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Deciphering mechanisms of production of natural compounds using inducer-producer microbial consortia☆

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A B S T R A C T

Living organisms produce a wide range of metabolites. Because of their potential antibacterial, antifungal, antiviral, or cytostatic properties, such natural molecules are of high interest to the pharmaceutical industry. In nature, these metabolites are often synthesized via secondary metabolic biosynthetic gene clusters that are silent under the typical culturing conditions. Among different techniques used to activate these silent gene clusters, co-culturing of "producer" species with specific "inducer" microbes is a particularly appealing approach due to its simplicity. Although several "inducer-producer" microbial consortia have been reported in the literature and hundreds of different secondary metabolites with attractive biopharmaceutical properties have been described as a result of co-cultivating inducer-producer consortia, less attention has been devoted to the understanding of the mechanisms and possible means of induction for production of secondary metabolites in co-cultures. This lack of understanding of fundamental biological functions and inter-species interactions significantly limits the diversity and yield of valuable compounds using biological engineering tools. In this review, we summarize and categorize the known physiological mechanisms of production of secondary metabolites in inducer-producer consortia, and then discuss approaches that could be exploited to optimize the discovery and production of secondary metabolites.

1. Introduction

Microbial secondary metabolites or microbial Natural Products (NPs) are compounds produced by microorganisms that, unlike primary metabolites, do not take part in the core cellular metabolic processes, such as growth or reproduction. Nevertheless, these molecules have important roles for specific physiological functions or participate in ecological interactions between organisms (O'Brien and Wright, 2011) and have many useful properties. NPs have important applications in pharmaceutical development (David et al., 2015; Atanasov et al., 2021), agriculture (Hüter, 2011), cosmetics (Gupta et al., 2019; Nowruzi et al., 2020) and food industry (Cleveland et al., 2001; Singh et al., 2017). Specific examples of NPs used in pharmaceutical industry include their use as antibiotics such as penicillin isolated from *P. notatum* (Roberts et al., 1943) and erythromycin isolated from *S. erythraea* (Minas, 2005), as well as antiviral compounds (Yasuhara-Bell and and Lu, 2010) including Macrolactin A that has shown an ability to protect immune system cells against HIV replication (Gustafson et al., 1989). In light of the rising concerns about antibiotic resistance (Maiden, 1998; Bottery et al., 2020) and economic challenges posed by the development of new antibiotics (Plackett, 2020), the discovery and analysis of novel microbial NPs has increased in importance (Lewis, 2013; Kealey et al., 2017) and also has profound implications for public health. In agriculture, NPs represent an alternative to chemical and physical pest control methods (Salwan and Sharma, 2021); for example, protease from *B. nematocida* has been shown to destroy the cuticle of nematodes (Niu et al., 2006). In the cosmetics industry, hyaluronic acid produced by various *Streptococci* including *S. zooepidermicus* is used for skin repair and in anti-aging (Gupta et al., 2019; Liu et al., 2011). Bacteriocins are antibacterial compounds produced by bacteria to kill other bacteria or inhibit their growth; nisin isolated from *L. lactis* has been used for food preservation (Cleveland et al., 2001). The total market for microbial products was estimated at 166 billion USD in 2021 and is expected to reach 248 billion USD in 2026 (MarketWatch, 2021). Consequently, the discovery and

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industrial production of microbial NPs are important areas of biotechnological research.

The industrial-scale synthesis of microbial NPs poses several major challenges. First, up to 99 % of the microorganisms inhabiting Earth can not be cultured in the laboratory using common laboratory techniques, which significantly limits efforts to discover novel NPs (Vartoukian et al., 2010; Overmann et al., 2017). Next, even if their producer strains are culturable, many of the microbial NPs of interest are expressed from cryptic biosynthetic gene clusters (BGCs) that are silent under typical laboratory conditions (Baral et al., 2018). Many secondary metabolites may also be assembled by mega-enzyme complexes such as non-ribosomal peptide synthetases and polyketide synthetases that impose a significant burden on the cellular resources (Kim et al., 2021) - consequently, microbial secondary metabolites are often synthesized only in the presence of specific environmental conditions, such as lack of nutrients (Jekosch and Kuck, 2000), competition with other organisms, and other physiological stress such as variations in pH and temperature (Shwab and Keller, 2008). Finally, the isolation and identification of novel NPs out of complex chemical extracts is difficult (Bertrand et al., 2014). The combination of these factors, which are often intertwined, makes the discovery of microbial NPs and increasing their yield per unit volume difficult. Scaling of bioproduction to industrial levels possesses its own set of major challenges, which range from the varying flow patterns in bioreactors, process considerations, to catalytic activity of the key enzymes (Schmidt, 2005; Du et al., 2022; Formenti et al., 2014).

Advances in biotechnology, genome sequencing, genome mining and synthetic biology have led to the development of a variety of techniques to discover new microbial NPs and increase their yields (Kim et al., 2021; Kenshole et al., 2021; Qian et al., 2020). Some of these techniques involve environmental perturbations, which may induce the production of a wide spectrum of secondary metabolites by a microorganism, that the organism would not produce under standard culturing conditions. The well-established method that utilizes environmental perturbations to culture conditions has been named "OSMAC": "One Strain MAny Compounds" (Bode et al., 2002). Another technique replaces native constitutive promoters with inducible promoters in the natural host cell to activate the production of NPs (Bergmann et al., 2007; Chiang et al., 2009). Other engineering techniques use CRISPR-based systems to induce production of silent BGCs (Mózsik et al., 2021; Jiang et al., 2021). Bioinformatics, genome sequencing, and genome mining can also help identify BGCs responsible for synthesis of NPs in the producer organism (Yaegashi et al., 2014; Baral et al., 2018; Kenshole et al., 2021), with the subsequent expression of secondary metabolites in a suitable host (Rutledge and Challis, 2015; Olano et al., 2014). However, each of these methods suffer from significant drawbacks: OSMAC-based approaches can require screening of a large space of culturing conditions, while genome mining and genome engineering methods also face a wide range of challenges, such as difficulties associated with heterologous expression and off-target effects (e.g. if employing CRISPRa-based activation (Jiang et al., 2021)). In addition to that, all the methods described above are limited by the fact that a thorough understanding of physiology of the producing organism is required.

On the opposite end of the complexity spectrum to genetic engineering-based techniques lie co-culturing of different microbial species, which is among the most attractive techniques for inducing synthesis of NPs. The co-culturing of two or more species can unlock a wealth of inter-species interactions, which may induce production of valuable secondary metabolites (Abdelmohsen et al., 2015). While co-culturing of different microbes may suffer from the same "curse of dimensionality" as variation of culture conditions does, given the number of possible inducer-producer pairs scales combinatorially, this method offers several advantages: it can be implemented in various physical settings (Nai and Meyer, 2018), does not require prior knowledge of physiology of individual species, does not necessitate genetic engineering of organisms (Kim et al., 2021), and may mimic ecological stress that organisms experience in their natural environment, which is often far from a typical, axenic culture (Bertrand et al., 2014; Marmann et al., 2014).

Over the last three decades, a vast number of papers described bioproduction of valuable compounds as a consequence of co-culturing of microorganisms in these "inducer-producer" consortia (Bertrand et al., 2014; Arora et al., 2020). Intriguingly, despite the plethora of culturing studies that have been conducted, a surprisingly small number of these have focused on explaining the mechanisms of inter-species interaction that lead to production of these NPs. Several reviews have discussed this topic; these studies also described subsets of mechanisms for the induction of secondary metabolites production in co-cultures, such as chemical interactions, physical contact, and bacterial competition (Abdelmohsen et al., 2015; Kim et al., 2021; Qian et al., 2020; Marmann et al., 2014; Zhuang et al., 2021). Nevertheless, a thorough categorization of the various means of inducing production of microbial NPs in inducer-producer co-cultures has not been conducted. Doing so is an important step toward biologically inspired rational design of microbial consortia producing NPs and efforts to optimize culturing conditions in order to maximize yields of valuable compounds or to improve their screening. This biomimetic approach is additionally important in the light of current trends in systems and synthetic biology, particularly the design of complex microbial consortia with applications in medicine (Landry and Tabor, 2017; Ozdemir et al., 2018), environmental bioremediation (Bhatt et al., 2021), and bioproduction (Zhou et al., 2015; Roell et al., 2019; Hong et al., 2020). Improved understanding of natural interactions in inducer-producer consortia could thus inspire the design of novel engineered microbial consortia with expanded functionality.

In this review, we provide a detailed overview of the methods of bioproduction of NPs in individual microbes and in inducer-producer consortia. We comprehensively classify the inter-species interactions leading to the production of NPs that have been previously described in the literature. We also describe physical configurations in which co-culturing of inducer-producer consortia can take place. Finally, we describe the key aspects of co-culturing systems that can be used to optimise bioproduction in inducer-producer consortia. Our work highlights the opportunities for bioproduction in inducer-producer consortia and opens possibilities for the rational design of such systems.

2. Methods of secondary metabolite discovery

2.1. Varying culturing conditions

Alteration of the chemical context and physical environment surrounding the target producer organism can lead to the activation of silent BGCs and production of a wealth of valuable microbial NPs (Fig. 1a). These might be, for example, produced as a result of abiotic stress (Romano et al., 2018). We note that co-culturing of producer organisms with other species was frequently included under the umbrella of OSMAC-based approach (Romano et al., 2018; Pan et al., 2019). However, other works draw a distinction between co-culturing of multiple different microbial species and variation of the external environment of a single species (Kim et al., 2021; Bertrand et al., 2014). Given the focus of this review on inducer-producer consortia, we follow the latter approach and draw a distinction between deliberate variation of the culturing conditions by experimenters and microbial co-cultures, where inter-species interactions drive the production of secondary metabolites.

A wealth of culturing conditions can be varied to elicit production of secondary metabolites in microbes; these include medium composition, growth on/in physical scaffolds in order to achieve the formation of unique microbial communities or disrupting agglomerations of fungal cells, addition of chemical elicitors that activate the expression from silent BGCs, as well as alteration of pH, temperature, light spectrum and intensity, air pressure, oxygen concentration and other factors (Pan et al., 2019; Tomm et al., 2019). For example, growth of *A. niger* on lactate or starch as the only source of carbon led to a low production of

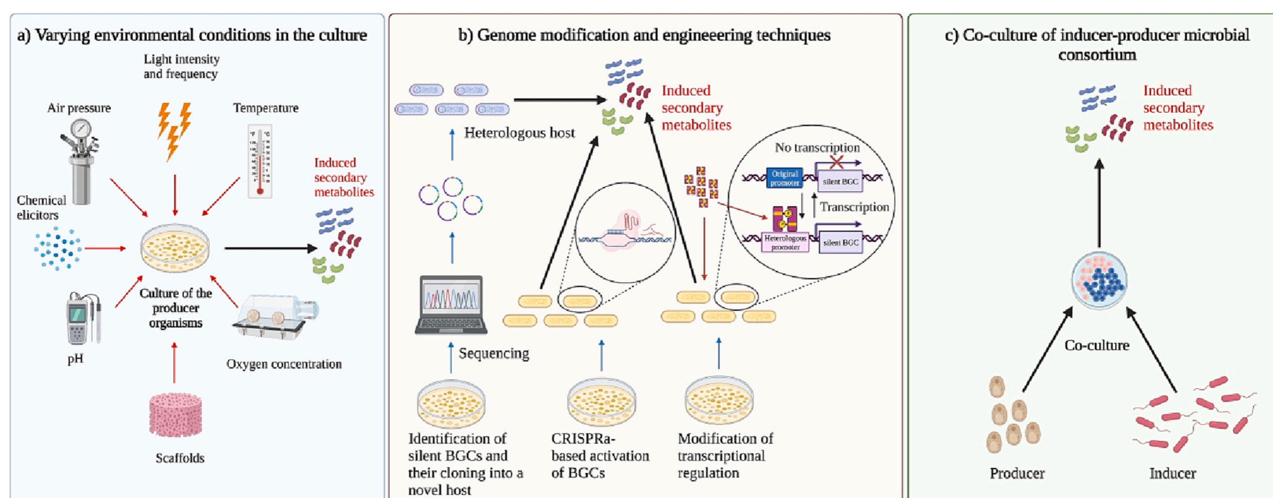

Fig. 1. Different ways of inducing production of valuable microbial NPs via activation of BGCs. **a)** Varying the environmental conditions in the culture, also known as OSMAC approach, requires modification of culturing conditions such as temperature, medium composition, pH, or addition of chemical elicitors. **b)** Genome modification and genetic engineering techniques combine the methods of bioinformatics, systems biology and synthetic biology to identify BGCs and to subsequently manipulate expression of these gene clusters to increase the production of microbial NPs. This include heterologous expression of the BGCs and activation of BGCs via manipulating transcription and translation processes. **c)** Co-culturing different microbial species exploits various types of inter-species interactions to induce production of secondary metabolites. Often, one species serves as an *inducer* which activates the production of valuable microbial NPs in the other, *producer*, organism. Created with BioRender.com.

NPs, while combination of these two nutrient sources resulted in increased production of fumonisins, ochratoxins and other secondary metabolites (Frisvad, 2012; Sørensen et al., 2009), indicating the possible importance of the carbon-to-nitrogen ratio in activating microbial NPs production (Frisvad, 2012; Pan et al., 2019). Culturing microorganisms with cotton scaffolds may facilitate biofilm formation and alter the metabolic state of microorganisms, while leading to increased production of NPs such as thiomarinol A and violacein (Timmermans et al., 2019). Addition of aluminium oxide nanoparticles or talc microparticles to the cultures of fungus *A. terreus* led to improved production of lovastatin, which is used to lower blood cholesterol (Boruta and Bizukojc, 2019; Gonciarz and Bizukojc, 2014).

Next, systematic variation of physical conditions is also a way to induce production of secondary metabolites (Tomm et al., 2019). pH variation in the culture of *S. coelicolor* increased the yield of prodignines like undecylprodignine and streptorubin B (Mo et al., 2013). Temperature variation induced pyomelanin production in deep-sea bacterium *Pseudoalteromonas* sp. SM9913 (Zeng et al., 2017). Light frequency and intensity variation stimulated production of lactobionic acid that is used in the cosmetics industry in *P. taetrolens*; interestingly, while red light promoted both growth of the bacterium and the yield of lactobionic acid, blue light only promoted growth (Shu et al., 2018). Variation in the partial pressure of oxygen has been shown to modulate the production of secondary metabolites produced by *S. parvulus*, such as Manumycin A and its precursors (Sattler et al., 1998). Increasing concentration of dissolved oxygen led to decrease of synthesis of aspinonene and increase in synthesis of aspyrone in *A. ochraceus* (Fuchser et al., 1995). All of these examples of OSMAC approach demonstrate the breadth and diversity of the culturing conditions that can be varied to achieve expression of novel NPs or overproduction of other NPs.

The OSMAC approach has several advantages, including a relatively simple implementation that can often be accomplished with affordable laboratory equipment. Furthermore, it is possible to tune culture conditions like medium composition with high precision, which allows for the collection of larger data sets and the optimization of medium culture for microbial production via statistical and mathematical approaches (Singh et al., 2017). Use of Genome-scale Metabolic Models (GSMM) that allow for the *in silico* simulation of perturbation of physiological status of the organism (Thiele and Palsson, 2010; Orth et al., 2010) can reduce the large screening space associated with the OSMAC method (Toro et al., 2018; Wei et al., 2021). However, the combinatorial space of possible culture conditions is still often enormous and its full screening is intractable. Furthermore, if an organism is present in an axenic culture, outside of its natural environment where it would interact with multiple different organisms, a number of its secondary metabolites that would be involved in the inter-species interplay may not need to be produced (Pan et al., 2019; Marmann et al., 2014; Nai and Meyer, 2018).

2.2. Genome mining and genetic engineering

A wide class of methods alternative to variation of culturing conditions employ techniques of genome sequencing and genome mining to identify BGCs for the synthesis of NPs in target microorganisms (Yaegashi et al., 2014; Baral et al., 2018; Kenshole et al., 2021; Rutledge and Challis, 2015). These techniques can be further supplemented with experimental techniques of genome engineering and synthetic biology that allow for the modification of microbes so as to increase the production of NPs (Lee et al., 2021) (Fig. 1b). Random mutagenesis, such as irradiation by ultraviolet (UV) light and use of mutagenic chemicals (Gao and Garcia-Pichel, 2011; Bose, 2016), as well as combination of physical and chemical mutagenesis (Bouassida et al., 2018) can be used enhance the production of target secondary metabolites. However, random mutagenesis often requires extensive time and resources devoted to high-throughput screening, with an uncertain result.

Ribosome engineering-based approaches focus on the discovery of the mutants with specific spontaneous mutations in their transcriptional and translational machinery through screening for antibiotic resistant mutants (Shima et al., 1996; Zhang et al., 2016). These mutants often exhibit a remarkable ability to produce secondary metabolites. Such screening can be used to identify mutations that might be responsible for such increased yield of secondary metabolites. For example, mutations in transcriptional and translational machinery can increase the level of protein translation during the later phases of growth and stimulate secondary metabolite production by regulating primary metabolism and global gene expression (Zhu et al., 2019). While ribosome engineering is attractive, given that it can be applied to organisms with limited availability of the genetic tools and does not require extensive understanding

of the regulatory and metabolic networks in living organisms, it still includes a significant amount of serendipity and might require deployment of significant resources to identify genotype-phenotype relationships (Zhang et al., 2016).

