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# Molecular Identification and Biochemical Characterization of Novel Marine Yeast Strains with Potential Application in Industrial Biotechnology

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**Abstract:** Cell-based agriculture is an emerging and attractive alternative to produce various food ingredients. In this study, five strains of marine yeast were isolated, molecularly identified and biochemically characterized. Molecular identification was realized by sequencing the DNA ITS1 and D1/D2 region, and sequences were registered in GenBank as *Yarrowia lipolytica* YlTun15, *Rhodotorula mucilaginosa* RmTun15, *Candida tenuis* CtTun15, *Debaryomyces hansenii* DhTun2015 and *Trichosporon asahii* TaTun15. Yeasts showed protein content varying from 26% (YlTun15) to 40% (CtTun15 and DhTun2015), and essential amino acids ranging from 38.1 to 64.4% of the total AAs (CtTun15-YlTun15, respectively). Lipid content varied from 11.15 to 37.57% with substantial amount of PUFA (>12% in RmTun15). All species had low levels of Na (<0.15 mg/100 g) but are a good source of Ca and K. Yeast cytotoxic effect was investigated against human embryonic kidney cells (HEK 293); results showed improved cell viability with all added strains, indicating safety of the strains used. Based on thorough literature investigation and yeast composition, the five identified strains could be classified not only as oleaginous yeasts but also as single cell protein (SCP) (DhTun2015 and CtTun15) and single cell oil (SCO) (RmTun15, YlTun15 and TaTun15) producers; and therefore, they represent a source of alternative ingredients for food, feed and other sectors.

**Keywords:** marine yeast; molecular identification; biochemical composition; cytotoxicity; SCO; SCP



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## 1. Introduction

Marine yeasts are ubiquitous micro-organisms, widely distributed in marine environments as they are isolated from marine water and sediments, estuaries, algae, seagrass, marine fauna and mangrove ecosystems [1–5]. The genera of obligatory identified marine yeasts are *Rhodotorula*, *Rhodosporidium*, *Candida*, *Debaryomyces*, *Cryptococcus*, *Yarrowia*, *Aureo basidiumfrigida* and *Guehomyces pullulans*, which are also widely distributed in Antarctic marine sediments [6,7]. However, various biotic and abiotic factors, including soil runoff, salinity, flora, fauna, temperature and human waste, affect the diversity of yeasts. Such conditions prevailing in natural habitats determine the growth, metabolic activity and survival rate of yeast populations [8]. The authentication and characterization of these populations are essential to carry out their cultures in controlled environments and to produce metabolites of interest.

The traditional identification and characterization of yeast species have been based on their morphological traits and physiological capacities. These methods are laborious and time-consuming, often producing random results depending on the culture conditions [9–12]. Advances in the development of molecular techniques with higher resolving power have led to more reliable characterization of yeasts, both at the species and strain level [13]. Research on ascomycete and basidiomycete yeasts [14] has led to the creation of a ribosomal DNA database with a large sub-region of the D1/D2 region, accessible from GenBank [15,16].

Marine yeasts are an inexhaustible source that can be exploited to produce various molecules of interest ranging from enzymes and pigments to antibiotics, via therapeutic metabolites and lipids [7–25]. Yeasts can generally accumulate more lipids than bacteria and microalgae on a given time scale. This is partly due to their faster growth [23,26], which provides significant potential for commercial triglyceride oil production [27]. Another peculiarity compared to algae and bacteria is that certain yeasts, such as *Saccharomyces cerevisiae*, *Candida utilis* and *Candida tropicalis* can be used for their unicellular protein with a digestibility rate generally greater than 80% [28,29].

However, investigation on marine yeast from the southern part of the Mediterranean basin is rather scarce [30–32]. Therefore, the aims of the present study are to isolate, purify, identify and characterize strains of marine yeasts from different marine sources for potential exploitation in the food sector.

## 2. Materials and Methods

### *2.1. Environmental Sampling*

In order to isolate marine yeasts, sampling was carried out targeting seawater, sediments, seaweeds, seagrass and co-products of fish and pink shrimp. Samples of seawater were collected at different levels (surface (A1 = 5 m), thermocline (A2 = 34 m), (A3 = 64 m) and (A4 = 80 m)) from the Golf of Tunis with precise geographical coordinates. The collection point was 37°01′14.1″ N; 10°38′44.4″ E. This sampling was carried out by the boat Scientist Hannibal and the temperature of the recorded day was 24.82 °C, using Niskin bottles of 5 L. For each sample, seawater was poured aseptically into 250 mL autoclaved and irradiated glass bottles.

In addition, further sediment and seawater collection was carried out in the La Goulette Neuve area (36°49′08.22″ N; 10°18′34.35″ E). Thus, to collect the sediment, it was necessary to use a distance of 0.5 to 1 m (about 40 cm deep) from the coast, and the sterile bottles were open under water near the bottom, where they were filled with sediment [33]. Concerning algae and sea grass, they were collected from the North Lake of Tunis (36°48′9.9 89″ N; 10°13′18.03″ E). The samples were put directly into plastic bags and were brought directly to the laboratory. To isolate yeast from farmed fish (*Dicentrarchus labrax* and *Sparus aurata*), waste, skin, scales and gills were aseptically taken and immersed in an already prepared culture medium. Chitin and shell waste of *Parapenaeus longirostris* caught from the canal of Sicily at different seasons were also used for the isolation of marine yeast.

### *2.2. Screening and Isolation*

Two methods were applied to isolate yeasts from different sources (Table 1).

The first is to filter the samples [15,33,34] while the second requires cultivating of samples in appropriate broths [26,35–39]. For the culture of the isolates, a loop of cells was transferred to 50 mL medium and kept in an orbital shaker at 30 °C with shaking at 150 rpm for 5 days. The growth of the cell culture was studied by determining the optical density (OD) at 600 nm using the UV-Vis spectrophotometer (LLG-uniSPEC2) at regular time intervals (24 h).

**Table 1.** Procedure of yeast isolation.

| Sample | Isolation Technique | Quantity (g; mL; cm) | Isolation Media |
|---|---|---|---|
| Sea water | Enrichment | 3 mL | MI; YNB; YPD |
| | Filtration | 500 mL | MI-Agar |
| Sediment | Enrichment | 2 g | MI; YPD |
| | Direct culture | 0.1 g | MI-Agar |
| Gills (*S. aurata/D. labrax*) | Enrichment | 0.450 g | YPD |
| Scales (*S. aurata/D. labrax*) | Enrichment | 0.120 g | YPD |
| Skin (*S. aurata/D. labrax*) | Enrichment | 0.780 g | YPD |
| Byproducts (*P. longirostris*) | Enrichment | 2 mL | YPD; YNB; MI |
| | Direct culture | 1 g | YPD-Agar |
| *P. longirostris* hydrolyses | Enrichment | 1 mL | YPD |
| Sea-grass and Algae | Enrichment | 1 g | YPD |
| | Direct culture | 4 cm | YPD-Agar |

The media used in this study are MI (20 g sucrose, 3 g peptone, 3 g yeast extract); YNB: yeast-nitrogen-base (DIFCO); YPD (20 g glucose, 10 g yeast extract, 20 g peptone), Min-YPD (2 g glucose, 1 g yeast extract, 10 g peptone), MI-agar (20 g sucrose, 3 g peptone, 3 g yeast extract, 20 g agar) and YPD-agar (20 g glucose, 10 g yeast extract, 20 g peptone, 20 g agar) prepared in 1 l of seawater, to which 100 ppm of antibiotic was added. Purified strains were conserved in 20 g glucose, 10 g yeast extract, 10 g peptone and 250 mL glycerol at −80 °C.

