ABSTRACT

Background: Recent interest in marine phospholipids rich in omega-3 polyunsaturated fatty acids has great potential to use in dietary supplements, cosmetics, food processing, and pharmaceutical applications. Lipids-rich heterotrophic marine microalga Thraustochytrids have great capability as a sustainable source of PUFAs. The components of the thraustochytrid single cell oils (SCOs) are mainly neutral lipids (NLs or triacylglycerols, TAGs), phospholipids (PLs) and unsaponifiable matter. Stress-induced fermentation optimization, including non-genetic techniques (such as adaptive laboratory evolution, ALE) can improve the thraustochytrid strains resulting in the production of high omega-3-enriched oils. ALE can efficiently develop beneficial phenotypes in industrial microorganisms without changing cell genetics. However, omega-3 enriched PLs have significant health applications compared to that of TAGs. Thus, the main objective of present study was to develop an improved thraustochytrid strain through ALE process that can produce omega-3 enriched-PLs for health applications.

Results: The cooperative two-stress ALE strategy with low temperature (4ºC) and high salinity (30 g/L NaCl) increased *Schizochytrium sp* DT3 strain and production. After 16 adaptation cycles, the most evolved strain ALE-13 had a maximal CDEW of 22.1 g/L without salt stress and lipid content of 49% with two cooperative stresses after 72h, which was 43% and 26% higher than parental strain. At 24 hours of fermentation, EPA, DHA, and n-3 PUFAs were 2%, 27.8% and 29.8% of total fatty acids (TFA) under two cooperative stress and 1.1% and 18.8% of TFA without salt stress. The values were 49%, 43%, and 40% higher than parent strain. Also, NLs showed n-3-enriched PLs lipid turnover within 48h.

Conclusion: This study shows that the cooperative two-stress ALE process increases DHA-rich lipid accumulation and TAG lipid turnover into PLs.