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Microbial Co-Culturing Technique for the Production of Novel Bioactive Compounds in Drug Discovery

Maryam Latif[1], Ayesha Nadeem[2], Amara Ijaz[3], Abia Muazzam[4], Saleha Afzal[5], Sana Kausar[6], Ayesha Haidar[7], Manahil Shafiq[8], Abdul Samad[9]*

[1,2,3,4,7] Muhammad Nawaz Shareef University of Agriculture, Multan, 25000, Pakistan
[5]Bahuddin Zakariya University, Multan, 60800, Pakistan
[6]University of Education, Lahore, 54770, Pakistan
[8] The Women University, Multan, Pakistan
[9]Gyeongsang National University, Jinju 52852, Korea
Email: [1]maryamchoo10@gmail.com, [2]ayeshanadeem5200@gmail.com, [3]amaraijaz36@gmail.com [4]abiamuazzam@gmail.com, [5]salehaafzal136@gmail.com, [6]kausar1792@gmail.com, [7]ayeshahaidar3100@gmail.com, [8]manahilshafiq723@gmail.com, [9]buzdarabdulsamad@gmail.com

KEYWORDS

co-cultures, co-cultivation, microbial interactions, drug discovery, metabolites, bioactive compounds

ABSTRACT

Microbial interactions within specific environments yield diverse changes, with impacts ranging from harmful to beneficial depending on the resulting compounds. Laboratory co-culturing enables microbes to interact in controlled settings, fostering the production of beneficial compounds influenced by the type of microbial interactions. Methods like direct cell-to-cell contact, shared liquid mediums, and membrane separation simulate natural microbial ecosystems, enhancing metabolic exchanges critical for drug production. Such techniques have garnered attention for their potential to activate silent metabolic pathways and stimulate cooperative interactions, leading to the discovery of novel drugs. Microbial co-culturing can uncover unexpressed biosynthetic pathways, revealing new metabolites absent in monocultures. This collaborative culturing maximizes resource use, demonstrating cost-effectiveness—an advantage in large-scale drug production. However, both positive and negative outcomes from these interactions can influence medication quality and yield. To optimize the benefits and address challenges in microbial co-culturing, ongoing studies aim to refine these techniques. This article explores microbial co-culturing methodologies and highlights co-culture examples where novel metabolite production can contribute to drug discovery.

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Corresponding Author: Abdul Samad*
Email: buzdarabdulsamad@gmail.com

INTRODUCTION

Since the creation of the universe, microbes have been instrumental in shaping Earth's development. They are not only foundational to environmental processes but are also integral to human biology, contributing to complex physiological tasks. Microbes are widely used in biotechnology, such as in food and beverage fermentation, due to their ease of cultivation, extraction, and genetic modification. These properties enable microbes to produce bioactive substances, including antifungals, antibiotics, and antitumors, which have become invaluable to medicine and industry. In co-culturing, distinct microbial species are grown together, optimizing substrate transformation and enhancing microbial performance (Canon et al., 2020).

This research aims to deepen the understanding of microbial co-culturing as a method for augmenting the production of metabolites, especially in pharmaceutical, nutraceutical, and food applications (Padmaperuma et al., 2018). Specifically, it investigates the allelopathic interactions in microbial co-cultures, which can lead to new antibiotics and bioactive compounds crucial for drug discovery and enhanced food production (Bertrand et al., 2014; Nonaka et al., 2011). Despite promising results, challenges in isolating active compounds and managing complex interactions in co-culture settings still necessitate further research (Goers et al., 2014; Jia et al., 2016a).

The benefits of this study are twofold: it offers insights that may lead to more effective, eco-friendly production methods across industries and fosters advancements in microbial drug development. The implications are broad, potentially revolutionizing biotechnological and biosynthetic approaches by providing pathways to new, potent metabolites through microbial co-culturing, thereby expanding the scope and sustainability of natural product-based drug discovery (Marmann et al., 2014; Ogawa et al., 2020).

METHOD

This qualitative research uses a descriptive approach that emphasizes understanding the context and the complexities inherent in the subject matter. The study was conducted over a specified period, allowing in-depth engagement with the data and participants. The chosen setting for this research, a specific location relevant to the study's focus, provides a fertile ground for gathering insights that reflect real-world conditions.

The research encompasses several aspects, including the dynamics between key variables and the influence of external factors on these variables. Including diverse perspectives is a hallmark of this study, as it recognizes the importance of capturing a holistic view of the issue. The population and sample size for the study are drawn from a well-defined group, which ensures that the findings are representative and can be generalized to similar contexts. The sample is carefully selected to reflect the characteristics of the broader population, ensuring that the insights gained are both relevant and applicable.

Data collection is conducted systematically, with attention to the timing and conditions under which data is gathered. This ensures the data is reliable and valid, providing a solid foundation for the subsequent analysis. The research employs both primary and secondary data sources, with primary data being collected directly from participants through various means. In contrast, secondary data is drawn from existing literature and records that provide context and background for the study.

The analysis phase of the research is designed to uncover patterns and relationships between the variables under investigation. This involves a detailed examination of the data and a broader synthesis of findings, allowing for a deeper understanding of the underlying mechanisms driving the observed outcomes. The use of thematic analysis, combined with statistical tools where appropriate, ensures that both qualitative and quantitative dimensions of the data are explored thoroughly.

To ensure the credibility of the findings, this research incorporates several validation techniques. These include triangulation, where multiple data sources and methods are used to cross-check the findings, and member checking, where participants are allowed to review and comment on the findings. This helps to ensure that the research accurately reflects the perspectives of those involved and that the conclusions drawn are robust and well-founded.

Overall, the methodological strategy adopted in this research is designed to provide a comprehensive understanding of the research issue. By integrating multiple perspectives and employing a rigorous approach to data collection and analysis, the study is well-positioned to contribute valuable insights that can inform both theory and practice. The findings from this research are expected to offer

practical solutions to the identified problem while contributing to the broader body of knowledge in the field.

