

## **Directed evolution of recombinant c-terminal truncated staphylococcus epidermidis lipase AT2 for the enhancement of thermostability**

### **ABSTRACT**

In the industrial processes, lipases are expected to operate at temperatures above 45 °C and could retain activity in organic solvents. Hence, a C-terminal truncated lipase from *Staphylococcus epidermis* AT2 (rT-M386) was engineered by directed evolution. A mutant with glycine-to-cysteine substitution (G210C) demonstrated a remarkable improvement of thermostability, whereby the mutation enhanced the activity five-fold when compared to the rT-M386 at 50 °C. The rT-M386 and G210C lipases were purified concurrently using GST-affinity chromatography. The biochemical and biophysical properties of both enzymes were investigated. The G210C lipase showed a higher optimum temperature (45 °C) and displayed a more prolonged half-life in the range of 40-60 °C as compared to rT-M386. Both lipases exhibited optimal activity and stability at pH 8. The G210C showed the highest stability in the presence of polar organic solvents at 50 °C compared to the rT-M386. Denatured protein analysis presented a significant change in the molecular ellipticity value above 60 °C, which verified the experimental result on the temperature and thermostability profile of G210C.

**Keyword:** Directed evolution; Staphylococcal lipases; Thermostability; Characterization; Circular dichroism