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Preview

No Need To Be Pure: Mix the Cultures!

In this issue of *Chemistry & Biology*, Angell et al. [1] demonstrate synergism between two bacterial species, isolated from the same sediment sample, to produce a secondary metabolite not found in their respective pure cultures.

In biology, the beneficial interaction between two organisms is usually called symbiosis, and there are several well-known examples where one of the partners is a microorganism (e.g., *Vibrio* and squid or *Bradyrhizobium* and legumes). Symbioses between two microorganisms are also common in nature as exemplified by lichens composed of algae and fungi, which work together to gain access to nutrients and to protect against desiccation and other environmental stresses.

The basis for all kinds of interactions is a common language of some sort, and in the microbial world, this language is based on chemistry. Some dual microbial systems have been characterized on the molecular level, and several small molecule "words" are known that are involved in counting members of the same species in a process called quorum sensing. At a certain cell density, specific processes like virulence, chemoluminescence or antibiotic production are induced [2]. Interestingly, even communication between different species is possible when they use the same signal, as shown for the autoinducer AI-2, which is widespread in the bacterial world [3, 4]. Furthermore, in cases where sensing the same compound leads to different reactions in different species, one organism can manipulate the behavior of a second one by interfering with its capability to respond properly to changes in population density [5]. In order to prevent manipulation of its chemical language, *Pseudomonas aeruginosa* produces the signaling molecule 2-heptyl-3-hydroxy-4-quinolone (PQS) together with a potent antibiotic that are secreted together in membrane vesicles that can also fuse to cells of other bacterial species. However, as these "other" cells would be killed by the antibiotic, misuse of the signaling compound is prevented and only *P. aeruginosa* cells receive and benefit from the message.

With respect to microbial natural product chemistry, most compounds described in the literature are produced by a single organism. However, there are several examples of natural products generated from symbioses [6]. While the role for each partner is not clear in most cases, one can speculate about a few examples where higher organisms like sponges provide shelter and a defined environment for bacteria, which in turn provide the higher organism with toxic compounds to protect them from parasites.

An unusual example of two bacteria that collaborate to produce a natural product that benefits both is described in this issue of *Chemistry & Biology* by Watanabe and co-workers [1]. In contrast to a classical screening approach that starts with the isolation of pure cultures that are then screened for bioactivity, Angell et al. use a mixed culture isolated from ocean floor sediments. A blue pigment with antibiotic activity was produced by this culture that was later identified as pyocyanin by chemical analysis. However, when pure bacterial cultures isolated from the mixed population were tested for pyocyanin formation, no production was detected, indicating the possibility of synergism between different bacterial species in the biosynthesis of this pigment. By mixing all possible combinations of the different bacterial strains isolated from the starting culture, Watanabe and co-workers were able to identify the two bacteria responsible for pyocyanin synthesis, Pup14A and Pup14B, which were classified as *Enterobacter* sp. and *Pseudomonas aeruginosa*, respectively.

Different cultivation experiments and genetic analyses revealed that the pyocyanin producer is *P. aruginoasa* Pup14B, a member of a well-known pyocyanin-producing genus. However, all known pyocyanin-producing *P. aeruginosa* strains are capable of self-inducing pyocyanin biosynthesis by quorum sensing (Figure 1A, **2**). Screening of four additional bacterial strains revealed that a second *Enterobacter* species was also capable of inducing pyocyanin biosynthesis in Pup14B.

Three small molecules are known to regulate secondary metabolite biosynthesis in *P. aeruginosa* [7, 8]. One can therefore postulate that Pup14B has lost the ability to produce the pyocyanin inducer molecule that is supplied by Pup14A instead, leading to the observed antitibiotic production (Figure 1B) that benefits both strains as they are both resistant to pyocyanin. This resistance might protect them from other bacteria that are sensitive to this compound. In accordance with this hypothesis, separation of Pup14A and Pup14B by a membrane permeable for small molecules still enables pyocyanin biosynthesis [1], excluding the involvement of direct cell-cell contact for signaling as found in myxobacteria [9]. However, it is evident that this simple explanation cannot be the whole story due to the result obtained from an experiment in which cell-free supernatant of the inducer Pup14A was added to growing cells of the producer Pup14B. Under this condition, Pup14B failed to produce pyocyanin, indicating that induction might be more than a unidirectional process. One possibility involves additional exchange of compounds from Pup14B to Pub14A resulting in the production of the inductor in Pub14A. The inductor itself would then stimulate the production of pyocyanin in Pup14B (Figure 1C). Alternatively, pyocyanin biosynthesis in Pup14B might depend on a compound produced by Pup14A that is fairly unstable in solution, but when produced constantly by Pup14A, the amount might be sufficient for induction. For example, autoinducer AI-2 is known to undergo spontaneous rearrangements [10] resulting in various derivatives with different bacteria showing strong preferences for only some of these derivatives [11].