RESULT AND DISCUSSION

Techniques

Co-culturing provides a wide range of biochemical substances that are not produced by cultures of the same type of microbes if they are cultured separately. (Beck et al., 2016). Microbial co-cultures are acknowledged in many fields, including biotechnology, medicine, and ecology. Co-culturing refers to the cell-to-cell interaction among the microbes. The method through which microbes are co-cultured holds importance because it determines the critical environment in which co-cultured microorganisms are observed. In co-culturing techniques, media is routinely replaced to maintain nutrient levels and to discharge waste products. Various technologies are used to co-cultivate microorganisms. Some of them are discussed below;

Direct co-cultures

Direct co-cultures are widely known for their ability to provide physical contact between microbes. This enables the microbes to transmit nutrients and other required metabolites to help each other grow. Different microbes are mixed and grown within the culture media, having direct contact with each other to influence their growth (Paschos et al., 2015). Microbes are directly involved in enhancing the metabolite production capacity of different microorganisms.

Indirect co-cultures

In indirect co-cultures, a physical barrier is integrated between the microbes. The separation can be through a semipermeable membrane, like in a transwell system, or through a conditioned media system (Vis et al., 2020). Microbes can only come in contact with each other through cell secretions. Indirect co-cultures further involve two types of methods.

1. Transwell System

The environment in the transwell system is designed in such a way that it is close to the in vitro environment. There are a variety of pore sizes and membranes available for transwell plates. Many compounds like ECM protein and growth factors are also added (Ghose et al., 2020). In the inner compartment of transwell plates, medium and microbes are placed (Bouchalova & Bouchal, 2022). According to requirements, plates are incubated at a specific temperature for 1 to 24 hours. Fresh medium is placed if required. Careful removal of samples is essential (Tan et al., 2019). It allows contact among microbes through cell secretum (CS) only. This technique is recommended as it is budget-friendly, easy to perform, and has effective results. Although, one of its disadvantages is that it allows low physical relevance (Katt et al., 2016).

2. Conditioned Media Exchange

In this type of indirect co-culturing, a conditioned medium is mainly used. The condition media consists of all essential metabolites required for the growth of microbes (Rouf et al., 2017). First, the conditioned medium is used to grow one type of microbes. Then, the same medium is used to grow another type of microbe. This medium contains cell secretoms (CS) of the first type of microbes, which are used by the second type of microbes. Thus, it provides contact between microbes through a conditioned medium containing cell secretoms. The cell secretoms consist of soluble particles. These cell secretoms can have both positive or negative effects on the second type of microbe depending on their adaptability and requirements (Katagiri et al., 2017). The secretions from the first microbes into the culture media are transferred to the second microbe. However, the physical interaction between microbes is less in conditioned media exchange than direct co-cultures which leads to less impact on

each other (Faheem et al., 2021). The effect of only one microbe on the other can be studied through this method.

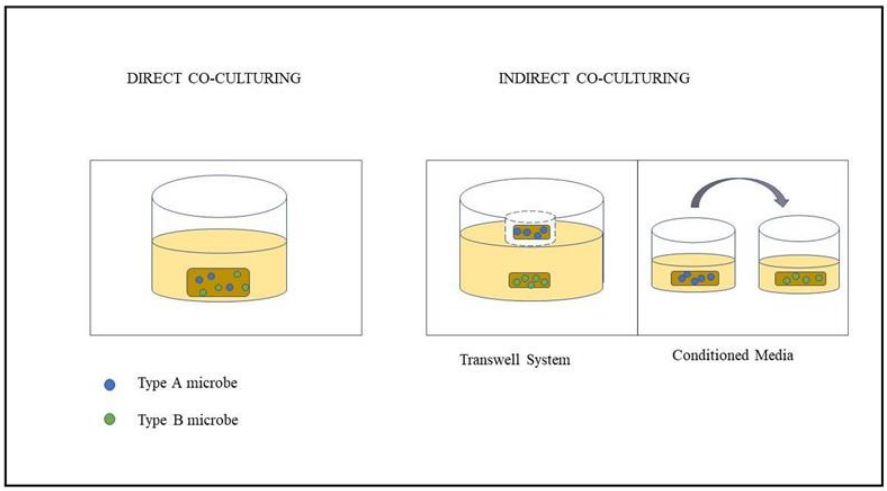

Figure 1. Different techniques for co-culturing

Interactions among Microbes

The potential of microbial interaction with each other depends on various factors. Metabolism and social activities can drive interactions in microbial communities (Suman et al., 2022). Among them, the co-culturing technique plays a vital role. If there are more types of bacterial colonies, there will be more chance of their interaction. Due to these interactions, they may have positive, negative, or neutral effects on each other. These interactions can be helpful for mankind as the products produced due to such interactions are of great importance. Each microbe is different from others in terms of its capability to survive in a specific environment, the toxins decomposed by it (Lindemann et al., 2016), and the natural products it produces (Nai & Meyer, 2018). The interaction among microbes allows them to distribute many metabolites like acetate, amino acids, glucose, or fixed nitrogen (Shin & Kim, 2018). Many types of interactions among microbes have been observed in co-culturing. Some of them are discussed below.

SMIT

Synthetic Mutualism In Trans is a type of interaction shown by various auxotrophs when co-cultured. In this type of interaction, when media is limited, microbes enhance the growth of each other through cross-feeding required metabolites. This interaction usually requires some external catalysts to start. These types of microbes show more growth as they help develop each other and, thus, accelerate their growth by extending essential metabolites (Zhang & Straight, 2019a).

Commensalism

In this type of interaction between two different microbes, one type is benefited while the other is not affected. The growth rate of one kind of microbe is increased by utilizing the metabolites produced by the second type of microbe (Cai et al., 2019). This doesn't affect the second type of microbes. Also, organism A can consume waste products organism B produces, while organism B is unaffected at all. This can be assumed as a food chain where one organism lives on the extra waste produced by the other. Mainly, LAB exhibits commensalism. For example, Lactobacillus bulgaricus and Streptococcus thermophilus show a commensal relationship (Canon et al., 2020).

