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Microbial Ecological Distribution of Marine Actinomycetes Isolated from Lagos Atlantic Ocean, South West Nigeria

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ABSTRACT: Actinomycetes are threadlike or filamentous, Gram positive bacteria that form branching filaments with hyphae and asexual spores. The hyphae are usually septate or coenocytic in nature. The aim of this study was to investigate the microbial ecological distribution of marine actinomycetes isolated from the Lagos Atlantic Ocean, South West Nigeria. Marine (soil and water) samples were collected from about 10 m deep from Lagos Lagoon and Atlantic Ocean. Starch Casein agar was prepared aseptically. Isolation and enumeration, and identification of actinomycetes were carried out using standard microbiological protocols, and Bergey's Manual of Determinative Bacteriology and Actinomycetes Atlas, respectively. It was discovered that marine actinomycetes were variably more abundant in Atlantic Ocean than in the Lagoon. Marine actinomycetes had a least count of $9 \pm 0.33 \times 10^2$ CFU/mL from site D and a highest count of $1.13 \pm 6.02 \times 10^4$ CFU/mL from site F. Most of the results obtained from the lagoon (site A, B, D, E. and F) were not significantly different ($p > 0.05$). Also, a total of 379 marine actinomycetes were identified from sediments the seashore and of which R*hodococcus equi* and *Dietzia* type strains had the least occurrence of 0.24% respectively. The second least actinomycetes are the species of *Nonomurea* which had an occurrence of 0.48%. *Streptomyces hydroscopicus* had the highest occurrence of 28.43%. This study discovered that marine actinomycetes were variably more in the Atlantic Ocean when compared to their counterpart in lagoon, and also there were more marine actinomycetes in sediments than in water samples. These organisms can be explored for the production of different industrial products ranging from pharmaceuticals to agroactive compounds and bio-corrosion compounds.

Keywords: Actinomycetes, Coenocytic, Sediments, Atlas, Lagoon, Bio-corrosion

Introduction

Actinomycetes are threadlike or filamentous Gram positive bacteria that form branching filaments with hyphae and asexual spores (Barakate *et al.,* 2002).The hyphae are usually septate or coenocytic in nature. They were originally incorrectly classified as fungi because they possess true aerial hyphae and form spores, both of which are considered to be fungal characteristics. They are among the most widely distributed microorganisms in nature and constitute a significant component of the microbial populations in most soils. They play a major role in the carbon cycle due to their ability to grow at low concentrations of carbon and to degrade recalcitrant organic compounds. Actinomycetes are found in terrestrial, aquatic, and marine environments. Some indigenous marine actinomycetes, like *Rhodococcus* and *Salinispora* species, have been identified (Barakate *et al.*, 2002).
However, most biologically active secondary metabolites are produced by filamentous marine actinomycetes. There is a continued need to bioprospect alternative sources of natural products, and one strategy is to harness the chemical

diversity of Actinomycetes genera, which were previously under-represented in natural product screening collections marine actinomycetes have been reported a good source of L- asparaginase. This can be ascribed to the fact that these microorganisms can adapt to an extreme marine environment (Gupta, 2007).
The existence of terrestrial actinomycetes has been reported in the relatively untapped marine ecosystem (Ward and Bora, 2006). The immense diversity of this habitat, along with its under-exploitation, is the fundamental reason for attracting researchers towards it for discovering novel metabolite producers. Actinomycetes comprise about 10% of the bacteria colonizing marine aggregates and can be isolated from marine sediments (Ward and Bora, 2006).
Thus, this study investigated the microbial ecological distribution of Actinomycetes isolated from the Lagos Atlantic Ocean

