: quaphotomics in biotechnology- new tools and opportunities

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IN BIOLOGICAL SCIENCES SQUAPHOTOMICS CAN BE USED FOR

- characterization of biological populations
- Microbial origin discrimination
- detection of fungal contamination
- bacterial detection
- chemical analysis
- differentiation of cells types
- intra and interspecific variation in algae and fungi
- characterization of biochemical response of living organisms to diverse process such as stress conditions and growing

Diverse advantages of FT -IR spectroscopy such as speed, minimal sample preparation and its non - destructive character are enhanced when multivariate analysis is applied on the spectra;
thus, chemical components of complex samples as biological samples can be evaluated by diverse multivariate calibration methods and this is also one of the most currently use of this technique now and in the

AQUAPHOTOMICS IN FERMENTATION PROCESSES

- for monitoring of fermentation processes as it provides simultaneous quantification of components and does not require sample preparation

AQUAPHOTOMICS IN FERMENTATION PROCESSES

- For biotechnological development of new industrial recombinant strains.
- For screening of recombinant strains (we can propose NIR spectroscopy with aquaphotomics as an effective and highly accurate screening method)
- For improved monitoring or control (on-line information on substrate, biomass, product, and metabolite concentrations in fermentation processes)

AQUAPHOTOMICS IN FERMENTATION PROCESSES

In the same time optimizations of fermentation processes can be made using methods of approaches of aquaphotomics.

Fermentation sample can be analyzed by NIR and the tools of aquaphotomics can be applied

The advantages of this approach are:

- It is a rapid. In each measurement (which last roughly only 1 minute) multiple components can be simultaneously determined.
- It is non -destructive. Fermentation samples can be studied directly, since no sample preparation is required. No waste and pollution are created.
- It is simple, highly precise and accurate.
- NIR technology can assist in identifying and fixing potential problems before the yield is compromised.
- NIR spectroscopy is an asset that allows plant to rapidly perform research on different protocols to optimize the fermentation process by changing enzymes, parameters or nutritional supplements.
- NIR analysis ensures considerable savings in raw materials, processing fuel, steam, labour , maintenance and equipment

AQUAPHOTOMICS IN FERMENTATION PROCESSES

Aquaphotomics, based on near infrared spectroscopy is time-efficient method, which allows rapid, chemical-free, non-invasive *in vivo* assessment, and it also provides an opportunity for researching live microorganisms in the cultivation process. The method is very sensitive to even traces of analytes. This makes it of first choice if the target components which affect the water structure and are important for the characterization of the studied objects are presented in very low concentrations.

AQUAPHOTOMICS IN FERMENTATION PROCESSES

Some considerations:

- ***Defining production scale***
- ***Growth factors***
- ***NIR Analysis Strategy***

Timely process information about the fermentation biomass, substrates, intermediates, and nutrients allows you to make better control decisions

AQUAPHOTOMICS AS A TOOL FOR MICROBIOLOGICAL MONITORING

- In vitro and in situ analysis of biofilm formation, structure and development by food-borne pathogens
- A rapid means of profiling microbial communities present in food products
- Development of food-borne pathogen spectra libraries

Aquaphotomics can constitute highly specific spectroscopic fingerprints of microorganisms by which they can be identified

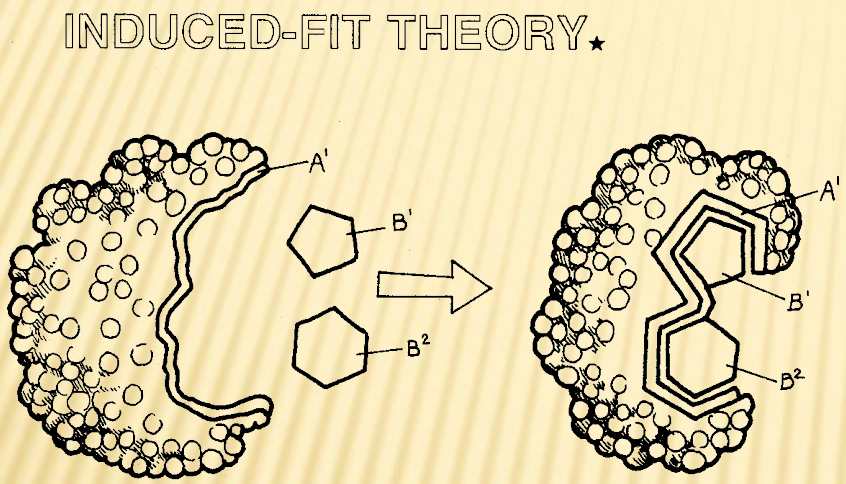
AQUAPHOTOMICS AS A TOOL IN THE REVEAL OF MOLECULAR MECHANISMS OF ENZYME REACTIONS

