PRODUCTION OF BIOACTIVE COMPOUNDS USING MARINE ISOLATES IN CO-CULTURING SYSTEMS

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Abstract— Co-culture is the simultaneous cultivation of two or more species of microorganisms in the same medium. Routine laboratory procedures practice culture of a single microorganism wherein only a fraction of the total genes are expressed. Co-cultivation of two or more different microbes tries to resemble the natural environment in which these organisms originally grow. Competition between microbes is induced deliberately and stressful conditions arise leading to enhanced production of compounds produced in pure cultures or production of novel compounds that are not detected in pure cultures. Present study deals with the production of bioactive compounds in co-culture of marine microorganisms. These compounds were purified and further screened for antimicrobial activity against multiple drug-resistant micro-organisms.

***Index Terms*—Bioactive compounds, co-culture, marine, multiple drug-resistant micro-organisms.**

I. INTRODUCTION

The marine environment covers almost 70% of the earth surface. Marine water bodies are a rich source of microorganisms which include a variety of fungus, bacteria, actinomycetes, etc. and these organisms represent a novel source of new bioactive compounds. Marine organisms are a potent source for new biologically active secondary metabolites. Marine-derived fungi and bacteria from various coasts have been isolated, characterised and exploited for the production of various drugs.

Co-culture systems have been used to study the interactions between cell populations and are fundamental to cell–cell interaction studies of any kind. A co-culture is a cell cultivation set-up, in which two or more different populations of cells are grown with some degree of contact between them. These techniques find myriad applications in biology for studying natural or synthetic interactions between cell populations. The main reason for conducting co-culture experiments and motivation for using such a set-up include: (1) studying natural interactions between populations, (2) improving cultivation success for certain populations, (3) establishing synthetic interactions between populations [4]. The importance of this study is to compare the rate of antibiotic production by the bacteria solely and when it is co-inoculated with another bacterium. Quorum sensing forms the basis for cell induced antibiotic production. Bacterial cells have the ability to show cell to cell communication in presence of another bacteria with their autoinducers. This allows the bacteria to sense a critical cell mass and in response activate or repress target genes [5].

Marine microorganisms are a major source for Marine Microbial Natural Products (MMNP) discovery [3]. Co-cultivation is also one of the techniques used for activation of the silent genes for the production of new compounds. Growing or cultivating of two or more microorganisms in the same broth is called co-cultivation, also referred to as "mixed fermentation". The present study deals with co-cultivation of marine microbial isolates. The extraction of bioactive compounds and further screening for antimicrobial activity was attempted against multiple drug-resistant micro-organisms isolated from clinical samples resistant against commonly used antibiotics.

II. MATERIALS AND METHODS

Marine microbial strains and media

The marine microbial strains used in this study are as follows: *Aspergillus fumigatus* (NCIM902), *Bacillus pumilus* (NCIM2327), *Candida albicans* (NCIM3100) and *Rhodococcus* sp. (NCIM5452). Strains were obtained from National Collection of Industrial Microorganisms (NCIM). *Bacillus* and *Rhodococcus* strains were cultured in nutrient broth (as suggested by NCIM) at 37°C while *Aspergillus* and *Candida* strains were cultured in yeast-malt extract broth at 37°C.

Test organisms

To test the antibiotic activity, clinical cultures of drug resistant strains, Methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeroginosa* (resistant to commonly used antibiotics) and MDR *E. coli,* isolated from clinical samples were used.

Pre-tests for microbial inhibition

To test the inhibition of one organism due to the bioactive compounds produced by another, cross streak method was used. Nutrient agar plates and yeast-malt extract agar plates were prepared. On these plates, one microorganism was streaked horizontally, while the other was streaked from the edge of the plate perpendicular to the first streak.

Combinations of organisms used for co-culture:

1. *Aspergillus fumigatus* – *Rhodococcus* sp. on nutrient broth agar plate
2. *Bacillus pumilus* - *Rhodococcus* sp. on nutrient broth agar plate
3. *Aspergillus fumigatus* - *Candida albicans* on yeast malt extract broth agar plate

Inhibition zones were observed at the intersection of two streaks.

Co-culture

The above mentioned combinations of microorganisms were grown in a medium to find out the production of antibiotic compound. Totally, three set of cultures of each combination were maintained as follows:

A. Live cells of 1st and 2nd strains
   In this, 10ml of 24 hours old broth cultures of both strains were added to the 100 ml of respective broths.
B. Live cells of 2nd strain alone (control)
   In culture system B, 10ml of 24 hours old culture of 1st strain alone was inoculated.
C. Live cells of 1st strain alone (control)
   In culture system C, 10ml of 24 hours old 2nd strain alone was inoculated.