Transporter engineering represents another attractive way to increase the yield of microbial secondary metabolites and increase the manufacturing capacity of microbes (van der Hoek and Borodina, 2020). Engineering of transporter proteins can increase the uptake of exogenous substrates that are required for the bioproduction of microbial products (Thomik et al., 2017). Furthermore, such transporter-focused manipulation can increase the efficacy of transfer of intermediates: either increase their re-uptake by original producer (Li et al., 2019) or, conversely, facilitate secretion of intermediates and hence increase their availability for further processing by another member of microbial consortium (Zhang et al., 2015). Finally, the lack of availability of product transporters and entrapping of the product within the cell may not only reduce the bioavailability of the product, but also cause the toxicity for the cell (van der Hoek and Borodina, 2020). The overexpression of product transporters has been shown to significantly increase the production of target metabolites, such as citric acid in *A. niger* (Steiger et al., 2019). However, transporter engineering has several difficulties, such as lack of understanding of their biology, intricacy of the global cellular transporter network, and challenges with the identification of transporter proteins via genome mining (Lv et al., 2022).

In global Transcription Machinery Engineering (gTME), the modification of transcription levels of multiple genes is used to obtain novel cellular phenotypes (Alper et al., 2006). This approach can be used to enhance levels of production of valuable secondary metabolites (Xue et al., 2019). Mutating components of RNA polymerase in engineered *E. coli* led to 114 % increase in the titre of L-tyrosine when compared to the parental strain that has already been engineered to produce high yield of L-tyrosine (Santos et al., 2012). Mutation libraries targeted to specific genes may significantly increase the yields of NPs, such as in the case of recombinant *E. coli* producing Hyaluronic acid with an increased yield (Yu et al., 2008). Despite its successes in both prokaryotes and eukaryotes, gTME presents several challenges: gTME methods focus only on a small subset of the cellular regulatory factors (Ke et al., 2020); tinkering with the global regulatory factors can lead to decreased overall fitness of the cells; and finally, current gTME techniques often employ plasmids for the construction of mutant libraries, which may not perform robustly when deployed at scale (Jiang et al., 2020).

An important intermediate step for producing valuable NPs is the identification of the corresponding BGCs. Numerous computational tools, such as antiSMASH (Skinnider et al., 2020) and PRISM (Blin et al., 2021) have been developed in order to predict BGCs for the synthesis of secondary metabolites. After the hypothetical BGCs for synthesis of a specific NP is identified via genome mining, various actions can be taken in order to activate or increase the production of the desired secondary metabolite. Probably the most obvious and direct strategy is the overexpression of BGCs or their regulators, either by manipulating the promoter of the selected operon or through increasing gene copy number (Liu et al., 2021; Wu et al., 2020; Zhang et al., 2016). Direct activation of BGCs may include modification of transcriptional regulation (Sidda et al., 2013; Wang et al., 2018; Laureti et al., 2011) as well as CRISPR-based activation of silent genes (Mózsik et al., 2021; Jiang et al., 2021). However, this approach requires not only the identification of the appropriate BGCs and their regulators, which is often difficult, but also poses challenges associated with the native negative feedback regulation of BGCs, which may hamper their overproduction (Wang et al., 2014; Xia et al., 2020). CRISPR-based editing might suffer from off-target effects (Jiang et al., 2021) and regulatory proteins might exhibit a cross-talk with other BGCs or physiological cellular processes (Bergmann et al., 2010).

Another way to increase the yield of the microbial secondary metabolites is metabolic engineering (Kim et al., 2016) and precursor engineering (Zhang et al., 2016). The availability of precursors, often products of the primary metabolism, is one of the deciding factors for the level of secondary metabolite production. Re-directing carbon flux through metabolic pathways can lead to increased production of microbial NPs, such as in the case of improved yield of chloramphenicol in recombinant *S. avermitilis* (Doi et al., 2020). Increasing the amount of precursors can also increase the yield of NPs; for example, the targeted disruption of glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) in *S. clavuligerus* led to accumulation of D-glyceraldehyde-3-phosphate, a precursor of clavulanic acid, the yield of which subsequently increased more than 2-fold when compared to the wild type (Li and Townsend, 2006). While metabolic engineering can reduce the levels of unwanted byproducts and increase resource flux towards expression of the target BGCs, major drawbacks remain (Zhang et al., 2016). These hurdles include the need for annotated, high-quality genome-scale metabolic network models, which are available only for a small subset of well-described organisms (Norsigian et al., 2020), as well as identification of precursors of target NPs (Blin et al., 2019; He et al., 2018).

If the identity of the BGCs has been confirmed, they also may be cloned into a heterologous host, not only to confirm the relationship between BGCs and NPs (Kenshole et al., 2021; Zhang et al., 2018; Liang et al., 2020), but also because a different host organism might be more suitable for their expression (Nielsen et al., 2013). For example, heterologous host organisms are often well-characterized, have a high growth rate, there are established tools for their genetic manipulation, and their genome and metabolome is better suited for recombinant bioproduction than that of natural producers (Zhang et al., 2010). Expression platforms such as HEx (Heterologous Expression), which allow for a scalable expression of fungal BGCs in *S. cerevisiae* have been developed (Harvey et al., 2018). However, heterologous expression has limitations, such as the need to clone genome sequences of excessive size and challenges with identifying suitable hosts for expression of a variety of BGCs Atanasov et al., 2021. In addition to that, the introduction of foreign genetic material might also place a burden on the cellular resources, and lead to reduced yield of the target NPs (Frei et al., 2020; Boo et al., 2019). Species that are suitable for heterologous expression of BGCs may be further genetically modified to optimize their expression properties, for instance, via modification of precursor flux and deletion of antibiotic downregulation genes (Kallifidas et al., 2018). In other study, the deletion of 15 non-essential native BGCs resulted in an increased yield of natural products produced in *Streptomyces* sp. (Myronovskyi et al., 2018), probably because of an increased availability of cellular metabolic resources for the synthesis of target NPs. In addition to that, integration of additional phage *phiC31 attB* sites enabled enhanced chromosomal integration of multiple BGCs copies, resulting into higher production yields (Myronovskyi et al., 2018). However, there are drawbacks associated with heterologous expression. Recombinant production requires cloning of recombinant DNA into host organisms (Atanasov et al., 2021). Furthermore, the correct assembly of NPs may require changes at the post-translational level (Szewczyk et al., 2008), which are difficult to infer solely from genome mining (Kenshole et al., 2021). Therefore, despite the advances in strain and pathway engineering approaches, the number of novel BGCs that could encode for new NPs still vastly exceeds the number of secondary metabolites identified and isolated (Pettit, 2011).

2.3. Co-culturing of different microbial species

Approaches involving variation of culturing conditions and genetic engineering methods focus on eliciting the production of novel microbial NPs or increasing their yields in isolated, axenic cultures. On the other hand, in their natural setting, microbes co-inhabit their respective ecosystems with other living organisms, which leads to wealth of direct or indirect inter-species interactions (Fig. 1c). Such interplay between organisms elicits production of numerous compounds, many of which are valuable secondary metabolites that are produced as the result of activation of cryptic BGCs, which are silent under the non-natural,

axenic culturing conditions (Nai and Meyer, 2018). If an organism is grown in an axenic culture, secondary metabolites often are not produced at all (Onaka et al., 2011). In many cases, the *inducer*, an organism that induces production of the target secondary metabolite by the other member of the pair, the *producer*, can be clearly distinguished (Onaka et al., 2011; Abdelmohsen et al., 2015). Hence, we name such mixed cultures *inducer-producer consortia*. The name "inducer-producer" consortia does not necessarily mean that one member of the consortium directly provides an "inducing compound" such as intermediate metabolite. Rather, "induction" is to be understood in a broader sense: inducer organisms are providing a general "input" or stimulus, which is required for upregulation of bioproduction in the producer species; the nature of such an input can range from a competition for nutrients to direct secretion of an intermediate small molecule. Such consortia might contain interactions within the same domain of life (i.e. fungus-fungus, bacterium-bacterium) or across different domains of life (bacterium-fungus), where both bacterium and fungus may serve as either the inducer or producer species (Kim et al., 2021). In bacterium-bacterium cultures, *actinomycetes*, bacteria from the order *Actinomycetales* are often co-cultivated to produce NPs; many co-cultivation studies were summarized in the past reviews (Abdelmohsen et al., 2015; Hoshino et al., 2019; Kim et al., 2021). *Streptomyces* species are often employed in the screening for NPs, as they devote significant portion of their genome to secondary metabolite production (Challis and Hopwood, 2003). For example, co-cultivation of an inducer species *Streptomyces viridochromogenes* with producer *Streptomyces coelicolor M145* led to production of a series of secondary metabolites, including antibiotic Actinorhodin (Traxler et al., 2013). Fungal inducer-producer consortia often include species from the genus *Aspergillus*, such as co-cultivation of *Aspergillus sulphureus KMM 4640* and *Isaria felina KMM 4639* that led to production of five new prenylated indole alkaloids (Afiyatullov et al., 2018). Mixed cultures of fungus and bacterium are also commonly explored for NPs production, for example, co-culture of *Streptomyces hygroscopicus* with *Aspergillus nidulans* (Schroeckh et al., 2009) that led to an overproduction of orsellinic acid. The richness of different organisms and co-culturing systems only highlights the opportunities for the discovery of novel secondary metabolites.

Co-culturing of microbes for novel or enhanced bioproduction has several major advantages when compared to OSMAC-based approaches and genetic engineering. Firstly, co-culturing the producer microbes with other species greatly enhances the diversity of chemical interactions to which the given organism is exposed (Marmann et al., 2014), which include not only the direct interactions such as exchange of small molecules (Rosero-Chasoy et al., 2021), but also indirect interactions, such as competition for a shared pool of the resources and nutrients (Kim et al., 2021; Lee et al., 2021; Lee et al., 2020). This significantly increases the search space when compared to OSMAC approaches (Pan et al., 2019). Secondly, mechanical setup for co-culturing is often simple and does not require expensive equipment, which opens up the possibility for high-throughput screening of various co-cultures, for instance, using microfluidic platforms (Hesselman et al., 2012; Ingham et al., 2007). Thirdly, production of secondary metabolites via mixed culturing does not require thorough understanding of the biology and physiology of the co-cultured organisms, which is in stark contrast with activation of NP synthesis via complex genetic engineering approaches (Romano et al., 2018). While co-culturing of microorganisms has certain drawbacks, such as the requirement for existence of appropriate culturing medium for all members of the microbial consortium, because of the need for maintenance of the co-existence of both co-cultured species, and the ability to detect and isolate synthesized NPs (Zhuang et al., 2021), co-cultivation represents an attractive approach to discovery and production of valuable microbial compounds.

3. Mechanisms of production of secondary metabolites in inducer-producer consortia

3.1. Classifying the mechanisms of production

Classifying mechanisms of bioproduction of secondary metabolites in inducer-producer consortia is not a simple endeavour. The most important reason is that the majority of experimental studies in which bioproduction of NPs by mixed cultures was studied did not explore the mechanisms of such bioproduction (Zhuang et al., 2021). This is largely because the discovery and isolation of secondary metabolites produced in mixed microbial co-cultures is easier than thorough understanding of the underlying mechanisms of induction of NP production or increase of its yield. An alternative reason why a more detailed classification of the mechanisms of bioproduction in microbial co-cultures has not been completed is the fact that disentangling complex interactions in microbial communities is often impossible at the time of conducting of the study. For example, Kurosawa and colleagues were able to demonstrate that horizontal gene transfer (HGT) of a large segment of DNA from *Streptomyces padanus* to *Rhodococcus fascians* led to the production of rhodostreptomycins in the latter organism (Kurosawa et al., 2008). The authors were not able to determined whether the route of HGT was via transformation or conjugation, although the authors ruled out transduction (Kurosawa et al., 2010). However, from the perspective of optimizing a co-culture of such species, the distinction between transformation and conjugation may be important: while during transformation, uptake of exogenous DNA from the external environment does not necessarily require physical contact between organisms, conjugation, which involves transfer of genetic material via the conjugation pilus, does require physical contact (Soucy et al., 2015). In such case, the underlying mechanism of HGT may pre-determine the selection of the best co-cultivation method (Kapoore et al., 2021).

We propose a classification which, on the highest level, distinguishes between the need for physical contact for activated bioproduction in a co-culture (Fig. 2). This is an important consideration for the selection of a culturing device and overall implementation. For contact-dependent elicitation of NP production, a distinction between contact-dependent secondary metabolite synthesis, such as in case of mycolic acid-containing bacteria (Onaka et al., 2011; Onaka et al., 2015) and a HGT via conjugation (Lu et al., 2017) is made because of the difference in the underlying biological processes. For contact-independent bioproduction of secondary metabolites, we differentiate between direct and indirect interactions: An indirect interaction involves the production of NPs that is induced via stimuli arising because of environmental changes due to presence of culturing partner such as competition for scarce nutrients. In contrast, direct inter-species interaction involve the exchange of small molecules, large molecules and oligonucleotides between the organisms. Given the richness of contact-independent interaction, we provide a more granular categorization for such inducer-producer consortia (Fig. 3). We also provide and analyze examples of co-cultures for each of the described mechanism of induction or enhancement of NP production (Table 1).

Our classification approach builds upon and extends approaches developed in prior work. For example, *Kim et al.*, proposed that induction mechanisms of NPs can be divided into three categories: physical interactions, chemical communications, and HGT (Kim et al., 2021). Our approach further subdivides these categories to avoid potential overlaps (for example, as illustrated in the above example, HGT may or may not involve direct physical interaction). *Abdelmohsen et al.*, suggested four possible putative mechanisms of bioproduction of secondary metabolites: physical cell-to-cell interactions, small molecule-mediated interactions, catalytic activation of metabolite precursor, and HGT (Abdelmohsen et al., 2015). However, such a classification does not take into consideration the increased production of secondary metabolites by a member of the co-culture due to inter-species competition environmental niche (Park et al., 2009; Lee et al., 2020). *Liu* and *Kakeya*

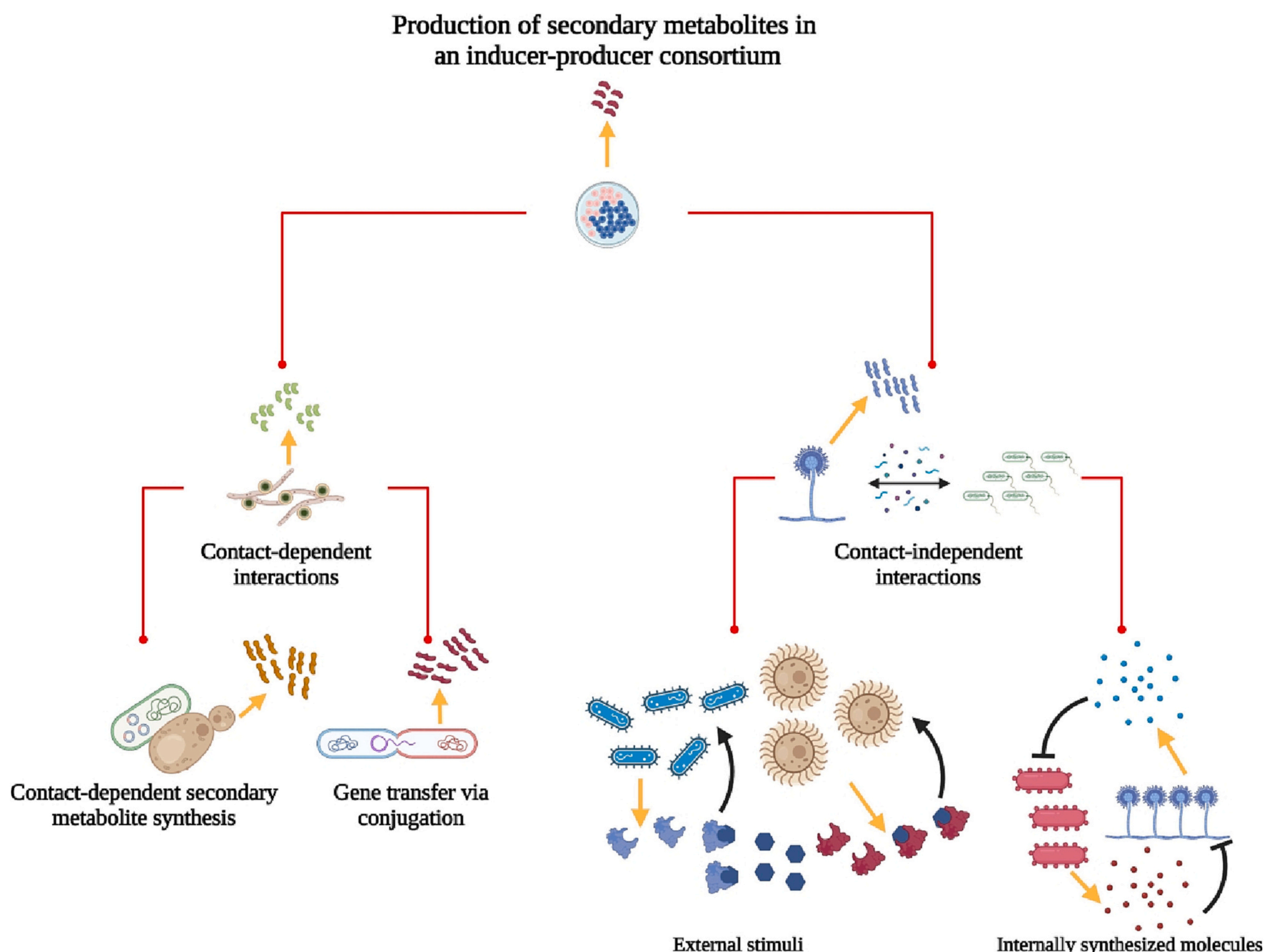

Fig. 2. Categorization of biological mechanisms that may lead to the production of microbial secondary metabolites in the naturally occurring (genetically unmodified) inducer-producer consortia. Fundamentally, a difference between the mechanisms of induction that require physical contact and those that only need chemical interaction is made. Next, physical interactions are split into two subcategories. Contact-dependent induction of secondary metabolites includes production caused by contact between mycolic acid-containing bacteria and fungi, as well as production induced due to contact-dependent secretion systems. Contact-dependent horizontal gene transfer methods include transfer of plasmids via conjugation and subsequent expression in a novel host. Chemical exchange-based methods of production of microbial NPs are versatile and include small molecule-mediated interactions, such as *quorum sensing*, enzymatic modifications of a precursor produced by a producer, direct or indirect competition between the species and non-contact genome modifications, for example, via gene loss or via transformation. Created with BioRender.com.

summarize several case studies of various mechanisms of induction of bioproduction in co-cultures (Liu and Kakeya, 2020); we build upon this work, forming it into a cohesive categorization system.