- Folding and stabilization of tertiary and quarterly structures are largely due to the creation of hydrogen bonds with the surrounding water molecules.
- Integral part of the protein structure is extensively hydrogen bonded with internal water, which determines protein stability and rigidity.
- In contrast, water molecules on protein surfaces are much more mobile with residence times on the order of tens of picoseconds
- Properties of protein hydration water are different to those of bulk water, i.e., the solvent structure in the presence of biomolecules differs from that of the bulk solvent

AQUAPHOTOMICS AS A TOOL IN THE REVEAL OF MOLECULAR MECHAN ENZYME REACTIONS

Aquaphotomics, based on visible-near infrared spectroscopy and multivariate analysis, could discover new water hydrogen bonds in protein/enzyme systems under various perturbations and relates water absorbance patterns to respective biofunctionalities.

AQUAPHOTOMICS AS A TOOL IN THE REVEAL OF MOLECULAR MECHANISMS OF ENZYME REACTIONS



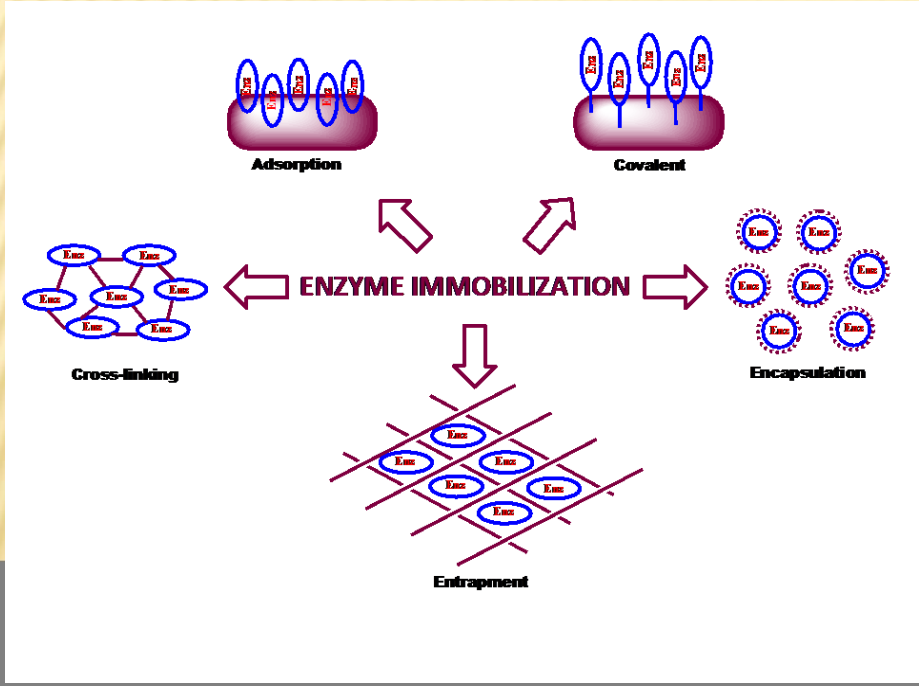
The approaches of aquaphotomics make it possible to reveal the mechanisms of enzyme action and to understand the nature of the enzyme - substrate interaction and experimentally prove so.

Induced -fit theory for enzyme action - the initial binding of a substrate induces certain changes in 3-D conformation of the enzyme, which felicitates the substrate to bind to the enzyme properly

AQUAPHOTOMICS IN MOLECULAR GENETICS

- To analyze DNA molecules
- To detect some genetically modified organisms by comparison of their DNA fragments
- To examine the activities and roles of a particular gene expression in some seed phenotypes with regard to their physiochemical composition
- For localization of some oxidative DNA damages

AQUAPHOTOMICS IN TECHNOLOGY OF IMMOBILIZED ENZYMES AND IMMOBILIZED CELLS



- Immobilized enzyme is an enzyme that is attached to an inert, insoluble material. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again. An alternative to enzyme immobilization is whole cell immobilization

