VI2-O-002

Effect of osmotic downshock treatment on the yield of ectoine synthesized by Halomonas sp. EG6
Omneya Osman *, Shoko Tanabe-Hosoi, Schinichi Nagata
Environmental Biochemistry Group, Research Center for Inland Seas, Kobe University, 1-1 Hikae, Higashinada-ku, Kobe 658-0022, Japan
E-mail address: nagata@maritime.kobe-u.ac.jp (O. Osman).

Halophilic bacterium strain EG6 was isolated from Burg el Arab solar saltern lake in Egypt. Strain EG6 can grow in a wide range of NaCl concentrations up to 4 M. Phylogenetic position was established by 16S rRNA gene sequencing as a member of the genus Halomonas. Morphological, physiological and biochemical tests were done to characterize strain EG6 Leifson (1963). Strain EG6 produced ectoine as the main compatible solute for osmotic stress adaptation (Csonka, 1989; Galinski and Trüper, 1994). Our aim was to establish the best condition of osmotic downshock required for high yield of ectoine production using strain EG6 and compare it with one of the type strains H. elongata IFO15536 (Nagata et al., 2008). We subjected strain EG6 to osmotic downshock treatment with different NaCl concentration from 2 M to 0–0.7 M to determine how much concentration of NaCl was optimum for high ectoine yield. HPLC and NMR analysis were used to quantify and confirm ectoine purity, respectively. It was interesting to notice that the cells of strain EG6 had a great flexibility to withstand the sudden change in NaCl concentration, especially when cells were subjected to osmotic downshock treatment from 2 M to 0.3 M NaCl. That advantage was due to cellular ability to compensate the loss of ectoine with high efficiency. This strain showed significant yield of ectoine, about 3.70 g/L was totally released after 7 days of osmotic downshock. H. elongata IFO15536 had a very low yield of total ectoine released, 2.89 g/L, than that of strain EG6 under same conditions (Table 1). In addition, growth of strain EG6 was highly increased during downshock treatment in comparison to H. elongata IFO15536. Thus, we concluded that 0.3 M NaCl was the optimum concentration for getting the maximum yield of ectoine from strain EG6 with high purity which can be used for large scale ectoine production. Accordingly, strain EG6 can be classified as a new species, Halomonas sp. EG6 which is of interest in the future studies.

VI2-O-004

An expressed sequence tag approach for cloning of phytochelatin synthase cdna clone from Eucheuma denticulatum (Rhodophyta)
Roohaida Othman 1,2, *, Diana Mohd Nor 1, Hasbullah Daud 1, Adura Mohd Adnan 2
1 Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
2 School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
E-mail address: roohaida@ukm.my (R. Othman).

Eucheuma denticulatum is an economically important carrageenophyte found in the Indo-Pacific region mainly algal reef areas of islands in Southeast Asia (Doty, 1987). Expressed sequence tags (EST) approach was undertaken to investigate gene expression in this red alga. Several cDNA libraries were constructed from whole plants where a total of 10,087 ESTs were obtained. Cluster analysis and contig assembly resulted in a total of 3649 unique transcripts. Comparison with the database showed that only 29% exhibited significant similarity to known sequences in the Genbank database and corresponded to a variety of genes associated with general cellular metabolism. Upon analysis of all the ESTs, a cDNA clone that codes for phytochelatin synthase (PCS) was found; no prior isolation in algae has been reported. PCS catalyses the synthesis of phytochelatins (PCs) which are cysteine-rich proteins with important role in heavy metal detoxification found in various types of plants (Cobbett, 2000). PCS uses glutathione (GSH) as a substrate in the presence of metal ions with Cd2+ as the strongest inducer. The
full-length clone for *E. denticulatum* PCS has been isolated using PCR and rapid amplification of cDNA ends (RACE) techniques. Total RNA was extracted from the whole plants and used as template in first strand cDNA synthesis 5' end-RACE (Othman et al., 2006). The full length cDNA clone with the size of 1.6 kb contains a single open reading frame which encodes a protein containing 218 amino acids. The expected amino acid sequence shared high similarity with PCS from *Allium sativum* (51%), *Cynodon dactylon* (51%) and *Arabidopsis thaliana* (50%). Multiple sequence alignment of the *E. denticulatum* cDNA clones with other clones showed presence of several protein motifs and binding sites typically found in PCS, suggesting structural similarity to other plant PCS. Phylogenetic analyses of this algal PCS sequence have also been performed for further characterization of its function and significance.

