

Marine actinomycetes from Madeira Archipelago preliminary taxonomic studies

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The oceans cover 70 % of the Earth's surface and harbor most of the planet's biodiversity. However the microbiological component of this diversity remains relatively unexplored. Marine actinomycetes, are a robust resource of chemically prolific novelty. Producing structurally unique biological active secondary metabolites, generating a valuable source for innovative biotechnology and drug discovery^[1,2]. As a consequence, the ecological role of actinomycetes and their marine ecosystems may no longer be neglected. It is crucial to move our research efforts into ocean regions for which we know little or nothing about the indigenous microbial diversity. The Portuguese Archipelago, Madeira is located in the Macaronesian Atlantic region, emerging from the African tectonic plate, found in the extreme south of the Tore-Madeira ridge, has a unique biogeography and biodiversity. These distinctive characteristics combined with the fact that Madeira have never been explored, as far as indigenous marine actinomycetes are concerned, makes it from the scientific point of view, the perfect target for our studies. From 662 marine sediment samples collected along Madeira Archipelago (Figure 1) during June of 2012, covering depths from 10-1310 m, a total of 421 actinomycete strains were isolated. In a previous study, an assemblage of 82 strains was selected for taxonomic identification, having into account representative morphological diversity characteristics of the actinomycetes, isolated from Madeira Archipelago. Based on 16S rRNA gene sequencing, it was observed that the genera *Streptomyces*, *Micromonospora* and *Salinispora* were predominant, 81% ^[3]. Additionally, in a recent study, our team selected 168 strains with *Salinispora* look-alike morphological features. From these 28 strains were identified as belonging to the seawater-obligate marine actinomycete genus *Salinispora*. Representing the first report of *Salinispora* spp. in the Macaronesian Atlantic Ocean region and suggesting a more globally distribution of this genus than previously supposed (unpublished results). In this study further 82 strains from Madeira Archipelago (out of 421) were selected for taxonomic identification, taking into account small groups of strains (1-4) evidencing very diverse morphological appearances, as exemplified in Figure 2. Using the same experimental microbiology identification tools, 8 genera were identified. However it was perceived that, the genera *Streptomyces*, *Nocardiopsis* and *Actinomycetospora* were predominant (93%), Figure 3. The phylogenetic trees built for the 82 taxonomically identified strains performed in this study are presented in Figures 4, 5 and 6. To date, having into account the present work and previous studies, our research group have identified from the actinomycetes isolated from Madeira's ocean sediments, genera *Streptomyces*, *Micromonospora*, *Salinispora*, *Nocardiopsis*, *Verrucosispora*, *Kocuria*, *Nonomuraea*, *Nocardia*, *Brevibacterium*, *Mycobacterium*, *Marinobacter*, *Actinomadura*, *Micrococcus*, *Actinomycetospora*, *Pseudonocardia*, *Gordonia* and *Millisia*. From which genera *Streptomyces*, *Micromonospora*, *Salinispora* evidence a major representation. Crude extracts were obtained from all 421 strains and tested for their ability to produce natural products with bioactive properties: (i) antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE) and *Candida albicans* strains; and (ii) cytotoxic activity against the HCT-116 cell line. A screening positive rate of 2.4% for antimicrobial MRSA and VRE assays and 3.2% for cytotoxic HCT-116 assay was obtained (submitted manuscripts). These studies demonstrate that the Macaronesian Atlantic Ocean region is a rich source of marine actinomycete biodiversity with potential industrial applications.

Figure 1. Marine actinomycetes sediment sampling locations at Madeira Archipelago.

Figure 2. Morphological diversity characteristics of the taxonomically identified 82 strains.

Figure 3. Identified genera from the 82 selected strains.

Figure 4. Neighbour-joining phylogenetic tree created from 63 partial 16S rRNA gene sequence from *Streptomyces* strains cultured from Madeira Archipelago, based on 1000 bootstrap replicates. BLAST matches (deposited in GenBank) are included with species and strain name followed by accession number. *Verrucosispora maris* and *Micromonospora aurantiaca* were used as outgroups.

Figure 5. Neighbour-joining phylogenetic tree created from 8 partial 16S rRNA gene sequence from *Nocardiopsis* strains cultured from Madeira Archipelago, based on 1000 bootstrap replicates. BLAST matches (deposited in GenBank) are included with species and strain name followed by accession number. *Streptomyces albolongus* and *Streptomyces exfoliatus* were used as outgroups.

Figure 6. Neighbour-joining phylogenetic tree created from 11 partial 16S rRNA gene sequence from actinomycete strains cultured from Madeira Archipelago, based on 1000 bootstrap replicates. BLAST matches (deposited in GenBank) are included with species and strain name followed by accession number. *Bifidobacterium* sp. and *Propionibacterium propionicus* were used as outgroups.

Figure 1



Figure 2

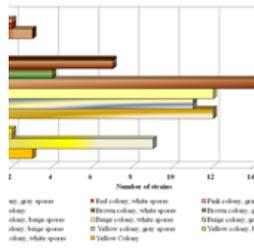


Figure 3

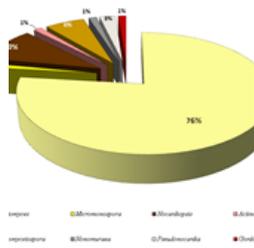


Figure 4



Figure 5

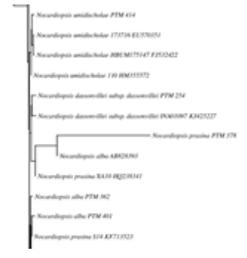
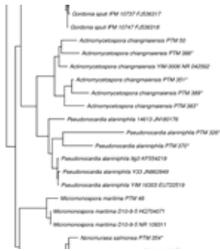


Figure 6



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References

- 1 Bérday, J. (2005) Bioactive microbial metabolites: A personal view. *J. Antibiot.* 58: 1-26.
- 2 Fenical, W., Jensen, P. R. (2006) Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* 2: 666-673.
- 3 Dias, T., Pereira, F., Millán, N., Gomes, S. E., Borralho, P. M., Rodrigues, C. M. P., Jensen, P. R., Sanches, I. S., Gaudêncio, S. P. (2013) Targeted search for actinomycetes from the Madeira Archipelago. MICROBIOTEC'13, 6th-8th December, Aveiro, Portugal.

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