A changing world: New approaches for the development of detection methods for safety and security-relevant bacterial pathogens

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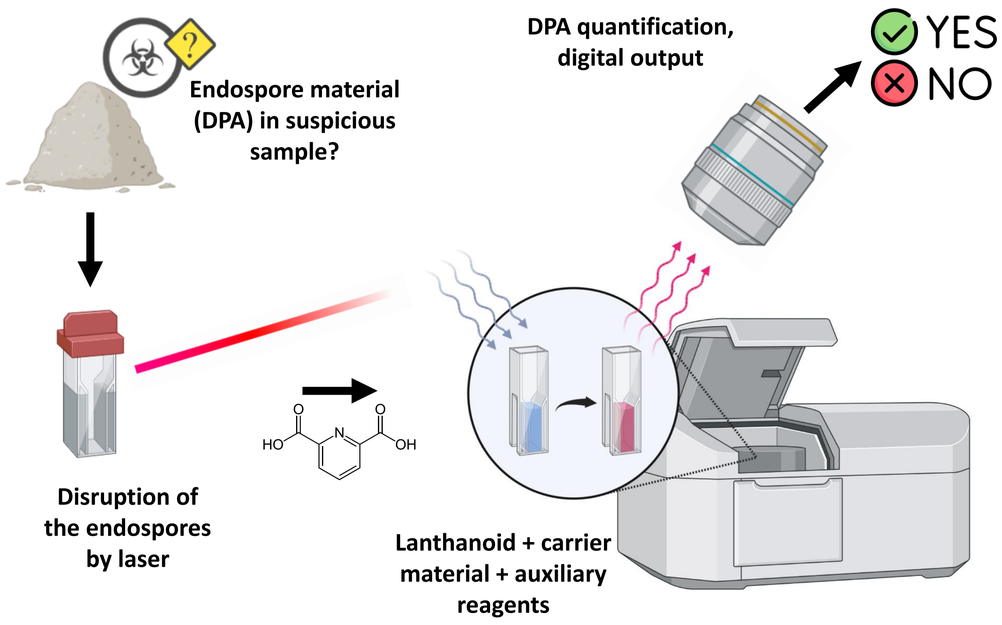
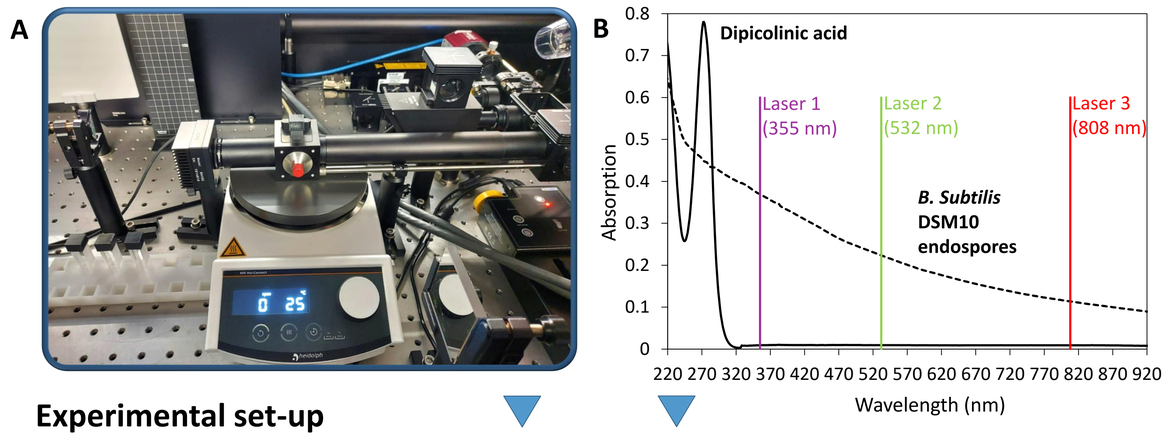
The effects of global warming, geopolitical tensions, radicalisation and demographic change are leading to an increasing challenge in different areas, affecting the stability of our community in many ways. This also includes the threat posed to our society by pathogenic microorganisms. In addition to the diverse scenarios based on the natural spread of new pathogens as well as their resistance to antibiotics, the threat posed by the deliberate release of harmful microorganisms is also becoming increasingly relevant. The first year of the corona pandemic has shown how vulnerable modern communities are and what kind of impact new pathogens can have on critical infrastructures. The raised awareness of this impact also leads to an increase in the risk of a terrorist attack involving biological agents or at least in the use of suspicious substances to spread fear. Both, for safety-related scenarios (e.g. the occurrence of a new pathogen due to global warming) and for security-relevant scenarios (e.g. suspicious powder in an envelope), the rapid and reliable detection and identification of microorganisms is therefore a top priority for civil protection. Depending on pathogen or the scenario at hand, the detection methods currently in use are often inadequate, as they either take too long, are too unspecific or prone to failure, or their level of automation is low and therefore presents a potential risk to the user. To avoid existing bottlenecks in the detection of various microorganisms in different scenarios, the Institute for the Protection of Terrestrial Infrastructures at the German Aerospace Center is working with partners to identify new and improved approaches. These include, for example, the rapid detection of bacterial endospores in suspicious samples (indication of the presence of pathogens of the genera *Bacillus* or *Clostridium*) as well as the aptamer-based detection of waterborne pathogenic bacteria by binding specific surface proteins.

