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Original Article

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Genetic analysis of polyketide synthase and peptide synthase genes of cyanobacteria as a mining tool for new pharmaceutical compounds

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Abstract

Cyanobacteria are considered a promising source for new pharmaceutical lead compounds and a large number of chemically diverse and bioactive metabolites have been obtained from cyanobacteria. To date, the majority of bioactive metabolites isolated from cyanobacteria have either been polyketide synthases (PKSs) or non-ribosomal peptide synthetases (NRPSs). Despite several worldwide studies on the prevalence of NRPSs and PKSs among the cyanobacteria, none of them included Iranian cyanobacteria of Kermanshah province. Therefore, the aim of this study was to amplify the NRPS and PKS genes, in order to predict a novel peptide compound and an amino acid activated by a specific unknown NRPS A module by three cyanobacteria isolated from freshwater and terrestrial cyanobacteria strains. These stains were the most frequent species of that zone. The DNA sequences of the 16s rRNA region were determined and the strains were named as Scytonema sp. N11, Calothrix sp. N42 and terrestrial Nostoc SP. N66). Lastly, to show that the presence of these genes correlated with natural product synthesis, we conducted biochemical assays to detect the presence of antimicrobial effects of organic extracts. The results of these experiments suggest that both NRPS and PKS genes are present in all three strains. The methanolic extract of Calothrix sp. N42 showed potent activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, whereas, there was no inhibitory effect against Escherichia coli and Staphylococcus aureus in two another strains. The different pattern of inhibition in the bacterial and fungal bioassays indicated that various antimicrobial substances are involved. Computer modeling and phylogenetic analysis were conducted to predict the putative amino acid recognized by the unknown adenylation domain in the NRPS sequences. Finally, the sequences presented in this study have been deposited in GenBank for getting the accession numbers (NRPS A domains) and (PKS KS domains). According to these results, it is concluded that the antibiogram bioassay and molecular detection of peptide synthetase and polyketide synthase genes in cyanobacterial strains may be useful techniques for the assessment of natural product -producing species and the possible role of peptide synthetase and polyketide synthase enzyme complexes in the biosynthesis of biologically active compounds.

Keywords: Peptide synthetase gene; Polyketide synthase gene; Cyanobacteria; Secondary metabolites.e

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Introduction

Cyanobacteria are well known for their capability to produce bioactive compounds, an important feature that might increase their survival in both marine and freshwater environments. These compounds demonstrate a diverse range of biological activities and chemical structures, including novel cyclic and linear lipopeptides, fatty acids and other organic chemicals. Many of these have potential pharmaceutical, nutraceutical, agricultural, and other applications (Wase and Wright 2008).

To date, the majority of bioactive metabolites isolated from cyanobacteria have either been polyketides, non-ribosomal peptides or a hybrid of two. This is a feature of their biosynthetic complexity, which they share with other bacteria such as Actinobacteria. Both NRPSs and PKSs are large (200- to 2,000-kDa), (Schwarzer, et al., 2001). Multimodular biocatalysts for natural product multifunctional enzymes that possess modular organization. NRPSs use amino acid monomers as substrates for synthesizing complex oligopeptides, whereas PKSs use acyl coenzyme, a monomers to form elaborate chemical structures along with a ketide backbone (Ehrenreich et al. 2005).

Discrete modules in the NRPSs and PKSs mediate the synthesis of their products. Similar to an assembly line, each enzymatic module is responsible for the addition of a single monomer to an elongating chain (Christiansen et al. 2001).

These modules, in turn, also possess modular organization, as they consist of a number of enzymatic active sites that can be partitioned into sequence domains at the level of primary structure. polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are exclusively involved in the biosynthesis of several important natural products that are currently in pre-clinical pharmaceutical development such as antibiotics, toxins, siderophores, and immune suppressants (Starcevic et al. 2012; Asthana et al., 2009 and Chetsumon et al. 1994).

Despite several worldwide studies on the prevalence of NRPSs and PKSs among the cyanobacteria, none of them included Iranian cyanobacteria of Kermanshah province. Therefore, the aim of this study was to amplify the NRPS and PKS genes, in order to predict a novel peptide compound and an amino acid activated by a specific unknown NRPS A module by cyanobacteria isolated from freshwater and terrestrial Iranian cyanobacteria of Kermanshah province.

Using molecular and phylogenetic techniques in combination with the analysis of the sequenced cyanobacterial genomes, we showed that NRPSs and PKSs were abundant across the cyanobacterial sections. The results of these experiments suggest that both NRPS and PKS genes are widespread and genetically diverse even among very closely related cyanobacterial species. Based on our results, the undifferentiated filamentous and heterocystous strains appeared to be the most likely sources of biochemically active natural products.

