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Phylogenetic Characterization and Detection of Polyketide Synthase Type I and Non-ribosomal Peptide Synthases Genes in *Micromonospora* Strains Isolated from Chilean Marine Sediments

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Abstract

There has been an increasing emphasis on the need to exploit un- and underexplored environments especially the marine environments for microbial and chemical diversity. Previous in-depth exploration of Chilean marine sediments have led to the isolation of members of the *Micromonosporaceae*, which require de-replication and characterization to establish taxonomic status along with screening of the isolates for the ability to produce bioactive compounds. This study was, therefore, embarked on with the aim of assessing phylogenetic relationship of the isolates and screening for novel polyketide synthases type I (PKS-I), non-ribosomal peptide synthases (NRPS) biosynthetic genes (BGC). This involved culture, de-replication by the traditional colour grouping to select representative strains, amplification of 16S rRNA, PKS-I and NRPS genes, sequencing and phylogenetic analysis. Thirty-four representative strains were selected from 66 *Micromonospora* species. Following the 16S rRNA gene sequence analysis, 11 belonged to the genus *Micromonospora*, 7 strains residing in the genera *Exiguobacterium* and *Bacillus*. The phylogenetic analysis of the 16S rRNA gene sequences of the strains inferred that these strains are novel members of this sub-order. The partial sequences of PKS-I and NRPS genes amplified from eight *Micromonospora* strains, produced matches with a variety of BGCs including *Streptomyces noursei*, *S. neyagawaensis* concanamycin A and *Streptomyces* sp. heptaene macrolide complex synthesis gene cluster. There exists an untapped microbial diversity in the Chilean marine sediments with great potential of been exploited for novel bioactive compounds as the search for newer and more potent natural products deepens.

Keywords: *Micromonosporaceae*, polyketide synthases type 1, non-ribosomal peptide synthases,

INTRODUCTION

Antimicrobial resistance is considered the silent pandemic, with increase in drug resistant infections becoming more difficult and expensive to treat. Despite the great impacts of natural products, there have been no new class of clinically viable antibiotics (Back *et al.*, 2021). As we are currently in the post-antibiotic era, there is a dire need for more potent drugs to combat emerging drug resistant infections caused by pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterobacter faecium* (VRE), fluoroquinolone resistant *Pseudomonas aeruginosa* (FRP), *Streptococcus pneumoniae,* multidrug resistant *Acinetobacter* spp, *Salmonella* spp and *Mycobacterium tuberculosis* which have led to a high morbidity and mortality especially amongst the immunocompromised and intensive care unit patients (Talbot *et al.*, 2006; Lautenbach and Polk, 2007, Payne *et al.*, 2007; Baker *et al.*, 2017). Consequently, there is need for emphasizing exploitation of more exotic natural environments such as the marine environments and extreme ecosystems that are largely untapped. Marine extremophiles are an important and attractive target for discovering novel natural product due to their innovative mechanisms of adaptation (Koehn & Carter, 2005, Back *et al.*, 2021). Molecular techniques have provided insights to the participation of sophisticated molecular enzymatic machines known as the non-ribosomal

