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Evaluation of Antibiotic Biosynthetic Potential of Actinomycete Isolates to Produce Antimicrobial Agents

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Authors' contributions

This research was carried out in collaboration between the two authors. Author MFA managed literature search, analyzed the study and compiled the manuscript. Author OOB conceptualized the idea, supervised the lead author and leads the project. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the antibiotic biosynthetic potential of actinomycete isolates from rhizospheric soil samples collected from Ngaka Modiri Molema district in North West Province of South Africa.
Study Design: The analysis of biosynthetic gene clusters through PCR-based approach presents a useful foundation for the discovery of bioactive compounds.
Place and Duration of Study: Microbial Biotechnology Laboratory, Department of Biological Sciences, North-West University, Mafikeng Campus, South Africa, between June 2011 and November, 2013.
Methodology: Through PCR-based approach 341 actinomycete isolates were screened for thepolyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) biosynthetic gene clusters. The amplification of the genes from some of the actinomycete isolates is an indication of

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their potential as antibiotic producers. Phylogenetic analysis using PKS-I, PKS-II and NRPS gene sequences were conducted.
Results: Sixteen isolates (4.69%) were identified as PKS-I gene positive strains, 15.25% for PKS-II and 13.48% for NRPS gene. Through the screening, it was found that *Streptomyces* have higher prevalence of PKS-I, PKS-II and NRPS genes compared to others genera. Phylogenetic analysis of the nucleotide sequences from the amplified biosynthetic genes confirmed that the isolates formed a close phylogenetic relationship with known antibiotic producers.
Conclusion: PCR-based approach using degenerative primers to screen for the presence of biosynthetic gene clusters responsible for the biosynthesis of bioactive secondary metabolites, is an effective approach for discovering diverse antibiotics from actinomycetes.

Keywords: Actinomycetes; secondary metabolites; biosynthetic gene cluster; PCR screening PKS; NRPS.

1. INTRODUCTION

Terrestrial actinomycetes are well known as prolific producers of secondary metabolites. These secondary metabolites exhibit pharmaceutical properties, such as antibacterial, antifungal, antiprotozoal, anticancer, anticholesterol and immunosuppressant. These bioactive compounds were synthesized through biosynthetic metabolic pathways. Polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) pathways are synthesized by various specific enzymatic steps [1]. These enzymes perform the repetitive chemical condensation of monomeric units, such as carboxylic acid and / or amino acid monomers. The vast structural complexity and functional diversity of PKS, NRPS or hybrid compounds are as a result of complex enzyme organization of the assembly lines and specific tailoring enzymes responsible for post-assembly modifications [2]. Recent advances in the areas of small molecule detection, isolation and structure elucidation, genome sequencing and molecular techniques have helped improve the understanding of the molecular genetics of secondary metabolite biosynthesis, rekindling interest in discovery platforms aimed at realizing the biosynthetic capacity of antibiotic producing microorganisms [2].

The PCR based approaches have been used successfully to amplify genes associated with secondary metabolite biosynthesis and therefore it is possible to predict whether secondary metabolite pathways are present within an organism [3]. It becomes feasible to envisage or estimate the number and novelty of bioactive compounds following phylogenetic analysis of the amplified and sequenced secondary metabolite genes. Secondary metabolites through PKS and NRPS pathways have proven to be one of the primary sources of new chemical scaffolds from which novel pharmaceutical agents have been developed [4].

The order Actinomycetales are renowned producers of bioactive metabolites with a track record of over 10,000 antimicrobial agents in clinical use [5]. Biosynthesis of these secondary metabolites is catalyzed by specific enzymes usually encoded by gene clusters. Polyketide synthases and non-ribosomal peptide synthetases are the major enzymes of secondary metabolites synthesis [6]. Examples of classes of antibiotics produced through this biosynthesis include ansamycins, tetracyclines, polyenes and glycopeptides [7].

In an effort to discover potentially novel bioactive compounds, degenerate primer sets were used to screen for the presence of biosynthetic gene clusters associated with PKS-I, PKS-II and NRPS pathways in genomic DNA of 341 actinomycete strains. The antibacterial potential of the actinomycete strains was based on the amplification of the expected size of the gene of interest. The PCR screening of the genomic DNA for specific antibiotic genes of interest allows for the rapid determination of the antibiotic biosynthetic potential of the isolates.

2. METHODOLOGY

2.1 Bacterial Strains and Cultivation

All actinomycete strains isolated and identified from rhizospheric soil samples were grown in Luria Bertani broth (Merck) at 30ºC with agitation in shaker incubator for 7 days [8].

2.2 Antibacterial Assays

Antagonistic activity of actinomycete isolates against Gram-negative and Gram-positive

bacteria was screened by using perpendicular streak method [8,9]. In perpendicular streak method, Mueller Hinton agar (Merck) was used and each plate was streaked with individual actinomycete isolates at the center/diameter of the plate and incubated at 30ºC for 7 days. Later, 24 h fresh sub-cultured test bacteria were prepared and streaked perpendicular to the isolates and incubated at 37ºC for 24 h. The experiment was carried out in triplicate.

2.3 DNA Extraction

The genomic DNA was extracted from all the actinomycete isolates using the cetyltrimethyl ammonium bromide (CTAB) method as previously described [10].

