been reported to promote the growth of BGA such as in cell size, biomass, growth rate, and productivity. Despite the remarkable diversity of the BGA-AHB, little is known concerning their potential contribution

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to the environmental role [4]

However, the distinct features of BGA along with their remarkable diversity, as well as, the diversity of the microbial community associated with BGA, make the last attractive microenvironment to be investigated. Therefore, the present study aimed to reveal the hidden potentials of the BGA-AHB. For that, different BGA were isolated from arid soil and identified. Heterotrophic bacteria associated with the obtained BGA were enriched, based on the utilization of BGA-released metabolites, and isolated, as well. Obtained bacterial isolates were characterized regarding their direct and indirect plant growth-promoting activities (PGPAs), such as nutrient availability and antifungal activity against some common phytopathogens. The taxonomic affiliation of BGA-AHB isolates, which displayed diverse PGPAs, were identified by employing 16S rRNA gene amplification and sequencing.

2. Materials and Methods

2.1. Isolation and purification of BGA from soil

Different BGA were isolated from desert soil at Sekem farm, Belbis, Egypt. Five grams of sieved soil (<2 mm) were transferred to an Erlenmeyer flask containing 45 ml of sterilized NaCl solution (0.85%). After thoroughly shaking, 1 ml of soil suspension was inoculated into a sterilized liquid nitrate-free BG-11_0 medium [4,5] and incubated at room temperature under continuous dark and sunlight periods for 3 weeks to enrich the BGA. Flasks were examined under a microscope and sub-cultured to a new sterilized solid nitrate-free BG-11_0 medium supplemented with 0.16 mM of cycloheximide to purify BGA from fungi and other eukaryotic microorganisms [7]. To obtain pure BGA isolates, sub-culturing step was repeated several times on solid-liquid alternate cultures. Pure isolates were grown in a sterile liquid BG-11_0 medium for further experimental processes.

2.2. Isolation of BGA-AHB

To obtain the cultivable bacterial isolates associated with BGA, cultures were prepared by inoculating 2 ml of BGA into 100 ml of sterilized tap water, where the BGA metabolites are the only source for bacterial nutrition. After 3 weeks of incubation at room temperature under continuous dark and sunlight periods, serial dilutions were prepared in a sterile NaCl solution (0.85%) from each BGA culture. One hundred microliters of each 10^{-3}, 10^{-4}, and 10^{-5} dilutions were plated onto nutrient agar (NA) medium. Bacterial colonies were picked, based on their colony morphology, after 48 – 72 h incubation at 30℃ and purified isolates were kept at -80℃ in a nutrient broth medium containing 20% glycerol for further study [8].

2.3. In vitro, qualitative bioassay for plant growth-promoting traits of the BGA-AHB isolates

2.3.1. Nitrogen fixation

All bacterial isolates were screened for nitrogen fixation ability using N-deficient combined carbon sources medium (CCM) [9]. Fresh bacterial isolates were streaked on CCM agar plates and incubated at 30℃ for 48 – 72 h. Successfully grown bacterial isolates were further sub-cultured on the corresponding medium up to three times. Bacterial isolates that were able to grow on N-deficient CCM medium were recorded as nitrogen-fixing bacteria.

2.3.2. Phosphate solubilization

Screening BGA-AHB isolates for phosphate solubilization capacity was carried out using National Botanical Research Institute Phosphate medium (NBRIP) [10], amended with tricalcium phosphate $Ca_3(PO_4)_2$ as an insoluble source of phosphorus. Fresh bacterial isolates were spot inoculated (5 µL) onto NBRIP agar plates with triplicates and incubated at 37℃ for 7 days [11]. Bacterial isolates with a clear halo zone were recorded as phosphate-solubilizing bacteria.

2.3.3. Potassium solubilization

Potassium solubilization capacity was tested using an Aleksandrov agar medium, amended with potassium feldspar powder as an insoluble source of potassium [12]. Fresh bacterial isolates were spot inoculated (5 µL) with triplicates onto Aleksandrov agar plates and incubated at 30℃ for 7 days [13]. Bacterial isolates with a clear halo zone were recorded as potassium-solubilizing bacteria.