3.2. *Contact-dependent inter-species interactions*

Contact-dependent inter-species interactions involve the class of systems in which a direct physical contact between species is required in order to elicit production of the NPs in the mixed microbial culture. Mechanisms and biosynthetic pathways leading to the synthesis of secondary metabolites are frequently unclear, and there is a need for a larger number of studies examining contact-dependent interactions between microbes. Nevertheless, induction of contact-dependent production of NPs has been associated with epigenetic modifications (Nützmann et al., 2011), presence of specific compounds in the outer membrane of inducer species (Onaka et al., 2011; Onaka et al., 2015; Hoshino et al., 2019), conjugation (Kommineni et al., 2015), and contact-dependent growth inhibition (Garcia et al., 2016; Ikryannikova et al., 2020; Garcia, 2018).

Contact-dependent secondary metabolite synthesis. The production of NPs in a microbial consortium might require a direct physical contact between the co-cultured microbes in order to activate biosynthetic pathways in the producing organism (Onaka et al., 2015; Kim et al., 2021). Co-culturing fungus *Aspergillus nidulans* with *Streptomyces rapamycinus* led to the production of several secondary metabolites, including orsellinic acid, lechanoric acid, and Cathepsin-K inhibitors (Schroeckh et al., 2009). When the authors treated *A. nidulans* with either supernatant of the bacterial culture, co-culture extracts, or heat-inactivated bacteria, the activation of polyketide synthase *orsA* required for production of orsellinic acid was reduced by several orders of the magnitude (Schroeckh et al., 2009). Similarly, when the bacterium and fungus were separated by a dialysis bag, only miniscule levels of expression were observed (Schroeckh et al., 2009). The direct contact between the fungus and bacterium was required for elevating expression of orsellinic acid (Schroeckh et al., 2009). Subsequent research revealed that physical interactions between the fungus and bacterium induced histone modification via main histone acetyltransferase complex Saga/Ada in the fungal species, which induced the production of orsellinic acid and lechanoric acid and that Saga/Ada was also required for triggering synthesis of other secondary metabolites (Nützmann et al., 2011). The mixed culture between *A. nidulans* and *S. rapamycinus* represents one of the few examples where biological mechanisms of contact-

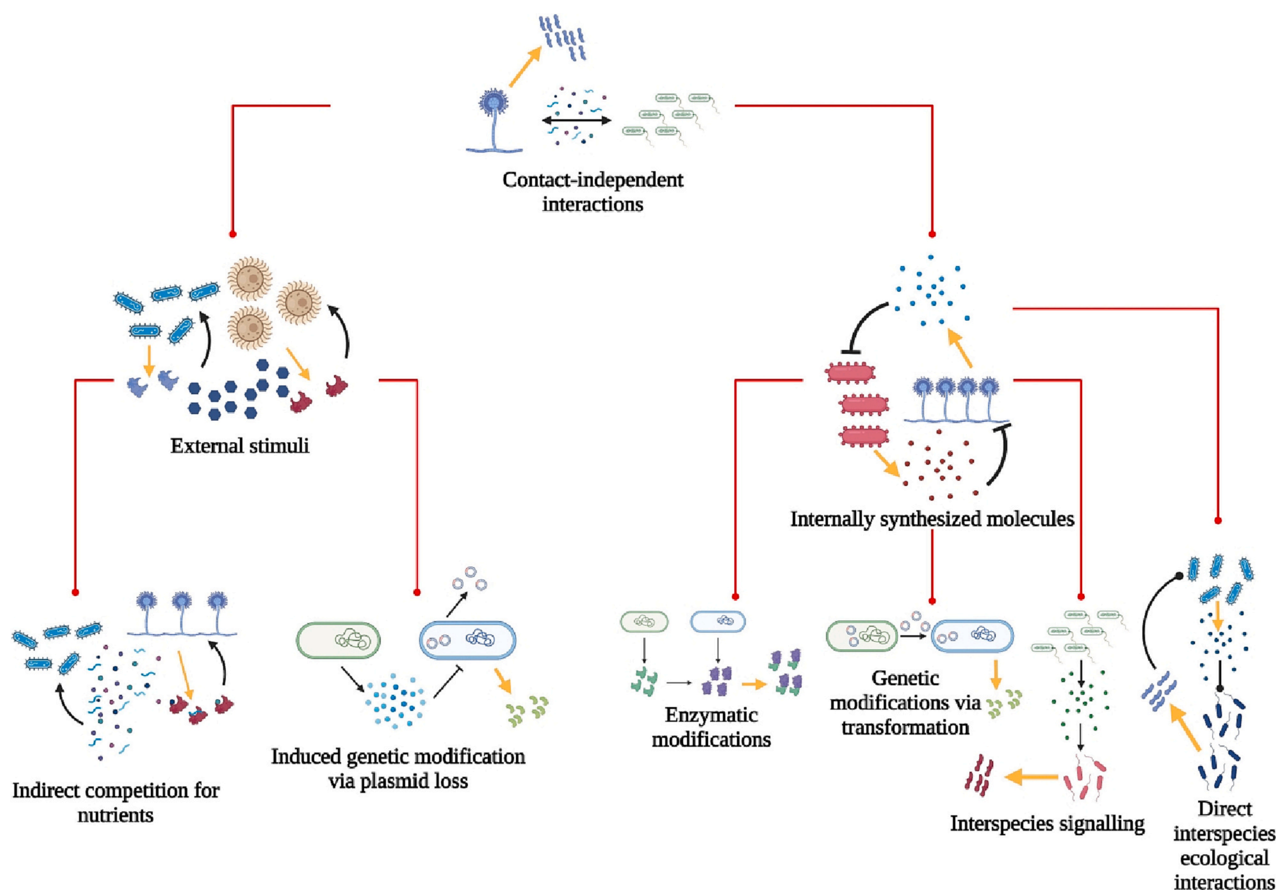

Fig. 3. Classification of contact-independent mechanisms of induction of secondary metabolites in microbial co-cultures. Bioproduction of NPs can be induced either via external stimuli, such as production of molecules increasing the nutrient uptake in response to the competition or non-HGT genetic modifications due to the modification of external environment by adverse species. Synthesis of secondary metabolites can also be induced as the consequence of small molecules, macro-molecules, or oligonucleotides directly produced by co-cultured microorganisms. Such interactions include enzymatic modification of precursors produced by producer strain by inducer species, genetic modification of producer species through non-contact HGT via transformation, direct inter-species signalling such as *quorum sensing*, and direct inter-species interactions, such as competition, commensalism and mutualism. Created with BioRender.com.

Table 1
Examples of different co-culturing systems and inducer-producer consortia for bioproduction of NPs and the mechanisms of bioproduction. I = inducer, P = producer, D = donor, A = acceptor.

Mechanism of induction	Interaction partners	Induced NPs	Activity	References
Contact-dependent secondary metabolite synthesis	*Tsukamurella pulmonis (I)* *Streptomyces lividans (P)* *Tsukamurella pulmonnis (I)* *Streptomyces endus (P)*	red pigment Alchivemycin A	pigment antibiotic	Onaka et al. (2011)
Gene transfer via conjugation	*Escherichia coli (D)* *Pseudomonas aeruginosa (A)*	gentamicin acetyltransferase	antibiotic resistance	Lu et al. (2017)
Indirect competition for nutrients	*Streptomyces coelicolor (I)* *Myxococcus xanthus (P)*	myxochelin (siderophore)	iron scavenging	Lee et al. (2020)
Induced genetic modification via plasmid loss	*Staphylococcus aureus (I)* *Streptomyces clavuligerus (P)*	holomycin	antibiotic	Charusanti et al. (2012)
Enzymatic modifications	*Pseudomonas maltophila* *Streptomyces griseorubiginosus*	biphenomycin A	antibiotic	Ezaki et al. (1992)
Genetic modifications via transformation	*Acinetobacter baylyi (D)* *Escherichia coli (A)*	Sh ble protein	zeomycin antibiotic resistance	Maeusli et al. (2020)
inter-species signalling	*Pseudomonas aeruginosa (I)* *Burkholderia cepacia (P)*	siderophore protease	iron sequestration proteolysis	McKenney et al. (1995) De Kievit and Iglewski (2000) Riedel et al. (2001)
Direct inter-species ecological interactions	*Bacillus subtilis (I)* *Fusarium tricinctum (P)*	lateropyrone enniatins fusaristatin A macrocarpon C	antibiotic	Ola et al. (2013)

dependent production were fully elucidated.

Another prominent class of contact-dependent production of NPs in inducer-producer consortia includes mycolic acid-containing bacteria. When bacterium *Streptomyces lividans* was co-cultured with Mycobacterium *Tsukamurella pulmonis*, production of red pigment was induced (Onaka et al., 2011). Subsequently, co-culturing *T. pulmonis* with *S. endus* led to production of a novel antibiotic, alchivemycin A, in *S, endus*. When the biosynthetic gene for synthesis of mycolic acid was disrupted, co-culture with *S. lividans* did not yield red pigment, while addition of mycolic acid extract did not induce production of red pigment in a monoculture of *S. lividans*. These results have shown that direct contact between viable mycolic acid-containing inducer and *Streptomyces* producer species was required for the production of secondary metabolites. A wealth of other secondary metabolites that have been discovered via co-culturing of producer strains with Mycobacteria have been summarized by Hoshino and colleagues (Hoshino et al., 2019). While Onaka and colleagues demonstrated that direct physical contact between the inducer and producer species was required, the exact biomolecular mechanisms of induction of NPs are unknown. The authors hypothesized that mycolic acid in the outer membrane of inducer microbes could stimulate the upper regulatory system in *Streptomyces* (Onaka et al., 2011). Alternatively, such inducer microbes could provide the digested nutrients and substrates required for the biosynthesis of the final secondary metabolites in the producer species (Onaka et al., 2015).

Contact-dependent growth inhibition may also induce production of antibiotic compounds, as well as gene expression and biosynthesis. Both Gram-positive and Gram-negative bacteria have developed systems for delivery of toxins into neighbouring microorganisms, which include widely conserved contact-dependent growth inhibition (CDI, type V secretion) and Type VI secretion system (T6SS) (Garcia, 2018; Blango and Mulvey, 2009), more unique Type IV secretion systems in *Xanthomonas* species (Souza et al., 2015), Type VII secretion systems (Esx pathway) and other secretion systems, such as surface-associated glycine-zipper toxins (Cdz) (García-Bayona and Comstock, 2018). In CDI, Gram-negative bacteria deliver a polymorphic toxin molecule into cytoplasm of neighbouring cells after direct cell-to-cell contact, which involves transfer of at least a portion of large exoprotein CdiA (or BcpA in *Burkholderia* and related species) into the target bacterium (Aoki et al., 2005) facilitated by proteins CdiB (BcpB in *Burkholderia*) (Garcia, 2018). On the other hand, T6SS is a contractile system that delivers protein toxins directly into neighboring cells of Gram-negative bacteria (Russell et al., 2014a). Numerous examples of contact-dependent secretion of toxins in the response to the presence of competing bacterial species have been reported; for example, *B. fragilis* employes T6SS to attack other *Bacteroides*, such as *B. thetaiotaomicron* in co-culture or in the mammalian gut when dietary polysaccharides are scarce (García-Bayona and Comstock, 2018; Russell et al., 2014b). Furthermore, gene expression in *Burkholderia thailandensis* was altered as the result of CDI; this phenomenon was named contact-dependent signalling (CDS) and led to cooperative changes, such as biofilm formation (Garcia et al., 2016). However, inter-species induction of secondary metabolites via CDS, though hypothesized, has not been observed.

Gene transfer via conjugation. Conjugation is a method of HGT that requires physical contact between the cells via a conjugation pilus, through which genetic material is transferred (Soucy et al., 2015). In addition to contact-dependent secondary metabolite synthesis, HGT via conjugation represents a viable mechanism for induction of production of secondary metabolites in co-cultures. Lu et al. co-cultured *E. coli* containing a mobilizable plasmid with the aacC1 gene cassette encoding gentamicin acetyltransferase, with *Pseudomonas aeruginosa* in the presence of antibiotic gentamicin (Lu et al., 2017). *P. aeruginosa* acquired gentamicin resistance via increased conjugation with *E. coli*. Interestingly, treatment of *P. aeruginosa* with *quorum sensing*-inhibiting antibiotic such as azithromycin or chloramphenicol showed a conjugation-promoting ability, which underlines the role of *quorum sensing* in conjugation. The role of *quorum sensing*, which does not require direct cell-to-cell contact in conjugative transfer, highlights the difficulties of developing an exhaustive and disjoint classification of mechanisms of bioproduction in co-culture. Another interesting case of HGT via conjugation has been demonstrated by Kommineni et al., who have shown that *Enterococcus faecalis* harbouring plasmid expressing bacteriocin 21 was able to transfer this plasmid to other *E. faecalis* strains via conjugation in a model of the mouse gut (Kommineni et al., 2015). HGT via conjugation offers an interesting opportunity for bioproduction of valuable NPs in rationally designed co-cultures, especially under the increased evolutionary pressure due to antibiotics. Such an approach may be used to allow for a bioproduction of valuable secondary metabolites in well-characterized hosts with better growth properties and with increased yield.

3.3. Contact-independent inter-species interactions

Contact-independent inter-species interactions represent the class of interactions in which direct physical contact between the populations of different organisms is not required. The first subcategory includes inter-species interactions due to external stimuli, chiefly indirect competition for nutrients and natural genetic modifications of producer strains via natural loss or acquisition of foreign genetic material, which do not include direct transfer of genetic material between the species. The second category encompasses interplay via molecules directly synthesized by individual species. Such interactions include enzymatic modifications, genetic modifications via direct transfer of oligonucleotides between different microbes, direct inter-species signalling via small molecules and direct ecological interactions that do not involve physical contact (Fig. 3).

Indirect competition for nutrients. The bioproduction of novel metabolites in microbial consortium can be induced because of the scarcity of the resources shared between the microorganisms. When different species of microorganisms inhabit the same niche, they often compete for the limited nutrients and environmental resources (Marmann et al., 2014; Zhang et al., 2018; Zengler and Zaramela, 2018). While such a competition may involve direct inter-species killing mediated by toxins (O'Brien and Wright, 2011), it may also demonstrate itself via synthesis of secondary metabolites by individual species that improve their nutrient uptake (Kim et al., 2021). In the co-culture of *Streptomyces coelicolor* with *Myxococcus xanthus*, both species compete for a shared iron pool (Lee et al., 2020). In the co-culturing process, *M. xanthus* increased its production of a siderophore myxochelin, which allowed it to sequester the iron and dominate iron scavenging. Such upregulation of iron sequestration by *M. xanthus* led to significant upregulation of production of antibiotic actinorhodin by *S. coelicolor*, which has the ability to repel *M. xanthus* and allows *S. coelicolor* to chelate iron.

Induced genetic modification via plasmid loss. Genetic modification of a microorganism in a co-culture with another microbe may induce a loss of certain genetic elements in the producer species, which may lead to induction of bioproduction of novel NPs. Evolutionary adaptation of microbial species to environmental challenge is one of the key mechanisms for their survival (Bottery et al., 2020). In the presence of an environmental challenge, such as other species competing for nutrients or in a presence of a pathogen, microbes have to develop mechanisms to face such threats. In a co-culture with methicilin-resistant *Staphylococcus aureus* (MRSA), *Streptomyces clavuligerus* acquired ability to constitutively produce antibiotic holomycin against MRSA, which could not be detected in the unevolved, wild type (Charusanti et al., 2012). Genomic analysis of evolved *S. clavuligerus* revealed the loss of a large, 1.8 Mbp megaplasmid pSCL4, as well as an acquisition of several single nucleotide modifications in the genes that influence synthesis of secondary metabolites. Prior analysis of megaplasmid pSCL4 has shown that it is a rich reservoir of BGCs for the potential synthesis of secondary metabolites, including putative antibiotics, such

as staurosporine and β-lactams (Medema et al., 2010). It is possible that the presence of pSCL4 led to repression of the BGCs for holomycin synthesis and its loss was needed to allow for its synthesis.