Materials and methods

Description of study area: Lagos State is located in Southwest Nigeria, with a total land area of 3,474 km^2 and 20 Local Government Areas. It is located on 6°35' N 3°45' E. Lagos lagoon is located on coordinates 6.5015814° N and 3.5224915° E, with a maximum length of about 50 km and a width of about 13 km, and an average surface area of 6, 3547 km^2 .The lagoon receives it's discharge from the Ogun and Osun rivers. The Lagos Atlantic Ocean is close to Victoria Island; it has coordinates of 6°25'25"N and 3°24'54" E.
Collection of marine samples: Marine sediments, soil, and water samples were collected from the Lagos lagoon and Atlantic Ocean at a depth of about 10 cm. Soil sample were collected specifically from the seashore and sea depth. Samples were collected using sterile universal containers.
Preparation of media: Starch Casein Agar (SCA) was prepared by dissolving 69 g in 1000 mL of distilled water. The agar was properly dissolved by using a thermostat hot plate (Gallenhamp)^{TM} at 150 °C for 20 mins. Sterilization was carried out by autoclaving at 121 °C for 15 mins according to the manufacturer's instruction.
Isolation of marine actinomycetes: Isolation and enumeration of actinomycetes were carried out by serial dilution and the pour plate method. Serial dilution was carried out by taking 25 g (marine sediments and soil samples) and 25 mL (water samples) of each sample and mixed with 225 mL of sterile distilled water. The mixture was agitated for 1 h at 1000 rpm using water bath (WHY -2 ^{Tm}), to enable the dislodge of microorganisms. The suspension was serially diluted by transferring 1 mL aliquots to a series of test tubes, each containing 9 mL of sterile distilled water. From each dilution, 1 mL of the sample was transferred to the sterile, labeled Petri plates, and the molten agar was poured onto it and allowed to solidify. The isolation media was supplemented with antibiotics cycloheximide (25 mg/mL) and nalidixic acid (25 mg/mL). The plates were inverted and incubated at 37 °C for 3- 4 days. Colony forming unit was calculated using the formula below:

$$\text{Counts/ml} = \frac{\text{Number of colonies on plates}}{\text{Amount of aliquot}} \times \frac{1}{\text{dilution factor}}$$

Isolation of pure culture: Pure **c**ultures of marine actinomycetes were obtained by using the streak plate method. The sterile straight wire was used to pick isolate and inoculated on a fresh Petri dish containing solidified starch casein agar. The pure cultures were streaked carefully in a zig zag pattern. The cultures were further incubated at 37 °C for 3–4 days.
Identification of isolated marine actinomycetes: Identification of isolated marine actinomycetes was carried out according to Bergey's manual of determinative bacteriology. Colony morphology, spore arrangement, Gram staining reaction, and the Actinomycetes ATLAS (Tamura *et al.,* 2015).

Results

The results of mean counts of marine actinomycetes obtained from sea depth, seashore, lagoon, and Atlantic Ocean are presented in Table 1. Samples E and F had the highest count of 2.62 ± 9.06^{d} $x10^4$ CFU//mL and 2.72 ± 1.45^{d} $x\ 10^4$ respectively, and site A had the least count of 1.5 ± 2.33^{a} $x\ 10^3$ CFU//mL. Samples from Atlantic Ocean showed that sample D had the least count of 9 ± 0.33^{a} $x10^2$ CFU//mL and highest count of 1.13 ± 6.02^{e} $x10^4$ CFU//mL

Table`1: Mean of marine Actinomycetes count obtained from sea depth, sea shore, Lagoon and Atlantic Ocean

Site/ Sample	Sea Depth (x10 ^{2}) Mean ± S.D.	Sea Shore (x10^{2}) Mean ± S.D.	Lagoon (x10^{2}) Mean ± S.D.	Atlantic Ocean (x10^{2}) Mean ± S.D.
A	1.5 ± 2.33^{a} x10^{3}	4.6 ± 7.26^{ab} x10^{3}	2.2 ± 1.45^{b} x10^{3}	1.0 ± 0.57^{a} x 10^{3}
B	8.5 ± 2.88^{c} x10^{3}	6.2 ± 1.73^{c} x10^{3}	2.0 ± 1.20^{b} x10^{3}	3.0 ± 5.77^{cd}x10^{3}
C	5.9 ± 3.05^{b}x 10^{3}	4.2 ± 2.33^{ab}x10^{3}	9 ± 0.33^{a} x10^{2}	2.3 ± 1.66^{bc}x10^{3}
D	5.7 ± 1.45^{b}x 10^{3}	4.9 ± 2.33^{b} x10^{3}	2.2 ± 1.45^{b} x10^{3}	9 ± 0.33^{a} x10^{2}
E	2.62 ± 9.06^{d} x10^{4}	3.6 ± 1.66^{a} x10^{3}	2.0 ± 0.57^{b} x10^{3}	1.5 ± 0.88^{ab} x10^{3}
F	2.72 ± 1.45^{d}x 10^{4}	$5.2.\pm1.52^{bc}$ x10^{3}	2.2 ± 1.00^{b} x10^{3}	1.13 ± 6.02^{e} x10^{4}

