Antibiotics on a plate

Over sixty years ago, a Russian soil scientist called Selman Waksman discovered that soil bacteria belonging to the Streptomyces *genus produce some very useful compounds. He isolated a species that produced streptomycin, which became the world's first anti-tuberculosis antibiotic. Suzy Moody of Swansea University describes how this work continues today.*

Scientists have made great progress since Waksman's time. Many other Streptomyces species have been isolated and their ability to make antibiotics, antifungals and even drugs to lower human blood cholesterol has been exploited. The centre spread picture (next page) is of Streptomyces coelicolor, which produces five different antibiotics. Some of the antibiotics are pigmented. These colonies have produced actinorhodin, which is blue and gives the species its name (coelicolor means heavenly colour or sky-coloured in Latin). The medium it was grown on (the agar in the plate) was originally very pale yellow, but the bacteria produce a red antibiotic called undecylprodigiosin that has diffused out and turned the whole plate pink. Beautiful, isn't it?

Knocking out genes

These colonies have been genetically modified. We have knocked out (or disabled) the gene for one of the other antibiotics, albaflavenone. We call this a knockout mutant, and these play a very important role in many areas of biology. Often, it is only by taking away a gene that you can work out exactly what it does. With bacteria such as Streptomyces, a knockout mutant may have a different morphology (it may look different) to the original bacteria, so taking photos of them is useful – see Image 1. Streptomyces, like many other bacteria, will look different depending on what medium you grow it on too. It might sound complicated, but it makes lab work interesting!

The albaflavenone mutant looks the same as the original. But if you look at the close up photo, you will notice that the colonies are similar but not identical. This can suggest that the mutant is unstable (although that does not seem to be the case with this one). Image 2 shows just how different the same bacteria look on different media – although the bacteria will grow well on it, they do not produce any antibiotics. So when new species are found, or new mutants made, it is important to know what they look like and how they behave, on a variety of media.

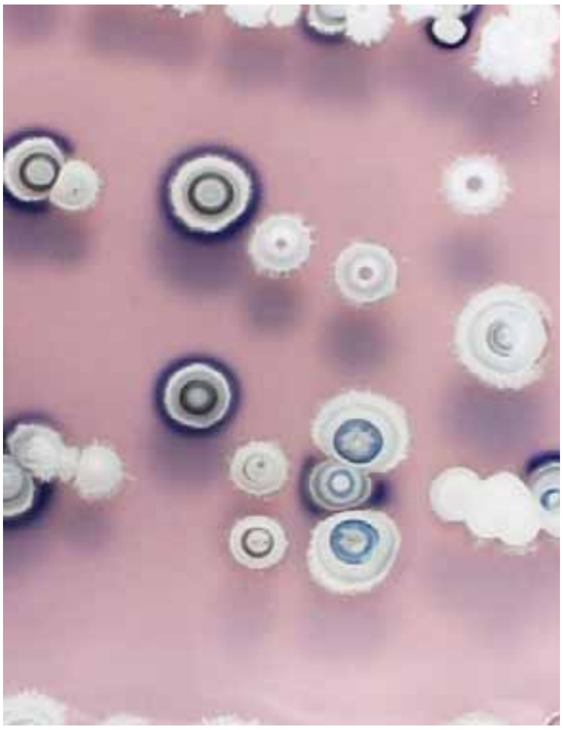

Image 1 Close-up of Streptomyces *colonies with differing morphologies. You can see that the colonies grow on the surface of the transparent medium; the red antibiotic they produce diffuses into the medium.*

Image 2 These colonies of S. Coelicolor are growing on a different medium to that shown in Image 1. This medium doesn't support antibiotic production.

Catalyst

www.sep.org.uk/catalyst

A knockout mutant of Streptomyces coelicolor growing on an agar plate; the pink colour shows that an antibiotic has been produced by the bacteria.

