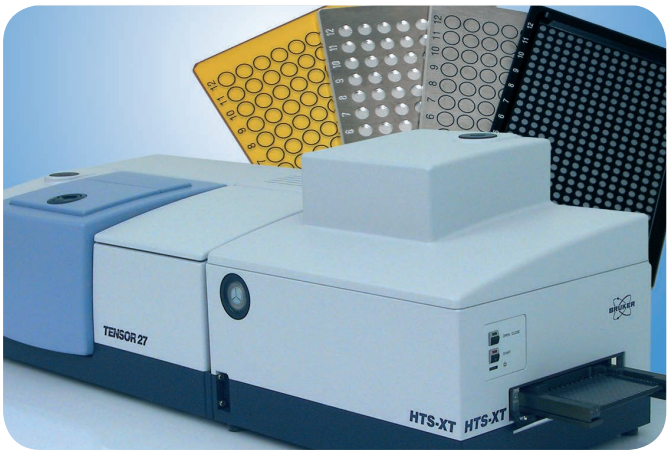

Application Note AN B405

Infrared Spectroscopic Bacterial Spore Detection

- Easy to handle and to apply
- Fast identification of spore formation
- Effective classification of sporeforming strains
- Simple sample preparation
- Easy system maintenance
- Marker bands of dipicolinic acid quantifiable

The formation of endospores is one of the most effective survival strategies that have been discovered by microorganisms over eons. Vegetative cells start dividing asymmetrically after receiving a trigger pulse whereby the smaller part of the cell develops into forespore. The final endospore body is build of a special protective coating (the cortex) and contains a high concentration of calcium-dipicolinate as well as a low quantity of water providing a chemical environment that guaranties survival under extreme conditions. Mainly two genera of Gram-positive bacteria have perfected the mechanism of spore formation under unfavorable growth conditions: Bacillus and Clostridium. Genetic profiling using PCR techniques is commonly applied to identify characteristic DNA fragments from genes encoding a sporulation function in spore forming bacteria.

Microscopic methods are also among the most often used detection and analysis tools ranging from classifications of visibly altered cell shapes to specific spore fluorescence. FT-IR spectroscopy does not only provide an easy and fast detection capability of spores and spore-formation, it also allows the classification of bacterial strains involved based on the infrared spectral profiles of the cells. This technique can be applied to spores, bacteria, and spore-containing bacteria as well as to their microcolonies. In cases where spores have been 'weaponized', FT-IR spectroscopy can easily identify the matrix used for this process such as clays or silica powders. This note illustrates the capabilities of FT-IR spectroscopy in the field of spore analysis.

Experimental

Two platinum loops of bacteria or two colonies harvested from an agar plate provide sufficient material for the standard infrared analysis of bacteria based on film technology using the microplate reader HTS-XT.

Optionally very small quantities of dried material (such as spores or stored powders of bacteria) can either be placed on a dedicated sample holder based on ATR design or under an infrared microscope.

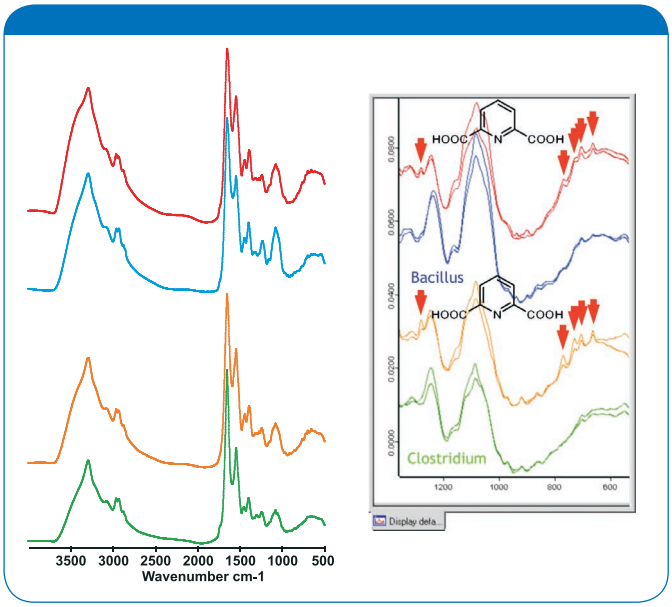

Figure 1: FT-IR survey spectra of Bacillus (blue) and Clostridium (green) without and during sporulation (red, orange). The enhanced view on the right illustrates the presence of marker bands for dipicolinic acid in the infrared spectrum of the entire bacteria indicating spore formation.

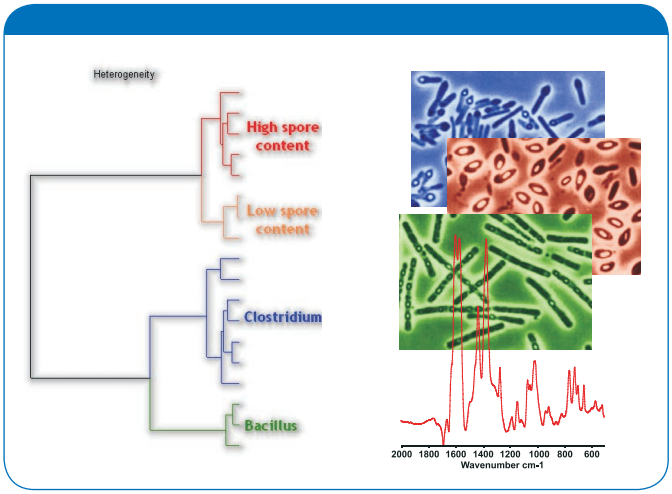

Figure 2: Dendrogram representation of the classification of Bacillus and Clostridium strains under conditions preventing and causing sporulation. The classification allows the separation of the two genera, however, during the sporulation process the dominance of dipicolinic acid can be used to roughly quantify the state of the spore development. On the right a typical infrared spectrum is shown illustrating the biochemical differences between Clostridium with and without spore. The background images display the different types of spore formation - terminal, central and sub-terminal.

Qualitative analysis

The significant chemical alteration in bacterial cells during spore formation is the production of high quantities of dipicolinic acid. This spore protective substance has a unique vibrational signature, which can easily be identified in the infrared spectra of bacteria. Marker bands allow the monitoring of the sporulation process (see Figure 1). During the formation of a spore inside the bacterium the concentration of dipicolinic acid increases over time, which can be seen in the infrared spectra of the intact bacteria. Multivariate statistics and/or cluster analysis can qualify the alterations that occur during the process of sporulation (see Figure 2). If the is no spore present the infrared spectra can be utilized to identify the bacterial species or strain. In case the onset of sporulation has already occurred, a classification of the infrared spectra can provide a more quantitative picture. Infrared spectra of bacteria with lower and higher spore content can be identified. Even dried cell material or spores can be investigated, since the infrared technology can obtain chemical information from freeze-dried powders.

Quantitative analysis

The sporulation process can also be monitored and quantified by simply following the relative intensities of the marker bands for dipicolinic acid. In liquid cultures aliquots can be obtained in time intervals and investigated for sporulation. It is also possible to test sensitivity of the bacteria towards external triggers in similar environments. Substances known to act as inhibitors of the spore forming process, can be tested and a reaction of the bacteria directly be observed due to the infrared detection of the chemical alteration in the cells.

System Configuration

The bacteria identification system is based on the Bruker Optics microplate reader HTS-XT. A stacking device can be coupled with the HTS-XT allowing automatic measurements of more than 30 microplates. The powerful OPUS LAB software performs the automatic measurement, evaluation and documentation of the spectra. This software also controls the stacking device.

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