Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:

Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia

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PEER REVIEW

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Comments

This is a good study in which the authors isolated and evaluated the actinomycetes which produce antibiotics from different freshwater habitats and tested against different Gram positive and Gram negative bacterial strains.
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ABSTRACT

Objective: To isolate, evaluate and characterize potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia. **Methods:** A total of 31 strains of actinomycetes were isolated and tested against Gram positive and Gram negative bacterial strains by primary screening. In the primary screening, 11 promising isolates were identified and subjected to solid state and submerged state fermentation methods to produce crude extracts. The fermented biomass was extracted by organic solvent extraction method and tested against bacterial strains by disc and agar well diffusion methods. The isolates were characterized by using morphological, physiological and biochemical methods. **Results:** The result obtained from agar well diffusion method was better than disc diffusion method. The crude extract showed higher inhibition zone against Gram positive bacteria than Gram negative bacteria. One-way analysis of variance confirmed most of the crude extracts were statistically significant at 95% confidence interval. The minimum inhibitory concentration and minimum bactericidal concentration of crude extracts were 1.65 mg/mL and 3.30 mg/mL against *Staphylococcus aureus*, and 1.84 mg/mL and 3.80 mg/mL against *Escherichia coli* respectively. The growth of aerial and substrate mycelium varied in different culture media used. Most of the isolates were able to hydrolysis starch and urea; able to survive at 5% concentration of sodium chloride; optimum temperature for their growth was 30 °C. **Conclusions:** The results of the present study revealed that freshwater actinomycetes of Lake Tana appear to have immense potential as a source of antibacterial compounds.

KEYWORDS

Actinomycetes, Solid state fermentation, Submerged state fermentation, Disc diffusion method, Agar well diffusion method, Crude extracts, Antibiotics, Antibacterial activity

1. Introduction

Actinomycetes are filamentous, antibiotics producing bacteria. They are found in freshwater and marine water habitats[1-3]. The dominant actinomycetes *Micromonospora* can be isolated from aquatic habitats such as streams, rivers, lake mud, river sediments, beach sands, sponge and marine sediments[4,5]. Several novel bioactive compounds were discovered from aquatic actinomycetes, for example rifamycin from *Micromonospora*[6]; salinosporamide-A, an anticancer metabolite from a *Salinispora* strain[7]; marinomycins from *Marinophilus* sp.[8]; abyssomicin-C from *Verrucosispora* sp. and marinopyrroles from *Streptomyces* sp[9,10].

Out of 22 500 biologically active compounds obtained from microbes, 45% are from actinomycetes, 38% from fungi and 17% from other bacteria[11]. Over 5 000 antibiotics have been identified from the culture of Gram positive, Gram negative bacteria and filamentous fungi[12]. Species of *Streptomyces*, account for more than 70% of the total antibiotic production and *Micromonospora* was less than one-tenth as many as *Streptomyces*[13]. Twenty seven actinomycetes were isolated from Mount Everest region soil samples and reported to have antibacterial activity against at least one tested bacteria among the two Gram positive and nine Gram negative bacteria[14]. Narendra Kumar *et al*. isolated 117 antibiotic

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Foundation Project: Supported by University of Gondar under Teaching and Learning Program (UoG/Budget code: 6417).

Article history:
Received 3 Feb 2013
Received in revised form 6 Feb, 2nd revised form 23 Feb, 3rd revised form 29 Feb 2013
Accepted 2 Apr 2013
Available online 28 Jun 2013

producing actinomycetes from non agricultural wasteland alkaline soils and compost rich garden soil in which most of the isolates inhibits Gram negative bacterial growth[15]. Four potential antibacterial actinomycetes were isolated from the aquatic environment[16]. Valli *et al*. isolated 21 potential actinomycetes from marine environment and reported that all the isolates were promising against at least one tested organisms[17]. Kalyani *et al*. isolated 20 species from marine soil samples in which three showed significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (*E. coli*)[18].