AQUAPHOTOMICS IN TECHNOLOGY OF IMMOBILIZED ENZYMES AND IMMOBILIZED CELLS

- Solvent perturbation spectroscopy can be used to evaluate if the incorporation process of enzymes into the gels has denaturated the incorporated proteins
- The absorption differences between the surrounded water of folded (native) and unfolded (denaturated) states of proteins can be used to investigate their stability
- In vivo studies of free and immobilized cells by approaches of aquaphotomics could provide very valuable information for the difference of the behavior between free and immobilized cells
- To characterize the effective diffusivity of nutrients in immobilized cell materials

USE OF AQUAPHOTOMICS TO ANALYZE SPECIFIC PROBIOTIC PROPERTIES OF MICROORGANISMS

Major issue - the selection of strains exhibiting strong probiotic characteristics.

Two main strategies have been applied for the selection of probiotic strains: selection of strains with particular genes and in vitro examination of strain growth under model conditions of the digestive tract.

These methods are time -consuming, require expensive equipment and consumables, and they give uncertain results.

Quick and cheap method, which allows fast, in vivo comprehensive evaluation, is needed.

USE OF AQUAPHOTOMICS TO ANALYZE SPECIFIC PROBIOTIC PROPERTIES OF MICROORGANISMS

Water spectral patterns of the living microorganisms present information about their functionality and could serve as a molecular mirror of cells phenotype. Therefore, replacing the phenotypic and genetic approach for probiotic bacteria selection with Aquaphotomics is an innovative strategy

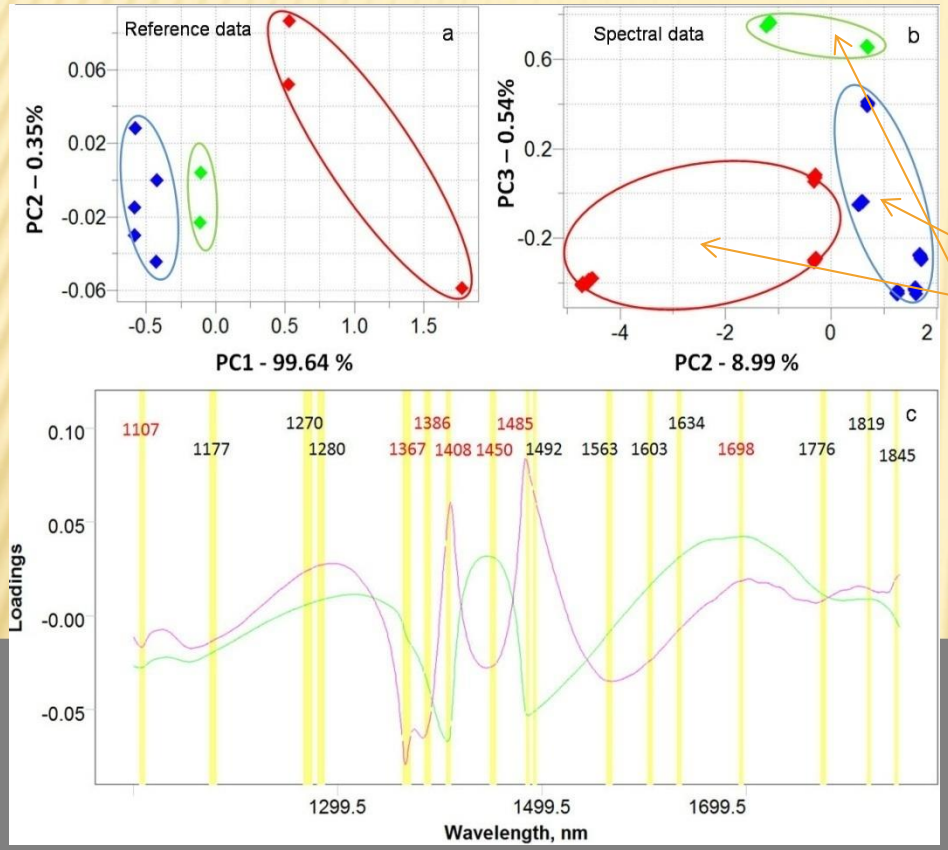
USE OF AQUAPHOTOMICS TO ANALYZE SPECIFIC PROBIOTIC PROPERTIES OF MICROORGANISMS

- The group of prof. Tsenkova at Kobe University NIR spectroscopy with Aquaphotomics was used as a quick non-destructive method to analyze ten strains – nine *L. bulgaricus* and one *L. gasseri.*
- Growth rate, low pH and bile tolerances were investigated in order to be further used as a reference data for the simultaneously acquired spectral data analysis.

