ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, 16 SECTIO D 2002

Department of Pulmonology, University School of Medicine, Lublin
Klinika Chorób Płuc i Gruźlicy Akademii Medycznej w Lublinie

SYLWIA CHOCHOLSKA, PAWEŁ KRAWCZYK,
MARTA ADAMCZYK-KORBEL, JANUSZ MILANOWSKI

Genetic methods used in detection and strain differentiation of the bacteria belonging to the Mycobacterium tuberculosis *complex for diagnosis and epidemiology*

Metody genetyczne stosowane do wykrywania i różnicowania szczepów należących do *Mycobacterium tuberculosis* complex dla celów diagnostycznych i epidemiologicznych

Tuberculosis remains a major worldwide health problem and it is estimated that the number of new occurrences of the disease will increase greatly in the next decade, especially because of its coexistence with human immunodeficiency virus (HIV) infections [38]. Every year approximately eight millions of people are affected by this disease. In Poland, 14 895 patients suffering from tuberculosis were registered in the year 2000, which gives the prevalence of tuberculosis of 38.5. Compared to the level of this coefficient in 1999 (i.e. 37.6) a slight increase was noticed [40].

Mycobacterium tuberculosis is an intracellular bacteria, which may live and proliferate mainly in the professional phagocytes (macrophages). This microorganism has a low virulence and proliferates slowly, therefore an inflammation process becomes prolonged lasting for months or even years. It results from the differences in the intensity of an immune response causing problems with diagnosis [34].

Therefore it is important to develop such diagnostic tests that allow a rapid, sensitive and specific detection and identification of *Mycobacterium tuberculosis* in clinical specimens as this will enable appropriate medical care decisions to be made. The standard methods used in diagnosis of tuberculosis have some important limitations. Although direct microscopy is a rapid diagnostic method used for detecting acid-fast mycobacteria, it is insensitive and does not permit identification of the species of identified bacteria. Mycobacterial culture is a sensitive but time-consuming procedure as it may require several weeks to obtain positive results. However, recent developments in molecular genetics have led to the methods for fast detection of mycobacteria based on the polymorphism of their DNA.

DNA POLYMORPHISM WITHIN THE SUBSPECIES OF THE *MYCOBACTERIUM TUBERCULOSIS* COMPLEX

The results obtained from different generic typing methods, like multilocus enzyme electrophoresis (MLEE), restriction fragment end labelling (RFEL) or multilocus sequencing, show that the bacteria belonging to the *Mycobacterium tuberculosis* complex (i.e. *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microtii* and *Mycobacterium canetii*) are genetically closely related [6, 8, 11, 23, 44]. However, a high degree of DNA polymorphism is observed and is associated with repetitive DNA such as insertion elements (IS) and short repetitive DNA sequences.

In *M. tuberculosis* complex strains four ISs, IS*6110*, *-1081*, *-1547* and the IS-like element, had been identified so far. IS*6110* is found in the members of the *M. tuberculosis* complex in numbers ranging from zero to more than twenty copies randomly distributed in the chromosome and is widely used as a genetic marker to differentiate clinical *M. tuberculosis* strains for epidemiological investigations [41]. IS*1081* is found in five to seven copies per genome and is associated with limited DNA polymorphism, whereas IS*1547* and IS-like element are present in one or two copies per genome [5, 7, 10, 26, 43].

In the genome of *M. tuberculosis* complex strains there are five types of short repetitive DNA sequences. Three of these, a repeat of a multimer $(GTG)_5$, the major polymorphic tandem repeat (MPTR) and the polymorphic GC-rich tandem repeat sequence (PGRS), are found in multiple chromosomal loci [19, 36, 45]. It is worth noticing that the multiple repeats of MPTRs and PGRSs are often a part of the so-called Pro-Glu and Pro-Pro-Glu multigene families, respectively, and are therefore postulated to be a probable result of antigenic variation [5]. The fourth type of short repetitive DNA, exact tandem repeat (ETR), has been identified in six loci in the bacterial genome. In contrast to the polymorphic MPTR and PGRS, each ETR loci contain large DNA repeats, ranging in size from 53 to 79 base pairs, with identical sequences in adjacent repeats [14]. Finally, in *M. tuberculosis* complex strains the direct repeat region has been identified [18]. It is composed of multiple direct variant repeats (DVRs), each of which is composed of a 36-bp direct repeat (DR) and a nonrepetitive, unique spacer sequence of similar size (35 to 41 bp).

