Overview of Mycobacterium: A Review
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Abstract: Mycobacterium is still one of the major causes of mortality, since two million people die each year from this malady. Currently, there are over 170 recognized species of Mycobacterium, the only genus in the family Mycobacteriaceae. Organisms belonging to this genus are quite diverse with respect to their ability to cause disease in humans; some are strict pathogens, while others are opportunistic pathogens or nonpathogenic. Similar to other major groups of bacteria, the mycobacteria have undergone an extraordinary expansion in the number of different species over the last 2 decades, due in large part to the discriminatory power of gene sequencing, which phenotypic methods cannot achieve. This discriminatory power is such that phenotypic traits, i.e., biochemical and cultural characteristics, are no longer acceptable for the identification of mycobacteria. M. tuberculosis strains are reportedly more virulent than others, as defined by increased transmissibility as well as being associated with higher morbidity and mortality in infected individuals. As more clinical laboratories use molecular or other methods, such as mass spectrometry, for identification, our understanding of clinical significance will change and evolve as the number of case reports regarding the “new” species increase; it may well be that the role of many of the newly described mycobacterial species has been underestimated either because of misidentification or because the species were unrecognized. Molecular typing methods have greatly improved our understanding of the biology of mycobacteria and provide powerful tools to combat the diseases caused by these pathogens. The utility of various typing methods depends on the Mycobacterium species under investigation as well as on the research question. Within this review, we summarize currently available molecular methods for strain typing of M. tuberculosis. For the various methods, technical practicalities as well as discriminatory power and accomplishments are reviewed.

General Description of Taxonomy and Nomenclature

The discovery of leprosy bacillus (originally named Bacillus leprosy) in 1880, and of tubercle bacillus (named Bacterium tuberculosis) in 1883, led to the first steps in the classification of mycobacteria. These organisms were renamed Mycobacterium leprae and Mycobacterium tuberculosis by Lehmann and Neumann and grouped within the genus Mycobacterium, which is the single genus within the Mycobacteriaceae family, in the Actinomycetales order and Actinomycetes class. Bacteria were first classified as plants constituting the class Schizomycetes, which along with the Schizophyceae (blue green algae/Cyanobacteria) formed the phylum Schizophyta. Despite there being little agreement
on the major subgroups of the *Bacteria*, Gram staining results were most commonly used as a classification tool, consequently until the advent of molecular phylogeny, the Kingdom *Prokaryotae* was divided into four divisions,[1] A classification scheme still formally followed by Bergey's manual of systematic bacteriology.[2]

A classification scheme still formally followed by Bergey's manual of systematic bacteriology.

Phylogenetic tree showing the relationship between the archaea and other forms of life. Eukaryotes are colored red, archaea green and bacteria blue. Adapted from Ciccarelli e

In 1987 Carl Woese divided the *Eubacteria* into 11 divisions based on 16S ribosomal RNA (SSU) sequences, which with several additions are still used today. While the three domain system is widely accepted some authors have opposed it for various reasons.[4]

One prominent scientist who opposes the three domain system is Thomas Cavalier-Smith, who proposed that the *Archaea* and the *Eukaryotes* (the *Neomura*) stem from Gram positive bacteria (*Posibacteria*), which in turn derive from gram negative bacteria (*Negibacteria*) based on several logical arguments,[5] which are highly controversial and generally disregarded by the molecular biology community (c.f. reviewers' comments on,[5] e.g. Eric Bapteste is "agnostic" regarding the conclusions) and are often not mentioned in reviews e.g. due to the subjective nature of the assumptions made.

