

Mycobacteria: Biology

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The bacterial genus *Mycobacterium* contains more than 120 members, several of which are extremely successful pathogens of man and animals including the agents of tuberculosis and leprosy. All mycobacteria are aerobic, nonmotile, nonspore forming, Gram-positive bacteria with a high genomic guanine + cytosine (GC) content. They possess a thick cell wall that contains unique lipids, called mycolic acids, which confer a characteristic acid-fast staining property and make the genus hardy and resistant to antimicrobials and host defences.

Introduction

The genus *Mycobacterium* belongs to the family Mycobacteriaceae within the order Actinomycetales. There are more than 120 naturally occurring species of mycobacteria. The majority of the mycobacteria are common saprophytic environmental organisms. They have been isolated from diverse environmental conditions including water, soil, biofilms, mosses, etc. However, the importance of the mycobacteria lies in the pathogenic ability of some members of the genus, hence, it is these mycobacteria that have been studied most intensively.

The mycobacteria were first discovered by Robert Koch who isolated the 'tubercle bacilli' in 1882. His research was driven by the desperate need to find both a cause and a cure for the most serious disease of his time, tuberculosis (TB):

If the number of victims which a disease claims is the measure of its significance, then all diseases, particularly the most dreaded infectious diseases such as bubonic plague, Asiatic cholera, etc. must rank far behind tuberculosis.

Robert Koch, during his lecture on 'The Aetiology of Tuberculosis', Berlin, 1882.

See also: Koch, Heinrich Hermann Robert

Records exist of TB occurring in China 2300 years ago, India 3300 years ago and the deoxyribonucleic acid (DNA) of *Mycobacterium tuberculosis* has been successfully amplified from the tissues of Egyptian mummies who were living as much as 5400 years ago. One theory, based on the separation of specific habitats of particular mycobacteria by continental land mass movements, even suggests that the mycobacteria were around in the Jurassic period of 150 million years ago.

As a group, the mycobacteria contain several of the most important pathogens of both man and animals. *M. tuberculosis* is the cause of TB and is one of the most successful and dangerous pathogens of all time. In Britain, the Medical Research Council was founded in 1913 purely to combat the threat of TB. Because of the importance of TB throughout history it is common to be 'TB centric'

Introductory article

Article Contents

- Introduction
- The Genus *Mycobacterium*
- Microbiology of the *Mycobacteria*
- The Mycobacterial Cell Wall
- The *M. tuberculosis* Complex
- Other Pathogenic Mycobacteria
- *Mycobacterium bovis* BCG – the Man-made Vaccine Strain
- Mycobacterial Genomics and Pathogenesis

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when discussing the mycobacteria and accordingly the mycobacteria are generally separated into those that cause TB and those that do not – the nontuberculosis mycobacteria (NTM).

Of the NTM *M. leprae*, which causes leprosy, has been a scourge for much of man's history attracting great stigma to those infected. In recent times, other members of the NTM, especially *M. avium* and *M. intracellulare*, are becoming even more important as agents of opportunistic infection in Acquired immunodeficiency syndrome (AIDS) patients infected with Human immunodeficiency virus (HIV). It is estimated that 11% of all HIV/AIDS-related deaths are due to mycobacterial infections. In addition to this, many of the other environmental mycobacteria have been documented as causing random, opportunist, infections in both humans and animals.

Great effort has been invested to unravel the biology of the mycobacteria and the pathogenic mechanisms by which they cause disease. Beginning with Koch's work in the late nineteenth century, the basic microbiology of the mycobacteria was gradually unravelled over the next 100 years. Work has continued until now when, in the post-genome era, the genome sequences of 10 mycobacteria have been completed with a further 20 ongoing. We are closer to understanding the biology and pathogenesis of the mycobacteria than ever before, yet we are still unable to conquer these formidable foes.