Data are presented as mean ± SD (n=3). Values with the same superscript letter(s) along the same column are not significantly different ($p > 0.05$), while values with different superscript along the same column are significantly different ($p > 0.05$).

Fig. 1 explains the percentage frequency of occurrence of marine actinomycetes from sea depth. *Rodococcus* spp. had the least percentage frequency of occurrence of 0.374 % and *Microbispora rosea* had the highest percentage of 33.7 %. *Streptomyces* spp. had percentage frequency of occurrence of 26.217 %.

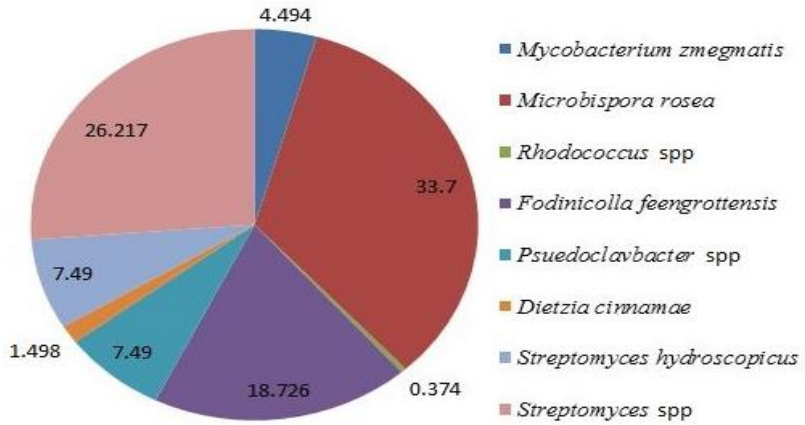

Figure 1: Percentage frequency of occurrence of marine actinomycetes from sea depth

Fig. 2 revealed more *Streptomyces hydroscopicus* (31.13 %) from sea shore

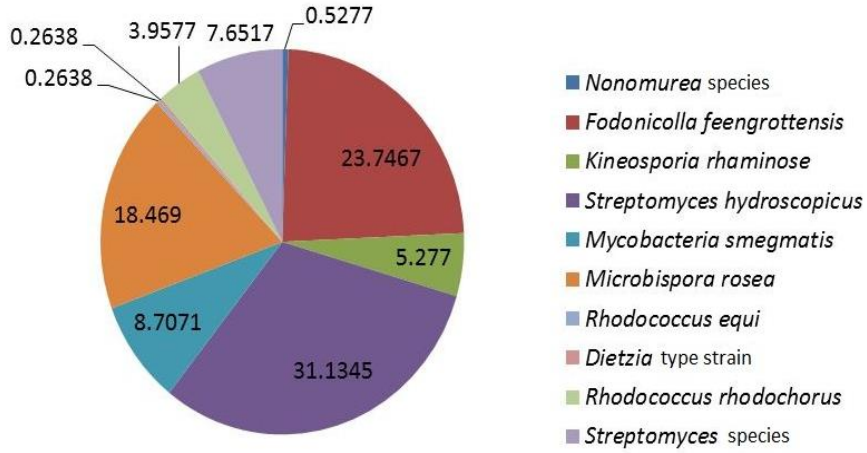

Figure 2: Percentage frequency of occurrence of marine actinomycetes obtained from sea shore