Materials and Method

Culture conditions, morphological characterization and preparation of extracts for bioactivity analyses

In 2015, soil and fresh water samples were collected from different agricultural and freshwaters areas in Kermanshah province, Iran. Samples were transferred to sterile Petri dishes with adequate quantities of liquid and solid media BG110 for culturing the soil and aquatic strains respectively (Rippka et al. 1979) without NaNO3. The pH was adjusted to 7.1 after sterilization. The Petri dishes were incubated in a culture chamber at 28°C and were provided with the continuous artificial illumination of approximately 1500-2000 lux for two weeks (Kaushik 1987). Three small fragments of growing colonies were then placed equidistantly onto an agar surface for purification. Morphological measurements were made by bright-field microscopy and by phase-contrast illumination of 10-day-old cultures using a Leica DM750 microscope. The following parameters

were selected to describe the morphology of heterocystous cyanobacteria: morphology of vegetative cells (including terminal cell), heterocytes, akinetes; presence or absence of terminal heterocytes; and the shape of the filament and its aggregation in colonies (Rajaniemi et al. 2005). The species were identified according to Desikachary (1959).

Three strains of heterocytous cyanobacteria which were the most frequently observed species in that areas were selected for evaluation of the presence of peptide synthetase and polyketide synthase genes and bioactivity potential. Cell mass was harvested by centrifugation at 7000 G for 7 min, resulting in a cell mass of 336 mg (wet weight). The harvested biomass was transferred into two separate sterile 1.5 mL microtubes, each containing 50 mg wet mass for DNA extraction and the remaining 236 mg for bioactivity analyses.

Genomic DNA extraction

Genomic DNA of ASN_M was extracted utilizing the E.Z.N.A.® SP Plant DNA kit (Omega Bio-Tek). The microtube containing 100 mg wet cells was supplemented with 300 mg of two differently sized glass beads (acid-washed, 180 μm and 425 – 600 μm, Sigma-Aldrich) as well as lysis buffer and RNase solution, both provided by the kit. In order to ensure proper disruption of the cells, tubes were homogenized three times for 20 s at a speed of 6.5 ms-1 with a FastPrep instrument (Savant Instruments). The extraction procedure was continued according to the kit's protocol, as supplied by the manufacturer. DNA was quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc).

PCR amplification

16S rRNA gene-based identification

Two oligonucleotide primers comprised of one forward primer (359F, 5'-GGGGAATYTTC-CGCAATGGG-3') and a reverse primer (781Ra, 5'-GACTACTGGGGTATCTA-ATCCCATT-3') (Nübel et al. 1997) were used for partial amplification of 16S rRNA gene. One PCR reaction was comprised of 1-time Buffer solution (DyNAzymeTM PCR buffer, Finnzymes), 0.5 μm forward primer, 0.5 μm reverse primer and 0.5 U Taq polymerase (DyNAzymeTM II DNA polymerase, Finnzymes) as well as 1 μL template DNA and sterile water in a total volume of 20 μL. The template DNA concentration of the three strains in the reaction accounted for approximately 140 ng. Sterile water was used as a negative control. The amplification reactions were conducted in a thermocycler (iCycler, Bio-Rad) with the following program: Initial denaturation at 94°C for 3 min, 30 cycles comprised of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and annealing at 72°C for 30 s, as well as a final annealing phase at 72°C for 5 min.

NRPS and PKS genes-based identification

The NRPS primers used for amplification were MTF2 and MTR. The PKS primers used were DKF and DKR (Ehrenreich et al. 2005). These NRPS and PKS primers target the A and KS domains, respectively. All PCRs were performed in either an Eppendorf (Westbury, NY) Mastercycler or a Bio-Rad (Hercules, CA) Icycler, and products were screened using 1% agarose gel electrophoresis. The following reaction mixtures and conditions were used for amplifying the NRPS A and PKS KS domains. The reaction mixture consisted of 33.8 μl of ddH 2O, 5 μl of Taq buffer A (MgCl2 included), 1 U of Taq DNA polymerase (Fisher, Pittsburgh, PA), 5 μl of 2 mM deoxynucleoside triphosphates, 2 μl of 50μM MTF2 or DKF primer,2 μl of 50μMMTRor DKR primer, and 5 μl of DNA template. The reactions were run with the following holds and cycles: 94°C for 5 min; 35 cycles of 94°C for 1 min, 50°C for 1 min for NRPS or 50.8°C for 1 min for PKS, and 72°C for 2 min for NRPS or 72°C for 1 min for PKS; and 72°C for 7 min.