Syntrophy

In syntrophy, one type of microbe benefits from the metabolites secreted by the other. In turn, it reduces the toxic effects of these metabolites for the second type of microbe. The metabolites produced by the second type of microbe sometimes reduce the growth and cause inhibition effects of the first type of microbe, which utilizes these metabolites as a food source (Scott & Segrè, 2024). This type of interaction is shown by sulfate-reducing and fermentative bacteria.

Mutualism

Mutualism refers to the interaction among microbes in which both interacting partners are benefited from each other. Both microbes produce some products and metabolites that help enhance the growth of others. In this way, both interacting partners grow independently. There is no competition as both types of microbes utilize different kinds of metabolites which are produced by the other ones. Mutualism is often observed between LAB and yeast.

Cooperation

This type of interaction is usually seen among microbes with similar phenotypes (Wang et al., 2018). Both interacting partners share metabolites produced by them and increase each other's health. The metabolites produced by these microbes are similar. Thus, they improve each other's growth capacity and rate, casting a positive effect (Piccardi et al., 2024). It is also referred to as a type of syntrophy as both types of microbes are served and improve the growth rate of each other. Nitrifying and denitrifying bacteria usually show this type of interaction.

Competition

Substrate competition is mostly seen amidst the microbial communities when they are co-cultured. In this interaction, two kinds of microbes compete with one another for the substrate and nutrition (Cressman et al., 2017). Due to the limited substrate, both interacting partners try to suppress the growth of the other (Sinsabaugh et al., 2016). When sources are limited, a decrease in overall growth is observed. This type of interaction is often seen between Streptococcus pneumoniae and Hemophilus influenza (X. Li et al., 2023).

No Interaction

Sometimes, when different types of microbes are co-cultured, they show no interaction. Two kinds of microorganisms utilize different metabolites and nutrients for growth and, thus, don't interfere with one another. The development of such microbes is independent of each other. Many microbes, including Escherichia coli and Saccharomyces cerevisiae, Bacillus subtilis, and Pseudomonas putida, are co-cultured with no significant interactions as they require distinct ecological niches (Treloar et al., 2020).

Predation

This type of interaction refers to synergism in which one partner is benefited while the other is harmed. Metabolites produced by the first type of microbe are utilized by the second type of microbe. While the metabolites produced by the second type of microbe are harmful to the first type of microbe and inhibit its growth (Jia et al., 2016b). Hence, the second type of microbe restricts the development of the first type of microbe while using its metabolites (Saleski et al., 2019). Eventually, it will cause the death of the first type of microbe. Escherichia coli's growth is inhibited when co-culturing with Pseudomonas aeruginosa due to antimicrobial compounds produced by Pseudomonas aeruginosa (Ortiz et al., 2021).

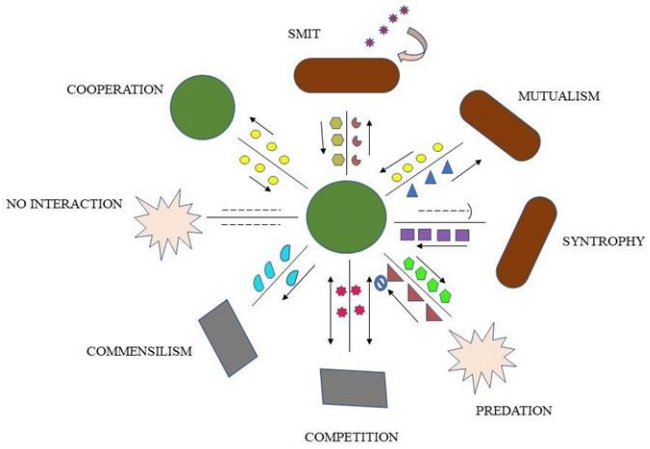

Figure 2. Types of interactions commonly observed between microbes when cocultured

Microbial co-culturing in drug discovery

Microbial co-culturing has been shown to activate the silent gene clusters, but the mechanism through which they are activated remains unknown. Microbes have the ability to produce compounds that can act as regulators for transcription or epigenetic modifiers. A study showed that co-culturing Aspergillus fumigatus with a bacterium activated similar silent pathways just like the pathways activated when an epigenetic modulator was incorporated (König et al., 2024). Co-culturing of microbes can have consequences like gene mutation in otherwise silent gene clusters (Hwang et al., 2014) or the interchange of entire pieces of the gene (horizontal gene transfer) can result in the generation of formerly undetected chemical structures (Abdelmohsen et al., 2015). Microbial co-cultures have been incorporated in quorum sensing (QS) (Kalia et al., 2018) in research of novel antibiotics because QS modifiers show the activity of these drugs. Quorum sensors effectively regulate the populations of bacteria by restyling their growth pattern according to the availability of nutrients. A quorum-sensing lactone generated through Pseudomonas aeruginosa halts the proliferation of cancer cells and causes cell death in human breast cancer (Balhouse et al., 2017).

Studies on microbial coculturing have shown that the metabolites produced due to interactions have various pharmacological functions like signals, siderophores, antibiotics, and other valuable medicines. The metabolites that are diffusible and produced by one organism may cause physiological stimulation and transformations of another organism. A study showed this kind of stimulatory activity among different species of Streptomyces. Bacillus subtilis was used as an indicator, and results showed that one species of streptomyces caused the sporulation of another co-cultured Streptomyces species. Bacillus subtilis formed a zone of inhibition, which showed the production of antibiotic metabolites when the former two Streptomyces species were in close adjacency. A more refined form of the experiment led to the discovery of a novel antibiotic (Zhang & Straight, 2019b).

