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ORIGINAL ARTICLE

ISOLATION AND IDENTIFICATION OF MARINE MICROALGAE FROM THE ATLANTIC OCEAN IN THE SOUTH OF MOROCCO

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ABSTRACT

Background: Among the large spectrum of marine organisms, microalgae are able to produce a wide diverse compounds through different pathways. These bioactive compounds give them a large number of applications in various fields such as human nutrition, aquaculture, pharmaceutical, cosmetics or biodiesel production. In Morocco, the study of marine microlagae for their bioactive potential has gained strength in recent years. Moreover, Morocco has a great potential for algae culture due to its specific geographical position and to its favorable climatic conditions. **Objective**: Thus, in the aim to isolate marine microalgae from the Atlantic Ocean (South of Morocco), several samples were collected from different locations (Agadir, Anza, Naïla Lagoon and Laâyoune). Fourteen strains were purified, identified and classified using morphological features. **Methods**: Microalgae isolation was done by the combination of two techniques: serial dilution and streaking. Purified marine microalgae strains were identified using their morphological features. **Results**: Diatoms were the most abundant among the isolated species (57%), followed by green algae (36%) then dinoflagellates (7%). **Conclusion:** The diatoms and green algae such *Navicula* sp., *Chaetoceros* sp., *Nitzschia* sp., *Chlorella* sp. and *Dunaliella* sp. present diverse applications. Hence the interest of the microalgae that we have isolated in this work.

Keywords: *Marine microalgae, diatoms, green algae, Morocco.*

1. INTRODUCTION

Marine algae, a multicellular or unicellular form of living organisms, are the primary producers of oxygen in the aquatic environment. They sit at the bottom of the marine food chain, serving all other organisms [1]. Marine algae can be divided into either macroalgae or microalgae based on its size, both being prolific sources of different biologically active metabolites [2]. Several macroalgal species have been systematically cultivated and harvested for over a thousand years, whereas microalgal exploitation is relatively new [3]. There are about 50 thousand species of microalgae described taxonomically underscoring their incredible diversity [4]. Traditionally, microalgae have been classified according to their photosynthetic pigments, life cycle, and basic cellular structures. Currently, classification systems take into consideration cytological and morphological characters, cell wall constituents and chemical nature of storage products. Then, the most employed methods to identify and classify algal species include morphological observations under a microscope, molecular-based classification using the hypervariable V4 region sequences of the 18S rDNA [5] or more recently by using a flow cytometer combined with computational methods [6,7,8]. Marine microalgae typically constitute the phytoplanktons. They can be categorized into three main groups: blue-green algae (Cyanobacteria), diatoms (Bacillariophyta), and dinoflagellates (Dinophyceae) [9]. In recent decades, the techno-economics of marine microalgae have become considerably more important [10]. Their applications range from simple biomass production for human nutrition and feed in aquaculture to valuable products for medical and cosmetics fields, more importantly, for clean energy (biodiesel, biogas, bioethanol) [2-10]. The microalgae sector is very dynamic throughout the world and it attracts last years an increased interest in Morocco specially for aquaculture and for third generation biofuels [11,12]. This country has important potential due to its geographical location: the North Atlantic Ocean to the west and the Western Mediterranean Sea to the north. This is essentially what affirms a new study of the Institute of prospective economic world of the Mediterranean (IPEMED) [11]. The aim of this study is to isolate and purify marine microalgae from different locations in the Atlantic Ocean in southern Morocco. The purified strains will be identified and classified using morphological features.

2. MATERIALS AND METHODS

2.1 Study site:

Marine water samples containing various populations of microalgae were collected in the South of Morocco from the Atlantic Ocean at the following different locations:

1. Agadir Beach (30° 25' 15.6" N 9° 36' 37.0" W),
2. Anza beach (30° 26' 57.5" N 9° 39' 36.5"W),
3. Naïla Lagoon (Khnifiss zone) (28° 02' 54" N 12° 13' 66" W),
4. Port of Laâyoune (27° 05' 45.8" N 13° 25' 31.8"W).

