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FULL LENGTH ARTICLE

Genetic and nutritional characterization of some macrophytes, inhabiting the Bardawil Lagoon, Sinai, Egypt

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Abstract The ecological and economical significances of macrophytes, inhabiting the Mediterranean Lagoon, Bardawil, northern Sinai, Egypt, are still ambiguous, due to lack of knowledge. This study focused on genetic and nutritional characterization of three dominant macrophyte species at Bardawil Lagoon. Genetic identifications were done through genomic DNA extraction, followed by PCR amplifications and sequencing of 18S rRNA genes of the studied species. Phylogenetic analyses indicated that two of the recorded species showed homologies with the seagrass species, *Posidonia oceanica* and *Halophila ovalis*, with nucleotide identities 94.5% and 96.8%, respectively. The third species showed a unique phylogenetic lineage, representing nucleotide identity average, 86.5%, among the brown seaweeds, Heterokontophyta. Nutritional analyses indicated that the recorded seaweed-like macrophyte had the highest recommended nutritional contents, crude protein, 24.67%, with a total amino acid composition of 6.64 g/100 g protein, and carbohydrate, 38.16%, besides a calorific value of 3.063 K cal/g, among the studied macrophytes. To the best of our knowledge, this is the first attempt to characterize macrophyte community in Bardawil Lagoon, using both genetic and biochemical approaches.
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Introduction

Macrophytes, including aquatic plants and macro-algae, grow in or near water and are either submergent, or floating. In shallow waters, macrophytes provide cover for fish and substrate for aquatic invertebrates, produce oxygen, and act as food for some fishes and wildlife.

Bardawil is a hypersaline Lagoon located at the north coast of the Sinai Peninsula, at longitudes, 32°40′ and 33°30′ E and latitudes, 31°03′ and 31°14′ N, Egypt. The Lagoon is separated from the Mediterranean Sea by a narrow sandbar and covering an area of about 700 km^2. The Lagoon is shallow, reaching a depth of about 3 m, and consequently, photosynthesis activity covers the whole water column, from the bottom to the surface (Touliabah et al., 2002). The floor of the Lagoon is covered with mats of macrophytes, in the form of seagrasses, which

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form large underwater meadows that are an important part of the ecosystem (El-Bana et al., 2002). Seaweeds are widely distributed at the surface water, near the west inlet of the Lagoon (El-Bana et al., 2002). The pollution-free water of the Lagoon displays a wide distribution of macrophytes, which may have several industrial applications.

Due to the reduced morphological traits and the high phenotypic plasticity, molecular methods might help to clarify the taxonomy and differentiation among different macrophytes, including seagrass and seaweed taxa. However, so far there is insufficient molecular data set for genotyping seagrass and seaweed species, and more work has to be done to close this gap of knowledge. Genetic analyses, based on rRNA genes, hold the promise of increased clarification of macrophyte identities, when joined with morphological and anatomical studies, as well as increased understanding of the species diversity and distribution worldwide (Nguyen et al., 2014). 18S rRNA gene has been utilized extensively in phylogenetic studies in many seagrass and seaweed families and has been particularly useful in improving our understanding of species relationships within these families (Baldwin et al., 1995; Verlaque et al., 2003).

The global diversity of seagrass species is low. There are 12 genera with some 58 species of known seagrasses (Kawaguchi and Hayashizaki, 2011; Papenbrock, 2012). The global distribution of seagrass genera is remarkably consistent in the northern and southern hemispheres, sharing ten seagrass genera and only has one unique genus each. Some genera are much more spacious than others. The genus Halophila has been considered as the most common seagrass species (Nguyen et al., 2014). The Mediterranean Sea has clear water with vast meadows of a moderate diversity of seagrasses (Pergent et al., 2014).

Seaweeds occupy a wide range of ecological niches. For example, brown algal flora, a target of this study, showed the greatest diversity in the North Atlantic; recording 127 genera (Wysor and De Clerck, 2003).

