



Research Article

Biological Properties of Actinomycetes Isolated from Marine Sponges in Madagascar

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Abstract: Marine actinomycetes are well known as a potential provider of novel bioactive compounds and currently considered as an important source of natural substances with unique chemical diversity. In this study, 20 marine actinomycetes were isolated from three *Demospongia* collected in the South East coast of Madagascar. Cultural, morphological, physiological and biochemical characteristics of the isolates showed that they belong to the genus *Streptomyces*. The Antimicrobial activity of the strains was performed using the agar cylinder technique against pathogens bacteria, yeast and fungi. It resulted that 90% of the isolates showed activity against at least one or more of the test germs. The isolates were more active against Gram-positive bacteria than Gram-negative bacteria. Simultaneously, ethanol extracts of the isolates were tested for their antioxidant activity using DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging test. Among tested extracts, those of *Streptomyces* M9 and M17 showed antioxidant activity against DPPH free radical with IC₅₀ values of 12.8µg/ml and 12.4µg/ml, respectively.

Keywords: Actinomycetes, Antimicrobial activity, Antioxidant activity, Marine sponges.

1. Introduction

Actinomycetes have received considerable attention of researchers, microbiologists and pharmaceutical industries for their ability to produce bioactive secondary metabolites with diverse chemical structures and biological activities. These bacteria are known to colonize a wide range of habitats (1) and represent the major microbes in the soil micro-ecosystem (2).

In recent years, it was found that pathogens became continually resistant to antibiotics (3). The rate of discovery of novel secondary metabolites from terrestrial actinomycetes has decreased considerably (4). Therefore, the isolation of actinomycetes from other biotops such as marine environment was conducted. This domain is actually getting better exploited. Approximately, 32.500 natural products were reported isolated from microbial sources which about 1000 were derived from marine microbes (5). At present, two-thirds of antibiotics available are obtained from marine actinomycetes. Several studies have

demonstrated the richness of actinomycetes from marine sediments (6,7,8,9) or sponges (10,11,12) in novel secondary metabolites endowed of different biological activities: antibiotic, anticancer, anti-inflammatory and antiviral.

In order to discover novel secondary metabolites and new producers' strains, actinomycete strains were isolated from marine organisms. The present work investigates three marine sponges (*Demospongia*) collected from the South East unexplored coast of Madagascar for actinomycetes isolation. The study was undertaken with the purpose to test the potential of the isolates to produce antimicrobial and antioxidant compounds.

2. Materials and Methods

2.1 Marine organisms' samples collection

Three samples of marine sponges were collected from the sea bottom of Vangaindrano in June 2014. The samples were put in sterile plastic bags containing a

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few quantity of seawater and stored in a cooler during their transport to the laboratory.

2.2 Marine actinomycetes isolation

Actinomycetes isolation was carried out by the serial dilution technique on four different solid media: MA, R2A, AIA, and ISP2 supplemented with three antibiotics: trimethoprim (20µg/ml), cycloheximide (50µg/ml) and nalidixic acid (50µg/ml). Plates were incubated at 30°C and daily examined for 4 weeks.

2.3 Antimicrobial activity

The marine actinomycete isolates were evaluated *in vitro* for their antimicrobial activity against eight human pathogens: *Klebsiella oxytoca* (ATCC 8724), *Enterobacter cloacae* (ATCC 700323), *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 13061), *Staphylococcus aureus* (ATCC 11632), *Streptococcus pneumonia* (ATCC 6301) and *Candida albicans* described by Acar and Goldstein (13). Test microorganisms were inoculated onto Mueller-Hinton agar. Disks (6mm in diameter) of mature actinomycetes, 5 days old, grown on starch casein (SCA) were transferred aseptically to the inoculated Mueller-Hinton agar plates. All the plates were first kept in a refrigerator +4°C for at least 4 hours to allow the diffusion of any antibiotics produced, then incubated at 37°C. Three replicates were done under completely randomized design (CRD) for this assay. Bioactivity was evaluated by measuring the inhibitory zones after 1-2 days and only isolates showing an inhibition zone greater than 8mm were considered as active isolates.

2.4 Antioxidant activity

2.4.1 Extraction of the secondary metabolite

ISP2 solid media were heavily inoculated with pure cultures of actinomycetes and incubated at 30°C for 8 to 14 days. 30ml of ethanol (90%) were, then, added into inoculated ISP2 agar and was settled at room temperature for two hours. Extracts were filtered to remove live cells, distributed in glass vials and dried in a SpeedVac for antioxidant test.