However, despite there being a wealth of statistically supported studies towards the rooting of the tree of life between the *Bacteria* and the *Neomura* by means of a variety of methods,[7] including some that are impervious to accelerated evolution—which is claimed by Cavalier-Smith to be the source of the supposed fallacy in molecular methods[67]—there are a few studies which have drawn different conclusions, some of which place the root in the phylum *Firmicutes* with nested archaea.[5]

Mycobacteria are defined as aerobic, acid-alcohol-fast, rod-shaped actinomycetes with occasional branching; aerial hyphae are normally absent, and the bacteria are nonmotile, nonsporulating organisms that contain arabinose, galactose, and meso-diaminopimelic in their walls. They have guanine and cytosine DNA base ratios in the range of 62-70 mol% (except for M. leprae with a GC base ratio of 58%), and have high-molecular-weight mycolic acids (60-90 carbons) lacking components with more than two points of unsaturation in the molecule[7]. Although historically defined as non-encapsulated organisms, mycobacteria are now known to contain a capsule-like structure[8] similarly, 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. Thus the minimum standards for including a species in the genus Mycobacterium are: its acid alcohol fastness, the presence of mycolic acids containing 60-90 carbons (which are cleaved to C22-C26 fatty acid methyl esters by pyrolysis), and a GC base ratio in the range of 61-71 mol%. The minimal standards for including a species in the genus Mycobacterium are i) acid-alcohol fastness, ii) the presence of mycolic acids containing 60-90 carbon atoms which are cleaved to C22 to C26 fatty acid methyl esters by pyrolysis, and iii) a guanine + cytosine content of the DNA of 61 to 71 mol %. Currently, there are 71 recognized or proposed species of Mycobacterium which can be divided into two main groups based on growth rate. The slowly growing species require > 7 days to form visible colonies on solid media while the rapidly growing species require < 7 days. Slowly growing species are often pathogenic for humans or animals while rapidly growing species are usually considered non-pathogenic for humans, although important exceptions exist.

**Introduction**

*Mycobacterium* is a genus of Actinobacteria, given its own family, the Mycobacteriaceae. The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*). The Greek prefix *myco* means fungus, alluding to the way mycobacteria have been observed to grow in a mold-like fashion on the surface of liquids when cultured.

*Mycobacteria* belong to the family *Mycobacteriaceae* and are members of the CMN group (*Corynebacteria, Mycobacteria and Nocardia*). The family *Mycobacteriaceae* is Gram positive, nonmotile, catalase-positive, have a rodlike to filamentous morphology and can be pleomorphic. As a group, they produce characteristic long chain fatty acids termed mycolic acids. Mycobacteria are acid-fast rods of variable appearance, approximately 0.2-0.6 by 1-10 micrometer.

*Mycobacterium tuberculosis* (Mt) is the most effective pathogen of mankind and is second only to human immunodeficiency virus/AIDS as the greatest cause of death worldwide due to a single infectious agent. Antibiotic resistance is a major reason why Tuberculosis (TB) is so difficult to eradicate and has become a challenge to overcome. Besides drug resistance, another major problem directly arises from the combination of Mt physiology and human immune response. The *Mycobacterium* genus belongs to the GC-rich
Gram-positive Actinobacteria phylum, order of Actinomycetales, and suborder of Corynebacterineae that also includes the Corynebacterium and Nocardia genera\textsuperscript{13}

Currently, there are over 170 recognized species of Mycobacterium, the only genus in the family Mycobacteriaceae. Organisms belonging to this genus are quite diverse with respect to their ability to cause disease in humans; some are strict pathogens, while others are opportunistic pathogens or nonpathogenic. Similar to other major groups of bacteria, the mycobacteria have undergone an extraordinary expansion in the number of different species over the last 2 decades, due in large part to the discriminatory power of gene sequencing, which phenotypic methods cannot achieve. This discriminatory power is such that phenotypic traits, i.e., biochemical and cultural characteristics, are no longer acceptable for the identification of mycobacteria\textsuperscript{14}

**Microbiological Representation of Mycobacterium**

**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, which protected the mycobacteria from host-mediated killing mechanisms. Subsequently, it was demonstrated that recycling of mycobacteria by the host permitted a better expression of this capsular material\textsuperscript{15}; 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 periphery 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\textsuperscript{1q} such as the outer layer (OL) or CAP, or by using a novel gelatin-Lowicryl embedding\textsuperscript{16}.

