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*Full Length Research Paper*

# Studies on the culturable marine actinomycetes isolated from the Nahoon beach in the Eastern Cape Province of South Africa

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**A total of 84 culturable marine actinomycetes belonging to 15 generic morphotypes were isolated from water and rock scrapings from the Nahoon beach, a coastal shore of Indian Ocean in the Eastern Cape Province of South Africa. The colonies showed diverse morphological characteristics and their occurrence and distribution varied with sample type. The rock scrappings yielded more actinomycetes morphotypes than the water samples. *Streptomyces* was most prevalent in both samples. *Actinoplane, Actinosynnema, Norcadia, Norcardiopsis, Streptosporagium* and *Intrasporagium* were present in rock scrapings but absent in water samples. *Kibdellosporium* was present in water sample and absent in rock scrapings. The *Streptomyces* isolates were selected and preliminarily screened for antimicrobial activity. The bioactive compounds of 10 most potent *Streptomyces* isolate was extracted twice with equal volume of ethyl acetate (1:1 v/v). The ethyl acetate extracts of the putative *Streptomyces* isolates showed activities against at least 6 and up to 26 of the 32 test bacteria screened. Inhibition zones were found to range between 9 and 32 mm diameters at a concentration of 10 mg/ml. The partial characterization of the crude extracts by IR spectra analysis revealed the possible presence of terpenoid, long chain fatty acids and secondary amine derivatives compounds in the extracts. We conclude that the Nahoon beach promises to be a veritable resource for diverse marine actinomycetes of potentials for bioactive compounds production and novel drug discovery.**

**Key words:** Morphotypes, occurrence, rock scrapings, IR spectral, bioactive compound.

## INTRODUCTION

Actinomycetes, the filamentous bacteria, are primarily saprophytic microorganisms of the soil, where they contribute significantly to the turnover of complex biopolymers, such as lignocellulose, hemicellulose, pectin, keratin, and chitin (Vijayakumar et al., 2007). The recently proposed class actinobacteria (Stackebrandt et al., 1997) is comprised of high G+C content gram-positive bacteria and includes the actinomycetes (order Actinomycetales), whose members have an unparalleled ability to produce diverse secondary metabolites (Mincer et al., 2002). The actinomycetes have provided many important bioactive compounds of high commercial value including antibiotics. Due to the special attributes of the marine environment, marine actinomycetes are thought to have distinct physiological, morphological and

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chemotaxanomical characteristics and unique production of secondary metabolite and bioactive compounds.

During the past 30 years, a large number of new compounds with structures completely different from those isolated from terrestrial organisms were successfully discovered from marine sources (Elyakov et al., 1994; El-Gendy et al., 2008b). Marine environment is a relatively untapped ecosystem with regards to isolation of indigenous *Streptomyces*, although existence of terrestrial origin has been reported (Solanki et al., 2008). 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.

Searching for previously unknown microbial strains is an effective approach for obtaining new biologically active substances. It is known that the antimicrobial activity of the metabolic products of aquatic bacterial strains is not weaker than the corresponding activity of soil strains. Water, bottom sediments, as well as sponges and other aquatic organisms are potential sources of producers of these metabolites (Sponga et al., 1999; Terkina et al., 2006).

Actinomycetes are the strongest antagonists among microorganisms. The antibiotic substances they produce display antibacterial, antifungal, antitumor, antiprotozoic, and antiviral properties. Of the ten thousand known antibiotics produced by microorganisms over a decade ago, about 70% are of actinomycete origin; of them, representatives of the genus *Streptomyces* account for two thirds (Miyadoh, 1993). Secondary metabolites produced from marine actinomycetes have distinct chemical structures, which may form the basis for the synthesis of new drugs (Solanki et al., 2008).

Some reports have shown that actinomycetes can be isolated from marine environment especially from marine sediment but little is known about occurrence and diversity of actinomycetes from marine water and most especially rock scrapings attached biofilms. Hence, in this paper, we report the culturable marine actinomycete morphotypes from water and rock surfaces of the Nahoon beach, the antimicrobial activities and partial characterization of bioactive compounds produced by some selected *Streptomyces* as part of our studies on bioprospecting of indigenous marine actinomycetes for important biomolecules in South Africa.

## MATERIALS AND METHODS

### Description of sampling site and samples collection

Samples were collected from the Nahoon beach, which is a coastal shore of the Indian Ocean located in East London in the Eastern Cape Province of South Africa at the geographical coordinates 32.99°S and 27.95°E. Several water samples and rock scrapings were aseptically collected from near-shore areas of the Nahoon beach into sterile nalgene bottles and transported to the laboratory in ice-pack. The samples were processed for actinomycetes isolation same day as collected.