2.2 Microalgae sampling and enrichment

Marine water samples were collected, at 0-1 m depth at each sampling stations and then they were stored in cool boxes for transportation to the laboratory. After collection, the samples were enriched by using two methods: **i**) a mixture of 10% of sample and 90% of BG11 culture medium; **ii**) 100 ml of water samples were filtered through a 0.45µm membranes. Subsequently, the membranes were taken, and directly placed in 250 ml erlenmeyer flasks containing 100 ml of BG11 medium [3-13]. This nutrient media was chosen to pre-select microalgae with rapid growth [13]. It was composed of (mg L^{-1}): 1.5×10^3 $NaNO_3$; 40 K_2HPO_4; 75 $MgSO_4.7H_2O$; 36 $CaCl_2.2H_2O$; 6 Citric acid.H_2O; 6 Iron and ammonium citrate; 1 $Na_2EDTA.2H_2O$; 20 Na_2CO_3; 2.86 H_3BO_3; 1.81 $MnCl_2.4H_2O$; 0.22 $ZnSO_4.7H_2O$; 0.39 $Na_2MoO_4.2H_2O$; 7.9×10^{-2} $CuSO_4.5H_2O$. The Erlenmeyer flasks were then incubated in batch process using continuous illumination of 150 µmol m^{-2} s^{-1} at 25 °C [14]. All cultures were with atmospheric CO_2 (no special CO_2 supply) [3].

2.3 Microalgae isolation

After an enrichment period, microalgae isolation was done by the combination of two techniques : serial dilution and streaking [15]. Serial dilution is the most common and established method of microalgae isolation [16]. A set of sterilized test tubes with BG11 media was used for dilution. Dilution sets was determined on the basis of estimated number of cells in the enriched culture. This number was evaluated using an optical microscope. After the dilution procedure, 100 µL of each dilution was streaked in BG11 solid medium in Petri dishes. Then, the plates were incubated for a period of 21 up to 30 days at 25°C and average continuous illumination of 150 µmol m^{-2} s^{-1}.

After the incubation period, morphologically distinct colonies were removed and streaked once again for an average period of (21 ± 2) days, in order to guarantee the purification of a single microalgae species [13].

2.4 Strain Identification

Purified marine microalgae strains isolated from the collected water samples were identified using their morphological features. The different purified colonies were examined using a light microscope. The identification of the algal strains was made using a field guides [17,18,19].

3. RESULTS AND DISCUSSION

3.1 Marine microalgae isolation and purification

In this study, 14 isolates of marine microalgae were obtained from the different samples collected in the South of Morocco from the Atlantic Ocean (Table 1). Seven isolates (S1 to S7) were obtained from samples taken on the beaches of Agadir and Anza, five isolates from the Naïla lagoon (S8 to S12) and two from the port of Laâyoune (S13 - S14). Then, the purification of the isolates was carried out using repeated streaking on the solid BG11 medium (figure 1a). The purification allowed monoalgal culture harvested on BG11 liquid medium (figure 1b). The BG11 medium was very useful in this work for the isolation of marine microalgae belonging to different groups (diatoms, green algae and dinoflagellates). While in the literature, this medium is described as more specific for the culture of cyanobacteria [20] and freshwater algae [10]. Microalgae isolation from seawater samples requires the use of several types of media containing different concentrations of many essential nutrients in order to allow the growth of the majority of microalgal species present in the samples. Common examples of media used for marine algae are Black Sea medium, ES Medium, ASP Medium, Aquil medium, Allen's Cyanidium Medium, CCAP Artificial Seawater, Chry medium, ESAW Medium, ESM Medium, f/2 Medium, K Medium, L1 Medium, MNK Medium, Pro99 Medium, SN Medium, etc. [10].

Table 1: The purified isolates from the different sampling sites.

Study site	Purified strains
Agadir Beach	4 (S1 to S4)
Anza Beach	3 (S5 to S7)
Naïla Lagoon	5 (S8 to S12)
Port of Laâyoune	2 (S13 - S14)