2.3.4. Zinc solubilization

Bacterial isolates were tested also for their zinc solubilization capacities. For this purpose, two insoluble sources of zinc, zinc carbonate ($ZnCO_3$) and zinc oxide (ZnO), were tested. Plates of tris-minimal salt medium supplemented with 0.1% insoluble zinc compound ($ZnCO_3$ or ZnO) were prepared and fresh bacterial cultures were spot-inoculated (5 µl) with triplicates. After incubation at 30°C for 7 days, bacterial isolates that showed a clear halo zone were recorded as zinc-solubilizing bacteria [14].

2.3.5. Siderophores production

Bacterial isolates were checked for siderophore-producing ability by universal chrome azurol sulfonate (CAS) assay. This assay was carried out using Hu and Xu's modified method [15]. One hundred ml of CAS reagent was mixed with 900 ml of sterilized Luria-Bertani agar medium to make CAS agar plates. Bacterial isolates inoculated onto CAS plates were incubated at 30°C with an un-inoculated plate that was used as a control. After 5–7 days, bacterial isolates that produced orange zone were recorded as siderophores-producing bacteria [16].

2.4. In vitro, assessment for antifungal activity of the BGA-AHB isolates

Bacterial isolates were tested for antifungal activity against three common phytopathogens, *Fusarium oxysporum*, *Fusarium* sp., and *Aspergillus niger* using a dual culture assay on a potato dextrose agar (PDA) (Merck KGaA, Darmstadt, Germany) medium. Mycelial agar discs (0.5 cm diameter) of the tested phytopathogens were taken from 7-day-old fungus plates and placed on one side of the PDA plates, about 3 cm from the edge. A loopful of each bacterial isolate was streaked 3 cm away from the mycelia disc from an overnight bacterial culture. Plates with only fungal mycelial discs for each of the tested phytopathogens were inoculated as controls. The antagonistic activity was calculated by measuring the size of the growth inhibition zone after 5–7 days of incubation at 25°C [17], and the percentage of growth inhibition (PGI%) was determined using the formula [18]:

$$PGI\% = (R_2-R_1)/R_1 \times 100$$

Where R_1 is the average radius of each fungus in the treatment, and R_2 is the average radius of each fungus in the control plate.

2.5. BGA and their AHB genomic DNA extraction and 16S rRNA gene-PCR amplification

2.5.1. DNA extraction

Pellets were prepared using 5 ml of each 2-week-old BGA culture by centrifugation at 8000 rpm for 5 min [19]. Genomic DNA was extracted from each BGA isolate using a QIAamp DNA mini kit (Qiagen Inc. Valencia CA) following the manufacturer's instructions.

Genomic DNA was obtained from each BGA-AHB isolate by boiling method [20]. Bacterial cells, grown on NA medium for 24 h, were harvested and washed three successive times with 1 ml 0.85% NaCl then pellets were prepared by centrifugation at 14000 rpm for 5 min. Obtained pellets were resuspended in 1 ml distilled water, boiled for 15 min in a water bath at 100℃, then centrifuged for 5 min at 14000 rpm. Five hundred microliters of the supernatant, which contains genomic DNA, were stored at -20℃.

2.5.2. PCR conditions

PCR amplification of 16S rRNA gene was performed using BGA-specific primers, CYA106F (5′-CGG ACG GGT GAG TAA CGC GTG A-3′) and CYA781R (5′-GAC TAC WGG GGT ATC TAA TCC CWT T-3′), while primers U8-27 (5′-AGA GTT TGA TC(AC) TGG CTC AG-3′) and R1494-1514 (5′-CTA CGG(T/C) TAC CTT GTT ACG AC-3′) were used for bacterial specific 16S rRNA gene amplification. The PCR reactions were carried out in a total volume of 25 µl reaction volume containing 2 µl of each forward and reverse primer, 12.5 µl PCR master mix (Fermentas, Thermo Scientific, MA, USA), 6.5 µl nuclease-free water, and 2 µl DNA template in addition to 2 µl nuclease-free water that was added instead of the DNA template and used as a negative control. Thermal cycling programs were performed according to Nübel [21] for BGA (5 min initial denaturing at 94℃, followed by 30 cycles of denaturation at 94℃ for 1 min, annealing at 60℃ for 1 min, and extension at 72℃ for 2 min, and a final extension period of 10 min at 72℃) and Topalović [22] for bacterial 16S rRNA gene (5 min initial denaturing at 94℃, followed by 30 cycles of