### Processing of samples

The method of Jensen et al. (2005) was used for processing of the water and rock scrapings samples. The water sample used for isolation of actinomycetes was concentrated by centrifugation in a step wise order to 20 ml each in two 50-ml centrifuge tubes. The rock scrapings were placed into two 50-ml centrifuge tubes containing 10 g of sterile glass beads (0.1 mm size Biospec Products, Inc) and 25 ml of sterile distilled water, and vortexed for 10 min. The tubes were left standing at room temperature for ten minutes to allow the beads, fibers and other solids to settle, after which the supernatant was decanted and severally diluted and used for actinomycetes isolation.

### Cultivation and Isolation of actinomycetes

The cultivation of actinomycetes from the samples was done in accordance with the description of Jensen et al. (1991) using M1 agar medium, [10 g of starch, 4 g of yeast extract, 2 g of peptone, 18 g of agar and 1 L of natural seawater] as described by Mincer et al. (2002). Cycloheximide (50 mg/L) and Nalidixic acid (20 mg/L) were added to the sterilized molten (at 45°C) media to prevent respectively, fungal and gram-negative bacterial growth. The cultivation medium was then poured into sterile 90 mm Petri dishes and allowed to set. Over 400 culture plates were prepared. Approximately 100 µl of the diluted samples were spread on the surface of the cultivation media and incubated aerobically at 28°C for 3 weeks. At the end of the incubation period, colonies of actinomycetes were isolated and purified using the same cultivation medium and conditions as described by Singh and Agrawal (2002). Identified isolates were stored in the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG), University of Fort Hare, Alice, South Africa.

### Identification of isolated cultures

Pure actinomycetes isolates were culturally characterized based on colour, dryness, rough, with irregular or regular margin, and generally convex colony morphology, tough leathery colonies, branched vegetative mycelia, and when present, aerial mycelia and spore formation as described by Ghanem et al. (2000) and grouped into generic morphotypes.

### Antibacterial screening

#### *Test bacteria*

The test bacteria that were used for antimicrobial screening include reference, environmental as well as clinical isolates. The typical reference strains were as follows:

**Gram-positive:** *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 29212, *Streptococcus pyogens* ATCC 10389, *Bacillus cereus* ATCC 10702, *Bacillus pumilus* ATCC 14884, *Acinetobacter calcaoceuticus* UP and *Acinetobacter. calcaoceuticus* subsp *anitratus* CSIR.

**Gram-negative:** *Escherichia coli* ATCC 8739, *Escherichia. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC7700, *Pseudomonas aeruginosa* ATCC 19582, *Proteus vulgaris* ATCC 6830, *Proteus. vulgaris* CSIR 0030, *Enterobacter cloacae* ATCC 13047, *Serratia marscens* ATCC 9986, *Klebsiella pneumoniae* ATCC 10131 and *Klebsiella. pneumoniae* ATCC 4352.

**Environmental isolates:** *Klebsiella pneumoniae*, *Bacillus subtilis*,

*Shigella flexineri*, *Salmonella* sp, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Proteus. vulgaris*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus. aureus* and *Bacillus stearothermophillus* were kindly provided by Dr. AO Olaniran of the University of KwaZulu-Natal.

**Clinical isolates:** Includes the following; staphylococcal strains isolated from septic wound abrasion: *Staphylococcus aureus* OKOH1, *Staphylococcus aureus* OKOH2A, *S. aureus* OKOH3 and *Staphylococcus sciuri* OKOH2B with Gene Bank accession numbers Eu244633, Eu244634, Eu244635 and Eu244636, respectively.

***Test Streptomyces species***

Of the 84 isolated actinomycetes, 30 isolates belonging to the *Streptomyces* morphotypes were selected for preliminary screening for antibacterial activity and these were coded as NB003, NB008, NB009, NB012, NB013, NB017, NB018, NB022, NB023, NB025, NB031, NB033, NB035, NB037, NB039, NB041, NB042, NB043, NB046, NB050, NB051, NB052, NB056, NB059, NB061, NB063, NB070, NB073, NB078 and NB084.

The test *Streptomyces* suspensions were prepared by suspending a loopful of pure *Streptomyces* colony in 2 ml sterile normal saline, vortexed to homogenize and stored at 4°C until ready for use. This suspension was used as *Streptomyces* inoculants in all cultivations.