2.4.2 DPPH radical scavenging activity

The Free radical scavenging activity of each extract was assessed according to the method reported by Kekuda *et al.*, (14). A range of concentration of the extracts (0.1-10mg/ml) and ascorbic acid (reference standard) was prepared. 2ml of DPPH solution (0.002% in methanol) were mixed with 2ml of each concentration of the extract and the reference standard. The preparation was incubated in dark at room temperature for 30min and the optical density was measured at 517nm using UV-Vis spectrophotometer. The absorbance of the DPPH control (without

extract/standard) was noted. The scavenging activity was calculated using the following formula:

$$\% \text{ Radical Scavenging activity} = [(A - B)/A] \times 100,$$

Where *A* is the absorbance of DPPH control; and *B* is the absorbance of DPPH in the presence of extract/standard. In addition, the inhibitory concentration that reduces 50% of free radicals (IC₅₀) was determined. The experiments were repeated three times.

2.5 Statistical analysis

ANOVA software was used for data analysis. Mean comparisons were conducted using a Least Significant Difference (LSD) test (*P* < 0:05).

3. Results and Discussion

3.1 Antimicrobial activity

The results of antimicrobial test of the marine actinomycetes isolated from Demospongia showed that among 20 tested isolates, 18 (90%) displayed antimicrobial activity against at least one test pathogen (Table 1). The isolate M2 exhibited antagonistic activity against all tested pathogens. The isolates were more active against Gram-positive bacteria than Gram-negative bacteria. The strain M2 showed high inhibition zone on *Streptococcus pneumoniae* and *Bacillus cereus* (Fig. 1). These results are similar to those found in several kinds of literature which reported that antagonistic activity of actinomycetes (especially terrestrial actinomycetes) is much higher for Gram-positive bacteria than for Gram-negative bacteria (15,16,17). However, it would be emphasized that six strains inhibited the growth of at least one tested Gram-negative bacteria. The isolates M2 and M20 showed antagonism against all tested Gram-negative bacteria (Fig. 2). This is in agreement with Thenmozhi and Kannabiran (18) who demonstrated that ethyl acetate extract of a marine actinomycetes *Streptomyces* sp. VITSTK7 possessed effects on all tested pathogens growths including Gram-negative bacteria.

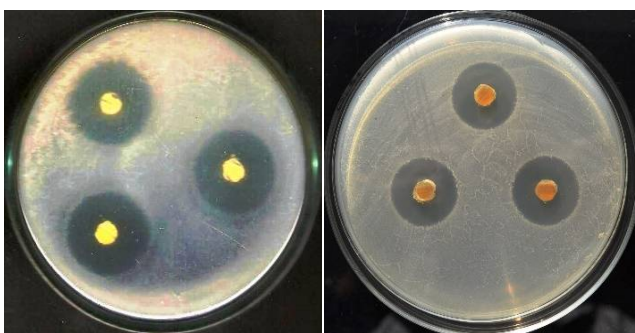


Fig. 1. Inhibition effect of the isolate M2 against *Streptococcus pneumonia* (A) and *Bacillus cereus* (B).

Table 1. Antimicrobial activity of actinomycete isolates.

Isolates	Diameter of inhibition zone (mm) ^(a)						
	Gram-negative bacteria				Gram-positive bacteria		
	<i>Enterobacter cloacae</i> ATTC 700323	<i>Escherichia coli</i> ATTC 25922	<i>Klebsiella oxytoca</i> ATTC 8724	<i>Salmonella enteritidis</i> ATTC 13076	<i>Staphylococcus aureus</i> ATTC 11632	<i>Streptococcus pneumoniae</i> ATTC 6301	<i>Bacillus cereus</i> ATTC 13061
M1	10.6 ± 0.5 ^c	0	15.3 ± 0.5 ^b	10 ± 1.0 ^c	0	0	0
M2	18.6 ± 0.1 ^a	16 ± 0.5 ^b	14.3 ± 0.5 ^{bc}	19.3 ± 0.5 ^b	11 ± 1.0 ^b	29 ± 1.0 ^a	21.3 ± 1.1 ^a
M3	0	0	0	0	10 ± 0.5 ^b	9.3 ± 0.5 ^{de}	0
M4	0	0	0	0	0	0	13.3 ± 0.5 ^{cd}
M6	0	0	0	0	0	0	10.6 ± 0.5 ^c
M8	0	0	0	0	0	21.3 ± 2.0 ^b	11.6 ± 1.5 ^{bc}
M9	10.6 ± 0.5 ^c	8.6 ± 0.5 ^c	13.3 ± 0.5 ^c	0	9.6 ± 0.5 ^c	0	12 ± 1.0 ^d
M10	0	0	9.6 ± 0.5 ^d	0	0	0	12.6 ± 0.5 ^{cd}
M11	0	0	0	0	0	10.6 ± 1.1 ^d	0
M12	0	0	0	0	0	0	16.3 ± 1.5 ^b
M13	0	0	0	0	0	10.3 ± 1.5 ^d	0
M14	0	0	0	0	0	10.6 ± 1.5 ^d	12.6 ± 1.1 ^b
M15	8.6 ± 0.5 ^d	0	0	0	0	9.3 ± 0.5 ^{de}	0
M16	0	0	0	0	0	0	13.3 ± 1.5 ^{cd}
M17	0	0	0	0	0	0	14.6 ± 1.1 ^{bc}
M18	0	0	0	0	0	0	13 ± 1.0 ^{cd}
M19	0	0	0	0	0	0	13 ± 1.7 ^{cd}
M20	13.6 ± 1.1 ^b	18 ± 1.1 ^a	22 ± 0.5 ^a	21 ± 1.0 ^a	19.3 ± 0.5 ^a	18.6 ± 0.5 ^c	20 ± 1.0 ^a

