Philippine Journal of Science
154 No. 1: 93-107, February 2025
ISSN 0031 - 7683
Date Received: 28 Aug 2024

In Silico Genome Mining of *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 Revealed Biofertilizer-essential Genes

Vienna Ai Marie V. dela Cerna*, Ernelea P. Cao, Libertine Rose S. Sanchez, Danica Pearl M. Untiveros, Maria Theresa T. Tengco, and Daisy May C. Santos

Plant Molecular Biology and Genetics Laboratory, Institute of Biology,
College of Science, University of the Philippines Diliman,
Quezon City 1101 the Philippines

The increasing price of inorganic fertilizers and their long-term detrimental effects on the environment accentuate the need for organic fertilizer supplementation. One emerging solution is the use of biofertilizers. Biofertilizers are microbial-based fertilizers that enhance plant growth, increase product yield, and produce better grain or fruit quality of crops while improving soil health. In this study, two cyanobacterial strains – namely *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 – were mined for biofertilizer-essential genes. The whole genomes of the strains were sequenced and assembled. The assemblies were then used for functional annotation, metabolic profiling, and biosynthetic gene cluster (BGC) mining. Downstream analyses revealed genes associated with nitrogen fixation and metabolism, nutrient solubilization and mobilization, phytohormone auxin production, and biostimulant/biocontrol compound production. Both strains contained the nitrogenase gene cluster *Nif* and genes that metabolize urea, nitrate/nitrite, cyanate, and allantoin into ammonia/ammonium (NH_3/NH_4^+). Genes that convert organophosphate to phosphate (PO_4^{3-}) and transport PO_4^{3-} and potassium (K^+) into and out of the cell were also identified. The detected auxin genes belong to the tryptophan gene cluster *Trp* and additional genes monoamine oxidase in PGN35 and aromatic-L-amino-acid decarboxylase in ULAP02. Predicted bioactive metabolites in *Nodosilinea* sp. PGN35 were terpene, phenazine, arylpolyene, and resorcinol, whereas in *Chlorogloeopsis* sp. ULAP02 were terpene, resorcinol, phosphonate, PKS/NRPS/hybrid, indole, and RiPPs. These metabolites are known to have agricultural roles as stimulants, biocides, and signaling molecules for plant-insect interaction. The presence of all these plant growth-stimulating and biocontrol genes in the two cyanobacterial strains indicates their potential as biofertilizers. This is the first study to explore the biofertilizer potential of *Nodosilinea* and *Chlorogloeopsis* using the genome-mining approach.

Keywords: biofertilizer, *Chlorogloeopsis*, *Nodosilinea*, plant growth-stimulating metabolites

*Corresponding author: vvdelacerna@up.edu.ph

INTRODUCTION

High agricultural input cost is one of the existing problems that affect farmers. The high price of inorganic fertilizers and the low product buying price in the market usually results in very low profits for the majority of small-scale farmers. At the same time, the use of inorganic fertilizers results in environmental degradation and unsustainable agriculture. One of the solutions that the Philippine government initiated and implemented is the Balanced Fertilization Strategy (BFP) program. This program pushes for the institutionalized use of organic fertilizers alongside inorganic fertilizers for more sustainable agricultural production (DA-FPA 2022). Aside from enhancing crop yield, organic fertilizers also contribute to soil health by providing organic matter and improving the soil's physical properties (Purbajanti *et al*. 2019). Organic fertilizers also ameliorate the negative effects of long-term inorganic fertilizer use such as soil acidification and salinization, nutrient leaching, decrease in soil total carbon and moisture content, and decrease in beneficial microbe diversity and abundance (Ramírez-López *et al*. 2019; Goemann *et al*. 2021; Hu *et al*. 2022; Pan *et al*. 2022).

Microbial fertilizers or biofertilizers are among the products recommended for use in the BFP program. They are more advantageous than conventional organic fertilizers (*e.g.* manure and agricultural waste) because they contain microorganisms that contribute to nutrient solubilization and mobilization, form biofilms in plant roots that protect the plant from biotic and abiotic stressors, produce phytohormones that improve plant growth, and secrete exopolysaccharides that improve soil aggregation and composition (Kholssi *et al*. 2021; Jan *et al*. 2023; Omer *et al*. 2023). Different microbial strains or consortia are now being studied worldwide as potential biofertilizers, especially those that belong to the plant-growth-promoting rhizobacteria (PGPR), the arbuscular mycorrhizal fungi (AMF), and the diazotrophic cyanobacteria (Múnera-Porras *et al*. 2020; Prasanna *et al*. 2021). Microbial species belonging to the genera *Bacillus*, *Pseudomonas*, *Rhodopseudomonas*, and *Azospirillum* are among the currently studied and marketed PGPR for agricultural use (Vejan *et al*. 2016). These microorganisms produce phytohormones and other secondary metabolites with antipathogenic properties, as well as increase the availability of nutrients such as phosphorus and potassium in soil (Surachat *et al*. 2022; Gupta *et al*. 2023; Iqbal *et al*. 2023). AMF (*e.g. Glomus*, *Rhizophagus*, *Acaulospora*, *Paraglomus*, and *Funneliformis*) establish a symbiotic relationship with plant roots by forming structures called arbuscules in root cells that serve as nutrient transfer sites between fungi and plants (Wilkes 2021). AMF also form networks of hyphae that stabilize soil structure and provide additional surface area for nutrient absorption. Marketed biofertilizers in the Philippines are Bio-N^{TM} and MYKOVAM$^{®}$, which contain *Azospirillum* and AMF, respectively. These biofertilizers were proven to enhance the growth and quality of common Philippine crops *Zea mays* (corn), *Carica papaya* (papaya), *Musa textilis* (abaca), and *Saccharum officinarum* (sugarcane) (Sumagaysay 2014; Aguilar *et al*. 2018; Lavelah *et al*. 2021). The use of biofertilizers, though, has drawbacks. Their dependence on microbial viability and activity results in a slower release of nutrients in the field compared with the water-soluble inorganic fertilizers. Application in the field exposes the microorganisms to various environmental stresses that may affect their biofertilization capacity, and their interaction with the existing microbial community is unpredictable whether synergistic or antagonistic. Different types of biofertilizers also have different mechanisms of action and tend to be crop- or area-specific, hence the need to identify more microbial strains with biofertilizer potential to develop tailor-fitting formulations (Rai *et al*. 2023).

Cyanobacteria biofertilizer is less studied in the Philippines compared to *Azospirillum* and AMF. Cyanobacteria are good biofertilizer candidates because they can fix the inert atmospheric nitrogen (N_2) into plant assimilable forms NH_4^+, nitrate (NO_3^-), and nitrite (NO_2^-) (Zhang *et al*. 2021; Shivay *et al*. 2022). They also produce phytohormones that enhance plant growth and improve crop or fruit yield and quality (Zhou *et al*. 2020; Bao *et al*. 2021). They produce extracellular polysaccharides and glomalin or glomalin-related soil proteins (GRSPs) that improve soil quality (Shivay *et al*. 2022; Jan *et al*. 2023). Cyanobacteria inoculation also changes the soil microbial community by promoting the growth of other beneficial microorganisms (Feng *et al*. 2022; Gay *et al*. 2022; Kokila *et al*. 2022). Some cyanobacterial genera are also capable of heavy metal bioremediation (Hu *et al*. 2022) and production of secondary metabolites with biocontrol potential (Toribio *et al*. 2021). In terms of mass production, growing cyanobacteria confers more advantages because they can utilize both agricultural wastewater and food waste as nutrient sources, can act as carbon sinks because of their photosynthetic properties, and may even reduce greenhouse gas emissions, thus contributing to a circular economy system (Torres-Franco *et al*. 2021; Cinq-Mars *et al*. 2022).

At present, most cyanobacteria biofertilizer studies are mainly focused on experimental applications in the field. Supplementation with genomic data will give us more insight into the biofertilization mechanisms of action at the molecular and genetic level, which can be used as a guide in formulating biofertilizer products. Genetic data can also be used to design molecular markers for screening potential microbial candidates. Hence, this study aims to explore the biofertilizer potential of the cyanobacteria

Nodosilinea sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 through whole genome mining of biofertilizer-essential genes. Specifically, this study aims to generate whole-genome assemblies of the two strains and use these assemblies to determine and identify genes associated with plant growth-stimulating and biocontrol mechanisms. Identification of these genes allows us to predict the possible mechanisms of action of cyanobacteria as a biofertilizer and may serve as the basis for future metabolomic and field application studies.

MATERIALS AND METHODS

Cyanobacterial Strains and Morphological Characterization

The cyanobacterial strains used in this study – namely *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 – were from the existing culture collection of the Plant Molecular Biology and Genetics Laboratory (PMBGL) of the Institute of Biology, University of the Philippines Diliman, Quezon City, the Philippines. The strains were isolated from sediment samples collected from mining sites in Benguet, the Philippines. Conventional microbiological techniques were used in isolation – namely serial dilution, filtration, inoculation of filtrates on liquid BG-11 (HiMedia, India) medium for enrichment, and streak plating on solidified BG-11 medium for separation of colonies. The isolated cultures were incubated at room temperature under white fluorescent lamps for a 12:12 h light: dark cycle. Morphological characterization was performed using a compound light microscope (Labomed LB-221, USA), and micrographs were processed using ScopeImage 9.0. Characterization was based on cell type, size, shape, and color. Features were compared with literature for morphological identification (Komárek *et al*. 2014).

