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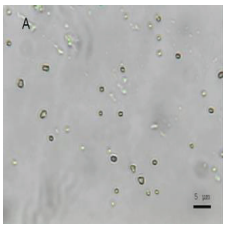
Raman Spectroscopy

Raman Investigation of Microorganisms on a Single Cell Level



Application Note

Biology RA59

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Introduction

Identification of contaminating microbes is an important public safety issue in many fields such as the control of food quality and the manufacture of pharmaceutical and cosmetic products. [1] Previous research has shown the potential for Raman spectroscopy in combination with chemometric methods to investigate bulk samples as well as a single bacterium and yeast cell for classification on the species and strain level. [2-8]

Analysis of Microorganisms

For Raman analysis the grown cells were taken from the agar plates and smeared by a diluting loop on a fused silica plate (see Fig. 1A).
The dark roundly shaped features correspond to single bacterial cells. The light features are caused by the blurred extra cellular matrix of the bacteria. Figure 1 B illustrates an image of separated yeast cells.

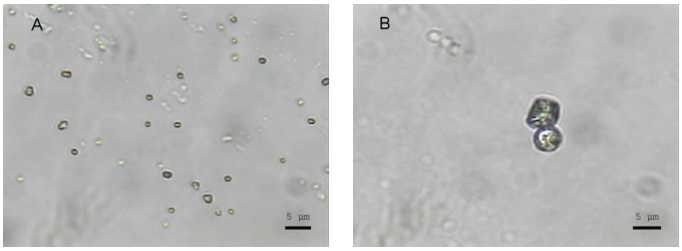

Figure 1. (A) Microscopic image of a smear of single bacterial cells of S. cohnii DSM 6669 on a fused silica plate. The dark roundly shaped features correspond to single bacterial cells and the light features to the blurred extra cellular matrix of the bacteria. (B) Microscopic image of two S. cerevisiae DSM 70449 cells adhering together.

In Figure 2 micro-Raman spectra from one single bacterial cell of two different strains from nine different species are shown exemplarily. Each spectrum was recorded with an accumulation time of 60 s with an excitation wavelength of 532 nm. [Due to the very low sample volume of one single cell (Micrococcus and Staphylococcus about 0.5 μm^3 and Bacillus and Escherichia 2.5 μm^3), signals of quartz can be observed at about 800 cm^{-1}]. The spectra contain important information about the complex structure of the investigated cell. Especially in the wavenumber region below 1800 cm^{-1} interesting vibrational features due to proteins (amide I, II, III), aromatic amino acids (phenylalanine (Phe)) and nucleic acid components (guanine (G), adenine (A)) are present.

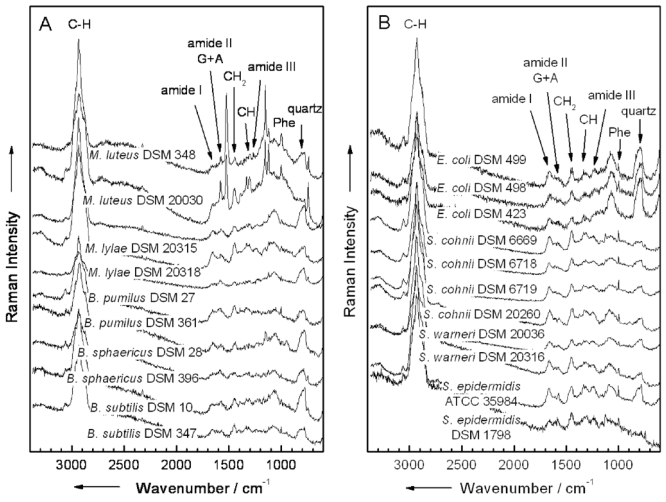

Figure 2. Micro-Raman spectra of different bacterial strains of the genus Bacillus and Micrococcus (A) and the genus Staphylococcus and Escherichia (B) measured with an integration time of 60 s and an excitation wavelength of 532 nm.

Due to the complexity of the spectra the differences are not easily visualizable making chemometric methods necessary in order to distinguish between different species and strains.

For classification a supervised method, the support vector machine (SVM) [9, 10] was applied. Raman spectra were preprocessed by a running median filtering and by centering to homogenize the distribution in all dimensions in the feature space. The classification is based on the wavenumber region of 330 – 3360 cm^{-1}. For the classification a nonlinear SVM with a Radial Basis Function (RBF)-Kernel was applied and for our data the one-versus-one test was used for training and classification.
For the experiments the gamma value was set to 7.5×10^{-7} while the cost value was set to 1×10^{6}. The result for classification is shown in table 1. From the 3806 Raman spectra 3346 spectra were correctly identified on strain level (average recognition rate 82.7 %) and 3731 were correctly identified on species level (average recognition rate 96.3 %). The lowest recognition rate for strains was obtained for E. *coli* DSM 499 with 49.4 % and on species level for *Staphylococcus cohnii* DSM 6718 with 86.2 %.

In addition to environmental contamination by bacteria, yeast can also contribute to contamination. However, recording their spectra on a Raman microscope requires taking into account the more extended size of these organisms and the fact that they are compartmentalized with numerous organelles such as the nucleus and cytoskeleton. In order to acquire spectra representative of the spatial heterogeneity of the eukaryotes, it is necessary to make about 10 measurements per cell from which one can to calculate the «average» spectrum.