Bacterial-Fungal co-cultures

While co-culturing bacteria and fungi, fungi are typically employed as the host strain, and bacteria come from outside as guests. Due to their distinctive culturing conditions, the environment is adjusted, such as the amount to be inoculated and the order of the experiment. Production of penicillin is among the major significant discoveries done on fungal-bacterial co-cultures (Devi et al., 2020) (Knowles et al., 2022). Many other pharmaceutically important bioactive molecules have been discovered by co-culturing bacteria and fungi. Such as Pestalones, which is a potent molecule to kill

resistant bacteria (Silber et al., 2016), Libertellenones D is a potent cytotoxic agent against HCT-116 human colon carcinoma cancer cell line (El-Hawary et al., 2018), Emericellamides A is an antibiotic against Staphylococcus aureus which is resistant to methicillin (Oppong-Danquah et al., 2020). They were discovered from interactions between marine bacteria and fungi. An anti-tumor metabolite, Glionitrin A, was reported while co-culturing bacteria and fungi from an acidic environment (Koning et al., 2021), and Secopenicillide C was reported by co-culturing two fungi from soil (Zhang & Straight, 2019c, 2019b).

Table 1.
Bioactive Molecules and Their Activity

BIOACTIVE MOLECULES	BIOLOGICAL ACTIVITY	References
PESTALONES	Antibiotic against resistant bacteria	Silber et al. (2016)
LIBERTELLNONES D	Cytotoxic activity against HCT-116 human colon cancer cells	(El-Hawary et al., 2018)
EMERICELLAMIDES A	Antibiotic against staphylococcus	(Oppong-Danquah et al., 2020)
GLIONNITRIN A	Anti-tumor metabolite	(Koning et al., 2021)

Coculturing of A. fumigatus KMC-901 and Sphingomonas species KMK-001 was done in broth culture known as Czapek-Dox, which produced two novel diketopiperazine disulfides and one of the compounds expressed muscular sub micromolar cytotoxic effects against human cancer cells (HCT116, A549, AGS, DU145) and showed notable antibiotic effects against various microbes including MRSA (Bhattacharya et al., 2021).

In a study, Aspergillus fumigatus was co-cultured through a process similar to the discovery of penicillin in a bacterial fungal interaction on agar plates. It showed significant inhibition with Streptomyces peucetius. By following that procedure, co-culturing of two different species was done which resulted in the generation of novel natural products, fumiformamide and N, N'- (1Z,3Z)-1,4-bis (4methoxyphenyl) buta-1,3-diene-2,3-diyl) diformamide, and two already known N-formyl derivatives and xanthocillin analog BU-4704 (Zhang & Straight, 2019b).

In a report, the co-culturing technique was used to produce various molecules, and their activity was also checked. (Pishchany et al., 2018). The experiment used solid-phase agar of nine Actinomycetes on transwell plates, allowing continuous screening without disturbing communities. The growing bacteria in the plate took necessary nutrients from liquid media and traded metabolites, causing them to diffuse in the fluid media. After co-culturing, the fluid media containing mixed colonies was tested for activity on S. aureus plates. Antimicrobial activity was seen. The sampling of the 16s rDNA was done to observe the composition of species in the culture mixture. This is a simple method and can potentially produce novel antimicrobial or signaling compounds. This report found that Streptomyces coelicolor M145 caused the stimulation of Amycolatopsis sp. AA4 produces a novel antibiotic, Amycomicin, which attacks the biosynthesis of fatty acid (Zhang & Straight, 2019b).

Fungal-Fungal co-cultures

Plant microbes are a promising source of new bioactive metabolites, and recent reports have shown that significant drugs generated by the plants are isolated from the co-cultivation of microbes. (Bachar et al., 2022; Gouda et al., 2016; Hardoim et al., 2015). In a report by Vinale et al. and colleagues, the co-culturing of two important plant fungi (T. harzianum M10 and T. pinophilus F36CF) was done to synthesize new secondary metabolites, which are usually not isolated from their separate cultures grown under a laboratory environment. Harziaphilic acid, a new compound produced by the fungal interaction, was taken and characterized. Apart from that, selective anti-proliferative activity of

HA and paraphilic acid, omitting the iso-HA, was shown with evidence on colorectal carcinoma cells. This was the first report clearly showing the important biological activity of such secondary metabolites. (Vinale et al., 2017) Microbial secondary metabolites (SMs) are significantly used to develop drugs, and almost 61.5% of microbial secondary metabolites are obtained using fungal strains. In the same way, co-culturing of plant microbes like fungal endophytes and Trichoderma spp. may be done to produce bioactive compounds (Xu et al., 2023).

Co-culturing of a species of Armillaria with a species of endophytic fungus Epicoccum was done in vitro, and cytotoxic effects against human cancer cell lines (HL-60, A549, MCF-7, SMMC7721, and SW480) were observed, and slight inhibitory effect against acetylcholinesterase (AChE) was also observed (H.-T. Li et al., 2020). Co-culturing of N. oryzae and Irpex lacteus obtained from seeds of Dendrobium officinale led to the formation of four novel secondary metabolites related to pulvilloric acid-type azaphilone and tremulant sesquiterpene showing anti-AChE properties (Xu et al., 2023).

The co-culturing of Penicillium fuscum and P. camembertii/clavigerum caused the formation of eight novel macrolides, one of the compounds showed antimicrobial effects against MRSA strains, and also Bacillus anthracis, Candida albicans, Streptococcus pyogenes and C. glabrata. A study on the mechanism of action of that compound suggested that it neither stopped the synthesis of bacterial protein nor attacked their ribosomes, showcasing a new method of antibiotic effects (Vinale et al., 2017).

Co-culturing of two plant fungi, Trichoderma harzianum M10 and Talaromyces pinophilus F36CF, produced an alkaloid called paraphilic acid which showed selective inhibition of cancer cells (Vinale et al., 2017). The co-culture of a Nigrospora species and a Stagonosporopsis species yielded a novel 10-membered lactone which showed an antifungal effect against P. janthinellum, Aspergillus fumigatus, Phomopsis sp., and Alternaria sp. (Chen et al., 2022).

