# Purification and characterization of the virus TSV1 in Thermococcus sp. TS1

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Thermococcus sp. TS1 isolated from the Suiyo Seamount changed its morphology during growth. A normal cocci with diameter of 1 micrometer were observed in the early stage of growth, but they were deformed to cocci with intracellular spot, and then to transparent cells with the spot by overnight culture. Since single colony isolation had been performed for TS1, this phenomenon should not be due to contamination of other microorganisms.

To get insight into mechanism of the morphological change, we measured growth curve of TS1 as well as the ATP concentration in the culture medium. The growth curve looked peculiar in that the density of cocci plus cocci with spot once decreased at 7.5 h after start of culture, then increased, and abruptly decreased from 9 h. In a mirror image to the decrease, the transparent cells increased, indicating the deformation of TS1 to transparent cells. The ATP concentration was mostly paralleled to the change in cocci plus cocci with spot except for the decrease at 7.5 h. This finding indicated that the transparent cells produce no ATP, and thus, they are likely to be dead or represent a resting form of TS1.

The decrease in the cell density at 7.5 h was reproducible, and we frequently observed large cocci with diameter of 3 micrometer after the decrease. These circumstantial evidences suggest that cell fusion may occur. Thus, we observed the cells 8 h after starting culture, fixing a filed of vision of phase contrast microscope. As expected, we found spontaneous cell fusion. We have also taken the video showing the cell fusion. This finding suggests that the cell fusion is the reason of the decrease of cell density at 7.5 h. Note that the diameter of the fused cell was about 3 micrometer, larger than that expected from the fusion of two cocci with 1-micrometer diameter. This finding suggests that swelling of the cell occurred along with the fusion. We frequently observed large cocci with spot as well as large transparent cells. These observations indicate that fused cells were also subject to the deformation.

In order to reveal what the spot is, we took transmission electron micrographs. Surprisingly, the spot was identified as a cluster of dense particles of about 50-nm diameter. Some particles looked hexagonal, suggesting the icosahedral structure characteristic to virus. The cytoplasm was present in the coccus and coccus with spot, but the cytoplasm of the transparent cells was almost vacant, which was consistent with the appearance of optical microscope. From these observations, we concluded that the spot represent virus that killed TS1, causing the deformation to the transparent cells. We named this virus TSV1.

TSV1 was liberated from the transparent cells of TS1 by sonication and collected as the precipitate of centrifugation at 30,000 rpm for 1 h at 4C. It was suspended in 100 mM Tris-HCl, pH 7.5, 10 mM MgCl2, and 3% NaCl (TMN medium), and treated with DNase I and RNase A, each at 10 microgram/ml, at 37 C for 20 min. The sample was subjected to sucrose density gradient centrifugation at 15,000 rpm for 1 h at 4C, where 10%, 20%, 30%, and 40% sucrose in TMN medium were stepwise layered. The virus particles were obtained from the 20% and 30% sucrose layers, diluted ten-fold with TMN medium, and collected by centrifugation at 30,000 rpm as precipitate. The virus was analyzed for the nucleic acid, protein components, and inter-domain infectivity to E. coli.