Expression of cocoa genes in Saccharomyces cerevisiae improves cocoa butter production

Background: Cocoa butter (CB) extracted from cocoa beans (Theobroma cacao) is the main raw material for chocolate production, but CB supply is insufficient due to the increased chocolate demand and limited CB production. CB is mainly composed of three different kinds of triacylglycerols (TAGs), 1,3-dipalmitoyl-2-oleoyl-glycerol (POP, C16:0–C18:1–C16:0), 1-palmitoyl-3-stearoyl-2-oleoyl-glycerol (POS, C16:0–C18:1–C18:0) and 1,3-distearoyl-2-oleoyl-glycerol (SOS, C18:0–C18:1–C18:0). In general, Saccharomyces cerevisiae produces TAGs as storage lipids, which consist of C16 and C18 fatty acids. However, cocoa butter-like lipids (CBL, which are composed of POP, POS and SOS) are not among the major TAG forms in yeast. TAG biosynthesis is mainly catalyzed by three enzymes: glycerol-3-phosphate acyltransferase (GPAT), lysophospholipid acyltransferase (LPAT) and diacylglycerol acyltransferase (DGAT), and it is essential to modulate the yeast TAG biosynthetic pathway for higher CBL production.

Results: We cloned seven GPAT genes and three LPAT genes from cocoa cDNA, in order to screen for CBL biosynthetic gene candidates. By expressing these cloned cocoa genes and two synthesized cocoa DGAT genes in S. cerevisiae, we successfully increased total fatty acid production, TAG production and CBL production in some of the strains. In the best producer, the potential CBL content was eightfold higher than the control strain, suggesting the cocoa genes expressed in this strain were functional and might be responsible for CBL biosynthesis. Moreover, the potential CBL content increased 134-fold over the control Y29-TcD1 (IMX581 sct1 triangle ale1 triangle lro1 triangle dga1 triangle with TcDGAT1 expression) in strain Y29-441 (IMX581 sct1 triangle ale1 triangle lro1 triangle dga1 triangle with TcGPAT4, TcLPAT4 and TcDGAT1 expression) further suggesting cocoa GPAT and LPAT genes functioned in yeast.

Conclusions: We demonstrated that cocoa TAG biosynthetic genes functioned in S. cerevisiae and identified cocoa genes that may be involved in CBL production. Moreover, we found that expression of some cocoa CBL biosynthetic genes improved potential CBL production in S. cerevisiae, showing that metabolic engineering of yeast for cocoa butter production can be realized by manipulating the key enzymes GPAT, LPAT and DGAT in the TAG biosynthetic pathway.