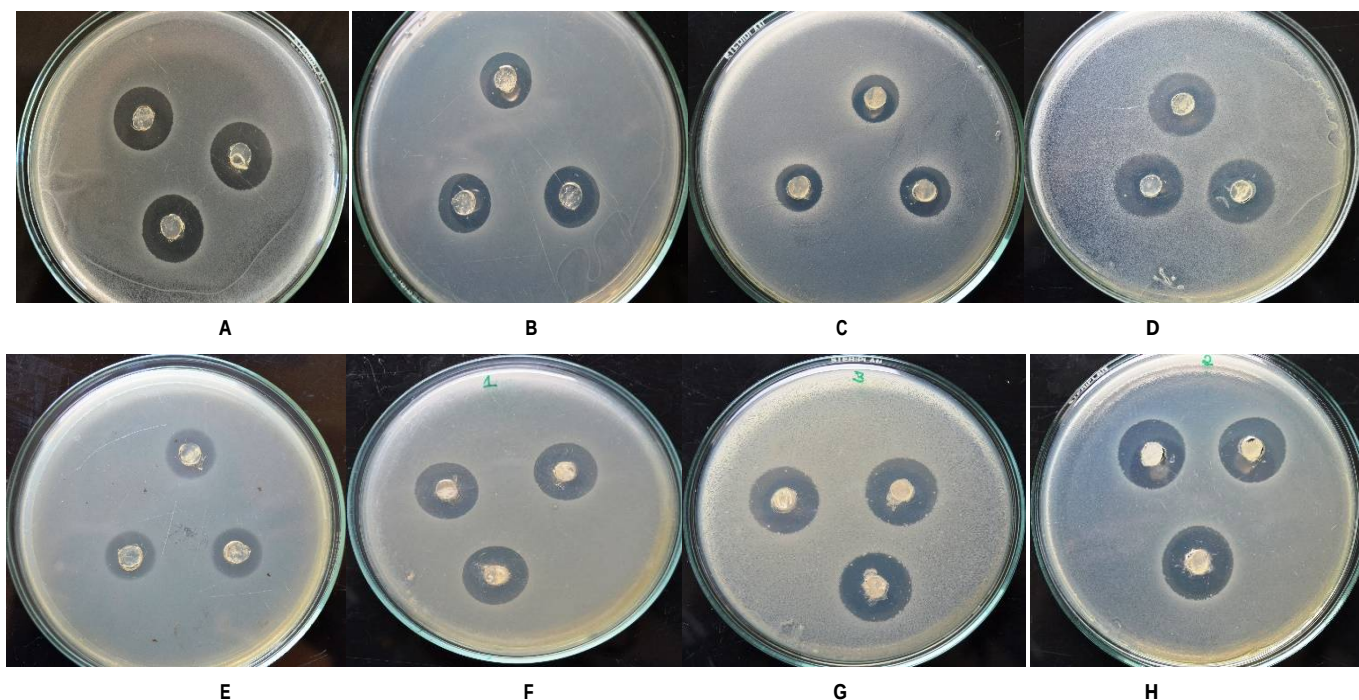
^aAverage ± standard error from triplicate samples

Fig. 2. Inhibition effect of the isolate M2 against *Enterobacter cloacae* (A), *Escherichia coli* (B), *Klebsiella oxytoca* (C) and *Salmonella enteritidis* (D); inhibition effect of the isolate M20 against *Enterobacter cloacae* (E), *Escherichia coli* (F), *Klebsiella oxytoca* (G) and *Salmonella enteritidis* (H).

