New rapid method for identification of non-tuberculosis mycobacteria

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Background

As the incidence of tuberculosis decreases regularly, the proportion of non-tuberculosis mycobacteria (NTM) infections increases. Microbiological diagnostic of NTM infection is a major challenge for at least two reasons.

First, many mycobacterial species and sub-species present important genetic similarities, limiting the utility of commercial multiplex PCR methods or home-made methods based on 16S RNA sequencing.

Second, culture of several epidemiologically relevant species such as Mycobacterium leprae, M. ulcerans or M. genavense is not feasible in conventional laboratory conditions and therefore identification techniques based on cultured specimen, such as MALDI-TOF MS, is not feasible.

Previous studies demonstrated the potential of rpoB gene sequencing for identification of Mycobacteria, but did not show sufficient clinical sensitivity and specificity to be introduced in routine.

Methods

The primers were designed as follow: two primers have been taken from the litterature, and four original primers were designed aligning 20 clinically relevant mycobacterial species. We further verified the specificity of primers using other genetically close bacterial species such as Propionibacterium.

DNA extraction from clinical specimen is performed using the commercial Fluorolyse kit (Hain Lifescience GmbH, Germany), while extraction from positive cultures can be done using conventional techniques.

A first step PCR touch-down amplification of the rpoB gene is done using the KOD Hot start master mix (Merck Millipore, Germany) with primers My9 and MycoR. These primers amplify a 800-bp region, presenting significant variations between mycobacterial species. Amplicons are then purified with the QIAquick PCR purification kit (QIAGEN, France) and diluted to 10ng/uL.

The sanger sequencing is then performed on ABI 3130 system (Life Technologies) using BigDye Terminator v3.1 Cycle Sequencing and BigDye XTerminator Purification Kit (Applied Biosystems, ref. 4376486) with primers My10, My11 and MycoF (Table 1).

Primers	Forward or Reverse	Sequence (5'- 3')	Location	Source
My 9	F	ATCGGCGCCGAGGTCCGCGAC	2356-2376	(Andre et al., 2015)
My 10	F	AAGGTGCCSCACGGTGAGTC	2494-2517	(Andre et al., 2015)
My 11	R	ATGTAGCCGACCGTSACCGGGT	3044-3065	(Andre et al., 2015)
MycoF	F	GGCAAGGTCACCCCGAAGGG	2392-2411	(Adekambi et al., 2003)
MycoR	R	AGCGGCTGCTGGGTGATCATC	3135-3152	(Adekambi et al., 2003)

Table 1 : Primers used for the amplification and sequencing of the rpoB gene, allowing identification of NTM species

Results

This technique allowed correct identification of NTM species from both clinical and culture specimen. Turn-around-time of this technique is 3 days compared to several weeks in some particular situations.

Conclusions

We propose a sequencing protocol allowing rapid identification mycobacteria that can be introduced in reference laboratories.

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