References


VI2-O-006

Phylogenetic relationship among mullidiae species in Turkish ichthyofauna

Emre Keskin1,2,*, Hasan Huseyin Atar2, Alp Can1

1 Ankara University Biotechnology Institute, Ankara, Turkey
2 Ankara University Agricultural Faculty Department of Fisheries and Aquaculture, Ankara, Turkey

E-mail address: keskin@ankara.edu.tr (E. Keskin).

DNA sequence comparisons of three mitochondrial DNA genes (Kocher et al., 1989) were used to reveal phylogenetic relationships among four species and a sub-species of mullidiae family. To our knowledge, this is the first report using mitochondrial DNA sequence data to infer phylogenetic relationships between *Mullus barbatus* and its subspecies; *Mullus barbatus ponticus*. Cytochrome *b*, 125 ribosomal RNA, and cytochrome oxidase II regions of 242 individuals belonging to species *Mullus barbatus*, *Mullus surmuletus*, *Upeneus moluccensis*, *Upeneus pori* and sub-species *Mullus barbatus ponticus* were sequenced and phylogenetic trees were constructed using four different methods: neighbor joining (NJ), minimum evolution (ME), maximum parsimony (MP) and unweighted pair-group method of arithmetic average (UPGMA) (Apostolidis et al., 2001). The phylogenetic trees constructed, support the existing taxonomic data of two mullid genera (*Mullus, Upeneus*). Molecular data shows no significant difference between same species of different geographical populations. The results suggest that the molecular difference is not large enough between *Mullus barbatus* and *Mullus barbatus ponticus* to consider them as subspecies.

Acknowledgements

The authors wish to thank Dr. Hilal Ozdag for helpful advise and comments. This work was supported by Ankara University Biotechnology Institute.

References


VI2-O-013

Expression studies of human antimicrobial peptide hepcidin

Farzana Rashid1,*, Ijaz Ali, Qipeng Yuan 1

1 Beijing University of Chemical Technology, Beijing 100029, China

E-mail address: fari_67@yahoo.com (F. Rashid).

Hepcidin, a small cysteine rich cationic peptide synthesized in hepatocytes and initially isolated from human plasma and urine is a key regulator of intestinal iron absorption (Krause et al., 2000; Park et al., 2001). We performed the cloning and expression of a low molecular weight human antimicrobial peptide hepcidin (hepc 20) in recombinant form. Full length synthesized gene of the hepcidin (20 amino acids) protein was cloned into different expression vectors and transformed into *E. coli* and *Pichia P. pastoris* strain GS115. The influence of different factors on biomass and hepcidin protein production during induction phase was subsequently studied. The expression level and activation were detected by Tricine SDS-PAGE and immuno blotting respectively. Studies revealed that active recombinant hepcidin peptide can be successfully expressed in *E. coli* and methylotrophic yeast *P. pastoris*. The BMMY medium was found to be optimal for recombinant hepcidin protein expression and growth of the recombinant yeast strains. The Dissolved Oxygen (DO) and the optimal concentration of methanol required for the growth of recombinant organisms were also recorded. The expressed hepcidin protein concentration was less than 10 mg/L. The recombinant protein had a high specificity against antibody. It also exhibited antibacterial activity against *S. aureus* and *Bacillus subtilis*. The study provides a basis for further researches on the early diagnosis of different peptides and the mechanism of their action.

References


VI2-O-001

Environmental adaptation: Genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP3

Fengping Wang 1, Jianbin Wang, Huahua Jian 1, Bing Zhang, Feng Wang 1, Jun Yu, Songnian Hu, Xiang Xiao 1

1 Key Laboratory of Marine Biogenic Resources, State Oceanic Administration, Xiamen 361005, PR China

Members of the genus *Shewanella* inhabit various environments, and are well known for their versatile respiratory capabilities, cou-