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Review Article

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Cocultivation-Powerful tool for the production of secondary metabolites

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ABSTRACT

Natural products produced by microorganisms have been considered the major resources for drug discovery. However, the production of new and active metabolites becomes difficult and getting gradually higher. Co-culture is one of the best methods for enhancing the production of cryptic metabolites by de silence the biosynthetic pathways. Microbial competition for resources like nutrients and space is proven to be the driving force for the production of bioactive secondary metabolites. It can create a competitive environment for limited space and nutrients may trigger the unexpressed pathways which result in the production of bioactive metabolites due to interspecies interaction. This review focuses on the production of different metabolites from bacteria and fungi by co-culture approach.

Key words: Interspecies interaction, silent genes, co-culture, natural products, biofilm

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INTRODUCTION

Natural products are the predominant source for drug discovery. Microorganisms have been produced more than 22,000 bioactive compounds. But determination of new and promising metabolites becomes difficult. Various approaches have been reported to induce the production of bioactive secondary metabolites[35]. In laboratory condition, biosynthetic pathways which produce secondary metabolites may remain silent. Several methods have been taken to express these silent biosynthetic pathways which results in the production of cryptic metabolites[31].One of the best and effective methods for enhancing method for enhancing the production of cryptic metabolites is co-culture [23]. In nature interspecies interaction is often exhibited by microorganisms. Competitive environment for space and nutrients results in interspecies interaction which leads to the production of secondary metabolites to enhance their own growth [5]. Due to chemical defense mechanism or microbial cross talk, biosynthetic genes are triggered when two different microbial strains are cultured together in one culture vessel [25]. Microbial competition for space and resources is proven to be the major driving force for the production of secondary metabolites[21].Under laboratory condition, two organisms can be co-cultured for the production of secondary metabolites that has higher benefits than pure or mono culture. Structurally unique and unusual bioactive metabolites have been produced such as lipoamino peptides, diketopiperazine and cytotoxic N- formyl alkaloids [15].

The co-culture method has been used to study various phenomenons such as 1.elucidation of symbiosis phenomena 2.natural communities' investigation in agriculture 3. Human micro biome interaction 4. Induction of pharmaceutical bioactive compounds 5.induced production of yield specific products[3].Many studies have been described about the application and advantages of co-culture such as increased yield of industrial products, production of useful consumer goods and the discovery of novel bioactive secondary metabolites.

MICROBIAL INTERACTIONS

Microbial interactions or Interspecies interactions are omnipresent that can be found in ecology [3]. Due competitive environment for nutrients and space organisms involve in interspecies interactions which results in the formation of secondary metabolites to enhance their own growth and to prevent from other organisms. Cell to cell communication can be occurred through chemical signaling molecules such as auto inducer or biofilm formations [2].

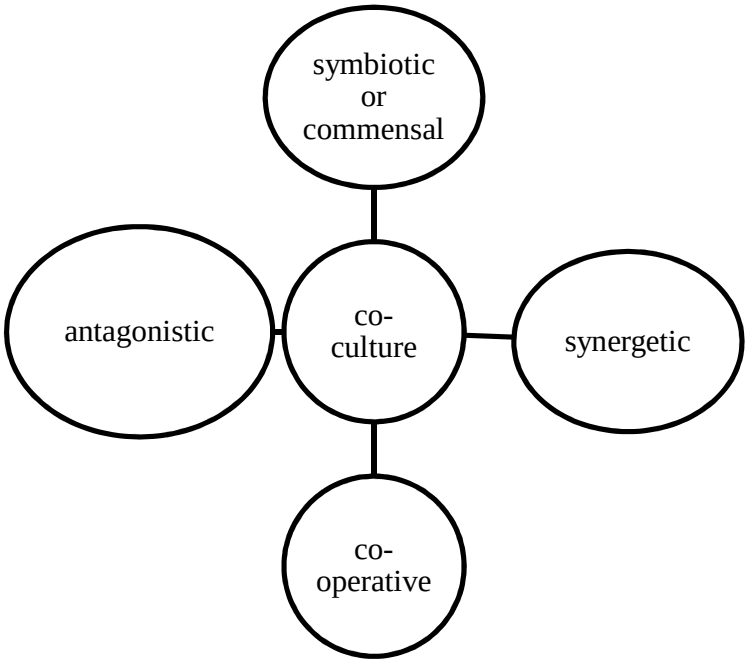

Figure 1: Types of interactions in co-culture [9]

BACTERIAL FUNGAL INTERACTIONS IN CO-CULTURE

Bacteria and fungi are the predominant source in secondary metabolite production[27]. The interaction between bacteria and fungi leads to the production of many secondary metabolites. The first antibiotic penicillin was discovered from the interaction of bacteria and fungi [26].The physical interaction between bacteria and fungi is mainly based on biofilm formation. The bacterial and fungal biofilm can be existed as mixed complex or fungi can be provided biotic support for the establishment of bacterial biofilm [11]. Different types of bacterial fungal interactions involved in secondary metabolites production such as 1. Interaction based on signaling 2.Antibiosis interaction 3. Cooperative metabolism interaction 4.Chemotaxis and cellular contacts [9].

