universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

"Characterization of marine pelagic fungi from the Atlantic Ocean"

verfasst von / submitted by

Magdalena Reitbauer, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2022 / Vienna, 2022

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 833
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Ecology and Ecosystems
Betreut von / Supervisor:	Assoz.-Prof. Dr. Federico Baltar,

Table of Contents

Acknowledgements

I would first like to thank my supervisor Federico Baltar who supported me throughout the whole process of working on this thesis. I am deeply grateful for the discussions we had and the constructive feedback I received. Furthermore I would like to thank the whole Fungi group for always exchanging knowledge and working together as a great team.

Especially, I want to thank Eva Breyer for the contribution in my project, where she gave me theoretical and practical input. I am generally very grateful that I could be part of such a great working group, where everyone has been supportive. Additionally, I would like to thank my parents for supporting me in a lot of different ways during the time where I have been studying and working on my thesis.

Abstract

More than 10.000 taxa of marine fungi are currently estimated to inhibit the ocean, potentially holding important roles for organic matter cycling and food web dynamics. However, there is a documentation of only 1901 species in the last 72 years. Thus, the diversity and ecological functions of marine fungi remain speculative and unexplored. Traditionally, it has been very popular to only describe fungi morphologically, which is limited. Identifying them genetically and additionally having them cultivated on plates leads to a more comprehensible documentation of species. This is crucial for studying fungal strains, and depicts the basis for further research on marine fungi. In this study pelagic fungi were collected across a transect through the Atlantic Ocean at different stations and depths, and were genetically identified. Furthermore, all isolates were used for physiological tests, exposing them to changing environmental parameters like salinity or temperature. Additionally metabolic experiments on the preference towards different types of carbon substrates were conducted. With this we were able to isolate, identify and characterize the yeasts *Rhodoturula mucilaginosa* and *Scheffersomyces spartinae*, and the hyphae *Sarocladium kiliense*. This study provides relevant information on the physiological, genetical and ecological properties of pelagic fungi, and ultimately contributes to understand the ecology and distribution of pelagic fungi in the oceanic water column.

Introduction

Fungi are phylogenetically, ecologically and morphologically a very diverse group. They are ubiquitous in terrestrial, freshwater and marine habitats (Hibbett et al. 2007). Phylogenetically, the Kingdom of fungi was traditionally classified into four main groups Ascomycota, Basidiomycota (forming the subdivision of Dikarya), Zygomycetes and the Chytrids. Ascomycota and Basidiomycota can both occur yeast like or filamentous and have been the major target for genetic investigations (Jones et al. 2011). Later on microsporidia (unicellular spore-building parasites) were also added to the Kingdom of fungi (Richards et al. 2011). However, many uncertainties concerning the taxonomic characterization of fungi are still remaining (Hibbett et al. 2007).

According to current estimations 2.2 to 3.8 million fungal species exist, whereas only 3% to 8% are described, resulting in a number of about 120.000 known fungal species (Hawksworth and Lucking 2017). Current studies report 1901 marine fungi species, consisting of 472 genera, that have been documented in the last 72 years. This classification includes 805 Ascomycota species (352 genera), 21 Basidiomycota species (17 genera), 26 Chytridiomycota species (14 genera), 3 Zygomycota species (2 genera) and Blastocladiomycota with 1 species. Besides that, 43 species (26 genera) of asexual morphs of filamentous fungi were described so far. Marine yeasts are represented as Ascomycota with 138 species and Basidimycota with 75 species (26 genera). The largest family of marine fungi are Halosphaeriaceae with 141 species (59 genera) (Jones et al. 2015).

Fungi are widely used in diverse industrial sectors (Raja et al. 2017). Yeasts are indispensable in the baking and brewing industry, and in the pharmaceutical sector, mostly related to the production of active secondary metabolites (Smith and Ryan, 2009). Over the last years various antibiotics and cholesterol lowering medicals have been developed based on fungal strains (Newman and Cragg, 2007). The importance of identifying fungi genetically is highlighted by a study of Raja et al. (2017), where they stated that “The Journal of Natural Products” published an average of 37 articles every year between 2000 and 2015. There they listed isolated secondary metabolites derived from fungal strains. According to Raja et al. (2017) around 28% of the fungal strains did not report any taxonomic identification and around 31% of them were solely identified by their morphology. Knowing the taxonomic and physiological background of these fungi is crucial to being able to ensure the cultivation of these strains and the production of their metabolites for further medical usage.

Fungi are not only very important in the medical field, but they also play a key role in the terrestrial and aquatic ecosystems. They are degraders and important remineralizers of organic matter (Grossart et al. 2019). Nevertheless, most fungal species are not described yet, and previous studies have mostly focused on terrestrial and freshwater fungi, causing marine fungi to be understudied.

Obligate aquatic fungi are defined by the fact that they rely on aquatic habitats for their whole life cycle (Grossart et al. 2019). However, mycologists face the challenge that many fungi that

are isolated from the ocean also occur in terrestrial systems (Amend et al. 2019). This points out the remarkable adaptive capabilities of fungi, but also generates the question of how many already described terrestrial species inhibit marine environments (Amend et al. 2019).

Marine fungi, especially yeasts, occur in almost every aquatic habitat on Earth (Grossart et al. 2019). A study by Hassett et al. 2020 states that in environments with an extraordinary high salinity like the Mediterranean and the Red Sea Chytridiomycota are the predominant phyla, whereas Dikarya dominate the open ocean. Within all areas explored in this study, the highest species richness was found in the Mediterranean and the Baltic Sea (Hassett et al. 2020). Recently, a very high abundance of Dikarya in the North Sea has been recorded by 18S rRNA gene tag sequencing (10^5 cells L^{-1}) (Priest et al. 2021). Fungi where also found in the Arctic, where chytrids dominate the fungal communities (Hassett and Gradinger 2016). Additionally, metagenomic analyses revealed that fungi are present in all oceans, dominated by Dikarya (Ascomycota and Basidiomycota), and exhibiting an increase in diversity with depth (Morales et al. 2019).

Due to their broad distribution marine fungi are also considered as being crucial participants in food web dynamics (Grossart et al. 2019). As saprophytes, they are decomposing recalcitrant substrates, making them more accessible for other organisms in the food web (Zeghal et al. 2021). Moreover, parasitic fungi, especially Chytrids, can have a high impact on food webs. Through killing their host or part of their host, substrates are released for microbial processing. These substrates provide nutrient-rich particles for the grazer food chain (Kagami, Amano and Ishii 2012).

Marine fungi have beeen isolated from different sources and also extreme marine habitats such as the deep sea, sub-sea floor, hydrothermal vents, marine coastal waters, lakes, ice and snow (Kutty and Philip 2008). As expected, the majority of fungi prefers oxic conditions, but some species are also found in oxygen minimum zones (OMZ) (Stief et al. 2014). It was also found that in coastal and surface waters marine fungi are able to degrade wood (Bucher et al. 2004) and animals debris (Kohlmeyer and Kohlmeyer, 1979). A recent study even indicates that they are also degrading plastics, such as polyethylene (Zeghal et al. 2021). Marine fungi might also act as saprotrophs on bathypelagic marine snow, as they were found to dominate the microbial biomass on it (Bochdansky 2017). Recently, it has been investigated that marine fungi show a high ratio of carbohydrate-active enzymes (CAZymes), indicating that pelagic fungi in all ocean basins and depths utilize different types of CAZymes, suggesting an active contribution in the carbon cycle (Baltar et al. 2021).

Traditionally, marine fungi have been classified based on their morphology, however this does not lead to an accurate representation of their genetical diversity, because the morphological appearance can depend on many factors (Inui et al. 1965). This is why there is the need of characterizing them to exclude any uncertainties concerning their taxonomic origin for cultivating them. Another problem is, that there are not many marine isolates in culture (Richards et al. 2015), leading to a gap between well-documented species descriptions and

dark matter fungal DNA sequences where almost no morphological or other information is available (Grossart et al. 2019).

To address this knowledge gaps, we characterized marine pelagic fungi at the species level and provide additional information on morphological characteristics by isolating and cultivating the fungi on agar plates. Furthermore, we performed experiments in liquid cultures to investigate their physiological features. We also had a look at the metabolic capacities of the strains by using MicroPlateTM. For the genetic characterization, we sequenced the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA (rRNA), which is well-known for being the official bar-code for the identification of fungal strains. If necessary, we also sequenced, the partial small subunit nuclear rDNA (SSU) and the partial large subunit nuclear rDNA (LSU), the translation elongation factor 1-alpha gene (TEF1- α), the protein coding gene RPB2 and the beta-tubulin (TUB) region. By comparing the sequences from the different gene regions we could successfully assign our replicates to the species that are described below. With this information we aimed to expand our understanding on the taxonomy, and the physiological and ecological properties of pelagic fungi in the ocean.

Material and Methods

Sample Collection and Isolation

In this study pelagic fungi were collected during the ANTOM1 cruise in 2020/21. The isolates were sampled across a transect through the Atlantic Ocean (from Spain to Chile) at 24 different stations (Table 1). The environmental parameters where measured with a CTD rosette. The sampling depth ranged from 5 to 2000m. After the samples were taken (400 to 500 ml of seawater) they were filtered through a GF/F filter and cultivated on agar plates. Additionally, we included one isolate from the Poseidon cruise (performed in the same waters in 2019) named “Poseidon”.

Table 1. The identified species and the according numbers of the replicates with their corresponding ID’s and the number of the station where the fungi were isolated. ‘Replicate’ refers to the original ID number given to the colonies formed in the isolation plates used during sampling.

Species	**ID**	**Station and Depth**
Scheffersomyces spartinae	Replicate 21, 13	10 (53m), 15 (5m)
Rhodoturula mucilaginosa	Poseidon	6 (93m)
Sarocladium kiliense	Replicate 15	12 (45m)

Culturing media

All isolates were cultured at room temperature on agar plates for four weeks containing the ingredients shown in Table 2. The plates were stored in the dark at room temperature.

Table 2. Chemicals used for agar plates in g/L

Agar	20
Glucose	10
Peptone	5
Yeast Extract	3
Malt extract	3
Sea salts	20
Chloramphenicol	0,5

DNA Extraction, PCR Amplification and Sequencing

The genomic DNA was extracted from living pure cultures using the following extraction protocol. The first step was to add 48µl MilliQ and 2µl 0.5M NaOH into a tube. With a sterile pipette tip a small colony was picked from the agar plate and resuspended in the NaOH mixture. Then the tube was kept in the -80°C freezer for 10 minutes. Afterwards the sample was incubated at 99°C for 10 minutes. This step was repeated if necessary.

To identify the fungal isolatesThe PCR thermal cycle programs were chosen as follows: for ITS, LSU and SSU: initial denaturation at 95 ◦C for 5 min, then 35 cycles of denaturation at 95 ◦C for 30 s, annealing at 55 ◦C for 30 s and extension at 72 ◦C for 30 s and final extension at 72 ◦C for 10 min; for TEF1-α: initial denaturation at 94 ◦C for 5 min, then 35 cycles of denaturation at 94 ◦C for 1 min, annealing at 55 ◦C for 50 s and extension at 72 ◦C for 90 s and final extension at 72 ◦C for 10 min. For RPB2 and TUB2: initial denaturation at 95 ◦C for 5 min, then 35 cycles of denaturation at 94 ◦C for 45 s, annealing at 55 ◦C for 45 s and extension at 72 ◦C for 1 min and final extension at 72 ◦C for 10 min.

Table 3. Samples with the investigated gene region and the corresponding primer pairs

Sample	**LSU**	**ITS**	**SSU**	**TEF**	**RPB2**	**TUB**
Poseidon	D1/D2	ITS1/ITS4	NL1/NL4	983F/2218R		
Replicate 13	D1/D2	ITS1/ITS4				
Replicate 15	LR0R/LR5	ITS1/ITS4	NL1/NL4	983F/2218R	5F/7R	2a/2b
Replicate 21	D1/D2	ITS1/ITS4				

The PCR products were checked in 2% agarose gels. The amplified PCR fragments were sent to the commercial sequencing provider Microsynth AG (Biotechnology company in Balgach, Switzerland).

Table 4. Sequences of the primers used for the amplification and sequencing of the fungal DNA (modified from Raja et al. 2017).

Primer Code	Sequence	Forward/Reverse	Location
D1	AGC GGA GGA AAA GAA ACT	Forward	LSU
D2	TAC TAG AAG GTT CGA TTA GTC	Reverse	LSU
LROR	GTA CCC GCT GAA CTT AAG C	Forward	LSU
LR5	TCC TGA GGG AAA CTT CG	Reverse	LSU
ITS1	TCCGTAGGTGAACCTGCGG	Forward	ITS
ITS4	TCCTCCGCTTATTGATATGC	Reverse	ITS
NL1	GCATATCAATAAGCGGAGGAAAAG	Forward	SSU
NL4	GGTCCGTGTTTCAAGACGG	Reverse	SSU
983F	GCY CCY GGH CAY CGT GAY TTY AT	Forward	TEF
2218R	AT GAC ACC RAC RGC RAC RGT YTG	Reverse	TEF
5F	GAY GAY MGW GAT CAY TTY GG	Forward	RPB2
7R	CCC ATW GCY TGC TTM CCC AT	Reverse	RPB2
2a	ACC CTC AGT GTA GTG ACC CTT GGC	Forward	TUB
2b	ACCCTCAGTGTAGTGACCCTTGGC	Reverse	TUB

Phylogenetic Analysis

The raw sequence data from the Sanger sequencing was edited and assembled in the program Geneious Prime ® 2022.0.1. After the assembling the generated sequences were blasted in the NCBI databank (https://blast.ncbi.nlm.nih.gov/BLAST.cgi) to identify closely related taxa. The similarity threshold of 97.0-98.5% which usually accounts for species level identification in fungi was used (Nilsson et al. 2019).

Sequences were downloaded from the GenBank. The output of the databank was used for building the phylogenetic trees. The sequences were aligned and trees were generated with the program DNA Star, Meg Align Pro (version 17.3.1).

Morphological Observations- Fluorescent microscopy

For investigating the morphology of the fungal strains, 20ml from each culture were sampled during the exponential phase. Subsequently the samples were fixed with glutaraldehyde (final concentration of 2%). 250μl sample were mixed with 5mL MilliQ in an Eppendorf tube and then filtered through a 0.2 μm GTTP filter. Afterwards the sampling tube was flushed with 5mL MilliQ and filtered again. The filter was dried and put on a microscopic slide. Two different methods were used for staining the fungal cells: Calcofluor and DAPI (4',6-

diamidino-2-phenylindole). Calcofluor White (Sigma-Aldrich, USA) is a non-specific fluorescent dye binding to chitin and cellulose. Here, the filter was stained with 25µl of the Calcofluor and 25µl of KaOH (10% mixed with 10% glycerin). The other staining method was done with a DAPI which is a fluorescent stain that binds strongly to the adenine- thymine-rich regions in the DNA, making the nucleus visible. 50µl of the DAPI mix were used to stain the filter. The samples were analyzed with a Zeiss Axio Imager 2 microscope using UV-light below 400 nm.

Physiological tests

Tests in liquid cultures

The growth of the fungal cells was traced by measuring optical density (OD), which determines the absorbance of a material and is a dimensionless quantity.

