**Note**

**Photocatalysis-dependent Inactivation of Lactobacillus Phage PL-1 by a Ceramics Preparation**

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A ceramics preparation (Cleansand-205), which was coated with a mixture of the oxides of Si, Al, Ti, and Ag, was found to inactivate *Lactobacillus* phage PL-1 suspended in a buffer solution. The inactivation of phage was dependent on the amounts of Cleansand-205 added, and the reaction obeyed almost first-order reaction kinetics. The phage inactivation was considerably accelerated by the presence of light.

**Key words:** ceramics, *Lactobacillus* phage; phage inactivation; photocatalysis; active oxygens

Recently, more attention has been given to the various biological activities of ceramics preparations, and some ceramics preparations have been shown to have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.[1-4] However, the detailed mechanism of the ceramics preparations to kill the bacterial cells has not yet been fully explained, although, in some cases, involvement of active oxygens generated in the water in the presence of light, has been suggested. In a previous paper, [5] we found that a granular ceramics preparation, Cleansand-205, inhibited somewhat the growth of algae in a domestic goldfish water tank and that no antialgal substances including heavy metal ions were dissolved in the water. In this paper, therefore, in one of the attempts to discover the action mechanism of a ceramics preparation in more detail, we have tested the virus-inactivating effect of Cleansand-205 using *Lactobacillus* phage PL-1, since the structure of this virus is much simpler than that of cellular organisms such as bacteria and algae.

The Cleansand-205 used was prepared by coating silica sand (40 kg) with a liquefied mixture (1 kg) of *SiO₂*, *Al₂O₃*, *TiO₂*, and Ag (wt %, 7.2, 22, 9, 3, and 1.7) and sintered at about 200°C for 2 h. The average particle diameter of Cleansand-205 was 2.0 ± 1.2 mm. Phage PL-1 of *Lactobacillus casei* ATCC 27095[5-7] was used as a representative virus, simply because it was available in our laboratory.

To test the effect of Cleansand-205 on PL-1 phages, various amounts of Cleansand-205 were added to the phage samples (2.6 × 10⁶ ph/ml) suspended in 5 ml of a sterilized buffer commonly used for the storage of PL-1 phage (10 mm phosphate buffer (pH 7.0), 1 mm MgSO₄, 17 mm NaCl, and 0.003% gelatin), and the mixture was incubated in a water bath (25°C) in a room, over which a fluorescent lamp (Toshiba FLR-40S type, 36 W, the direct brightness over the container (test tube) of the reaction mixture was about 10,000 lux) was illuminated from 9:00 am to 7:00 pm every day. As a control, some of the reaction mixture was covered with aluminum foil to shield it from light. In this case, since Cleansand-205 added was deposited at the bottom of test tube, the test tube was shaken gently once a day to make the Cleansand-205 particles come into contact with the PL-1 phages. At regular times, a 0.05 ml sample was taken aseptically from the supernatants and the numbers of surviving phages were assayed by the usual soft-agar layer method[6] and expressed as plaque-forming units per ml (pfu/ml).

The course of the phage inactivation is shown in Fig., where the relative number of surviving phages against that at zero time incubation is plotted semilogarithmically as a function of time. The values of the relative survivors were the average of three experiments, and the standard deviations were depicted by bars only with 5% (g/v) Cleansand-205 as an example as shown in Fig. (A). The standard deviations were in general negligibly small in the early stages of incubation, but in the later, from the time when the relative survival fell to less than 10⁻⁷, they became progressively greater with the passage of incubation time. As Fig. (A) shows, when PL-1 phages were incubated with Cleansand-205 in the presence of light, the phages were inactivated according to almost first-order reaction kinetics, similar to the process of one-hit mechanism by the dart-target theory of Stahl.[9] In this case, the phage inactivation occurred spontaneously to some extent even in the absence of Cleansand-205, but it did more extensively with the increase in the amounts of Cleansand-205 added, showing that Cleansand-205 itself has an activity to inactivate PL-1 phages.

With 5% (g/v) Cleansand-205, the relative survivors fell to 1.5 × 10⁻² after 100 days, 2.8 × 10⁻² after 200 days, and 1.0 × 10⁻³ after 300 days, respectively.

On the other hand, as shown in Fig. (B), when the reaction mixture was not illuminated, namely covered with aluminum foil to shield it from light, Cleansand-205 had very little activity. The relative survivors with 5% (g/v) Cleansand-205 after 300 days maintained the level of 1.3 × 10⁻², which was the same value as that without both Cleansand-205 and light. These results showed that Cleansand-205 inactivated PL-1 phages and that the phage inactivation was accelerated by the presence of light. The solution of any harmful heavy metal ions from Cleansand-205 was not observed as shown in a previous paper.[5]

Then, the generation of superoxide anion (O₂⁻) in the Clean-
sand-205-containing water was examined, since it is known that some metal oxides like titanium oxide having the properties of semiconductors reduce the oxygen dissolved in water photocatalytically to give what are called active oxygens, and that active oxygens are responsible for various kinds of physiological phenomena including DNA breakage.[10-12] The concentration of superoxide anion was measured by the method of Beauchamp and Fridovich.[13] As the result, the amounts of O₂⁻ measured as ΔOD₅₅₀nm/min × 100 in the water, to which final 5% (g/v) Cleansand-205 were added and kept at 25°C with illumination for various times from 0 h to 10 days, were all only about 0.00011, which was the same value as that of the background without both Cleansand-205 and light. Therefore, at present, it is not clear why Cleansand-205 inactivated PL-1 phages and why the phage inactivation was accelerated by the presence of light.

In the cases of some DNA-breaking agents such as L-ascorbic acid,[14] mitomycin C,[15] and β-glucosamine,[16] the generation of active oxygens was detected easily in their water solutions, possibly because these substances are water-soluble and are freely dispersed in their water solutions, thereby generating active oxygen constantly in the surroundings. On the other hand, since
Cleansand-205 is a water-insoluble solid material, even if the active oxygen would have been generated photocatalytically on the surface of Cleansand-205, they might have been quenched soon by the surrounding water and would not diffuse into the surroundings as widely and homogeneously as in the cases of water-soluble substances. To confirm the generation of active oxygen and their involvement in the antiviral activity of Cleansand-205 in the presence of light, use of the inhibitors or quenchers of active oxygen may be useful.

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References