The co-culture of *Aspergillus fumigatus* and *Streptomyces bullii* can produce the Ergo sterol one and seven metabolites belong to the family of diketopiperazine. None of these compounds were produced when these two microorganisms cultured separately. Ergo sterol 1 and seven metabolites showed antibacterial and antiprotozoal activity against *S.aureus, E.coli, Trypanosomabrucei brucei and Leishmania donovani* [25].In similar approach, Co-cultivation of *Aspergillus fumigatus* KMC-901 and *Sphingomonas sp*. KMK-001 produced a new Diketopiperazine, glionitrin B. The suppression of D0145 cell invasion can be caused by glionitrin B [24].

The cytotoxic activity of Libertellenones A-D (diterpenoid metabolites) against HCT-116 human adenocarcinoma cell line. Libertellenones were produced by marine derived fungus *Libertellasp* when it co-cultured with marine alpha proteo bacterium [21].The isolation of endophytic fungus *Fusarium tricinctum* was done and co-cultured with bacterial strain of *Bacillus subtilis* for the induced production of secondary metabolites included lateropyrone(5), enniatin type cyclic depsipeptides (6-8) and the lipopeptide fusaristatin A(9). These compounds shown antibacterial activity against *Enterococcus faeclis, Streptococcus pneumonia* and *Staphylococcus aureus* [22].

In similar approach, induced the production of Emericellmides A and B by the co-culture of marine derived fungus *Emericella sp* and *Salinispora arenicola* was discovered. Emericellamides A and B showed antibacterial activity

against *Staphylococcus aureus* which is methicillin resistant [20].The production N-formyl alkaloids from the co-culture of *Aspergillus fumigatus* and *Streptomyces peucetius* was reported andcytotoxic activity of N-formyl alkaloids were carried out on NCI-60 cell line and it has shown significant activity against cell line [35].

The antibiotic activity in the co-culture of *Bacillus subtilis* and *Rhizopus peka* were expressed both in pure culture and co-culture in which co-culture shown higher activity than mono culture. The production of antibiotic from *Rhizopus peka* can be influenced in the presence *Bacillus subtilis* [10].

BACTERIAL INTERACTION IN CO-CULTURE

Marine bacteria have been produced many bioactive secondary metabolites [30]. The competition for resources like nutrients and space leads to the production of metabolites by the help of microbial interactions. Bacterial communications occurs by chemical signaling molecules such as auto inducers [32]. It has proven to be an abundant source for the production of structurally distinctive bioactive secondary metabolites [29].

Co-cultivation of *Actinokineospora sp.* EG 49 and *Nocardiopsis sp*.RV163 induced the production of known ten compounds such as angucycline, diketopiperazine and beta carboline derivatives (1-10) and also induced the production of three natural products N-(2-hydroxyphenyl)-acetamide(11)1,6-dihydroxyphenazine (12) and 5a, 6, 11a, 12-tetrahydro-5a,11a-dimethyl[1,4]benzoxazino[3,2-b][1,4]benzoxazine(13a).Among these, only phenazine(12) was shown activity against *Trypanosomabrucei and bacillus sp* P25 also against *Actinokineospora sp*.EG49 [6].

The co-culture system between *Streptomyces sp.* and *Tsukamurella pulmonis* for antibiotic production. Antibiotic A33853 and aclacynomycin were produced by *S.albogriseolus* S430 and *S.panayensis* TT1712 when co-cultured with *T.pulmonis* [12].Ina similar approach, the cross species interaction of *Streptomyces padanus* and *Rhococcus facians* resulted in the production of rhodostreptomycin A(1) and B (2), two isomers of a new class of aminoglycosides. These antibiotic exhibited activities against *H.pylori*. None of them produced activity against *S.cerevisiae* and human leukemia cell (HL-60)[16]. Similar to this work, isolation of four marine epibiotic bacteria *Bacillus sp*.S3, *B.licheniformis* D1,*B.pumilus* S8, and *Serratia marcescens4* were carried out andco-cultured with pathogens and bio fouling strains. It enhanced the antifungal activity against *Y.lipolytica* and antibacterial activity against *P.aeruginosa* [8].