The methods used for visualising the DNA polymorphism in *M. tuberculosis* complex strains are: restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), DNA hybridisation and combinations of these methods.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

Restriction fragment length polymorphism (RFLP) analysis has become a very important tool in the diagnosis of tuberculosis. Various DNA probes such as IS*6110*, IS*1081*, the polymorphic GC-rich sequence and the direct repeat have proven to be useful for this purpose. In 1992, an internationally accepted consensus for the stand-

ardised use of IS*6110* DNA fingerprinting was established, with *Pvu*II as the resctriction enzyme. This standardised technique proceeds as follows: genomic DNA is digested with restriction endonuclease *Pvu*II, electrophoresed through an agarose gel and vacuum blotted onto a nylon membrane. After blotting, the membrane is hybridised with a digoxigenin-labelled IS*6110* probe. The IS*6110* probe was synthesised in digoxigenin-labelled form by gene amplification with primers INS1 and INS2 [42]. A limitation of IS*6110*-RFLP typing is that it requires culture of bacteria prior to DNA extraction and the results can be obtained only weeks or even months after the initial presentation of the patient at the clinic. In addition, some strains of *M. tuberculosis* contain only one or two copies of the IS*6110* insertion element; therefore fingerprinting provides poor discrimination among such strains.

PCR-BASED ASSAYS

In comparison with the earlier phage-typing method [39], IS*6110* RFLP is a rapid method that gives the results in several weeks. However, compared with other molecular methods, like PCR-based assays, the IS*6110* RFLP is slow and requires more bacterial material for an analysis. PCR methods produce DNA fingerprint results quickly (i.e. within 1–2 days), they do not rely on growing cultures or viable cells and in some of the procedures there is no need to purify DNA. PCR is a good test for detecting *M. tuberculosis* DNA even when small numbers of bacteria are present in the clinical specimens. Several large studies have found that a sensitivity of PCR-based approach used for rapid diagnosing of tuberculosis infections is equivalent to or somewhat less than that of mycobacterial culture [2, 4, 9, 12, 27, 37].

Most of the PCR-based techniques have been developed with the purpose of amplifying polymorphic DNA regions flanking IS*6110* with oligonucleotide primers complementary to the end of IS*6110* (the method is known as IS*6110*-PCR) [35]. In a modified IS*6110*-PCR technique, called IS*6110*-inverse-PCR (IPCR), the primers oriented in the reverse direction of the usual orientation are used. The template for the reverse primers is a restriction fragment that has been ligated upon itself to form a circle [29, 30, 32]. Other approaches combine the variability of IS*6110* with the conserved sites of MPTR (this PCR-based method is called IS*6110*-ampliprinting) or PGRS (this method is called double-repetitive-element PCR — DRE-PCR) for differentiating *M. tuberculosis* strains [13, 33]. Another technique based on IS*6110* polymorphism is known as mixed-linker PCR (ML-PCR). The method uses one primer specific for IS*6110* and a second primer complementary to a linker ligated to the restricted genomic DNA [1, 17]. For most of these methods sequential DNA manipulations such as digestion, ligation and then amplification are necessary, which complicate their use.

A different approach employed to detect polymorphisms for PCR-based analysis of *M. tuberculosis* isolates is characterised by the use of nonspecific oligonucleotide primers. The methods based on random amplified polymorphism DNA analysis, know as arbitrarily primed PCR (APPCR), show, however, that standardisation, reproducibility and comparison between patterns are difficult [24, 31].

For strain differentiation and evolutionary studies in bacteria belonging to the *M. tuberculosis* complex typing of loci containing variable number of tandem repeats (VNTR loci) is useful. Tandem repeat loci (i.e. MPTR and ETR loci), found in the *M. tuberculosis* genome, can be analysed by PCR using specific primers complementary to the flanking regions [14].