PCR products were checked by electrophoresis on 1% agarose gels (SeaPlaque® GTG®, Cambrex Corporation) at 100 V, followed by 0.10 μg mL-1 EtBr (Ethidium bromide, Bio-Rad) staining. PCR products were visualized in the gel by UV light utilizing the Molecular Imager® Gel DocTM

XR system (Bio-Rad). A digital gel image was obtained utilizing the QUANTITY ONE® 1-D V 4.6.7 analysis software. The size of the products was estimated by comparing with marker DNA (/HinfIII + x/HaeIII, Finnzymes). The products were purified using the Geneclean® Turbo kit (Qbiogene, MP Biomedicals) and were quantified with a NanadropTM ND-1000 spectrophotometer (Thermo Scientific). All the primers purchased and sequenced by Roobin Teb company.

Analysis of sequences

BLAST N searches (http://www.ncbi.nlm.-nih.gov/BLAST) of the partial 16S rRNA gene of three strains and BLAST X search were used to identify similar sequences deposited in the GenBank™ database of NCBI. The 16S rRNA gene sequences obtained in this study, as well as reference sequences retrieved from GeneBank, were first aligned with CLUSTAL W (Thompson et al. 1994) with the default settings and were then manually edited in BioEdit version 7.0 (Hall 2004). The positions with gaps, as well as undetermined and ambiguous sequences were removed for subsequent phylogenetic analyses.

Identifications of the predicted amino acid activated by a specific unknown NRPS A module was performed using software located at http://www.tigr.org/ Jravel/nrps (Challis et al. 2000; Bachmann and Ravel 2009). The activation of amino acids by the identification A-domain motif will indicate their presence, unmodified or modified in the final natural product structure.

Phylogenetic trees using the maximum Likelihood (Jones–Taylor–Thornton model) analyses was constructed by the MEGA version 5.1 (Tamura et al. 2007). The robustness of the tree was estimated by bootstrap percentages using 1000 replications. Complete deletion handling of gaps and confidence levels were calculated via bootstrapping using a resampling number of 1,000. Reference sequences were obtained from GenBank (NCBI).

Evaluation of antimicrobial activity

The microorganisms used in the antimicrobial assay were collected from the Iranian Research Organization for Science and Technology (IROST). Extracted compounds of the three strains were examined for bioactivity against a Gram-positive bacteria Staphylococcus aureus (PTCC 1112), and two Gram-negative bacteria Escherichia coli (PTCC 1047) and Pseudomonas aeroginosa (PTCC 1310) by disk diffusion assays. Müller-Hinton agar plates were inoculated with a standardized quantity of suspension containing 1.5 108 cfu ml−1 bacteria corresponding to 0.5 MacFarland standards according to NCCLS (now CLSI 2008) (1997). 100 μl of the methanolic cyanobacterial extracts and 100 μl MeOH as negative controls were pipetted into the 6 mm diameter filter paper discs in assay plates. Plates were incubated overnight at 37°C for a period of 18 to 24 h for bacteria. The diameter of the zones with complete inhibition of growth was measured to the nearest centimeter using a ruler and expressed in mm. All the tests were performed under sterile conditions and repeated three times (Fish and Codd 1994; Issa 1999; Qstensvik et aL. 1998 and Radhakrishnan at al. 2009).

Results

Evaluation the antimicrobial activity of three strains

The methanolic extract of Calothrix sp. N42 showed potent activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, whereas, there was no inhibitory effect against Escherichia coli and Staphylococcus aureus in two another strains. (Data not shown). However, there was significant inhibitory effect against Pseudomonas aeruginosa from three strains. The response of inhibitory zone of extracts grown against Pseudomonas aeruginosa was nearly similar. For example Nostoc sp. N66 and Scytonema sp. N11 had a maximum inhibitory (1.9 centimeters) and Calothrix sp. N42 had a lower zone of inhibition (1.6 centimeters) (Figure 1).

