South African Journal of Botany xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Review

Microbial communication: A significant approach for new leads

M.A. Abdalla [a,b,*], S. Sulieman [c], L.J. McGaw [a,**]

[a] Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[b] Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, 13314 Khartoum North, Sudan
[c] Department of Agronomy, Faculty of Agriculture, University of Khartoum, 13314 Khartoum North, Sudan

ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form 28 September 2017
Accepted 3 October 2017
Available online xxxx

Edited by A Romano

Keywords:
Co-culture
Secondary metabolism
Cryptic pathways
Microbial interactions

ABSTRACT

A significant number of naturally occurring secondary metabolites from plants and microbes have been explored for their pharmacological properties and used as drugs, for example to combat infectious diseases, in human and animal health. Unfortunately, antibiotic resistance is growing faster than the discovery of new antibiotics. With this in mind, a more targeted search for lead compounds by investigating new sources and applying alternative approaches and strategies is necessary. Many recent studies confirm that most secondary metabolite gene clusters in microorganisms, especially in fungi, are silent under laboratory growth conditions. These findings lead to a better understanding of the basic principles of the chemical communication between different microorganisms in nature as they form close communities, such as interactions between fungi — bacteria, fungi — fungi, bacteria — bacteria and microorganisms existing as endophytes within their host plants. The influences of these associations in nature establish, restore and sustain the great biosynthetic potential of secondary metabolite formation. Mixed fermentation or co-cultivation represents an important approach of inducing secondary metabolism by providing appropriate physiological conditions, including competition and communication between microorganisms. This report reviews several relevant co-culturing experiments and their influences on natural product biosynthesis and methods recently used to identify the compounds afforded by co-cultivation. The medicinal importance of microbial co-cultures is also discussed.

Contents

1. Introduction

In recent years, a wide range of biologically active secondary metabolites produced by bacteria and fungi have attracted considerable interest (Abdalla et al., 2010; Jumpathong et al., 2010; Abdalla et al., 2011a, 2011b; Zinad et al., 2011; Abdalla and Matasyoh, 2014). Microorganisms co-exist in close associations, where they interact and communicate with each other (Strobel and Daisy, 2003; Aly et al., 2011). The chemical communication between different microbes in their respective habitats is based mainly on the presence of natural products, which act as signaling molecules involved in interaction, competition and different defense mechanisms (Ola et al., 2013). Two types of microbial competition can occur in the ecological system: interference and scramble competition. These can take place either within or between species.

* Corresponding author at: Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.
** Corresponding author.
E-mail addresses: muna.mohamed@up.ac.za (M.A. Abdalla), lyndy.mcgaw@up.ac.za (L.J. McGaw).

https://doi.org/10.1016/j.sajb.2017.10.001
0254-6299/

Interference competition takes place when one type of microorganism keeps nutrients away from another. Scramble competition takes place when one type of microorganism consumes nutrients before another. Of course, competition becomes more intense when nutrients are scarce. Microbial co-culture holds much potential for the discovery of sustained production of secondary metabolites, which can be achieved under laboratory conditions. Mixed fermentation or microbial co-culture together with microbial transformation, genome mining and unculturable microorganisms have been recently discovered to be important reservoirs of novel antibiotics (Wilson et al., 2014; Ling et al., 2015).

The role of secondary metabolites is important in acting as defense molecules against different predators of the producers, and inhibiting the growth of competitors (Davies, 1990). Several recent studies have discussed numerous co-culturing combinations such as fungal-bacterial, fungal-fungal and bacterial-bacterial interactions and their positive impact on new compound formation. Successful co-cultivation experiments can be mediated through signaling molecules or intimate contact, where the fungal mycelia and bacterial filaments of both microorganisms connect together as shown in Fig. 1. An excellent example of the intimate physical interaction of fungus and bacterium has been observed in a co-culture of *Aspergillus nidulans* and *Streptomyces hygroscopicus* (Schroeckh et al., 2009). This study discovered that silent gene clusters of secondary metabolism of *A. nidulans* are only stimulated when the fungus is co-cultivated with a bacterium and the microorganisms could physically interact. These findings support the view that not only diffusible molecules can induce cryptic biosynthetic genes in microbial communication, but that close physical interaction can have the same effect.

Fungal-fungal co-cultures and their promising effects on inducing natural products have been extensively discussed (Li et al., 2011; Zhu et al., 2013). Moreover, fungal-fungal interactions are potentially significant when mycelia of different species meet and closely interact in the area of physical contact, known as the interaction zone. These responses enhance modifications in the morphology of the mycelium, as well as production of extra-cellular enzymes and natural products (Griffith et al., 1994; Rayner et al., 1994; Boddy, 2000; Woodward and Boddy, 2008). It is important to note that sub-elements, such as protein domains, from bacterial and fungal systems have potential use in many applications, such as using sequences of the protein domain of a fungal non-ribosomal peptide synthetase (NRPS) to convert a similar gene in *Bacillus subtilis*. This application induced the production of novel secondary metabolites (Frey-Klett et al., 2011). In general, through possible gene activation, a number of different combinations of mixed fermentation have proven their capabilities to enhance natural product biosynthesis.

Herein, we report various previously investigated microbial co-culturing experiments and highlight the medicinal significance of microbial mixed fermentation as a powerful tool for discovering new bioactive secondary metabolites.