Other possibilities for this unusual result of interspecies communication are possible and cannot be

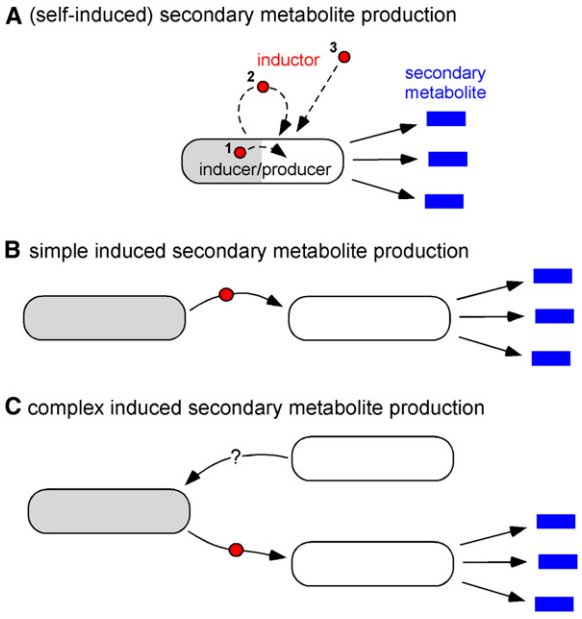

Figure 1. Possible Ways for Microbial Secondary Metabolite Production in Pure or Mixed Culture

(A) Pure culture. (B and C) Mixed culture. In (A) induction might be intracellular (1), by a self-produced compound (2) or by an extracellular component from the environment (3).

excluded by the authors at this stage. Clearly the next, however advanced, step would be the elucidation of the detailed mechanism of the induction process.

Fortuitously, uncovering the example by Watanabe and co-workers was greatly facilitated by the easy visual detection of the compound produced, and their work shows that pure cultures will not always lead to secondary metabolite production. A recent example of symbiosis and secondary metabolism was found for the biosynthesis of the rice seedling blight-causing compound rhizoxin, which was shown to be produced by endosymbiotic *Burkholderia* bacteria living inside the *Rhizopus* fungus that was originally thought to be the producer of this cytotoxic compound [12]. Another example involving two bacterial species was described for strains of the myxobacterium *Chondromyces crocatus*. Here, several strains have been isolated from all over the world, most of them associated with a Gram-negative bacterium named "*Candidatus* comitans" [13, 14]. *Chondromyces crocatus* strains have been described as multiproducers of secondary metabolites, which for practical reasons have been isolated from one of the few nonassociated strains, but which are all produced by the associated strains as well [15, 16, 17]. However, in case of the associated strains, neither pure cultures of the *Chondromyces* nor the "*Candidatus* comitans" strains could be maintained, indicating a close symbiotic relationship between the two partners. In this case as well, working with mixed cultures might enable the isolation of additional compounds that are not produced by the nonassociated strain and therefore will be missed otherwise.

In this context, it is worth mentioning that genome sequencing projects have revealed the presence of many more biosynthesis gene clusters than known secondary metabolite classes [18]. One reason why we have not succeeded in inducing the production of the corresponding compounds might be that we tried really hard with the pure culture by changing almost all accessible cultivation parameters [19], but probably should have used the original mixture of micro-organisms from the original environment as inducer instead.

Helge B. Bode[1]
[1] Institut für Pharmazeutische Biotechnologie
Universität des Saarlandes
P.O. Box 151150
66041 Saarbrücken
Germany

Selected Reading

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