Photonics-based rapid detection of bacterial endospores

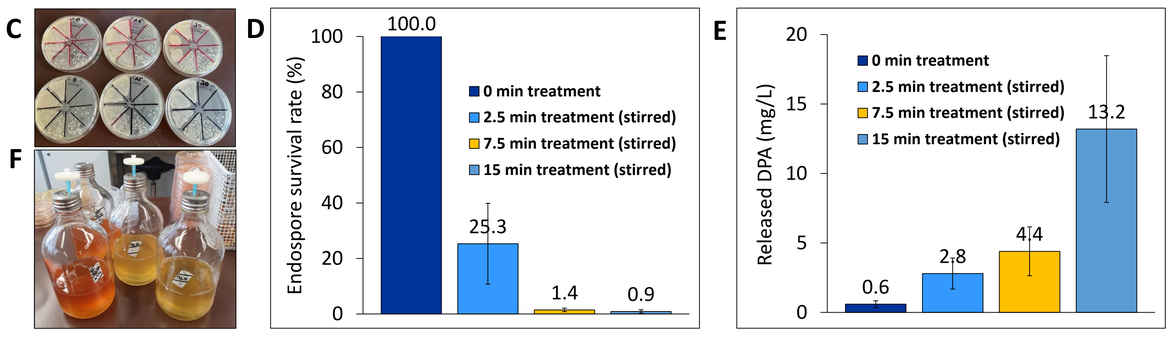
In various situations (hidden laboratory, suspicious letters) it is of great importance to find out very quickly whether a sample at hand contains bacterial endospores in larger quantities. The aim of this first analysis is to at least confirm or exclude the presence of endospores (independent of the 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.





Experimental set-up

The setup allows continuous mixing of the endospore suspension during laser exposure (**A**). Based on the findings from the recorded spectra (**B**), a focused 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. 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.

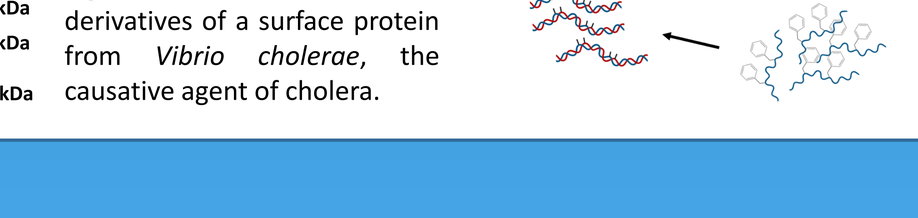


Laser-induced sporicidal effect and DPA release

After each irradiation experiment, *Bacillus subtilis* DSM10 endospores were heat treated and plated on LB medium for CFU determination (**C**). It was found that already after 7.5 min > 98 % of the endospores could be inactivated by irradiation in a continuously mixed system (**D**), while DPA accumulates in the supernatant during the process (**E**). Future work will also focus on the lytic effect in regard to anaerobic endospore formers (**F**).

