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The mycobacteria: an introduction to nomenclature and pathogenesis

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Summary

Tuberculosis, caused by *Mycobacterium tuberculosis*, and leprosy, caused by *M. leprae*, are diseases known since antiquity. In developing countries, tuberculosis is still the leading cause of mortality due to an infectious disease. Taxonomically, mycobacteria belong to the genus *Mycobacterium*, which is the single genus within the family of Mycobacteriaceae, in the order Actinomycetales. Actinomycetales include diverse micro-organisms, but mycobacteria and allied taxa are easily distinguished on the basis of the ability to synthesise mycolic acids. Mycobacterial species are traditionally differentiated on the basis of phenotypic characteristics, and the authors provide an updated list of the biochemical tests currently employed and the culture properties that help to discriminate among various species of mycobacteria. However, as the phenotypic characteristics do not allow precise identification of all species, recent molecular taxonomical approaches for mycobacterial classification and phylogeny are also described. Mycobacteria are also a leading cause of infection in various domesticated animals and wildlife. The authors briefly describe the mycobacteria involved in animal infections, the wildlife reservoirs and strategies to control bovine tuberculosis, and the use of molecular tools for diagnostics and epidemiology of mycobacterial infections in animals. The characteristic of intracellular parasitism is discussed, in addition to the fate of pathogenic mycobacteria that have the ability to grow inside phagosomes and phagolysosomes of infected host macrophages. The mycobacterial cell envelope, which is a complex tripartite structure containing a high proportion of lipids (approximately 30% to 40% of the total weight) could play a crucial role in the adaptation of mycobacteria to intracellular growth and survival, immune modulation and drug resistance.

Keywords

Animals – Cell envelope – Disease – Disease control – Mycobacterium – Nomenclature – Pathogenicity – Phylogeny – Taxonomy.

Introduction

The genus *Mycobacterium* contains a number of strict and opportunistic pathogens that afflict humans and animals alike. Among the strict pathogens, the principal pathogens of humans include *Mycobacterium tuberculosis*, the causative agent of tuberculosis, and *M. leprae*, which causes leprosy. Opportunistic pathogens comprise a variety of mycobacterial species, including *M. avium*, *M. simiae*, *M. kansasii* and *M. haemophilum*, which are more common among immunocompromised patients. Other opportunistic mycobacterial infections are caused by *M. ulcerans*, which produces a destructive, primarily tropical skin disease which, if not treated rapidly, produces chronic ulcers with necrotic centres (also known as Buruli ulcer); *M. marinum*, responsible for fish-tank or swimming-pool granuloma which essentially concerns people exposed to fish or water; *M. scrofulaceum*, which has been associated with cervical lymphadenitis in children and may also cause pulmonary tuberculosis in adults; *M. szulgai*, which has been associated with pulmonary disease; *M. xenopi*, which was initially isolated from a skin lesion of a South African toad (*Xenopus laevis*), and is implicated in chronic pulmonary diseases as well as non-pulmonary infections in immunocompromised patients;

and *M. malmoense*, which is associated with pulmonary disease and cervical adenitis. Recently described opportunistic pathogens include *M. celatum* and *M. genavense*, which are relatively more common among immunocompromised patients. Medically important rapidly growing mycobacteria are essentially limited to *M. fortuitum*, *M. chelonae* and *M. abscessus* and are associated with traumatic and surgical wound infections, skin and soft tissue infections and pulmonary disease. Lastly, the principal mycobacterial pathogens of animals include *M. bovis*, the causative agent of bovine tuberculosis, *M. paratuberculosis*, which causes Johne's disease or paratuberculosis in cattle, and *M. avium*, which is often associated with disease in pigs and poultry. However, of these many species of mycobacteria, the species that has been most troublesome, and about which current knowledge is the most extensive, is *M. tuberculosis*.

Suspected as the cause of death in some of the Egyptian mummies 3,000 years ago, tuberculosis is known to have been present since antiquity (108). Recently, evidence for pulmonary tuberculosis, including the presence of acid-fast organisms, was also detected in a New World mummy from Peru, dated 700 AD. The importance of rest and the need for fresh air were suspected to be essential elements of treatment by Hippocrates, Celsus and Galen. Recognised as a contagious disease in Mediterranean culture during the 16th Century, and initially termed phthisis, pathological and anatomical characteristics of tuberculosis were reported in *Opera Medica* by Sylvius in 1679, and this was followed by the description of pathological features of miliary tuberculosis by Manget in 1702. The microbial nature of tuberculosis was suspected by the French military doctor Jean-Antoine Villemin as early as 1865, when a phthisis-like infection was successfully reproduced in rabbits and guinea-pigs which had been inoculated with a homogenate prepared from tuberculous lesions. Although air-borne infectiousness was also suspected by the English physician Benjamin Marten in 1720, according to Austin Flint, the non-communicability of tuberculosis was a general belief as late as 1881.

Also known as 'white plague', tuberculosis was a leading cause of death in Europe and the United States of America (USA) in the 19th Century (the estimated mortality rate from tuberculosis was as high as 400/100,000 in the USA in 1830). In this grim context, the Norwegian doctor G. Armauer Hansen identified, in 1873, the bacterium responsible for leprosy, and this was subsequently found to have a close resemblance to the tubercle bacillus, discovered nine years later by Koch, who was able to culture the tubercle bacillus on coagulated serum, and visualise the bacterium using a specialised staining method in 1882. Other important advances in tuberculosis research include the discovery of the acid-alcohol resistance of these organisms and characteristic Ziehl-Neelsen staining that is still routinely used (contributions of Ehrlich, Ziehl and Neelsen), the differentiation between avian and human tubercle bacillus (Rivolta 1889, Maffucci 1890), description of Koch's postulate and preparation of tuberculin in 1891 (Koch), and the description of the bovine tubercle bacillus by Smith in 1902.

Although the tuberculosis mortality rate had fallen to 200/100,000 by 1900, due to the improvements in social and sanitary conditions, a wider availability of adequate nutrition, and a wider use of sanatoria throughout Europe and the USA, the active therapy was still limited to surgical methods. However, the discovery of X-rays made it possible to detect and follow the progression of disease for the first time. During the years 1908 to 1920, Calmette and Guérin from the Pasteur Institute, France, used specific culture media to lower the virulence of the bovine tubercle bacillus (*M. bovis*). This work provided the background for the development of the bacillus Calmette-Guérin (BCG) vaccine that was used for the first time in 1921, and is still widely used. Another major breakthrough was achieved by Waksman in 1944, through the discovery of streptomycin, an antibiotic that is still used for tuberculosis chemotherapy. The subsequent discovery of other antimycobacterial drugs, such as para-amino salicylic acid in 1949, isoniazid in 1952, and rifampin in 1967, provided the basis for the standard antituberculous chemotherapy, in which three to four drugs are administered for a period of six to nine months.

As a result of efficient chemotherapy, BCG vaccination programmes and improved living conditions, a steady decline in tuberculosis notification rates occurred in industrialised countries (from 200/100,000 in 1900 to less than 10/100,000 in 1980 [79]). However, a re-emergence of tuberculosis has been reported, with a higher percentage of drug resistant isolates since 1985, which has been associated with drug abuse, human immunodeficiency virus (HIV) infection, young patients, and a foreign origin of the patients (principally from the developing countries of Africa, South-East Asia, Latin America and the Caribbean, where tuberculosis remains an enormous health problem). The worsening socio-economic conditions in the industrialised world and the dismantling of the public health infrastructure to control tuberculosis have certainly played a crucial role in this re-emergence. In the developing world, tuberculosis remains an immense health and economic problem, causing approximately eight million new cases and three million deaths annually, making it the leading cause of mortality due to an infectious disease in these countries. Co-infection with HIV further complicates management of tuberculosis and considerably increases the mortality due to the disease in this group (130).

Nomenclature

The genus *Mycobacterium* is one of the oldest defined. The generic name *Mycobacterium* initially designated a group of organisms that grew as mould-like pellicles on liquid media (90). At the beginning of last century, the characteristics used to define mycobacteria were the absence of motility, the

morphology of the bacilli (slightly curved and rod-shaped), and the characteristic resistance to acid-alcohol following coloration with phenicated fuchsin (Ziehl-Neelsen stain). In the order Actinomycetales, mycobacteria belong to the genus *Mycobacterium*, which is the single genus within the family of Mycobacteriaceae (160). Mycobacteria are defined as aerobic, acid-alcohol fast, rod-shaped actinomycetes with occasional branching; aerial hyphae are normally absent, and the bacteria are non-motile, non-sporulating organisms that contain arabinose, galactose, and *meso*-diaminopimelic in the cell wall; the guanine and cytosine (GC) deoxyribonucleic acid (DNA) base ratios are in the range of 62 mol % to 70 mol % (except for *M. leprae*, which has a GC base ratio of 58%); mycolic acids of high molecular weight (sixty to ninety carbons) are present, which lack components with more than two points of unsaturation in the molecule (63). Although historically defined as unencapsulated organisms, mycobacteria are now known to possess a capsule-like structure (120). Similarly, although initially considered as obligate aerobes, some species and strains are microaerophilic and grow as a narrow band under the surface of a semi-solid medium (63). Actinomycetes include diverse micro-organisms, in both ecological or morphological terms (63). Differentiation between the mycobacteria and allied taxa (e.g. the members of the *Corynebacterium*, *Mycobacterium* and *Nocardia* [CMN] group which also includes the genera *Rhodococcus*, *Gordona* and *Tsukamurella*) can be easily performed, on the basis of the ability to synthesise mycolic acids that are high molecular weight β-hydroxy fatty acids with a long α-side chain. Mycobacterial mycolic acids usually occur as complex mixtures of components that have oxygen functions such as carboxy, keto or methoxy groups, in addition to the 3-hydroxy acid system and combinations of *cis* or *trans* double bands or cyclopropane rings; methyl branches are also encountered. The members of the CMN group are the only micro-organisms that are able to synthesise mycolic acids (63), however, on the basis of the number of carbon atoms and pyrolysis esters of the mycolic acids (as well as the GC content of the DNA), discrimination among the various CMN group members is possible (Table I). Consequently, the genus *Mycobacterium* is defined by the length of the carbon backbone, the number of unsaturated links, the presence of supplementary oxygenated functions, and the esters produced on pyrolysis.

Historically, the nomenclature of mycobacterial infections, irrespective of origin, was limited to tuberculous or non-tuberculous mycobacteria (also termed atypical mycobacteria or 'mycobacteria other than *M. tuberculosis* complex'). The former include *M. tuberculosis*, which is responsible for human tuberculosis, *M. bovis*, which is responsible for bovine tuberculosis, *M. africanum*, which causes human tuberculosis and is essentially limited to Africa, *M. microti*, which is a pathogen of small rodents, and the vaccinal strain *M. bovis* BCG. Although this classification is sufficient for practical purposes, the exact taxonomic status of a number of newly-described species, subspecies or subtypes is difficult to specify. This is best illustrated by the example of the pig or poultry pathogen belonging to the *M. avium-intracellulare* complex which, although known for years, suddenly became a focus of attention when acquired immune deficiency syndrome (AIDS) patients harbouring these bacteria were first diagnosed in the 1980s (130, 132).

General structure of mycobacteria

The cell envelope plays a crucial role in the adaptation of mycobacteria to intracellular growth, for example by promoting the adhesion of mycobacteria to host macrophages and the acquisition of essential nutrients inside host cells, by inhibiting the microbicidal properties of the host and by determining eventual cell death (120). The architecture and the constituents of the cell envelope not only intervene in intracellular survival and immune modulation, but are also instrumental in conferring drug resistance (120). An

Table I
Chemical characteristics of *Mycobacterium* and allied taxa

Taxon	GC content of DNA (mol %)	Fatty acids	Mycolic acids	
			Overall size (number of carbons)	Ester released on pyrolysis
Corynebacterium	48-59	S, U	22-38	8-18
Gordona	63-73		48-66	16-18
Mycobacterium	62-70	S, U, T	60-90	22-26
Nocardia	64-69	S, U, T	46-60	12-18
Rhodococcus	63-73	S, U, T	34-52	12-16
Tsukamurella	63-73		64-78	20-22

DNA: deoxyribonucleic acid
GC : guanine and cytosine
S : straight-chain
T : tuberculostearic
U : unsaturated
Source: adapted from Goodfellow and Wayne (63) and Vincent (187)

understanding of these cell envelope constituents is also important as a means of developing potential drugs and/or treatment strategies to control resulting infection. Finally, these cell envelope constituents are among the initial factors contributing to host responsiveness. In the following section the term cell envelope is used to describe the bacterial cytoplasmic membrane, the cell wall and the mycobacterial capsule.

The structure-function relationships of the cell envelope have been extensively explored by chemical, ultrastructural, cytochemical and immunological methods. Although historically considered as unencapsulated organisms, recent electron microscopic data, using improved methods of embedding and immunocytochemical localisation of bacterial antigens, have provided evidence that pathogenic mycobacteria do contain a 'capsular structure' which not only contributes towards the permeability barrier of the mycobacterial cell envelope, but also protects the mycobacteria from the microbicidal activities of the host macrophage (120). As underlined recently, a better understanding of the structure of the mycobacterial cell envelope and the biosynthesis and cell wall location of antigens, will help to define the specific roles of complex surface molecules and will also permit the development of specific inhibitors that are capable of interfering with drug resistance and virulence properties (13, 87).

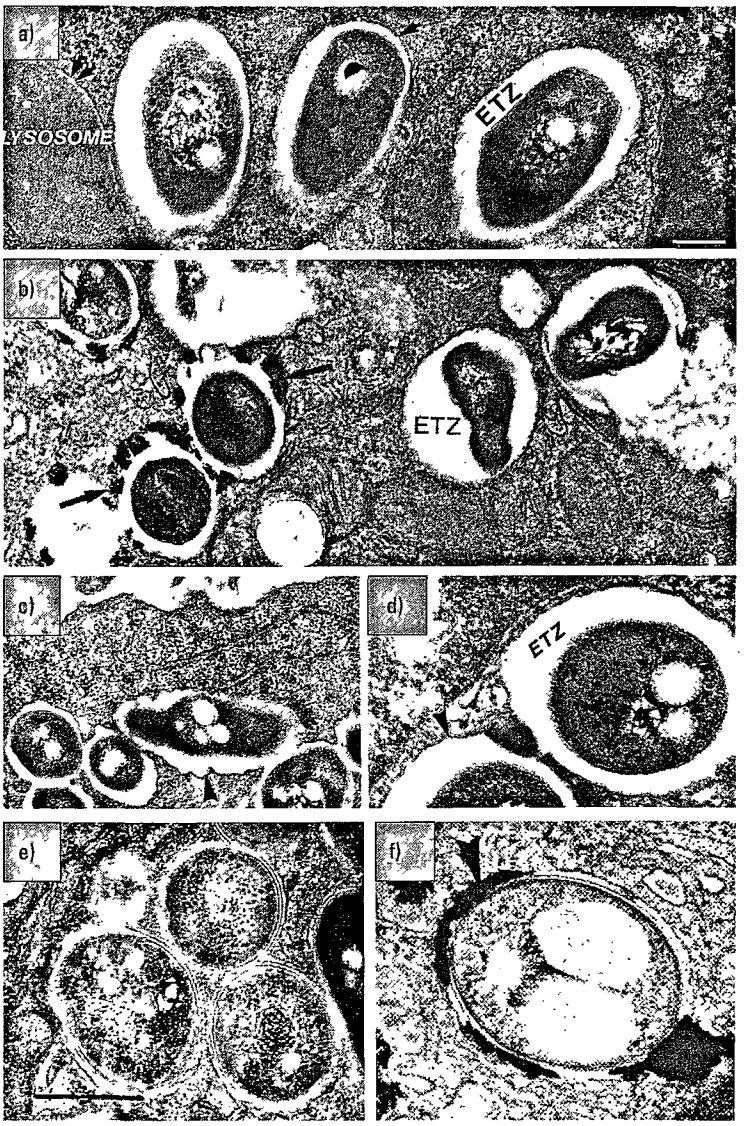
Mycobacterial capsule

Initial mycobacteria-macrophage interaction studies had revealed that intracellular mycobacteria were surrounded by a 'capsular structure' (CAP) or 'electron-transparent zone' of 70 nm to 100 nm (54), which protected the mycobacteria from host-mediated killing mechanisms (55). Subsequently, it was demonstrated that recycling of mycobacteria by the host permitted a better expression of this capsular material (58), implying that the synthesis of the capsular material is controlled, at least partially, by host-dependent regulatory mechanisms in pathogenic mycobacteria. The classical fixation methods used were presumed to cause the peripheral capsular material to collapse, hence protection of this material was necessary, either by saturating surface antigens of the bacteria with antisera raised against surface antigens such as the outer layer (OL) or CAP, or by using a novel gelatin-Lowicryl embedding (57). This protective capsular material was concluded to be an integral part of the mycobacterial cell envelope in the pathogenic species *M. avium* and *M. tuberculosis*, but not in the non-pathogenic species *M. smegmatis*. Using immunogold labelling under the electron microscope, the location of this capsular material was confirmed by locating specific surface antigens in ultra-thin sections of *M. avium-intracellulare* organisms (127). These observations were in agreement with the findings that phagocytised *M. avium* are encapsulated, inhibit phagosome-lysosome fusions, and are able to grow intracellularly, whereas non-pathogenic *M. aurum* are uncapsulated, do not inhibit the phago-lysosome fusions and are rapidly degraded within phagolysosomes (54, 55). Some of these observations are illustrated in Figure 1.