peptide synthases (NRPS) and polyketide synthases type I (PKS-I) in the biosynthesis of secondary metabolites (Donadio *et al.*, 2007). They also catalyse a number of discrete biochemical reactions with typical examples such as rapamycin PKS and cyclosporin NRPS which catalyse 51 and 40 steps respectively, in their assembly line production (Hutchinsons, 2003; Kroken *et al.*, 2003). The presence of novel PKS and NRPS genes in an organism especially those indigenous to marine environments are an indication of the production of novel bioactive compounds from such organisms. Micromonosporae and Streptomycetes are two most prolific producers of bioactive metabolites (Hifnawy *et al.*, 2020). Micromonosporae are widely distributed in diverse environments such as peat swamp forest soil, water, root nodules, marine sediments, plant tissues, sea sands, and marine sponge (Supong *et al.*, 2013). Novel strains have been isolated from marine sediments collected from different sites such as Andaman Sea of Thailand (Supong *et al.*, 2013), deep sea sediment (Carro *et al.*, 2019) and southern Black Sea coast, Ordu, Turkey (Veyisoglu *et al.*, 2020). The members of the family *Micromonosporaceae* are an important source of novel metabolites e.g. *Micromonospora galareinsis* produce perimycin with activity against multi-resistant Gram-positive bacteria and also has antifungal activity against a spectrum of pathogenic fungi (Hifnawy *et al.*, 2020). Recently, a new Micromonospora strain, designated 28ISP2-46T recovered from the microbiome of a mid-Atlantic deep-sea sponge was reported to produce a diverse array of natural products, including kosinostatin and isoquinocycline B, which exhibit both antibiotic and antitumour properties (Back *et al.*, 2021). Following in-depth exploration of Chilean marine sediments which led to the isolation of members of the *Micromonosporaceae* (Pathom-aree *et al.*, 2006), this study was embarked with the aim of de-replicating and characterising novel members of the *Micromonosporaceae* from the Chilean marine sediments and screening of the isolates for PKS-I and NRPS genes.

MATERIALS AND METHODS

Culture and de-replication of isolates: Sixty-six *Micromonosporaceae* strains recovered from the Chilean marine sediments from two different locations (Canal Lemuy; Latitude 42º 31.8, Longitude 73º 45.7 and depth 60 m and Bahia Tic Toc; Latitude 43º 32', Longitude 72º 52 and depth 20 m) from a previous study (Pathom-aree, 2005) were subcultured from 20 % glycerol stocks onto the designated media for isolating members of the Micromonosporaceae. The prepared media included oat meal agar, ISP4 media, raffinose-histidine agar, starch-casein agar and glucose yeast extract agar (Qiu *et al.*, 2008; Supong *et al.*, 2013). All media were prepared according to the manufacturer's instructions but amended with artificial sea water (33g/1000ml; Aquarium systems) to facilitate isolation (Supong *et al.*, 2013). The inoculated plates were incubated at 28ºC for three- four weeks after which the micro- and macromorphology of the characteristic colonies were recorded using the Inter-Society Colour Council and the National Bureau of Standards (ISCC-NBS) Centroid Colour Charts Standard Sample Number 2106 and a stereomicroscope (Nikon; Japan) (Supong *et al.*, 2013). De-replication of their isolates was based on comparing the morphological and cultural characteristic such as the colour, shape and consistency of the colonies (Donadio *et al.*, 2002). Apart from using the colour codes as a criterion, isolation media was used as criteria for de-replication in this study. Only representative strains were used for the subsequent analysis.

Identification of bacteria using 16S rRNA sequencing: Genomic DNA extraction was from the 3 weeks old plates using the Gen Elute™ bacterial genomic DNA kit (Sigma-Aldrich, UK). The protocol was modified by bead beating with sterile glass beads (≤ 106µm) to facilitate DNA extraction for the *Micromonosporaceae* isolates. The presence and integrity of the genomic DNA extracts were assessed by agarose gel electrophoresis. The eluates were stored at -20ºC until required. For the amplification of the 16S rRNA gene, 50µl reaction mixture containing 5.0µl of 10X NH_4 buffer, 1.0µl of dNTP mixture, 0.5µl of forward primer 27F (20µM), 0.5µl of reverse primer 1525R (20µM), 40µl of sterile water, 0.5µl of Taq polymerase, 1.5µl of Magnesium chloride were prepared in a microtube. For the 16S rRNA, the following conditions were used; initial denaturation at 94ºC for 5 minutes followed by 30 cycles of denaturation step at 94˚C for 30 seconds, primer annealing step at 56˚C for 30 seconds; to optimize the reaction and extension step at 72˚C for 2 minutes. The PCR products were assessed by agarose gel electrophoresis (1.0% w/v with 0.5µg/ml Ethidium bromide, 30 minutes at 100V, 0.5X TBE buffer) and stored at -20ºC until required for purification. Purification was carried out using the GenElute™ PCR clean up kit in accordance with the manufacturer's protocol.

