Streptomycetes and beyond

In terms of commercial value, the streptomycetes rank high among the bacteria, having been the principal source of numerous and valuable therapeutic agents for more than half a century.

The current world market for antibiotics is upwards of $US 25 million/year, of which a significant proportion are the products of bacterial fermentation, mainly from *Streptomyces* species. In terms of the industrial exploitation of microbial products in general, only the yeasts are more important, having provided humans with sustenance and pleasure for thousands of years.

The development of streptomycetes as sources of antibiotics and as industrial microorganisms was spearheaded by Selman Waksman who was the first to identify their extraordinary capacity to make antibiotics [1]. Incidentally, this discovery also led to the modern pharmaceutical industry, as we know it now.

It has been estimated that the streptomycetes alone may produce upwards of 250,000 different biologically active molecules of different classes [2]. The complex biosynthetic pathways of many of these molecules have been elucidated, the gene clusters cloned, and a number have been expressed in heterologous hosts for more detailed studies. There must remain many more small molecule products to be isolated since analyses of the completed genome sequences of several *Streptomyces* species reveal the presence of some 25 different small molecule biosynthetic pathways, in each genome!

Like most biosynthetic pathways, the evolutionary origins of the gene clusters involved in small molecule syntheses are little understood. Interestingly, there is recent evidence that some of the pathway genes were acquired by lateral gene transfer involving bacterial, fungal and plant sources [3].

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In more recent years, the search for small molecules with pharmacologic activity has been extended to other bacterial genera, as the discovery of new and useful molecules from the streptomycetes has become more and more infrequent. One problem might be that many of the biosynthetic pathways are cryptic and conditions for their expression have not been found.

Are there any specific phenotypic characteristics that might define certain classes of bacteria as good producers of small molecules? Might it be possible to identify entirely new bacterial genera useful in this sense?

In fact (perhaps not surprisingly), a very large number of bacterial species are able to produce biologically-active small molecules, many of which have demonstrated antibiotic activity. These include Gram-negative bacteria such as myxobacteria, pseudomonads and even *Escherichia coli.* Among the Gram-positives, *Bacillus* sp. are prolific producers of antibiotics and several of these molecules are still on the market.

Perhaps the most distinctive characteristics of bacteria producing a great diversity of small molecules are large genomes (often 8Mb or larger), with a high G+C content (>70%), complex cell development, and a cosmopolitan distribution as befits saprophytic organisms with the capacity to metabolise many different substrates [4] (Figure 1).

However, if the search for organisms producing novel and useful chemicals is to be successfully continued, it will be necessary that the quest for producing organisms be expanded to include new families of bacteria, both cultivatable and non-cultivatable. As a result of work by several leading scientists in the field, there have been major advances in the studies of bacterial phylogeny, and the identification of novel and unsuspected taxonomic groups lends credence to the probability that this approach will help in the identification of new classes of antibiotic-producing bacteria. Thus the streptomycetes are only the tip of the iceberg of small molecule discovery.

This becomes obvious when we realise that the Streptomycetaceae are but one of the members of the order Actinomycetales, of which there may be at least 40 known families and well over 100 different genera (and if the estimates of the number of non-cultivatable bacteria are correct, there will be many more!). Many have large genomes with DNA of high G+C content and have already been shown to produce small molecules with useful biological activities. However, we can expand this chemical and biological horn-of-plenty even further; since the order Actinomycetales is but one of the groups within the class Actinobacteria, a recently characterised and growing family that has been assigned its own branch of the prokaryotic tree. Many of the

