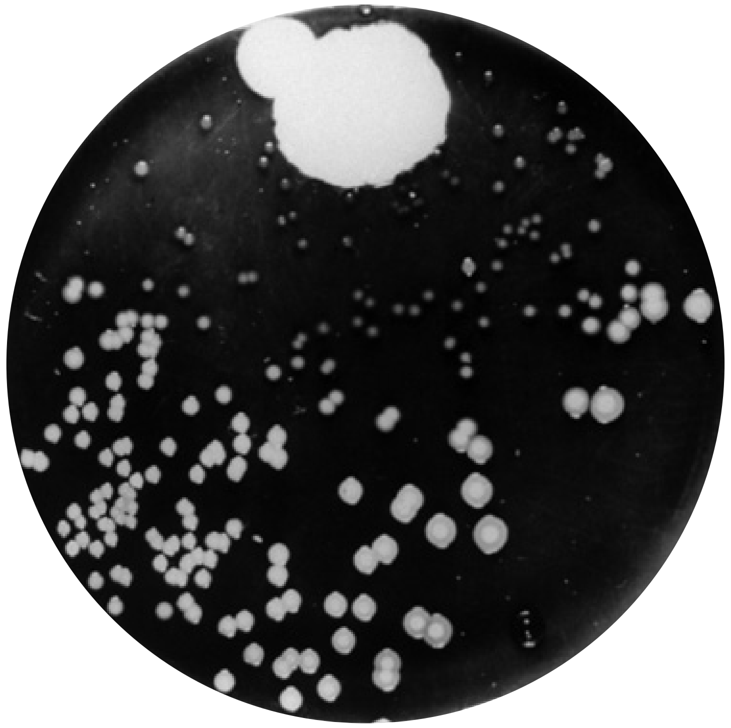


When antibiotics were discovered, they were considered only as miracle cures for infections. But as **Julian Davies** describes, these and other natural products also have important roles in microbial physiology as intercellular signalling agents.

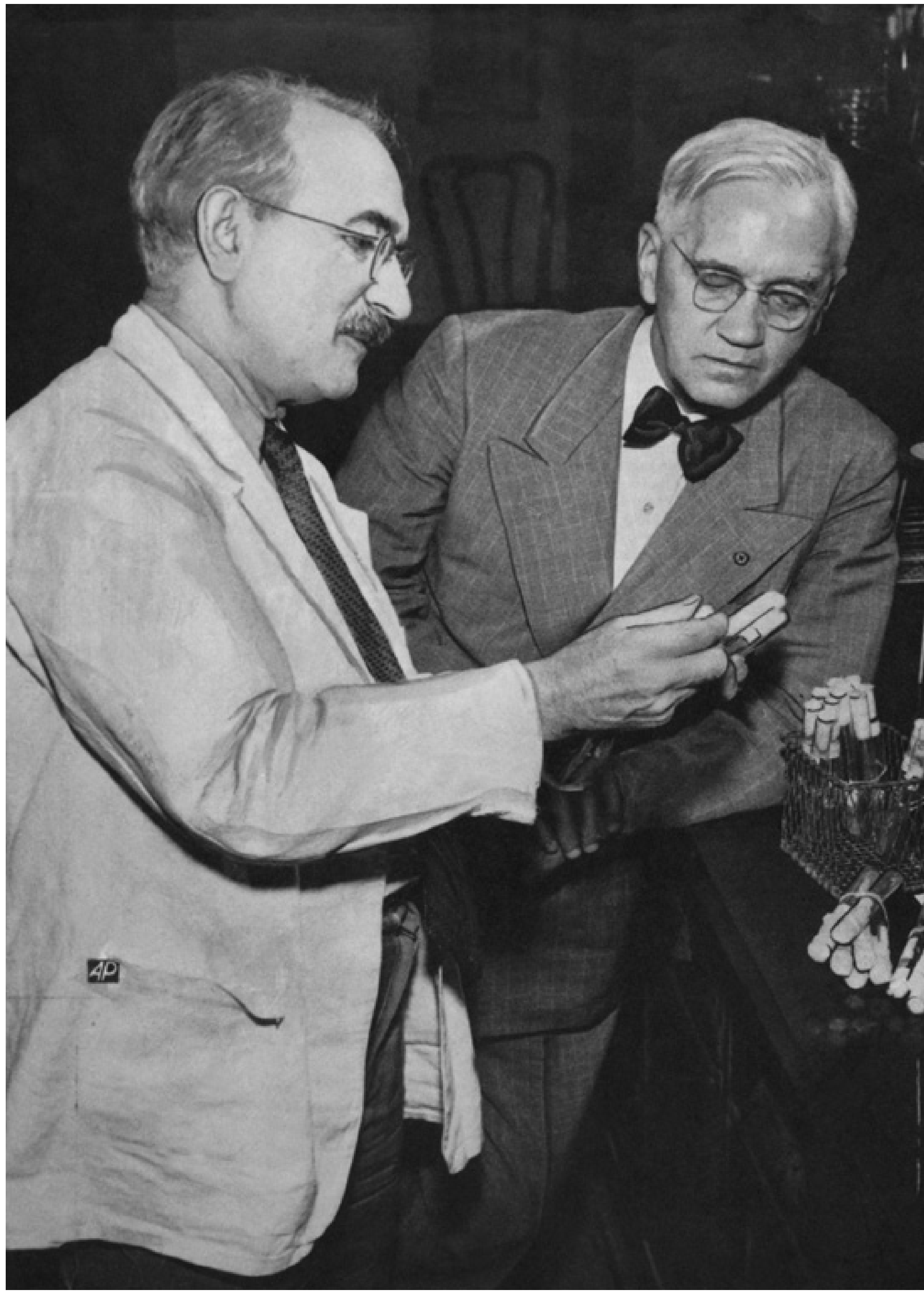
Look who's talking!

