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Co-culturing of Marine and Terrestrial Actinomycetesto Obtain Novel Secondary Metabolites

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Abstract

Co-culturing of actinomycetes is a classical approach used to enhance bioactivity. In this study, co-culturing of terrestrial and marine actinomycetes was performed to obtain increased anticancer cytotoxic activity. Crude extract prepared from co-cultured isolates showed increased anticancer activity. Although normal and healthy actinomycetes filament was observed during co-culture, spore formation was suppressed greatly by co-culturing. Crude extract of co-culture isolates demonstrated the IC_{50} of 20 µg/ml, while the monoculture of isolates showed the IC_{50} of 40 µg/ml. Both the isolate were greatly diverse from each other, morphologically and biochemically. To the best of our knowledge, this is the first report on co-culture of marine and terrestrial actinomycetes enhance the bioactivity. During co culturing organisms compete each other for their survival rather individually. However, further studies are required to identify the variation in the composition of secondary metabolites produced by them during co-culturing.

Introduction

Co-culturing is a technique that is being used by microbiologists for various applications. Co-culturing of two bacteria could have either antagonistic or symbiotic or neutral association, and could result in formation of new by-products (secondary metabolites). Co-culturing of two marine actinomycetes, isolated from sponge, resulted in production of new secondary metabolites namely N-(2-hydroxyphenyl)-acetamide; 1,6-dihydroxyphenazine; and 5a,6,11a,12-tetrahydro-5a,11a-dimethyl [1,4] benzoxazino[3,2-b] [1,4] benzoxazine [1]. These three secondary metabolites were not produced by isolates, when cultured individually. I t suggest that, co-culturing of actinomycetes colonies, results in production of new secondary metabolites, due to the cross-link between the extra cellular enzymes and reactive by-products. It was recently reported that, co-culturing of marine actinomycetes with pathogenic bacteria, increased the antagonistic activity of the actinomycetes [2]. Uzzal, et al. [3] has analyzed 5 different marine actinomycetes isolates for their antibacterial activity, against *Escherichia coli* and *Staphlycoccus aureus*. Results of the study indicated that, the antibacterial activity of the marine isolates was considerably increased, when the same isolates were grown with the pathogenic bacteria. This proves that, co-culturing of actinomycetes with non-actinomycete bacteria would result in increased antagonistic activity of the actinomycete isolate. It was reported that, co-culturing of actinomycetes isolate with pathogenic bacteria, resulted in production of a modified antibiotic, with increased antibacterial activity [3]. *Streptomyces* strain FXJ2.014 produced quinomycin-A antibiotic during mono culture, but when the isolate was co-cultured with bacterial pathogen *Bacillus subtilis*, produced a modified qunimycin-A like molecule named as FXJ2.014-HB. It was reported that co-culturing of actinomycetes, might trigger the latent genes present in the actinomycete genome, to produce a modified or new antagonistic molecules.

Even though few reports are available on the impact of co culturing of actinomycetres in enhancing the bioactivity, further studies are needed to ascertain the changes in the genome especially in the biosynthetic gene clusters (BGCs) need to be studies. In order to address this issue, a study was undertaken to co-culture terrestrial actinomycete and marine actinomycete isolates together to obtain new and effective anticancer cytotoxic secondary metabolites.

Results

Morphology of co-culturing

Terrestrial and marine isolates were inoculated onto International Streptomyces Project No.1 (ISP.No.1) agar media. Morphological appearance of the isolates on 7th day is shown in Figure 1.

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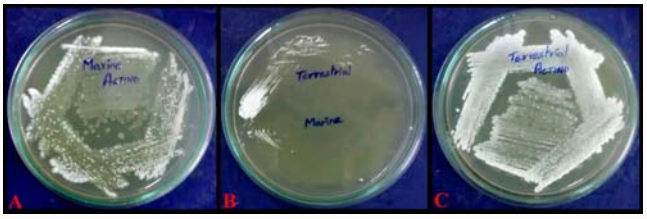

Figure 1: Morphological appearance of marine and terrestri alactinomycete isolates. A) Marine actinomycete; B) Co-cultured isolates; C) Terrestrial actinomycete.