Strains of Trametes versicolor and Ganoderma applanatum were co-cultured in glucose (10 g/L), KH2PO4 (1 g/L), MgSO4 (0.5 g/L), and peptone (2 g/L) containing medium. Two new formamide compounds were isolated, and one of the compounds showed the ability to increase the viability of the cells of human preserved bronchial epithelial cell line (Bao et al., 2017). Two mangrove endophytic fungi (strain Nos. 1924 and 3893) were co-cultured in a medium containing glucose 10 g/L, peptone 2 g/L, yeast extracts 1 g/L, crude marine salt 3.5 g/L, and water 1 L, which produced two novel antibiotic 1-isoquinoline analogs (C. Li et al., 2014).

Two mangrove fungi, Phomopsis specie K38 and Alternaria specie, were cocultured in a liquid medium containing glucose 10 g/L, yeast extract 1 g/L, peptone 2 g/L, NaCl 30 g/L), and a novel diimide derivative and three new cyclic peptides were found and characterized, one of the compounds showed slight cytotoxic effects against Hep-2 and HepG2 cells while other compounds showed medium to high antifungal effects comparatively to the positive control (Ketoconazole) (Lane & Lundy, 2018) (Huang et al., 2014).

Bacteria-Bacterial co-cultures

The indole and (Phe-Pro) diketopiperazines production was observed to have increased when coculturing of two dissimilar Bacillus species was done. This caused a buildup of diketopiperazines with a simultaneous antibiotic effect against one of the Bacillus strains ("B. megaterium"). When coculturing of the other bacillus strain, "B. thuringiensis," was done with Staphylococcus sciuri) there was no production or buildup of diketopiperazine. Also, the indole and (Phe-Pro) diketopiperazines did not exhibit any kind of antibiotic effects against S. sciuri (Marmann et al., 2014).

In a report, 78 bacterial strains were taken, and coculturing was done to analyze their antibiotic activity. Out of 78, 9 co-cultured extracts exhibited antibiotic effects, and 2 strains, MH46 and SSE20, were taken for further culturing into broths which were cell-free, of E. coli, B. subtilis or of P.

aeruginosa and also unidentified MH1, MH2, and MH3 bacterial strains. The product extracts were inquired for antibiotic activity against B. subtilis. Two activities were observed, the MH46, which was antibiotically inactive expressed antibiotic effects when cultured in broth of P. aeruginosa. Additionally, the strains MH1, MH2 and MH3, and SSE20, when cultured in broth containing MH1, MH2 and MH3, and B. subtilis, exhibited increased antibiotic effects (Marmann et al., 2014; Satheesh et al., 2016).

In another report the co-culturing of Streptomyces Tenjimariensis and 53 other bacteria was done, in order to investigate the Istamycin A and B buildup by S. Tenjimariensis. 12 off of the 53 co-cultures exhibited a minimum of two times greater induction of Istamycins comparatively to the axenic culturing of S. tenjimariensis. This activity only happened when inoculation of S. tenjimariensis was done 24 hours before the inoculation of the other bacteria, and when both of them were put into cultures at the same time, a decreased Istamycin generation was seen (Marmann et al., 2014).

Mycolic acid-generating bacteria were observed to cause, in other bacteria, a buildup of natural products. A coculturing of S. lividans with mycolic acid-generating bacteria Tsukamurella pulmonic produced a pigment that was red in color in S. lividans (Hoshino et al., 2017) To produce pigment; it was necessary to have a cell to cell contact between S. lividans and T. pulmonis. In that report, Streptomyces endus produced a novel antibiotic, Alchivemycin A when its coculturing was done with T. Pulmonis. (Marmann et al., 2014).

Another study showed that Streptomyces specie generates novel bioactive metabolites when cocultured with the bacterium Tsukamurella pulmonic containing mycolic acid. Various new natural products, Arcyriaflavin E (Hoshino, Okada, et al., 2015), Chojalactones A-C (Hoshino, Wakimoto, et al., 2015), Niizalactams A−C (Hoshino, Zhang, et al., 2015), and 5-alkyl-1,2,3,4-tetrahydroquinolines (Sugiyama et al., 2015) were isolated by co-culturing Streptomyces specie With T. pulmonis. Adnani et al. and his companions reported a novel anthracycline antibiotic, Keyicin, when co-culturing of Micromonospora species was done with sympatric Rhodococcus species taken from the microbiome of sea squirt. Keyicin shows activity against Gram-positive bacteria (Adnani et al., 2017; Zhang & Straight, 2019b).

CONCLUSION

Co-culturing of different species is a technique that is usually done to discover novel bioactive metabolites and their metabolic activities for use in various industries. But, the addition of more than one specie into a culture medium makes the isolation of a single active compound complex. Moreover, for the observation of interactions among multiple species usually requires plate-based or other structured-culture formats which are unmanageable for typical microbiology. In spite of the challenges, recent reports on competitive interactions among different species show the possibility to gain access to novel metabolites. These studies showed that co-cultivation can lead to the isolation of pharmaceutically important bioactive metabolites but the mechanism of these bioactive metabolites is yet to be demonstrated. The principal mechanisms of interactions between species can show new methods to induce specialized metabolites and further improve biomedical discovery efforts. Co-culturing is promising but still in its infancy. We cannot generalize situations in this technique, every experiment requires individual attention before we draw any general conclusion. Hopefully researchers will further conduct researches utilizing this approach in the future.

REFERENCES

Abdelmohsen, U. R., Grkovic, T., Balasubramanian, S., Kamel, M. S., Quinn, R. J., & Hentschel, U. (2015). Elicitation of secondary metabolism in actinomycetes. *Biotechnology Advances*, *33*(6), 798–811. https://doi.org/10.1016/j.biotechadv.2015.06.003

Adnani, N., Chevrette, M. G., Adibhatla, S. N., Zhang, F., Yu, Q., Braun, D. R., Nelson, J., Simpkins, S. W., McDonald, B. R., & Myers, C. L. (2017). Coculture of marine invertebrate-associated bacteria and interdisciplinary technologies enable biosynthesis and discovery of a new antibiotic, keyicin. *ACS Chemical Biology*, *12*(12), 3093–3102.

Bachar, S. C., Kadir, A. K. M. S., Wahab, S. M. R., & Al Hasan, A. (2022). Heterocyclic Anti-cancer Compounds Derived from Natural Sources with their Mechanism of Action. In *Key Heterocyclic Cores for Smart Anticancer Drug–Design Part I* (pp. 1–56). Bentham Science Publishers.