Previously, various subcellular fractions of *M. avium* organisms were isolated and used to raise antisera in rabbits. Upon immunolabelling of the bacteria using specific antisera, the location of OL epitopes was observed to be closer to the cell surface than the diffused labelling observed with CAP antisera, indicating that although the majority of the structural antigens in *M. avium* remained close to the wall, some protruded outwards (probably because of the size of these antigens) forming a glycocalyx-like arrangement of the epitopes inside CAP (119, 128). As discussed below, ultrastructural studies suggest that the OL and CAP are linked to the rest of the mycobacterial wall through lipid-lipid interactions (119, 128).

The tripartite nature of the mycobacterial cell wall

Mycobacterial cell walls have a complex tripartite structure and contain a high proportion of lipids (approximately 30% to 40% of the total weight), a significant number of which are 'loosely bound', i.e. extractable using organic solvents, as opposed to the 'firmly bound lipids' which can be extracted only after the saponification of the previously extracted residues. A complete description of various mycobacterial lipids can be obtained from the recent review by Asselineau and Lanéelle (6). Most of the biologically active lipids of mycobacteria are present in the loosely bound fraction, whereas the firmly bound fraction essentially contains mycolic acid residues esterified to arabinose residues in the arabinogalactane (7), constituting the 'cell wall skeleton' (81). Chemical analysis of saponified and delipidated cell wall residues revealed arabinose, galactose, muramic acid, glucosamine, alanine, diaminopimelic acid and glutamic acid. In 1968, Imaeda *et al.* attempted to explain how the loosely-bound lipids were placed over the cell wall skeleton (74). In the proposed model, the cell wall was assigned three distinct layers: an outer lipopolysaccharide layer (LPS), an intermediate LPS-lipid-protein complex and an inner LPS-mucopeptide layer. This model was later slightly modified by Barksdale and Kim (8), who retained the multilayered concept with distinct L1, L2, L3 and the innermost murein layers. However, controversy remained over the existence of the mycobacterial outer layer, due to the inability to observe this layer using classical electron microscopy methods. Using ruthenium red cytochemical staining, a regularly structured outer layer of 10 nm to 12 nm (Fig. 2a), containing acidic polysaccharides, was detected in *M. avium* (121), and subsequently in all of the eighteen species of mycobacteria studied (122). Despite being a structured monolayer, mycobacterial OL can behave as a functional bilayer by excluding some substrates and drugs (119, 126). Specific inhibition of the surface amphiphils that form this layer, e.g. inhibition of surface glycopeptidolipids (GPLs or C-mycosides) in *M. avium* by *m*-fluoro-phenylalanine, resulted in OL being released into the



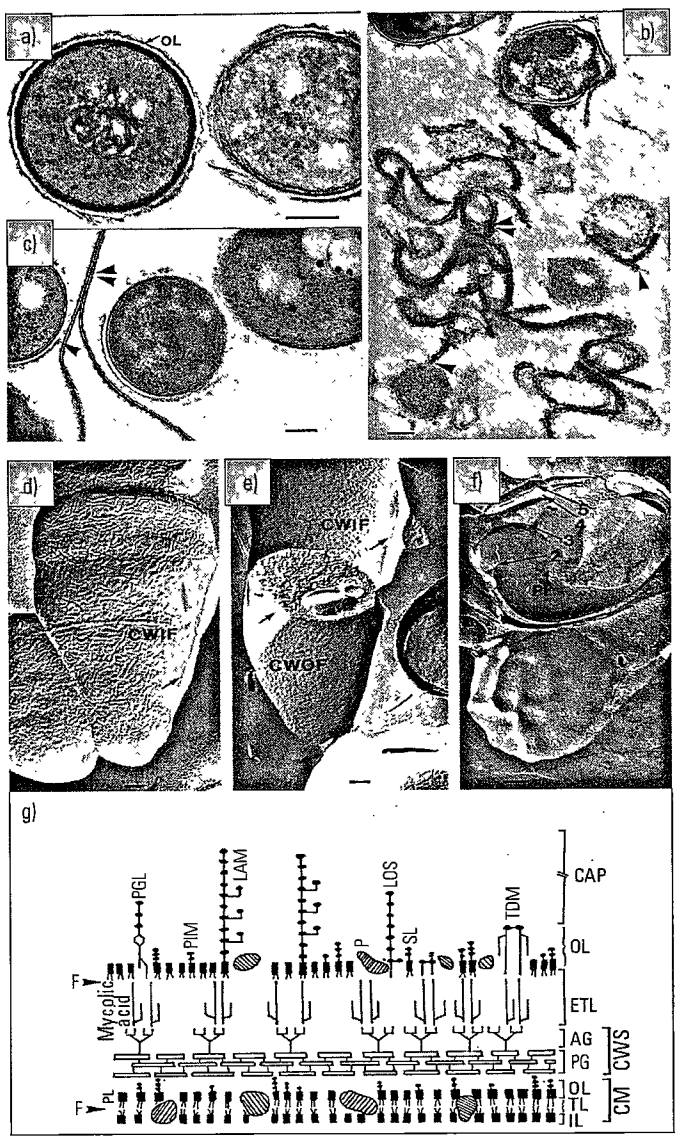
a) In *M. avium*-infected cells, observed at 4 h post infection, the bacteria are surrounded by a capsular structure of 60 nm to 100 nm (electron transparent zone [ETZ] indicated by a single arrow). The ETZ prevents direct contact between the bacilli and the lysosomes (double arrow)
b) During a phagosome-lysosome fusion event, the ETZ effectively protects the bacteria against the host microbicidal mechanisms, this can be visualised by acid-phosphatase (AcPase) cytochemistry of infected cells at 4 h post infection (electron-dense AcPase deposits, shown by arrows, are not in direct contact with the bacterial surface)
c) and d) The *M. avium* ETZ is effectively maintained in infected macrophages for long periods, and nearly all bacilli that multiply intracellularly post infection are surrounded by the ETZ, observed at 4 days c), and at 6 days d), post infection, and in mouse bone-marrow-derived macrophages that were maintained for as long as 21 days post infection (results not shown)
e) non-pathogenic mycobacteria, *M. aurum*, 24 h post infection (not surrounded by capsular material upon infection of macrophages), does not inhibit phagosome-lysosome fusion
f) *M. aurum*, the lysosomal AcPase is in direct contact with the bacterial surface (arrowhead)

Fig. 1
Selected electron microscope images illustrating the interaction of pathogenic and non-pathogenic mycobacteria with macrophages
The macrophages were infected with pathogenic *Mycobacterium avium* organisms (a to d), and the non-pathogenic species *M. aurum* (e to f)
Bar shown in a) represents 200 nm for a), b) and d), and 100 nm for c), whereas the bar shown in e) represents 500 nm for e) and f)
Source: adapted from Fréhel *et al.* (54, 55, 58), Rastogi and David (123) and N. Rastogi, unpublished findings

surrounding medium and reorganised to form a bilayer (34; Figs 2b and 2c), with a subsequent increase in the activity of a number of antimicrobials against these organisms (125, 134).

Organisation of lipids in the mycobacterial cell envelope: a proposed model

During freeze-etching and freeze-fracture studies, the preferential plane of fracture passes through a plane of hydrophobic-hydrophobic interactions (e.g. both through the outer membrane and the cytoplasmic membrane [CM] in the case of Gram-negative organisms, and through the CM only in the case of Gram-positive bacteria). Among mycobacteria (17), the preferential plane of fracture passes through the cell wall rather than the CM with visible periseptal annuli and characteristic fibrillar and filamentous structures on the cell wall inner fracture face (Fig. 2d). However, when the bacteria were treated with polymyxin-E or phenethyl alcohol (which have reversible effects on the mycobacterial CM when used at



a) regularly structured outer layer (OL) of 10 nm to 12 nm in width, in *M. avium*, containing acidic polysaccharides. Ruthenium red cytochemical staining
b) and c) specific inhibition of surface glycopeptidolipids in *M. avium* by *m*-fluorophenylalanine results in the release of OL in the surrounding medium with reorganisation to form a bilayer
d) the preferential plane of fracture during freeze-etching and -fracture in mycobacteria passes through the cell wall rather than the cytoplasmic membrane (CWIF: cell wall inner fracture face)
e) and f) bacteria treated with polymyxin-E or phenethyl alcohol, respectively. Both the inner and outer fracture faces (CWIF and CWOF) were devoid of the characteristic fibrillar network with additional fractures in the cytoplasmic membrane, showing the organisation of the mycobacterial cell envelope as successive layers (the layers exposed are shown by arrows)
g) a schematised model of the mycobacterial cell envelope. In the cytoplasmic membrane (CM = 7 nm), the mannose-containing phospholipids (PIM) are placed in the outer layer (OL = 3 nm), which is thicker than the inner layer (IL = 2 nm). In the cell wall skeleton (CWS = 13 nm) made up of arabinogalactan (AG = 4 nm) and peptidoglycan (PG = 9 nm), the PG is shown by layers. The mycolic acids in the electron transparent layer of the cell wall (ETL = 8 nm) are shown esterifying the AG, or upside down in the lipid-lipid interaction region of the ETL (shown by F, the plane of fracture). In the cell wall outer layer (OL = 12 nm), a matrix of phospholipids (PL and PIM) and some complex amphiphils (sulpholipid = SL; phenolic glycolipid = PGL; trehalose dimycolate = TDM) are represented with their fatty acid moieties facing the plane of fracture. Although not represented in the Figure, the PL in the wall OL are esterified by tuberculostearic acid which is not the case for PL in the CM. The 'capsular structure' (CAP) is represented as protrusions of the sugar moieties of the long-sized amphiphils (e.g. lipoarabinomannan = LAM) and mannose-derived lipooligosaccharides (LOS) anchored by the fatty acid ends to the ETL. Wall proteins (P), are represented arbitrarily embedded in the cell wall OL matrix

Fig. 2
Transmission electron microscopy, freeze-etching and freeze-fracture studies of mycobacteria, and a generalised view of the mycobacterial cell envelope architecture
Bar = 100 nm
Source: adapted from Benedetti *et al.* (17), David *et al.* (34) and Rastogi *et al.* (119, 121, 122, 126, 128)

sublethal concentrations [119]), both the inner and outer fracture faces were devoid of the characteristic fibrillar network (Fig. 2e); additional fractures in the CM were also observed (Fig. 2f), with up to four additional layers in the bacterial envelope, suggesting that the mycobacterial cell envelope is organised in successive layers.

A schematised model of the mycobacterial cell envelope, based on the studies described above, is represented in Figure 2g. In this model, the CAP is represented as a protrusion of sugar moieties of long-sized amphiphils (e.g. lipoarabinomannan [LAM] and lipooligosaccharides) anchored by the fatty acid ends to the bacterial

electron-transparent-layer (ETL). However, McNeil and Brennan have suggested that LAM protrudes through the envelope from a covalent phospholipid anchor in the CM (94). Both these models are based on a generalised chemical model proposed earlier by Minnikin (101), who assigned a multilayered arrangement to the mycobacterial cell envelope, and favoured a partial structural analogy with the bilayered membrane of Gram-negative bacteria (102). Nikaido and Jarlier recently reported that X-ray diffraction analysis of purified mycobacterial cell walls produced a reflection at 0.42 nm, which is consistent with the lipid bilayer model (110).

Permeability barrier located in the mycobacterial cell wall

Most of the chemically-defined amphipathic substances implicated in pathogenicity, virulence and characteristic multiple drug resistance of mycobacteria have been located in the cell wall (119, 123, 128), which functions as an effective permeability barrier, rendering these organisms resistant to most antimicrobial agents (121, 133). This low permeability is not solely due to the large proportions of unusual lipids in the mycobacterial cell wall, but also to the physical arrangement of these lipids. X-ray diffraction studies of purified *M. chelonei* cell walls confirmed the physical organisation of mycobacterial lipids, the hydrocarbon chains being predominantly arranged in a direction perpendicular to the cell surface, producing a bilayer structure (110, 111). This highly 'organised' and 'compact' structure is responsible for the exceptionally low permeability of the mycobacterial cell wall. The role of the outer parts of the mycobacterial cell envelope, as permeability barriers, has been recently reviewed (48). The susceptibility to drugs of the multiple drug-resistant atypical mycobacteria can be significantly enhanced by compounds known to disrupt the cell envelope (126) and diffusion of drugs through the cell envelope can be facilitated by lipophilic carriers (124, 125). Unlike the genetically-related multiple drug resistance that develops as the result of inadequate treatment of *M. tuberculosis*, drug resistance in atypical mycobacteria is apparently associated with the cell envelope, particularly the refractory nature of the cell envelope towards drug penetration (135). Specific inhibition of cell envelope constituents has been successfully used to circumvent the natural resistance of these bacteria; for example, *m*-fluorophenylalanine, an inhibitor of GPL biosynthesis, and ethambutol, an inhibitor of arabinogalactan biosynthesis, have been reported to enhance the activity of macrolides, aminoglycosides, fluoroquinolones and rifamycins against *M. avium* (129, 131). Similarly, the inhibitors of glycosylation (2-deoxy-D-glucose, bacitracin and ethambutol), fatty acid biosynthesis (cerulenin) and peptide biosynthesis (*N*-carbamyl-L-isoleucine and *m*-fluorophenylalanine), which affect the biosynthesis of GPLs, led to enhanced action of macrolide, aminoglycoside and fluoroquinolone drugs against *M. avium* (11, 134).

The above findings supported the view that exclusion might be an important mechanism of drug resistance in mycobacteria (33, 75, 121). In fact, the interplay between cell wall barrier and β-lactamase activity was shown to determine the high resistance to β-lactam drugs in *M. chelonei* (76). Although the terms permeability and exclusion have often been used interchangeably, they denote two different situations. The lipid-barrier is a non-selective exclusion barrier, whereas a permeability barrier denotes a more precise situation (e.g. that of a hydrophilic solute traversing a porin) (119). The lipophilic pathway appears to be the major pathway in mycobacteria, as correlation between higher drug activity and increased hydrophobicity of drugs has been observed for macrolides, tetracyclines, fluoroquinolones and rifamycins (77). Conclusive evidence for lipophilic solute transport is provided by the observation that the addition of a C_{16} fatty acyl chain to the otherwise hydrophilic molecule of isoniazid (which is completely inactive against *M. avium*), significantly increased the activity of the drug (124, 125).

