Visualization of superoxide generated from colonies of *Candida albicans*

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Chemiluminescent (CL) visualization of superoxide generated by *Candida albicans* was conducted. The wild type strain K of *Candida albicans* cultured overnight in liquid PYG medium at 37 °C with shaking was diluted and 0.1ml of dilutions containing 50 cells was spread on PYG agar plates. After incubation at 37 °C for one to 5 days, reactive oxygen species (ROS) generation by colonies of *C. albicans* on the PYG agar plate was examined with a ultralow light image analyzer equipped with a photon-counting CCD camera.

To visualize ROS generated by *C. albicans*, methyl-Cypridina-luciferin analogue (MCLA) as a CL probe and the herbicide paraquat (PQ) as a respiration-dependent ROS generator were used. After taking photographs of colonies under light, a mixture of 0.1M PQ and 0.05mM MCLA(1:1) was gently dropped onto the colonies. To examine the effects of antioxidants, SOD or L-cystein was added to the PQ-MCLA mixture. The MCLA-dependent CL due to ROS generated by the colonies was recorded for 5 min in a light-tight box.

CL from the colonies was observed at 1 day after incubation and whole of the colonies were weakly luminous. In parallel with the increase in the colony size after incubation for 3 and 5 days, the marginal regions of the colony were strongly bright. These results indicate that *Candida* colonies expand by division of metabolically active cells in the marginal regions, leaving aged cells in the central regions. CL from colonies almost completely vanished by the addition of SOD and/or L-cystein. In addition, we also succeeded the CL visualization of ROS generated by hyphal form cells of *C. albicans* in 96-well plate. To our knowledge, the present report is the first CL visualization of ROS including superoxide generated by *C. albicans*. 