ABSTRACT

Over the past few years, a great deal of research has been implemented to develop yeast as a lipid-producing platform for biofuel or nutraceutical applications. Although many natural oleaginous (storage lipid content > 20% of dry cell weight) yeast species have been studied for this purpose, lack of substantial exploration of lipogenesis, scarcity of suitable genetic transformation tools, and extensive cost of scale-up processes have limited the scope of such developments in most of them. In this work, the prospects of cost-effective lipid production by a novel oleaginous isolate SY005 of the yeast *Candida tropicalis* was investigated, as a host for developing a lipid-producing platform. The lipogenesis and xylose utilization processes were investigated in-depth utilizing non-oleaginous and non-xylose utilizer *Saccharomyces cerevisiae* as heterologous expression system with an objective to identify important targets for genetic engineering, as SY005 displayed its efficiency in both of these attributes. Two sugar transporter proteins- CtStp1 and CtStp2 were identified and their functions in efficient xylose transport were established. While studying the lipid packaging system in SY005 strain, a novel lipid droplet protein (CtLdp1) was characterized for its role in lipid droplet proliferation. As a part of lipid metabolism in SY005, a transcription factor homolog of the repressor activator protein (Rap1) of *S. cerevisiae* was identified and studied for its function in upregulating lipid biogenesis. To engineer the natural isolate SY005 for augmented lipogenesis, a uracil auxotrophic strain and a genome-integrative transgene expression system were developed by employing SY005-specific genetic elements. With the help of this transformation system, the endogenous *CtRAP1* gene was ectopically expressed in SY005, leading to a substantial increase (60% *w/w*) in lipogenesis in the engineered strain. Enhanced expression of *CtRAP1*, in turn, upregulated the expression of key regulatory genes of triacylglycerol biosynthesis, justifying the enhancement of total lipid content in recombinant SY005 strain. The identified targets, genetic tools, and the recombinant yeast strain developed in this study successfully establish *C. tropicalis* SY005 as a suitable platform for further exploring and designing cost-effective processes for large-scale lipid production from cheap lignocellulosic substrates.

Keywords: *Candida tropicalis*, genetic engineering, heterologous expression, lipogenesis, oleaginous yeast, xylose utilization