Enzymatic modifications. Another mechanism of production of valuable compounds in inducer-producer consortia is a modification of the precursor produced by the producer strain by an enzyme produced by the inducer microbe (Abdelmohsen et al., 2015). Such mode of production of NPs has been demonstrated in a co-culture of *Streptomyces griseorubiginosus* and *Pseudomonas maltophilia*, where *S. griseorubiginosus* produced a precursor of biphenomycin A, an antibiotic, and *P. maltophilia* subsequently converted this precursor into the final product (Ezaki et al., 1992). A subsequent structural and cultivation study revealed the structure of the precursor produced by *S. griseorubiginosus*, which was named biphenomycin C (Ezaki et al., 1993). This elegant case of cooperative bioproduction bears a significant similarity to approaches where an engineered microbial consortium is used to split the engineered metabolic pathway among multiple species in a co-culture (Roell et al., 2019). In another study, Liang et al., co-cultured *Streptomyces sp. RKBH-B178* with heat-killed *P. aeruginosa* and *M. smegatis*. *Streptomyces sp. RKBH-B178* was able to transform *P. aeruginosa quorum sensing* molecules pseudomonas quinolone signal (PQS) and 2-heptyl-4-quinolone (HHQ) into their glucoronidated versions, which led to deactivation of their *quorum sensing* capability (Liang et al., 2019).

Genetic modification via transformation. Contactless HGT via transformation can lead to the bioproduction of novel microbial compounds in heterologous host. Unlike HGT via conjugation, neither HGT via transformation (delivery of the genetic material via uptake of plasmid DNA), nor via transduction (transfer of genetic material, phage predation and integration of exogenous genes into the phage genome) require direct contact between microbes in the consortium (Soucy et al., 2015). Transformation has been shown to lead to HGT of ble protein conferring resistance against zeomycin from *Acinetobacter baylyi* into *E. coli* during *in vitro* co-culture, on lettuce, and in BALB/c mice (Maeusli et al., 2020). *A. baylyi ADP1* is a great candidate as a member of an engineered microbial consortium due to its high natural transformation competence, recombination efficiency, as well as metabolic diversity (Santala and Santala, 2021); hence, the frequency of its use in co-culturing studies involving HGT is expected to rise. In another study, *Cooper et al.*, co-cultured *A. baylyi* with *E. coli* carrying a kanamycin resistance plasmid. The authors showed that *A. baylyi* killed *E. coli* in a contact-dependent fashion via the T6SS system, while subsequent transfer of plasmids with kanamycin resistance into *A. baylyi* via natural transformation increased up to 100-fold (Cooper et al., 2017). Importantly, the authors developed and quantified a population-dynamic mathematical model of natural plasmid transformation as well as contact-dependent killing (Cooper et al., 2017). This is a particularly important result, given that mathematical modelling of dynamics in co-cultures has been neglected in many studies that were only limited to phenomenological observations. However, this co-culturing study reflects the difficulties of developing a rigorous classification of mechanisms of production of inducer-producer consortia. While expression of antibiotic resistance proteins was due to HGT via transformation, the killing of *E. coli* by *A. baylyi* was enhanced by contact-dependent killing, which makes such a system difficult to classify.

Inter-species signalling. Inter-species signalling via uni-directional or multi-directional exchange of molecules is one of the key mechanisms of intra-species and inter-species communication (Mehta et al., 2009; Shank and Kolter, 2009). Exchange of the signalling molecules in a co-culture can lead to the upregulation of secondary metabolite synthesis (Bertrand et al., 2014; Marmann et al., 2014), as well as other physiological phenomena, such as biofilm formation (Oliveira et al., 2015). One of the best understood intercellular communication systems is *quorum sensing*, a cell-to-cell communication system in which microbes produce and detect chemical compounds to monitor the population density in their environment (Ng and Bassler, 2009). Autoinducer-2 (AI-2) *quorum sensing* system has a particularly important role because of its involvement in inter-species signalling (Pereira et al., 2013; Bivar Xavier, 2018). In an inducer-producer consortium, the inducer may synthesize signalling molecules that are transported into extracellular space and subsequently interact with producer species, either through interaction with the surface receptors or via direct diffusion into the cells, where they activate gene expression and synthesis of NPs (Abdelmohsen et al., 2015). Multiple studies have shown the ability of quorum-sensing molecules to induce or enhance production of secondary metabolites. For example, addition of *quorum sensing* molecule N-butyryl-DL-homoserine lactone to culture medium in which *A. fumigatus* was grown led to isolation of emestrins A-B. Synthesis of these species was not observed in the pure fungal culture (Rateb et al., 2013). While production of Emestrins A-B was induced by addition of a *quorum sensing* molecule into the medium, they were not detected in co-culture of producer *Aspergillus fumigatus* and inducer *Streptomyces bullii*, though other novel secondary metabolites were detected (Rateb et al., 2013). Unfortunately, bacteria that naturally produce N-butyryl-DL-homoserine lactone such as *Salmonella* and *E. coli* were not co-cultivated with *A. fumigatus* in this experiment, but co-cultivation of *E. coli* and *A. fumigatus* could lead to activation of bioproduction of emestrin A and B.

One of the prominent examples of *quorum sensing*-mediated inter-species signalling is found in the co-culture of *P. aeruginosa* and *Burkholderia cepacia*, species that are the major pathogens in cystic fibrosis and cause several nosocomial infections in immuno-compromised patient populations (Eberl and Tümmler, 2004). The addition of concentrated cell-free material from stationary phase cultures of *P. aeruginosa* to the culture of *B. cepacia* led to significant upregulation of protease and siderophore synthesis by *B. cepacia* (McKenney et al., 1995). Subsequently, homologs of luxRI have been identified in *B. cepacia* (Lewenza et al., 1999). Co-culturing experiments between *B. cepacia* and *P. aeruginosa* demonstrated that *B. cepacia* was able to sense N-acylhomoserine lactone molecules synthesized by *P. aeruginosa* (Riedel et al., 2001). Furthermore, experiments have shown that the inter-species signalling is unidirectional and *B. cepacia* responds to signals from *P. aeruginosa*, while *P. aeruginosa* does not respond to signals from *B. cepacia* (Riedel et al., 2001; Bragonzi et al., 2012). Together, these results demonstrate the role of small molecule-mediated signalling as the mechanism of induction of NPs production. *Quorum sensing*-mediated interactions are a particularly interesting tool for engineering microbial interactions in rationally engineered microbial consortia, given their modularity and relatively good biological understanding (McCarty and Ledesma-Amaro, 2019). Numerous examples of inter-species interactions engineered using *quorum sensing* molecules have been presented (Liao et al., 2019; Miano et al., 2020; Mee et al., 2014; Kong et al., 2018).

Direct inter-species ecological interactions. In the microbial consortia, the bioproduction of secondary metabolites may be a result of direct ecological interactions between microbes. Direct killing, in particular, is one of the natural mechanisms that microorganisms employ to secure their survival in an environment with limited nutrients or in the presence of a pathogen (Marmann et al., 2014; Netzker et al., 2015; García-Bayona and Comstock, 2018). Multiple examples of interactions in which the inducer stimulates production of a producer's secondary metabolite that is toxic to the inducer exist; such co-cultures have been particularly useful for the discovery of novel antibacterial (Gonzalez et al., 2011; Sun et al., 2022) and antifungal compunds (Chagas et al., 2013). Many such systems have been described elsewhere (Bertrand et al., 2014; Kim et al., 2021; Abdelmohsen et al., 2015). Therefore, we describe two particularly interesting examples that demonstrate the importance of optimization of co-culturing conditions. Co-culturing fungus *Fusarium trinctum* with bacterium *Bacillus subtilis* led to up to 78-fold increase of constitutively produced fungal NPs, including increase in production of lateropyrone, which exhibited inhibitory effect against *B. subtilis* (Ola et al., 2013). Interestingly, the upregulation of fungal secondary metabolism only occurred if *B. subtilis*

was inoculated several days before *F. trinctum* (Ola et al., 2013; Marmann et al., 2014), which suggests that optimization of the microbial growth dynamics while taking into account inter-species ecological relationship is an important factor for improving the yield of the secondary metabolites in co-cultures.

Another interesting example that illustrates the complexity of relationships in inducer-producer consortia is co-cultivation of *Streptomyces sp. CMB-M0423* and *Aspergillus sp. CMB-AsM0423* isolated from Heron Island, Queensland, Australia (Khalil et al., 2019). In this work, the authors described a microbial community in which *Aspergillus sp.* produced a bacteriostatic metabolite *cyclo*-(L-Phe-trans-4-hydroxy-L-Pro) that induced production of nitric oxide (NO), which, in turn, mediated activation of a silent biosynthetic gene cluster (BGCs) in *Streptomyces sp.* that led to production of fungistatic heronapyrrole B (Khalil et al., 2019). This work demonstrates not only the unique example of thorough investigation of the mechanism of activation of NPs production in inducer-producer consortium, but also an intriguing feedback loop constructed by the mutual relationship between species. The ecological significance of such a complex interaction pattern is yet to be understood.

4. Setting up inducer-producer consortia for bioproduction

One of the key considerations for the successful discovery and production of novel NPs in the inducer-producer co-cultures is the nature of the culturing system and the physical setting in which such bioproduction occurs. The different co-culturing systems include 1) communal liquid medium growth cultures (Padmaperuma et al., 2018; Shuler and Kargi, 2001), 2) solid–liquid interface systems (Covarrubias et al., 2012), 3) membrane-separated systems (Briand et al., 2016), 4) spatially separated systems (Kim et al., 2011; Kim and Chung, 2004), and 5) microfluidic systems (Barkal et al., 2016) (Fig. 4). These co-culturing systems have recently been summarized in an excellent review by Kapoore and colleagues (Kapoore et al., 2021). Communal liquid medium growth methods consist of co-culturing microorganisms in a shared culturing medium and involve direct mixing, pelletization and floculation, as well as biofilm formation by the microbial community. In the case of pelletization and flocculation, one of the microorganisms releases bioflocculants, which lead to formation of aggregates by the other member of the co-culture, while in biofilm formation, the members of the consortia secrete substances that form a composite network. Solid–liquid interface systems require that the co-culture is trapped in a porous container suspended in a liquid or gas and include methods such as encapsulation and formation of cell droplets. Membrane-separated systems employ semipermeable membranes that prevent direct contact between cultured systems, but allow for the communication between co-culture partners through the exchange of signalling molecules. On the other hand, spatially separated systems prevent the exchange of materials via direct contact, but allow for an indirect communication through contact of different phase, such as liquid–solid and gas–liquid phases (Kapoore et al., 2021). Microfluidic systems allow for the precise control of the fluid flow, microenvironment composition and spatial arrangement in microbial co-cultures at a small scale, which allows for a better mimicking of the natural environment of microbial consortium (Kapoore et al., 2021).

All the co-culturing systems for microbial consortia posses certain advantages and disadvantages, which are summarized in Table 2. While applications of high-throughput microfluidic technology are useful for high-throughput screening of microbial secondary metabolites (Grünberger et al., 2015; Inoue et al., 2001), their potential for scaling up the bioproduction is small. Spatially separated systems might be ideal for studying microbial co-cultures in which the interaction is mediated via small molecule, however their use for co-culturing of systems that require physical contact for bioproduction would not be useful. While co-culturing using microscale capillary flow may be selected if physical contact is not required; submersive cultivation may be chosen if physical contact is essential (Nai and Meyer, 2018). Consequently, the selection of an appropriate co-culturing system depends on the specific biological properties of the microbial consortium under investigation, as well as on the objective that is to be achieved by the specific bioprocess. Furthermore, if a discovery of a novel secondary metabolite in a co-culture is suspected to be due to a HGT that occurs on a longer timescale, a sufficient amount of nutrient has to be provided to sustain the prolonged co-cultivation and the appropriate mode has to be employed if a bioreactor is used (e.g. batch/fed-batch/continuous) (Mandli and Modak, 2011). Therefore, the nature of inter-species interactions in the investigated microbial consortia may have a major impact on the physical setting and the infrastructure required for the optimal production of NPs.

The key design and operational considerations vary across these

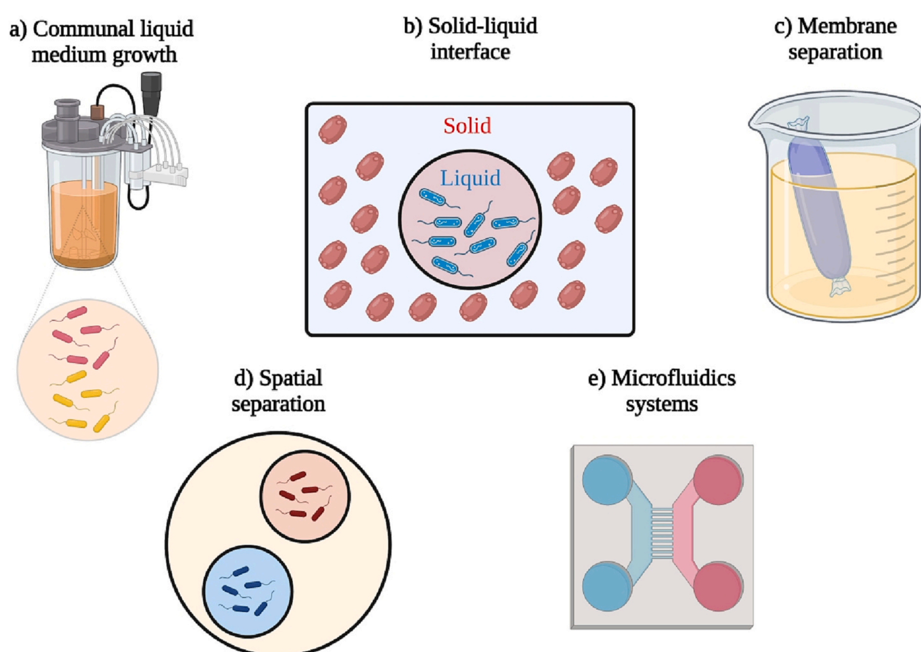

Fig. 4. The different physical setups for co-culturing of microbial consortia. **a)** Communal liquid medium growth cultures involve growth of microorganisms in a shared liquid medium. **b)** Solid–liquid interface co-culturing methods require trapping of co-culture within a porrous vessel suspended in a liquid or gaseous medium. **c)** Membrane separation systems entail segregation of co-culturing partners by a semipermeable membrane. **d)** In spatial separation systems, individual members of a microbial consortium are not in a direct contact and no direct material exchange is possible. **e)** Microfluidics systems allow for the precise manipulation of the co-cultured systems at a small scale. Created with BioRender.com.

Table 2
The summary of different co-cultivation techniques, their advantages, disadvantages, and scale-up ability (Kapoore et al., 2021; Nai and Meyer, 2018).

Co-culturing method	Description	Advantages	Disadvantages	Scale-up ability
Communal liquid medium growth	Growth of microorganisms in a shared liquid medium	Simplicity Low cost	Need for inoculation timing and inoculation ratio Requires ability of organisms to grow on a shared substrate	Medium to high
Solid–liquid interface	Trapping co-culture within a porous vessel suspended in a liquid or gaseous medium	Protecting organisms from environmental stress Reduce substrate competition Co-culturing organisms with dissimilar growth characteristics	Potentially reduced growth Reduced biomass production Low scalability Possible leaks of cultured organisms into environment	Low
Membrane separation	Separating co-culturing partners by a semipermeable membrane	Improved population density monitoring Easy setup Potentially larger culture volumes are possible	Pre-optimization might be required Not suitable for consortia requiring physical contact	Low to medium
Spatial separation	Spatial separation prohibiting a direct exchange of materials	Eliminated nutrient competition Application for analysis of impact of microbial volatile compounds Possibly long-term consortium preservation	Not a true reflection of interactions between organisms in natural habitat Mass transfer limitations	Low to medium
Microfluidics	Manipulation with co-cultures at a small scale	Better control over fluids and microenvironment Simple to use Rapid workflow	Operations at low volumes	Low to medium

culturing systems. Communal liquid growth media systems require optimization of the inoculation ratio of co-cultured microbes, as well as its inoculation timing. Solid–liquid interface systems must achieve adequate surface for an effective contact and chemical exchange and sufficient culture densities. Membrane separation systems require a pre-optimization setup and tuning of the membrane permeability in order to allow for the appropriate level of exchange of the desired molecules. Spatial separation setups require careful optimization of the matrix and medium composition to avoid restricting mass transfer. Finally, microfluidic systems have design requirements such as an appropriate spatial orientation and optimal fluid flow to maximize interactions between culturing partners (Kapoore et al., 2021). Therefore, elucidation of microbial interactions in inducer-producer consortia is not the only challenge that complex microbial communities possess; physical culturing setups represent a major factor in the successful discovery and bioproduction of novel NPs.