The Genus *Mycobacterium*

Superficially the mycobacteria and their closest relatives (the *Corynebacterium*, *Gordonia*, *Nocardia*, etc. – whose members include the agents of diphtheria and nocardiosis) are difficult to distinguish. Therefore, for an organism to be classified in the genus *Mycobacterium* the minimal set of requirements are thus; (1) it must be acid fast, (2) it must contain mycolic acids of 60–90 carbon atoms in length and (3) its genome must have a guanine + cytosine (G/C) content of 61–71%. Currently, there are more than 120 species of *Mycobacterium* that fit these criteria and are shown to be closely related through 16s ribosomal RNA (rRNA) gene

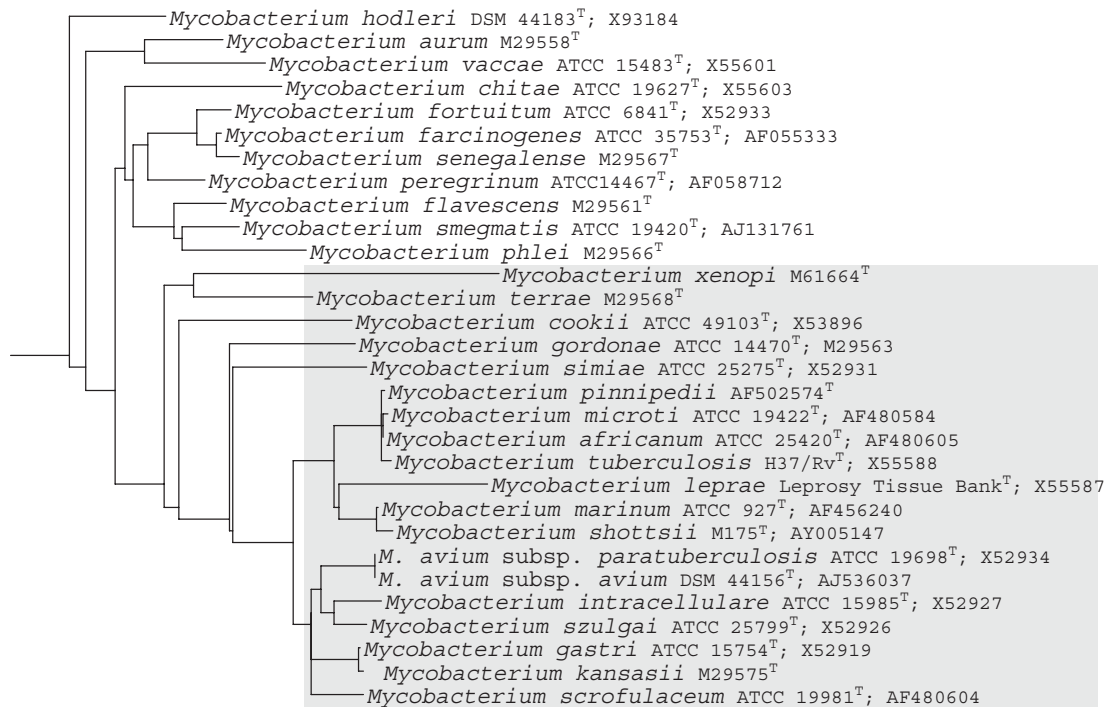


Figure 1 A phylogenetic tree showing a selection of the mycobacteria. The tree was calculated based on the similarity of the mycobacterial 16S rRNA gene sequences. A clear separation of the fast and slow growers (grey shading) is apparent. Tree created using the tree builder tool and 16S rRNA gene sequences from the Ribosomal Database Project (<http://rdp.cme.msu.edu/>). Strain name is followed by the strain designation (^T indicates a Type Strain) and 16S rRNA gene accession numbers. The tree is rooted using *Nocardia farcinica* ATCC 3318 (X80595).

sequencing, which is a common modern method of determining the identity of a species (see **Figure 1**).

Ever since the mycobacteria were first isolated, they have been troublesome organisms to work with and the genus is characterized by slow to very slowly growing organisms. The mycobacteria can be divided into two groups, the 'slow'-growing mycobacteria (SGM) and the 'fast'-growing mycobacteria (FGM). Traditionally, FGM are distinguished from SGM by their ability to produce visible colonies on solid media within 7 days but we can see that these distinct groups are clearly differentiated when the mycobacteria are classified based on the similarity of their 16S rRNA gene sequences (see **Figure 1**). The major mycobacterial pathogens are all slow growers, including the *M. tuberculosis* complex, *M. leprae* and the *M. avium* complex organisms.