Genomic DNA Extraction and Sequencing

Cyanobacterial cells were harvested from 2-mo-old cultures grown in liquid BG-11 medium. DNA samples were previously extracted and sent to Macrogen (South Korea) for whole-genome sequencing (Untiveros *et al*. 2023). Genomic DNA extraction was performed using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA), following the manufacturer's protocol. Agarose gel electrophoresis was performed to check for the presence of the extracted DNA. The concentration and purity of the DNA were also checked using the NanoDrop spectrophotometer (EpochTM). Extracted genomic DNA with concentrations greater than 50 ng/μL and purity greater than 1.5 (A260/A280) were sent for whole genome shotgun sequencing (2 x 150 PE) using the Illumina NovaSeq platform.

Whole-genome Assembly

Whole-genome assembly was conducted using the different molecular biology bioinformatics tools in the web-based platform KBase (United States Department of Energy Systems Biology Knowledgebase) (Allen *et al*. 2017). The raw sequence reads were filtered and trimmed using PRINSEQ (Preprocessing and INformation and SEQuence) v0.20.4 (Schmieder and Edwards 2011) and Trimmomatic v0.36 (Bolger *et al*. 2014). The qualities of the raw and trimmed sequence reads were checked and compared using FastQC v0.12.1 (Andrews 2010). Trimmed reads were used for *de novo* genome assembly using Unicycler v0.4.8 (Wick *et al*. 2017). Unicycler is a SPAdes-optimiser that uses a wide range of k-mer sizes (27, 47, 63, 77, 89, 99, 107, 115, 121, and 127) and selection of the best size based on contig length and graph connectivity. Assembled contigs were checked for completeness and contamination using CheckM v1.0.18 (Parks *et al*. 2015). Contigs were further taxonomically binned using MaxBin2 v2.2.4 (Wu *et al*. 2016), MetaBAT2 v1.7 (Kang *et al*. 2015), and CONCOCT v1.1 (Alneberg *et al*. 2014). DAS Tool v1.1.2 (Sieber *et al*. 2018) was used to optimize the results from the three binning tools. The optimized bins were used for taxonomic identification using GTDB-tk (Genome Taxonomy Database Toolkit) v2.3.2 (Chaumeil *et al*. 2020). Bins identified as cyanobacteria were extracted as assemblies and assessed using QUAST v4.4 (Gurevich *et al*. 2013) and CheckM. Assemblies with similar features to the type species (*e.g.* genome size and GC content) in the National Center for Biotechnology Information database were used for functional annotation and genome mining downstream analyses.

Genome Annotation and Mining of Plant-growth-promoting and Biocontrol Genes

The draft genome assembly was annotated using the RASTtk v1.073 (Rapid Annotation using Subsystem Technology Toolkit) pipeline (Brettin *et al*. 2015). Plant-growth-promoting and biocontrol genes were identified based on the following RASTtk functional categories: [1] nitrogen metabolism, [2] phosphorus metabolism, [3] potassium metabolism, and [4] secondary metabolism. Identified genes were cross-referenced with the metabolic model constructed using the ModelSEED v2.1.1 pipeline (Seaver *et al*. 2021). The metabolic model presents the compounds and chemical reactions associated with the identified genes. Fama Genome Profiling v1.1 (Kazakov and Novichkov 2019) was used to search for proteins or enzymes involved in nitrogen fixation. AntiSMASH 7.0 (Antibiotic and Secondary Metabolite Analysis Accessory) (Blin *et al*. 2023) was used for predicting biosynthetic gene clusters (BGCs) with potential agricultural biocontrol applications. AntiSMASH detects secondary metabolite

signature protein domains in BGCs using profile Hidden Markov Models (pHMMs) and cross-references these with BGC databases including ClusterBlast and MIBiG (Minimum Information about a Biosynthetic Gene Cluster) (Blin *et al*. 2023). Genome assembly and annotation were inputted in the antiSMASH website (https://antismash.secondarymetabolites.org), relaxed detection strictness was selected, and extra features KnownClusterBlast, MIBiG cluster comparison, Cluster Pfam analysis, ClusterBlast, ActiveSiteFinder, Pfam-based gene ontology term annotation, and SubClusterBlast were turned on. Circular genome maps were generated through Proksee (https://proksee.ca) (Grant *et al*. 2023) using the RASTtk annotated genome and antiSMASH-predicted BGCs.

RESULTS

Morphological Characteristics of Cyanobacteria Strains

Two morphologically distinct cyanobacterial strains – namely *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 – from the culture collection of PMBGL were used for phenotypic characterization, whole-genome sequencing, and genome mining for plant-growth-promoting and biocontrol genes. *Nodosilinea* sp. PGN35 is a homocystous (non-heterocystous) filamentous cyanobacterium that forms dense biofilm when cultured in liquid BG-11 medium. It has blue-green filaments that are uniseriate and isopolar, unbranched, and covered with a thin, transparent sheath (Figure 1A). Filaments form loose spirals (Figure 1B, black arrow). The barrel-shaped or cylindrical cells are longer (3.71 ± 0.86 µm) than wide (2.77 ± 0.51 µm) and undergo transverse binary fission. *Chlorogloeopsis* sp. ULAP02 forms aggregates when cultured in a liquid BG-11 medium. Dark green cells are sometimes arranged in multiseriate filaments (Figure 2A) or are sometimes observed as unicellular or colonial cells (Figure 2B). Filament branching is still unresolved whether true, false, or no branching. Spherical cells with an average diameter of 5.04 ± 0.79 µm divide *via* fission in multiple planes (Figure 2, black arrows).

Draft Whole-genome Assembly Features

Whole-genome sequencing results generated 22,265,312 raw reads for PGN35. After quality control, the total read count was reduced to 20,771,936, which was used for *de novo* assembly using the Unicycler tool. Taxonomic binning resulted in only a single bin identified as *Nodosilinea* sp., with a high multiple sequence alignment of amino acids (MSA AA) percentage at 90.69%. The extracted *Nodosilinea* bin assembly generated 58 contigs with a total length of 6,739,130 bp and 58.98% GC content. Quality control of the assembly showed 99.18% completeness of the genome and 1.63% contamination. Whole-genome sequencing results for ULAP02 generated a total of 23,723,010 raw reads. Quality control reduced the read count to 23,185,152. *De novo* assembly and taxonomic binning generated six optimized bins, in which Bin 5 was the only bin belonging to the cyanobacteria lineage. Bin 5 was taxonomically identified as *Chlorogloeopsis* sp. with an MSA AA percentage of 89.04%. The extracted Bin 5 assembly generated 90 contigs with a total length of 7,715,057 bp and 40.93% GC content. The assembled genome showed 99.54% completeness and 3.77% contamination. Genome assembly features for *Nodosilinea* sp. PGN35 (GenBank accession number: NZ_JAQKQJ000000000.2) in comparison with the type species *Nodosilinea nodulosa* PCC 7104 (GenBank accession number: NZ_ALVP00000000.1)

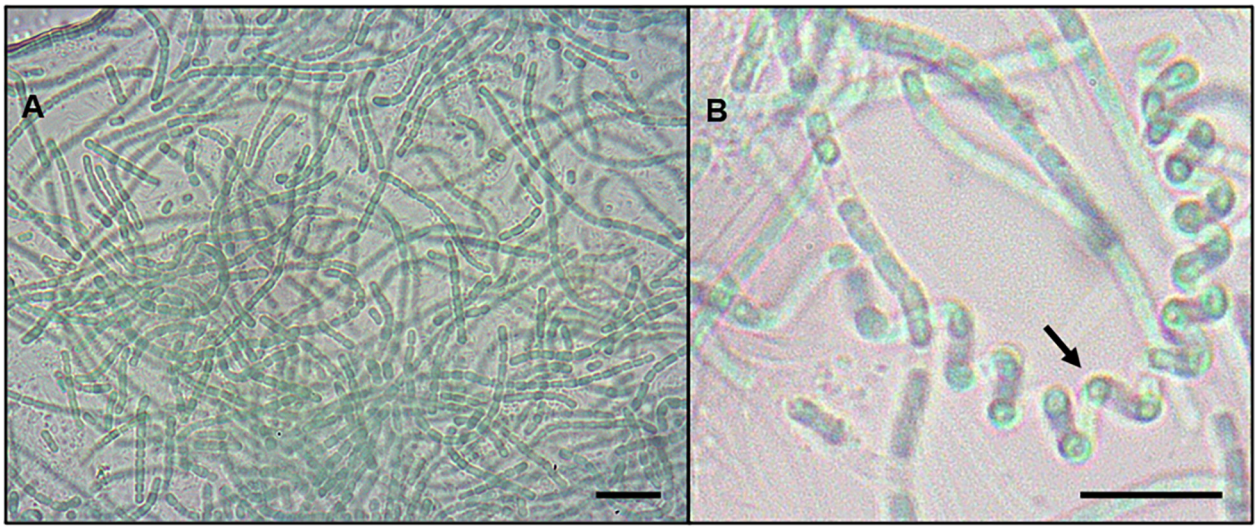

Figure 1. Blue-green uniseriate filaments (A) and loose spiral (black arrow) (B) of *Nodosilinea* sp. PGN35. Scale bar = 20 µm.

