Laser-assisted indirect detection of bacterial endospores in suspicious macroscopic samples

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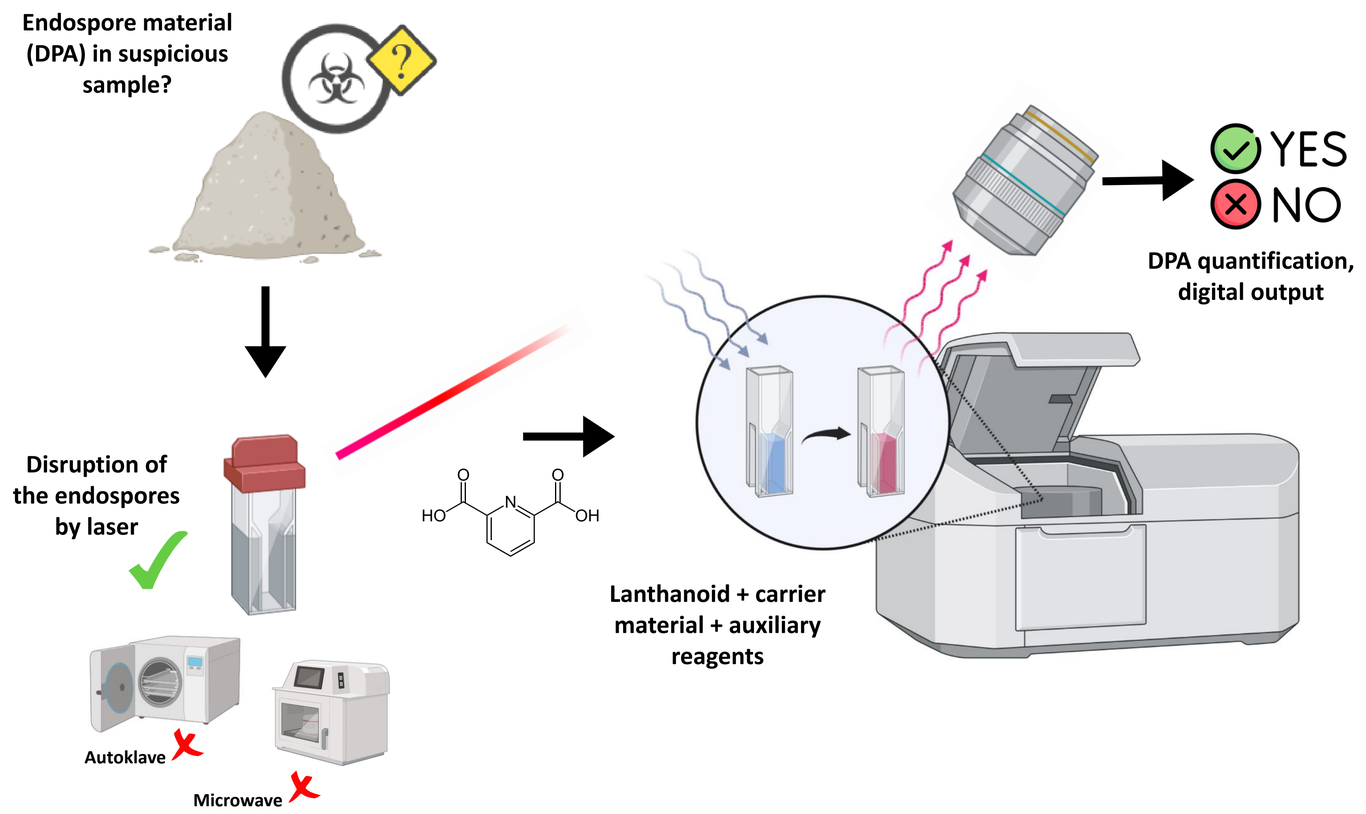
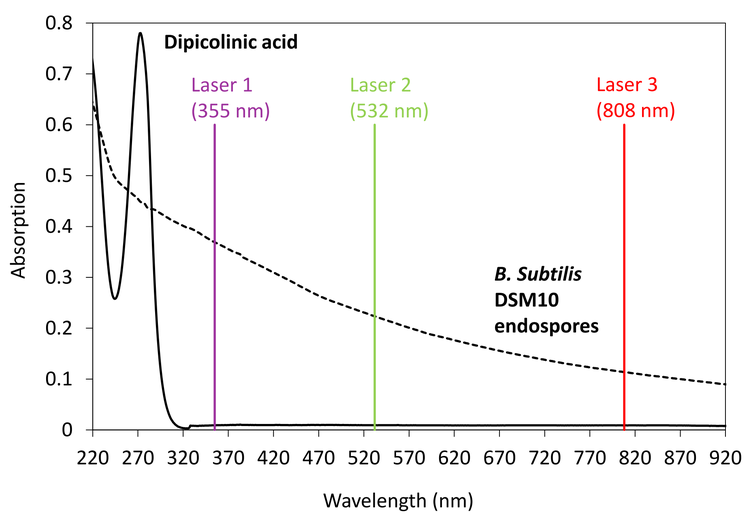
Globalisation, climate change, population growth, geopolitical tensions, radicalisation - these factors influence the security and stability of our society in a variety of ways and pose an increasing challenge in different areas. This also includes the threat posed by pathogenic microorganisms to our society and its security. In addition to multiple scenarios based on the natural spread of new or dangerous pathogens and their resistance towards antibiotics, the threat of deliberate release of pathogens is also of increasing importance. Rapid, reliable detection of organisms released during a terrorist attack or present in a clandestine laboratory as an indication of an imminent terrorist act are of the highest priority for the protection of the population and the emergency services. However, the specificity of currently used pathogen detection methods often correlates inversely with the detection speed. Furthermore, the detection principle cannot be automated in most cases, which would further increase the protection of involved and uninvolved persons during the operation. The aim of the present work was therefore to develop a test set-up in which certain groups of pathogens were to be detected or excluded at high detection speed. The concept focuses on the rapid detection of bacterial endospores in suspicious samples as an indication of the presence of pathogens of the genera *Bacillus* (e.g. *Bacillus anthracis*, *Bacillus cereus*) or *Clostridium* (e.g. *Clostridium perfringens*). The aim here is to quickly obtain a first statement about the biological hazard potential of the suspect sample and thus to be able to take initial protective measures at a very early stage.