Identification of putative polyketide synthase

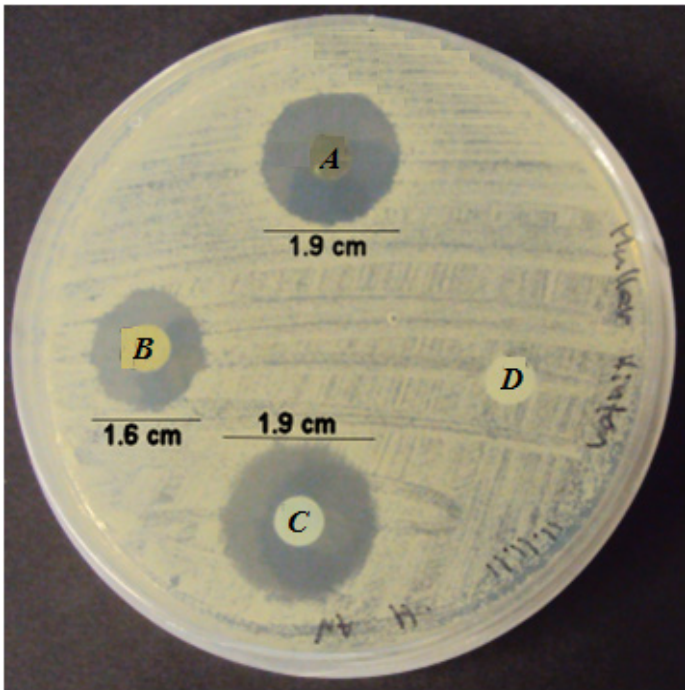

Figure: 1. Zone of inhibition exhibited by methanolic extracts of Nostoc sp. N66 (A), Calothrix sp . N42 (B) and Scytonema sp. N11 (C) against of Pseudomonas aeruginosa. D is negative control.

(KS) and peptide synthetase (NRPS) regions

Here, we present the study the presence of the aminoacyl-adenylation and ketoacyl domain of NRPS and PKS genes using two sets of degenerate oligonucleotide primers set MTF2/MTR and DKF/DKR respectively (Figure 2).

To verify that the amplified products were NRPS A and PKS KS domains, (~1 kb for NRPS A domains; ~700 bp for PKS KS domains) were sequenced. BLAST analysis in GenBank (tBlastn) confirmed that the sequences were cyanobacterial NRPS A or PKS KS domains, as all sequences were most similar to cyanobacterial A or KS domains already present in GenBank. BLASTx sequence analysis of these strains showed varying similarities to other known cyanobacterial NRPS and PKS sequences.

Sequenced A domains (~297 amino acids and 923 bp long for Nostoc sp. N66 and ~468 amino acids and 901 bp for Scytonema sp. N11 and ~ 148 amino acids and 596 bp for Calothrix sp . N42) (KY548060, KY548064 and KY548066) and KS domains (~220 amino acids long and 563 bp for Nostoc sp. N66 and ~ 193 aa and 435 bp for Scytonema sp. N11 and ~ 191 aa and 427 bp for Calothrix sp . N42) (KY548061, KY548063, and KY548067) were submitted in genebank.

Putative conserved domains have been detected and searched for details on NCBI site and five domain hits for the aminoacyl-adenylation domain of NRPS gene has been found. The details were similar to three strains. 1: The adenylation (A) domain of NRPS recognizes a specific amino acid or hydroxy acid and activates it as an (amino) acyl adenylate by hydrolysis of ATP. 2: Amino acid adenylation domain represents a domain responsible for the specific recognition of amino acids and activation as adenylyl amino acids. 3: PRK12467 is responsible for peptide synthase, 4: EntF (Non-ribosomal peptide synthetase component F) is responsible for secondary metabolites biosynthesis, transport, and catabolism and the 5 hits belong to AMP-binding enzyme.

Moreover, the list of domain hits for ketoacyl domain of PKS gene have been detected and searched for detail on NCBI site. The details were similar to Nostoc sp. N66 and Scytonema sp. N11 and it was different for some hits with Calothrix

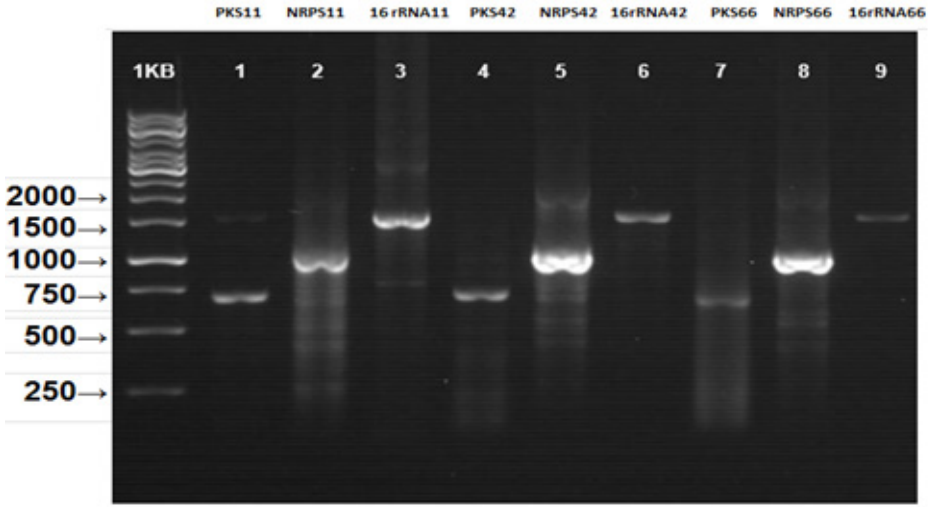

Figure 2: Agarose gel electrophoresis of degenerate PCR products from DNA isolated from three species. Lanes 3, 6 and 9, Selective amplification of ~1 kb fragments using primers specific for NRPS adenylation sequences and Lanes 1, 4 and 7, Selective amplification of ~700 bp fragments using specific primers for PKS KS domains of Scytonema sp. N11, Calothrix sp . N42 and Nostoc sp. N66 respectively. Marker: Thermo Scientific GeneRuler 100 bp Plus DNA Ladder.