### 2.3. Molecular Identification

DNA extraction was carried out using the method described by Sambrook (1989) with some modification [40]. Thus, an overnight pre-culture in YPD media (1 ml; OD = 1) was centrifuged. The cell pellet was washed by distilled water and suspended in 100 µL of 0.2 M of lithium acetate (LiOAc) in aqueous solution of 1% sodium dodecyl sulfate (SDS). The solution was mixed thoroughly and incubated at 90 °C for 1 h, followed by adding ethanol to the mix. The solution was then mixed and centrifuged. The cell pellet was dried at 60 °C for 30 min, then mixed with sterile water. The mix was centrifuged and the supernatant containing DNA was collected.

Primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and NL4 (5′-GGTCCGTGTTTCAA GACGG-3′) were used to amplify the conserved region of yeast DNA in order to identify the species of yeast [41]. Primers ITS1 and NL4 target ITS1 and D1/D2 region. The length of each region is the same 500 bp.

The amplified PCR products were purified using innuPREP DOUBLE pure kit (Analytical Jena). In total, 5 µL of the purified DNA sample was mixed with 0.5 µL ITS1 primer. The blend was sent for sequencing to WMG Operon Company (Germany). The sequencing order was aligned using Basic Local Alignment Tool (BLAST) analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 9 July 2015).

### 2.4. Phylogenetic Analysis

Sequences generated after a Basic Alignment Search Tool (BLAST) were aligned using the CLUSTALW program and phylogenetic analyses and molecular evolution were conducted using MEGA version 11.0. The Tamura model was used to estimate the evolutionary distance. The phylogenetic reconstruction was performed using the join algorithm (NJ), with start values calculated from 1000 replicated runs, using the software routines included in the MEGA 11.0 software [42,43].

### 2.5. Biochemical Characterization

in double distilled water at a concentration of 100 mg/100 mL and used as the standard

*Ash content:* The mineral content of the samples was determined by incineration of organic biomass according to the official AOAC (1995) method [44]. The method is accredited in ISO/IEC 17025:2017.

*Mineral content:* The discovery of the composition of minerals was carried out in Green-Lab laboratory (Tunis, Tunisia). The identified minerals are: calcium, sodium, potassium, magnesium, iron, copper, manganese, nickel and selenium. The assay was carried out according to Standard NT.09.193 (2010) by optical emission ICP [45].

*Carbohydrate content:* The determination of the carbohydrate concentration was conducted according to the method of Dubois (1956), with some modifications [46]. Thus, to 20 mg of the lyophilized biomass of each strain, 20 mL of the hydrogen chloride solution HCl (2 M) was added to tubes and placed in an ultrasound bath for 5 min. The tubes were then incubated in a water bath at 100 °C for 30 min with a vortex for 1 min every 10 min. After centrifugation, sulfuric acid and phenol were added to the supernatant. The carbohydrate determination was carried out using a UV-Visible spectrophotometer at a wavelength λ = 490 nm. The method is accredited in ISO/IEC 17025:2017.

*Lipid content:* The extraction was carried out according to the method of Folch (1957), with some modifications according to the internal methodology (MO/02, accredited ISO/IEC 17025:2017) [47].

*Fatty acid content:* The fatty acid methylation was carried out to obtain the methyl ester derivatives for analysis by gas chromatography (GC) [48]. GC analysis was carried out using a GC-HP model N6890 fitted with a flame ionization detector (FID) and an HP-INNWAX capillary column (30 m × 0.25 µm). The sample (1 µL) was injected at a split-rate of 1:50. At the beginning, oven temperature was 150 °C, and then it rose to 200 °C with a gradient of 15°/min. To reach 250 °C, a gradient of 2 °C/min was applied. The temperatures of the injector and detector were 220 and 275 °C, respectively. The run was 30 min with a flow rate of 1 ml/min. The retention time and the areas of the peaks of FAME were determined by ChemStation software. The identification and the quantification of FAMEs (g of FA/100 g of sample) were performed by comparing the retention times of the samples against PUFA3-SUPLECO methyl esters standards (Sigma, Germany). The method is accredited in ISO/IEC 17025:2017.

*Protein content:* The samples were digested with sulfuric acid ($H_2SO_4$) concentrated in the presence of a catalyst ($H_2O_2$) and hot bath (100 °C) as described by Lourenço et al. (2004), with some modifications [49]. To each freeze-dried yeast sample (30 mg), 2 mL of sulfuric acid in a screw tube was added. All of the tubes were then incubated for 60 min in a water bath at 100 °C. After cooling (5 min.), 3 mL of $H_2O_2$ was added to each tube. The tubes were again incubated for 30 min at 100 °C. This step was repeated three times (final volume of $H_2O_2$ was 9 mL). The digestion product was cooled to room temperature and then injected into a Flow Injection Analysis system. The quantity of nitrogen determined was then multiplied by 6.25 in order to estimate the quantity of protein in each species.

*Amino Acid profile:* Amino Acid (AA) extraction was performed after hydrolysis of the sample with a concentrated (6N) HCl solution [50,51] and analyzed using a High-Performance Liquid Chromatography (HPLC, Agilent 1260 infinity) system equipped with a DAD detector.

*Biogenic amines content:* Histamine (HIS), cadaverine (CAD), agmatine (SPD), tyramine (TYR) and spermine (SPM) were dissolved working solution. The chromatographic determination (HPLC, Knauer, UV detection) of biogenic amines was carried out according to ISO standard 19343: 2017 [52].

### 2.6. Cytotoxicity of Yeast Strains on HEK293 Cell Viability

*Cell lines and cultures:* The discovery of the morphological changes and viability of human embryonic kidney (HEK) cells (HEK293) was performed as described in Elloumi-Mseddi et al. (2015) [53]. The HEK293 cells were grown in Dulbecco's Modified Eagle's

Medium (DMEM) supplemented with 10% fetal bovine serum, 50 UI/mL penicillin and 50 mg/mL streptomycin at 37 °C in a humidified 5% $CO_2$ atmosphere. The cells used in this work (HEK293) were kindly provided by "Pasteur Institute-Tunis, Tunisia".

*Cell viability:* The cells were plated in 96-well plates (density of 80,000 cells/mL) and allowed to adhere for 24 h, then the testing materials TaTun15, RmTun15, YlTun15, DhTun2015, and CtTun15 were added to the culture medium. After 24 h of exposure, the medium was removed and MTT (bromide of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) solution (5 mg/mL) was then added to each well containing 100 µL of fresh medium and incubated for 4 h; the precipitate of formazan was dissolved in 100 µL of SDS at 10%. The absorbance was measured in an ELISA reader at 570 nm. The cell viability ratio was calculated by the subsequent formula:

$$\text{Cell viability ratio (\%)} = [\text{A\_HEK293treated}/\text{A\_HEK293untrated}] \times 100 \quad (1)$$

### 2.7. Statistical Analysis

The data were subjected to 5% variance analyses using SPSS 24.0 software and the Tukey test was performed to identify differences between the means.

## 3. Results and Discussions

### 3.1. Screening and Isolation of Marine Yeast

The method of sample filtering, followed by the application of the nitrocellulose filters on the agar plates [33,34], allowed us to obtain different microbial strains. Thus, the incubations of such samples, which lasted 10 days, showed that colonies begin to appear from the 4th day, and then the invasion of the culture by different types of microbial strain, mainly bacteria and fungi, make transplanting and purification of yeast strains impossible. Similar results were obtained even after several replication assays; it was than necessary to seek another faster method such as the enrichment method. Table 2 summarizes the growth of strains following the enrichment method of the various samples in different culture media during 5 days while monitoring the growth and contamination of the cultures.

The 32 isolated purified strains were cultured in YPD medium containing an antibiotic for 5 days, incubated in YPD agar plates and then stored at 4 °C for subsequent analyses.