Balhouse, B. N., Patterson, L., Schmelz, E. M., Slade, D. J., & Verbridge, S. S. (2017). N-(3-oxododecanoyl)-L-homoserine lactone interactions in the breast tumor microenvironment: Implications for breast cancer viability and proliferation in vitro. *PLoS One*, *12*(7), e0180372.

Bao, J., Wang, J., Zhang, X., Nong, X., & Qi, S. (2017). New furanone derivatives and alkaloids from the co-culture of marine-derived fungi Aspergillus sclerotiorum and Penicillium citrinum. *Chemistry & Biodiversity*, *14*(3), e1600327.

Beck, A. E., Hunt, K. A., Bernstein, H. C., & Carlson, R. P. (2016). Interpreting and Designing Microbial Communities for Bioprocess Applications, from Components to Interactions to Emergent Properties. In *Biotechnology for Biofuel Production and Optimization* (pp. 407–432). Elsevier. https://doi.org/10.1016/B978-0-444-63475-7.00015-7

Bertrand, S., Bohni, N., Schnee, S., Schumpp, O., Gindro, K., & Wolfender, J.-L. (2014). Metabolite induction via microorganism co-culture: A potential way to enhance chemical diversity for drug discovery. *Biotechnology Advances*, *32*(6), 1180–1204. https://doi.org/10.1016/j.biotechadv.2014.03.001

Bhattacharya, D., Lai, T. K., Saha, A., Selvin, J., & Mukherjee, J. (2021). Structural elucidation and antimicrobial activity of a diketopiperazine isolated from a Bacillus sp. associated with the marine sponge Spongia officinalis. *Natural Product Research*, *35*(14), 2315–2323.

Bouchalova, P., & Bouchal, P. (2022). Current methods for studying metastatic potential of tumor cells. *Cancer Cell International*, *22*(1), 394.

Cai, P., Sun, X., Wu, Y., Gao, C., Mortimer, M., Holden, P. A., Redmile-Gordon, M., & Huang, Q. (2019). Soil biofilms: microbial interactions, challenges, and advanced techniques for ex-situ characterization. *Soil Ecology Letters*, *1*, 85–93.

Canon, F., Nidelet, T., Guédon, E., Thierry, A., & Gagnaire, V. (2020). Understanding the mechanisms of positive microbial interactions that benefit lactic acid bacteria co-cultures. *Frontiers in Microbiology*, *11*, 2088.

Chen, J.-X., Xia, D.-D., Yang, X.-Q., Yang, Y.-B., & Ding, Z.-T. (2022). The antifeedant and antifungal cryptic metabolites isolated from tobacco endophytes induced by host medium and coculture. *Fitoterapia*, *163*, 105335. https://doi.org/10.1016/j.fitote.2022.105335

Cressman, R., Halloway, A., McNickle, G. G., Apaloo, J., Brown, J. S., & Vincent, T. L. (2017). Unlimited niche packing in a Lotka–Volterra competition game. *Theoretical Population Biology*, *116*, 1–17. https://doi.org/10.1016/j.tpb.2017.04.003

Devi, R., Kaur, T., Guleria, G., Rana, K. L., Kour, D., Yadav, N., Yadav, A. N., & Saxena, A. K. (2020). Fungal secondary metabolites and their biotechnological applications for human health. In *New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 147–161). Elsevier. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

El-Hawary, S. S., Sayed, A. M., Mohammed, R., Khanfar, M. A., Rateb, M. E., Mohammed, T. A., Hajjar, D., Hassan, H. M., Gulder, T. A. M., & Abdelmohsen, U. R. (2018). New Pim-1 kinase inhibitor from the co-culture of two sponge-associated actinomycetes. *Frontiers in Chemistry*, *6*, 538.

Faheem, M. S., Dessouki, S. M., Abdel- Rahman, F. E. S., & Ghanem, N. (2021). Physiological and molecular aspects of heat-treated cultured granulosa cells of Egyptian buffalo (Bubalus bubalis). *Animal Reproduction Science*, *224*, 106665. https://doi.org/10.1016/j.anireprosci.2020.106665

Ghose, R., Rice, A. J., Cortes, E., Ghose, U., Lachowski, D., & del Rio Hernandez, A. (2020). *Implementation of a basement membrane invasion assay using mesenteric tissue* (pp. 99–122). https://doi.org/10.1016/bs.mcb.2019.10.001

Goers, L., Freemont, P., & Polizzi, K. M. (2014). Co-culture systems and technologies: taking synthetic biology to the next level. *Journal of The Royal Society Interface*, *11*(96), 20140065.

Gouda, S., Das, G., Sen, S. K., Shin, H.-S., & Patra, J. K. (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in Microbiology*, *7*, 1538.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3), 293–320.

Hoshino, S., Okada, M., Awakawa, T., Asamizu, S., Onaka, H., & Abe, I. (2017). Mycolic acid containing bacterium stimulates tandem cyclization of polyene macrolactam in a lake sediment derived rare actinomycete. *Organic Letters*, *19*(18), 4992–4995.

Hoshino, S., Okada, M., Wakimoto, T., Zhang, H., Hayashi, F., Onaka, H., & Abe, I. (2015). Niizalactams A–C, multicyclic macrolactams isolated from combined culture of Streptomyces with mycolic acid-containing bacterium. *Journal of Natural Products*, *78*(12), 3011–3017.

Hoshino, S., Wakimoto, T., Onaka, H., & Abe, I. (2015). Chojalactones A–C, cytotoxic butanolides isolated from Streptomyces sp. cultivated with mycolic acid containing bacterium. *Organic Letters*, *17*(6), 1501–1504.

Hoshino, S., Zhang, L., Awakawa, T., Wakimoto, T., Onaka, H., & Abe, I. (2015). Arcyriaflavin E, a new cytotoxic indolocarbazole alkaloid isolated by combined-culture of mycolic acid-containing bacteria and Streptomyces cinnamoneus NBRC 13823. *The Journal of Antibiotics*, *68*(5), 342–344.