Discussion

The marine environment is a unique habitat that has features that terrestrial area do not have, like high hydrostatic pressure, high salinity, and a low concentration of organic matter. Thus microorganisms that survive in both the marine (salt water) and terrestrial environment are expected to be totally different. This leads to the fact that marine environment are excellent sampling point as microbes in them (sea water and marine sediments) are diverse. However, media formulated with sea water has shown that terrestrial strains could grow well. Halo-tolerance of NaCl-sensitive strain could be induced by stepwise exposure to increasing NaCl concentration (Imada *et al.*, 2007). In this study, it was discovered that marine actinomycetes were variably more abundant in the Atlantic Ocean when compared to their counterparts in lagoons. Marine actinomycetes had a least count of $9 \pm 0.33 \times 10^2$ CFU/mL from site "D" and a highest count of $1.13 \pm 6.03 \times 10^4$ CFU/mL from site F .

Most of the results obtained from the lagoon (site A, B, D, E and F) were not significantly different ($p>0.05$), indicating that the Null hypothesis is accepted. There is no significant difference between mean counts in the results obtained from the different sites. However, we could attribute the higher actinomycetes population in the Atlantic Ocean to higher salinity in the ocean, lower water temperature, less terrestrial interference, and so on.

Meanwhile, the existence of indigenous marine actinomycetes had been reported by Barka *et al.* (2016) based on abundant actinomycetes isolated from deep sea sediments. Jensen *et al. (*2005) had also reported that the maximum number of actinomycetes isolates from near shore sediment in both shallow and deep sampling sites showed a bimodal distribution in relation to depth. This was characterized by an obvious decrease in *Streptomycetes* and *Actinoplanetes* increment as depth increased. Furthermore, 98% of the *Streptomycetes* were obtained from a depth ≤ 3 m and the percentage decreased radically with increasing depth, and the number of *Actinoplanets* increased with up to a 33 m of depth increment. However, in this study, the population of marine actinomycetes was higher at sea depth than at seashore, and there were no significant difference in count (actinomycetes) obtained. This could indicate that the sea depth marine actinomycetes are well-adapted and functional members of the marine microbial community. It could also be concluded that the isolates from seashore were mostly *Streptomycetaceae.*

According to Ghanem *et al.* (2000), marine actinomycetes isolated from sediments far exceeded those found in sea water. Thus, sediments are still the best supplier of Actinomycetes. In this study, there were relatively more actinomycetes in sediments (seashore and sea depth) than in sea water (lagoon and Atlantic Ocean). Although, a sampling site on the Atlantic Ocean tends to have a count of $1.13 \pm 6.02 \times 10^4$ CFU/mL which is significantly different from other results obtained from water, this could be attributed to the fact that sediments have more concentration of organic matter than sea water, and this type of organism can breakdown these organic matter in order to grow. Examples of organic matter are dead fish shells, which contains chitin, cellulose, and lignin.

Hakvag *et al.* (2008) isolated a total of 217 marine actinomycetes from sea surface microlayer. All these strains resembled *Streptomyces* species. However, in this study 379 marine actinomycetes were identified from sediments of seashore and of which *Rhodococcus equi* and *Dietzia* type strain had an occurrence of 0.24 % respectively. The second least actinomycetes are the species of *Nonomurea* which had an occurrence of 0.48%. *Streptomyces hydroscopicus* had the highest occurrence of 28.43 %. Bioprospecting of these marine actinomycetes could lead to the production different metabolites such as plant-promoting agents which aid in the production of plant growth hormone indole 3–acetic acid), bio pesticides agents, antifungal compounds, bio corrosion, and a source of agroactive compounds.

Conclusion

This study discovered that marine actinomycetes were variably more in the Atlantic Ocean when compared to their counterpart in lagoon, and also there were more marine actinomycetes in sediments than in water samples. These organisms can be explored for the production of different industrial products ranging from pharmaceuticals to agroactive compounds and bio-corrosion compounds.

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