

Abstract

Oleaginous microorganisms are capable of producing > 20% of their dry biomass as lipid, which is mostly triacylglycerol (TAG). There are several reasons to promote oleaginous microbes as complementary sources to plants and animals for lipid feedstock required for food and non-food industrial applications. In order to explore the novel oleaginous fungi including yeasts and molds, several isolates were screened from rhizosphere and plants. Four isolates of endophytic fungi and six isolates of rhizospheric yeasts were found to be oleaginous as revealed through lipid-specific Nile red staining. Initially, two endophytic fungi i.e., *Colletotrichum* sp. DM06 and *Alternaria* sp. DM09 along with one rhizospheric yeast i.e., *Candida tropicalis* SY005 were chosen for in-depth investigation on lipid productivity. Significant variations in lipid contents, ranging from 30–58% of their dry biomass were found in liquid culture using various carbon sources and nutrient-stress conditions. The fatty acid profiles of fungal lipids highlight their potential utilities as biodiesel feedstock, whereas the yeast lipid enriched with stearic acid makes it a potential substitute of coco-butter. Furthermore, investigation was carried out on the regulation of four key genes involved in TAG accumulation in *C. tropicalis* strain, where a drastic overexpression of diacylglycerol acyl transferase (DGAT) gene was found to be correlated with a particular nitrogen-stress (of C:N ratio 150:1) yielding highest lipid content. Cloning and sequence analysis revealed two homologues of type-2 DGAT gene, designated as *CtDGAT2a* and *CtDGAT2b* are differentially expressed in *C. tropicalis* strain during various nitrogen-stress conditions, and are responsible for enhanced TAG accumulation. Interestingly, the *CtDGAT2b* isozyme was found to be structurally different and catalytically ~12% more efficient compared to the *CtDGAT2a* for storage lipid production as revealed through heterologous expression in *Saccharomyces cerevisiae* mutant strain H1246 that is inherently defective in TAG biosynthesis. Therefore, in order to genetically improve the endophyte *Colletotrichum* sp. DM06 for enhanced TAG production, the *CtDGAT2b* gene was incorporated into the fungal genome through *Agrobacterium*-mediated transformation. Artificial neural network model-based optimization was carried out to maximize the lipid yield in the wild type and genetically modified stable transformant of *Colletotrichum* fungus. In optimized condition, the lipid titer of genetically modified *Colletotrichum* strain increased to ~2.9 fold over the wild type due to functional activity of the *CtDGAT2b* transgene. Taken together, the present study documents the proof of concept that a pivotal gene of lipid accumulation i.e., the *CtDGAT2b* from an oleaginous yeast has been successfully utilized for enhancement of lipid productivity in an oleaginous fungus through genetic modification coupled with bioprocess optimization.