denaturation at 94℃ for 1 min, annealing at 56℃ for 1 min, and extension at 72℃ for 2 min, and a final extension period of 10 min at 72℃). The yield of the PCR products was checked via electrophoresis using 0.8% agarose gel and visualized after staining with ethidium bromide using a gel documentation system (Phase, Lubeck, Germany) [23]. PCR products were purified following the QIAquick gel extraction kit (Qiagen) protocol. Purified PCR products were sequenced at the Animal Health Research Institute (AHRI), Giza, Egypt. The 16S rRNA sequences of each BGA as well as BGA-AHB isolates were deposited in the NCBI GenBank database.

2.6. Sequence data analysis

Obtained sequences, BGA and BGA-AHB, were analyzed using the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/) [24]. Phylogenetic trees comprise 16S rRNA gene nucleotide sequences of two different BGA or 10 BGA-AHB isolates were constructed via a neighbor-joining method using MEGA v5.2. Included in the tree are 4 and 28 nucleotide sequences from the NCBI GenBank database, representing the closest hits with BGA and BGA-AHB, respectively. To visualize the total 63 BGA-AHB with their PGPA(s) and the differences on a phylogenetic basis (for BGA and BGA-AHB), the tree was saved in Newick format and visualized with an interactive tree of life (iTOL v3) (https://itol.embl.de) [25].

3. Results

3.1. BGA isolation and identification

Two morphologically different BGA, designated as AnHu and NoHu, were isolated from desert soil and characterized as nitrogen-fixers, based on their ability to grow in liquid nitrate-free BG-11_0 medium. 16S rRNA gene was amplified from both BGA isolates and sequenced. A total of 584 and 411 bp were subjected to GenBank BLAST search analysis and revealed 100% identity and 95.86% similarity for the isolates AnHu and NoHu with the strains *Anabaenopsis circularis* and *Nostoc carneum*, respectively. A neighbor-joining phylogenetic tree was constructed with the 16S rRNA gene sequences for both BGA obtained in the present study and the four closest hits from the NCBI GenBank database and confirmed via bootstrap analysis (Figure 1). However, AnHu- and NoHu-BGA isolates are affiliated to Aphanizomenonaceae and Nostocaceae families, respectively.

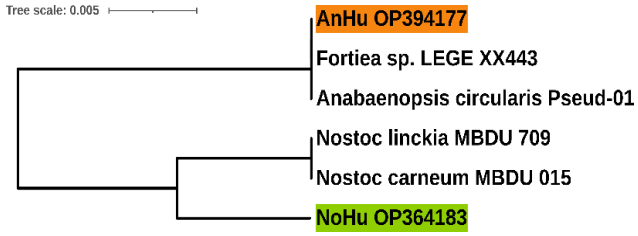

Fig. (1): Phylogenetic tree based on 16S rRNA gene sequences showing the relationships among the two BGA strains with previously isolated strains from NCBI. The analysis was conducted with MEGA using the neighbor-joining method. BGA strains were submitted to GenBank as AnHu and NoHu, as shown in orange and green highlights, respectively, with their accession number using iTOL.

3.2. Isolation of cultivable BGA-AHB

Heterotrophic bacteria, enriched by exploiting the metabolites of AnHu and NoHu-BGA strains as a sole source of nutrition, were isolated. Based on the colony morphology, a total of 51 and 12 bacterial isolates were picked from liquid cultures of BGA strains AnHu and NoHu, respectively.