Microbiology of the *Mycobacteria*

The genus name, *Mycobacterium*, is derived from the Greek 'Myces' meaning a fungus, and 'Bakterion' denoting a small rod; literally meaning, a bacteria of fungal appearance. Although the mycobacteria can appear filamentous and branched in liquid culture, they do not produce a true mycelium and are easily fragmented back to individual cells ~1–10 µm in length and 0.5 µm in diameter appearing as

elongated rods, see **Figure 2a**. In laboratory liquid culture, the nonmotile cells often clump, forming long stringy cords (see the section on The mycobacterial cell wall) causing the resemblance to a fungal mycelium.

The majority of the mycobacteria are easily cultivated, with patience, in the laboratory. A notable exception is *M. leprae*, which has still to be grown on media *in vitro*. Koch first isolated mycobacteria using coagulated blood serum, however most mycobacteria are now grown on laboratory media that is rich in inorganic ions and supplemented with albumin to sequester toxic fatty acids, catalase to remove toxic peroxides and glycerol and dextrose as carbon/energy sources. Tween 80 is an additional supplement that has two features; first, it acts as a detergent to prevent clumping of the mycobacterial cells. Second, Tween 80 is a long-chain polysorbate esterified to a fatty acid (oleic acid). As mycobacteria are thought to use fatty acids as their carbon source during infection, some argue that the presence of Tween 80 more readily resembles conditions pathogenic mycobacteria are likely to encounter within the host and so use Tween 80 as the sole carbon source.

On solid media, the general colony morphology is compact and appears dry and wrinkled, although some mycobacteria produce smooth round glistening colonies, see **Figure 2b**. This is further complicated by the fact that

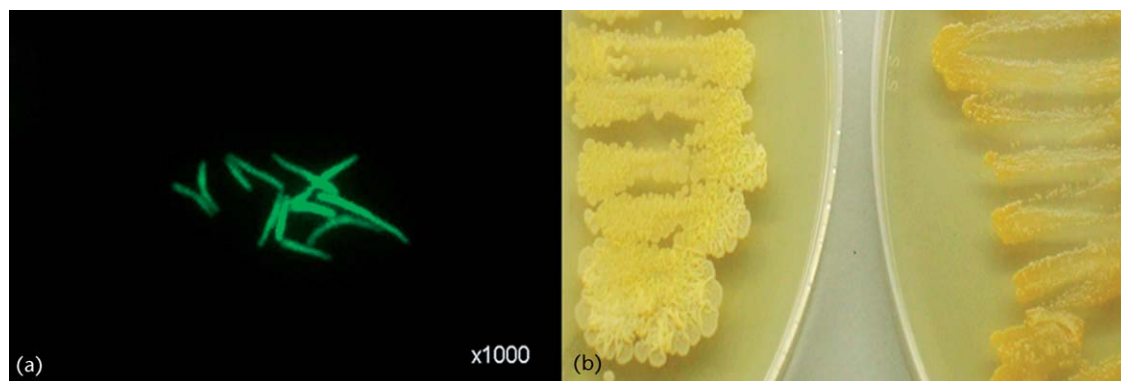


Figure 2 The cell and colony morphology of *M. smegmatis* is typical of that of many mycobacteria. (a) depicts the characteristic rod shape of *M. smegmatis* shown expressing the green fluorescent protein (GFP), visualized using confocal microscopy. The cells are $\sim 10\ \mu\text{m}$ long (Taken by B. Sidders, RVC). (b) depicts *M. smegmatis* colonies growing on solid agar and the differences that can be seen in colony morphology. Colonies shown are growing in the presence (left) and absence (right) of glycerol (Courtesy of S. Kendall, RVC).

some species produce differing colony types dependent on the growth conditions and nutrients available. Some mycobacteria, but not the members of the *M. tuberculosis* complex (see the section on The *M. tuberculosis* complex), have yellow colonies due to the production of a yellow carotenoid pigment.

The mycobacteria grow optimally in the range of $28\text{--}45^\circ\text{C}$ and are generally aerobic, although they can happily persist in microaerophilic conditions such as those found within a host granuloma produced during *M. tuberculosis* infections. The mycobacteria do not produce spores in response to any environmental condition.

