Thermotolerance of *Thermococcus* sp. strain Tc-1-95 and its key mechanism for thermal death

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**Purpose of study**

Thermotolerance of hyperthermophiles at temperatures above the maximum limits for growth is basic information for estimating the ecological distribution of hyperthermophiles in submarine and terrestrial hydrothermal systems, as well as for understanding the mechanism of their thermal adaptation. Recently we developed a flow-type apparatus aiming to precisely measure the thermal stability of hyperthermophiles and their biological molecules (1). This apparatus is a unique system capable of exposing aqueous samples to high temperatures and pressures (up to 400°C and 30 MPa, respectively) under aerobic or anaerobic condition. Heat-treatment time is accurately controlled within the range of only a few seconds to some tens of minutes.

In this study, employing this apparatus, we investigated the thermotolerance of *Thermococcus* sp. strain Tc-1-95 at temperatures above the maximum limit for growth. Moreover, we examined its key mechanism for thermal death.

**Methods**

*Thermococcus* sp. strain Tc-1-95 is a hyperthermophilic archaeon, which was isolated from an active deep-sea hydrothermal field (water depth 2,422 m) in the Central Indian Ridge (2). It grows at <80 to 100°C (optimum 90°C). The cell culture at the late-exponential growth phase was heated at temperatures between 95 and 125°C for 1.5 sec. Experiments were performed at 0.5 MPa and 25 MPa, the latter of which corresponds to the hydrostatic pressure at the depth of isolation. The survival rate was estimated by the three-tube most-probable-number technique. Change of cell morphology was observed by epifluorescent and electron microscopies.

**Results**

At 0.5 MPa, no decrease of the survival rate was observed even at 114 °C. The survival rates decreased with temperature above 114 °C, and no viable cell was detected at 122°C. The thermostable temperature was raised up to 118 °C, when the hydrostatic pressure of 25 MPa was applied.

Interestingly, it was found that the thermal death of *T.* sp. strain Tc-1-95 is associated with the rupture of the cell envelope structure. The deformation of surface-layer (S-layer) due to the lethal heat treatment was observed by transmission electron microscopy.

**Conclusion**

The majority of archaea lacking a rigid wall layer, S-layer proteins are the only cell envelope component and they form natural two-dimensional protein array, covering the cell completely. The inactivation of *T.* sp. strain Tc-1-95 at lethal high temperatures is most possibly attributed to the rupture of cell envelope structure, which is triggered by the deformation of S-layer. This is in marked contrast to the thermal death of meso- and thermophilic bacteria and yeast, where the thermal denaturation of ribosome is the primary process. To our best knowledge, this is the first study revealing the key molecular mechanism for the thermal death of a hyperthermophile. Furthermore, It is predicted that the same thermal killing mechanism might be true for the majority of hyperthermophilic archaeal organisms as they share the same cell envelope structure.

High hydrostatic pressure may induce the elevated thermal stability of the cell envelope system and this effect may primarily contribute to the pressure-induced thermal adaptation of the microorganism.

**References**