3.3. In vitro, qualitative bioassay for plant growth-promoting traits of the BGA-AHB isolates

3.3.1. Nitrogen fixation

Among the tested 63 bacterial isolates, only 6 showed the ability to grow on nitrogen-free CCM medium. All potential nitrogen fixers were among the AnHu-AHB isolates, representing 11.8%, while none of the NoHu-AHB isolates was able to grow in the absence of nitrogen.

3.3.2. Phosphate, potassium and zinc solubilization

The capacity of 63 BGA-AHB for solubilizing inorganic phosphate and potassium was tested through the formation of a clear halo zone around their colonies on NBRIB and Aleksandrov agar media, respectively. Amongst, phosphate solubilizing capacity was recorded for 11 AnHu-AHB and 2 NoHu-AHB isolates, representing 21.6% and 16.7%, respectively. Potassium solubilization ability was exhibited by 8 AnHu-AHB and 1 NoHu-AHB isolate, representing 15.7% and 8.3%, respectively. However, none of the tested bacterial isolates showed zinc solubilization ability.

3.3.3. Siderophores production

The siderophore production ability was examined for 63 BGA-AHB using CAS blue agar assay. The orange-colored zone was recorded for 20 and 3 AnHu- and NoHu-AHB isolates, representing 39.2% and 25%, respectively.

3.4. In vitro, assessment for antifungal activity of the BGA-AHB isolates and morphological observation

3.4.1. The antagonistic test

Antifungal activity of obtained BGA-AHB isolates was tested against *F. oxysporum*, *Fusarium* sp., and *A. niger* fungi. The dual culture plate assay revealed that 60.3% of the tested BGA-AHB isolates inhibited mycelial development of at least one of the tested phytopathogens at different rates. However, a total of 34, 25, and 20 bacterial isolates showed antagonistic activity against *F. oxysporum*, *Fusarium* sp., and *A. niger*, respectively. The highest number of antagonists was recorded against *F. oxysporum*. The greatest antagonism rates were shown by the DE7AnHu, DE43AnHu, and DE33AnHu bacterial isolates against *F. oxysporum*, *Fusarium* sp., and *A. niger* representing 73.33%, 62.42%, and 61.82%, respectively, as shown in (Table 1).

Table (1): The table shows the most promising bacterial isolates with antifungal activities against three different phytopathogens.

Phytopathogens	Bacterial code	PGI%	Inhibitory activity
F. oxysporum	DE7AnHu	73.33%	++
	DE45AnHu	72.73%	++
	DE39AnHu	71.52%	++
Fusarium sp.	DE43AnHu	62.42%	++
	DE6AnHu	52.73%	+
	DE39AnHu	50.91%	+
A. niger	DE33AnHu	61.82%	++
	DE39AnHu	54.55%	+
	DE43AnHu	52.12%	+

+, ++ and +++ represent relative mycelial growth inhibition rates for each fungal colony on potato dextrose agar medium. Inhibition rates were recorded as follows: +, <60%; ++, 60-80%; and +++, >80%.

3.4.2. Morphological observation of the BGA-AHB isolates

Further characterization, based on colony morphology description, was performed for the selected 10 BGA-AHB isolates that exhibited more than a single PGPA. Most of the AnHu-AHB isolates displayed circular, raised, undulate, opaque, confined, paste-like, and pigmented/non-pigmented colony morphology, while most of the NoHu-AHB isolates showed circular, convex, entire, translucent, confined, shiny, and pigmented as shown in (Table 2).

3.5. 16S rRNA Identification of selected BGA-AHB isolates

All the PGPA results of the obtained 63 BGA-AHB isolates are shown in (Figure 2), the most potent 10 bacterial isolates were then selected for further identification, and visualized in a neighbor-joining phylogenetic tree including the 16S rRNA gene sequences obtained from the selected 10 BGA-AHB strains and the 28 closest hits from the NCBI GenBank database, as shown in (Figure 3).