The Mycobacterial Cell Wall

A unique characteristic of the mycobacteria is an extremely thick, lipid-rich and hydrophobic cell wall, which is quite different to that of other bacteria, see **Figure 3**. Although mycobacteria possess Gram-positive-type cell envelopes, they have such a high lipid content that they do not take up the primary crystal violet dye of the Gram stain. Although Gram staining is ineffective, mycobacteria can be identified based on their ability to resist the bleaching effects of acid. This property forms the basis of the Ziehl–Neelsen stain, which is used throughout the world to positively identify mycobacteria. The staining procedure first drives the red dye fuchsin into the cell envelope, where it interacts with the mycobacterial-specific lipids. The cells are then washed with acid-alcohol to remove the fuchsin from nonmycobacterial cells and then counter stained with the dye methylene blue. Mycobacteria resist the acid washing and appear red after staining; hence, mycobacteria are commonly referred to as being ‘acid-fast’.

The mycobacterial cell wall is in large part responsible for the general hardness of the genus, their ability to persist in the environment and to resist desiccation, antimicrobials and host-immune mechanisms. The key component of this cell wall is the mycolic acids. These are long-chain high

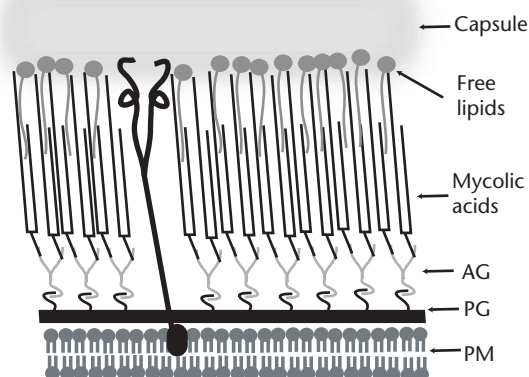


Figure 3 The mycobacterial cell wall. The mycobacteria possess a uniquely thick, lipid-rich cell wall that forms a near impermeable barrier. Atop a typical Gram-positive membrane is the MAP complex, consisting of peptidoglycan (PG) linked to arabinogalactan (AG) which acts to anchor the mycolic acids in place. Outside this are a layer of ‘free’ or covalently bound lipids including the phthiocerol dimycoserates (PDIMs), phenolic glycolipids (PGLs), etc. The mycobacteria are also surrounded by a loosely attached capsule made of various polysaccharides. Interspersed through all of this are various cell wall proteins (porins, etc.) and components such as lipoarabinomannan (LAM, shown) a major glycolipid that plays a key role in modulating the host–immune response.

molecular weight lipid molecules that readily ‘stack’ side by side to produce a near impermeable barrier and are responsible for the acid-fastness of the mycobacteria. There are several important varieties of mycolic acids, the most extensively studied being trehalose-6,6'-dimycolate (TDM) from *M. tuberculosis* and other pathogenic mycobacteria which is otherwise known as Cord Factor. The name Cord Factor is derived from the property that TDM confers upon mycobacteria growing in liquid medium – colonies exhibit a filamentous or cord-like structure. Aside from this, TDM has also been shown to play an important role in modulating the interaction between the host and the bacterial cell during an infection.

Many frontline antimycobacterial drugs target mechanisms involved in cell wall and fatty acid biosynthesis. Both isoniazid and ethionamide are active against a key component of the mycolic acids synthesis pathway, whereas ethambutol prevents synthesis of arabinogalactan – the major polysaccharide of the cell wall. **See also:** Bacterial Cell Wall

The *M. tuberculosis* Complex

The mycobacteria that cause human and/or animal TB are grouped together in what is known as the *M. tuberculosis* complex (MTC). Originally, the MTC consisted of *M. tuberculosis*, *M. africanum*, *M. microti* and *M. bovis* (including the vaccine strain *M. bovis* bacille Calmette-Guérin (BCG), see the section on *M. bovis* BCG – the man-made vaccine strain). Recent additions to this group include *M. pinnipedii*, *M. canettii* and *M. caprae*. All of these mycobacteria have identical 16s rRNA gene sequences and their genome sequences are 99.9% homologous. *M. canettii* displays considerable phenotypic differences to the rest of the MTC, but the rest are very similar both biochemically and phenotypically. All of the MTC members are pathogenic, yet they have widely differing host preferences, mechanisms of disease and pathologies.

