Nondestructive chemical analysis of Lactobacillus plantarum biofilms with time- and space-resolved Raman microspectroscopy

Abstract of Master's Thesis, 2018

Nondestructive chemical analysis of *Lactobacillus plantarum* biofilms with time- and space-resolved Raman microspectroscopy

Graduate School of Science and Technology, Kwansei Gakuin University

Department of Chemistry, Shigeto Lab. , Himanshu Kumar Yadav

Biofilms are communities of microbial cells that are adherent to a surface and usually enclosed in extracellular polymeric substances (EPS). Biofilms formed by *Lactobacillus* species are, in many cases, relevant to food production and preservation, as well as probiotics. It is therefore highly important to study *Lactobacillus* biofilms from a chemical point of view. Here, we used confocal Raman microspectroscopy for chemical component analysis of *L. plantarum* biofilms. Raman spectroscopy is a noninvasive, nondestructive optical technique and is very powerful for looking into biological systems including biofilms as they are. In present study, we measured time- and space-resolved Raman spectra of *L. plantarum* biofilms under aerobic and anaerobic conditions and *L. plantarum* planktonic cells under aerobic conditions in order to elucidate how their chemical compositions change depending on culture time and growth phases.

Biofilms of *L. plantarum* JCM1149 (provided by the Nomura group at the University of Tsukuba) were formed on a glass bottom dish containing MRS broth.[1] Pouch-Bags with AneroPouch-Anaero were used for preparing samples under anaerobic conditions, whereas glass bottom dishes sealed with a parafilm tape was used for preparing samples under aerobic conditions. After cell culture, the liquid medium in the glass bottom dish was removed, and the remaining biofilm was rinsed thrice and supplied with PBS(-). *L. plantarum* planktonic cells were cultured in the MRS broth. After cell culture, a 1 mL portion of the medium was centrifuged thrice at 10000 rpm for 1 min, supplied with 1 mL of PBS(-), and eventually transferred to a glass bottom dish for Raman measurements. Time- and space-resolved Raman spectra of the biofilm samples were recorded with a laboratory-built confocal Raman microspectrometer. The excitation wavelength was 632.8 nm, and the laser power at the sample point was ~4.0 mW. The acquisition time used for biofilm measurements was 150 s. For imaging experiments of *L. plantarum* biofilms, a high-precision piezoelectric translation stage was used as a scanning device.

The observed Raman spectra exhibit Raman bands at 1575, 1480, 1247, 813, 783, and 723cm^{-1}, all of which are assigned to nucleic acids. We found that the concentration of nucleic acids in anaerobic conditions of biofilm increases in going from the lag phase to the log phase (0-10 h), and then decreases in going from the log phase to the stationary phase (10-30 h). The rise in concentration of nucleic acid during log phase is due to rapid multiplication of *L. plantarum* cells during log phase. In the sharp contrast in aerobic condition of biofilm the concentration of nucleic acid decreases from lag phase to stationary phase, in planktonic cell nucleic acid trend is same as biofilm in anaerobic conditions. The observed Raman spectra also exhibit Raman bands of proteins at around 1650 and 1003 cm^{-1}, which are assigned to the amide I mode and the ring-breathing mode of the phenylalanine residue, respectively. The concentration of protein did not change as much in any of these cases. We observed broad Raman bands at ~920, ~1155, and 1370–1410 cm^{-1}, which may be assigned to polysaccharides. The concentrations of polysaccharides increase under both anaerobic and aerobic conditions, but they do not vary significantly in planktonic cells . This finding suggests that polysaccharide concentration is higher in biofilms than in planktonic cells. We also use stable isotope labelling (in present case ^{13}C glucose is used) to see isotopic shift in 48 hours of biofilm spectra and found only isotopic shift for polysaccharides like 913 cm^{-1} band shifted to 895cm^{-1} , 855 cm^{-1} shifted to 825 cm^{-1}. We use univariate analysis to construct Raman images, with this univariate approach using a specific band area directly reflect molecular distributions within biofilms. We also use multivariate curve resolution-alternating least square analysis which is a quite powerful tool for separating pure component spectra from complex spectra of biofilms.

In conclusion, we have demonstrated that Raman microspectroscopy enables us to reveal different trends in culture-time dependence for *L. plantarum* biofilms with and without the presence of oxygen and their planktonic cells.

Keywords confocal Raman microspectroscopy, biofilms, growth phase, univariate analysis, multivariate curve resolution-alternating least square analysis.