**Preparation of test bacteria**

All test bacteria were grown in Nutrient broth (Biolab, Merck) and incubated at 37°C for 24 h. A total of 10 ml of the pure culture was centrifuged to pellet out the cells, washed twice with sterile physiological saline and the suspension adjusted to optical density 0.1 at 600 nm which is equivalent to a cell population of about $10^6$ cells/ml on the McFarland standard. Bacteria suspension was stored in test tubes and refrigerated at 4°C. These test bacteria were frequently checked for viability and re-prepared when appropriate.

**Primary screening of actinomycetes for antibiotic production**

***Preparation of preliminary test organism***

The test organisms used for preliminary screening were *E. coli* ATCC 8739, *B. cereus* ATCC 10702, *B. subtilis* KZN and *Candida albicans.* Test bacteria were grown in 4.50 ml Nutrient broth for 18 h, and then standardized to McFarland standard of 0.1 at OD 600 nm. *C. albicans* was grown on potato dextrose agar (PDA) plate for 48 h, then re-suspended in nutrient broth and standardized to McFarland standard of 0.1 at OD 600 nm before use.

***Preliminary screening***

Yeast malt broth (YMB) was prepared and 50 ml dispensed into 250-ml capacity flask, autoclaved, allowed to cool and inoculated with 0.5 ml actinomycetes suspension. Flasks were incubated at 28°C at 230 rpm for 8 days. Cultures were harvested by centrifugation at maximum speed for 15 min to obtain cell free extract which was used for preliminary screening for antibacterial activity. The preliminary screening was carried out by streaking method. Ten milliliter of the cell free extract was incorporated into double strength 10 ml sterilized molten MHA at 50°C, plates poured, allowed to set and then streaked upon by test bacteria and incubated at 37°C for 24 h. The same regimen was done with PDA for the test yeast, but incubation was at 28°C for 24 - 48 h. Control experiments were carried out using sterile nutrient broth in place of the cell free extracts.

**Secondary screening of fermentation product for antibiotic production**

***Bulk fermentation and preparation of crude ethyl acetate extracts***

Based on the results of preliminary screening, 10 putative *Streptomyces* isolates were selected for the fermentation and assessment of antibiotic production. The putative *Streptomyces* isolates included NB003, NB008, NB009, NB012, NB017, NB022, NB046, NB063, NB078 and NB084. Fermentation for production of antibiotic and subsequent extraction of the antibiotics was done as described by Ilic et al. (2007) with modification. Yeast malt extract broth (YMB) was prepared and 20 ml dispensed into 100-ml Erlenmeyer flask capacity, sterilized, allowed to cool and inoculated with 0.5 ml *Streptomyces* isolate suspension and incubated at 28°C for 48 h at 230 rpm. About 500 ml of YMB was prepared in 1 L Erlenmeyer flask and inoculated with the 48 h old pre-culture of *Streptomyces* isolate and incubated for 10 days at 28°C and 230 rpm. At the end of the incubation period, the culture was harvested by centrifugation at maximum speed for 15 min. The culture supernatant was extracted twice with equal volumes of ethyl acetate (1:1 v/v) and vaporized to dryness in a rotary evaporator at 50°C. The extract was re-constituted in 50% filter sterilized methanol to obtain the desired concentration at every stage of screening.

***Screening for antibacterial activity***

The crude ethyl acetate extracts was screened for antibacterial activity using the cup well agar diffusion method as described by Pandey et al. (2004). For this purpose 20 ml of sterilized molten Mueller Hinton agar (MHA) in McCartney bottles was seeded with 50 µl of standardized test bacteria, swirled gently and aseptically poured into Petri dishes and allowed to solidify. Sterile cork borer (6 mm diameter) was used to bore holes in the plate. About 100 µl of the crude ethyl acetate extract at a concentration of 10 mg/ml was carefully dispensed into bored holes. This was done in duplicate. Extracts were allowed to diffuse for about 2 h before incubating. Plates were incubated at 37°C for 24 h. The presence of zone of inhibition around each well was indicative of antibacterial activity. Control experiment was carried out by loading 10% methanol into control well against each test organism to ensure that it does not have activity against test bacteria.

**Characterization of crude extracts**

***Infrared spectroscopy***

The infrared (IR) spectra of the crude extracts were measured (as KBr discs) between 400 – 4000 $cm^{-1}$ on Perkin Elmer 2000 FT-IR spectrophotometer. The important IR bands, such as ţ (C-N), ţ (O-H), ţ (C-H), ţ (C=C), ţ (N-H), ţ (C-O) and (C-H) symmetric and asymmetric stretching, and stretching frequencies were studied to determine the presence of functional group in the ethyl acetate crude extracts.