However, the penetration rate of cephalosporins across the cell wall of *M. chelonei* was neither strictly dependent on the hydrophobicity of the molecules, nor on the temperature. A hydrophilic pathway involving 'porins' was therefore suggested by Jarlier and Nikaido (75). By reconstituting detergent extracts of *M. chelonei* cell walls into proteoliposomes, a protease-sensitive, channel-forming activity was detected, which led to the identification of a 59 kDa porin protein (178). This minor cell wall protein of relatively lower permeability produced slightly cation-selective channels of a defined size upon reconstitution into planar bilayers, with a 2 nm channel diameter (178). A porin with a 3 nm pore diameter and negative charges at the mouth (cation-selective) was subsequently characterised in *M. smegmatis* (179). Wide pore diameters and the negatively charged channel-mouths in the two porins so far characterised tend to suggest that positively-charged hydrophilic molecules may permeate through these porins more rapidly than those which are negatively-charged. As discussed previously (110), three distinct pathways may exist for solute penetration in mycobacteria, the lipophilic and hydrophilic pathways, and a self-promoted uptake which may involve absorption to the cell wall of a compound, leading to the subsequent disorganisation of the cell wall, thus permitting penetration. This may be the case with fairly large polycationic drugs that are active against mycobacteria (e.g. aminoglycosides). One of the pathways may be perturbed without affecting the others.

A gene coding for a porin-like protein of the ompA family from the virulent H37Rv strain of *M. tuberculosis* was recently studied (150). This protein (ompATb, 38 kDa, pore diameter of approximately 1.8 nm) was identified as a porin of low specificity, and appeared to be different from mycobacterial porins previously described. Another class of porin protein (100 kDa) with a high channel-forming activity, encoded via the *mspA* gene was recently identified in *M. smegmatis* (109).

Using Southern blotting, the authors demonstrated that several fast-growing mycobacterial species contained homologous *mspA* sequences in their chromosomes, whereas the slowly growing species did not appear to harbour similar sequences, suggesting that major permeability-related differences may exist between the rapidly growing mycobacterial saprophytes and slowly growing pathogenic and opportunistic mycobacteria.

Mycobacterial taxonomy

With the advent of genotyping and sequencing technologies, an improved correlation of phenotypic and genetic characteristics has permitted the redefinition of existing species, as well as the description and subsequent addition of new species. However, the naming of bacteria is controlled by the *International Code of Nomenclature of Bacteria* and the correct name of a bacterial taxon is based on valid publication, legitimacy, and the priority of publication. Since 1 January 1980, priority of bacterial names has been based upon the Approved Lists of Bacterial Names (155), and names that were not included in the Approved Lists lost standing in bacterial nomenclature (42). Valid publication of new names and new nomenclatural combinations can be made by publication in the *International Journal of Systematic Bacteriology (IJSB)*, renamed as the *International Journal of Systematic and Evolutionary Microbiology (IJSEM)* since January 2000, either as an original article or in the 'Validation Lists' that appear regularly in this journal. The Validation Lists constitute valid publication of new names and new combinations that were previously effectively published outside the *IJSB/IJSEM*. Names not considered to be validly published should no longer be used or should be used in quotation marks (e.g. '*Bacillus mesentericus*') to denote that the name is not validly published (42). According to clinical importance, mycobacteria – currently about eighty-five species (Table II) – can be classified into the following three principal groups:

a) strict pathogens, including the human pathogens *M. tuberculosis* and *M. leprae*, and the animal pathogen *M. bovis*

b) opportunistic (or potential) pathogens, including *M. simiae*, *M. avium* and *M. xenopi*

c) rare pathogens, including saprophytes such as *M. smegmatis* and *M. phlei*.

Among potential pathogens, the term *M. avium-M. intracellulare-M. scrofulaceum* (MAIS) complex was previously used to denote a group of slowly growing mycobacteria that were phenetically similar and sometimes difficult to differentiate. However, the use of the term MAIS is not encouraged, since *M. scrofulaceum* is now relatively easily differentiated from *M. avium* and *M. intracellulare*, using DNA-hybridisation, antigenic analysis or the ability of the organisms to hydrolyse urea. Another widely used term is 'M. *avium* complex' (MAC), which contains the two species *M. avium* and *M. intracellulare*, the former being composed of three distinct subspecies, namely: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum*. Within the *M. avium* group, the mycobactin-dependence and slow growth pattern of *M. paratuberculosis* are remarkable (mean division time of 48 h and cultures held for sixteen weeks).

For practical purposes, mycobacteria are sometimes also differentiated into two groups known as the 'M. *tuberculosis* complex' (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti* and the newly described species *M. canetti*) (114) and 'mycobacteria other than the *M. tuberculosis* complex' (MOTT). The terms 'non-tuberculous' or 'atypical' mycobacteria are synonymous with MOTT. Usually, though not necessarily, the strict and opportunistic mycobacterial pathogens are slow growers (a mean division time of 12 h to 24 h, with a fully-grown culture requiring approximately fifteen to twenty-eight days), except *M. leprae* which does not grow on artificial media, and is known to multiply every seven to fourteen days in experimental animal hosts. In contrast, most of the rare pathogens or saprophytic mycobacterial species are rapid growers (mean division time of ≤ 2 h to 6 h, with a culture available within two to seven days).

Identification based on phenotypic characteristics

Mycobacterial species are traditionally differentiated on the basis of speed of growth, optimal growth temperature, colony morphology, pigment and niacin production and serotyping. Other growth characteristics, such as the ability of some species to grow in media other than Löwenstein-Jensen, the most widely used medium for mycobacterial growth (e.g. on ordinary gelose or MacConkey broth), or the ability to grow in the presence of inhibitors such as *p*-nitrobenzoate and hydroxylamine, are used for species determination. In addition, specific biochemical properties are useful for discriminating among various species, for example the presence or absence of some enzymes such as urease, arylsulphatase and catalase, and/or the specific characteristics of enzymes, such as the thermoresistance of catalase.

The mycobacteria were initially divided into the *M. tuberculosis* complex and the non-tuberculous or atypical mycobacteria, as this discrimination could be easily performed in most microbiological laboratories. For practical purposes, Runyon proposed a classification of atypical mycobacteria in four groups (147), based on phenotypic characteristics such as pigmentation and speed of growth. Groups I, II and III included only slowly growing mycobacteria, i.e. organisms which require more than one week to grow, whereas group IV included rapidly growing mycobacteria which require one week or less for culture. Runyon group I includes photochromogenic species, in which colonies acquire pigmentation in the presence of light only (medically important species include *M. kansasii* and

M. marinum). Group II includes scotochromogenic species (colonies that are pigmented in the presence or absence of light), namely *M. gordonae* and *M. scrofulaceum*. Group III comprises non-chromogenic species (non-pigmented colonies) including *M. avium*, *M. intracellulare* and *M. xenopi*. Finally, the Runyon group IV includes rapid growers (medically important species include *M. fortuitum* and *M. chelonae*).

An updated list of the biochemical tests currently employed and the cultural properties helpful in discriminating among various species of slowly growing mycobacteria are summarised in Table III. This Table also incorporates the distribution of mycolic acid profiles upon thin layer chromatography among the species illustrated, as this remains an important chemotaxonomic marker for mycobacterial identification (35). Results for selected rapidly growing mycobacteria are summarised in Table IV. Until the 1980s, the above phenotypic characteristics were the only tool available to classify various mycobacterial species, despite the fact that these characteristics do not allow precise identification of all the species. The results based on phenotypic characteristics may be variable at the subspecies level or may give the same results for completely different species, thus leading to false identification results in a clinical microbiology laboratory. In this context, recent molecular taxonomical approaches have provided an opportunity to improve the understanding of mycobacterial classification and phylogeny. New categories of information of potential taxonomic value have become available (e.g. chemo-taxonomy, DNA base composition, DNA-DNA hybridisation) which allow very fine distinctions to be made between organisms and reveal previously undetected dissimilarities (42). New phylogenetic relationships are attracting increasing attention and have become an important basis of the taxonomy and nomenclature of actinomycetes in general, and mycobacteria in particular; for example sequences of 16S ribosomal ribonucleic acid (RNA) have provided actinomycetologists with a phylogenetic tree that allows the investigation of the evolution of actinomycetes and also provides a basis for classification (49). Nevertheless, the distribution of some morphological and chemotaxonomic traits, such as types of peptidoglycan, menaquinone, phospholipids, cell wall sugars and fatty acids, greatly facilitates the phenotypic delineation of genera within each clade, and combinations of phenotypic properties allow the prediction of whether a new organism is likely to be a member of an established or a novel taxon (49). However, phenotypic traits are mostly polyphyletic (with the exception of mycolic acids), and hence are an unreliable indicator of phylogenetic relationships that tend to be unpredictable and less conserved at higher taxonomic levels (e.g. at the family level) (49). A brief discussion of the way in which the integrated use of phenotypic and genotypic characteristics is currently changing mycobacterial taxonomy is provided below.

Identification based on genotypic characteristics

For a rapid diagnosis of tuberculosis, the slow growth of the tubercle bacillus used to be a limiting factor. Because early diagnosis is highly important for the global control of tuberculosis, rapid assays based on molecular biology were developed and have been applied since the mid-1980s. The important developments that permitted a better and more rapid identification of mycobacteria are briefly reviewed below.

DNA/DNA hybridisation

The DNA/DNA hybridisation technique is a reference method for species determination which directly mirrors the homology between two entire genomes. The results are expressed as the percentage of hybridisation or as ΔTm (temperature difference between the homo- and the hetero-duplex). However, standardisation of the results and comparison between studies require the determination of ΔTm to precisely evaluate the thermal stability of hybrids obtained, which should be less than 6°C for strains belonging to the same species (159). This value is independent of the method used and of the size of the genomes, whereas the percentage of hybridisation may vary between experiments and/or methods.

Species-specific sequences

A reliable identification may be obtained by characterising a species-specific DNA sequence by polymerase chain reaction (PCR) or hybridisation. Various PCR targets for *M. tuberculosis* identification include the gene encoding the MPB64 protein (98), the gene encoding the 38 kDa protein (154), and the *mtp40* sequence (39), whereas others, such as the DT1 and DT6 sequences allow differentiation between *M. intracellulare* and *M. avium*, two MAC species (173). Various mycobacterial species also contain a number of insertion sequences (IS) integrated in the genome. The IS belong to a number of distinct families, and studies of mycobacteria have so far revealed approximately fifty IS belonging to about ten major IS families (Table V). A number of IS elements are present in numerous copies in mycobacterial genomes, and are species-specific, thereby increasing the sensitivity of the assays, for example IS*6110* for the *M. tuberculosis* complex (172), IS*900* for *M. avium* subsp. *paratuberculosis* (66), and IS*1245* and IS*1311* for *M. avium* (68, 141).

Commercialised intragenic hybridisation probes

One of the most widely used and valuable systems exploits the polymorphisms of 16S ribosomal RNA (105). Specific probes for *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii* and *M. gordonae* are commercially-available. Other systems use a PCR-based amplification of the 16S ribosomal (r)DNA, followed by hybridisation (36). Such a system was developed to directly detect *M. tuberculosis* in sputum specimens (32, 43).

Table II
List of mycobacterial species

Species	Status	Described by	Reference [a]	Hazard	Type strains group [b]
M. abscessus	NC	Moore and Frerichs, 1993; Kusunoki and Ezaki, 1992	*IJSB* 42: 244	2	ATCC 19977, DSM 43491
M. africanum [c]	AL	Castets *et al.*, 1969	*IJSB* 30: 325	3	ATCC 25420
M. agri	NS	Tsukamura,1981	*IJSB* 31: 256	1	ATCC 27406
M. aichiense	NS	Tsukamura,1981	*IJSB* 31: 274	1	ATCC 27280, NCTC 10820
M. alvei	NS	Ausina *et al.*, 1992	*IJSB* 42: 531	1	CIP 103464
M. asiaticum	AL	Weiszfeiler *et al.*, 1971	*IJSB* 30: 325	2	ATCC 25276
M. aurum	AL	Tsukamura, 1966	*IJSB* 30: 325	1	ATCC 23366, DSM 43999
M. austroafricanum	NS	Tsukamura *et al.*, 1983	*IJSB* 33: 467	1	ATCC 33464
M. avium subsp. *avium*	AL	Chester, 1901	*IJSB* 30: 325	2	ATCC 25291
		emended by Thorel *et al.*, 1990	*IJSB* 40: 258		
M. avium subsp. *paratuberculosis*	NSS	Thorel *et al.*, 1990	*IJSB* 40: 259	2	ATCC 19698
M. avium subsp. *silvaticum*	NSS	Thorel *et al.*, 1990	*IJSB* 40: 259	2	CIP 103317
M. bohemicum	NS	Reischl *et al.*, 1998	*IJSB* 48: 1354	–	DSM 44277
M. bovis	AL	Karlson and Lessel, 1970	*IJSB* 30: 325	3	ATCC 19210
M. brumae	NS	Luquin *et al.*, 1993	*IJSB* 43: 411	1	CIP 103465
M. celatum	NS	Butler *et al.*, 1993	*IJSB* 43: 547	2	ATCC 51131, CDC 89-0899
M. chelonae subsp. *chelonae*	AL	Bergey *et al.*, 1923; Kubica *et al.*, 1972	*IJSB* 30: 325	2	NCTC 946, DSM 43804
M. chitae	AL	Tsukamura, 1967	*IJSB* 30: 325	1	ATCC 19627
M. chlorophenolicum	NC	Apajalahti *et al.*, 1986; Häggblom *et al.*, 1994	*IJSB* 44: 491	1	DSM 43826
M. chubuense	NS	Tsukamura, 1981	*IJSB* 31: 274	1	ATCC 27278, NCTC 10819
M. confluentis	NS	Kirschner *et al.*, 1992	*IJSB* 42: 261	1	DSM 44017
M. conspicuum	NS	Springer *et al.*, 1996	*IJSB* 46: 362 [d]	2	DSM 44136
M. cookii	NS	Kazda *et al.*, 1990	*IJSB* 40: 220	1	ATCC 49103, DSM 43922
M. diernhoferi	NS, RN	Tsukamura, 1983	*IJSB* 33: 468	1	ATCC 19340, DSM 43524
M. duvalii	AL	Stanford and Gunthorpe, 1971	*IJSB* 30: 325	1	NCTC 358
M. fallax	NS	Levy-Frébault *et al.*, 1983	*IJSB* 33: 342	1	CIP 8139
M. farcinogenes	AL	Chamoiseau, 1973	*IJSB* 30: 325	2	NCTC 10955, DSM 43637
M. flavescens	AL	Bojalil *et al.*, 1962	*IJSB* 30: 326	2	ATCC 14474, DSM 43219
M. fortuitum subsp. *acetamidolyticum*	NS	Tsukamura *et al.*, 1986	*IJSB* 36: 489 [d]	2	ATCC 35931
M. fortuitum subsp. *fortuitum*	AL	da Costa Cruz, 1938	*IJSB* 30: 326	2	ATCC 6841, DSM 46621
M. gadium	AL	Casal and Calero, 1974	*IJSB* 30: 326	1	ATCC 27726
M. gastri	AL	Wayne, 1966	*IJSB* 30: 326	2	ATCC 15754, DSM 43505
M. genavense	NS	Böttger *et al.*, 1993	*IJSB* 43: 842	2	ATCC 51234
M. gilvum	AL	Stanford and Gunthorpe, 1971	*IJSB* 30: 326	1	NCTC 10742
M. gordonae	AL	Bojalil *et al.*, 1962	*IJSB* 30: 326	1	ATCC 14470
M. haemophilum	AL	Sompolinsky *et al.*, 1978	*IJSB* 30: 326	2	ATCC 29548
M. hassiacum	NS	Schröder *et al.*, 1997	*IJSB* 47: 90	1	DSM 44199
M. heidelbergense	NS	Haas *et al.*, 1998	*IJSB* 48: 627 [d]	–	ATCC 51253
M. hiberniae	NS	Kazda *et al.*, 1993	*IJSB* 43: 355	1	ATCC 49874
M. hodleri	NS	Kleespies *et al.*, 1996	*IJSB* 46: 686	1	DSM 44183
M. interjectum	NS	Springer *et al.*, 1995	*IJSB* 45: 197 [d]	2	ATCC 51457, DSM 44064
M. intermedium	NS	Meier *et al.*, 1993	*IJSB* 43: 207	2	DSM 44049
M. intracellulare	AL	Cuttino and McCabe, 1949; Runyon, 1965	*IJSB* 30: 326	2	ATCC 13950, DSM 43223
M. kansasii	AL	Hauduroy, 1955	*IJSB* 30: 326	2	ATCC 12478
M. komossense	AL	Kazda and Müller, 1979	*IJSB* 30: 326	1	ATCC 33013
M. lentiflavum	NS	Springer *et al.*, 1996	*IJSB* 46: 836 [d]	2	ATCC 51985, DSM 44195
M. leprae	AL	Hansen, 1880; Lehmann and Neumann, 1896	*IJSB* 30: 326	3	Non-cultivable
M. lepraemurium	AL	Marchoux and Sorel, 1912	*IJSB* 30: 326	2	Difficult to grow
M. madagascariense	NS	Kazda *et al.*, 1992	*IJSB* 42: 526	1	ATCC 49865
M. mageritense	NS	Domenech *et al.*, 1997	*IJSB* 47: 539	1	CIP 104973
M. malmoense	AL	Schröder and Juhlin 1977	*IJSB* 30: 326	2	ATCC 29571, DSM 44163

