

## Prevalence of Culturable Marine Actinomycetes Genera in Near-Shore Sediments of Algoa Bay in the Eastern Cape Province of South Africa

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The present study was carried out to assess the culturable actinomycetes diversity of near-shore sediments of Algoa Bay collected at depths ranging from 5.91 to 7.51 m and approximately 500 m distance from shore. Counts of the actinomycetes ranged in the orders  $10^1$  to  $10^2$  cfu/g using CSPY-ME agar and  $10^2$  to  $10^3$  cfu/g using M1 agar. A total of 326 actinomycetes isolates belonging to sixteen (16) genera were isolated from sediment samples and includes *Actinoplane* spp. (4.9%); *Actinopolyspora* spp. (3.68%); *Amycolata* spp. (0.92%); *Actinosynema* spp. (1.53%); *Ampularia* spp. (3.37%); *Amycolaptosis* spp. (2.45%); *Catellospora* spp. (6.14%); *Intrasporangium* spp. (3.37%); *Kibdellosporium* spp. (2.45%); *Kitasatospora* spp. (2.15%); *Micromonospora* spp. (7.98%); *Norcadia* spp. (2.45%); *Salinispora* spp. (2.15%); *Saccharopolyspora* spp. (0.92%); *Streptovercillium* spp. (19.33%); and *Streptomyces* spp. (36.20%). Depths of collection of sediment samples does not appear to affect the distribution of the actinomycetes genera, but choice of media appears to be a contributing factor to consider in cultivating marine actinomycetes as the M1 agar appears to support actinomycetes growth than CSPY-ME agar. We conclude that Algoa Bay sediment promises to be an important reservoir of marine actinomycetes of potentially biotechnological relevance.

**Key words:** Culturable marine actinomycetes, sediment, Algoa Bay.

Actinomycetes are Gram-positive eubacteria with high G+C content and are comprised of a group of branching unicellular microorganisms<sup>1, 2</sup>. They are historically best known as soil bacteria and were originally believed to occur in the ocean largely as dormant spores that were washed into the sea<sup>1</sup>. They are economically and biotechnologically valuable organisms and produce approximately half the discovered bioactive secondary metabolites, such

as antibiotics, anti-tumor agents, immune-suppressive agents and enzymes<sup>3</sup>. Because of the excellent track record of actinomycetes in this regard, a significant amount of efforts have been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs in the past fifty years<sup>4</sup>, while less effort has been focused on the marine environments.

Until recently, very little was known about the microbial diversity of marine sediments compared to that of terrestrial environment. However, some reports<sup>5, 6</sup> have shown that marine sediments contain a wide range of microorganisms which are unique and are not found in terrestrial environment. The marine environment conditions

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are found to be extremely different from that of the terrestrial environment and suggest that marine actinomycetes have different characteristics from those of their terrestrial counterparts, and consequently may produce unique types of bioactive compounds<sup>6</sup> different from those of terrestrial origin, more so, considering the conditions under which the marine actinomycetes survive, e.g. extremely high pressure and anaerobic conditions at temperatures below 0°C at the deep sea floor to high acidic conditions at temperatures of over 100°C, near the hydrothermal vents at mid ocean ridges. All these reflect on the genetic and metabolic diversity of marine actinomycetes which are currently poorly understood<sup>7</sup>.

It has been noted that actinomycetes are increasingly being isolated from marine environments thus showing that they are ubiquitous in marine sediments<sup>8</sup> and their distribution is said to vary depending on the depth from which samples are collected<sup>9</sup>. This has led to increased exploration of the marine environment for new actinomycetes taxa with unique metabolic and physiological capabilities.

There is paucity of information on the actinomycetes diversity of the South African marine environment, as very scanty reports exist in literature in this regards<sup>10</sup>. Hence, in this paper, we report on the culturable marine actinomycetes diversity of the bottom sediments of Algoa Bay in the Eastern Cape Province of South Africa as part of our extensive exploration for new bioactive compounds.

## MATERIALS AND METHODS

### Description of study site

Algoa Bay is a wide inlet along the South African East Coast and situated within the coordinates 33° 50S and 25° 50E. It is bounded in the West by Cape Recife and in the East by Cape Padrone. The bay is up to 436 m deep.