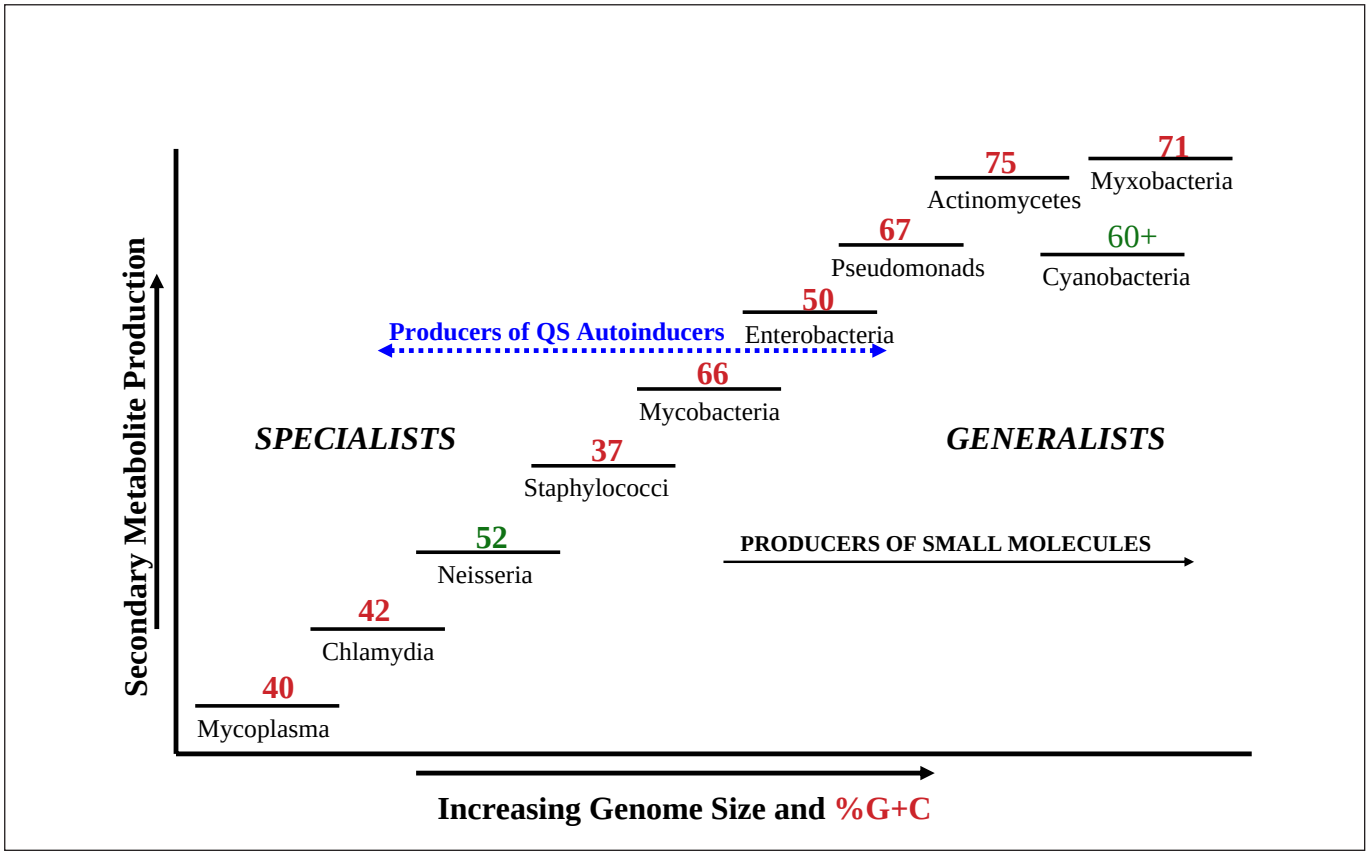

Figure 1. Genome size, G+C content and small molecules [modified from L Shimkets].

Actinobacteria have been identified only from 16S rRNA sequences obtained from environmental DNA and it can be assumed that there are a large number of unidentified members of this class. We do not know how many of the Actinobacteria have the genetic capacity to produce small molecules but they may be a veritable cornucopia of therapeutically active compounds.

An obvious question to be asked is: "What are the roles of so many compounds of diverse chemical structures in the biology of their producing organisms and the communities that produce them?"

The full extent of the microbial population of the biosphere is very poorly appreciated. Microbes define the limits of the conditions of life in terms of temperature (<0–>140^0), pH (1–12.8), pressure and chemical parameters. In fact, the growth of bacteria defines the limits of all forms of life, since there is no other form of life without bacteria.

Why have so few of the microbial population been identified? This is due mostly to the lack of the right techniques to isolate and identify bacteria, especially from more complex environments. Some habitats, such as soils, are rich in microbial content, whereas others, such as marine environments and those presenting extremes of chemical and physical stress, appear to have significantly lower microbial diversity.

The past 20-30 years have seen great advances in the cataloguing of bacterial populations in selected situations by the use of molecular tools such as DNA isolation, cloning and sequencing. New approaches using metagenomic analysis offer great promise, especially for non-expressed pathways, but there are many technical challenges that need to be overcome before these approaches can be applied generally. The results so far are encouraging but, in reality, only serve to emphasise the enormity of the problem of identifying the components of the microbiosphere[5].

