Retraction Notice

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The Editorial Board would like to extend its sincere apology for any inconvenience the paper withdrawal may have caused.

Open Access Library Journal

Isolation of Antibiotic Vibrionaceae Bacteria from a Community Marine Silvo-Fishery Farm along Mtwapa Creek, Kenya

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Abstract

Bacterial strains inhibitory towards pathogenic bacteria were isolated from a marine fish pond culture facility in Mtwapa Creek, Kenya. 12 of these antibacterial isolates were assigned to the Vibrionaceae family. The purpose of the present study was to investigate the antibacterial activity of five Vibrionaceae strains with pronounced antibacterial activity. These were identified as *Vibrio coralliilyticus* (two strains), *V. neptunius* (two strains), and *Photobacterium halotolerans* (one strain). The two related *V. coralliilyticus* and *V. neptunius* strains were isolated from marine fish pond culture system. Bioassay identified two known antibiotics as being responsible for the antibacterial activity, andrimid (from *V. coralliilyticus*) and holomycin (from *P. halotolerans*). Despite the isolation of already known antibiotics, our findings show that marine Vibrionaceae are a resource of antibacterial compounds and may have potential for future natural product discovery.

Keywords

***Vibrio coralliilyticus*, *Vibrio neptunius*, *Photobacterium*, Andrimid, Holomycin**

Subject Areas: Microbiology

1. Introduction

Bioactive secondary metabolites are believed to play a key role in microbial interactions by mediating antagonistic activity and intercellular communication [1]. In addition, many microbial natural products have biotechnological potential as antibiotics, biosurfactants, antifungal, or anticancer agents [2]. Sequences of microbial genomes revealed that only a small fraction of the natural product diversity was known, highlighting the potential for finding novel bioactive compounds in environmental microorganisms [3]. The need for novel antimicro-

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bials to combat increasing antibiotic resistances in pathogenic bacteria has stimulated the exploration of other than the traditional sources, such as terrestrial actinomycetes or fungi [4]. Bioactive bacterial strains predominantly belong to *Pseudoalteromonas* spp., the *Roseobacter* clade and *Actinobacteria* [5]. A number of marine-derived antimicrobials have been characterized in greater detail, including halogenated and sulfuric compounds, depsipeptides and lipopeptides, glycolipids, as well as high molecular weight structures such as amino acid oxidases [6], also the *Vibrionaceae* family, Gram-negative *Gammaproteobacteria* ubiquitous in marine and brackish environments, harbors strains with antagonistic activity. The family comprises eight genera, with *Vibrio* and *Photobacterium* constituting the majority of species [7]. Antibacterial activities have been described from *V. alginolyticus*, *V. parahaemolyticus*, *V. anguillarum*, and several unidentified *Vibrio* spp. [8]. However, the nature and frequency of antagonism among vibrios is still largely unknown, and only a few antibiotic *Vibrio* compounds have been structure elucidated to date [9]. The study describes the analysis of bioactive *Vibrionaceae* strains collected during the study period. The purpose was to 1) isolate *Vibrionaceae* strains with the strongest antibacterial activity and 2) isolate and elucidate the bioactive metabolites.

2. Materials and Methods

2.1. Study Area and Study Site

The Kenyan Coast is situated immediately south of the equator; it covers a distance of about 500 km while the actual length of the seafront is about 600 km. The coastline forms part of the western border of the Indian Ocean and has an almost continuous fringing coral reef. Other features of the Kenyan coast include mangrove forests and estuaries as well as a number of islands to the south, which protect several embayments and harbours [10]. Approximately three million people inhabit the Kenyan coastal areas, at a density of 300 - 400 persons/km^2. The marine environment provides this population with employment and food in the form of shell and finfish. Fish contributes over 70% of the protein consumed by the coastal inhabitants [11]. Artisanal fishery lands 95% of the total marine catch, contributing 6% to the coastal economy, and this is the main source of livelihood for more than 60,000 households [10]. Mariculture in the Kenyan coast at the moment is still at its infancy stage. It is thus important to understand the likely ecological changes that mariculture may introduce and their remedies so that the farmers and policy makers can be guided accordingly.