Table 5. Estimation of the growth phase according to the optical density (OD) of the fungi

Growth phase	**Optical density (660nm)**
Adaptation	0.070 to 0.399
Exponential	0.400 to 1.199
Stationary	>1.20

Influence of temperature on fungal growth

For testing the temperature affinity for each species a liquid medium was prepared containing 2g of glucose, 2g of peptone, 2g of yeast and 35g of sea salts in a 1L Schott bottle. After the pH was set to 8, the medium was autoclaved and 0.5g of chloramphenicol were added. This medium was distributed in nine smaller Schott bottles (150 ml) to get triplicates of the three different temperatures. The cultures were mixed with MilliQ until it reached an optical density (OD) of approximately 1. Afterwards the fungus was inoculated (1ml fungi/100 ml media) in all nine bottles. All incubations were in the dark, at 5°C, 35°C and at room temperature (the laboratory had a temperature control system settled at 23°C. The remaining triplicate was kept on a shaker with room temperature.

The optical density was measured on the inoculation day (day 0) with the UV-1800 Shimadzu Spectrophotometer at a wavelength of 660 nm. Subsequently the OD of all the triplicates was measured until the fungal cultured reached the stationary phase (Table 5).

Influence of salinity on fungal growth

Four different salinity concentrations were tested. The liquid medium consisted of 2g of glucose, 2 g of peptone, 2 g of yeast and either 0g, 20g, 35g or 40g of seal salts per liter. The pH was again set to 8, the medium was autoclaved and 0.5 g of chloramphenicol were added.

Triplicates were prepared for each salinity, making a total of twelve bottles, 150 ml each. As mentioned above for the temperature sensitivity experiments, the fungi from the pure culture were mixed with MilliQ to get an OD value of one. Subsequently the cultures were incubated at room temperature and OD values were taken on a regular base until the stationary phase was reached.

Metabolic activity and assimilation of carbon compounds by fungal cultures

FF MicroPlateTM (© Copyright, Biolog, Inc.) plates were used for investigating how the fungal isolates assimilate compounds of different carbon sources, which can lead to a "Metabolic Fingerprint" of each species. A plate that contained 96 wells of different carbon sources was chosen for the investigations. These carbon sources include compounds such as amines/amides, carbohydrates, amino acids, carboxylic acids, polymers and others (alcohols, nucleotides, nucleosides, amines, amides, etc.) (Table 6). The first well (A1) is the blank without any compounds, which can be used as a reference.

First a pure culture is isolated on 2% malt and agar extract. Then the agar plates are incubated at 26°C. For filamentous fungi the incubation period is usually between 5-10 days until sporulation occurs, whereas it takes between 24 and 48 hours for yeast species to sporulate. The next step is to prepare a uniform suspension as follows: In a safety hood the spores are removed from the agar plate with a sterile swab. The swab with the fungal material on it is pressed against the inside surface of the tube on the dry glass above the fluid line. Afterwards the tube has to be mixed gently to avoid air bubbles as they will interfere with the absorbance reading. The cell suspension is quickly poured into a multichannel pipet reservoir. Subsequently a multipipette is used to fill each well of the plate with 100ul of the suspension fluid. Afterwards the FF MicroPlates were incubated for three days, at 26°C. During this incubation time the absorption was measured every day at a wavelength of 490 nm with the Tecan M200 Infinite Pro. Increased mitochondrial activity and therefore consumption of the substrate can be detected by the formation of a reddish-orange color. This is caused by the reduction of colorless tetrazolium chloride turning into red formasane (Islam et al. 2011). The highest OD was detected on day three, therefore this values are displayed in the graphs.

Table 6. The 95 different compounds of the MicroPlateTM that were uses for observing the metabolic activity.

Compound	**Category**
Tween 80	Polymer
N-Acetyl-D-Galactosamine	Carbohydrate
N-Acetyl-D-Glucosamine	Carbohydrate
N-acetyl-β-D- Mannosamine	Carbohydrate
Adonitol	Alcohol
Amygdalin	Glycoside

D-Arabinose	Carbohydrate
L-Arabinose	Carbohydrate
D-Arabitol	Sugar alcohol
Arbutin	Glycoside
D-Cellobiose	Carbohydrate
α-Cyclodextrin	Polymer
β-Cyclodextrin	Polymer
Dextrin	Carbohydrate
i-Erythritol	Carbohydrate
D-Fructose	Carbohydrate
L-Fucose	Carbohydrate
D-Galactose	Carbohydrate
D-Galacturonic Acid	Carbohydrate
Gentiobiose	Carbohydrate
D-Gluconic Acid	Carboxylic acid
D-Glucosamine	Amino sugar
α-D-Glucose	Carbohydrate
α-D-Glucose-1-Phosphate	Carbohydrate
Glucuronamide	Carbohydrate
D-Glucuronic Acid	Sugar acid
Glycerol	Trihydroxyalcohol
Glycogen	Polymer
m-Inositol	Carbohydrate
2-Keto-D-Gluconic Acid	Ketoaldonic acid
D-Lactose	Carbohydrate
Lactulose	Carbohydrate
Maltitol	Carbohydrate
Maltose	Carbohydrate
Maltotriose	Carbohydrate
D-mannitol	Carbohydrate
D-Mannose	Carbohydrate
D-Melezitose	Carbohydrate
D-Melibiose	Carbohydrate
α-Methyl-D-Galactoside	Carbohydrate
β-Methyl-D-Galactoside	Carbohydrate
α-Methyl-D-Glucoside	Carbohydrate
β-Methyl-D-Glucoside	Carbohydrate
Palatinose	Carbohydrate
D-Psicose	Carbohydrate
D-Raffinose	Carbohydrate
L-Rhamnose	Carbohydrate
D-Ribose	Carbohydrate
Salicin	Salicyl alcohol

Sedoheptulosan	Carbohydrate
D-Sorbitol	Sugar alcohol
L-Sorbose	Carbohydrate
Stachyose	Carbohydrate
Sucrose	Carbohydrate
D-Tagatose	Carbohydrate
D-Trehalose	Carbohydrate
Turanose	Carbohydrate
Xylitol	Sugar alcohol
D-Xylose	Carbohydrate
γ-Aminobutryic Acid	Amino acid
Bromosuccinic Acid	Dicarboxylic acid
Fumaric Acid	Butenedioic acid
β-Hydroxybutyric Acid	Monocarboxylic acid
γ-Hydroxybutyric Acid	Carboxylic acid
p-Hydroxy-phenylacetic Acid	Monocarboxylic acid
α-Ketoglutaric Acid	Oxo dicarboxylic acid
D-Lactic Acid Methyl Ester	Methyl lactate
L-Lactic Acid	Alpha hydroxy acid
D-Malic Acid	2-hydroxydicarboxylic acid
L-Malic Acid	2-hydroxydicarboxylic acid
Quinic Acid	Cyclohexanecarboxylic acid
D-Saccharic Acid	Glucuronic acid derivatives
Sebacic Acid	Dicarboxylic acid
Succinamic Acid	Dicarboxylic acid
Succinic Acid	Dicarboxylic acid
Succinic Acid Mono-Methyl Ester	Dicarboxylic acid monoester
N-acetyl-L-Glutamic Acid	Glutamic acid and derivatives
L-Alaninamide	Amino acid
L-Alanine	Amino acid
L-Alanyl-Glycine	Acetic acid
L-Asparagine	Amino acid
L-Aspartic Acid	Amino acid
L-Glutamic Acid	Amino acid
Glycyl-L-Glutamic Acid	Dipepptide
L-Ornithine	Amino acid
L-Phenylalanine	Amino acid
L-Proline	Amino acid
L-Pyroglutamic Acid	Amino acid
L-Serine	Amino acid
L-Threonine	Amino acid
2-Aminoethanol	Monoethanolamine
Putrescine	Amino acid

Adenosine	Ribonucleoside
Uridine	Nucleoside
Adenosine-5'-Monophosphate	Nucleotide

Statistical Analysis

All statistical analysis were done in R.Studio® (version 3.6.1). The data was visualized with the package ggplot2 (version 3.3.5). For evaluating the statistical significances of the compound usage an ANOVA was conducted (version 4.0.2). Additionally a post-hoc test was performed to compare the different in the utilization of compound groups between each other. The p-values of the boxplots were displayed with the package ggpubr (version 0.4.0).

Results & Discussion

Three different fungal species were identified and characterized based on the genetic and physiological methods explained above: *Scheffersomyces spartinae, Rhodoturula mucilaginosa and Sarocladium kiliense.*

Scheffersomyces spartinae

Phylogenetic analysis

The phylogenetic tree shows that the two replicates 13 and 21 can be classified as *Scheffersomyces spartinae* (Fig. 1). Our results are supported by a bootstrap value of 100 indicating a high reliability (Fig. 1).

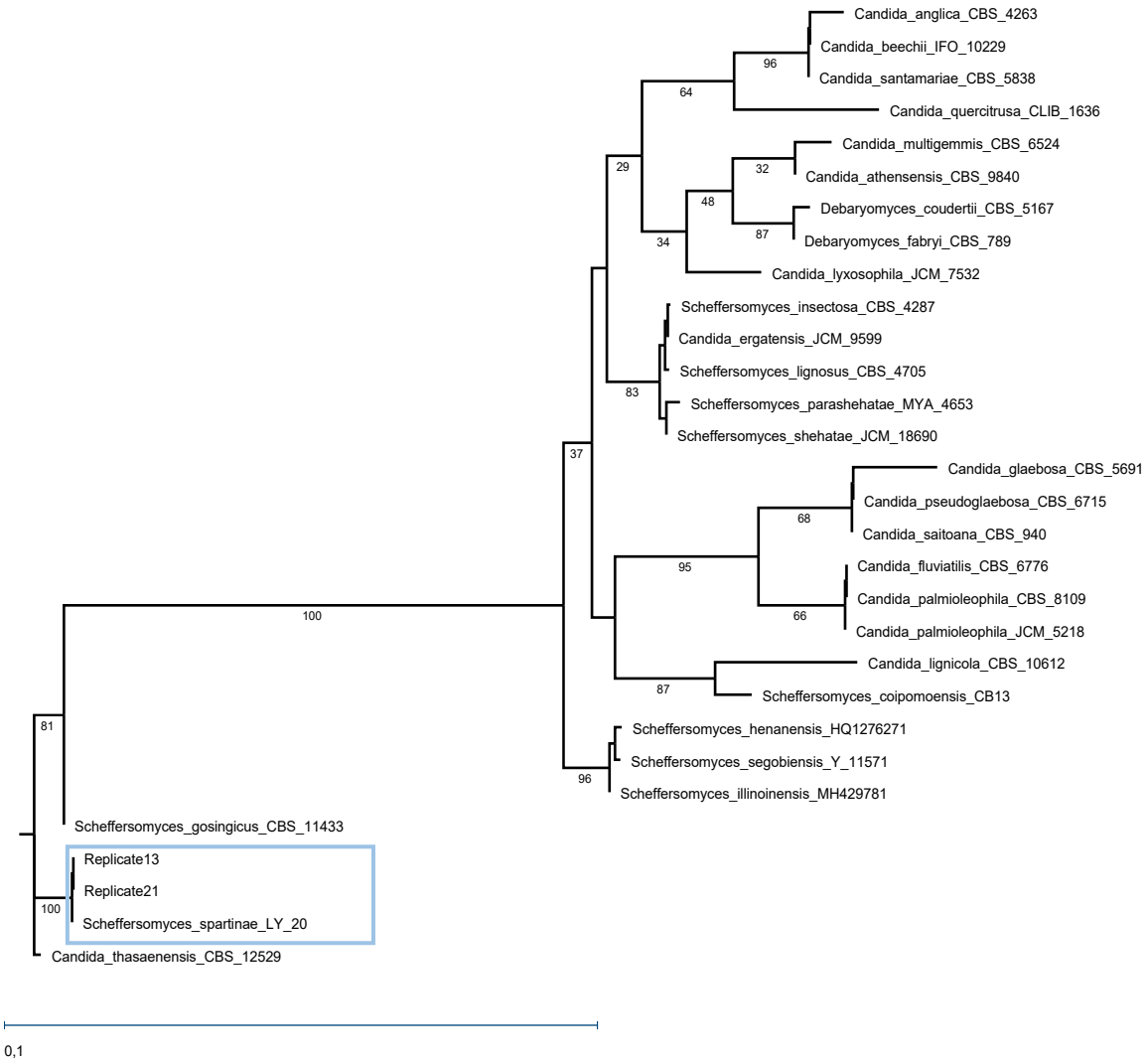

Figure 1. Phylogenetic relationships represented as RAxML tree. The Replicates 13 and 21 were identified as *S. spartinae* and are shown with related taxa based on the nucleotide sequences of the combined ITS and LSU rDNA. The bootstrap values for maximum likelihood are indicated by the numbers under the branches. The tree is rooted to *Candida thasaenensis* (CBS 12529).

The identified fungal taxon named *S. spartinae* belongs to the Division of Ascomycota. Taxonomically, they are in the Class of Saccharomycetes. They belong to the Family of Debaryomycetaceae which is classified in the Order of Saccharomycetales (Kurtzman and Suzuki 2010). Based on the phylogenetic similarities with the genus Pichia, *S. spartinae* was previously classified as *Pichia spartinae* in 1970 by Ahearn, Yarrow & Meyers. After genomic analysis this taxon was described as *Scheffersomyces spartinae* in 2010 (Kurtzman and Suzuki 2010) . *S. spartinae* is exclusively known from aquatic environments. This fungi could be isolated before from habitats like brackish waters in association with marsh grass. Moreover it is referred for being the predominant yeast species associated to the rhizoids and surface

of roots of the cordgrass *Spartina alterniflora* (Nakamura et al. 1991). The occurrence of this fungi has been also documented in biofilms where this taxon acts as an acidophile (Gillings et al. 2007). *S. spartinae* is known for being a highly salt-tolerant yeast species. Moreover, a study by Tan et al. (2016) indicates that this fungus is even able to degrade and detoxify azo dyes. Those azo dyes are commonly used in the textile industry and are known for polluting the aquatic environment. Thus, *S. spartinae* also shows a great potential in the treatment of wastewaters. They are toxic for aquatic biota and are also considered as being carcinogenetic for humans (Tauber, Gubitz and Rehorek 2008).

Macroscopic morphology

After one week of incubation at room temperature, the cells of *S. spartinae* have relatively thin branches and show some wobbly structures. They appear in a very bright white (Fig. 2; A). The colonies are rather flat, but show some wobbly structures. The coloration changed slightly after one month of incubation to a more yellowish tone. The shape of the cultures got in general flatter and has edges that are a bit more toothed (Fig. 2; B).