sp. N42. 5 similar hits were: 1. polyketide synthases (PKSs) polymerize simple fatty acids into a large variety of different products and called polyketides, by successive decarboxylating Claisen condensations. 2. Beta-ketoacyl synthase, the structure is similar to the thiolase family and also chalcone synthase. 3. PksD, Acyltransferase domain in polyketide synthase (PKS) enzymes are responsible for secondary metabolites biosynthesis, transport, and catabolism. 4. omega_3_PfaA is involved in omega-3 polyunsaturated fatty acid biosynthesis, such as the protein PfaA from the eicosapentaenoic acid biosynthesis operon in Photobacterium profundum strain SS9. 5, 3-oxoacyl-(acyl carrier protein) synthase II.

Two hits of ketoacyl domain are unique for Calothrix sp. N42. One of them is acetyl-CoA acetyltransferases that represent a large family of enzymes which catalyze the thiolysis of a linear fatty acid CoA (or acetoacetyl-CoA) using a second CoA molecule to produce acetyl-CoA and a CoA-ester product, two carbons shorter (or, alternatively, the condensation of two molecules of acetyl-CoA to produce acetoacetyl-CoA and CoA) and the other is Thiolase that is reported to be strauvtully related to beta-ketoacyl synthase, and also chalcone synthase.

Phylogenetic analysis of gene homologs and prediction of amino acid recognition by NRPS adenylation domains

Two Phylogenetic trees using the Maximum Likelihood analyses were constructed via MEGA5 software package (version 5.0) (Figure 3 and 4). Phylogenetic analysis was performed on the putative peptide synthase protein sequences from the three strains species screened from the present study and additional sequences of enzymes from species that showed high similarity to the sequences identified.

The Phylogenetic analysis clustered the non-ribosomal peptide synthetase sequence of Nostoc sp. N66, Calothrix sp. N42 and Scytonema sp. N11 in a strongly supported subclade group (bootstrap value of 100% ML). The signature sequence and the name of the compound and the predicted amino acid of three species were the same for each subclade group (Table 1). Clustering of these NRPS sequences and the predicted amino acid illustrates a lack of taxonomic affiliations between cyanobacteria and A-domains. For