2.4 PCR Primers

Four sets of primers were used: F1: 5'-AGAGTTTGATCITGGCTCAG-3' and R5: 5'-ACGGITACCTTGTTACGACTT-3' targeting 16S rDNA gene [11]. AHBA-F: 5'-CCSGCSTTCACSTTCATCTC-3' and AHBA-R: 5'-AISYGGAICATIGCCATGTAG-3' targeting 3-amino-5-hydroxyl-benzoic acid (AHBA) synthase gene [3]. ARO-PKS-F: 5'-GGCAGCGGITTCGGCGGITTCCAG-3' and ARO-PKS-R: 5'-CGITGTTIACIGCGTAGAACCAGGCG-3' targeting the ketosynthase alpha (KS_α) and ketosynthase beta (KS_β) gene pair [3]. NRPS-A3F: 5'-GCSTACSYSATSTACACSTCSGG-3' and NRPS-A7R: 5'-SASGTCVCCSGTSCGGTAS-3' targeting the targeting NRPS adenylation (A) domains [12]. All the oligonucleotide primers were synthesized by Integrated DNA Technologies (South Africa).

2.5 PCR Amplification Conditions

PCR was performed in a total volume of 50 µl containing 30-50 ng DNA, 100 mM of each primer, 0.05 U/µl *Taq* DNA polymerase, 4 mM $MgCl_2$, and 0.4 mM of each dNTP (Fermentas, USA). The amplification reaction was performed with a DNA Engine DYAD Peltier thermal cycler (BioRad, USA). The PCR cycling programme was as follows: initial denaturation (96ºC for 2 min); 30 cycles of denaturation (96ºC for 45 s), annealing (56ºC for 16S rRNA and AHBA synthase genes/64ºC for the KS_α–KS_β gene pair/57ºC for NRPS gene, for 30 s), and extension (72ºC for 2 min); and a final extension (72ºC for 5 min). The PCR amplicons were analyzed by electrophoresis on 1% (w/v) agarose gel. The gel containing ethidium bromide (10 µg/ml) was viewed under Syngene Ingenius Bioimager (UK) to confirm the expected size of the PCR products. The remaining mixture was purified using NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel, Germany).

2.6 DNA Sequencing

Sequencing of the purified PCR products was conducted at the facilities of Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa using ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems, USA).

2.7 Phylogenetic Analysis

All nucleotide sequences for the 16S-rDNA, PKS and NRPS phylogenetic trees were obtained from GenBank, except for the sequences that were determined in this study. Nucleotide sequences were analyzed and edited using BioEdit software package version 7.1. The obtained sequences were compared to sequences in the NCBI GenBank database with the Basic Local Alignment Search Tool (BLAST) [13]. Multiple alignments of the sequences were carried out by Mafft program 7.050 [14] against corresponding nucleotide sequences retrieved from GenBank. Phylogenetic and molecular evolutionary analyzes were conducted using software in MEGA version 5.2.2 [15]. Evolutionary distance matrices were generated and a phylogenetic tree was inferred by neighbor-joining method [16]. Tree topologies were evaluated by bootstrap analysis [17] based on 1000 resamplings of the neighbor-joining data set. Manipulation and tree editing were carried out using TreeView [18].

2.8 Nucleotide Sequence Accession Numbers

The GenBank accession numbers of the nucleotide sequences generated in this study are shown in parentheses beside the isolate codes on the phylogenetic trees.

3. RESULTS

3.1 Bioactivity of Actinomycete Isolates

All the actinomycete isolates were tested for antibacterial activity using cross streak method. The result shows that 54% of the isolates exhibited antibacterial activity against one or more of the test organisms [8]. Analysis of the bacterial isolates showed that the genus *Streptomyces* is the most active in exhibiting broad spectrum antimicrobial activities [19].

3.2 Detection of Biosynthetic Gene in Actinomycete Isolates

Degenerate primers targeting genes encoding for polyketide synthases (PKS-I and PKS-II) and non-ribosomal peptide synthetases (NRPS) were used to screen the biosynthetic potential of 341 actinomycete isolates, as identification of these genes provides indirect evidence of potential chemical diversity among these actinobacteria in terms of natural product drug discovery. The antibiotic producing actinomycete isolates were the subject of 16S rDNA gene sequencing which is used for the identification of the strains by BLAST search program. It is apparent that the actinomycete isolates can be assigned to the genera *Streptomyces* spp, *Nocardia* spp, *Rhodococcus* spp, *Pseudonocardia* spp, *Saccharothrix* spp, *Promicromonospora* spp, *Arthrobacter spp*, *Micrococcus* spp, *Nocardiopsis* spp, *Streptosporangium* spp, *Nonomuraea* spp and *Actinomadura* spp.

PCR screening for the biosynthetic gene involved in the production of antimicrobial agents depends on the amplification of the predicted size of the DNA fragments. As expected, PCR screening of the genome of the actinomycete isolates for the biosynthetic gene yielded DNA fragments of the predicted size from the potential producers. PCR screening for the PKS-I, yielded DNA fragments of approximately 500 bp. The results indicate 4.69% (16) positive strains for the presence of PKS-I biosynthetic gene cluster from the 341 actinomycete isolates screened. Molecular identification of the actinomycete isolates using 16S rDNA shows that *Streptomyces* are the most prominent genus containing the AHSA biosynthetic gene. A standard nucleotide BLAST search was carried out using the partial nucleotide sequences of the positive strains against reference sequences in the GenBank database. This showed that the actinomycete isolates were most similar to other PKS-I producers such as *Amycolatopsis mediterranei*, *Micromonospora* spp, and *Streptomyces* spp. Fig. 1 shows the results of the PCR screening for the PKS-II gene pair, KS_{α} and KS_{β} with an expected amplified DNA fragment of approximately 500 bp. Out of 341 actinomycte isolates screened 15.25% were found to contain the biosynthetic gene for PKS-II. A BLAST search against the GenBank database using the nucleotide sequences generated from this study showed that the positive strains were most similar to the corresponding sequences from aromatic polyketide producers. An approximately 750 bp DNA fragment was amplified from the positive strains for NRPS gene. Of all the 341 actinomycete isolates screened, 13.48% (46) were positive for the presence of the NRPS pathway. The NRPS gene sequences were generated and compared to sequences in the GenBank by BLAST analysis. The result showed that majority of the strains belong to the genus *Streptomyces*.