## RESULTS

### Culturable actinomycetes

A total of 84 isolates belonging to 15 genera were

**Table 1.** Occurrence and distribution of marine actinomycetes genera in water and rock surfaces samples.

| Sampling point | | Actinomycetes genera | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Water | 24 | 6 | 1 | - | 3 | 2 | 2 | 2 | 4 | 1 | - | - | - | - | - | 2 |
| Rock scrapings | 61 | 24 | 4 | 2 | 8 | 2 | 1 | 5 | 6 | - | 2 | 1 | 1 | 1 | 2 | 2 |
| Total | 84 | 30 | 5 | 2 | 11 | 4 | 3 | 7 | 10 | 1 | 2 | 1 | 1 | 1 | 2 | 4 |
| Frequency (%) | | 35.7 | 5.9 | 2.4 | 13.1 | 4.8 | 3.5 | 8.3 | 11.9 | 1.2 | 2.4 | 1.2 | 1.2 | 1.2 | 2.4 | 4.8 |

obtained from this study (as shown in Table 1) with higher occurrence from the rock scrapings than from the water samples suggesting high species richness in the former. The prevalence of the actinomycetes isolates differ significantly ($P < 0.001$) among the two sources. Most of the isolates belong to the *Streptomyces* genera (39.3% from the rock scrapings samples and 25% from the water samples). Five of the genera isolated from the rock scrapings which include *Actinoplane, Actinosynnema, Nocardia, Norcadiopsis, Streptosporangium* and *Intrasporangium* were absent in the water samples, while Kibdellosporium isolated from the water sample was absent in the rock scrapings samples.

## Preliminary screening of the putative *Streptomyces* isolates

The result of preliminary screening is shown in Table 2. Of the thirty putative *Streptomyces* screened, twelve showed varying levels of antimicrobial activities against four test organisms including *E. coli* ATCC 8739, *B. cereus* ATCC 10702, *B. pumilus* ATCC 14884 and *C. albicans.* NB025 and NB042 showed activity against *C. albicans* only, while NB003, NB008, NB009, NB012, NB017, NB046, NB063 and NB078, showed broad spectrum activity against the tested bacteria. Isolate NB022 showed only a narrow spectrum activity against the test gram positive, and isolate NB084 was active against all test bacteria and *C. albicans*. Eighteen of the isolate viz. NB013, NB018, NB023, NB031, NB033, NB035, NB037, NB039, NB041, NB043, NB050, NB051, NB052, NB056, NB059, NB061, NB070 and NB073 did not show any activity against the test organisms.

## Screening of fermentation products for antibacterial activity

The result of the screening of the ethyl acetate crude extracts of the selected ten putative *Streptomyces* for antibacterial activities is shown in Table 3. These extracts showed activities against a minimum of 6 test bacteria and maximum of 26 among the 32 test bacteria amounting to approximately 18.8 to 81.3% antibacterial activities (Table 3). NB003 extracts showed activity against twenty-six test bacteria with zones of inhibition ranging from 10 to 22 mm, while NB008 and NB009 extracts showed activity against 25 and 15 test bacteria respectively (Table 3). Isolates NB012, NB017, NB022, NB046, NB063, NB078 and NB084 extracts were active against 19, 6, 17, 19, 18, 20 and 12 test bacteria respectively with zones of inhibition ranging from 9 - 32 mm diameter (Table 3).

## Characterization of crude extract

### *Infrared (IR) analysis*

The presence of some functional group as revealed by IR spectral is shown in Table 4. The FTIR Spectral analyses of extracts NB003, NB008, NB009**,** NB012, NB046, NB063, NB078 and NB084 show certain common absorption bands between 1700 and 1730 $cm^{-1}$ which are characteristics of a C=O stretching vibration of a carboxylate functional group. The peaks between 3390 and 3420 $cm^{-1}$ are characteristics of hydroxyl $\upsilon$ (O-H) and $\upsilon$ (N-H) vibrational frequency which are interchangeable. A common 2 bands vibrational peak between 2855 and 2979 $cm^{-1}$ are characteristic of a $\upsilon$ (C-H) symmetrical vibration of saturated hydrocarbon.

