J Biochem Tech (2010) 2(5):S120-S121
ISSN: 0974-2328

Applications of MS-MALDI–TOF for quick identification of microorganisms

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Received: 25 October 2010 / Received in revised form: 13 August 2011, Accepted: 25 August 2011, Published: 25 October 2011

Abstract

From the beginning of microbiology scientists have discussed the issue of how to easily, quickly but also accurately identify unknown microorganisms. Today there is a number of precise but time–consuming and costly molecular techniques available. The MS MALDI–TOF–based method offers a suitable alternative for currently used methods. This method allows comparing different isolates according to the characteristic profile of ribosomal proteins and selecting those which are identical, or it enables accurate direct identification of the samples using a commercial database. Measurements that we did showed that the identification accuracy by MALDI–TOF mass spectrometry is comparable to the methods of 16S rRNA gene analysis, which is used as a standard method for identification of microorganisms. The disadvantage of using commercial databases for identification is a small scale of such database. It is possible to say that method of MALDI–TOF mass spectrometry offers a fast and suitable alternative for the identification of microorganisms.

Keywords: MALDI-TOF MS, bacteria, 16S rRNA gene

Introduction

Identification of unknown microorganisms is one of the main goals, which microbiology has dealt with from its inception. There are many methods that are time consuming and technically demanding. The method of using whole cells with MALDI–TOF MS is a suitable alternative to currently used methods because it is fast and simple (Strupat et al. 1991).

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Materials and methods

For the identification of microorganisms, we used the “whole cell” methodology. This method involves the growth of microorganisms on agar Luria-Bertani medium (Difco, USA) from which one colony is isolated. It is then spread on the target plate and covered with layer of 3,5-dimethoxy-4-hydroxycinnamic acid in mixture of acetonitril and 5% trifluoroacetic acid 70:30 (v/v). Crystallized samples are analyzed with mass spectrometer Biflex IV (Bruker Daltonics Inc., Billerica, USA) in linear positive ion mode with constant potential of 19 kV and pressure lower than 9.10^{-5} Pa. Each spectrum is the sum of the ions from 200 laser shots coming from different regions of the same well. The spectrum is analyzed in the range from m/z 2000-20000. The spectra are evaluated visually as well as using program BioTyper (Bruker Daltonics Inc., Billerica, USA) by creating correlation composition index.

Results and Discussion

In the measurement, we compared accuracy of the methods for the identification of microorganisms.

Table 1: Identification of microorganisms

MALDI-TOF	**16SrRNA**
Rhodoccocus opacus	*Rhodoccocus opacus*
Rhodoccocus opacus	*Rhodococcus sp.*
Rhodococcus sp.	*Unknown*
Rhodococcus sp.	*Rhodococcus sp.*
Rhodococcus sp.	*Rhodoccocus opacus*
Rhodococcus sp.	*Rhodoccocus opacus*
Rhodoccocus opacus	*Rhodoccocus opacus*
Achromobacter xylosooxidans	*Achromobacter sp.*
Rhodoccocus opacus	*Rhodoccocus opacus*
Rhodoccocus opacus	*Rhodoccocus opacus*
Rhodococcus sp.	*Rhodococcus sp.*
Rhodococcus sp.	*Rhodoccocus opacus*
Rhodococcus sp.	*Unknown*
Rhodococcus sp.	*Rhodococcus sp.*
Rhodococcus sp.	*Unknown*

We compared MALDI–TOF MS and analysis of 16S rRNA genes. The results are shown in the Table 1. They show that MALDI–TOF methodology is satisfactory for the identification on the species level. In cases, where identification of the isolates by analysis of 16S rRNA genes failed due to low concentration of DNA in the sample, identification by MS MALDI–TOF was successful.

Conclusion

MALDI–TOF MS is suitable to identify unknown microorganisms and the current results also show that the identification accuracy is comparable to the classically used method for analysis of 16S rRNA genes. The disadvantage of this method is the high cost of equipment and database.

Acknowledgement

The work has been supported by the MSM 6046137305, MSMT No.21/2011, MSMT 2B06156

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