### Sample collection and processing

Five different marine sediment samples were randomly collected from approximately 500 m off shore the Algoa Bay and from depths ranging from 5.91 m to 7.51 m using a Smith-McIntyre sediment sampler courtesy the Elwandle Node of South African Environmental Observation Network (SAEON) in Grahamstown. Collected samples were

transported in ice to the AEMREG laboratory of the University of Fort Hare, Alice, South Africa, dried overnight at 28°C followed by sieving through a 2 mm mesh to remove large sand particles.

### Isolation of marine actinomycetes

A protocol for efficient isolation of actinomycetes was devised following the methods of Jensen *et al.*<sup>9</sup>. Briefly, one gram of each dried sieved sample was suspended in 4 ml sterilized sea water and vortexed for 30 sec. Ten-fold serial dilutions of the suspensions were made using sterile sea water and 100 µl of each dilution were inoculated onto the surface of agar plates and spread evenly with sterile glass spreader. The media used for isolation of actinomycetes include Casein-Starch-Peptone-Yeast extract-Malt extract (CSPY-ME) agar and M1 media. The CSPY-ME medium was composed of [0.5 g of dipotassium hydrogen phosphate, 3.0 g of casein, 10.0 g of starch, 1.0 g of peptone, 10.0 g of malt extract, 15.0 g of agar, and 1 litre of natural sea water] as described by Devi *et al.*<sup>11</sup> and M1 medium composed of [10 g of starch, 4 g of yeast extract, 2 g of peptone, 18 g of agar and 1 litre of natural seawater] as described by Mincer *et al.*<sup>8</sup> and Ogunmwonyi *et al.*<sup>10</sup>. To eliminate fungal and bacterial contamination, the media were supplemented with Cycloheximide (50 mg/l) and Nalidixic acid (20 mg/l) respectively<sup>10</sup>. The inoculated Petri dishes were incubated at 28 °C and were monitored over a period of two weeks for actinomycetes growth. At the end of the incubation period, colonies on agar plates were enumerated<sup>12</sup>.

### Purification and identification of actinomycetes isolates

After a period of two weeks incubation, fully grown colonies were observed and selected for purification by streaking on fresh media and incubated at 28°C for a week after which the isolates were identified and grouped into generic morphotypes. Identification was based on morphological and cultural characteristics<sup>13</sup>. These morphological characteristics were based on colony colour, form/configuration, margin, elevation, pigmentation colour if present and the size of the colony. Identified isolates were stored in the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG), University of Fort Hare, Alice, South Africa.

**RESULTS**

The culturable actinomycetes densities in the sediment samples generally ranged in the order of 10<sup>1</sup> - 10<sup>3</sup> cfu/g, with the M1 agar supporting more actinomycetes growth (10<sup>2</sup> to 10<sup>3</sup> cfu/g) than the CSPY-ME agar which yielded counts in the order of 10<sup>1</sup> to 10<sup>3</sup> cfu/g (Table 1). Sampling depths does not appear to affect the counts of actinomycetes.

A total of 326 actinomycetes isolates belonging to sixteen genera were isolated from the five different samples collected and from the two media used (CSPY-ME and M1 medium). These includes *Actinoplane* spp. (4.9%), *Actinopolyspora* spp. (3.68%), *Amycolata* spp. (0.92%), *Actinosynema* spp. (1.53%), *Ampularia*

spp. (3.37%), *Amycolaptosis* spp. (2.45%), *Catellospora* spp. (6.14%), *Intrasporangium* spp. (3.37%), *Kibdellosporium* spp. (2.45%), *Kitasatospora* spp. (2.15%), *Micromonospora* spp. (7.98%), *Norcadia* spp. (2.45%), *Salinispora* spp. (2.15%), *Saccharopolyspora* spp. (0.92%), *Streptoverticillium* spp. (19.33%), and *Streptomyces* spp. (36.20%). *Streptomyces* and *Streptoverticillium* species were the most prevalent genera while *Actinosynema* spp., *Amycolata* spp., and *Saccharopolyspora* spp., were least prevalent (Table 2). Also, the prevalence of the different genera does not appear to be influenced by sampling depth, but by media type as the M1 agar yielded more actinomycetes morphotypes.

