1B2b

A new experimental approach to determine freezing temperature of micrometer-sized aqueous droplets containing ice-nucleation proteins

Kohei Fukugi, Shoji Ishizaka

Department of Chemistry, Graduate School of Science, Hiroshima University

1, Introduction

Xanthomonas campestris is a type of bacteria found in diseased barley plants [1]. It has been reported that *Xanthomonas campestris* triggers intracellular ice formation in tissues of frost-sensitive plants and gives rise to subsequent frost injury. The bacteria have also been found in the atmosphere, the role of them in cloud formation and precipitation is a topic of increasing interest [2]. However, the molecular mechanism to increase ice nucleation probability in water is still unclear. In order to quantitatively evaluate ice nucleating activity of ice-nucleation bacteria in cloud water droplets, it is necessary to observe freezing events of water droplets in air without contacting a solid surface. In this study, the freezing temperature of micrometer-sized aqueous droplets containing *Xanthomonas campestris* was measured by using laser trapping technique.

2, Experimental

Ultraviolet lightsterilized and lyophilized *Xanthomonas campestris* (Wako Pure Chemical Industries Ltd.) was ground and used for experiments. Aqueous ammonium sulfate droplets containing *Xanthomonas campestris* were generated by an ultrasonic nebulizer (Omron, NE-U07) and introduced into a sample chamber set on the stage of an inverted optical microscope (Olympus, IX71). Single droplets were trapped by focused laser beam (532 nm, Coherent, Verdi) through an objective lens (×60, NA=0.6). Temperature of trapped droplets was controlled by tuning the flow rate of nitrogen gas stream passed through a glass tube dipped in a liquid N_2 bath.

3, Results and discussion

Single aqueous ammonium sulfate droplet in air is shown in Fig.1a. The droplet was frozen in -53 °C (Fig.1b). Since the trapping laser beam can be used simultaneously as an excitation light source for Raman spectroscopy, *in situ* characterization of ice particles levitated in air can be achieved by means of Raman spectroscopy. As shown Fig.2, intense peak was observed at around 3145 cm^{-1}, which is OH stretching vibration of ice. Aqueous droplets containing ice-nucleation bacteria were frozen at temperature on the same level with pure water.

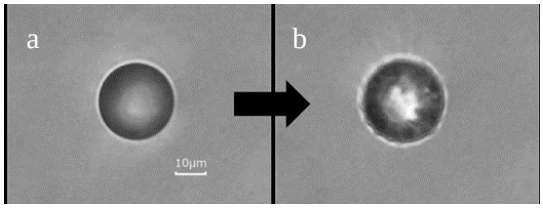

Fig.1 Micrometer-sized aqueous droplet was frozen at -53 °C

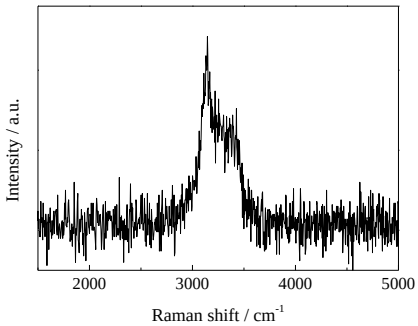

Fig.2 Raman spectrum of ice in air

[1] Kim, H. K., Orser, C., Lindow, S.E., and Sands, D.C., 1987. Plant Disease 71:994-997

[2] Janine Frohlich-Nowoisky, et al., Atmospheric Research 182 (2016) 346-376