The purity of the 16S rRNA products were assessed by agarose gel electrophoresis and pure products were sent for sequencing at Macrogen, United Kingdom. Only strains (20) that were identified following 16S rRNA gene amplification and sequencing were subjected to the amplification of their PKS-I and their NRPS genes.

Phylogenetic analysis

The chromatograms of 16S rRNA, NRPS and PKS-I gene sequences were viewed using FinchTV, the subsequent contigs were aligned using DNAbaser. Phylogenetic relatedness of the 16S rRNA genes of some *Micromonospora* strains were compared with those of 50 representative species of the Micromonosporaceae downloaded from the GenBank/EMBL/DDBJ databases. Sequences of poor quality were excluded during the phylogenetic analysis. The sequences were aligned by multiple alignment using CLUSTALX version 1.83 and a phylogenetic tree constructed by the neighbor joining (Supong *et al.,* 2013)) using MEGA version 4 with *Dactylosporangium aurantiacum (D. aurantiacum)* used to root the tree. The reliability of the trees were estimated by a bootstrap analysis based on 1000 re-samplings. A similarity matrix was generated following a pair-wise alignment of the closest strains using the CLUSTALX program.

Based on the similarity matrix, the pair of *Micromonospora* strains with the highest 16S rRNA gene similarity was subsequently used as a reference point in analysing the data in the similarity matrix. The partial sequence data obtained for the amplified products of the NRPS and PKS-I genes were identified following a search on NCBI for related BGCs using the BLASTN algorithm.

Amplification of Polyketide Synthase Type I and Non-ribosomal Peptide Synthases genes

The PKS-I and NRPS genes were amplified in a 50µl reaction containing 1X NH_4 buffer, 1.0µl of 0.2Mm dNTP mixture, 0.4µM primers (PKS primers; K1F/M6R and NRPS primers; A3F/A7R), 22.5µl sterile water, 0.2U Taq polymerase, 5mM Magnesium Chloride and 10% DMSO. The master mix was dispensed into the micro tubes containing 1µl of genomic DNA. Each tube was vortexed and microfuged for a few minutes then placed in the Biometra Tgradient PCR cycler (Thistle scientific). PKS1 genes and NRPS genes were amplified with the following conditions; initial denaturation at 95ºC for 5 minutes followed by 40 cycles of denaturation step at 95˚C for 30 seconds, primer annealing step at 55˚C for 2 minutes for PKS primers; K1F/M6R and 59˚C for 2 minutes for the NRPS primers; A3F/A7R followed by an extension step at 72˚C for 10 minutes. The PCR products were assessed with agarose gel electrophoresis and purified prior to sequencing as previously described.

RESULTS

Culture and de-replication of strains: The isolates displayed variations in their colonial appearance; few exhibited diffused pigmentation in the growth media. Based on the macro-morphology, there was a high degree of consistency in form, elevation and margin of the colonies. All the colonies were circular, convex and filamentous but varied in their colour codes (orange, red, brown or black colonies). Only thirty-four representative strains were selected following de-replication.

Identification and phylogenetic relations of the isolates

Following the 16S rRNA sequence analysis, 11 strains; JH24, JH37, JH66, JH72, JH231, JH235, JH270 and JH280, JH87, JH252 and JH268 were confirmed to be members of the family *Micromonosporaceae* but only 8 strain were used for the phylogenetic analysis. Figure 3 showed the phylogenetic relatedness of the 16s rRNA genes of the bacterial strains *as* compared with related bacteria from the GenBank. . Six strains; JH37, JH66, JH231, JH235, JH270 and JH280 formed subclades with *M. carbonacea*, and a polyphyletic clade with *M. chokoriensis*, *M. matsumotoense*, *M. rifamycinica*, *M. rosaria*, *M. siamensis* and *Micromonospora saelicesensis* while JH24 and JH72 formed a subclade with *M. chalcea* (Figure 3). Based on the similarity matrix, the highest 16S rRNA gene similarity of 99.4% was between *M. auratinigra* and *M. chaiyaphumensis* with a corresponding 9 nucleotide differences at 1433 locations. Strain JH24 isolated from Canal Lemuy was most closely related to *M. chalcea* with a percentage similarity of 97.6% while JH37 and JH66 both isolated from Bahia Tic Toc, were closest to *M. carbonaceae* with a value of 97.0% and 98.0%. JH270 and JH280 isolated from Canal Lemuy and Bahia Tic Toc respectively, were also closely related to *M. carbonaceae* with a percentage similarity of 98.5% and 99.2%. Although JH24 and JH72 had similar colonial morphology and formed a cluster with a high bootstrap value of 85%, they were isolated from different sediments collected from Canal Lemuy and Bahia Tic Toc respectively. It is noteworthy of mention that JH231 and JH235 which formed a cluster, were both isolated from the same sediment (Canal Lemuy) as strain JH24 and were all very similar in their macro-morphology.