Antimicrobial drugs used for prophylactic or therapeutic purposes in human, veterinary and agricultural purposes were favoring the survival and spread of resistant organisms[19]. The appearances of multidrug resistant pathogenic strains caused substantial morbidity and mortality especially among the elderly and immuno-compromised patients. To overcome this situation, there is an interest to improve or discover novel class antibiotics that have different mechanisms of action worldwide[20,21]. The continuous screening of secondary microbial products produced from potential bacterial taxa was important to discover novel chemicals for the development of new therapeutic agents[22]. Recently, many scientists are searching new antibiotics from different untouched habitats to find out for their productions of antibiotics[23]. In Ethiopia, no significant studies have been conducted so far to isolate and evaluate actinomycetes from different freshwater habitats that could produce useful antibiotics. Therefore, present study is intended to isolate, screen and characterize antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia.

2. Materials and methods

2.1. Sampling area

The samples of water and sediments were collected from Lake Tana, Amhara regional state, Ethiopia. The sampling area was located at a latitude of 12 (12° 0′ 0 N) and a longitude of 37.33 (37° 19′ 60 E). The lake was the source of Blue Nile with a total surface area of 3 600 km^2, a volume of 28.00 km^3 and an average elevation of 1 911 m above the sea level[24].

2.2. Sample collection

Totally 12 water and 12 sediment samples were collected from Gorgora site of Lake Tana which was located at 65 km North West direction of Gondar town. The water samples were collected in 500 mL sterile screw capped bottles and sufficient space was provided for aeration and thorough mixing. The sediments were collected by sterilized spatula and transferred to wide mouth sterilized bottle. All samples were labeled and transported to Microbiology Laboratory, Department of Biology, University of Gondar and stored at 4 °C for further studies.

2.3. Pretreatment of samples

The samples were subjected to various physical and chemical pretreatment methods in order to facilitate isolation of actinomycetes[25]. The sediment samples were air dried; heated aseptically, which stimulates the isolation of actinomycetes by eliminating most unwanted Gram negative bacteria. Appropriate selective media such as starch casein agar, glycerol yeast extract agar and antifungal antibiotics (amphotericin B) at 25 μg/mL were used for actinomycetes growth promotion and also for prevention of fungal contamination[26,27].

2.4. Actinomycetes isolation and maintenance

Actinomycetes were isolated by serial dilution method from sediments[28]. Stock solution was prepared by diluting 1 g of sediment in 9 mL of sterile saline water and shaken well by using vortex mixer. From the stock solution, 1 mL was used to prepare the final volume of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} by serial dilution method. Finally, 0.1 mL of suspension from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were used to spread on starch-casein agar medium aseptically. In water samples, 1 mL of aliquot was taken and spread evenly over the sterilized starch casein agar plates by using L-shaped glass rod. For each sample three plates were used and incubated at 30 °C for 7 to 14 d. The plates were observed periodically for the growth of actinomycetes. The pure colonies were selected, isolated and maintained in starch casein agar slants at 4 °C for subsequent studies.

2.5. Bacterial strains

Pathogenic bacterial strains such as *E. coli* (ATCC2592), *Salmonella typhi* (ATCC9289) (*S. typhi*), *Staphylococcus aureus* (ATCC2923) (*S. aureus*), *Pseudomonas aeruginosa* (ATCC27853) (*P. aeruginosa*) and *Klebsiella pneumonia* (ATCC7000603) (*K. pneumonia*) were obtained from Gondar College of Medical Science, University of Gondar.

2.6. Preparation of 0.5 McFarland standard

McFarland standard was prepared by adding 0.5 mL of 0.048 mol/L $BaCl_2$ (1.17% w/v $BaCl_2 \cdot 2H_2O$) in to 99.5 mL of 0.18 mol/L H_2SO_4 (1% w/v) with constant stirring. The equal volume of standard solution was distributed into same sized screw capped test tubes. These test tubes were tightly sealed and stored at room temperature to prevent from evaporation and light. Before use, turbidity standard was vigorously agitated by using vortex mixer[29].