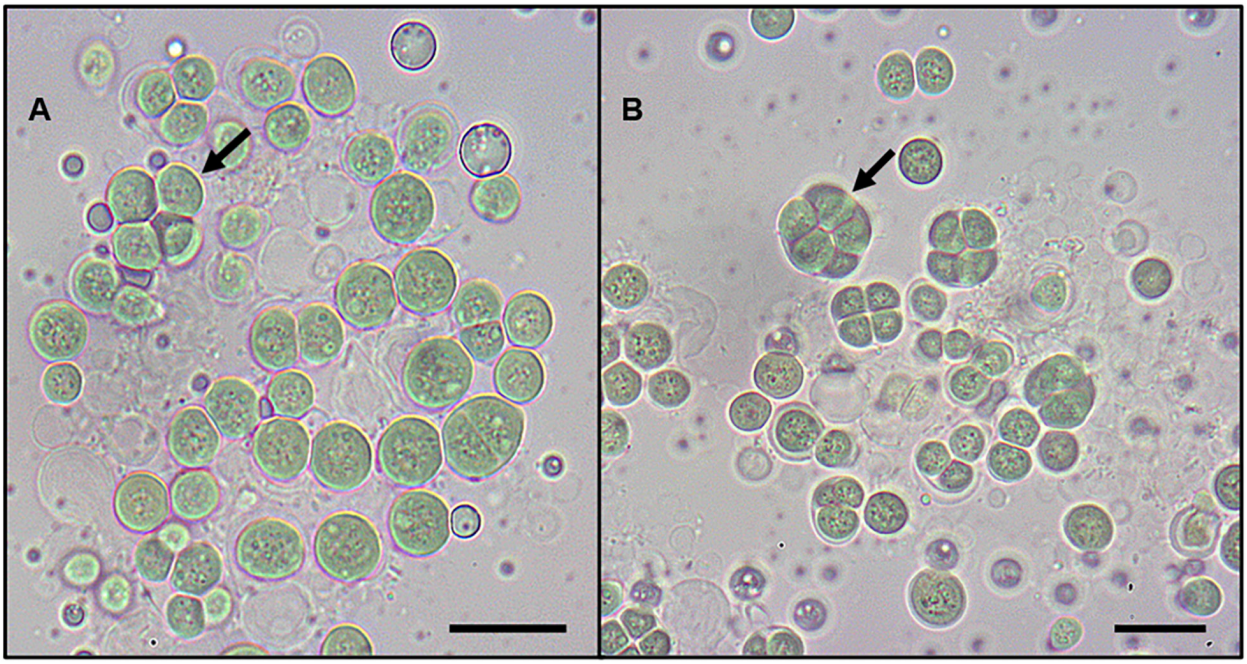

Figure 2. Dark green multiseriate filament (A) and unicellular (B) *Chlorogloeopsis* sp. ULAP02 with cells undergoing fission in multiple planes (black arrow). Scale bar = 10 μm.

Table 1. Genome assembly features of *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 in comparison with type species *Nodosilinea nodulosa* PCC 7104 and *Chlorogloeopsis fritschii* PCC 6912, respectively.

Genome assembly features	*Nodosilinea* sp. PGN35	*Nodosilinea nodulosa* PCC 7104	*Chlorogloeopsis* sp. ULAP02	*Chlorogloeopsis fritschii* PCC 6912
Estimated genome size (Mb)	6.7	6.9	7.7	7.8
GC content (%)	58.98	57.5	40.93	41.5
Number of contigs	58	62	90	154
Contig N50 (kb)	239.9	211.6	171.4	115.4
Total number of predicted genes	6,026	6,306	6,757	6,963
Completeness (%)	99.18	98.73	99.54	99.37
Contamination (%)	1.63	4.08	3.77	2.18
Sequencing technology	Illumina NovaSeq	Illumina	Illumina NovaSeq	Illumina MiSeq
Assembly method	Unicycler v0.4.8	Newbler v.2.3	Unicycler v0.4.8	Velvet v.1.21.1

and *Chlorogloeopsis* sp. ULAP02 (GenBank accession number: NZ_JAYKTT000000000.2) in comparison with the type species *Chlorogloeopsis fritschii* PCC 6912 (GenBank reference number: NZ_RSCJ00000000.1) were summarized in Table 1.

Biofertilization and Biocontrol Genes and Gene Clusters

Functional annotation, metabolic profiling, and BGC mining of the assembled genomes of *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 showed potential biofertilizer-essential genes and gene clusters, specifically those involved in nitrogen fixation, nutrient solubilization and mobilization, phytohormone auxin production, and biostimulant/biocontrol compound production. These genes were distributed throughout the whole genomes of PGN35 and ULAP02, categorized under different plant-growth-promoting functional subsystems – "nitrogen metabolism," "phosphorus metabolism," "potassium metabolism," "plant hormone biosynthesis," and "other secondary metabolite biosynthesis" (Figures 3A and B, outermost ring).

Nitrogen fixation genes under the "nitrogen metabolism" subsystem were found in both strains forming distinct

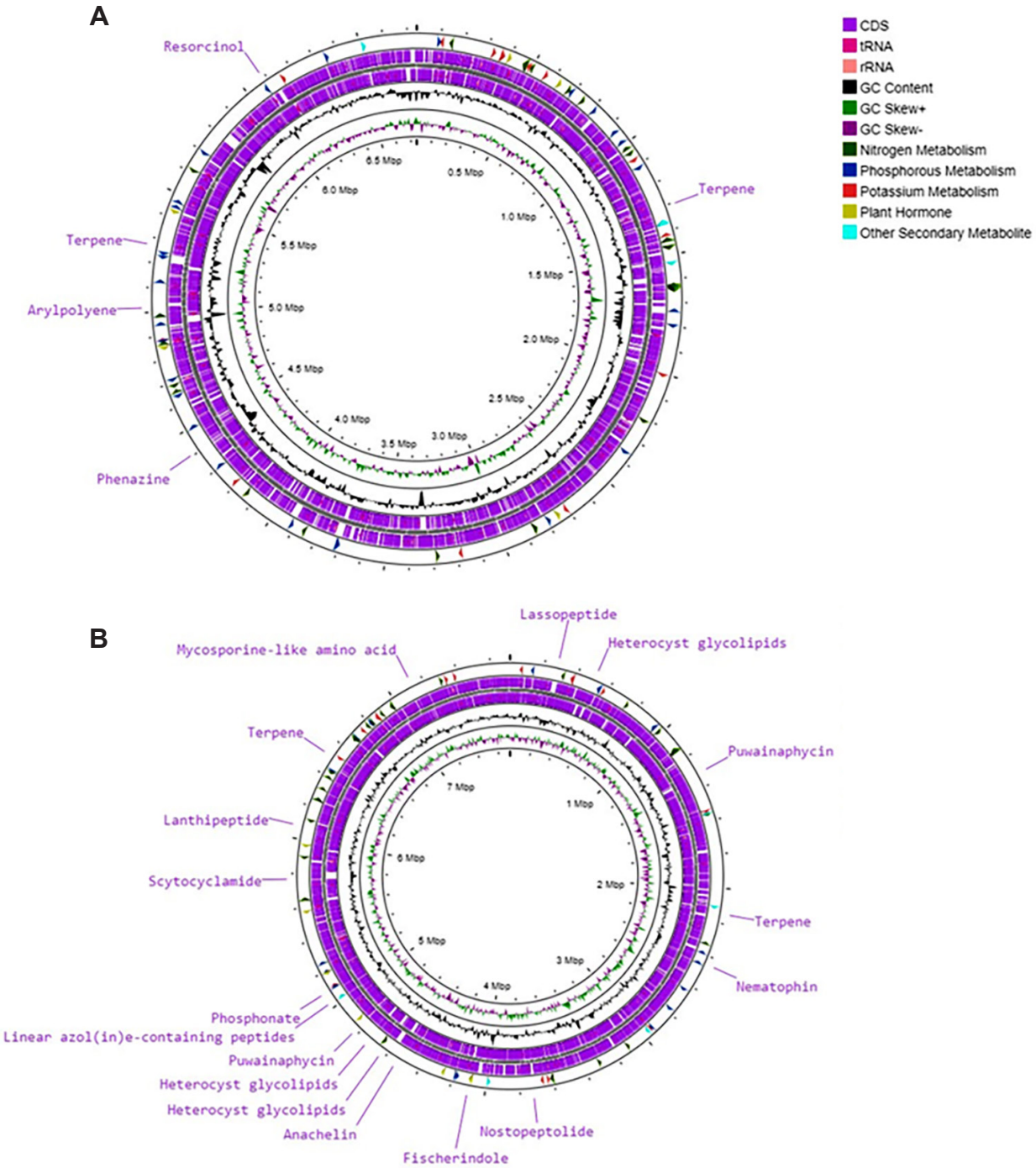

Figure 3. Circular genome representations of *Nodosilinea* sp. PGN35 (A) and *Chlorogloeopsis* sp. ULAP02 (B) showing nitrogen, phosphorus, and potassium metabolism genes, plant hormone and other secondary metabolite biosynthesis genes (outermost ring), and BGCs predicted by antiSMASH.