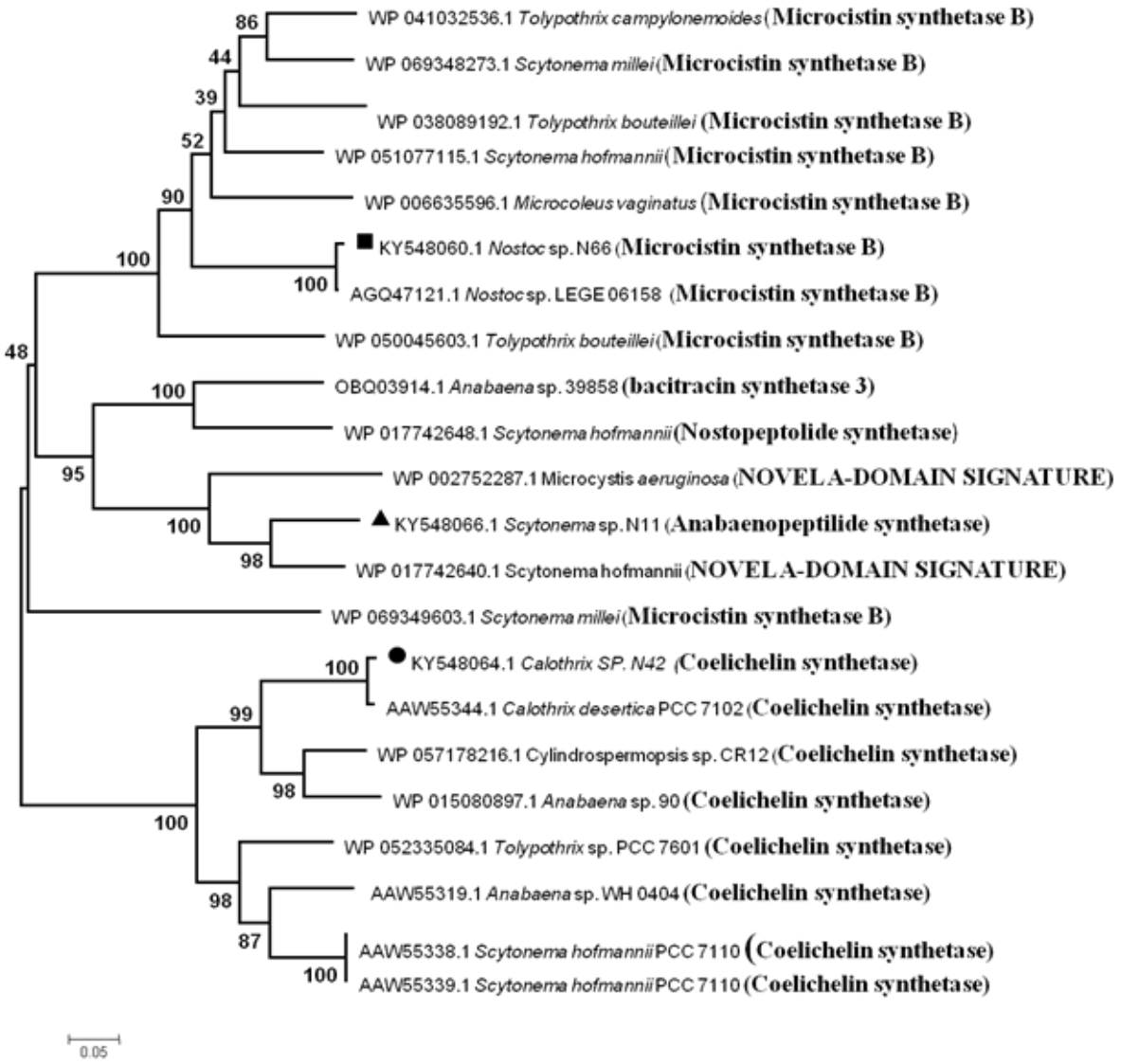

Figure 3: Phylogenetic analysis of A domains sequenced in this study or taken from the GenBank. The PCR-fragment is ~1 kb long. The significant bootstrap percentages obtained from 100 replicates using maximum likelihood (ML), greater than 50%, indicated in the near of nodes respectively. The studied starins, Scytonema sp. N11, Calothrix sp . N42 and Nostoc sp. N66 are shown in full triangle, Full circle and full Square respectively. The scale indicates 0.05 mutations per amino acid position.

example, few instances of the close relationship between the taxonomic status and the predicted compound were evident (Figure 3). Analysis showed that each strain screened possessed 2 or more domains that were responsible for multiple independent compounds into the same species.

Phylogenetic analysis of the detected PKS KS domains showed that all the obtained KS domains were 66, 96 and 100 percent similar to referenced sequences retrieved from the GenBank database for Calothrix sp. N42, Scytonema sp. N11 and Nostoc sp. N66 respectively based on the AA level. The sequences isolated from three species each tend to cluster in independent clades on the phylogenetic tree. although Calothrix sp. N42 showed about 66% identities to KS domains