Several strains have been identified with the method of enriching samples [32]; in particular, the methanogens isolated from deep marine sediments [54] and probiotics with phytase activity isolated from sea cucumbers [55]. Such a technique was equally adopted for the isolation and characterization of various marine yeasts including *Candida membranifaciens sub* sp. *flavinogeny* W14-3 [56]; *Cryptococcus aureus* G7a [57], and 32 yeast species sampled at different depths [38,39]. Recently, the marine yeast *Sporidiobolus pararoseus* ZMY-1 was isolated from the mud of the mangrove reserve of Zhangzhou, Fujian using a nutritive broth based on dextrose (nutritive broth 8 g, yeast extract 5 g, dextrose 10 g in 1000 mL water distilled) [58]. Similarly, Senthil Balan (2019) was able to isolate the marine yeast *Cyberlindnera saturnus* from the sediments obtained in the coastal regions of Tamil Nadu, in India (0.3% yeast extract, 0.3% d malt extract, 0.5% peptone and 1% glucose, prepared with 100 mL of sea water) [59]. Ahmed (2019), using the nutrient medium YPD and YPD-Agar, identified *Candida parapsilosis*, *Debaryomyces hansenii*, *Debaryomyces fabryii*, *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Shizosaccharomyces pombe* from marine sediments of the mangrove ecosystem of the Makran coast in Gwadar, Pakistan [8].

To conclude, the present trials identified this technique as being more effective and suitable for our working conditions than that of filtration. Thus, during the culture period, we were able to monitor all the parameters and conditions that can influence the growth of yeasts. The daily microscopic monitoring permits one to detect any type of contamination. On the other hand, this technique was faster (5 days less than the filtration method). However, this does not negate the performance of the filtration technique, since in some studies, such a technique was chosen for the isolation of marine yeasts [60], while others have combined both methods [61,62].

**Table 2.** Growth of isolated yeasts from different Tunisian marine sources in different broth media according to their biomass productivity (BP).

| Sample | YPD | Min-YPD | YNB |
|---|---|---|---|
| A1 | ++ | + | + |
| A2 | +++ | + | + |
| A3 | +++ | + | + |
| A4 | ++ | + | + |
| Sediment | + | + | − |
| Sea water La Goulette | ++++ | + | + |
| *P. oceanica* | ++ | + | + |
| *Z. marina* | ++ | + | + |
| Red Algae | +++ | − | + |
| *P. longirostris* coproduct | ++ | + | + |
| * Raw Spring | ++ | + | − |
| * Raw Summer | ++ | + | − |
| * Raw Autumn | ++ | + | − |
| * Raw Winter | ++ | + | − |
| Chitin | ++ | + | − |
| Chitosan | − | − | − |
| Hydrolysis of *P. longirostris* | ++ | − | − |
| Skin (*Sparus aurata*) | +++ | ++ | + |
| Skin (*Dicentrarchus labrax*) | +++ | ++ | + |
| Scales (*Sparus aurata*) | ++++ | ++ | + |
| Scales (*Dicentrarchus labrax*) | ++++ | ++ | + |
| Gills (*Sparus aurata*) | ++++ | ++ | + |
| Gills (*Dicentrarchus labrax*) | ++++ | ++ | + |

* Raw: milled shell of *P. longirostris*; − : no growth; +: BP = 0.05 mg/mL/h; ++: BP = 0.15 mg/mL/h; +++: BP = 0.25 mg/mL/h; ++++: BP = 0.35 mg/mL/h.

### 3.2. DNA Identification

Over the past two decades, the extensive application of nucleotide sequences of the D1/D2 region of the large ribosomal RNA gene (26 S rDNA) subunit and internal transcribed spacer regions (ITS) between domains rDNA 18 S, 5.8 S and 26 S greatly facilitated the identification of yeasts, leading to the discovery of new yeast species [8,16,26,33,38,39,58,59,63–65]. The molecular identification of the purified strain has gone through several optimizations in order to extract a representative DNA fraction for all the strains. In the first place, the experimental protocol consists of all incubations lasting 30 min. Thus, the measurement of the optic density at 230, 260, 280 and 320 nm reveals the presence of DNA while the gel of the PCR product has other results. In order to improve this method, the Genomic DNA Buffer Kit (QIAGEN) for DNA extraction was tested and 8 bands appeared from 16 inoculated products on the agarose gel. For this, we increased the performance of the initial protocol by introducing some modifications. Doubling the incubation time with LiOAc/SDS solution allowed a better lysis of cell wall strains. Moreover, the repetition of washing of the cell pellet to drain the remaining LiOAc/SDS solution provided a purer nucleic fraction than previous tests. Thus, this improvement was confirmed by the profile observed on the agarose gel where all inoculated DNA appeared. The sequencing result of those products is shown in Table 3.

**Table 3.** Molecular identification of marine yeast.

| Species | Substrates |
|---|---|
| *Cryptococcus curvatus* | Seawater (−34 m) |
| *Meira nashicola* | Seawater (−64 m) |
| *Meira* sp. | Seawater (−64 m) |
| *Rhodotorula mucilaginosa* | Seawater (−64 m), Seawater (−0.4 m) |
| *Sporobolomyces roseus* | Chitin *P. longirostris* |
| *Sporobolomyces aff. Ruberrimus* | Chitin *P. longirostris* |
| *Sporobolomyces ruberrimus* | Chitin *P. longirostris* |
| *Trichosporon asahii* | *P.longirostris coproduct* |
| *Debaryomyces hansenii* | Scale of *D. labrax* |
| *Candida parapsiolis* | Posidonia, red algae, Zostera, skin of *D. labrax* |
| *Yarrowia lipolytica* | Sediment, scale of *S. aurata*, gills of *D. labrax*, *P. longirostris coproduct* |
| *Candida tenuis* | *S. aurata gills and skin* |

The identified species are distributed between two yeast groups, the ascomycetes (*Candida*, *Yarrowia* and *Debaryomyces*) and basidiomycetes (*Rhodotorula*, *Sporobolomyces*, *Meira*, *Cryptococcus*, *Trichosporon*). Basidiomycete species are mainly present with a percentage of 63%.

According to their phyla, the different genera have specific ecological characteristics and properties [66,67], mainly as follows. (i) The composition of the cell wall: the majority compound is chitin in basidiomycetes and β-glucans in ascomycetes. (ii) DNA: the level of guanine and cytosine nucleic acids tends to be more than 50% in basidiomycetes and less than 50% in ascomycetes. (iii) Ascomycete yeasts are generally more fermentative, more fragrant and mainly hyaline, while basidiomycete yeasts more often form mucoid colonies, exhibit intense carotenoid pigments and tend to use a wide range of carbon at lower concentrations. Ascomycete yeasts are isolated from specialized niches involving interactions with plants and insects or other invertebrates on which they depend for their dispersion. Basidiomycete yeasts seem to be adapted to the colonization of solid surfaces poor in nutrients.

According to these characteristics, the frequency and the genus of marine yeasts vary depending on the isolation medium and the molecular identification technique. A comparison was made with other works transcribing the ITS and D1/D2 regions for the identification of strains, revealing the abundance and the presence of the same species found in the present work. Table 4 reports a distribution of marine yeast isolated from different marine and aquatic environments.

### *3.3. Phylogenic Analysis*

In this study, the phylogenetic tree is based on rDNA sequences from the ITS1 and D1/D2 region with a scale bar representing 0.1 nucleotide substitution per sequence position. The tree was constructed using the maximum likelihood phylogenetic analysis method (Mega 11.0).

Phylogenetic analysis of the isolates strains in this study revealed that the sequences show a similar evolutionary relationship. Based on the evolutionary distance between yeast strains, phylogenetic research indicates that these strains are linked (Figure 1). This can be explained by the fact that these strains have some similar physiological characteristics such as lipid accumulation and the secretion of certain bioactive substances [26,77].