One community property that has excited interest in the past few years is the intense cooperativity of microbial populations. This has been realised for some time in the sense that the survival of all life forms is absolutely dependent of bacterial interactions and, in another sense, the nature of host-pathogen relationships. This cooperativity is omnipresent in environmental communities and it has been proposed that microbial species always exist in consortia[6], even though a limited number of strains can be grown in isolation under laboratory conditions.

However, true microbial life is not represented on Petri plates! There must be many biochemical signalling or communication processes that maintain microbial communities in stable forms (even when subjected to fluctuation due to the pressures of environmental change). It can be assumed that most, if not all, microbial interactions have a chemical basis. Thus the chemical ecology of microbial populations is a very important field of study.

There has been considerable interest in the phenomenon of quorum-sensing in Gram-positive and Gram-negative pathogens, in which a variety of simple organic pheromones are used to sense population density and regulate metabolic functions (in the microbe and the host). As mentioned earlier, most bacterial producers of biologically-active small molecules must exist within cosmopolitan communities which are likely subject to many fluctuations; a variety of chemical signals are therefore needed to regulate metabolic functions in microbes under these conditions. Both intercellular signalling and contact-dependent regulation must be investigated.

Recent studies identifying a range of activities of antibiotics and other small molecules demonstrate that they possess two separate classes of biological activities depending on whether they are tested at low (sub-inhibitory) or high (inhibitory) concentrations [7]. The former are characterised by the modulation (up regulation or repression) of some 5% of the cellular transcripts, while the latter show strong repression of most transcripts with the exception of a small, specific number of functions. This is a clear example of chemical hormesis which is defined as low dose stimulation followed by higher dose inhibition of a biological function [8]. In this respect, it is worth noting that most well known antibiotics, when used therapeutically at optimum concentrations, are static and not cidal agents.

Studies of this type have been performed with different bacterial species with compounds that have distinct macromolecular targets (transcription, translation, cell-wall metabolism). Thus it appears that several different forms of cell-regulatory processes operate in environmental communities; small molecules (produced at low concentrations) act on other members of the microbial community through intracellular macromolecular targets to modulate their metabolic activity and so influence the stability of the community.

At the present time, the pharmaceutical industry is suffering from a 'pipeline-drought' due to the failure of the combinatorial chemistry/high-throughput screening approaches that were so heavily promoted (at great cost) over the past 10 years. Concurrently, the pace of discovery of naturally-occurring compounds slowed considerably, largely due to the low rate of return in terms of novel active compounds. This is in spite of the fact that natural products represent an astronomical number of compounds that, by definition, must be biologically active!

The fault obviously does not lie in the sources but in the creativity of the searchers; the traditional screening approaches for active molecules must be replaced. It may be that the newly recognised hormetic activities of naturally-occurring small molecules and improved methods for the isolation and characterisation of microbes from different environments, in combination with imaginative use of phylogenetic relationships, will provide new and successful approaches to the discovery of antibiotics and other pharmaceutically-active molecules. One thing is certain, the drugs are there for us to find.

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References

1. Waksman SA & Woodruff HB. Bacteriostatic and bactericidal substances produced by soil actinomyces. *Proc. Soc. Exp. Biol. Med.* 1940; 45:609-614.
2. Watbe MG, Tickoo R, Jog MM & Bohle BD. How many antibiotics are produced by the genus *Streptomyces? Arch. Microbiol.* 2001; 176:386-390.
3. Bode HB & Muller R. Possibility of bacterial recruitment of plant genes associated with the biosynthesis of secondary metabolites. *Plant Physiology* 2003; 132:1153-1161.
4. Bentley SD *et al.* Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* 2002; 417:141-147.
5. Curtis TB, Sloan WT, & Scannell JW. Estimating prokaryotic diversity and its limits. *Proc. Natl. Acad. Sci. USA* 2002; 99:10494-10499.
6. Buckley MR. *Microbial communities: advantages of multicellular cooperation.* Am. Acad. Microbiol Colloquium, Washington, DC, 2003. Available at http://www.asmusa.org
7. Goh EB, Yim G, Tsui W, McClure J, Surette MG & Davies J. Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. *Proc. Natl. Acad. Sci. USA* 2002; 99: 17025-17030.
8. Conolly RB & Lutz WK. Nonmonotonic dose-response relationships: mechanistic basis, kinetic modeling and implications for risk assessment. *Toxicol. Sci.* 2004; 77:151-157.