Abstract

ABSTRACT

An appropriate balanced intake of omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs) promotes proper health, growth, brain & retinal development and homoeostasis of inflammatory responses. Plant seed oils are poor sources of n-3 PUFAs while marine fishes and fish oil are major source of n-3 PUFAs. Dietary habits, cost, biomagnifications of toxic compounds limit the use of marine fishes. Hence search of alternative sources of n-3 PUFAs is desirable. Yeasts might be the best competitor among different microbes for production of essential n-3 PUFAs. In the present work, seventy yeast isolates from different sources were screened for presence of omega-3 fatty acid desaturase gene (*fad*-3) by PCR, hybridization analysis and further for their ability to convert n-6 PUFAs to n-3 PUFAs mainly LA to ALA. Nineteen *fad*-3 positive isolates were identified that belonged to different genera *viz. Candida, Issatchenkia, Kodamaea, Meyerozyma, Pichia, Arxula* and *Rhodotorula.* Omega-3-fatty acid desaturase (Ct-*fad*-3) gene was isolated from *C. tropicalis* PS-2 by a two-step cloning strategy. Ct-*fad*-3 was subcloned and expressed in a yeast secretory expression vector pGAL-MF as a fusion protein with the yeast mating factor-α and inducible from GAL L promoter, in order to achieve extracellular expression. However the expressed protein was neither detected in culture supernatant nor in the cell extracts by SDS-PAGE gel. Functional expression of Ct-*fad*-3 gene was found to be cell associated. Heterologous expression of Ct-*fad*-3 was successfully achieved in *E. coli*, but was not functionally active. Polyclonal anti-FAD-3 antibodies against purified FAD-3 protein were generated in rabbit. The expression of FAD-3 was detected by ELISA and Western blot analysis using purified anti-Ct-FAD-3 IgG antibodies in the total proteins of recombinant *S. cerevisiae* (pSP-*fad*-3) but neither in the cell free supernatant nor in the vector control strain, which indicated its cell associated nature. Desaturase activity of Ct-FAD-3 was confirmed by the presence of ALA in the recombinant yeast as analysed by GC-MS of fatty acids. Ct-FAD-3 activity was found to be C18 specific and resulted in production of 12.2 mg/g dry cell weight ALA with 1:3-4 final ratios of n-3 and n-6 PUFAs. Our findings may give an insight regarding diversity in the yeasts containing PUFAs and their probable biotechnological utilization as source of n-3 PUFAs. The concept of obtaining essential n-3 PUFAs from *S. cerevisiae* in sustainable quantities for human consumption can be of special attraction and may be explored further for probiotic preparations.