For thousands of years mankind has used natural products, chemicals produced by plants, fungi, bacteria and other living organisms, in a variety of applications: drugs, food, hallucinogens, etc. In more modern times this has led to investigations of the active compounds and their chemistry. The analysis of natural products, the basis of organic chemistry, contributed greatly to development of the methodology of structure determination and the art of total synthesis. Since the early 1900s, many organic chemists have honed their skills in natural product chemistry by characterizing plant compounds with a wide range of real and proposed curative properties. Microbial products were little studied until Alexander Fleming's fortuitous discovery (or was it rediscovery?) of their antibacterial properties, published in 1929. The chance finding of a stray fungal spore that produced a substance (subsequently shown to be penicillin) that caused lysis of a *Staphylococcus* strain became the all-time most famous photograph of a Petri plate (Fig. 1)!

The introduction of penicillin and streptomycin as the first potent antimicrobial agents derived from natural products in the early 1940s had global implications for the treatment of infectious disease. Penicillin was remarkably successful in saving lives in the Second World War, and streptomycin, discovered in the laboratory of Selman Waksman in 1944, turned out to be the first successful treatment for tuberculosis, the 'white plague' that has been a major cause of morbidity and mortality throughout human history. These two events also heralded the antibiotic era, when bioactive molecules produced by microbes became the silver bullets that completely transformed the treatment of disease. Since then, tens of thousands of antibiotic compounds have been discovered and tested, and medicine currently has an armamentarium of more than 100 approved antibiotics of some dozen different chemical classes. The legacy of Fleming and Waksman (Fig. 2) has been survival from the ravages of infectious diseases for billions of humans and animals over the past 60 years.