Table II (contd)

Species	Status	Described by	Reference [a]	Hazard	Type strains group [b]
M. marinum	AL	Aronson, 1926	*IJSB* 30: 327	2	ATCC 927, NCTC 2275
M. microti	AL	Reed, 1957	*IJSB* 30: 327	3	NCTC 8710
M. moriokaense	NS	Tsukamura, 1986	*IJSB* 36: 333	1	ATCC 43059
M. mucogenicum	NS	Springer *et al.*, 1995	*IJSB* 45: 266	2	ATCC 49650
M. murale	NS	Vuorio *et al.*, 1999	*IJSB* 49: 25	–	DSM 44340
M. neoaurum	AL	Tsukamura, 1972	*IJSB* 30: 327	1	ATCC 25795
M. nonchromogenicum	AL	Tsukamura, 1965	*IJSB* 30: 327	1	ATCC 19530
M. novocastrense	NS	Shojaei *et al.*, 1997	*IJSB* 47: 1206	2	DSM 44203
M. obuense	NS	Tsukamura and Mizuno, 1971, 1981	*IJSB* 31: 274	1	ATCC 27023, NCTC 10778
M. parafortuitum	AL	Tsukamura *et al.*, 1965	*IJSB* 30: 327	1	ATCC 19686, DSM 43528
M. peregrinum	NS, RN	Kusunoki and Ezaki, 1992	*IJSB* 42: 244	1	ATCC 14467, DSM 43271
M. petroleophilum	–	Nippon Oil Co. Ltd	US Patent 3888736	2	ATCC 21497, DSM 44182
M. phlei	AL	Lehmann and Neumann, 1899	*IJSB* 30: 327	1	ATCC 19249, NCTC 8151
M. porcinum	NS	Tsukamura *et al.*, 1983	*IJSB* 33: 164	2	ATCC 33776
M. poriferae	NS	Padgitt and Moshier, 1987	*IJSB* 37: 189	1	ATCC 35087
M. pulveris	NS	Tsukamura *et al.*, 1983	*IJSB* 33: 811	1	ATCC 35154
M. rhodesiae	NS	Tsukamura, 1981	*IJSB* 31: 274	1	ATCC 27024, NCTC 10779
M. scrofulaceum	AL	Prissick and Masson, 1956	*IJSB* 30: 327	2	ATCC 19981, DSM 43992
M. senegalense	AL	Chamoiseau, 1973, 1979	*IJSB* 30: 327	2	NCTC 10956, DSM 43656
M. septicum	NS	Schinsky *et al.*, 2000	*IJSEM* 50: 575	2	ATCC 700731, DSM 44393
M. shimoidei	NS	Tsukamura, 1982	*IJSB* 32: 67	2	ATCC 27962
M. simiae	AL	Karassova *et al.*, 1965	*IJSB* 30: 327	2	ATCC 25275
M. smegmatis	AL	Trevisan, 1889; Lehmann and Neumann, 1899	*IJSB* 30: 327	1	ATCC 19420, NCTC 8159
M. sphagni	NS	Kazda, 1980	*IJSB* 30: 81	1	ATCC 33027
M. szulgai	AL	Marks *et al.*, 1972	*IJSB* 30: 328	2	NCTC 10831
M. terrae	AL	Wayne *et al.*, 1966	*IJSB* 30: 328	1	ATCC 15755, DSM 43227
M. thermoresistibile	AL	Tsukamura, 1966	*IJSB* 30: 328	1	ATCC 19527
M. tokaiense	NS	Tsukamura, 1981	*IJSB* 31: 274	1	ATCC 27282, NCTC 10821
M. triplex	NS	Floyd *et al.*, 1997	*IJSB* 47: 601 [d]	2	ATCC 700071
M. triviale	AL	Kubica, 1970	*IJSB* 30: 328	1	ATCC 23292
M. tuberculosis	AL	Zopf, 1883; Lehmann and Neumann, 1899	*IJSB* 30: 328	3	ATCC 27294
M. tusciae	NS	Tortoli *et al.*, 1999	*IJSB* 49: 1839	2	DSM 44338
M. ulcerans	AL	McCallum *et al.*, 1950	*IJSB* 30: 328	3	ATCC 19423
M. vaccae	AL	Bönicke and Juhasz, 1964	*IJSB* 30: 328	2	ATCC 15483, DSM 43292
M. xenopi	AL	Schwabacher, 1959	*IJSB* 30: 328	2	NCTC 10042, DSM 43995

AL : approved lists
NC : new combination
NS : new species
NSS : new subspecies
RN : revived name

a) *International Journal of Systematic Bacteriology*, refer to the journal and/or Deutsche Sammlung von Mikroorganismen und Zellkulturen (42) and Skerman *et al.* (155) for further details
b) The risk group classification is based on the list produced by the German Occupational Safety and Benefit Authority of the Chemical Industry available on the Deutsche Sammlung von Mikroorganismen und Zellkulturen website (42)
c) Basonym
d) Validation list
Source: adapted from Deutsche Sammlung von Mikroorganismen und Zellkulturen (42) and Skerman *et al.* (155)

Polymerase chain reaction-restriction fragment length polymorphism

Other methods rely on amplification and subsequent detection of species-specific restriction fragment length polymorphisms (RFLP), for example the PCR-RFLP of *hsp*65 (44, 168, 169), the internal transcribed spacer 16S-23S (ITS region [144, 145]), or the 16S rDNA (183). The most commonly used method involves the amplification of a 439 base-pair (bp) portion of *hsp*65, followed by digestion with *Bst*EII and *Hae*III. The resulting data permit identification of thirty-four mycobacterial species in a single experiment (Fig. 3).

Deoxyribonucleic acid sequencing

For taxonomical and phylogenetic studies, one of the most important targets is the gene coding for 16S rRNA (139, 140).

Table III
Phenotypic characteristics and chemotaxonomic markers of slowly growing mycobacteria

Characteristic	*M. tuberculosis*	*M. bovis*	*M. africanum*	*M. microti*	*M. avium*	*M. paratuberculosis*	*M. intracellulare*	*M. scrofulaceum*	*M. simiae*	*M. genavense*	*M. interjectum*	*M. triplex*	*M. conspicuum*	*M. malmoense*	*M. gastri*	*M. nonchromogenicum*	*M. triviale*	*M. terrae*	*M. ulcerans*	*M. xenopi*	*M. asiaticum*	*M. kansasii*	*M. marinum*	*M. gordonae*	*M. szulgai*	*M. tusciae*	*M. haemophilum*	*M. lepraemurium*
Enzyme activity																												
Niacin	+	−	v	+	−	−	−	−	v	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	−	−	−	−
Nitrate reductase	+	−	v	v	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	−
Catalase 68°C	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+		−	−
Peroxydase	+	+	+	+		−		+							−				v			+	+	v				
Tween hydrolysis (10 days)	v	v	v	v	−	v	−	−	−	−	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−
Urease	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	−	−	+	+	−	+	+	−	−
Nicotinamidase	+	−	v	+	+	+	+	+	+		+		−	+	+	+	−	−	−	+	−	+	+	−			+	−
Pyrazinamidase	+	−	v	+	+	+	+	+	+	+	+		−	+	−	+	−	−	−	+	−	−	+	−			+	−
Acid phosphatase	−	−	v	−	−	−	−	−	−		−		v	−	+	+		+	v	−	+	+	+	v				−
α-esterase	+	+	+	+	+		+	v			+			−	−	−		v		+	+	−	−	+				
β-esterase					+		+	v			−			−	−	v		−		+		v	+	+				
β-galactosidase					−	−	−	−			v		−		−	+		+	−	−	−	−	−	−			−	
Arylsulphatase within 10 days					−		+	−	−	−	−	v	+	−	+	+	+	v		+	+	+	+	v	v	+		
Pigmentation	N	N	N	N	N	N	N	S	P/N	N	N	N	N	N	N	N	N	N	v	v	P	P	P	S	S	S	N	N
Growth																												
at 30°C					+		+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	v	+	+	v	−	−
at 42°C	−	−	−	−	+	v	+	+	v	+	−	−	−	−	−	−	−	−	−	+	v	−	−	−	−	−	−	−
Resistance to (µg/ml)																												
p-nitrobenzoate (500)	−	−	−	−	+		+	+	+					+	−	v	+	+		v	+	+	v	+	+	−	−	−
p-aminosialicylate (1)					+		+	−					+		+	+		+		v		−	+	−			−	
p-aminosialicylate (200)	−	−	−	−				−					−		−					−		−	+	−			−	
Oleic acid (250)	−	−	−	−	+		+	+							−	v		v		−	+	−	−	v	+	−		
Toluidine blue (300)	−	−	−	−	+		+								+	+		+		+								
Hydroxylamine (125)	−	−	−	−	+		+	+	+				+	+	+	+	+	+		+	+	+	+	+	+			−
Hydroxylamine (250)	−	−	−	−	+		+	+	+				+	+	v	+	+	+		v	+	−	+	+	+			−
Hydroxylamine (500)	−	−	−	−	v		+	+	+				+		+	+		+		−	+	−	+	v				−
Thiophene 2-carboxylic acid hydrazide	+	−	−	−	+	+	+	+	+			+	+	+	+	+	+	+		+	+	+	+	+	+	+		
Thiacetazone (10)					+		+	v	+						−	+	+	+		+	+	−	v	+	−	+		
Isoniazid (1)	−	−	−	−	+	+	+	−	+	−	+	+	+		−	+	+	+	+	−	+	−	+	+	−	−	+	
Isoniazid (10)	−	−	−	−	+		v	−		−	+		−		−	+		+	+	−		−		v	−	−	+	
Ethambutol (1)					+	+	+	+	+		+	+	+	−	−	−	−	−	+	+	−	+	+				+	
Ethambutol (5)	−	−	−	−	+	+	+	+			+	+	+	−	−	−	−	−		+	−			v			+	
Mycolic acid patterns on thin layer chromatography																												
I	+	+	+		+	+	+	+	+		+			+	+	+	+	+	+	+	+	+	+	+	+		+	
II	−	−	−		−	−	−	−	+		−			+	−	−	−	−	−	−	−	−	−	−	−		−	
III	+	+	+		−	−	−	−	−		−			−	+	−	−	−	+	−	+	+	+	+	+		+	
IV	+	+	+		+	+	+	+	+		+			+	+	−	−	−	+	−	+	+	+	+	+		+	
V	−	−	−		−	−	−	−	−		−			−	−	−	−	−	−	−	−	−	−	−	−		−	
VI	−	−	−		+	+	+	+	−		+			−	−	+	−	+	−	+	−	−	−	−	−		−	

+ : positive
− : negative
N : non-pigmented
P : photochromogenic
S : schotochromogenic
v : variable
Source: adapted from David *et al.* (35) and Goodfellow and Wayne (63) with the addition of data from Floyd *et al.* (53), Springer *et al.* (156, 157) and Tortoli *et al.* (177)

In mycobacteria, ribosomal genes are linked into an operon in the following order: 5'-*rrs* (16S rRNA) - *rrl* (23S rRNA) - *rrf* (5S rRNA) -3'. Each intergenic region inside this operon encodes for transfer RNA. This operon is present as a single copy in slowly growing mycobacteria, whereas two copies are generally present in rapidly growing species (18). The 16S rDNA gene contains two hypervariable regions (regions A and B) that contain species-specific signatures localised on a 138 bp portion of the region A and a 70 bp portion of the region B. However, a significant proportion of the species-specific variability is located within the region A, which makes this region an interesting target for mycobacterial gene sequencing studies (83, 158). In the phylogenetic trees so far created on the basis of 16S rDNA, a distinction has been maintained between slow and rapid growers (152). By feeding all the available sequences through the GenBank (62), an updated version of a single 16S rDNA-phylogenetic tree has been created, incorporating data for slow and rapid growers (Fig. 4). As illustrated in Figure 4, the distinction between slow and rapid growers can be easily made on the basis of a specific signature in the 16S rDNA (a 21 bp hairpin loop in the helix 18 region for rapid growers, compared to a longer hairpin loop of 27 bp for slow growers). Nevertheless, *M. simiae*, *M. triviale*, *M. intermedium*, *M. interjectum* and *M. genavense*, which constitute a genetically close group, are an exception to this rule, as these species harbour a short loop (Fig. 4; 161).

Another interesting target is the ITS 16S-23S region, which is more variable than the 16S rDNA gene, and allows further discrimination amongst organisms at the sub-species level (e.g. the various sequevars of *M. avium* [41, 59, 60] and *M. kansasii* [1]). Sequencing of other genes, such as *hsp*65 (88, 138, 162, 164, 165), *dnaJ* (166), *sod* (46), and *gyrB* (84) has also been used to construct phylogenetic trees. All these genes can be considered as molecular clocks and may provide interesting and essentially compatible data on phylogeny and molecular evolution of mycobacteria. Nevertheless, uncertainties in branchings for some species or families still exist.

The *hsp*65 gene of *M. simiae* was recently compared with reported sequences of thirty-nine mycobacterial species, and a phylogenetic tree was constructed, based on the neighbour-joining method (Fig. 5; 88). This tree, based on *hsp*65 sequences, is the first attempt in literature to group both the rapidly and slowly growing mycobacteria. With the exception of *M. nonchromogenicum*, this phylogenetic tree also shows distinct branches for slow growers (on the left), and the rapid growers (on the right). Among slow growers, *M. simiae* I, *M. habana* and *M. simiae* III form a distinct cluster, easily discriminated from related species such as *M. avium*, *M. intracellulare*, *M. malmoense*, *M. asiaticum* and *M. shimoidei*. Although numerical taxonomy studies generate distinct clusters corresponding to the above mentioned species, satisfactory key biochemical and/or cultural tests permitting easy differential identification of these species in clinical laboratories may sometimes be problematic, due to the variability of the test characteristics. The tree illustrated in Figure 5 also differentiated *M. avium* and *M. avium* subsp. *paratuberculosis* from *M. intracellulare*, species that share a number of common features within the broad group previously termed as the *M. avium-intracellulare* complex.