Molecular identification of 16S rRNA gene was performed for 10 selected isolates, 6 AnHu-AHB and 4 NoHu-AHB, which displayed multifunction related to direct and indirect plant growth promotion. All the identified 10 AHB strains are shown in (Table 3) with their most similar strain from NCBI. Therefore, the strains represented a diverse group of AHB in both AnHu and NoHu. Analysis of 16S rRNA gene fragments (between 492 - 691 bp) showed affiliation of AnHu-AHB within 4 phyla (Proteobacteria, Bacillota, Pseudomonadota, and Actinomycetota) while the NoHu-AHB were affiliated to 2 phyla (Pseudomonadota and Actinomycetota). At the genus level, almost all bacterial isolates exhibited identical 16S rRNA gene sequences with species belonging to 3 and 4 different genera for AnHu- and NoHu-AHB, respectively. However, the majority of AnHu-AHB strains were assigned to three different genera, *Bacillus* (3 out of 6) followed by *Pseudomonas* (2 out of 6), whereas a single strain was assigned to *Kocuria*. As for NoHu-AHB, the four strains represented four different genera *Achromobacter*, *Rhizobium*, *Sphingomonas*, and *Gordonia*.

Table (2): Colony morphology of selected potent BGA-AHB isolates with AnHu and NoHu.

BGA	Bacterial Strains	Appearance	Elevation	Margin	Transparency	Spreading	Consistency/ Texture	Pigmentation
Strain AnHu	DE11AnHu	circular	raised	entire	translucent	confined	shiny	pigmented (pale red)
	DE6AnHu	irregular	raised	undulate	opaque	confined	paste-like	non-pigmented (white)
	DE10AnHu	circular	raised	undulate	transparent	confined	shiny	pigmented (pale yellow)
	DE1AnHu	irregular	growth into medium	undulate	translucent	semi-spreading	dry	pigmented (pale yellow)
	DE2AnHu	curled	umbonate	entire	opaque	semi-spreading	paste-like	non-pigmented (white)
	DE7AnHu	circular	raised	filamentous	opaque	confined	paste-like	non-pigmented (creamy)
Strain NoHu	ED3NoHu	circular	raised	entire	translucent	confined	shiny	non-pigmented (pale white)
	ED8NoHu	circular	convex	entire	translucent	confined	shiny	pigmented (yellowish orange)
	ED5NoHu	circular	convex	entire	translucent	confined	shiny	non-pigmented (creamy)
	ED9NoHu	circular	convex	entire	translucent	confined	shiny	pigmented (pale pink)