Comparisons between the genomes of the MTC organisms have revealed an evolutionary timeline for the MTC and are helping us to understand how these organisms cause disease. Scientists have analysed deletions from the genomes of the MTC organisms (regions of DNA which have been lost over time), and in a sequential manner can trace which deletions (and therefore which genomes) came first. Current opinion is that the ancestor of the MTC most closely resembled *M. tuberculosis* who, via losses from the genome, gave rise to the other members. The major pathogens of the MTC are described in more detail later, while **Table 1** summarizes some of the important characteristics for all the members of this group including *M. microti*, *M. pinnipedii* and *M. caprae*, the agents of tuberculosis in the vole, seal and goat, respectively.

Mycobacterium tuberculosis

M. tuberculosis is the aetiological agent of TB, and is the most intensively studied of all the mycobacteria. The bacilli are strongly acid-fast, and when grown on solid media the colonies appear rough and wrinkled with an off-white colour and are usually visible after 3 weeks of growth. In liquid culture, *M. tuberculosis* grows in long serpentine cords and has a doubling time of 14–15 h.

M. tuberculosis is spread by aerosols exhaled by infected individuals. These aerosols are inhaled and enter the alveolar spaces within the lungs where host macrophages engulf the mycobacteria. *M. tuberculosis* then blocks maturation of the phagosome in which it is interred, preventing activation of host antimicrobial responses and its own destruction. The immune system, unable to kill the bacteria,

wards the bacteria off in immune cell-surrounded structures called granulomas. Here the bacilli can remain for many years, until they reactivate to cause active disease once more. Reactivation can be spontaneous, but often appears to occur in response to a weakening of the immune system, either through age or disease (AIDS). Active TB is characterized by a chronic fever, weight loss, malaise and cough. The cough can contain blood in advanced cases, helping to spread *M. tuberculosis* via aerosol once more.

It is thought that nearly a third of the world's population is latently infected with *M. tuberculosis*. In the year 2000, this resulted in an estimated 8.3 million active cases of disease and ~2 million deaths from *M. tuberculosis* infection worldwide. Other articles within this volume explore tuberculosis as human disease in more detail. **See also:** Tuberculosis

Mycobacterium bovis

M. bovis is the aetiological agent of bovine tuberculosis, a similar disease to that of tuberculosis in humans. *M. bovis* bacilli are generally shorter than other mycobacteria, and when grown on solid media form thin, flat colonies that appear corded in the early stages of growth. Colonies are often visible after 3 weeks of growth.

Unlike *M. tuberculosis*, *M. bovis* has a wide host range. Before the pasteurization of milk was introduced, *M. bovis* accounted for a large percentage of human cases of disease. *M. bovis* represents a significant economic burden to the agriculture industry as any cattle found to react positively to skin test is slaughtered to prevent spread of the disease. The UK spent £90.5 million on bovine TB testing and control in 2005.

Mycobacterium africanum

M. africanum is very closely related to *M. tuberculosis* and causes very similar pathology. The bacilli produce flat, dull, rough colonies on solid media and have a doubling time similar to that of *M. tuberculosis* (~15 h). In many other respects, its biochemical and cultural features fall halfway between those of *M. tuberculosis* and *M. bovis*.

This organism was isolated from a patient in Senegal, and so far it is generally found in both western and central African countries. There is some debate as to whether *M. africanum* is a pathogen solely of humans (like *M. tuberculosis*) or whether the human infections are zoonotic and represent the spillover from a predominantly animal pathogen (like *M. bovis*).

Other Pathogenic Mycobacteria

Aside from the MTC, many other mycobacteria are capable of causing infections. The most important of these is *M. leprae*, another obligate pathogen. Initially it was thought that only the obligate pathogens of the mycobacteria represented a serious threat of disease. However, in 1953