USE OF AQUAPHOTOMICS TO ANALYZE SPECTROSCOPIC PROPERTIES OF MICROORGANISMS

- The obtained spectral data was subjected to various multivariate data analyses – PCA, SIMCA, OPLS-DA, PLSR showed consistently and with high accuracy, never obtained until now, that it is possible to separate and classify bacterial strains according to their probiotic strength on the basis of their growth spectral patterns in the region of 1100-1850nm

USE OF AQUAPHOTOMICS TO ANALYZE SPECIFIC PROBIOTIC PROPERTIES OF MICROORGANISMS



PCA score plots

- a) calculated on the reference data (strains growth rates, Bile MIC and the yield of biomass after three hours stay at pH 1.80 in presence of pepsin
- b) calculated on spectral data (n = 100) using the 1100-1850 nm wavelength interval. Probiotic, moderate and non-probiotic groups
- c) loadings of PC2 (purple line) and PC3 (green line) of PCA calculated on the spectral data highlighting the bands with red color (numbers with black color refers to SIMCA results)

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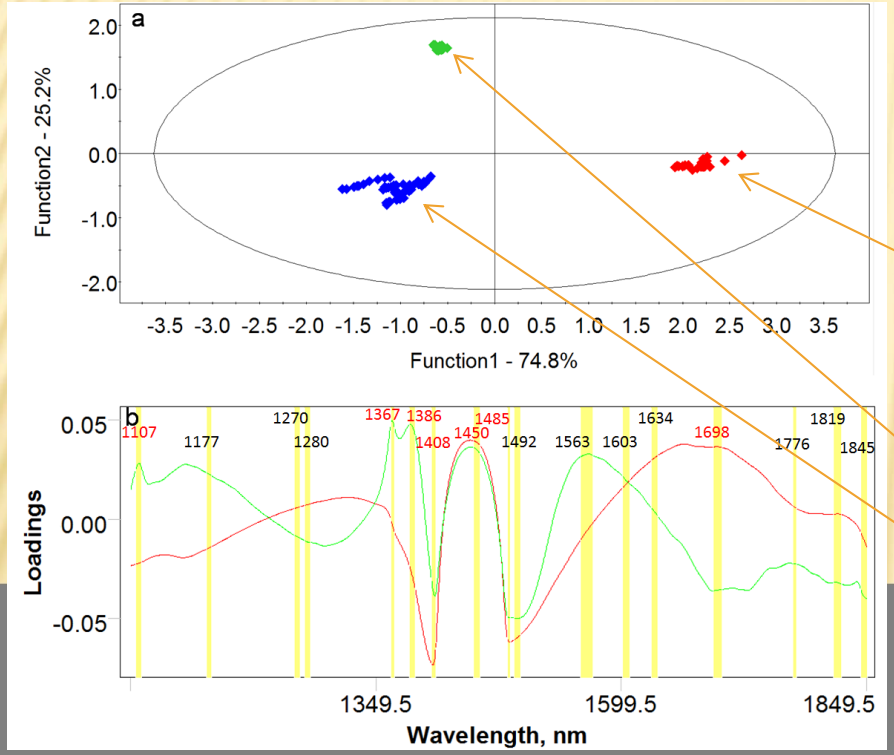

- a) score plot and
- b) loadings plot of OPLS-DA model built on the data of the 10 strains in the monitoring time between 10.5-11.2 h (n=100) using the 1100-1850 nm wavelength interval to classify the probiotic, moderate and non-probiotic groups, highlighting the bands with red color most important for the discrimination

USE OF AQUAPHOTOMICS TO ANALYZE SPECIFIC PROBIOTIC PROPERTIES OF MICROORGANISMS

- The NIR spectral analyses allowed high accurate qualitative and quantitative analysis of bacteria. Both of them reveal the importance of the first overtone range of water (1300 -1600 nm) for the classification and prediction of the different phenotypic properties of potential probiotic candidates of genus Lactobacillus .
- These results demonstrate the potential for application of Aquaphotomics as a holistic approach in the selection of probiotic microorganisms.