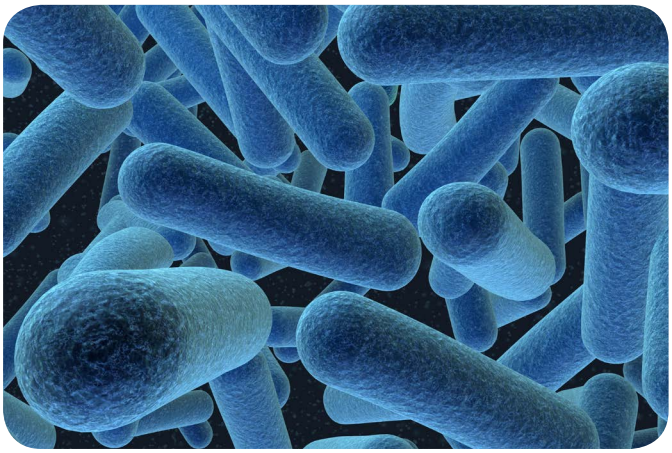


Application Note AN B406

Identification of Yeasts and Bacteria in Food Microbiology

General

Microorganisms are not only essential for the production of food like dairy products, bread, beer and wine but they play also a major role in modern industrial processes such as the fermentation of enzymes, pigments, antioxidants etc. and increase the digestibility and stability of food. However microorganisms can cause serious problems in the production process like spoilage or even poisoning. An effective microbial quality management is essential for the safety of the product. The reliable identification of microorganisms allows to optimize production processes and to track down sources of contaminations.

Method

The Fourier-Transform-Infrared (FT-IR)-Spectroscopy is already established as a fast and cost-efficient method in many areas of food analysis. Qualitative tests like the control of raw materials, as well as quantitative evaluation of complex compositions are possible using this technique. In the field of microbiology a FT-IR spectrum reveals a fingerprint from microorganisms. Its pattern comprises the vibrational characters of the cell constituents as mainly proteins, lipids, DNA/RNA and carbohydrates (Fig. 1).

Therefore a FT-IR spectrum is like a characteristic fingerprint from an organism. The high sensitivity and accuracy of the measurement facilitates microorganisms to be identified even on strain level.

Sample Preparation and Measurement

Only a few hours of training are needed to master the sample preparation and only a few seconds to prepare each sample for the measurement.

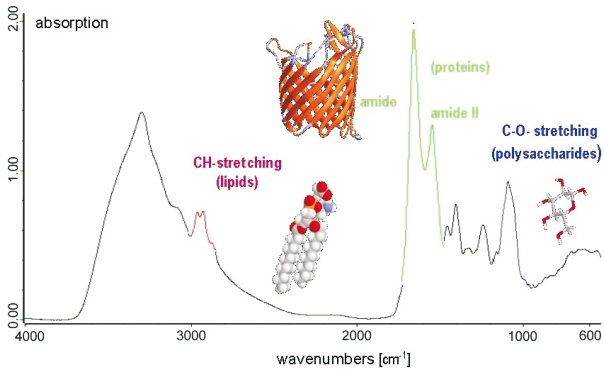


Fig. 1: Typical FT-IR spectrum of a bacterium. Spectral ranges that are dominated by bands of certain cell constituents are indicated.

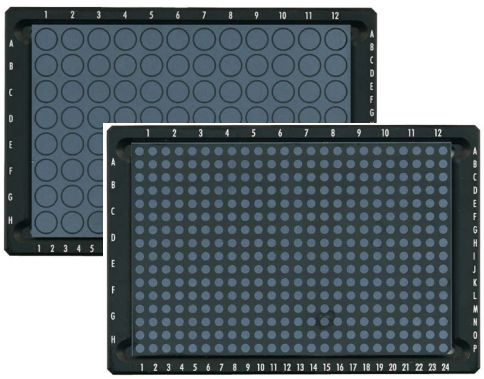


Fig. 2: Sample plates are available in the standarized 96 and 384 well format. The plates are easy to clean and can be reused very often.