3.2 Antioxidant activity test

Out of the 20 ethanol extracts from marine isolates assayed for DPPH radical scavenging activity, 02 extracts from the isolates M9 and M17 displayed antioxidant activity. M9 extract showed 37.54% DPPH free radical scavenging activity and M17 extract showed 38.74% at 10mg/ml with IC_{50} values of 12.8 μ g/ml and 12.4 μ g/ml respectively (Fig. 3). At the same concentration, ascorbic acid showed 97.46% DPPH radical scavenging activity with IC_{50} values of 5.2 μ g/ml. Then, the antioxidant activity of both extracts

was 2.5 times lower than that of the reference standard (Fig. 4).

Thenmozhi *et al.*, (19) demonstrated that extracellular extracts of *Streptomyces* species VITTK3 isolated from Puducherry Coast, India possesses significant DPPH scavenging activity compared to standard ascorbic acid. However, several authors working on the antioxidant properties of marine actinomycetes extracts have been found that they presented antioxidant activity. Most of them showed lower activity than the reference standard (18, 20) which occurs with our results.

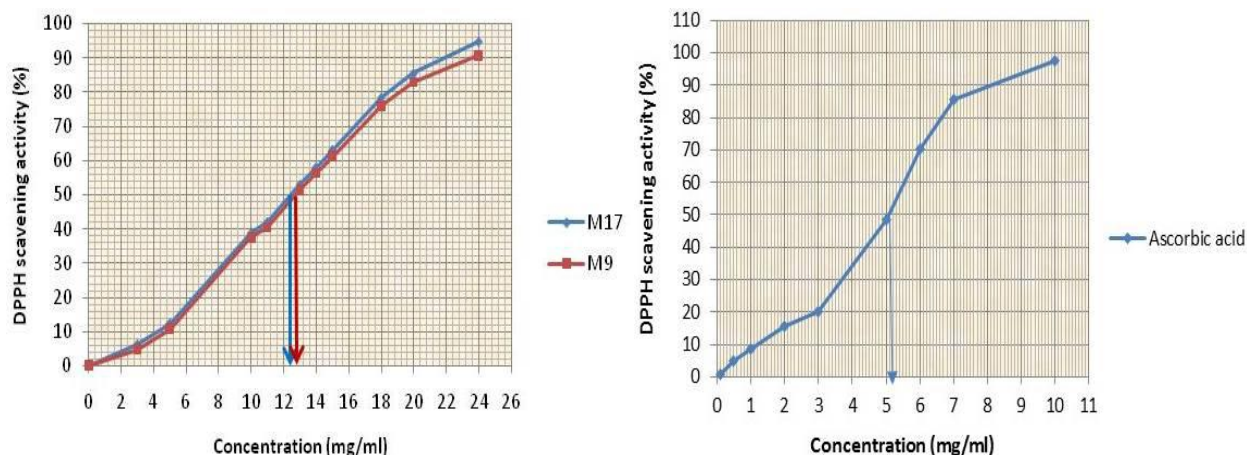


Fig. 3. DPPH radical scavenging activity of the ethanol extracts from *Streptomyces* M9, M17, and ascorbic acid.

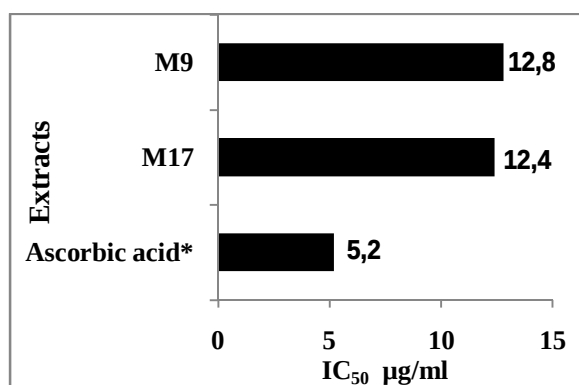


Fig. 4. IC_{50} of the ethanol extracts from *Streptomyces* M9, M17 and ascorbic acid (*reference standard).

4. Conclusion

From this work, it would be concluded that actinomycetes isolated from marine sponges (Demospongia) of the South East coast of Madagascar (Vangaindrano) are prolific sources of diverse metabolites such as antimicrobial and antioxidant compounds. However, further work is necessary to investigate the chemical nature of the secondary metabolites produced by these active marine

actinomycetes strains and to assess other biological activity of isolated strains.

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