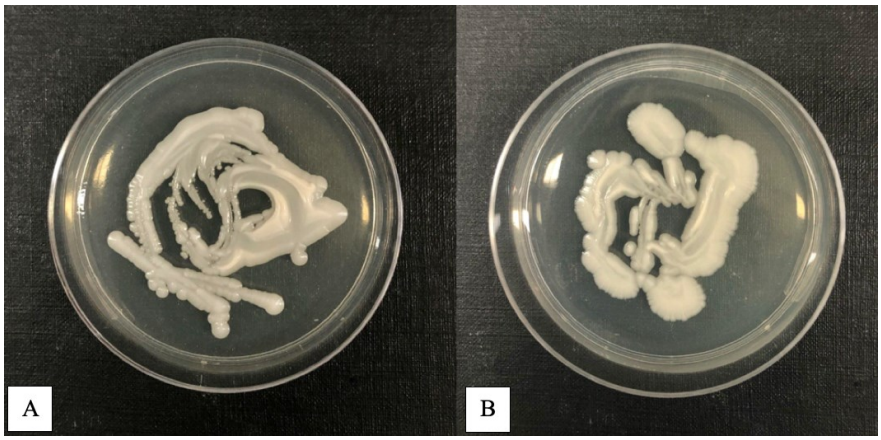

Figure 2. Representative images of the morphology of *Scheffersomyces spartinae* on an agar plate A) a colony that is 1 week old B) a colony that is 4 weeks old.

Microscopic morphology

The cells are mostly oval and ellipsoidal and show a rather uniform size of 1.9-2.2 x 4.1-5.3 um. The cells occur singly, but some are also attached to each other indication asexual reproduction through budding (Fig. 3; A-D).

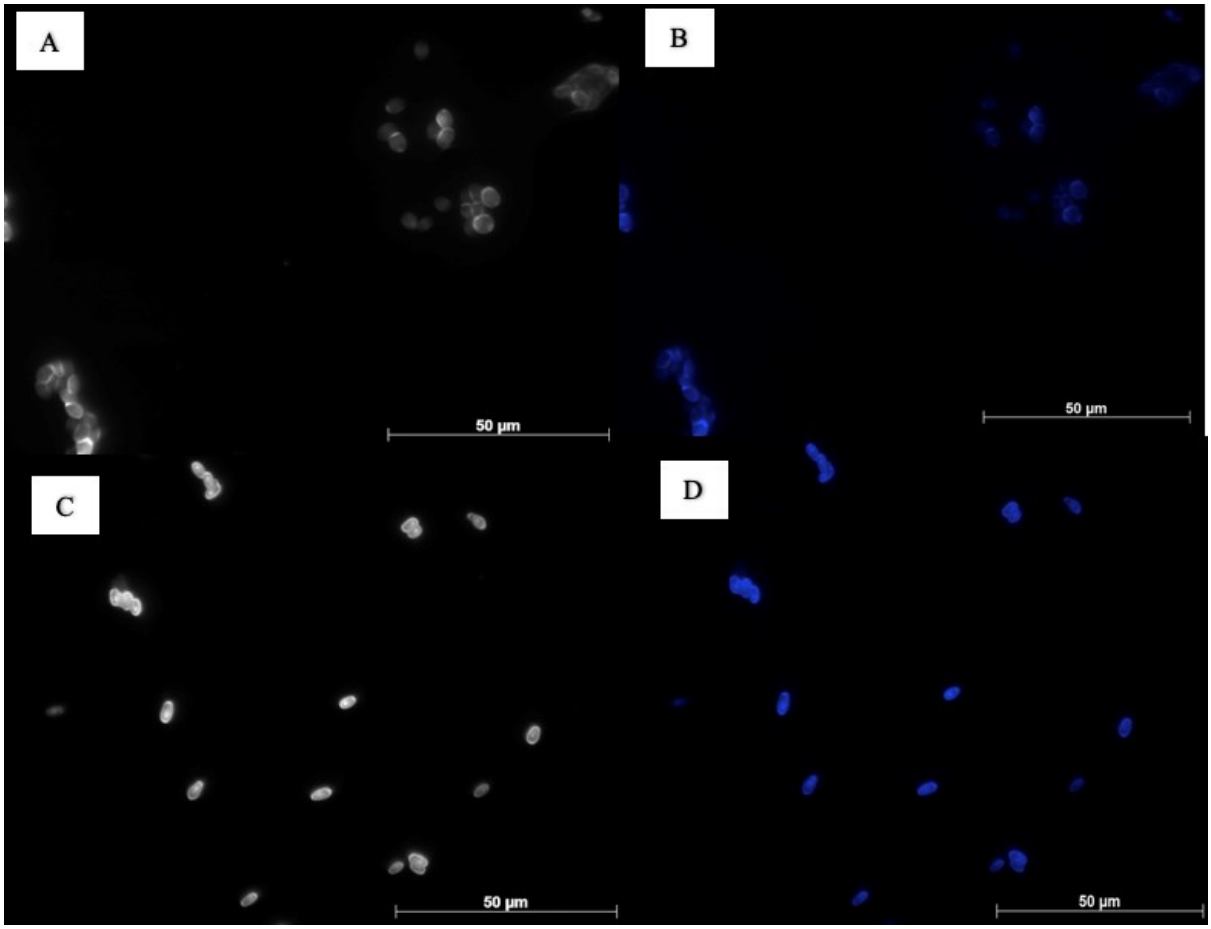

Figure 3. Fluorescent microscopical images of *S. spartinae* under UV-light below 400 nm. Samples were taken from liquid cultures in the exponential growth phase; A,B) Fungal cells stained with calcofluor making the cell walls visible; C,D) Fungal cells stained with the DAPI mix making the nucleus visible.

Physiological tests in liquid media

Influence of temperature on fungal growth

The samples incubated at 35°C already reached the exponential phase on day 1 with an OD of 0.71 ± 0.01 (Fig. 4). On the second day the stationary phase was reached showing an OD value of 1.24 ± 0.16 (Fig. 4). The value was still increasing until an OD value of 1.66 ± 0.16 on day 11. Under these conditions the OD increased the most compared to the other temperatures in the first 24 hours, indicating a faster growth in warmer waters. However the cells reached the highest OD density values at room temperature (Fig. 4), pointing out a preference of moderate water temperature. The exponential phase was reached on the second day of incubation with an OD of 0.41 ± 0.08. On the fourth day these samples increased to an OD of 1.46 ± 0.03, the stationary phase (Fig. 4). The OD still increased to 1.79 ± 0.08 on day 11 which was the highest value measured in this experiment. The samples incubated at 5°C never reached the exponential phase (Fig. 4). The OD ranged from 0.15 ± 0.01 to 0.19 ± 0.01. This leads to the assumption that *S. spartinae* is not adapting very well to

colder temperatures. Consistently, *S. spartinae* has also never been documented to occur in colder waters.

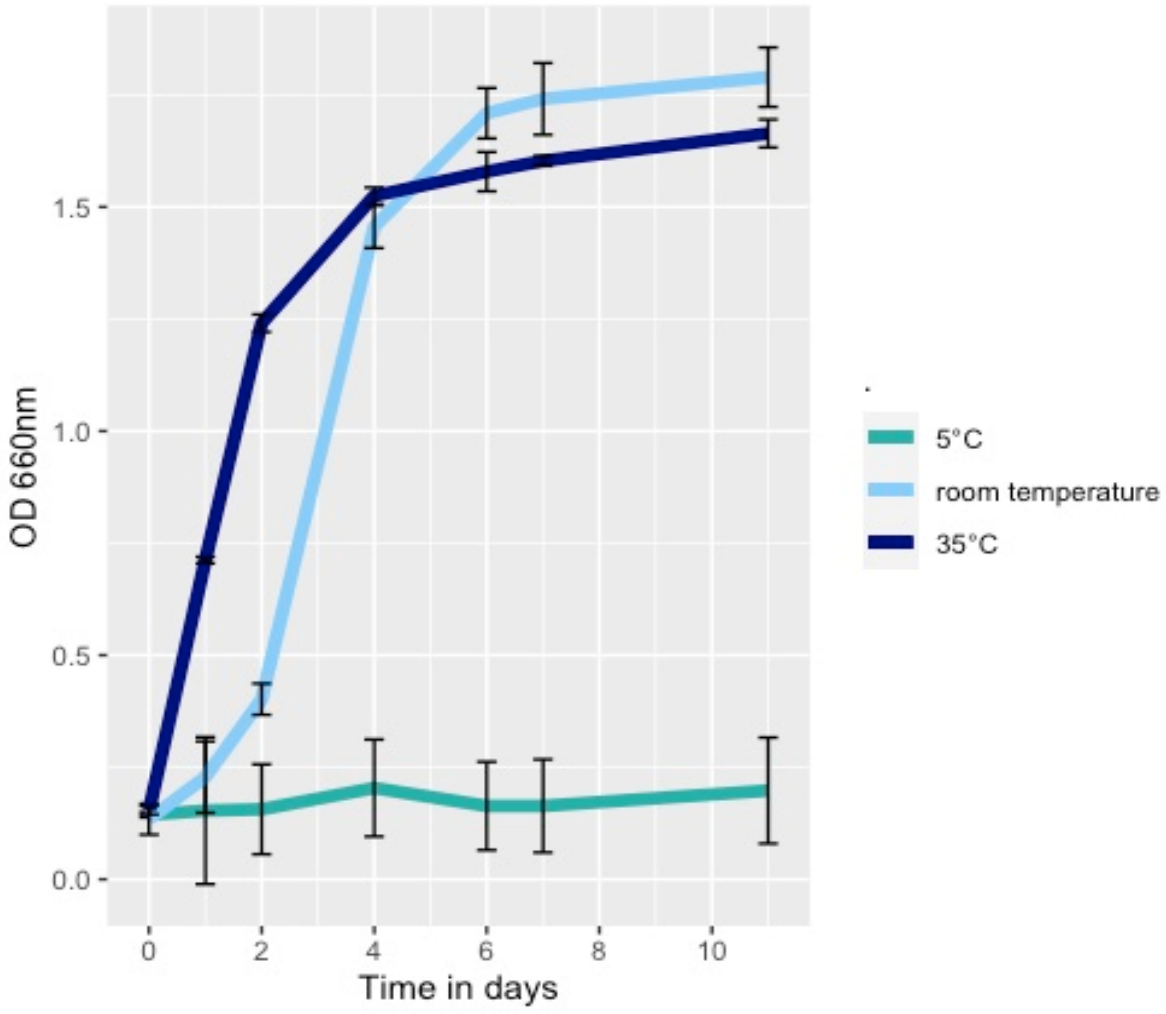

Figure 4. Optical density (OD) at a wavelength of 660 nm of *S. spartinae* on each day. The three different temperatures measured are 35°C, room temperature (ca. 23°C) and 5°C. Erros bars indicate the standard deviation (SD).

Influence of salinity on fungal growth

The highest OD values were measured at a concentration of 35g/L (Fig. 5). There samples reached the exponential phase on day 2 with an OD of 0.42 ± 0.08 and the stationary phase on day 4 (OD 1.46 ± 0.05). The OD increased to 1.74 ± 0.06 until day 7 (Fig. 5). These results are consistent with the notion that the salinity concentration of 35g/L reflects the normal range of ocean salinity, which lays between 33g and 37g per liter of seawater. Surprisingly the samples incubated in the media that had 0 g/L of sea salts exhibited an increase in OD from 0.06 ± 0.01 to 1.41 ± 0.02 already after the first 24 hours of incubation (Fig. 5). On day 2 they exhibited a slight decrease to an OD of 1.39 ± 0.02. However, their OD values still increased

to 1.47 ± 0.03 (Fig. 5). *S. spartinae* is not yet documented to occur in freshwater systems, but our results indicate that it can also grow under non-saline conditions. Yet, this species has been isolated from brackish waters, where the salinity is usually very low (0.1-1%) caused by the mixing of fresh- and seawater (Kurtzman and Suzuki, 2010).

The samples with the salinity concentration of 20g/L reached the exponential phase on day 2 (OD 0.66 ± 0.09) and the stationary phase on day 3 (OD 1.34 ± 0.03). On day seven they showed an OD of 1.46 ± 0.03. In the media that had 40g/L of sea salts the fungal cells entered the exponential phase on day 2 with an OD of 0.77 ± 0.11 (Fig. 5). The stationary phase was reached on day 3 (OD 1.32 ± 0.03). The value increased to 1.43 ± 0.03 until the seventh day (Fig. 5). *S. spartinae* is known to be a salt tolerant species (Tan et al. 2016). According to Frigon and Liu (2016) in study on wastewater treatment, *S. spartinae* can even tolerate concentrations of salinity up to 120 g/l of NaCl. Based on these studies, it does not seem unexpected that *S. spartinae* showed similar OD values in the media with 20g and 40g per liter (Fig. 5), since it can occur in a broad range of saline environments.

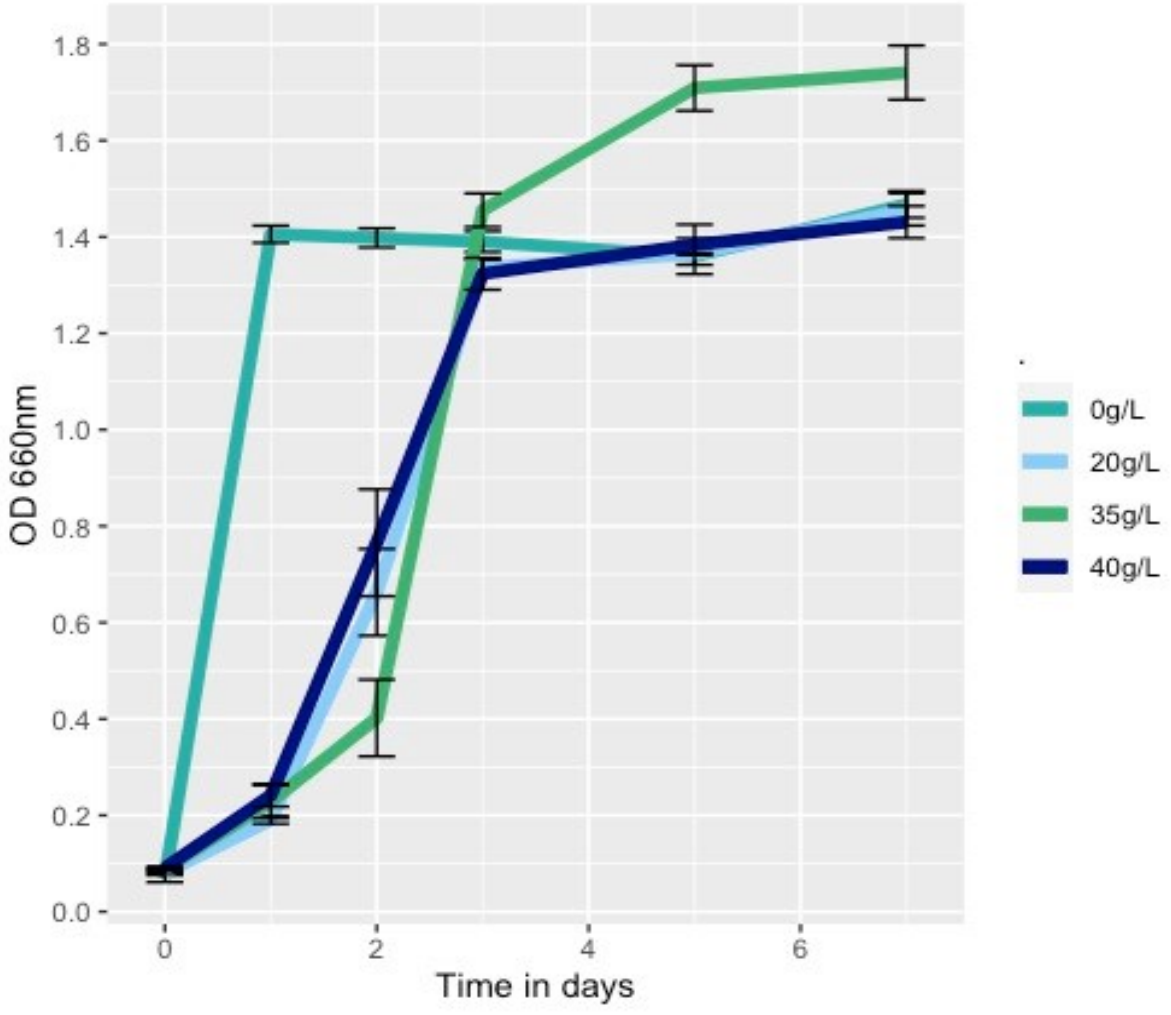

Figure 5. Optical density (OD) at a wavelength of 660 nm of *S. spartinae* on each day. The four different salt concentrations measured are 0, 20, 35 and 40 g/L. Erros bars indicate the standard deviation (SD).

