To grasp water molecule structure reflecting bacterial growth ability by NIRS and Aquaphotomics

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Summary: There is not an established technology to grasp and predict the bacterial state and movement. Our final objective is to elucidate bacterial communication and establish a technology to predict the bacterial state and movement regarding "Bottle Effect [1]", by means of NIRS and Aquaphotomics [2]. This technology is expected to be applied to various fields such as medical, food and agriculture. In this experiment, we used 4 bacterial strains and observed the growth phases by NIRS to grasp the differences between bacteria with fast and slow growth at very early stage in mineral water. As a result, it was possible to classify them at very early stage with high accuracies. Moreover, effective spectral patterns that classify bacteria were identified.

Introduction: Bacteria exist everywhere. They are in soils, on our skins, in foods, and in water. However, there is no established technology for non-invasive monitoring and measurement of the state and movement of bacteria and predicting subsequent changes. In this study, we focus on "Bottle Effect" and observe bacterial growth stages. "Bottle Effect" means the phenomenon that after bottling, the number of viable cells increases rapidly, attaining 104 ~ 105 CFU/ml within 3〜7 days [1]. Our final object is to grasp the state and movement of bacteria and to predict subsequent changes by using NIRS and Aquaphotomics which observes the water molecule's structure comprehensively [2]. The results so far showed that each bacterial growth stage has specific spectral pattern. There is a possibility that contamination of bacteria and growth initiation can be identified by respective spectral patterns [3]. In this presentation, we examined whether it is possible to define specific spectral patterns for bacteria with fast and slow growth to be further used for prediction.

Materials & Methods: In these experiments, we used 4 bacterial strains (*Sphingomonas*, *Acidovorax*, *Pseudomonas*, and *Curvibacter*) to inoculate Suntory mineral water (50 ml). The initial number of bacteria was about 102 CFU/ml. As control, spectra of sterilized mineral water samples were measured. NIR spectra were acquired in the range of 400 ~ 2500 nm using XDS - Rapid Liquid Analyzer (FOSS. Co., Ltd) with 1 mm optical path length. All samples were measured with 3 consecutives spectral measurements. We counted the number of viable cells by a standard plate count method. Culture medium was R2A agar. In this presentation, we show results of the following 2 types of experiments.

- Experiment I (Term 1, 2)
  Strains: *Sphingomonas*, *Acidovorax*, *Pseudomonas*
  Cultivate & Measurement temperature: 25 ℃
  Measurement points: 7 points in 72 hours
- Experiment II (Term 3, 4)
  Strains: *Acidovorax*, *Pseudomonas*, *Curvibacter*
  Cultivate & Measurement temperature: 29 ℃
  Measurement points: 11 points in 800 minutes

For understanding the differences between bacteria with fast and slow growth, we analyzed lag phase and log phase separately in 600 ~ 1090 nm (the second and third overtone of water), 1110 ~ 1300 nm (the first overtone of water combination bands) and 1300 ~ 1600 nm (the first overtone of water) wavelength ranges. SIMCA classification method and subtracted spectra (averaged spectra of bacteria with fast growth – averaged spectra of

bacteria with slow growth) were used to define the specific spectral patterns of the two investigated types of bacteria.

Results and Discussion: High classification accuracy was obtained for all investigated spectral regions, Fig1. Especially noteworthy results were obtained for the 1300 ~ 1600 nm (1st overtone of water). These accuracies are averaged values of 4 experiment results. Discriminating Powers of SIMCA in the range of 1300 ~ 1600 nm showed that there were common peaks which were considered to be effective to classify the bacterial growth ability on the short wavelength range during lag phase and long wavelength range during log phase as shown in Fig2. When observing subtracted spectra, there were common peaks with Discriminating Powers. When observing both Discriminating Power and subtracted spectra in the range of 1300~1600 nm, common peaks in lag phase were in the less hydrogen bonded water region. On the other hand, common peaks in log phase were seen in the hydrogen bonded water region. It has been discovered that bacteria spectral pattern even in the lag phase can be used to predict further bacterial growth. The results of assignment of the Discriminating Power are shown in Table1. The water structures shown in Table1 reflect the differences between bacteria with fast and slow growth. In particular, many wavelengths were assigned to protonated water structures.

From the above, we can state that NIRS and Aquaphotomics could classify bacteria with fast and slow growth by analyzing the water molecule structure in each initial stage of development.

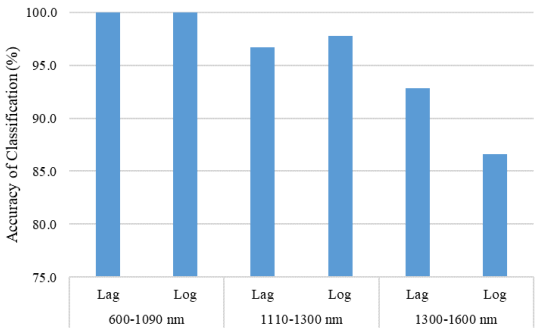

Fig1. Accuracy of classification for bacteria that increased and did not increased using SIMCA at each growth stage

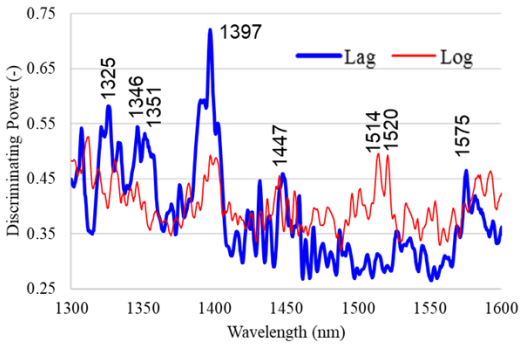

Fig2. Averaged Discriminating Power at each growth stage for identifying the bacterial growth ability (1300 ~ 1600 nm)

Table1. Main peak assignments of Discriminating Power in lag phase (1300~1600nm)

Peaks	Assignment
1325 nm, 1346 nm, 1351 nm	Protonated water
1397 nm	Trapped water
1447 nm	Water hydration shell

References:

[1] H. Leclerc, A. Moreau, Microbiological safety of natural mineral water, FEMS Microbiology Reviews 26, 207-222, (2002)

[2] Tsenkova R, Aquaphotomics: dynamic spectroscopy of aqueous and biological systems describes peculiarities of water, J Near Infrared Spec 17, 303–314 (2009)

[3] Yuki Nakagawa, R. Tsenkova, Development of a technology for detecting bacterial growth by means of NIRS and Aquaphotomics, Proceedings of the 33rd NIR Forum, 116 (2017)