STANDOFF LASER INDUCED FLUORESCENCE OF LIVING AND INACTIVATED BACTERIA

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INTRODUCTION

Biological hazards, such as bacteria, represent a non-assessable threat in case of an accident or a terroristic attack. Rapid detection and highly sensitive identification of released, suspicious substances at low false alarm rates are challenging requirements which one single technology cannot cope with. It has been shown that standoff detection using laser-induced fluorescence (LIF) can provide information on the class of bioorganic substances in real-time[1]. In combination with traditional, highly sensitive, but non-standoff methods, the time for identification of the threat can be optimized. This work is aimed at the selectivity of LIF technology for different bacterial strains. A second important aspect examines how to deal with inactivated bacteria and how their fluorescence signature changes after deactivation. LIF spectra of closely and more distantly related bacterial strains are presented as well as spectra of bacteria treated by different inactivation methods.

EXPERIMENTAL

1.1 Cultivation and Sample preparation

Bacterial strains: *B. thuringiensis* Berliner var. kurstaki originated from an insecticide (Dipel ES, Cheminova Deutschland, Stade, Germany), *Escherichia coli* K12 J53, *Bacillus atrophaeus* (DSM 7264) and *Bacillus subtilis* (DSM 1917) were obtained from the Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ) Cultivation of the bacteria species was carried out on blood agar plates (nutrient agar 1 (Sifin, Berlin, Germany) supplemented with 7.5 % cattle or sheep blood) at 37 °C for 24 h. Colony material was harvested and suspended in phosphate buffered saline (PBS) and stored at 4 °C. The concentration of colony forming units (CFU) per ml was determined by cultivation of 100 µl serially diluted solution in PBS with 0.4 % agar on agar plates at identical conditions. Bacterial suspensions were diluted with PBS to the concentrations measured. Deactivation of

bacteria was carried out either by heating at 95 °C for 10 min, autoclaving at 134 °C for 30 min, dilution in ethanol, or gamma radiation at 30 kGy (±10%).

1.2 LIF detection setup

The equipment used in this work has been designed for outdoor, eye-safe standoff detection. Here, the setup will be described briefly and a full description can be found in Ref. [1]. The system (see Figure 1) consists of a modified Nd:YAG laser which provides alternating, 280 and 355 nm laser pulses at 10 Hz. UV light is directed onto the samples placed in a distance of 22 m. Fluorescence in backward direction is collected by a Newtonian telescope, guided by an optical fiber into an ICCD spectrometer. The complete measurement procedure is controlled by a personal computer including correction background scattering and calibration of intensity of spectra and - if required - classification of measured data by pattern recognition software.