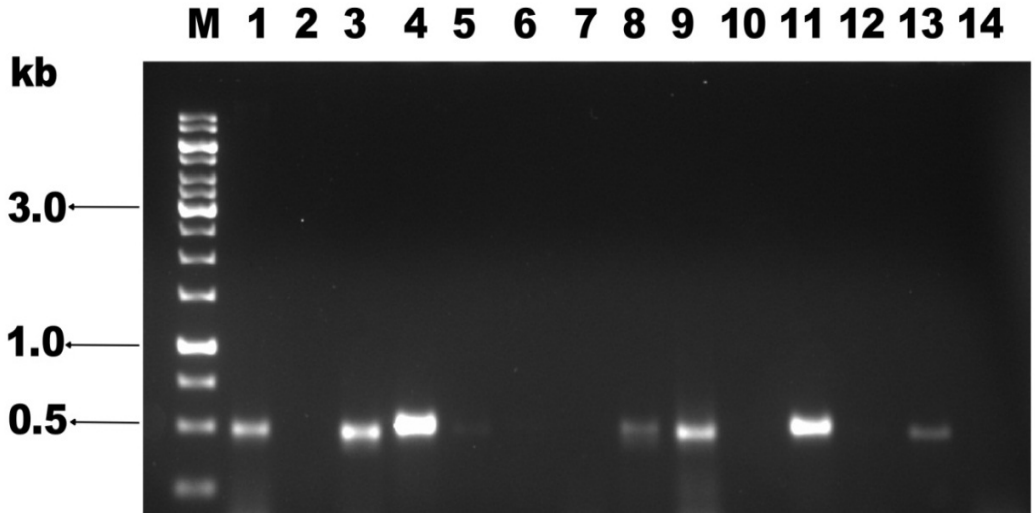

Fig. 1. Agarose gel electrophoresis of PCR products from selective amplification of 500 bp fragment using ARO-PKS-F/ARO-PKS-R specific for PKS-II sequences

