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Mini review

Future potential for anti-infectives from bacteria – How to exploit biodiversity and genomic potential

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ABSTRACT

The early stages of antibiotic development include the identification of novel hit compounds. Since actinomycetes and myxobacteria are still the most important natural sources of active metabolites, we provide an overview on these producers and discuss three of the most promising approaches toward finding novel anti-infectives from microorganisms. These are defined as the use of biodiversity to find novel producers, the variation of culture conditions and induction of silent genes, and the exploitation of the genomic potential of producers via "genome mining". Challenges that exist beyond compound discovery are outlined in the last section.

Introduction

After the golden era of antibiotics in the third quarter of the last century it is increasingly evident that therapy of human infectious diseases faces numerous daunting challenges. Some widely accepted reasons for the existence of a gap in the pharmaceutical pipeline of antibiotics are the rapid development and spread of resistance in causative agents, the occurrence of new and re-emerging infectious diseases, loss of interest in large pharma-companies to develop anti-infectives, and strict drug regulatory issues.

In this article we will focus on the early stages in the drug development pipeline where novel hit compounds are urgently required for the development of pharmaceuticals. Most of the currently used antibiotics are derived from microorganisms, either as natural products themselves or as semi-synthetically optimized derivatives (Newman and Cragg, 2012). Although the identification of derivatives of known compounds is of significant importance, most researchers aim to identify novel basic structures ideally exhibiting new modes of action. We define novel basic structures as novel secondary metabolite scaffolds not sharing significant similarity to those of known compounds. Often a novel compound class cannot be developed into a pharmaceutical due to a lack of required pharmaceutical properties that include a reasonable ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) profile. However, it may be modified chemically to identify new targets (Bielecki et al., 2012; Nagaraj et al., 2012) that can subsequently be addressed using the methods of structure or target-based medicinal chemistry.

Often the question is raised whether microorganisms may continue to provide modern medicine with new antibiotics, a question closely connected to the remaining potential of these organisms after decades of exploitation. How can we judge whether this resource is exhausted or still promising for the identification of novel scaffolds for development? In this review we will argue for microorganisms as the most promising source for the future from two perspectives: First, from the amazingly low percentage of biodiversity among microorganisms which has been harvested and second from current genomic knowledge showing enormous potential for the production of an almost inexhaustible number of new secondary metabolites (Baltz, 2006, 2008; Berdy, 2005; Bull et al., 2000; Clardy et al., 2006; Demain, 2006; Donadio et al., 2010; Gullo et al., 2006; Harvey, 2008; Li and Verdas, 2009; Bode and Müller, 2005; Wenzel and Müller, 2009; see Fig. 1).

Here we focus on research with actinomycetes and gliding bacteria as consideration of all potential bacterial sources of novel anti-infectives is outside the scope of this review. However, it should emphasized that approaches for natural product and anti-infective discovery outlined here are also generally applicable to almost every type of microorganism. Promising results are also provided by fungi and other bacteria such as pseudomonads, bacilli, burkholderiae or insect-pathogenic bacteria, just to name a few (Demain, 1999; Ishida et al., 2012; Visca et al., 2006; Forst et al., 1997; Piel, 2004; Sahl and Bierbaum, 1998). Unfortunately the

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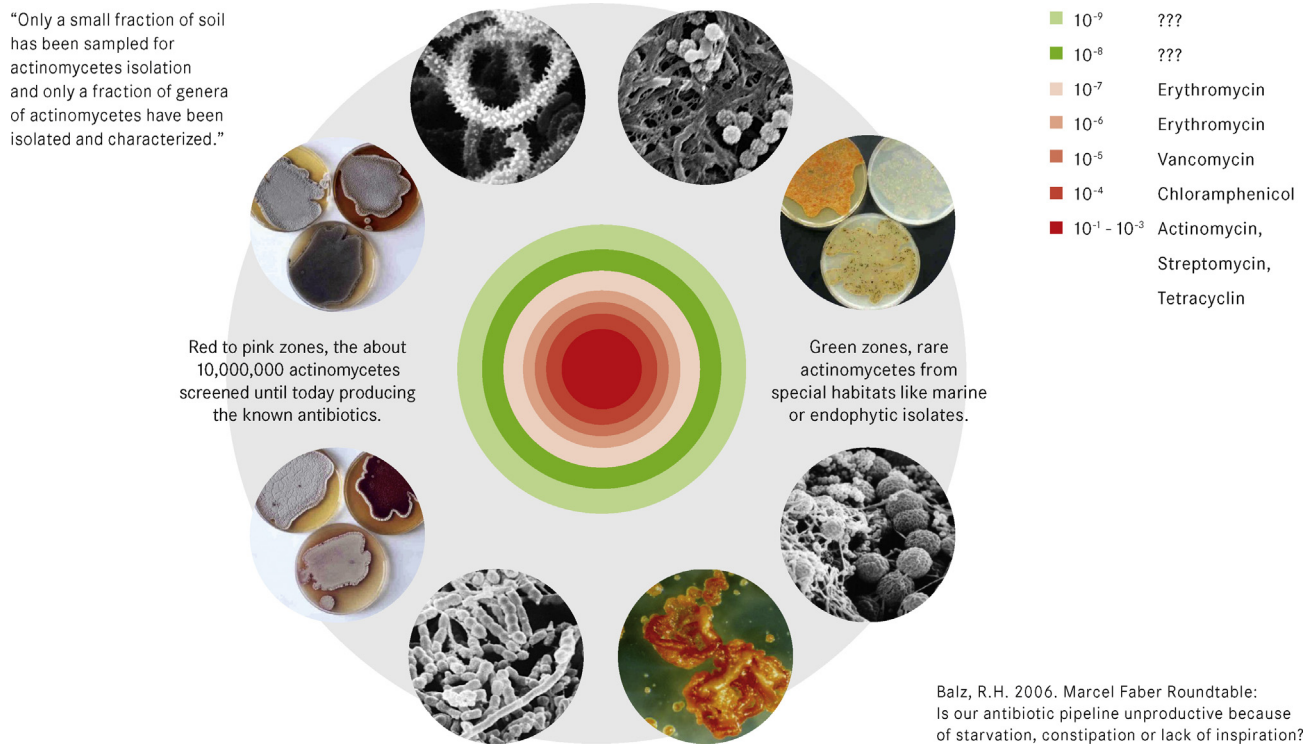