Table 1 Summary of important and common mycobacterial species

Group	Organism	Growth rate	Type of organism	Most common disease	Host range	Theory behind the name	Natural environment
M.tb complex	<i>M. tuberculosis</i>	SGM	Obligate pathogen	Tuberculosis	Humans	Lesions in lungs originally described as small swellings or 'tubercles'	Human population
	<i>M. bovis</i>	SGM	Obligate pathogen	Bovine tuberculosis	Cattle, humans and small mammals	Isolated originally from tubercles in cattle. Bovis, of the Ox	Cattle, humans and small mammals
	<i>M. africanum</i>	SGM	Obligate pathogen	Tuberculosis-like disease	Humans	Originally isolated from a patient in Senegal and an agent of human TB throughout Africa	Human population
	<i>M. microti</i>	SGM	Obligate pathogen	Tuberculosis-like disease	Voies, rodents and small mammals	Originally isolated from voies which are members of the genus microtus	Rodent population
	<i>M. pinnipedii</i>	SGM	Obligate pathogen	Tuberculosis-like disease	Seal, humans, tapir and cattle	Originally isolated from seals (Pinnipeds)	Seal population
	<i>M. caprae</i>	SGM	Obligate pathogen	Tuberculosis-like disease	Goats	Originally isolated from a goat (Capra)	Goat population
Nontuberculosis mycobacteria	<i>M. leprae</i>	SGM	Obligate pathogen	Leprosy – cutaneous, nerve lesions	Humans	Leprae, the cause of leprosy. Also known as Hansen's Bacillus after discoverer	Human population
	<i>M. marinum</i>	SGM	Opportunistic pathogen	Cutaneous 'swimming pool granulomas'	Humans/fish/amphibians	Marinus, of the sea. Isolated from diseased fish	Water
	<i>M. fortuitum</i>	FGM	Opportunistic pathogen	Pulmonary, cutaneous and disseminated	Humans/other mammals	Appears to cause infection fortuitously as result of other ailments, e.g. post-op wounds	Soil, dust, water
	<i>M. kansasii</i>	SGM	Opportunistic pathogen	Pulmonary lesions	Humans	Isolated from a human pulmonary lesion in the US state of Kansas	Water

(Continued)

Table 1 Continued

Group	Organism	Growth rate	Type of organism	Most common disease	Host range	Theory behind the name	Natural environment
	<i>M. smegmatis</i>	FGM	Environmental isolate	Rarely causes opportunistic infections	-	Originally isolated from smegma – an oily effusion. Smegmatis, infecting smegma	Soil
	<i>M. bovis BCG</i>	SGM	Vaccine	-	-	A descendant of <i>M. bovis</i> , the Bacillus created by Calmette and Guérin (BCG)	-
M. avium complex	<i>M. avium</i> ssp <i>avium</i>	SGM	Frequent opportunist	Pulmonary/ disseminated	Humans/birds	Isolated from TB lesions in fowl. Avium, of birds	Avian population
	<i>M. avium</i> ssp <i>paratuberculosis</i>	SGM	Obligate pathogen	Johne disease and Crohn disease (?)	Ruminants/ humans(?)		Ruminants, soil, milk
	<i>M. intracellulare</i>	SGM	Frequent opportunist	Pulmonary/ disseminated	Humans/birds/ cattle/swine		In lesions, soil, water

a report was made of an 'infection with atypical acid-fast organisms', which later became known as *M. kansasii*, the first of the NTM shown to cause disease. With the knowledge that disease could be caused opportunistically by mycobacteria that were not obligate pathogens, environmental mycobacteria were soon discovered to be everywhere. They are present in water, biofilms, soil and aerosols and are natural inhabitants of the urban environment, especially drinking water–distribution systems. NTM organisms that cause a significant amount of disease include the members of the *M. avium* complex – *M. avium* and *M. intracellulare*, *M. marinum* and *M. ulcerans*.

Mycobacterium leprae

M. leprae is the aetiological agent of leprosy (or Hansen disease). The bacilli are strongly acid fast and tend to be found in clumps. Currently, there is no repeatable method for the continued growth of *M. leprae* *in vitro*. It was discovered that the organism grows readily in the nine-banded Armadillo which has been used to propagate organisms ever since; however they still have to be isolated directly from the tissues. *M. leprae* has a doubling time of 20–40 days.