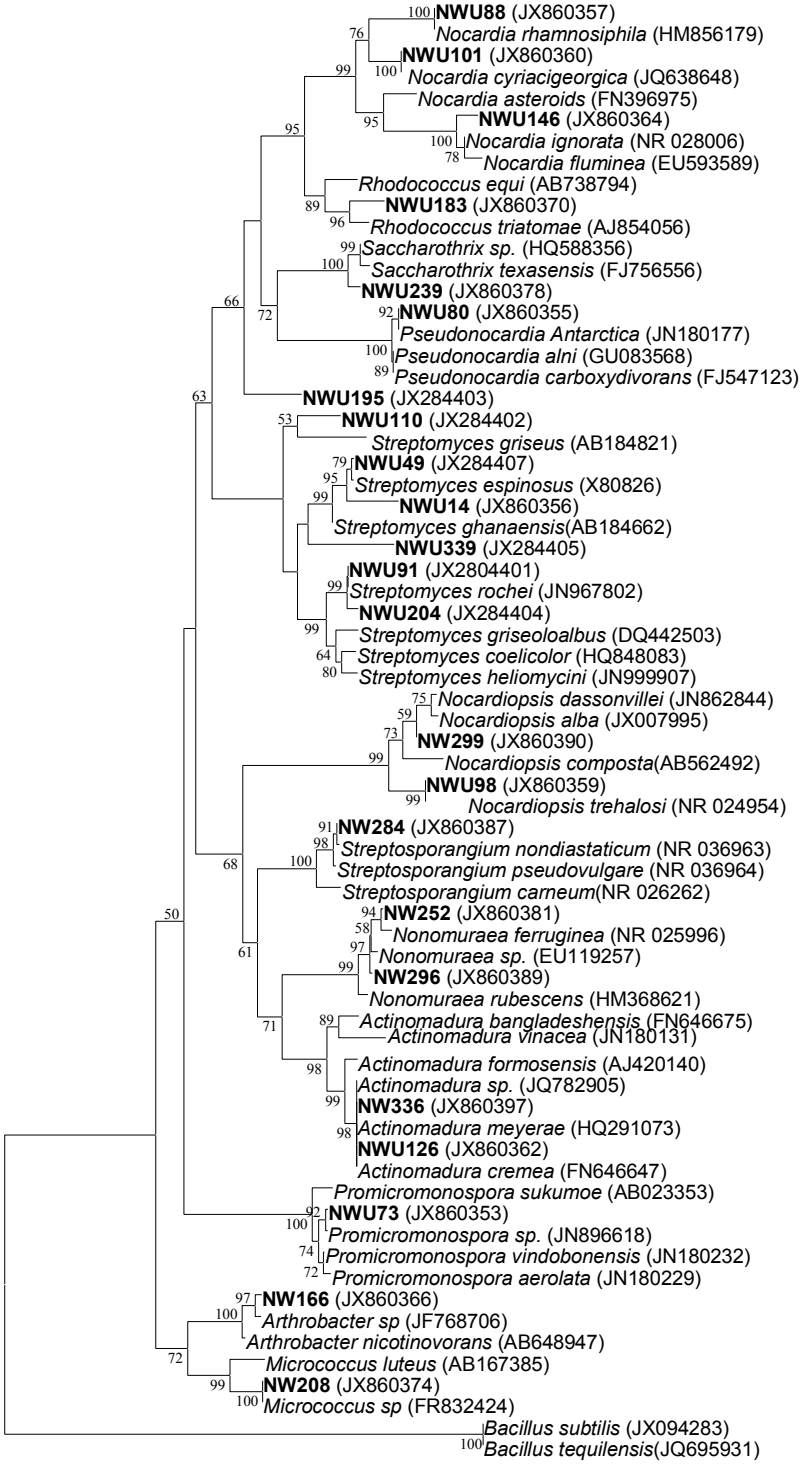

Fig. 2. Neighbor-joining tree (Saitou and Nei, 1987) based on 16S rRNA gene sequences showing relationships between actinomycetes strains and representatives of related taxa. Bootstrap value (>50%) based on 1000 resampled datasets are shown at branch nodes. *Bacillus subtilis* and *Bacillus tequilensis* are used as outgroup

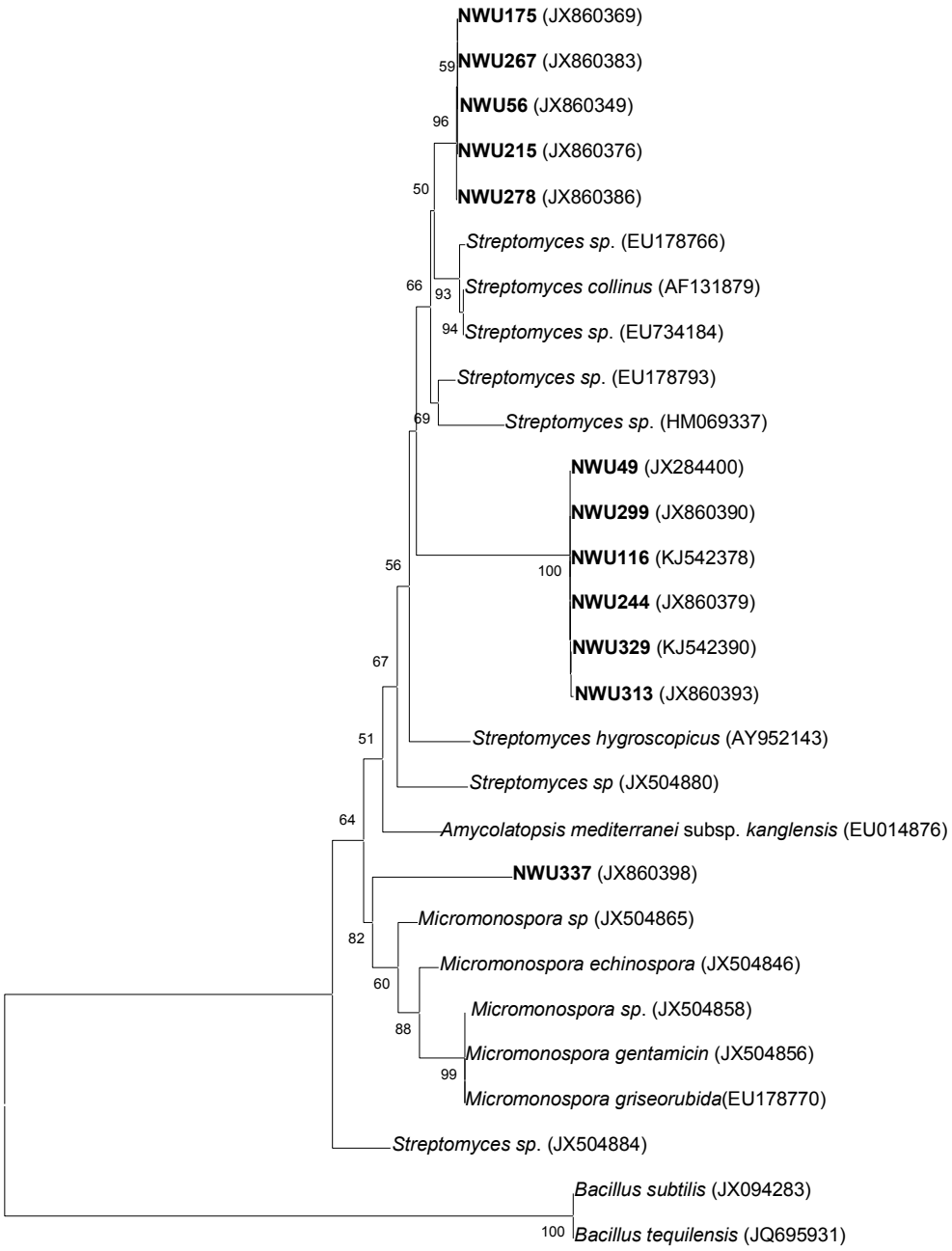

Fig. 3. Neighbor-joining tree (Saitou and Nei, 1987) based on AHSA gene sequences showing relationships between actinomycetes strains and representatives of related taxa. Bootstrap value (>50%) based on 1000 resampled datasets are shown at branch nodes. *Bacillus subtilis* and *Bacillus tequilensis* are used as outgroup