Metabolic activity (FF-Plates)

S. Spartinae utilized all of the organic compounds displayed in Table 6. We could find significant differences in the utilization of the organic compounds between amino acids and carbohydrates (p=0.00088), amino acids and other substrates (p=0.024) and amino acids and polymers (p=0.018), as well as between carboxylic acids and carbohydrates (p=0.19) (Fig 6). The organic compound type utilized the most by this species were amino acids (AC) with a median OD value of 1.3 ± 0.6 (Fig. 6). Consistently, a study conducted by Shi et al. (2021) found that the metabolic activity of *S. spartinae* can be enhanced by adding amino acids to the growth media. The second highest utilized carbon compound type was carboxylic acids (CA). A study by Fiebig (1992) also showed that aquatic fungi utilized carboxylic acids to a big extent from labile components of DOM. The substrate class that was defined as others (OT) showed an OD of 0.49 ± 0.5 (Fig. 5). Carbohydrates (CB) reached a median value of 0.47 ± 0.33, which is relatively low compared to the other substrate groups (Fig. 6). The group of polymers exhibited a median OD of 0.35 ± 1.9 (Fig. 6), which was the lowest value measured in this experiment. Recently it has been demonstrated that adding carbon sources, especially lactose, sucrose and glucose, to a growth medium can increase the degrading ability and therefore the metabolic activity of S. *spartinae* (Shi et al. 2021). Interestingly, our results indicate that *S. spartinae* exhibits a relatively lower consumption of carbohydrates, whereas amino acids and carboxylic acids seem to be preferentially utilized.

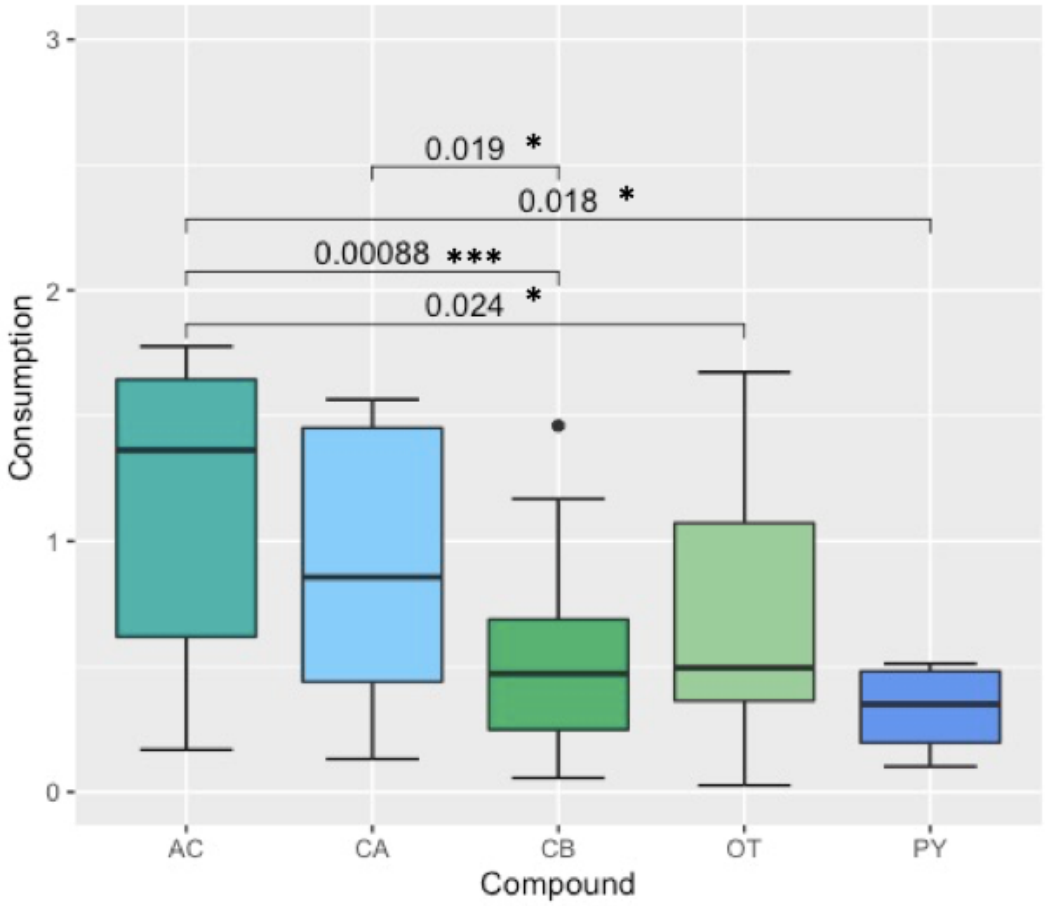

Figure 6. The consumption of various compounds measured as absorption (OD) of *S. spartinae* at a wavelength of 490nm. The compounds are: AC-amino acids, CA-carboxylic acids, CB- carbohydrates, OT- others, PY- polymers. The statistical significance is indicated by the p-values (* = p ≤0.05, ** = p ≤0.01, *** = p ≤0.001).

Rhodoturula mucilaginosa

Phylogenetic analysis

The results of the phylogenetic analysis show that the fungi we named “Poseidon” can be classified as *Rhodoturula mucilaginosa*. These findings are highly supported by a bootstrap value of 89 (Fig. 7).

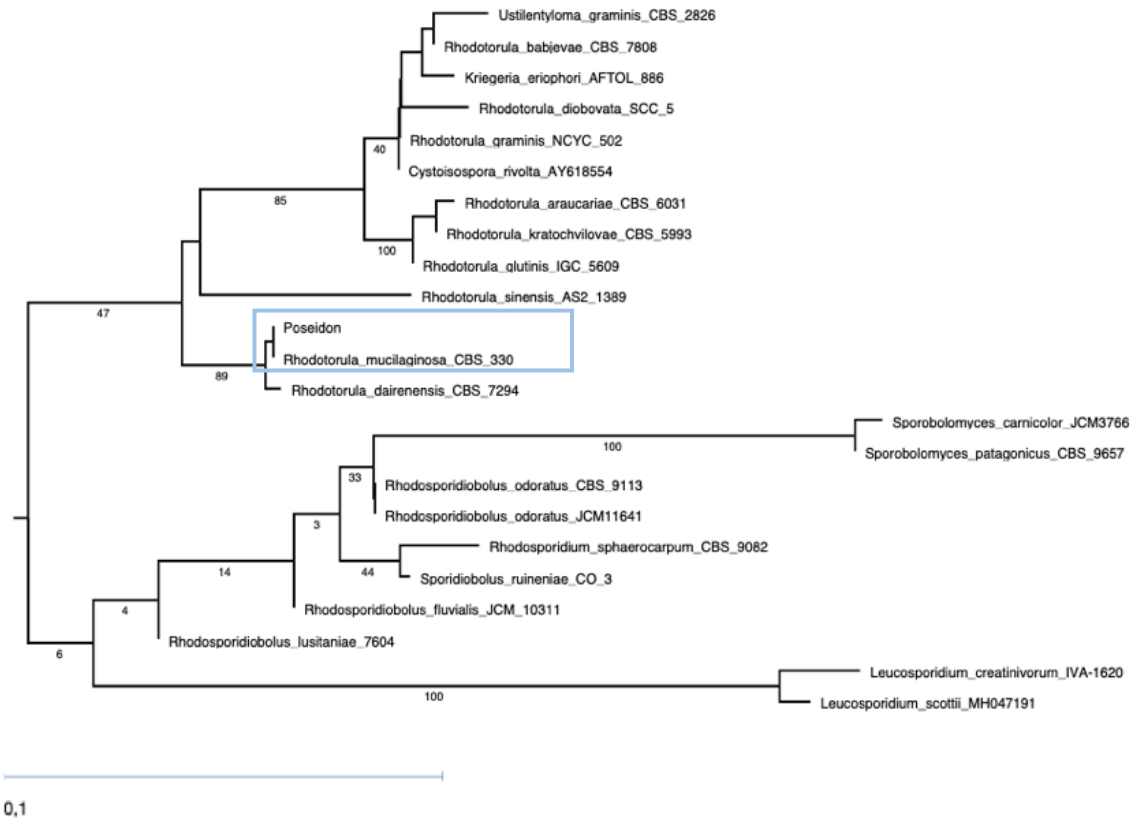

Figure 7. Phylogenetic relationships represented as RAxML tree. "Poseidon" was identified as *R. mucilaginosa* and is shown with related taxa based on the nucleotide sequences of the combined ITS, LSU, SSU and TEF region. The bootstrap values for maximum likelihood are indicated by the numbers under the branches. The tree is rooted to Leucosporidium *scottii* (MH047191).

This taxon belongs to the Division of Basidiomycota and the Class of Microbotryomycetes. They are classified in the Order of Sporidiobolales and the Family of Sporidiobolaceae (Harrsison, 1928). It was first described in 1928 by F.C. Harrison. *R. mucilaginosa* has been reported to occur worldwide and is one of the most omnipresent basidiomycetous yeast species. This taxon was already isolated from a great variety of terrestrial and aquatic environments, including marine and freshwater. In the marine environment they can occur in all different kind of habitats. This species was found in estuaries (de Almeida 2005), coastal waters as well as open oceans (Gadanho and Sampaio 2005). Moreover, their distribution was also detected in more extreme environments like the deep sea and ultra-acidic waters (Gadanho, Libkind and Sampaio 2006). *R. mucilaginosa* also holds important features such as the ability of degrading plasticizers (Gartshore, Cooper and Nicell 2003). Moreover, this species is also of great interest in matters of medical research. According to epidemiological studies *R. mucilaginosa* is suggested to be included into the list of opportunistic pathogens (Perniola et al. 2006). *R. mucilaginosa* is involved in fungal infections, such as fungemia (Anatoliotaki et al. 2003), and also in lymphadenitis in HIV-positive patients (Fung et al. 2009).

Macroscopic morphology

The most distinct morphological feature of *R. mucilaginosa* is the bright color. A colony that was about 1 week old showed a bright pinkish, coral to saffron orange coloration (Fig. 8; A). This appearance changed after approximately 1 month of incubation time at room temperature, where the color changed to a lighter nuance of orange (Fig. 8; B). In general the colonies have a shiny appearance and a very smooth structure. They are mainly flat, but can develop a wobbly texture (Fig. 8; A, B).

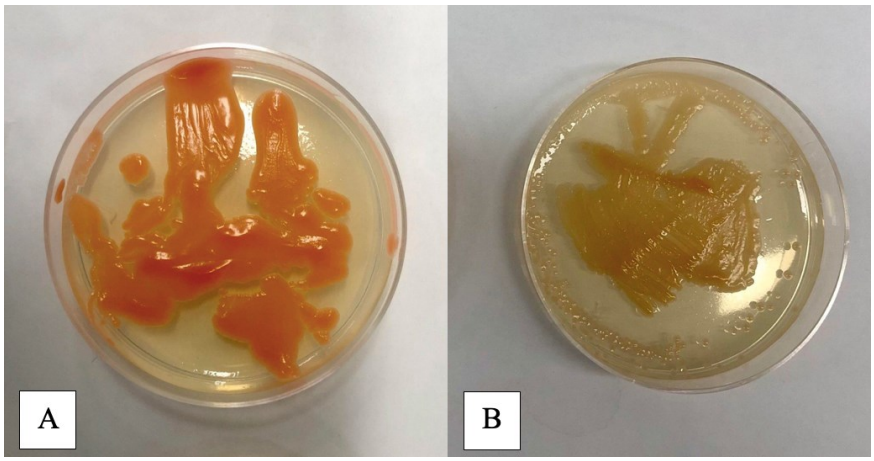

Figure 8. Representative images of the morphology of *Rhodothurula mucilaginosa* on an agar plate A) a colony that is one week old B) a colony that is 4 weeks old

Microscopic morphology

The cells of *R. mucilaginosa* appear to be ellipsoid or oval (Fig. 9; A-D) with an average size of 1.5-2 x 4.2-5.4 um. The cells occur mainly single, showing a very low morphological variation. Some cells seem to be attached to each other suggesting that the most common form of asexual reproduction, budding, happens (Fig. 9; A,B).

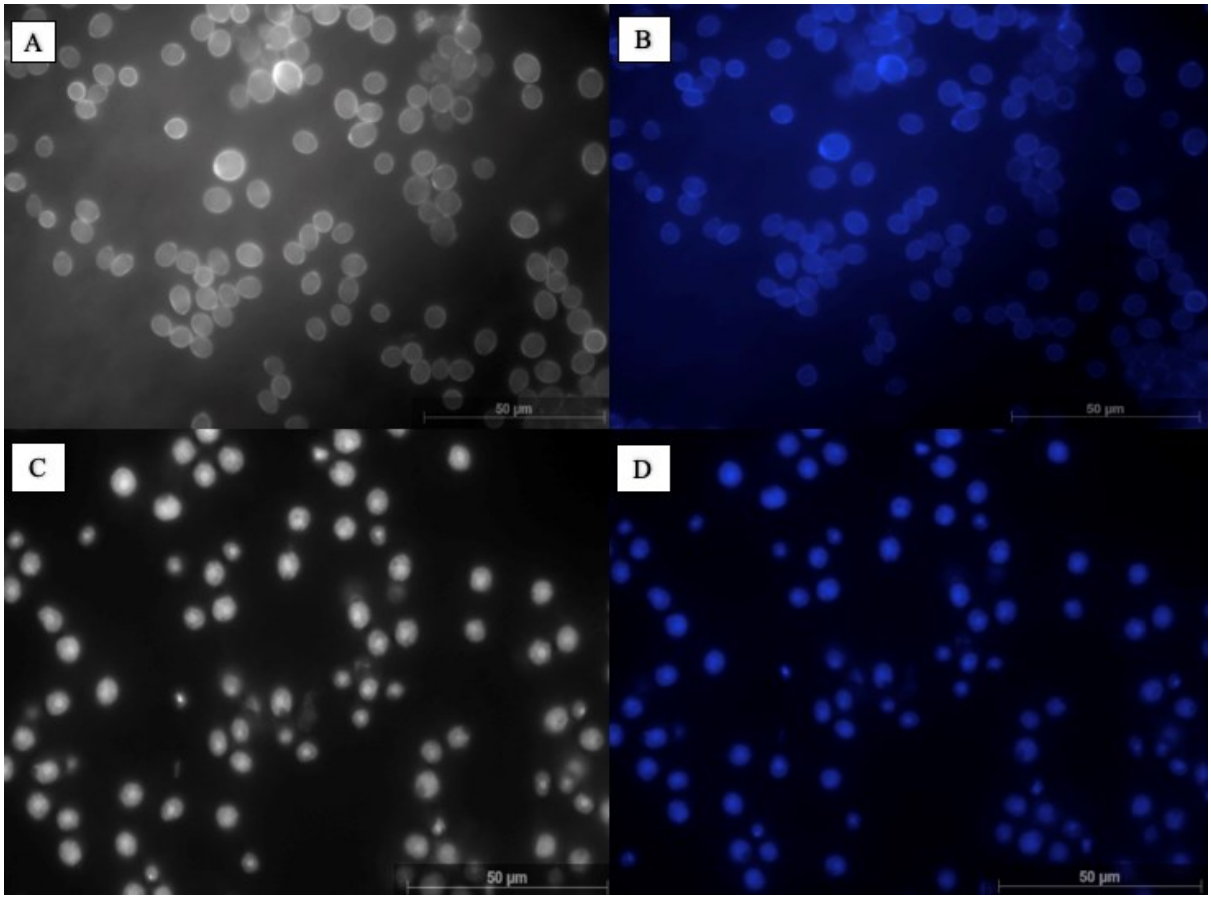

Figure 9. Fluorescent microscopical images of *R. mucilaginosa* under UV-light below 400 nm. Samples were taken from liquid cultures in the exponential growth phase. A,B) Fungal cells stained with calcofluor making the cell walls visible; C,D) Fungal cells stained with the DAPI mix making the nucleus visible.