operons *Nif*HDK, *Nif*ENXW, *Nif*VZT, and *Nif*B-*fdx*N-*Nif*U that together make up the entire nitrogenase gene cluster *Nif* (Table 2). BGC mining also supported the nitrogen-fixing capacity of the strain ULAP02 by revealing four different gene clusters associated with nitrogen fixation (three heterocyst glycolipid BGCs and one terpene BGC) (Figure 3B; Figures 4A–D). Heterocyst glycolipids are components of the cell envelope of heterocysts, which are the specialized nitrogen-fixing cells of cyanobacteria. The terpene BGC contained two core biosynthetic genes squalene synthase and squalene-hopene/-hopanol cyclase that synthesize hopanoids (Figure 4D). These are lipid membrane components of vesicles that protect nitrogenase, the enzyme responsible

Table 2. Nitrogen fixation genes identified in *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02.

Gene	Synthesized protein
*Nif*H	Nitrogenase (molybdenum-iron) reductase and maturation protein[a,b]
*Nif*D	Nitrogenase (molybdenum-iron) alpha chain[a,b]
*Nif*K	Nitrogenase (molybdenum-iron) beta chain[a,b]
*Nif*E	Nitrogenase FeMo-cofactor scaffold and assembly protein[a,b]
*Nif*N	Nitrogenase FeMo-cofactor scaffold and assembly protein[b]
*Nif*X	Nitrogenase FeMo-cofactor carrier protein[a,b]
*Nif*W	Nitrogenase stabilizing/protective protein[a,b]
*Nif*V	Homocitrate synthase[a,b]
*Nif*Z	NifZ protein[a,b]
*Nif*T	NifT protein[a,b]
*Nif*B	Nitrogenase FeMo-cofactor synthesis FeS core scaffold and assembly protein[a,b]
*fdx*N	Nitrogenase-associated 4Fe-4S ferredoxin[b]
*Nif*U	Iron-sulfur cluster assembly scaffold protein[a,b]

[a]Gene was identified in *Nodosilinea* sp. PGN35
[b]Gene was identified in *Chlorogloeopsis* sp. ULAP02

include alkaline phosphatase, polyphosphate kinase, exopolyphosphatase, and inorganic pyrophosphatase. Phosphate transporter genes *PstS*, *PhoR* (*SphS*), *PhoB* (*SphR*), and *PhoU* and potassium channel protein genes that transport phosphate and potassium ions from inside to outside the cell and *vice versa* were also present in both strains.

Phytohormone biosynthetic genes specific for auxin were also found in both PGN35 and ULAP02. These include the tryptophan BGC (*Trp*), monoamine oxidase, and aromatic-L-amino acid decarboxylase. *Trp* synthesizes the auxin precursor molecule tryptophan, whereas monoamine oxidase and aromatic-L-amino acid decarboxylase are genes involved in the tryptamine (TAM) pathway of auxin biosynthesis. The identified *Trp* genes in both strains were the chorismate pyruvate-lyase (has similar function with anthranilate synthase *Trp*EG), anthranilate phosphoribosyl transferase *Trp*D, *N*-(5-phospho-β-D-ribosyl)-anthranilate isomerase *Trp*F, 1-(2-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate (has similar function with indole-3-glycerol phosphate synthase *Trp*C), and tryptophan synthase α and β chains *Trp*AB.

Different BGCs with possible biostimulating and biocontrol activities were also identified in PGN35 and ULAP02.

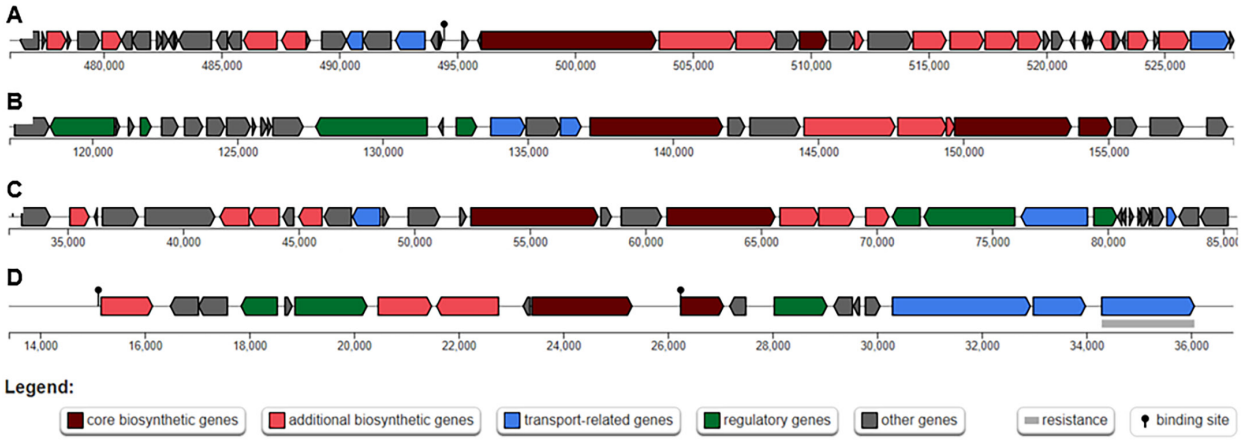

Figure 4. Different heterocyst glycolipids biosynthetic gene clusters (BGCs) (A–C) and hopanoid BGC (D) predicted by antiSMASH from *Chlorogloeopsis* sp. ULAP02 genome assembly.

for nitrogen fixation, from the toxic effects of oxygen. Aside from nitrogen fixation, genes involved in other nitrogen metabolism functions were also identified including ammonia assimilation, nitrate and nitrite ammonification, dissimilatory nitrite reduction, cyanate hydrolysis, nitrosative stress and denitrification, and allantoin utilization.

Nutrient solubilization and mobilization genes were found in both strains under the "phosphorus metabolism" and "potassium metabolism" subsystems. These genes

Terpenes, resorcinol, phenazine, and arylpolyene were predicted from the PGN35 assembly, whereas terpene, resorcinol, phosphonate, polyketide synthase (PKS)/ nonribosomal peptide synthetase (NRPS)/ PKS-NRPS hybrid, indoles, and ribosomally synthesized and post-translationally modified peptide (RiPPs) were predicted from the ULAP02 assembly (Figure 5). PKS/NRPS gene clusters detected in ULAP02 had high similarity scores with biocidal natural products puwainaphycin (30%), nematophin (13%), nostopeptolide (50%), anachelin

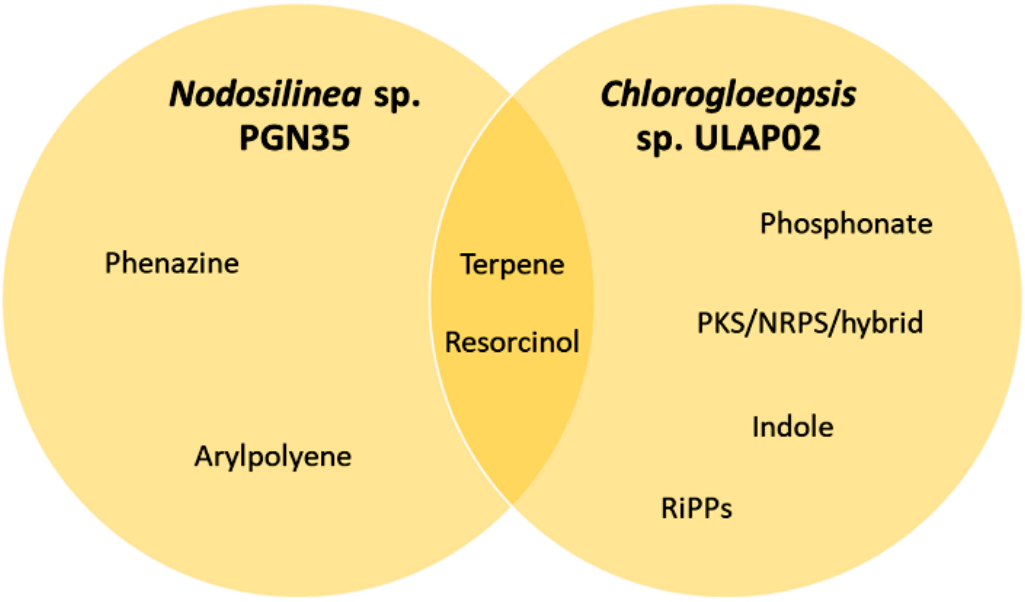

Figure 5. Venn diagram showing biostimulant/biocontrol metabolite BGCs in *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02.