**Table 2.** Results of preliminary screening of antimicrobial activities of the *Streptomyces* isolates.

| Test streptomyces | Antimicrobial activity | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***B. cereus*** | ***B. subtilis*** | ***C. albicans*** |
| NB003 | + | + | + | _ |
| NB008 | + | + | + | _ |
| NB009 | + | + | + | _ |
| NB012 | + | + | + | _ |
| NB013 | _ | _ | _ | _ |
| NB017 | + | + | + | _ |
| NB018 | _ | _ | _ | _ |
| NB022 | _ | + | + | _ |
| NB023 | _ | _ | _ | _ |
| NB025 | _ | _ | _ | + |
| NB031 | _ | _ | _ | _ |
| NB033 | _ | _ | _ | _ |
| NB035 | _ | _ | _ | _ |
| NB037 | _ | _ | _ | _ |
| NB039 | _ | _ | _ | _ |
| NB041 | _ | _ | _ | _ |
| NB042 | _ | _ | _ | + |
| NB043 | _ | _ | _ | _ |
| NB046 | + | + | + | _ |
| NB050 | _ | _ | _ | _ |
| NB051 | _ | _ | _ | _ |
| NB052 | _ | _ | _ | _ |
| NB056 | _ | _ | _ | _ |
| NB059 | _ | _ | _ | _ |
| NB061 | _ | _ | _ | _ |
| NB063 | + | + | + | _ |
| NB070 | _ | _ | _ | _ |
| NB073 | _ | _ | _ | _ |
| NB078 | + | + | + | _ |
| NB084 | + | + | + | + |

Legend: + = extract active against test organism; - = extract inactive against test organism.

The vibrational frequency υ (C-O) was observed in the spectra of all the extracts around 1100 $cm^{-1}$. Deviation from this region to a higher wave number was observed which is indicative of a secondary amide. These peaks were sharper than the υ (O-H) peaks due to reduction in hydrogen bonds which increases with electronegativity.

In extract NB084, the absence of vibrational peaks between 1600 and 1640 cm-1 was an indication of the absence of unsaturation. Vibrational peaks between 1620 and 1680 cm-1 in extract NB022 and NB078 signify the possibility of an aromatic compound. Based on the physical state (oily) of the extracts and the characteristic features of the infrared vibrational peaks in the spectra, terpenoids, long chain fatty acids and secondary amine derivatives are possible compounds in the extracts.

## DISCUSSION

Actinomycetes are indeed well adapted and are functional members of the aquatic microbial community (Jensen et al., 1991). The result of this study corroborates the report of Rifaat (2003) that actinomycetes are widely spread in various bodies of water and attached biofilms, where they play a great part in the carbon cycle due to their ability to grow at low concentration of carbonaceous substance and to degrade recalcitrant organic matter. Actinonmycetes especially *Stretopmyces*, have been reported from the marine sub habitats such as marine sediments (Takizawa et al., 1993; Vijayakumar et al., 2007); marine soil