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\textsuperscript{17}. 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\textsuperscript{18}

**Cell Wall:** Mycobacterium 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 Asse lineau and Lanéelle \textsuperscript{19}. 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 constituting \textsuperscript{20}. Mycobacterial cell wall: 1-outer lipids, 2-mycolic acid, 3-polysaccharides (arabinogalactan), 4-peptidoglycan, 5-plasma membrane, 6-lipoarabinomannan (LAM), 7-phosphatidylinositol mannoside, 8-cell wall skeleton.

**Culture and growth**

Mycobacteria are aerobic and non motile bacteria (except for the species *Mycobacterium marinum*, which has been shown to be motile within macrophages) Many *Mycobacterium* species adapt readily to growth on very simple substrates, using ammonia or amino acids as nitrogen sources and glycerol as a carbon source in the presence of mineral salts. Optimum growth temperatures vary widely according to the species and range from 25 °C to over 50 °C.\textsuperscript{11}

Some species can be very difficult to culture (i.e. they are fastidious), sometimes taking over two years to develop in culture. Further, some species also have extremely long reproductive cycles — *M. leprae*, may take more than 20 days to proceed through one division cycle (for comparison, some *E. coli* strains take only 20 minutes), making laboratory culture a slow process. \textsuperscript{21}
A natural division occurs between slowly- and rapidly-growing species. Mycobacteria that form colonies clearly visible to the naked eye within seven days on subculture are termed rapid growers, while those requiring longer periods are termed slow growers.

**Clinical Representation and significance of Mycobacterium**

The mycobacterial species have long been divided into two major groups: (1) the M. tuberculosis complex, and (2) nontuberculous mycobacteria or atypical mycobacteria (also termed "mycobacteria other than Mycobacterium tuberculosis" complex or MOTT). The former includes: M. tuberculosis, responsible for human tuberculosis; M. bovis, responsible for bovine tuberculosis; M. africanum, which causes human tuberculosis essentially limited to Africa; M. microti, a pathogen for small rodents; and the vaccinal strain M. bovis BCG [151. Although this distinction is still sufficient for practical purposes, it remains vague with regard to the exact taxonomic status of a number of newly described species, subspecies, or subtypes [22]. Depending on their clinical importance, mycobacteria have been classified into three major groups: 1. Strict pathogens that include human pathogens M. tuberculosis and M. lepra and animal pathogen M. bovis (group 3 of hazard risk group classification) 2. Opportunistic or potential pathogens that include M. avium, M. simiae, M. kansasi, and M. haemophilum which are more common among immunocompromised patients (group 2 of hazard risk group classification) 3. Rare pathogens that include saprophytes such as M. smegmatis and M. phlei (group 1 of hazard risk group classification)

The most common sites where mycobacterial disease occurs are the lungs, the lymph nodes and skin. However, as *M. tuberculosis* is mostly known to cause the well-established pulmonary manifestation but is capable of infecting virtually all tissue types, the NTM species follow the same behaviour: the range of clinical manifestations is extensive [23]. Mycobacterial infections are complex diseases and are even more in individuals suffering from immune-mediated inflammatory diseases (IMIDs). Mycobacterial infections are generally a potential long-term complication of patients with defects in cell-mediated immunity and tend to develop subacutely. The risk of *Mycobacterium tuberculosis* infection is strongly dependent on the ethnic background and country of origin of the patient, and tuberculosis should be considered in at-risk patients with a cell-mediated immune defect, patchy or nodular lung shadowing from high-risk ethnic backgrounds. Nontuberculous mycobacterial infections (e.g., *Mycobacterium kansasi* or *Mycobacterium avium-intracellulare* complex) are infrequent complications in immunocompromised patients.
Exclusion of the diagnosis by negative culture takes too long to be clinically useful in patients with progressive disease. Therefore, if there is significant clinical suspicion of mycobacterial infection, invasive investigations are necessary to obtain material for a rapid diagnosis by identification of acid-fast bacilli or specific histologic changes.