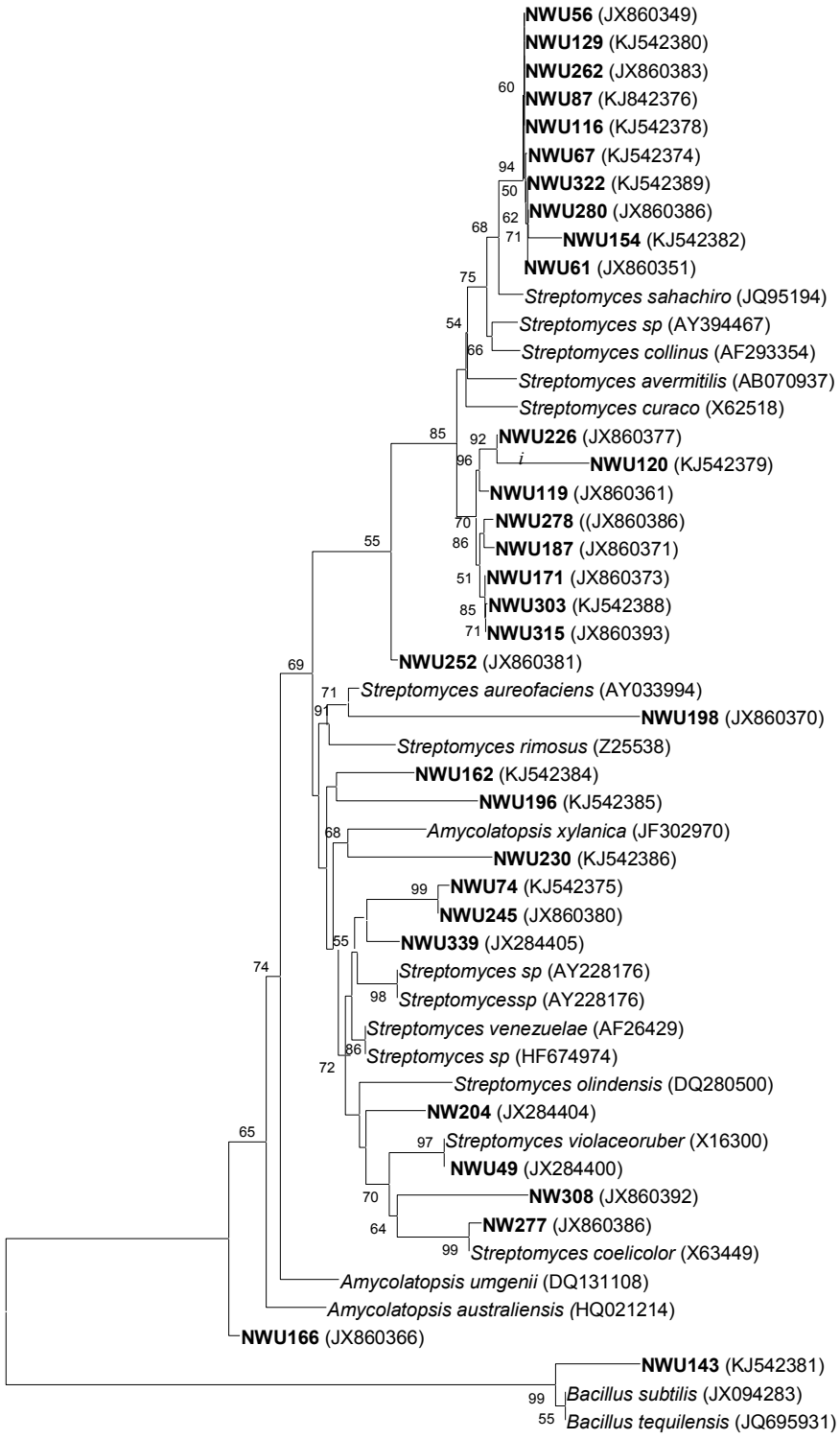

Fig. 4. Neighbor-joining tree (Saitou and Nei, 1987) based on PKS-II gene sequences showing relationships between actinomycetes strains and representatives of related taxa. Bootstrap value (>50%) based on 1000 resampled datasets are shown at branch nodes. *Bacillus subtilis* and *Bacillus tequilensis* are used as outgroup

