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# Daily lamination formed by sulfur oxidizing bacteria and chloroflexus in a hot spring stromatolite

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Travertines are carbonate precipitates from hot-spring water containing a sufficient amount of calcium and carbon dioxide. Most of travertines show sub-mm order laminations that were resemble to ancient stromatolites. Recently, daily microbial processes were identified in some travertines precipitated from some sulfide-poor and mid temperature (<55 degree C) springs. In the processes, daily growth of biofilms consisting cyanobacteria and heterotrophic bacteria, which inhibited inorganic mineral precipitation [1]. While, lamination is less common in the travertines at higher-temperature (>60 degree C) and sulfide-rich springs [2] likely because such cyanobacteria-associated daily processes might not be occurred. To understand the geomicrobiological system in high temperature and sulfide-rich spring, this study investigates a travertine in Sipoholon, Northern Sumatra, Indonesia.

Sipoholon hot spring forms the hugest travertine mound among the hot springs in Tarutung area located about 30 km south from the Lake Toba. The travertine mound spread in total area of 50,000 $m^2$, which has three main vent areas. In all area, sulfur-turf and sulfur-rich yellow sediments were formed near the vent, while laminated sediment was formed from midstream to downstream. The surface color of the laminated travertine was changed with water temperature and $H_2S$ concentration; pale pink around 55 degree C and 1-6 μM-$H_2S$, while green below 50 degree C and <1 μM-$H_2S$. The pale pink travertine exhibited lamination despite sulfide-rich condition.

Sequences of water and travertine samples collected from a pale pink travertine sites every 4 hours during 48 hours showed that the dark colored biofilm layer was formed during daytime and light colored crystalline layer was formed during nighttime. No daily variation was detected in pH, water temperature, Ca ion concentration, alkalinity, and flow. While dissolved oxygen concentration showed the daily variation, which was higher during the daytime and lower during the nighttime. Phylogenetic analysis on 16S rRNA gene showed that *Tiofava,* an obligatory chemolithoautotophic sulfur-oxidizing bacteria was dominated in the pale pink travertine. The pale pink color likely resulted from the pigment of *Chloroflexus* at the heterotrophic growth [3]. Thermophilic coccoidal cyanobacteria were identified as a minor component, and locally distributed in the biofilm.

These results suggest that *Thiofaba-Chloroflexus* biofilm formation likely responsible for the lamina formation of the pink travertine, which was stimulated by daytime increment of oxygen concentration.

This is a novel microbial process forming stromatolite. This could be occurred in ancient stromatolites formed under the suboxic sulfide-rich ancient ocean.

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