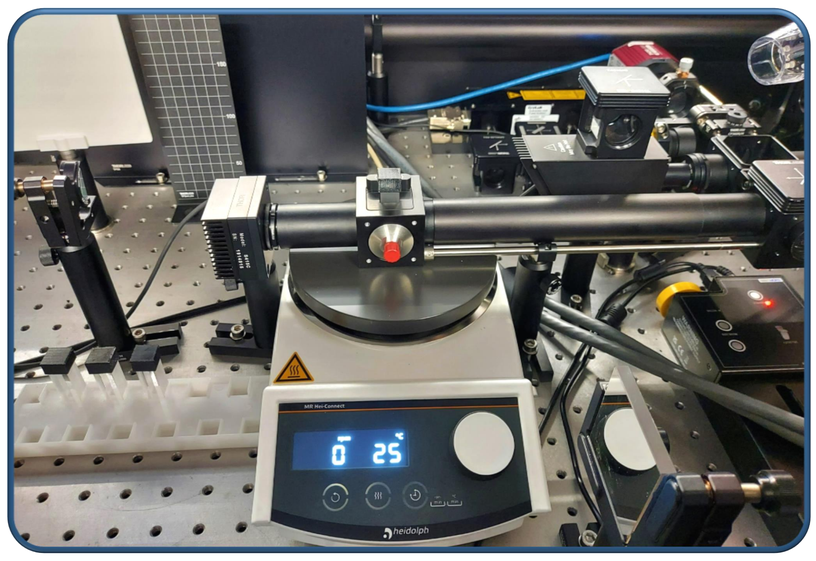
The principle:

In various situations (hidden laboratory, suspicious letters) it is of great importance to find out very quickly whether a sample at hand contains bacterial endospores in larger quantities. The aim of this first analysis is to at least confirm or exclude the presence of endospores (independent of the organism) after only a few minutes, so that follow-up steps can be initiated and the risk for the emergency services can be kept low. Dipicolinic acid (DPA) is used as a marker molecule for potentially present endospores in the examined sample, which is released from the spore core applying laser energy before the actual detection. A downstream reaction produces an antenna effect resulting from the interaction of DPA with lanthanides. Due to this effect, a shift in the wavelength of the emitted light can be measured after excitation in the UV range if DPA is present in the sample, from which conclusions can also be drawn about the concentration of DPA present in the suspect sample. The design of the detection concept is aimed at simplified automation at a later stage, both in sampling and in digital optical evaluation.