5. Constructing inducer-producer consortia for success

Optimization of the discovery of novel microbial NPs in inducer-producer consortia or maximizing their yield represents a multifaceted, multidimensional challenge. Nevertheless, we note that despite challenges associated with bioproduction of novel mocrobial secondary metabolites and increasing their yield, numerous successful examples of co-culturing were summarized in several excellent reviews (Kapoore et al., 2021; Zhuang et al., 2021; Arora et al., 2020; Bertrand et al., 2014; Kim et al., 2021). In this section, we present four key considerations for culture optimization: 1) selection of the inducer-producer pair, 2) co-culturing setup, 3) selection of the method for compound isolation and identification, 4) optimization of the culturing conditions (Fig. 5). To successfully exploit the bioproduction potential of inducer-producer microbial co-cultures, all of these optimization variables deserve adequate attention.

Firstly, appropriate co-culturing partners must be selected. The choice of inducer and producer has often been informed by prior research endeavours, or based on a random screening (Marmann et al., 2014). The key considerations include the culturability of both inducer and producer on the selected medium, which already signficantly reduces the spectrum of microbes that can be considered for a co-culture (Vartoukian et al., 2010; Overmann et al., 2017), as well as the potential of microorganism to produce novel secondary metabolites, which can be indicated by the presence of silent BGCs in the genomes of these microbes (Rutledge and Challis, 2015).

Secondly, an appropriate co-culturing setup has to be selected in order to allow for the appropriate interaction of the microbial populations (Kapoore et al., 2021). If a culturing setup that does not correspond to underlying biological nature of microbial interactions is selected (for example, if an inducer-producer consortium that requires intimate spatial contact is spatially separated), the impact on the production of novel secondary metabolites and their yield might be devastating. Even the specific implementation of the particular co-culturing setup might be critical. For example, establishing a batch process is often less complicated than setting up a continuous culture; on the other hand, continuous culture allows for an increased yield of the

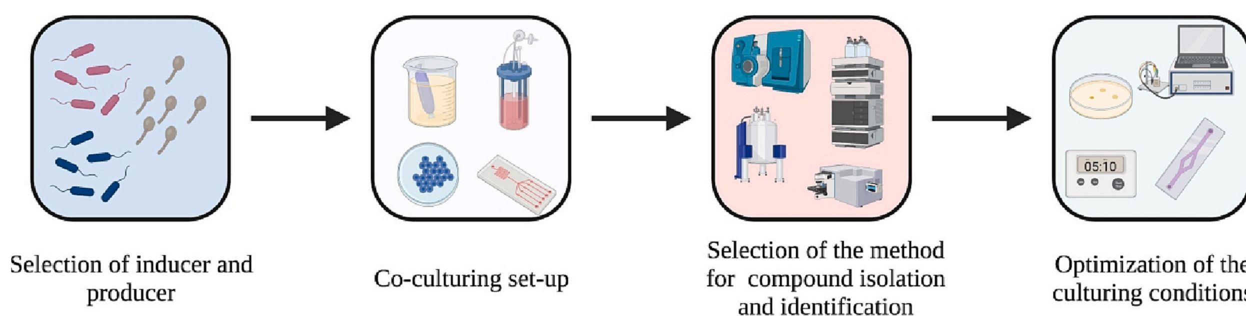

Fig. 5. The key considerations for successful setup of microbial co-cultures include selection of the appropriate inducer-producer pair, specific physical co-culturing setup, selection of the method for compound isolation and identification, and optimization of the culturing conditions. Created withBioRender.com.

product with smaller equipment (Liu, 2016). With the advent of new, small-scale continuous reactors with multiple control inputs such as *Chi. Bio*, prototyping of continuous cultures for optimal co-culturing microbial consortia may be simplified (Steel et al., 2020).

Thirdly, a suitable technique for the targeted compound isolation and identification has to be employed. Though not the focus of this work, we note that such techniques include high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS) or nuclear magnetic resonance spectroscopy (NMR) and other methods (Zhang et al., 2018).

Finally, culturing conditions, such as inducer-to-producer inoculation ratio, induction time, as well as the physical setups should be tuned in order to achieve maximum over-expression or diversity of the NPs produced in an inducer-producer consortium (Kapoore et al., 2021). Given that, to date, many of the co-culturing studies fall short of an investigation of the mechanisms of induction of secondary metabolite production (Arora et al., 2020), this significantly reduces the opportunities for analytical, model-based optimization of bioproduction in such systems. This is a missed opportunity given the fact that multiple frameworks for modelling of inter-species interactions in the ecological systems including microbe-microbe interactions and microbe-plant interactions systems have been developed (van den Berg et al., 2022; Schulte et al., 2021). In addition to that, the combination of methods of feedback control theory and systems biology can allow not only for the quantitative description and understanding of the microbial systems (Ren and Murray, 2019; Manhart and Shakhnovich, 2018), but also, potentially their rational design and optimization of discovery of novel NPs and their yield maximization (Arpino et al., 2013; Harris et al., 2015; Hsiao et al., 2018).

Additional key challenges associated with co-culturing of inducer-producer consortia include difficulties with observing metabolic induction in co-culture, poor reproducibility of the results of co-culturing studies, need for selection of the appropriate time to observe the induction, and challenges of compound isolation and identification (Arora et al., 2020). Furthermore, successful deployment of inducer-producer consortia for bioproduction faces additional bottlenecks: accumulation of potentially toxic by-products in the co-culture; difficulties with increasing the efficacy of material transfer between co-culture members; issues with stability of the microbial co-cultures, and, importantly, effective identification of the underlying biological mechanisms of interaction in the mixed cultures.

6. Conclusion

Inducer-producer microbial consortia have a massive potential to enrich the repertoire of natural products available for use in various fields, including in healthcare, food industry, cosmetics and other areas (Bertrand et al., 2014; Arora et al., 2020). In addition to that, co-culturing of directly or indirectly interacting microbial species can lead to a drastic increase in the level of biosynthesis of biologically attractive secondary metabolites (Bertrand et al., 2014; Onaka et al., 2015; Arora et al., 2020; Zhuang et al., 2021).

It is, therefore, unsurprising that numerous studies have focused on the discovery of novel NPs and over-expression of natural secondary metabolites produced by microbes. However, remarkably little attention has been devoted to the investigation of mechanism beneath bioproduction in inducer-producer consortia. In this review, we attempted to exhaustively categorize the different mechanisms that govern bioproduction in natural microbial consortia. Classifying interactions in microbial systems is difficult because of their convoluted nature, as well as the potential complex interplay between different mechanisms of interaction. Our categorization system provides a set of distinctions not only between contact-dependent and contact-independent relationships, but also further dissects contact-independent interactions and develops functional differentiation between their various types. Note that in our analysis, we purposefully omitted engineered microbial systems, in which biological mechanisms of bioproduction, such as division of labour, are often well-understood (McCarty and Ledesma-Amaro, 2019; Rafieenia et al., 2022). Notwithstanding, our classification system can be used to categorize engineered microbial systems as well.

We believe that this approach opens up future research avenues with potential practical implications for the design of novel co-culturing systems. In particular, we believe that use of the modelling techniques of systems biology to quantitatively describe inducer-producer consortia mechanisms will advance our understanding of such systems. In addition to that, methods of control theory can be applied to inducer-producer consortia setups, which could allow for model-based optimization of such co-culturing systems. With progress in the characterization of biological systems, development of new co-culturing set-ups and bioreactors, improvement of methods for identification, analysis and quantification of secondary metabolites, and progress in biological control, inducer-producer consortia will increasingly represent an attractive and powerful tool for future bioproduction efforts. Certain challenges associated with the stability of inducer-producer systems and their long-term evolutionary adaptation remain (D'souza and Kost, 2016), as do challenges associated with up-scaling of the co-cultures (Arora et al., 2020). Nevertheless, the importance of bioproduction of valuable compounds in mixed microbial communities will continue to emerge; and so will the research and commercial opportunities associated with the investigation of inducer-producer consortia.

Data availability

No data was used for the research described in the article.

References

Abdelmohsen, U.R., Grkovic, T., Balasubramanian, S., Kamel, M.S., Quinn, R.J., Hentschel, U., 2015. Elicitation of secondary metabolism in actinomycetes. Biotechnol. Adv. 33, 798–811. https://doi.org/10.1016/j.biotechadv.2015.06.003.

Afiyatullov, S.S., Zhuravleva, O.I., Antonov, A.S., Berdyshev, D.V., Pivkin, M.V., Denisenko, V.A., Popov, R.S., Gerasimenko, A.V., von Amsberg, G., Dyshlovoy, S.A., Leshchenko, E.V., Yurchenko, A.N., 2018. Prenylated indole alkaloids from co-culture of marine-derived fungi Aspergillus sulphureus and Isaria felina. J. Antibiot. 71, 846–853. https://doi.org/10.1038/s41429-018-0072-9.

Alper, H., Moxley, J., Nevoigt, E., Fink, G.R., Stephanopoulos, G., 2006. Engineering yeast transcription machinery for improved ethanol tolerance and production. Science (New York, N.Y.) 314, 1565–1568. https://doi.org/10.1126/SCIENCE.1131969.

Aoki, S.K., Pamma, R., Hernday, A.D., Bickham, J.E., Braaten, B.A., Low, D.A., 2005. Microbiology: Contact-dependent inhibition of growth in Escherichia coli. Science 309, 1245–1248. https://doi.org/10.1126/SCIENCE.1115109/SUPPL_FILE/AOKI-SOM.PDF.

Arora, D., Gupta, P., Jaglan, S., Roullier, C., Grovel, O., Bertrand, S., 2020. Expanding the chemical diversity through microorganisms co-culture: Current status and outlook. Biotechnol. Adv. 40, 107521 https://doi.org/10.1016/J.BIOTECHADV.2020.107521.

Arpino, J.A., Hancock, E.J., Anderson, J., Barahona, M., Stan, G.B.V., Papachristodoulou, A., Polizzi, K., 2013. Tuning the dials of synthetic biology. Microbiology (United Kingdom) 159, 1236–1253. https://doi.org/10.1099/mic.0.067975-0.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Orhan, I.E., Banach, M., Rollinger, J.M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E.A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G.K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., Diederich, M., Dinkova-Kostova, A.T., Efferth, T., El Bairi, K., Arkells, N., Fan, T.P., Fiebich, B.L., Freissmuth, M., Georgiev, M.I., Gibbons, S., Godfrey, K.M., Gruber, C.W., Heer, J., Huber, L.A., Ibanez, E., Kijjoa, A., Kiss, A.K., Lu, A., Macias, F.A., Miller, M.J., Mocan, A., Müller, R., Nicoletti, F., Perry, G., Pittalà, V., Rastrelli, L., Ristow, M., Russo, G.L., Silva, A.S., Schuster, D., Sheridan, H., Skalicka-Woźniak, K., Skaltsounis, L., Sobarzo-Sánchez, E., Bredt, D.S., Stuppner, H., Sureda, A., Tzvetkov, N.T., Vacca, R.A., Aggarwal, B.B., Battino, M., Giampieri, F., Wink, M., Wolfender, J.L., Xiao, J., Yeung, A.W.K., Lizard, G., Popp, M.A., Heinrich, M., Berindan-Neagoe, I., Stadler, M., Daglia, M., Verpoorte, R., Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. Nat. Rev. Drug Discov. 203 (20), 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Baral, B., Akhgari, A., Metsä-Ketelä, M., 2018. Activation of microbial secondary metabolic pathways: Avenues and challenges. Synth. Syst. Biotechnol. 3, 163–178. https://doi.org/10.1016/J.SYNBIO.2018.09.001.

Barkal, L.J., Theberge, A.B., Guo, C.J., Spraker, J., Rappert, L., Berthier, J., Brakke, K.A., Wang, C.C., Beebe, D.J., Keller, N.P., Berthier, E., 2016. Microbial metabolomics in open microscale platforms. Nat. Commun. 7 (1), 1–11. https://doi.org/10.1038/ncomms10610.

van den Berg, N.I., Machado, D., Santos, S., Rocha, I., Chacón, J., Harcombe, W., Mitri, S., Patil, K.R., 2022. Ecological modelling approaches for predicting emergent properties in microbial communities. Nat. Ecol. Evol. 6 (7), 855–865. https://doi.org/10.1038/s41559-022-01746-7.

Bergmann, S., Funk, A.N., Scherlach, K., Schroeckh, V., Shelest, E., Horn, U., Hertweck, C., Brakhage, A.A., 2010. Activation of a silent fungal polyketide biosynthesis pathway through regulatory cross talk with a cryptic nonribosomal peptide synthetase gene cluster. Appl. Environ. Microbiol. 76, 8143–8149. https://doi.org/10.1128/AEM.00683-10.

Bergmann, S., Schümann, J., Scherlach, K., Lange, C., Brakhage, A.A., Hertweck, C., 2007. Genomics-driven discovery of PKS-NRPS hybrid metabolites from Aspergillus nidulans. Nat. Chem. Biol. 3 (4), 213–217. https://doi.org/10.1038/nchembio869.

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. Biotechnol. Adv. 32, 1180–1204. https://doi.org/10.1016/J.BIOTECHADV.2014.03.001.

Bhatt, P., Bhatt, K., Sharma, A., Zhang, W., Mishra, S., Chen, S., 2021. Biotechnological basis of microbial consortia for the removal of pesticides from the environment. Crit. Rev. Biotechnol. 41, 317–338. https://doi.org/10.1080/07388551.2020.1853032.

Bivar Xavier, K., 2018. Bacterial interspecies quorum sensing in the mammalian gut microbiota. C.R. Biol. 341, 297–299. https://doi.org/10.1016/J.CRVI.2018.03.006.

Blango, M.G., Mulvey, M.A., 2009. Bacterial Landlines: Contact-dependent Signaling in Bacterial Populations. Curr. Opin. Microbiol. 12, 177. https://doi.org/10.1016/J.MIB.2009.01.011.

Blin, K., Kim, H.U., Medema, M.H., Weber, T., 2019. Recent development of antiSMASH and other computational approaches to mine secondary metabolite biosynthetic gene clusters. Brief. Bioinform. 20, 1103–1113. https://doi.org/10.1093/BIB/BBX146.

Blin, K., Shaw, S., Kloosterman, A.M., Charlop-Powers, Z., Van Wezel, G.P., Medema, M.H., Weber, T., 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res. 49, W29–W35. https://doi.org/10.1093/NAR/GKAB335.

Bode, H.B., Bethe, B., Hofs, R., Zeeck, A., 2002. Big Effects from Small Changes: Possible Ways to Explore Nature's Chemical Diversity. ChemBioChem 3, 619–627. https://doi.org/10.1002/1439-7633.

Boo, A., Ellis, T., Stan, G.B., 2019. Host-aware synthetic biology. Curr. Opin. Syst. Biol. 14, 66–72. https://doi.org/10.1016/j.coisb.2019.03.001.

Boruta, T., Bizukojc, M., 2019. Application of aluminum oxide nanoparticles in aspergillus terreus cultivations: Evaluating the effects on lovastatin production and fungal morphology. BioMed Res. Int. 2019 https://doi.org/10.1155/2019/5832496.

Bose, J.L., 2016. Chemical and UV Mutagenesis. Methods Mol. Biol. (Clifton, N.J.) 1373, 111–115. https://doi.org/10.1007/7651_2014_190.

Bottery, M.J., Pitchford, J.W., Friman, V.P., 2020. Ecology and evolution of antimicrobial resistance in bacterial communities. ISME J. 154 (15), 939–948. https://doi.org/10.1038/s41396-020-00832-7.

Bouassida, M., Ghazala, I., Ellouze-Chaabouni, S., Ghribi, D., 2018. Improved Biosurfactant Production by Bacillus subtilis SPB1 Mutant Obtained by Random Mutagenesis and Its Application in Enhanced Oil Recovery in a Sand System. J. Microbiol. Biotechnol. 28, 95–104. https://doi.org/10.4014/JMB.1701.01033.

Bragonzi, A., Farulla, I., Paroni, M., Twomey, K.B., Pirone, L., Lorè, N.I., Bianconi, I., Dalmastri, C., Ryan, R.P., Bevivino, A., 2012. Modelling Co-Infection of the Cystic Fibrosis Lung by Pseudomonas aeruginosa and Burkholderia cenocepacia Reveals Influences on Biofilm Formation and Host Response. PLoS ONE 7. https://doi.org/10.1371/JOURNAL.PONE.0052330.

Briand, E., Bormans, M., Gugger, M., Dorrestein, P.C., Gerwick, W.H., 2016. Changes in secondary metabolic profiles of Microcystis aeruginosa strains in response to intraspecific interactions. Environ. Microbiol. 18, 384–400. https://doi.org/10.1111/1462-2920.12904.

Chagas, F.O., Dias, L.G., Pupo, M.T., 2013. A Mixed Culture of Endophytic Fungi Increases Production of Antifungal Polyketides. J. Chem. Ecol. 39, 1335–1342. https://doi.org/10.1007/S10886-013-0351-7/FIGURES/3.

Challis, G.L., Hopwood, D.A., 2003. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species. Proc. Natl. Acad. Sci. U.S.A. 100, 14555–14561. https://doi.org/10.1073/PNAS.1934677100.

Charusanti, P., Fong, N.L., Nagarajan, H., Pereira, A.R., Li, H.J., Abate, E.A., Su, Y., Gerwick, W.H., Palsson, B.O., 2012. Exploiting Adaptive Laboratory Evolution of Streptomyces clavuligerus for Antibiotic Discovery and Overproduction. PLOS ONE 7, e33727. https://doi.org/10.1371/JOURNAL.PONE.0033727.