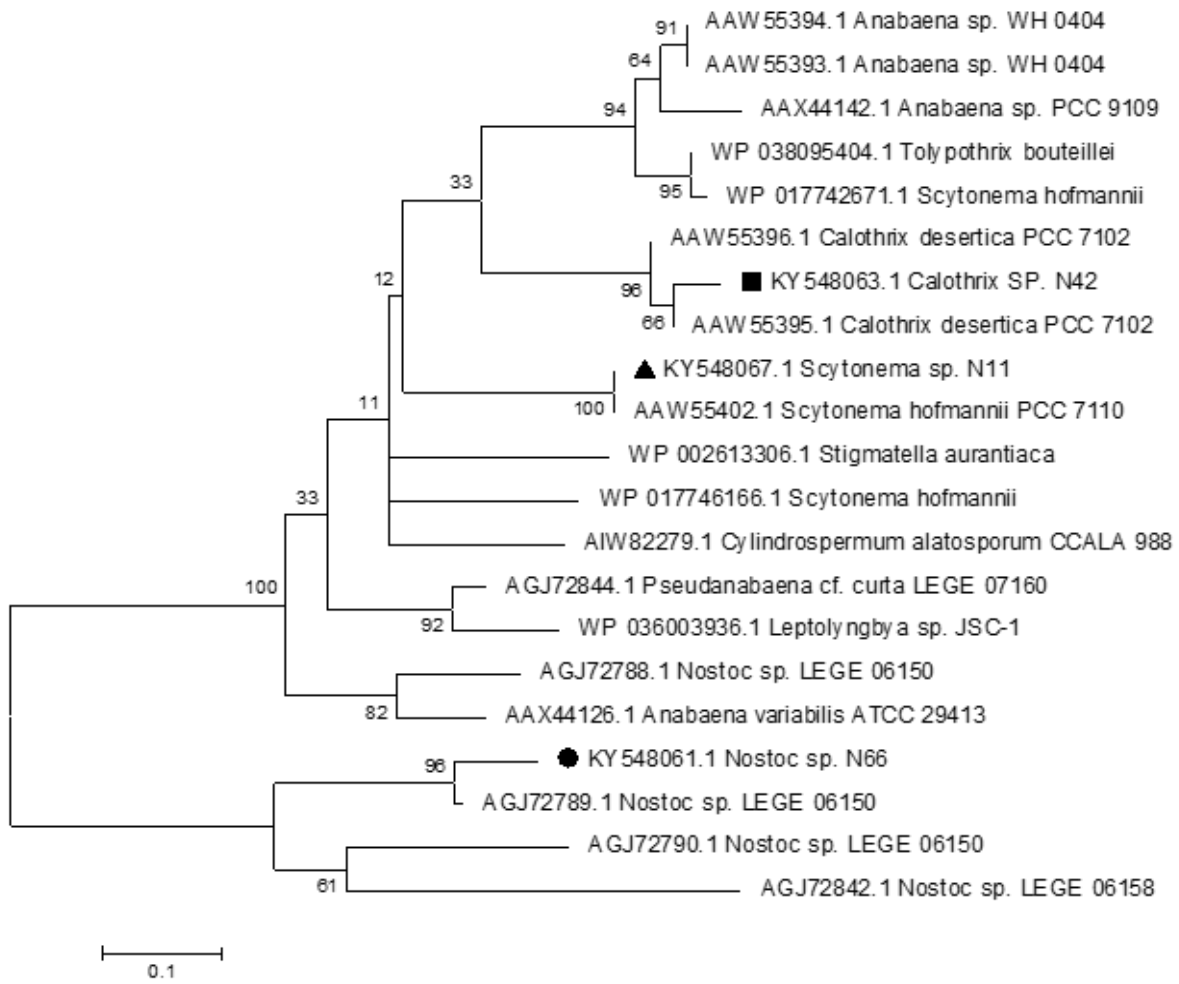

Fig 4. Phylogenetic analysis of KS domains from sequenced species in this study or taken from the GenBank. The PCR-fragment is ~700 bp long. The significant bootstrap percentages obtained from 100 replicates using maximum likelihood (ML), greater than 50%, indicated in the near of nodes respectively. The studied strains, Scytonema sp. N11, Calothrix sp . N42 and Nostoc sp. N66 are shown in full triangle, Full square and full corcle respectively. The scale indicates 0.1 mutations per amino acid position.

from various cyanobacteria. The low similarity prohibited the prediction of possible biosynthesis pathways and substrates by the alignment of KS domains to homologous fragments (Figure 4).

The cyanobacteria strains were identified and registered under DDBJ as Nostoc sp. N66 KY548059, Calothrix sp. N42 KY548062 and Scytonema sp. N11 KY548065. Moreover, the sequences from amplification of NRPS and PKS genes were deposited in GenBank and had accession numbers KY548060, KY548064 and KY548066 (NRPS A domains) and KY548061, KY548063 and KY548067 (PKS KS domains) for Nostoc sp. N66, Calothrix sp. N42 and Scytonema sp. N11 respectively.

Discussion

In these two decades, cyanobacteria have been known to produce a wide range of new

Table 1: Cyanobacterial NRPS sequences analyzed using the BLASTp tool for natural product biosynthesis. Sequences identified in this study are marked in bold and the other sequences were retrieved from the GenBank.

a Accession numbers corresponding to the NCBI website for amino acid sequences.

c Eight variable amino acids of the signature sequences.

d Nomenclature of the reference compounds.

e The compound here is for illustrative purposes, in that the domain is similar. This does not imply the strain makes this exact compound.