The most common infection caused by mycobacterium are Pulmonary infections, Skin infections, Lymphadenitis, Disseminated diseases, Skeletal infections, Gastrointestinal infections and Foreign body related and nosocomial infections

**Phenotypic and Genotypic Characteristics**

Mycobacterial species are traditionally differentiated on the basis of phenotypic characteristics. The most commonly used tests for slow-growing species include: growth at 25°C, 30°C, 33°C, 37°C, 42°C, 45°C; pigmentation; resistance to a number of substances (isoniazid, 1 and 10 Ilg/ml; thiophene-2-carboxylic hydrazide, Ilg/ml; hydroxylamine, 500 Ilg/ml; p-nitrobenzoic acid, 500 Ilg/ml; sodium chloride, 5% wt/vol; thiacetazone, 1 Ilg/ml; picrate 0.2% wt/vol; oleate, 250 Ilg/ml); characteristic mycolic acid profiles and pyrolysis esters; and the activities of heat-resistant and/or semiquantitative catalase, tween-80 hydrolysis, urease, niacin production, nitrate reductase, acid phosphatase, 3-day arylsulfatase, pyrazinamidase, a-esterase, α-esterase, and α-galactosidase.

**Pathogenesis and host defences**

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 and experimentally infected mice, and of virulent strains of M. tuberculosis in rabbit alveolar macrophages, these observations have not been confirmed by other investigators for M. avium and M. leprae-infected macrophages.

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, M. leprae and M. avium). In the case of the tubercle bacillus, this property has been attributed to
bacterial sulpholipids that are located on the bacterial cell surface. Other important mycobacterial virulence factors include the lipoglycans (such as LAM) which are able to modulate cytokine secretion and macrophage effector functions. 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. 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. The components, structure and reported biological activities of the mycobacterial wall have been reviewed in detail else.

The host defense against *M. avium* is primarily dependent on CD4+ T lymphocytes and natural killer cells. Activated macrophages can inhibit or kill intracellular bacteria by mechanisms that are currently unknown, but *M. avium* can invade resting macrophages and suppress key aspects of their function by triggering the release of transforming growth factor β and interleukin 10. Co-infection with HIV-1 appears to be mutually beneficial, with both organisms growing faster.

**Methods of Genetic Analysis in Mycobacteria**

The complete sequence and annotation of the *M. tuberculosis* genome has allowed many new genetic approaches to studies of the physiology and pathogenicity of this organism, but much important work was done in this area before the genome-sequencing project was initiated. These pregenome approaches dealt largely with developing methods for creating mutations in specific genes. The choice of which genes to use and ultimately inactivate in order to study virulence was frequently based on the existence of naturally mutations occurring in normally virulent strains that affected pathogenicity, e.g., *sigA* and *katG*, or predictions of which genes should be important in some aspect of *M. tuberculosis* virulence and/or physiology by inference from studies of other bacterial pathogens, e.g., genes encoding sigma factors and iron acquisition regulators. The following discussion addresses methods used for genetic analysis of mycobacterial species. Their application to the identification and characterization of *M. tuberculosis* genes that play a role in virulence is discussed later in this review. There are some methods, such as bacterial conjugation and generalized bacteriophage-mediated transduction that have been used for genetic studies of *M. smegmatis* but are not discussed here because they have not yet been successfully applied to *M. tuberculosis.*
**Initial genetic studies.**

Early studies on the creation of mutations in mycobacteria concentrated on the faster-growing nonpathogenic species because of the relative ease of working with these bacteria. There is no requirement for biosafety level 3 containment facilities, and the experiments are relatively rapid; e.g., *M. smegmatis* has a 3-h generation time while that of *M. tuberculosis* is 20 to 24 h. Several techniques were developed to inactivate genes in these bacteria, with the first reports of *M. smegmatis* and *M. bovis* BCG transformation being published in 1988 (268) and the subsequent development of a highly transformable *M. smegmatis* strain, MC\textsuperscript{2}155, being published in 1990 \textsuperscript{31}. These and more recent articles have been reviewed. While these methods had some success in investigating *M. tuberculosis*, the process was still difficult. In addition to its extremely slow growth, which makes it time-consuming to do the standard types of gene inactivation (it takes 3 weeks for a single *M. tuberculosis* cell to become a visible colony on solid media), this bacterium was thought to have lower rates of homologous recombination and higher rates of illegitimate recombination than other mycobacteria, which would complicate gene disruption by standard gene replacement techniques\textsuperscript{31}