Chiang, Y.M., Szewczyk, E., Davidson, A.D., Keller, N., Oakley, B.R., Wang, C.C., 2009. A gene cluster containing two fungal polyketide synthases encodes the biosynthetic pathway for a polyketide, asperfuranone, in aspergillus nidulans. J. Am. Chem. Soc. 131, 2965–2970. https://doi.org/10.1021/JA8088185/SUPPL_FILE/JA8088185_SI_002.PDF.

Cleveland, J., Montville, T.J., Nes, I.F., Chikindas, M.L., 2001. Bacteriocins: safe, natural antimicrobials for food preservation. Int. J. Food Microbiol. 71, 1–20. https://doi.org/10.1016/S0168-1605(01)00560-8.

Cooper, R.M., Tsimring, L., Hasty, J., 2017. Inter-species population dynamics enhance microbial horizontal gene transfer and spread of antibiotic resistance. eLife 6. https://doi.org/10.7554/ELIFE.25950.

Covarrubias, S.A., De-Bashan, L.E., Moreno, M., Bashan, Y., 2012. Alginate beads provide a beneficial physical barrier against native microorganisms in wastewater treated with immobilized bacteria and microalgae. Appl. Microbiol. Biotechnol. 93, 2669–2680. https://doi.org/10.1007/S00253-011-3585-8/FIGURES/5.

David, B., Wolfender, J.L., Dias, D.A., 2015. The pharmaceutical industry and natural products: historical status and new trends. Phytochem. Rev. 14, 299–315. https://doi.org/10.1007/S11101-014-9367-Z/TABLES/1.

De Kievit, T.R., Iglewski, B.H., 2000. Bacterial quorum sensing in pathogenic relationships. Infect. Immun. 68, 4839–4849. https://doi.org/10.1128/IAI.68.9.4839-4849.2000/FORMAT/EPUB.

Doi, S., Komatsu, M., Ikeda, H., 2020. Modifications to central carbon metabolism in an engineered Streptomyces host to enhance secondary metabolite production. J. Biosci. Bioeng. 130, 563–570. https://doi.org/10.1016/J.JBIOSC.2020.08.006.

D'souza, G., Kost, C., 2016. Experimental Evolution of Metabolic Dependency in Bacteria. PLoS Genet. 12, 1006364. https://doi.org/10.1371/journal.pgen.1006364.

Du, Y.H., Wang, M.Y., Yang, L.H., Tong, L.L., Guo, D.S., Ji, X.J., 2022. Optimization and Scale-Up of Fermentation Processes Driven by Models. Bioengineering 9, 473. https://doi.org/10.3390/BIOENGINEERING9090473.

Eberl, L., Tümmler, B., 2004. Pseudomonas aeruginosa and Burkholderia cepacia in cystic fibrosis: genome evolution, interactions and adaptation. Int. J. Med. Microbiol. 294, 123–131. https://doi.org/10.1016/J.IJMM.2004.06.022.

Ezaki, M., Iwami, M., Yamashita, M., Komori, T., Umehara, K., Imanaka, H., 1992. Biphenomycin A production by a mixed culture. Appl. Environ. Microbiol. 58, 3879–3882. https://doi.org/10.1128/AEM.58.12.3879-3882.1992.

Ezaki, M., Shigematsu, N., Yamashita, M., Komori, T., Umehara, K., Imanaka, H., 1993. Biphenomycin C, a precursor of biphenomycin A in mixed culture. J. Antibiot. 46, 135–140.

Formenti, L.R., Nørregaard, A., Bolic, A., Hernandez, D.Q., Hagemann, T., Heins, A.L., Larsson, H., Mears, L., Mauricio-Iglesias, M., Krühne, U., Gernaey, K.V., 2014. Challenges in industrial fermentation technology research. Biotechnol. J. 9, 727–738. https://doi.org/10.1002/BIOT.201300236.

Frei, T., Cella, F., Tedeschi, F., Gutiérrez, J., Stan, G.B., Khammash, M., Siciliano, V., 2020. Characterization and mitigation of gene expression burden in mammalian cells. Nat. Commun. 11, 4641. https://doi.org/10.1038/s41467-020-18392-x.

Frisvad, J.C., 2012. Media and growth conditions for induction of secondary metabolite production. Methods Mol. Biol. (Clifton, N.J.) 944, 47–58. https://doi.org/10.1007/978-1-62703-122-6_3.

Fuchser, J., Thiericke, R., Zeeck, A., 1995. Biosynthesis of aspinonene, a branched pentaketide produced by Aspergillus ochraceus, related to aspyrone. J. Chem. Soc., Perkin Trans. 1 (1), 1663–1666. https://doi.org/10.1039/P19950001663.

Gao, Q., Garcia-Pichel, F., 2011. Microbial ultraviolet sunscreens. Nat. Rev. Microbiol. 9 (11), 791–802. https://doi.org/10.1038/nrmicro2649.

Garcia, E.C., 2018. Contact-dependent interbacterial toxins deliver a message. Curr. Opin. Microbiol. 42, 40–46. https://doi.org/10.1016/J.MIB.2017.09.011.

Garcia, E.C., Perault, A.I., Marlatt, S.A., Cotter, P.A., 2016. Interbacterial signaling via Burkholderia contact dependent growth inhibition system proteins. Proc. Natl. Acad. Sci. U.S.A. 113, 8296–8301. https://doi.org/10.1073/PNAS.1606323113/-/DCSUPPLEMENTAL/PNAS.1606323113.SAPP.PDF.

García-Bayona, L., Comstock, L.E., 2018. Bacterial antagonism in host-associated microbial communities. Science 361. https://doi.org/10.1126/SCIENCE.AAT2456/ASSET/30D68E51-4A02-4613-AF39-4FAA097D19E2/ASSETS/GRAPHIC/361_AAT2456_F3.JPEG.

Gonciarz, J., Bizukojc, M., 2014. Adding talc microparticles to Aspergillus terreus ATCC 20542 preculture decreases fungal pellet size and improves lovastatin production. Eng. Life Sci. 14, 190–200. https://doi.org/10.1002/ELSC.201300055.

Gonzalez, D.J., Haste, N.M., Hollands, A., Fleming, T.C., Hamby, M., Pogliano, K., Nizet, V., Dorrestein, P.C., 2011. Microbial competition between Bacillus subtilis and Staphylococcus aureus monitored by imaging mass spectrometry. Microbiology 157, 2485–2492. https://doi.org/10.1099/MIC.0.048736-0/CITE/REFWORKS.

Grünberger, A., Probst, C., Helfrich, S., Nanda, A., Stute, B., Wiechert, W., von Lieres, E., Nöh, K., Frunzke, J., Kohlheyer, D., 2015. Spatiotemporal microbial single-cell analysis using a high-throughput microfluidics cultivation platform. Cytometry Part A 87, 1101–1115. https://doi.org/10.1002/CYTO.A.22779.

Gupta, P.L., Rajput, M., Oza, T., Trivedi, U., Sanghvi, G., 2019. Eminence of Microbial Products in Cosmetic Industry. Nat. Products Bioprospect. 9, 267–278. https://doi.org/10.1007/S13659-019-0215-0/TABLES/3.

Gustafson, K., Roman, M., Fenical, W., 1989. The Macrolactins, a Novel Class of Antiviral and Cytotoxic Macrolides from a Deep-Sea Marine Bacterium. J. Am. Chem. Soc. 111, 7519–7524. https://doi.org/10.1021/JA00201A036.

Harris, A.W., Dolan, J.A., Kelly, C.L., Anderson, J., Papachristodoulou, A., 2015. Designing Genetic Feedback Controllers. IEEE Trans. Biomed. Circuits Syst. 9, 475–484. https://doi.org/10.1109/TBCAS.2015.2458435.

Harvey, C.J., Tang, M., Schlecht, U., Horecka, J., Fischer, C.R., Lin, H.C., Li, J., Naughton, B., Cherry, J., Miranda, M., Li, Y.F., Chu, A.M., Hennessy, J.R., Vandova, G.A., Inglis, D., Aiyar, R.S., Steinmetz, L.M., Davis, R.W., Medema, M.H., Sattely, E., Khosla, C., Onge, R.P., Tang, Y., Hillenmeyer, M.E., 2018. HEx: A heterologous expression platform for the discovery of fungal natural products. Sci. Adv. 4 https://doi.org/10.1126/SCIADV.AAR5459.

He, Y., Wang, B., Chen, W., Cox, R.J., He, J., Chen, F., 2018. Recent advances in reconstructing microbial secondary metabolites biosynthesis in Aspergillus spp. Biotechnol. Adv. 36, 739–783. https://doi.org/10.1016/J.BIOTECHADV.2018.02.001.

Hesselman, M.C., Odoni, D.I., Ryback, B.M., de Groot, S., van Heck, R.G., Keijsers, J., Kolkman, P., Nieuwenhuijse, D., van Nuland, Y.M., Sebus, E., Spee, R., de Vries, H., Wapenaar, M.T., Ingham, C.J., Schroën, K., Martins dos Santos, V.A., Spaans, S.K., Hugenholtz, F., van Passel, M.W., 2012. A Multi-Platform Flow Device for Microbial (Co-) Cultivation and Microscopic Analysis. PLOS ONE 7, e36982. https://doi.org/10.1371/JOURNAL.PONE.0036982.

van der Hoek, S.A., Borodina, I., 2020. Transporter engineering in microbial cell factories: the ins, the outs, and the in-betweens. Curr. Opin. Biotechnol. 66, 186–194. https://doi.org/10.1016/J.COPBIO.2020.08.002.

Hong, J., Im, D.K., Oh, M.K., 2020. Investigating E. coli Coculture for Resveratrol Production with 13C Metabolic Flux Analysis. J. Agric. Food. Chem. 68, 3466–3473. https://doi.org/10.1021/ACS.JAFC.9B07628.

Hoshino, S., Onaka, H., Abe, I., 2019. Activation of silent biosynthetic pathways and discovery of novel secondary metabolites in actinomycetes by co-culture with mycolic acid-containing bacteria. J. Ind. Microbiol. Biotechnol. 46, 363–374. https://doi.org/10.1007/S10295-018-2100-Y.

Hsiao, V., Swaminathan, A., Murray, R.M., 2018. Control Theory for Synthetic Biology: Recent Advances in System Characterization, Control Design, and Controller Implementation for Synthetic Biology. IEEE Control Syst. 38, 32–62. https://doi.org/10.1109/MCS.2018.2810459.

Hüter, O.F., 2011. Use of natural products in the crop protection industry. Phytochem. Rev. 10, 185–194. https://doi.org/10.1007/S11101-010-9168-Y/FIGURES/12.

Ikryannikova, L.N., Kurbatov, L.K., Gorokhovets, N.V., Zamyatnin, A.A., 2020. Contact-Dependent Growth Inhibition in Bacteria: Do Not Get Too Close! Int. J. Mol. Sci. 21, 7990. https://doi.org/10.3390/IJMS21217990.

Ingham, C.J., Sprenkels, A., Bomer, J., Molenaar, D., Van Den Berg, A., Van Hylckama Vlieg, J.E., De Vos, W.M., 2007. The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms. Proc. Natl. Acad. Sci. U.S.A. 104, 18217–18222. https://doi.org/10.1073/PNAS.0701693104/SUPPL_FILE/IMAGE869.GIF.

Inoue, I., Wakamoto, Y., Moriguchi, H., Okano, K., Yasuda, K., 2001. On-chip culture system for observation of isolated individual cells. Lab Chip 1, 50–55. https://doi.org/10.1039/B103931H.

Jekosch, K., Kuck, U., 2000. Loss of glucose repression in an Acremonium chrysogenum beta-lactam producer strain and its restoration by multiple copies of the cre1 gene. Appl. Microbiol. Biotechnol. 54, 556–563. https://doi.org/10.1007/S002530000422.

Jiang, C., Lv, G., Tu, Y., Cheng, X., Duan, Y., Zeng, B., He, B., 2021. Applications of CRISPR/Cas9 in the Synthesis of Secondary Metabolites in Filamentous Fungi. Front. Microbiol. 12, 164. https://doi.org/10.3389/FMICB.2021.638096/BIBTEX.

Jiang, T., Li, C., Teng, Y., Zhang, R., Yan, Y., 2020. Recent advances in improving metabolic robustness of microbial cell factories. Curr. Opin. Biotechnol. 66, 69–77. https://doi.org/10.1016/J.COPBIO.2020.06.006.

Kallifidas, D., Jiang, G., Ding, Y., Luesch, H., 2018. Rational engineering of Streptomyces albus J1074 for the overexpression of secondary metabolite gene clusters. Microb. Cell Fact. 17, 1–14. https://doi.org/10.1186/S12934-018-0874-2/FIGURES/7.

Kapoore, R.V., Padmaperuma, G., Maneein, S., Vaidyanathan, S., 2021. Co-culturing microbial consortia: approaches for applications in biomanufacturing and bioprocessing. Crit. Rev. Biotechnol. https://doi.org/10.1080/07388551.2021.1921691.

Ke, T., Liu, J., Zhao, S., Wang, X., Wang, L., Li, Y., Lu, Y., Hui, F., 2020. Using Global Transcription Machinery Engineering (GTME) and Site-Saturation Mutagenesis Technique to Improve Ethanol Yield of Saccharomyces cerevisiae. Appl. Biochem. Microbiol. 56, 563–568. https://doi.org/10.1134/S0003683820050087/FIGURES/4.

Kealey, C., Creaven, C.A., Murphy, C.D., Brady, C.B., 2017. New approaches to antibiotic discovery. Biotechnol. Lett. 39, 805–817. https://doi.org/10.1007/s10529-017-2311-8.

Kenshole, E., Herisse, M., Michael, M., Pidot, S.J., 2021. Natural product discovery through microbial genome mining. Curr. Opin. Chem. Biol. 60, 47–54. https://doi.org/10.1016/J.CBPA.2020.07.010.

Khalil, Z.G., Cruz-Morales, P., Licona-Cassani, C., Marcellin, E., Capon, R.J., 2019. Inter-Kingdom beach warfare: Microbial chemical communication activates natural chemical defences. ISME J. 13, 147–158. https://doi.org/10.1038/s41396-018-0265-z.

Kim, H.J., Du, W., Ismagilov, R.F., 2011. Complex function by design using spatially pre-structured synthetic microbial communities: degradation of pentachlorophenol in the presence of Hg(ii). Integr. Biol.: Quant. Biosci. Nano Macro 3, 126–133. https://doi.org/10.1039/C0IB00019A.

Kim, H.U., Charusanti, P., Lee, S.Y., Weber, T., 2016. Metabolic engineering with systems biology tools to optimize production of prokaryotic secondary metabolites. Nat. Prod. Rep. 33, 933–941. https://doi.org/10.1039/C6NP00019C.

Kim, J.H., Lee, N., Hwang, S., Kim, W., Lee, Y., Cho, S., Palsson, B.O., Cho, B.K., 2021. Discovery of novel secondary metabolites encoded in actinomycete genomes through coculture. J. Ind. Microbiol. Biotechnol. 48, 1. https://doi.org/10.1093/JIMB/KUAA001.

Kim, P.I., Chung, K.C., 2004. Production of an antifungal protein for control of Colletotrichum lagenarium by Bacillus amyloliquefaciens MET0908. FEMS Microbiol. Lett. 234, 177–183. https://doi.org/10.1016/J.FEMSLE.2004.03.032.

Kommineni, S., Bretl, D.J., Lam, V., Chakraborty, R., Hayward, M., Simpson, P., Cao, Y., Bousounis, P., Kristich, C.J., Salzman, N.H., 2015. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. Nature 526 (7575), 719–722. https://doi.org/10.1038/nature15524.

Kong, W., Meldgin, D.R., Collins, J.J., Lu, T., 2018. Designing microbial consortia with defined social interactions. Nat. Chem. Biol. 14, 821–829. https://doi.org/10.1038/s41589-018-0091-7.

Kurosawa, K., Ghiviriga, I., Sambandan, T.G., Lessard, P.A., Barbara, J.E., Rha, C., Sinskey, A.J., 2008. Rhodostreptomycins, antibiotics biosynthesized following horizontal gene transfer from Streptomyces padanus to Rhodococcus fascians. J. Am. Chem. Soc. 130, 1126–1127. https://doi.org/10.1021/JA077821P/SUPPL_FILE/JA077821P-FILE001.PDF.

Kurosawa, K., MacEachran, D.P., Sinskey, A.J., 2010. Antibiotic biosynthesis following horizontal gene transfer: new milestone for novel natural product discovery? Expert Opin. Drug Discov. 5, 819–825. https://doi.org/10.1517/17460441.2010.505599.

Landry, B.P., Tabor, J.J., 2017. Engineering Diagnostic and Therapeutic Gut Bacteria. Microbiol. Spectr. 5 https://doi.org/10.1128/microbiolspec.bad-0020-2017.

Laureti, L., Song, L., Huang, S., Corre, C., Leblond, P., Challis, G.L., Aigle, B., 2011. Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in Streptomyces ambofaciens. Proc. Natl. Acad. Sci. U.S.A. 108, 6258–6263. https://doi.org/10.1073/PNAS.1019077108/-/DCSUPPLEMENTAL.