Indole and diketopiperazine were obtained from competitive induction between Bacillus isolate (UA-094) and bacillus strain (UA-089) [30]. In further study, the cell to cell communication between *Halobacillus salinus* and *Vibrio harveyi* which leads to the production of two phenethylamide metabolites. These nontoxic metabolites can be acted as antagonist for bacterium quorum sensing molecules with N-acyl homoserine [29].

The microbial competition between *Streptomyces tenjimariesis* with different bacterial isolates can induce the production of istamycin. Among 53 bacterial isolates only 12 bacterial species induced the istamycin production in *S.tenjimariensis* [28]. Alchivemycin A was produced from the co-culture of *T.pulmonis* which is mycolic acid containing bacteria and *Streptomyces endus* S-522. Mycolic acid presents in the outer layer of the bacterium induced the natural product biosynthesis in *Streptomyces endus* S-522[23].

FUNGAL INTERACTION IN CO-CULTURE

Fungi are prodigious sources for the production of natural products [5]. Antibiotics like penicillin, cyclosporine were produced by fungi which have distinctive and unusual pathways for the production of secondary metabolites[14]. In a competitive environment fungi will synthesis enzymes and secondary metabolites for enhancing their own growth. Interconnecting network of fungi can be formed as multicellular filaments (mycelia). Two mycelia can interact with each other in various ways like neutralistic and mutualistic which leads to the formation of bioactive compounds [3]. The genes which are responsible for the production of secondary metabolites are formed as clusters and remain silent in fungi. These genes can be expressed by fungal interactions [4].

When two different fungi *Fusarium tricintum* and *Fusarium begonia* were co-cultured, two linear depsipeptides, subenniatins A and B(1-2) were found. These compounds were allowed for antibiotic activity against *Pseudomonas aeruginosa, E.coli* and *S.Aureus* . But none of them were active at the concentration of 64 μg/ml [31].In another approach, the interaction of two marine derived fungal strains *Penicillium sp* and *Trichoderma sp* for the production of two new polyketides 1 and 2. These polketides were undergone for ESI-MS [15].

In further study, the production of new alkaloid aspergicin and known compounds ergosterol and neoaspergillic acid by two mangrove derived epiphytic fungi in mixed fermentation. These compounds shown activity against *B.dysenteriae, E.coli, S.aureus B.subtilis S.epidermis* and *B.proteus*,[34]. The production of novel xanthone derivative by the co-culture of two epiphytic unidentified mangrove fungi. Xanthone derivative antifungal activity was obtained against *gloeosporium musae* and *peronophthora cichoralearum* [17].The new lipoamino peptides and acremostatin A, B and C were produced by the co cultivation of *Acremonium sp* Tbp-5 and *Mycogone rosea* DSM [7].

INTERACTION OF OTHER ORGANISMS IN COCULTURE

The co-culture of *Saliva miltiorrhiza bunge* (lamiacee) and *Bacillus cereus* bacteria which induced the production of tanshinone by 12 folds and yield of tanshinone was also increased from 1.40mg/l to 10.4 mg/l [33].The co cultivation of the suspension cells of *Taxus chinensis var.nairei* and endophytic fungi *Fusarium mairei* was produced paclitaxel which is 38 fold higher than mono culture. The productivity of paclitaxel was 25.63mg/l in co-culture [18].Co cultivation of *Haliotis discus hannai* and sea cucumber *stichopus japonicas* can reduce the inorganic nitrogen in water and enhance their growth [13].

In further study, seventy five marine bacterial strains associated with four sponges(*Mycale mannarensis*, *Spongia sp, Echinodictyum sp* and *Sigmadocia fibulatus*) were isolated for antibiotic production against *Bacillus subtils , E.coli, Vibrio harveyi* and fungal strain of *Candida albicans*. Among these, 20% of strains were antibiotic producers with activity from broad to species specific [1].

CONCLUSION

The studies reported in this review clearly described that the co-culture is an effective method for the production of secondary metabolites by bacteria and fungi. Co-cultivation could be leads for the biomedical research. This promising method can be used for the induction of novel structures and bioactive compounds in the field of drug discovery. But the interspecies interaction of microbes is complex and still clearly unknown. The clear expression of silent pathways due to cross species interaction and chemical defense mechanism are needed to be found in the future.

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