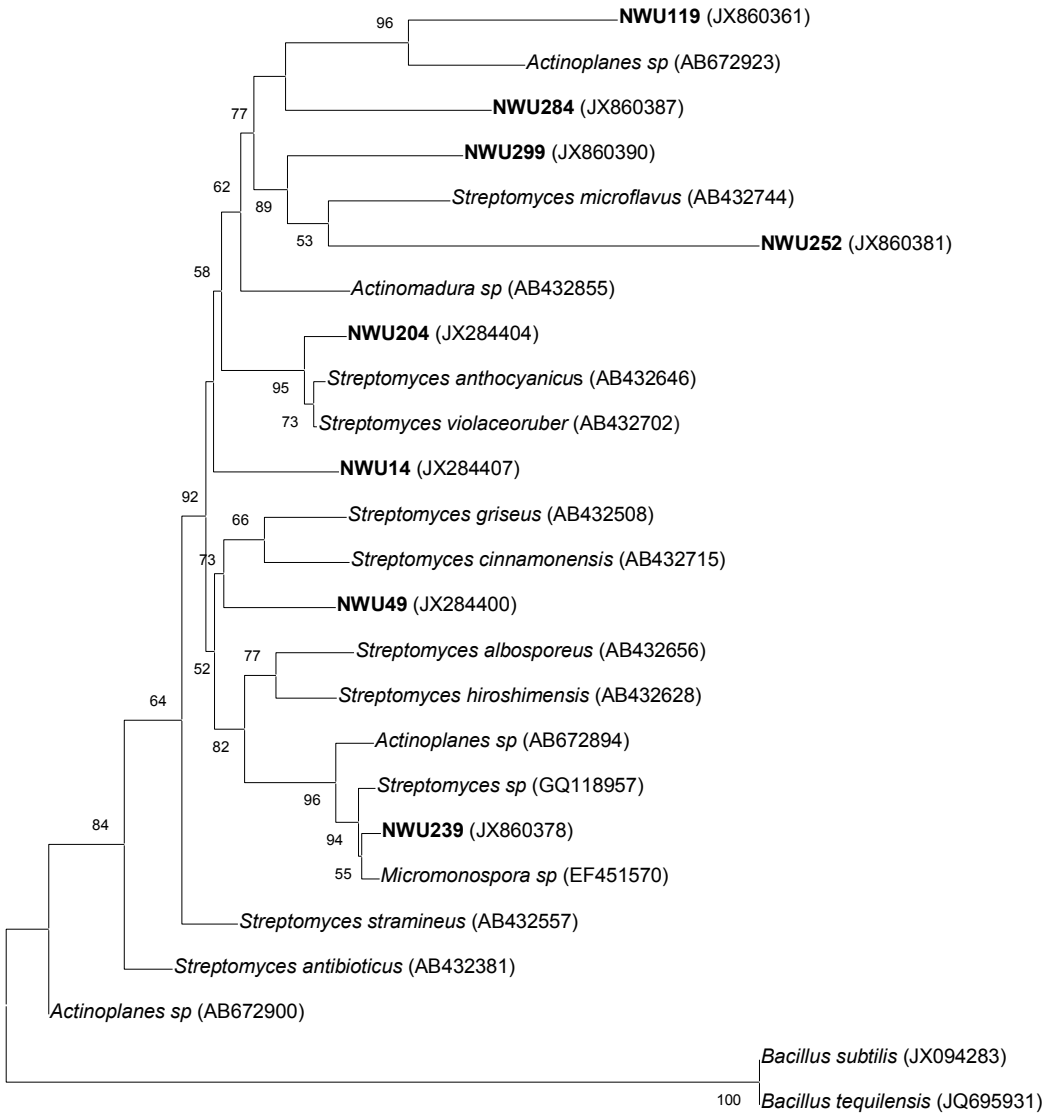

Fig. 5.Neighbor-joining tree (Saitou and Nei, 1987) based on NRPS gene sequences showing relationships between actinomycetes strains and representatives of related taxa. Bootstrap value (>50%) based on 1000 resampled datasets are shown at branch nodes. *Bacillus subtilis* and *Bacillus tequilensis* are used as outgroup

3.3 Phylogenetic Analysis of Antibiotic Producing Actinomycete Isolates

The phylogenies based on the 16S rDNA and biosynthetic gene nucleotide sequences from the actinomycete isolates constructed by the neighbor-joining method are shown in Figs. 2-5 above. Trees constructed by the UPGMA and maximum parsimony method gave similar topology (data not shown). Actinomycete isolates for the 16S rDNA were picked for the phylogenetic tree based on the exhibition of inhibitory activities against at least one test organism. Analysis based on the 16S rDNA gene alignments showed that the actinomycete isolates shared between 89-100% similarities with their closest relatives in the GenBank database that are known antimicrobial agent producers. It was also found that the actinomycete isolates formed a clade or cluster with already known antibiotic producing actinomycete reference sequences from the

GenBank. As seen from the phylogenetic tree (Fig. 2), depicting also bootstrap values, the 23 antibiotic producing actinomycete isolates were sorted into 14 main clusters with highest similarity to already known antibiotic producing actinomycete reference sequences from the GenBank. The isolates selected for the phylogenetic tree were representative samples of the antibiotic producing actinomycetes from this study. A scrutiny of the 16S rRNA phylogenetic trees showed that isolate NWU195 formed a distinct phyletic line.

Further phylogenetic analysis was carried out on the positive strains for the biosynthetic gene cluster with similarity to known antibiotic producing actinomycetes. The neighbor-joining phylogenetic tree was constructed using the nucleotide sequences of AHSA synthase gene obtained in this study and the gene segment from other PKS-I producers reference sequences from the GenBank (Fig. 3). The nucleotide sequences of the AHSA synthase gene from the positive strains previously identified as *Streptomyces* isolates formed a distinct clade from other known antibiotic producer of the genus *Streptomyces*. The *Micromonospora* spp cluster with NWU337 forming a phylogenetically distinct lineage. Fig. 4 shows the phylogenetic position of the isolates with known aromatic polyketide producers based on the analysis of the PKS-II gene nucleotide sequences. The nucleotide sequences of NRPS gene from the positive isolates and selected ones from the GenBank were used in constructing the phylogenetic tree (Fig. 5). Clustering with other known producers is evident. The lineage relationships of branches composed of the individual isolates are supported by bootstrap values that are based on neighbor-joining analyses.