Physiological tests in liquid media

Influence of temperature on fungal growth

The samples incubated at 35°C reached the exponential phase on day one with an OD of 0.62 ± 0.01 (Fig. 10). The fungi were growing very rapidly in the first 24h, pointing out that the high temperature leads to an increased growth. Gadanho and Sampaio (2005) found that *R. mucilaginosa* seems to be adapted to very hot waters, since this taxon has been isolated from the hydrothermal fields in the Mid-Atlantic ridge. On the second day the samples entered the stationary phase (1.21 ± 0.09), where the values still increased (OD 1.28 ± 0.09) until day 11 (Fig. 10). At 5°C the samples grew relatively slowly. On day 9 the exponential phase was reached (OD 0.81 ± 0.07), followed by the stationary phase on day 10 with an OD value of 1.17 ± 0.05 (Fig. 10). The values still increase a bit until day 11 to an OD of 1.36 ± 0.04. Still, this fungi culture managed to reach the stationary phase also at 5°C, indicating that they are able to grow also in colder temperatures. This finds are in line with the results of a study

conducted by Butinar, Spencer-Martins and Gunde-Cimerman (2007), which isolated *R. mucilaginosa* from melt ice from Arctic glaciers.
However, the highest growth yield was reached at room temperature (about 23°C) (Fig. 10). On day 1 the exponential phase was already reached (OD 0.49 ± 0.01) and on day 2 the value increased already to an OD of 1.21 ± 0.01, which accounts for the stationary phase (Fig. 10). Until day 11 the culture could still grow to an OD value of 1.53 ± 0.06. These findings suggest that this taxon is able to tolerate a wide range of temperatures, but still prefers warmer than colder ones.

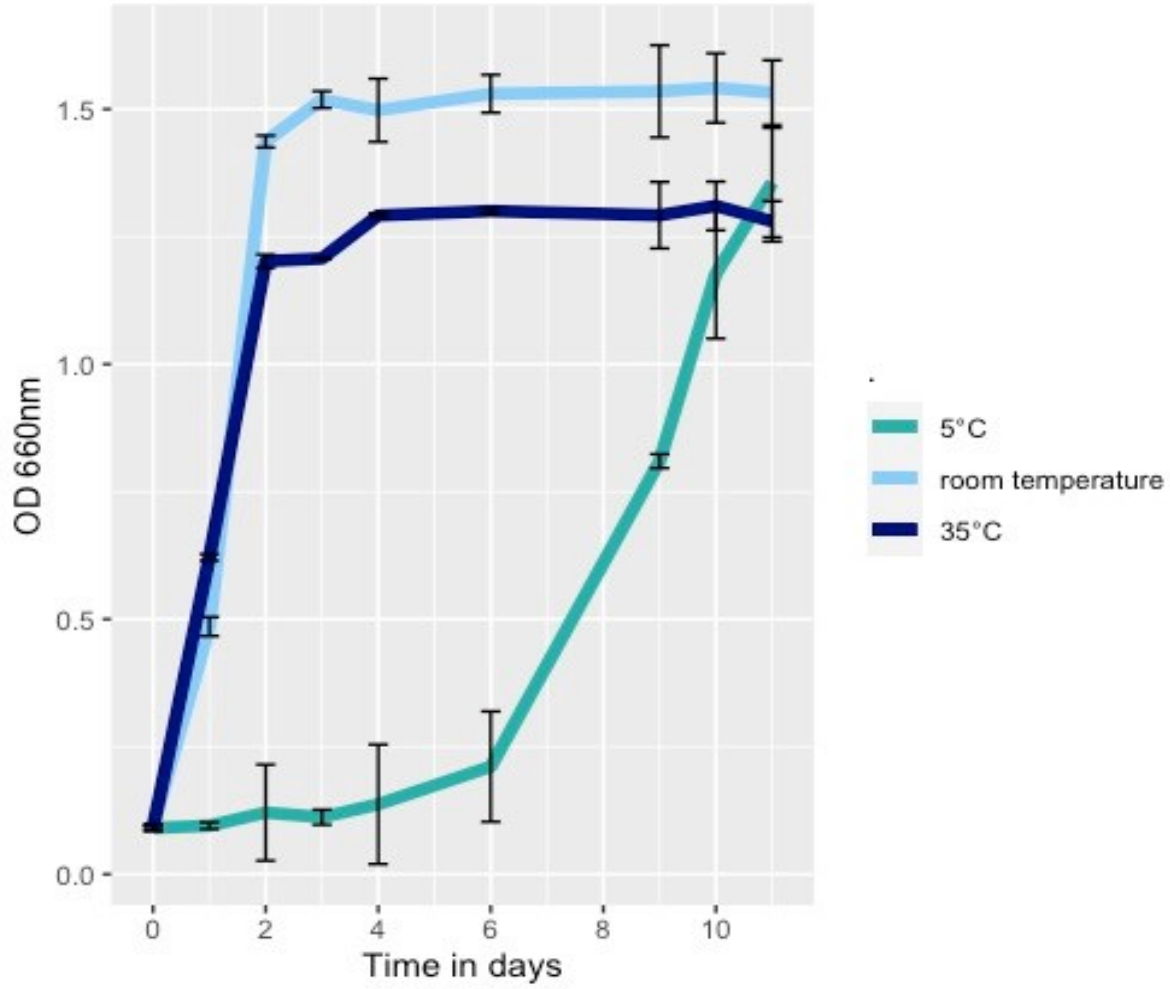

Figure 10. Optical density (OD) at a wavelength of 660 nm of *R. mucilaginosa* on each day. The three different temperatures measured are 35°C, room temperature (ca. 23°C) and 5°C. Erros bars indicate the standard deviation (SD).

Influence of salinity on fungal growth

The highest growth yield was reached at a concentration of 35g/L (Fig. 11). On day 1 the exponential phase was reached (OD of 0.41 ± 0.02) and on day 2 the cultures already entered

the stationary phase (OD of 1.34 ± 0.01) (Fig. 11). The cultures still grew until day 6 to an OD of 1.43 ± 0.09. The samples with the concentration of 20 g/L reached the exponential phase on the second day of the experiment showing an OD of 0.41± 0.02 (Fig. 11). The stationary phase was entered on day 2 (OD 1.23 ± 0.01). On day 6 the OD values increased to 1.28 ± 0.01. The growth with 40g/L of exhibited an OD value of 0.25 ± 0.01on day 1 and increased to an OD of 1.23 ± 0.01 on day 2 (Fig. 11). The cultures in the stationary phase still increased to an OD of 1.31± 0.01 until day six (Fig. 11). Since the media with the concentration of 20g/L and 40g/L reached quite similar OD values, we assume that this taxon is not really sensitive to salinity. This assumption is also supported by the findings of de Almeida (2005), which detected the presence of *R. mucilaginosa* in estuaries. In this habitat they are also exposed to changing salinity due to ebb and flood. The media that contained no sea salts (0 g/L) showed the lowest growth yield (Fig. 11), still the culture was still developing a normal growth pattern. On day 1 the OD value was 0.24 ± 0.01 increasing to an OD of 1.12 ± 0.06 on the sixth day (Fig. 11). *R. mucilaginosa* occurs as well in freshwater systems (Libkind et al. 2003), which explains the growth under these conditions.

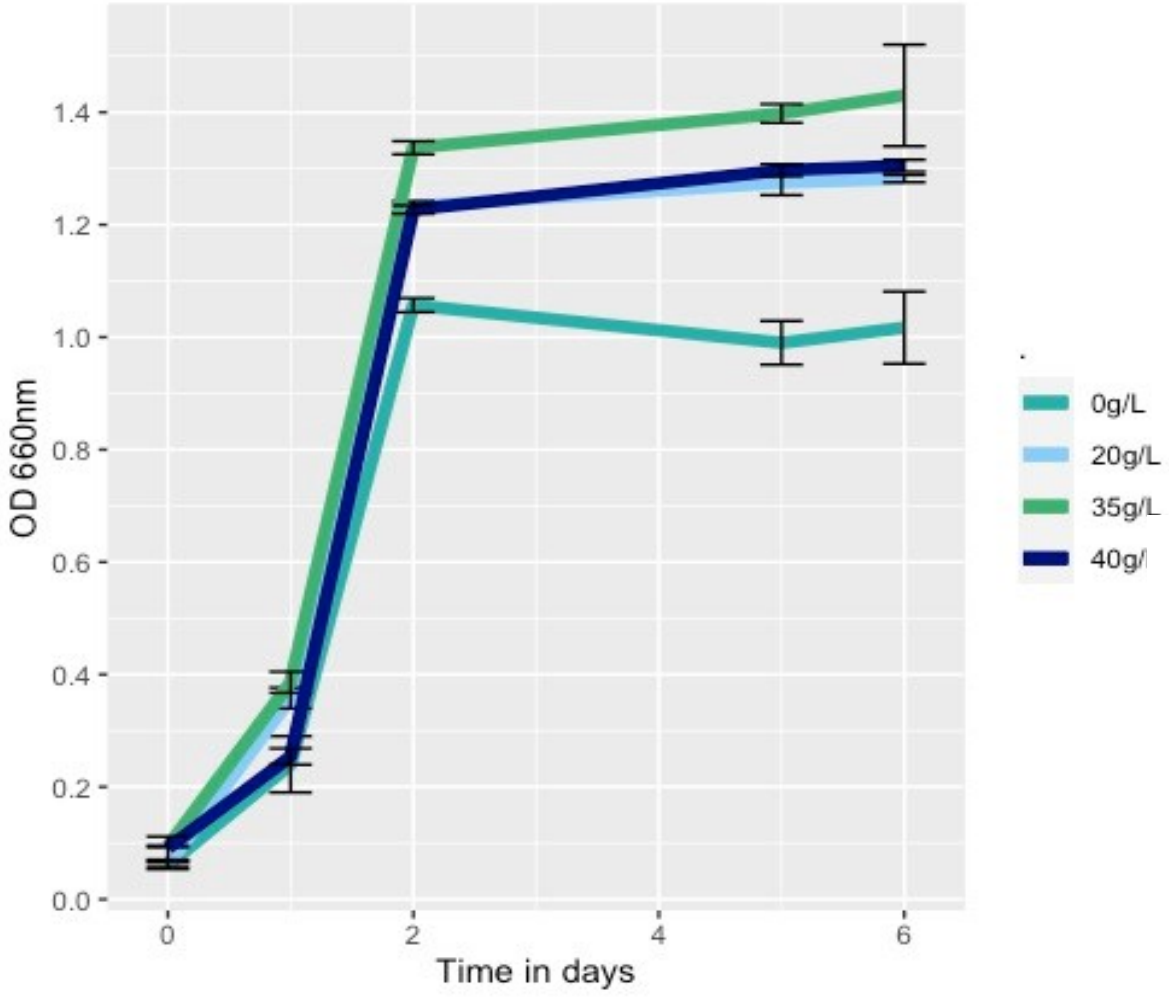

Figure 11. Optical density (OD) at a wavelength of 660 nm of *R. mucilaginosa* on each day. The four different salt concentrations measured are 0, 20, 35 and 40 g/L. Erros bars indicate the standard deviation (SD).

Metabolic activity (FF-Plates)

Out of the 95 substrate compounds (Table 6) *R. mucilaginosa* used 94. Significant differences were found in the utilization of amino acids and all other compound groups like carboxylic acids (p=0.029), carbohydrates (p=0.0013), other compounds (p= 0.0011) and polymers (p=0.026) (Fig. 12). The most highly utilized compounds were the group of amino acids (AC) with a median OD of 1.15 ± 0.48 (Fig. 12). According to Nishida et al. (2016) amino acids are a substantive nutritional source for fungi in general. The compound group of carbohydrates (CB) showed a median OD density of 0.39 ± 0.42 (Fig. 12), which does not really indicate a high metabolically uptake of carbohydrates. The compounds that account for other (OT) showed a value of 0.36 ± 0.44 (Fig. 12), similar to Carboxylic acids (CA) which showed a consumption 0.31 ± 0.5 (Fig. 12). The group of polymers (PY) exhibited the lowest consumption of 0.09 ± 0.23 (Fig. 12).