2.2. Facility Design

The study was carried out in ponds constructed at Kwetu Training Centre, Mtwapa Creek (**Figure 1**). The fish pond culture system consists of the water column and surface sediment in the ponds. The water from the creek moves through the mangrove ecosystem before getting into the ponds. The ponds were constructed in such a way that there is regulated inflow and out flow of water which is controlled by a sluice gate at the main channel entry. Water enters the ponds when the tide level rises above 3.4 m but when the tide is below 3.4 m no water enters the ponds. This means that the ponds are subjected to periods of no water exchange alternating with periods where there is water exchange during the high spring tide. The length of these periods varies with behavior of tides but on average water exchange takes place between 10 - 14 days.

2.3. Bacteriological Culture Techniques: Microscopy, Culture and Identification

Culture and identification of bacterial types was performed using slightly modified methods described by Buller [12]. Total Bacterial Counts (TBCs) were performed using Nutrient agar (NA) with 2% NaCl. Samples were stored at 4˚C during transport and while waiting to be analyzed in the laboratory. The procedure involved pipetting 100 µl (or 0.1 ml) of each dilution into the centre of an agar plate (using a separate plate for each dilution), and spread evenly using a sterile inoculating cotton swab to obtain individual colonies for counting. Plates were then placed in sealed plastic containers, incubated at room temperature and counting done after 24 and 48 hours. Appropriate colonies were selected and sub-cultured on to Nutrient Agar (NA) with 2% NaCl, Triple Sugar Indole (TSI), Blood Agar (BA) and Thiosulphate Citrate Bile Salt Agar (TCBS). They were then incubated for a further 24 h for sufficient growth. Pure subculture growths were used for the inoculation of biochemical identification sets which were then used to identify the bacteria into families/genera/species. All strains were retested for antibacterial activity against fish pathogen *Vibrio anguillarum* strain 90-11-287 and the human pathogen *Staphylococcus aureus* strain 8325 by spotting colony mass on pathogen-seeded agar [7]. Activity was assessed