Fig. 1. "What should we screen" in the actinomycetes for novel antibiotics (compare Baltz, 2006). The diagram shows the frequencies of actinomycetes that produce antibiotics. The different circles represent the numbers of isolates which had to be screened for isolating the different antibiotics in the past (e.g. $10–10^3$ *Actinomycetes* for the isolation of actinomycin, 10^4 for chloramphenicol, 10^5 for vancomycin, 10^6 for erythromycin, 10^7 for daptomycin). To isolate novel antibiotics the number of screened strains has to be enlarged to 10^8 or more, or novel and unusual isolates, which have never been screened have to be used. This is shown by the examples in the green zone.

non-comprehensive nature of this review prevents us mentioning many important results and studies of numerous research groups active in this field.

Actinomycetes and myxobacteria as antibiotic factories

Since the 1940's the history of antibiotic discovery and -development is inseparably connected to microorganisms. Today we know that bacteria exhibiting large genomes (often more than 8 MB) also show highest potential for rich secondary metabolism. Actinobacteria of diverse genera, such as the *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes*, are the producers of clinically used antibiotics belonging to different chemical classes (for instance, the carbapenemes, anthracyclines, macrolides, glycopeptides, ansamycins, lipopetides and aminoglycosides (Omura, 1992)). Actinobacteria are a group of gram-positive bacteria characterized by DNA with high GC content. Many of them are soil bacteria, but pathogenic or saprophytic organisms also belong to this group. Many show a characteristic differentiation by forming endospores which are arranged in spore chains, sporangia or are found as single spores on sporophores. Approximately 2500 species in 238 genera, 37 families and 9 suborders are known (Approved List of Bacterial Names).

Already in 1946 Oxford and coworkers described the first bacteriolytic effect based on an antibiotic produced by a myxobacterium (Oxford and Singh, 1946). The myxobacteria are gram negative bacteria which are also ubiquitously found in soil; most of them degrade biomacromolecules such as cellulose or prey on other microorganisms. Similar to most actinobacteria, myxobacteria are characterized by a differentiation process culminating in the formation of fruiting bodies and more than 50 species in 20 genera, 6 families and 3 suborders are known. In the 1980's sorangicin was isolated from a *Sorangium* strain as the first myxobacterial antibiotic with high potential for market development (Irschik et al., 1987). Since then many new compounds have been described from this fascinating group of bacteria that have considerably increased the structural diversity found in natural product libraries. Intriguingly, and in contrast to the actinobacterial secondary metabolites, many compounds from myxobacteria are highly active against fungi (e.g. soraphen is an example of a potent antifungal with a novel mode of action (Gerth et al., 1993) see Fig. 3). Today we know that myxobacteria harbor a large potential for the production of novel secondary metabolites, which holds true for antibacterials and antifungals (Weissman and Müller, 2009; Plaza and Müller, 2014), particularly if we look for unexploited isolates or for the still almost unexplored genetic potential of their large genomes. In conclusion actinobacteria and myxobacteria are believed to be potent resources for novel anti-infectives, a probable consequence of their habitat where they live in competition with other bacteria and fungi, making the production of bioactive compounds an obvious strategy for survival.

Although the isolation of novel species and families significantly increases the chances of the discovery of novel chemical entities, most of the already known and well described species also harbor a huge and so far almost untapped "hidden" biosynthetic potential in their genome (see below). An understanding of the biosynthesis of the antibiotics is the basis for taking a more direct approach. With the identification of many different biosynthetic pathways and the organization of the corresponding genes in gene clusters, new possibilities for enhancing the production of known metabolites or their modification, as well as the induction of the so called "silent" genes for the production of novel metabolites have opened up. Interestingly, in the actinomycetes and myxobacteria, only a limited number of potential secondary metabolite gene clusters can be correlated to compound production after analysis under standard laboratory conditions (Udwary et al., 2007). The genome sequencing of the first microorganisms