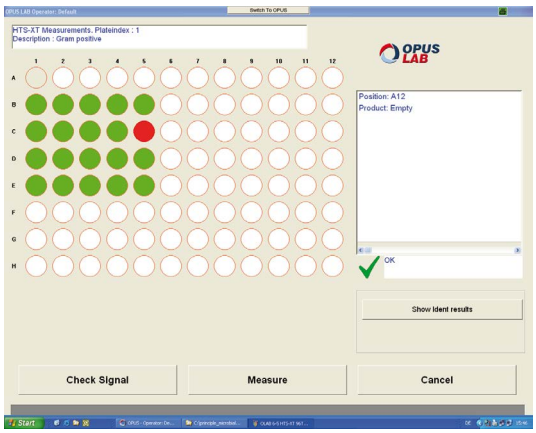


Fig. 3: The OPUS Lab software facilitates automatic measurement, data quality testing, data evaluation and data documentation.

The sample preparation consists of three steps. Pure cultures of microorganisms are grown typically for 24 h under standardized conditions. Some colonies are picked from the agar plate and suspended in distilled water. This suspension is then loaded on special reusable microplates in the 96 or 384 well format (Fig. 2).

In contrast to other methods no further time consuming cultivation step is necessary. After drying (ca. 15 minutes) the plate is inserted into the microplate reader HTS-XT. No reagents or consumables are required for the measurement.

The data acquisition as well as the data evaluation and data reporting is performed automatically (Fig. 3). One sample is measured and identified within about one minute. The microplates can be easily cleaned and reused very often. The identification results are summarized in a table format showing the identified strain or species as well as the hit quality. This value allows to judge the result easily (Fig. 4 and 5).

Databases

The quality of data bases is of fundamental importance for the reliable identification. The spectrum of each unknown microorganism is compared against all spectra from this data base. Therefore, a complete library has to cover all relevant species, but also the spectral variances of different strains from one species.

This allows to identify micro-organisms even below species level. In the existing data bases of food-relevant microorganisms not only strains from reference stocks are included but also microorganisms from different production sites. By adding samples from product specific habitats the method was further improved for its robustness and reliability.

All microorganisms were identified by reliable reference methods before adding them into the libraries. The existing data bases were built up and validated by the

Technical University Munich, Zentralinstitut für Ernährungs- und Lebensmittelforschung Weihenstephan (ZIEL, Munich, Germany), Department of Microbiology (Prof. Dr. Scherer).

They are used here for routine identification for several years. (Complete library list see Fig.6).

Results are documented in a convenient table format. The system software also allows to depict the similarities of spectra of different microorganisms graphically in form of a dendrogram.

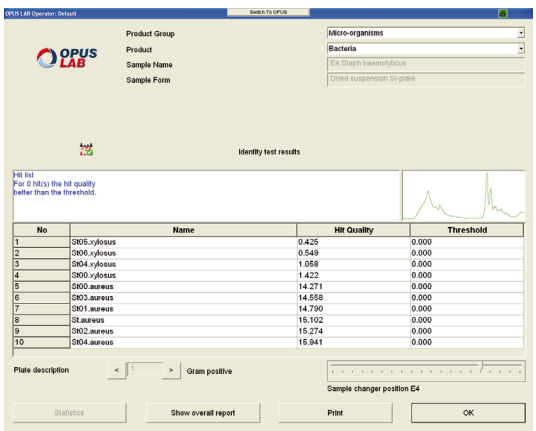


Fig. 4: For each identification result a hit list will be generated showing the species or strains with the greatest similarities.