Lee, N., Hwang, S., Kim, W., Lee, Y., Kim, J.H., Cho, S., Kim, H.U., Yoon, Y.J., Oh, M.K., Palsson, B.O., Cho, B.K., 2021. Systems and synthetic biology to elucidate secondary metabolite biosynthetic gene clusters encoded in Streptomyces genomes. Nat. Prod. Rep. 38, 1330–1361. https://doi.org/10.1039/D0NP00071J.

Lee, N., Kim, W., Chung, J., Lee, Y., Cho, S., Jang, K.S., Kim, S.C., Palsson, B., Cho, B.K., 2020. Iron competition triggers antibiotic biosynthesis in Streptomyces coelicolor during coculture with Myxococcus xanthus. ISME J. 14, 1111–1124. https://doi.org/10.1038/S41396-020-0594-6.

Lewenza, S., Conway, B., Greenberg, E.P., Sokol, P.A., 1999. Quorum sensing in Burkholderia cepacia: Identification of the LuxrI homologs CepRI. J. Bacteriol. 181, 748–756. https://doi.org/10.1128/JB.181.3.748-756.1999/ASSET/698A7E69-4CC3-499A-A16A-F0C226794ED6/ASSETS/GRAPHIC/JB0390750006.JPEG.

Lewis, K., 2013. Platforms for antibiotic discovery. Nat. Rev. Drug Discov. 12, 371–387. https://doi.org/10.1038/nrd3975.

Li, R., Townsend, C.A., 2006. Rational strain improvement for enhanced clavulanic acid production by genetic engineering of the glycolytic pathway in Streptomyces clavuligerus. Metab. Eng. 8, 240–252. https://doi.org/10.1016/J.YMBEN.2006.01.003.

Li, Y.P., Yu, P., Li, J.F., Tang, Y.L., Bu, Q.T., Mao, X.M., Li, Y.Q., 2019. FadR1, a pathway-specific activator of fidaxomicin biosynthesis in Actinoplanes deccanensis Yp-1. Appl. Microbiol. Biotechnol. 103, 7583–7596. https://doi.org/10.1007/S00253-019-09949-Y.

Liang, L., Sproule, A., Haltli, B., Marchbank, D.H., Berrué, F., Overy, D.P., McQuillan, K., Lanteigne, M., Duncan, N., Correa, H., Kerr, R.G., 2019. Discovery of a new natural product and a deactivation of a quorum sensing system by culturing a producer bacterium with a heat-killed inducer culture. Front. Microbiol. 10, 3351. https://doi.org/10.3389/FMICB.2018.03351/BIBTEX.

Liang, M., Liu, L., Wang, W., Zeng, X., Liu, J., Karthik, L., Zhu, G., Bai, L., Hou, C., Chen, X., Ouyang, L., Liu, X., Hu, B., Xia, X., Tong, Y., Lou, C., Alterovitz, G., Tan, G.Y., Zhang, L.X., 2020. Simple cloning of large natural product biosynthetic gene cluster by CRISPR/Cas12a-mediated fast direct capturing strategy. bioRxiv, 2020.06.25.170191. doi:10.1101/2020.06.25.170191.

Liao, M.J., Din, M.O., Tsimring, L., Hasty, J., 2019. Rock-paper-scissors: Engineered population dynamics increase genetic stability. Science 365, 1045–1049. https://doi.org/10.1126/science.aaw0542.

Liu, C., Kakeya, H., 2020. Cryptic Chemical Communication: Secondary Metabolic Responses Revealed by Microbial Co-culture. Chem. – Asian J. 15, 327–337. https://doi.org/10.1002/ASIA.201901505.

Liu, K., Hu, X.R., Zhao, L.X., Wang, Y., Deng, Z., Taoa, M., 2021. Enhancing Ristomycin A Production by Overexpression of ParBLike StrR Family Regulators Controlling the Biosynthesis Genes. Appl. Environ. Microbiol. 87, 1–19. https://doi.org/10.1128/AEM.01066-21/SUPPL_FILE/AEM.01066-21-S0002.XLSX.

Liu, L., Liu, Y., Li, J., Du, G., Chen, J., 2011. Microbial production of hyaluronic acid: current state, challenges, and perspectives. Microb. Cell Fact. 10, 1–9. https://doi.org/10.1186/1475-2859-10-99/TABLES/1.

Liu, S., 2016. Bioprocess Engineering: Kinetics, Sustainability, and Reactor Design: Second Edition.

Lu, Y., Zeng, J., Wang, L., Lan, K., Shunmei, E., Wang, L., Xiao, Q., Luo, Q., Huang, X., Huang, B., Chen, C., 2017. Antibiotics promote Escherichia coli-Pseudomonas aeruginosa conjugation through inhibiting quorum sensing. Antimicrob. Agents Chemother. 61 https://doi.org/10.1128/AAC.01284-17/SUPPL_FILE/ZAC012176739S1.PDF.

Lv, X., Xue, H., Qin, L., Li, C., 2022. Transporter Engineering in Microbial Cell Factory Boosts Biomanufacturing Capacity. BioDesign Res. 2022 https://doi.org/10.34133/2022/9871087.

Maeusli, M., Lee, B., Miller, S., Reyna, Z., Lu, P., Yan, J., Ulhaq, A., Skandalis, N., Spellberg, B., Luna, B., 2020. Horizontal Gene Transfer of Antibiotic Resistance from Acinetobacter baylyi to Escherichia coli on Lettuce and Subsequent Antibiotic Resistance Transmission to the Gut Microbiome. mSphere 5. https://doi.org/10.1128/MSPHERE.00329-20/SUPPL_FILE/MSPHERE.00329-20-ST002.DOCX.

Maiden, M.C., 1998. Horizontal genetic exchange, evolution, and spread of antibiotic resistance in bacteria. Clin. Infect. Dis. 27, S12–S20. https://doi.org/10.1086/514917.

Mandli, A.R., Modak, J.M., 2011. Evolutionary Algorithm for the Determination of Optimal Mode of Bioreactor Operation. Ind. Eng. Chem. Res. 51, 1796–1808. https://doi.org/10.1021/IE2006142.

Manhart, M., Shakhnovich, E.I., 2018. Growth tradeoffs produce complex microbial communities on a single limiting resource. Nat. Commun. 9, 1–9. https://doi.org/10.1038/s41467-018-05703-6 arXiv:1802.05774.

MarketWatch, 2021. Microbial Products Market Size.

Marmann, A., Aly, A.H., Lin, W., Wang, B., Proksch, P., 2014. Co-cultivation: A powerful emerging tool for enhancing the chemical diversity of microorganisms. Mar Drugs 12, 1043–1065. https://doi.org/10.3390/md12021043.

McCarty, N.S., Ledesma-Amaro, R., 2019. Synthetic Biology Tools to Engineer Microbial Communities for Biotechnology. Trends Biotechnol. 37, 181–197. https://doi.org/10.1016/j.tibtech.2018.11.002.

McKenney, D., Brown, K.E., Allison, D.G., 1995. Influence of Pseudomonas aeruginosa exoproducts on virulence factor production in Burkholderia cepacia: evidence of interspecies communication. J. Bacteriol. 177, 6989. https://doi.org/10.1128/JB.177.23.6989-6992.1995.
Medema, M.H., Trefzer, A., Kovalchuk, A., Van Den Berg, M., Müller, U., Heijne, W., Wu, L., Alam, M.T., Ronning, C.M., Nierman, W.C., Bovenberg, R.A., Breitling, R., Takano, E., 2010. The sequence of a 1.8-mb bacterial linear plasmid reveals a rich evolutionary reservoir of secondary metabolic pathways. Genome Biol. Evol. 2, 212–224. https://doi.org/10.1093/GBE/EVQ013.
Mee, M.T., Collins, J.J., Church, G.M., Wang, H.H., 2014. Syntrophic exchange in synthetic microbial communities. Proc. Natl. Acad. Sci. U.S.A. 111 https://doi.org/10.1073/pnas.1405641111.
Mehta, P., Goyal, S., Long, T., Bassler, B.L., Wingreen, N.S., 2009. Information processing and signal integration in bacterial quorum sensing. Mol. Syst. Biol. 5, 325. https://doi.org/10.1038/MSB.2009.79.
Miano, A., Liao, M.J., Hasty, J., 2020. Inducible cell-to-cell signaling for tunable dynamics in microbial communities. Nat. Commun. 11, 1193. https://doi.org/10.1038/s41467-020-15056-8.
Minas, W., 2005. Production of Erythromycin With Saccharopolyspora erythraea. Microb. Process. Prod. 18, 65–90. https://doi.org/10.1385/1-59259-847-1:065.
Mo, S., Ki, J.H., Oh, C.H., 2013. Different effects of acidic pH shock on the prodiginine production in Streptomyces coelicolor M511 and SJM1 mutants. J. Microbiol. Biotechnol. 23, 1454–1459. https://doi.org/10.4014/JMB.1307.07067.
Mózsik, L., Hoekzema, M., de Kok, N.A., Bovenberg, R.A., Nygård, Y., Driessen, A.J., 2021. CRISPR-based transcriptional activation tool for silent genes in filamentous fungi. Sci. Rep. 11 (1), 1–13. https://doi.org/10.1038/s41598-020-80864-3.
Myronovskyi, M., Rosenkränzer, B., Nadmid, S., Pujic, P., Normand, P., Luzhetskyy, A., 2018. Generation of a cluster-free Streptomyces albus chassis strains for improved heterologous expression of secondary metabolite clusters. Metab. Eng. 49, 316–324. https://doi.org/10.1016/J.YMBEN.2018.09.004.
Nai, C., Meyer, V., 2018. From Axenic to Mixed Cultures: Technological Advances Accelerating a Paradigm Shift in Microbiology. Trends Microbiol. 26, 538–554. https://doi.org/10.1016/J.TIM.2017.11.004.
Netzker, T., Fischer, J., Weber, J., Mattern, D.J., König, C.C., Valiante, V., Schroeckh, V., Brakhage, A.A., 2015. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front. Microbiol. 6, 299. https://doi.org/10.3389/FMICB.2015.00299/BIBTEX.
Ng, W.L., Bassler, B.L., 2009. Bacterial Quorum-Sensing Network Architectures. Annu. Rev. Genet. 43, 197–222. https://doi.org/10.1146/ANNUREV-GENET-102108-134304.
Nielsen, M.T., Nielsen, J.B., Anyaogu, D.C., Holm, D.K., Nielsen, K.F., Larsen, T.O., Mortensen, U.H., 2013. Heterologous Reconstitution of the Intact Geodin Gene Cluster in Aspergillus nidulans through a Simple and Versatile PCR Based Approach. PLoS ONE 8, e72871. https://doi.org/10.1371/ANNOTATION/C05C3FD1-DD00-4840-891D-693C614AAAF9.
Niu, Q., Huang, X., Zhang, L., Li, Y., Li, J., Yang, J., Zhang, K., 2006. A neutral protease from Bacillus nematocida, another potential virulence factor in the infection against nematodes. Arch. Microbiol. 185, 439–445. https://doi.org/10.1007/S00203-006-0112-X.
Norsigian, C.J., Fang, X., Seif, Y., Monk, J.M., Palsson, B.O., 2020. A workflow for generating multi-strain genome-scale metabolic models of prokaryotes. Nat. Protoc. 15, 1–14. https://doi.org/10.1038/s41596-019-0254-3.
Nowruzi, B., Sarvari, G., Blanco, S., 2020. The cosmetic application of cyanobacterial secondary metabolites. Algal Res. 49, 101959 https://doi.org/10.1016/J.ALGAL.2020.101959.
Nützmann, H.W., Reyes-Dominguez, Y., Scherlach, K., Schroeckh, V., Horn, F., Gacek, A., Schümann, J., Hertweck, C., Strauss, J., Brakhage, A.A., 2011. Bacteria-induced natural product formation in the fungus Aspergillus nidulans requires Saga/Ada-mediated histone acetylation. Proc. Natl. Acad. Sci. U.S.A. 108, 14282–14287. https://doi.org/10.1073/PNAS.1103523108.
O'Brien, J., Wright, G.D., 2011. An ecological perspective of microbial secondary metabolism. Curr. Opin. Biotechnol. 22, 552–558. https://doi.org/10.1016/J.COPBIO.2011.03.010.
Ola, A.R., Thomy, D., Lai, D., Brötz-Oesterhelt, H., Proksch, P., 2013. Inducing secondary metabolite production by the endophytic fungus Fusarium tricinctum through coculture with Bacillus subtilis. J. Nat. Prod. 76, 2094–2099. https://doi.org/10.1021/np400589h.
Olano, C., García, I., González, A., Rodriguez, M., Rozas, D., Rubio, J., Sánchez-Hidalgo, M., Braña, A.F., Méndez, C., Salas, J.A., 2014. Activation and identification of five clusters for secondary metabolites in Streptomyces albusJ1074. Microb. Biotechnol. 7, 242–256. https://doi.org/10.1111/1751-7915.12116/SUPPINFO.
Oliveira, N.M., Martinez-Garcia, E., Xavier, J., Durham, W.M., Kolter, R., Kim, W., Foster, K.R., 2015. Biofilm Formation As a Response to Ecological Competition. PLOS Biol. 13, e1002191 https://doi.org/10.1371/JOURNAL.PBIO.1002191.
Onaka, H., Mori, Y., Igarashi, Y., Furumai, T., 2011. Mycolic acid-containing bacteria induce natural-product biosynthesis in Streptomyces species. Appl. Environ. Microbiol. 77, 400–406. https://doi.org/10.1128/AEM.01337-10.
Onaka, H., Ozaki, T., Mori, Y., Izawa, M., Hayashi, S., Asamizu, S., 2015. Mycolic acid-containing bacteria activate heterologous secondary metabolite expression in Streptomyces lividans. J. Antibiot. 68, 594–597. https://doi.org/10.1038/ja.2015.31.
Org Overmann, J., Abt, B., Sikorski, J., 2017. Present and Future of Culturing Bacteria. Annu. Rev. Microbiol. https://doi.org/10.1146/annurev-micro-090816.
Orth, J.D., Thiele, I., Palsson, B.O., 2010. What is flux balance analysis? Nat. Biotechnol. 28, 245–248. https://doi.org/10.1038/nbt.1614.
Ozdemir, T., Fedorec, A.J., Danino, T., Barnes, C.P., 2018. Synthetic Biology and Engineered Live Biotherapeutics: Toward Increasing System Complexity. Cell Syst. 7, 5–16. https://doi.org/10.1016/j.cels.2018.06.008.
Padmaperuma, G., Kapoore, R.V., Gilmour, D.J., Vaidyanathan, S., 2018. Microbial consortia: a critical look at microalgae co-cultures for enhanced biomanufacturing. Crit. Rev. Biotechnol. 38, 690–703. https://doi.org/10.1080/07388551.2017.1390728.
Pan, R., Bai, X., Chen, J., Zhang, H., Wang, H., 2019. Exploring structural diversity of microbe secondary metabolites using OSMAC strategy: A literature review. Front. Microbiol. 10, 294. https://doi.org/10.3389/FMICB.2019.00294/BIBTEX.
Park, H.B., Kwon, H.C., Lee, C.H., Yang, H.O., 2009. Glionitrin A, an antibiotic - Antitumor metabolite derived from competitive interaction between abandoned mine microbes. J. Nat. Prod. 72, 248–252. https://doi.org/10.1021/np800606e.
Pereira, C.S., Thompson, J.A., Xavier, K.B., 2013. AI-2-mediated signalling in bacteria. FEMS Microbiol. Rev. 37, 156–181. https://doi.org/10.1111/J.1574-6976.2012.00345.X.
Pettit, R.K., 2011. Small-molecule elicitation of microbial secondary metabolites. Microb. Biotechnol. 4, 471–478. https://doi.org/10.1111/J.1751-7915.2010.00196.X.
Plackett, B., 2020. Why big pharma has abandoned antibiotics. Nature 586, S50–S52. https://doi.org/10.1038/d41586-020-02884-3.
Qian, X., Chen, L., Sui, Y., Chen, C., Zhang, W., Zhou, J., Dong, W., Jiang, M., Xin, F., Ochsenreither, K., 2020. Biotechnological potential and applications of microbial consortia. Biotechnol. Adv. 40, 107500 https://doi.org/10.1016/J.BIOTECHADV.2019.107500.
Rafieenia, R., Atkinson, E., Ledesma-Amaro, R., 2022. Division of labor for substrate utilization in natural and synthetic microbial communities. Curr. Opin. Biotechnol. 75, 102706 https://doi.org/10.1016/J.COPBIO.2022.102706.
Rateb, M.E., Hallyburton, I., Houssen, W.E., Bull, A.T., Goodfellow, M., Santhanam, R., Jaspars, M., Ebel, R., 2013. Induction of diverse secondary metabolites in Aspergillus fumigatus by microbial co-culture. RSC Adv. 3, 14444–14450. https://doi.org/10.1039/C3RA42378F.
Ren, X., Murray, R.M., 2019. Cooperation enhances robustness of coexistence in spatially structured consortia. In: 2019 18th European Control Conference, ECC 2019. Institute of Electrical and Electronics Engineers Inc., pp. 2651–2656. https://doi.org/10.23919/ECC.2019.8796069
Riedel, K., Hentzer, M., Geisenberger, O., Huber, B., Steidle, A., Wu, H., Høiby, N., Givskov, M., Molin, S., Eberl, L., 2001. N-acylhomoserine-lactone-mediated communication between Pseudomonas aeruginosa and Burkholderia cepacia in mixed biofilms. Microbiology 147, 3249–3262. https://doi.org/10.1099/00221287-147-12-3249/CITE/REFWORKS.
Roberts, E.C., Cain, C.K., Muir, R.D., Reithel, F.J., Gaby, W.L., Van Bruggen, J.T., Homan, D.M., Katzman, P.A., Jones, L.R., Doisy, E.A., 1943. Penicillin B, An Antibacterial Substance From Penicillium Notatum. J. Biol. Chem. 147, 47–58. https://doi.org/10.1016/S0021-9258(18)72411-3.
Roell, G.W., Zha, J., Carr, R.R., Koffas, M.A., Fong, S.S., Tang, Y.J., 2019. Engineering microbial consortia by division of labor. Microb. Cell Fact. 18, 35. https://doi.org/10.1186/s12934-019-1083-3.
Romano, S., Jackson, S.A., Patry, S., Dobson, A.D., 2018. Extending the One Strain Many Compounds (OSMAC) Principle to Marine Microorganisms. Marine Drugs 16. https://doi.org/10.3390/MD16070244.
Rosero-Chasoy, G., Rodríguez-Jasso, R.M., Aguilar, C.N., Buitrón, G., Chairez, I., Ruiz, H. A., 2021. Microbial co-culturing strategies for the production high value compounds, a reliable framework towards sustainable biorefinery implementation – an overview. Bioresour. Technol. 321, 124458 https://doi.org/10.1016/j.biortech.2020.124458.
Russell, A.B., Peterson, S.B., Mougous, J.D., 2014a. Type VI secretion system effectors: poisons with a purpose. Nat. Rev. Microbiol. 12 (2), 137–148. https://doi.org/10.1038/nrmicro3185.
Russell, A.B., Wexler, A.G., Harding, B.N., Whitney, J.C., Bohn, A.J., Goo, Y.A., Tran, B. Q., Barry, N.A., Zheng, H., Peterson, S.B., Chou, S., Gonen, T., Goodlett, D.R., Goodman, A.L., Mougous, J.D., 2014b. A Type VI Secretion-Related Pathway in Bacteroidetes Mediates Interbacterial Antagonism. Cell Host Microbe 16, 227–236. https://doi.org/10.1016/J.CHOM.2014.07.007.
Rutledge, P.J., Challis, G.L., 2015. Discovery of microbial natural products by activation of silent biosynthetic gene clusters. Nat. Rev. Microbiol. 13 (8), 509–523. https://doi.org/10.1038/nrmicro3496.
Salwan, R., Sharma, V., 2021. Current trend and future prospects of secondary metabolite-based products from agriculturally important microorganisms. In: Biocontrol Agents and Secondary Metabolites. Woodhead Publishing, pp. 239–255. https://doi.org/10.1016/B978-0-12-822919-4.00010-7.
Santala, S., Santala, V., 2021. Acinetobacter baylyi ADP1—naturally competent for synthetic biology. Essays Biochem. 65, 309–318. https://doi.org/10.1042/EBC20200136.
Santos, C.N.S., Xiao, W., Stephanopoulos, G., 2012. Rational, combinatorial, and genomic approaches for engineering L-tyrosine production in Escherichia coli. Proc. Natl. Acad. Sci. U.S.A. 109, 13538–13543. https://doi.org/10.1073/PNAS.1206346109/SUPPL_FILE/PNAS.1206346109_SI.PDF.
Sattler, I., Thiericke, R., Zeeck, A., 1998. The manumycin-group metabolites. Nat. Prod. Rep. 15, 221–240. https://doi.org/10.1039/A815221Y.
Schmidt, F.R., 2005. Optimization and scale up of industrial fermentation processes. Appl. Microbiol. Biotechnol. 68, 425–435. https://doi.org/10.1007/S00253-005-0003-0/FIGURES/1.
Schroeckh, V., Scherlach, K., Nützmann, H.W., Shelest, E., Schmidt-Heck, W., Schuemann, J., Martin, K., Hertweck, C., Brakhage, A.A., 2009. Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in Aspergillus nidulans. Proc. Nat. Acad. Sci. 106, 14558–14563. https://doi.org/10.1073/PNAS.0901870106.