(60%), and scytocyclamide (61%). The predicted indole had the highest similarity with fischerindole (26%). The RiPPs detected were lassopeptide, linear azol(in) e-containing peptide (LAP), and lanthipeptide class V.

DISCUSSION

The use of *Nodosilinea* and *Chlorogloeopsis* in agriculture has not yet been explored. Most of the well-studied biofertilizer genera were the heterocystous *Anabaena* and *Nostoc* (Zhou *et al*. 2020; Prasanna *et al*. 2021; Shivay *et al*. 2022). Most of the published applications of *Nodosilinea* and *Chlorogloeopsis* were more on their potential in biofuel production because of their high lipid content and in the cosmetic industry for their antioxidant properties and ability to produce UV-screening compounds (Candelo and Llewellyn 2023; Passos *et al*. 2023). Genome-mining of the two cyanobacterial strains *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 showed agricultural biocontrol and plant-growth-promoting genes that predicted their possible mechanisms of action as biofertilizers. These mechanisms were nitrogen fixation, nutrient solubilization and mobilization, phytohormone production, and biostimulant/biocontrol compounds production.

Most cyanobacteria are capable of nitrogen fixation because they synthesize the enzyme nitrogenase. Nitrogenase is an enzyme complex that is made up of the dinitrogenase and dinitrogenase reductase subunits, FeMo-cofactors, and P-clusters, which are synthesized by the gene cluster *Nif* composed of distinct operons *Nif*HDK, *Nif*ENXW, *Nif*VZT, and *Nif*B-*fdx*N-*Nif*SU (Stal 2015; Esteves-Ferreira *et al*. 2017). Functional annotation of both *Nodosilinea* sp. PGN35 (homocystous) and *Chlorogloeopsis* sp. ULAP02 (heterocystous) verified the presence of the *Nif* gene cluster (Table 2), implying the high probability that both can fix N_2. Even if most of the studied biofertilizer strains are heterocystous, some homocystous and unicellular strains such as *Leptolyngbya* and *Synechococcus*, respectively, were also included in biofertilizer formulations. These strains, however, were often studied in consortium with the heterocystous strains (Ramírez-López *et al*. 2019). It is interesting to observe these strains separately since they might be more efficient nitrogen fixers. This efficiency was especially observed in oligotrophic oceans where the homocystous *Trichodesmium* dominates over heterocystous strains as the most abundant diazotroph (Zehr 2011). Many studies have already shown that cyanobacteria inoculation increases nitrogen fixation and improves crop growth and yield. Inoculation of *Anabaena* spp. in soil increased the nitrogenase activity in *Cicer arietinum* (chickpea) roots, which correlated with enhanced nodule formation and plant growth (Bidyarani *et al*. 2016). Inoculation of *Aliinostoc* sp. YYLX235 increased the *Nif*H gene copies in soil which correlated with increased *Oryza sativa* (rice) yield (Hu *et al*. 2022). The use of N-fixing cyanobacteria biomass as fertilizers increased the total nitrogen and nitrate contents in the soil, which correlated with increased rice grain and straw yield (Zhang *et al*. 2021).

Other major limiting nutrients needed for plant growth and reproduction are phosphorus and potassium. These elements, however, exist naturally in organic forms (*e.g.* organophosphate) or are bound in soil minerals (*e.g.* rock phosphate and mica), making them unavailable for direct assimilation of plants (Afkairin *et al*. 2021; Olaniyan *et al*. 2022). There are diverse microorganisms known as phosphate-solubilizing microorganisms and potassium-solubilizing bacteria that can convert these unavailable forms into assimilable ionic forms (Bagyalakshmi *et al*. 2017; Afkairin *et al*. 2021). Cyanobacteria – especially those belonging to the genera *Anabaena* and *Westiellopsis* – are known to produce enzymes such as alkaline phosphatase, exopolyphosphatase, and pyrophosphatase that can solubilize both organic and mineral forms of phosphorus into PO_4^{3-} (Yandigeri *et al*. 2011; Lin *et al*. 2018; Sanz-Luque *et al*. 2020; Afkairin *et al*. 2021; Hong *et al*. 2021). *Anabaena* was even capable of solubilizing more phosphorus than the commercial microbial biostimulant Mammoth P^{TM} composed of *Citrobacter freundii*, *Enterobacter cloacae*, *Pseudomonas putida*, and *Comamonas testosteroni* (Afkairin *et al*. 2021). Genome mining discovered that genes involved in the synthesis of these enzymes were found in both *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 (Figures 3A and B). Alkaline phosphatase hydrolyzes organophosphate into PO_4^{3-} (Lin *et al*. 2018). Polyphosphate kinase synthesizes polyphosphate, a polymer that acts as PO_4^{3-} reservoir. Polyphosphate is then degraded into PO_4^{3-} by exopolyphosphatase and one of the other products of degradation, pyrophosphate, is further degraded into PO_4^{3-} by pyrophosphatase (Sanz-Luque *et al*. 2020). Genes involved in the transport of PO_4^{3-} and K^+ into and out of the cell were also found in both strains, implying that these strains are capable of releasing metabolized PO_4^{3-} and K^+ into the soil environment to compensate for the limited available nutrients for plant assimilation, hence contributing to nutrient mobility. This, however, is an assumption that further needs experimental validation.

Production of phytohormones in cyanobacteria and their roles in increasing plant growth are already well-established in many studies. Auxin, cytokinin (CK), ethylene (ET), abscisic acid (ABA), salicylic acid (SA), and gibberellins (GA) are the known phytohormones detected in cyanobacterial extracts or biomass (Toribio *et al*. 2020; Zhou *et al*. 2020; Bao *et al*. 2021; Kholssi *et al*. 2021). *Anabaena cylindrica* and *Calothrix* sp. produced auxin that increased root and shoot growth of *Triticum aestivum* (wheat) (Kholssi *et al*. 2021). *Calothrix* sp. and *Trichormus* sp. produced CK and SA that stimulated *Cucumis sativus* (cucumber) seedling growth (Toribio *et al*. 2020). *Nostoc* and *Fischerella* also produced phytohormones that improved plant growth and yield in common crops – including rice, corn, and *Colocasia esculenta* (taro) (Ashok *et al*. 2017; Mishra *et al*. 2019; Suresh *et al*. 2019). Despite the abundant evidence supporting cyanobacteria phytohormone synthesis, information about their biosynthetic pathways is lacking relative to plants. There are cyanobacterial genes involved in phytohormone synthesis that are unique in structure and function making their biosynthetic pathways way more diverse (Tan *et al*. 2021). Auxin, the most studied phytohormone, is also chemically and physiologically diverse and is synthesized *via* multiple metabolic pathways. There are two general pathways for auxin biosynthesis – the tryptophan-dependent and -independent pathways, which are mainly differentiated by the utilization of tryptophan as a precursor in the former and indole or indole-3-glycerophosphate in the latter. The tryptophan-dependent pathway is further categorized into four pathways – indole-3-pyruvic acid (IpyA), tryptamine (TAM), indole-3-acetaloxime (IAOx), and indoleacetamide (IAM) pathways, differentiated based on the enzymes and intermediate compounds formed before auxin (Tan *et al*. 2021). The presence of the *Trp* gene cluster, the additional monoamine oxidase in PGN35 and aromatic-L-amino-acid decarboxylase gene in ULAP02, and the absence of genes involved in the other pathways imply the possibility of the strains utilizing the tryptophan-dependent TAM pathway for auxin biosynthesis. The TAM pathway begins with the conversion of tryptophan to TAM by tryptophan decarboxylase, followed by the hydroxylation of TAM to N-hydroxyl-TAM (HTAM) with flavin monooxygenases, and finally the conversion of HTAM into auxin by currently unknown enzymes that may or may not be involved in other tryptophan-dependent auxin biosynthetic pathways. Despite knowing the intermediates of the pathway, some of the enzymes involved, their catalytic actions, and their incorporation in the other pathways remain unclear and need to be elucidated. Cyanobacteria auxin biosynthesis is a promising avenue for research especially since most studies are mainly about spectrophotometric validation of phytohormone production. Genome mining might reveal novel enzymes or provide insights into the possible functions of identified phytohormone biosynthetic genes.