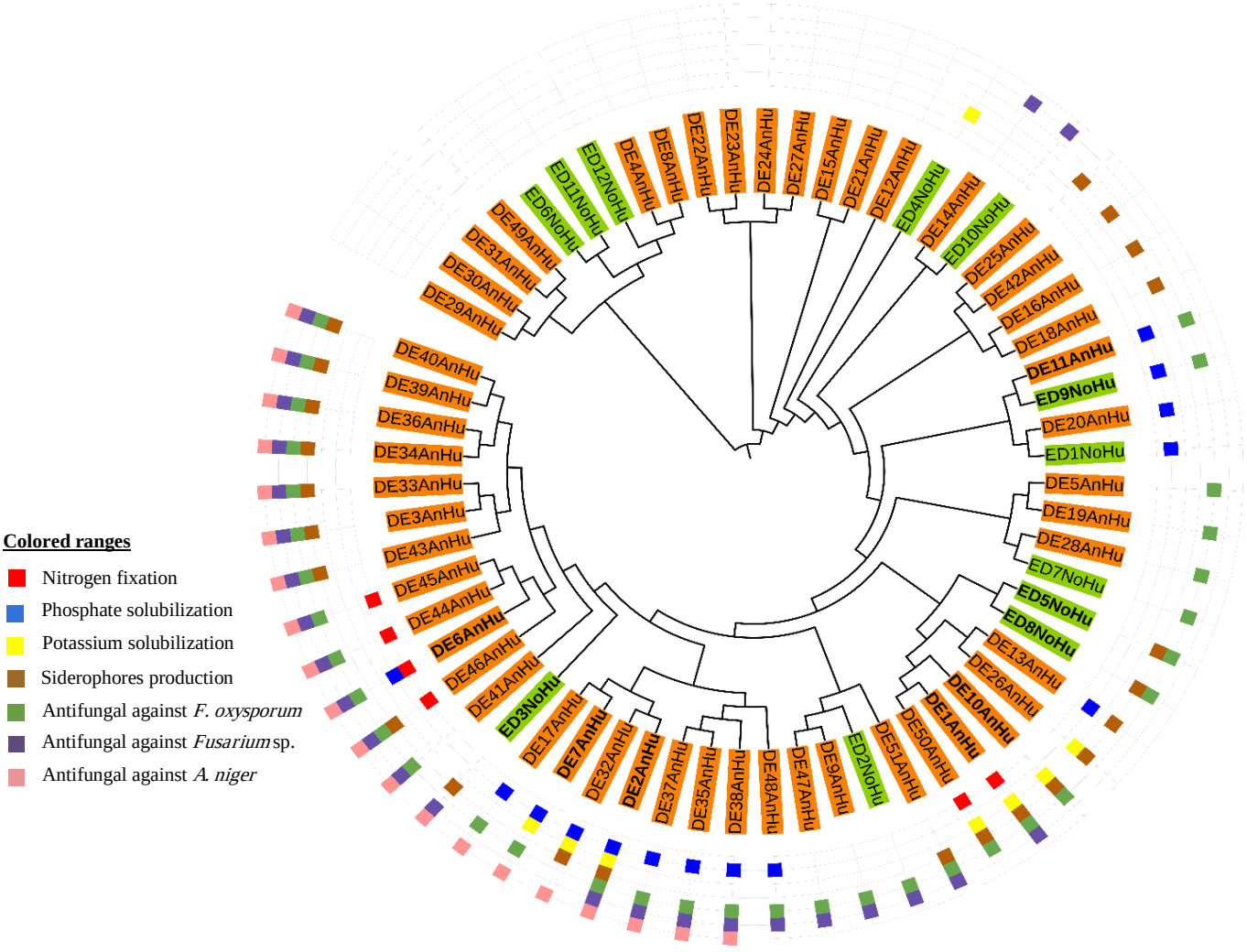

Fig. (2): Phylogenetic tree was constructed from a total 63 bacterial isolates based on their PGPA(s) using iTOL. AHB isolated from AnHu and NoHu are shown in orange and green highlights, respectively. The identified multifunctioning isolates are presented in Bold. PGPAs are displayed as rectangular shapes with different colors in consecutive rings outside the tree.