**Current genetic methods.**

Despite the problems mentioned in the previous section, several current techniques have been successful in inactivating *M. tuberculosis* genes. Gene disruption techniques in mycobacteria, as described below, can be divided into directed and global methods but generally require a selectable phenotype, usually resistance to an antibiotic. The most frequently used antibiotic resistance cassettes in mycobacteria are those conferring resistance to kanamycin, hygromycin, and streptomycin. These antibiotics are also useful for selection in *E. coli*, allowing most cloning procedures to take place in this organism with appropriate plasmid vectors. Selection for kanamycin resistance (Kan\textsuperscript{r}) is favored in many mycobacteria like *M. smegmatis* because of the generally low levels of spontaneous Kan\textsuperscript{r} mutations and the high stability and low cost of the antibiotic.\textsuperscript{32} However, kanamycin resistance is not a good selective marker in *M. tuberculosis* because there is a high spontaneous mutation rate that results from the presence of only one rRNA (*rrn*) cistron in which the 16S rRNA gene can undergo mutations to Kan\textsuperscript{r} at a significant level. *M. smegmatis* has two *rrn* cistrons, and mutations to Kan\textsuperscript{r} in one of these is masked by the dominance of the second, sensitive *rrn* cistron \textsuperscript{33}. 
(i) Directed gene disruption.

Directed gene inactivation entails the insertion of an antibiotic resistance cassette in the middle of the gene of interest and then the transformation of this DNA into mycobacteria as a linear or circular molecule, using electroporation. The desired result is allelic replacement of the chromosomal gene by the mutated one. In members of the *M. tuberculosis* complex, directed gene disruptions have been made with long linear molecules, up to 40 kb (9), or shorter ones, in the range of 4 kb \(^{34}\). The use of single-stranded linear DNA increases allelic replacement by homologous recombination in *Streptomyces*, and this effect is also observed in gene inactivation experiments in *M. smegmatis* and in the *M. tuberculosis* complex\(^{35}\). The advantage of the linear-DNA method is that cloning is relatively easy, especially when short DNA fragments are used. A disadvantage is that unique restriction enzyme sites are required unless one employs more elaborate manipulation of the DNA sequence of interest, and this problem is magnified as the DNA increases in length.

(ii) Global gene inactivation.

The principle of global gene inactivation is the insertion of foreign DNA, usually a transposable element, into many sites in the bacterial genome, ideally on a completely random basis. These events require a selectable phenotype, generally an antibiotic resistance marker carried within the transposable element. Two groups have developed efficient transposition systems that produce integration events in the genomes of mycobacterial species, including *M. tuberculosis*. These systems use transposable elements carried by vectors that cannot replicate at temperatures above 39°C: in one case, a *ts* plasmid that carries Tn1096 \(^{36}\), and in the other, a *ts* bacteriophage, similar to the one described in the previous section, that carries Tn 5367 or Tn 5370. The advantage of these systems is that one can start with a transformed or infected population of cells and easily obtain many integrative events by passage at the restrictive temperature.

(iii) Complementation.

Genetic complementation has also been used to identify *M. tuberculosis* virulence genes. These studies use *M. tuberculosis* strains that are known to be avirulent or nonpathogenic as recipients for genes that can be selected on the basis of encoding a virulence phenotype, using assays described earlier in this review. In vivo complementation has been made possible by using integration-proficient vectors, which allow the stable propagation of genomic libraries as well as individual genes in bacteria during animal infections \(^{36}\). It was shown that the
random cloning of an *M. tuberculosis* cosmid library into an avirulent *M. bovis* strain localized the attenuating mutation in *sigA*, encoding the major mycobacterial sigma factor, since the wild-type *sigA* restored virulence in a guinea pig morbidity (spleen focus) assay. A similar *M. tuberculosis* cosmid library transformed into the avirulent *M. tuberculosis* strain H37Ra permitted the isolation of a DNA fragment that increased bacterial survival in mouse spleens but not lungs. The nonpathogen *M. smegmatis* was also used as the host in similar experiments. In one case, a cosmid library made with *M. bovis* BCG DNA was transformed into *M. smegmatis*, allowing the identification of a chromosomal fragment that modestly increased bacterial survival in mouse spleens and in mouse peritoneal macrophages.

