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Full Length Article

Comparative efficacy of macrolides containing marine actinomycetes formulation versus ciprofloxacin ophthalmic solution in controlling *Pseudomonas aeruginosa* induced conjunctivitis on rabbit model

Femina Wahaab, Kalidass Subramaniam *, Morris Jawahar

Department of Biotechnology, School of Biotechnology & Health Sciences, Karunya University, Coimbatore, India

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Abstract The main objective of this study was to evaluate the antimicrobial activity and anti-inflammatory activity of marine actinomycetes extract against ocular pathogen *Pseudomonas aeruginosa*. Actinomycetes isolated from Rameswaram coastal region, Tamilnadu, India were initially screened by primary screening and secondary screening against ocular pathogen *P. aeruginosa*. Followed by anti-conjunctivitis efficacy of actinomycetes ethyl acetate extract formulation versus ciprofloxacin ophthalmic solution was evaluated using rabbit as animal model. The bioactive compounds present in the best actinomycetes extract was identified by HPTLC and GC–MS analysis. Finally the screened best actinomycetes was identified by 16S rRNA sequencing method. In primary screening 28 actinomycetes that inhibited the growth of *P. aeruginosa* were taken for secondary screening. In secondary screening RAM24C2 extract had maximum activity against *P. aeruginosa*. *In vivo* study of conjunctivitis developed rabbits treated with RAM24C2 extract formulation showed the best clinical cure than ciprofloxacin ophthalmic solution. The RAM24C2 extract was chromatographically characterized and found to contain macrolides. In addition, the effective major pivotal molecule in the extract was detected as 1, 2 benzene dicarboxylic acid and Bis (2-ethylhexyl) phthalate by GC–MS analysis. The RAM24C2 strain was identified as *Streptomyces* sp. MAD01 and the sequence was submitted in NCBI with accession number **JX050218**. From our study it is found that the ethyl acetate extract obtained from marine actinomycetes is effective against ocular pathogen

* Corresponding author. Tel.: +91 0422 2614 499 (O), +91 9443022508; fax: +91 0422 2615 615.
E-mail addresses: feminabiotech@gmail.com (F. Wahaab), kalidass@karunya.edu, kallidass@gmail.com (K. Subramaniam), morrisjawahar@gmail.com (M. Jawahar).

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P. aeruginosa. Compared to ciprofloxacin ophthalmic solution our RAM24C2 extract formulation hastens the cure of conjunctivitis developed rabbits and need less dosage frequency.

1. Introduction

Conjunctivitis is a prevalent disease all over the world especially higher rate of infection was found in developing countries. Every people at least once in their lifetime gets affected by conjunctivitis. Conjunctivitis commonly described by red eye which shows symptoms like conjunctival congestion, eyelid edema, irritation in eye with discharge, sometimes in the morning difficult to open the eye due to sticking of the eyelid together by discharge. Conjunctiva is a mucous membrane which covers the bulbar conjunctiva and palpebral conjunctiva and safeguard the eye against invading microorganisms. Conjunctivitis caused by various external factors like bacteria, virus and fungi. Among these 80% of conjunctivitis occurred by bacteria [1]. Among the different bacteria *Pseudomonas aeruginosa* is one of the common pathogen causing conjunctivitis with severe infection including capable of producing different toxins and proteases thereby damages ocular tissues [2].

There are many topical antibiotics are available to cure for *P. aeruginosa* conjunctivitis. Fluoroquinolones like ciprofloxacin, ofloxacin, levofloxacin and aminoglycosides like tobramycin, gentamicin and some other antibiotics like polymyxin B and neomycin are used as ophthalmic eye drops in different concentration. These topical antibiotics possessed broad spectrum activity as antimicrobial agents. But the resistance problem of ophthalmic pathogens, side effects and expensive of antibiotics making the researchers to search for new antibiotics as ophthalmic solution. Especially *P. aeruginosa* highly resistant to most of the antibiotics [3,4].

In this regard here we have taken marine actinomycetes for our research purpose. Because marine actinomycetes reveals as source of natural products for human ailments. Marine actinomycetes exhibit different biological activity especially broad spectrum activity against gram positive and gram negative bacteria. Among marine actinomycetes, *Streptomyces* are the chief producer of novel antibiotics. Each day many new antibiotics are identified from actinomycetes. Among actinomycetes 80% of antibiotics were derived from *Streptomyces* family [5,6].

The present study was carried out to evaluate the biological activity (anti-conunctivitis) of actinomycetes isolated from marine coastal regions of Rameswaram, Tamilnadu, India. Their potential to control ocular pathogen *P. aeruginosa* both *in vitro* and *in vivo* animal model was analyzed. The efficacy of actinomycetes extract formulation, with available quinolone ophthalmic solution ciprofloxacin was also compared.

2. Materials and methods

2.1. Isolation and identification of Actinomycetes

Marine soil samples were collected from coastal regions of Rameswaram, Tamilnadu, India. Selective isolation of actinomycetes from soil samples was carried out by spread plate technique using Actinomycetes isolation agar medium. The isolation medium was supplemented with 0.2 mg/ml fluconazole [7] and 20 mg/l nalidixic [8] to inhibit fungal and bacterial colonization respectively. After incubation, actinomycetes colonies appeared on the agar medium were pure cultured by restreaking in the same agar medium and used for further assays.

2.2. Bacterial culture

The authenticated ophthalmic pathogen *P. aeruginosa* obtained from Aravind eye Hospital, Coimbatore, Tamilnadu, India. The pathogen was isolated form clinical specimen in Clinical Microbiology laboratory of the hospital.

2.3. Antibiotic susceptibility of the test organism

The antibiogram pattern of the ophthalmic pathogen *P. aeruginosa* was checked against the commercially available antibiotic discs (Himedia) using the standard disc diffusion test [9]. The log phase culture broth was seeded in Muller Hinton agar plate by sterile swab. The antibiotics discs with the following concentrations were placed in equidistant with uniform contact between the antibiotic discs and the surface of the Muller Hinton agar plates, Amoxyclav (30 mcg), Ceftazidime (30 mcg), Chloramphenicol (30 mcg), Gentamicin (10 mcg), Nalidixic acid (30 mcg), Nitrofurantoin (30 mcg), Norfloxacin (10 mcg), Tetracycline (30 mcg), Kanamycin (30 mcg), Imipenem (15 mcg), Ampicillin (15 mcg) and Ciprofloxacin (15 mcg). The plates were then incubated at 37 °C in upright position for overnight. Inhibition zone diameters were measured and tabulated.

2.4. Screening of actinomycetes for antimicrobial activity

The primary screening of all 131 actinomycetes isolates were done by streaking perpendicular to the ophthalmic pathogen *P. aeruginosa* with four isolates streaked parallel per petridishes. The zones of inhibition were measured and tabulated [10]. The actinomycetes isolates that which exhibited good antibiosis effect against *P. aeruginosa* were pure cultured respectively in culture flask. The culture was extracted by solvent extraction method [11] using ethyl acetate as solvent. The actinomycetes culture extracts were secondary screened for their antagonistic activity against ophthalmic pathogen. The secondary screening was done by well diffusion method.

2.5. Determination of stability and shelf life of RAM24C2 ethyl acetate extract

The best actinomycetes culture extract that had maximum antagonistic activity against ophthalmic pathogen was subjected for stability and shelf life test. The extract was aliquot