Most of the macrophytes have varieties of chemical compositions, characters to be applied in food, phycocolloids, fertilizers and production of bioactive compounds (Mišurcová, 2012). The biochemical contents of some macrophytes have been studied in some area of the Mediterranean Sea (Rotini et al., 2013). The nutritional values of the two seagrasses, *Cymodocea nodosa* and *Posidonia oceanica*, and their potential use as fertilizers were evaluated in five sites along the western Egyptian Mediterranean coast (Shams El Din and El-Sherif, 2013).

Recently, seaweed consumption is increasing due to their high nutritional values. Red and brown seaweeds have been used as human and animal food sources. However, they contain 80–90% moisture and their dry weights contain 50% carbohydrates, 1–3% lipids, and 7–38% minerals (Burtin, 2003). Seaweeds have variable protein contents, 10–47%, showing high proportions of essential amino acids (García-Casal et al., 2007).

Molecular characterization and nutritional values of macrophytes, mainly seagrasses and seaweeds, in Bardawil Lagoon have not been studied, and consequently, the beneficial tasks of the species are still unknown. In this study, dominated macrophyte species in Bardawil Lagoon were characterized based on two approaches. The first approach included molecular characterization based on 18S rRNA gene analyses. The objective of the second approach was measuring the nutritional values, including crude protein, amino acid composition, lipid, ash, crude fiber and carbohydrate of the studied macrophytes.

Materials and methods

Sample collection

Three dominant macrophyte species were collected during summer season from Bardawil Lagoon (Fig. 1). The first species, Macrophyte.1, (Fig. 2a) grows in dense meadows at depth of average 1 m. The leaves were ribbon-like, appearing in tufts of up to 1 m long. Average leaf width was around 10 mm. The leaves were green perhaps turning brown, in some parts, with age, and had 13–17 parallel veins. The second species, Macrophyte.2, (Fig. 2b) looks like a small herbaceous plant that occurs in the Lagoon bed. The leaves were ovate in outline, appearing light green. The roots are up to 800 mm long and covered in fine root hairs. The third species, Macrophyte.3, (Fig. 2c) was recorded floating on the surface of the water, near the west inlet of the Lagoon. The species was typically long, with hollow rope-like brown fronds about 5 mm in diameter, but can reach to lengths of 2 m.

The macrophytes were collected from the field by hand-picking and washed thoroughly by using filter sterile seawater to remove epiphytes, sand and debris, and then transported to the laboratory.

Molecular analyses

The collected macrophytes were cut into pieces and treated with SET buffer, 20% sucrose, 50 mM EDTA, 50 mM Tris–hydrochloride, pH 7.6 (Somerville et al., 1989) for molecular analyses. Genomic DNAs were extracted by homogenizing with glass beads. The homogenates were chemically lysed, using a mixture of 5 M guanidine thiocyanate and 10% SDS at 80 °C for 20 min with a continuous vigorous shake. Genomic DNAs were purified from the crude lysate, using Power-Plant Pro DNA Isolation Kit (catalog no. 13400-50, Mo Bio Laboratories, Carlsbad, CA). The purified DNAs, extracted from all samples, were run on 0.9% agarose gel electrophoresis, followed by staining with ethidium bromide and UV visualization.

PCR amplifications of the macrophyte 18S rRNA gene, from the purified genomic DNAs, were carried out using the primer sets, Eukarya-63F, 5′-ACG CTT GTC TCA AAG ATT A-3′; and Eukarya-1818R, 5′-ACG GAA ACC TTG TTA CGA-3′ (Lepere et al., 2011). PCR reaction mixture, 50 μl, contained 10× EX taq buffer II (Mg^{2+} plus), 0.2 μM primer, 400 μM dNTP each, 2.25U Takara EX-Taq Polymerase (Takara, Japan) and 5–30 ng DNA template. PCR was performed with an initial denaturation step of 5 min at 95 °C. The PCR reaction continued with 30 cycles of 1 min at 95 °C, 40 s at the annealing temperature, 55 °C, and 1 min extension at 72 °C. The PCR products were cut from the gel and eluted using QIAquick Gel Extraction Kit, catalog no. 28704, Qiagen, USA. The purified 18S rRNA gene amplicons were analyzed directly by sequencing, using 3500 series genetic analyzer (Life Technology, CA, USA).

The sequences were submitted to FASTA screening to determine their similarity with known macrophyte sequences in the DNA database. Construction of phylogenetic trees

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