Schulte, C.C., Borah, K., Wheatley, R.M., Terpolilli, J.J., Saalbach, G., Crang, N., de Groot, D.H., Ratcliffe, R.G., Kruger, N.J., Papachristodoulou, A., Poole, P.S., 2021. Metabolic control of nitrogen fixation in rhizobium-legume symbioses. Sci. Adv. 7 https://doi.org/10.1126/SCIADV.ABH2433/SUPPL_FILE/SCIADV.ABH2433_SM.PDF.

Shank, E.A., Kolter, R., 2009. New Developments in Microbial Interspecies Signaling. Curr. Opin. Microbiol. 12, 205. https://doi.org/10.1016/J.MIB.2009.01.003.

Shima, J., Hesketh, A., Okamoto, S., Kawamoto, S., Ochi, K., 1996. Induction of actinorhodin production by rpsL (encoding ribosomal protein S12) mutations that confer streptomycin resistance in Streptomyces lividans and Streptomyces coelicolor A3(2). J. Bacteriol. 178, 7276–7284. https://doi.org/10.1128/JB.178.24.7276-7284.1996.

Shu, C.H., Tseng, K., Jaiswal, R., 2018. Effects of light intensity and wavelength on the production of lactobionic acid from whey by Pseudomonas taetrolens in batch cultures. J. Chem. Technol. Biotechnol. 93, 1595–1600. https://doi.org/10.1002/JCTB.5528.

Shuler, M.L., Kargi, F., 2001. Bioprocess Engineering: Basic Concepts, second ed. Prentice Hall.

Shwab, E.K., Keller, N.P., 2008. Regulation of secondary metabolite production in filamentous ascomycetes. Mycol. Res. 112, 225–230. https://doi.org/10.1016/J.MYCRES.2007.08.021.

Sidda, J.D., Song, L., Poon, V., Al-Bassam, M., Lazos, O., Buttner, M.J., Challis, G.L., Corre, C., 2013. Discovery of a family of γ)aminobutyrate ureas via rational derepression of a silent bacterial gene cluster. Chem. Sci. 5, 86–89. https://doi.org/10.1039/C3SC52536H.

Singh, R., Kumar, M., Mittal, A., Mehta, P.K., 2017. Microbial metabolites in nutrition, healthcare and agriculture. 3 Biotech 7. https://doi.org/10.1007/S13205-016-0586-4.

Skinnider, M.A., Johnston, C.W., Gunabalasingam, M., Merwin, N.J., Kieliszek, A.M., MacLellan, R.J., Li, H., Ranieri, M.R., Webster, A.L., Cao, M.P., Pfeifle, A., Spencer, N., To, Q.H., Wallace, D.P., Dejong, C.A., Magarvey, N.A., 2020. Comprehensive prediction of secondary metabolite structure and biological activity from microbial genome sequences. Nat. Commun. 11 (1), 1–9. https://doi.org/10.1038/s41467-020-19986-1.

Sørensen, L.M., Lametsch, R., Andersen, M.R., Nielsen, P.V., Frisvad, J.C., 2009. Proteome analysis of Aspergillus niger: lactate added in starch-containing medium can increase production of the mycotoxin fumonisin B2 by modifying acetyl-CoA metabolism. BMC Microbiol. 9 https://doi.org/10.1186/1471-2180-9-255.

Soucy, S.M., Huang, J., Gogarten, J.P., 2015. Horizontal gene transfer: building the web of life. Nat. Rev. Genet. 16 (8), 472–482. https://doi.org/10.1038/nrg3962.

Souza, D.P., Oka, G.U., Alvarez-Martinez, C.E., Bisson-Filho, A.W., Dunger, G., Hobeika, L., Cavalcante, N.S., Alegria, M.C., Barbosa, L.R., Salinas, R.K., Guzzo, C.R., Farah, C.S., 2015. Bacterial killing via a type IV secretion system. Nat. Commun. 6 (1), 1–9. https://doi.org/10.1038/ncomms7453.

Steel, H., Habgood, R., Kelly, C.L., Papachristodoulou, A., 2020. In situ characterisation and manipulation of biological systems with Chi. Bio. PLOS Biol. 18, e3000794 https://doi.org/10.1371/journal.pbio.3000794.

Steiger, M.G., Rassinger, A., Mattanovich, D., Sauer, M., 2019. Engineering of the citrate exporter protein enables high citric acid production in Aspergillus niger. Metab. Eng. 52, 224–231. https://doi.org/10.1016/J.YMBEN.2018.12.004.

Sun, Y., Shi, X., Xing, Y., Ren, X.X., Zhang, D.Y., Li, X., Xiu, Z.L., Dong, Y.S., 2022. Co-culture of Aspergillus sydowii and Bacillus subtilis induces the production of antibacterial metabolites. Fungal Biol. 126, 320–332. https://doi.org/10.1016/J.FUNBIO.2022.01.002.

Szewczyk, E., Chiang, Y.M., Oakley, C.E., Davidson, A.D., Wang, C.C., Oakley, B.R., 2008. Identification and characterization of the asperthecin gene cluster of Aspergillus nidulans. Appl. Environ. Microbiol. 74, 7607–7612. https://doi.org/10.1128/AEM.01743-08/SUPPL_FILE/ASPERTH_SUPPLEMENTAL_DOC.ZIP.

Thiele, I., Palsson, B., 2010. A protocol for generating a high-quality genome-scale metabolic reconstruction. Nat. Protoc. 5, 93–121. https://doi.org/10.1038/nprot.2009.203.

Thomik, T., Wittig, I., Choe, J.Y., Boles, E., Oreb, M., 2017. An artificial transport metabolon facilitates improved substrate utilization in yeast. Nat. Chem. Biol. 13 (11), 1158–1163. https://doi.org/10.1038/nchembio.2457.

Timmermans, M.L., Picott, K.J., Ucciferri, L., Ross, A.C., 2019. Culturing marine bacteria from the genus Pseudoalteromonas on a cotton scaffold alters secondary metabolite production. Microbiologyopen 8. https://doi.org/10.1002/MBO3.724.

Tomm, H.A., Ucciferri, L., Ross, A.C., 2019. Advances in microbial culturing conditions to activate silent biosynthetic gene clusters for novel metabolite production. J. Ind. Microbiol. Biotechnol. 46, 1381–1400. https://doi.org/10.1007/S10295-019-02198-Y.

Toro, L., Pinilla, L., Avignone-Rossa, C., Ríos-Estepa, R., 2018. An enhanced genome-scale metabolic reconstruction of Streptomyces clavuligerus identifies novel strain improvement strategies. Bioprocess Biosyst. Eng. 41, 657–669. https://doi.org/10.1007/S00449-018-1900-9.

Traxler, M.F., Watrous, J.D., Alexandrov, T., Dorrestein, P.C., Kolter, R., 2013. Interspecies interactions stimulate diversification of the Streptomyces coelicolor secreted metabolome. mBio 4. https://doi.org/10.1128/MBIO.00459-13/ASSET/AF4D134B-137D-4DCB-8230-CD820407E047/ASSETS/GRAPHIC/MBO0041315990006.JPEG.

Vartoukian, S.R., Palmer, R.M., Wade, W.G., 2010. Strategies for culture of 'unculturable' bacteria. FEMS Microbiol. Lett. 309, 1–7. https://doi.org/10.1111/J.1574-6968.2010.02000.X.

Wang, B., Guo, F., Dong, S.H., Zhao, H., 2018. Activation of silent biosynthetic gene clusters using transcription factor decoys. Nat. Chem. Biol. 15 (2), 111–114. https://doi.org/10.1038/s41589-018-0187-0.

Wang, J.B., Zhang, F., Pu, J.Y., Zhao, J., Zhao, Q.F., Tang, G.L., 2014. Characterization of AvaR1, an autoregulator receptor that negatively controls avermectins production in a high avermectin-producing strain. Biotechnol. Lett. 36, 813–819. https://doi.org/10.1007/S10529-013-1416-Y.

Wei, Q., Bai, J., Yan, D., Bao, X., Li, W., Liu, B., Zhang, D., Qi, X., Yu, D., Hu, Y., 2021. Genome mining combined metabolic shunting and OSMAC strategy of an endophytic fungus leads to the production of diverse natural products. Acta Pharm. Sin. B 11, 572–587. https://doi.org/10.1016/J.APSB.2020.07.020.

Wu, Y.J., Yang, S.B., Zhang, Z.Y., Chen, S.X., 2020. Improvement of Nemadectin Production by Overexpressing the Regulatory Gene nemR and Nemadectin Biosynthetic Gene Cluster in Streptomyces Cyaneogriseus. Pharmaceut. Fronts 2, 151–159. https://doi.org/10.1055/s-0040-1722746.

Xia, H., Li, X., Li, Z., Zhan, X., Mao, X., Li, Y., 2020. The Application of Regulatory Cascades in Streptomyces: Yield Enhancement and Metabolite Mining. Front. Microbiol. 11, 406. https://doi.org/10.3389/FMICB.2020.00406/BIBTEX.

Xue, T., Chen, D., Su, Q., Yuan, X., Liu, K., Huang, L., Fang, J., Chen, J., He, W., Chen, Y., 2019. Improved ethanol tolerance and production of Saccharomyces cerevisiae by global transcription machinery engineering via directed evolution of the SPT8 gene. Food Biotechnol. 33, 155–173. https://doi.org/10.1080/08905436.2019.1572517.

Yaegashi, J., Oakley, B.R., Wang, C.C., 2014. Recent advances in genome mining of secondary metabolite biosynthetic gene clusters and the development of heterologous expression systems in Aspergillus nidulans. J. Ind. Microbiol. Biotechnol. 41, 433–442. https://doi.org/10.1007/S10295-013-1386-Z.

Yasuhara-Bell, J., Lu, Y., 2010. Marine compounds and their antiviral activities. Antiviral Res. 86, 231–240. https://doi.org/10.1016/J.ANTIVIRAL.2010.03.009.

Yu, H., Tyo, K., Alper, H., Klein-Marcuschamer, D., Stephanopoulos, G., 2008. A high-throughput screen for hyaluronic acid accumulation in recombinant Escherichia coli transformed by libraries of engineered sigma factors. Biotechnol. Bioeng. 101, 788–796. https://doi.org/10.1002/BIT.21947.

Zeng, Z., Cai, X., Wang, P., Guo, Y., Liu, X., Li, B., Wang, X., 2017. Biofilm formation and heat stress induce pyomelanin production in deep-sea Pseudoalteromonas sp. SM9913. Front. Microbiol. 8, 1822. https://doi.org/10.3389/FMICB.2017.01822/BIBTEX.

Zengler, K., Zaramela, L.S., 2018. The social network of microorganisms - How auxotrophies shape complex communities. Nat. Rev. Microbiol. 16, 383–390. https://doi.org/10.1038/s41579-018-0004-5.

Zhang, B., Wang, K.B., Wang, W., Bi, S.F., Mei, Y.N., Deng, X.Z., Jiao, R.H., Tan, R.X., Ge, H.M., 2018. Discovery, Biosynthesis, and Heterologous Production of Streptoseomycin, an Anti-Microaerophilic Bacteria Macrodilactone. Org. Lett. 20, 2967–2971. https://doi.org/10.1021/ACS.ORGLETT.8B01006/SUPPL_FILE/OL8B01006_SI_001.PDF.

Zhang, H., Boghigian, B.A., Armando, J., Pfeifer, B.A., 2010. Methods and options for the heterologous production of complex natural products. Nat. Prod. Rep. 28, 125–151. https://doi.org/10.1039/C0NP00037J.

Zhang, H., Pereira, B., Li, Z., Stephanopoulos, G., Demain, A.L., 2015. Engineering Escherichia coli coculture systems for the production of biochemical products. Proc. Natl. Acad. Sci. U.S.A. 112, 8266–8271. https://doi.org/10.1073/PNAS.1506781112/SUPPL_FILE/PNAS.1506781112.SAPP.PDF.

Zhang, M.M., Wang, Y., Ang, E.L., Zhao, H., 2016. Engineering microbial hosts for production of bacterial natural products. Nat. Prod. Rep. 33, 963–987. https://doi.org/10.1039/C6NP00017G.

Zhang, S., Merino, N., Okamoto, A., Gedalanga, P., 2018. Interkingdom microbial consortia mechanisms to guide biotechnological applications. Microb. Biotechnol. 11, 833–847. https://doi.org/10.1111/1751-7915.13300.

Zhou, K., Qiao, K., Edgar, S., Stephanopoulos, G., 2015. Distributing a metabolic pathway among a microbial consortium enhances production of natural products. Nat. Biotechnol. 33 (4), 377–383. https://doi.org/10.1038/nbt.3095.

Zhu, S., Duan, Y., Huang, Y., 2019. The Application of Ribosome Engineering to Natural Product Discovery and Yield Improvement in Streptomyces. Antibiotics 8, 133. https://doi.org/10.3390/ANTIBIOTICS8030133.

Zhuang, L., Zhang, H., Pfeifer, B.A., Zhang, G., 2021. Utilizing cross-species co-cultures for discovery of novel natural products. Curr. Opin. Biotechnol. 69, 252–262. https://doi.org/10.1016/j.copbio.2021.01.023.