**Table 4.** Distribution of marine yeast species by source using transcription of the ITS and D1/D2 region.

| | Source | Specie | Reference |
|---|---|---|---|
| **Sediment** | Pacific Ocean | *R. minuta*; *R. mucilaginosa* | [68] |
| | South China Sea | *Cryptococcus aureus* | [57] |
| | Pit of Japan | *Dipodascus tetrasporeus* | [69] |
| | Veraval, India | *Candida* sp. | [70] |
| | South East coast of India | *Candida albicans*, *C. tropicalis*, *D. hansenii*, *Geotrichum* sp., *Pichia capsulata*, *Pichia fermentans*, *Pichia salicaria*, | [71] |
| | South East coast of India | *R. minuta* *C. dimennae* *Y. lipolytica* | [72] |
| | Tamil Nadu, India | *Cyberlindnera saturnus* | [59] |
| | Mangrove of Makran, Gwadar, Pakistan | *Candida parapsilosis*, *Debaryomyces hansenii*, *Debaryomyces fabryii*, *Saccharomyces cerevisiae*, *Saccharomyces bayanus Shizosaccharomyces pombe* | [8] |
| **Seawater** | Northwest Pacific Ocean | *R. pacifica* | [16] |
| | Mid-Atlantic ridge (−2300 m) | *Candida sp* | [72] |
| | Coastal waters of northeast Taiwan | *Candida tropicalis* *Pichia anomala* *Issatchenkia orientalis* *C. glabrata* *Saccharomyces vakushimaensis* *Kodamaea ohmeri* *Hanseniaspora uvarum* *Kazachstania jiainicus* *Torulaspora delbrueckii* | [34] |
| | Veraval, India | *Candida* sp. | [70] |
| | Queens Cliff, Victoria region, Australia | *Rhodotorula sp* | [26] |
| | Medit. Sea. Alexandria, Egypt | *Candida viswanathii* | [73] |
| | Red Sea. Ismailia, Egypt | *Candida tropicalis* | |
| | Irish Sea. | *Candida tropicalis* | |
| | Wales, U.K. | *Candida tropicalis* | |
| | English Channel, Plymouth, U.K. | *Saccharomyces cerevisiae* *Wickerhamomyces anomalus* *Pichia kudriavzevii* *Candida glabrata* | |
| **Fisheries coproduct** | Gastropod gills *Ifremeria nautilei* | *Debaryomyces hansenii* | [38] |
| | Shrimp and Mussel | *R. mucilaginosa* | [38] |
| | Fish intestine, Chili | *R. mucilaginosa* *Debaryomyces hansenii* | [74] |
| | *Abramis brama*, *Rutilus*, *Perca fluviatilis* | *Cr. uniguttulatus*; *R. mucilaginosa*; *R. glutinis* | [61] |
| **Marine plant** | Marine Plant, Chili | *Rhodotorula mucilaginosa* | [75] |
| | Antarctic Algae | *R. mucilaginosa* | [76] |

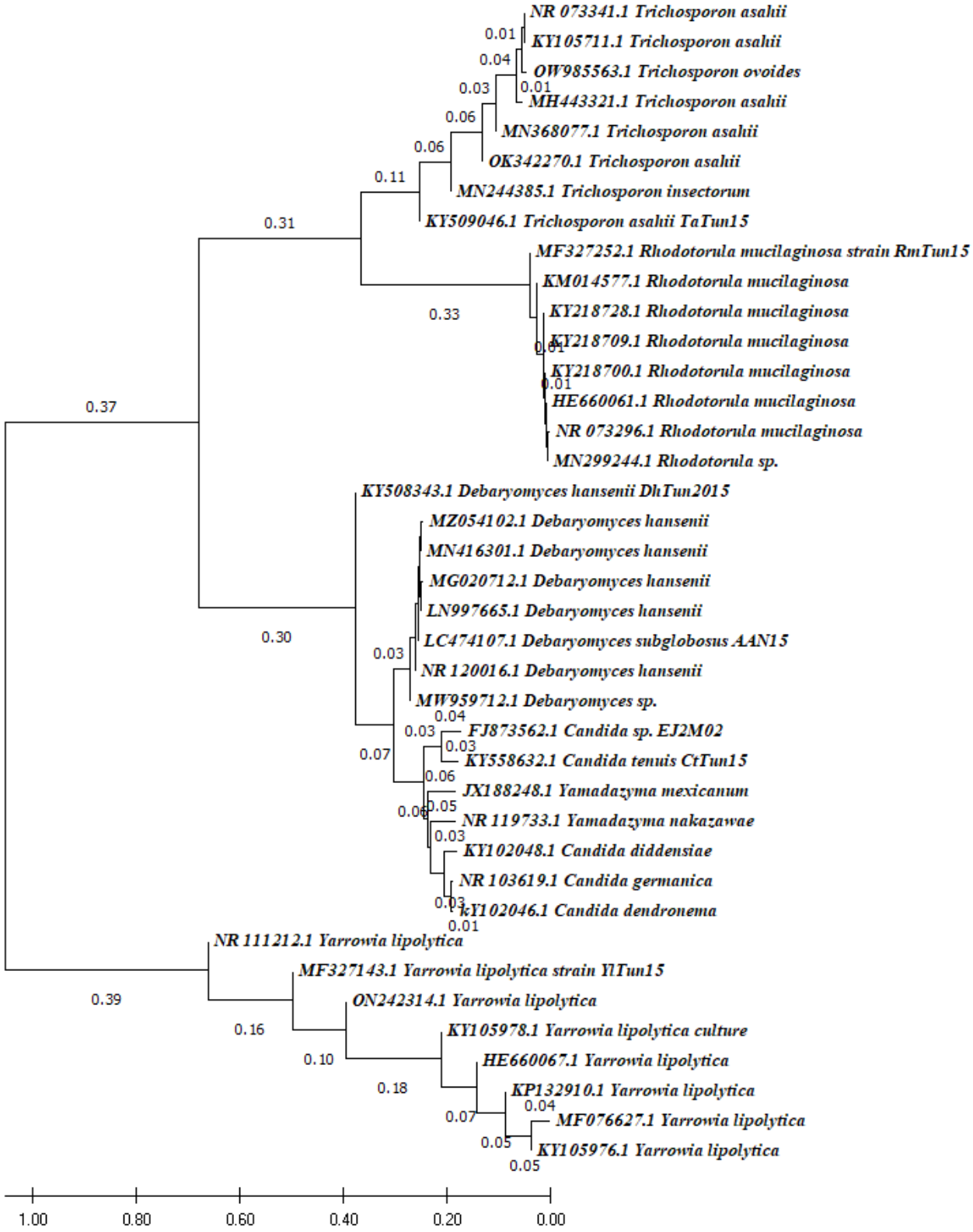


**Figure 1.** Molecular phylogenetic tree by neighbor-joining method, Mega 11.0.

### *3.4. Biochemical Characterization*

Table 5 summarizes the biochemical composition of *Rhodotorula mucilaginosa* RmTun15, *Yarrowia lipolytica* YlTun15, *Trichosporon asahii* TaTun15, *Candida tenuis* CtTun15 and *Debaryomyces hansenii* DhTun2015.

**Table 5.** Biochemical composition of the 5 identified marine yeasts ($n$ = 3 for each strain in each parameter; letter, symbol for statistical analysis ($p < 0.05$).