**Table 3.** Antibacterial activities of fermentation products of the selected putative *Streptomyces* isolates

| Test bacteria | Gram reaction | Antibacterial activity (zone of inhibition in mm) (Mean ± SD) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NB003 | NB008 | NB009 | NB012 | NB017 | NB022 | NB046 | NB063 | NB078 | NB084 |
| *Escherichia coli* ATCC 8739 | - | 12 ± 0.2 | 0 ± 0 | 15 ± 0.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| *Escherichia coli* ATCC 25922 | - | 13 ± 0.1 | 12 ±0.4 | 0 ± 0 | 17 ± 1.2 | 10 ± 0.6 | 11 ± 1.2 | 0 ± 0 | 13 ± 1.5 | 0 ± 0 | 0 ± 0 |
| *Pseudomonas aeruginosa* ATCC 19582 | - | 10 ± 0.2 | 13 ± 0.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 18 ± 0.5 | 18 ± 0.1 | 0 ± 0 | 0 ± 0 |
| *Staphylococcus aureus* ATCC6538 | + | 10 ± 1.2 | 0 ± 0 | 0 ± 0 | 22 ± 0.2 | 0 ± 0 | 13 ± 0.4 | 10 ± 1.2 | 0 ± 0 | 0 ± 0 | 10 ± 1.0 |
| *Streptococcus faecalis* ATCC 29212 | + | 0 ± 0 | 12 ± 1.1 | 0 ± 0 | 17 ± 1.5 | 0 ± 0 | 0 ± 0 | 16 ± 1.4 | 16 ± 0.5 | 0 ± 0 | 14 ± 1.0 |
| *Bacillus cereus* ATCC 10702 | + | 13 ± 0.2 | 14 ± 0.4 | 18 ± 1.5 | 15 ± 1.4 | 12 ± 0.1 | 14 ± 0.1 | 15 ± 1.0 | 15 ± 0.2 | 12 ± 0.5 | 12 ± 1.0 |
| *Bacillus pumilus* ATCC 14884 | + | 14 ± 1.2 | 11 ± 0.8 | 18 ± 0.5 | 19 ± 2.1 | 0 ± 0 | 14 ± 1.0 | 15 ± 1.2 | 15 ± 0.5 | 13 ± 0.2 | 16 ± 0.1 |
| *Pseudomonas aeruginosa* ATCC 7700 | - | 15 ± 2.1 | 12 ± 0.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 10 ± 0.4 | 16 ± 0.8 | 0 ± 0 | 0 ± 0 |
| *Enterobacter cloacae* ATCC 13047 | - | 14 ± 0.9 | 11 ± 0.5 | 0 ± 0 | 17 ± 1.4 | 0 ± 0 | 15 ± 1.5 | 0 ± 0 | 16 ± 1.1 | 13 ± 0.5 | 0 ± 0 |
| *Klebsiella pneumoniae* ATCC 10031 | - | 12 ± 0.8 | 13 ± 1.1 | 15 ± 1.5 | 18 ± 2.0 | 12 ± 0.1 | 13 ± 0.5 | 0 ± 0 | 17 ± 1.5 | 12 ± 0.6 | 16 ± 0.8 |
| *Klebsiella pneumoniae* ATCC 4352 | - | 12 ± 0.2 | 13 ± 0.4 | 15 ± 0.5 | 18 ± 0.9 | 12 ± 0.5 | 10 ± 0.4 | 18 ± 1.2 | 0 ± 0 | 12 ± 1.1 | 17 ± 1.5 |
| *Proteus vulgaris* ATCC 6830 | - | 14 ± 0.9 | 12 ± 0.8 | 13 ± 0.7 | 17 ± 0.3 | 0 ± 0 | 0 ± 0 | 17 ± 0.4 | 0 ± 0 | 14 ± 0.7 | 0 ± 0 |
| *Proteus vulgaris* CSIR 0030 | - | 0 ± 0 | 12 ± 0.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 12 ± 1.2 | 12 ± 0.9 | 11 ± 0.5 | 0 ± 0 |
| *Serratia marscens* ATCC 9986 | - | 10 ± 0.5 | 12 ± 0.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 15 ± 0.4 | 15 ± 0.1 | 13 ± 0.5 | 9 ± 0.1 |
| *Acinetobacter calcaoceuticus* UP | + | 15 ± 0.2 | 12 ± 0.1 | 16 ± 0.5 | 17 ± 1.1 | 0 ± 0 | 10 ± 0.5 | 14 ± 0.8 | 0 ± 0 | 13 ± 0.6 | 14 ± 0.2 |
| *Acinetobacter calcaoceuticus* subsp *anitratus* CSIR | + | 15 ± 1.1 | 13 ± 1.2 | 0 ± 0 | 18 ± 1.4 | 0 ± 0 | 0 ± 0 | 15 ± 1.0 | 15 ± 1.2 | 13 ± 0.5 | 10 ± 0.2 |
| *Klebsiella pneumoniae* KZN | - | 12 ± 0.4 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 12±0.2 | 12±0.5 | 20 ± 2.1 | 0 ± 0 |
| *Bacillus subtilis* KZN | + | 15 ± 1.0 | 12 ± 0.5 | 14 ± 0.5 | 22 ± 2.0 | 0 ± 0 | 12 ± 0.5 | 0 ± 0 | 10 ± 0.5 | 15 ± 0.4 | 0 ± 0 |
| *Shigella flexineri* KZN | - | 0 ± 0 | 0 ± 0 | 11 ± 0.7 | 11 ± 0.8 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| *Salmonella* sp KZN | - | 12 ± 0.5 | 12 ± 0.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 12 ± 0.1 | 0 ± 0 | 15 ± 0.5 | 0 ± 0 |
| *Staphylococcus epiderdemis* KZN | - | 10 ± 0.8 | 0 ± 0 | 0 ± 0 | 20 ± 1.5 | 0 ± 0 | 12 ± 0.9 | 0 ± 0 | 17 ± 0.9 | 0 ± 0 | 0 ± 0 |
| *Pseudomonas aeruginosa* KZN | - | 0 ± 0 | 12 ± 0.6 | 10 ± 0.1 | 10 ± 0.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 17 ± 0.7 | 11 ± 1.2 | 0 ± 0 |
| *Proteus vulgaris* KZN | - | 14 ± 0.5 | 15 ± 1.0 | 12 ± 1.0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 12 ± 0.9 | 0 ± 0 |
| *Enterococcus Faecalis* KZN | - | 0 ± 0 | 0 ± 0 | 10 ± 0.9 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| *Escherichia coli* KZN | - | 15 ± 0.1 | 14 ± 0.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| *Staphylococcus aureus* KZN | + | 17 ± 0.2 | 9 ± 0 | 0 ± 0 | 22 ± 0.5 | 0 ± 0 | 20 ± 0.8 | 16 ± 0.4 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| *Staphylococcus aureus* OKOH1 | + | 22 ± 0 | 12 ± 0.5 | 0 ± 0 | 0 ± 0 | 10 ± 0.1 | 22 ± 0 | 0 ± 0 | 0 ± 0 | 10 ± 0.5 | 13 ± 0.9 |
| *Staphylococcus aureus* OKOH 2A | + | 0 ± 0 | 0 ± 0 | 0 ± 0 | 29 ± 2.0 | 0 ± 0 | 12 ± 0.5 | 18±0.9 | 32 ± 1.5 | 0 ± 0 | 0 ± 0 |
| *Staphylococcus sciuri* OKOH 2B | + | 12 ± 0.8 | 10 ± 0.5 | 11 ±0.9 | 0 ± 0 | 0 ± 0 | 13 ±0.4 | 17±0.6 | 17±0.2 | 0 ± 0 | 0 ± 0 |
| *Staphylococcus aureus* OKOH 3 | + | 20 ± 1.2 | 17 ± 0.5 | 10 ± 0.2 | 14 ± 1.0 | 14 ± 0.8 | 27 ± 1.5 | 12 ± 0.5 | 0 ± 0 | 12 ± 0.9 | 15±0.1 |
| *Micrococcus kristinae* | + | 19 ± 0.9 | 15 ± 0.8 | 12 ± 0.5 | 0 ± 0 | 0 ± 0 | 25 ± 1.5 | 16 ± 0.9 | 12 ± 0.2 | 11 ± 1.0 | 12 ± 1.2 |
| *Micrococcus luteus* | + | 14 ± 0.8 | 10 ± 0.5 | 0 ± 0 | 27 ± 1.2 | 0 ± 0 | 10 ±0.5 | 0 ± 0 | 0 ± 0 | 13 ± 0.1 | 0 ± 0 |
| % of susceptible* bacteria | | 81.3 | 78.1 | 46.9 | 59.4 | 18.8 | 53.1 | 59.4 | 56.3 | 62.5 | 37.5 |