AntiSMASH predicted BGCs in both *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 with known uses in agriculture (Figure 5). Terpenes, for example, are currently being studied as signaling molecules that mediate plant-to-insect interactions as attractants of beneficial insects (*e.g.* pollinators) and as repellants of herbivores and pathogens (Boncan *et al*. 2020; Ninkuu *et al*. 2021). Terpenes also ameliorate the negative effects of both abiotic and biotic stressors. Phenazine enhances plant yield and has antibacterial and antifungal properties (Chin-A-Woeng *et al*. 2003; Sakhtah *et al*. 2013). Arylpolyenes are structurally and physiologically similar

to carotenoids and protect cells against oxidative stress and photodamage (Cimermancic *et al*. 2014; Nelkner *et al*. 2019). Resorcinol acts both as a biostimulant and antipathogenic compound. It enhanced seed germination, plant growth, chlorophyll content, and enzyme activities in *Lycopersicon esculentum* (tomato) (Bibi *et al*. 2023) and inhibited the growth of the mango black spot-causing fungus *Aternaria alternata* (Cojocaru *et al*. 1986). Phosphonates are commonly used in agriculture as fungicides and herbicides (Manghi *et al*. 2021). PKS/NRPS/hybrid, indole (alkaloid), and RiPPs such as lassopeptide, LAPs, and lanthipeptide are known to have antibacterial, antifungal, and antiparasitic properties. Cyanobacteria are rich in BGCs and are known producers of bioactive compounds. Current studies, however, are mainly focused on the utilization of cyanobacteria natural products in the medical and pharmaceutical fields. However, we can also apply these studies in the field of agriculture. The presence of these BGCs in strains PGN35 and ULAP02 implies their potential to be utilized in agriculture as both biostimulant and biocontrol agents.

CONCLUSION

In silico genome mining of the two cyanobacterial strains *Nodosilinea* sp. PGN35 and *Chlorogloeopsis* sp. ULAP02 revealed biofertilizer-essential genes involved in nitrogen fixation and metabolism, phosphorus and potassium solubilization and mobilization, phytohormone production, and biostimulant/biocontrol compounds production. Identified nitrogen fixation and metabolism genes included the nitrogenase gene cluster *Nif* and the genes involved in urea, nitrate/nitrite, cyanate, and allantoin metabolism into NH_3/NH_4^+. Nutrient solubilization and mobilization genes included the phosphate-solubilizing genes and the intracellular-extracellular (*vice versa*) transport genes for PO_4^{3-} and K^+. Identified phytohormone genes belonged to the tryptophan BGC *Trp* and the additional genes monoamine oxidase in PGN35 and aromatic-L-amino-acid decarboxylase in ULAP02. Biostimulant/biocontrol BGCs were also discovered in both strains – including terpene, phenazine, arylpolyene, resorcinol, phosphonate, PKS/NRPS/hybrid, indole (alkaloid), and RiPPs. Identification of these genes is only the first step in evaluating the potential of cyanobacteria as a biofertilizer. The functionality of these genes – whether actively transcribed, translated into proteins, or metabolically operational – can be verified in future studies using transcriptomics, proteomics, or metabolomics. The mechanisms of action predicted from these genes can be tested on plants for verification. Since biofertilizers tend to be specific to certain crops and have different mechanisms of action, it is important to identify more microbial strains with biofertilizer potential that can be tested for tailor-fitted formulations. This study is the first to use the genome-mining approach to explore the biofertilizer potential of *Nodosilinea* and *Chlorogloeopsis*.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Science and Technology–Advanced Science and Technology Institute–Computing and Archiving Research Environment or DOST-ASTI-COARE for allowing access to their high-performance computing services and the University of the Philippines Office of the Vice-President for Academic Affairs or UP OVPAA for the funding under the Emerging Inter-Disciplinary Research Program (EIDR-C09-08).

REFERENCES

AFKAIRIN A, IPPOLITO JA, STROMBERGER M, DAVIS JG. 2021. Solubilization of organic phosphorus sources by cyanobacteria and a commercially available bacterial consortium. Applied Soil Ecology 162: 103900. https://doi.org/10.1016/j.apsoil.2021.103900

AGUILAR EA, ELLEVA LIF, FABRO DMA, GARCIA GR, DEVINA II FAD, AGGANGAN N. 2018. Arbuscular mycorrhizal fungi increased root-mycorrhizal association and enhanced seedling growth of abaca, papaya, and sugarcane. Journal of ISSAAS (International Society for Southeast Asian Agricultural Sciences) 24(2): 22–29.

ALLEN B, DRAKE M, HARRIS N, SULLIVAN T. 2017. Using KBase to assemble and annotate prokaryotic genomes. Current Protocols in Microbiology 46: 1E.13.1–1E.13.18. https://doi.org/10.1002/cpmc.37

ALNEBERG J, BJARNASON BS, DE BRUIJN I, SCHIRMER M, QUICK J, IJAZ UZ, LAHTI L, LOMAN NJ, ANDERSSON AF, QUINCE C. 2014. Binning metagenomic contigs by coverage and composition. Nature Methods 11(11): 1144–1146. https://doi.org/10.1038/nmeth.3103

ANDREWS S. 2010. FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

ASHOK AK, RAVI V, SARAVANAN R. 2017. Influence of cyanobacterial auxin on sprouting of taro (*Colocasia esculenta* var. Antiquorum) and corm yield. The Indian Journal of Agricultural Sciences 87(11): 1437–1444. https://doi.org/10.56093/ijas.v87i11.75670

BAGYALAKSHMI B, PONMURUGAN P, BALAMURUGAN A. 2017. Potassium solubilization, plant growth promoting substances by potassium solubilizing bacteria (KSB) from southern Indian tea plantation soil. Biocatalysis and Agricultural Biotechnology 12: 116–124. https://doi.org/10.1016/j.bcab.2017.09.011

BAO J, ZHUO C, ZHANG D, LI Y, HU F, LI H, SU Z, LIANG Y, HE H. 2021. Potential applicability of a cyanobacterium as a biofertilizer and biopesticide in rice fields. Plant and Soil 463: 97–112. https://doi.org/10.1007/s11104-021-04899-9

BIBI S, ULLAH R, BURNI T, ULLAH Z, KAZI M. 2023. Impact of resorcinol and biochar application on the growth attributes, metabolite contents, and antioxidant systems of tomato (*Lycopersicon esculentum* Mill.). ACS Omega 8(48): 45750–45762. https://doi.org/10.1021/acsomega.3c06233

BIDYARANI N, PRASANNA R, BABU S, HOSSAIN F, SAXENA AK. 2016. Enhancement of plant growth and yields in chickpea (*Cicer arietinum* L.) through novel cyanobacterial and biofilmed inoculants. Microbiological Research, 188–189: 97–105. https://doi.org/10.1016/j.micres.2016.04.005

BLIN K, SHAW S, AUGUSTIJN HE, REITZ ZL, BIERMANN F, ALANJARY M, FETTER A, TERLOUW BR, METCALF WW, HELFRICH EJN, VAN WEZEL GP, MEDEMA MH, WEBER T. 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures, and visualisation. Nucleic Acids Research 51: W46–W50. https://doi.org/10.1093/nar/gkad344

BOLGER AM, LOHSE M, USADEL B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15): 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

BONCAN DAT, TSANG SSK, LI C, LEE IHT, LAM HM, CHAN TF, HUI JHL. 2020. Terpenes and terpenoids in plants: interactions with environment and insects. International Journal of Molecular Sciences 21(19): 7382. https://doi.org/10.3390/ijms21197382

BRETTIN T, DAVIS JJ, DISZ T, EDWARDS RA, GERDES S, OLSEN GJ, OLSON R, OVERBEEK R, PARRELLO B, PUSCH GD, SHUKLA M, THOMASON JA, STEVENS R, VONSTEIN V, WATTAM AR, XIA F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Scientific Reports 5(1): 8365. https://doi.org/10.1038/srep08365

CANDELO V, LLEWELLYN CA. 2023. Separating and purifying mycosporine-like amino acids from cyanobacteria for application in commercial sunscreen formulations. BioTech 12(1): 16. https://doi.org/10.3390/biotech12010016

CHAUMEIL PA, MUSSIG AJ, HUGENHOLTZ P, PARKS DH. 2020. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36(6): 1925–1927. https://doi.org/10.1093/bioinformatics/btz848

CHIN-A-WOENG TF, BLOEMBERG GV, LUGTENBERG BJ. 2003. Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytologist 157: 503–523. https://doi.org/10.1046/j.1469-8137.2003.00686.x

CIMERMANCIC P, MEDEMA MH, CLAESEN J, KURITA K, BROWN LCW, MAVROMMATIS K, PATI A, GODFREY PA, KOEHRSEN M, CLARDY J, BIRREN BW, TAKANO E, SALI A, LININGTON RG, FISCHBACH MA. 2014. Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. Cell 158(2): 412–421. https://doi.org/10.1016/j.cell.2014.06.034

CINQ-MARS M, BOURDEAU N, MARCHAND P, DESGAGNÉ-PENIX I, BARNABÉ S. 2022. Characterization of two microalgae consortia grown in industrial wastewater for biomass valorization. Algal Research 62: 102628. https://doi.org/10.1016/j.algal.2021.102628

COJOCARU M, DROBY S, GLOTTER E, GOLDMAN A, GOTTLIEB HE, JACOBY B, PRUSKY D. 1986. 5-(12-Heptadecenyl)-resorcinol, the major component of the antifungal activity in the peel of mango fruit. Phytochemistry 25(5): 1093–1095. https://doi.org/10.1016/S0031-9422(00)81560-5

[DA-FPA] Department of Agriculture–Fertilizer and Pesticide Authority. 2022. Balanced fertilization strategy (BFS) program: the FPA commitment in the OneDA approach. 64p.

