Application of Aquaphotomics to learn more about the rules of water

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Summary: All living creatures are made of cells, small membrane-bounded compartments filled with a concentrated aqueous solution of chemicals [1]. The relationship between the different water structures in the cells and its functionality has not been fully understood yet. NIR spectra of the water provide very stable signal and immense information for the whole system directly related to its functionality. Aquaphotomics is a novel scientific approach for the exploration of aqueous systems through rapid and comprehensive analysis of water-light interaction as a potential source of information for better understanding of the biological world using spectroscopy [2]. In this concept water is described as multi-element system providing multi-dimensional spectra. Water absorbance bands and absorbance patterns can provide important information on water structure and intrinsic interactions between water and other components of solution.

Introduction: Aquaphotomics, a new scientific discipline has been successfully applied in the research and diagnosis of various species [3]–[6] and for identification and discrimination of bacterial species [7]. It has been proven that extracellular metabolites played more signi cant role in successful spectral qualitative model performance [8]. In our previous research Aquaphotomics and NIR spectroscopy were successfully applied to discover the water structure in different biological systems including plant cells and bacteria strains. Results of a study conducted to monitor the growth of Lactobacillus strains proved that water spectral pattern of the solution as a molecular fingerprint of the cell culture system holds lots of information which can be used for accurate classification of Lactobacillus strains according to their probiotic strength [7]. It has been proven in many studies the absorption regions of water provides important spectral information about the different biological systems [7], [9], [10]. The successful application of the Aquaphotomics concept has been proven in many different application. It is worth to develop further specific supporting tools for the application of the Aquaphotomics evaluation methodology and for demonstration of the water spectral patterns. The objective of our work in one hand is to develop methods to provide easy to comprehend tools for the presentation of the ratio of the water species that provides us numerical results on a clearly defined scale related to temperature. On the other hand, our goals are also to use Aquaphotomics to explore the possibility of using different growth stages for in vivo discrimination of resistant and non-resistant *Lactobacillus* strains, and prediction of their low pH and bile resistances based, furthermore, to determine the water structural changes occurred during cell development in plant cells by monitoring the wavelengths activated during the cell development.

Materials and Methods: Model experiments were performed on Milli-Q water in the temperature range between 20 and 70°C at every 2 °C resulting in 26 temperature steps and with aqueous solutions of KCl in the range between 1 and 1000 mM to provide dataset for the development of the specific supporting tools for the Aquaphotomics data evaluation.

The growing characteristic of five probiotic and four non-probiotic strains (genus *Lactobacillus*) possessing different bile salt tolerance and ability to resist low pH (pH 1.80) in presence of pepsin was monitored with their NIR transflectance spectra in the spectral region between 400 and 2500 nm at every 4 min for 24 hours.

Growing of somatic and callus rice (*Oryza sativa*) cells were monitored by Visible and Short Wavelength Near InfraRed (Vis/SW-NIR, 660-960 nm) spectrometer from the forth to the 26th day of the cell development.

Results and Discussion:

The effect of temperature on the spectral pattern of Milli-Q water acquired in the temperature range of 20-70°C can be presented with the temperature based aquagram calculation method. The method provides the presentation of the phenomena demonstrating the blue shift in the first OH overtone range with increasing temperature. Furthermore, the scale of the aquagram expresses the effect of perturbation in degree Celsius equivalent. Aqueous solutions of potassium-chloride was analyzed as it has no absorption in the NIR region, thus, its effect on the water spectral pattern can be clearly evaluated. The aquagrams display how salts change the surrounding water molecular structure by changes in the OH bonding of the water molecular system. The higher concentrations of potassium-chloride (0.1 - 1M) show the highest difference from the pattern of Milli-Q water resulting in less H-bonded water structure and a decrease in the water-species referring to the more H-bonded water structure in the aquagram. The new temperature based aquagram calculation method provides further information about the magnitude of the change it shows how much a given sample would have had to be warmed up or cooled down in each of the single water coordinates to achieve the same result as the actual measurement, while all measurements were performed at precisely the same temperature.

The application of Aquaphotomics for in-vivo evaluation of probiotic and non-probiotic bacteria strains provided very accurate, fast and non-invasive identification of probiotic bacteria strains based on spectral monitoring of their bacterial growth. The multivariate methods applied for the spectral data assessment in regards to phenotype identification showed several common absorbance bands, with high importance. Most of the bands with high variations of their absorbance were consistent with the described 12 water matrix coordinates (WAMACS) [2].

The results of the plant cell experiments showed the Aquaphotomics approach is appropriate to determine the different growing stages of the cells based on the changes occurring in their water structure. Furthermore, it can provide more knowledge to understand different biological phenomena better based on the information available through the water mirror.

Conclusion: The results acquired with the development of new tools and application of the Aquaphotomics concept has been leading us to further understanding of the rules of water in biology. Presented results achieved in various fields open a new venue to think on water from different perspectives.

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