Strain	Compound e	Signature Sequencec c	Predicted amino acid d
KY548064.1_Calothrix_SP._N42	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
AAW55344.1_Calothrix_desertica_PCC_7102	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
KY548060.1_Nostoc_sp._N66	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
AGQ47121.1_Nostoc_sp._LEGE	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
KY548066.1_Scytonema_sp._N11	Anabaenopeptilide synthetase	D L A F T G C V	AdpB-M1-Hty
WP_051077115.1_Scytonema_hofmannii	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
WP_069348273.1_Scytonema_millei	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
WP_017742640.1_Scytonema_hofmannii	NOVEL A-DOMAIN SIGNATURE	D S A S G G C V	NO HIT
WP_017742648.1_Scytonema_hofmannii	Nostopeptolide synthetase	D A S T V A A V	NosD-M1-Tyr
WP_069349603.1_Scytonema_millei	Anabaenopeptilide synthetase D	D A F F L G X X	AdpD-M1-Ile
AAW55338.1_Scytonema_hofmannii_PCC_7110	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
AAW55339.1_Scytonema_hofmannii_PCC_7110	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
WP_041032536.1_Tolypothrix_campylonemoides	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
WP_050045603.1_Tolypothrix_bouteillei	Microcistin synthetase B	D A W F L G N V	McyB-M1-Le
WP_038089192.1_Tolypothrix_bouteillei	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
WP_052335084.1_Tolypothrix_sp._PCC_7601	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
WP_006635596.1_Microcoleus_vaginatus	Microcistin synthetase B	D A W F L G N V	McyB-M1-Leu
WP_002752287.1_Microcystis_aeruginosa	NOVEL A-DOMAIN SIGNATURE	D S A S G G C V	NO HIT
WP_015080897.1_Anabaena_sp._90	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
AAW55319.1_Anabaena_sp._WH_0404	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr
OBQ03914.1_Anabaena_sp._39858	bacitracin synthetase 3	D A F T I A A -	BacC-M2-Phe
WP_057178216.1_Cylindrospermopsis_sp._CR12	Coelichelin synthetase	D F W N I G M V	CchH-M2-Thr

bioactive compounds having pharmacological significance (Dittmann et al. 2001; Kreitlow et al. 1999). These active compounds exhibit a wide range of bioactivities that may be relevant to the natural environment such as antibacterial, antifungal, antiviral and cytotoxic activities and sometimes immunomodulatory and protease inhibitory activities which are not related to ecological significance (Biondi et al. 2004; Juttner et al. 2001; Kaushik et al. 2009; Portmann et al. 2009 and Dahms et al. 2006). Many different active substances, mainly belonging to the chemical classes of cyclic peptides, alkaloids, and macrocycles, have been already identified in different species of cyanobacteria (Rezanka and Dembitsky 2006; Dembitsky et al. 2005). The NRPS and PKS are two classes of large modular enzymes in which modules incorporate building blocks into the growing chain like in an assembly line. Interestingly, Cyanobacteria dedicated about 5% of their genomes for these pathways, with an average of five NRPS/PKS clusters per genome (Calteau et al., 2014).

The above findings indicate that the search for bioactive secondary metabolites from cyanobacteria is providing an increasing number of useful and structurally characteristic compounds from the pharmaceutical point of view (Abedin et al. 2008; Lerena et al. 2007 and Skulberg 2000).

In this study, we screened three species of cyanobacteria families for both NRPS and PKS genes and analyzed using PCR by amplifying NRPS, PKS genes with degenerate primers (Figure 3 and 4). Sequencing these gene fragments encoding for adenylation domain could allow the prediction of the probable name of compound (Table 1). Phylogenetic tree constructed with the A domains taken from the two characterized gene were concatenated together with other amino acid sequences from several cyanobacteria retrieved from the GenBank appeared to be quite divergent from one another, implying that domains in the same gene or pathway can differ substantially in primary structure.

The phylogenetic analysis of NRPS gene using maximum likelihood method revealed that each strain positioned in a separate internal branch within a supported major clade by high bootstrap value (bootstrap value of 100% (ML).

Our analysis showed that clustering of the full A-domain is possible when substrate conferring tendencies are taken into account. These results confirm those previously described by Burns et al. 2005; Stachelhaus et al. 1999; Genuario et al. 2010; Moffitt and Neilan 2001; Zhao et al. 2008).

Analysis of the specificity of A-domains towards particular amino acids (Table 1) shows the presence of homo tyrosine (Hty), Threonine, (Thr) and Leucine, (Leu) for Scytonema sp. N11, Calothrix sp. N42 and Nostoc sp. N66 respectively. Moreover, we can discern branches specific to Anabaenopeptilide synthetase, Microcystin synthetase B, and Coelichelin synthetase, however, this does not imply the strain makes these exact compound.

The results of this paper showed that three species Nostoc sp. N66 KY548059, Calothrix sp. N42 KY548062 and Scytonema sp. N11 assayed for natural products had both NRPS and/or PKS genes with biologically detectable activities. However, until now, there isn't any evidence to determine if NRPS and/or PKS genes underlie the detected activities. Ehrenreich et al. 2005 found that both Lyngbya sp. strain PCC7419 and Cyanothece sp. strain WH8904 were negative for NRPS and PKS genes but both had detectable activities. Actually, further studies have to be made on connecting the biochemical activities and the existence of the NRPS and PKS genes. The strains examined provided the basis for further studies on the isolation and identification of bioactive compounds. Moreover, exploiting these metabolites and their powerful biological activities will improve our understanding of natural phenomena on a molecular level and can contribute to the development of molecular strategies to the control of human pathogens and other diseases.

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