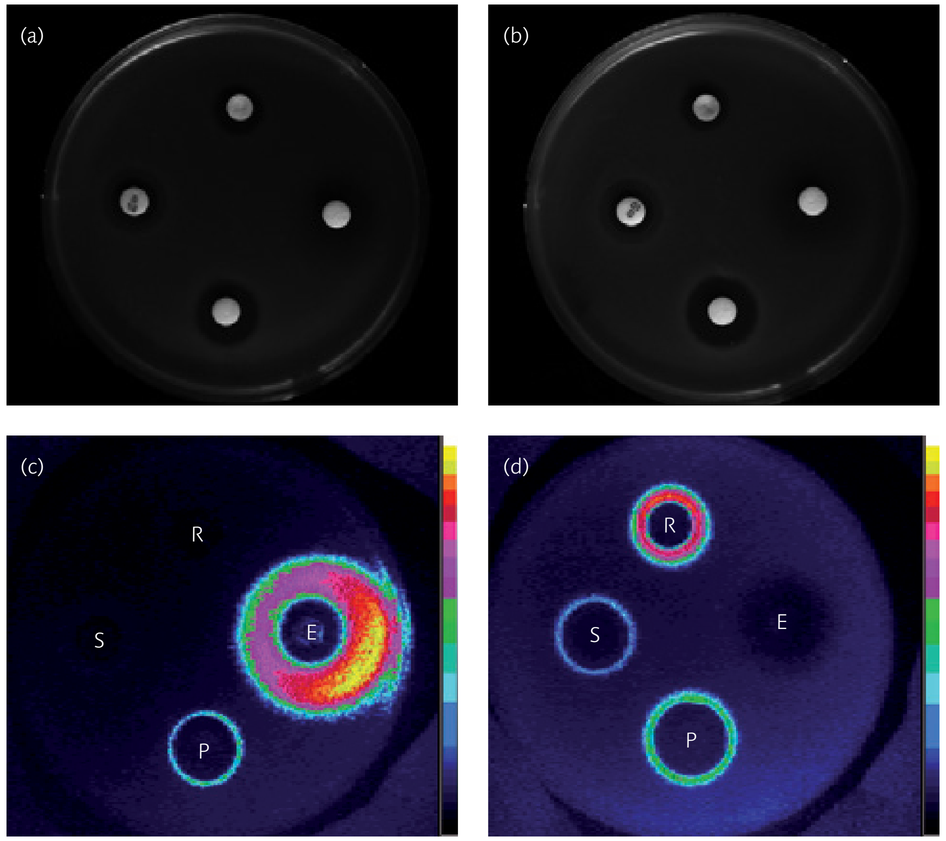
'Antibiotics'

It was Waksman who coined the word antibiotic: 'a chemical substance of microbial origin that possesses antimicrobial properties'. The word 'antibiosis' had been used previously but not in reference to purified chemicals. Waksman's definition has not been strictly adhered to and frequently includes compounds from any living source and even completely synthetic molecules. Microbial products with antibiotic activity exhibit many other types of bioactivity; the word antibiotic describes a specific function, but the compounds are multifunctional. The fact that a particular product has inhibitory activity in the laboratory does not mean that it plays such a role in nature. Antibiotics have a number of effects on bacterial physiology; for example they may affect the ability to swarm or form biofilms, or act as mutagens and induce bacterial lysogens to produce phage. Less well-appreciated is the fact that they also affect the function of plant cells and those of human hosts, and may cause undesirable side reactions; these secondary effects often occur at sub-inhibitory concentrations. Our laboratory has long been interested in the roles of antibiotics and other bioactive compounds of low molecular mass (<3000 Da) produced by microbes, etc., in biochemical evolution, and in the early 2000s we decided to examine their diverse activities by looking at transcription effects using promoter-reporter bacteria. The results were surprising (and colourful) Fig. 3; for example, rifampicin, the well-known antibiotic inhibitor of bacterial transcription (used for the treatment of TB), actually stimulated the transcription of a significant number of bacterial genes at low concentrations. This led us into detailed studies of



◄ Fig. 1. A photograph of Fleming's original culture plate, showing the zone of inhibited staphylococcal growth around the *Penicillium notatum* colony. *St Mary's Hospital Medical School / Science Photo Library*

▲ Fig. 2. Selman Waksman (left) and Alexander Fleming (right) in 1951. *Science Source / Science Photo Library*



▲ Fig. 3. Antibiotic growth inhibition (top) and transcription responses (luminescence) (bottom) of *Salmonella* Typhimurium 14028 carrying *ilvLG::luxCDABE* (a, c) and *nafC::luxCDABE* (b, d) transcriptional reporters. The colour scale on the right of (c) and (d) ranges from white, indicating high *lux* expression, to dark blue, indicating low *lux* expression). *Julian Davies*