Huang, S., Ding, W., Li, C., & Cox, D. G. (2014). Two new cyclopeptides from the co-culture broth of two marine mangrove fungi and their antifungal activity. *Pharmacognosy Magazine*, *10*(40), 410.

Hwang, K.-S., Kim, H. U., Charusanti, P., Palsson, B. Ø., & Lee, S. Y. (2014). Systems biology and biotechnology of Streptomyces species for the production of secondary metabolites. *Biotechnology Advances*, *32*(2), 255–268. https://doi.org/10.1016/j.biotechadv.2013.10.008

Jia, X., Liu, C., Song, H., Ding, M., Du, J., Ma, Q., & Yuan, Y. (2016a). Design, analysis and application of synthetic microbial consortia. *Synthetic and Systems Biotechnology*, *1*(2), 109–117.

Jia, X., Liu, C., Song, H., Ding, M., Du, J., Ma, Q., & Yuan, Y. (2016b). Design, analysis and application of synthetic microbial consortia. *Synthetic and Systems Biotechnology*, *1*(2), 109–117. https://doi.org/10.1016/j.synbio.2016.02.001

Kalia, V. C., Prakash, J., Koul, S., & Ray, S. (2018). *Quorum sensing and its inhibition: biotechnological applications*. Springer.

Katagiri, W., Sakaguchi, K., Kawai, T., Wakayama, Y., Osugi, M., & Hibi, H. (2017). A defined mix of cytokines mimics conditioned medium from cultures of bone marrow-derived mesenchymal stem cells and elicits bone regeneration. *Cell Proliferation*, *50*(3), e12333.

Katt, M. E., Placone, A. L., Wong, A. D., Xu, Z. S., & Searson, P. C. (2016). In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. *Frontiers in Bioengineering and Biotechnology*, *4*, 12.

Knowles, S. L., Raja, H. A., Roberts, C. D., & Oberlies, N. H. (2022). Fungal–fungal co-culture: a primer for generating chemical diversity. *Natural Product Reports*, *39*(8), 1557–1573.

König, S., Schroeder, J., Nietzsche, S., Heinekamp, T., Brakhage, A. A., Zell, R., Löffler, B., & Ehrhardt, C. (2024). The influenza A virus promotes fungal growth of Aspergillus fumigatus via direct interaction in vitro. *Microbes and Infection*, *26*(3), 105264. https://doi.org/10.1016/j.micinf.2023.105264

Koning, N. R., Sundin, A. P., & Strand, D. (2021). Total Synthesis of (−)-Glionitrin A and B Enabled by an Asymmetric Oxidative Sulfenylation of Triketopiperazines. *Journal of the American Chemical Society*, *143*(50), 21218–21222.

Lane, A. L., & Lundy, T. A. (2018). Interactions Between Microorganisms as Modulators of Natural Product Biosynthesis. In *Chemical Ecology* (pp. 261–290). CRC Press.

Li, C., Wang, J., Luo, C., Ding, W., & Cox, D. G. (2014). A new cyclopeptide with antifungal activity from the co-culture broth of two marine mangrove fungi. *Natural Product Research*, *28*(9), 616–621.

Li, H.-T., Tang, L.-H., Liu, T., Yang, R.-N., Yang, Y.-B., Zhou, H., & Ding, Z.-T. (2020). Protoilludane-type sesquiterpenoids from Armillaria sp. by co-culture with the endophytic fungus Epicoccumsp. associated with Gastrodia elata. *Bioorganic Chemistry*, *95*, 103503. https://doi.org/10.1016/j.bioorg.2019.103503

Li, X., Xu, H., Li, Y., Liao, S., & Liu, Y. (2023). Exploring diverse bioactive secondary metabolites from marine microorganisms using co-culture strategy. *Molecules*, *28*(17), 6371.

Lindemann, S. R., Bernstein, H. C., Song, H.-S., Fredrickson, J. K., Fields, M. W., Shou, W., Johnson, D. R., & Beliaev, A. S. (2016). Engineering microbial consortia for controllable outputs. *The ISME Journal*, *10*(9), 2077–2084.

Marmann, A., Aly, A., Lin, W., Wang, B., & Proksch, P. (2014). Co-Cultivation—A Powerful Emerging Tool for Enhancing the Chemical Diversity of Microorganisms. *Marine Drugs*, *12*(2), 1043–1065. https://doi.org/10.3390/md12021043

Nai, C., & Meyer, V. (2018). From axenic to mixed cultures: technological advances accelerating a paradigm shift in microbiology. *Trends in Microbiology*, *26*(6), 538–554.

Nonaka, K., Abe, T., Iwatsuki, M., Mori, M., Yamamoto, T., Shiomi, K., Ômura, S., & Masuma, R. (2011). Enhancement of metabolites productivity of Penicillium pinophilum FKI-5653, by co-culture with Trichoderma harzianum FKI-5655. *The Journal of Antibiotics*, *64*(12), 769–774. https://doi.org/10.1038/ja.2011.91

Ogawa, M., García-Martínez, T., Bisson, L., Mauricio, J. C., Moreno, J., & Moreno-García, J. (2020). Mapping the intracellular metabolome of yeast biocapsules - Spherical structures of yeast attached to fungal pellets. *New Biotechnology*, *58*, 55–60. https://doi.org/10.1016/j.nbt.2020.05.003

Oppong-Danquah, E., Budnicka, P., Blümel, M., & Tasdemir, D. (2020). Design of fungal co-cultivation based on comparative metabolomics and bioactivity for discovery of marine fungal agrochemicals. *Marine Drugs*, *18*(2), 73.

Ortiz, A., Vega, N. M., Ratzke, C., & Gore, J. (2021). Interspecies bacterial competition regulates community assembly in the C. elegans intestine. *The ISME Journal*, *15*(7), 2131–2145.