Finally, a method based on the nature of the DNA polymorphism in the spacer regions between the DR has been developed. This procedure is referred to as spacer oligotyping or "spoligotyping" because it involves PCR amplification of the DR region from each strain, followed by hybridisation to a membrane with covalently associated oligonucleotides corresponding to various spacer sequences [20]. There is no need to purify extracted DNA before performing this assay and ten femtograms of chromosomal *M. tuberculosis* DNA, an amount corresponding to about two mycobacterial genomes, has been reproducibly detectable. It is noteworthy that this method allows the detection of *M. tuberculosis* and strain differentiation at the same time, whereas some of the methods described above (especially DRE-PCR and APPCR) are not suitable for routine simultaneous detection and typing of *M. tuberculosis* in clinical specimens. That is because of polymorphism in the target for PCR primers or because of too much variation in the size of the DNA target to be flanked by the primers used in these procedures.

The variation of spacers within the conserved DRs has been used earlier by Groenen et al. to differentiate *M. tuberculosis* strains by PCR-based method called direct variable repeat PCR (DVR-PCR) [16]. This procedure, however, is not appropriate for routine use in a clinical laboratory because the method is technically demanded.

In comparison with spoligotyping, IS*6110* RFLP analysis demonstrates a greatly higher level of discrimination. Spoligotyping, on the other hand, is particularly useful for analysis of isolates with low IS*6110* copy numbers, can be applied directly to clinical samples by PCR and provides an important tool for rapid detection of *M. tuberculosis* isolates.

At present spoligotyping is recommended as a method of choice for routine PCR-based simultaneous detection and strain differentiation of the *M. tuberculosis* complex having a particular application as a first-line typing technique because it can be performed with extracts of sputum or bronchoalveolar lavage samples [15, 30].

There is also another, worth mentioning, PCR-based technique, which is useful in solving the problem that not all clinical samples, which are culture positive are also positive by PCR and that only a minority of culture-negative samples from patients eventually shown to have tuberculosis are positive by this approach. There are two main obstacles limiting the sensitivity of this approach in diagnosis of tuberculosis, especially of paucibacillary forms of the disease. First, high levels of DNA in the sample can inhibit PCR, and many clinical specimens contain large numbers of immune and inflammatory cells, a source of large amounts of DNA. Thus, it is necessary to dilute these samples (and also the mycobacterial DNA) prior to amplification. Second, to obtain optimal sensitivity, it is necessary to eliminate inhibitors of PCR present in clinical samples. The problem is that the multistep processes required to obtain

highly purified DNA are difficult to apply in routine practice. To overcome these problems, Mangiapan et al. have developed a technique known as sequence capture-PCR. This method permits the specific capture of mycobacterial DNA in crude samples containing large numbers of human cells prior to amplification, thereby concentrating the target sequences and removing irrelevant DNA and other potential inhibitors. The biotinylated oligonucleotides are hybridised to mycobacterial DNA in solution and subsequently bound to avidin-coated beads (two-step capture). Afterwards, the amplification of two different sequences specific for organisms of the *M. tuberculosis* complex (IS*6110* and DR region) is performed. This approach allows for detecting as little as one genome of mycobacterial DNA in samples containing up to 750 μg of total DNA, which gave a 10- to 100-fold increase in sensitivity compared with that obtained by purifying total DNA prior to amplification [25].

For the detection and analysis of PCR products agarose gel electrophoresis in the presence of ethidium bromide and Southern blot hybridisation with an internal probe (mainly a digoxigenin labelled one) are usually used [21, 28]. However, hybridisation procedures on membranes are time-consuming and the readings are subjective. Therefore the quicker and simpler to perform methods, used for the detection of amplicons from the *M. tuberculosis* complex, were developed [3, 22]. These techniques are known as microwell hybridisation assays.