Further applications

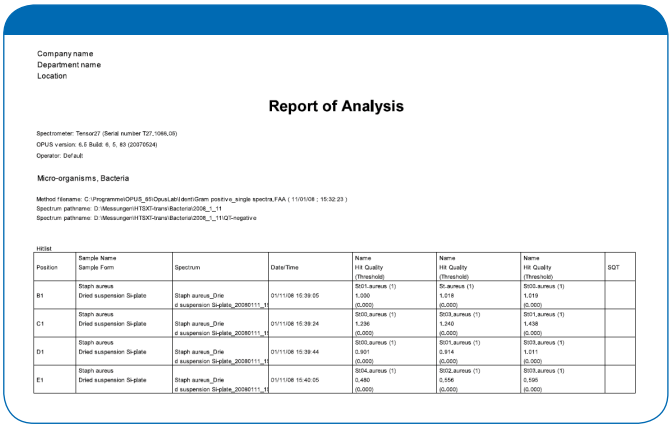
In food producing companies the analysis and characterization of microbial populations is an important part of the quality management.

The ease of use and its cost efficiency combined with quick and reliable results as well as the possibility to differentiate down to strain level makes the method an effective tool to monitor the house flora of any production plant. This specific house flora library may help for:

- Tracking down routes of microbial contaminations
- Specific control of cultures
- Early recognition of changes in the flora
- Improved troubleshooting (detection of the source/ responsibilities of a contamination)
- Improved hygiene monitoring

Efficiency and Economy

- No consumables or reagents
- Minimal maintenance
- Identification down to strain level
- Complete libraries for food-relevant microorganisms
- Universally applicable
- Library build up and modification by customer
- Integrated data management
- Minimal hands-on time
- Faster than other identification methods (only one cultivation step)



Company name
Department name
Location

Report of Analysis

Spectrometer: Tensor27 (Serial number T27_1066_05)
OPUS version: 6.5 Build: 6, 5, 83 (20070524)
Operator: Default

Micro-organisms, Bacteria

Method filename: C:\Programme\OPUS_65\OpusLab\Ident\Gram positive_single spectra_FAA (11/01/08 ; 15:32:23)
Spectrum pathname: D:\Messungen\HTSXT-trans\Bacteria\2008_1_11
Spectrum pathname: D:\Messungen\HTSXT-trans\Bacteria\2008_1_11\21-negative

HitList

Position	Sample Name Sample Form	Spectrum	Date/Time	Name Hit Quality (Threshold)	Name Hit Quality (Threshold)	Name Hit Quality (Threshold)	SQT
B1	Staph aureus Dried suspension Si-plate	Staph aureus_Drie d suspension Si-plate_20080111_1(	01/11/08 15:39:05	St01_aureus (1) 1.000 (0.000)	St_aureus (1) 1.016 (0.000)	St03_aureus (1) 1.019 (0.000)	
C1	Staph aureus Dried suspension Si-plate	Staph aureus_Drie d suspension Si-plate_20080111_1(	01/11/08 15:39:24	St03_aureus (1) 1.236 (0.000)	St03_aureus (1) 1.240 (0.000)	St01_aureus (1) 1.438 (0.000)	
D1	Staph aureus Dried suspension Si-plate	Staph aureus_Drie d suspension Si-plate_20080111_1(	01/11/08 15:39:44	St03_aureus (1) 0.901 (0.000)	St01_aureus (1) 0.914 (0.000)	St03_aureus (1) 1.011 (0.000)	
E1	Staph aureus Dried suspension Si-plate	Staph aureus_Drie d suspension Si-plate_20080111_1(	01/11/08 15:40:05	St04_aureus (1) 0.480 (0.000)	St02_aureus (1) 0.556 (0.000)	St03_aureus (1) 0.595 (0.000)	

Fig. 5: The identification results are summarized in a table. Any failure of the spectra quality test would be indicated in the right column.

Group	Spectra (ca.)	Genera (ca.)	Species (ca.)
Yeasts	2940	44	178
Coryneforms, Micrococci, other gram positive bacteria	1130	53	254
Bacilli	402	5	68
Pseudomonas	314	46	88
Acetic acid bacteria	483	6	27
Lactic acid bacteria, Cocci	467	14	105
Bifidobacteria, Clostridia, Anaerobic bacteria	176	4	46

Fig. 6: Libraries for food relevant microorganisms.

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