All the cultures were incubated at 37°C for 5 days. After the incubation period, the cultures were centrifuged at 2500 rpm for 20 minutes. The supernatant was collected and subjected to antibacterial assay with multiple drug resistant test strains.

Screening for antibiotic activity

Antibiotic activity was assayed using a standard agar well diffusion method [2]. Nine test tubes were prepared each containing 1ml of LB. After autoclaving, the pathogenic strains were inoculated in it. Nutrient agar plates were flooded with test strains. Wells were created using a cork borer on plates and the supernatants of co-culture experiments were introduced into the wells. The plates were then incubated for 24 hours and the inhibition zones were observed.

Totally, three sets of plates were maintained for each combination. Each plate in a set was flooded with different test strains. All plates contained three wells which included two supernatants from control flasks and one supernatant from co-culture flask.

Agar Well Diffusion:

Following groups were made and each group was tested against the test organisms:

Group I: In yeast-malt extract broth

1. *Aspergillus fumigatus* (control)

2. *Candida albicans* (control)
3. *Aspergillus* + *Rhodococcus* (Co-culture supernatant)

Group II: In nutrient broth

1. *Aspergillus fumigatus* (control)
2. *Candida albicans* (control)
3. *Aspergillus* + *Rhodococcus* (Co-culture supernatant)

Group III: In nutrient broth

1. *Bacillus pumilus* (control)
2. *Rhodococcus sp.* (control)
3. *Bacillus* + *Rhodococcus* (Co-culture supernatant)

These three groups were tested on the following organisms:

1. MDR *E. coli*
2. Methicillin resistant *Staphylococcus aureus* (MRSA)
3. *Pseudomonas aeroginosa* (resistant to commonly used antibiotics)

III. RESULTS

Pre-tests for microbial inhibition- Cross streak method

The tests showed that *C. albicans* completely inhibits *A. fumigatus* (Fig. 1{a}). Cross streak analysis for *B. pumilus – Rhodococcus* sp. combination showed that both organisms can grow together (Fig. 1{b}) while in *A. fumigatus – Rhodococcus* sp. combination, *Rhodococcus* sp. strongly (but not completely) inhibits *A. fumigatus* (Fig. 1{c}). Hence, the latter two combinations were selected for co-culture studies.

Screening for antibiotic activity

Antibiotic activity was studied using agar well diffusion method. In all cases, supernatants sfrom co-culture showed higher antibiotic activity than those from individual controls which proved that co culturing would help bring out higher antibiotic activity (Fig. 2). Supernatant from *A. fumigatus – Rhodococcus* sp. co-culture maximally inhibited MDR *E.coli* while supernatant from *B. pumilus – Rhodococcus* sp. co-culture maximally inhibited MDR *E.coli* and methicillin resistant *Staphylococcus aureus* (Fig. 3).

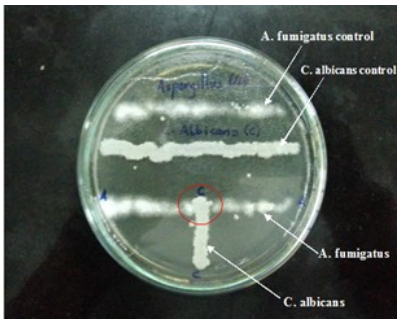
IV. CONCLUSION

Co-cultivation is one of the techniques used for activation of the silent genes for the production of new compounds [1].

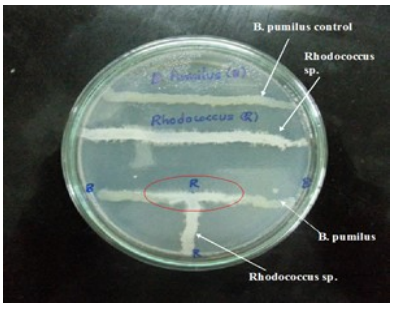
An effort was made to co-cultivate marine derived fungi and bacteria and isolation of crude bioactive compounds capable of acting on clinically resistant strains of infectious organisms.

Co-cultivation (also called mixed fermentation) of two or more different microorganisms tries to mimic the ecological situation where microorganisms always co-exist within complex microbial communities. The competition or antagonism experienced during co-cultivation is shown to lead to an enhanced production of constitutively present compounds and/or to an accumulation of cryptic compounds.

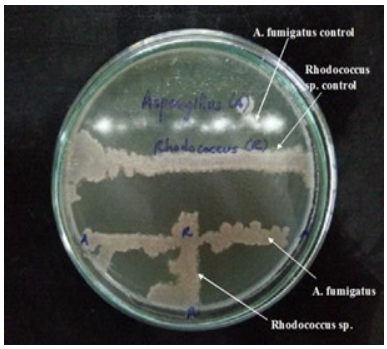
The present study provides a platform for further studies of interaction between marine bacteria and the exploration of their antibiotic property towards MDR bacteria.