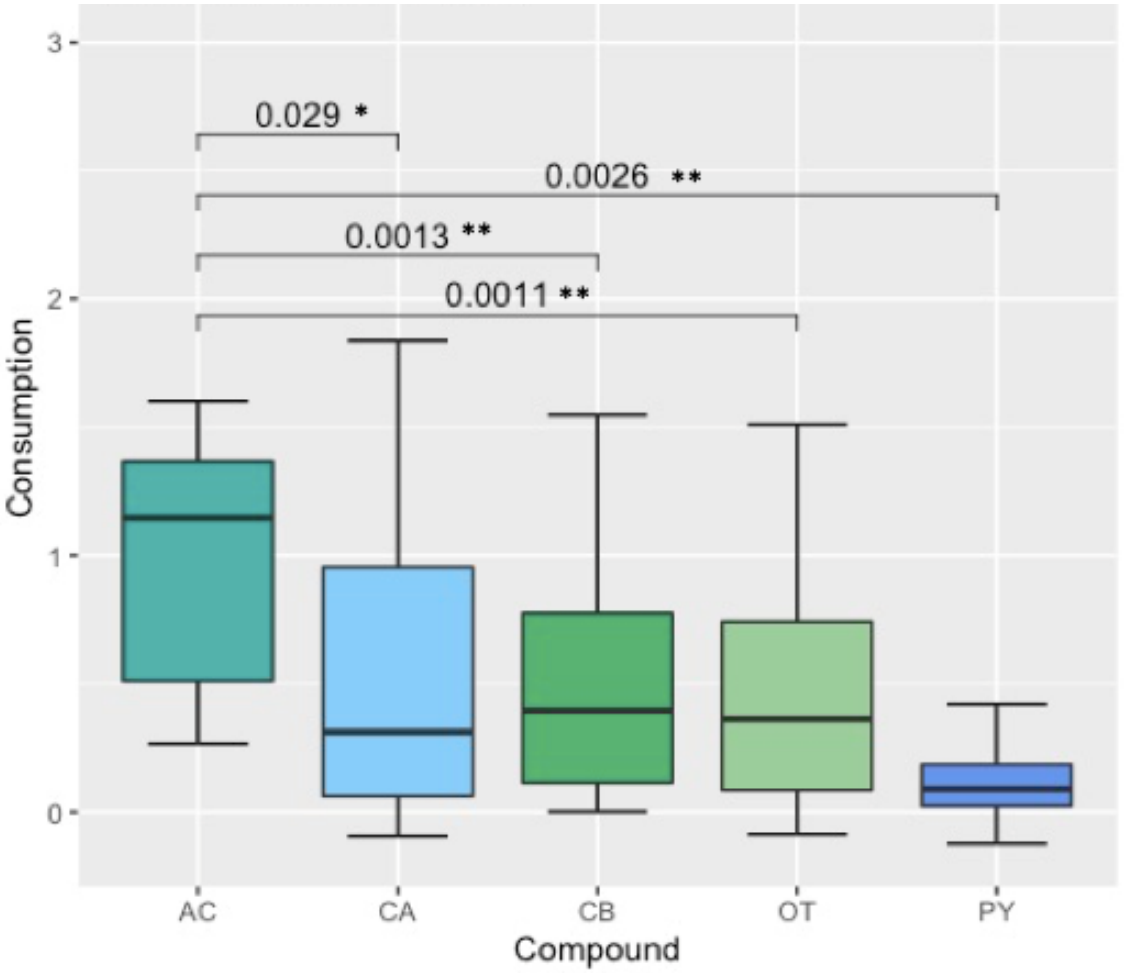

Figure 12. The consumption of various compounds measured as absorption (OD) of *R. mucilaginosa* at a wavelength of 490nm. The compounds are: AC-amino acids, CA-carboxylic acids, CB-carbohydrates, OT- others, PY- polymers. The statistical significance is indicated by the p-values (* = p ≤0.05, ** = p ≤0.01, *** = p ≤0.001).

Sarocladium kiliense

Phylogenetic analysis

The fungal culture we named Replicate 15 was classified as *S. kiliense* also strongly supported through a bootstrap value of 100 (Fig. 13).

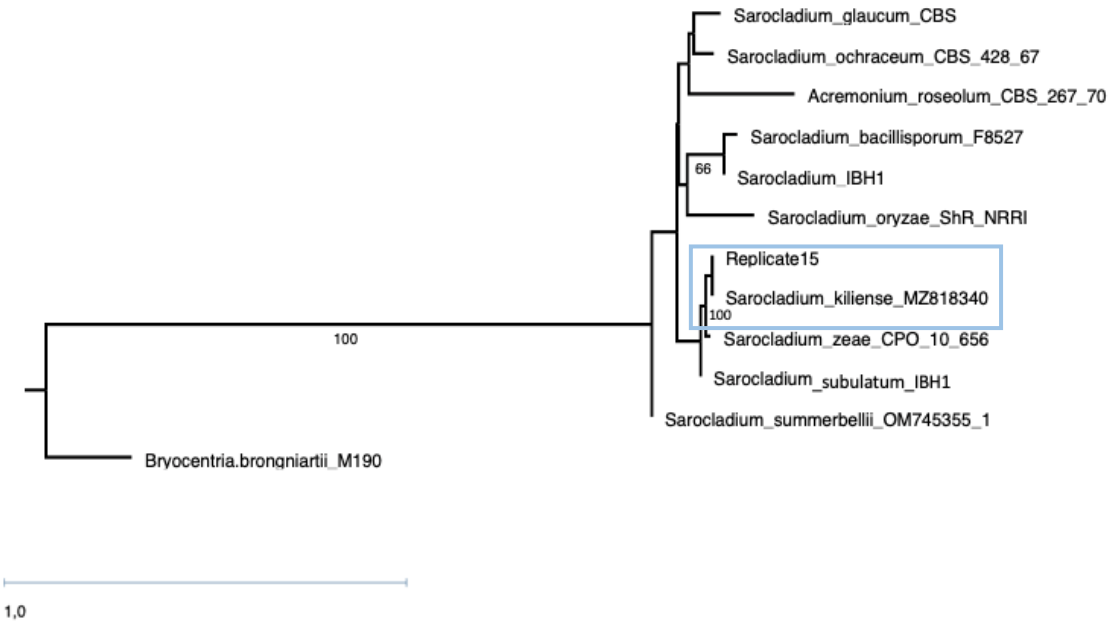

Figure 13. Phylogenetic relationships represented as RAxML tree. Replicate 15 was identified as S. kiliense and is shown with related taxa based on the nucleotide sequences of the combined ITS, LSU, SSU, RPB2 and TUB region. The bootstrap values for maximum-likelihood (ML) are indicated by the numbers under the branches. The tree is rooted to Bryocentria brongniartii (M190).

This taxon was first described as *Acremonium kiliense* by Grütz in 1925. In 2011 it was reclassified as *Sarocladium kiliense* by Summerbell. *S. kiliense* belongs to the Divison of Ascomycota. This fungi is classified in the Order of Hypocreales which lays inside the Class of Sordariomycetes (Summerbell et al. 2011). This taxon appears worldwide, however it has been mainly isolated from soils. This species has been isolated from very alkaline environments with a pH of 10-12. It is described as being alkalotolerant meaning that it can occur in both alkaline and acidic environments (Bondarenk et al. 2019). Recently, *S. kilinese* has been detected to also occur in the deep sea at a depth of 5070 m (Fan et al. 2019). Additionally, this study also revealed that this fungi has a remarkable cytotoxicity against various cancer cells (Fan et al. 2019). Moreover a study from Li et al. (2019), where *S. kiliense* has been isolated from mangrove forests, also indicates that this species contains bioactive compounds that show cytotoxic activity. This taxon has been also reported as being an opportunistic human pathogen and is therefore causing a wide range of human infections (Perdomo et al. 2011). Thus, this species is of great medical interest in matters of fungi induced infections as well as in cancer research.

Macroscopic morphology

S. kiliense is a hyphae that exhibits relatively rapid growth and develops fairly flat colonies that can slightly raise in the center. After 1 week of incubation the colony is white to cream (Fig. 14; A) and changes its appearance after around 1 month of incubation to a yellowish to light orange tone (Fig. 14; B). Apart from the color this taxon shows wrinkled and furry structures on the plate.

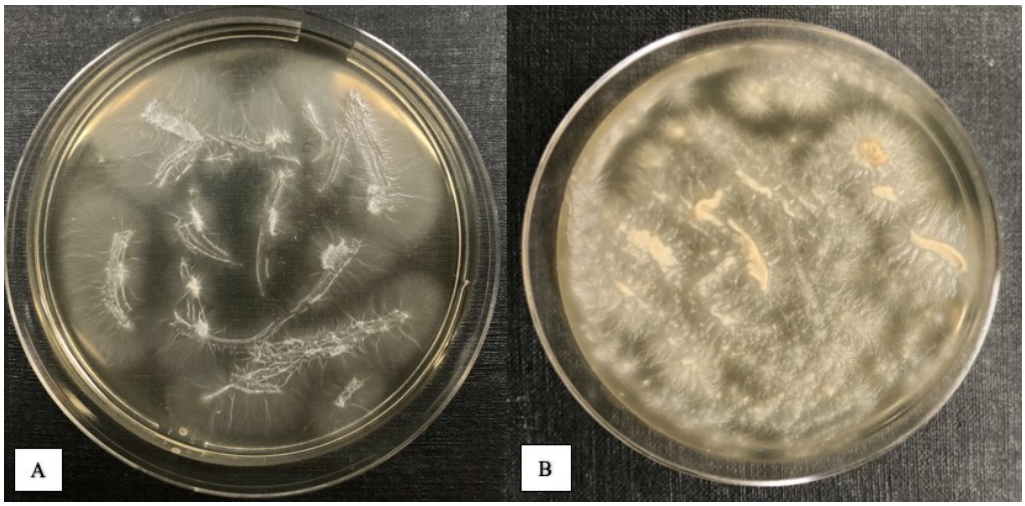

Figure 14. Representative images of the morphology of *Sarocladium kiliense* on an agar plate A) a colony that is 1 week old B) a colony that 4 weeks old

Microscopic morphology

The most common asexual reproductive spores of *S. kiliense* are called conidia and emerge from conidiophores or hyphae. Conidia can be found accumulated around the tip of hyphae (Fig. 15; E,F). However, they can also appear dispersed (Fig. 15; C,D) and are also found in slimy masses (Fig. 15; A, B). Single-celled conidia are shaped either oval or ellipsoidal (Fig. 15; C, D) with an average size of 3-5 x 2-3 um.

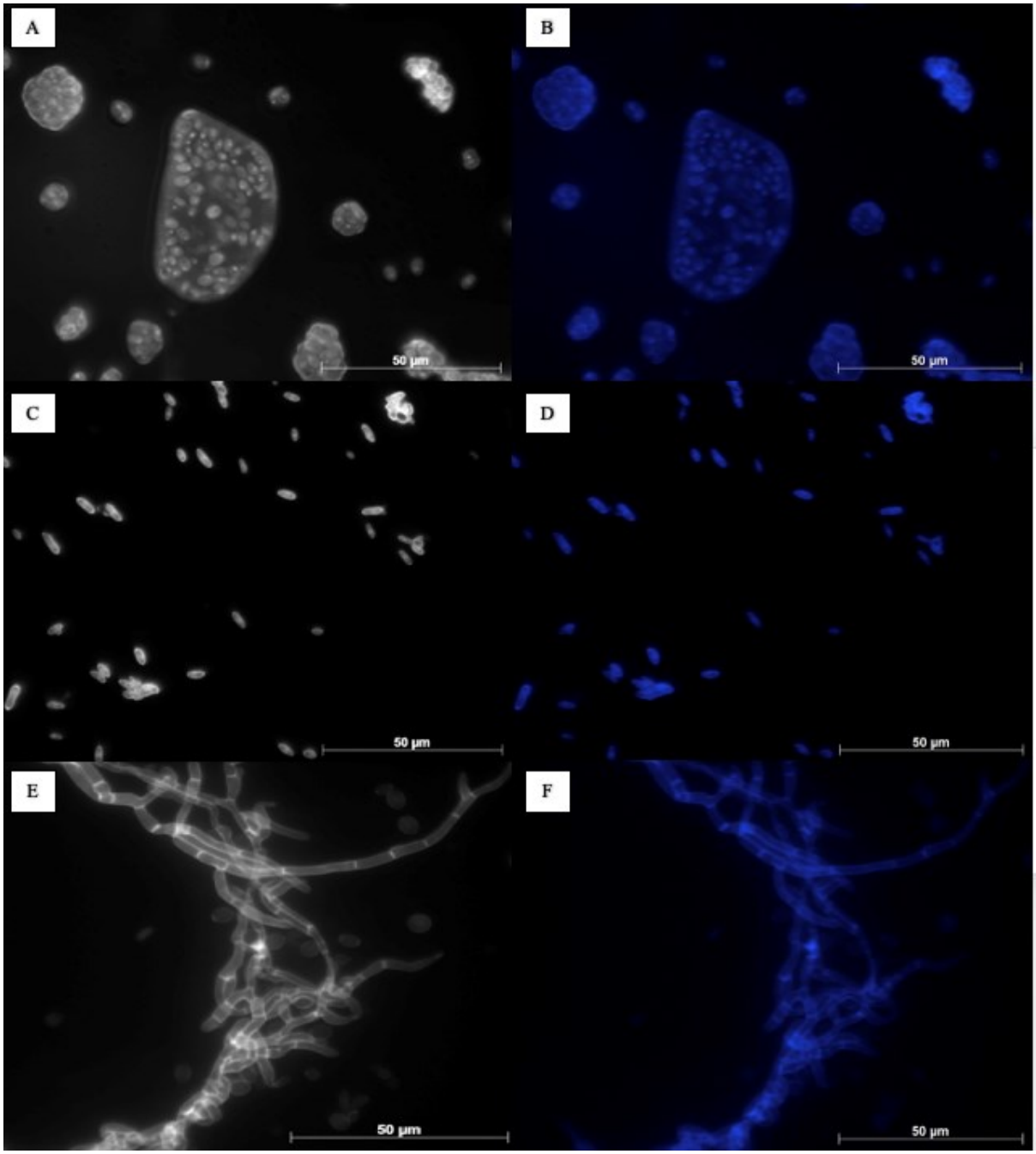

Figure 15. Fluorescent microscopical images of *S. kiliense* under UV-light below 400 nm; Samples were taken from liquid cultures in the exponential growth phase. A,B) Conidia in slimy heads of *S. kiliense* stained with the DAPI mix, making the nucleus visible; C, D) ellipsoidal conidia stained with DAPI mix making the nucleus visible; E, F) Hyphae entangled and some free, single celled conidia stained with calcofluor making the cell wall visible

Physiological tests in liquid media

Influence of temperature on fungal growth

The highest growth yield was reached at room temperature (Fig. 16). There, *S. kiliense* reached the exponential phase with an OD of 0.66 on the second day of incubation (Fig. 16). On day 3 the stationary phase was reached with an OD of 1.38. Until day 14 the OD still increased until an OD of 1.54 (Fig. 16). The treatment at 35°C showed a similar growth curve, reaching the exponential phase on day 2 (Fig. 16). The values increased until the stationary phase was reached on day 10 (Fig. 16). The OD values increased until day 14 to an OD of 1.21 (Fig. 16). The lowest growth yield was obtained at 5°C (Fig. 16). The exponential phase was reached on the seventh day of incubation (OD 0.45), and the stationary phase was reached on day 14 (Fig. 16).

According to a study of Summerbell et al. (2011) colonies of *S. kiliense* show an enhanced growth under 30°C compared to 20°C. Here, we found that the highest growth was observed at room temperature, which was around 23°C. Although in this case the highest growth yield were not measured at the highest temperature, yet, the cultures incubated at 35°C exhibited a higher growth than the ones at 5°C (Fig. 16).