4. DISCUSSION

As part of bioprospecting programme, actinomycete isolates from rhizospheric soil samples were screened in order to assess their antimicrobial potential, using degenerate primers to detect the presence of PKS-I, PKS-II and NRPS genes. The PCR-based approach offers a method by which to quickly screen a large collection of isolates in order to identify strains with the potential to produce specific structural classes of secondary metabolites [2]. The potential of actinomycetes to produce bioactive compounds has been exploited for years. Recent genomic sequence analyses of the biosynthetic gene clusters have revealed a previously unrecognized biosynthetic potential and diversity in actinomycetes [12].

The PCR-based approach can only indicate when a gene is present or absent through the amplification of the gene or not. Some isolates such as NWU (14, 49, 119, 204, 220 and 339) exhibited antibacterial activity against selected test organisms and these corrected well with successful amplification of either one or more of the targeted genes from the genomes of the isolates. The PCR-based approach helps to expedite the screening of a large collection of isolates in order to identify strains with the potential to produce bioactive secondary metabolites [2,20,21]. It can be inferred that these isolates contain at least one complete biosynthetic gene cluster for bioactive secondary metabolite production. Some actinomycete isolates such as NWU (73, 91, 110, 208, 252, 284 and 336) exhibited antimicrobial activity but there were no amplification products. The absence of PCR amplicons in some of the isolates suggests the lack of the biosynthetic genes although it cannot be concluded that the isolates in question lack such a gene. While it is possible that the isolates do not harbor any PKS or NRPS genes, this might also be as a result of the less conserved domain sequences sharing low homology with the primers in strains not well-known to produce secondary metabolites of pharmaceutical importance [20]. Also according to Wood et al. [3] this might be as result of variations in primer target sequences preventing the primers from binding efficiently. A limitation in this kind of study is that the design of degenerative primers is based on the available sequences in the GenBank of more abundant and well-studied organisms such as *Streptomyces*, unlike the rare actinomycetes that have few available sequences on their biosynthetic gene clusters in database library. As the number of whole genome sequences for rare actinomycetes increases, this will help in designing primers to amplify diverse biosynthetic genes. Some isolates such as NWU (87, 154, 196, 229, 267 and 322) also did not exhibit activity but there is amplification of the biosynthetic gene cluster. According to Finking and Marahiel [17] not all biosynthetic gene clusters are involved in the biosynthesis of bioactive secondary metabolites. It is also possible that the genes detected by the PCR are non-functional or the isolates in question might have different nutritional requirements for the production of bioactive secondary metabolites

[21,22]. Also Wood et al. [3] concluded that positive PCR amplification does not indicate that the genes are expressed nor does it show that the strain possesses the full suite of biosynthetic gene cluster for the biosynthesis of that class of antibiotic. The detection of PKS-I, PKS-II and NRPS genes in some isolates such as NWU (119, 204, 230 and 299) are indicators of their potential natural product diversity and divergent genetic evolution. Isolates with amplification of at least 2 of the primers used but with no antimicrobial activity will be induced for the production of bioactive secondary metabolites. This might lead to the discovery of novel compounds especially among the rare actinomycetes.

A phylogenetic approach using the nucleotide sequences generated from the DNA fragment of the biosynthetic gene clusters (PKS-I, PKS-II and NRPS) of the positive actinomycete isolates elucidated how nucleotide sequence information can be used to predict the number and novelty of possible bioactive compounds through the phylogenetic relatedness to known antibiotic producers. Phylogenetic analysis showed that most isolates have high bootstrap values with known antibiotic producers. The study correlates with the finding of other researchers [23-25] who isolated and screened for biosynthetic genes from actinomycetes.

As seen in Fig. 3, the isolates formed 2 distinct clades from other organisms on the phylogenetic tree; this indicates the possibility of novel bioactive compounds biosynthesis from the isolates. Although NWU337 is identified as *Streptomyces* sp, it clustered with *Micromonospora* spp which are known producers of aminoglycoside antibiotics. In Fig. 4, NWU198 clustered with *S. aureofaciens*and *S. rimosus* with bootstrap value of 91, these organisms are known producers of tetracycline [7,26]. Isolates NWU (49, 204, 277 and 308) clustered with *S. olindensis, S. violaceoruber*and *S. coelicolor*, known producers of antibiotics [27]. NWU277 has a bootstrap value of 99 with *S. coelicolor*, a prolific producer of antibiotics such as actinorhodin, methylenomycin, undecylprodi-grosin, and perimycin [7,28] in Fig. 5, isolate NWU14 formed a distinct phyletic line suggesting it might a produce novel bioactive compound. Isolate NWU49 clustered together with *S. griseus* and *S. cinnamonensis* with a bootstrap value of 77, showing that it is close relative to these organisms which are known producers of streptomycin and monensin respectively [29,30]. Isolates in close phylogenetic relationships with known antibiotic producers might also be involved in the biosynthesis of the same or similar bioactive compounds since they share similar features [31].

5. CONCLUSION

The PCR-based approach helps identify actinomycete isolates with biosynthetic gene clusters for the production of bioactive secondary metabolites. The presence of a particular gene gives an indication of the type of biosynthetic pathway to be explored. From the result generated in this study, there is a clear relationship between the occurrence of biosynthetic gene clusters and production of antimicrobial agents. The advantage of the PCR-based approach for novel drugs is that it provides knowledge about the type of prospective compounds that can be produced by the strain having the biosynthetic gene. While the information is expedient, phylogenetic analysis of biosynthetic genes associated with the production of secondary metabolites can give an insight into both the novelty and diversity of pathways present within an isolate. All these give an insight about the applicable extraction and purification technique to be applied for that class of bioactive compound. Fermentation extracts from some of the actinomycete isolates are currently being analyzed chemically in order to identify the bioactive compounds and assess their novelty.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wenzel SC, Müller R. Recent developments towards the heterologous expression of complex bacterial natural product biosynthetic pathways. Curr Opin Biotechnol. 2005;16(6):594-606.