ESTEVES-FERREIRA AA, CAVALCANTI JHF, VAZ MGMV, ALVARENGA LV, NUNES-NESI A, ARAÚJO WL. 2017. Cyanobacterial nitrogenases: phylogenetic diversity, regulation, and functional predictions. Genetics and Molecular Biology, 40: 261–275. https://doi.org/10.1590/1678-4685-gmb-2016-0050

FENG Y, ZHANG H, SONG X, GE T, ZHU J, ZHOU C, COBB K, YAN X, RUAN R, CHENG P. 2022. Microalgae as a potential conditioner for continuous cropping obstacles for taro (*Colocasia esculenta* L. Schott) production. Journal of Cleaner Production 369: 133356. https://doi.org/10.1016/j.jclepro.2022.133356

GAY JD, GOEMANN HM, CURREY B, STOY PC, CHRISTIANSEN JR, MILLER PR, POULTER B, PEYTON BM, BROOKSHIRE ENJ. 2022. Climate mitigation potential and soil microbial response of cyanobacteria-fertilized bioenergy crops in a cool semi-arid cropland. GCB Bioenergy 14(12): 1303–1320. https://doi.org/10.1111/gcbb.13001

GOEMANN HM, GAY JD, MUELLER RC, BROOKSHIRE ENJ, MILLER P, POULTER B, PEYTON BM. 2021. Aboveground and belowground responses to cyanobacterial biofertilizer supplement in a semi-arid, perennial bioenergy cropping system. GCB Bioenergy 13(12): 1908–1923. https://doi.org/10.1111/gcbb.12892

GRANT JR, ENNS E, MARINIER E, MANDAL A, HERMAN EK, CHEN C, GRAHAM M, VAN DOMSELAAR G, STOTHARD P. 2023. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Research 51: W484–W492. https://doi.org/10.1093/nar/gkad326

GUPTA RK, FUKE P, KHARDENAVIS AA, PUROHIT HJ. 2023. *In silico* genomic characterization of *Bacillus velezensis* strain AAK_S6 for secondary metabolite and biocontrol potential. Current Microbiology 80(2): 81. https://doi.org/10.1007/s00284-022-03173-0

GUREVICH A, SAVELIEV V, VYAHHI N, TESLER G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29(8): 1072–1075. https://doi.org/10.1093/bioinformatics/btt086

HONG S, PAN Q, CHEN S, ZU Y, XU C, LI J. 2021. Identification and characterization of alkaline phosphatase gene phoX in *Microcystis aeruginosa* PCC7806.3. Biotech 11(5): 218. https://doi.org/10.1007/s13205-021-02774-z

HU T, CHEN A, JIANG Y, SUN C, LUO S, SHAO J. 2022. Application of a newly recorded diazotrophic cyanobacterium in acidified and Cd contaminated paddy soil: promotes rice yield and decreases Cd accumulation. Science of The Total Environment 814: 152630. https://doi.org/10.1016/j.scitotenv.2021.152630

IQBAL S, QASIM M, RAHMAN H, KHAN N, PARACHA RZ, BHATTI MF, JAVED A, JANJUA HA. 2023. Genome mining, antimicrobial, and plant growth-promoting potentials of halotolerant *Bacillus paralicheniformis* ES-1 isolated from salt mine. Molecular Genetics and Genomics 298(1): 79–93. https://doi.org/10.1007/s00438-022-01964-5

JAN Z, ALI S, RAHIM HU, AKBAR WA, SEHRISH AK, TAJ A, RAHIM T, IQBAL M. 2023. Cyanobacterial strains reclaimed induced-salinity stress attributes and improved the physicochemical properties of induced-saline soil. Acta Ecologica Sinica 43(6): 1074–1079. https://doi.org/10.1016/j.chnaes.2023.03.003

KANG DD, FROULA J, EGAN R, WANG Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ 3: e1165. https://doi.org/10.7717/peerj.1165

KAZAKOV A, NOVICHKOV P. 2019. Fama: a computational tool for comparative analysis of shotgun metagenomic data. Great Lakes Bioinformatics conference (poster presentation). https://iseq.lbl.gov/mydocs/fama_glbio2019_poster.pdf

KHOLSSI R, MARKS EAN, MIÑÓN J, MATÉ AP, SACRISTÁN G, MONTERO O, DEBDOUBI A, RAD C. 2021. A consortium of cyanobacteria and plant growth promoting rhizobacteria for wheat growth improvement in a hydroponic system. South African Journal of Botany 142: 247–258. https://doi.org/10.1016/j.sajb.2021.06.035

KOKILA V, PRASANNA R, KUMAR A, NISHANTH S, SHUKLA J, GULIA U, NAIN L, SHIVAY YS, SINGH AK. 2022. Cyanobacterial inoculation in elevated CO_2 environment stimulates soil C enrichment and plant growth of tomato. Environmental Technology & Innovation 26: 102234. https://doi.org/10.1016/j.eti.2021.102234

KOMÁREK J, KAŠTOVSKÝ J, MAREŠ J, JOHANSEN JR. 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86: 295–335.

LAVELAH OM, EDAÑO MLS, STA. CRUZ PC, DIZON JT. 2021. Growth, yield, and grain quality of three maize varieties and mungbean under different cropping systems inoculated with Mykovam®. International Journal of Research and Innovation in Social Science 05(04): 330–337. https://doi.org/10.47772/IJRISS.2021.5415

LIN W, ZHAO D, LUO J. 2018. Distribution of alkaline phosphatase genes in cyanobacteria and the role of alkaline phosphatase on the acquisition of phosphorus from dissolved organic phosphorus for cyanobacterial growth. Journal of Applied Phycology 30(2): 839–850. https://doi.org/10.1007/s10811-017-1267-3

MANGHI MC, MASIOL M, CALZAVARA R, GRAZIANO PL, PERUZZI E, PAVONI B. 2021. The use of phosphonates in agriculture: chemical, biological properties, and legislative issues. Chemosphere 283: 131187. https://doi.org/10.1016/j.chemosphere.2021.131187

MISHRA SK, SINGH J, PANDEY AR, DWIVEDI N. 2019. Indole-3-acetic acid production by the

cyanobacterium *Fisherella muscicola* NDUPC001. Current Science 116(7): 1233. https://doi.org/10.18520/cs/v116/i7/1233-1237

MÚNERA-PORRAS LM, GARCÍA-LONDOÑO S, RÍOS-OSORIO LA. 2020. Action mechanisms of plant growth promoting cyanobacteria in crops in situ: a systematic review of literature. International Journal of Agronomy 2020: 1–9. https://doi.org/10.1155/2020/2690410

NELKNER J, HENKE C, LIN TW, PÄTZOLD W, HASSA J, JAENICKE S, GROSCH R, PÜHLER A, SCZYRBA A, SCHLÜTER A. 2019. Effect of long-term farming practices on agricultural soil microbiome members represented by metagenomically assembled genomes (MAGs) and their predicted plant-beneficial genes. Genes 10(6): 424. https://doi.org/10.3390/genes10060424

NINKUU V, ZHANG L, YAN J, FU Z, YANG T, ZENG H. 2021. Biochemistry of terpenes and recent advances in plant protection. International Journal of Molecular Sciences 22(11): 5710. https://doi.org/10.3390/ijms22115710

OLANIYAN FT, ALORI ET, ADEKIYA AO, AYORINDE BB, DARAMOLA FY, OSEMWEGIE OO, BABALOLA OO. 2022. The use of soil microbial potassium solubilizers in potassium nutrient availability in soil and its dynamics. Annals of Microbiology 72(1): 45. https://doi.org/10.1186/s13213-022-01701-8

OMER RM, HEWAIT HM, MADY E, YOUSIF SKM, GASHASH EA, RANDHIR R, ASHMAWI AE, EL-TAHER AM, AL-HARBI NA, RANDHIR TO. 2023. Chemical, anatomical, and productivity responses of cowpea (*Vigna unguiculata* L.) to integrated biofertilizer applications with PGPR, cyanobacteria, and yeast. Sustainability 15(9): 7599. https://doi.org/10.3390/su15097599

PAN Y, BORJIGIN S, LIU Y, WANG H, WANG Y, WU Y, HAO G, GONG D, YANG Q, GENG Z, LI Y, HAN D. 2022. Role of key-stone microbial taxa in algae amended soil for mediating nitrogen transformation. Science of the Total Environment 823: 153547. https://doi.org/10.1016/j.scitotenv.2022.153547