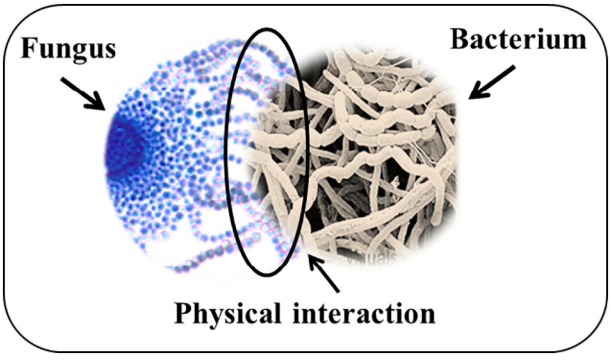

Fig. 1. Intimate physical contact between fungus and bacterium induces the cryptic biosynthetic pathway of secondary metabolism.

2. Influences of microbial co-cultures on production of secondary metabolites

2.1. Bacterial-fungal co-culture as a dynamic way to induce silent gene clusters

Several studies of bacterial-fungal interactions have confirmed co-culturing as a potential tool for inducing secondary metabolites (Abdalla and Sulieman, 2017). In the early 2000s, Fenical et al. discovered a chlorinated benzophenone named pestalone (**1**), which is produced when the marine fungus *Pestalotia* is co-cultivated with a unicellular marine bacterium, strain CNJ-328 (Cueto et al., 2001). Compound **1** showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* and against vancomycin-resistant *Enterococcus faecium*, in addition to *in vitro* cytotoxicity in the National Cancer Institute's 60 Human Tumor Cell Line Screen. Co-culturing the marine bacterium *α-proteobacterium* (Strain CNJ-328) with the marine-derived fungus *Libertella* sp. induced the synthesis of the novel diterpenoids, libertellenones A–D (**2**–**5**) (Oh et al., 2005). Terpenoids are generally known as fungal and plant metabolites and are rarely produced by bacteria (Turner and Aldridge, 1983; Hefter et al., 1993), and there is strong evidence that these diterpenoids were produced by the fungus. It can be assumed that the fungal biosynthetic gene cluster of compounds **2**–**5** was activated in the presence of the bacterium in the same culture.

Several co-cultures of *Aspergillus fumigatus* and various *Streptomyces* spp., such as *S. peucetius*, induced the biosynthesis of formyl xanthocillin analogues (Zuck et al., 2011). Fumiformamide (**6**) and *N,N′*-(1Z,3Z)-1,4-bis(4-methoxyphenyl)buta-1,3-diene-2,3-diyl)diformamide (**7**) are new compounds produced by this co-culture (Table 1). *A. fumigatus* was co-cultivated with the soil-derived *S. bullii*, isolated from the Atacama Desert, in South America, producing 10 compounds, including seven diketopiperazine alkaloids in addition to pseurotins 11-*O*-methylpseurotin A and its new isomer, 11-*O*-methylpseurotin A2 (**8**) (Rateb et al., 2013).

Mixed fermentation of *A. fumigatus* and *Streptomyces rapamycinicus* yielded the new fungal-derived polyketides fumicyclines A (**9**) and B (**10**) (König et al., 2013), confirming that close intimacy of the fungus and bacterium is very important in inducing the fungal metabolites. The study concluded that the presence of *S. rapamycinicus* modifies the gene expression in *A. fumigatus* by modulating its regulatory processes. Another study demonstrated that physical interaction between *A. nidulans* and *S. hygroscopicus* ATCC 29253 activated the expression of the fungal genes that were responsible for the synthesis of the polyketide metabolites known as orsellinic acid (**11**), lecanoric acid (**12**), F-9775A (**13**), and F-9775B (**14**) (Schroeckh et al., 2009). Interestingly, compounds **13** and **14**, which were partially derived from orsellinic acid (**11**), had previously been isolated from *Paecilomyces carneus* (Satou et al., 1999) and were not known as metabolites of *A. nidulans*. This confirmed that the fungal silent genes were induced to produce these compounds only in the presence of the bacterium in a co-culture, but not in a fungal mono-culture. Compared with a mono-culture of *Fusarium tricinctum*, its co-culture with the bacterium *Bacillus subtilis* 168 trpC278 induced the production of three new secondary metabolites, macrocarpon C (**15**), N-(carboxymethyl)anthranilic acid (**16**), and (−)-citreoisocoumarinol (**17**), along with a 78-fold increase in the production of known fungal metabolites, including lateropyrone, fusaristatin, and enniatins A1, B, and B1 (Ola et al., 2013). Interestingly, compounds **15**–**17** were not detected when *F. tricinctum* was co-cultured with *S. lividans* or in fungal and bacterial mono- cultures, confirming that expression of genes in *F. tricinctum* is enhanced only by *B. subtilis* specifically. Co-cultivation of the marine-derived fungus *A. fumigatus* and the marine-derived bacterium *Sphingomonas* sp. yielded a cytotoxic, antibacterial diketopiperazine disulfide: glionitrin A (**18**). This

Please cite this article as: Abdalla, M.A., et al., Microbial communication: A significant approach for new leads, South African Journal of Botany (2017), https://doi.org/10.1016/j.sajb.2017.10.001