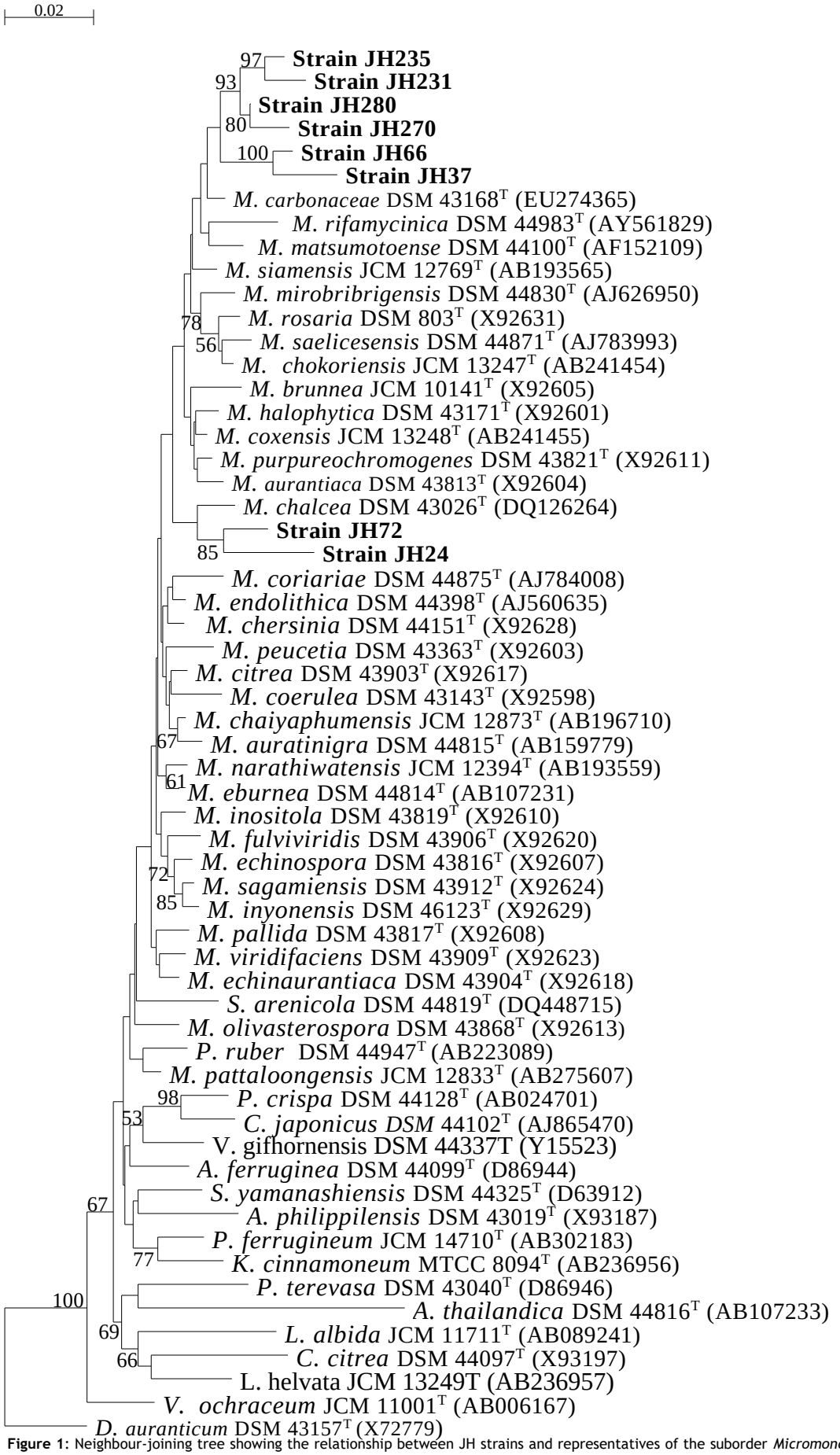

Figure 1: Neighbour-joining tree showing the relationship between JH strains and representatives of the suborder *Micromonosporaceae*. The scale bar indicates 0.02 substitutions per nucleotide position.

Presence of PKS-I and NRPS genes in the Isolates

The PKS-I and NRPS gene amplicons of the isolates from the marine sediments are shown in Figures 2 and 3 respectively. The presence of PKS-I (1200-1400bp) and NRPS (700bp) genes in the identified strains are also presented in Table 1. There was a degree of similarity with a variety of BGCs from different organisms especially the *Streptomyces* spp. Some matches from the BLAST results of the sequences and the percentage similarity are shown in Table 2.