| | | **RmTun15** | **YlTun15** | **TaTun15** | **CtTun15** | **DhTun2015** |
|---|---|---|---|---|---|---|
| | | mg/100 g (dry biomass) | | | | |
| Mineral | Calcium | 1.15 | 1.9 | 1.75 | 2.62 | 1.82 |
| | Magnesium | 0.823 | 0.896 | 0.637 | 1.92 | 0.573 |
| | Potassium | 8.68 | 11.7 | 7.03 | 18.9 | 5.8 |
| | Sodium | 0.12 | 0.05 | 0.116 | 0.15 | 0.0948 |
| | Iron | 0.0884 | 0.0713 | 0.0523 | 0.111 | 0.0656 |
| | Manganese | 0.0061 | 0.00547 | 0.00259 | 0.00668 | 0.00245 |
| | Nickel | 0.00164 | 0.00328 | 0.00243 | | |
| | Selenium | 0.00446 | 0.00453 | 0.00113 | 0.00065 | 0.00046 |
| Biogenic Amine | Putrescine | 12 | 15 | - | 1 | 2 |
| | Cadaverine | 37 | 94 | 6 | 23 | 18 |
| | Histamine | 14 | 13 | 9 | 12 | 8 |
| | Tyramine | 56 | 20 | 20 | 30 | 13 |
| | Spermidine | 84 | 61 | 12 | 44 | 22 |
| | BAI | 0.74 | 1.97 | 1.15 | 0.80 | 1.21 |
| | | g/100 g (dry mass) | | | | |
| Ash | | 2.43 ± 0.01 | 2.56 ± 0.06 | 1.82 ± 0.10 | 2.36 ± 0.35 | 3.01 ± 0.14 |
| Carbohydrate | | 25.19 ± 0.27 $^{m}$ | 23.21 ± 0.74 $^{n}$ | 33.12 ± 0.35 | 23.21± 0.51 $^{n}$ | 26.19 ± 0.58 $^{m}$ |
| Lipid | | 26.63 ±0.19 $^{1}$ | 37.57 ± 0.41 $^{2}$ | 11.15 ± 0.42 $^{3}$ | 15.25 ± 0.24$^{4}$ | 14.33 ± 0.35 $^{5}$ |
| Protein | | 28.67 ± 0.11 $^{S}$ (33%) | 24.37 ± 0.20 $^{T}$(26%) | 30.76 ± 0.03 (35%) | 30.50 ± 0.09 (40%) | 32.12 ± 0.28 $^{V}$ (40%) |
| Fatty Acid | C14:0 | - | 0.50 ± 0.04 | 0.48 ± 0.05 | 0.03 | 0.04 |
| | C15:0 | 7.34 ± 1.44 | 6.88 ± 0.45 | 2.16 | 4.14 ± 0.26 | 6.78 ± 1.04 |
| | C16:0 | 1.21 ± 0.41 | 12.11 ± 0.43 | 3.56 ±0.52 | 0.26 | 0.68 ± 0.06 |
| | C16:1 W7 | 0.83 ± 0.03 | 0.05 ± 0.006 | - | 0.26 | 0.07 |
| | C16:2 W4 | 7.94 ± 1.03 | 2.21 ± 0.44 | 0.34 ± 0.06 | 2.37 ± 0.20 | 1.31 ± 0.07 |
| | C16:3 W4 | - | - | - | 0.04 | 0.15 |
| | C18:0 | 1.75 ±0.18 | 7.56 ± 0.21 | 1.42 ± 0.07 | 0.57 ± 0.11 | 1.97 ± 0.67 |
| | C18:1 W9 | 0.24 ± 0.07 | 0.21 ± 0.006 | 0.29 ± 0.02 | 0.47 ± 0.21 | 2.13 ± 0.03 |
| | C18:1 W7 | - | 0.15 ± 0.004 | - | - | - |
| | C18:2 W6 | 2.18 ± 0.13 | 1.41 ± 0.41 | 2.12 ± 0.61 | 2.33 ± 0.95 | 0.84 ± 0.09 |
| | C18:3 W4 | 0.75 | 1.41 ± 0.03 | 0.25 ± 0.04 | - | - |
| | C18:3 W3 | - | - | - | 0.27 ± 0.11 | 0.24 ± 0. 29 |
| | C18:4 W3 | - | - | - | - | 0.07 ±0.017 |
| | C20:1 W9 | - | 0.18 ± 0.02 | - | - | - |
| | C20:4 W6 | - | - | - | 0.13 ± 0.02 | - |
| | C20:4 W3 | - | - | - | - | - |
| | C20:5 W3 | - | - | - | - | - |
| | C22:5 W3 | 0.47± 0.04 | 0.50 ± 0.04 | - | | - |
| | C22:6 W3 | 0.76 ± 0.07 | 1.09 ± 0.14 | 0.34 ± 0.01 | - | 0.03 ± 0.003 |
| | SFA | 10.30 ± 0.52 $^{b}$ | 27.04± 0.28 $^{a}$ | 7.61 ± 1.35 | 5 ± 0.62 | 9.47 ± 1.46 $^{b}$ |
| | MUFA | 1.07 $^{\alpha}$ | 0.58 ± 0.05 $^{\beta}$ | 0.29 ± 0.03 $^{\gamma}$ | 0.73 ± 0.08 $^{\lambda}$ | 2.20 ± 0.78 $^{\Omega}$ |
| | PUFA | 12.10 ± 0.07 ** | 6.61 ± 0.10 * | 3.06 ± 0.58 | 5.14 ± 1.41 * | 2.64 ± 0.15 |
| | ∑ FA | 23.47 ± 0.02 $^{A}$ | 34.24 ± 0.13 $^{B}$ | 10.95 ±0.78 | 10.86 ± 1.37 | 14.32 ± 0.77 $^{C}$ |

**Table 5.** *Cont.*

| | | RmTun15 | YlTun15 | TaTun15 | CtTun15 | DhTun2015 |
|---|---|---|---|---|---|---|
| Amino Acid | Aspartate | 1.41 ± 0.11 | 1.90 ± 0.36 | 2.16 ± 0.12 | 8.67 ± 0.02 | 1.91 ± 0.04 |
| | Glutamate | 1.56 ± 0.41 | 0.92 ± 0.01 | 0.98 ± 0.02 | 1.63 ± 0.69 | 1.86 ± 0.37 |
| | Serine | 2.05 ± 0.08 | 1.73 ± 0.03 | 1.32 ± 0.03 | 1.23 ± 0 | 1.88 ± 0.02 |
| | Asparagine | 0.14 ± 0.00 | - | 0.14 | - | 0.14 |
| | Glutamine | 0.20 ± 0.00 | 0.2 | 0.2 | - | 0.2 |
| | Histidine | 0.58 ± 0.01 | 0.72 ± 0.1 | 0.48 ± 0.05 | 0.81 | 0.50 ± 0.01 |
| | Glycine | 1.48 ± 0.02 | 1.73 | 1.22 ± 0.01 | 1.04 | 1.23 ± 0.03 |
| | Threonine | 1.37 ± 0.02 | 1.72 ± 0.13 | 1.24 ± 0.16 | 1.11 | 1.49 ± 0.13 |
| | Arginine | 1.84 ± 0.09 | 0.94 | 1.15 ± 0.05 | 0.68 | 1.67 ± 0.10 |
| | Alanine | 1.40 ± 0.27 | 1.09 ± 0.25 | 2.87 ± 0.14 | 1.67 ± 0.07 | 1.82 ± 0.04 |
| | Tyrosine | 0.87 ± 0.05 | 0.76 ± 0.03 | 0.62 ± 0.02 | 0.51 | 0.73 |
| | Valine | 0.45 ± 0.02 | 2.31 ± 0.16 | 0.26 ± 0.16 | 0.18 | 0.23 ± 0.17 |
| | Methionine | 2.17 ± 0.04 | 0.53 ± 0.05 | 1.08 ± 0.05 | 0.61 ± 0.05 | 0.28 ± 0.02 |
| | Tryptophan | 0.10 ± 0.02 | 0.10 ± 0.04 | 0.08 ± 0.03 | 0.8 | 3.48 ± 0.14 |
| | Phenylalanine | 0.97 ± 0.06 | 0.98 ± 0.09 | 0.78 ± 0.06 | 1.35 | 1.07 ± 0.03 |
| | Isoleucine | 1.01 ± 0.01 | 1.06 ± 0.08 | 0.86 ± 0.03 | 0.14 | 1.12 ± 0.02 |
| | Leucine | 2.21 ± 0.14 | 1.64 ± 0.13 | 1.74 ± 0.10 | 1.29 | 1.82 ± 0.02 |
| | Lysine | 2.55 ± 0.35 | 2.54 ± 0.04 | 1.94 ± 0.03 | 2.54 ± 0.03 | 2.59 ± 0.01 |
| | Hydroxyproline | 0.71 ± 0.35 | 2.05 ± 0.49 | 2.42 ± 0.09 | 1.29 ± 0.02 | 2.85 ± 0.59 |
| | Proline | 2.11 ± 0.02 | 2.94 ± 0.13 | 1.71 ± 0.14 | 1.69 ± 0.44 | 2.57 ± 0.27 |
| | ∑ AA | 25.18 ± 0.10 $^{R}$ | 23.86 ± 0.11 | 23.25 ± 0.06 | 27.24 ± 0.06 $^{S}$ | 29.44 ± 0.10 $^{T}$ |
| | EAA | 15.26 ± 0.07 (61%) | 15.38 ± 0.09 (64%) | 11.24 ± 0.08 $^{X}$ (48%) | 10.4 ± 0.05 $^{Y}$ (38%) | 13.34 ± 0.07 $^{Z}$ (45%) |
| | NEAA | 9.92 ± 0.13 (39%) | 8.48 ± 0.12 (36%) | 12.01 ± 0.04 (52%) | 16.84 ± 0.08 (62%) | 16.1 ± 0.12 (55%) |

* and ** are symbols used for the statistical analysis to show a significant difference between stains in the same parameter (PUFA content). Letters shows significant difference between studied strains in each analyzed parameters.

