Supplemental Material

Isolation, and molecular characterization of ZLPs

Cells of *A. hospitalis* were grown aerobically at 75℃, pH 3, in the presence of elemental sulfur, as described previously (1, 2). Single strains of *A. hospitalis* were isolated from the culture by plating on colloidal sulfur-containing Gelrite (Kelco, San Diego, USA), as described by Zillig et al (3). Single isolates were screened for ZLP production. The strain YS8 of *A. hospitalis* was selected as a source for ZLPs.

YS8 were grown to OD_{600} = 0.1 and after removal of cells by low speed centrifugation (4500 rpm in a Beckman rotor JA-10) for 15 min, the cell-free supernatant was sequentially filtered through filters with pore sizes of 0.80, 0.45 and 0.20 µm (Supor®, Pall Gelman Laboratory, Ann Arbor, USA). The ZLP were collected from the filtrate and concentrated to the volume of 1.5 ml by filtration though Polyethersulfon ultrafilter Omiga™ with the exclusion size of 100 kDa (Pall Gelman Laboratory, Ann Arbor, USA). The filter was washed three times with 1 ml of H_2O and the resulting 3 ml were added to 1.5 ml concentrate. The ZLPs were collected from the suspension by ultracentrifugation in a Beckman rotor SW 60Ti, at 42000 rpm for 3.5 hours, re-suspended in 50 µl of 20 mM Tris-acetate, pH 6.

Analysis of the protein constituents of ZLPs by SDS-PAGE revealed two major bands of proteins with apparent molecular masses of 55±5 and 110±10 kDa and three minor bands of proteins with molecular masses exceeding 200 kDa (Figure 2A). As a result of prolonged treatment of the protein preparation with SDS and β-mercaptoethanol (10 min instead of 5 min, in the buffer containing 15% (w/v) of β-mercaptoethanol and 10% (w/v) SDS, at 95℃) prior to electrophoresis, the ratio between the intensities of the bands significantly increased in favour of the 55 kDa band.

Transmission electron microscopy

Negatively stained ZLPs were observed by transmission electron microscopy as described previously (2). Briefly, samples were examined using a CM12 transmission electron microscope (TEM) (FEI, Eindhoven, The Netherlands) operated at 120 keV.

The magnification was calibrated using catalase crystals, negatively stained with uranyl acetate. The images were digitally recorded using a slow-scan CCD-camera that was connected to a PC running TVIPS software (TVIPS GmbH, Gauting, Germany).

Quantification of the ZLP production

For quantification, ZLPs were collected from cell-free growth medium, as described above, suspended in H_2O and protein amount in the suspension was determined by measuring the absorption at 280 nm.

The induction of ZLP production resulting from cell treatment with mitomycin C (1 µg/ml, final concentration), UV-light (7 min in the layer of 3 mm, with mild agitation) and freezing in liquid nitrogen and quick thawing, was verified by comparing the amount of ZLPs in cultures of treated and untreated cells.

The decrease of ZLP production as a result of adaptation of cells to growth conditions was verified by comparing the amount of ZLPs in cultures after successive steps of dilution 1:1000 followed by further growth to the standard OD_{600} = 0.1. In these experiments, the ZLP amount was measured in 50 ml cultures as follows. After reaching OD_{600} = 0.1, a 50 µl aliquot of the culture was used for inoculation of 50 ml medium, and from the rest ZLPs were purified. For this, cells were removed by low speed centrifugation (4000 rpm for 15 min in a Beckman rotor JA-10) and the cell-free supernatant was filtered through Acrodisc® PF 0.8/0.2 µm filter (Pall Gelman laboratory, Ann Arbor, USA). Nine ml of the filtrate were subjected to ultracentrifugation at 42000 rpm for 3.5 hours in a Beckman rotor SW 60Ti. The pellet was suspended in H_2O, lyophilized, re-suspended in 50 µl H_2O and A_{280} of the suspension was determined. In six successive steps of dilution, the ZLP amount continuously decreased, and eventually, after the sixth round of dilution the presence of ZLPs could not be detected. If the adapted cells were treated with stress-inducing factors as described above, the production of ZLPs was resumed.

References

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2. **Rachel, R., M. Bettstetter, B. P. Hedlund, M. Haring, A. Kessler, K. O. Stetter, and D. Prangishvili.** 2002. Remarkable morphological diversity of viruses and virus-like particles in hot terrestrial environments. Arch Virol **147:**2419-2429.
3. **Zillig, W., A. Kletzin, C. Schleper, I. Holz, D. Janekovic, J. Hain, M. Lanzendorfer, and K. J.** 1993. Screening for *Sulfolobales*, their plasmids and their viruses in Icelandic solfataras. Syst Appl Microbiol **16:**609-628.