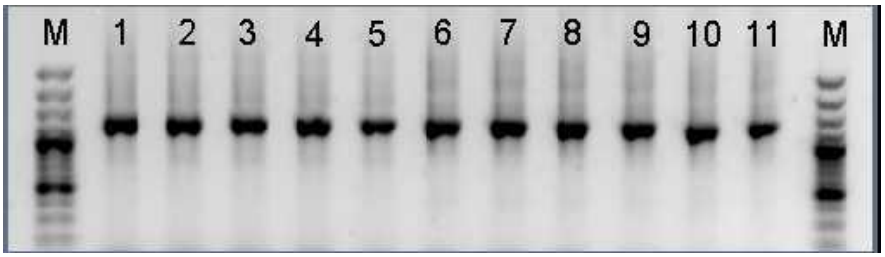

Figure 2: Bands (1200-1400bp) of PKS-1 genes amplified from the isolates from Chilean marine sediments. Lanes M, DNA marker; 1, JH24; 2, JH37; 3, JH66; 4, JH72; 5, JH87; 6, JH196; 7, JH270; 8, JH275; 9, JH277; 10, JH278; 11, JH280.

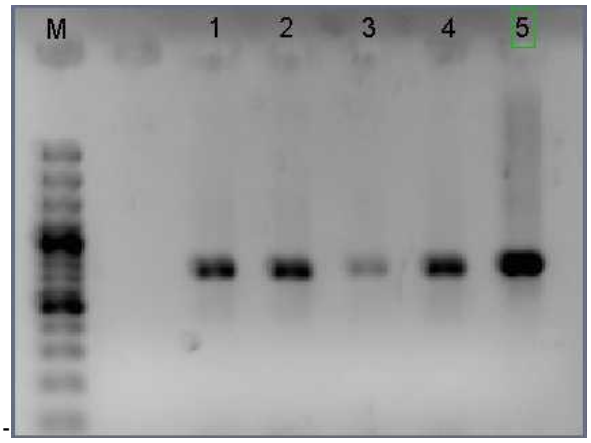

Figure 3: Bands (700bp) of NRPS genes amplified from some isolates from Chilean marine sediments. Lanes M, DNA marker; 1, JH24; 2, JH37; 3, JH66; 4, JH72; 5, JH87.

Table 1: Identified bacteria using 16S rRNA sequencing with presence of PKS-I and NRPS genes.

