1. Introduction

Many pathogenic bacteria are fast developing resistance to existing antibiotics. Hence, the urgency in the need for new drugs becomes apparent to control the incidence of infections caused by these antibiotic resistant pathogens as well as treat cancer related diseases which could be life threatening[1].

Methicillin resistance in Staphylococcus aureus (S. aureus) has been recognized globally since it was first reported in the United Kingdom by British scientists in 1961[2,3]. However, there has been increased difficulty combating methicillin resistant S. aureus because of emerging resistance to available antibiotics[4]. To solve the problem of antibiotic-resistant pathogens by periodic replacement of existing antibiotics with novel antibiotics, unique environments need to be constantly exploited for novel bioactive compounds. Bioactive compounds synthesized by microorganisms seem to be the most promising source of novel antibiotics[5].

A mongst the prokaryotes, members of the phylum Actinobacteria, particularly those which belong to the genus Streptomyces have been recognized as prolific sources of novel bioactive metabolite with a wide
spectrum of activities[6,7]. The search for novel natural products have focused on the terrestrial environment but in recent times, the marine environment, which remained unexplored with regard to isolation of antibiotic producing actinomycetes have now been considered as a new source of novel drugs. However, evidence in literature has shown that indigenous actinomycetes of the marine environment in West Africa have not been explored for their antibiotic production potentials. Therefore, the marine actinomycetes diversity of West Africa could be a potential source of novel antimicrobial compounds that can combat rapidly emerging drug-resistant pathogens that have become issues of important public health concern in Nigeria and world wide.

2. Materials and methods

2.1. Sample collection and isolation of Actinomycetes

Soil samples were collected from different locations of Lagos lagoon using pre-sterilized grab. The samples were kept in sterile polythene bags and transported immediately to the laboratory. They were air-dried for 2 weeks after which the Actinomycetes were isolated by serial dilution using spread plate method on Starch Casein and Kuster’s Agar supplemented with 80 µg/mL of cycloheximide to prevent fungal growth[8]. The plates were incubated at 28 °C for 1-2 weeks. Isolates were selected based on their cultural characteristics as well as their Gram reaction and subcultured. Pure cultures were maintained on nutrient agar slants at 4 °C[9].

2.2. Biochemical characterization of isolates

Biochemical studies were carried out on the suspected actinomycetes isolates using API 20A kit (BioMerieux, France). The tests were carried out according to the manufacturer’s instructions, incubated at 28 °C for 24-48 h and were later read. All the positive and negative tests were recorded on the result sheet. Other biochemical tests such as starch hydrolysis and casein hydrolysis were carried out using standard methods[10].

2.3. DNA extraction, amplification and sequencing

DNA was extracted from the isolates and stored at -20 °C. The primers S-C-Act-0235-a-S-20 5’CCG TAC TCC CCA GGC GGG G 3’ and S-C-Act-0878-s-a-19 5’CGC GGC CTA TCA GCT TGG TTG 3’, specific for Actinobacteria were used to amplify an 640-bp stretch of the 16S rRNA gene of all the strains using PCR method[10]. The PCR conditions were an initial denaturation stage at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60.5 °C for 45 seconds, extension at 72 °C for 50 seconds and a final extension at 72 °C for 5 min. Extension controls with no DNA template were included in all PCR experiments. Amplification was detected by agarose gel electrophoresis and UV fluorescence after ethidium bromide staining and purified PCR products were sequenced (ABI 3730 DNA Analyzer) and sequences run on basic local alignment search tool for identification of the isolates[11].

2.4. Screening of secondary metabolites for antimicrobial activity

A loopful of each pure actinomycete culture was inoculated into 30 mL sterile starch casein broth and incubated for 8 d at 28 °C. After incubation, the culture was centrifuged at 5 000 r/min for 20 min. Using agar well diffusion method, the cell-free supernatant was assayed for antimicrobial activity against the following microorganisms: methicillin resistant S. aureus, S. aureus ATCC 29213, Escherichia coli ATCC 25922 (E. coli), Pseudomonas aeruginosa ATCC 27853, Candida albicans (C. albicans), Enterococcus faecalis ATCC 29212. Coagulase-negative staphylococci isolated from HIV patients were also used (Staphylococcus warneri, Staphylococcus xylosus and Staphylococcus epidermidis). Sterile Mueller–Hinton and Sabouraud dextrose agar plates were seeded with test bacteria and yeast respectively and incubated at 37 °C for 24 h while those seeded with the yeast were incubated at 37 °C for 48 h[12].

2.5. Gas chromatography-mass spectrometer (GC-MS) analysis of crude extract

Extraction of secondary metabolites was carried out using the method of Mohkam et al. with modifications[13]. Twenty millilitre of cell-free crude extract was mixed with a combination of ethyl acetate/methanol (1:1) in a separating funnel and shaken vigorously for 30 min and afterwards, was allowed to stand without any disturbance for 15 min. The lower aqueous phase was discarded and the organic phase was collected into a glass beaker and concentrated to 1 mL. A standard (pure) for the antibiotic combinations was first injected into the GC to set its equivalent peak area and retention time profiles of the individual antibiotics. Afterwards, 0.1 ul was injected in to GC 6890 series (Hewlett Packard) with specification (column size 0.25 mm × 30 m, carrier gas nitrogen, flow rate 22 mL/min, injection temperature at 220 °C, acceleration and reflector temperature 100 °C/min, initial column temperature at 50 °C, holding time 2 min). The peak area of the standard antibiotics were compared to those of the test samples.