*Diameter of zone of inhibition reported on susceptibility was based on the recommended guidelines by Clinical and Laboratory Standards Institute (2005).

**Table 4.** IR spectral of functional groups present in crude ethyl acetate extracts of putative *Streptomyces* isolate.

| Extracts | υ (N-H) ($cm^{-1}$) | υ (C=O) ($cm^{-1}$) | υ(C-O) ($cm^{-1}$) | υ (O-H) ($cm^{-1}$) | υ (C-N) ($cm^{-1}$) | υ (C=C) ($cm^{-1}$) | υ (C-H) ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|
| NB003 | | 1709, 1665 | 1090, 1016 | 3392 | | | 2975 |
| NB008 | 3415 | 1702 | | | 1270 | | 2961 |
| NB009 | | 1712 | 1121 | | 3411 | | 2902 |
| NB012 | 3420 | 1724 | 1052 | | | 1638 | 2924 |
| NB017 | | | 1100 | 3435 | | 1648 | 2959 |
| NB022 | | 1726 | | 3417 | | 1662 | 2926 |
| NB046 | | 1664 | 1077 | 3400 | | | 2962 |
| NB063 | | 1712 | 1091 | 3392 | | | 2975 |
| NB078 | | 1709 | 1091 | 3397 | | 1655 | 2979 |
| NB084 | 3415 | 1709 | | | 1217 | | 2964 |

Legend: υ(N-H) Vibrational frequency of Nitrogen-Hydrogen bond; υ(C=O) Vibrational frequency of Carbon oxygen double bond; υ(C-O) Vibrational frequency of Carbon oxygen single bond; υ(O-H) Vibrational frequency of hydroxyl group; υ(C-N) Vibrational frequency of carbon nitrogen bond; υ(C=C) Vibrational frequency of carbon- carbon unsaturated bond; υ(C-H) Vibrational frequency of saturated Carbon hydrogen bond, ($sp^3$ hybridised).

