A high-throughput microcultivation protocol for FT-IR spectroscopy of microorganisms

A. Wold Åsli[a] , V. Shapaval[a] , T. Møretrø[a], H.P. Suso[b], U. Böcker[a,c], J. Schmitt[e], A. Kohler[a,c,d]

[a]Nofima Mat, Centre for Biospectroscopy and Data Modelling, Osloveien 1, 1430 Ås, Norway
[b]Elopak AS, Spikkestad, Norway
[c]CIGENE – Center for Integrative Genetics, University of Life Sciences, 1432 Ås, Norway
[d]Department of Mathematical Sciences and Technology (IMT), Norwegian University of Life Sciences, Ås, Norway
[e]Synthon GmbH, Heidelberg, Germany

FTIR spectroscopy of microorganisms is very sensitive to all kind of variations: Strain storage, cultivation of strains, sample preparation. Among the different parameters determining the cultivation conditions, variation of the medium composition is the most important factor in terms of reproducibility and discrimination ability, whereas typical variations in incubation time and temperature influenced the IR-spectra only slightly [1].

In the present study we wanted (1) to increase throughput and automatization of cultivation for FTIR spectroscopy of yeasts and (2) to investigate the possibilities for increasing discrimination ability of FTIR spectroscopy for yeasts by using different media at the same time. The composition of agar containing media is - in comparison with broth media - hard to control, it is in addition not suitable for high-throughput applications. In order to increase throughput, cultivation in liquid media in the automated Bioscreen C system [2] in combination with high-throughput FTIR was applied. The developed approach allowed the analysis of 200 samples per day and reduced variations of agar media. Automated plate washing (washing of 96 samples at the same time) was applied to increase automatization of the analysis. The use of the automated Bioscreen C system allowed us to apply different media in one run.

Fifty-nine food-related yeasts were used to test five liquid media: YPD broth, Yeast Malt Extract Broth (YMB), Sabouro Broth (SAB), YEPD broth, Synthetic Nutrient Broth (SD) were used. Our results show that use of more than one media gives higher discrimination ability in FTIR spectroscopy. It turned out that the use of organic liquid media is more preferable than synthetic since it provides enough biomass for FTIR spectroscopy.

References:

[1] H. Haag, H.-U. Gremlich, R. Bergmann, J.-J. Sanglier, *Journal of Microbiological Methods* 27, 157-163, (1996).
[2] S.-M. Tauk-Tornisielo, J.M. Vieira, J.S. Govone, *Brazilian Journal of Microbiology* 38, 113-117 (2007).