Influenza virus inactivation by effective micro-organisms fermented liquid (EM・1)
The 62nd Annual Meeting of the Japanese Society for Virology, Yokohama, 2014

Sairenji, T. [1), 2)], Fujii, H. [2)], Kunihiro, M. [1), 2)], Tanaka, R. [2)], Tanaka, Y. [2)], Shintani, M.[1)], Okumoto, S. [1)], Higa, T. [3)]

1) EM Research Organization, Inc.
2) Department of Immunology, Graduate School of Medicine, University of the Ryukyus.
3) Meio University.

[Objective]
An effective prevention of viral infection is to inactivate the virus. EM-1 is a microbial inoculant and consists of lactic acid bacteria, yeast and phototrophic bacteria. EM-1 is used in various fields, such as in agriculture, animal husbandry, aquaculture and environmental conservation. Also, EM・1 is told to have the preventive effects against animal and plant pathogens. We reported that EM・1 inactivates the herpes simplex virus (The 61st Annual Meeting of the Japanese Society of Virology, 2013). In this time, we present that EM・1 is also able to inactivate the influenza virus.

[Materials and Methods]
Commercial EM-1 was used. Influenza virus (A/PR/8) was propagated in embryonated chicken eggs. EM-1 was diluted with sterilized water or physiological saline and mixed with the virus solution and reacted to the indicated time and temperature. The reaction was stopped by the addition with the ice cold DMEM culture medium containing 1% fetal bovine serum. The remained virus infectivity in the solution was measured by $TCID_{50}$ (tissue culture infective dose for 50%) /ml, which was determined by the cytopathic effect (CPE) induced with virus infection to MDCK cells. The solution was serially 10-fold diluted and the 0.1 ml of each dilution / well was added to the MDCK cell culture on 96-well microplates. After being cultured for 4 - 5 days, the CPE appearance was determined using an inverted microscope.

[Results]
The virus infectivity was suppressed by the reaction of virus and EM-1 diluted with sterilized water at room temperature for 10 minutes. The suppression depended on EM-1 concentration. The undiluted EM-1 was pH 3.3 and the pH increased gradually with the dilution and the 10,000-fold dilution was still pH5.3. The viral infectivity was completely suppressed with 100-fold dilution of EM・1 for 10 minutes at room

temperature, and the partial suppression was observed with 1,000- fold dilution. When EM-1 was diluted with phosphate buffered saline (pH 7.2), or neutralized by alkaline solution, no suppression of the virus infectivity was observed. The suppression of the virus infectivity by EM-1 occurred when the pH level was lower than pH 4.3 at both room temperature and in ice bath within 5 minutes.

[Discussion]

We confirmed that EM・1 has the activity of the acidic pH dependent influenza virus inactivation. EM-1 is registered as a stockbreeding feed of Japanese Agricultural Standard (JAS) and also used as an odor eliminator for poultry houses and barns. These results suggest the possibility that EM-1 may prevent the epidemic influenza by suppressing of the avian and swine flu.