**Table 1.** Actinomycete counts in the bottom sediment of Algoa Bay samples

Sample code	Sample depth (m)	Actinomycetes counts (cfu/g)	
		CSPY-ME agar	M1 agar
1	7.194	9.5 × 10 <sup>2</sup>	4.1 × 10 <sup>2</sup>
2	7.259	5.8 × 10 <sup>3</sup>	5.2 × 10 <sup>3</sup>
3	7.51	2.0 × 10 <sup>1</sup>	2.3 × 10 <sup>2</sup>
4	5.91	1.3 × 10 <sup>2</sup>	4.4 × 10 <sup>2</sup>
5	7.061	1.2 × 10 <sup>2</sup>	2.1 × 10 <sup>2</sup>

**DISCUSSION**

Terrestrial actinomycetes have played a significant role in the areas of science and medicine particularly as producers of antibiotics and other chemotherapeutic compounds such as anti-tumor agents<sup>3,4</sup>. This present study has further corroborated previous reports of marine environments as reservoirs of actinomycetes, and that the choice of media could be a contributing factor to the isolation of culturable actinomycetes from marine sediments.

From the 16 actinomycetes genera isolated in this study, *Streptomyces* spp. dominated followed by *Streptoverticillium* spp. and *Micromonospora* spp in that order (Table 2). Acquiescent with our results is the findings of Suthindhiran and Kannabiran<sup>14</sup> who reported *Streptomyces* spp. as the major species isolated in

their study. Similar findings were reported by Bredholt *et al.*<sup>3</sup> on bottom sediments of the Trondeheim Fjord.

Though *Streptoverticillium* spp was the second dominating actinomycetes observed in this study, however, Witt and Stackebrandt<sup>15</sup> had unified *Streptoverticillium* and *Streptomyces* genera in their study, based on phylogenetic similarities of partial 16S rRNA gene sequences thus suggesting very close relatedness amongst the two genera. Also, although *Micromonospora* spp. ranked third in terms of dominance in this present study, contrary reports have been made elsewhere, which suggest *Micromonospora* spp. to be dominant at sea<sup>16</sup>.

A number of other actinomycetes were isolated from the sediment samples (Table 2) some of which are considered to be rare actinomycetes. Example includes *Actinoplanes* species. According

Table 2. Prevalence and distribution of actinomycetes genera from Algoa Bay bottom sediment

Actinomycetes Genera	Proportion of tentatively identified actinomycetes genera per sample												Total Abundance (Number of Isolates) actinomycetes Genera
	1 (7.194 m)		2 (7.259 m)		3 (7.51 m)		4 (5.91 m)		5 (7.061 m)		M1		
	CSPY	M1	CSPY	M1	CSPY	M1	CSPY	M1	CSPY	M1			
<i>Actinoplane</i>	-	-	-	3	-	-	3	5	-	2	3	16	4.9
<i>Actinopolyspora</i>	3	-	2	5	-	-	-	2	-	-	-	12	3.68
<i>Amycolata</i>	2	-	1	-	-	-	-	-	-	-	-	3	0.92
<i>Actinosynema</i>	-	-	-	-	-	2	2	1	-	1	1	5	1.53
<i>Ampularia</i>	-	-	1	-	-	4	2	2	-	2	-	11	3.37
<i>Amycolaptosis</i>	-	-	-	1	-	-	-	-	-	-	4	8	2.45
<i>Catellospora</i>	-	2	1	4	1	7	3	1	1	1	-	20	6.14
<i>Intrasporangium</i>	-	2	-	3	-	2	-	-	-	-	4	11	3.37
<i>Kibdellosporium</i>	-	-	-	-	-	4	-	-	-	-	-	8	2.45
<i>Kitasatospora</i>	-	-	-	-	-	-	3	-	-	-	2	7	2.15
<i>Micromonospora</i>	3	2	-	6	-	5	3	3	2	2	-	26	7.98
<i>Norcadia</i>	-	-	2	3	-	-	-	-	3	-	-	8	2.45
<i>Salinospora</i>	1	-	1	2	-	2	-	-	-	-	-	7	2.15
<i>Saccharopolyspora</i>	1	-	-	-	-	2	-	-	-	-	-	3	0.92
<i>Streptoverticillium</i>	5	5	4	6	3	13	6	6	6	6	7	63	19.33
<i>Streptomyces</i>	11	7	8	19	2	23	9	9	12	12	10	118	36.20
Total Prevalence / medium	26	18	20	52	6	70	31	31	29	29	31	326	
Total Prevalence / sample	44	72	76	74	60								
% Prevalence / sample	13.5	22.09	23.31	22.70	18.40								