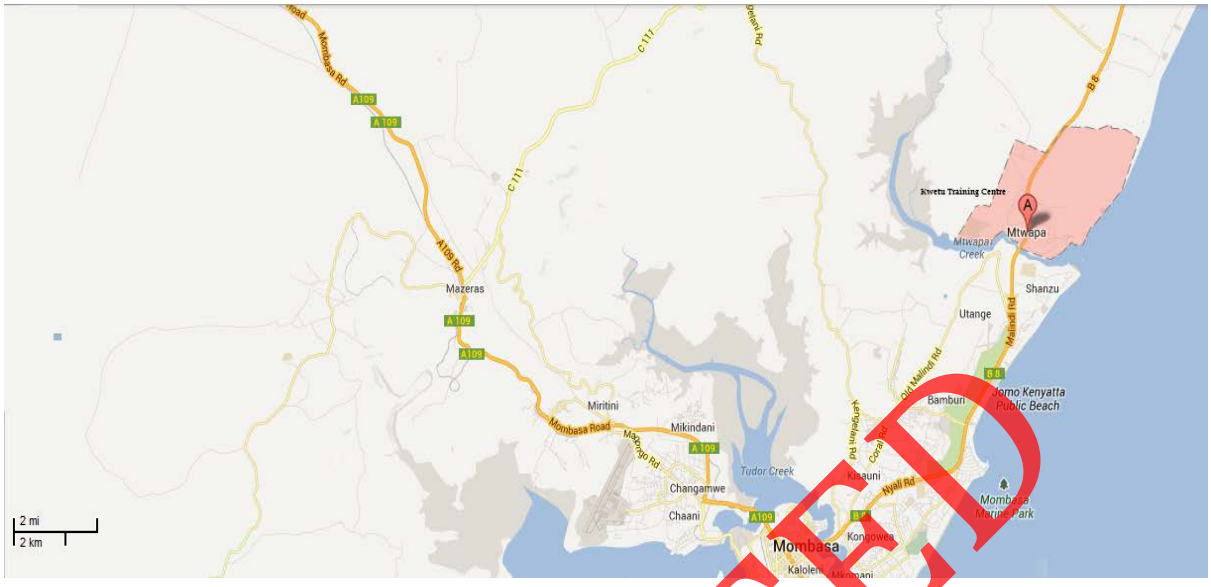

Figure 1. Map of Kenyan coast showing the study site. (Source: www.googlemaps.com).

by the formation of clearing zones around spotted colony mass. To identify the compounds responsible for the observed activity, large-scale cultivations and fractionations were undertaken for *V. coralliilyticus* S2052 and *P. halotolerans* S2753, representing two distant *Vibrionaceae* species with different metabolite profiles. All fractionation steps were guided by activity testing against *V. anguillarum* strain 90-11-287.

3. Results and Discussion

Bacterial strains inhibitory towards pathogenic bacteria were isolated from a marine fish pond culture facility in Mtwapa Creek, Kenya. They included Actinobacteria, the *Vibrionaceae* and *Pseudoalteromonas*. The potential of marine bacteria to produce antimicrobial compounds has been known for decades [13]-[15]. Subsequent studies have also shown that the marine environment indeed comprises a multitude of bacterial species producing bioactive metabolites [16] [17]. Twenty one strains were assigned to *Vibrionaceae* and were isolated based on their ability to antagonize the fish pathogen *Vibrio anguillarum* strain 90-11-287 [7]. Out of the 21 strains, only 12 retained antibacterial activity, being a small fraction compared to other antagonistic marine bacteria [18]. Eight strains causing pronounced inhibition (diameter of clearing zones larger than 10 mm) were retested using the same set-up, resulting in a subselection of 5 strains with reproducible strong antibacterial activity when spotted on pathogen-seeded agar. This subselection was inoculated in liquid cultures and extracted with ethyl acetate to determine if antibacterial compounds were extractable with organic solvent. Activity was seen in ethyl acetate extracts from five strains, which were selected for further analyses. Initial solid-phase extraction (E-SPE) indicated that andrimid (Rt = 10.02 min) could be responsible for the antibacterial activity. This compound, a hybrid nonribosomal peptide-polyketide antibiotic, was first described from an insect endosymbiont and later found in other microbial species including marine vibrios [9]. Andrimid acts as an acetyl-CoA carboxylase inhibitor [19], and we extended its broad antibiotic spectrum [20] by showing inhibition of the bacterial pathogens *Salmonella Enteritidis*, *Bacillus cereus*, *Yersinia enterolitica*, *Yersinia ruckeri*, *Vibrio harveyi*, and *Vibrio vulnificus*. Production of andrimid was also confirmed for the other isolated *V. coralliilyticus* strain, S4053. Our study is the first linking andrimid production to a specific *Vibrio* species, with production occurring in two strains isolated from the study site. The antibacterial compound of *P. halotolerans* S2753 was identified as holomycin (Rt = 1.70 min, a compound belonging to the pyrrothine class of antibiotics acting by interference with RNA synthesis [21]. Holomycin has until now only been found in Gram-positive *Streptomyces* [22], and the present study is the first demonstrating production of this antibiotic in a Gram-negative heterotrophic bacterium.

4. Conclusions and Recommendations

The present study adds to the knowledge of *Vibrionaceae* bioactivity and physiology by showing occurrence of

marine strains producing antibacterial compounds in marine pond culture system. The discovery of known antibiotics that are also produced by evolutionary distant microbes suggests an involvement of horizontal gene transfer, and indicates that these compounds are fundamental to compete and communicate in the natural habitat. The cosmopolitanism of identical antibiotics has major implications for natural product discovery strategies. An alternative approach could be the screening for largely untested bioactivities, for instance, interference with quorum sensing or modulation of gene expression.

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