Strain-ID	PKS-I†	NRPS§	Genus/species	Percentage Similarity
JH5	-	-	*Bacillus* spp	98.0
JH20	-	-	*Bacillus* spp	98.0
JH24	+	+	*Micromonospora* spp	97.6
JH29	-	-	*Exiguobacterium* spp	98.0
JH37	+	+	*Micromonospora* spp	98.9
JH66	+	+	*Micromonospora* spp	98.0
JH72	+	+	*Micromonospora* spp	97.9
JH87	-	+	*Micromonospora* spp	97.0
JH196	+	+	*Bacillus* spp	98.0
JH231	-	-	*Micromonospora* spp	98.9
JH235	-	-	*Micromonospora* spp	98.0
JH252	+	-	*Micromonospora* spp	98.0
JH268	-	-	*Micromonospora* spp	98.0
JH270	+	+	*Micromonospora* spp	98.0
JH275	+	+	*Exiguobacterium* spp	98.9
JH277	-	-	*Exiguobacterium* spp	98.9
JH278	+	+	*Bacillus* spp	97.9
JH280	+	+	*Micromonospora* spp	98.9

† (+) Presence of PKS-1 gene, (-) Absence of PKS-1 gene
§ (+) Presence of NRPS gene, (-) Absence of NRPS gene

Table 2: BLAST results obtained after analysing partial sequences of the PKS-1 and NRPS genes from *Micromonospora* strains isolated from Chilean marine sediments.

Strain ID	BLAST results for partial PKS-I sequence	BLAST results for partial NRPS sequence
JH24	*Streptomyces avermitilis* oligomycin BGC (55%)	Malate dehydrogenase (77%)
JH37	*Streptomyces* sp. FR-008 heptaene macrolide complex synthesis gene cluster (70%), *Streptomyces griseus* (70%)	*Streptomyces collinus* kirromycin (63%), *Streptomyces avermitilis* peptide-1 BGC (63%), *Amycolatopsis lactamdurans* isolate AAL29 NRPS gene (62%), *Streptomyces atroolivaceus* leinamycin BGC (62%), *Streptomyces virginiae* virginiamycin synthetic gene cluster (61%), *Streptomyces fradiae* lipopeptide (61%), *Stigmatella aurantiaca* myxochromide S, BGC (61%)
JH66	*Streptomyces neyagawaensis* concanamycin A, BGC (61%), *Streptomyces nanchangensis* nanchangmycin BGC (56%), *Streptomyces hygroscopicus* geldanamycin BGC (58%), *Streptomyces* sp. FR-008 heptaene macrolide complex gene cluster (56%), *Saccharopolyspora erythraea* PKS gene cluster (58%)	Malate dehydrogenase (89%)
JH72	*Streptomyces noursei* (61%), *Streptomyces neyagawaensis* concanamycin A, BGC (60%), *Streptomyces* sp. heptaene macrolide complex synthesis gene cluster (59%), *Streptomyces natalensis* primaricin BGC (58%), *Streptomyces nanchangensis* nanchangmycin BGC (57%), *Streptomyces avermitilis* oligomycin BGC (57%)	No significant similarity
JH87	NS*	No significant similarity
JH252	No significant similarity	NS*
JH270	*Micromonospora megalomicea subsp. nigra* megalomicin BGC (69%), *Streptomyces noursei nystatin* BGC (61%), *Streptomyces rimosus* rimocidin synthase gene	No significant similarity
JH280	*Streptomyces venezuelae* methymycin/pikromycin gene cluster (22%), *Streptomyces avermitilis* oligomycin BGC (23%), *Streptomyces noursei nystatin* BGC (23%), *Amycolatopsis mediterranei* rifamycin BGC (22%), *Streptomyces neyagawaensis* concanamycin A, BGC (23%)	No significant similarity

NS*-Gene amplification was not successful.

