Exploiting and expanding actinomycete diversity for antibiotic discovery

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Actinomycetes are soil microbes well known for their ability to produce a wide variety of bioactive compounds, including antibacterial, antifungal, antitumour and immunosuppressants agents. Close to 50% of the known microbial products are produced by actinomycetes. In particular, the discovery, development and clinical use of antibiotics has been one of the most significant medical advances in the 20th century, and antibiotics are probably the most prescribed class of drugs. However, the effectiveness of many antibiotics has been severely diminished by the insurgence and spreading of many antibiotic-resistant pathogens, with the consequent need for novel and better antibiotics.

Discovery of novel antibiotics from natural sources represents quite a challenge. *Streptomyces* spp. have long been recognised as the best antibiotic-producing bacteria, and it can be estimated that several million strains have been extensively screened by the pharmaceutical industry. Consequently, the chances of isolating a novel *Streptomyces* strain have substantially diminished. This implies that the chances of discovering a novel antibiotic from a *Streptomyces* strain by traditional approaches will require a substantially larger effort [1].

Therefore, in order to decrease the probability of rediscovering known compounds, novel strategies are required in the search for new antimicrobial products [2]. These strategies must not ignore the probabilistic nature of a screening approach – a significant number of microbes must be screened to have a reasonable chance to discover a new antibiotic with useful properties.

In the last decade, significant advances in molecular genetics and genomics have suggested alternative routes to antibiotic discovery from natural sources. Current estimates indicate that only 1% of microbial strains are related to known taxa, leading to the proposal that these uncultured strains, or simply their DNA expressed in a convenient host, could represent a novel source of bioactive compounds [3]. In addition, genomic studies have indicated that the potential to produce secondary metabolites is not uniformly distributed among bacteria, with some taxa possessing few or no genes for secondary metabolism. Interestingly, it can be assumed that the average *Streptomyces* strain [4, 5] and possibly other actinomycete genera [6] may have the genetic potential to produce a dozen or so different secondary metabolites. Since many of these clusters are apparently unexpressed under normal conditions, they could represent an additional source for novel antibiotics [7].

An alternative approach, which is currently pursued at Vicuron Pharmaceuticals, would be to concentrate efforts on unusual or difficult to isolate microbes that are phylogenetically related to good producers of secondary metabolites. According to this strategy, we have prepared a proprietary collection of over 60,000 strains, mostly non-*Streptomyces* actinomycetes and slow-growing filamentous fungi. Since these strains are hard to isolate, they are unlikely to have been screened in large numbers in the past.

Because they are phylogenetically related to good producers of secondary metabolites, they are likely to share the same large genetic potential for producing bioactive compounds. This strategy rests on the assumption that the ability to produce large numbers of bioactive metabolites is a hallmark of filamentous actinomycetes, and that strains distantly related to cultured and heavily exploited taxa offer a higher probability of possessing clusters containing novel combinations of secondary metabolism genes, and hence a higher probability of yielding novel compounds.

In this respect, molecular tools can greatly help in the identification of promising sources of poorly described actinomycete genera and in the quick recognition of as-yet uncultured representatives of these bacteria. Isolation programmes can be oriented by prescreening soil samples for the presence of DNA derived from uncommon genera of actinomycete [8], while the extent of the genetic diversity of newly isolated strains can be established through rapid fingerprints [9]. Our results also indicate that the so-called 'rare actinomycetes' are relatively abundant in the soil, and they can be retrieved in large numbers if a suitable isolation method is available.

In the long run, however, if isolation programmes are successful and uncommon actinomycete strains are isolated in large numbers, these taxa are eventually going to become part of the

exploited groups of strains, leading to a decreased result from equivalent effort. Through analysis of soil DNA, we observed 16S rRNA sequences ascribable to as yet-uncultured groups of actinomycetes[8]. We reasoned that many uncultured actinomycetes exist in the environment, and they could be cultured under appropriate conditions. To this end, soil samples showing an interesting diversity of actinomycete DNA can be recognised and aliquots plated on a variety of different conditions. Morphologically unusual strain can be rapidly classified through 16S rDNA sequencing, leading to their phylogenetic assignment within the *Actinobacteria.* Next, their genetic potential to produce secondary metabolites could be rapidly established.