REFERENCES

1. *Butler W.R., Haas W.H., Crawford J.T.*: Automated DNA fingerprinting analysis of *Mycobacterium tuberculosis* using fluorescent detection of PCR products. J. Clin. Microbiol., 1996; 34: 1801-1803.
2. *Chin D.P., Yajko D.M., Hadley W.K., Sanders C.A., Nassos P.S. et al.*: Clinical utility of a commercial test based on the polymerase chain reaction for detecting *Mycobacterium tuberculosis* in respiratory specimens. Am. J. Respir. Crit. Care Med., 1995; 151: 1872-1877.
3. *Cho S.N., van der Vliet G.M.E., Park S., Baik S.H., Kim S.K. et al.*: Colorimetric microwell plate hybridisation assay for detection of amplified *Mycobacterium tuberculosis* DNA from sputum samples. . J. Clin. Microbiol., 1995; 33: 752-754.
4. *Clarridge J.E. III, Shawar R.M., Shinnick T.M., Plikaytis B.B.*: Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol., 1993; 31: 2049-2056.
5. *Cole S.T., Brosch R., Parkhill J., Garnier T., Churcher C. et al.*: Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature, 1998; 393: 537-544.
6. *Collins D.M., de Lisle G.W.*: DNA restriction endonuclease analysis of *Mycobacterium tuberculosis* and Mycobacterium bovis BCG. J. Gen. Microbiol., 1984; 130: 1019-1021.
7. *Collins D.M., Stephens D.M.*: Identification of insertion sequence, IS*1081*, in Mycobacterium bovis. FEMS Lett., 1991; 83: 11-16.
8. *Eisenach K.D., Crawford J.T., Bates J.H.*: Genetic relatedness among strains of the *Mycobacterium tuberculosis* complex: analysis of restriction fragment heterogeneity using cloned DNA probes. Am. Rev. Respir. Dis., 1986; 133: 1065-1068.
9. *Eisenach K.D., Sifford M.D., Cave M.D., Bates J.H., Crawford J.T.*: Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis., 1991; 144: 1160-1163.
10. *Fang Z., Morrison N., Batt B., Doig C., Forbes K.J.*: IS*6110* transposition and evolutionary scenario of the direct repeat locus in a group of closely related *Mycobacterium tuberculosis* strains. J. Bacteriol., 1998; 180: 2102-2109.