DISCUSSION

The global antimicrobial resistance burden has resulted in an intense search for new drug leads especially from natural products produced microorganisms to tackle drug resistant infections. Following culture, de-replication and molecular analysis of *Micromonospora* strains previously isolated from Chilean marine sediments, only 11 strains out of 20 strains were identified as *Micromonospora* spp based on 16S rRNA gene sequence analysis. Also, PKS-I and NRPS genes were detected in strains JH24, JH37, JH66, JH72 and JH87 but not for JH231, JH235 and JH268. Phylogenetic analysis showed clustering of the strains with other *Micromonospora* species including *M. chalcea, M. carbonaceae, M. auratinigra* and *M. chaiyaphumensis*. Four isolates were confirmed as *Bacillus* spp with PKS-I and NRPS BGC present in two strains while three strains were identified as *Exiguobacterium* spp with PKS-I and NRPS BGC detected in only one strain.

The colonial characteristics of the strains (Table 1) match those of members belonging to the Family Micromonosporaceae which possess carotenoid mycelial pigments with yellow, orange, red, purple, brown or black colonies (Hifnawy *et al.*, 2020). There are previous reports of the isolation of *Micromonospora* species from diverse geographical habitats including soil, mangrove sediments, marine sediment, plants, and extreme habitats (hyper-arid deserts, deep-sea sediments and hypersaline lakes) (Hill, 2003; Hifnawy *et al.*, 2020; Back *et al.*, 2021). The clustering of the strains with other *Micromonospora* species including *M. chalcea, M. carbonaceae, M. auratinigra* and *M. chaiyaphumensis* which have been isolated from marine sediments, infer their phylogenetic relatedness and potential for the production of natural products. For example, a novel dipeptide, *N*-(2, 6-diamino-6-hydroxymethylpimelyl)-L-alanine which inhibits cell wall biosynthesis of *E. coli* was obtained from marine-derived *M. chalcea*. Also, *M. carbonacea* produces an unusual sulfur-rich antibiotic, Sch 40832 (Hifnawy *et al.*, 2020). The isolation of *Micromonospora* strains of different cultural and phylogenetic characteristics from the different Chilean marine sediments is a reflection of the microbial diversity that exists in the environment (Hifnawy *et al.*, 2020). The marine environment is rich in microbial diversity despite its oligotropic nature which has impact primary production in waters away from the coastal areas and the organic matter that reaches the sea floor (Gartner *et al.*, 2016). The isolation and characterization of *Micromonospora* strains from marine sediments at different depths (Bahia Tic Toc; 20 m and Canal Lemuy; 60 m) is supported by different reports of an increasing diversity of *Micromonospora* compared with Streptomyces as the water depth increases (Gartner *et al.*, 2016). Micromonospora isolates are the major group of actinobacteria in the deeper sediment samples (Bredholt *et al.*, 2008). Presence of *Exiguobacterium* and *Bacillus* spp. may have been due to contamination and/or selective isolation failure, as they have similar nutrient requirements as *Micromonospora* strains. *Bacillus* species are known to be an important bacterial group and primary competitors of actinobacteria in marine sediments. It is expected that the production of bioactive compounds such as antibiotics might enhance the persistence and growth of the *Micromonospora* community with antibiotic producing potential. Consequently, successful isolation of *Micromonospora* species would require more complex media, pre-treatment methods and the growth conditions (hydrostatic pressure and low temperature) (Gartner *et al.*, 2016).