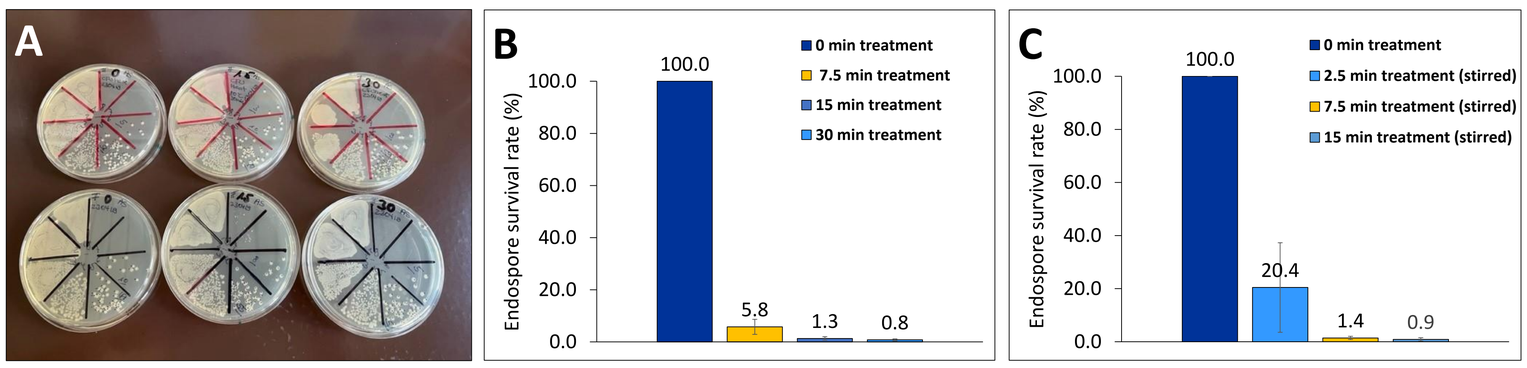
Choosing the right wavelength

In order to achieve an accumulation of DPA in the supernatant during laser-assisted spore lysis, it is essential to expose the suspect sample to a suitable wavelength. On the one hand, this should be maximally harmful to the endospore and, on the other hand, have only a minimal photolytic effect on DPA. In our experiments, a wavelength of 355 nm proved to be the most suitable compromise between a lytic effect on the endospore and a low negative effect on DPA.



Experimental set-up

Based on the findings from the recorded spectra, a UV laser with a wavelength of 355 nm at a power of 2.4 W and a pulse rate of 60 kHz is used in the current experiments. The setup allows continuous mixing of the endospore suspension during laser exposure. This setup is for testing purposes only, while efforts will be made in the future to miniaturise it and thus enable the development of a handheld system.



First results regarding the laser-induced sporicidal effect

After each irradiation experiment, *Bacillus subtilis* DSM10 endospores were heat treated and plated on lysogeny broth (LB) medium for CFU determination (A). At the same time, a microscopic assessment of possible effects on spore morphology was carried out (not shown). It was found that already after 7.5 minutes > 95% of the endospores could be inactivated by irradiation (B), in the continuously mixed system even > 98 % (C). First effects (approx. 40 % inactivation) were already measured after 1 min of irradiation (not shown).

Upcoming tasks and experiments:

- Monitoring and optimisation of DPA release efficiency during laser-induced lysis of endospores
- Enhancement of laser-induced lysis as a time-limiting step through the application of specific supportive agents (chemical and biological).
- Testing of the setup with different endospore-producing species and evaluation of the efficiency
- Development of a customised lanthanoid-based assay for the rapid detection of the marker molecule DPA at low concentrations
- Development of a digital result display based on an automated optical evaluation of the assay after UV light irradiation
- Miniaturisation and improvement of user-friendliness with focus on the development of a rapid handheld endospore detector

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