3. Results

Three isolates (ULS12, ULS13 and ULK3) suspected to be actinomycetes grew on the starch casein and Kuster’s agar supplemented with cycloheximide. The mycelia of ULS12 colonies were white and turned grey with age and produced brown pigment in agar while that of ULS13 were white, powdery turning grey with age and produced yellow pigment. ULK3 colonies were faint green and powdery with no pigment in agar.

The result of the physicochemical characteristics of the suspected actinomycetes isolates was shown in Table 1. The organisms were non-sporulal and showed ability to utilize glucose, lactose, saccharose, maltose while none of the isolates were able to utilize urease, cellobiose, gelatine, mannose, melezitose, rhamnose. All isolates were however found to be catalase negative but able to hydrolyze starch.

The species-specific primers used confirmed the strains to be actinomycetes based on the amplification of the 640-bp stretch of the 16S rRNA gene. The results of the sequences analysed using basic local alignment search tool showed ULS12 having 99% similarity
to *Streptomyces albus* J1074 but ULK3 showed a higher similarity (100%) to *Streptomyces albus* J1074 while ULS13 showed 100% similarity to *Streptomyces fulvissimus* DSM 40593.

The crude extracts from the isolates were screened for antimicrobial activity against pathogenic microorganisms and it was observed that isolate ULS12 and ULS13 displayed significant inhibitory activity against bacterial isolates such as *E. coli* ATCC 29522 (Figure 1) while ULS13 showed the highest antifungal activity against *C. albicans* (Figure 2). ULK3 showed activity against *C. albicans* only as shown in Table 2.

| Isolates | Zone of inhibition (mm) |
|----------|--------------------------|
| S. warneri | Methicillin-resistant S. aureus | S. xylosus | S. epidermidis | P. aeruginosa ATCC 27853 | E. coli ATCC 29522 | E. faecalis | S. aureus ATCC 29213 | C. albicans |
| ULS12 | 12 | 29 | 18 | 12 | 9 | 15 | 26 | 36 | 3 |
| ULS13 | 13 | 20 | 8 | 14 | 10 | 18 | 27 | 29 | 18 |
| ULK3 | - | - | - | - | - | - | - | - | 3 |

*S. warneri*: *Staphylococcus warneri*; *S. xylosus*: *Staphylococcus xylosus*; *S. epidermidis*: *Staphylococcus epidermidis*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *E. faecalis*: *Enterococcus faecalis*.
Table 1. Temperature program:

| Solvent/0.36 | Init temp | hold | Ramp | Final temp |
|--------------|-----------|------|------|------------|
|             |           |      |      |            |

Figure 4. Detection of antibiotics present in the crude extract of ULS13.

Figure 5. Detection of antibiotics present in the crude extract of ULS12.
The reduction in the frequency of novel drug discovery from well-explored terrestrial environment has given rise to exploration of the marine environment, which has been largely overlooked, for novel broad spectrum antibiotics. An attempt was therefore made in this study to isolate actinomycetes from sediment of Lagos Lagoon to evaluate their antimicrobial potential.

Molecular approaches such as PCR amplification of genes for identification have been used over many decades because identification of microorganisms based on biochemical characteristics has been found to be inadequate. All the isolates showed positive results as amplification using the species specific primers confirmed the presence of the marine actinomycetes of the Lagos lagoon in the West African Coast is an unexplored area for novel drug discovery. Therefore, the findings from this study highlights the potentials of the marine actinomycetes of the Lagos lagoon in the West African Coast to produce novel antibiotics. The identified antibiotics were extracts from ULS12 and ULS13 with the exception of the extract from isolate ULK3 were found to contain rifamycin B & SV as well as tubelactomicin and resistoflavin. Tetracemycin was also found in ULS12. Therefore in all, the extracts from isolates ULS12 and ULS13 were found to have the highest number of both identified and unidentified peaks.

4. Discussion

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marine environment as a source of novel antimicrobial compounds which could contribute to current efforts aimed to control drug-resistant pathogens.

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**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Background**

Antimicrobial secondary metabolites of *Streptomyces* sp., isolated from Lagos lagoon soil were screened for antimicrobial activity against antimicrobial resistance strains. Antimicrobial resistance is a global threat and this study proposed antimicrobial activity against methicillin resistant *S. aureus*, which is of prime significance.

**Research frontiers**

The present research article proposes antimicrobial efficacy of *Streptomyces* sp. ULS12 and ULS13 against methicillin resistant *S. aureus*. There identified metabolites could pose as a treatment strategy against methicillin resistant *S. aureus*.

**Related reports**

The observation are in line with previous study carried out by Singh et al. who isolated an actinomycete which displayed antibacterial activity against methicillin-resistant *S. aureus*[7].

**Innovations and breakthroughs**

The extracts from ULS12 and ULS13 were found to contain rifamycin B&SV as well as tubelactomicin A, which has already been reported in previous studies. Tests and methods used in study are well established.

**Applications**

The extracts from ULS12 and ULS13 were found to contain rifamycin B&SV as well as tubelactomicin A. The major findings from this study highlight the potentials of the marine actinomycetes of the Lagos marine environment as a source of novel antimicrobial compounds. These peptides have potential to be explored against antimicrobial resistance strains with application of recombinant DNA technology and bulk production.

**Peer review**

This is a valuable research work in which authors have demonstrated the antimicrobial efficacy of actinomycetes isolated from Lagos marine environment. GC-MS studies identified rifamycin as well as tubelactomicin as the inhibitory peptides.