Non-pathogenic bacteria as targets in antimicrobial high-throughput screening

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Abstract

Antimicrobial screening usually analyses the effects of natural or synthetic molecules against pathogens. McAuley *et al.* (*Cell Chem. Biol*., 2019) changed this paradigm, testing the effect of synthetic compounds against the sporulation of the non-pathogenic bacterium *Streptomyces venezuelae*. They discovered a novel DNA-targeting antibiotic effective against pathogens.

Keywords: Antimicrobials; high-throughput screening; *Streptomyces*; sporulation

The screening of new secondary metabolites was tremendously productive during the "Golden Age of Antibiotics" (1940s–1960s) [1], which yielded important economic and social benefits revolutionising the treatment of microbial infections. Drug discovery became challenging once the most common antibiotics were discovered. In fact, most chemical scaffolds from which antibiotics are derived were discovered between 1930 and 1960 (Fig. 1A). During the past 30 years only three new classes of antibiotics have been brought to the clinic (mutilins, lipopeptides and oxazolidinones) (Fig. 1A) [1]. At the same time, microbial resistance to existing antibiotics has increased dramatically, rendering some microbial infections extremely hard to treat. The current scenario looks gloomy, and there is a high risk of medicine returning to the pre-antibiotic era, with high mortality from routine surgical or chemotherapeutic procedures because of infections by antibiotic-resistant pathogens. While better sanitation procedures, rational use of the already available antimicrobials and alternative therapeutic options (e.g. phages or vaccines) can ameliorate the antibiotic crisis, there is a general consensus that new antibiotics are urgently required to fight resistance.

High-throughput screening consists of testing natural or synthetic compounds against specific targets to detect the desired activities. Both the correct compound library and the correct target assay(s) are essential for a successful outcome (Fig. 1B).

In the case of antimicrobials, natural compound libraries, especially those coming from streptomycetes, were the most successful ones. Streptomycetes produce two-thirds of the bioactive secondary metabolites used in the clinical setting (mainly antibiotics, but also antitumorals, immunosupressors, etc.) [1]. No valid alternatives to screening natural strains have emerged to find new scaffolds and families of antibiotics. Non-natural synthetic antibiotics, obtained by chemical or combinatorial biosynthesis, exist, but most of them are variations of natural molecules. New antimicrobials can be found screening already tested natural microbial strains, activating secondary metabolite pathways that are not expressed in the laboratory (cryptic pathways), which indeed are the majority of pathways (Fig. 1B) [2, 3]. Strains from niches that have not been well explored or new synthetic compound libraries can also be screened (Fig. 1B) [3].

New antimicrobials can also be discovered changing the target used to test the biological activity. The most commonly used targets in antimicrobial screening are the pathogens that we want to fight (Fig. 1B). McAuley *et al.* have changed this paradigm using, for the first time, a non-pathogenic bacterium, *Streptomyces venezuelae*, as target [4]. *Streptomyces* is a soil bacterium characterised by its complex life cycle, which includes mycelial differentiation, programmed cell death and sporulation [5]. *Streptomyces* sporulation consists of the synchronous chromosomal DNA division and the formation of 1-µm unigenomic compartments, at the tips of the non-compartmentalised reproductive hyphae, that differentiate into spore chains [6]. The motivation of McAuley *et al*. to use *Streptomyces* to screen antimicrobials was simple and elegant: alteration in the highly regulated and easily recognisable *Streptomyces* sporulation might reveal antimicrobial

activity, especially DNA targeting antibiotics interfering chromosomal DNA replication during sporulation. *S. venezuelae* sporulation can be observed macroscopically by the characteristic green pigmentation of the spores. Defective sporulation is observed by the white pigmentation of the reproductive hyphae. To test their hypothesis, McAuley *et al.* first demonstrated that DNA-binding antibiotics, such as novobiocin, ciprofloxacin, mitomycin C and bleomycin, indeed blocked *S. venezuelae* sporulation [4]. Subsequently, they screened a library of 3,705 synthetic compounds using *S. venezuelae* sporulation as the target. They identified the EN-7 compound as a potent inhibitor of sporulation. The final aim was to discover antimicrobials effective against pathogens. Consequently, the authors tested EN-7 antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli.* EN-7 was highly active against *S. aureus*, but not against *E. coli*. They discovered that EN-7 targets DNA gyrase and further characterised the EN-7 chemical structure. Consequently, *Streptomyces*, the most important source for antimicrobials in nature, can also be used to test antimicrobial activities [1].

Overall, McAuley *et al.* demonstrated that *Streptomyces* sporulation is useful to find novel DNA targets [4]. This is the first high-throughput screen against the sporulation of a non-pathogenic bacterium. There are other bacteria showing sporulation (*Bacillus*), complex development (myxobacteria, cyanobacteria, etc.) or activities regulated by quorum sensing (for instance luminescence in *Vibrio fischeri*) that might also be potential targets in antimicrobial assays. The use of non-pathogenic microbes and bacteria differentiation as antimicrobial assay targets is an important advance that will contribute to find novel antimicrobials in high-throughput screening campaigns. Multidisciplinary approaches combining all the strategies described above are being applied to face the current fall in antimicrobial discovery. According to the Pew Charitable Trusts foundation[i] as of June 2019, 42 new antibiotics with the potential to treat serious bacterial infections are in clinical development globally.

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Resources

[i]https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2014/antibiotics-currently-in-clinical-development

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Figures

Figure 1. Major classes of antibiotics and a typical antimicrobial high-throughput screening. (A) Discovery of the major classes of antibiotics over the years. (B) Outline of a typical antimicrobial high-throughput screening process. Synthetic or natural compound libraries are tested against specific bacteria. Innovations that can lead to the identification of novel antimicrobials are highlighted in red.