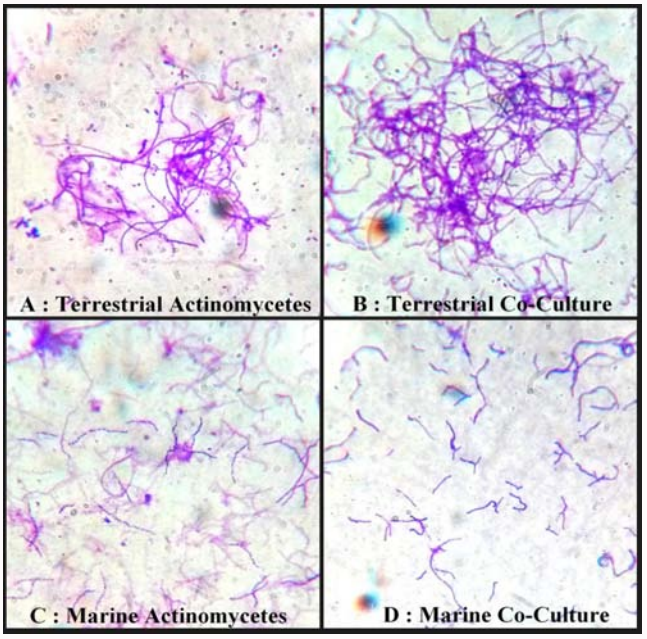

Figure 2: Gram's staining of isolates observed under light microscope (100 X magnification). A) Terrestrial actinomycete; B) Terrestrial actinomycetes in co-culture; C) Marine actinomycete; D) Marine actinomycete in co-culture).

As observed, in the co-culture, aerial mycelium was not white in colour, as it was observed in the individual inoculum. Further, the isolates were subjected for Gram's staining under the monoculture and co-culture condition. The results of the Gram's staining are shown in Figure 2. It was observed that, the isolates had normal cell morphology in both monoculture and co-culture condition, but the spore production was greatly suppressed in co-culture condition. Both the marine and terrestrial isolate, demonstrated retarded spore production, indicating the slow maturation of the isolates.

Anticancer activity

Crude chloroform extract prepared from the isolates was extracted and tested for its anticancer cytotoxic activity. The co-

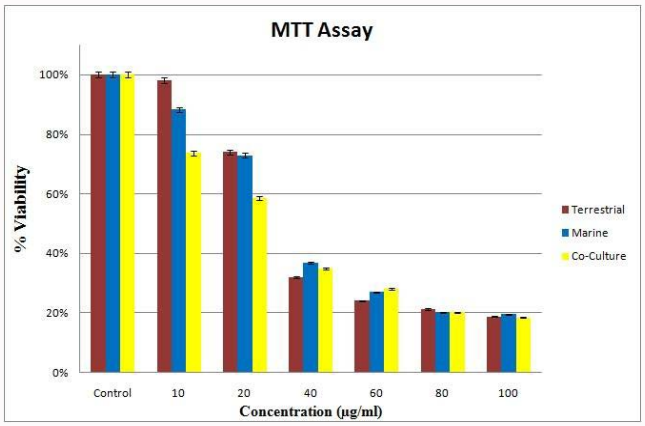

Figure 3: Anticancer cytotoxic activity of crude extract (Terrestrial; Marine; Co-culture).

cultured extract demonstrated an IC_{50} value of 20 µg/ml, while the monoculture of marine and terrestrial isolates, showed IC_{50} of 40 µg/ml (Figure 3). Suggesting that, the anticancer activity of the isolates was increased due to co-culturing indicated that new metabolite or modified secondary metabolite might be produced by the isolates during co-culture.

Co-culturing of terrestrial actinomycetes with marine actinomycetes which differ with their environmental origins is a new idea to enhance the bioactivity of potential actinomycetes. This idea could also be used to increase the production of useful bioactive products from actinomycetes. However a detailed genomic study would certainly help us to understand the influence of co-culturing on BGCs.

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