Mycobacteria involved in infections of animals

Mycobacterial infections of animals are of primary importance for both economic and public health reasons (91, 113). Many mycobacterial species have been recognised as causes of zoonoses, among which the most important are as follows:

– *M. bovis* (Table VI), which essentially infects cattle, sheep, goats and deer; other hosts include badgers, possums and other marsupials, European hares, primates, elephants, horses, pigs and camels; for further details refer to the papers by Cousins (29) and de Lisle *et al.* (38) in this issue of the *Review*

– *M. tuberculosis* in primates, elephants, aquatic mammals, horses, pigs, cattle and deer; for further description refer to Montali *et al.* in this issue (103)

– *M. avium*, which essentially infects pigs and poultry. Other hosts include cattle, sheep, goats, deer/antelope, marsupials, primates and horses; for more details refer to Tell *et al.* (170) and Thorel *et al.* (176) in this issue

– *M. paratuberculosis*, which affects a broad range of domestic and non-domestic ruminant species; for further details refer to Manning and Collins (99) and Kennedy and Benedictus (85) in this issue.

In addition to these principal mycobacterial animal pathogens, a number of other species have also been implicated in animal infections, as follows:

– *M. intracellulare* (amphibians, reptiles, birds including domestic poultry, marsupials, primates, pigs and cattle)

– *M. chelonae* (causes lesions in fish, reptiles, aquatic mammals, and also in primates, pigs and cattle)

– *M. fortuitum* (essentially linked to disease in amphibians, primates and pigs)

– *M. lepraemurium* (also called the rat leprosy bacillus; essentially infects rodents)

– *M. marinum* (the causative agent of fish-tank or fish-breeder granuloma which affects people in contact with tropical fish, a causative agent of lesions in fish and molluscs)

– *M. microti* (essentially a pathogen of small rodents; also causes disease in hedgehogs and voles)

– *M. scrofulaceum* (cattle, buffaloes and pigs)

– *M. xenopi* (amphibians and pigs)

Table IV
Phenotypic characteristics and chemotaxonomic markers of rapidly growing mycobacteria

Characteristics	*M. chelonae*	*M. abscessus*	*M. chitae*	*M. diernhoferi*	*M. flavescens*	*M. fortuitum*	*M. parafortuitum*	*M. peregrinum*	*M. phlei*	*M. senegalense*	*M. smegmatis*	*M. thermoresistibile*	*M. vaccae*
Pigment production													
In the dark	−	−	−	−	+	−	+	−	+	+	−	+	+
In response to light	−	−	−	−	−	−	+	−	−		−	−	+
Enzyme activity													
Acid phosphatase	+	+	+	+	−	+	−	+	+		−	−	−
Arylsulphatase after 3 days	+	+	−	−	−	+	−	+	−		−	−	−
Arylsulphatase after 1 week	+	+	+	+	v	+	+	+	v		+	−	+
β-glucosidase	−	−				+		+					
Hippurate hydrolysis	+	v	−	+	v	+	−	+	+		−	−	−
Nitrate reductase	v	−	+	+	+	+	+	+	+	+	+	+	v
Penicillinase	+	+				−		−					
Putrescine oxidase	+	+	+	+	−	+	+		+		+	−	v
Tween hydrolysis (5 days)	−	v	+	v	+	v	+	v	+		+	+	+
Growth													
in <5 days	+	+	+	+	−	+	+	+	+	+	+		+
at 30°C	+	+			+	+		+	+	+	+	−	+
at 37°C	v	v			+	+		+	+	+	+	+	−
at 42°C	−	−	−	−	v	+	+	v	+		+	+	+
at 45°C	−	−	−	−	−	−	−	−	+		+	+	−
at 52°C	−	−	−	−	−	−	−	−	+		−	+	−
Growth on a single carbon source													
Benzoate	−	−	+	−	−	−	−	−	−	+	+	−	+
Citrate	+	v	−	+	−	+	+	+	+	+	+	−	+
Malonate	−	−	−	−	−	−	v	−	+	+	+	−	+
Mucate or oxalate	−	−	−	−	−	−	−	−	−		+	−	−
Propanol	−	−	+	−	v	+	v	+	+		+	−	−
Growth on a single nitrogen/carbon source													
Acetamide	−	−	+	−	−	+	−	v	+		+	−	−
Pigment production													
Benzamide	−	−	−	−	−	−	−	−	−		+	−	−
Trimethylenediamine	−	+	−	+	−	+	+	+	+		+	−	+
Growth in the presence of													
Deoxycholate (1% w/v)	−	+	−	−	−	+	−	v	+		+	−	−
MacConkey agar	+	+	−	−	−	+	−	+	−		−	−	−
Methyl violet	+	+	−	−	−	+	−	+	−		v	−	−
NaCl (5% w/v)	v	+	+	−	+	+	+	+	+		+	+	+
Pyronine B (0.01% w/v)	+	+	−	−	−	+	−	+	−		+	−	−
Degradation of													
p-aminobenzoate	+	+	v	−	−	−	−	−	−		−	−	−
Sialicylate	+	+	−	−	−	v	−	v	−		−	−	−
Acid production from													
Glucose	+	+	+	+	+	+	+	+	+	+	+	−	+
Arabinose	−	−	−	+	−	−	v	−	+		+	−	+
Dulcitol	−	−	−	−	−	−	−	−	−		v	−	−
Fructose	v	−	+	+	v	+	+	+	+	+	+	+	+
Galactose	−	−	−	−	−	−	v	−	+		+	−	−
Inositol	−	−	+	+	−	−	v	v	−		+	−	+
Mannitol	−	−	+	+	v	−	+	+	+	+	+	v	+
Rhamnose	−	−	−	−	−	−	v	−	−		+	−	+
Sorbitol	−	−	−	−	v	−	v	−	+		+	−	+
Sucrose	−	−	−	−	−	−	v	−	−		−	−	+
Threhalose	+	+	+	+	+	+	+	+	+		+	−	+
Xylose	−	−	−	+	−	−	+	−	+		v	−	+

Table IV (contd)

Characteristics	*M. chelonae*	*M. abscessus*	*M. chitae*	*M. diernhoferi*	*M. flavescens*	*M. fortuitum*	*M. parafortuitum*	*M. peregrinum*	*M. phlei*	*M. senegalense*	*M. smegmatis*	*M. thermoresistibile*	*M. vaccae*
Other tests													
Iron uptake from ferric ammonium sulphate	–	–	–	+	–	+	+	+	+		+	–	+
Acid phosphatase, at 70°C for 30 min	v	–	v	–	–	+	–	+	–		–	–	–
Amidase													
Acetamidase	v	v	+	+	–	+	+	+	–	+	+	–	+
Allantoinase	–	–	–	–	–	–	–	+	–	+	v	–	+
Benzamidase	–	–	–	–	–	+	–	–	–	+	v	–	+
Isonicotinamidase	–	–	–	–	–	+	–	–	–	+	+	–	+
Nicotinamidase	+	v	+	+	v	v	+	–	+	+	+	+	+
Pyrazinamidase	v	+	+	+	+	v	+	–	+		+	+	+
Succinamidase	–	–	–	–	–	–	–	–	–		+	–	+
Mycolic acid patterns on thin layer chromatography													
I	+	+			+	+		+	+	+	+	+	+
II	+	+			–	+		+	–	–	–	+	+
III	–	–			–	–		–	–	–	–	+	–
IV	–	–			+	–		–	+	–	–	+	+
V	–	–			–	+		+	–	+	+	–	–
VI	–	–			+	–		–	+	–	–	–	+

+: positive
–: negative
v: variable
Source: adapted from David *et al.* (35) and Goodfellow and Wayne (63)

– *M. silvaticum* (also called the wood-pigeon bacillus; essentially a pathogen of birds).

Naturally occurring mycobacterioses of animals are extremely common. In a survey of mycobacterioses among feral pigs in the Northern Territory of Australia, 47.7% of 751 feral pigs examined had macroscopic abscesses, and of these, 80.2% were suspected to be caused by mycobacteria (27). Out of 193 pigs examined bacteriologically, a total of ninety-three mycobacterial strains were isolated, and those typed conclusively were *M. bovis* (thirty-seven strains), *M. avium-intracellulare* complex (fifteen), *M. scrofulaceum* (eight), *M. gordonae* (two), *M. simiae* (two), *M. szulgai* (two), *M. xenopi* (two), *M. vaccae* (one) and *M. kansasii* (one). The authors concluded that the feral pig was probably an end host for both *M. bovis* and atypical mycobacteria and not a significant source of infection for cattle. Although *M. bovis* was not considered a significant cause of mortality in feral pigs, the authors emphasised that mycobacterioses were a significant cause of morbidity (27). Thus, the study of potential wildlife reservoirs is of great interest and the overall epidemiology of these opportunistic pathogenic mycobacteria remains poorly understood, due to the great variety of potential environmental reservoirs.

Of the mycobacterial infections found more rarely in wild animals, one striking example concerns the naturally occurring leprosy-like infections of wild nine-banded armadillos (*Dasypus novemcinctus*). Although armadillos have been used since 1971 as experimental models of infection with *M. leprae* (a species that remains non-cultivable *in vitro*), several wild animals captured in 1975 were found to have a disease identical to the experimental *M. leprae* infection of armadillos (52). A retrospective study of sera taken from 182 armadillos between 1960 and 1964, and predating the use of these animals in leprosy research, showed that seventeen of 182 samples were positive for phenolic glycolipid-I antigen, which is specific for *M. leprae* (180). Therefore, *M. leprae* appears to be enzootic in wild armadillos. As shown previously (78), lepromatous placentitis and intrauterine foetal infections exist among pregnant lepromatous armadillos, and congenital infection is possible in leprosy of armadillos. Lepromatous armadillos have an increased susceptibility to other cultivable mycobacteria, and a number of species, including *M. gordonae*, *M. fortuitum* and *M. avium*, have been isolated from *M. leprae*-infected animals (45). A detailed description of *M. leprae* as an animal pathogen is presented in the paper by Rojas-Espinosa and Løvik in this issue (142).

Table V
Characteristics of insertion sequences from various species of *Mycobacterium*

Element	Species	Length (base pairs)	Family	Number of copies	IR	DR	Accession number
IS*219*	*M. fortuitum*	1653	Unknown		8	2	
IS*900*	*M. paratuberculosis*	1451	IS*110*	14-18	0	0	X16293
IS*901*	*M. avium*	1472	IS*110*	10-14	0	0	X59272
IS*902*	*M. silvaticum*	1470	IS*110*	10-14	0	0	X58030
IS*1081*	*M. bovis*	1435	IS*256*		19/26	8	X61270
IS*1096*	*M. smegmatis*	2259	IS*L3*		24/26	8	M76495
IS*1110*	*M. avium*	1457	IS*110*	1-5	0	0	Z23003
IS*1137*	*M. smegmatis*	1361	IS*3*		22/24	3	X70913
IS*1141*	*M. intracellulare*	1588	IS*3*		18/23		L10239
IS*1245*	*M. avium*	1313	IS*256*	0-27	31/40		L33879
IS*1311*	*M. avium*	1259	IS*256*	0-27	15		U16276
IS*1407*	*M. celatum*	>1399	IS*256*				X97307
IS*1408*	*M. branderi*	>1325	IS*256*				U62766
IS*1511*	*M. gordonae*	1142	IS*256*				U95315
IS*1512*	*M. gordonae*	1428	IS*256*				U95314
IS*1532*	*M. tuberculosis*	2609	IS*21*	1	48	4	Z77165
IS*1533*	*M. tuberculosis*	2212	IS*21*	1	54	5	Z83858
IS*1534*	*M. tuberculosis*	2129	IS*21*	1	49	5	Z95436
IS*1535*	*M. tuberculosis*	2322	IS*1535*	1	17		Z95210
IS*1536*	*M. tuberculosis*	1391	IS*1535*	1			Z97182
IS*1537*	*M. tuberculosis*	1889	IS*1535*	1			Z97188
IS*1538*	*M. tuberculosis*	2055	IS*1535*	1			Z83018
IS*1539*	*M. tuberculosis*	2057	IS*1535*	1			Z74024
IS*1540*	*M. tuberculosis*	1162	IS*3*	1			Z95389
IS*1547*	*M. tuberculosis*	1351	IS*110*		0	4	Y13470
IS*1549*	*M. smegmatis*	1634	IS*4*		11		
IS*1552'*	*M. tuberculosis*	844	IS*256*	1			Z95389
IS*1553*	*M. tuberculosis*	1292	IS*256*	1	13		Z95436
IS*1554*	*M. tuberculosis*	1435	IS*256*	1	15		Z95210
IS*1555*	*M. tuberculosis*	398	IS*L3*	1			Z81331
IS*1556*	*M. tuberculosis*	1468	Unknown	1			Z73966
IS*1557*	*M. tuberculosis*	1513	IS*L3*	3	20/28		Z73419
IS*1558*	*M. tuberculosis*	1212	IS*110*	2	13		Z81451
IS*1560*	*M. tuberculosis*	1567	IS*5*	2	25	2	AL009198
IS*1561'*	*M. tuberculosis*	1319	IS*L3*	1			AL009198
IS*1602*	*M. tuberculosis*	2052	IS*1535*	1			AL008967
IS*1603*	*M. tuberculosis*	1327	IS*30*	1	63		AL021646
IS*1604*	*M. tuberculosis*	1408	IS*3*	1			Z81331
IS*1605'*	*M. tuberculosis*	287	IS*1535*	1			AL022004
IS*1606'*	*M. tuberculosis*	330	IS*L3*	1			AL022004
IS*1607*	*M. tuberculosis*	1227	IS*110*	1			Z74025
IS*1608'*	*M. tuberculosis*	1031	IS*110*	2			AL009198
IS*1613*	*M. avium*	1453	IS*110*	1-8			AJ0011837
IS*6100*	*M. fortuitum*	880	IS*6*		14		X53635
IS*6110*	*M. tuberculosis*	1354	IS*3*	0-25	25/28	3/4	X1748
IS*6120*	*M. smegmatis*	1486	IS*256*		21/24	9	M69182
ISMk*1*	*M. kansasii*	947	ISNCY				L11041
ISMt*1*	*M. tuberculosis*	969	IS*5*		16/17	4	X65618
ISMt*2*	*M. tuberculosis*	>2200	IS*21*				Z77165
ISMt*3*	*M. tuberculosis*	2213	IS*21*		40/50	5	Z83858

IR : invert repeat (base pairs) in the terminal part of insertion sequence
DR : direct repeat (base pairs) in the target sequence
Source: from Fang *et al.* (51), Gordon *et al.* (64), Mahillon and Chandler (95) and Waskar *et al.* (189)