to Laatsch<sup>17</sup> the density of rare actinomycetes like *Actinoplanes*, *Rhodococcus* or *Actinomadura*, is obviously increasing in sediments of the open sea. According to Jensen *et al.*<sup>9</sup> *Actinoplanes* species were found dominating together with *Streptomyces* genera contrary to our findings.

Marine actinomycetes over the past years have demonstrated tremendous potential in diverse fields including biotechnology, pharmaceuticals industry and medicine through discovery of new bioactive compounds. Typical examples include butenolides four varieties of which were found to be produced by *Streptoverticillium luteoverticillatum*<sup>18</sup>. These butenolides showed cytotoxicity against the murine lymphoma P388 and human leukemia K562 cell lines, and it is the first report of isolation of cytotoxic butenolides from the marine ecosystem.

Also, a halophilic *Actinopolyspora* species AHI was isolated from the sediments of Alibag coast of Maharashtra which produces very active antimicrobial metabolites against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Aspergillus niger*, *Aspergillus umigatus*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium* sp. and *Trichoderma* sp.<sup>19, 20</sup>. *Actinopolyspora* specie isolated from marine shellfish collected from the Mediterranean coast of Port-Said, Egypt showed antibacterial activities against *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio* specie, *Shigella boydii*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus* sp.<sup>21</sup>.

*Micromonospora* is well known as marine microbial symbionts and has been reported to exist in symbiosis with some China Sea sponges including *Hymeniacidon perleve*, *Haliclona* sp., sea hare *Aplysia dactylomela* as well as the marine algae *Gracilaria verrucosa*<sup>22</sup>. A *Micromonospora* specie isolated from Chinese mangrove exhibited appreciable antagonistic activities against *Candida albicans*, *Staphylococcus aureus*, tumor cell, Caspase 3 and protein tyrosine phosphatase (PTP1B) inhibitions<sup>23</sup>.

A significant cytotoxicity of metacycloprodigiosin against human cancer cell lines has been reported before<sup>24</sup> which was isolated from a *Saccharopolyspora* sp. found associated with the sponge *Mycale plumose* consequently leading to the isolation of two known prodigiosin

analogs metacycloprodigiosin (1) and undecylprodigiosin (2). These compounds exhibited significant cytotoxic activities against five cancer cell lines: P388, HL60, A-549, BEL-7402, and SPCA4<sup>22, 24</sup>. Also, a halophilic *Saccharopolyspora salina* VITSDK4, isolated from marine sediment samples in the Marakkanam, coast of the Bay of Bengal, India produces an extracellular bioactive metabolite, which inhibits the proliferation of HeLa cells as well as antagonistic to fungal and bacterial pathogens. The cytotoxic activity of VITSDK4 on HeLa cells suggests that the strain could be clinically important for its anticancer properties<sup>14</sup>.