Patients are infected with *M. leprae* via the mucosal membranes where the bacilli are taken up by monocytes that migrate to the body's extremities. Here, at areas of lower body temperatures, *M. leprae* shows a marked preference for host Schwann cells – specialized cells that encase axons of the peripheral nervous system. Here, they multiply until the hosts immune system is activated and forced to destroy the infected nerves. Swollen lesions appear on the skin, major nerves become swollen and progressive nerve damage leads to muscle weakening and reduced sensory perception. Other articles in the present volume explore leprosy as a disease in more detail. **See also:** Leprosy

The *M. avium* Complex

In recent times, members of the mycobacteria other than the MTC and *M. leprae* are becoming ever more important as agents of opportunistic infections. This is linked very closely to the rise of infections with HIV. It is estimated that 11% of all HIV/AIDS-related deaths are due to mycobacterial infections, and the majority of these are from *M. avium* (MAC) complex organisms.

The MAC consists of *M. avium* and *M. intracellulare*. *M. avium* is a common cause of TB in the avian (bird) population, whereas *M. intracellulare* is isolated from human patients. Neither is commonly found in the environment. *M. avium* and *M. intracellulare* are very closely related and exhibit very similar phenotypical and biochemical characteristics. They are so similar that recent moves have been made to classify them both as *M. avium* subspecies, e.g. *M. avium* subsp. *avium* and *M. avium* subsp. *intracellulare*. Both organisms produce colonies on solid media within 7 days that are usually smooth and non-pigmented, although it is common to see rough and opaque colonies also.

These organisms have the ability to cause a rapid disseminated disease in immunocompromised individuals, yet they are rarely found as the aetiological agent of tuberculosis in healthy individuals. When they are, the disease is usually confined to the pulmonary system and associated with predisposing lung conditions. In the immunocompromised, disease is often rapid and those diagnosed with a MAC infection have considerably reduced survival times. The spread of MAC infections appears to be via aerosol in a similar manner to *M. tuberculosis*, although a more likely route of infection is via the gastrointestinal tract as person-to-person spread has yet to be shown. The pathogenicity of MAC organisms appears to be dependent on the presence of a plasmid within the bacterial cell. This is interesting because only rarely have mycobacterial species have been shown to harbour plasmids naturally.

Other pathogens of note

M. ulcerans is the causative agent of Buruli ulcer, a cutaneous infection commonly found in parts of Africa. It is thought to be the fourth most common mycobacterial infection after TB, leprosy and MAC infections. *M. ulcerans* poses a severe threat because drugs are unable to penetrate the lesion and patients do not respond to treatment. Often, the only course of treatment is for the lesion to be surgically removed.

M. marinum is primarily a pathogen of fish and other aquatic animals; however, it is the causative agent of 'swimming pool granulomas' in humans. *M. marinum* causes a cutaneous infection associated with previous wounds that is contracted from infected water sources, hence, this infection is common among fisherman and divers. *M. marinum* is the most closely related of the NTM to *M. tuberculosis*.

***Mycobacterium bovis* BCG – the Man-made Vaccine Strain**

Current cures for mycobacterial infections remain costly and arduous for the patient. A preferred alternative, which has led to the eradication of many infectious diseases, is immunization. In the early 1900s, Albert Calmette and Camille Guérin working at the Institut Pasteur in France undertook a Herculean task to try and attenuate the *M. bovis* bacillus so that it might be used as a vaccine strain.

Between 1906 and 1919, *M. bovis* was serially cultured, or 'passaged', on potato slices soaked in glycerol and Ox bile and repeated observations were made of the gradual loss of the organism's ability to infect various animals over time. Eventually, after 230 passages they began to use the 'Bacillus of Calmette and Guérin' successfully as a vaccine in humans. It has become apparent that BCG, while effective in certain geographical areas and at certain ages (e.g. in early childhood), is not the perfect vaccine required to eradicate TB in the same manner as smallpox and polio. Repeated attempts have been made to improve on the protective ability of BCG as a vaccine, but so far none have been successful.

A study of the genomes of *M. bovis* and *M. bovis* BCG identified several regions that have been lost from the *M. bovis* BCG genome. The most important of these, from a virulence point of view, contains genes that code for a secretion system used by the bacteria to excrete certain proteins which are known to be important for causing *M. tuberculosis* infections and this appears to account for the attenuation of BCG. This secretion system is the subject of intensive study and may well yield the key to future preventative measures against tuberculosis.