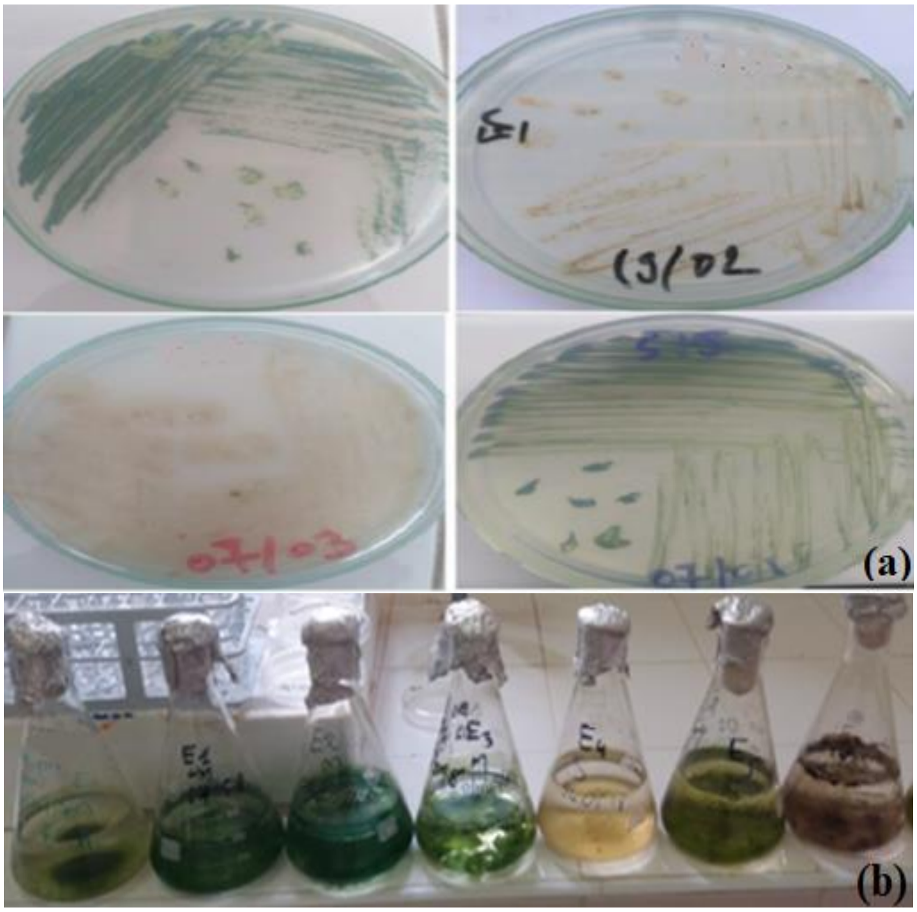

Figure 1: Examples of the purification of the isolated strains **(a)** and some purified monoalgal cultures on BG11 liquid medium **(b)**.

3.2 Strains Identification

The microscopic study of the purified microalgae resulted in the identification of several taxa (Table 2). The identified species represent 11 families and 4 classes (Bacillariophyceae, Chlorophyceae, Dinophyceae and Trebouxiophyceae) (Table 2). Diatoms were the most abundant among the isolated species (57%), followed by green algae (36%) while dinoflagellates represent only 7% (Figure 2).

Table 2: Strains identification and classification using morphological features [21].

Strain	Study site	Identification	Classification
S1	Agadir Beach	*Bracteacoccus* sp.	**Phylum** Chlorophyta **Class** Chlorophyceae **Order** Sphaeropleales **Family** Bracteacoccaceae **Genus** *Bracteacoccus*
S2	Agadir Beach	*Coelastrella* sp.	**Phylum** Chlorophyta **Class** Chlorophyceae **Order** Sphaeropleales **Family** Scenedesmaceae **Genus** *Coelastrella*
S3	Agadir Beach	*Coscinodiscus* sp.	**Phylum** Bacillariophyta **Class** Bacillariophyceae **Order** Coscinodiscales **Family** Coscinodiscaceae **Genus** *Coscinodiscus*
S4	Agadir Beach	*Nitzschia* sp.	**Phylum** Ochrophyta **Class** Bacillariophyceae **Order** Bacillariales **Family** Bacillariaceae **Genus** *Nitzschia*
S5	Anza Beach	*Gymnodinium* sp.	**Phylum** Miozoa **Class** Dinophyceae **Order** Gymnodiniales

			Family Gymnodiniaceae **Genus** *Gymnodinium*
S6	Anza Beach	*Dictyochloropsis* sp.	**Phylum** Chlorophyta **Class** Trebouxiophyceae **Order** Trebouxiales **Family** Trebouxiaceae **Genus** *Dictyochloropsis*
S7	Anza Beach	*Chlorella* sp.	**Phylum** Chlorophyta **Class** Trebouxiophyceae **Order** Chlorellales **Family** Chlorellaceae **Genus** *Chlorella*
S8	Naïla Lagoon	*Nitzschia* sp.	**Phylum** Ochrophyta **Class** Bacillariophyceae **Order**: Bacillariales **Family** : Bacillariaceae **Genus**: *Nitzschia*
S9	Naïla Lagoon	*Navicula* sp.	**Phylum** Ochrophyta **Class** Bacillariophyceae **Order** Naviculales **Family** Naviculaceae **Genus** *Navicula*
S10	Naïla Lagoon	*Chaetoceros* sp.	**Phylum** Bacillariophyta **Class** Bacillariophyceae **Order** Chaetocerotales **Family** Chaetocerotaceae **Genus** *Chaetoceros*
S11	Naïla Lagoon	*Coscinodiscus* sp.	**Phylum** Bacillariophyta **Class** Coscinodiscophyceae **Order** Coscinodiscales **Family** Coscinodiscaceae **Genus** *Coscinodiscus*
S12	Naïla Lagoon	*Pleurosigma* sp.	**Phylum** Bacillariophyta **Class** Bacillariophyceae **Order** Naviculales **Family** Pleurosigmataceae **Genus** *Pleurosigma*
S13	Port of Laâyoune	*Navicula* sp.	**Phylum** Ochrophyta **Class** Bacillariophyceae **Order** Naviculales **Family** Naviculaceae **Genus** *Navicula*
S14	Port of Laâyoune	*Dunaliella* sp.	**Phylum Chlorophyta** **Class** Chlorophyceae **Order** Dunaliellales **Family** Dunaliellaceae **Genus** *Dunaliella*