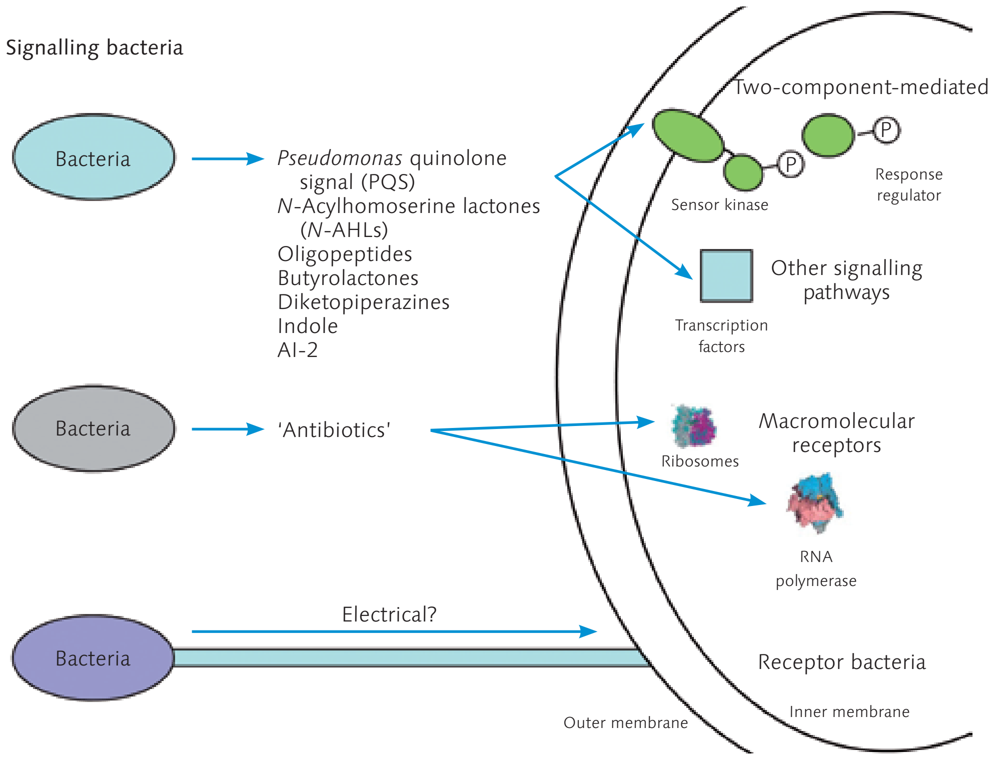
the effects of different inhibitors on pathogenic bacteria such as *Salmonella* Typhimurium and *Staphylococcus aureus*; to our surprise we found that at low concentrations all antibiotics modulate bacterial transcription in compound- and promoter-dependent ways. What does this tell us about the natural roles that antibiotics play in cell physiology? What might be their properties in the environment? The fact that these effects were seen at quite low concentrations suggested to us that so-called antibiotics act as intercellular signalling agents. We have been working on this ever since.

Signalling in bacterial communities

Bacteria in the environment exist in immense numbers, variety and genetic diversity. The microbial content of the biosphere approaches 10^{31}, a number beyond comprehension even when compared to the US national debt. The content of 1 gram of a typical soil is around a billion microbes. (These numbers are not yet accurate and vary with environment.) What is astonishing is that more than 95% of the population cannot be grown in the laboratory and cannot be classified (named) correctly; it is estimated that there are upwards of 1,000 different species in a gram of a rich soil. Our gastrointestinal tract contains a correspondingly huge number of microbes, most unidentified.

Bacterial populations normally grow as stable communities unless they are disturbed in some way. How do such mixed cultures maintain their stability with environmental changes? For example, therapeutic concentrations of antibiotics seriously deplete the bacterial population in the human GI tract, but once treatment stops, the population returns to its original state in a relatively short time. (Just imagine the burst of activity in soil bacteria after rain!) More and more evidence suggests that intercellular signalling maintains community growth and metabolism in a homeostatic manner, but what are the signals that modulate this? How do different types of bacteria communicate? And why? It is most likely that bacterial cross-talk occurs through the medium of small molecule and/or electrical signalling. An illustration of the signalling processes is shown in Fig. 4. In many cases the process involves modulation of transcription (RNA synthesis) either directly or indirectly, but other mechanisms may exist. The ribosome is an excellent example of a macromolecular receptor, possessing specific RNA and protein binding sites for small molecules that modulate metabolic processes essential for bacterial growth. On the other hand, at high concentrations these same molecules lead to translation inhibition, the basis of their antibiotic activity. Signalling by sub-inhibitory concentrations of natural compounds is mediated through interactions between ligands (small molecules) and different types of receptors. These take place at both the molecular and macromolecular level as indicated.