Mycobacterial Genomics and Pathogenesis

The genome sequence

Starting with the laboratory strain of *M. tuberculosis*, H37Rv, in 1998, 10 mycobacterial genome sequences have been completed to date. The most useful to researchers striving to understand the mechanisms by which this group of organisms causes disease are those of *M. tuberculosis* H37Rv, *M. tuberculosis* CDC1551 (a clinical isolate), *M. leprae* and *M. bovis* AF2122/97.

Major insights have been gained from observations of the genomic content of these pathogens. For example, almost all bacteria possess genes that code for the biosynthesis and degradation of fatty acids. Uniquely, the genome of *M. tuberculosis* contains nearly 240 of these lipid metabolism genes while *Escherichia coli*, for example, has just 50. This strongly suggests that *M. tuberculosis* has a preference for lipids as its energy source and reflects the importance of lipids in maintaining the mycobacterial cell wall.

Another interesting feature observed in the genomes of the *M. tuberculosis* complex organisms is the presence of a family of 170 genes, representing 9% of the genome, which have a repetitive proline and glutamate-rich motif at one end. This family is known as the PE and PPE family of genes, all of which are highly homologous and unique to the mycobacteria. They have been proposed to function as cell wall components, components of antigen presenting pathways and as a means to induce antigenic diversity to the mycobacterial cell wall; however their complete range of functions remains unclear.

Finally, after all of the genes in the *M. tuberculosis* genome had been annotated just under 40% had to be classified as having an 'unknown' function. There are no known homologues of these genes in any other organism which is not a part of the MTC. Therefore, these genes represent key targets for future studies as they could hold the reasons as to why the mycobacteria cause disease and in the manner by which they do it. With this knowledge targeted attempts can be made to interfere with the disease process leading to a cure.

Genome comparisons

Genomic comparisons between the different mycobacteria have also shed light on how these organisms are likely to

cause disease. By comparison to the *M. tuberculosis* genome, *M. leprae* has undergone considerable loss from its genome. *M. tuberculosis* has a genome of 4.4 million DNA base pairs (Mb), whereas the *M. leprae* genome is over 1 Mb smaller at 3.27 Mb. Functions that have been lost include many metabolic pathways, which likely accounts for the difficulty that researchers have found in culturing *M. leprae* in the laboratory.

The *M. bovis* genome is just 60 000 DNA bases smaller than that of *M. tuberculosis* and they are 99.95% identical at the sequence level. *M. bovis* does not have any extra genes compared to *M. tuberculosis*, in fact, the only significant differences between them are the regions of difference (RD), which have been lost from the *M. bovis* genome. This poses an interesting conundrum as it was hoped the genome sequence would reveal why *M. tuberculosis* has such specificity for humans, while *M. bovis* by comparison infects quite a diverse range of hosts. Without any unique host-specific genes the difference in pathogenicity must be the result of how their common genes are used, or regulated, during an infection.

Functional genomics

Since the sequencing of the mycobacterial genomes, many new technologies have been developed to identify the functions of the 'unknown' genes coded for in them. The most successful of these has been the Microarray. Microarrays have allowed researchers to identify the genes that are switched on in response to specific environmental stimuli, including during an infection. One of the most important discoveries to date is that of the *dosRS* regulon. When aerobically grown bacteria were compared to bacteria grown in low-oxygen conditions a specific set of genes, the regulon, were shown to have increased levels of expression. DosS is a sensor protein that monitors the environmental conditions outside the cell. When the level of oxygen reaches very low levels (such as the levels found inside host granulomas) DosS activates its partner DosR which increases the expression of the genes in the regulon. In this way the organism adapts to low levels of oxygen, preparing itself for entry into a latent phase of existence and a prolonged infection of the host. **See also:** Multispot Array Technologies

Another method of identifying genes that are important in mycobacterial infections was developed using an approach called TraSH that randomly inactivated all of the genes in the genome one at a time. Each of these gene-inactivated mutants were then tested for their ability to grow as well as the wild-type strain and to cause infection in a mouse model. Researchers were able to identify cases where a mutant with a specific inactivated gene was no longer able to cause an infection. In this way, 614 genes were identified that are needed for *M. tuberculosis* to grow as well as it would normally and 194 were required for the organism to cause disease as severely as it does naturally. Interestingly, the majority of these genes are present in the reduced genome of *M. leprae* suggesting that they have been evolutionarily conserved as their importance for

infection is high. These genes will be very good potential drug targets once their function is known.

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