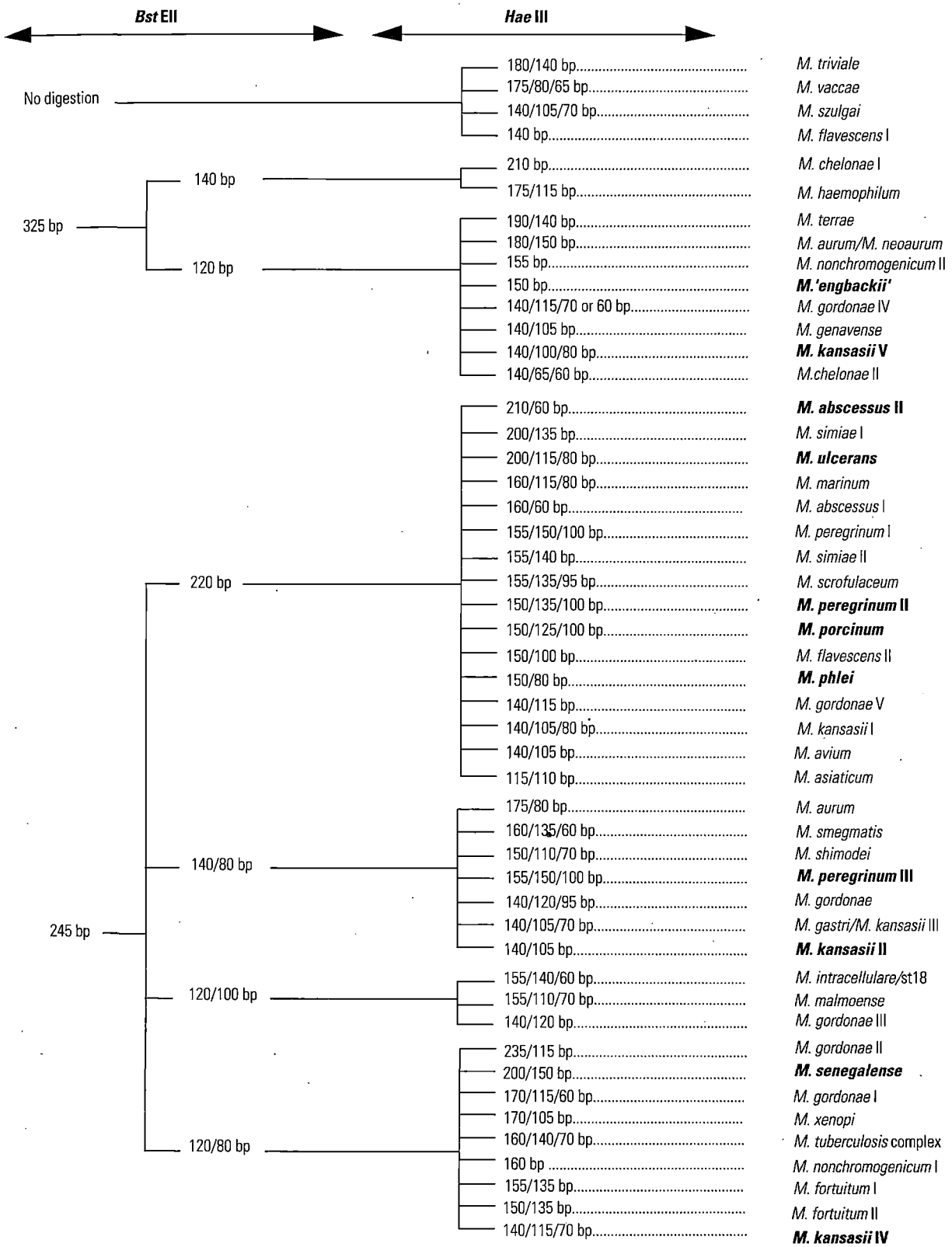
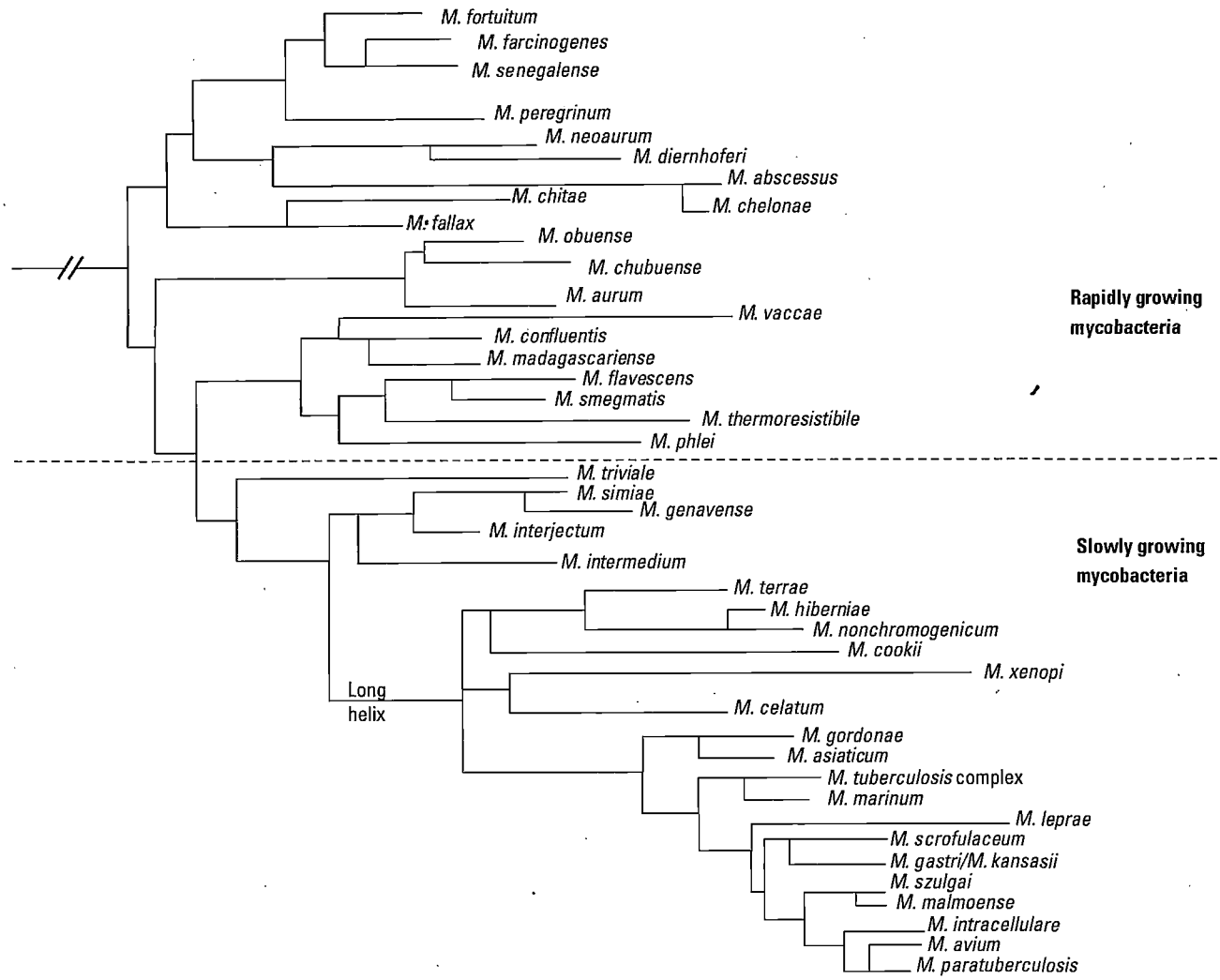


Fig. 3
Algorithm of polymerase chain reaction restriction fragment length polymorphism of *hsp*65 (PRA) profiles for identification of thirty-four mycobacterial species
Species in bold illustrate newer PRA patterns that were not reported in previous algorithms of Taylor *et al.* (168) and Telenti *et al.* (169) (adapted from Devallois *et al.* [44])

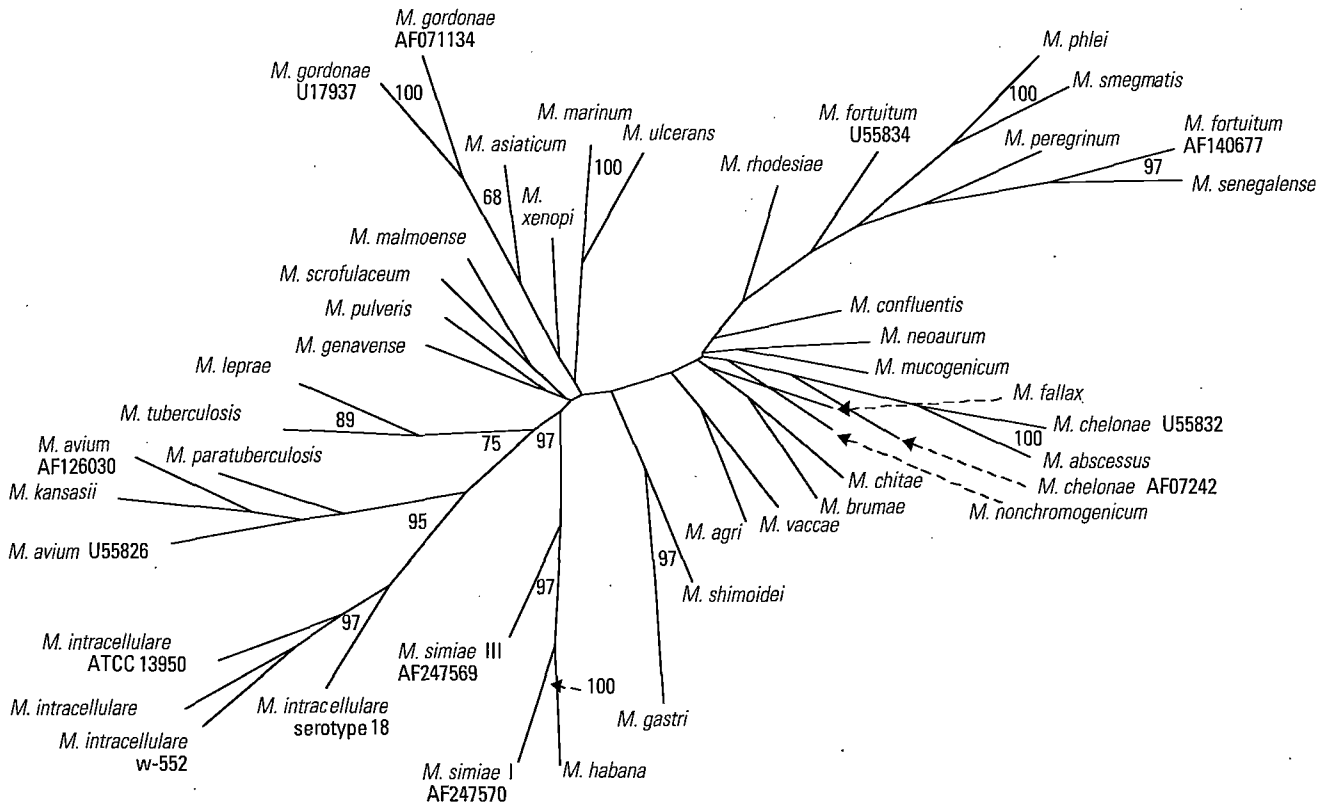


The 16S rDNA sequences from 44 mycobacterial species (with corresponding accession numbers) were as follows: *M. abscessus* (X82235), *M. asiaticum* (M29556), *M. aurum* (M29558), *M. avium* subsp. *avium* (X52918), *M. avium* subsp. *paratuberculosis* (X52934), *M. celatum* (L08170), *M. chelonae* (X82236), *M. chitae* (X67874), *M. chubuense* (X55596), *M. confluentis* (X63608), *M. cookii* (M59278), *M. diernhoferi* (X55593), *M. fallax* (M29562), *M. farcinogenes* (X55592), *M. flavescens* (M29561), *M. fortuitum* (X52921), *M. gastri* (X52919), *M. genavense* (X60070), *M. gordonae* (X52923), *M. hiberniae* (X67096), *M. interjectum* (X70961), *M. intermedium* (X67847), *M. intracellulare* (X52927), *M. kansasii* (X15916), *M. leprae* (X55587), *M. madagascariense* (AJ011335), *M. malmoense* (X52930), *M. marinum* (X52920), *M. neoaurum* (M29564), *M. nonchromogenicum* (X52928), *M. obuense* (X55597), *M. peregrinum* (AF058712), *M. phlei* (M29566), *M. scrofulaceum* (X52924), *M. senegalense* (M29567), *M. simiae* (X52931), *M. smegmatis* (X52922), *M. szulgai* (X52926), *M. terrae* (X52925), *M. thermoresistibile* (X55602), *M. triviale* (M29571), *M. tuberculosis* (AJ131120), *M. vaccae* (X55601) and *M. xenopi* (X52929)

Fig. 4
Neighbour-joining 16S rDNA phylogenetic tree of rapidly and slowly growing mycobacteria
The accession numbers are from GenBank (62)

Animal infections due to the *Mycobacterium tuberculosis* complex

This topic is covered in detail in subsequent papers in this issue (29, 38, 103), but a brief overview is presented here. The control of bovine tuberculosis in Europe was historically conducted by tuberculin-testing of cattle and slaughtering of diseased animals, and the industrial process of milk pasteurisation. In France, before milk pasteurisation, *M. bovis* was considered to be responsible for 10% of all cases of tuberculosis in humans. In 1937, *M. bovis* was isolated in 6.9% of human tuberculosis cases, whereas between 1951 and 1965, this percentage declined to 4.3%. In 1992, in England and Wales, *M. bovis* was estimated to represent only 1% of all mycobacterial isolates from human patients (69). Strict regulations for cattle trade were adopted at the European Community level in 1957; to earn the cachet of 'official tuberculosis-free herd', all animals had to be free of clinical signs of tuberculosis and show negative results in two tuberculin tests which were performed by an official veterinarian at a six-month interval (24). In Canada, bovine tuberculosis in Cervidae was recognised as a potential problem in the 1990s (50). In the USA, the eradication of bovine tuberculosis was not completed until the mid-1990s; the prevalence of herds infected with bovine tuberculosis was estimated to be 0.003%, and bovine tuberculosis still affected Puerto Rico and at least eight states of the USA in 1994 (50).



The numbers on the dendrogram are the percentage of occurrence in 100 bootstrapped trees (only values above 50% are shown). The *hsp*65 sequences from 44 mycobacterial isolates representing 39 species (with corresponding accession numbers) were as follows: *M. avium* subsp. *avium* (U55826), *M. avium* subsp. *paratuberculosis* (U55827), *M. chelonae* (U55832), *M. fortuitum* (U55834), *M. intracellulare* serotype 18 (U55830), *M. marinum* (U55831), *M. tuberculosis* (AL021932), *M. habana* (AF129011), *M. agri* (U17920), *M. asiaticum* (U17921), *M. fallax* (U17930), *M. gastri* (U17931), *M. genavense* (U17932), *M. gordonae* (U17937), *M. intracellulare* (U17924), *M. kansasii* (U17947), *M. malmoense* (U17948), *M. neoaurum* (U17950), *M. nonchromogenicum* (U17951), *M. phlei* (U17952), *M. pulveris* (U17953), *M. rhodesiae* (U17954), *M. scrofulaceum* (U17955), *M. shimoidei* (U17956), *M. vaccae* (U17958), *M. xenopi* (U17959), *M. avium* subsp. *avium* (AF126030), *M. intracellulare* strain (ATCC13950), *M. leprae* (M14341), *M. abscessus* (AF071139), *M. brumae* (AF071129), *M. chelonae* (AF072242), *M. chitae* (AF071131), *M. confluentis* (AF071132), *M. fortuitum* (AF140677), *M. gordonae* (AF071134), *M. mucogenicum* (AF071135), *M. peregrinum* (AF071136), *M. senegalense* (AF071137), *M. smegmatis* (AF071138), *M. ulcerans* (U34034), *M. intracellulare* strain w-552 (U85638). This tree also incorporates the two *M. simiae* sequences from the recent study by Legrand *et al.* (88), which bear GenBank accession numbers AF247570 (*M. simiae* type I) and AF247569 (*M. simiae* type III)

Fig. 5
Neighbour-joining unrooted *hsp*65 phylogenetic tree for rapidly and slowly growing mycobacteria
Source: adapted from Legrand *et al.* (88); the accession numbers are from GenBank (62)

Most of the infected and exposed herds in the above case were detected during epidemiological investigations, rather than by routine testing.