(Peela et al., 2005; Vijayakumar et al., 2007) and also from almost all parts of the world. Also, the dominance of *Streptomyces* among the actinomycetes especially in soils has been reported by many workers (Jensen et al., 1991; Peela et al., 2005). Hence they have worldwide distribution, which indicate their plasticity and adaptability to extremely varied environment. Also, a higher number of isolates were recovered from the rock scrapings compared to water sample, thus suggesting that actinomycetes can adhere to natural or artificial surfaces and within sessile multicellular communities known as biofilms. Biofilms is a complex microbial community including diverse species with a variety of functions.

Significant attention is currently being paid to the isolation and characterization of *Streptomyces* from poorly researched habitats given the premise that screening such organisms raises the prospect of discovering new natural products that can be developed as a resource for biotechnology (Eccleston et al., 2008). This reasoning appears to be sound as novel *Streptomyces* isolated from unexplored marine habitats are proving to be a valuable source of new bioactive metabolites (Bull and Stach, 2007). It seems timely, therefore, to extend this approach to another poorly studied environment, such as the Nahoon beach ecosystem in the Eastern Cape of South Africa.

It was not intended in the present investigation to assemble a detailed record of the kinds of *Streptomyces* found in the Nahoon beach environment or to isolate and characterize novel bioactive compounds, but the study was designed to prepare the ground for such studies by isolating, partially characterizing and screening a diverse range of *Streptomyces*. The antimicrobial activities of the extracts were tested against a wide panel of microorganisms (Table 3). These extracts showed activities against a minimum of 6 test bacteria and maximum of 26 among the 32 test bacteria amounting to approximately 18.8 to 81.3% antibacterial activities (Table 3). This percentage is higher than those described by Barakate et al. (2002) studying the activity of Moroccan soil *Streptomyces*. These results were also different from those of other authors showing 16% in soil of Turkey (Oskay et al., 2004); 53 – 61% in Algerian soil (Sabaou et al., 1998) and 44.5% in soils of South-Eastern Serbia (Illic et al., 2005, 2007). On the other hand, similar inhibition pattern was demonstrated by the ethyl acetate extracts of marine *Streptomyces* RM17 and RM42 (Remya and Vijayakumar, 2008); marine *Streptomyces* isolates from the Andaman Coast of the Bay of Bengal (Peela et al., 2005); and marine *Streptomyces* strain Merv 1996 and Merv 7409 (El-Gendy et al., 2008b); although more bacteria species were screened in this study. The antibacterial spectrum exhibited by all extracts highlights their potentials and suggests that they could be important candidates for antibiotics in this regard. Further studies on the bioactive metabolites produced by these extracts which exhibits broad spectrum activity is under progress. Overall the study gives first hand information on the antimicrobial activity of putative indigenous *Streptomyces* isolated from the Nahoon beach environment.

IR spectra of crude extracts showed some similarities in their content due to the presence of some functional groups, but the different vibrational peaks of these functional groups in these extracts depicts that the extracts were different hence the diverse activity they exhibited against test organisms during the susceptibility screening. The IR spectrum of an antifungal compound: 4' phenyl -1-napthyl –phenyl acetamide from *Streptomyces* sp. DPTB16 indicates that the compound had $NH_2$ and -OH group (Dhanasekaran et al., 2008). Similar functional group and spectra profiles were exhibited by these extracts suggesting similarity with these extracts and this could be attributed to antifungal activity demonstrated by some of the extracts during preliminary screening (Table 2). The distribution of the antibiotic inhibition phenotype of *Streptomycetes* with

great antibacterial and antifungal activity which gave a similar spectra profile has also been reported (Illic et al., 2007). Nevertheless, further investigation is needed in order to purify and determine the structure of the active components in the extracts.

## Conclusion

The present finding highlights the importance of further investigation towards the goal of obtaining novel antimicrobial agent from the *Streptomyces* from this untapped habitat. The Nahoon beach habitat appears to be an unexplored area in this environment, with unique ecological niches and rich in biodiversity. The microbial ecology of the Nahoon beach environment has to be further explored in order to get benefit out of the precious biowealth, moreso as it has great demand from the perspective of health care especially as regards combating existing and emerging drug resistant pathogens.


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