11. *Feizabadi M.M., Robertson I.D., Cousins D.V., Hampson D.J.*: Genomic analysis of *Mycobacterium bovis* and other members of the *Mycobacterium tuberculosis* complex by isoenzyme analysis and pulsed-field gel electrophoresis. J. Clin. Microbiol., 1996; 34: 1136-1142.
12. *Forbes B.A., Hicks K.E.S.*: Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. J. Clin. Microbiol., 1993; 31: 1688-1694.
13. *Friedman C.R., Stoeckle M.Y., Johnson W.D. Jr, Riley L.W.*: Double-repetitive-element PCR method for subtyping *Mycobacterium tuberculosis* clinical isolates. J. Clin. Microbiol., 1995, 33, 1383-1384.
14. *Frothingham R., Meeker-O'Connell W.A.*: Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable number of tandem DNA repeats. Microbiology, 1998; 144: 1189-1196.
15. *Goyal M., Saunders N.A., van Embden J.D.A., Young D.B., Shaw R.J.*: Differentiation of *Mycobacterium tuberculosis* isolates by spoligotyping and IS6110 restriction fragment length polymorphism. J. Clin. Microbiol., 1997; 35: 647-651.
16. *Groenen P.M., Bunschoten A.E., van Soolingen D., van Embden J.D.*: Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*; application for strain differentiation by a novel typing method. Mol. Microbiol., 1993; 10: 1057-1065.
17. *Haas W.H., Butler W.R., Woodley C.L., Crawford J.T.*: Mixed-linker polymerase chain reaction: a new method for rapid fingerprinting of isolates of the *Mycobacterium tuberculosis* complex. J. Clin. Microbiol., 1993; 31: 1293-1298.
18. *Hermans P.W.M., van Soolingen D., Bik E.M., de Haas P.E.W., Dale J.W. et al.*: The insertion element IS987 from *Mycobacterium bovis* BCG is located in a hot spot integration region for insertion elements in *Mycobacterium tuberculosis* complex strains. Infect. Immun., 1991; 59: 2695-2705.
19. *Hermans P.W.M., van Soolingen D., van Embden J.D.A.*: Characterisation of a major polymorphic tandem repeat in *Mycobacterium tuberculosis* and its potential use in the epidemiology of *Mycobacterium kansasii* and *Mycobacterium gordonae*. J. Bacteriol., 1992; 174: 4157-4165.
20. *Kamerbeek J., Schouls L., Kolk A., van Agterveld M., van Soolingen D. et al.*: Simultaneous detection and strain diferentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol., 1997; 35: 907-914.
21. *Kolk A.H., Schuitema, A.R.J, Kuijper S., van Leeuwen J., Hermans P.W. et al.*: Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. J. Clin. Microbiol., 1992; 30: 2567-2575.
22. *Kox L.F.F., Noordhoek G.T., Kunakorn M., Mulder S., Sterrenburg M. et al.*: Microwell hybridisation assay for detection of PCR products from *Mycobacterium tuberculosis* complex and the recombinant *Mycobacterium smegmatis* strain 1008 used as an integral control. J. Clin. Microbiol., 1996; 34: 2117-2120.
23. *Kremer K., van Soolingen D., Frothingham R., Haas W.H., Hermans P.W.M. et al.*: Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. J. Clin. Microbiol., 1999; 37: 2607-2618.
24. *Linton C.J., Jalal H., Leeming J.P., Millar M.R.*: Rapid discrimination of *Mycobacterium tuberculosis* strains by random amplified polymorphic DNA analysis. J. Clin. Microbiol., 1994; 32: 2169-2174.
25. *Mangiapan G., Vokurka M., Schouls L., Cadranel J., Lecossier D. et al.*: Sequence capture-PCR improves detection of mycobacterial DNA in clinical specimens. J. Clin. Microbiol., 1996; 34: 1209-1215.
26. *Mariani F., Piccolella E., Colizzi V., Rappuoli R., Gross R.*: Characterisation of an IS-like element from *Mycobacterium tuberculosis*. J. Gen. Microbiol., 1993; 139: 1767-1772.
27. *Nolte F.S., Metchock B., McGowan J.E. Jr, Edwards A., Okwamabua O. et al.*: Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridisation. J. Clin. Microbiol., 1993; 31: 1777-1782.