Photo: Suzy Moody

How streptomycetes grow

Streptomyces are unusual bacteria as they have three distinct life stages. While most bacteria grow as rods or cocci (spheres), streptomycetes grow as long branching filaments called vegetative mycelia. When the colony is mature, aerial hyphae (stalks) grow up from the mycelia, and spores are made at the end of each hypha. Some species grow spores in curled chains, so they end up looking a bit like a twister lolly. Images 3 and 4 are taken down a microscope, and show the mycelial growth. Antibiotic production is associated with a mature colony, that is one producing aerial hyphae and spores. The big question is: why do these bacteria produce antibiotics in the first place?

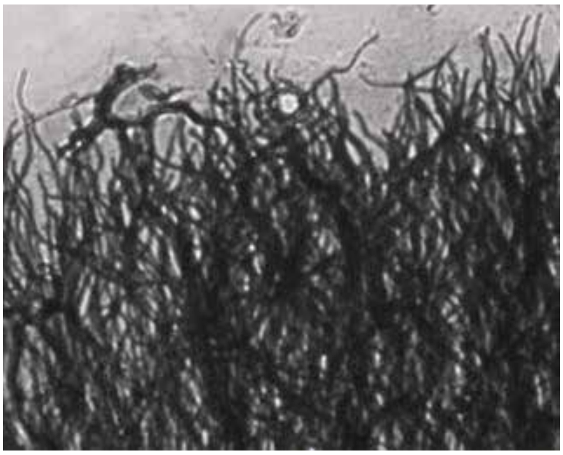

Image 3 *A microscope image of dense mycelial growth*

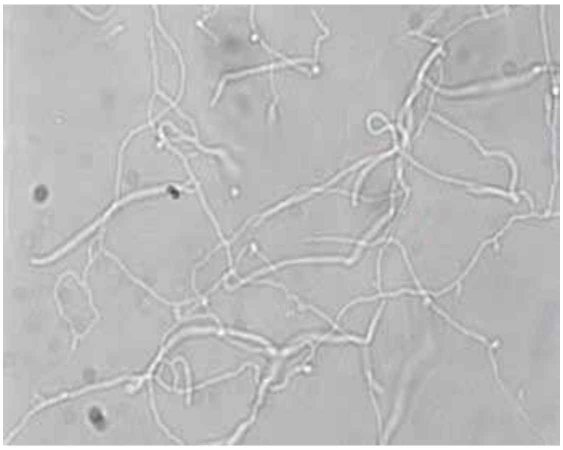

Image 4 *When the sample in Image 3 is diluted a little, vegetative mycelia with branching chains can be seen*

Why antibiotics?

It has long been thought that the main reason for antibiotic production is to give the producing bacteria a competitive advantage over their rivals. Soil is a particularly complex chemical and biological environment, with lots of different species of bacteria, fungi, plants and other organisms, all competing for space, nutrients and energy. Streptomycetes may produce antibiotics to kill off their bacterial competitors, thereby giving them more space and energy with which to grow. This effect can be demonstrated on agar plates.

Image 5 *An agar plate showing competition between B. licheniformis and S. coelicolor.*

Image 5 shows a mature *S. coelicolor* colony producing antibiotics that are inhibiting the growth of the bacteria on the left side. This is *Bacillus licheniformis*, another common soil microbe. We call the gap caused by the antibiotics a 'zone of inhibition'.

Image 6 *Close up of the zone of inhibition*

If you look more closely at what is going on, the story gets even more interesting. Image 6 shows the same plate, with the zone of inhibition still very clear even down a microscope. But streptomycetes only produce antibiotics as a mature colony. So when we plated the same two species against each other, but with a young streptomycete colony, we saw a different pattern emerging, as shown in Image 7. Here the *B. licheniformis* at the top, is growing out towards the streptomycete colony with no problem. *Streptomyces coelicolor* hasn't begun its antibacterial warfare yet.

Image 7 *B. licheniformis and S. coelicolor growing towards each other*

Suzy Moody is a microbiology research student at Swansea University. She loves her streptomycetes and enjoys growing pretty colonies to photograph!