Due to the economic importance of exports for the cattle industries of Australia and New Zealand, tuberculosis testing programmes were initiated rapidly and eradication programmes commenced in the dairy districts at an early stage (182). Extended eradication programmes for cattle were launched in the 1970s, resulting in a decline in prevalence of tuberculosis-infected animals in Australia. Epidemiological evidence indicated that the Australian brushtail possum (*Trichosurus vulpecula*), an introduced species in New Zealand, had become a major vector of tuberculosis for cattle, and later for farmed deer (182). Consequently, possum control, tuberculosis surveillance programmes, zoning of the country, and movement control restrictions for cattle (similar to quarantine) were introduced to solve this problem. In Latin America and the Caribbean, eleven countries, harbouring nearly 75% of the total bovine population, constitute an area of high bovine tuberculosis prevalence, and the need for improved control measures has been underlined (37).

Wildlife reservoirs and strategies to control bovine tuberculosis

Although enhanced control efforts in many industrialised countries have led to a huge reduction in the incidence of both bovine and human tuberculosis due to *M. bovis*, the total eradication of bovine tuberculosis has yet not been achieved, principally because of the existence of wildlife reservoirs of infection, which vary greatly according to geographical area. Consequently, knowledge of wildlife tuberculosis may be important in the search for strategies for total elimination of animal tuberculosis (96, 97). This could be achieved by wildlife control (82) and/or immunological approaches (107). Wildlife control is highly dependent on knowledge of the ecology of the territories surrounding cattle production areas. This varies according to the areas studied, for example in New Zealand and Ireland, the knowledge of herd-specific risk factors is fairly comprehensive (40, 82), whereas further

Table VI
A non-exhaustive list showing the distribution of *Mycobacterium bovis* among domesticated and wildlife hosts

Host (common name)	Host (species)	Countries	Population (year), country, remark
Baboon, wild	*Papio cynocephalus*	KEN	–
Badger, European	*Meles meles*	IRL, GBR, CHE	230,000, IRL; 250,000, GBR
Bison, park	*Bison bison athabascae*	USA, CAN	From approximately 15,000 (1940) to 5,000 (1968)
Buffalo	*Syncerus caffer*	UGA	–
Buffalo, Indian and European domestic	*Bubalus bubalis*	IND	–
Buffalo, river	*Bubalus bubalis*	AUS	300,000 (1994)
Camel	*Camelus dromedarius*	MRT, EGY	Potential long distance vector
Deer, axis (chital)	*Cervus axis* and *Axis axis*	GBR	Sporadic cases
Deer, farmed	*Cervus dama*	GBR, DNK	40,000 (1989) GBR; 25,000 (1989) DEN
Deer, farmed fallow	*Cervus dama*	SWE	–
Deer, farmed red	*Cervus elaphus*	NZL	1.2 million (1994) NZL
Deer, feral red	*Cervus elaphus*	NZL	–
Deer, mule	*Odocoilus hemionus*	USA	5 million (1993) USA
Deer, roe	*Capreolus capreolus*	CHE	–
Deer, sika	*Cervus nippon*	GBR	Sporadic cases
Deer, white-tailed	*Odocoileus virginianus*	USA, IRL, CAN, NZL	18.4 million (1991) USA
Dog, cat	*Canis familiaris, Felis catus*	–	Sporadic cases
Elk, American	*Cervus elaphus* var. *nelsoni*	USA	Wildlife cases
Elk, American (wapiti)	*Cervus elaphus* var. *canadensis*	CAN, USA	714,000 (1991) USA
Ferrets, wild	*Mustela putorius furo*	NZL	–
Goat, farmed	*Capra hircus*	GBR, ESP, UGA	–
Goat, feral	*Caprae* sp.	NZL	Up to 31% prevalence
Guinea-pig	*Cavia porcelus*	–	Animal model for tuberculosis immunology
Horse	*Equus* sp.	–	Of no epidemiological significance
Llama	*Lama glama*	NLD, GBR	Imported llamas from Peru
Ostrich	*Struthio camelus*	AUS	Sporadic cases
Pig (feral and wild swine)	*Sus scrofa*	–	–
Possum, brush-tailed	*Trichosurus vulpecula*	NZL	70 million
Sheep	*Ovis aries*	NZL	–
Warthog, African	*Phacochoerus aethiopicus*	UGA	–

AUS : Australia
CAN : Canada
CHE : Switzerland
DNK : Denmark
EGY : Egypt
ESP : Spain
GBR : Great Britain
IND : India
IRL : Ireland
KEN : Kenya
MRT : Mauritania
NLD : The Netherlands
NZL : New Zealand
SWE : Sweden
UGA : Uganda
USA : the United States of America
Source: adapted from O'Reilly and Daborn (113)

research is required in countries of South and Central America in which heavily infected areas still persist (37).

Concerning the possible reservoirs of *M. bovis*, the Eurasian badger (*Meles meles*) is known to be an important susceptible host in Great Britain, whereas in New Zealand, the Australian brushtail possum is the principal source of new cases of bovine tuberculosis in cattle (82). In Australia, marsupials have been recognised as susceptible hosts (23). In Sweden, deer herds may be subjected to *M. bovis* infection, and control programmes have been established (188). In contrast, a recent study in Texas failed to identify non-bovine reservoirs of *M. bovis*, although the region investigated had the highest number of tuberculosis-infected cattle herds of any state in the USA (115). Between 1987 and 1997, none of the 670 mammalian, avian or environmental (soil, water and air) samples collected and cultured from the premises of the cattle herds (twelve infected and two non-infected) resulted in a positive culture for *M. bovis*. Furthermore, none of the 119 human samples obtained from the employees of the dairies were positive (115). Only 48 of 124 dairy-farm workers showed positive tuberculin skin-tests, and no difference was found in percentages of positive tuberculin skin-test results between farms with and without a history of bovine tuberculosis within the previous two years (115). These results suggest that non-bovine reservoirs may not be

implicated in the tuberculosis of cattle in Texas, suggesting that the vectors of bovine tuberculosis may vary highly among different regions of the world (115).

Another cause for concern is tuberculosis among captive wild animals, a subject that was recently discussed by Altwood (2). Some examples include *M. bovis* infection of the white-tailed deer (*Odocoileus virginianus*) in Michigan, USA, and the return of *M. bovis* to buffalo herds in the Kruger National Park (a game reserve in South Africa which covers over 20,000 km^2 and contains over 137 species of mammal). In the former case, an outbreak of *M. bovis* infection in wild white-tailed deer was identified in a portion of Michigan, a state bordering the Great Lakes region of the USA. Following an extensive surveillance programme, the same strain of *M. bovis* was isolated from multiple species, including the white-tailed deer and five carnivore species that prey on these deer. Infected animals were also diagnosed in one farmed deer herd, one dairy herd and seven beef herds.

Among potential control strategies, the vaccination of badgers was adopted in England as a means of reducing the risk of transmission to cattle (107), and the effect of a badger control programme on the incidence of tuberculosis in a cattle population was assessed in Ireland (96). The latter study showed that cattle herds present in the area from which badgers were removed had a significantly lower proportion of new confirmed tuberculosis cases, compared to those in areas where no systematic badger control was attempted (96). Efficient vaccination for control of bovine tuberculosis has long been proposed, but carries the potential of compromising existing diagnostic tests. However, the understanding of protective immunity against *M. bovis* infection remains an important priority for bovine tuberculosis research (107). In a recent experimental infection study in cattle, the immune responses in the peripheral blood and at the site of active disease were compared twenty weeks after infection with *M. bovis*, and the results obtained suggested that responses occurring in the peripheral blood may correctly reflect those at the site of the disease (136). Another study suggested that a cocktail of antigens, rather than individual antigens, should be used for immunodiagnosis of bovine tuberculosis (137).

Infections in animals due to the *Mycobacterium avium* complex organisms

Mycobacterium avium complex organisms (*M. avium*, *M. paratuberculosis* and *M. silvaticum*) are major pathogens of animals. *Mycobacterium avium*, originally detected in birds, has been implicated as a pathogen of mammals, *M. paratuberculosis* is the aetiological agent of Johne's disease of cattle, and *M. silvaticum* essentially causes disease in birds (89). Although these three organisms were initially considered as separate species within the *M. avium* complex, more recent numerical taxonomy studies and molecular typing approaches using DNA-DNA hybridisation, RFLP and pulsed-field gel electrophoresis, revealed that these organisms could be distinguished as three different subspecies within the single species *M. avium*, namely: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum* (149, 175). Phenotypic and genetic differences have been found to exist between the three subspecies, for example mycobactin-dependent growth and its stimulation by pyruvate on solid medium, in addition to the presence of IS*900* in *M. paratuberculosis*, the absence of IS*901* and IS*902* in *M. paratuberculosis* (present in *M. avium* and *M. silvaticum*) and the specific presence of IS*1613* in *M. avium* (89). Furthermore, the three subspecies show different epidemiologies, animal hosts and reservoirs (89). The combination of these parameters not only permits discrimination between the three subspecies, but also justifies the distinction between the three. Aspects concerning *M. avium* infections in birds and mammals are discussed in greater detail in the papers by Tell *et al.* (170) and Thorel *et al.* (176) in this issue.

Recently, a significant increase in the incidence of caseous lesions in the lymph nodes of slaughter pigs prompted an extensive investigation in the Netherlands (86); of the animal groups examined (2,899 groups, 158,763 animals in total), 5% showed caseous lesions in the submaxillary and/or mesenteric lymph nodes in at least one pig. In 91.5% of the positive groups, the number of pigs affected was equal to or less than five, whereas in 8.5% of groups more than five animals were affected. Acid-fast bacteria were detected in 41% of cases by microscopic examination of Ziehl-Neelsen stained smears. Isolation and investigation of strains by IS*1245*-RFLP revealed that 90 of 91 isolates were *M. avium*, whereas one pig isolate harboured the bird-type RFLP pattern. When patterns were compared to those obtained for MAC isolates from 191 human patients, 75% similarity was detected, suggesting that pigs may be a vector of *M. avium* for humans, or alternatively that both pigs and humans share common environmental sources of infection (86). Determination of confirmed epidemiological associations for MAC organisms is problematic because of the ubiquitous nature of the organisms. However, surface and drinking water, soil and foods, as well as direct contact with pet birds have been proposed as possible sources of infection. Although transmission of *M. avium* from poultry to humans does not appear to be a significant risk, the extensive similarities between pig and human isolates do suggest potential epidemiological links (100).

Infection by *M. paratuberculosis* causes Johne's disease, or paratuberculosis, a slowly developing granulomatous enteritis which is eventually fatal (174) and is responsible for important economic losses (25, 26). All ruminant species are likely to be susceptible to infection by *M. paratuberculosis*, as suggested by the reports of Johne's disease in domestic agriculture (e.g. cattle, sheep and goats), free-ranging wildlife (e.g. elk, bison and Bighorn sheep) and captive wildlife (e.g. addax, springbok and oryx). Further information and

references are supplied in the paper by Manning and Collins in this issue (99). The greatest economic impact of this infection has been felt by domestic agriculture through production losses and premature culling. A discussion of Johne's disease control programmes is provided by Kennedy and Benedictus in this issue (85).

Mycobacterium paratuberculosis is shed by infected animals into manure, milk and colostrum; kids and calves thus acquire the organism orally. Young animals are the most susceptible, acquiring the infection directly from the dam or from a contaminated environment. The majority of strains of the organism appear to be transmitted among different species (25, 26, 174). In Scotland, the same strain has been isolated from rabbits and their predators, foxes and stoats (lesions consistent with Johne's disease were detected), and the strain was the same as that obtained from dairy herds in the area. In a recent study, differentiation between the isolates from wild rabbits and those from cattle was not possible using molecular methods (67). If wildlife (both ruminant and carnivorous species) serves as a reservoir for this infection, test and slaughter control programmes for domestic agriculture may not be effective and vaccination programmes may also be necessary.

Molecular tools for diagnostics and epidemiology of mycobacterial infections in animals

Spoligotyping is a recently described fingerprinting technique that allows discrimination between *M. bovis* isolates from cattle and human *M. tuberculosis* (80). In an initial study in Spain, 182 *M. bovis* clinical isolates from farmed or feral animals were genotyped using this technique (3). Results suggested that spoligotyping was useful as an initial genotyping technique, to be used prior to IS*6110*-RFLP. This study also showed identical spoligotypes in wild boar, deer and cattle, suggesting inter-species transmission and the existence of animal reservoirs of bovine tuberculosis in this country (3). Using genotyping, inter-species transmission of *M. bovis* among wild animals (deer and badgers) and domestic livestock (cattle, sheep, goats and pigs) was suggested in Ireland (28), and transmission between cattle and wild boar was recently suspected in northern Italy (151).

Another study on *M. bovis* isolates from Australia, Canada, Ireland and Iran compared four genotyping methods, namely: IS*6110*-RFLP, direct repeat (DR)-RFLP, polymorphic GC-rich sequences (PGRS)-RFLP, and spoligotyping (30). The study concluded that PGRS was the most discriminative marker (77 types identified among 273 isolates), followed by DR-RFLP and spoligotyping (35 types) and IS*6110* (23 types). Another study performed on 128 *M. bovis* isolates from Spain proposed various typing strategies (4); given the importance of geographical variations obtained on *M. bovis* IS*6110* copy number and insertion sites, a standardised typing procedure was proposed (31). However, a recent study argued against the use of the standardised typing protocol using IS*6110*-RFLP as an initial test (191), and suggested that the use of spoligotyping as a first-line test might easily and cost-effectively help to define primary clusters to be studied further using PGRS and IS*6110*-typing for confirmatory epidemiology. A recent study performed in Ireland underlined the numerous advantages of spoligotyping compared to IS*6110* and confirmed the consistency of the results obtained with both techniques for epidemiological studies on *M. bovis* (143). Spoligotyping has also been used to define subspecies-specific signatures within the various members of the *M. tuberculosis* complex. Some of these specific signatures are illustrated in Figure 6; for example, *M. bovis* shows a characteristic absence of spacers 39 to 43, whereas the recently described *M. bovis* subsp. *caprae* shows the absence of spacer 1-16.