PARKS DH, IMELFORT M, SKENNERTON CT, HUGENHOLTZ P, TYSON GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Research 25(7): 1043–1055. https://doi.org/10.1101/gr.186072.114

PASSOS LS, DE FREITAS PNN, MENEZES RB, DE SOUZA AO, SILVA MFD, CONVERTI A, PINTO E. 2023. Content of lipids, fatty acids, carbohydrates, and proteins in continental cyanobacteria: a systematic analysis and database application. Applied Sciences 13(5): 3162. https://doi.org/10.3390/app13053162

PRASANNA R, HOSSAIN F, SAXENA G, SINGH B, KANCHAN A, SIMRANJIT K, RAMAKRISHNAN B, RANJAN K, MUTHUSAMY V, SHIVAY YS. 2021. Analyses of genetic variability and genotype x cyanobacteria interactions in biofortified maize (*Zea mays* L.) for their responses to plant growth and physiological attributes. European Journal of Agronomy 130: 126343. https://doi.org/10.1016/j.eja.2021.126343

PURBAJANTI ED, SLAMET W, FUSKHAH E, ROSYIDA. 2019. Effects of organic and inorganic fertilizers on growth, activity of nitrate reductase, and chlorophyll contents of peanuts (*Arachis hypogaea* L.). IOP Conference Series: Earth and Environmental Science 250: 012048. https://doi.org/10.1088/1755-1315/250/1/012048

RAI PK, RAI A, SHARMA NK, SINGH T, KUMAR Y. 2023. Limitations of biofertilizers and their revitalization through nanotechnology. Journal of Cleaner Production 418: 138194. https://doi.org/10.1016/j.jclepro.2023.138194

RAMÍREZ-LÓPEZ C, ESPARZA-GARCÍA FJ, FERRERA-CERRATO R, ALARCÓN A, CAÑIZARES-VILLANUEVA RO. 2019. Short-term effects of a photosynthetic microbial consortium and nitrogen fertilization on soil chemical properties, growth, and yield of wheat under greenhouse conditions. Journal of Applied Phycology 31(6): 3617–3624. https://doi.org/10.1007/s10811-019-01861-2

SAKHTAH H, PRICE-WHELAN A, DIETRICH LEP. 2013. Regulation of phenazine biosynthesis. In: Microbial phenazines: biosynthesis, agriculture, and health. Chincholkar S, Thomashow L eds. Berlin: Springer-Verlag. p. 19–42. https://doi.org/10.1007/978-3-642-40573-0_2

SANZ-LUQUE E, BHAYA D, GROSSMAN AR. 2020. Polyphosphate: a multifunctional metabolite in cyanobacteria and algae. Frontiers in Plant Science 11: 938. https://doi.org/10.3389/fpls.2020.00938

SCHMIEDER R, EDWARDS R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27(6): 863–864. https://doi.org/10.1093/bioinformatics/btr026

SEAVER SMD, LIU F, ZHANG Q, JEFFRYES J, FARIA JP, EDIRISINGHE JN, MUNDY M, CHIA N, NOOR E, BEBER ME, BEST AA, DEJONGH M, KIMBREL JA, D'HAESELEER P, MCCORKLE

SR, BOLTON JR, PEARSON E, CANON S, WOOD-CHARLSON EM, COTTINGHAM RW, ARKIN AP, HENRY CS. 2021. The ModelSEED Biochemistry Database for the integration of metabolic annotations and the reconstruction, comparison, and analysis of metabolic models for plants, fungi, and microbes. Nucleic Acids Research 49(D1): D575–D588. https://doi.org/10.1093/nar/gkaa746

SHIVAY YS, PRASANNA R, MANDI S, KANCHAN A, SIMRANJIT K, NAYAK S, BARAL K, SIROHI MP, NAIN L. 2022. Cyanobacterial inoculation enhances nutrient use efficiency and grain quality of basmati rice in the system of rice intensification. ACS Agricultural Science & Technology 2(4): 742–753. https://doi.org/10.1021/acsagscitech.2c00030

SIEBER CMK, PROBST AJ, SHARRAR A, THOMAS BC, HESS M, TRINGE SG, BANFIELD JF. 2018. Recovery of genomes from metagenomes *via* a dereplication, aggregation, and scoring strategy. Nature Microbiology 3(7): 836–843. https://doi.org/10.1038/s41564-018-0171-1

STAL LJ. 2015. Nitrogen fixation in cyanobacteria. In: Encyclopedia of life sciences (eLS). John Wiley & Sons, Ltd. p. 1–9. https://doi.org/10.1002/9780470015902.a0021159.pub2

SUMAGAYSAY CL. 2014. Bio N fertilization on corn. In: Proceedings book of ICETSR, handbook on the emerging trends in scientific research. p. 316–320.

SURACHAT K, KANTACHOTE D, DEACHAMAG P, WONGLAPSUWAN M. 2022. In silico genomic analysis of *Rhodopseudomonas palustris* strains revealed potential biocontrol agents and crop yield enhancers. Biological Control 176: 105085. https://doi.org/10.1016/j.biocontrol.2022.105085

SURESH A, SOUNDARARAJAN S, ELAVARASI S, OSCAR FL, THAJUDDIN N. 2019. Evaluation and characterization of the plant growth promoting potentials of two heterocystous cyanobacteria for improving food grains growth. Biocatalysis and Agricultural Biotechnology 17: 647–652. https://doi.org/10.1016/j.bcab.2019.01.002

TAN CY, DODD IC, CHEN JE, PHANG SM, CHIN CF, YOW YY, RATNAYEKE S. 2021. Regulation of algal and cyanobacterial auxin production, physiology, and application in agriculture: an overview. Journal of Applied Phycology 33(5): 2995–3023. https://doi.org/10.1007/s10811-021-02475-3

TORIBIO AJ, SUÁREZ-ESTRELLA F, JURADO MM, LÓPEZ MJ, LÓPEZ-GONZÁLEZ JA, MORENO J. 2020. Prospection of cyanobacteria producing bioactive substances and their application as potential phytostimulating agents. Biotechnology Reports 26: e00449. https://doi.org/10.1016/j.btre.2020.e00449

TORIBIO AJ, JURADO MM, SUÁREZ-ESTRELLA F, LÓPEZ MJ, LÓPEZ-GONZÁLEZ JA, MORENO J. 2021. Seed biopriming with cyanobacterial extracts as an eco-friendly strategy to control damping off caused by *Pythium ultimum* in seedbeds. Microbiological Research 248: 126766. https://doi.org/10.1016/j.micres.2021.126766

TORRES-FRANCO AF, ZULUAGA M, HERNÁNDEZ-ROLDÁN D, LEROY-FREITAS D, SEPÚLVEDA-MUÑOZ CA, BLANCO S, MOTA CR, MUÑOZ R. 2021. Assessment of the performance of an anoxic-aerobic microalgal-bacterial system treating digestate. Chemosphere 270: 129437. https://doi.org/10.1016/j.chemosphere.2020.129437

UNTIVEROS DPM, SANCHEZ LRS, CAO EP. 2023. Whole genome sequence analysis of the filamentous *Nodosilinea* sp. PGN35 isolated from a mining site in Tuba, Benguet, Philippines. Frontiers in Ecology and Evolution 11: 1205557. https://doi.org/10.3389/fevo.2023.1205557

VEJAN P, ABDULLAH R, KHADIRAN T, ISMAIL S, BOYCE AN. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability – a review. Molecules 21(5): 573. https://doi.org/10.3390/molecules21050573

WICK RR, JUDD LM, GORRIE CL, HOLT KE. 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology 13(6): e1005595. https://doi.org/10.1371/journal.pcbi.1005595

WILKES TI. 2021. Arbuscular mycorrhizal fungi in agriculture. Encyclopedia 1(4): 1132–1154. https://doi.org/10.3390/encyclopedia1040085

WU YW, SIMMONS BA, SINGER SW. 2016. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. Bioinformatics 32(4): 605–607. https://doi.org/10.1093/bioinformatics/btv638

YANDIGERI MS, MEENA KK, SRINIVASAN R, PABBI S. 2011. Effect of mineral phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria. Indian Journal of Microbiology 51(1): 48–53. https://doi.org/10.1007/s12088-011-0081-x

ZEHR JP. 2011. Nitrogen fixation by marine cyanobacteria. Trends in Microbiology 19(4): 162–173. https://doi:10.1016/j.tim.2010.12.004

ZHANG J, SONG X, WEI H, ZHOU W, PENG C, LI D. 2021. Effect of substituting nitrogen fertilizer with nitrogen-fixing cyanobacteria on yield in a double-rice cropping system in southern China. Journal of Applied Phycology 33(4): 2221–2232. https://doi.org/10.1007/s10811-021-02455-7

ZHOU Y, BAO J, ZHANG D, LI Y, LI H, HE H. 2020. Effect of heterocystous nitrogen-fixing cyanobacteria against rice sheath blight and the underlying mechanism. Applied Soil Ecology 153: 103580. https://doi.org/10.1016/j.apsoil.2020.103580