Detection of *Bacillus subtilis* spores in air samples

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1 Introduction

Detection of microorganisms in the air is a challenging procedure. The main problems in the measurements of bio-aerosols are usually the very low amount of organisms which can be collected from the air sample and also the presence of spores. Various biosensors have been developed for the detection of harmful air spores, such as Bacillus anthracis, a potential biological threat agent (Edwards et al., 2006). Being causative agent for anthrax B. anthracis is difficult to work with, and therefore the close related species, Bacillus subtilis (Stachowiak et al., 2007) and B. cereus (Arakawa et al., 2003), are often used in the development of detection strategies.

The primary strategies for detection of Bacillus spores include polymerase chain reaction based techniques, immunoassays, spectrometry, chromotography and protein profiling. Recently some autonomous pathogen detection systems for aerosol collection, sample preparation and detection were developed. The methods for direct analysis are based on DNA or protein detection and cannot distinguish between viable and dead spores. Furthermore, most of them have low sensitivity or require an amplification step like PCR. With RNA based detection methods it is possible to specifically analyze only viable spores. Sandwich hybridization assay (SHA) is one of methods suitable for a rapid and quantitative detection of RNA (Rautio et al., 2003) based on the hybridization of the target RNA with two specific oligonucleotide probes. The capture probe is used to immobilize the target on magnetic beads; the detection probe is labelled with a marker molecule which generates a fluorescent signal proportional to the amount of target molecule. By comparing the signals obtained with a target molecule and artificial nucleic acid with a known concentration, exact amount of the target molecule can be determined. Oligonucleotide probes required for this assay can be designed for almost any RNA and can easily be modified for another target. It means that the developed detection system can be applied for different organisms with just some small adaptations. Sandwich hybridization is relatively sensitive (10-16-10-15 moles of a specific molecule) and can be performed with crude biological samples without any RNA purification. The possibility to use various markers makes the method applicable for different read-out systems, such as fluorescence meter (Rautio et al., 2003) or electrical biochip reader (Gabig-Ciminska et al., 2004).

2 Objectives of the research

Here we describe an RNA based diagnostic method for the detection of bacterial spores e.g. in air samples. The procedure was optimized for Bacillus subtilis spores but is applicable to other organisms.

3 Results

3.1 Results 1

The present method was developed for detection of B. subtilis spores using enrichment cultivation and RNA based sandwich hybridization (SHA) (Rautio et al., 2000). The method includes the following steps: the sampling (filtration) of spores from the air, the activation of spores, the germination of spores and enrichment cultivation (if needed), cell disruption and analysis of RNA with SHA. After the activation

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spores rapidly start to synthesize RNA, and at this moment the most abundant RNAs (rRNAs), are used as specific markers in the detection system.

SHA probes for the detection of B. subtilis 16S rRNA were designed on basis of general eubacterial probes with some modifications of sequences specific for B. subtilis. The probes were tested using in vitro transcribed fragment of B. subtilis 16S rRNA as the target molecule. The detection limit of the method was estimated as 108 target molecules per assay, and the linear range was between 108 and 3 x 1010 molecules.

3.2 Results 2

The sensitivity of the method composed of activation, enrichment cultivation and 16S rRNA analysis of B. subtilis spores was studied. Germination medium was inoculated with 101 – 105 activated B. subtilis spores / ml. The germination and growth of the cells were monitored by measurement of the optical density OD600 (Figure 1A). The cells were collected, disrupted and analyzed with sandwich hybridization assay using the probes for 16S rRNA (Figure 1B). The detectable signals were observed after 110, 140, 245, 340 and 370 minutes of cultivation for the samples with initial number of spores 105, 104, 103, 102 and 101 respectively.

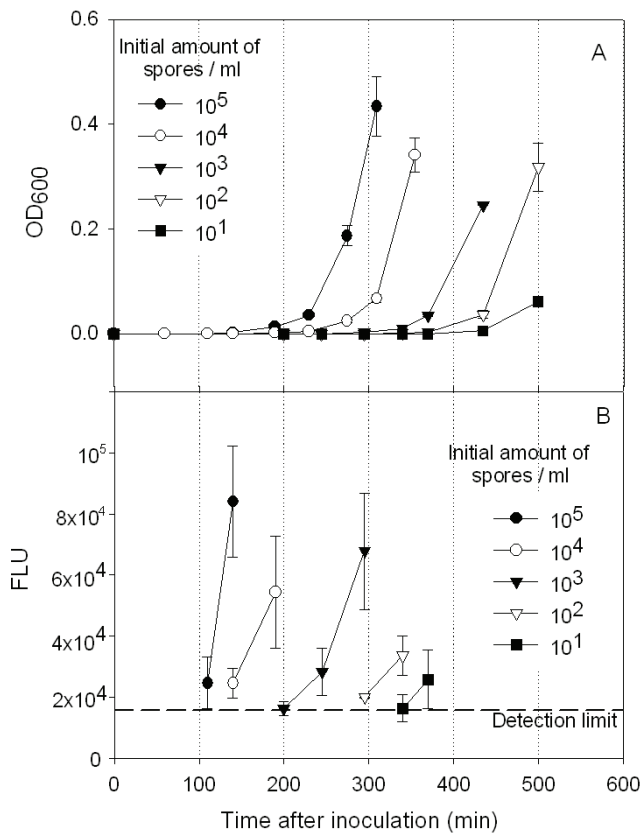

Figure 1 *(A) Growth curves of B. subtilis cells after the spore activation. The initial number of the spores was 101-105 spores / ml of germination medium. (B) Detection of B. subtilis 16S rRNA with the sandwich hybridization assay after the enrichment cultivation. Error bars show ±SD of three independent experiments.*

4 Relevance of the research

The method for the detection of spores developed here includes activation of spores, their germination, enrichment cultivation, and RNA detection using a sandwich hybridization assay. The method was developed for B. subtilis, a model organism capable of spore formation and quite abundant in aerosols. The new method can easily be modified for other target RNAs and is applicable for the specific detection of spores from known hierarchic groups of organisms.

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