The diversity of marine microalgae is vast and represents an intact resource [10]. The most of microalgae can grow in fresh water and seawater, whereas several other species of microalgae grow in extremely saline environments, such as the green algae *Dunaliella salina* which can survive even at 4.5 M NaCl [22]. Within these aqueous habitats, some algae populate the subsurface water column, others grow inside a few hundred micrometers of the water layer, and a few grow at 200-300 m below the water surface, that is the limits of the photic zone [10]. Currently, microalgae are the topic of a growing interest in Morocco due to their high and numerous potentialities applications especially in aquaculture and biofuels production. Moreover, Morocco has a great potential for algae culture due to its specific geographical position and to its favorable climatic conditions.

In aquatic food chain, microalgae play a vital role and they are valuable in aquaculture and have been used as live natural feeds for all bivalve mollusks and for larval or juvenile crustaceans and finfish, as well as for raising the zooplanktons required for feeding of juvenile [23,24]. Diatoms and green algae are largely used in aquaculture especially for larval shrimp and in fish hatcheries [23]. The most diatoms and green algae used in aquaculture are *Navicula* sp., *Chaetoceros* sp., *Nitzschia* sp., *Chlorella* sp. and *Dunaliella* sp. [24,25,26]. Hence, the interest of the microalgae that we have isolated in this work. Moreover, microalgae are one of the most promising sources of biomass for biofuel production due to their fast growth, high photosynthetic efficiency and high lipid content [27]. The screening of biodiesel producing microalgae has been done in some works in Morocco [14-28,29,30]. The microalgae tested belong to different genera (*Nannochloropsis*, *Chlorella*, *Dunaliella*, *Isochrysis*, *Phaeodactylium, Chaetoceros, Navicula,* and *Tetraselmis*). According to El Arroussi *et al.* (2017) the marine species *Nannochloropsis* sp., *Dunaliella tertiolecta, Isochrysis* sp. and *Tetraselmis* sp. have a good potential for biodiesel production. Several regions of the south of Morocco (from Agadir to Dakhla) present suitable climatic conditions to exploit in order to develop microalgae-based biodiesel [12]. Nevertheless, no large-scale project has been carried out until know probably due to the lack of funding structure for pilot/industrial projects subvention [12].

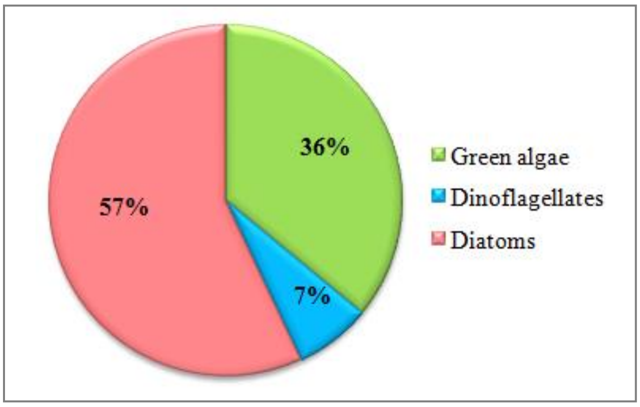

Figure 2: The proportion of the three groups of the identified microalgae.

4. CONCLUSION

Interest in microalgae research has increased last decades in various fields and the microalgae sector is very dynamic throughout the world. In Morocco, the exploitation of marine microalgae for their bioactive substances is still limited, even if the potential remains important due to its favorable climatic conditions and to its specific geographical position. In this work, fourteen marine microalgae were purified, identified and classified using morphological features. The isolates obtained belong to important groups of microalgae (diatoms, green algae). These groups include species that are highly exploited on an industrial scale for the production of different molecules of interest or used as food or feed. This study is only an initiation of research on marine microalgae which requires more support to be developed in Morocco.

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