28. *Noordhoek G.T., Kaan J.A., Mulder S., Wilke H., Kolk A.H.*: Routine application of the polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Pathol., 1995; 48: 810-814.
29. *Ochman H., Gerber A.S., Hartl D.L.*: Genetic applications of an inverse polymerase chain reaction. Genetics, 1988; 120: 621-623.
30. *Otal I., Samper S., Asensio M.P., Victoria M.A., Rubio M.C. et al.*: Use of a PCR method based on IS6110 polymorphism for typing *Mycobacterium tuberculosis* strains from BACTEC cultures. J. Clin. Microbiol., 1997; 35: 273-277.
31. *Palittapongarnpim P., Chomyc S., Fanning A., Kunimoto D.*: DNA fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates by arbitrarily primed polymerase chain reaction. J. Infect. Dis., 1993; 167: 975-978.
32. *Patel S., Wall S., Saunders N.A.*: Heminested inverse PCR for IS*6110* fingerprinting of *Mycobacterium tuberculosis* strains. J. Clin. Microbiol., 1996; 34: 1686-1690.
33. *Plikaytis B.B., Crawford J.T., Woodley C.L., Butler, W.R., Eisenach K.D. et al.*: Rapid, amplification-based fingerprinting of *Mycobacterium tuberculosis*. J. Gen. Microbiol., 1993; 139: 1537-1542.
34. *Pożarowski P., Roliński J.*: Rola odpowiedzi immunologicznej w infekcjach układu oddechowego wywołanych bakteriami żyjącymi wewnątrz komórek. W.: Emeryk A., *Mycoplasma pneumoniae* i *Chlamydia pneumoniae* w zakażeniach dróg oddechowych i chorobach obturacyjnych płuc. Medycyna praktyczna 2001, 22-27.
35. *Ross B., Dwyer B.*: Rapid, simple method for typing isolates of *Mycobacterium tuberculosis* by using the polymerase chain reaction. J. Clin. Microbiol., 1993; 31: 329-334.
36. *Ross B.C., Raios K., Jackson K., Dwyer B.*: Molecular cloning of a highly repeated DNA element from *Mycobacterium tuberculosis* and its use as an epidemiological tool. J. Clin. Microbiol., 1992; 30: 942-946.
37. *Schluger N.W., Rom W.N.*: The polymerase chain reaction in the diagnosis and evaluation of pulmonary infections. Am. J. Respir. Crit. Care Med., 1995; 152: 11-16.
38. *Schürmann D., Nightingale S.D., Bergmann F., Ruf B.*: Tuberculosis and HIV infection: a review. Infection, 1997; 25: 274-280.
39. *Snider D.E. Jr, Jones W.D. Jr, Good R.C.*: The usefulness of phage typing *Mycobacterium tuberculosis* isolates. Am. Rev. Respir. Dis., 1984; 130: 1095-1099.
40. *Szczuka I.*: Gruźlica i choroby układu oddechowego w Polsce w 2000 roku. Instytut Gruźlicy i Chorób Płuc, Warszawa 2001, 1-10.
41. *Thierry D., Brisson-Noël A., Vincent-Lévy-Frébault V., Nguyen S., Guesdon J. et al.*: Characterisation of a *Mycobacterium tuberculosis* insertion sequence, IS*6110*, and its application in diagnosis. J. Clin. Microbiol., 1990; 28: 2668-2673.
42. *van Embden J.D., Cave M.D., Crawford J.T., Dale J.W., Eisenach K.D. et al.*: Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol., 1993; 31: 406-409.
43. *van Soolingen D., Hermans P.W.M., de Haas P.E.W., van Embden J.D.A.*: Insertion element IS*1081*-associated restriction fragment length polymorphism in *Mycobacterium tuberculosis* complex species: a reliable tool to recognize Mycobacterium bovis BCG. J. Clin. Microbiol., 1992; 30: 1772-1777.
44. *van Soolingen D., Hoogenboezem T., de Hass P.E.W., Hermans P.W.M., Koedam M.A. et al.*: A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, *canetii*: characterisation of an exceptional isolate from Africa. Int. J. Syst. Bacteriol., 1997; 47: 1236-1245.
45. *Wiid I.J.F., Werely C., Beyers N., Donald P., van Helden P.D.*: Oligonucleotide $(GTG)_5$ as a marker for *Mycobacterium tuberculosis* strain identification. J. Clin. Microbiol., 1994; 32: 1318-1321.

STRESZCZENIE

Mycobacterium tuberculosis jest wewnątrzkomórkową bakterią bytującą w fagocytach. Charakteryzuje się małą zjadliwością i powolnym procesem rozmnażania, a jej patogenność uzależniona jest

od aktywności układu immunologicznego. Wymienione cechy tego drobnoustroju wymagają specjalistycznych metod stosowanych obecnie w diagnostyce gruźlicy. Wśród nich należy wymienić dwie podstawowe techniki genetyczne: polimorfizm długości fragmentów restrykcyjnych (ang. *restriction fragment length polymorphism* — RFLP) i łańcuchowa reakcja polimerazy (ang. *polymerase chain reaction* — PCR). RFLP jest czułą metodą przydatną nie tylko do wykrywania, ale i do różnicowania szczepów bakterii należących do *Mycobacterium tuberculosis* complex. Technika PCR pozwala wprawdzie na słabsze zróżnicowanie poszczególnych szczepów, ale ze względu na szybkość wykonania i czułość jest bardziej użyteczna we wczesnej diagnostyce gruźlicy. Za najbardziej wartościowe odmiany PCR należy uznać następujące metody: amplifikacja i hybrydyzacja regionu bezpośredniego powtórzenia (ang. *direct repeat* — DR) i analiza loci o zmiennej liczbie tandemowych powtórzeń (ang. *variable number of tandem repeats* — VNTR).