### iv) Antisense methods.

Antisense RNAs are used to reduce the expression of specific genes because they prevent the translation of the mRNAs to which they are complementary. They are especially useful in systems where gene inactivation is difficult and also when genes are essential because antisense inhibition of translation is rarely, if ever, complete. A general system for conditionally controlling the production of antisense RNA in mycobacteria was developed, using the regulatable acetamide/acetamidase system. In a demonstration of the usefulness of this method, a prototrophic *M. smegmatis* strain was made into a histidine auxotroph when a *hisD5* antisense RNA was induced by acetamide. Other applications of the antisense method were in *M. bovis* to lower the levels of AhpC and in *M. tuberculosis* H37Rv to reduce the amounts of SodA. These are discussed later in this review. A related antisense approach, but using phosphorothioate antisense deoxyoligonucleotides, was used to decrease levels of the *M. tuberculosis* glutamine synthetase in growing cells.

### Other (Non genetic) methods.

Gene inactivation, either directed or global, and the subsequent analysis of mutant phenotypes is the most straightforward way to identify and characterize genes and proteins that are involved in a specific process and, for *M. tuberculosis*, is virulence. A major problem with this approach is that some genes may be essential and cannot be disrupted. Thus, methods that do not rely on the absence of a function are also useful, and these “nongenetic” screens usually rely on the differential expression of genes and their products in different environments. As in the genetic methods, they can be used to characterize the expression of individual genes identified by other means or can function in global searches for genes that show the desired pattern of gene expression. The output of these methods can be enzyme
activity using reporter genes, levels of RNA or proteins, and in some cases direct selection of genes using a selectable or screenable phenotype.

**FUTURE RESEARCH**

**Genetic Approaches**

As shown in this review, much work has been performed in the search for *M. tuberculosis* virulence factors. Various targets have been identified, e.g., enzymes involved in the synthesis of unique cell wall structures and secreted proteins. More work has to be performed so that every potential gene is systematically inactivated in the *M. tuberculosis* genome and the virulence of these mutants can be assayed. Ideally, the mariner transposon system can be modified to incorporate signature tagging so that many potential mutants can be tested in vivo at the same time. In addition to this global search, there are more specific approaches that can be undertaken.

**New Ways To Study *M. tuberculosis*-Host Interactions**

As an intracellular pathogen, *M. tuberculosis* has developed global strategies to survive and grow in macrophages and granulomas formed in various organs of its host. In the same way, the infected phagocytic cell and surrounding tissue respond in a global sense to the presence of an intruding pathogen. As discussed earlier in this review, DNA arrays have been used to study the global expression of genes in wild-type and mutant *M. tuberculosis* strains grown under different conditions. Proteomic techniques have also been used to measure the levels of large numbers of bacterial proteins when *M. tuberculosis* is in different environments. The purpose of this section is to discuss how new global methods are currently being used and could be used in the near future to study the interactions between *M. tuberculosis* and its host.

**Conclusion**

From this study it is concluded that initially Taxonomy was totally based on phenotypic characteristics but later on as genetic knowledge increased new tool and techniques for classifying microorganisms discovered. Today Numerical approaches, genetic analysis of genes and molecular similarities are primer tools for classification.
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 synthesize mycolic acids. Mycobacterial species are traditionally differentiated on the basis of phenotypic characteristics, and this study 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. 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.

As described above, there is a wide range of methods available for genotyping of *M. tuberculosis* complex. Each method has its own benefits and shortfalls, and none of them have proven clearly superior to any of the others. The choice of the optimal typing system depends heavily on the sample under investigation, the setting in which typing is performed, and the expected outcome.
References