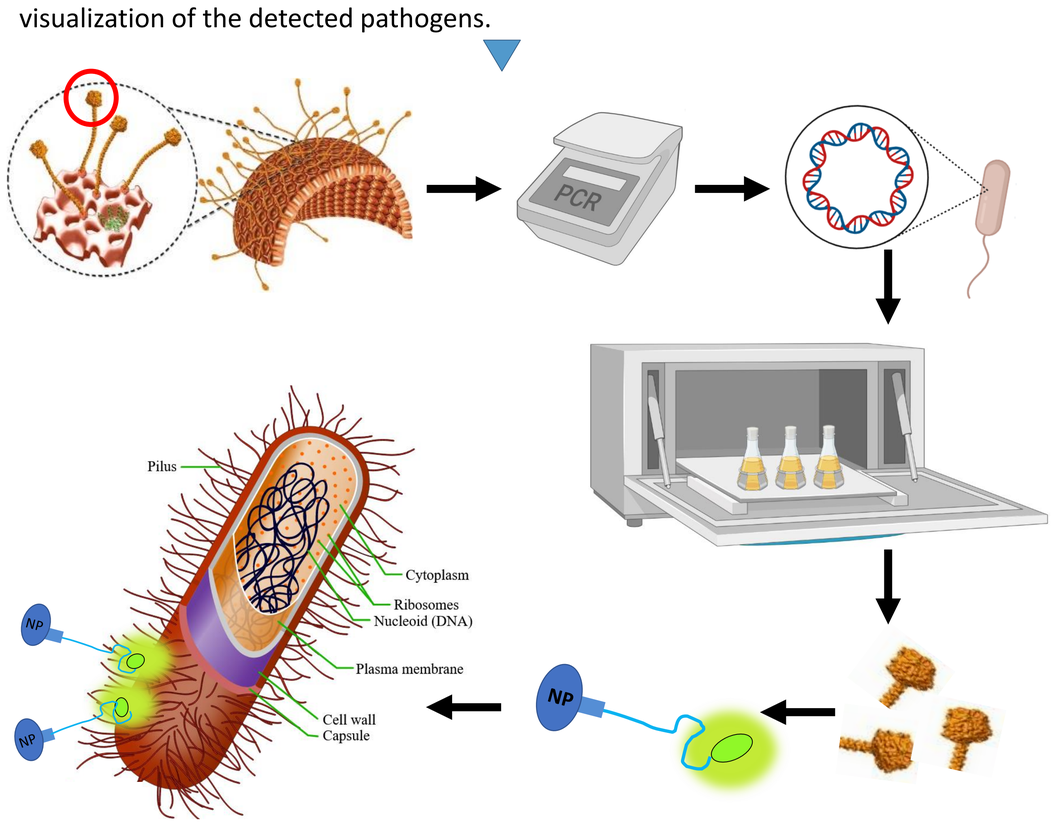
Aptamer-based qualitative and sensitive detection of waterborne pathogens

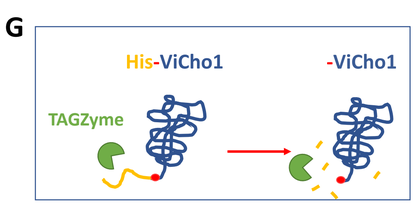
Pathogenic microorganisms that are deliberately or naturally transmitted through waterways have a huge impact on public health, which makes their monitoring and detection particularly important. Current technologies for detecting these either take a very long time, are highly susceptible to failure and often cannot be used on site (POC). In this context, we address the development of an innovative detection technology that enables the rapid, specific and sensitive detection of pathogenic microorganisms. The basic principle of the biosensor will be based on the affinity of clickmers to surface proteins of the selected pathogens. Clickmers, as modified aptamers, offer significant advantages compared to conventionally used antibodies and can be effectively coupled to various nanostructures (e.g. nanoparticles; NP), enabling sensor optimization and direct visualization of the detected pathogens.



Principle of the molecular switch

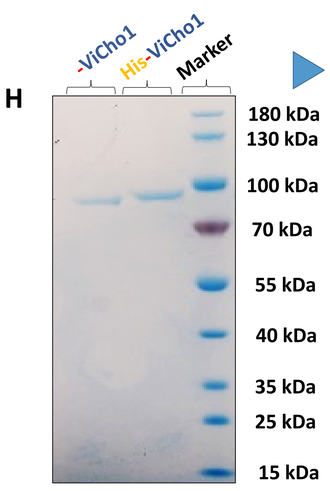
In the absence of the target protein, the aptamer-based recognition sequence is present in a hybridized state. Hybridization with the blocking strand brings a quencher molecule into spatial proximity to the fluorescent dye so that no signal can be measured (“switch off”). In the presence of the target protein, the blocking strand is displaced due to the greater affinity to the protein, the quencher dissociates and fluorescence becomes measurable (“switch on”).





Click-SELEX process

A Click-SELEX (**S**ystematic **E**volution of **L**igands by **EX**ponential Enrichment) is carried out to select specific binding clickmers. A large number of different clickmers are incubated with the target structure (**1**). Subsequently, all binding sequences are eluted (**2**), amplified, sequenced (**3**) and chemically modified (**4**).



Target protein preparation

For clickmer production, suitable surface proteins without membrane anchors are produced recombinantly. To ensure that the His-tag does not interfere with SELEX, it is removed after production (**G**). Fig. **H** shows two modified derivatives of a surface protein from *Vibrio cholerae*, the causative agent of cholera.