2. Gontang EA, Gaudêncio SP, Fenical W, Jensen PR. Sequence-based analysis of secondary-metabolite biosynthesis in marine actinobacteria. Appl Environ Microbiol. 2010;76(8):2487-99.
3. Wood SA, Kirby BM, Goodwin CM, Le Roes M, Meyers PR. PCR screening reveals unexpected antibiotic biosynthetic potential in *Amycolatopsis* sp. strain UM16. J Appl Microbiol. 2007;102(1):245-53.
4. Gontang EA. Phylogenetic diversity of gram-positive bacteria and their secondary metabolite genes: ProQuest; 2008.
5. Berdy J. Bioactive microbial metabolites. J Antibiot. 2005;58(1):1-26.
6. Ichikawa N, Sasagawa M, Yamamoto M, Komaki H, Yoshida Y, Yamazaki S, et al. DoBISCUIT: A database of secondary metabolite biosynthetic gene clusters. Nucleic Acids Res. 2013;41(D1):408-14.
7. Adegboye M, Babalola O. Actinomycetes: a yet inexhaustive source of bioactive secondary metabolites. *In*: Microbial pathogens and strategies for combating them: science, technology and education. A. Méndez Vilas, ed. Formatex Research Center, Badajoz, Spain; 2013.
8. Adegboye MF, Babalola OO. Isolation, characterization and antibacterial activity of streptomycetes from rhizosphere soils in North West Province, South Africa. Asia Life Sci. 2013;9(2):403-21.
9. Zin N, Tasrip NA, Desa M, Kqueen CY, Zakaria ZA, Hamat RA, et al. Characterization and antimicrobial activities of two streptomyces isolates from soil in the periphery of Universiti Putra Malaysia. Trop Biomed. 2011;28:651-60.
10. Manfioletti G, Schneider C. A new and fast method for prearing high quality lambda DNA suitable for sequencing. Nucleic Acids Res. 1988;16(7):2873-84.
11. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991; 173(2):697-703.
12. Ayuso-Sacido A, Genilloud O. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: Detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microb Ecol. 2005;49(1):10-24.
13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10.
14. Katoh S. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772-80.
15. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731-9.
16. Saitou N, Nei M. The neighbor-joining method—a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
17. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evol. 1985;39:783–91.
18. Page RDM. TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci. 1996;12: 357-8.
19. Adegboye MF, Babalola OO. Phylogenetic characterization of culturable antibiotic producing *streptomyces* from rhizospheric soils. Mol Biol. 2013;1:2.
20. Lee LH, Zainal N, Azman AS, Eng SK, Goh BH, Yin WF, et al. Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. Scientific World J. 2014;2014.
21. Qin S, Li J, Chen HH, Zhao GZ, Zhu WY, Jiang CL, et al. Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol. 2009;75(19):6176-86.
22. Janso JE, Carter GT. Biosynthetic potential of phylogenetically unique endophytic actinomycetes from tropical plants. Applied Environ Microbiol. 2010;76(13):4377-86.
23. Bredholdt H, Galatenko OA, Engelhardt K, Fjaervik E, Terekhove LP, Johsen G, et al. Rare actinomycete bacteria from the shallow water sediments in Trondheim Fjord Norway: Isolation, diversity and biological activity. Environ Microbiol. 2007; 9:2756-64.
24. Bai Y, Yang D, Wang J, Xu S, Wang X, An L. Phylogenetic diversity of culturable bacteria from alpine permafrost in the Tianshan Mountains, Northwestern China. Res Microbiol. 2006;157(8):741-51.
25. Hwang YB, Lee MY, Park HJ, Han K, Kim ES. Isolation of putative polyene-producing actinomycetes strains via PCR-based genome screening for polyene-specific hydroxylase genes. Process Biochem. 2007;42(1):102-7.

26. Arias AA, Craig M, Fickers P. Science against microbial pathogens: *In*communicating current research and technological advances. Badajoz, Spain: Formatex Research Center; 2011.
27. de Lima Procópio RE, da Silva IR, Martins MK, de Azevedo JL, de Araújo JM. Antibiotics produced by *Streptomyces*. Braz J Infect Dis. 2012;16(5):466-71.
28. Wang G, Hosaka T, Ochi K. Dramatic activation of antibiotic production in *Streptomyces coelicolor* by cumulative drug resistance mutations. Appl Environ Microbiol. 2008;74(9):2834-40.
29. Li C, Florova G, Akopiants K, Reynolds KA. Crotonyl-coenzyme A reductase provides methylmalonyl-CoA precursors for monensin biosynthesis by *Streptomyces cinnamonensis* in an oil-based extended fermentation. Microbiol. 2004;150(10):3463-72.
30. Ohnishi Y, Ishikawa J, Hara H, Suzuki H, Ikenoya M, Ikeda H, et al. Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. J Bacteriol. 2008;190(11):4050-60.
31. Wang HX, Chen YY, Ge L, Fang TT, Meng J, Liu Z, et al. PCR screening reveals considerable unexploited biosynthetic potential of ansamycins and a mysterious family of AHBA-containing natural products in actinomycetes. J Appl Microbiol. 2013; 115(1):77-85.

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