Based on the phylogenetic analysis, the strains JH24, JH37, JH66, JH72, JH231, JH235, JH270 may be considered as novel species of the family *Micromonosporaceae* as their nucleotide difference exceeded the reference value of 9 nucleotides out of 1433 for the two most closely related type strains, *M. auratinigra* and *M. chaiyaphumensis*. Furthermore, the detection of PKS-1 and NRPS BGC is supported by the previous findings that majority of BGC-families belong to the polyketide synthases type I and the non-ribosomal peptide synthetases (Zhu *et al.*, 2007; Hifnawy *et al.*, 2020). However other BGC families include Terpene and PKS and NRPS hybrids (Zhu *et al.*, 2007; Hifnawy *et al.*, 2020). The BLAST results from the PKS-I and NRPS sequences suggests that the strains JH24, JH37, JH66, JH72, JH270, and JH280 analyzed, possessed BCGs with sequence similarity with those from other organisms (Table 1). This may infer structural and functional similarity. Members of the family *Micromonosporaceae* and other actinomycetes are an invaluable source of novel metabolites. Some *Micromonospora* strains produce ECO-4601, a farnesylated dibenzodiazepinone with antibacterial, anti-inflammatory and anti-tumour activity. Other examples include *Verrucosispora* strain which produces Abyssomicins (Maldonado *et al.*, 2005), arenicolide A, saliniketal B and cyclomarin D from *Salinispora arenicola*, lomaviticin A, cyanosporaside A and cyanosporaside B from *Salinispora pacifica* (Fenical and Jensen, 2006). The Streptomycetes including those presented as BLAST matches are known to be a rich and renewable source of secondary metabolites include antibiotics such as macrolide antibiotics such as methymycin and pikromycin. Series macrolides from the pikromycin biosynthetic system in *S. venezuelae,* and bioactive compounds invaluable to human and veterinary medicine, agriculture are unique biochemical tools (Omura *et al.*, 2001). These matches include those of *S. noursei* and *S. neyagawaensis* concanamycin A which are known to be potent producers of bioactive secondary metabolites of invaluable use (Stratmann *et al.*, 1999; Huss *et al.*, 2002; Hirsch and Valdes, 2010).

A typical example is the antibiotic rifamycins from *Amycolatopsis mediterranei* which although are primarily used against *M. tuberculosis* and *M. leprae*, aetiological agents of tuberculosis and leprosy, respectively, are also known to be effective against other organisms, including bacteria and viruses (Huss *et al.*, 2002).

The absence of significant hit for NRPS gene sequences from JH24, JH66, JH72, JH270 and JH280 following the BLASTN analysis may imply a degree of novelty associated with their genes but further analysis by the amplification of the entire gene cluster, cloning and sequencing would be required to establish any novelty. Although NRPS and PKS genes may be present in some organisms, the successful amplification of such genes does not necessarily imply their involvements in the biosynthesis of bioactive compounds as their products may be involved in other functions such as iron metabolism and quorum sensing or the genes may be non-functional (Pathom-aree *et al.*, 2006). Consequently, these *Micromonospora* strains could be studied and exploited for the production of bioactive compounds with potential for drug leads.

Apart from the use of culture-independent techniques, improved culture techniques has led to the isolation of quite a number of novel actinobacteria which were thought to be viable but non-culturable (Janssen *et al.*, 2002 and Jensen *et al*, 2005). For this study, selection isolation was achieved using the amended media as previously mentioned. Poor isolation also confirmed the fact the strains may have been dormant, injured or non-viable hence the need for longer incubation periods since the members of the family *Micromonosporaceae* are known to be slow growers (Maldonado *et al.*, 2008 and Back *et al.*, 2021). Other limitations of this study include the poor DNA extraction and sequence data quality for some strains. The unsuccessful PCR amplification in the remaining 14 out of 34 isolates may be attributed to a number of factors including DNA inhibition or degradation (due to the presence of PCR inhibitors such as salts, ethanol, ionic detergents and polysaccharides) and DNA shearing during DNA extraction (Pathom-Aree, 2005). Due financial constraints, the PKS-I and NRPS biosynthetic gene clusters were partially sequenced and hence phylogenetic trees were not constructed.

CONCLUSION

The phylogenetic analysis of the 16S rRNA gene sequences of the presumptive *Micromonospora* strains JH24, JH37, JH66, JH231, JH235, JH270 and JH280 with representative members inferred that theyare novel members of Micromonosporaceae. BLASTN analysis of the partial sequences of their PKS-I and NRPS genes produced matches to a variety of biosynthetic gene clusters of the *Streptomyces* and other actinomycetes known to be proficient at the synthesis of bioactive compounds. This implied that the *Micromonospora* strains have a potential for the production of novel bioactive compounds required to tackle the emergence of drug resistant pathogens. Further analysis such as whole genome sequencing or DNA-DNA pairing and the analysis of chemotaxonomic properties such as fatty acid profiles, cell wall composition and phospholipid types and morphological features (presence or absence of sporangium and spore motility) would be required to establish their novelty.

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