in orden to determine the phylogenetic status of the populations. Approximately 25% of bands recovered resulted in operational taxonomic units (OTUs) with 100% identity to *Cycloclasticus* sp., a versatile PAH-degrading bacterium not reported at these coasts, and 10% showed high identity with *Shewanella* sp. Other OTUs were related to *Vibrio*, *Pseudoalteromonas*, *Pseudomonas* and *Rhodobacter*. DNA samples were also used as template for amplification of partial PAH dioxygenase iron sulfur protein (ISP) genes. Primers cyc-p 372f and cyc-p 854r (Lozada et al., 2008), and primers nahAc 149F and nahAc 1014R (Ferrero et al., 2002) designed with GC clamp for DGGE were used for these assays. The presence of *phnA1*-like genes of *Cycloclasticus* was observed in five of seven sediments analyzed and *nah*-like genes of *Pseudomonas* or *Sphingomonas* in the others. Only one band was obtained of *nah*-like gene, but several bands were observed at bacterial populations amplified for *phnA1*-like genes. Amplification products identified as *nah*-like genes reveal the potential intrinsic capability of degrading PAHs, nevertheless, presence of important amount of OTUs related to the genus *Cycloclasticus* and their characteristic type *phnA1* gene suggests that it could play an important role in the marine degradation of PAHs as it was demonstrated by studies in several sites of the northern hemisphere (Kasai et al., 2002).

References

Ferrero, M., Llobet-Brossa, E., Lalucat, J., Garcia-Valdes, E., Rossello-Mora, R., Bosch, R., 2002. Coexistence of two distinct copies of naphthalene degradation genes in Pseudomonas strains isolated from the western Mediterranean region. Appl. Environ. Microbiol. 68, 957–962.

Kasai, Y., Kishira, H., Harayama, S., 2002. Bacteria belonging to the genus *Cycloclasticus* play a primary role in the degradation of aromatic hydrocarbons released in a marine environment. Appl. Environ. Microbiol. 68, 5625–5633.

Lozada, M., Riva Mercadal, J.P., Guerrero, L.D., Di Marzio, W.D., Ferrero, M.A., Dionisi, H.M., 2008. Novel aromatic ring-hydroxylating dioxygenase genes from coastal marine sediments of Patagonia. BMC Microbiol. 8, 50.

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Construction of reproduction-specific cDNA microarrays for the black tiger shrimp (*Penaeus monodon*)

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Microarrays are an important technique to simultaneously measure gene expression levels (Schena et al., 1995). Commercial DNA arrays are widely available for many organisms, but not for the black tiger shrimp (*Penaeus monodon*). To construct the first in-house version of cDNA microarrays for *P. monodon*, ovary and testes EST libraries (Leelatanawit et al., 2004; Preechaphol et al., 2007) were used as templates for reproduction-specific cDNA microarray (ReproArray) fabrication. ReproArray is aimed to analyze large-scale gene expression of reproductive genes. A total of 4992 cDNA from ovaries and testes of *P. monodon* broodstock were amplified and printed onto amino propyl silane coated glass slides using an automatic spotter.

In order to validate the capacity of ReproArray, the gene expression analysis throughout four stages of ovarian maturation in the black tiger shrimp was performed. Several differential expression patterns during the development were identified. Among highly induced genes, the nuclear autoantigenic sperm protein gene (*NASP*) was further investigated and characterized for its importance in female reproduction. The successful identification of this reproduction-important gene demonstrates that ReproArray is an effective tool for high-throughput gene screening for further characterization.

References

Leelatanawit, R., Klinbunga, S., Puanglarp, N., Tassanakajon, A., Jarayabhand, P., Hirono, I., Aoki, T., Menasveta, P., 2004. Isolation and characterization of differentially expressed genes in ovaries and testes of the giant tiger shrimp (*Penaeus monodon*). Mar. Biotechnol. 6, S506–S510.

Preechaphol, R., Leelatanawit, R., Sittikankeaw, K., Klinbunga, S., Khamnamtong, B., Puanglarp, N., Menasveta, P., 2007. Expressed sequence tag analysis for identification and characterization of sex-related genes in the giant tiger shrimp *Penaeus monodon*. J. Biochem. Mol. Biol. 40 (4), 501–510.

Schena, M., Shalon, D., Davis, R.W., Brown, P.O., 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467–470.

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Optimization of RAPD–PCR for intraspecies polymorphism in *Pampus argenteus* in Kuwait

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Pampus argenteus (Silver pomfret) or Zobaidy is one of the most popular fish in Kuwait that holds a high economical value. The available data indicate that zobaidy in the northern Gulf region is comprised of one stock and its migration is confined to the sea area between Kuwait, Iraq and Iran. In the recent years, a drastic decline in the fish catch has been noted, that is mostly due to over fishing and ecological changes due to the decrease of rivers discharges (Al-Hussaini et al., 2003).

The fish stock decline phenomenon, calls for detailed studies on population identification and assessment. One of the approaches would be through the use of RAPD–PCR technique to investigate the polymorphism in the fish strains in an aim to determine stock variation in the northern Gulf region. DNA (RAPD–PCR) fingerprinting techniques have been applied widely in fish and other species, and are ideal for population identification, structure, variations assessment and management (Waldman et al., 1996; Tagaart et al., 1995; Ruzzante et al., 1996).

In this study, fish samples were collected from different stations in the Kuwaiti and Iranian waters. Genomic DNA (gDNA) extraction was optimized using several protocols for different tissues such as liver, muscle and fin tissues towards the selection of an optimum method to produce the best quality of gDNA that is suitable for molecular studies. In addition, most parameters (PCR reaction components concentration and thermal profiles) affecting RAPD–PCR were examined, in an effort to produce the most distinctive fingerprint patterns.

Our results suggest that the optimum conditions for zobaidy DNA-PCR amplification are 50 ng of DNA template, 5 mM of $MgCl_2$, 2.5 u of the enzyme Ultima polymerase, 1 μM of microsatellite primer and 30 thermal cycles. The optimized factors yielded the best genetic polymorphism of the closely related Zobaidy strains, by obtaining highly complex band profiles.

The RAPD–PCR study resulted in banding profiles that can be used to differentiate between the Zobaidy strains with a poten-