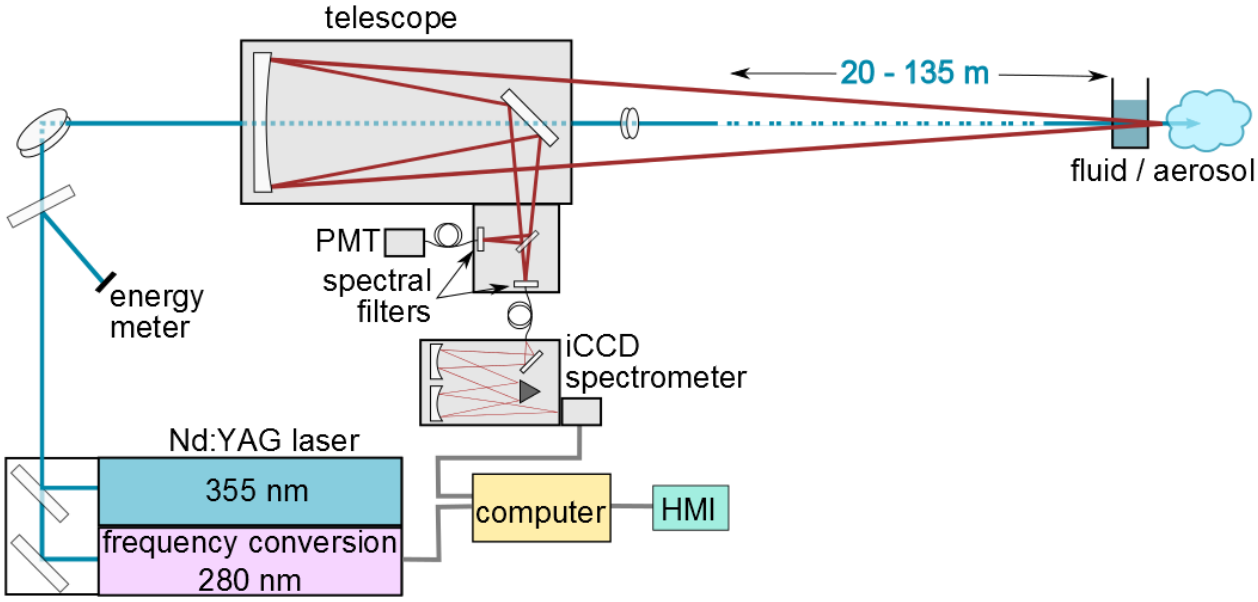

Figure 1: Experimental setup for standoff detection

RESULTS

1.1 LIF Spectra of Bacteria from Different Strains

It is well known that organisms which have a close phylogentic relationship are more similar in their morphological and chemical properties than those more far related. An attempt has been made to LIF spectral features of bacteria from closely and more distantly related bacteria. From the *Bacillus* genus, *B. thuringiensis*, *B. subtilis* and *B. atrophaeus* have been investigated. Additionally, *E. coli* as an example for a distant relative to the *Bacillus* genus has been examined. The comparison of resulting LIF spectra reveals differences in shape and overall intensity depending on the excitation wavelength. At 355 nm excitation, the LIF

spectra express similar shapes dominated by NADH, tryptophan and tyrosine fluorophores but with a different integral intensity within an order of magnitude for the same concentration (10^9 CFU). In contrast, the bacteria spectra excited at 280 nm can be distinguished more clearly. Here, *E. coli* shows a strong fluorescence signal between 300 nm and 450 nm, whereas *B. thuringiensis* shows a broader, less pronounced signal. Fluorescence of *B. subtilis* and *B. atrophaeus* show similar spectral features compared to *B. thuringiensis*, e.g. broad spectra and a strong emission in the region above 450 nm.

1.2 Deactivation

In a standard laboratory environment, only low risk bacterial species can be examined for legal and responsibility reasons. This restriction is invalid for hazardous, but inactivated bacteria.