(a) *A.fumigatus - C.albicans*

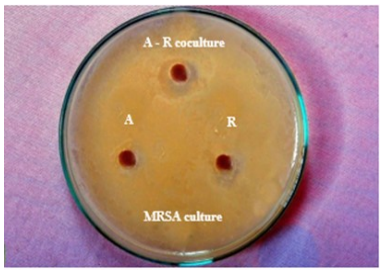


(b) *B.pumilus – Rhodococcus* sp.

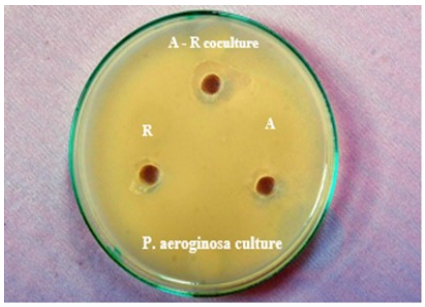


(c) *A.fumigatus – Rhodococcus* sp.

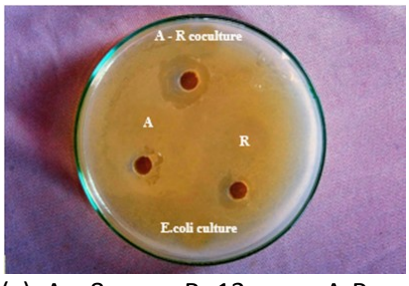
Fig. 1: Inhibition tests by cross streak method



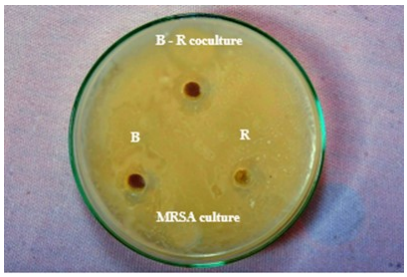
(a) A= 9 mm, R=8 mm, A-R co-culture= 14 mm



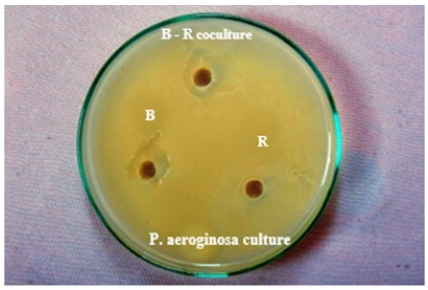
(b) A= 10 mm, R=9 mm, A-R co-culture= 14 mm



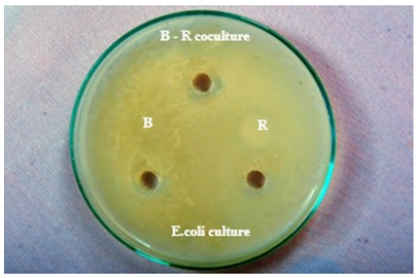
(c) A= 8 mm, R=13 mm, A-R co-culture= 20 mm



(d) B= 11 mm, R=8 mm, B-R co-culture= 15 mm

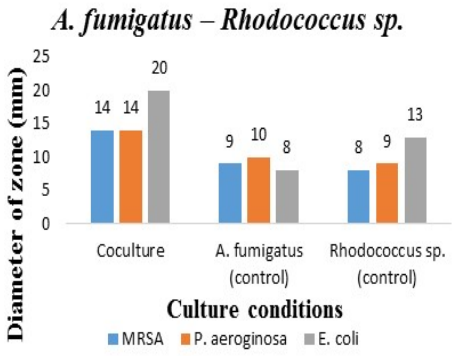


(e) B= 12 mm, R=8 mm, B-R co-culture= 14 mm



(f) B= 10 mm, R=0 mm, B-R co-culture= 15 mm

Fig. 2: Screening for antimicrobial activity using agar well diffusion method and the inhibition diameters (A- *A.fumigatus*, B- *B.pumilus,* R- *Rhodococcus* sp.)



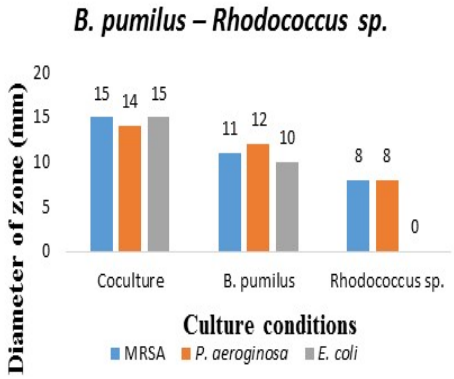


Fig. 3: Effect of culture conditions on production of antibiotic activity and tested against multiple drug resistant organisms.

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