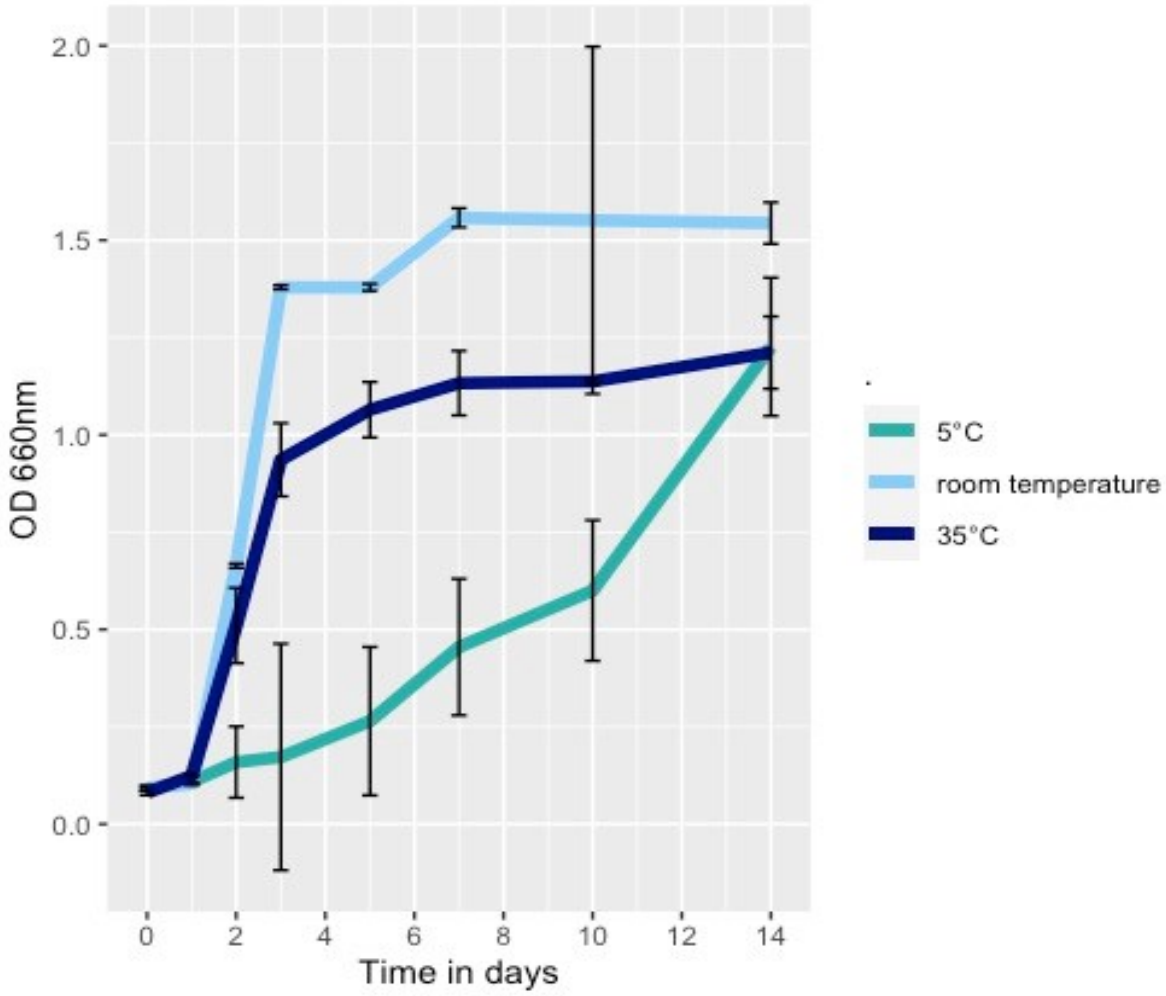

Figure 16. Optical density (OD) at a wavelength of 660 nm of *S. kiliense* on each day. The three different temperatures measured are 35°C, room temperature (ca. 23°C) and 5°C. Erros bars indicate the standard deviation (SD).

Influence of salinity on fungal growth

S. kiliense did not exhibit strong differences between salinity treatments (Fig. 17). The fungal cultures in all treatments reached the exponential phase on the second day of incubation (OD-0 g/L: 0.69; OD-20 g/L: 0.59; OD-35 g/L: 0.67; OD-40 g/L: 0.68), and already on the third day all reached the stationary phase (OD-0 g/L: 1.35; OD-20 g/L: 1.29; OD-35 g/L: 1.37; OD-40 g/L: 1.29) (Fig. 17). This lack of strong sensitivity of *S. kiliense* to salinity is consistent with the widespread nature of this fungi, which has been recovered from marine habitats (Fan et al. 2019, Perdomo et al. 2011) as well as in freshwater environments such as extremely alkaline lakes (Bondarenk et al. 2019).

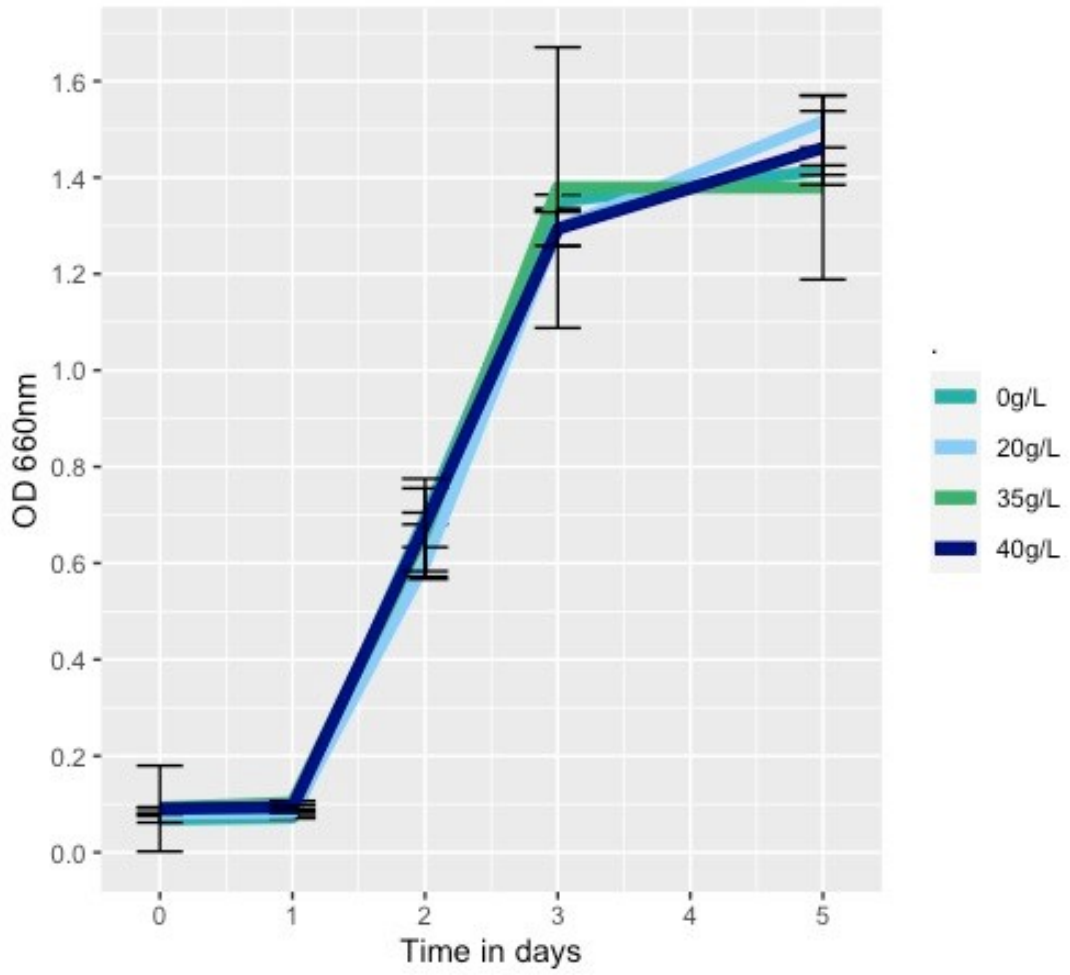

Figure 17. Optical density (OD) at a wavelength of 660 nm of *S. kiliense* on each day. The four different salt concentrations measured are 0, 20, 35 and 40 g/L. Erros bars indicate the standard deviation (SD).

Metabolic activity (FF-Plates)

All of the 95 organic compounds (Table 6) were metabolized by *S. kiliense.* Significant differences were found between the utilization of amino acids and carboxylic acids (p= 0.022), amino acids and other compounds (p=0.011), amino acids and polymers (p=0.026), carbohydrates and polymers (p=0.017) and other compounds and polymers (p=0.025) (Fig. 15). The most utilized substrate group by *S. kiliense* was found was the amino acids (AC) with a median OD of 1.8 ± 0.2 (Fig. 18). This is in line with the findings of other studies where amino acids are indicated as being one of the most important nutritional sources for fungi (Nishida et al. 2016). The second highest utilized group of substrates was the carbohydrates with a median OD of 1.76 ± 0.54 (Fig. 18). It was noted that species belonging to this genus efficiently degrade carbohydrates such as polysaccharides, pectin, and precisely *S. kiliense* was shown to exhibit high degradation rates of starch (Summerbell et al. 2011). The category of other compounds (OT) had a median of 1.55 ± 0.31 (Fig. 18). Carboxylic acids showed a median of their consumption of 1.47 ± 0.46 (Fig. 18). Interestingly, *S. kiliense* reached the highest utilization rates (OD values) for all investigated substrate groups compared to *S. spartinae* (Fig. 6) and *R. mucilaginosa* (Fig. 12), indicating that this fungi might present higher rates of carbon cycling than the other fungi strains in the environment.

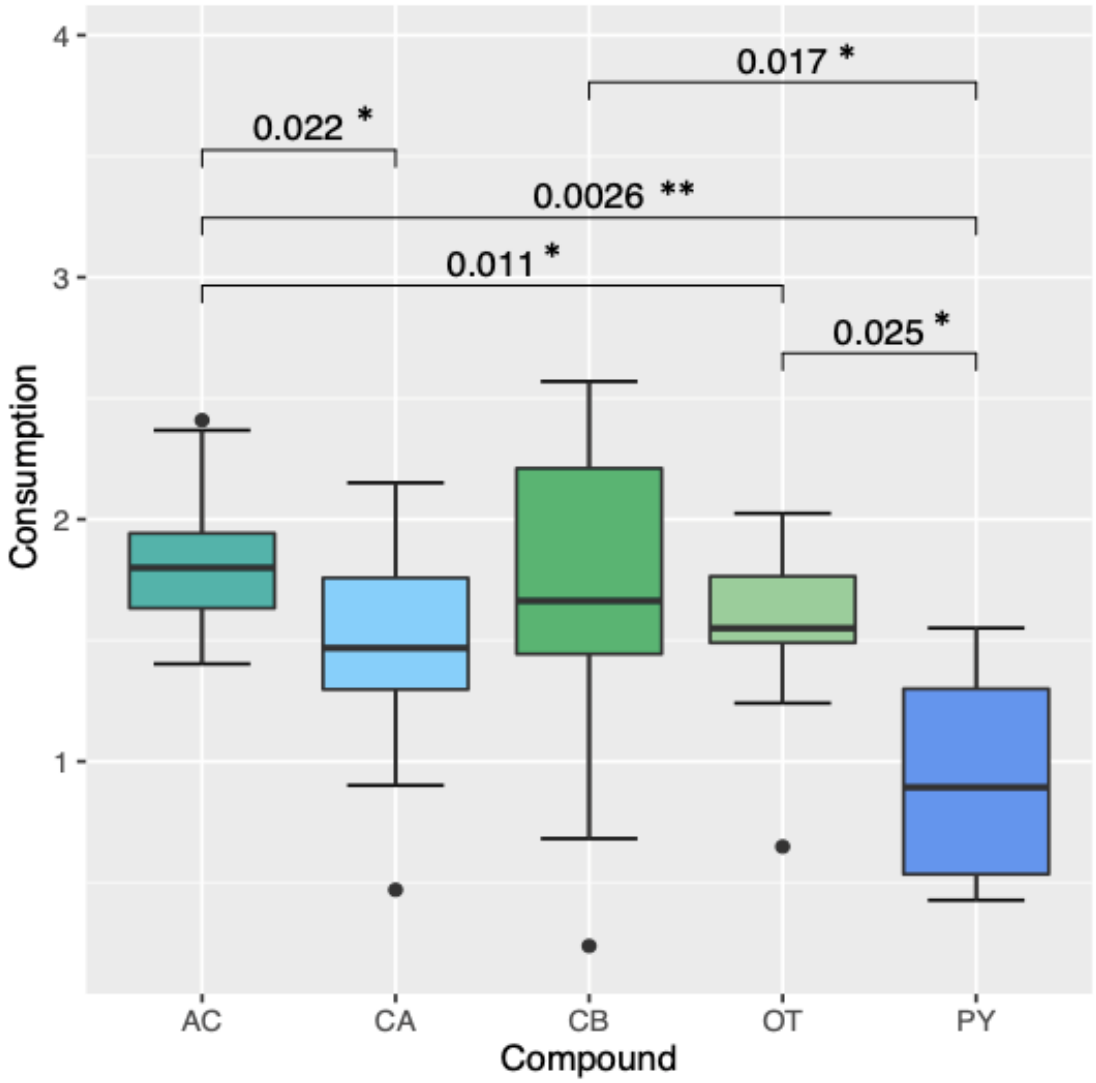

Figure 18. The consumption of various compounds measured as absorption (OD) of *S. kiliense* at a wavelength of 490nm. The compounds are: AC-amino acids, CA-carboxylic acids, CB- carbohydrates, OT- others, PY- polymers. The statistical significance is indicated by the p-values (*= P ≤0.05, **= p ≤0.01, ***= p≤0.001).

Conclusion

We isolated and characterized four fungal strains from the open Atlantic Ocean. We managed to phylogenetically identify these fungi down to the species level by the combination of different set of gene regions. Identifying fungi only morphologically is a very limited approach, since the morphological appearance can depend on various factors. Despite the strong taxonomical differentiation between our fungal strains (belonging to different Divisions: Ascomycetes and Basidiomycetes), we found that all the marine pelagic fungi examined in this study share some physiological characteristic. For instance, all fungal isolates grow faster and reached higher growth yields under 23 °C (the temperature closer to the *in situ* conditions where the fungi were isolated from), compared to the other temperatures. Yet, *R.*

mucilaginosa and *S. kiliense* were still able to grow moderately well at 5°C, whereas *S. spartinae* did not reach the exponential phase during our incubations. Clearly, fungi respond to differences in temperature suggesting that shifts in temperature through climate change could potentially have unpredictable impacts on marine fungal communities. Furthermore, all studied species seem to be very tolerant regarding the salinity concentration of their growth medium (much wider than the salinity range generally observed in the open ocean waters), indicating a remarkable capability for pelagic oceanic fungi to thrive in a vast range of salinity. Still, for *R. mucilaginosa* and *S. spartiane* the highest growth yields were achieved in the media with the concentration of 35g/L, which is the closest to the *in situ* oceanic salinities. Concerning the metabolic activity, all three fungi exhibited the highest utilization rates for the amino acids group. The lowest substrate consumption investigated was associated with polymers. We also found that the uptake of these two substrate types "amino acids" and "polymers" was significantly different from each other for all three fungal species. Yet, our data revealed that the utilization of carbohydrates was about 4 times higher in *S. kiliense* than in *S. spartinae* and *R. mucilaginosa*, indicating potential strong differences in the cycling of carbon between the different pelagic taxa.

As mentioned above marine fungi are contributing in a lot of different ways to a healthy and smoothly running ecosystem and their importance is not only limited by their ecological roles. Hence, we emphasize to put the spotlight on characterizing marine pelagic fungi on a species level to better understand the role of the individual fungal members, populations and communities in the marine ecosystem. For future studies on marine mycological taxonomy and marine microbial ecology the main objective should be to establish a standardized set of primer pairs that is used for identifying marine fungi. This is necessary to enable further research and develop new approaches for using marine fungi medically and study their ecological roles.