#### 3.4.1. Ash Content

The determination of ash content revealed that the five marine yeast species are rich in mineral, which is similar to the values reported for the three yeasts *Debaryomyces hansenii* (S8 and S100); *Candida* sp. (S186) and *Saccharomyces cerevisiae* S36 [78,79]. For *Rhodotorula glutinis*, Andlid (1995) reported that the ash contents decreased from 7% to 3% from 16 h to 161 h of culture in an environment where the supply is limited in nitrogen and with glucose as the source of carbon and energy [80].

The ash represents between 10 and 17% of the dry weight of different species studied. These results are similar to those found by Brown (1996) for the marine yeasts *Debaryomyces hansenii* ACM 4783 and *Candida utilis* ACM 4774 but are higher than those found for *Dipodascu* ssp. ACM 4780 (6.8%), *Dipodascu* ssp. ACM 4779 (9.3%), *Dipodascu ssp*. ACM 4778 (4.7%), *Dipodascu* ssp. ACM4781 (4.9%), *Dipodascu* ssp. ACM4782 (2.5%) and *Saccharomyces cerevisiae* ACM 4775 (7.7%) [81].

#### 3.4.2. Mineral Profile

The analysis of the dry biomass of the different species revealed that potassium represents 80% of the mineral for YlTun15, RmTun15 and CtTun15; 73% for TaTun15 and 69%. For calcium, this percentage was 11% for the species RmTun15 and CtTun15, 13% for YlTun15, 18% for TaTun15 and 22% for DhTun2015. The magnesium level is on average 7% for the five strains analyzed.

Potassium, magnesium, calcium and zinc are cationic nutrients which play an essential structural and functional role in yeast cells and are particularly important in the

fermentation processes. Potassium is the most abundant cellular cation in yeasts, constituting 1 to 2% of the dry weight of yeast cells and constitutes the main electrolyte essential for osmoregulation and the absorption of divalent cations. It is also a major cofactor for an enzyme involved in oxidative phosphorylation, protein synthesis and carbohydrate catabolism [82].

Magnesium is the most abundant intracellular divalent cation in all living cells and is absolutely essential for yeast growth. It represents approximately 0.3% of the dry weight of yeast cells and constitutes an essential cofactor of enzymes. Along with potassium, magnesium can neutralize the electrostatic force in nucleic acids, polyphosphate and proteins. Calcium binds to the walls of yeast cells and plays a key role in flocculation, essential in brewing fermentation processes. Calcium also antagonizes the absorption of magnesium and can block the metabolic process dependent on essential magnesium. It is important to note the calcium-magnesium antagonism, in particular with regard to the fermentation of yeast. As for the other trace elements, iron, zinc, nickel, copper, cobalt, manganese, they are necessary in metalloenzymes, redox pigments, heme proteins and vitamins as essential structural stabilizers and cofactors [83–87].

#### 3.4.3. Carbohydrate Content

The carbohydrate content in yeasts ranged from 21 to 39% [76]. In the present study, the amount of carbohydrates showed variable rates depending on the species, ranging from 23 to 25% for, respectively, (CtTun15 and YlTun15)—(RmTun15 and DhTun2015) to 33% for TaTun15. These results are consistent with those reported in other studies, notably for the yeasts *Debaryomyces hansenii* (21%) and *Saccharomyces cerevisiae* (39%). The highest carbohydrate content was found in *Torulopsis* and *Candida* 57 and 55% [88]. Generally, marine yeasts are rich in carbohydrates [81]. Yeasts also act as immunostimulants due to their high content of carbohydrates (β-1,3 glucans) [79] and can therefore be involved as feed in aquaculture.

#### 3.4.4. Lipid Content

The ability of certain microorganisms to accumulate large amounts of lipids is well established, but it is only in recent decades that real efforts have been made to elucidate the underlying biochemical pathways, including the pathway synthesis of lipids, factors influencing the accumulation of lipids and genetic modifications [89]. In oleaginous yeasts, certain ones can accumulate lipids at rates greater than 20% of their dry cell weight. The genera include *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon* and *Lipomyces* [90].

The YlTun15 and RmTun15 species have the highest fat contents, with respective levels of 37.57 ± 0.14 and 26.63 ± 0.19 g/100 of dry matter. These contents are statistically higher than that of *Trichosporon asahii* TaTun15 20.63 ± 0.17 g/100 g of dry matter. CtTun15 and DhTun2015 have the lowest level of lipids with an average of 14.79 ± 0.29 g/100 g.

The efficient extraction of lipids and the culture medium have the greatest impact on the quantity of microbial lipids and are considered as the main limiting factors for large-scale culture [91,92]. Thus, for certain species of marine yeast cultivated under the same conditions as this study (substrate: glucose; temperature) and the method of lipid extraction (Folch), the lipid contents vary according to the species. Bommareddy (2015) reported that the lipid level in *Rhodosporidium toruloides* was 30% [93]. *Mortierella isabella* ATCC 4613 and *Rhodotorula mucilaginosa* have contents of 0.360 and 0.445 g/g of material, respectively [94,95]. Several studies have maintained glucose as a substrate for culture and have introduced certain modifications in the Folch method and they have detected an increase in the lipid contents of *Y. lipolytica* DSM8218 (33%), *Cryptococcus albidus* (41%), *Candida sp.* (43%) and *Y. lipolytica* M7 (49%) [96,97].

The possibility of industrial production of lipids using oleaginous yeasts has already been considered [7,90,98–101].

3.4.5. Fatty Acid Profile

Saturated fatty acids (SFA) are predominant in the five species including C15; C16 and C18 represent 95% of the SFA identified and from 30% (RmTun15 and CtTun15) to more than 50% (TaTun15; DhTun2015 and YlTun15) of the total FA (Table 5). C16: 2; C18: 2 and C18: 3 are the majority PUFAs and they represent 45.71; 43.09; 22.53; 16.64 and 10.55% of the total FA identified respectively in CtTun15, RmTun15, TaTun15, DhTun2015 and TaTun15.

DHA represents 3% of the total FA identified for YlTun15, RmTun15 and TaTun15. According to the literature, oleaginous yeasts are rich in fatty acids from the 16 and 18 carbon atoms [102]. A similar profile has been reported for Brown (1996) [81]; Chi (2008) [29]; Kang (2006) [103]; Enshaeieh (2013 and 2014) [96,97]; Wang (2017) [104]; Dalmas Neto (2019) [92]. The lipids accumulated by oleaginous yeasts are mainly composed of long chain fatty acids, in particular oleic acid (C18: 1), palmitic acid (C16), linoleic acid (C18: 2) and stearic acid (C18) similar to the composition of vegetable oils and can be converted into biodiesel by enzymatic or inorganic catalysis [105–108].

