LVII SAIB Meeting - XVI SAMIGE Meeting

SAIB - SAMIGE Joint Meeting
2021 *on line*

REGULATION OF K-RAS GENE EXPRESSION. INFLUENCE OF POST-TRANSCRIPTIONAL PROCESSING VARIANTS ON THE SUBCELLULAR LOCALIZATION

Mercedes Agustina Montani, Martín García Solá, Omar Adrián Coso
Universidad Nacional de Buenos Aires, Facultad de Ciencias Exactas y Naturales, IFIBYNE-CONICET, Argentina
E-mail: ma.montani@gmail.com

Cell proliferation is regulated by multiple factors in a healthy cell and the deregulation of any of the mechanisms in which these factors intervene can cause dysregulated proliferation of cells and constituting the disease we know as cancer. These signal transduction mechanisms, known as proliferative pathways, share the Ras oncogene product as a main component. This oncogene has been widely reported in the plasma membrane. However, recent studies demonstrated the importance of RAS in other membrane systems such as the Golgi Complex, where Ras isoforms were linked to apoptosis processes as a mechanism for preventing cell transformation. Also the K-Ras isoform has been reported in the mitochondrial outer membrane associated with another protein, being this union an apoptotic inducer. In the last two decades, other processes involved in the regulation of gene expression have gained interest in scientific studies, such as: mRNA stability, alternative polyadenylation or the subcellular distribution of proteins. Recent results from our laboratories show that the stability of messenger RNAs and alternative polyadenylation contribute to modulating the quantity and / or quality of these molecules to be translated. This mechanism leads alternative messenger RNA subpopulations to different subcellular compartments, contributing with a new functional regulation factor. In the present work, we evaluated the existence of different mRNAs generated by alternative polyadenylation and the subcellular distribution in murine SVEC and NIH3T3 lines of the transcription products of the KRas oncogene, which is known for its implication in the development of a wide variety of tumors. The results in control cells were compared with those that stably express the viral oncogene vGPCR, a G protein-coupled receptor that triggers tumorigenic effects in both endothelial cells and fibroblasts. By 3'RACE assays we have found that this oncogene presents alternative polyadenylation and differential expression of the isoforms between the lines evaluated. Moreover, we designed expression vectors that allowed us to observe by fluorescence microscopy, that different isoforms are found distributed in the plasma membrane, cytoplasm and endoplasmic reticulum. These results revealed that the alternative polyadenylation mechanism generates different isoforms of KRas messenger RNA, which vary according to the cell line and that this mechanism could be associated with the regulation of the subcellular location of the protein.

BIOTECHNOLOGY

BT-P01-17

LOW TEMPERATURE INDUCES PUFAs PRODUCTION IN A NATIVE-MICROALGA CRYPTOPHYTE *PLAGIOSELMIS* SP.

Almeyda MD [1,2], Scodelaro Bilbao PG [1, 2], Constenla D [3,4], Leonardi PI [1, 2].
1.Dpto. Biología, Bioquímica y Farmacia-UNS, 2. CERZOS, UNS-CONICET, 3. Dpto. Ingeniería Química-UNS, 4 PLAPIQUI UNS-CONICET. Bahía Blanca, Argentina.
E-mail: mdalmeyda@cerzos-conicet.gob.ar

The nutritional value of cryptophytes is of great importance due to the presence of high amounts of polyunsaturated fatty acids (PUFAs), sterols and amino acids. Therefore, native cryptophytes have biotechnological potential as a source of high-value products for nutraceutical and aquaculture industries. The synthesis of these metabolites is conditioned by both the strain and the cultivation conditions, being temperature one of the main factors for PUFAs synthesis. The objective of this work was to evaluate the effect of low temperature stress on the production of PUFAs and sterols in the marine cryptophyte *Plagioselmis* sp. cultivated in a photobioreactor. *Plagioselmis* sp. was isolated from Bahía Blanca's Estuary. Cultures were carried out for 10 days under two temperature conditions: 1) continuously at 20°C (Control) and 2) lowered to 11°C during the stationary growth phase (Low Temperature Stress, LTS). TAG and sterols were separated through thin layer chromatography (TLC) and quantified spectrophotometrically. Lipid extraction and fractionation into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) were performed. These fractions were analyzed by gas chromatography. LTS significantly increased lipid production by ≈40%. Both temperature conditions showed TAG and sterol accumulation within the days of cultivation. NL was the main lipid fraction (≈63% of Total Lipids, TL) followed by GL (≈32% of TL) and PL (≈5% of TL) for both temperature conditions. PUFA content (expressed as % of total FAME) was significantly higher in the LTS condition (41.3%) than in the control (35.71%), mainly due to PUFAs from the NL fraction. Omega-3 fatty acids (ω-3 FAs) represented 19.62% of the Control and 22.72% of the LTS condition, while ω-6 FAs comprised 16.09% (Control) and 18.65% (SLT). The most abundant PUFAs were eicosapentaenoic (EPA) and docosapentaenoic (DPA), which significantly increased due to LTS from 14.6% to 18.17% (EPA) and from 5.35 to 9.29% (DPA). Under LTS the production of PUFAs was of 13.5 mg L^{-1} being 7.41 mg L^{-1} ω-3 FAs and 6.09 mg L^{-1} ω-6 FAs. The production of EPA and DPA FAs was 5.93 mg L^{-1} and 3.03 mg L^{-1}, respectively. The results point out the potential of the native microalga *Plagioselmis* sp. to develop a sustainable biotechnological system for the production of PUFAs with nutraceutical and aquaculture applications.

BT-P02-40