	Total number of spectra	Number of wrongly classified strain spectra	Recognition rate for strains (%)	Number of wrongly classified species spectra	Recognition rate for species (%)
B. *pumilus* DSM 27	57	11	80.7	7	87.7
B. *pumilus* DSM 361	69	10	85.5	5	92.8
B. *sphaericus* DSM 28	53	8	84.9	3	94.3
B. *sphaericus* DSM 396	42	6	85.7	6	85.7
B. *subtilis* DSM 10	306	7	97.7	5	98.4
B. *subtilis* DSM 347	42	3	92.9	3	92.9
E. *coli* DSM 423	94	19	79.8	0	100.0
E. *coli* DSM 429	90	29	67.8	0	100.0
E. *coli* DSM 498	134	25	81.3	4	97.0
E. *coli* DSM 499	83	42	49.4	1	98.8
E. *coli* DSM 613	86	23	73.3	0	100.0
E. *coli* DSM 1058	71	15	78.9	0	100.0
E. *coli* DSM 2769	108	30	72.2	0	100.0
M. *luteus* DSM 348	619	3	99.5	3	99.5
M. *luteus* DSM 20030	48	4	91.7	3	93.8
M. *lylae* DSM 20315	45	4	91.1	4	91.1
M. *lylae* DSM 20318	20	1	95.0	1	95.0
S. *cohnii* DSM 6669	67	1	98.5	1	98.5
S. *cohnii* DSM 6718	65	11	83.1	9	86.2
S. *cohnii* DSM 6719	63	10	84.1	5	92.1
S. *cohnii* DSM 20260	65	4	93.9	1	98.5
S. *epidermidis* 195	74	3	96.0	3	96.0
S. *epidermidis* 2682	141	5	96.5	0	100.0
S. *epidermidis* DSM 1798	112	46	58.9	1	99.1
S. *epidermidis* DSM 3269	93	33	64.5	0	100.0
S. *epidermidis* DSM 3270	110	48	56.4	0	100.0
S. *epidermidis* DSM 20042	106	42	60.4	0	100.0
S. *epidermidis* ATCC 35984	805	4	99.5	4	99.5
S. *warneri* DSM 20036	71	9	87.3	4	94.4
S. *warneri* DSM 20316	67	4	94.0	2	97.0
Average recognition rate	3806		82.7		96.3

Table 1: Result of SVM classification of micro-Raman spectra from single bacterial cells (532 nm)

In Figure 3 average micro-Raman spectra from single yeast cells of from four different species are illustrated exemplarily. For classification of the yeast cells 10 to 18 average Raman spectra of each strain were applied to the support vector machine. The classification is based on the wavenumber region of 560 – 3150 cm^{-1}. For the classification a Radial Basis Function (RBF)-Kernel was applied and the gamma value was set to 1.1 x 10^{-7} and the cost value to 3.9 x 10^{6}. The result for classification is listed in table 2. From the 92 Raman spectra 80 spectra were correctly identified on strain level (average recognition rate 86.2 %) and 87 were correctly identified on species level (average recognition rate 96.3 %). The lowest recognition rate for strains was obtained for C. glabrata DSM 70615 with 60.0 %. On species level the strain with the lowest recognition rate is S. cerevisiae DSM 1334 with 81.8 % (9/11).

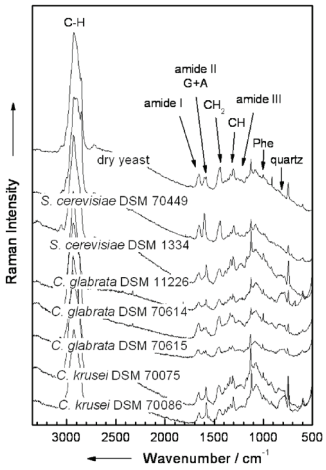

Figure 3: The average micro-Raman spectra of different yeast strains measured with an integration time of 60 s and an excitation wavelength of 532 nm.

Conclusions

Raman spectroscopy and a supervised classification method, the support vector machine, have been used for the identification of bacterial and yeasts on a single cell level. It could be shown that it is possible to reach a promising recognition rate not only for bacteria, but also for yeast cells that exhibit in contrast to bacteria a spatial heterogeneity making it necessary to analyze average spectra.

Acknowledgement

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	Number of average spectra	Number of wrong classified strain spectra	Recognition rate for strains (%)	Number of wrong classified species spectra	Recognition rate for species (%)
C. glabrata DSM 11226	10	1	90.0	0	100.0
C. glabrata DSM 70614	10	0	100.0	0	100.0
C. glabrata DSM 70615	10	4	60.0	1	90.0
C. krusei DSM 70075	10	1	90.0	0	100.0
C. krusei DSM 70086	10	1	90.0	0	100.0
S. cerevisiae DSM 1334	11	3	72.7	2	81.8
S. cerevisiae DSM 70449	13	1	92.3	1	92.3
Dry yeast	18	1	94.4	1	94.4
Average recognition rate			86.2		94.8

Table 2: Result of SVM classification of averaged micro-Raman spectra from single yeast cells (532 nm)

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