Padmaperuma, G., Kapoore, R. V., Gilmour, D. J., & Vaidyanathan, S. (2018). Microbial consortia: a critical look at microalgae co-cultures for enhanced biomanufacturing. *Critical Reviews in Biotechnology*, *38*(5), 690–703.

Paschos, N. K., Brown, W. E., Eswaramoorthy, R., Hu, J. C., & Athanasiou, K. A. (2015). Advances in tissue engineering through stem cell-based co-culture. *Journal of Tissue Engineering and Regenerative Medicine*, *9*(5), 488–503.

Piccardi, P., Ulrich, E., Garcia-Garcerà, M., Martino, R. Di, Testa, S. E. A., & Mitri, S. (2024). The evolution of reduced facilitation in a four-species bacterial community. *Evolution Letters*, qrae036.

Pishchany, G., Mevers, E., Ndousse-Fetter, S., Horvath Jr, D. J., Paludo, C. R., Silva-Junior, E. A., Koren, S., Skaar, E. P., Clardy, J., & Kolter, R. (2018). Amycomicin is a potent and specific antibiotic discovered with a targeted interaction screen. *Proceedings of the National Academy of Sciences*, *115*(40), 10124–10129.

Rouf, A., Kanojia, V., Naik, H. R., Naseer, B., & Qadri, T. (2017). An overview of microbial cell culture. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 1923–1928.

Saleski, T. E., Kerner, A. R., Chung, M. T., Jackman, C. M., Khasbaatar, A., Kurabayashi, K., & Lin, X. N. (2019). Syntrophic co-culture amplification of production phenotype for high-throughput screening of microbial strain libraries. *Metabolic Engineering*, *54*, 232–243. https://doi.org/10.1016/j.ymben.2019.04.007

Satheesh, S., Ba-akdah, M. A., & Al-Sofyani, A. A. (2016). Natural antifouling compound production by microbes associated with marine macroorganisms — A review. *Electronic Journal of Biotechnology*, *21*, 26–35. https://doi.org/10.1016/j.ejbt.2016.02.002

Scott, H., & Segrè, D. (2024). Metabolic Flux Modeling in Marine Ecosystems. *Annual Review of Marine Science*, *17*.

Shin, W., & Kim, H. J. (2018). *Pathomimetic modeling of human intestinal diseases and underlying host-gut microbiome interactions in a gut-on-a-chip* (pp. 135–148). https://doi.org/10.1016/bs.mcb.2018.05.006

Silber, J., Kramer, A., Labes, A., & Tasdemir, D. (2016). From discovery to production: biotechnology of marine fungi for the production of new antibiotics. *Marine Drugs*, *14*(7), 137.

Sinsabaugh, R. L., Turner, B. L., Talbot, J. M., Waring, B. G., Powers, J. S., Kuske, C. R., Moorhead, D. L., & Shah, J. J. F. (2016). Stoichiometry of microbial carbon use efficiency in soils. *Ecological Monographs*, 172–189.

Sugiyama, R., Nishimura, S., Ozaki, T., Asamizu, S., Onaka, H., & Kakeya, H. (2015). 5-Alkyl-1,2,3,4-tetrahydroquinolines, New Membrane-Interacting Lipophilic Metabolites Produced by Combined Culture of *Streptomyces nigrescens* and *Tsukamurella pulmonis*. *Organic Letters*, *17*(8), 1918–1921. https://doi.org/10.1021/acs.orglett.5b00607

Suman, A., Govindasamy, V., Ramakrishnan, B., Aswini, K., SaiPrasad, J., Sharma, P., Pathak, D., & Annapurna, K. (2022). Microbial community and function-based synthetic bioinoculants: a perspective for sustainable agriculture. *Frontiers in Microbiology*, *12*, 805498.

Tan, Z. Q., Leow, H. Y., Lee, D. C. W., Karisnan, K., Song, A. A. L., Mai, C. W., Yap, W. S., Lim, S. H. E., & Lai, K. S. (2019). Co-culture systems for the production of secondary metabolites: Current and future prospects. *The Open Biotechnology Journal*, *13*(1).

Treloar, N. J., Fedorec, A. J. H., Ingalls, B., & Barnes, C. P. (2020). Deep reinforcement learning for the control of microbial co-cultures in bioreactors. *PLoS Computational Biology*, *16*(4), e1007783.

Vinale, F., Nicoletti, R., Borrelli, F., Mangoni, A., Parisi, O. A., Marra, R., Lombardi, N., Lacatena, F., Grauso, L., & Finizio, S. (2017). Co-culture of plant beneficial microbes as source of bioactive metabolites. *Scientific Reports*, *7*(1), 14330.

Vis, M. A. M., Ito, K., & Hofmann, S. (2020). Impact of culture medium on cellular interactions in in vitro co-culture systems. *Frontiers in Bioengineering and Biotechnology*, *8*, 911.

Wang, C., Liu, Y., Gao, X., Chen, H., Xu, X., & Zhu, L. (2018). Role of biochar in the granulation of anaerobic sludge and improvement of electron transfer characteristics. *Bioresource Technology*, *268*, 28–35. https://doi.org/10.1016/j.biortech.2018.07.116

Xu, S., Li, M., Hu, Z., Shao, Y., Ying, J., & Zhang, H. (2023). The Potential Use of Fungal Co-Culture Strategy for Discovery of New Secondary Metabolites. *Microorganisms*, *11*(2), 464. https://doi.org/10.3390/microorganisms11020464

Zhang, C., & Straight, P. D. (2019a). Antibiotic discovery through microbial interactions. *Current Opinion in Microbiology*, *51*, 64–71. https://doi.org/10.1016/j.mib.2019.06.006

Zhang, C., & Straight, P. D. (2019b). Antibiotic discovery through microbial interactions. *Current Opinion in Microbiology*, *51*, 64–71. https://doi.org/10.1016/j.mib.2019.06.006

Zhang, C., & Straight, P. D. (2019c). Antibiotic discovery through microbial interactions. *Current Opinion in Microbiology*, *51*, 64–71. https://doi.org/10.1016/j.mib.2019.06.006