Zusammenfassung

Aktuellen Schätzungen zufolge gibt es mehr als 10.000 marine Pilzarten. Sie sind wesentlich daran beteiligt organisches Material zu zersetzen, wodurch sie wichtige Bestandteile mariner Nahrungsketten sind. In den letzten 72 Jahren sind jedoch nur 1901 Arten dokumentiert worden. Somit bleiben die taxonomische Vielfalt und die ökologischen Funktionen von marinen Pilzen weitgehend unerforscht. Üblicherweise wurden Pilze nur nach ihren morphologischen Charakteristiken beschrieben. Dies führt nicht zu einer tatsächlichen Repräsentation der Artenvielfalt. Eine vollständige Dokumentation der Arten kann nur erfolgen, wenn marine Pilze genetisch analysiert und zusätzlich auf Platten kultiviert werden. Eine solche Dokumentation bildet die Grundlage für die Forschung an marinen Pilzen. In dieser Studie wurden pelagische marine Pilze entlang eines Transekts durch den Atlantik in verschiedenen Tiefen gesammelt und danach genetisch identifiziert. Darüber hinaus wurden alle Pilzkulturen für physiologische Tests herangezogen, welche in einem Flüssigmedium mit verschiedenem Salzgehalt oder Temperaturen untersucht wurden. Zusätzlich wurden metabolische Experimente durchgeführt, um die präferierten Substrate der Pilze zu eruieren. Wir konnten die Hefepilze *Rhodoturula mucilaginosa* und *Scheffersomyces spartinae* und die Hyphe *Sarocladium kiliense* charakterisieren. Diese Studie bietet relevante Informationen über die physiologischen, genetischen und ökologischen Eigenschaften von pelagischen Pilzen und trägt dazu bei, deren Ökologie und Verbreitung in der Wassersäule zu verstehen.

Literature

Amend, A., G. Burgaud, M. Cunliffe, V. P. Edgcomb, C. L. Ettinger, M. H. Gutierrez, J. Heitman,E. F. Y. Hom, G. Ianiri, A. C. Jones, M. Kagami, K. T. Picard, C. A. Quandt, S. Raghukumar, M. Riquelme, J. Stajich, J. Vargas-Muniz, A. K. Walker, O. Yarden & A. S. Gladfelter (2019) Fungi in the Marine Environment: Open Questions and Unsolved Problems. mBio, 10.

Anatoliotaki, M., E. Mantadakis, E. Galanakis & G. Samonis (2003) Rhodotorula species fungemia: a threat to the immunocompromised host. Clin Lab, 49, 49-55.

Arnosti, C., M. Wietz, T. Brinkhoff, J. H. Hehemann, D. Probandt, L. Zeugner & R. Amann (2020) The Biogeochemistry of Marine Polysaccharides: Sources, Inventories, and Bacterial Drivers of the Carbohydrate Cycle. Ann Rev Mar Sci.

Baltar, F., Z. Zhao & G. J. Herndl (2021) Potential and expression of carbohydrate utilization by marine fungi in the global ocean. Microbiome, 9, 106.

Bochdansky, A. B. (2017) Eukaryotic microbes, principally fungi and labyrinthulomycetes, dominate biomass on bathypelagic marine snow. ISME J, 11, 362-373.

Bondarenko, S.A., M.L. Georgieva, L.Y. Kokaeva, E.N. Bilanenko (2019) The First Discovery of Alkali-Resistant Fungi on the Coast of Chloride Lake Baskunchak. Moscow Univ. Biol.Sci. Bull.74, 57–62

Bucher, V., K. Hyde, S. Pointing, and C. Reddy (2004) Production of wood decay enzymes, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs. Fungal Div, 15, 1–14.

Butinar, L., I. Spencer-Martins & N. Gunde-Cimerman (2007) Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. Antonie Van Leeuwenhoek, 91, 277-89.

de Almeida, J. M. (2005) Yeast community survey in the Tagus estuary. FEMS Microbiol Ecol, 53, 295-303.

Fan, S. Q., C. L. Xie, J. M. Xia, C. P. Xing, Z. H. Luo, Z. Shao, X. J. Yan, S. He & X. W. Yang (2019) Sarocladione, a unique 5,10:8,9-diseco-steroid from the deep-sea-derived fungus Sarocladium kiliense. Org Biomol Chem, 17, 5925-5928.

Fiebig D.M.(1992) Fates of dissolved free amino acids in groundwater discharged through stream bed sediments. Hydrobiologia 235/236, 311–319.

Frigon, M. D. & D. Liu (2016) Effect of high salinity on yeast activated sludge reactor operation. Water Sci Technol, 74, 2124-2134.

Fung, H. B., C. A. Martyn, A. Shahidi & S. T. Brown (2009) Rhodotorula mucilaginosa lymphadenitis in an HIV-infected patient. Int J Infect Dis, 13, e27-9.

Gadanho, M., D. Libkind & J. P. Sampaio (2006) Yeast diversity in the extreme acidic environments of the Iberian Pyrite Belt. Microb Ecol, 52, 552-63.

Gadanho, M. & J. P. Sampaio (2005) Occurrence and diversity of yeasts in the mid-atlantic ridge hydrothermal fields near the Azores Archipelago. Microb Ecol, 50, 408-17.

Gartshore, J., D. G. Cooper & J. A. Nicell (2003) Biodegradation of plasticizers by Rhodotorula rubra. Environ Toxicol Chem, 22, 1244-51.

Gillings, M. R., M. P. Holley and M. Selleck (2006) Molecular identification of species comprising an unusual biofilm from a groundwater treatment plant. Biofilms 3, 19–24.

Grossart, H. P., S. Van den Wyngaert, M. Kagami, C. Wurzbacher, M. Cunliffe & K. Rojas-Jimenez (2019) Fungi in aquatic ecosystems. Nat Rev Microbiol, 17, 339-354.

Hawksworth, D. L. & R. Lucking (2017) Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiol Spectr, 5.

Harrison, F.C. (1928) Rhodoturula mucilaginosa. The yeasts: A Taxonomic Study, 155.32, 1913.

Hassett, B.T., T. R. Vonnahme, P. Xuefeng, E. B. G. Jones, and C. Heuzé (2020) Global diversity and geography of planktonic marine fungi. *Botanica Marina*, 63,121-139.

Hibbett, D. S., M. Binder, J. F. Bischoff, M. Blackwell, P. F. Cannon, O. E. Eriksson, S. Huhndorf, T. James, P. M. Kirk, R. Lucking, H. Thorsten Lumbsch, F. Lutzoni, P. B. Matheny, D. J. McLaughlin, M. J. Powell, S. Redhead, C. L. Schoch, J. W. Spatafora, J. A. Stalpers, R. Vilgalys, M. C. Aime, A. Aptroot, R. Bauer, D. Begerow, G. L. Benny, L. A. Castlebury, P. W. Crous, Y. C. Dai, W. Gams, D. M. Geiser, G. W. Griffith, C. Gueidan, D. L. Hawksworth, G. Hestmark, K. Hosaka, R. A. Humber, K. D. Hyde, J. E. Ironside, U. Koljalg, C. P. Kurtzman, K. H. Larsson, R. Lichtwardt, J. Longcore, J. Miadlikowska, A. Miller, J. M. Moncalvo, S. Mozley-Standridge, F. Oberwinkler, E. Parmasto, V. Reeb, J. D. Rogers, C. Roux, L. Ryvarden, J. P. Sampaio, A. Schussler, J. Sugiyama, R. G. Thorn, L. Tibell, W. A. Untereiner, C. Walker, Z. Wang, A. Weir, M. Weiss, M. M. White, K. Winka, Y. J. Yao & N. Zhang (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res, 111, 509-47.

Inui T., Y. Takeda, H. Iizuka (1965) Taxonomical studies on genus Rhizopus. J Gen Appl Microbiol, 11,1–121.

Islam, M. R., P. S. Chauhan, Y. Kim, M. Kim, and T. Sa (2011) Community level functional diversity and enzyme activities in paddy soils under dierent long-term fertilizer management practices. Biol. Fertil. Soils 47, 599–604.

Jones, M. D., I. Forn, C. Gadelha, M. J. Egan, D. Bass, R. Massana & T. A. Richards (2011) Discovery of novel intermediate forms redefines the fungal tree of life. Nature, 474, 200-3.

Jones, E. B. G., S. Suetrong, A. H. Bahkali, M. A. Abdel-Wahab (2015) Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. Fungal Diversity, 73, 1–72

Jones, G. E. B., K.L. Pang, M. A., Abdel-Wahab, B. Scholz, K. D. Hyde, T. Boekhout, R. Ebel, M. E. Rateb, L. Henderson, J. Sakayaroj, S. Suetrong, M. C. Dayarathne, V. Kumar, S. Raghukumar, K. R. Sridhar, A. H. A. Bahkali, F.H. Gleason, C. Norphanphoun (2019) An online resource for marine fungi. Fungal Diversity, 96, 347–433.

Kagami, M., Y. Amano & N. Ishii (2012) Community structure of planktonic fungi and the impact of parasitic chytrids on phytoplankton in Lake Inba, Japan. Microb Ecol, 63, 358-68.

Kohlmeyer, J., and E. Kohlmeyer (1979c) 18 - Fungi in Animal Substrates. in Marine Mycology, eds J. Kohlmeyer and E. Kohlmeyer (Cambridge, MA: Academic Press), 137–142.

Kurtzman, C.P., M. Suzuki (2010) Scheffersomyces. The yeasts: A Taxonomic Study, 65, 775.

Kutty, S. N. & R. Philip (2008) Marine yeasts-a review. Yeast, 25, 465-83.

Li, F., W. Guo, L. Wu, T. Zhu, Q. Gu, D. Li & Q. Che (2019) Correction to: Saroclazines A-C, thio-diketopiperazines from mangrove-derived fungi Sarocladium kiliense HDN11-84. Arch Pharm Res, 42, 376.

Libkind, D., S. Brizzio, A. Ruffini, M. Gadanho, M. van Broock & J. Paulo Sampaio (2003) Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. Antonie Van Leeuwenhoek, 84, 313-22

Morales, S.E., A. Biswas, G. J. Herndl, F. Baltar (2019) Global Structuring of Phylogenetic and Functional Diversity of Pelagic Fungi by Depth and Temperature. *Front. Mar. Sci.*, *6*, 131.

Mueller G. M., J. P. Schmit (2007) Fungal biodiversity: What do we know? What can we predict? Biodiversity Conservation, 16, 1–5

Nakamura,T., T. Murakami, M. Saotome, K. Tomita, T. Kitsuwa and S. P. Meyers (1991) Identification of Indole-3-Acetic Acid in Pichia spartinae, an Ascosporogenous Yeast from Spartina alterniflora Marshland Environments. Mycologia , 83, 662-664.

Newman, D. J., G. M. J. Cragg (2007) Nat. Prod. 70, 461−477.

Nilsson, R. H., S. Anslan, M. Bahram, C. Wurzbacher, P. Baldrian & L. Tedersoo (2019) Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol, 17, 95-109.

Nishida, I., D. Watanabe, A. Tsolmonbaatar, T. Kaino, I. Ohtsu & H. Takagi (2016) Vacuolar amino acid transporters upregulated by exogenous proline and involved in cellular localization of proline in Saccharomyces cerevisiae. J Gen Appl Microbiol, 62, 132-9.

Perdomo, H., D. A. Sutton, D. Garcia, A. W. Fothergill, J. Cano, J. Gene, R. C. Summerbell, M. G. Rinaldi & J. Guarro (2011) Spectrum of clinically relevant Acremonium species in the United States. J Clin Microbiol, 49, 243-56.

Perniola, R., M. L. Faneschi, E. Manso, M. Pizzolante, A. Rizzo, A. Sticchi Damiani & R. Longo (2006) Rhodotorula mucilaginosa outbreak in neonatal intensive care unit: microbiological features, clinical presentation, and analysis of related variables. Eur J Clin Microbiol Infect Dis, 25, 193-6.

Priest, T., B. Fuchs, R. Amann & M. Reich (2021) Diversity and biomass dynamics of unicellular marine fungi during a spring phytoplankton bloom. Environ Microbiol, 23, 448-463.

Raghukumar S. (2004) The role of fungi in marine detrital processes. In Marine Microbiology: Facets and Opportunities, Ramaiah, 91–101.

Raja, H. A., A. N. Miller, C. J. Pearce & N. H. Oberlies (2017) Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. J Nat Prod, 80, 756-770.

Richards, T. A., M. D. M. Jones, G. Leonard, and D. Bass (2012) Marine Fungi: Their Ecology and Molecular Diversity. Annual Review of Marine Science, Vol. 4, 495-522.

Richards, T. A., G. Leonard, F. Mahe, J. Del Campo, S. Romac, M. D. Jones, F. Maguire, M. Dunthorn, C. De Vargas, R. Massana & A. Chambouvet (2015) Molecular diversity and distribution of marine fungi across 130 European environmental samples. Proc Biol Sci, 282.

Shi, Y., Z. Yang, L. Xing, X. Zhang, X. Li & D. Zhang (2021) Recent advances in the biodegradation of azo dyes.World J Microbiol Biotechnol, 37, 137.

Smith, D., M. J. Ryan (2009) Fungal sources for new drug discovery. McGraw-Hill Yearbook of Science and Technology, 131-133.

Stief, P., S. Fuchs-Ocklenburg, A. Kamp, C. S. Manohar, J. Houbraken, T. Boekhout, D. de Beer & T. Stoeck (2014) Dissimilatory nitrate reduction by Aspergillus terreus isolated from the seasonal oxygen minimum zone in the Arabian Sea. BMC Microbiol, 14, 35.

Summerbell, R. C., C. Gueidan, H. J. Schroers, G. S. de Hoog, M. Starink, Y. A. Rosete, J. Guarro & J. A. Scott (2011) Acremonium phylogenetic overview and revision of Gliomastix, Sarocladium, and Trichothecium. Stud Mycol, 68, 139-62.

Szabo, L. J. & W. R. Bushnell (2001) Hidden robbers: the role of fungal haustoria in parasitism of plants. Proc Natl Acad Sci U S A, 98, 7654-5.

Tan, L., M. He, L. Song, X. Fu & S. Shi (2016) Aerobic decolorization, degradation and detoxification of azo dyes by a newly isolated salt-tolerant yeast Scheffersomyces spartinae TLHS-SF1. Bioresour Technol, 203, 287-94.

Tauber, M. M., G. M. Gubitz & A. Rehorek (2008) Degradation of azo dyes by oxidative processes--laccase and ultrasound treatment. Bioresour Technol, 99, 4213-20.

Vetrovsky, T., M. Kolarik, L. Zifcakova, T. Zelenka & P. Baldrian (2016) The rpb2 gene represents a viable alternative molecular marker for the analysis of environmental fungal communities. Mol Ecol Resour, 16, 388-401.

Vu, D., M. Groenewald, M. de Vries, T. Gehrmann, B. Stielow, U. Eberhardt, A. Al-Hatmi, J. Z. Groenewald, G. Cardinali, J. Houbraken, T. Boekhout, P. W. Crous, V. Robert & G. J. M. Verkley (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol, 92, 135-154.

Wang, Y., D. Barth, A. Tamminen & M. G. Wiebe (2016) Growth of marine fungi on polymeric substrates. BMC Biotechnol, 16, 3.

Zeghal, E., A. Vaksmaa, H. Vielfaure, T. Boekhout and H. Niemann (2021) The Potential Role of Marine Fungi in Plastic Degradation – A Review. Frontiers Marine Science, 8, 738877.