3.4.6. Protein Content

Proteins are essential biomolecules as they perform diverse functions in living cells. In the present study, different protein contents ($p < 0.05$) were found in *Y. lipolytica* YlTun15 and *R. mucilaginosa* RmTun15 strain (24.36 ± 0.20 and 28.67 ± 0.11 g/100 gDW, respectively). Higher but similar ($p > 0.05$) protein levels were found in CtTun15 and TaTun15 strains with a mean value of 30.63 ± 0.06 g/100 gDW; the highest content was found in *Debaryomyces hansenii* DhTun2015 strain with 32.13 ± 0.28 g/100 g corresponding to 40% of the yeast dry mass. Such levels were close to values reported in other studies on marine yeasts such as *Debaryomyces hansenii* ACM4784, *Dipodascuscapitatus* ACM4779, *Dipodascu* ssp. ACM4780 and *Candida utilis* which contained 23, 32, 36 and 42% of crude protein, respectively [81]; and therefore, can be considered as single cell protein (SCP). As SCP, yeast cells are better than any other microorganism because of their high protein and other nutrient contents and also their low nucleic acid contents [109]. However, it is important to emphasize that higher protein contents can be reached following mutagenesis [110] or fermentation [35,111] in *Y. lipolytica* SWJ-1b (53.7%) and C. *aureus* G7a (65.3%) respectively. Initially, marine yeasts such as *D. hansenii* and *Candida austromarina* were suggested to be used as a food source for the cultivation of the crustacean *Moinamacrocopa* [103] or as feed for aquaculture [29]. However recently, research has focused interests on using yeast as a source of alternative protein [112], but research on marine yeast is rather lacking.

3.4.7. Amino Acid Content

Chromatographic analysis revealed that the marine yeast strains contain significant levels of essential amino acids (EAAs), especially threonine, arginine, proline, lysine and leucine. Thus, the EAAs represented 64.4; 60.6; 48.3; 45.3 and 38.1% of the total amino acids identified in the species YlTun15; RmTun15; TaTun15; DhTun2015 and CtTun15, respectively. This result may encourage the use of these species in animal feeding. Such EAAs were higher than values reported for *Hanseniaspora uvarum* YA03a strain (7.8% lysine and 8.9% leucine) and for *Cryptococcus aureus* G7a strain containing 10.2% lysine and 6.0% leucine [29]. Brown et al. (1996) reported that *C. utilis* ACM4774, widely used in the diet of terrestrial animals, contained only 3.8% lysine and 7.8% leucine of total amino acids, while the marine yeast strains *Debaryomyces hansenii* ACM4784, *Dipodascus capitatus* ACM4779 and *Dipodascus* sp. ACM4780 contained 4.8% lysine and 7.5% leucine, 4.6% lysine and 7.5% leucine, 3.9% lysine and 7.9% leucine, respectively. Therefore, the wild marine yeasts isolated in this study can be considered a good source of EAAs [81].

3.4.8. Biogenic Amine Content

Biogenic amines are low molecular weight organic bases that have biological activity. Several biogenic amines play an important role in many physiological human and animal functions, such as regulating body temperature, stomach volume and pH, and brain

activity [113]. They can be formed and degraded due to normal metabolic activity in animals, plants and microorganisms, and are generally produced by the decarboxylation of amino acids. The absence of published work did not allow us to carry out a comparative study. However, the occurrence of biogenic amines is not only a risk factor for intoxication but is also an indicator of food quality. Di- and polyamines (Cad and Put) are considered as indicators of food freshness and quality. According to the BAI value, CtTun15 and RmTun15 have a BAI < 1 and they can be considered to be of good quality. Regarding their biogenic amine's composition, these strains may be used in several applications such as agriculture [114], aviculture [115] and medicine as a supplement [116].

### *3.5. Cytotoxicity Effect of F5 Peptide on HEK293 Cells*

As a new source of alternative protein, it was important to have an insight on the cytotoxicity of the isolated wild marine yeasts. Cytotoxicity assay is an experimental method used to evaluate the effect/toxicity measurement on the normal cell line such as human embryonic kidney cells [117]. In this study, the cytotoxic effect of TaTun15, RmTun15, YlTun15, DhTun2015, and CtTun15 samples was tested based on MTT assay using HEK293 cells.

Figure 2A showed that all the tested samples increased the cell survival with a significant distinction compared with the control. In fact, the activity of mitochondrial succinate dehydrogenase of HEK293 cells, related to MTT assay, has been well improved. In addition, the microscopic observation of untreated and treated HEK293 cells (Figure 2B) with TaTun15, RmTun15, YlTun15, DhTun2015, and CtTun15 samples confirmed the non-toxic effect of the samples. On the other hand, the samples can regulate the function of the body's cellular activity; however, further investigation to establish the exact scheme of cellular induction will be performed.

### *3.6. Potential Application of Marine Yeast*

Yeasts are single-celled organisms with a solid outer cell wall and can generally survive in various biotopes where they generate different biomolecules [118]. In this work, a thorough literature survey was realized to identify the potential interest of marine yeast. Thus, oils, enzymes, bioactive natural products, unicellular proteins and nanoparticles have been extracted from yeasts. Such biomolecules have interesting potential applications in the food, chemical, agricultural and pharmaceutical sectors (Table 6).

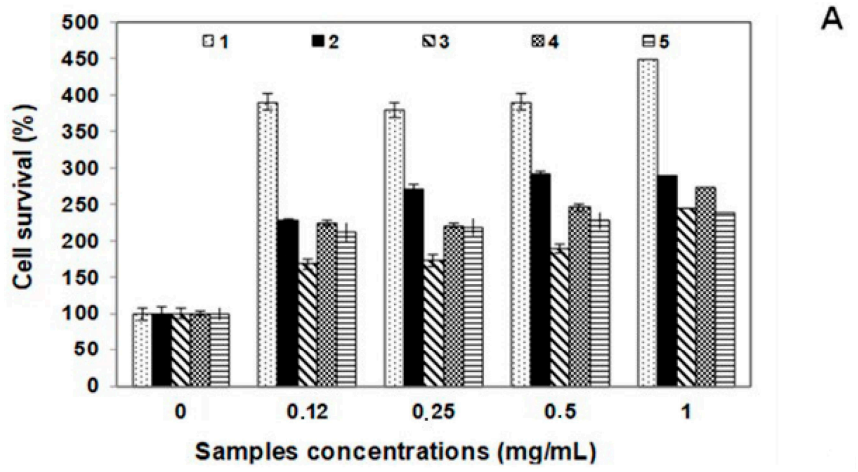


**Figure 2.** *Cont.*

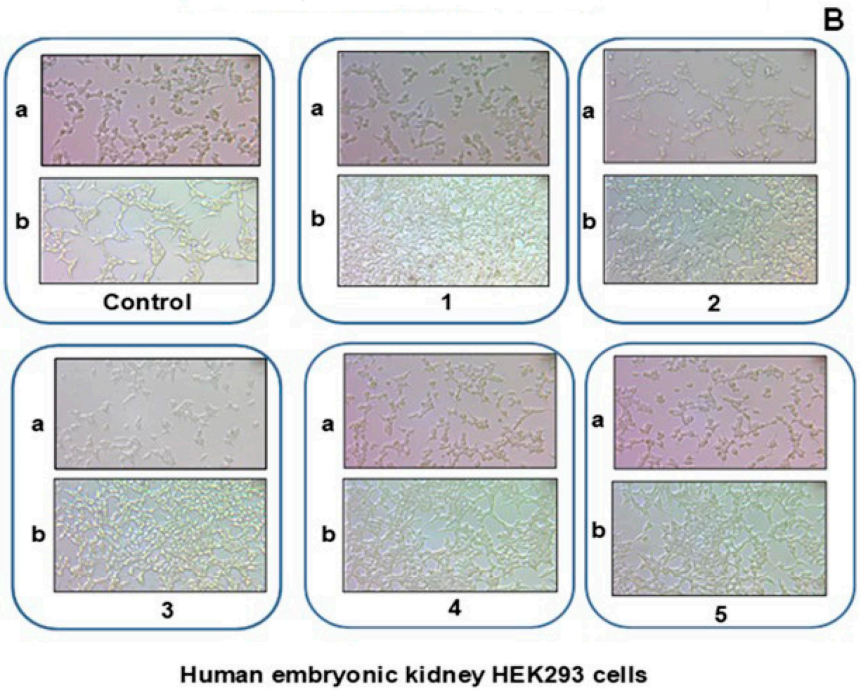


**Figure 2.** Effect of 1 (TaTun15), 2 (RmTun15), 3 (YlTun15), 4 (DhTun2015) and 5 (CtTun15) samples on HEK293 cells. Effect of different 1, 2, 3, 4, and 5 samples concentration on HEK293 cells survival. All data are expressed as mean of at least three independent experiments (**A**). Analysis of HEK293 cells morphology in untreated (**a**) and treated HEK293 cells (**b**) with the marine yeast 1 (TaTun15), 2 (RmTun15), 3 (YlTun15), 4 (DhTun2015), and 5 (CtTun15) after 24 h of incubation (**B**).