The genus *Streptomyces* alone produces an overwhelming majority of the bioactive molecules discovered amongst microbial organisms<sup>25</sup>. In their review article, Solanki *et al.*<sup>25</sup> reported that a marine inhabitant known as *Streptomyces psommoticus* produced an antibiotic (SBR-22) which showed antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Also, Piericidins C7 and C8 produced by a marine *Streptomyces* sp showed selective cytotoxicity against rat glia cells transformed with the adenovirus E1A gene and neuro-2a mouse neuroblastoma cells<sup>26</sup>. Tetracenomycin D an anthraquinone antibiotic produced by *Streptomyces corchorusii* AUBN(1)/7 showed cytotoxicity against cell line HMO2 (gastric adenocarcinoma) and HepG2 (hepatic carcinoma) and possesses weak antibacterial activities against gram-positive and gram-negative bacteria<sup>27</sup>. Other important bioactive compounds accredited to the *Streptomyces* include Resistoflavine<sup>28</sup>, Bonactin<sup>29</sup> Chinikomycins A and B<sup>30</sup>, Trioxacarcins<sup>25</sup>, Methylpyridine Streptokordin<sup>31</sup>, several enzyme inhibitors such as Pyrostatins A and B and Pyrizinostatin<sup>32</sup>.

In a previous report<sup>12</sup>, the *Salinospora* genus was suggested to exhibit a pantropical distribution having been isolated from marine sediments collected around the world including the Caribbean Sea, the Sea of Cortez, the Red Sea, and the tropical Pacific Ocean off Guam. Mincer *et al.*<sup>8</sup> has earlier reported that this actinomycetes genus is distinct from other actinomycetes in that it resides in ocean sediments and is the first bacterium of its type to require seawater for growth thus providing opportunities to study marine

adaptations in Gram-positive bacteria. Members of this genus have been reported to produce antibacterial compound similar to rifamycin<sup>33</sup>, structurally unique secondary metabolites such as Salinosporamide A<sup>34</sup>, a potent inhibitor of the 20S proteasome; enzymes inhibitors; Saliniketol A and B which are inhibitors of ornithine decarboxylase biosynthesis<sup>25</sup>.

*Kitasatospora* genus has been implicated in the production of compounds that inhibited growth of the P388 lymphocytic leukemia and a minipanel of human cancer cell lines<sup>35</sup> and identified as cyclodepsipeptides 1, 2 and 3. In addition to the human cancer cell line activity, cyclodepsipeptide 1 had activity against the pathogenic fungus *Cryptococcus neoformans* and cyclodepsipeptide 2 had marginal activity against the opportunistic bacterium *Micrococcus luteus*. Cyclodepsipeptides 3 had marginal activity against *C. neoformans* and *Enterococcus faecalis*<sup>35</sup>. In another report, the metabolic extracts of *kitasatospora* specie isolated from marine shellfish *Donax trunculus anatinus* specimens collected from the Mediterranean coast of Port-Said, Egypt showed both antimicrobial and antitumor activities<sup>21</sup>. In this same report, metabolic extracts of a *Intrasporangium* specie showed antibacterial properties. In like manner, a strain of *Actinoplanes* isolated from a soil sample collected in the Indole region (India) produced antimicrobial compounds identified as a chlorinated phenylpyrrole compound (A 15104 Y) as well as another compound identified as a chlorophenol derivative (A 15104 Z)<sup>36</sup>. This is the first example of a chloropyrrole derivative isolated from an actinomycete<sup>36</sup>. Also, Arizonins, a novel complex of antibiotics related to kalafungin, were discovered in the fermentation broth of *Actinoplanes arizonaensis* sp. nov. isolated from a soil sample collected near Tuba City, Arizona, U.S.A. Two members of the complex, arizonins A1 and B1, exhibit moderate to potent *in vitro* antimicrobial activity against pathogenic strains of Gram-positive bacteria<sup>37</sup>. Not much is known about bioactive compounds from the *Actinosynnema* genus, but *Actinosynnema pretiosum* has been reported to produce Maytansinoids which are a family of 19-membered macrocyclic lactams having extraordinary cytotoxic and antineoplastic activities<sup>38</sup>.

## CONCLUSION

The bottom sediments of Algoa Bay appears to be a reservoir of important marine actinomycetes taxa with potentials for producing new important bioactive metabolites and further corroborates previous suggestion that marine sediments could be valuable for the isolation of novel strain of actinomycetes, which could potentially yield useful new bioactive products. Extensive assessment and characterization of unique bioactive compounds produced by the actinomycetes diversity of this marine habitat is a subject of on-going investigation in our group.

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