Mycobacterial pathogenicity

The host microbicidal functions may vary depending on the location of phagocytised bacteria in different intracellular loci (phagosomes, phagolysosomes and the cytoplasm), as may the means by which bacteria protect themselves. Although studies have reported the extraphagosomal location of *M. leprae* in the tissue of leprosy patients (20) and experimentally infected mice (104), and of virulent strains of *M. tuberculosis* in rabbit alveolar macrophages (106), these observations have not been confirmed by other investigators for *M. avium* and *M. leprae*-infected macrophages (54, 55, 56, 153). In 1993, McDonough *et al.* re-examined the dynamics of phagolysosome fusion and its effect on intracellular replication of virulent (H37Rv) and avirulent (H37Ra) strains of *M. tuberculosis*, and *M. bovis* BCG (93). In all cases, by 2 h post infection, approximately 85% of the bacteria clearly resided in fused vacuoles. However, at four days post infection, fusion levels for viable H37Rv and H37Ra were reduced by half, whereas the fusion profiles of BCG and of heat-killed H37Rv and H37Ra were unchanged. A comparison of the numbers of bacteria per fused and non-fused vacuole suggested both a net transfer of bacteria out of fused vacuoles and preferential bacterial multiplication in non-fused vacuoles, and in some cases, the bacteria appeared to be free in the cytoplasm (93). The authors concluded that viable tubercle bacilli, but neither the heat-killed bacteria nor *M. bovis* BCG, had the capacity to escape from fused vesicles as the infection progressed. Furthermore, after extrusion from the phagolysosomes, H37Rv, but not H37Ra, was able to multiply. Thus, virulent *M. tuberculosis* may elude the microbicidal mechanisms of macrophages by escaping from fused phagolysosomes into non-fused vesicles or the cytoplasm (93). Recently, Russell *et al.* investigated the vacuoles inhabited by viable *M. avium* and *M. tuberculosis*, which show limited fusion with endosomal and lysosomal compartments in experimentally-infected murine macrophages (148). The ability to regulate the maturation of the phagosomal compartments and restrict

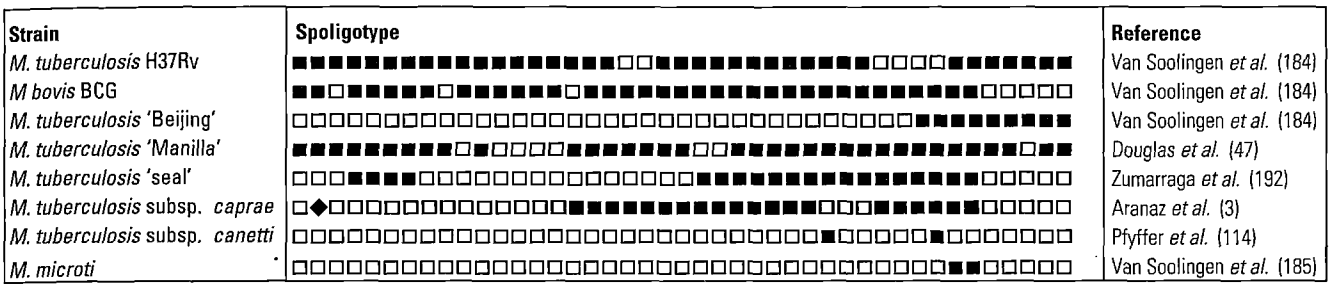


Fig. 6
Subspecies-specific signatures within the various members of the *Mycobacterium tuberculosis* complex, as defined by spoligotyping

differentiation into hydrolytically active vacuoles in infected macrophages appeared to correlate with the survival of the bacilli. Using cholera toxin B subunit which binds to GM1 ganglioside on the macrophage plasmalemma, these authors demonstrated that rather than being fusion-incompetent, mycobacterial vacuoles were highly dynamic, fusion-competent vesicles that behaved like an extension of the recycling endosomal apparatus. Thus, the results of Russell *et al.* (148), and those of Fréhel *et al.* (54), suggest that instead of being fusion-incompetent vesicles, mycobacterial vacuoles may be considered as extremely selective regarding the intracellular compartments with which they mix; thus, the latter may possess selected constituents of the endosomal network, yet may be deficient in others.

In summary, pathogenic mycobacteria are intracellular pathogens with the ability to grow inside phagosomes and phagolysosomes, and are able to inhibit the fusion of bacteria-containing phagosomes with lysosomes upon macrophage infection (e.g. *M. tuberculosis* [5], *M. leprae* [56] and *M. avium* [54, 55]). In the case of the tubercle bacillus, this property has been attributed to bacterial sulpholipids (65) that are located on the bacterial cell surface (70). Other important mycobacterial virulence factors include the lipoglycans (such as LAM) which are able to modulate cytokine secretion and macrophage effector functions (186). The ability of mycobacteria to survive and modulate immune responses in the host is clearly related to the architecture of the cell envelope and the constituents contained therein (117, 132). For example, surface products such as phenolic glycolipids, LAM and sulpholipids may also protect these organisms from intracellular killing by scavenging reactive oxygen molecules or by modulating macrophage activation (123). The components, structure and reported biological activities of the mycobacterial wall have been reviewed in detail elsewhere (116, 118, 123). A brief description of biological activities of selected compounds of mycobacterial origin is presented in Table VII.

Given the major differences observed among pathogenic and non-pathogenic mycobacteria regarding the ability to trigger cytokine secretion by human macrophages (16), it is important to identify specific cell envelope components of various pathogenic and non-pathogenic species, and characterise their ability to modulate immune responses in the infected host. A recent review discussed aspects relating to the interaction of *M. tuberculosis* with macrophages, and subsequent activation of tuberculocidal activity of macrophages (112). A model has been proposed involving *M. avium* and a group of amphipathic substances referred to as the GPL antigens, which are one of the key elements in the cell envelope of these organisms. These serovar-specific *M. avium* antigens (21) localised in the exterior of the cell envelope (9, 171) tend to accumulate within the phagosomal compartments following long-term infection of macrophages (146). Relatively inert to macrophage degradation (70, 71, 190), the GPLs not only accumulate in host macrophages, but may persist as the infection continues. The interaction of the macrophages with biological membranes induces alterations (92). The immunosuppressive role of *M. avium* lipids as a result of interaction with human monocytes (181), and of a GPL in *M. smegmatis* (similar to the apolar lipids of *M. avium*), was demonstrated to modify membrane permeability (163). These effects on biological membranes may be extremely important, hindering the ability of immunocompetent cells to function properly in immune reactions such as cell-to-cell recognition, phagolysosomal fusion, and electron transfers, and may be deleterious for the host. Consequently, GPLs and related lipids may act as important immunomodulators of host responsiveness. *Mycobacterium avium* GPLs result in a non-specific inflammatory response when injected intraperitoneally into mice (72). Brownback and Barrow have suggested that GPL-metabolites may also be responsible for the immunomodulatory events in the host (e.g. modification of the helper subsets of mice splenic cells) (22). Using the β-elimination procedure to remove the serovar-specific oligosaccharides from *M. avium* GPL (21), two major fragments are produced, namely: a residual lipid (referred to as β-lipid) and a reduced oligosaccharide (r-olig). Using mouse splenic cells, the β-lipid was demonstrated to be the active moiety with regards to the ability to suppress mitogen-induced lymphoproliferative responses (167). In connection with these findings, the ability of total lipids and purified GPLs from *M. avium* serovars 4 and 8 (which predominate as opportunistic infection among AIDS patients), was demonstrated to affect T helper (TH)1-type responses in human peripheral blood mononuclear cells (PBMC) from healthy donors (73). Exposure of PBMC to total

Table VII
Biological activities attributed to compounds of mycobacterial origin

Compounds	Biological activities
Mycolic acid-containing glycolipid	Induce granuloma formation ('foreign-body' type?)
Mannose-containing phospholipid	Immunogen; adjuvant activity
Mycosides of the glycopeptidolipid type	Antigens related to serotyping in the *M. avium-intracellulare* complex; implicated in the formation of the 'capsule-substance' around phagocytised bacteria
Mycosides of the phenoglycolipid type	Specific antigen in *M. tuberculosis*, *M. leprae* and *M. kansasii*; implicated in the formation of the 'capsule-susbtance' in phagocytised bacteria
Wax-D	Adjuvant activity; induces arthritis in mice and rats; induces granuloma ('foreign-body' type?)
Cord factor (TDM)	Toxicity in mice (peritonitis, acute haemorrhage); virulence factor; inhibition of PMN migration; depression of NAD-linked microsomal enzymes; depression of muscle and liver glycogen synthesis; affects pyruvate metabolism; immunogen; adjuvant activity; induces granuloma ('infection' type)
Sulphatides	Virulence factor; toxicity in mice; inhibits phagosome-lysosome fusion in infected macrophages
Muramyl-dipeptide (MDP)	Adjuvant activity; immunomodulator
Cell wall skeleton	Adjuvant activity; induces granuloma ('foreign-body' type); immunomodulator
Mycobactins and exochelins	Iron chelators; pathogenicity factor

NAD : nicotinamide acid dinucleotide
PMN : polymorphonuclear neutrophil
TDM : trehalose 6,6'-dimycolate
Source: adapted from Rastogi and David (123)

lipids significantly suppressed phytohaemagglutinin-p/phorbol myristate acetate (PHA/PMA)-induced secretion of interleukin (IL)-2 and gamma interferon, as determined by enzyme-linked immunosorbent assay (ELISA). The GPL4 was found to be more efficient at inhibiting TH1 responses than GPL8. This study suggested that the accumulation of *M. avium* lipids in the chronic stages of infection may play an important role in the pathogenesis of HIV infection (73).

Phenylalanine-containing lipopeptides from rough and smooth colony-forming *M. avium* serovars with immunosuppressive activity have also been identified (10). Although the lipopeptide core has not yet been demonstrated to be a product of GPL degradation *in vivo* or *in vitro*, some of the immunosuppressive effects may be due to other lipids structurally-related to the GPL, or to metabolic products of GPL. A total lipid extract from *M. avium* serovar 4 not only has the capacity to reduce mitogen-induced lymphoproliferative responses of human monocytes, but may also interfere with the ability of human monocytes to control the intracellular growth of mycobacteria, probably because of the ability of the lipid extract to induce substantial prostaglandin-E2 (PGE2) secretion by cells (10). Similar immunosuppressive properties were also observed for the β-lipid fragment of the serovar 4 GPL, but not for the r-olig or purified GPL (10). In the case of serovar 8, both the total lipid and serovar-specific GPL fractions induced significant levels of tumour necrosis factor alpha (TNF-α) as well as PGE2 in human monocytes (12). These findings further suggest the differing ability of mycobacterial lipids to affect immune functions. However, the role of the carbohydrate substituents of the GPL antigens versus that of the 'lipid core' should be further investigated. Rough colony variants of *M. avium* that do not contain the regular GPL antigens, but instead contain novel lipopeptides that are devoid of carbohydrate moieties are now available for such comparisons (15); these newly discovered lipopeptides are identical to the fatty acyl-tripeptide-amino alcohol 'core' component of the GPLs of the *M. avium* complex. The isolation of different sets of spontaneous mutants of *M. avium* that are differentially defective in the capacity to synthesise GPL antigens will permit the exploration of the biosynthesis of GPLs and their roles in opportunistic pathogenesis. Immunomodulatory properties have been demonstrated in other mycobacterial lipids, but some of these have covalent links (e.g. LAM [94]), and hence may not be as easily released and accumulated within host macrophages as non-covalently-linked GPLs and related lipids.

The induction of cytokines by *M. avium* appears to be related to the colony type of the organism, which in turn is related to the difference in the cell envelope architecture (117, 118). Thus, the more virulent smooth transparent (SmT) phenotype induces the secretion of small quantities of TNF-α, whereas the less virulent smooth dome (SmD) phenotype induces high levels of TNF-α (61). Similarly, SmT variant of *M. avium* induces less secretion of IL-1 and IL-6 than the SmD phenotype (19). In this context, Belisle and Brennan have reported that the SmD morphotype always produces more GPL in total than the SmT morphotype (14). Variability in the composition of the cell envelope is therefore likely to play an important role in the inherent ability of a particular mycobacteria to modulate immune responses in a host.

Consequently, a multiplicity of factors contribute to mycobacterial pathogenicity (133).

Concluding remarks

Since the 1980s, research into the molecular biology and genetics of mycobacteria has provided an enormous amount of information, stimulating renewed interest in medical and veterinary mycobacteriology. Molecular tools now permit a rapid diagnosis of mycobacterial infections, novel taxonomical and phylogenetic approaches, and an improved comprehension of the mechanisms of mycobacterial pathogenicity and virulence. Effective control of mycobacterial infections in animals and humans will rely heavily on knowledge of the molecular aspects underlying structure and function relationships of the mycobacterial cell envelope and the various biologically active substances and antigens contained therein, the subsequent events leading to the development of specific immunity against mycobacteria, the application of recombinant DNA technology for preparing new vaccines, and the selective production of compounds used in immunotherapy. The concerted efforts of many, as reviewed in this article, have already contributed an impressive amount of data which show promising results. However, improvements in rapid diagnostic tests and in vaccine programmes are required, particularly in terms of the organisation of field-applications, and the global co-ordination of research, diagnosis and epidemiological investigations.

■

Introduction à la nomenclature et à la pathogénie des mycobactéries

N. Rastogi, E. Legrand & C. Sola

Résumé

La tuberculose, due à *Mycobacterium tuberculosis*, et la lèpre, due à *M. leprae*, sont connues depuis l'Antiquité. Dans les pays en développement, la tuberculose reste la principale cause de mortalité imputable à une maladie infectieuse. Du point de vue taxonomique, les mycobactéries appartiennent au genre *Mycobacterium*, genre unique de la famille des Mycobactériacées, ordre des Actinomycétales. Les Actinomycétales comprennent divers micro-organismes, mais les mycobactéries et autres taxons apparentés sont aisément reconnaissables par leur aptitude à synthétiser les acides mycoliques. Les espèces mycobactériennes se distinguent en général par leurs caractéristiques phénotypiques ; les auteurs présentent une liste actualisée des tests biochimiques utilisés ainsi que des caractères culturaux qui aident à différencier les diverses espèces de mycobactéries. Cependant, comme les caractéristiques phénotypiques ne permettent pas une identification précise de toutes les espèces, les auteurs décrivent également les méthodes récentes de taxonomie moléculaire qui permettent de classifier les mycobactéries et d'en établir la phylogénie. Les mycobactéries sont également les premières responsables d'épizooties chez plusieurs espèces d'animaux domestiques et sauvages. Les auteurs décrivent brièvement ces mycobactéries, les réservoirs existants chez les animaux sauvages et les stratégies de lutte contre la tuberculose bovine, ainsi que l'utilisation de techniques moléculaires pour le diagnostic et l'épidémiologie des infections mycobactériennes chez les animaux. Les caractéristiques du parasitisme intracellulaire font l'objet de la discussion ainsi que l'évolution des mycobactéries pathogènes qui se développent dans les phagosomes et les phagolysosomes des macrophages de l'hôte infecté. La membrane cellulaire

mycobactérienne, structure tripartite complexe comportant une proportion élevée de lipides (environ 30 % à 40 % du poids total), pourrait jouer un rôle décisif dans l'adaptation des mycobactéries à la croissance et à la survie intracellulaires, ainsi que dans la modulation immunitaire et la résistance aux médicaments.

Mots-clés
Maladie – Membrane cellulaire – Mycobacterium – Nomenclature – Pathogénie – Phylogénie – Prophylaxie – Taxonomie.

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Introducción a la nomenclatura y la patogénesis de las micobacterias

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Resumen
La tuberculosis (causada por *Mycobacterium tuberculosis*) y la lepra (*M. leprae*) son enfermedades conocidas desde la antigüedad. En países en desarrollo, la tuberculosis es todavía la primera causa de mortalidad por enfermedad infecciosa. Las micobacterias se adscriben taxonómicamente al género *Mycobacterium*, único de la familia Micobacteriaceae, perteneciente al orden Actinomicetales. Aunque este orden comprende microorganismos diversos, las micobacterias y taxones emparentados con ellas se distinguen fácilmente del resto por su capacidad de sintetizar ácidos micólicos. Hasta hace poco, la diferenciación entre especies micobacterianas se basaba principalmente en sus rasgos fenotípicos; los autores presentan una lista actualizada de las actuales pruebas bioquímicas y las propiedades en cultivo de las especies micobacterianas que ayudan a distinguirlas entre sí. Sin embargo, no todas las especies pueden identificarse con precisión atendiendo sólo a sus rasgos fenotípicos, y por ello los autores describen también recientes sistemas de taxonomía molecular que se aplican a la clasificación y el estudio filogenético de las micobacterias. Además de su capacidad de infectar al hombre, estos microorganismos son también una de las grandes causas de infección de diversos animales domésticos y salvajes. Los autores hacen una breve descripción de las micobacterias que infectan a los animales, sus reservorios entre la fauna salvaje, las estrategias de lucha contra la tuberculosis bovina y el uso de técnicas de biología molecular para el diagnóstico y el estudio epidemiológico de las micobacteriosis animales. Los autores examinan asimismo el característico fenómeno del parasitismo intracelular, y consideran la evolución que pueden seguir las micobacterias patógenas capaces de crecer dentro de los fagosomas y fagolisosomas de los macrófagos del huésped. La membrana celular micobacteriana, compleja estructura tripartita con una elevada proporción de lípidos (de un 30% a un 40% del peso total), podría desempeñar una función básica en la adaptación de esos microorganismos al crecimiento y la supervivencia en medio intracelular, la modulación inmunitaria y la resistencia a los antibióticos.

Palabras clave
Control – Enfermedad – Filogenia – Membrana celular – Mycobacterium – Nomenclatura – Patogenicidad – Taxonomía.

■

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