**Table 6.** Application field of marine yeasts.

| Application | Species | Reference |
|---|---|---|
| Biodiesel (SCO) | *Trichosporon fermentans* | [119] |
| | *Rhodotorula glutines* | [120] |
| | *Rhodotorula mucilaginosa* | [121] |
| | *Rhodotorula graminis* | [122] |
| | *Rhodotorula toruloides NBRC 0559* | [123] |
| | *Yarrowia lipolytica* | [124] |
| | *Debaryomyces etchellssi* | [125] |
| | *Trichosporon* sp. | [126] |
| | *Trichosporon cutaneum* | [127] |
| | *Yarrowia lipolytica NCIM3589* | [128,129] |
| | *Rhodotorula kartochvitovae SY89* | [130] |
| | *Rhodotorula glutinis* | [131] |
| | *Yarrowia lipolytica* | [132] |
| | *Y. lipolytica YlTun15; MY-2 et MY-3* | [31] |
| | *R. mucilaginosa RmTun15; MR-2 et MR-3* | |
| | *T. asahii TaTun15; MT-2 et MT-2* | |

**Table 6.** *Cont.*

| Application | Species | Reference |
| --- | --- | --- |
| Bioethanol | *Rhodotorula minuta*<br>*Yarrowia lipolytica* | [133] |
| | *Candida albicans*<br>*Candida tropicalis*<br>*Debaryomyces hansenii* | [71] |
| | *Candida* sp. | [70] |
| | *Yarrowia lipolytica Po1g* | [134] |
| | *Yarrowia lipolytica* | [135] |
| | *Yarrowia lipolytica* | [136] |
| | *Yarrowia lipolytica* | [12] |
| | *Yarrowia lipolytica* | [137] |
| | *Yarrowia lipolytica* | [138] |
| | *Debaryomyces hansenii* | [139] |
| | *Debaryomyces hansenii CBS004* | [140] |
| | *Trichosporon asahii* | [141] |
| | *Y. lipolytica RmTun15* | [28] |
| | *Rhodotorula mucilaginosa* | [142] |
| Carotenoids (Torulene, torularhodin,β-carotene) | *Rhodotorula mucilaginosa* | [143] |
| | *Rhodotorula RY1801* | [144] |
| | *Rhodotorula mucilaginosa URM7409* | [145] |
| | *Rhodotorula glutinis* | [146] |
| | *R. mucilaginosa ATCC 66034*<br>*R. gracilis ATCC*<br>*R. glutinis LOCKR13* | [147] |
| | *Rhodotorula mucilaginosa C2.5t1* | [148] |
| | *Rhodotorula sp* | [149] |
| | *Rhodotorula glutinis CCY 20-2-26* | [150] |
| | *Yarrowia lipolytica W29* | [151] |
| | *Rhodotorula glutinis* | [152] |
| | *Rhodotorula mucilaginosa AY-01* | [153] |
| | *Yarrowia lipolytica* | [154] |
| | *Rhodotorula mucilaginosa* | [155] |
| | *Rhodotorula mucilaginosa* | [156] |
| | *Rhodotorula glutinis DM28* | [157] |
| | *Rhodotorula glutinis* | [158] |
| Pharmaceutical | *Debaryomyces hansenii* | [159] |
| | *Yarrowia lipolytica* | [160] |
| | *Yarrowia lipolytica NCYC 789* | [161] |
| | *Candida tropicalis* | [162] |

**Table 6.** *Cont.*

| Application | Species | Reference |
|---|---|---|
| Nanoparticles | *Candida albicans*, *C. tropicalis*, *Debaryomyces hansenii*, *Rhodotorula minuta*, *Yarrowia lipolytica* | [163] |
| | *Rhodotorula sp ATL72* | [164] |
| | *Yarrowia lipolytica IMUFRJ 50682* | [165] |
| Alimentation | *Candida* sp. | [35] |
| | *Rhodotorula mucilaginosa* | [166] |
| | *Rhodotorula* sp. | [80] |
| | *Debaryomyces hansenii S8* *Debaryomyces hansenii S100* *Candida tropicalis S186* | [79,87] |
| | *Debaryomyces hansenii* | [167] |
| | *Debaryomyces hansenii* | [168] |
| | *Debaryomyces hansenii* | [169] |
| | *Yarrowia lipolytica* | [170] |
| | *Rhodotorula* sp. *H26* | [171] |
| Bio-surfactant | *Yarrowia lipolytica* NCIM 3589 | [172] |
| | *Yarrowia lipolytica IMUFRJ50682* | [173] |
| | *Candida glabrata* | [174] |
| | *Candida tropicalis* | [175] |
| | *Candida tropicalis* | [176] |
| | *Rhodotorula mucilaginosa KUGPP-1* | [177] |
| | *Candida lipolytica* | [178] |
| | *Candida bombicola* | [179] |
| | *Candida tropicalis* UCP 1613 | [180] |

This prompted us to extract and characterize these bio-molecules, which can be synthesized by RmTun15, YlTun15, TaTun15, CtTun15 and DhTun2015. Previous work revealed that *Yarrowia lipolytica* YlTun15 secretes an alkaline protease, which does not belong to the group of metalloproteins [30] and may have potential application in food; and RmTun15, TaTun15 and YlTun15 can generate triglyceride-based oils [31]. Based on the biochemical analysis, the present investigation suggests that these species can be used in agriculture and medicine.

## 4. Conclusions

In conclusion, the isolation of marine yeasts from various Tunisian aquatic environments and marine waste products allowed the purification of 32 strains of yeasts. The optimization of the isolation conditions, culture and extraction of DNA enabled the identification of the following marine yeast strains: *Cryptococcus curvatus*; *Meira nashicola*; *Meira* sp.; *Rhodotorula mucilaginosa*; *Sporobolomyces roseus*; *Sporobolomyces aff. Ruberrimus*; *Sporobolomyces ruberrimus*; *Trichosporon asahii*; *Debaryomyces hansenii*; *Candida parapsiolis*; *Yarrowia lipolytica* and *Candida tenuis*.

Among them, five species have been registered in the GenBank and have been the subject of biochemical characterization: *Yarrowia lipolytica* YlTun15 (GenBank Acc. N°MF327143), *Rhodotorula mucilaginosa* RmTun15 (GenBank Acc. N°MF327252), *Candida*

*tenuis* CtTun15 (GenBank Acc. N°KY558632), *Debaryomyces hansenii* DhTun2015 (GenBank Acc. N°KY508343) and *Trichosporon asahii* TaTun15 (GenBank Acc. N°KY509046).

The biochemical investigation of the five identified marine yeast strains showed that these species are rich in mineral elements, lipids, protein and EAAs with no cytotoxicity effects. Therefore, these yeasts can be used in food industries and other sectors such as the nutraceutical sector.

**Author Contributions:** B.B. and S.S. designed the experiment and wrote the manuscript; B.B. conducted the practical work; the molecular identification of the marine yeast strain was made in IBK laboratory under the supervision of T.B.; the harmonization of the analytical procedure was conducted by A.S. and C.M.M.; MTT assay and cell viability were conducted under the supervision of B.J. All authors have read and agreed to the published version of the manuscript.

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