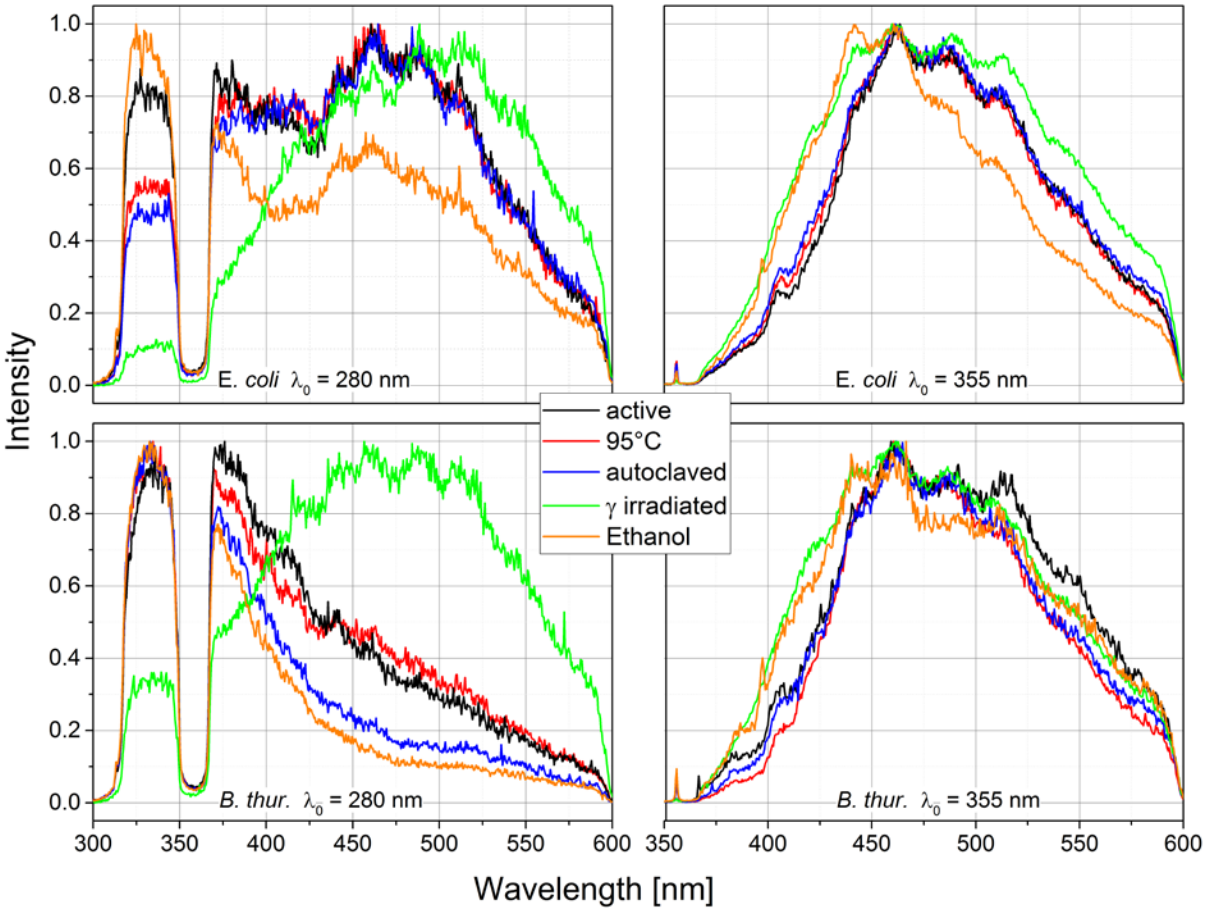

Figure 2. LIF spectra of living and deactivated *E.coli* and *B. thuringiensis* (10^9 CFU, top left: *E. coli* λ_0=280 nm, top right: *E. coli* λ_0=355 nm, bottom left: *B. thur.* λ_0=280 nm, bottom right: *B. thur.* λ_0=355 nm).

In order to see how different deactivation methods affect the fluorescing properties of bacteria, results are presented for two types, *E. coli* and *B. thuringiensis*; both of them have been deactivated by methods given in section 1.1. The resulting LIF spectra excited at 280 and 355 nm (left and right panels, respectively) are normalized to maximum intensity and their shapes can be compared from Figure 2. Upon heating to 95 °C, the 280 nm spectrum shows minor changes for *E. coli* and *B. thuringiensis*, namely a decrease below and a minor increase above 350 nm. The spectrum excited at 355 nm remains almost unchanged. Autoclaved, the spectra show similar changes with a larger increase in overall fluorescence intensity. The pronounced spectral fluorescence shape changes for both excitation wavelengths upon ethanol treatment and especially gamma irradiation and basically rule out these methods of deactivation for preparation of low risk bacterial substituents with similar fluorescent behavior. However, upon both heating to 95 °C and autoclaving, the spectral changes are far less significant for 280 nm spectra and negligible at 355 nm.

CONCLUSION AND OUTLOOK

From the above work two main statements can be extracted:

- The spectral diversity within three species of a *Bacillus* genus is demonstrated to be large enough for safe distinction of bacteria from this genus.
- Resulting LIF spectra are strongly influenced by the method of inactivation for both excitation wavelengths, indicating changes in the morphological structure and chemical composition. Thus, LIF measurements of living bacterial samples are indispensable as input and training data for a classifier development.

In order to realize a distinct classification of bacteria, the existing spectral database will be extended. This includes also investigations on bacterial strains produced by different cultivation methods and kinds of deployment of bacteria as well as more relevant, hazardous bacteria.

REFERENCE

[1] Fischbach et al., *Standoff detection and classification procedure for bioorganic compounds by hyperspectral laser-induced fluorescence.* In: SPIE Conference on Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XVI, 2015 (9455), 945508.