Given the large number and diversity of bacteria, one might expect that there would be myriads of different signalling molecules, so it is not surprising that bacteria have evolved many processes for small-molecule biosynthesis. The biosynthetic gene clusters are everywhere! In some prokaryotic families, such as the actinobacteria, pseudomonads and bacilli, each individual strain has devoted hundreds of kilobases of DNA to encode the production of dozens of different polyketides, non-ribosomal peptides, and hybrids of



◀ Fig. 4. Mechanisms of intermicrobial signalling. *Julian Davies*

these types, plus other unknown compounds. What is important is that signalling takes place at low concentrations.

Interbacterial signalling is essential in the formation of biofilm communities in disease and health, as has been demonstrated in the case of quorum-sensing agents. Signalling within the microbial communities of the GI tract is essential to human health; these complex microbiomes play important roles in protection against disease and their disruption is associated with a number of human pathologies. The gut bacteria cross-talk with their environment, which includes the host cells in the gut mucous membranes.

What about natural antagonistic reactions resulting from antibiotic activity? These have been clearly demonstrated in certain bacterial and fungal interactions in the lifestyles of ants and other insect species. Some natural products, such as the enediynes, are the most cytotoxic compounds known; they kill bacteria at nanomolar concentrations. One wonders if this is their only function in nature. Nevertheless, compounds with a range of activities are common; the bioactivity of natural products is exquisitely dose-dependent, yet we have no idea of their active concentrations in nature.

Antibacterial preparations from microbes underwent testing and even clinical use long before the introduction of penicillin, a good example being the substance 'pyocyanase', a small-molecule product of a *Pseudomonas* strain. Only recently have we begun to understand how they work. Diane Newman's laboratory has shown that the pyocyanins influence redox responses in bacterial populations; they are essentially acting as interbacterial signals in this case.

Conclusion

In the year in which we celebrate the anniversary of Darwin's *The Origin of Species* and Fleming's publication of the discovery of penicillin, it is interesting to consider how these monumental events have influenced our understanding of microbiology. Microbes evolved cell-to-cell interactions to ensure that their complex communities operate most efficiently for function and survival and for the benefit of their hosts. And in a typical Darwinian genetic response to antagonism (antibiosis), resistance developed in bacterial pathogens such as *S. aureus* – by mutation or by inheritance of antibiotic resistance mechanisms found in environmental bacteria. This is another evolutionary story; Darwin knew little about microbes and never envisioned horizontal gene transfer. Likewise, Fleming and Waksman could not have anticipated that small molecules and the antibiotics they discovered would prove to be so vital in the life of microbes!

Julian Davies, FRS
Emeritus Professor of Microbiology and Immunology
Grace Yim & **Dorothy Davies**
Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, Vancouver, Canada (e jed@interchange.ubc.ca)

Further reading

Davies, J., Spiegelman, G.B. & Yim, G. (2006). The world of subinhibitory antibiotic concentrations. *Curr Opin Microbiol* 5, 445–453.

Dietrich, L.E.P., Teal, T.K., Price-Whelan, A. & Newman, D.K. (2008). Redox-active antibiotics control gene expression and community behavior in divergent bacteria. *Science* 321, 1203–1206.

Fajardo, A. & Martínez, J.L. (2008). Antibiotics as signals that trigger specific bacterial responses. *Curr Opin Microbiol* 11, 161–167.

Florey, H.W., Chain, E., Heatley, N.G., Jennings, M.A., Sanders, A.G., Abraham, E.P. & Florey, M.E. (1949). *Antibiotics. A Survey of Penicillin, Streptomycin, and Other Antimicrobial Substances from Fungi, Actinomycetes, Bacteria and Plants*, Vols I and II. London: Oxford University Press.

Nicolaou, K.C. & Montagnon, T. (2008). *Molecules That Changed the World*. Weinheim: Wiley.

Yim, G., Wang, H.H. & Davies, J. (2006). The truth about antibiotics. *Int J Med Microbiol* 296, 163–170.