According to this scheme, illustrated in Figure 1, strains belonging to new actinomycete taxa were isolated and identified (unpublished results). Interestingly, many of them possess the typical genes for secondary metabolism that make *Streptomyces* strains successful antibiotic producers, and thus these strains constitute a potential source of secondary metabolites worth of further investigation. The success of this approach depends on the use of isolation methods that counterselect rapidly growing actinomycete strains and on the application of objective methods for establishing strain identity.

In conclusion, opportunities exist to exploit the genetic capability of microbes for discovering valuable bioactive metabolites. The success of new approaches will ultimately depend on the ability to rapidly assemble and effectively screen a large diversity of gene clusters for secondary metabolism. The molecular structures observed today from natural sources represent the results of million of years of evolution. It is hard to imagine that future drug discovery can be effective without tapping into the rich source of chemical diversity offered by microbial products.

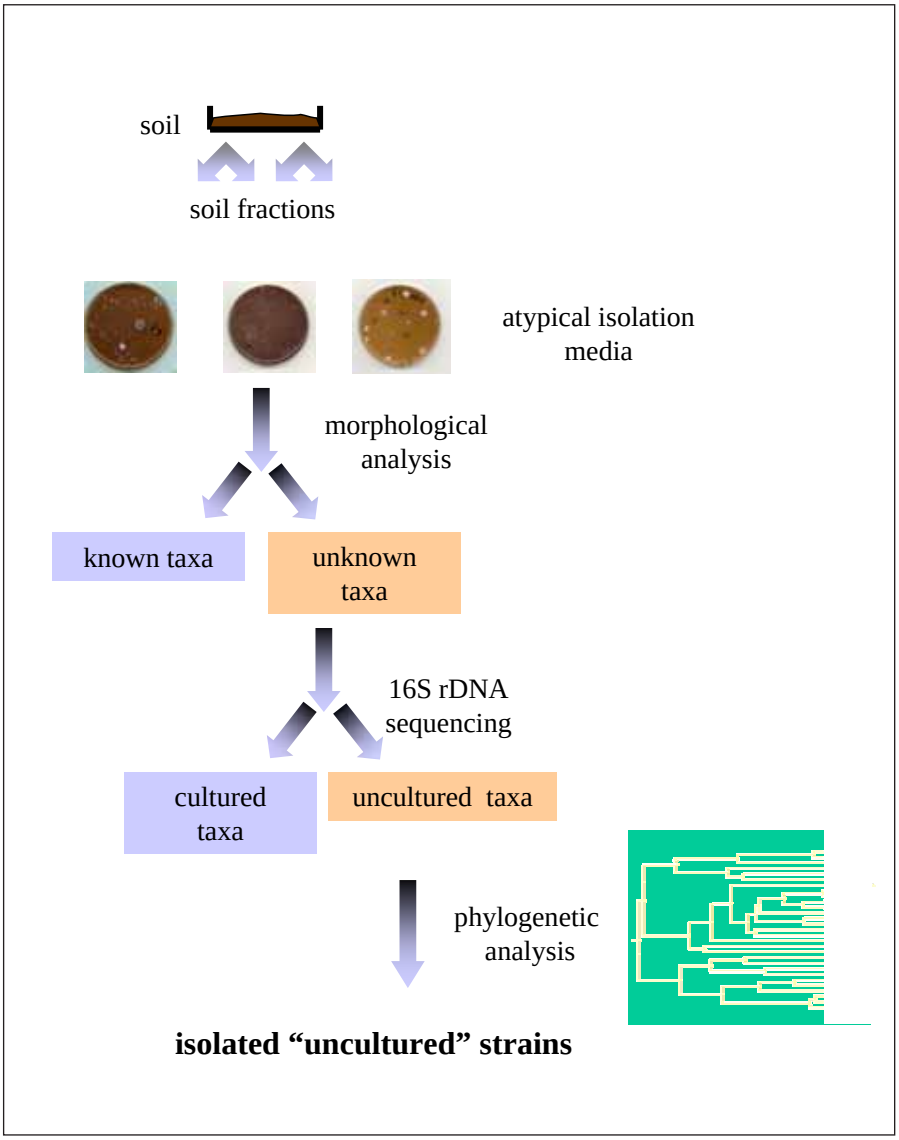

Figure 1. Scheme for identification of isolated ‘uncultured’ actinomycetes.

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