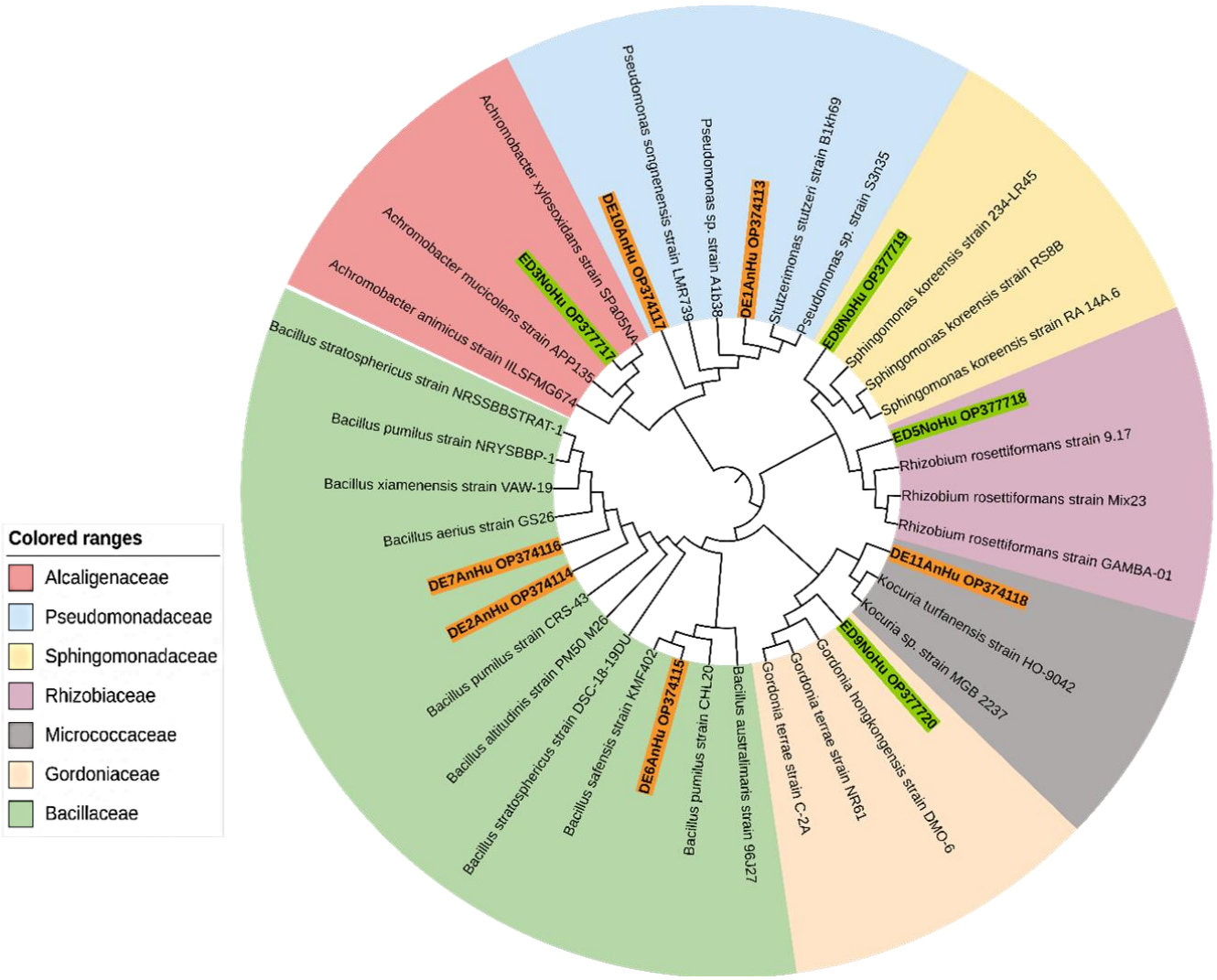

Fig. (3): Phylogenetic tree showing the relationships among different bacterial strains with previously isolated strains from NCBI. Bacterial strains were submitted to GenBank. The analysis was conducted with MEGA using the neighbour-joining method. Highlighted bacterial strains with their accession number were used in the current study. BGA-AHB strains isolated from AnHu and NoHu are in bold and highlighted in orange and green colors, respectively, using iTOL. Different families are indicated with different

Table (3): Similarity of the AHB strains with previously isolated strains from NCBI and their accession number

BGA	AHB strains	Similar with NCBI	Accession number
AnHu	DE1AnHu	*Pseudomonas stutzeri* (99.84%)	OP374113
	DE2AnHu	*Bacillus stratosphericus* (100%)	OP374114
	DE6AnHu	*Bacillus safensis* (100%)	OP374115
	DE7AnHu	*Bacillus stratosphericus* (100%)	OP374116
	DE10AnHu	*Pseudomonas songnenensis* (100%)	OP374117
	DE11AnHu	*Kocuria turfanensis* (100%)	OP374118
NoHu	ED3NoHu	*Achromobacter xylosoxidans* (100%)	OP377717
	ED5NoHu	*Rhizobium rosettiformans* (100%)	OP377718
	ED8NoHu	*Sphingomonas koreensis* (100%)	OP377719
	ED9NoHu	*Gordonia terrae* (100%)	OP377720

