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Isolation and identification of bacteria by means of Raman spectroscopy☆

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ABSTRACT

Bacterial detection is a highly topical research area, because various fields of application will benefit from the progress being made. Consequently, new and innovative strategies which enable the investigation of complex samples, like body fluids or food stuff, and improvements regarding the limit of detection are of general interest. Within this review the prospects of Raman spectroscopy as a reliable tool for identifying bacteria in complex samples are discussed.

The main emphasis of this work is on important aspects of applying Raman spectroscopy for the detection of bacteria like sample preparation and the identification process. Several approaches for a Raman compatible isolation of bacterial cells have been developed and applied to different matrices. Here, an overview of the limitations and possibilities of these methods is provided. Furthermore, the utilization of Raman spectroscopy for diagnostic purposes, food safety and environmental issues is discussed under a critical view.

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1. Introduction

Bacterial detection is an issue-area which is of great importance for various aspects of modern life. The industrial production of food or pharmaceutical substances requires test procedures which will prevent spoiled or contaminated products from being placed on the market. The supply system for drinking water can be subject to bacterial contamination, too, and constant controls are necessary in order to ensure that the consumer won't be exposed to any health risk. More examples of high significance can be found in the medical field. Of course, a timely identification of the source of an infection can be lifesaving for patients. But also strategies for improving hospital hygiene in general have received considerable attention lately. In addition, bacterial detection methods are of peculiar interest for the environment and agriculture, because the spread of plant pathogens for instance can not only endanger whole ecosystems, but can cause serious economic damage as well.

These examples highlight the meaningfulness of strategies which allow identifying bacteria in different environments. According to the relevance of this research area, several approaches have been developed. The current gold standard is to culture the bacteria and identify them based on their morphological and metabolically characteristics. Even though this technique is well established and reliable, it comes along with two disadvantages which are quite severe. Firstly, depending on the bacterial species culturing can take several days. Secondly, it is assumed that the vast majority of bacteria are not culturable at all and therefore not accessible with this approach. Within this context it should be noted, that in some cases only cells which are in a viable but not culturable (VBNC) state might be available. Nucleic acid based detection methods for bacteria, which usually rely on the amplification of DNA or RNA via polymerase chain reaction (PCR), provide a high sensitivity and specificity and are also very rapid. Depending on the primer design and the follow up method used for analyzing the amplified sequence, the identification of the pathogens can be achieved on genus, species or strain level. Even though PCR is a precise, efficient and rapid method, some difficulties can arise when real-world-samples are investigated. If the sample matrix contains substances inhibiting the PCR reaction, more or less complicated procedures will be necessary for extracting the target DNA. The method is also sensitive to contamination and experimental conditions.

Despite the fact that culturing and PCR are well established and widely recognized techniques for pathogen detection, further research and development of more sophisticated approaches are desirable. As previously mentioned numerous fields of applications will benefit from advances made in this challenging territory. Among other alternatives to PCR and culturing, Raman spectroscopy can be a valuable and attractive tool in pathogen diagnostics [1]. The possibility to acquire suitable spectra of single cells within seconds and the high specificity of the Raman signal are the most important characteristics of this approach and imply an enormous potential for various applications. Most importantly, Raman spectroscopy enables the culture independent detection of bacteria, since single cell measurements can be conducted. Being able to omit the culturing step completely, huge amounts of time can be saved, which is quite an important factor in many fields of application. Of course, it is also possible to apply Raman spectroscopy to cultured bacteria, if desired. The measurements can be either performed on single cells or in bulk phase. A further advantage of Raman spectroscopy is that the sample generally is not destroyed and remains available for additional investigations. This potential has been recognized quite a while ago; accordingly the detection of microbes by means of Raman spectroscopy is a well discussed topic. But, although it was often shown that vibrational spectroscopy can provide results rapidly and accurately, especially in the section of bacterial detection, it is still a method in the early stage of development [2].

The requirements for establishing a new detection system are well known. Important parameters like the time, required until the result is available, sensitivity, specificity, size, costs and handling have to be optimized and defined. Those specifications are particularly important with regard to the proposed application of the system. It is often desirable to develop a device, which can be used directly on-site. It should be kept in mind that for virtually any application not only the Raman measurements have to be conducted, but also sample preparation and the analysis of the spectra are vital parts of the process chain. In Fig. 1 a typical work flow, which involves multiple steps from sampling to the final identification, is depicted.

Raman spectroscopy exploits the effect that laser light is scattered inelastically to a small extent, when interacting with a sample [3–7]. During this process energy is transferred between the incident photons and the sample molecules. The amounts of energy correspond to specific molecule vibrations. The fact that the Raman spectrum displays the molecular composition of the investigated sample with unique specificity makes this spectroscopic technique highly attractive for various analytes. By combining a Raman setup with a microscope even very small sample volumes within the range of a few μm^3, including single cells, can be investigated separately. Additionally, Raman measurements can be performed unimpededly on microbial cells, since water is a very weak Raman scatterer. Usually, visible wavelengths are used to probe microorganisms. Eventually fluorescence can hamper the measurements and dominate the spectra, since the laser light used for the Raman measurements can also excite fluorescence emission in the samples. The rather weak, but very sharp Raman bands can be masked by a broad and intense fluorescence background, appearing in the same spectral region. The choice of an appropriate excitation wavelength is one option to overcome this problem. On the one hand the wavelength can be chosen so that the excitation is far away from any absorption process, but on the other hand it is useful to select a wavelength which matches with an absorption to partially get a resonant effect. This phenomenon, known as resonance Raman spectroscopy, is additionally a method to enhance the intrinsically weak Raman process [8]. Surface enhanced Raman spectroscopy (SERS) is another well-known possibility to face this problem [9–14]. Here, metallic nanoparticles or nanostructured metal surfaces are used in order to enhance the intrinsically weak Raman signal by several orders of magnitude.

Regarding SERS based detection of bacteria, there are several possibilities, some of which are schematically depicted in Fig. 2. For example the samples can be analyzed using nanostructured arrays as shown in Fig. 2a [15–18]. Various types of metallic nanoparticles or colloids are also frequently employed for acquiring SERS spectra of bacteria (Fig. 2b) [19–29]. It is also possible to deposit the nanoparticles directly onto the bacterial cell wall as indicated in Fig. 2c [30,31]. While all the previously mentioned approaches usually aim to detect the chemical fingerprint spectrum of the bacterial cells, it is also possible to use SERS tags (see Fig. 2d, for example [32–35]), which enable a highly sensitive detection of the cells via the spectra of the Raman reporter molecules. A more detailed discussion concerning SERS based detection of bacteria and spores can be found in other reviews [36–41]. Even though using SERS for the detection of bacteria can be advantageous, it also causes some challenges. For example providing SERS substrates with reliable reproducibility is an extremely demanding task, but if