4. Discussion

In the present study, two morphologically different BGA isolates were obtained from desert soil and identified as Anabaenopsis circularis strain AnHu and Nostoc sp. strain NoHu, based on 16S rRNA sequencing. Both strains are widely tolerated saline and drought stresses. Their application for enhancing crop productivity in agricultural saline soil remediation was previously reported [24-27]. BGA are extensively colonized by a high diversity of AHB that provide BGA with key nutrients [30]. Several studies have investigated the microbial community structure associated with BGA and their role in driving ecosystem functionality, focusing in particular on BGA from aquatic systems [1,28,29]. However, there is a gap concerning the beneficial heterotrophic bacteria grown in association with terrestrial BGA and their potential role as plant growth promoters. Therefore, in the present study, heterotrophic bacteria associated with two different terrestrial BGA were isolated, characterized, and identified.

Almost 74.6% of the obtained bacterial isolates showed at least a single plant growth promotion-related trait, while 11.8% and 33.3% from strains AnHu and NoHu, respectively, displayed multifunction. Although different bacterial species were identified as plant growth promoters, their action is commonly unstable under field conditions [33]. The low competition of the introduced bacterial isolates in the soil stressful conditions is a proposed reason behind the limited efficiency. Interestingly, almost all the obtained bacterial isolates showed the closest similarity or even identity to strains known for their functionality as plant growth promoters under harsh environmental conditions. For instance, strains Pseudomonas songnenensis, Achromobacter xylosoxidans, Bacillus safensis, Pseudomonas stutzeri, and Kocuria turfanensis, were reported before as good candidates for developing effective biofertilizers for crops in saline soils [34]. In addition, Rhizobium rosettiformans was reported to facilitate the growth of plants under normal and drought conditions [32,33], while Tanveer & Ali [37] reported the potency of Pseudomonas songnenensis to enhance plant growth in alkaline soil. Sphingomonas koreensis was isolated before from Egyptian oily soil and was found to degrade aliphatic and polyaromatic hydrocarbons [38].

In addition to the plant growth promotion properties, all obtained bacterial isolates have shown antifungal activity against F. oxysporum, Fusarium sp., and A. niger phytopathogens. Bacillus stratosphericus was found in a previous.

A study that has a broad spectrum in inhibiting the growth of some common phytopathogens [39]. Thus, the results indicate that BGA-AHB exhibit an indirect plant growth promotion mode of action.

Despite numerous plant growth-promoting bacteria having been isolated from various plant species, including wild and cultivated plants still, the efficiency of these bacteria is commonly limited under field conditions [33]. Therefore, the frequently reported effects of these strains in saline and alkaline soil suggest their great potential as effective biofertilizers in Egyptian agricultural soil, particularly in arid areas.

The 16S rRNA sequences analysis showed that the obtained bacterial isolates are affiliated to diverse phyla, classes, orders, families, genera, or even species. The affiliation of the bacterial isolates into different families suggests the high diversity of cultivable bacteria that can grow in association with different BGA strains. Moreover, Pseudomonas songnenensis showed 16S rRNA identity to the newly described bacterial strain. However, obtained results suggest that beneficial BGA have great potential as an unlimited source for diverse valuable and/or novel bacterial species.

5. Conclusions

Our results shaded the light on the PGP potentials of the BGA-AHB that might contribute along with BGA to their remarkable role in enhancing plant growth. *In vitro*-based characterization of BGA-AHB, isolated in the present study, revealed a different PGP capacity as 74.6% of the cultivable BGA-AHB isolates exhibited at least a single plant growth-related activity. Interestingly, 16S rRNA gene sequence analysis revealed the notable diversity of cultivable AHB, indicated by their affiliation to 4 different phyla, in addition to the BGA species-dependent diversity. Moreover, BGA-AHB isolates showed antifungal activities against some common phytopathogens, suggesting the valuable role of BGA-AHB in enhancing plant growth performance either directly or indirectly. The 16S rRNA sequence identity of BGA-AHB with known soil stresses

tolerant, as well as, newly isolated bacteria propose their great potential as biofertilizers adequate for arid soils. However, the above-mentioned results strongly suggest the need of exploring the hidden and valuable potentials of BGA-AHB.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

No funding sources.

8. References

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